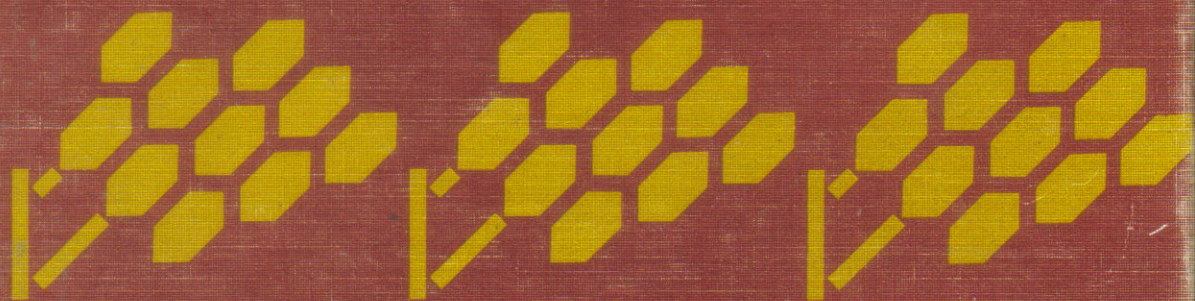


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# Developments in Agricultural Engineering 1



## Controlled Atmosphere Storage of Grains

J. Shejbal (editor)

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Elsevier



Developments in Agricultural Engineering, 1

# **Controlled Atmosphere Storage of Grains**



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# Controlled Atmosphere Storage of Grains

An International Symposium held from 12 to 15 May 1980  
at Castelgandolfo (Rome) Italy

edited by

**J. SHEJBAL**

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## PREFACE

The International Symposium on Controlled Atmosphere Storage of Grains was held at Castelgandolfo, near Rome, Italy, from 12th to 15th May 1980. It was organized by ASSORENI (Association of ENI Companies for Scientific Research) and co-sponsored by FAO (Food and Agriculture Organization of the United Nations), ICC (International Association for Cereal Chemistry) and the Italian Ministry of Foreign Affairs. Nearly one hundred participants from 26 countries were present.

The Symposium was characterized, (as Dr. Calverley put it in his final address on behalf of the participants) "by the absence of the professional convention attendants and, on the contrary, by the attendance of those people who can bring experience and erudition to the discussion of the controlled atmosphere storage technology". It was the first international symposium ever held on this particular subject and therefore the whole breadth of the field of grain storage in controlled atmospheres was included in the six sessions, from naturally produced oxygen-poor atmospheres in underground pits to sophisticated automatic inert gas industrial storage facilities.

Already during this Symposium, several participants called for further meetings on some of the more problem-loaded aspects of modern controlled atmosphere storage and disinfestation of grains: gas-tight facilities, sealing practices, inert gas production, high moisture grain storage. I do hope that the ASSORENI initiative in organizing this meeting will therefore be followed elsewhere in the world to permit a further diffusion of this environment-friendly, economical and safe preservation technique in modern storage facilities.

I should like to acknowledge the important support given to me by Prof. E. Cernia, President of ASSORENI, and Dr. G. Nencini, Vice-Director of the Basic Research Laboratory of ASSORENI, during



the organization of the Symposium.

Thanks are due to Mr. G.G. Corbett and Dr. H.R. Shuyler of FAO for the many suggestions during the preparation of the meeting and to Harlan and Sedley Shuyler for proof-reading of the Session 7 manuscript.

The Symposium was made possible by financing by ASSORENI and the Italian Ministry of Foreign Affairs made a grant for the participation of some representatives from Developing Countries.

The contributions given to the organization of the meeting and the typing of the Proceedings by Miss Marilina Frondaroli, Miss Serenella Gambini, Mrs. Maria Grazia Nobili and Miss Emanuela Soldati of ASSORENI were invaluable.

The Proceedings volume follows the lay-out of the Symposium itself and the 7 chapters correspond to the 6 Sessions of original papers and the Round Table Discussion Session.

The papers are reproduced by off-set, direct photographic reproduction of the manuscripts, as they were submitted by the authors for publication. The wrap-up reports were retyped from the rapporteurs' manuscripts and the Round Table Discussion was completely transcribed from a tape recording.

Finally, I should like to thank my wife and my sons, Mira, Martin and Christian, for their understanding during the many months in which they saw so much less of me than usual.

Rome, July 1980

Jindřich Shejbal



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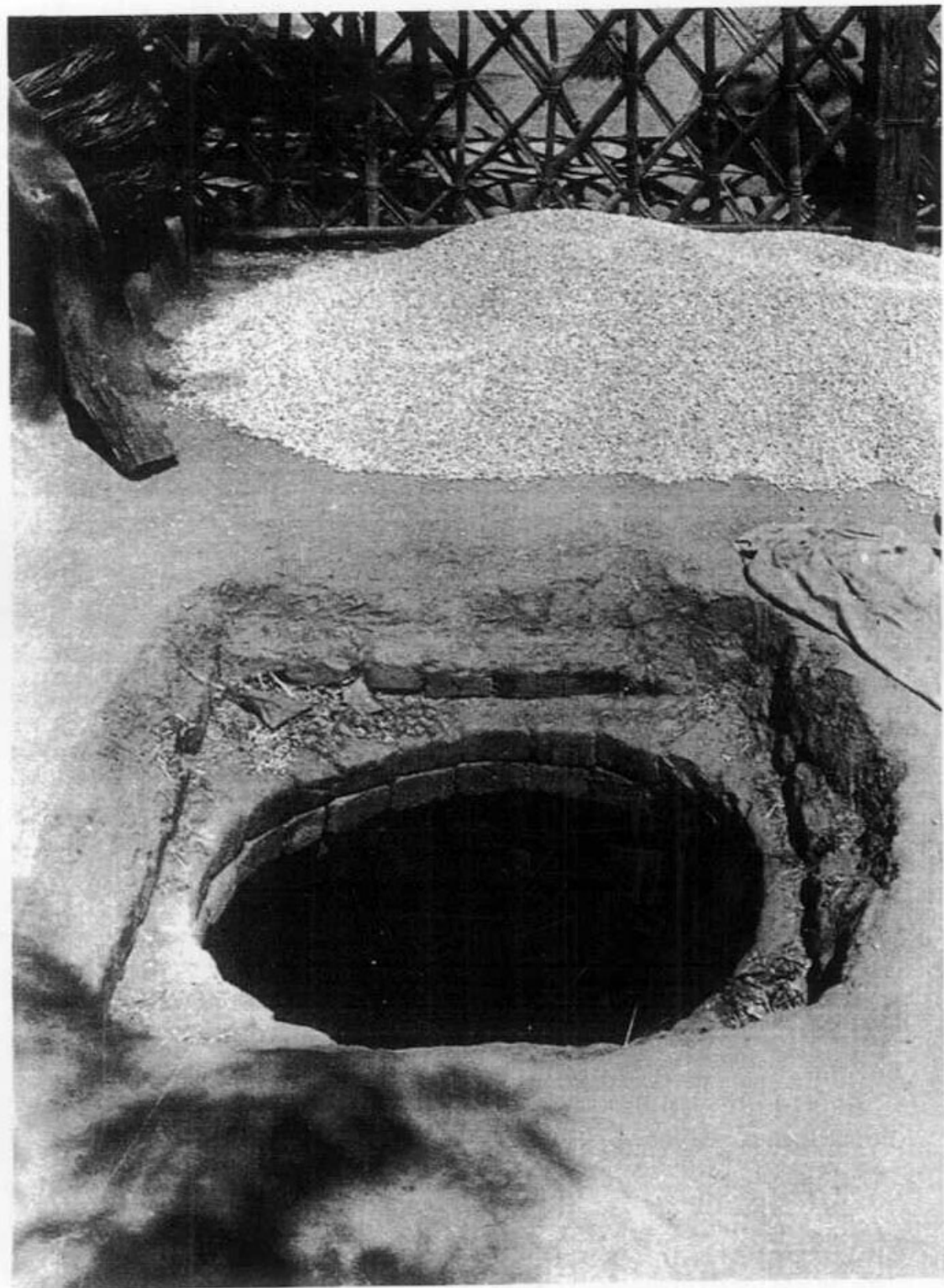
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I S E S S I O N :

NATURAL AIR-TIGHT STORAGE





## SIGNIFICANCE OF UNDERGROUND STORAGE IN TRADITIONAL SYSTEMS OF GRAIN PRODUCTION

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Until the early nineteenth century, Underground Grain Storage (UGS) has been one of the main methods of long-term preservation for large bulks of grain, if not the main one, in most cereal-growing societies. Its use was common throughout an immense area stretching from Spain and Morocco in the West, up to India and China in the East, including Southeast Europe (from Hungary to the Caucasus), South Arabia, etc. In addition, UGS was also known in East and South Africa (including Madagascar), and, before European settlement, in North America from Mexico to the Upper Missouri valley. A map of all the areas where UGS was currently used by 1800 would probably include more than a half of the grain-growing regions of the world. Conversely, only four large areas appear to have ignored UGS completely by that date, or with inconsiderable exceptions: Northwest Europe, Southeast Asia (including Austronesia and Japan), West and Central Africa, and South America. Yet in some of those areas, Britain and France for example, UGS had been important in former times, from at least the Late Neolithic to the Upper Middle Ages. To sum up all this, UGS has been, and still is in some areas, of so basic significance that it is difficult to understand how and why it was so largely neglected by both historians and technicians alike.

But is it possible to assess more precisely what was exactly this significance? And it is possible to use the relevant evidence for a better understanding of what this significance may become in the future? This paper aims at answering these two questions. Evidence for it has been gathered in the course of a research programme that we were able to launch late in 1975, owing to financial support by the CORDES (Comité des Recherches Appliquées sur le Développement Economique et Social). Since then, three conferences have been or are being organized in France on the history and technology of grain storage: Sénanque, March 1977; Arudy, June 1978; and Levroux, November 1980. Four publications have been or are being issued: Sigaut 1978; Gast and Sigaut (eds) 1979; Sigaut 1979; and Gast and Sigaut (eds) in press. This paper must be considered as a summary of all these publications.

## 1. HISTORICAL EVIDENCE AND SOURCES.

Evidence on grain storage in pre-industrial societies is to be found in four major kinds of sources: (1) archaeological reports; (2) travellers' accounts and

other similar writings; (3) monographs written by ethnographers, geographers, etc.; and (4) the technical literature, old and new. With the exception of a few short mentions by the Latin or Arab writers, very little information is to be found in the literature before the XVIth century.

### 1.1. Archaeology.

Until the Middle Ages, archaeology is practically our only source of information. Of course, the main problem facing archaeologists is the precise identification of the structures that have been used for grain storage. In the case of elevated granaries, this identification has to be made with the only help of post-holes patterns, and therefore has to remain largely conjectural. Evidence concerning underground structures, such as grain silos, is of course better preserved. However, silos are always or nearly always found empty, or filled only with earth or rubbish. Rubbish can be helpful to date them, but tells precious little on what had been their real use and purpose. Finds of underground silos still containing carbonized grain are quite rare. Fortunately, archaeologists are now developing new methods, such as experimental archaeology or micro-stratigraphy of the infill, that enable us to derive much more information from the preserved evidence than was formerly thought possible.

Underground grain silos are now known to have existed from pre-neolithic contexts in the Middle East (Natufian, 9000 to 7000 BC) and in neolithic Europe (from 4500 BC on). However, these older silos are essentially small and shallow pits with no specifically recognizable form, which makes difficult to differentiate them from pits dug for other purposes. Only with the advent of iron, it seems, does the "classic" underground silo appear: more than 2 m deep, bottle-formed with a narrow neck as entrance (man hole), and of at least 1 to 3 tonnes in capacity. There can be no doubt that these "bottle-" or "gourd-"silos were designed to be airtight. It is sometimes argued that they were in fact developed as an alternative for the big storage jars used in the Mediterranean. "Bottle" silos have been found by the hundreds on Iron Age settlements sites in Britain and Northeast France. In central and southern France, many more were used in medieval times; their use was discontinued between the XIth and the XVIth centuries, except in the Southwest (Haute-Garonne, Gers, Tarn-et-Garonne), where it survived till the XVIIIth century. In some parts of Italy (Tuscany, Apulia, Sicily...) and of Spain, UGS lived on well into the XIXth century.

### 1.2. Travellers' accounts and ethnographical evidence.

Written evidence begins to appear during the XVIth century. But detailed descriptions of UGS become available in significant numbers only from the XVIIIth century on. By the 1800s, a rough idea of its areal distribution and of the role of airtightness in grain preservation had been acquired by the most knowledgeable agri-

cultural writers of western Europe (in France and Italy at least, and probably also in Germany, Sweden, Switzerland, etc.; the British and Dutch do not seem to have been much interested).

The identification of airtightness as a determining factor of grain preservation is an important advance of the time. It was first explicitly made in 1708 by a writer named Reneaume, in one of the *Mémoires* issued by the French Académie Royale des Sciences in Paris. This realization was important, although it has already been done long before by the Roman writer Varro, living in the first century BC. The only thing that Reneaume added to Varro's was the result of his own observations on the loss of germinating power with time in stored seeds. Subsequent ethnographical literature only confirms how general was the awareness of airtightness as a factor of successful grain preservation in pre-industrial societies. Details given on the ways silos were dug out, lined, filled and stopped up, as well as on the danger of suffocation when entering them too soon when opened to be emptied (a burning lamp could be used as a means to detect "foul air"), show without a doubt that airtightness was consciously acknowledged and looked for by UGS users everywhere.

### 1.3. Experiments on UGS and AGS since 1819.

So, around 1800, the growing body of agricultural writers in Northwest Europe had acquired a roughly correct idea of UGS functioning essentially as AGS (Airtight Grain Storage). But this idea was still far from being precise enough to be operative. Local circumstances and skills making for successful long term preservation of grain underground were unsufficiently understood to be replicated elsewhere. The very few experiments attempted before 1800 were complete failures, as far as it is possible to tell. For the first experiments to be properly recorded began only in 1819. They were made by an industrialist named Ternaux at Saint-Ouen, a suburb lying a few miles north of Paris. Ternaux's experiments gave mixed results.

In fact, they were made under so unfavourable circumstances — the silos were dug into a wet alluvium, and grain was stored in them without caring for its humidity — that the mere fact that the grain was not completely rotten after several years is by itself a remarkable result! But although not quite rotten, except for some spots in direct contact with the walls, mouth or bottom of the silos, the grain had plainly deteriorated. The bread made from it was eatable, but somewhat bitter and acrid in taste. For practical use, therefore, the Ternaux's experiments were a failure. But it is from them on that a really scientific literature on grain storage can be said to have been born. Their impact was considerable. It is at that time that the word "silo", from Spanish origin, was introduced in the international literature. Ternaux's experiments were not the only ones, besides. At least nine different series of experiments are known from the period 1819-1830, in France only. In some of them, airtightness was achieved by the use of containers or ordinary storage rooms lined with sheets of lead welded together.



Although by and large successful, this way of achieving airtightness proved self-evidently too clumsy and expensive for current use (in addition, lead sheets teared up easily, and the danger of lead poisoning was already known).

Nevertheless, the idea of AGS was not given up. It proved (it still proves) so attractive that new experiments were attempted again and again ever after, up to the present. A chronological list of these experiments, as far as they are known to us (Table 1) strongly conveys the impression of a wavelike pattern. Every 30 years or so, it seems, i.e. at each new generation, a fresh wave of interest for AGS rises and subsides, leaving behind some more results, more or less favourable, but remarkably few actual implementations. The only AGS capacities ever built, to my knowledge, are gathered in Table 2. Except in Argentina, they were usually small, and even negligible statistically. In Argentina, AGS capacities in 1977 did not amount to more than about 14% of the total grain storage capacity of the country.

How are we to explain this wavelike pattern of interest and disinterest for AGS in the last 160 years — with perhaps another wave now, just about 20 years after the preceding one? There is no doubt a time factor. Each generation tends to ignore or to underrate what has been done by the preceding one. After 20 to 30 years, experimental results, if not quite forgotten, are always looked on with some suspicion, rightly or wrongly. But this generation factor is certainly not sufficient for an explanation. For, if AGS did not answer its supporters' expectations, why was it tested again and again? And if it did, how is it that it could never be industrially developed? The answer, or answers, obviously require a systematic comparison between AGS and other available methods of grain preservation in various contexts. One would expect such comparisons to have been a matter of course in all the experiments listed in Table 1. But in fact, they never were. With rare exceptions, like Kondo and Okamura in the 1930s, or Pixton, Hyde *et al.* at Slough in the 1960s, a precise comparison between AGS and alternative methods under controlled circumstances ("everything else being equal") was never really achieved. Granted, to maintain "equal" circumstances in long-term grain preservation experiments is a tricky business. (One has first to decide which parameters can and must be maintained "equal", of course.) But the fact remains that it was infrequently attempted, and still more rarely achieved. As a rule, results of AGS experiments were assessed, not against results of parallel experiments under conditions as similar as possible, but either against samples of the stored grain kept in the office or laboratory, or against some standard criteria of grain quality.

That is not to say that all AGS experiments have been valueless. Quite the contrary. Usually indeed, AGS has been tested under exceptionally unfavourable conditions, either out of sheer enthusiasm for the idea (as in the case of Ternaux), or because those conditions were a new problem, to be solved only by new methods anyway — as in the case of AGS of damp grain for fodder in the last 15 years. Two conclusions can be drawn from all this; 1, that AGS was never seriously considered as a potential

TABLE 1. MAIN EXPERIMENTS ON UNDERGROUND GRAIN STORAGE, 1819-1960 (Dry grain).

<u>Period</u>	<u>Authors</u>	<u>Countries</u>	<u>Comments</u>
1819-1830	Ternaux, and a number of others	France, mainly Paris & suburbs	At least 9 series of experim. recorded, most of them large scale and over several years
1852-1862	Doyère	France, Algeria, several places	Large scale exp. Emphasis on underground silos lined with sheet-iron. First assessments of upper limit of grain humidity for safe preservation
1878-1880	Müntz	France, Paris	Large, elevated bins of sheet-iron; fodder-grains (oats, maize, horse beans, etc.). First accurate measurements of grain respiration at various humidity levels.
1918-1920	Dendy and Elkington	Great Britain	Exp. on insect in airtight containers, not on AGS proper. First idea of AGS from India, authors quite unaware of former French experiments.
1926-1935	Kondo and Okamura	Japan	First AGS exp. with systematic checking of temperature, humidity, gaseous composition, etc.
1936-1939	Blanc	France, Le Chesnoy	Half scale exp., metal and concrete bins.
1942-1947	Lopez	Argentina	Emergency UGS, large scale
1952-1960	Oxley, Hyde and others	Great Britain, Slough	A number of various laboratory and large scale field experim.
1956-1965	CNEEMA	France, Antony	Various, mostly half scale exp.
Source: Sigaut & Gast 1979: 26; Sigaut 1979: 38-43, 51, 57-58.			

alternative for traditional storage methods under customary circumstances; and 2, that "everything else being equal" (insofar as it makes any sense to say so), AGS has a wider range of possible use than non-AGS methods.

To make complete this historical survey of AGS experiments, it must be added that the new possibilities offered by airtight receptacles were soon explored. Experiments, or at least explicit proposals, of grain storage under vacuum or in desoxygenated atmospheres (burnt air, nitrogen) were made in France as early as the 1860s. But of course, like AGS proper, none of those methods proved suitable for large scale applications.

## 2. IDENTIFICATION AND COMPARISON OF GRAIN STORAGE TECHNIQUES.

### 2.1. Identification as a method.

At this point, what is needed is a way to identify each technique of grain storage with precision, and without any ambiguity. This is the only means to compare these techniques to each other, and perhaps to better understand what were, or are, the real alternatives facing people of different societies in their choice of one or several among them.

A system of identification for some agricultural and food preservation techniques, including grain storage, has been proposed (*in Sigaut and Gast in press*). The basic idea is that any one technique can be unambiguously defined by crossing the two following criteria: (1) the specific thing or *product*, to which (2) a specific mode of *action* is applied. This normally results in a double-entry table, where the various possible products are listed vertically, and the various modes of action are listed horizontally. In such an identification table, each square contains one "family" or "kind" of techniques. Some square may be void, of course, either because we still do not know actual examples of the corresponding technique, or because this technique appears physically improbable or impossible.

This identification table for grain storage techniques is rather complex. It cannot be presented here, if only for typographical reasons. Nor is it possible to mention here all the grain storage and preservation techniques thus obtained (for this, cf. *Gast and Sigaut, in press*). Only some results can be given here. But it should be clear that these results would be valueless speculations if they were not based on this systematic work of identification.

### 2.2. AGS and the storage of loose grain in bulk.

Grain products are very numerous, and each of them is or has been an important item of storage somewhere or sometime. For our purpose here, however, only three products are to be considered: (1) spikes, ears or cobs; (2) sheaves; and (3) loose grain. The point is that in pre-industrial societies, only loose grain was stored underground. Three exceptions to this rule only are known to me so far: in Somalia,

in Mauritania, and in pre-settlement America; but they do not seem to be very important. This fact means that UGS could be possible and useful under two sets of circumstances only. First, when grain had to be transported over large distances, which implies the existence of large consumption centres, i.e. towns. Or second, within peasant societies themselves, when grain was customarily threshed immediately after harvest. The latter was mainly the case in most parts of the huge area alluded to before, from Spain/Morocco up to China and India. Throughout this area, the use of animals for treading out the grain has been known for millenia. With large quantities of loose grain to be disposed of by the middle of the summer, UGS was there an obvious solution. It was safer and cheaper than any possible alternative devised ever since, except fully mechanized systems.

Outside this area, threshing was traditionnally done by human labour only, with the help of sticks, mallets, pestles or flails. This hand-threshing was rarely done all at once after the harvest (as, for example, in western France), but rather little by little, according to the peoples' needs for food or money. The main part of the grain had therefore to be stored unthreshed, that is on the ear or cob, or in sheaves.

### 2.3. AGS and surrounding techniques.

So, threshing techniques are a crucial factor in the choice of storage techniques, as is well known anyway by every European farmer old enough to remember what meant the change from harvester-binder to combine in the 1950s. We may ask, for instance, if the decline of UGS in Britain and northern France after the Iron Age does not point to a replacement of animal treading by hand threshing. However it may be, the fact is that the big barns used both to store the grain in sheaves and to thresh it during the winter months are a new feature of the European landscape in the High Middle Ages. They do not seem to have ever been known outside Europe.

This consideration of threshing techniques, therefore, directly leads to building techniques. It is self-evident that the building of big barns required considerable amounts of labour, money and skills. But whereas barns are characteristic of the storage of sheaves, elevated post-granaries are often characteristic of storage of grain on the ear or cob. Maize everywhere, but also millets and spelt in pre-industrial Europe, rice in Indonesia, and most tropical small grains, are harvested ear by ear. This may be due, either to the morphology of the plant itself (maize), or because of the lack of more efficient tools (sickles), or because the straw is not wanted or cannot be transported, or to a lot of other reasons. But as a rule, harvesting ear by ear goes with delayed threshing and storage of the ears. The elevated post-granaries, so conspicuous in the African landscapes, but also to many parts of Asia and even of Europe (northwestern Spain, Balkan peninsula) are very often linked with this mode of storage. In humid and cool climates, they are built with perforated walls, in order to let the air in, to allow for a progressive drying of



the ears or cobs. Cribs are only the modern variant of a much older pattern.

#### 2.4. Climatic factors.

Climate is of course a crucial factor. After all, the stored grain is an eco-system, and the problem of grain preservation is basically an ecological one. But climate never affects any one technique single-handedly, in a simple, one-way fashion. Without a previous awareness of the complex relationships between neighbouring techniques (storage, threshing, harvesting, building, etc.), a true appraisal of the impact of climatic parameters is hardly possible.

The first of these parameters is the setting of the growing cycle against the rainy/dry periods of the year. Cereals harvested at the beginning of a rainy season are never stored underground. This was the case in mid-latitude Europe with millets, spelt, buckwheat and later maize, and in northern Europe of every kind of cereals. And conversely, millet was stored underground in ancient China, in astonishing large quantities, because fall and winter are dry seasons there. So, the main areas of UGS are not simply "dry" areas. They are areas where harvest can take place under such conditions as will ensure a sufficient dryness of the grain. Doyère has shown by studying UGS in southern Spain in the 1850s, that the humidity level of the grain stored in silos rose slowly, finally reaching levels incompatible with its further preservation. Grain had then to be taken out and dried in the sun. Of course, the lower its humidity when first buried, the longer grain could stay in one silo. In Almendralejo (Estremadura), Doyère reported humidity rates of 6 to 9% after harvest (presumably wet basis), and of 12 to 15% after two years underground. It is unfortunate that studies of comparable precision on traditional UGS systems have not been replicated since Doyère's time.

This regular rise in grain humidity was so well-known that in many parts of Italy, the "increase" of the grain kept in *fosse* or *buche* — 1 to 2% in volume per year — was the rent paid to their owners (it being sometimes the state itself). Water vapour entered the grain from the walls, bottom and mouth of the silo, so that after some time, the outer grain layers were quite damper than the inner layers: extremes of 12% and 19% have been reported by Doyère. Although quite detrimental in a way, soil humidity had also perhaps its necessity, up to a point, for achieving a sufficient degree of airtightness. For an extremely dry soil would have been too permeable to air.

#### 2.5. Food habits and grain quality.

Another point of interest in this process of slow grain humidification in confined atmosphere, is the fermentations it caused. The exact nature of these fermentations is not known. They began with the outer layers, of course, and reached gradually toward the centre of the mass. Sometimes, a silo was drowned by exceptionally heavy rains, and all the grain was fermented. But this fermented grain was by no means

lost grain. In North Africa, it was called *hamum* ("hot"), and sold at a rather higher price than ordinary grain for making *couscous* (Holtz, in Sigaut 1978: 118; Vignet-Zunz, in Sigaut and Gast 1979: 216). In some parts of India, rice is put into underground silos, not so much for preserving it than for "curing" or "ripening" it more quickly, a process said to be necessary to improve its cooking and digestive qualities (Ramiah 1937, in Sigaut 1978: 18).

Clearly, "cured" grain, whether accidentally or intentionally, can only be valued in societies where it can be consumed otherwise than made into bread. This is the case with rice anyway, but in the Far East, there is a sharp contrast between areas like the Indochinese Peninsula, where freshly harvested rice is highly preferred over old rice, and areas like several parts of the Indian subcontinent, where conversely "cured" rice is preferred, to the point that fresh rice is deemed unfit for human consumption. A precise delimitation of the two contrasted areas has not been attempted, as far as I know, nor is it possible as yet to propose any tentative explanation. In the Mediterranean, the problem looks simpler since bread-making is clearly incompatible with grain fermentation. The gradual taking over of bread at the expense of other modes of grain consumption was certainly an important factor in the decline of UGS in Europe.

No serious and recent studies of grain "cured" or "ripened" in UGS are available, so far as is known to me. But the old experiments by Ternaux in the 1820s, by Doyère in the 1850s and by Blanc in the 1930s perhaps give us a clue. These experiments were very complete, including milling and bread-making. It was observed, for instance, that the somewhat "deteriorated" grain yielded at milling less flour, less bran and more grits than usual, so that it had to be milled one or two more times than usual in order to give the same quantity of flour. The flour from Blanc's experiments was analyzed; it proved all the richer in minerals (and bran symmetrically all the poorer) than "deterioration" had gone farther (in Gast and Sigaut 1979: 33-34). Such results tentatively suggest some analogy with parboiling. Lacking proper experiments on the two processes, one has to be very careful with this analogy. But the idea that the "curing" of grain by fermentation in UGS may resemble a kind of slow and cool process of parboiling is presently the only hint that can be done.

## 2.6. Insects.

This is the last, but obviously not the least factor to be mentioned in connexion with UGS. Our means of controlling storage pests have so fantastically improved during the last 50 years, that it is hard to imagine how people were and felt unarmed and hapless against insects before. In West and central France in the XVIIIth century, the arrival of the Angoumois Grain Moth (*Sitotroga cerealella* Oliv.) spelled disaster. Keeping grain in sheaves had been the best means to control the grain beetles (*Sitophilus* sp.). But now grain in sheaves also was attacked by the new moth. What could be done next?

This is the question to which Réaumur, and later Duhamel du Monceau and Tillet tried to find an answer. It provided the impetus for the first experiments on grain storage by Duhamel in the 1750s. A century later, in the 1850s, a fresh burst of activity by the same pest directly led to Doyère's studies and experiments on UGS and AGS. From an analysis of the literature of the time, it can be concluded that in areas without cold winters, there was no efficient way to protect large bulks of grain from insect attack, except UGS, or AGS.

#### CONCLUSION

Although many details are still lacking, the picture of UGS in pre-industrial societies is by now pretty clear. It was practically the only means to keep large quantities of loose grain free from insect attack for significant lengths of time in areas with mild winters. It was also cheaper than alternative methods requiring either large buildings (sheaves stored in barns) or both large buildings and much manpower (loose grain in layers with frequent shovelling). But UGS had the specific drawback that a progressive dampening of the grain could not be prevented. This dampening could be coped with when the grain was buried in a very dry state, or it could be tolerated and even looked for when food habits made fermented grain a valuable item in the diet. But in any case, fermented grain was unfit for making bread of the quality required by European town-dwellers by the XVIIIth century, and this is probably one of the main reasons why UGS could never find a wide acceptance in northern Europe.

What can be concluded from this historical survey for the future?

The future of AGS, if any, may lie in two directions. First, as a general storage method in hot countries, where cooling the grain by aeration is impossible. The main problems to be solved in such countries are perhaps those of the skills necessary to operate AGS on a routine basis. An extensive study of the knowledge and skills of craftsmen in countries where UGS lives on would certainly be helpful. The second direction would perhaps be to look at AGS as a residueless method of desinsectisation. But is it possible, with the use of atmospheric gases only, to achieve the complete desinsectisation of cool grain in a sufficiently short time? The main drawback of desoxygenated atmospheres is that they kill insects only insofar as they are active, i.e. at comparatively high temperatures (18° to 20° C and more). Cool grain takes a long time to be made insect-free by AGS alone. But there is a kind of gaseous combination that has never been tried, as far as I know: mixtures containing more than the usual 20% of oxygen. What would be the effects of such superoxygenated atmospheres on insects at their various development stages (and also of course, on the grain itself)? Every conceivable mixture of atmospheric gases seems to have been extensively studied, except this one. This will be my last concluding remark.

TABLE 2. AIRTIGHT STORAGE CAPACITIES, MODERN

<u>Country, date</u>	<u>Type of buildings</u>	<u>Total capacity</u>
France, around 1860	Silos "Doyère" (underground bins, lined with sheet-iron)	550 t
France, around 1880	Elevated iron bins of the Cie des Omnibus (Paris); fodder grains for tramway horses.	21000 m3
Argentina, 1947-	Horizontal bins, half underground, lined with concrete, etc.	2500000 t
France, Venezuela, Morocco..., 1952-60	Vertical steel bins, built by French firms or expertise	(?) 60000 t
Cyprus, Kenya, 1960-1970	Invert. conical bins, half underground, concrete lining and roof, built by British firms or expertise	148000 t
Source: Sigaut 1979: 39, 56. Figures compiled from various kinds of sources, sometimes second-hand, not to be considered as exhaustive.		

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La Piazza delle Fonti a Foggia (Vittorio 1877).

## STUDIES ON THE PRESERVATION OF FOODGRAINS UNDER NATURAL AIRTIGHT STORAGE

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### ABSTRACT

Studies were undertaken to determine the losses and eco-climatic conditions of foodgrains viz temperature, oxygen concentration, moisture content, germination, damaged grain, insect fauna, free fat acidity and alcoholic acidity in some of the underground storage structures.

Two types of underground structures (1) made up of brick masonry and plastered with Cement ("Pucca Khattis") and (2) structures without any brick masonry or cement plaster ("Kaccha Khattis") were selected for these studies. It is concluded from the results that there was an increase in temperature from top to bottom layers of both types of structures. The grain in "Kaccha Khattis" remained cooler as compared to the grain in "Pucca Khattis". The oxygen content in both types of structures was much lower than normal. More spoilage was observed in top layers. The stored grain pests were present only in the top layers. Continuous decrease in FFA and alcoholic acidity was observed from top to bottom layers in both structures.

### INTRODUCTION

Preservation of foodgrains under natural airtight underground storage has long been practised in India and other countries. Absence of insect attack has been reported to be a good feature of underground storage structures. In fact, this easy way of solving the insect problems has caught the imagination of many workers in different parts of the world and many investigations have been carried out to understand the cause of insect death in airtight condition.

There are various types of underground storage structures in India

viz Khatti (U.P. & Punjab), Khani (Orissa), Patra (Andhra), Kondi and Banda (M.P) etc. Studies were undertaken to determine the losses and eco-climatic conditions of foodgrains in some of the underground storage structures.

## MATERIALS AND METHODS

Description of Khattis (underground storage structures): where the sub-soil water is low, underground cellars known as "Khattis" are used for storage of foodgrains. A "Khatti" is an underground pit with a narrow circular opening at the top, about 2.5 feet in diameter, through which a man can pass. It is usually 15 ft to 16.5 ft deep and can usually take 24 to 32 tons.

Generally wheat, barley, peas, gram and maize are stored. This method of storage is extremely cheap. A "Khatti" once made, is serviceable for years. In some parts where this method is prevalent, "Khattis" as old as 100 years have been observed.

Before putting in the grain, a layer of straw is placed at the bottom and against the walls. When it is full, its mouth is closed with straw and plastered with mud in flush with the surface of the ground, a man goes down into the "Khatti" with a basket, which is pulled up by means of two thick ropes at opposite ends on the rim of the basket.

The most serious defect in this storage is that the grain absorbs moisture from the walls and turns black and becomes sometimes unfit for human consumption.

During the present investigations four Khattis made up of brick masonry with cement (Pucca Khattis) at Shamli and ten "Kaccha Khattis" (underground pits without any brick masonry or cement plaster) at Hapur were selected for the studies. The "Khattis" were filled with grain during April and May and the observations were made during November and December just before removal of grain for sale by the traders. The general information about these "Khattis", is given in Table 1. The "Khattis" were opened and oxygen concentration and temperature in the bulk at different depths were recorded. Samples of grain were withdrawn from the same places using a "Thermosampler". The grain samples were

analysed for moisture content, germination, damaged grain, insect fauna, free fat acidity and alcoholic acidity. All the observations were made at the top of the "Khattis" and at depth of 0.46, 1.0, 2.0 and 2.5 meters.

#### Recording of temperature:

Temperature measurements were made by using a "Telemex" temperature recording unit. The probe was inserted in the grain at desired depths and was left undisturbed for 3 minutes at the same place and the temperature was recorded on the scale.

#### Determination of moisture:

The samples of grain from desired depths were taken out and the moisture percentage was recorded using a moisture meter (Ceratester).

#### Determination of oxygen:

Oxygen concentrations were estimated using a Beckman oxygen analyzer model D2. The air intake tube of the oxygen analyzer was inserted in the grain bulk at desired depths with the help of an iron rod. Air was sucked and oxygen percentage was recorded on the scale.

#### Determination of germination:

The grain samples were mixed thoroughly and 100 grains were taken in a Petri dish for testing. They were washed with 0.1 per cent mercuric chloride solution followed by distilled water. The grains were kept on moist filter paper at 90 percent humidity and 30°C temperature for 7 days. After 7 days the sprouted grains were counted and the germination percentage was recorded. There were three replications for each sample.

#### Determination of percentage damaged grain:

One hundred grains from thoroughly mixed samples were taken in an enamelled plate and the damaged grains (damaged by moulds, heating and insects) were picked out, counted and expressed as damage percentage. There were three replications for each sample.



Estimation of alcoholic acidity:

The alcoholic acidity was estimated according to ISI method using 5 gms sample 11.

Estimation of free fat acidity:

FFA was estimated according to AACC method 12.

## RESULTS AND DISCUSSIONS

In these studies four "Pucca Khattis", two filled with wheat and another two with gram and ten "Kaccha Khattis", eight filled with wheat and one each with maize and peas were examined after a storage period from 7-9 months (Table 1).

TABLE 1

General information about the "Khattis" which were studied

S. No.	Place	Type of Khatti	Capacity (quintals)	Commodity stored	Variety	Duration of storage (months)	Remarks
1	Shamli	Pucca	180	Wheat	K. 68	8	Average of two Khattis
2	"	"	180	Gram	--	8	- do -
3	Hapur	Kaccha	160	Wheat	Dara	7	Average of four Khattis
4	"	"	160	Maize	--	9	
5	"	"	160	Wheat	S. 227	9	Average of four Khattis
6	"	"	160	Peas	--	9	

The "Kaccha Khattis" are located in a well drained area where some gain in weight in grain after a normal storage period is observed by the traders. The results obtained are summarised in Tables 2 and 3.

Temperature:

There is a slight increase in temperature from top to bottom layers

in both type of "Khattis" (Pucca and Kaccha). The temperature in "Pucca Khattis" ranged from 35.5 to 42.5°C, while in "Kaccha Khattis" it ranged from 24.5 to 36.0°C. However, the temperature difference in the bottom layers was not significant in any "Khatti". The data indicate that the grain in "Kaccha Khattis" remained cooler as compared to the grain stored in "Pucca Khattis". The lower temperature in the top layers may be due to the lower atmospheric temperatures.

#### Moisture:

In the "Pucca Khattis" the moisture content of grain in the top layers was less than in the middle and bottom layers, while in "Kaccha Khattis" it was reverse.

In the "Kaccha Khattis" Nos. 4, 5 and 6 the grain in the top layers had very high moisture contents as compared to the remaining lots. The grain near the side walls of "Khattis" also showed higher moisture contents. This is perhaps due to the fact that they are in the open and rain water might have percolated into the "Khattis" and the top layers of grain absorbed the water. The lower moisture content in the grain of top layers in "Pucca Khattis" may be because of driage and the construction is such that no rain water can enter in these "Khattis".

#### Oxygen:

The oxygen content in the "Khattis" of both types, was much lower than normal. The oxygen percentage in "Pucca Khattis" ranged from 3.5 to 10.0 per cent, while in "Kaccha Khattis" it ranged from 0.0 to 18.5 per cent. In the deeper layers slightly more oxygen was present in "Pucca Khattis" as compared to "Kaccha Khattis" as in "Khatti" No. 5 (Kaccha Khatti) there was only 2.0 per cent oxygen at a depth of 1.0 meter, while even in the deepest layers i.e. at a depth of 2.5 meters, there was 3.5 to 4.0 per cent oxygen in "Pucca Khattis" (Khatti Nos. 1 and 2).

The lowering of oxygen in both types of "Khattis" is due to respiratory activities of grain. More reduction in oxygen content in "Kaccha Khattis" was perhaps due to higher respirative activities as

TABLE 2

Various observations in the "Pucca Khattis".

Khatti No.	Depth of observation (meters)	Temperature (°C)	Moisture content (%)	Oxygen (%)	Damaged grain (%)	Germination (%)	FFA mgs./100 gms	Alcoholic acidity (%)	No. of insects in 100 gms-sample	Remarks
1	Top	35.5	10.1	10.0	24	9	32	0.072	10 larvae	(T.granarium)
	0.4	40.0	10.8	5.5	8	62	19	0.072	---	
	1.0	40.0	10.9	4.5	2	91	18	0.072	---	Average of two Khattis
	2.0	41.0	10.9	4.5	1	96	16	0.070	---	
	2.5	41.0	11.0	3.5	2	94	15	0.068	---	
2	Top	36.5	7.9	5.0	3	85	25	0.127	3 adults	(Alphitobius)
	0.4	40.0	9.4	5.0	1	86	23	0.098	"	"
	1.0	42.5	9.4	5.0	0	92	22	0.108	"	"
	2.0	42.5	10.0	4.5	1	94	23	0.117	"	"
	2.5	42.5	10.1	4.0	0	96	23	0.078	"	"

TABLE 3

Various observation in the "Kaccha Khattis".

Khatti No.	Depth of observation (meters)	Temperature (°C)	Moisture content (%)	Oxygen (%)	Damaged grain (%)	Germination (%)	FFA mgs./100 gms	Alcoholic acidity (%)	No. of insects in 100 gms-sample adults larvae		Remarks
3	Top	29.5	13.2	9.0	35	0	46	0.116	11		(Alphitobius)
	0.4	32.5	11.8	5.0	15	10	25	0.107	---		
	1.0	35.0	11.1	3.5	8	78	20	0.083	---		
	2.0	36.0	9.7	3.0	7	87	19	0.068	---		Average of
	2.5	36.0	9.7	2.0	3	94	16	0.066	---		four Khattis
4	Top	24.5	26.5	18.5	35	0	135	0.210	---		
	0.4	25.5	16.5	13.0	20	0	118	0.196	---		
	1.0	32.0	15.5	4.0	8	10	104	0.186	---		
	2.0	32.5	13.5	0.0	4	10	88	0.173	---		
	2.5	32.5	13.5	0.0	0	16	80	0.150	---		
5	Top	25.0	19.5	15.5	31	0	70	0.144	6	4	(Alphitobius)
	0.4	29.0	19.0	4.5	17	6	63	0.085	---		
	1.0	31.0	13.5	2.0	12	68	36	0.057	---		
	2.0	33.5	11.0	1.0	4	71	28	0.055	---		Average of
	2.5	34.0	11.0	0.5	3	92	16	0.049	---		four Khattis
6	Top	25.0	15.0	7.0	100	0	59	0.178	---		
	0.4	26.5	7.5	5.5	4	8	48	0.127	---		
	1.0	28.0	7.1	2.5	4	20	42	0.116	---		
	2.0	30.0	6.1	1.0	2	68	34	0.125	---		
	2.5	32.0	5.6	0.0	0	96	23	0.082	---		



a result of more moisture content in the grain in these "Khattis" than in "Pucca Khattis".

#### Damaged grain and insect infestation:

In general there was more spoilage in the grain in the top layers as compared to deeper layers in both types of "Khattis.". In "Khatti" No. 2 (Table 2) the damaged grain was only 3 per cent even in the top layers. The damage was comparatively more in "Kaccha Khattis." than in "Pucca Khattis.". More damage in "Kaccha Khattis " may be due to high moisture content in the top layers.

The stored grain insect pests were present only in the top layers of both types of "Khattis ". No insect was present in the deeper layers due to insufficient supply of oxygen. The insect pests found on the top were adults and larvae of Alphitobius, adults of Tribolium castaneum, larvae of Trogoderma granarium and adults of Rhizopertha dominica.

#### Germination:

Lower germination percentage was recorded in the grain in the top layers as compared to deeper layers due to more insect infestation and mould damage in both types of "Khattis ". The difference observed in germination from top layers to bottom layers was from 11 to 85 per cent and 16 to 96 per cent in "Pucca" and "Kaccha Khattis " respectively. The loss in germination in maize in "Khatti" No. 4 (Table 3) was very high. The loss in germination in grain stored in "Pucca Khatties" was comparatively less than in "Kaccha Khattis ".

#### F. F. A.:

Maximum free fat acidity was observed in the grain of top layers in both type of "Khattis". There was continuous decrease in FFA from top to bottom layers. The FFA range in "Pucca Khattis " was from 15 to 32 mgs/100 gms, while it was from 16 to 135 mgs/100 gms. in "Kaccha Khattis ". The difference in FFA from top to bottom layers was from 2 to 17 and 30 to 55 gms./100 gms in "Pucca Khattis" and "Kaccha Khattis " respectively. High fat acidity values have been shown to be

associated with high content of damaged kernels and low viability 13, 14. Ordinarily, fat acidity values increases and viability decreases significantly long before external physical evidence of wheat deterioration appears.

#### Alcoholic acidity:

There was a decrease in alcoholic acidity, estimated in the samples collected from top to bottom layers in both types of "Khattis". The alcoholic acidity ranged from 0.068 to 0.127 per cent and 0.049 to 0.210 per cent in "Pucca" and "Kaccha Khattis" respectively. The difference in alcoholic acidity in the grain from top to bottom layers was from 0.004 to 0.049 per cent in "Pucca Khattis" and from 0.050 to 0.096 per cent in "Kaccha Khattis".

These studies indicate that the grain stores better in "Pucca Khattis" than in "Kaccha Khattis". Except in top layers, the damage in the grain and loss in viability also is less. The slight insect infestation also can be controlled with proper fumigation. The "Kaccha Khattis" allowed seepage of moisture into the top layers of grain which resulted in more damage and loss in viability. The increase in free fat acidity and alcoholic acidity was also more, indicating the damage in the grain in the top layers. The insect infestation in these "Khattis" can be controlled by proper fumigation but provision to make it water proof at the top is necessary.

## UNDERGROUND STORAGE IN SOME ARAB COUNTRIES

By

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### ABSTRACT

The traditional methods of grain storage on the farm level in the Peoples Democratic Republic of Yemen, the Arab Republic of Yemen, the Democratic Republic of Somalia, the Democratic Republic of Sudan and the Arab Republic of Egypt, were surveyed with special details about underground storage.

The different underground pits, their merits, drawbacks, description and methods of their up-grading were included.

### INTRODUCTION

During a trip in some of the Arab Countries to study the traditional methods existing and used for grain storage in the Arab Peninsula, Somalia and Sudan, underground storage was found to be one of the most common. A review of the types, environmental conditions affecting such storage, and its efficiency, as well as other similar traditional methods are presented in detail for each of the countries visited.

#### The Peoples Democratic Republic of Yemen

In the Peoples Democratic Republic of Yemen, and on the farm level, some farmers store their grains in baskets made of date-palm leaves completely compact with a capacity not exceeding 150 kg. Such baskets are kept in underground pits and covered with soil. Grains stored by this method last for a long time in a good condition provided that their moisture content is low and the soil is dry.

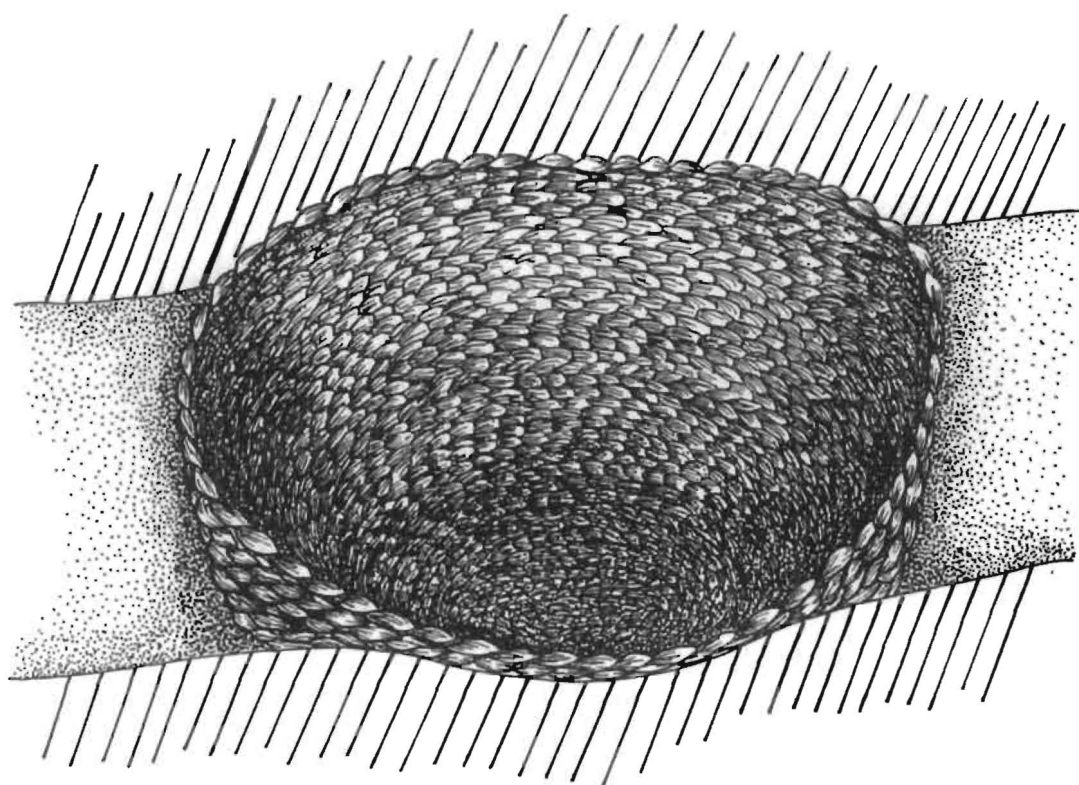
Arab Republic of Yemen

On the farm level, farmers store corn, which is one of the most important cereal crops produced, in pits under ground. Pits excavated in the ground for this purpose are locally called "madfans". The total capacity of madfans existing now in the YAR reaches about 200,000 tons. A madfan is dug either in heavy clay soil or in rocks. It has a funnel shape, 4 meters deep and ranging between 4 and 8 meters top diameter. The bottom is flat, of  $\frac{1}{2}$  meter diameter. The depth and diameter depend on the quantity of grains supposed to be kept in the madfan. The inner surface has a very gentle slope to prevent the structure from collapsing. The soil must be compact and hard to avoid, as far as possible, the infiltration of water. Before the madfans are filled with grains, their inner surfaces are lined with a thick layer of corn stalks or any other dry plants or herbs. After filling to the top, the grains are covered with a thick layer of dried plants as mentioned before and then with a thick layer of soil having a dome shape. Grains are kept in such madfans relatively safely for as long as 4 years. When the madfans are opened and the grains have to be sold, their prices are 10% lower than the fresh crop. The percentage in the reduction in prices corresponds positively with the storage period in madfans.

The efficiency of madfans as a grain storage method in the YAR is correlated mostly with the quantity of rainfall in the area. In Hodeida, in the South West of the YAR where the rainfall is at its lowest level, the loss in stored grains in madfans is very limited, compared with grains stored in madfans in other governorates having high rainfall. High rainfall results in caking, especially near the walls, and the level of damage might reach in some rare cases about 30%, besides tainting of the grains and loss of their bright colour. In such severe cases, the grains are most probably unfit for either human or animal consumption. Spoiled grains which are seemingly good, when consumed result in symptoms of stomach aches and intestinal troubles.

On the other hand, when the soil is dry, the grain is dry and there is no rainfall, the madfans keep grains in a very good condition for as long as 10 years.





Basket of date palm leaves buried under  
ground existing in P.D.Yemen R.

The total loss of grains kept in madfans is, in some rare cases, considerable, but when there is no water seepage or infiltration and there is no infection or infestation at all the loss is negligible. This may lead us to state that "madfans" as a method of storage are fit for the purpose and their success depends on:

- a. the locality where the madfans are dug
- b. the type of soil
- c. the period of storage
- d. the moisture of grains should not exceed 12%
- e. the walls should be dry; otherwise, burning dry plants inside before storing the grains will help in drying up the inner surfaces.

The madfans are used permanently for grain storage in the arid zones with very limited protection. Protection involves getting rid of the inner surface of the madfan which may carry various spores from the old crop and which may badly affect the new crop.

This type of storage is considered airtight storage and depends mainly on the  $\text{CO}_2$  build-up to the level which is lethal to all organisms. Therefore, if implemented properly, insect infestation does not increase and mould damage could be kept at its minimum level.

To avoid spoilage of grains due to soil water infiltration it is suggested that cracks in the walls should be plastered and walls should be lined with thick polyethylene welded sheets. This suggestion as well as others should be tested on some madfans mainly located in the rainy regions.

It is also advisable that storage in madfans should be shortened to the least possible number of years.

On the other hand, the farmers' houses generally consist of three flats, the basement is used for grain storage either in sacks or in buld. Most of the farmers dig some underground pits of limited size to store grains for their own consumption.

Underground pits in the YAR are in some cases conical and very limited in size, especially when dug in the rocks or inside houses.

To overcome the problem of storage in madfans by small producers, the use of barrels for grain storage is now being used on a limited scale. The number of barrels used now for this purpose reaches about 50,000. The barrel has a 40 gallon capacity and has a screw cover. To make these barrels always available in the market, a special plant of the barrel industry is to be constructed.

#### Democratic Republic of Somalia

##### A - Underground Pits

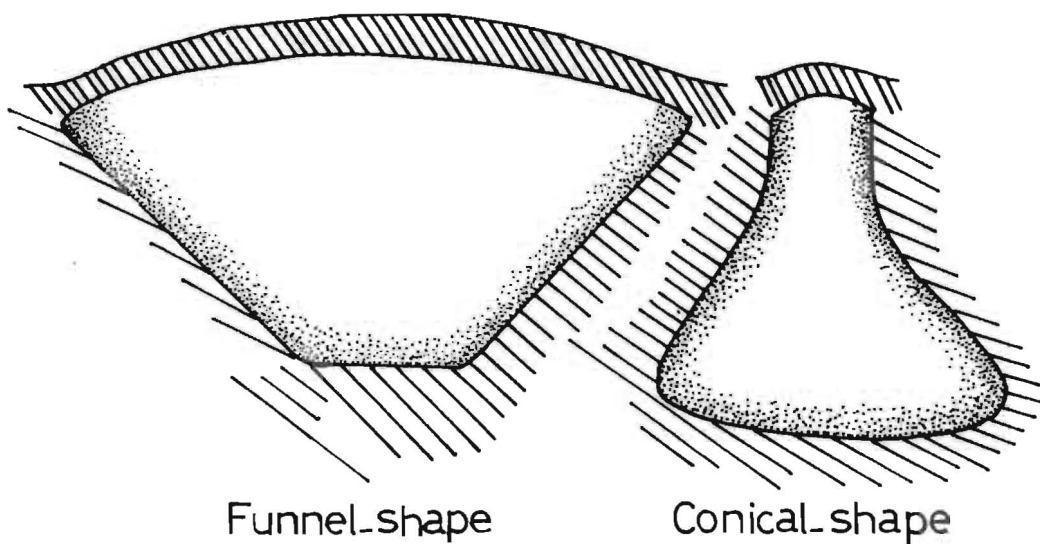
About 50% of the total production of grains in Somalia is kept on the farm. In the South, most of the quantity is stored in underground pits excavated directly in the soil. The size of the pit varies from as low as 5 tons (mainly pits owned by the farmers) to 250 tons (pits owned by merchants). Due to shortage of threshing machines, farmers in some cases store their own corn on cobs in pits. Storing maize on cobs in underground pits does not meet the requirements of hermetic storage due to the sufficient amount of air still present and consequently insect infestation might show and build up considerably.

The shape of the underground pits in Somalia is variable; they are either rectangular or boat-like, or conical with a small upper hole.

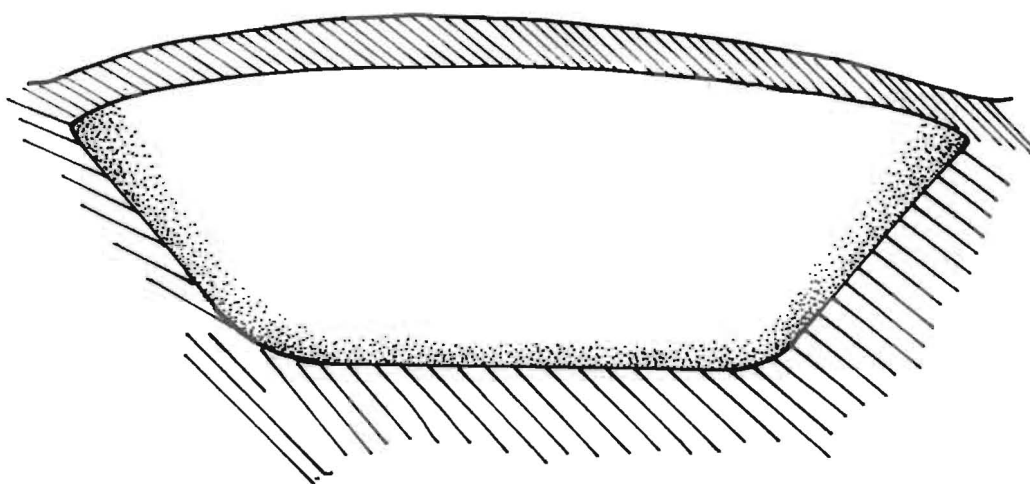
In all cases, before the pits are filled, they are lined carefully with dry stalks of corn or sorghum plants, dry plants or seaweeds and then covered with the same lining and then with a thick layer of soil.

Grains are kept in such pits for several years, reaching in some cases 10 years.

The main problem of pit storage in Somalia is the high moisture content of grains when placed in the pits. In many cases a thick layer of caked grains shows up near the walls and on the top, as well as tainting and discolouration of kernels. The other draw-



Under ground pits existing in A.R.Yemen & Somalia



Boat like shape under ground pit  
existing in Somalia



back is the infiltration of soil water into the pit, especially after heavy rainfall reaching the aforementioned bad conditions.

Lining the inside of the pits with a thick layer of overlapping polyethylene sheets could be considered a remedy in the case of water infiltration, but in general this type of storage in Somalia needs a research program based on studying the soil profiles in the different areas and their compatibility with this type of storage.

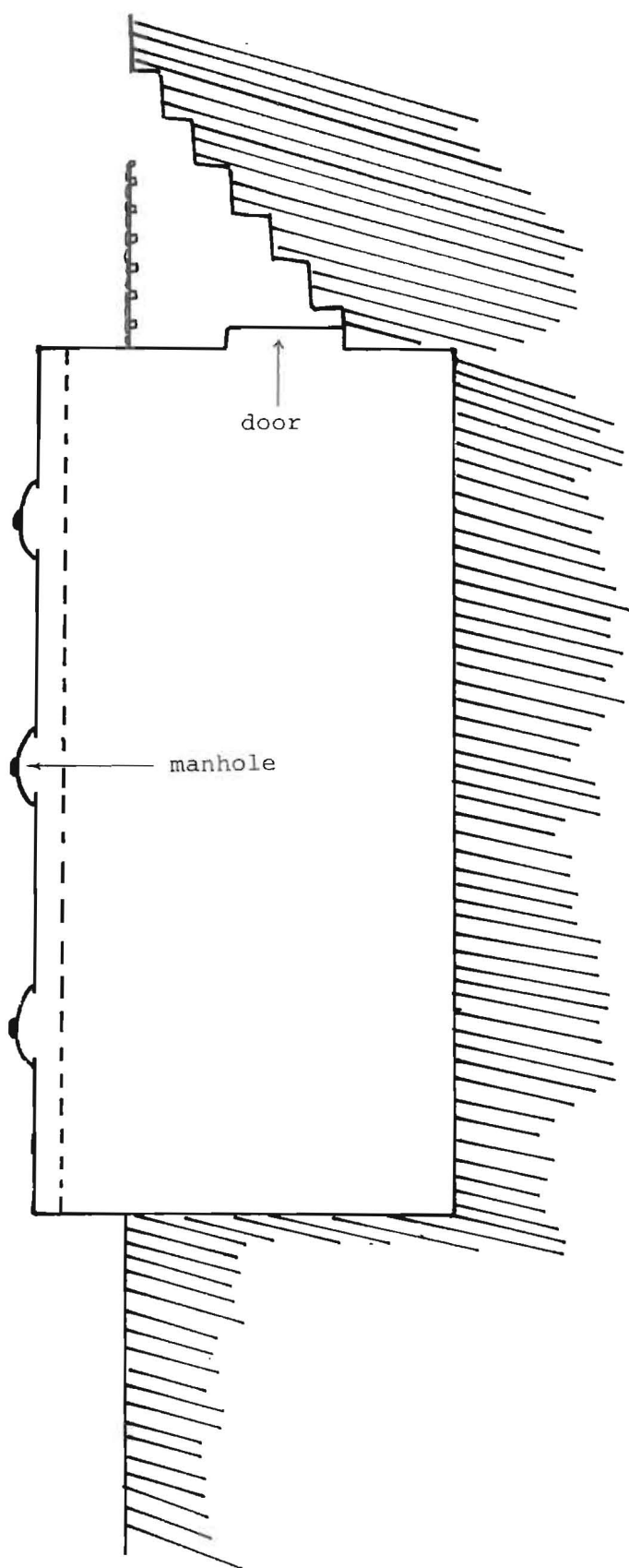
#### B - Underground Bins

In Somalia, underground bins occur widely and these bins are owned by the Agricultural Development Corporation (ADC).

The bins are constructed underground. The dimensions and capacities are variable and in many cases reach more than a thousand tons. They are rectangular in shape, the height is about 5 meters, 4 of which are below ground level. They are built either from concrete or local stones, the roof being of concrete. Each bin has several manholes at the top and one side door with stairs going down for unloading. The manhole covers are either conical or flat. When filling, a space of less than one foot is left free from grains over the top surface of the grains.

This type of storage could be considered the best existing in Somalia. However, to up-grade this method the following points should be applied:

1. Grains should be at a maximum of 12.5% m.c., otherwise high moisture content might result in moulding and deterioration.
2. To overcome the problem of translocation of moisture in the bin it is suggested that each bin be supplied with a certain amount of slick lime (raw calcium oxide) to be put in shallow containers on the surface of the grain underneath every manhole. This slick lime should be replaced periodically, as soon as it changes to the powder form which indicates its inability to react with more water (calcium hydroxide).
3. Repairing and cleaning and disinfecting the inner surfaces before the entry of the new crop.



Under ground bin existing in Somalia

4. Periodical inspection is very essential in order to refumigate as soon as any number of live insects shows up.

#### The Democratic Republic of the Sudan

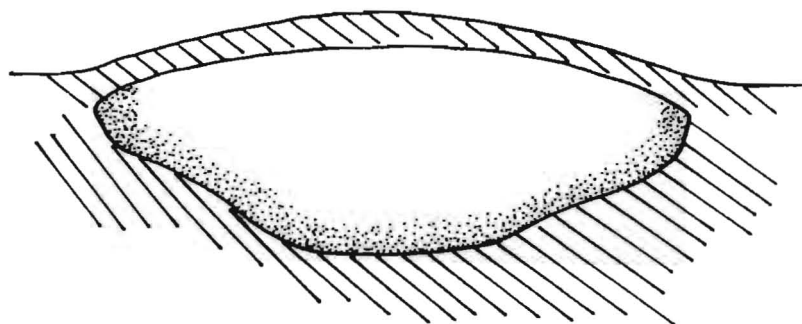
The underground pits in the Sudan, locally called "matmura" are a traditional storage method and are mainly concentrated in Central Sudan where local cultivators depend on this method of storage entirely to keep their produce.

Matmuras belonging to small producers are dug under ground in dry land and vary in size and capacity, very rarely exceeding 25 tons. They are cylindrical in shape, having a depth equal to that of the diameter. Before being filled, they are plastered on the inside surface with cow dung mixed with clay and then a fire is lit inside the pit until the walls become dry. Dura (Sorghum) is kept in such pits very successfully for at least three years and probably up to ten years. It was also noted that the success of matmuras for grain storage depends positively on their sizes. In small matmuras, loss in germination of seeds is expected.

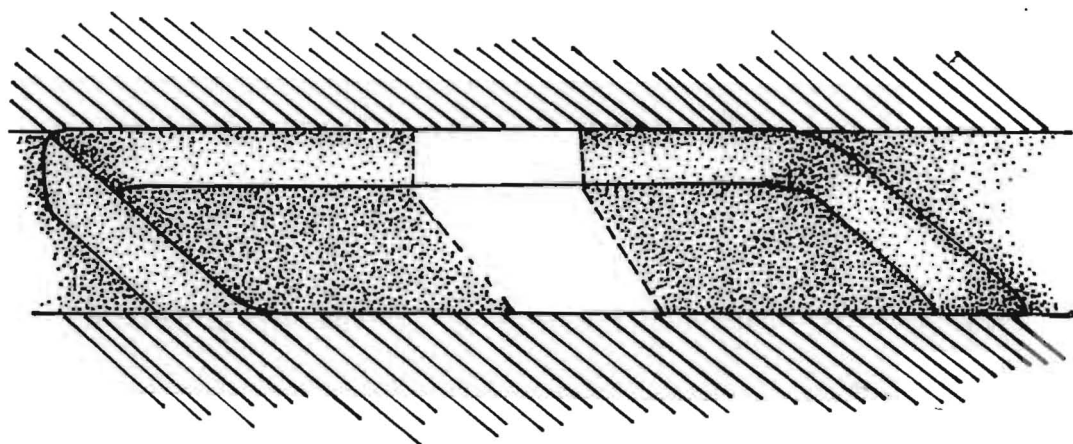
A matmura belonging to traders or as a governmental storage is large enough to hold up to 700 tons of grains. Places where such matmuras are dug have to be chosen carefully, taking into consideration that the area must be high enough and very far from water infiltration. The soil must be heavy clay so as to be compact. Such matmuras are dug in a shallow funnel shape with a flat bottom, boat-like shape or as a trench ranging between 50 and 150 meters in length, 11 meters wide and 2.5 meters deep. Grains if kept dry in these matmuras (9% m.c.) and stored during the dry season may last very safely up to 18 months inspite of rainfall.

When pits get old, after several years of continuous use, the internal surfaces become so hard that they become susceptible to cracking and consequently infiltration water may seep in and severe deterioration may occur. In such conditions, the locality of the pits has to be changed.

Losses due to storage in matmuras do not exceed 2% within 18 months, and are due to caking of the periphery within 3 cm.



Shallow funnel-shape



Shallow-rectangular

(50-150) x 11 x 2.5 m

Under ground pits existing in D.R.of Sudan



## Egypt

Underground storage has been used in Egypt since ancient days (3000 - 4000 years ago). At that time grains were stored underground in deep ditches, either between two layers of straw or in baskets made of stems of bamboo (Arundo donax). The size of each ditch depended on the quantity of grains stored, but in general never exceeded two or three tons.

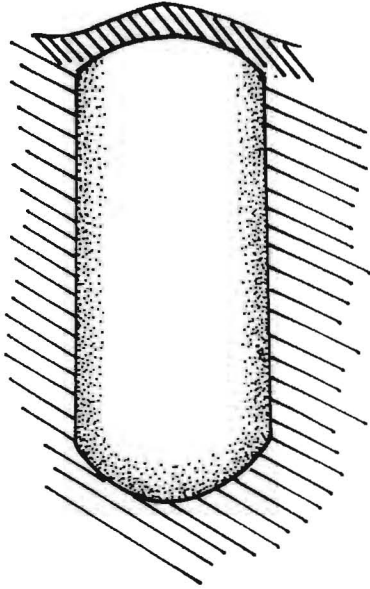
Nowadays, this method exists in rainless regions far from infiltration water and regions free from termites as well as in the desert areas and in the oases. It is very essential that grains, before being stored, be dry. Covering with sand keeps the grains in a very good condition and free from insect infestation. In its simplest form, the underground store, in such places, is nothing but a deep ditch sufficient to keep a quantity of grains used for the owners' own consumption.

South of the Nile Delta, in lower Egypt, Barheem village (Mounoufia Governorate) is the only village in which faba beans (Vicia fabae) are stored on a commercial basis in underground pits. This method of storage started in this village during the First World War in 1919.

This village has a storage capacity of about 45,000 tons in 5000 pits scattered over an area of about 30 feddans (a feddan equals 4200 m<sup>2</sup>).

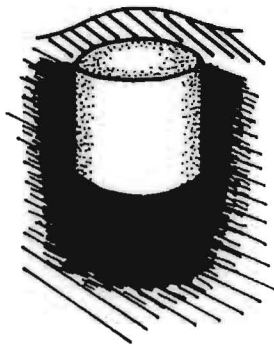
When first excavated, the pit is of about three meters bottom diameter and two meters deep with a flat bottom and an entrance hole of one meter diameter and two meters deep. With these dimensions, it holds about 6 tons of beans. After being dug, the walls are well pressed and smoothed. Before the second and third storage seasons, the pit is enlarged yearly and gradually until it reaches its full capacity of about 11 tons (3.25 m diameter and 3 m deep).

1. Under ground ditch  
existing in Egypt.



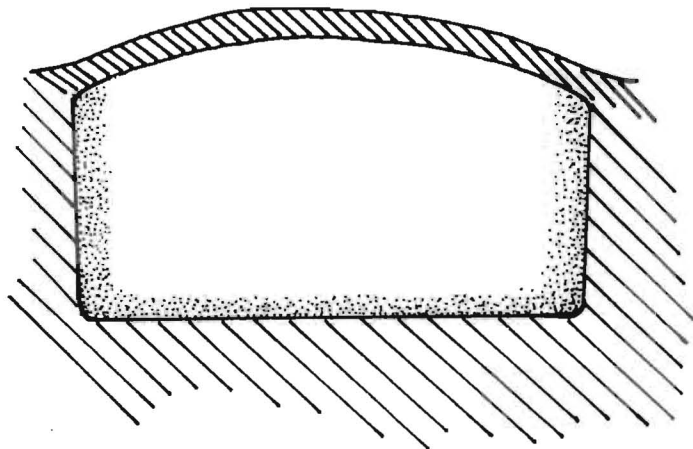
1

2. Under ground pit (Matmura )  
existing in Sudan.

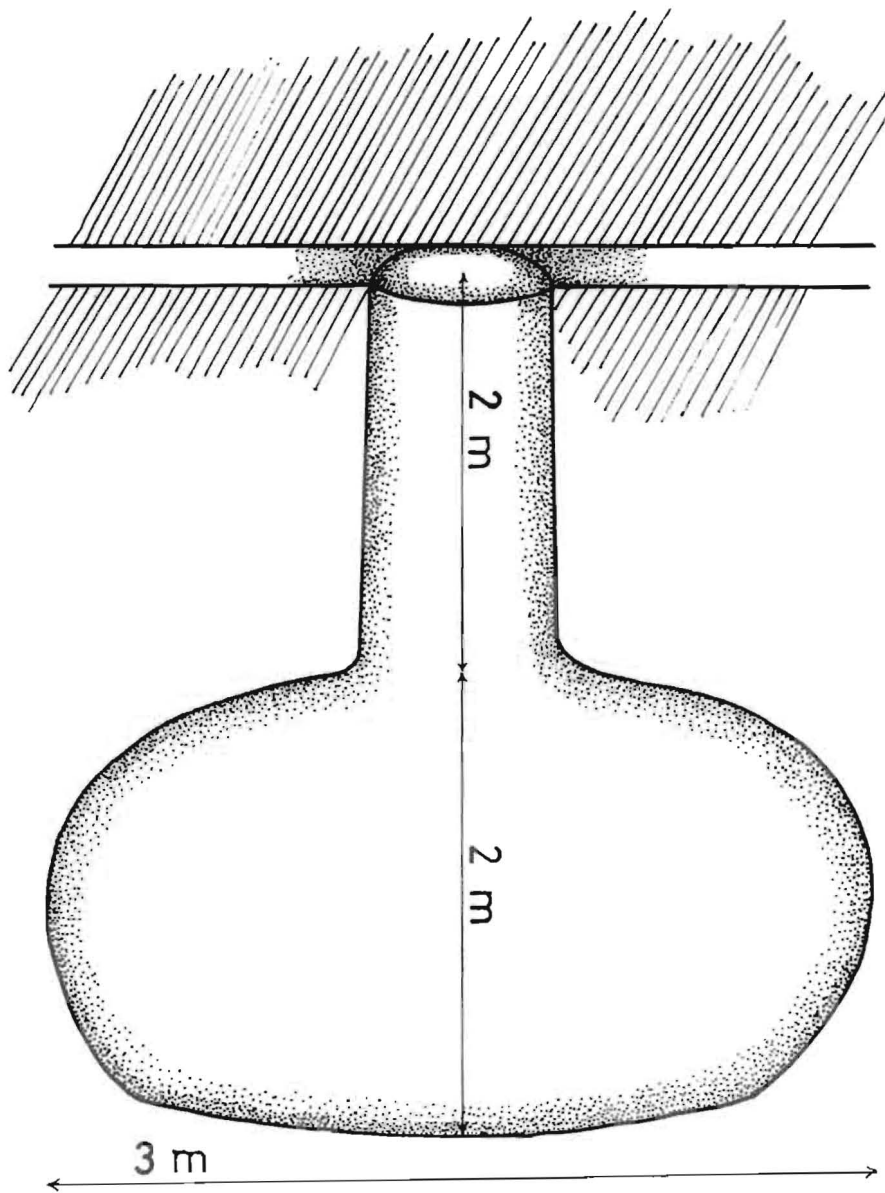


2

3. Rectangular underground  
pit existing in Somalia.



3



Pitcher like under ground pit  
existing in Egypt

Through the only hole at the top, grain can be either introduced or removed. As the pits are filled, the bottom and walls are lined with faba bean straw or helba (Trigonella foenum-graecum) scraw and finally covered and sealed with mud.

Beans are either introduced or removed from the pit by labourers. Because carbon dioxide is produced by the beans and thus accumulates in the pit, it is carefully aerated for safety before anyone can get in. Safety for labourers to get inside is detected by a candle flame. If it continues to burn, entrance for labourers is allowed and safe.

The percentage of infestation in stored beans never increases, and the testa remains white instead of turning brown as in the case of beans stored in the open air, where they are exposed to the different weather and light conditions. This method of bean storage also results in better cooking properties for beans. Consequently beans stored in such a way are sold for prices 25% above those of beans stored by other means.

The storage season starts early in May, directly after harvest of the beans, and might last for two months to one year, according to the market demand. Losses under such conditions never exceed 1%, these being due to direct contact of some beans with the walls.



FIELD EXPERIENCE WITH HERMETIC STORAGE OF GRAIN IN EASTERN AFRICA WITH  
EMPHASIS ON STRUCTURES INTENDED FOR FAMINE RESERVES

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1. INTRODUCTION

Tropical countries are expanding agricultural production to meet the increasing food requirements of their people. More food is becoming available and requires storage for longer periods. With the uncertainties of climate, and the need for self-reliance, consideration must be given to storage systems that enable safe storage for long periods at low cost and with minimum use of pesticides.

Low cost storage can be achieved on a large scale with the use of semi-underground hermetic structures. Traditional underground semi-hermetic storage pits have been in use in Eastern Africa and elsewhere for several centuries. In recent years, large hermetic structures have been successfully used in Kenya for storage of famine reserves.

2. TRADITIONAL AND EXPERIMENTAL UNDERGROUND SEMI-HERMETIC STORAGE PITS IN  
EASTERN AFRICA

Underground pits have been traditionally used in Ethiopia and Somalia for semi-hermetic storage of grain (Gillman and Boxall, 1974). In Ethiopia the pits are generally conical but cylindrical pits are found in the South near the Somalia border and in Somalia. The pits hold 5 tonnes or less on average, although larger pits of 25-50 tonnes are constructed by traders. The main crops stored are sorghum, millet and maize and in some areas of Ethiopia wheat and barley. Occasionally beans are stored for temporary periods during the dry season.

A limited amount of experimental work was done in the mid-1950's on underground concrete pits lined with bitumen. These pits of approximately 120 tonnes capacity (35 x 18 ft at the top and 10 ft deep) were constructed in Tanzania and Malawi (Hall et al. 1956).

Underground pit storage is attractive because of the relative ease of construction, safety from theft, better thermal insulation, protection from rodent attack, less insect activity and protection from insect re-infestation. Disadvantages are that pits are difficult to empty and are subject to moisture damage if there is a rise in the water table. There is often more severe mould infestation of the grain due to moisture migration from the soil on the sides and condensat-

ion on the top. The grain is not very suitable for seed as the viability is reduced. Gilman and Boxall (1974) indicate that traditional underground pit storage is on the decline in parts of Southern Africa because of improvements in the marketing system and change in social habits creating a demand for better quality grain.

### 3. SEMI-UNDERGROUND HERMETIC STORAGE ON A LARGE SCALE

Some of the benefits of traditional underground pits have been made use of in planning for large scale storage of grain in Argentina, Cyprus and Kenya (Hyde et al. 1973). Advantage has been taken of modern methods of construction and suitable waterproof membranes have been used to prevent ingress of ground water. When good quality grain has been put into these structures it has been stored without loss of quality for several years and with the minimum of attention.

### 4. THE NEED FOR LARGE SCALE HERMETIC STORAGE IN KENYA

Although Kenya has been largely self-sufficient in producing her staple food maize, there have been occasions when poor rainfall has resulted in near famine especially in the marginal areas. As a long-term remedy it was decided to have a large scale reserve of grain suitably preserved in good condition for minimum periods of 3 years.

Following the successful development of hermetic bins in Cyprus (Oxley et al. 1960) similar structures were considered for Kenya. Construction was begun in 1966 and completed in 1967 and 1968 at two sites. 40 Bins were built at Kitale (approx. 250 miles from Nairobi) and 30 Bins at Nakuru (approx. 100 miles from Nairobi) giving a total capacity of 100,000 tons of maize.

### 5. THE DESIGN AND CONSTRUCTION OF THE "CYPRUS" BINS

The design and constructional details have been fully given by Hyde et al. (1973). Each bin is a concrete lined cone 20 ft in the ground, covered by a concrete shell dome 20 ft high. The underground cone gives rise to a reinforced concrete ring beam at ground level (diameter 64 feet) on which the dome rests.

#### 5.1 The underground cone

The sites at which the bins are built have a water table 30 ft below ground level giving a 10 ft margin of safety. After excavation and formation of the correct slope, the slope was blinded with 3 inches of weak mix concrete and then treated with 4-5 coats of bituminous emulsion embedded (for binding) with a scrim of nylon mesh or hessian. Care was taken to ensure that the membrane was completely continuous. The membrane was then blinded with sand and, when dry,

covered with a half inch coat of cement mortar. Reinforcing rods were fitted in position, and a 4 inch thick layer of structural concrete was placed in the slope of the cone. The ring beam was then formed at ground level.

## 5.2 The dome roof

The dome is required to support its own weight and withstand other forces such as the weight of operatives on the surface and internal grain pressures and for this reason it was necessary to form a corrugated shell (Fig. 1), the construction of which required considerable care and skill. A temporary formwork of 12 arch ribs was prepared and covered with a hessian envelope. After an initial  $\frac{1}{2}$  inch coat of sand/cement mortar was placed on the hessian, reinforcing rods were placed in the valleys and the crest. Two further coats of mortar  $1\frac{1}{4}$  and  $\frac{1}{2}$  inches thick were then applied. When the dome was thoroughly dry, the



Fig. 1. View of the "Cyprus" bins at Kitale. In the foreground, a bin is being pressure tested for leaks.

bitumen membrane was applied using at least 4 coats of bitumen emulsion and nylon mesh scrim or lightweight hessian for binding. The membrane was continued through the gutters and over the kerb of the ring beam to join with that placed under the base cone. Two final reflective coats of white paint were applied.

Each bin has two man-holes: one at the apex of the dome for filling and one at ground-level for emptying. Air-tight hatch covers are provided for each man-hole.

## 6. CORRECTION OF STRUCTURAL FAULTS AFTER CONSTRUCTION

### 6.1 The ring beam

In 1971, approximately 3 years after the bins were constructed, a small amount of damage was observed to grain adjacent to the ring beam due to seepage of water through the external membrane of the bin and the structural joint at the point of connection of the dome to the ring beam.

Initial repairs consisted of renovation of the external bituminous membrane. This later proved ineffective mainly because the dome pivots about the point where it is connected to the ring beam. This movement is caused by the expansion and contraction of the thin shell due to solar radiation.

An alternative repair procedure developed in 1973, has since proved effective and is still in use on a routine basis. This consists of removing the bitumen membrane around the gutter area on the ring beam. The structural joint is then located, by chiselling away some of the concrete if necessary. The joint is then sealed using a suitable mastic sealant (Reebdeck mastic). This is done by placing the bin under negative pressure (6 in. wg) with a test fan and applying the paint over and about 3 inches on either side of the joint. The paint is drawn in until the crack is filled. A second coat is given to the joint (under negative pressure) and to the whole gutter area. The joint at the kerb of the ring beam is similarly treated. The effectiveness of the seal is tested by applying positive pressure (6 in. wg) and leaks detected by splashing water over the joints. Any leaks discovered are repaired and a final coat of white PVA emulsion is applied to protect the Reebdeck mastic from solar radiation. The bins are routinely tested for leaks after each emptying (Figure 1).

### 6.2 Hatch covers

The original steel hatch covers did not provide sufficient insulation against diurnal temperature variations. This resulted in a considerable amount of condensation on the underside of the steel cover. In addition, the cover could not be adequately sealed to prevent the entry of a small quantity of air into the bin. New hatch covers manufactured of fibre glass were installed and used with a sealant to provide an air-tight seal. The inspection cap was made air-tight with a rubber gasket.

## 7. OPERATION AND MONITORING OF GRAIN CONDITION

### 7.1 Operation

Only fresh good quality grain is accepted for storage in the Bins. This grain (which may arrive in bags) is passed through a cleaning and drying plant (where dust is removed and grain dried to below 12.5% moisture) before being conveyed in bulk carriers to the bin site. Before filling, each bin is thoroughly cleaned and disinfested.

The bins are rapidly filled to capacity (within 3 weeks) and before sealing, tablets releasing 30 gm of hydrogen phosphide gas or a strip releasing 17.7 gm dichlorvos are placed on the surface of the grain at the top and side hatches. This treatment is for the control of superficial infestation by Sitotroga cerealella and Ephestia cautella.

The bins remain sealed for a period of 6 months before the first sampling is done through the inspection cap (diameter 10 cm).

### 7.2 Monitoring of grain condition

Each bin is fitted with thermistors for monitoring temperatures and gas extraction tubes for recording oxygen levels. These measurements are taken remotely using the Telemax and Servomax instruments respectively. Grain is also sampled through the inspection cap of the top hatch using a sampling probe. Details of monitoring positions are shown in Figure 2.

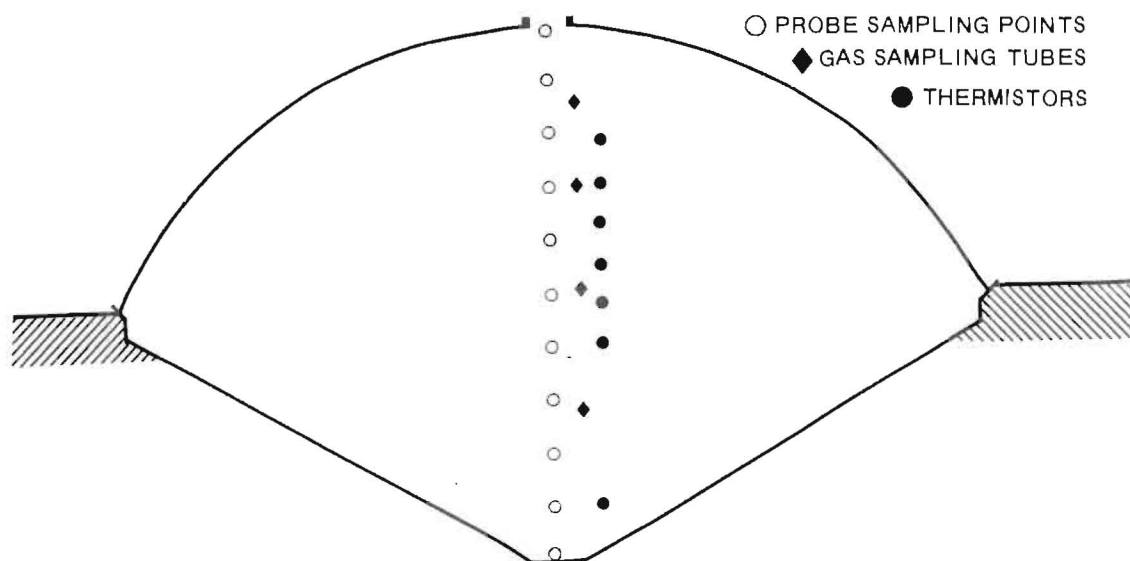


Fig. 2. Monitoring positions for grain samples temperature and oxygen levels in a "Cyprus" Bin.



### 7.3 Temperature

The grain temperature during loading is generally in the region of 20-25°C. Seven thermistors are located in each bin along the central axis, one each at the following depths; 9'9", 12'9", 15'9", 18'9", 21'9", 24'9", 36'9".

Temperatures are recorded daily after filling until the readings are fairly constant around 25°C. Thereafter records are taken at weekly intervals unless a sudden change is observed. In the event of a temperature rise to 35°C or 10°C above normally recorded values, daily measurements are taken and the bin sampled using a suction probe.

### 7.4 Oxygen

The oxygen concentration is measured using a Servomex Oxygen Analyser. Gas sampling tubes are installed at 6', 12', 20' and 30'. Records are taken as soon as the bin is sealed and thereafter at weekly intervals until the oxygen level is less than 6% or until a constant value is reached. When this happens further records are taken at intervals of 4 weeks.

### 7.5 Grain samples

Grain samples are taken by passing a Probe-A-Vac suction sampler through the inspection cap (diameter 10 cm) of the top hatch cover. Sampling is done immediately after the bin is filled and subsequently at 6 month intervals. More frequent sampling may be done in the event of a rapid increase in grain temperature.

Samples of at least 1 kg each are taken from the surface and at 4 ft. intervals through the central axis to the bottom of the bin. Sampling depths are 0', 4', 8', 12', 16', 20', 24', 28', 32', 36' and 40'.

Each sample is examined separately. A mercury glass thermometer is used to record the temperature of each sample as soon as it is removed from the probe. In the laboratory, each sample is weighed and then sieved.

The thrash passing through the sieve (including insects) is weighed and recorded. The moisture content of the sample is then taken using a meter (Supermatic or Burroughs). The insects present in the thrash are identified and the number of live and dead individuals recorded as numbers per kg. Each sample is then divided into approximately 4 sub-samples of 250 gm each using a box divider. One sub-sample is incubated at room temperature (approx. 20-22°C at both sites) for 8 weeks and the numbers of adult insects emerging recorded.

A second sub-sample is examined for damage. This is done in two operations. First, the grains are separated into four classes: broken, holed, tunnelled and whole. The weight of each class is recorded. The sub-sample is then re-constituted and the grains separated once again into discoloured and normal and a record made of the weight of the discoloured fraction.

## 8. OBSERVATIONS AND RESULTS

### 8.1 Routine monitoring

Some observations from the routine monitoring of Bin No. 13 in Kitale are shown in Figure 3. The results are fairly typical of the bins loaded with good quality maize using the chain conveyor. Oxygen levels remained between 17.5% and 19% over the first 18 months of storage then dropped gradually to 5-6% in the following 18 months before emptying.

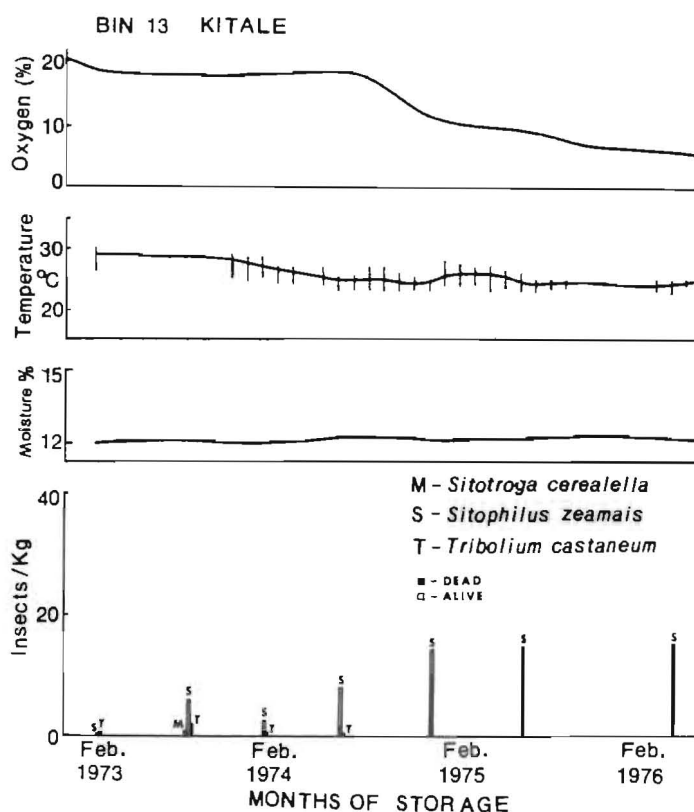


Fig. 3. Summary of routine monitoring data for oxygen, temperature, moisture and insect population levels in grain in Bin 13 at Kitale over 42 months storage.

Temperatures varied between 25°C and 30°C over the 3 years. The average grain moisture was below 12.5% for the entire storage period. Probe samples showed that infestations by *Sitophilus zeamais*, *Sitotroga cerealella* and *Tribolium castaneum* were kept in check by the hermetic conditions. The insects are mainly in the centre where sampling is done and so the infestation given as numbers/kg. is unrepresentative of the distribution of insect population through the grain bulk. However, for monitoring purposes, the central axis of the bin is an ideal place to sample.

### 8.2 Oxygen

Since the bins are loaded with grain that comes directly from the farm, there is a small amount of initial infestation by insects and fungi. The cleaning and drying process causes some mortality to the insects; and development of fungi and insects is further reduced by drying the grain to below 12.5%. Oxygen levels therefore remain high for a fairly long period of time and often for more than 12 months. When the concentration of oxygen begins to fall, this is associated with the increase in fungal growth. Daily fluctuations in temperature cause a small amount of condensation on the top of the dome. This moisture drops on the top layer of grain and enhances fungal development. Fungal development causes discolouration of the grain and is reflected as "discoloured" grain in samples. The association between the decrease in oxygen concentration with increased fungal growth on the surface layer of grain is shown in Figure 4.

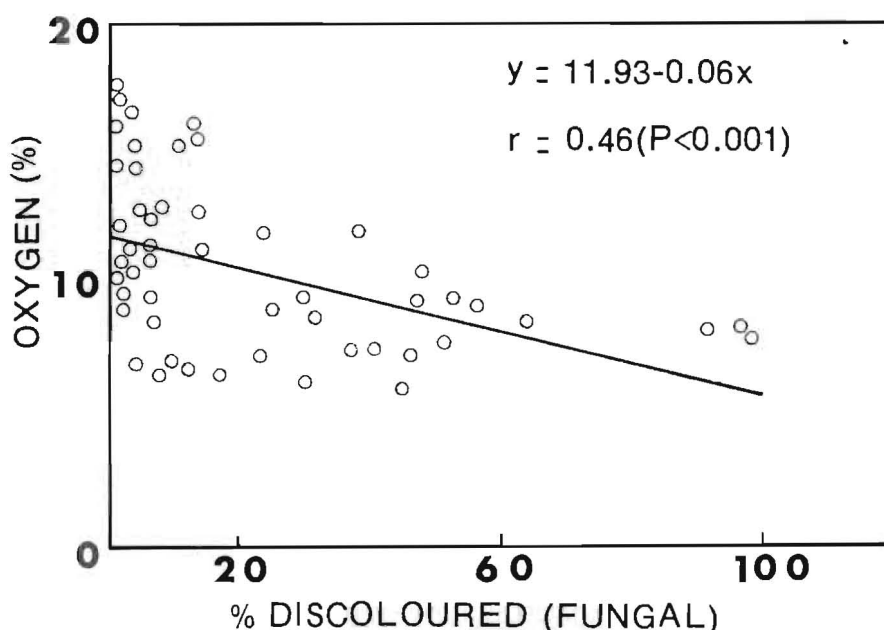


Fig. 4. Relationship between oxygen concentration and fungal growth.

Each point on the graphs represents the relevant observations made on individual bins at the Kitale site over the 1972-1980 period.

### 8.3 Moisture and Microflora

The increase in the amount of fungus infested grain was directly related to the amount of grain moisture (Figure 5). The average moisture content of the top 4 feet was above 14% and had fairly high fungal damage. The greatest fungal damage was in the top 2 ft. of grain and generally this had to be discarded as

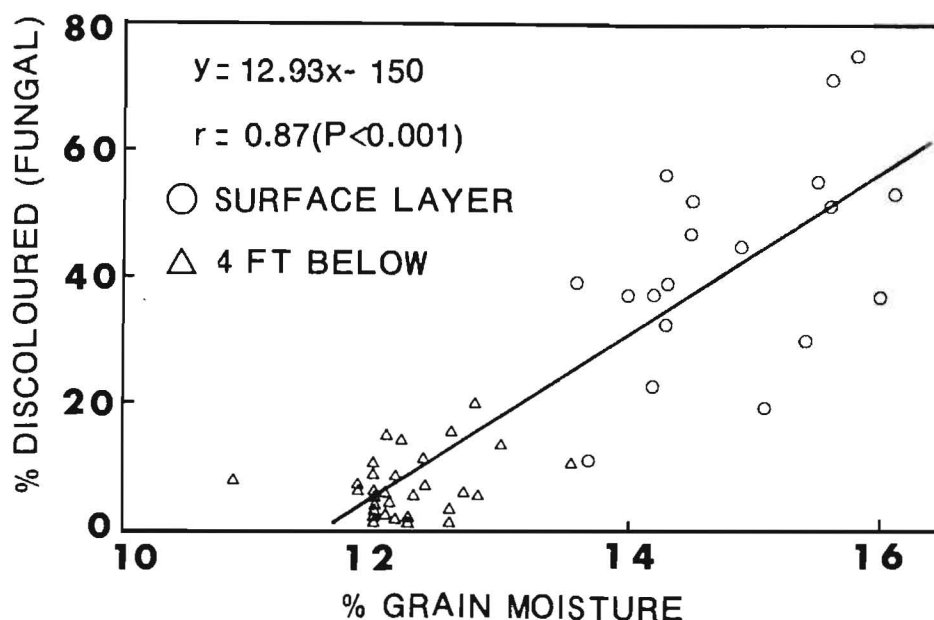


Fig. 5. Fungal growth and grain moisture at the top and 4 ft below.

rotten grain. As is explained later (Section 9.2) this represents a very insignificant amount of loss. The grain below this for another 2-3 ft. often required aeration and drying and was generally graded as animal feed. The average moisture content of grain in the 4-8 ft. layer was between 12 and 13 percent and showed little or no fungal discolouration. Grain below this level was entirely free of fungal discolouration except for small amounts at the ring beam where cracks had occurred and sometimes at the very bottom (40 ft. deep). The main fungi were species of Aspergillus, Pencillium and Rhizopus.

#### 8.4 Temperature

Average daily temperatures over the year rarely exceed 25°C at both, the Kitale and Nakuru, bin sites. However, there is often a large difference between day and night temperatures. This difference, which usually exceeds 10°C, is transferred through the thin shell of the dome. The night temperatures on the inner surface of the bin dome are generally below the dew point temperature of the internal atmosphere. Condensation therefore takes place and the resulting moisture drops on the surface layer of the grain.

Average temperatures through the grain bulk are approximately 25°C. They are usually about 22°C through most of the grain bulk and slightly higher than average in the top 4 feet. The amount of dust in the grain has a very significant effect on temperatures. The original conveying machinery installed on

the site in 1968 and used up to 1972 was pneumatic in design and caused breakage of grain and accumulation of dust especially along the central core of the bin. This resulted in severe heating and grain had to be transferred to other bins to arrest the problem (Figure 6.). For example, the grain in Bin 19 was transferred to Bin 35 when temperatures approached  $40^{\circ}\text{C}$  in the first 8 months after filling and within 6 months had reached  $47^{\circ}\text{C}$  in Bin 35. It was then re-cleaned and transferred to Bin 38 where it remained for a further 18 months without severe problems.

In the second filling of Bin 38 the chain and flight conveying equipment was used and no serious heating problems arose. The grain remained in a satisfactory condition for nearly 40 months.

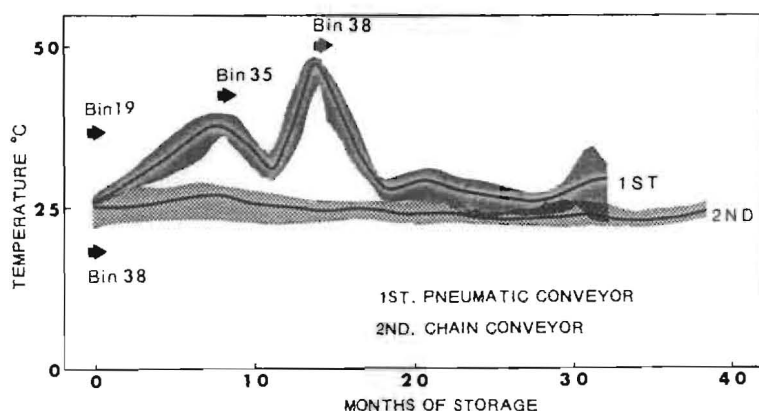


Fig. 6. Pneumatic conveyor causes grain breakage and heating results from dust accumulation. No breakage and heating with chain conveyor.

### 8.5 Insect infestation

The main insect species infesting the produce in the bins were Sitophilus zeamais (Motschulsky), Sitotroga cerealella (Olivier), Ephestia cautella (Walker) and Tribolium castaneum (Herbst). Sitophilus and Sitotroga usually accompany the grain from the farm. Ephestia and Tribolium are present in conventional stores and mills near the bins and infest the produce when the bins are open especially during extended filling operations.

The insect infestation is often fairly low in fresh grain but has the potential of building up to alarming levels within 2-3 months if not kept in check. When a bin is filled, a limited amount of disinfestation at the top and side hatches is carried out as explained earlier (Section 7.1). The amount of



hydrogen-phosphide used (30 gm) is 1/300th of what would normally be required for complete disinfestation in a conventional non-hermetic store.

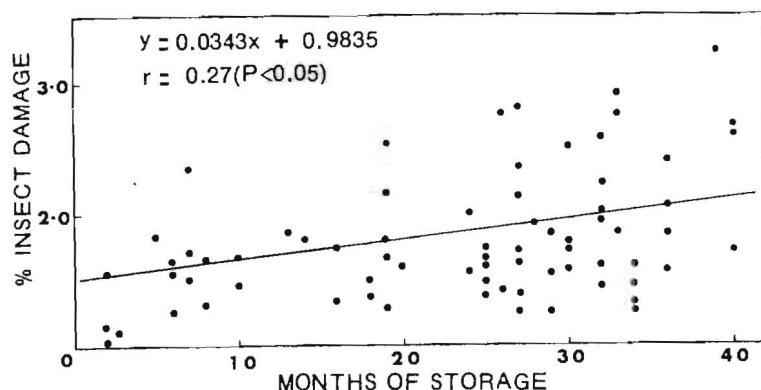


Fig. 7. Percentage of insect damaged grain in final samples before emptying the bins.

The hermetic conditions and the low grain moisture kept the damage caused by the insects in check. This is illustrated in Figure 7 which is a plot of insect damage found in final sampling before emptying each bin. Insect numbers rarely exceeded 10/Kg along the central axis, where the probability of finding insects is highest, and this was reflected in the very limited amount of insect damage to grain samples collected at intervals along the central axis. Insect damage represented by the percentage of grains showing exit holes and internal tunneling is on average below 3% after 3 years of storage.

## 9. EMPTYING OPERATIONS AND DETERMINATION OF LOSSES

### 9.1 Emptying

Each bin is normally emptied after 3 years of continuous storage. Both (top and side) hatches are opened and the produce well ventilated before unloading begins. Usually a small quantity of wet and rotten grain is found below the top hatch on the underside of the dome, and sometimes around the ring beam and in the bottom of the bin. This is removed separately, weighed and destroyed. Damp and mouldy grain adjacent to the wet and rotten maize is also removed separately, dried and aerated in the cleaning plant, weighed and stored separately for inspection and grading by the National Cereals Marketing Board. This produce may be sold as undergrade stock for animal feed. All the good maize emptied from the bin is weighed and graded. If the maize is passed through the cleaner, the weight of the dust obtained is also recorded.

## 9.2 Determination of losses

In the early (1972) filling operations, records of the quantity of maize loaded into individual bins were not maintained. An average capacity was determined for the total number of bins filled. This was calculated as 15,474 (90 kg) bags per bin. Since there are variations in individual bin capacities, some bins registered fewer than 15,474 bags on unloading. Other bins registered more. This information provided some (unforseen) confusion in the determination of losses. It was therefore decided that separate records would be maintained of the weight of grain being loaded into each bin.

Losses were therefore determined on the actual quantities of grain unloaded. The contents of each bin when unloaded were separated into rotten grain, grain for animal feed, dust and good grain and then weighed. An analysis of these losses for the bins at the Kitale site are summarised in Table 1. In all the data presented in this paper, the results for Bin No. 16 (for the 1972 filling only) at Kitale are omitted because of an unaccountable discrepancy. The data in Table 1 shows the results from using the 40 bins at Kitale 78 times. The average period of storage (after sealing) was 26.5 months and a total quantity of 108,777 tonnes of maize (equivalent to 1,208,637 x 90 kg bags) was stored from January 1972 to March 1980. As a consequence of a current shortage of maize all the grain has been removed for consumption. The average period of storage (over this 9 year period) was also shortened because security problems at the border necessitated removal of the food reserve to safety in 1976.

TABLE 1

Analysis of losses at Kitale site (1972-1980)

Period of storage (months after sealing)	Number of bins	Rotten grain (%)	Animal feed (%)	Dust (%)	Good grain (%)
< 12	16	0.027	0.027	0.015	99.932
13-24	13	0.154	0.110	0.107	99.628
25-36	28	0.224	0.294	0.204	99.288
> 37	21	0.280	0.335	0.347	99.038

The loss in weight obtained as described above is the "gross" value and for the entire 9 years that the bins have been used the maximum loss of 2.17% occurred in Bin 19 after 42 months of storage (Figure 8). In general, average annual weight losses were below 0.3%. It should be pointed out that these weight losses do not imply monetary loss as part of the "losses" include

animal feed.

The losses due to breakage caused by the pneumatic conveyor, classified as "dust", were successfully reduced by installation of the chain and flight conveyor.

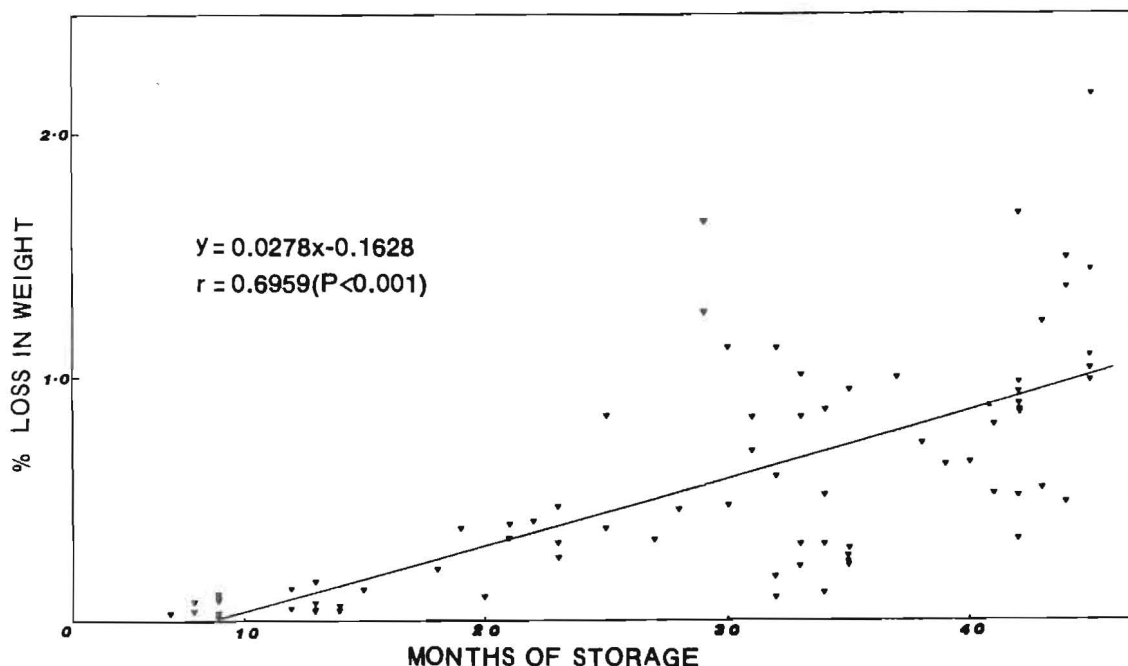


Fig. 8. Loss in weight (gross value) after hermetic storage.

The amount of "dust" in the gross value of losses dropped from 39% with the pneumatic conveyor to barely 5% with the chain conveyor (Figure 9). It is interesting to note that animal feed and rotten grain are approximately in the 1:1 ratio and with the present almost negligible amount of loss due to dust and breakage, true losses mainly due to rotten grain are in the region of 0.15% annually. In contrast annual losses in conventional storage are often in excess of 2% by weight despite regular insecticide and fumigation treatments.

## 10. DISCUSSION

Semi-underground hermetic storage has considerable potential for future use in the tropics especially for the preservation of national famine reserves of grain. The "Cyprus" bin may not be the most appropriate structure especially in countries with a shortage of trained personnel.

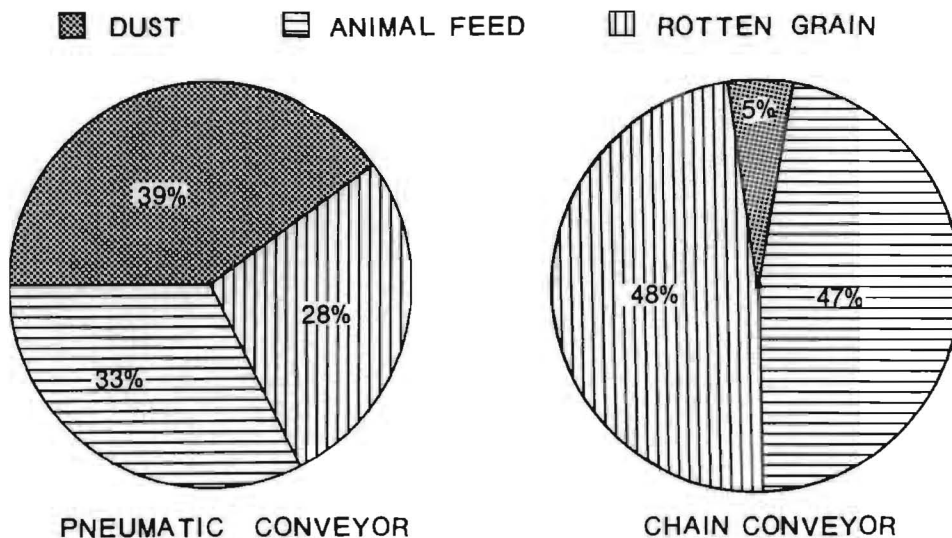


Fig. 9. Losses resulting from use of pneumatic and chain conveyors.

The experience reported here shows that a fixed roof for a semi-underground hermetic structure is not ideal and confirms previous experience in Argentina (Hyde et al., 1974). It would appear that temperature variations in the tropics is the most important aspect to be taken into consideration in the design of a suitable roof for a semi-underground hermetic silo. Presently a new set of hair-line cracks are appearing on the ring beam leading from the ridges of the dome. Although these are satisfactorily repaired following the procedures mentioned earlier, they are nevertheless a source of concern and an indication that future designs will have to be more carefully evaluated.

Moisture condensation on the inner surface of a semi-underground structure is inevitable in the tropics but can be reduced to fairly insignificant levels. The resulting fungal infestation is generally negligible and in a properly air-tight structure may be used to advantage in that a more rapid reduction of the oxygen concentration is achieved to limit insect development. Structural modifications to reduce condensation problems in the "Cyprus" bins are not economically justifiable.

At the national level, planning for the storage of food grain should take an "integrated" approach (De Lima, 1979). A certain proportion of the national harvest should be reserved for hermetic storage and retained for periods of not less than 12 months and preferably for 36 months. As mentioned earlier, minimal use of pesticides is required in hermetic storage and with proper

planning, national grain marketing organisations can make satisfactory use of modern hermetic storage technology.

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## EFFECT OF AIRTIGHT STORAGE ON INSECT PESTS OF STORED PRODUCTS

N.J. BURRELL

## 1 INTRODUCTION

Many workers have shown that removal of oxygen by respiration causes insects to die in a sealed container (Dendy and Elkington, 1920). Thus over a period of 14 days at temperatures of 29° to 30°C, Bailey (1955, 1956, 1957, 1965) found a concentration of oxygen below about 2%, killed six important species of grain infesting beetles. Since, however, the susceptibilities of the various adult and immature stages were not equally affected, it seems likely that when a mixed population of insects is sealed into an airtight bin, some species will be eliminated sooner than others and some might even survive. In practice also there is some risk of oxygen diffusing into containers and permitting some survival (Oxley and Wickenden, 1963). In any event, low temperatures prolong insect life and so delay the process of disinfection.

The test described here was set up at short notice, when a farm infestation was discovered, to find if butyl-rubber airtight grain storage bins could be used in practical farm conditions in the U.K. to destroy an existing infestation in grain.

## 2 METHODS

2.1 Farm store

The investigation was carried out between March and July using 40 tonnes of heavily infested barley of 15.5-16% moisture content from a farm store in which a temperature of 42°C was recorded and in consequence seeds were sprouting on the surface in the centre of the bulk and some 0.5 tonnes was visibly mouldy. The badly damaged barley was destroyed and the rest was transferred into two airtight bins. The five abundant species of insects in the barley bulk were Oryzaephilus surinamensis L., Sitophilus granarius L., Cryptolestes ferrugineus Steph., Ahasverus advena Waltl. and Typhaea stercorea L.

2.2 Airtight bins

The two airtight bins, each consisted of a cylinder of welded-steel mesh and a cylindrical lining bag made of black butyl-rubber 0.75 mm thick which could be sealed at the top. These were erected in the open about 7 m from the farm store. Bin 1 was 4 m in diameter and 2.7 m high; Bin 2, was 3.4 m diameter and 3.4 m

high. Twenty tonnes of barley, mainly from the heated core in the farm store including some of the damp barley from near the surface, was placed in Bin 1 and 20 tonnes from the cooler periphery of the original farm bulk were placed in Bin 2. The grain was moved by shovel onto a rubber belt elevator into the bins.

The bins had already been used for storage and were slightly damaged and dirty. All holes found were patched but 3 weeks later when Bin 1 was inspected at the end of the test, prior to the final sampling, three further slits, 2-4 mm long, were found in the top sheet and were repaired after sampling the grain.

### 2.3 Temperatures

Three thermocouples were placed in a vertical row up the centre of the bins at distances of 1.0, 1.8 and 2.6 m above floor level and temperatures were measured during each weekly visit to the farm.

### 2.4 Gas sampling

Two rigid pvc air sampling tubes of 5 mm internal diameter were placed in each bin during loading, one at the centre and one in the small air space below the top sheet. The inner end of each tube was guarded by wire mesh to prevent blockage and the outer end was sealed with rubber tubing closed by a screw clip.

Air samples were drawn from the sealed bins, initially at daily intervals and later at weekly intervals, by a double-action pump and stored in 30 ml metal cylinders at a pressure of 10-20 bars, for later analysis at the laboratory using a modified Haldane apparatus.

### 2.5 Sampling for insects and moisture content

Immediately after loading, before the bins were sealed, barley samples were taken from selected locations with a 200 g gravity-filled sampling spear. The sampling points were spaced vertically at intervals of 0.3 m below 7 points evenly spaced over the upper surface in Bin 1 and below 6 in the narrower Bin 2 yielding 56 samples from Bin 1 and 63 from Bin 2. Some samples were omitted from the sampling pattern where there was a possibility of displacing thermocouples or gas sampling tubes. Every sample was sieved to remove all free-living stages of insects but because the insects were so numerous, and counting was carried out under difficult conditions on the farm premises, only adults were counted. At the end of storage, because there were so many insects initially, the number of sampling points in the bulk was reduced by increasing the vertical spacing to 0.6 m. However, inspection of the bins before opening revealed three small punctures in Bin 1 so a sample was taken from the surface grain close to each puncture and further 14 surface samples were taken from each bin. These

samples were sieved to remove free living and dead adults and then all the samples from each depth from Bin 2 were collected together in lots of about 1.2 kg and kept in a room at 25°C. At intervals of 2-13 days thereafter, the barley samples were sieved and adults were removed, recorded and destroyed but all other debris sieved off was replaced.

Bin 1 was opened for insect sampling after 3 weeks and then closed for gas sampling for another 5 weeks until the grain in the bin was removed for feeding to cattle. Bin 2 was kept closed for gas sampling for as long as possible and sampled for insects after 15 weeks immediately before the grain was removed for cattle food.

## 2.6 Removal of oxygen from Bin 2

Five weeks after sealing, Bin 2 still had an oxygen concentration near 10%. Therefore, the bin was opened sufficiently for 100 kg of damp barley at 23% moisture content to be added on a polythene sheet and the bin was rapidly resealed.

## 3 RESULTS

### 3.1 Temperatures

Immediately after loading, the temperatures in the centre of Bin 1 varied from 16.5°C to 35°C and those in Bin 2, from 12°C to 16°C. The maximum temperature recorded in Bin 1 reached 40°C during the first day but fell to 35°C during the first week and to 23°C after three weeks when the bin was opened and sampled. No temperature below 15°C was recorded in this bin during these three weeks but the minimum temperature recorded reached 13°C by the time emptying was started.

Grain temperatures in Bin 2 scarcely changed during storage.

### 3.2 Gas analysis

The two oxygen concentrations obtained from each bin on each visit never differed by more than 1% so the means are presented in Fig. 1.

In Bin 1 the oxygen concentration fell to 4% in 2 days and reached a minimum of 0.3% after about 10 days (Fig. 1 upper). After 3 weeks, when the bin was opened for 6 hours for grain sampling, the oxygen concentration rose to nearly 15% but when the bin was resealed it began to fall steadily though more slowly than before. This is evidence that the bin still contained respiring organisms, possibly micro-organisms, in the patches of damp grain transferred from around the farm hot spot into the test bins.

The oxygen level in Bin 2 had fallen only to 10% in about 4.5 weeks and even when damp grain was added, it fell only slightly faster but it reached a minimum of 1% after 2 months and remained below 5% until the bin was opened after 15 weeks.

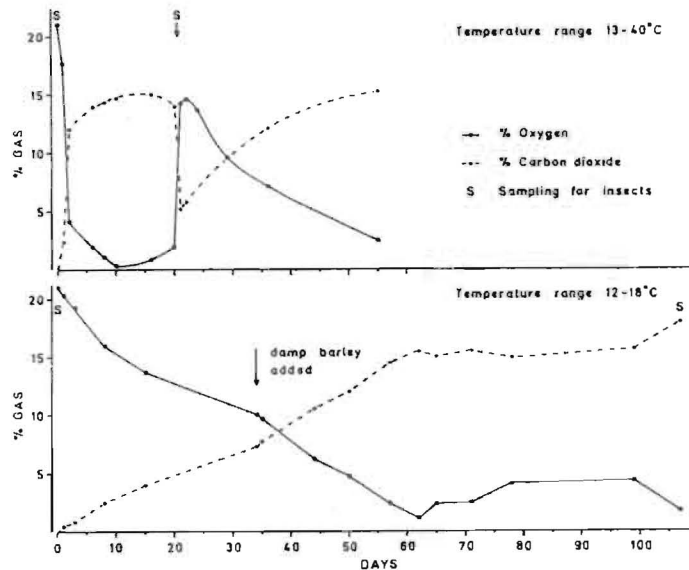


Fig. 1. Diagram showing changes in gas concentration with time and sampling occasions in airtight Bin 1 (upper) and Bin 2 (lower).

### 3.3 Insect numbers

Approximately 5000 live adult insects were removed from each bin in the initial samples indicating a population of about 8 million adults per bin. The initial numbers for each species were similar in both bins.

#### Bin 1

The initial 56 samples contained a mean of 91 adults per sample (range 5-392) with all species spread fairly evenly throughout the bin. There were only 33 live adults in the final 38 samples and of these 26 were in the 18 samples from the upper surface, 17 being in the three samples taken close to the visible small punctures. This represents a survival of only 0.6%. The majority of these survivors were C. ferrugineus, which, unlike the other species present, had a high rate of survival near the punctures (Table 1.)

#### Bin 2

The initial 63 samples each contained a mean of 78 live adults (range 16-255) again fairly evenly distributed though slightly more abundant in the top half of the bin (Table 2.) The final 51 samples contained a total of 10 live adults of which 7 were found in 21 samples from the upper surface although no obvious

leaks were found. This represents a survival of only 0.2%. However, 13 S. granarius, five C. ferrugineus and one O. surinamensis emerged from the grain samples at 25°C (Table 3) and had survived exposure to concentrations of 1-4% oxygen and 15% CO<sub>2</sub> maintained during the last 8 weeks of the test. No A. advena or T. stercorea were found alive after airtight storage.

TABLE 1

Number of live adult insects from Bin 1 samples

A. Before airtight storage		Insect	<u>O.s</u>	<u>C.f</u>	<u>A.a</u>	<u>S.g</u>	<u>T.s</u>
Samples	Number	Numbers per sample					
Bulk	49	Max	378	83	34	6	6
		Min	11	0	0	0	0
		Mean	70	18	5	1	1
Top surface	7	Max	101	10	2	3	3
		Min	0	0	0	0	0
		Mean	53	3	1	2	1
Total	56	Total	3824	902	249	86	57
B. After airtight storage for 3 weeks							
Bulk	20	Max	0	2	0	1	0
		Min	0	0	0	0	0
		Mean	0	0.2	0	0.1	0
Top surface	18	Max	1	7	0	1	0
		Min	0	0	0	0	0
		Mean	0.1	1.2	0	0.1	0
Total	38	Total	2	27	0	4	0



TABLE 2

Numbers of live adult insects from Bin 2 samples

A. Before airtight storage							
Samples		Insect	O.s	C.f	A.a	S.g	T.s
Position	Number	Numbers per sample					
Bulk	58	Max	232	45	14	4	10
		Min	13	0	0	0	0
		Mean	60	12	2	0.8	1.3
Top surface	5	Max	143	18	10	1	3
		Min	30	1	0	0	0
		Mean	71	9	4	0.2	1.4
Total	63	Total	3876	768	165	45	83

B. After airtight storage for $3\frac{1}{2}$ months							
Bulk	30	Max	1	1	0	0	0
		Min	0	0	0	0	0
		Mean	0.03	0.1	0	0	0
Top surface	21	Max	2	3	0	0	0
		Min	0	0	0	0	0
		Mean	0.14	0.19	0	0	0
Total	51	Total	4	6	0	0	0

TABLE 3

Breeding out tests at 25°C on bulk samples from Bin 2

Depth (m)	0	0.6	1.2	1.8	2.4	3.0	
Insect	Numbers per sample						Total
<u>O.s</u>	0	0	0	0	0	1	1
<u>C.f</u>	3	0	0	1	0	1	5
<u>S.g</u>	0	3	8	1	1	0	13

#### 4. DISCUSSION

Although insufficient oxygen leaked into the small airspace at the top of Bin 1 to raise the concentration above that in the middle of the bin it was enough to permit the survival of a few adult insects for nearly 3 weeks, indicating that higher concentrations than those measured existed close to the punctures. The mortality of C. ferrugineus adults reached over 97% in the bulk but most of the survivors of this species were found in the vicinity of these leaks. Only 2 live O. surinamensis and 4 S. granarius were found after storage so mortality was almost complete. However there were sufficient survivors, particularly of C. ferrugineus to recolonise the bulk if oxygen and temperature later became suitable. The Bin 1 test was completed in March, exposing the insects around the periphery to low ambient temperatures which may have promoted their survival by reducing their activity.

Because oxygen remained above 5% for 2 months in Bin 2, final sampling was delayed till summer and in this bin, only 6 adult C. ferrugineus and 4 adult O. surinamensis were found alive in 51 final samples. Despite the higher kill of adults in Bin 2, the breeding out tests showed that survival of the three granivorous species had occurred in the bulk.

The damp grain at 23% moisture content placed at the top of Bin 2 to absorb oxygen and speed disinfestation was estimated to be sufficient to produce an anaerobic atmosphere in the bin within a week under the prevailing temperature conditions assuming a dry matter loss near 0.01% per day (Burrell, 1974, pp. 424-7) and assuming that gaseous diffusion was not a limiting factor. Because deoxygenation occurred more slowly than expected, a further test was set up at the laboratory in a similar butyl-rubber bin, 3.4 m diameter, holding 10 tonnes of uninfested wheat 1.5 m deep at 15.3% moisture content. In this test 60 kg of grain wetted to 30% moisture, with an estimated dry matter loss near 0.1% per day, was spread in a layer 2.5 cm deep on a plastic sheet covering 35% of the upper grain surface and the bin was then sealed. The atmosphere at the top of the bin reached 2% oxygen in less than 2 days but took 8 days to reach the same oxygen concentration at the centre of the bin only 0.75 m below the top.

#### 5 CONCLUSIONS

The procedure of sealing infested grain into an airtight bin to kill the insects when their own respiration has removed oxygen from the bin can be satisfactory for a heavy infestation in warm grain but is likely to prove uneconomic for light infestations or cool grain because of the long storage period required. Even when the conditions provide a sufficiently low concentration

of oxygen it appears, in practice, that sufficient insects are likely to survive to provide an inoculum for reinfestation if the grain is removed from the bin.

The method, therefore, has some limitations and the replacement of oxygen by flushing out the air with CO<sub>2</sub>, described in later papers, may prove more satisfactory. However, the use of fungal respiration or fermentation to achieve anaerobic conditions shows considerable promise, seems highly cost effective and merits further investigation.

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## II S E S S I O N :

ENTOMOLOGY OF CONTROLLED ATMOSPHERE STORAGE





## LOW TEMPERATURES: EFFECTS ON CONTROL OF SITOPHILUS ORYZAE (L.) WITH MODIFIED ATMOSPHERES

EDWARD JAY\*

### INTRODUCTION

A considerable amount of information has been obtained in recent years concerning the effects of modified or controlled atmospheres on stored-product insects. However, most laboratory studies have been carried out at temperatures ranging from 26° to 33° C. Bailey and Banks (1974), in a summary of 10 of the more recent laboratory studies, found only one that was conducted at temperatures as low as 15.6° C, and this one (Harein and Press, 1968) had to do only with adult Tribolium castaneum (Herbst). Again, when Banks (1978) summarized results of field studies with modified atmospheres, he noted only one low temperature large-scale field study (Banks et al., 1978). This was conducted at 13.5° C, and 77 to 79% mortality of all life stages of Sitophilus oryzae (L.) was obtained by a 10-day exposure to atmospheres containing from 47 to 68% carbon dioxide (CO<sub>2</sub>) (balance of modified atmosphere was air).

Earlier Banks and Annis (1977) had suggested when a storage facility containing grain at 15° C is purged with nitrogen (N<sub>2</sub>) for insect control, the exposure time must be greater than 24 wk for complete disinfestation, even when the oxygen (O<sub>2</sub>) level is held at 0-1.2%.

Because of the lack of information on the combination of low temperature and modified atmospheres, the following study was undertaken.

### METHODS AND MATERIALS

The insects used in this study were immature S. oryzae that are continuously reared at the Savannah laboratory on 12% m.c. soft red winter wheat at 26.7° C and 60% RH. The cultures for the tests were established by seeding 360 g of this wheat with ca. six hundred 1- to 3-wk-old adults for 72 hr. At the end of this period, the adults were sieved off leaving immature insects 0 to 3 days old in the cultures. S. oryzae used in these tests were therefore from 0 to 3, 7 to 10, 14 to 17, 21 to 24, or 28 to 31 days old at the beginning of the exposure. Wheat containing insects of these ages was blended together for 5 min in a ball mill, and 5 g of this blend was placed in a 16 x 14 (to cm)

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mesh wire screen cage measuring 6.4 cm high x 1.9 cm diam. An adequate number of cages was filled to provide a group of three 5-g cages for each exposure plus another cage that was used as the control for each group.

The exposure chambers were similar to those described by Harein and Press (1968) and consisted of 2.8-liter glass jars that were partly submerged in laboratory baths filled with water. These baths were equipped with refrigeration systems so the water temperature could be reduced to levels below the ambient temperature. Four such baths were used, and they were individually set to maintain the water temperature at ca. 1.6°, 4.4°, 10.0°, or 15.0° C. The jars were closed with metal screw-top lids fitted with 23- and 2.5-cm lengths of 0.6-cm o.d. copper tubing; these were used as gas inlet and gas exit tubes, respectively. The lids were also fitted with a neoprene stopper so a humidity sensor could be inserted. During exposure, the cages were suspended in the exposure chambers from a 5-cm length of steel wire hung from the underside of the neoprene stopper.

The gas mixtures were released from the cylinders through two-stage regulators and flowed through a micrometering valve and flowmeter into gas washing bottles that contained a glycerin-water mixture that adjusted the RH of the gases to ca. 56%. The gases then flowed into the exposure chambers. A flow rate of 200 cc/min was used for the first hour to purge the chambers. A rate of 30 cc/min was used for the balance of the exposure periods. The RH was monitored with an electric hydrometer (model 15-2001 humidity indicator and narrow range humidity sensors, Hygro-dynamics, Inc.), and the temperature was recorded daily.<sup>1/</sup>

A Fisher-Hamilton model 29 gas partitioner equipped with dual columns was used for daily analysis of ternary mixtures. A Vidar model 6300 digital integrator was used to measure the areas under the peaks. A Beckman model E-2 oxygen analyzer was used for daily analysis of binary mixtures.

Insects and grain at four temperatures were exposed to gases from cylinders containing air, 100% N<sub>2</sub>, 100% CO<sub>2</sub>, or a blend containing ca. 60% CO<sub>2</sub>, 9% O<sub>2</sub>, and 31% N<sub>2</sub>. (Small leaks in the system reduced the actual concentrations of 100% N<sub>2</sub> to ca. 99% and 100% CO<sub>2</sub> to ca. 98%; this was expected). Exposures were for periods of 1, 2, 3, or 4 wk. Controls were held in similar cages in a room maintained at 26.7°±1°C. and 60±5% RH. Four replicates (3 cages per replicate) were tested at each of the four temperatures and four exposure periods for normal air and with the CO<sub>2</sub> mixture. Three replicates (3 cages per replicate) were tested similarly with the ca. 100% CO<sub>2</sub> flow and two with the ca. 100% N<sub>2</sub> flow.

At the end of the exposure period, the contents of the cages were placed in 120-ml glass containers with filter paper lids and held at 26.7°C. for 1 wk. Emergence counts were made at 1 wk and weekly thereafter for 5 more wk.

Effectiveness of treatment was determined by dividing the total number of insects that emerged after treatment by the total number that emerged in the controls and converting this to percent reduction in emergence (RIE).

## RESULTS AND DISCUSSION

Samples of atmosphere analyzed for all replicates had the following percentages ( $\pm$  S.D.) of carbon dioxide, oxygen, and nitrogen: normal air -  $20.9 \pm 0.1\%$   $O_2$ , balance  $N_2$  and rare gases;  $CO_2$  mixture -  $60.4 \pm 0.7\%$   $CO_2$ ,  $8.9 \pm 0.2\%$   $O_2$ , and  $30.7 \pm 0.6\%$   $N_2$ ; 100%  $CO_2$  -  $97.7 \pm 0.9\%$   $CO_2$ ,  $0.4 \pm 0.2\%$   $O_2$ , and  $1.9 \pm 0.6\%$   $N_2$ ; and 100%  $N_2$  -  $0.5 \pm 0.1\%$   $O_2$  and  $99.6 \pm 0.1\%$   $N_2$ . The mean ( $\pm$  S.D.) of the relative humidities were: natural air -  $55.9 \pm 3.1\%$ ; in  $CO_2$  mixture -  $56.0 \pm 3.3\%$ ; 100%  $CO_2$  -  $56.2 \pm 3.2\%$ ; and in 100%  $N_2$  -  $55.3 \pm 2.8\%$ . Mean temperatures ( $\pm$  S.D.'s) in the exposure chambers are given in Tables 1 through 4.

Tables 1 through 4 present results of exposures to the normal air and to three modified atmospheres at the four temperatures. At  $1.6^\circ C$  (Table 1) there was no emergence of insects exposed to normal air or to 98%  $CO_2$  after 2 wk of exposure. Exposures of this length to the  $CO_2$  mixture or to 99%  $N_2$  gave a 99% or higher RIE, but increasing the exposure time to 3 or 4 wk did not produce a 100% RIE when insects were exposed to the  $CO_2$  mixture. Those exposed 4 wk to 99%  $N_2$  had a 100% RIE.

At  $4.7^\circ C$ , the only atmospheres producing 100% RIE were: normal air after a 3-wk exposure and 98%  $CO_2$  after a 4-wk exposure (Table 2); however, these two atmospheres gave a 99% RIE after an exposure of 2 wk. *S. oryzae* exposed to the  $CO_2$  mixture had RIE of over 99% after a 3-wk exposure; those exposed 4 wk to 99%  $N_2$  had a 99.9% RIE.

At  $10.4^\circ C$ , the only 100% RIE occurred when insects were exposed to the  $CO_2$  mixture for 4 wk (Table 3). However, the RIE was over 99% when insects were exposed to the  $CO_2$  mixture for 3 wk or 98%  $CO_2$  for 4 wk. When the insects were exposed to air or to 99%  $N_2$ , mortality gradually increased with increasing exposure time and reached about 98% after 4 wk.

At  $15.7^\circ C$ , the RIE was 99% after a 2-wk exposure to the  $CO_2$  mixture and 100% after a 3-wk exposure (Table 4). Insects exposed for 3 or 4 wk to 98%  $CO_2$  or to 98%  $N_2$  for 4 wk had an RIE above 99%.

Low numbers of insects emerged from the 60%  $CO_2$  and 99%  $N_2$  atmospheres after 2- or 3-week exposures at  $1.6^\circ C$ . The fact that no emergence occurred from the samples exposed to only air is assumed to be the result of the cold reducing the respiration rate of the insects to a low level and thus preventing significant, but not total, venting of the abnormal atmospheres. This venting in response to atmospheres high in  $N_2$  or in response to atmospheres high in  $CO_2$  but with some oxygen present prevents complete anoxia which has been shown to be one of the major causes of death due to exposure to these atmospheres. This venting

TABLE 1

Percent reduction in emergence (% RIE) when immature *S. oryzae* were exposed to air or to one of three modified atmospheres at  $1.6^{\circ}\pm 0.1^{\circ}$  C (S.D.) for indicated periods.\*

Atmosphere	% RIE after exposure of (wk)			
	1	2	3	4
Air	98.7	100.0	100.0	100.0
60% CO <sub>2</sub>	95.6	99.4	99.8	99.9
98% CO <sub>2</sub>	99.8	100.0	100.0	100.0
99% N <sub>2</sub>	94.5	99.1	99.9	100.0

TABLE 2

Percent reduction in emergence (% RIE) when immature *S. oryzae* were exposed to air or to one of three modified atmospheres at  $4.7^{\circ}\pm 0.2^{\circ}$  C (S.D.) for indicated periods.\*

Atmosphere	% RIE after exposure of (wk)			
	1	2	3	4
Air	93.3	99.8	100.0	100.0
60% CO <sub>2</sub>	71.0	94.6	99.7	99.9
98% CO <sub>2</sub>	90.1	99.3	99.9	100.0
99% N <sub>2</sub>	50.3	91.0	97.6	99.9

\*Mean and S.D. for adult emergence for all controls ( $26.7^{\circ}$  C) were 1 wk -  $63.8\pm 15$ ; 2 wk -  $89.6\pm 45.5$ ; 3 wk -  $172.8\pm 41.5$ ; and 4 wk -  $239.5\pm 35.9$ .

TABLE 3

Percent reduction in emergence (% RIE) when immature *S. oryzae* were exposed to air or to one of three modified atmospheres at  $10.4^{\circ}\pm 0.6^{\circ}$  C (S.D.) for indicated periods.\*

Atmosphere	% RIE after exposure of (wk)			
	1	2	3	4
Air	89.3	99.4	95.0	97.9
60% CO <sub>2</sub>	72.6	92.4	99.4	100.0
98% CO <sub>2</sub>	76.1	89.5	95.6	99.6
99% N <sub>2</sub>	64.3	72.5	84.6	98.1

TABLE 4

Percent reduction in emergence (% RIE) when immature *S. oryzae* were exposed to air or to one of three modified atmospheres at  $15.7^{\circ}\pm 0.5^{\circ}$  C (S.D.) for indicated periods.\*

Atmosphere	% RIE after exposure of (wk)			
	1	2	3	4
Air	67.1	88.5	89.3	89.1
60% CO <sub>2</sub>	80.5	99.0	100.0	100.0
98% CO <sub>2</sub>	97.4	97.2	99.6	99.9
99% N <sub>2</sub>	41.7	86.0	82.7	99.7

\*Mean and S.D. for adult emergence for all controls ( $26.7^{\circ}$  C) were 1 wk -  $63.8\pm 15$ ; 2 wk -  $89.6\pm 45.5$ ; 3 wk -  $172.8\pm 41.5$ ; and 4 wk -  $239.5\pm 35.9$ .

is associated with spiracular control and body weight loss. The majority of the weight loss is assumed to be water, and death is partially caused by desiccation in high  $N_2$  atmospheres (Navarro, 1978). Jay and Cuff (unpublished manuscript) confirmed this relationship in experiments that showed a high weight loss in larvae, pupae, and adults of I. castaneum when they were exposed to a ca. 60%  $CO_2$  concentration similar to the one used in these tests. Therefore, we assumed that the S. oryzae in the present tests, even in the presence of low temperatures, reacted enough to actively engage in the venting or pumping of their respiratory systems. Their metabolic rate was thereby increased, and their body temperature was slightly increased so they were actually protected from the direct effects of the low temperatures, but, the magnitude of this activity was not enough to induce heavy water losses and desiccation causing death.

Jay and Cuff (unpublished manuscript) also showed that in very high  $CO_2$  concentrations, similar to the 98%  $CO_2$  used in these studies, the body weight of I. castaneum was not reduced as mortality increased. Friedlander and Navarro (1979) studied the mode of action of high  $CO_2$  concentrations on pupae of Ephestia cautella (Walker). They found that treatments with 99%  $CO_2$  drastically reduced ATP levels in these insects. In the tests reported here, 98%  $CO_2$  gave 100% RIE after 2, 3, and 4 wk of exposure at 1.6° C, but cold alone did the same when the insects were exposed to normal air. I therefore believe that the insects exposed to 98%  $CO_2$  were completely anesthetized, could not raise their body temperature by venting, and were therefore killed by a combination of low temperature and  $CO_2$ .

From a practical standpoint then, there is little need to use modified atmospheres or any other control techniques against S. oryzae when grain temperatures are below 10.4° C. The cold alone will produce a high mortality, almost 98% RIE, after 4 wk. At a temperature of 15.7° C, the mixture with 60%  $CO_2$  will give a RIE of 99% in 2 wk and 100% in 3 wk, and an atmosphere containing 98%  $CO_2$  will give a RIE of over 99% in 3 wk but would be more expensive to use. Moreover, at this temperature, cold alone gave an RIE of almost 90% in 2 wk, though it did not increase its effectiveness by additional exposures of up to 4 wk. The data reported here compare favorably with those of Banks (1978) who obtained 77 to 79% mortality with a 10-day exposure to 47 and 68%  $CO_2$  at 13.5° C. For example, an exposure to the mixture contained 60%  $CO_2$  at 10.4° C for 1 wk gave 72.6% RIE; a 2-wk exposure to this concentration and temperature gave a 92.4% RIE (Table 3). However, they cannot be compared with the estimated mortality of Banks and Annis (1977) for an exposure time of more than 24 wk at 15° C for high  $N_2$  (low  $O_2$ ) concentrations. Since Table 4 shows that a 4-wk exposure at 15.7° C to 99%  $N_2$  will give a 99.7% RIE, a 100% RIE would probably be obtained by an additional exposure of 1 to 2 wk, not 20 wk.



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## FOOTNOTE

1/Mention of a commercial product in this paper does not constitute an endorsement of this product by the USDA.

## INTEGRATED APPROACH TO THE USE OF CONTROLLED ATMOSPHERES FOR INSECT CONTROL IN GRAIN STORAGE\*

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### INTRODUCTION

Significant progress has been made in recent years in using modified atmospheric gas concentrations for the control of stored product insects (Banks, 1979; Navarro et al., 1979; Shejbal, 1979). Contributions on this subject have shown the feasibility of using low oxygen concentrations in nitrogen atmospheres, high carbon dioxide concentrations or a mixture of these gases as efficient non-chemical control methods for the prevention of insect damage (Jay and Pearman, 1973; Shejbal et al., 1973; Navarro et al., 1979). However, it should be kept in mind that the atmospheric gas composition is only one of the factors acting on insects breeding in the grain bulk ecosystem (Banks and Annis, 1977). Other environmental factors affecting the survival of insects found in bulk-stored grain are temperature and relative humidity (Navarro, 1978; Storey, 1975). Therefore, the efficient use of controlled atmosphere storage should require the careful consideration of data concerning these two factors, which in some cases could be modified to increase the efficacy of this control method.

In this paper the effect of temperature and relative humidity on the effectiveness of atmospheric gas mixtures as an integrated approach to the control of stored grain pests, is described and discussed.

### INSECTICIDAL EFFECT OF MODIFIED ATMOSPHERES

The effect of low  $O_2$  or high  $CO_2$  concentrations on insect mortality was demonstrated many years ago (Bailey, 1955; Bailey, 1965). A limit of about 2%  $O_2$  has been indicated (Banks, 1979) for the mortality of stored product insects so far tested. These findings provided the basis of the revived ancient method of hermetic storage (Bailey and Banks, 1974). However, the concentrations of low  $O_2$  or high  $CO_2$  needed for the control of different stored product insect species

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\* Contribution from the Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel. No. 186-E, 1980 series.

differ widely (Harein and Press, 1968; Marzke et al., 1970).

Direct comparison of the many results given by numerous researchers in this field is difficult, since the insect mortalities obtained in various gas compositions at different temperature and humidity levels are so diverse (Bailey and Banks, 1974; Harein and Press, 1968). The effect of modified atmospheres on immature stages varies also, and this has been the subject of recent investigation and current research (Storey, 1977).

Recently, the synergistic effect of the combination of low  $O_2$  and high  $CO_2$  tensions on Tribolium castaneum adults has been demonstrated (Calderon and Navarro, 1979). This work opens the possibilities of using relatively high  $O_2$  concentrations when supplemented by  $CO_2$ , as a modified atmosphere formula in controlling storage insects. Further research along these lines is required.

#### ROLE OF TEMPERATURE

The time required to obtain a certain level of insect mortality exposed to a given atmospheric gas composition is dependent on the temperature of the environment. In fact, from the physiological aspects at normal atmospheres, development of insects can only occur within a fairly narrow range of temperatures. Developmental thresholds have been determined for a number of stored product insects at different stages of development (Birch, 1945; Howe, 1960). For example, for the egg-to-adult stage of Sitophilus oryzae, the threshold limits for development are between  $15^{\circ}$  and  $34^{\circ}C$ , while for Rhyzopertha dominica these limits are between  $18^{\circ}$  and  $40^{\circ}C$  (Birch, 1945). Below or above these temperatures, complete mortality of insects is obtained, as determined by failure to hatch or emerge. Within the above mentioned limits, the rate of development is greatly affected by temperature (Howe, 1965).

It is well accepted that the effect of insecticidal treatment (especially for insecticides acting through the respiratory system like fumigants) is much more pronounced at higher temperatures (Monro, 1969). The effect of modified atmospheres seems to be very similarly dependent on the ambient temperature.

Fig. 1 demonstrates that at given atmospheric gas compositions, the higher the temperature - the shorter the exposure time needed to achieve 95% mortality of insects (Person and Sorenson, 1970; Storey, 1975 ; Storey, 1977). In an atmosphere of  $<1.0\%$   $O_2$  and  $9-9.5\%$   $CO_2$ , R. dominica adults were more tolerant than T. castaneum adults. The decrease in the number of hours required to obtain 95% mortality of the tested species, from  $15^{\circ}$  to  $21^{\circ}C$ , is very pronounced.

This effect is shown to be similar in tendency for the three insect species depicted in Fig. 1, which differ in their susceptibility in the given gas concentration. In practice, these data should be considered very carefully. It is clear that much longer exposures will be needed, at a given gas composition,

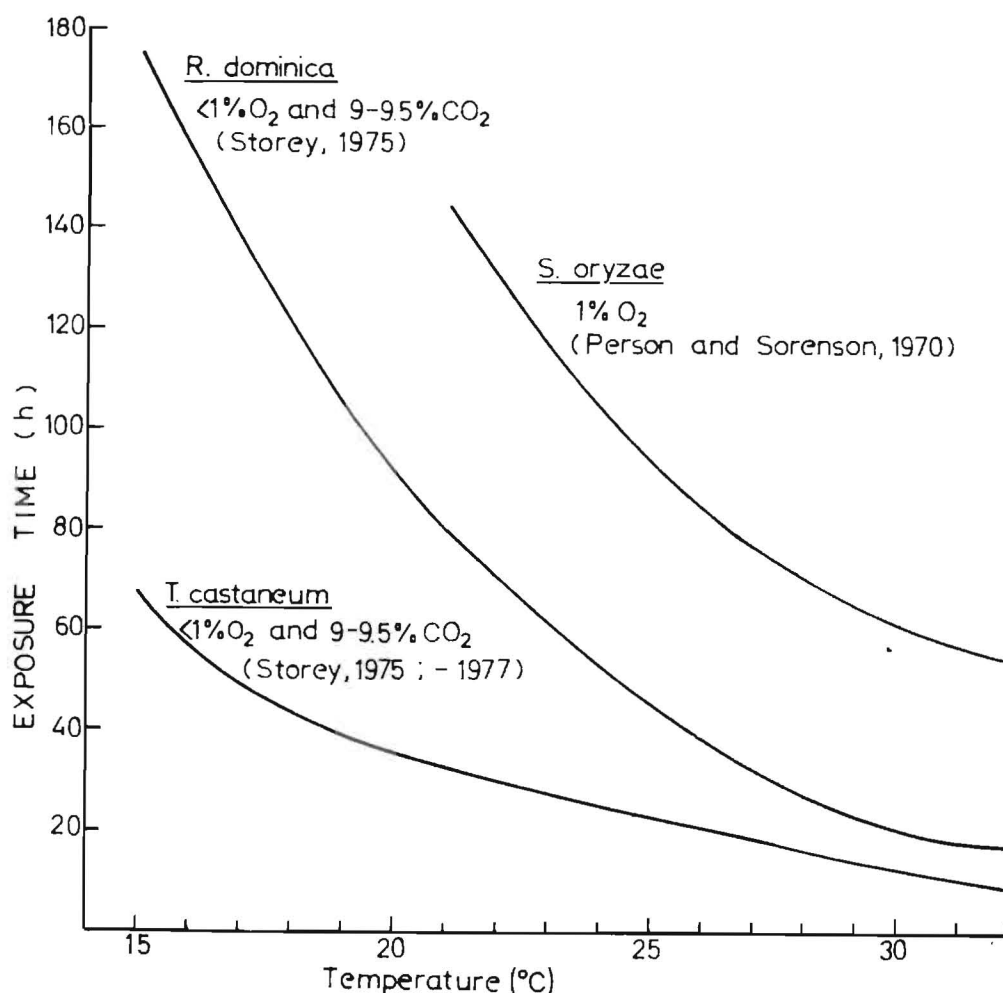


Fig. 1. Effect of temperature on exposure time required to produce 95% mortality of three stored product insects (adults) exposed to two atmospheric gas compositions.

to obtain effective control when low temperatures prevail in the grain bulk. Furthermore, since differences in temperature exist in the various parts of the grain bulk, the lowest temperature recorded should determine the length of the exposure time required for the treatment. In the use of controlled atmosphere treatment, the most resistant insect species found in the grain bulk to be treated should be considered.

#### ROLE OF RELATIVE HUMIDITY.

Humidity influences the survival of insects mainly through the effect on their water content. Dry conditions appear to be generally unfavourable for the reproduction of most insects. However, most stored product insects can survive at very low humidities (Howe, 1965). The survival of insects under dry conditions depends on their maintaining a balance between the losses and gains of water (Edney, 1967).

Therefore, the means of maintaining water within certain limits in stored product insects is an important aspect of their structure and physiology (Navarro, 1978).

The role of relative humidity in producing mortality in insects by desiccation alone, when exposed to controlled atmospheres, requires further clarification.

The pronounced dependence of low  $O_2$  or elevated  $CO_2$  tensions on the relative humidity of the environment for producing a lethal atmosphere for several stored product insects, has been investigated (Jay et al., 1971; Navarro, 1978; Navarro and Calderon, 1974). Figure 2 demonstrates the role of relative humidity on adult emergence of *Ephestia cautella* pupae exposed to two atmospheric compositions (Navarro, 1974a). These data indicate that at 20-24% relative humidity, 3.2%  $O_2$  or 4.3%  $CO_2$  in air is needed to achieve complete mortality. However, at the same gas compositions but at higher relative humidities, *E. cautella* pupae could survive. These results, as well as data obtained by other authors (Jay et al., 1971; Navarro, 1978), indicate that the controlled atmosphere treatments could be more effective when the moisture content of the treated grain is low.

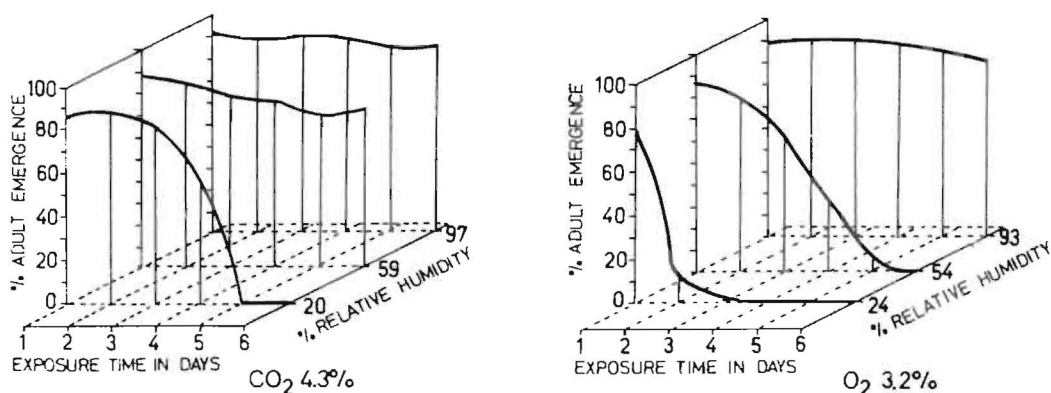


Fig. 2. The effect of  $O_2$  and  $CO_2$  at different relative humidities on adult emergence from *E. cautella* pupae at  $26^{\circ}C$ .

The moisture content of a stored product may vary over a considerable range and it is in equilibrium with the relative humidity of the intergranular air space of the grain bulk ecosystem. Due to the phenomenon of moisture migration, the moisture content of grain in some parts of the bulk may be considerably higher than in other remaining parts of the bulk. This also requires careful consideration so that extreme concentrations of gas should be applied to achieve effective control of insects found in grain of high moisture content.

#### PRACTICAL CONCLUSIONS

A number of field trials have been conducted using different atmospheric gas compositions (Banks, 1979). The effectiveness of the experimental trials using

controlled atmospheres has been high, but complete mortality has not always been obtained (Jay and Pearman, 1973; Navarro et al., 1979; Shejbal et al., 1973). The reasons for the observed incomplete insect mortality have not been properly analyzed.

In field trials with controlled atmospheres, the approach has been based on maintaining the required gas composition within acceptable limits in a reasonably gastight silo. Efforts have been made to seal the silos to a degree which will prevent excessive use of gas mixtures (Banks and Annis, 1977). However, in practice, it is difficult and expensive to render a large structure completely gastight. Therefore, there is the possibility that surviving insect populations will aggregate around the leaks (Navarro et al., 1979).

One major problem that may arise from surviving insect populations is the development of resistance to modified atmospheres. Although resistant insect species have not yet been recorded from field populations, there are data indicating that stored grain pests have the genetic potential to develop resistance to CO<sub>2</sub> (Bond and Buckland, 1979).

Factors other than gastightness may also contribute to the observed incomplete insect control in large-scale modified atmosphere treatment. Prolonged exposures of several weeks are required for obtaining satisfactory results at low temperatures. Under certain conditions it seems to be impractical to maintain the required gas concentrations for such long periods of time, especially when the temperature is below the lower threshold limit for the development of the insect species infesting the grain.

Damp grain pockets in the bulk are also the cause of insect survival after controlled atmosphere treatment. Drying of the grain bulk for obtaining efficient insect control with modified atmospheres is not a practical proposition. Therefore, the moisture content of the grain should be seriously considered in determining the required gas concentrations and exposure times.

From the above discussion the following integrated insect control method is suggested: Controlled atmosphere treatment should be applied when high temperatures prevail in the grain, followed by aeration for reducing grain temperature. This sequence of treatments will provide a reasonable degree of insect control: The controlled atmosphere treatment will aim at obtaining the maximum possible insect mortality, while the cooling of the grain bulk (Navarro, 1974b) will contribute further protection by reducing the reproductive rate of the possible survivors of an insect population.

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SYNERGISTIC EFFECT OF CO<sub>2</sub> AND O<sub>2</sub> MIXTURES ON TWO STORED GRAIN INSECT PESTS\*

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## ABSTRACT

Tribolium castaneum and Rhyzopertha dominica adults and eggs were exposed to atmospheres containing 2%-8% O<sub>2</sub>, supplemented with 5%-30% CO<sub>2</sub> at 26°C and 55% r.h. for 24-144 h. Eggs of both species were more susceptible than their adult stage to low O<sub>2</sub> concentrations per se as well as to the O<sub>2</sub> and CO<sub>2</sub> mixtures tested. R. dominica eggs were more tolerant than T. castaneum eggs to the above treatments.

Addition of CO<sub>2</sub> to low O<sub>2</sub> atmospheres resulted in a synergistic effect on adult mortality of both species, while there was only an additive effect on the eggs exposed to the same treatments.

These results provide additional information to be considered in the use of controlled atmospheres for grain storage.

## INTRODUCTION

The effect of various combinations of atmospheric gases on stored product insects has been investigated extensively by numerous workers and interesting results have been recorded (Bailey, 1965; Harein and Press, 1968; Jay and Pearman, 1971; Navarro and Calderon, 1974). In many of these studies a clear indication of the enhancing effect of CO<sub>2</sub> on insect mortality was found. One of the most interesting and surprising findings was by Harein and Press, 1968, who showed that for T. castaneum and Plodia interpunctella adults, atmospheres containing up to 15% O<sub>2</sub> supplemented with 36% CO<sub>2</sub> were lethal after 10 days of exposure.

In a test of the effectiveness of atmospheric gas mixtures containing up to 5% O<sub>2</sub> and 5-35% CO<sub>2</sub>, a significant interaction was found between the levels of O<sub>2</sub> and CO<sub>2</sub> tested and the exposure period (Calderon and Navarro, 1979). These results indicated a clear synergistic effect of the two gases on the mortality of Tribolium castaneum adults.

The following reports an experiment to detect this interaction in gas mixtures containing up to 8% O<sub>2</sub>, and to study the effect of the mixtures on the adults and eggs of T. castaneum and R. dominica.

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\* Contribution from the Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel, No. 195-E, 1980 series.

## MATERIALS AND METHODS

Tribolium castaneum was reared on wheat feed mixed with 5% brewer's yeast (by weight), and Rhyzopertha dominica on whole wheat (11% moisture content), in a controlled temperature ( $26 \pm 1^{\circ}\text{C}$ ) and relative humidity ( $70 \pm 5\%$ ) room. Groups of 50 adults, 7-10 days old, were collected from cultures and each species was exposed, on 3 g of appropriate food, to different atmospheric gas mixtures in a chamber of 100 ml capacity (Navarro and Donahaye, 1972).

Eggs, 0-24 h old, were collected and confined in individual incubation cells devised for this purpose (Navarro and Gonen, 1970). Groups of 100 eggs confined in these cells were exposed in the same chambers to the same treatment as the adults.

In each experiment one group of insects was exposed to the given gas mixture for 24, 48, 72, 96, 120 and 144 h, at  $26 \pm 1^{\circ}\text{C}$  and an average r.h. of 55%. Adult mortality was determined as follows: after exposure, adults were transferred to vials of 100 ml capacity containing 3 g of appropriate food, and kept under controlled temperature and r.h. as in the insects' rearing room. Dead and live insects were counted 14 days after the end of each treatment. Egg mortality was determined by the failure to hatch 14 days after the hatch of the last egg of the same group. During the incubation period eggs were kept in the same exposure chambers - at normal atmosphere and ambient conditions - as the adults.

Mortality results were prepared for analysis by stepwise multiple regression analysis (Snedecor and Cochran, 1969). For determination of interaction among the variables, a factorial design was adopted using analysis of variance.

## RESULTS AND DISCUSSION

### Effect on adults

Data on the mortality of the two species exposed to different combinations of  $\text{O}_2$  and  $\text{CO}_2$  are shown in Fig. 1. Results obtained from tests with different exposure times were recorded, but for demonstration purposes only the results of the 96-h exposure are given. The curves in Fig. 1 show the enhanced toxicity of the gas mixture when  $\text{CO}_2$  was added to the given  $\text{O}_2$  atmosphere.

For T. castaneum, exposure to the different  $\text{O}_2$  concentrations alone, did not result in substantial mortality, except with 2%  $\text{O}_2$ . However, when 15%  $\text{CO}_2$  was added, the lethal effect of the reduced  $\text{O}_2$  concentrations was considerably increased. Even at 6%  $\text{O}_2$ , 90% mortality was achieved when 30%  $\text{CO}_2$  was added.

Similar results were obtained with R. dominica (Fig. 1), showing increased toxicity of reduced  $\text{O}_2$  concentrations supplemented by  $\text{CO}_2$ . The combination of the two gases seemed to affect R. dominica adults considerably more: at 2%  $\text{O}_2$ , 100% mortality was obtained by adding only 10%  $\text{CO}_2$ .

The data obtained for the adult stage of the two species tested indicate the existence of a strong interaction between the effects of the two gases. This was expressed in increased toxicity of the gas mixtures containing  $\text{CO}_2$  added to

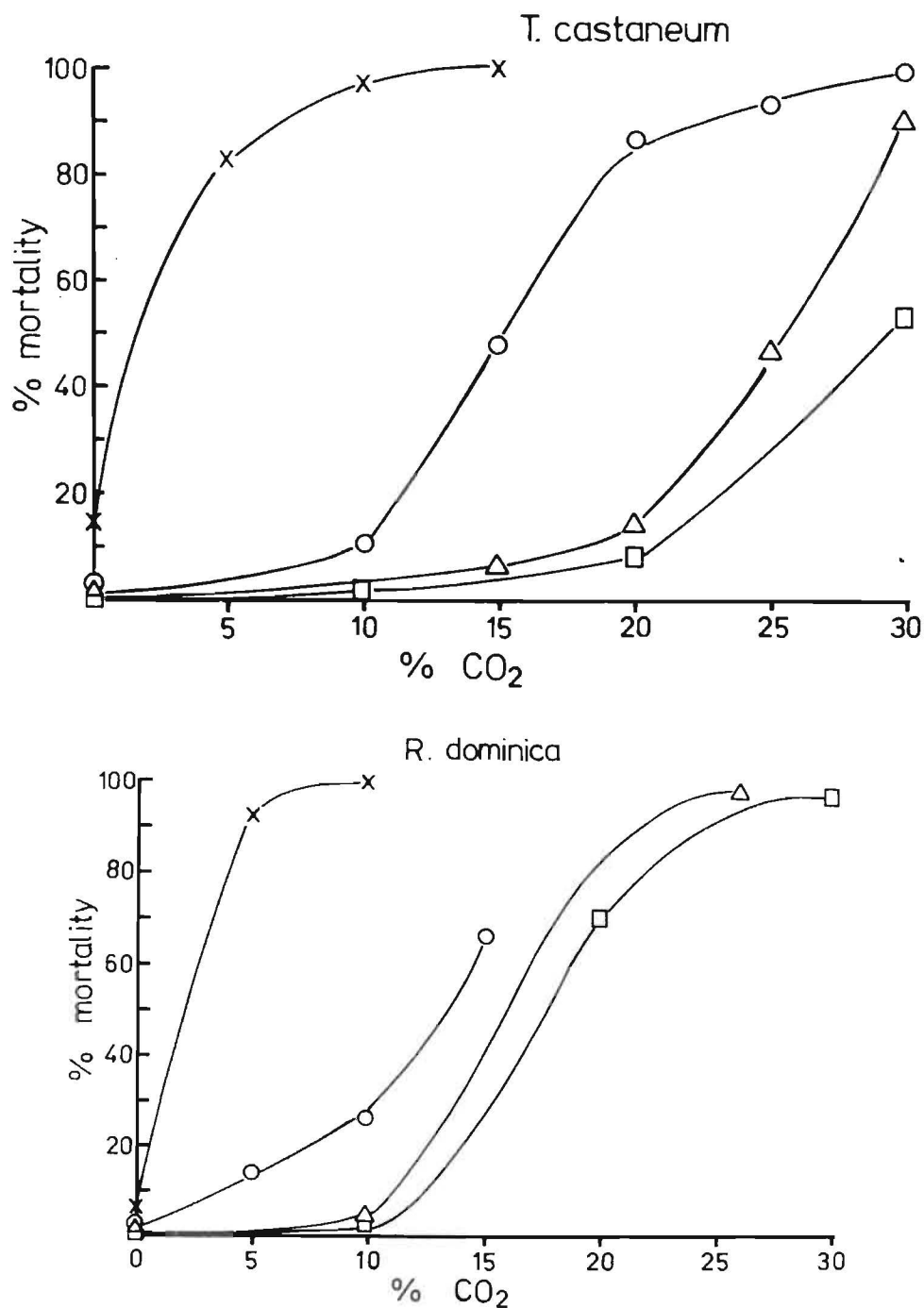


Fig. 1. Mortality of *T. castaneum* and *R. dominica* adults exposed for 96 h to different O<sub>2</sub> and CO<sub>2</sub> mixtures at 57% r.h. and 26°C (O<sub>2</sub> levels were: 2%—X, 4%—O, 6%—Δ and 8%—□.).

reduced O<sub>2</sub> atmospheres. Analysis of variance performed on the data revealed the significant level of this interaction, which explains the synergistic effect of the two gases.

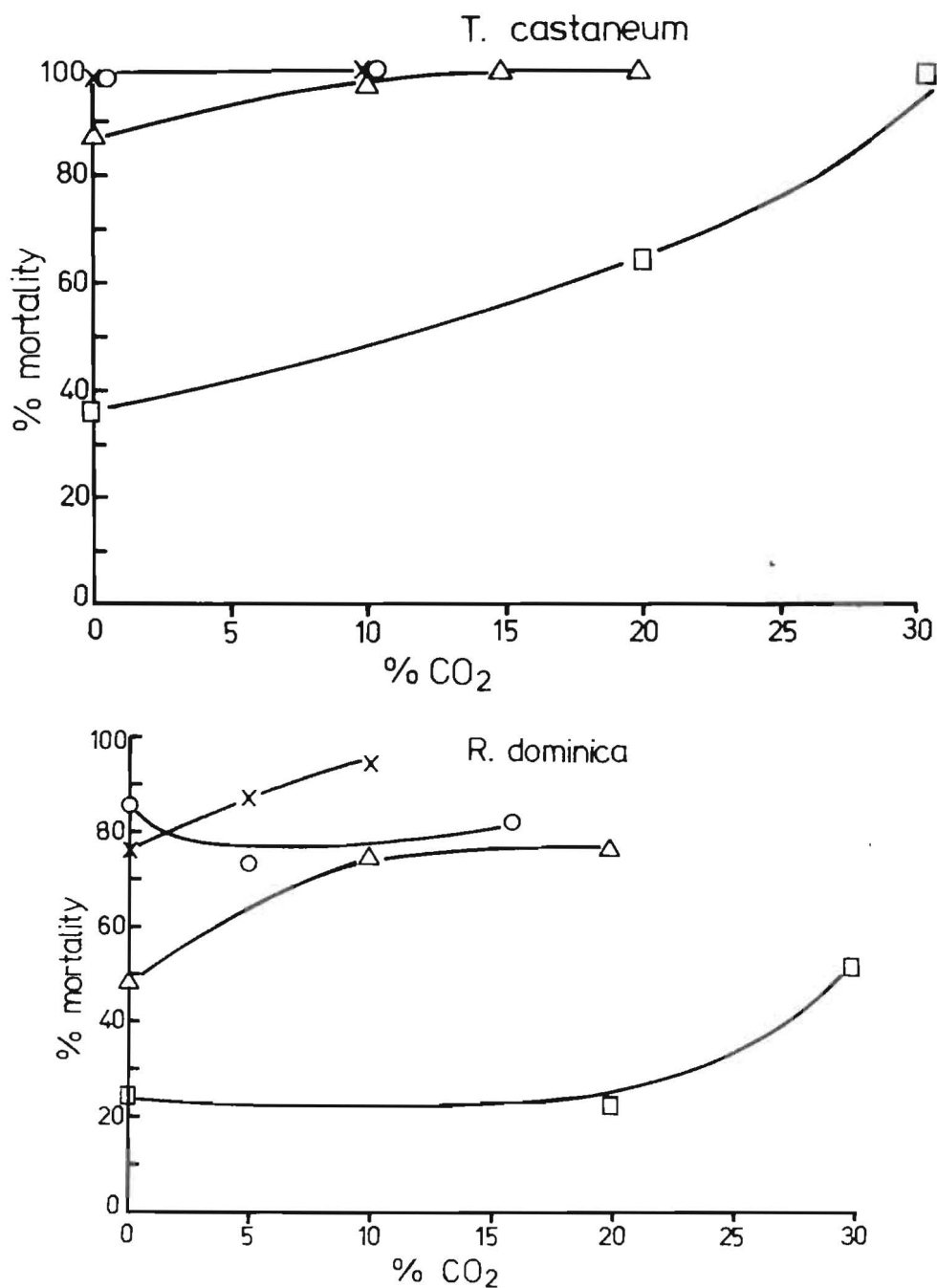


Fig. 2. Mortality of *T. castaneum* and *R. dominica* eggs exposed for 96 h to different O<sub>2</sub> and CO<sub>2</sub> mixtures at 57% r.h. and 26°C (O<sub>2</sub> levels were: 2%—X, 4%—O, 6%—Δ and 8%—□).

#### Effect on eggs

Figure 2. shows a different pattern of response of the eggs exposed to the tested atmospheric gas combinations. First, there was a marked difference in susceptibility to reduced O<sub>2</sub> atmospheres, compared with that of adults. For

T. castaneum eggs exposed for 96 h, 100% mortality was recorded at 2% and 4%  $O_2$  concentrations without  $CO_2$ . Exposure to 6%  $O_2$  resulted in about 87% egg mortality, and even at 8%  $O_2$  up to 47% of the exposed eggs failed to hatch. In contrast to the effect on adults (Fig. 1)  $O_2$  concentrations of 4% and higher, no adult mortality was obtained. Rhyzopertha dominica eggs (Fig. 2) were less susceptible to the above treatments than T. castaneum eggs, but the adult was again found to be more tolerant than the egg.

These findings appear to be in disagreement with conclusions reported by other authors (Ali Niaze, 1971; Lindgren and Vincent, 1970; Storey, 1977), whose results indicate relative tolerance of the egg as compared with the adult and the larva. However, those results cannot be compared with ours, since in the other investigations eggs were exposed to either very low  $O_2$  of 0.1-0.5% (Storey, 1977), or to pure  $N_2$ ,  $CO_2$  or He atmospheres (Ali Niaze, 1971; Lindgren and Vincent, 1970). Moreover, in most cases the above treatments were tested on 3-day-old eggs, while in our study the eggs were 0-24 h old. We consider our findings on the high susceptibility of the egg stage to low  $O_2$  atmospheres, interesting and of practical importance. More work on this subject is in progress.

Results on the effect on eggs of  $CO_2$  added to reduced  $O_2$  atmospheres show again a different pattern of response, to that of the adults. The addition of  $CO_2$  to the given  $O_2$  atmospheres had little effect on egg mortality. At the  $O_2$  concentrations tested, increasing  $CO_2$  concentrations did show an additive effect on egg mortality and did not indicate any synergism of these two gases on egg hatch.

We find it difficult to explain the above difference in response based on the physiology of insect eggs versus adults. However, we can speculate that the loss of water in adults which takes place at atmospheres of higher  $CO_2$  content, does not apparently occur at the same rate in the exposed eggs. Unpublished data have indicated that E. cautella and T. castaneum eggs are significantly less affected by low r.h. than are the adults. Further research is needed to elucidate this interesting point.

The findings described herein may have important practical implications in the use of controlled atmospheres for insect control in stored products.

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## MORTALITY OF VARIOUS STORED PRODUCT INSECTS IN LOW OXYGEN ATMOSPHERES PRODUCED BY AN EXOTHERMIC INERT ATMOSPHERE GENERATOR

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### ABSTRACT

Atmospheres produced by an exothermic inert atmosphere generator and composed of less than 1% oxygen, about 9-9.5% carbon dioxide, and the balance principally nitrogen were found to be lethal to all stages of both internally and externally developing stored grain insects. Time periods required for control varied between species and between life stages within the same species. Pupae and mature larvae of internally developing species were generally the most tolerant stages and immature larvae and adults of externally developing species the most susceptible. Eggs of most species were not especially tolerant of exposure to the low oxygen atmosphere, but did exhibit a tendency to become increasingly tolerant after the first day of development. Tolerance during larval-pupal development of most species also increased with each successive period of growth after hatching. The maximum period of treatment at 27°C required to give control of the most tolerant insect species was about 10 days; however, most species were either killed or suffered severe physiological damage after 3 to 5 days of exposure. As treatment temperature decreased the length of exposure time required to obtain effective control significantly increased.

### INTRODUCTION

Insect infestations are one of the principal factors that adversely affect the quality of stored commodities. The food storage, transportation and processing industries, and the consumer suffer large monetary losses from stored product insects that cause damage, lower nutritional value, and make products unfit for human consumption. The presence of insects and the damage they cause also discredits the quality of agriculture products in highly competitive foreign markets. Present chemical methods used to control insects may leave objectionable residues in the treated commodity and are generally hazardous to handle and apply; also, some stored product insects are developing resistance to chemical treatment. There is therefore urgent need for acceptable and effective methods of preventing insect damage and contamination of agriculture products that would reduce or eliminate dependence on chemical pesticides during storage, handling, processing, packaging, transportation, and retail.

The insecticidal activity of modified atmospheres of oxygen, carbon dioxide and nitrogen has long been recognized and research programs to develop the use of these atmospheres for insect control are in progress in several locations throughout the world.

Four factors are essential to the use of modified atmospheres for control of insects in stored commodities; 1) the atmosphere must be easily obtained in sufficient volume to displace existing atmosphere in large bulk storage; 2) the atmosphere must be lethal to storage pests within a reasonable time; 3) the atmosphere must have no harmful effect on the quality of the treated commodity, and 4) the storage structure must have an adequate gas holding capability (Storey 1979).

One technique for producing high volumes of an oxygen-deficient atmosphere is the ignition of air and a fuel gas in an exothermic inert atmosphere generator. Such generators are used for metallurgical and chemical processes that require low oxygen environments and also by the food industry to retard metabolism and preserve freshness of fruits and vegetables. The feed industry has used generated atmospheres for the preservation of vitamin content, color, weight and palatability of alfalfa pellets (Kruger 1960). Composition of the generated atmosphere varies with the fuel-to-air ratio and composition of fuel gas burned, but usually consists of less than 1% oxygen, 9-9.5% carbon dioxide, 86-89% nitrogen, 1% argon, and 1.5% or less each of hydrogen and carbon monoxide.

Research was undertaken at the Science and Education Administration-Agricultural Research, U.S.D.A. Grain Marketing Research Laboratory to explore the use of inert atmosphere generators in regulating insect populations in stored products. Data presented here were obtained from the following references: Storey 1975a, b, c; Storey and Soderstrom 1977, Storey 1977, and Storey 1978.

## MATERIALS AND METHODS

Tests to determine the toxicity of the generated atmosphere to various life stage of the major insect pests infesting stored products were conducted in a pilot inert atmosphere treatment system consisting of a laboratory-scale generator, a series of cylindrical metal silos 45 cm in diameter by 4.27 m high, a series of incubators modified to permit treatment of the insects at selected temperatures, and pneumatic grain handling equipment for loading and unloading the silos. The small scale generator was built by Gas Atmospheres, Inc., Port Washington, Wisconsin and produce about  $2.83 \text{ m}^3/\text{hr}$  (100 cu. ft/h) of combustion atmosphere in which the concentration of oxygen measured by a Seromex<sup>®</sup> paramagnetic oxygen analyzer was generally between 0.1 and 0.25% and rarely exceeded 0.5%.

During operation, natural gas and air are ignited under controlled pressure at a ratio of ca. 1 part gas to 10 parts air. Combustion takes place in a water-cooled, refractory-lined combustion chamber. The exhaust gas from the chamber passes through

a water-cooled plate-coil and into a condensate separator. Water removed from the gas leaves the separator through a seal leg located beneath the separator (Fig. 1).

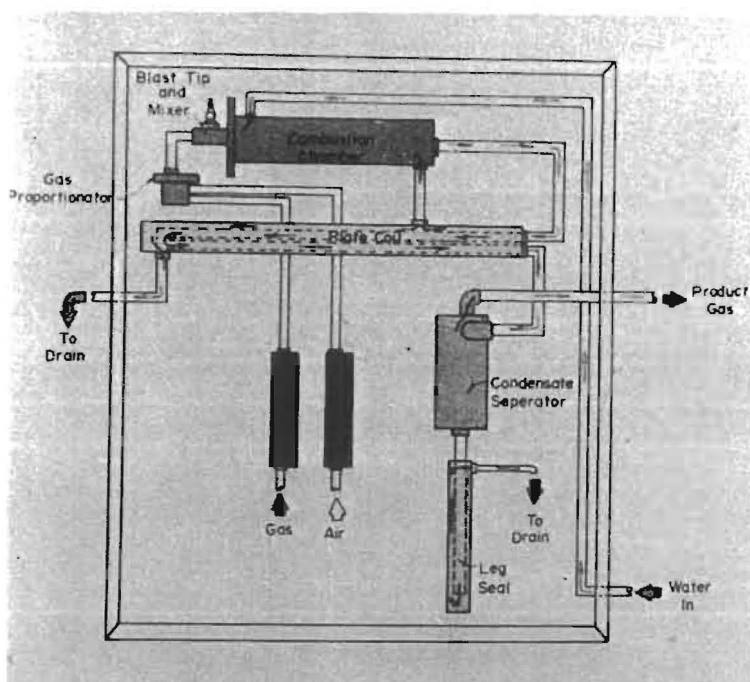


Fig. 1. Flow chart of laboratory scale inert atmosphere generator. During operation natural gas and air are ignited under controlled pressure at a ratio of about 1 part gas to 10 parts air. Combustion takes place in a watercooled refractory-lined combustion chamber. The exhaust gas from the chamber passes through a water-cooled platecoil and into a condensate separator

The RH of the inert atmosphere was maintained at  $50 \pm 5\%$  by passing the inert atmosphere through a self-draining manifold immersed in a refrigerated constant temperature water bath to lower the temperature of the inert atmosphere below the dewpoint and then reheating the gas to room temperature before release into the silos or temperature-regulated incubators.

Silos used in the testing program were equipped with perforated metal tubes about 2 cm dia placed crosswise through the silos at intervals of 60 cm (Fig. 2). Threaded fittings on the end of the tubes permitted insertion of screened cages containing test insects and also served as sample points for analysis of the atmosphere within the silos. Each silo was loaded with 545 kg of hard winter wheat with a moisture content of about 12%. The inert atmosphere was released at the base of the silos and passed upward through the grain mass. Air exhausting from the top of the silos was vented to the outside. The rate of flow of inert atmosphere into each silo was 1.5 liter/min. Oxygen levels in the inert atmosphere were measured



Fig. 2. Cages of test insects are inserted in metal silos (45 cm in diameter by 4.27 m high) containing 545 kg of wheat. Generated atmosphere is passed upward through the silos.

periodically throughout the testing period.

Test insects were also exposed to the generated atmosphere in 0.47-liter jars placed in the incubators. Each jar was equipped with a lid fitted with a rubber stopper. Plastic lines inserted through the stoppers served as inlet and outlet tubes for the generated atmosphere. The rate of gas flow through the jars ranged from 25 to 50 cc/min. Airflow rates were controlled by purge meters that adjusted and monitored the air movement through each jar. Test insects together with a suitable food supply were placed in separate small screen cages in the jars or directly into food medium held in the jar and exposed to the atmosphere flowing through the jar. Tests were conducted over a temperature range of  $15$  to  $32 \pm 2^\circ\text{C}$  for exposure periods of 1 to 336 hours depending on the species and stage of development. Most treatments were replicated three times. Parallel control experiments were conducted in atmospheric air for each combination of species, stage of development, exposure time and treatment temperature. Mortalities of some

immature stages were based on the relative numbers of adults emerging from treated and untreated samples; other mortality data were based on direct counts of living insects from specific numbers of insects exposed to the treatment.

Times required to kill 95% of the treated insects were estimated by transforming mortality data to probits and calculating the regression of probits on time. Lethal times (LT) were then estimated by using the linear calibration technique described by Snedecor and Cochran (1967).

## RESULTS

The effectiveness of the generated inert atmosphere varied between insect species and between the development stages of the same species. A comparison by species and stage of the time periods required for 95% (or 100%) mortality when the temperature was 27°C and the R.H. was 50 ± 5% is shown in Table 1. Pupae and mature larvae of internally developing species such as the weevils (*Sitophilus* spp.) and lesser

TABLE 1.

A comparison of time (hours) required for 95 or 100% mortality of various stages of insects exposed at 27°C and 50 ± 5% RH to an atmosphere produced by an exothermic atmosphere generator: Composition, 1.0% O<sub>2</sub> and 9.0-9.5% CO<sub>2</sub>, the balance principally N<sub>2</sub>.

Insect	Eggs <sup>1/</sup>	Larvae <sup>2/</sup>	Pupae <sup>1/</sup>	Adults
Rice weevil ( <i>Sitophilus oryzae</i> )	70	79-246	107-241	48
Granary weevil ( <i>Sitophilus granarius</i> )	85	38-137	120-148	55
Lesser grain borer ( <i>Rhyzopertha dominica</i> ) <sup>3/</sup>	72	72-192	144-216	36
Angoumois grain moth ( <i>Sitotroga cerealella</i> ) <sup>3/</sup>	48	72-120	120	24
Navel orangeworm ( <i>Amyelois transitella</i> )	8-28	13-27	35-38	17
Confused flour beetle ( <i>Tribolium confusum</i> )	30-40	7-20	24-53	17
Red flour beetle ( <i>Tribolium castaneum</i> )	25-40	8-23	17-47	18
Indianmeal moth ( <i>Plodia interpunctella</i> ) <sup>3/</sup>	24	8	24	8
Almond moth ( <i>Ephestia cautella</i> ) <sup>3/</sup>	48	8	24	8
Cowpea weevil ( <i>Callosobruchus maculatus</i> ) <sup>3/</sup>	96	120-192	192	48

1/ Range over each day of development.

2/ Range over each week of development.

3/ Minimum exposure time for 100% mortality.

grain borer (*Rhyzopertha dominica* (F.)) were generally the most tolerant stages and early-instar larvae and adults of externally developing species such as the flour beetles (*Tribolium* spp.), the Indianmeal moth (*Plodia interpunctella* (Hubner)), and almond moth (*Ephestia cautella* (Walker)) were the most susceptible. Eggs, which often exhibit a high degree of resistance to chemical fumigation, were not especially tolerant of the generated atmosphere, but the eggs of some species such as the flour beetles tended to become more tolerant after the first day of development. For example, 1-day-old eggs of *T. castaneum* (Herbst) required only 25 hours



of exposure to cause 95% mortality, but 4-day-old eggs required nearly 40 hours of exposure to achieve the same level of mortality. Age response to the treatment among eggs of the navel orangeworm, Amyelois transitella (Walker) showed an opposite effect with 0 to 1-day-old eggs needing only 8 hours of exposure. Tolerance during the larval-pupal period of most species tended to increase with each successive period of development after hatching. Among Sitophilus spp. the period of greatest tolerance appeared to correspond with the cessation of feeding by the mature larvae and to end midway through the pupal development period. Susceptibility of Tribolium spp. during the pupal period followed a U-shaped course that more closely paralleled the oxygen uptake and the productions of carbon dioxide common during pupal metabolism, that is, there were high uptake-production values and increased susceptibility during the early and late pupal periods and low uptake-production values and decreased susceptibility near mid-pupal life.

Temperature had a pronounced influence on the length of time it took to kill insects exposed to the oxygen deficient generated atmosphere. The time required to kill 95% of various species of adult stored-product insects was reduced by about 90% when the treatment temperature was increased from 15° to 32°C (Table 2). Within

TABLE 2.

Time (hours) required to obtain 95% mortality of adult stored-product insects exposed at 4 temperatures to a modified atmosphere produced by an exothermic inert atmosphere generator: composition, 1.0% O<sub>2</sub> and 9.0-9.5% CO<sub>2</sub>, the balance principally N<sub>2</sub>.

Temperature	<u>S. oryzae</u>	<u>S. granarius</u>	<u>R. dominica</u>	<u>T. castaneum</u>	<u>O. surinamensis</u>
32°C	19	20	17	8	4
27°C	48	55	31	17	11
21°C	200	145	79	32	18
15°C	297	228	175	67	47

this temperature range the treatment times were reduced more for Sitophilus spp. as temperature was increased from 21 to 27°C. Other species responded most to an increase from 15 to 21°C. Treatment times for developing stages of S. oryzae (L.) ranged from 48 hours for adults to 246 hours for 4th-instar larvae when the temperature was 27°C, and was 108 hours for 1st-instar larvae and 653 hours for 1-day-old pupae when the temperature was 21°C (Table 3).

Although death was the principal criterion used in evaluating the effectiveness of the generated atmosphere, other responses were observed. Insects were immobilized soon (generally in less than 60 seconds) after exposure to the atmosphere and did not become active throughout the period of exposure. Some immobilized insects exposed for sublethal subsequently had only a delay in development time approximately equal to the period of exposure. Others similarly exposed developed partial paralysis, particularly in the posterior segments, and appeared incapable of

TABLE 3.

Time required (hours) to obtain 95% mortality of developing stages of rice weevil (*Sitophilus oryzae*) exposed at two temperatures to a modified atmosphere produced by an exothermic, inert atmosphere generator: composition 1.0% O<sub>2</sub> and 9.0-9.5% CO<sub>2</sub>, the balance principally N<sub>2</sub>.

Temperature	Eggs	Larvae	Pupae	Adult
27°C	70	79-246	245-107	48
21°C	162	108-562	653-537	200

defecation. Larvae, so stricken, were often observed with dried fecal material protruding from the abdomen; none survived to the pupal stage. Some adults emerging from exposed pupal stages of the flour beetle, *T. castaneum*, and the cowpea weevil, (*Callosobruchus maculatus* (F.)) were less than normal size and had rudimentary wings; others never developed adult characteristics in the posterior segments. In addition, sublethal exposures of the adult navel orangeworm reduced the number of progeny produced by surviving adults (Table 4): exposures of 1/2 to 3 hours did not kill

TABLE 4.

Percent mortality and number of progeny of adult navel orangeworms exposed at 27 ± 1°C to low O<sub>2</sub> atmosphere produced by an exothermic inert atmosphere generator.

Exposure hours	% Mortality <sup>a</sup> in 48 hours	Progeny/25 adults <sup>a</sup>
Control (untreated)	0	122.0
1/2	0	123.0
1	0	88.0
2	0	1.3
3	0	1.0
4	6	1.0
8	26	0.0
12	64	0.0
24	100	0.0

<sup>a</sup>Avg of 3 replicates.

any exposed adults, but the number of 2nd-generation adult progeny declined from 123 among adults exposed for 1/2 hour to only 1 progeny from adults exposed for 3 hours. Adults surviving 4-hour exposure deposited viable eggs, but the newly hatched larvae had limited mobility and most died during the first week of development. Some eggs were deposited by adults surviving 8-hour exposures, but the red coloration typical of developing eggs of the navel orangeworm was not observed and none of the eggs hatched. None of the adults surviving 12-hour exposures deposited eggs.

## CONCLUSIONS

Four general conclusions can be drawn from results obtained in studies of the insecticidal activity of generated low oxygen atmospheres: (1) the atmosphere is lethal to all life stages (egg, larva, pupa and adult) of the common beetles and moths that infest stored-products, (2) susceptibility to the atmosphere varies substantially between species and between the various stages of development within each species, (3) effectiveness of the generated atmosphere is primarily a product of exposure time and treatment temperature; time periods required for control decrease as treatment temperature increases, and (4) sublethal exposures to the generated atmosphere often result in morphological abnormalities and physiological damage that disrupts or prevents normal development.

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SUSCEPTIBILITY OF THE LIFE STAGES OF SITOPHILUS ZEAMAI AND TROGODERMA GRANARIUM LARVAE TO NITROGEN ATMOSPHERE IN MINISILOS

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ABSTRACT

The traditional method of storage and the use of insecticidal protectants on grains in Nigeria have attendant problems and risks both to the consumer and operator. Silo usage in Nigeria was also discouraged because of physical problems of moisture migration and condensation and consequently spoilage of the stored commodity. The possible use of nitrogen as insect control method in minisilos to replace the traditional methods of insect control in storage was tested.

Tests were done to determine the exposure time required to kill all the life stages of maize weevils, Sitophilus zeamais, and the larvae of khapra beetles Trogoderma granarium. They were carried out in airtight minisilos of 0.65 m<sup>3</sup> capacity, in the environment produced by maize of 13.5% moisture content stored under near to pure nitrogen.

Various exposure times required to kill developing maize weevils and larvae of khapra beetles at 27°C ± 1°C were observed in the conditions representing real inert gas silo storage practices. The eggs and the adult stages of maize weevils were more susceptible to anoxia than the larval and pupal stages. The duration of exposure required to kill the eggs, larvae, pupae and adults of maize weevils were 4.0, 7.5, 8.0 and 3.0 days respectively, while 5.0 days exposure to nitrogen killed all the larvae of khapra beetle.

The number of live insects at the beginning and the end of a ten weeks storage trial of maize at 13.5% was assessed in minisilos under

nitrogen and controls in air. A full kill of all stored product insects was achieved in nitrogen, while a very high insect infestation persisted in the controls.

## INTRODUCTION

About 10 million tons of Nigerias' major staple food crop viz. sorghum, millet, maize and rice are produced annually; 10-25% of the grains are however lost annually to stored product insects.

Various methods used to control insect infestation include the traditional methods of smoking, use of local plants, sand and ashes and recently modern technology ushered in the use of chemicals which are mainly organic poisons. The use of chemical protectants on grains in storage was immediately accepted because they are cheap, effective in small quantities, persistent and broadly toxic and require very little labour for their employment, until attendant problems and risks both to the consumer and operator were identified. Some insecticides leave undesirable taint effect on seed and also damage the viability of the seeds. Cases of insects developing resistance to insecticides are very frequent. Consequently new products keep coming into the storage business and this requires more extension time to convince our farmers to adopt the new insecticides. To replace the use of insecticides in our grains therefore, a cue was taken from the claim of Assoreni (Shejbal, 1978, 1979) that storing grains in silos purged with nitrogen will eliminate:

- (a) Toxicity of insecticides to both consumer and operators
- (b) Insect resistance, developing in response to insecticides
- (c) Problems of insect reinfestation, since the storage structure is airtight.

A small scale trial of controlled atmosphere storage of maize in minisilos was jointly conducted by NSPRI Nigeria and Assoreni of Rome to determine the effect of Nitrogen on the life stages of maize weevils and larvae of khapra beetles, in Nigeria.

A summary of the exposure time required to kill adults, pupae, larvae and egg stages of rice weevil was given to be 2, 18.4, 8.9 and 9.3 days respectively (Lindgren and Vincent, 1977).

## MATERIALS

The atmosphere for testing the effect of nitrogen on the life stages of maize weevil and the larvae of khapra beetle was provided by 0.3 tonnes of 13.5% moisture maize stored in minisilos of 0.5 m<sup>3</sup> capacity. Two minisilos were used and each consists essentially of two main parts, a long cylindrical body contains the insect cage introduction point which is a perforated metal tube reaching 2/3rd of the diameter of the minisilo, it is equipped with a rapid closing valve. By this arrangement the composition and relative humidity of the atmosphere inside the minisilos and in the insect cages was maintained the same.

The insect cages were of cylindrical shape made of plexiglas. The cage was 2 inches long and 1 inch in diameter. Both ends of the cylindrical cage were covered with 60 mesh per inch nylon nets to allow free movement of air. The lid of the cage was equipped with a handle for easy and quick introduction and removal of the insect cages in the minisilos.

The maize weevils and khapra beetles used were from laboratory cultures reared on sorghum and cowpeas respectively at  $26 \pm 1^{\circ}\text{C}$  and  $70\% \pm 5\%$  r.h.

The eggs, larvae and the pupae of maize weevils were obtained by exposing disinfested sorghum (sorghum kernels were deep frozen for 7 days before use) to egg laying adult weevils for 5 days in the Nigerian Stored Products Research Institute's rearing room. The life stages of the developing maize weevil progeny were estimated on the basis of developmental activities reported in earlier papers (Shariff and Mills, 1971) who correlated days from oviposition with developmental stages by using daily radiographing of infested kernels and confirmed the stages by measurements of larvae head capsules. For this test, therefore, ages of the life stages of maize weevils in sorghum kernels were taken as follows: 7 days after start of oviposition = eggs, 16 days after start of oviposition = larvae, 28 days after start of oviposition = pupae.

The larvae of khapra beetle used were from laboratory cultures. The adult beetles were reared on disinfested "Black-eye" beans under  $27^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and  $70\% \pm 5\%$  r.h. conditions. Forty khapra beetle larvae



were selected (active) for this test. The larvae were put on disinfested "Black-eye" beans in the special insect cages that fitted the insect cage compartment the of the minisilos.

#### EXPERIMENTAL PROCEDURE

Forty adult maize weevils were put into each insect cage containing disinfested sorghum and introduced into the minisilos. The cages were left in the minisilos for periods ranging from 24 hours to 120 hours. The first batch in nitrogen was removed after 14 hours, removals were made every twenty four hours thereafter and the last batch of the insect cages were removed 120 hours after introduction into the minisil purged with nitrogen.

One hundred kernels of sorghum containing different life stages of maize weevil were put into each of the insect cages and introduced into the minisilos at different periods. Each of the cages containing different designated life stages of maize weevils were purged with nitrogen in the minisilos for periods varying from 24 hours to 240 hours, the insect cages were removed every twenty-four hours.

Forty larvae of khapra beetle were used per each insect cage and introduced for periods varying from 24 to 240 hours in minisilo purged with nitrogen.

Parallel control experiments were conducted in 0.14 tonnes of maize stored in non-air-tight tanks.

After removal from the minisilos the cages containing the designated life stages of maize weevil were kept in the rearing room alongside with their controls to observe progeny emergence. The samples were kept at 27°C and 70% r.h. and examined periodically. Mortality in the immature stages of maize weevils was based on the relative number of adults emerging from treated and untreated samples. The adults of maize weevil and larvae of khapra beetles were also studied under the binocular microscope for a period of time sufficient to confirm the killing effect of nitrogen, since it is known that mortality may be delayed in some fumigants and some fumigants only cause temporary paralysis (F.A.O., 1969).

## PRE-PURGING AND POST-PURGING ASSESSMENT OF INFESTATION

The number of live insects at the beginning and the end of the 10 week storage trial of maize at 12.7% moisture was assessed in minisilos under nitrogen and controls in air. This was done by sieving one kilogramme of the shelled maize with 10 mesh per inch sieves. The 1 kg samples were taken at random. The sieved insects were each identified and counted.

Three hundred kernels were selected randomly and incubated at the start and end of the storage period in the rearing room of 27°C and 70% r.h. for 6 weeks. Emerged adults were sieved periodically from the samples and the number was recorded. Counting was terminated at 6 weeks so as not to include the second generation of insects.

## RESULTS AND DISCUSSION

Data in Table 1 show a variation in the exposure time required to kill all the live stages of maize weevil and larvae of khapra beetles in nitrogen. The egg and the adult stages of maize weevil were more susceptible in nitrogen atmosphere than the larval and pupal stages. All the larvae of khapra beetle tested were killed after 5 days of exposure to nitrogen. Exposing the adults of maize weevil and larvae of khapra beetle to nitrogen for periods below that required to kill them, only caused temporary paralysis and some of the insects recovered after they were restored to fresh air. Adult emergence was delayed (compared with control in air) in samples containing immature stages of maize weevils when exposed to nitrogen for periods below the required time to kill them.

TABLE 1

Time in days required to kill all adults and developing stages of maize weevil and larvae of khapra beetles in minisilos under 99.9% nitrogen.

Species	Designated stages	Temperature (°C)	Exposure time (days)	Commodity
<u>Sitophilus zeamais</u>	adults	26.5	3.0	sorghum
	eggs	26.5	4.0	"
	larvae	27.0	7.5	"
	pupae	26.0	8.0	"
<u>Trogoderma granarium</u>	larvae	26.5	5.0	beans

TABLE 2

Assessment of insect infestation of maize stored in minisilos at start and end of a ten weeks preservation trial (1 kg samples).

Time (weeks)	Gas	Station	Number of adults					Total
			Sitophilus zeamais	Cryptolestes sp	Carpophilus sp	Tribolium castaneum	Lasioderma serricorne	
0	Nitrogen	Top	10	0	5	9	1	25
		Middle	6	0	3	6	3	18
		Bottom	8	1	6	8	6	29
	Air	Mean sample	9	0	8	4	2	26
	Nitrogen	Top	0	0	0	0	0	0
		Middle	0	0	0	0	0	0
		Bottom	0	0	0	0	0	0
	Air	Mean sample	307	7	21	12	15	362

In Table 2, data indicate that a full kill of all stored product insects was achieved in nitrogen while a very high insect infestation persisted in the controls. From the initial samples of the shelled maize (not yet purged with nitrogen), 1 kg-samples from the top, middle and bottom of the minisilos showed that the maize was very heavily infested by stored product insects which were all eliminated at the end of the storage period in nitrogen.

Table 3 data indicates that 132 maize weevil adults emerged from pre-treatment samples and no emergence at all from samples stored in nitrogen, after incubating samples for six week period in the rearing room, was observed.

TABLE 3

Number of adult insects emerged from incubated samples of maize stored in nitrogen and in air (200 kernels incubated from each treatment).

Treatment	No. of emerged insects	Infestation level, by adult insect counts
Samples of maize before storage in nitrogen	132	heavily infested
Samples of maize after storage in nitrogen	0	not infested at all

Quality deterioration in stored maize is known to be due, in large measure, to insect damage. Storing maize in nitrogen, therefore, has a great potential of residue free control of stored product insects and simultaneous maintenance of quality of stored maize in airtight storage facilities.

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## A REVIEW OF RECENT STUDIES OF THE EFFECTS OF CONTROLLED ATMOSPHERES ON STORED PRODUCT PESTS

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### ABSTRACT

Controlled atmosphere storage of grain involves the alteration of the proportion of the normal atmospheric gases, nitrogen, CO<sub>2</sub> and oxygen, to give an atmosphere in the storage lethal to stored grain pests. Progress in the laboratory study of the action of controlled atmospheres on such pests is reviewed with particular emphasis on recent developments. Lethal and sublethal effects are considered. Sublethal effects include delayed or disturbed development, other deleterious phenotypic effects and behavioural phenomena. The literature is apparently conflicting on the relative susceptibilities of various species, the relative speed of action of nitrogen-oxygen mixtures and those containing CO<sub>2</sub> and the temperature dependence of the action of air-CO<sub>2</sub> mixtures. The effects of controlled atmospheres on stored product mites and predators are reviewed and the possible development of tolerance to such atmospheres is discussed.

### INTRODUCTION

Controlled atmosphere storage of cereal grains and grain products is generally understood to consist of the medium or long term storage of such commodities in atmospheres consisting only of the major gaseous components of the earth's atmosphere: that is storage without the introduction of any gases or vapours normally considered to be poisons. In a world that is currently very conscious of pollution by toxic materials, it is this latter fact that has drawn so much attention to the technique. Nevertheless, in view of the great potential of the method in providing long term solutions to storage problems, the number of research workers involved is very small. We urgently need more accurate information on the biological mode of operation of controlled atmosphere storage. The aims of this section of the symposium are to discuss our current knowledge of the biological effects on the arthropod fauna of stored grain and to point out where our greatest deficiencies in our knowledge occur in the hope that they will receive effective study in the near future.

This review is intended to update that of Bailey and Banks (1975), which dealt largely with the acute mortality response of stored product insects to controlled atmospheres. It aims to present a broader outline of the effects of controlled atmospheres including sublethal effects and refers to the earlier literature where necessary to give a complete picture.



The major gaseous components of the earth's atmosphere are nitrogen, oxygen, argon and carbon dioxide. Of these gases argon has been considered to be totally inert biologically and, in the context of controlled atmosphere storage, has received no attention from researchers. Oxygen and carbon dioxide, the obviously biologically active components, have received much attention but, more recently, attention has been focussed on the interaction of water vapour concentration with the other components. Nitrogen, four-fifths of the earth's atmosphere, has been largely looked upon, probably correctly so, as a diluent, since its action has been shown to be similar to that of the inert gas, helium (Lindgren and Vincent, 1970; Aliniaze, 1972).

#### LETHAL EFFECTS OF CONTROLLED ATMOSPHERES

##### Mixtures of nitrogen and oxygen

Since the last general review in 1974 (Bailey and Banks, 1975) few investigations of the effects of changing the proportions of nitrogen and oxygen on mortality have been conducted. Shejbal (1979) has tabulated current knowledge on this. Such studies are very relevant to practical use of low oxygen atmospheres, as they help define the commercial target oxygen level. This is an important consideration because, the lower the target level, the more difficult it is to maintain the specified atmosphere in the face of inevitable leakage. It appears that different pest species have different responses to low oxygen atmospheres and that the available data on the subject may even be conflicting. In the most comprehensive study to date Navarro (1978a) found the lethal response of S. oryzae adults was dependent largely on exposure time, being almost independent of oxygen concentration below 3%, with 1% oxygen atmospheres being slightly more rapid in action than 0.2% or 2% atmospheres. The lethal effect on I. castaneum adults or E. cautella pupae was strongly dependent on oxygen content with more rapid kill occurring with lower oxygen levels (see Fig. 1). Person and Sorenson (1973a,b) found 0.5% O<sub>2</sub> to be only slightly more effective than 1% O<sub>2</sub> particularly at lower temperatures against S. oryzae adults and immatures, but considerably more effective against "flat grain beetle" adults. Careri et al. (1972) found no difference between the speed of action of technical (< 0.5% O<sub>2</sub>) or pure nitrogen against I. confusum adults but a slightly more rapid action of pure nitrogen against S. granarius adults. Shejbal et al. (1973) found an increase in speed of action for adults of I. confusum, I. castaneum and S. granarius as the oxygen was progressively reduced from 1% to 0.1%. The apparent difference between the action on the two Sitophilus species adults is notable and, as Navarro (1978a) concludes, it may be dangerous to generalise from one species to another. A possible reason for wide variations in the results of different studies may be explained by the findings of Shejbal et al. (1973) who demonstrated the interesting phenomenon that the flow rate of

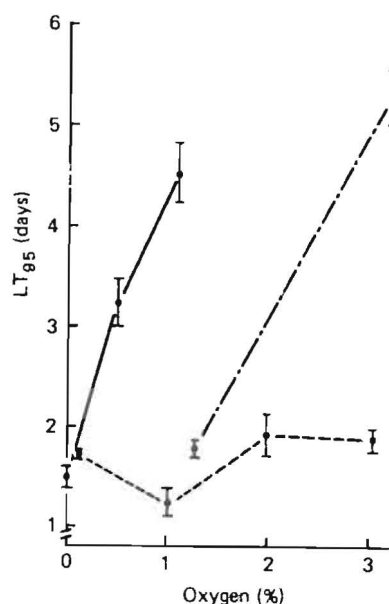


Fig. 1. Variation of  $LT_{95}$  on oxygen concentration in an oxygen-nitrogen mixture at 26°, 54% for *S. oryzae* adults (---), *I. castaneum* adults (—) and *E. cautella* pupae (-.-). Redrawn from Navarro (1978a).

gas mixture through the grain has an effect on insect mortality, a faster rate enabling insects to survive longer. This result suggests that localised regions of still lower oxygen concentration may form at low flow rates resulting in increased mortality under such conditions.

#### Studies on high CO<sub>2</sub> atmospheres

Several studies of the effects of carbon dioxide-air mixtures have been carried out recently. Stoyanova and Shikrenov (1976) determined the time required for a 50% and 99.9% kill of 4 species at 20°C and 28°C using the relatively low concentrations of 20% and 40% CO<sub>2</sub> in air. The longest time necessary was 19.5 days for 99.9% kill of *S. granarius* at 20°C and 20% CO<sub>2</sub>. They suggested the lower observed tolerance of *S. oryzae* than *S. granarius* may be due to a higher respiratory rate.

Press and Flaherty (1973) exposed adult moths of *Ephestia khueniella*, *E. cautella* and *Plodia interpunctella* to a 96% CO<sub>2</sub> atmosphere for 2 hours per day for 6 days. They found a large reduction in oviposition in all 3 species and observed that egg hatch was entirely eliminated in *E. khueniella*, was less than 2% normal in *E. cautella* and was 5% normal in *P. interpunctella*.

Banks and Sharp (1979) reported a trial CO<sub>2</sub> fumigation of wheat and rye using an atmosphere of about 60% CO<sub>2</sub> generated from dry ice under plastic sheeting at 11 to 13°C. The CO<sub>2</sub> level was maintained for 22 days. The pest infestation was predominantly *R. dominica* with small numbers of *I. castaneum*,

Cryptolestes ferrugineus, E. cautella and S. oryzae. Small numbers of R. dominica only survived the treatment, emerging as adults four weeks or more after the start of incubation: they were presumably early instar larvae at the time of gassing. Cantwell and Neidhardt (1978) working on the disinfection of hospital food carts, found that 8 hours exposure to  $> 35\% \text{CO}_2$  at about  $32^\circ\text{C}$  gave complete kill of adult Blatella germanica and prevented hatching from oothecae.

The mortality of stored product pests found in field trials using high  $\text{CO}_2$  atmospheres has been summarised by Banks (1979). It was noted that while very high mortalities were often attained, complete mortality of added test insects or natural populations seldom occurred. This was attributed to a failure to provide sufficient exposure time to  $\text{CO}_2$ .

There have been a few studies on the relative toxicities of high  $\text{CO}_2$  and low oxygen atmospheres. More are required to provide full details of their relative effects, especially against particular species, and to define the speed of action of the two atmospheres. It is well known that  $\text{CO}_2$  acts in the presence of oxygen and that mixtures of  $\text{CO}_2$  and air (e.g.  $80\% \text{CO}_2$ ,  $4\% \text{O}_2$ ,  $16\% \text{N}_2$ ) are more speedily lethal to some species (e.g. Sitophilus spp. (Lindgren and Vincent, 1970)) than pure  $\text{CO}_2$ . However studies on the relative rates of action of  $\text{CO}_2$  or  $\text{N}_2$ -rich atmospheres under conditions where the oxygen concentration is low enough to kill stored product species ( $> 2\% \text{O}_2$ ) give a complex picture. Press and Harein (1966) found pure nitrogen to be slightly more effective against I. castaneum adults and P. interpunctella larvae than pure  $\text{CO}_2$ . Lindgren and Vincent (1970) found no difference between  $100\% \text{CO}_2$  and  $100\% \text{N}_2$  against different developmental stages of both S. oryzae and S. granarius whereas Zakladnoi (1976) showed pure  $\text{CO}_2$  to be more rapidly toxic than pure nitrogen to the adults of S. granarius, S. oryzae, R. dominica and I. castaneum, at all three experimental temperatures ( $20^\circ$ ,  $25^\circ$  and  $35^\circ\text{C}$ ). Verma (1977) and Verma and Wahdi (1978) exposed a number of different stored product pests to pure  $\text{CO}_2$  and  $\text{N}_2$  atmospheres. The action of the  $\text{CO}_2$  atmosphere was significantly slower than the nitrogen one against I. castaneum adults, O. mercator eggs, larvae and pupae and E. cautella eggs and larvae; significantly faster against O. mercator adults, E. cautella adults and I. granarius larvae and not significantly different for I. castaneum eggs, larvae and pupae, E. cautella pupae and I. granarius pupae. As may be expected under oxygen levels that are only just insecticidal in pure nitrogen mixtures, the replacement of nitrogen by  $\text{CO}_2$  gives a more toxic mixture, presumably combining the effect of the low oxygen level with  $\text{CO}_2$ 's specific toxic effect on the insect. Thus Aliniaze (1971) obtained complete mortality of I. confusum and I. castaneum adults at  $27^\circ/38\%$  r.h. in less than 1.5 days with  $2\% \text{O}_2$  in  $\text{CO}_2$ , but it took 4 days using  $2\% \text{O}_2$  in nitrogen.

The possible synergistic effects of  $\text{CO}_2$  and chemical pesticides feature in two studies. Young and McDonald (1970) examined the effect of  $\text{CO}_2$  anaesthesia on the toxicity of malathion to adults of I. castaneum, I. confusum, and Lasioderma serricorne and to larvae of Attagenus megatoma. Anaesthesia for 60 minutes increased the toxicity of malathion to all four species. Anaesthesia for as little as five minutes increased the toxicity to all species except I. confusum. The study arose from concern over the use of  $\text{CO}_2$  anaesthesia during the handling of experimental insects. Bond and Buckland (1978) tested the effect of adding  $\text{CO}_2$  to a number of fumigants at  $0^\circ$ ,  $10^\circ$  and  $25^\circ\text{C}$  using adult S. granarius and I. castaneum and fourth-instar larvae of Tenebrioides mauritanicus. The fumigants used were acrylonitrile, methyl bromide, a mixture of these two, and phosphine and hydrogen cyanide. Results varied ranging from a seven-fold increase of the toxicity to I. castaneum of acrylonitrile in the presence of 30%  $\text{CO}_2$  to no increase at all with hydrogen cyanide. The authors suggest the use of low concentrations of fumigants in controlled atmospheres employing  $\text{CO}_2$  as a means of reducing the concentration of  $\text{CO}_2$  required, or of reducing the exposure times.

#### Controlled atmospheres based on mixtures of $\text{CO}_2$ and nitrogen

Lindgren and Vincent (1970) found no difference in the mortality response of S. granarius and S. oryzae to pure  $\text{N}_2$ ,  $\text{CO}_2$  or helium. Accordingly, it was assumed (e.g. Bailey and Banks, 1975) that at low oxygen levels the proportion of  $\text{CO}_2$  and nitrogen present is unimportant. Recently this has been shown to be untrue and it is now clear that, at low oxygen levels, the presence of a few per cent of  $\text{CO}_2$  accelerates the lethal action of the atmosphere compared with pure nitrogen-oxygen mixtures.

Calderon and Navarro (1979) found that at  $< 5\%$   $\text{O}_2$  the mortality of adult I. castaneum was dependent on the quantity of  $\text{CO}_2$  also present. For instance at 2% oxygen,  $> 90\%$  mortality was attained with 5%  $\text{CO}_2$  present compared with about 50% mortality in the pure nitrogen-oxygen mixture ( $30^\circ\text{C}$ , 57% r.h.). Fig. 2 shows the overall variation of effectiveness of given oxygen levels with different combinations of  $\text{CO}_2$  and  $\text{N}_2$  at  $30^\circ\text{C}$ . A similar variation was found at  $26^\circ\text{C}$ . Girish (1978) using 1.1 to 1.2% oxygen found the  $\text{LT}_{50}$  for I. granarium larvae at  $35^\circ\text{C}$  was 27, 30, 32 and 39 hours for atmospheres containing 15, 11, 6 and 0%  $\text{CO}_2$  respectively (balance nitrogen).

Storey (1975a, 1975b, 1975c, 1977, 1978) subjected a number of species of stored product pests to a gas mixture produced by burning natural gas and containing about 9%  $\text{CO}_2$ ,  $< 1\%$   $\text{O}_2$  and with balance  $\text{N}_2$  and some carbon monoxide and other gases. Zakladnoi et al. (1974) and Zakladnoi (1976) report similar work using a burner producing an atmosphere containing from 0.4 to 2% oxygen and up to 13%  $\text{CO}_2$ . It is not possible to compare the results directly with

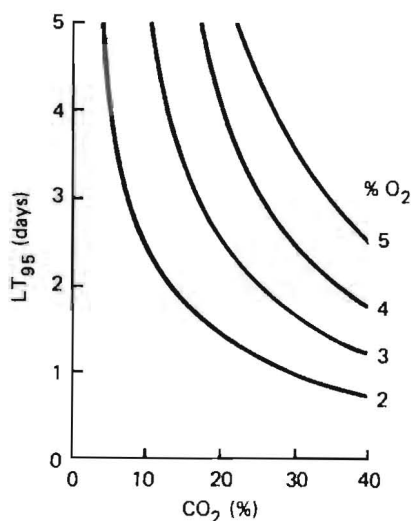


Fig. 2. Variation of  $LT_{95}$  with %  $CO_2$  in atmosphere at various oxygen levels (balance nitrogen) at  $30^{\circ}$ , 57% r.h. for *T. castaneum* adults. Redrawn from Calderon and Navarro (1979).

those obtained using pure gases because the influence of the minor components, carbon monoxide, oxides of nitrogen and other combustion products, is not known and the oxygen level was not precisely regulated. The general trends are similar to that found by other workers using low oxygen atmospheres (see Bailey and Banks, 1975), with *S. oryzae* being the most tolerant species both as adult and immature forms. *S. oryzae* adults gave an  $LT_{95}$  of 12.4 days at  $15^{\circ}C$ , falling to 0.8 days at  $32^{\circ}C$  (50% r.h.) (Storey, 1975a). It is notable that Zakladnoi (1976) found *S. granarius* to be much more tolerant than *S. oryzae*. Moth species (Storey, 1975b) were relatively susceptible to the gas mixture with all stages of *Sitotroga cerealella* killed after five days, of *E. cautella*, after two days and of *P. interpunctella* within one day at  $27^{\circ}C$ , 50% r.h. Some immature stages of *S. oryzae* were more tolerant than the adults to the gas mixture, with the greatest tolerance being shown by the fourth-instar larvae and pupal stage. The  $LT_{95}$  of prepupae was found to be 27 and 10 days at  $21^{\circ}$  and  $27^{\circ}C$  (50% r.h.) respectively. There was no survival of immature *S. oryzae* exposed for 14 days in maize, wheat or sorghum at  $27^{\circ}C$ , 50% r.h. This exposure period is similar to that needed to obtain complete mortality using 1% oxygen in nitrogen under the same conditions (Banks and Annis, 1977). Thus, although the results of Storey and those given above suggest that under commercial conditions there may be some advantage in using a proportion of  $CO_2$  in low oxygen atmospheres, the reduction in exposure time allowable will not be substantial if it is necessary to ensure that *Sitophilus* spp. are eliminated.

### Relative susceptibility of various species to controlled atmospheres

It is important to know what is the most tolerant stored product pest in a given atmosphere in order to be able to set the exposure limit to control it and thus all others too. It is usually assumed for both high-CO<sub>2</sub> and low oxygen atmospheres that immature stages of S. oryzae are the most tolerant form of the most tolerant pest likely to be encountered but that stages of S. granarius may also be as tolerant. However there are no comparative studies on immature stages of other species and the evidence based on the adult forms is conflicting. Table 1 summarises the order of susceptibility to various controlled atmospheres found by various workers for the main stored grain pests. There is general agreement that T. confusum is similar in susceptibility or slightly more tolerant than T. castaneum both as adults and immature stages to all types of controlled atmosphere (Jay and Pearman, 1971; Storey, 1977; Aliniaze, 1971; Jay et al., 1971; Shejbal et al., 1973). Bearing this information in mind, it can be seen from Table 1 that Sitophilus granarius is consistently rated as a tolerant species, but authorities are divided as to the degree of susceptibility of S. oryzae. Whether the apparent inconsistencies result from strain differences, difference in technique or a true change in relative susceptibility in different gas mixtures is yet to be determined.

The susceptibility of Trogoderma granarium larvae to pure nitrogen and CO<sub>2</sub> atmospheres, reported by Verma and Wadhi (1978), is similar to that of Sitophilus larvae (Lindgren and Vincent, 1970; Storey, 1975a). A detailed comparison should be made to determine which is the more tolerant species to various modified atmospheres.

### Influence of relative humidity on action of controlled atmospheres

A component of controlled atmospheres that has been largely neglected so far as its biological action is concerned is water vapour. Pearman and Jay (1970) reported a marked increase in the mortality of T. castaneum exposed for 46% CO<sub>2</sub> where the relative humidity was decreased to 33% or lower. Jay, Arbogast and Pearman (1971) exposed adult T. castaneum, T. confusum and O. surinamensis to atmospheres containing less than 1% oxygen with the balance as nitrogen at relative humidities ranging from 9% to 68%. All three species showed a marked increase in mortality as the relative humidity decreased. Also with essentially similar relative humidities used in conjunction with atmospheres containing about 38% carbon dioxide they showed that the mortality of all three species increased with decreasing humidity. The authors suggested that desiccation at low humidities was an essential factor in mortality in both instances. Zakladnoi (1976) demonstrated a more rapid mortality at low relative humidity in assessments employing "dry grain, moderately dry grain and wet grain".



Subsequently, Navarro and Calderon (1973) examined the inter-relationship of  $\text{CO}_2$  and relative humidity as it affects pupae of E. cautella. They confirmed the finding of high mortality at lower relative humidity and found that for this species death appeared to be associated with a critical water loss representing about 30% of weight of the pupae. The higher the concentration of  $\text{CO}_2$  and the lower the humidity the more pronounced was water loss. In a further study using pupae of E. cautella, Navarro and Calderon (1974) showed that there was little difference in rate of action of  $\text{CO}_2$  concentration between 21-88% at 21 and 55% r.h. but a difference became apparent at high r.h. (95%) with lower  $\text{CO}_2$  levels being slower in action. At 21 and 55% r.h. mortality appeared to be correlated with weight loss and an inverse relationship between  $\text{CO}_2$  concentration and relative humidity was found for a given mortality level. At the highest relative humidity used, 95%, weight loss was small and a direct toxic effect of  $\text{CO}_2$  acting as a fumigant appeared to be involved.

Navarro (1978a) reported that in the absence of  $\text{CO}_2$  a relationship existed between oxygen tension and relative humidity for a set level of mortality. With pupae of E. cautella and adults of I. castaneum the higher the relative humidity the lower the oxygen concentration necessary to produce 95% mortality.

#### Action of controlled atmospheres on mites

There is little published work on the influence of controlled atmospheres on stored product mites. Stepien (1979, 1975) investigating the action of 99.5%  $\text{CO}_2$  on Tyrophagus putrescentiae Schrank found the 0-24 hour old eggs to be the most tolerant stage of development with complete mortality attained only after six days (25°C, 85% r.h.) but with other stages controlled in less than one day.  $\text{CO}_2$  under high pressure is rapidly toxic to I. putrescentiae (? adults only) (Mitsura et al., 1973) requiring 30 minutes exposure at 16 atm pressure at 30°C. Nitrogen even at 100 atm was ineffective at 30°C even after one hour. The lethal effect was dependent on  $\text{CO}_2$  pressure and exposure time requiring 15, 30 and 55 minutes exposure at 20°C at 26, 21 and 16 atm respectively for complete mortality. Hughes (1943) found Acarus siro (? adults only) was killed by an exposure of four days at 20°C (high r.h.) to either pure  $\text{N}_2$  or  $\text{CO}_2$  but could survive four days at 0.4%  $\text{O}_2$  in  $\text{N}_2$ . These observations suggest that controlled atmospheres may be potential control agents for stored product mites, but additional data, notably on A. siro, is required to confirm this and extend the range of atmospheres tested.



### Action of controlled atmospheres on beneficial insects associated with grain storage

Banks and Sharp (1979) found that significant numbers of the hymenopteran parasitoids Anisopteromalus calandrae and Choetospila elegans survived conditions (11-13°C, 25-90% CO<sub>2</sub> over 22 days) under which a high level of mortality was attained for their host, Rhyzopertha dominica. There is a possibility that this differential susceptibility could be exploited for suppression of R. dominica under conditions where an incomplete kill was achieved in the presence of these parasitoids. A similar situation does not appear likely to occur with the predator Xylocoris flavipes under low oxygen atmospheres as it is very susceptible: 97% CO<sub>2</sub>, 1% O<sub>2</sub>, 2% N<sub>2</sub> at 30°C, 60% r.h. gave complete mortality of eggs and adults, the most tolerant stages, within 24 hours (Press and Flaherty, 1978).

### Temperature dependence

There is general agreement in the literature (see Bailey and Banks, 1975) that the action of low oxygen atmospheres is strongly dependent on temperature, with action at low temperatures being slow. This generalisation includes those low oxygen atmospheres containing CO<sub>2</sub> produced by burning hydrocarbons (Storey 1975c, 1977). However there is a general lack of information on the temperature dependence of high CO<sub>2</sub> atmospheres containing substantial quantities of oxygen (e.g. 60% CO<sub>2</sub> in air) and little information on the action of low oxygen at between 20 and 15°C, a range in which death from cold does not occur, but in which low oxygen systems are likely to be very slow acting. Banks and Annis (1977) by extrapolating the available data for temperatures at > 20°C, predicted that about 15 weeks exposure to 1% O<sub>2</sub> in nitrogen at 18°C was required for complete mortality of all species and developmental stages of stored product insect pests.

As an extreme case of temperature dependence under oxygen conditions only just adequate for insect control, the response of mixed cultures of S. granarius containing approximately equal numbers of various developmental ages was studied (Bailey and Banks, unpublished data) at three temperatures at 1.3% O<sub>2</sub> in nitrogen under an r.h. not likely to cause additional stress (70%). The cumulative emergence curves for this experiment are given in Fig. 3 for various periods of exposure to the low oxygen gas. Control cultures yielded 5000 to 7000 insects each before the start of emergence of the next generation. Almost complete mortality (> 99.5%) was attained in 2 weeks at 29.4°, in 3 weeks at 23.9°, but at 18.3°C only 78% mortality was attained after 12 weeks exposure. Numbers emerging at various times after various exposures are given in Table 2 for the latter experiment (see below).

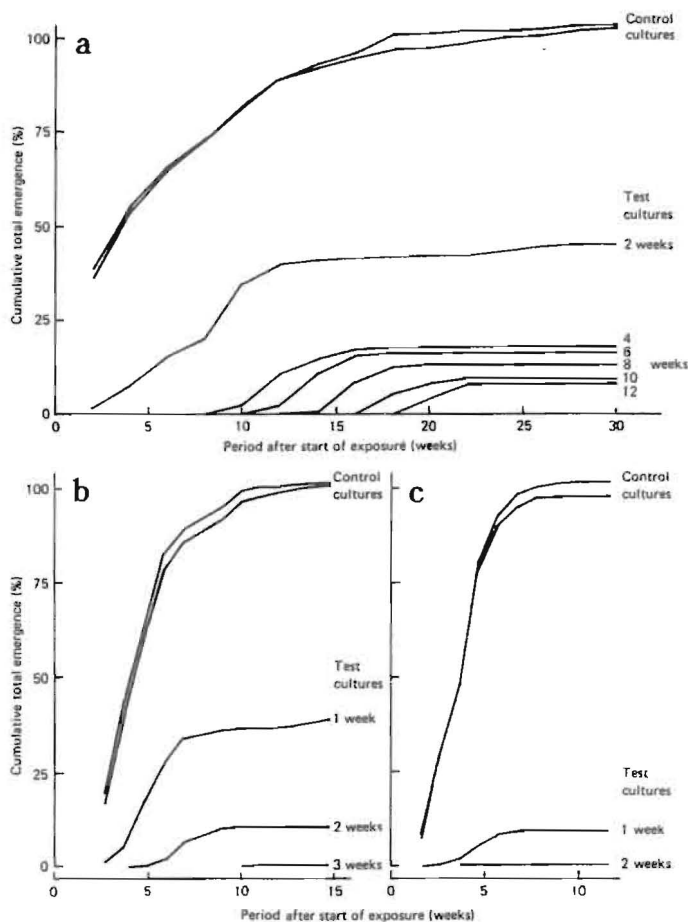


Fig. 3. Cumulative total emergence of *Sitophilus granarius* cultures containing approximately equal numbers of each developmental age expressed as a percentage of the average total observed numbers in the control cultures. Emerged adults removed after each inspection. Cultures continuously exposed to 1.3% O<sub>2</sub> in nitrogen at 70% r.h. at (a) 18.30, (b) 23.90 and (c) 29.40°C for various periods and then incubated at the same temperature.

There are no published studies on the temperature dependence of mortality to atmospheres containing mixtures with 60-90% CO<sub>2</sub> in air. However, Harein and Press (1968) found little difference in speed of action of 47-49% CO<sub>2</sub> in air against *Tribolium castaneum* larvae exposed at 16, 27 or 39°C (61-64% r.h.). With adults, exposure at 27°C appeared slightly less effective than at the other two temperatures. It appears that, in contrast to that of low oxygen atmospheres, there may be only a small temperature dependence of atmospheres containing high CO<sub>2</sub> with air. With pure gases, Zakladnoi (1976) demonstrated little temperature dependence in 100% CO<sub>2</sub> but a marked temperature dependence in 100% nitrogen. By contrast, Aliniyazee (1971) found a substantial positive temperature effect in the lethal action of 100% CO<sub>2</sub> on both *I. confusum* and *T. castaneum*.

TABLE 1

Relative susceptibility of common stored product pests (beetles) to various controlled atmospheres as reported by various authorities.

Order of susceptibility	Stage	Gas Mixture	Temperature (°C)	Approximate r.h. (%)	Reference
S.g. < S.o. <sup>a</sup> < T.co. < R.d.	adult	100% N <sub>2</sub>	20,25,35	75	Zakladnoi, 1976
T.ca. = S.o.	adult	100% N <sub>2</sub>	26	54	Navarro, 1978a
S.o. < S.g.	all stages	100% CO <sub>2</sub> or N <sub>2</sub>	27	65	Lindgren & Vincent, 1970
S.g. < S.o. < T.ca.	adult	100% CO <sub>2</sub>	20	?	Busvine, 1942
S.g. < T.co. < S.o. = R.d.	adult	100% CO <sub>2</sub>	20,25,35	75	Zakladnoi, 1976
T.ca. < S.o.	adult	1% O <sub>2</sub> /N <sub>2</sub>	26	54	Navarro, 1978a
R.d. = T.ca.	adult	2% O <sub>2</sub> /N <sub>2</sub>	32	70	Bailey, 1965
S.g. < T.co. = T.ca.	adult	low oxygen	22	70	Shejbal et al., 1973
S.o. = or < S.g.	all stages	b	27	50	Storey, 1975a
S.o. < S.g. < R.d. = T.ca.	adult	b	21,27,32	50	Storey, 1975c
S.o. < S.g. < R.d. < T.ca.	adult	b	15	50	Storey, 1975c
T.ca. < R.d.	adult	50% CO <sub>2</sub> /21% O <sub>2</sub> /N <sub>2</sub>	32	70	Bailey, 1965
S.g. < S.o.	adult	high CO <sub>2</sub>	27	65	Lindgren & Vincent, 1970
S.g. < T.co. < R.d. < S.o.	adult	CO <sub>2</sub> /O <sub>2</sub> mixtures			
		20,40% CO <sub>2</sub> /air	20,28	?	Stoyanova & Shikrenov, 1976

<sup>a</sup> A < B signifies species A is less susceptible than B.

<sup>b</sup> low oxygen atmosphere (< 1% O<sub>2</sub>) produced by burning natural gas

S.g. = *Sitophilus granarius*; S.o. = *S. oryzae*; T.ca. = *Tribolium castaneum*; T.co. = *T. confusum*; R.d. = *Rhyzopertha dominica*.

## SUBLETHAL EFFECTS OF CONTROLLED ATMOSPHERES

### Behavioural effects associated with controlled atmospheres

There have been two reports (Shejbal et al., 1973; Navarro et al., 1976) of stored product Coleoptera, presumably within the grain bulk, congregating around leaks in stores under low-oxygen atmospheres. Bailey (unpublished data) observed a similar phenomenon in an hermetic storage. The high concentration of insects observed suggests that movement had occurred and thus that the insects can sense and move up the oxygen concentration gradient towards the leak. Navarro (1978b) was unsuccessful in his attempts to demonstrate this aggregation under laboratory conditions with various species using gradients of either oxygen or carbon dioxide.

Barrer and Jay (1980) demonstrated that E. cautella females found a source of wheat odour more attractive if containing 30% CO<sub>2</sub> than if mixed with pure air. A source of odour with 60% CO<sub>2</sub> was as attractive as pure air while that from a low oxygen source (1% O<sub>2</sub>) was significantly repellent. Oviposition close to the source was increased in the presence of 30% CO<sub>2</sub> relative to air. Willis and Roth (1954) found 60% CO<sub>2</sub> to be repellent to T. castaneum.

Aggregation at leaks both from within and outside a storage are relevant to the controlled atmosphere technique as such abilities could allow localised survival in a treated grain bulk or infestation of a region around a leak from external sources.

The 'anaesthetic' effect of carbon dioxide or nitrogen on insects is well-known. For stored product insects, immobilisation in response to low oxygen atmospheres is rapid after a brief period of hyperactivity (Aliniaze, 1972; Storey, 1975c). At about 40% CO<sub>2</sub> in air, immobilisation may take some hours for some species (Stoyanova and Shikrenov, 1976) but be rapid for others (Oosthuizen and Schmidt, 1942). Individual S. granarius remain active to varying degrees (Bond and Buckland, 1979). The latter effect appears to be related to the ability to develop tolerance to 42% CO<sub>2</sub>.

### Influence of controlled atmospheres on development

An important and neglected aspect of the action of both low oxygen and high-CO<sub>2</sub> atmospheres concerns the influence of these gas mixtures on development rate. With both types of atmosphere, a significant retardation of development occurs. The effect is more easily observed under slower rates of development. Table 2 gives the observed emergence during successive 2-week periods of S. granarius cultures set up as described above and exposed to 1.3% oxygen in nitrogen for various periods. In this case the time at which peak numbers emerge increases directly with the period of exposure to the low oxygen atmosphere. The pattern is consistent with the survival of one developmental

age group by some form of slowing or suspension of development, thus presumably lessening its oxygen consumption and partially avoiding the effects of anoxia. The number emerging between 8-10 weeks after 2 weeks of exposure to low oxygen is significantly higher than expected from the numbers observed emerging each fortnight from the controls between 4 and 12 weeks ( $\bar{x} = 399$ , s.d. = 75,  $n = 8$ ,  $P < 0.01$ ) suggesting that some development occurred into the tolerant stage, increasing the total numbers surviving for that emergence period. It is clear from the declining overall numbers that even the tolerant stage was slowly killed by the low oxygen atmosphere under these conditions.

		Assay date (weeks after start)													
		2	4	6	8	10	12	14	16	18	20	22	24	26	
Exposure (weeks)	0	1709	816	501	332	424	338	169	125	$F_2$ 165	54	50	55	37	
	2	73	290	353	219	672	231	58	20	11	10	11	57	56	
	4		0	0	1	119	378	186	117	14	2	1	3	2	
	6			0	0	1	118	388	212	19	1	0	1	3	
	8				0	0	0	23	373	182	24	4	0	0	
	10					0	0	0	0	246	134	52	1	4	
	12						0	0	0	6	184	165	15	2	

Table 2. Numbers of adult *S. granarius* emerging and removed at fortnightly intervals from cultures held at 18.3°C/70% r.h. under 1.3% O<sub>2</sub> in nitrogen for various periods and subsequently in air under the same conditions. Bold figures denote maximum emergence for a given period of exposure to low oxygen conditions.

A delay in developmental period corresponding to that spent under high-CO<sub>2</sub> (46-53%) was observed by Oosthuizen and Schmidt (1942) for *Callosobruchus chinensis*. Intermittent exposure of *Blatella germanica* to high CO<sub>2</sub> levels even for only three minutes a week can substantially slow development (Brooks, 1957). Unspecified delays in development have been noted for *T. castaneum* pupae and larvae under pure nitrogen and CO<sub>2</sub> (Aliniasee, 1971, 1972) and for their eggs in 20% CO<sub>2</sub> in air (Aliniasee and Lindgren, 1920), for *S. granarius* and *oryzae* immature stages under pure nitrogen and CO<sub>2</sub> (Lindgren and Vincent, 1970), of *T. granarium* larvae under high CO<sub>2</sub> and low oxygen gas mixtures (Bailey, 1965) and *T. castaneum*, *T. confusum*, *S. oryzae*, *S. granarius* and *C. maculatus* in low oxygen atmospheres from hydrocarbon burning (Storey, 1975a, 1977, 1978). Spratt (1979a) found continuous exposure of *S. zeamais* to 10% O<sub>2</sub>, 10% CO<sub>2</sub>, 80% N<sub>2</sub> (30°C, 71% r.h.) resulted in a delay in development of about 11 days compared with development in air. The intrinsic rate of increase,  $r$ , fell from 0.32 in

air to 0.12 in the gas mixture (Spratt, 1979b) with the fall in rate caused by a combination of delay in development and other factors. The general effect on development rate merits further study as it is an important component of the effect of controlled atmospheres. Enhancement of the ability to delay development could lead to a form of tolerance to the treatment. If the phenomena is not recognised, it may also lead to errors in laboratory or field assessment of controlled atmosphere applications.

There have been a number of observations of sublethal effects of controlled atmospheres apparently common to both high CO<sub>2</sub> and low oxygen atmospheres which influence the overall fitness of the exposed population and thus reduce the rate of increase. These are perturbed metamorphosis (Storey, 1977, 1978; Aliniaze, 1971, 1972; Stepien, 1979), decrease in fecundity (Press and Flaherty, 1973; Spratt, 1979a; Stepien 1979), decrease in longevity (Spratt, 1979b; Stepien, 1979) and partial paralysis (Storey, 1975c, 1977). A general review of sublethal effects of CO<sub>2</sub> on insects was given by Brooks (1957).

#### RESISTANCE OR INCREASED TOLERANCE TO CONTROLLED ATMOSPHERES

It has been stated (e.g. Shejbal, 1978) that resistance to controlled atmospheres, specifically low oxygen systems, is unlikely to develop. It is indeed, difficult to envisage any stored product insect pest rapidly becoming capable of continuing to develop under low oxygen conditions (< 0.5% O<sub>2</sub>) as this would entail a substantial change in its biochemistry of metabolism. However many insects possess anerobic metabolic pathways (Gilmour, 1965) and some are capable of continued development under very low oxygen conditions, although none are known which will complete its life cycle in such a situation. In practical terms, resistance does not entail continued development under a control measure but only survival until it no longer operates. Thus an accentuation of the delay in development observed when stored product insects are treated with low oxygen atmospheres together with an increased overall tolerance would result in a form of resistance. There appears no reason why such a pattern could not be selected for and why it could not eventually become significant in commercial practice, particularly when short exposures (e.g. 1 month) are used.

A similar form of tolerance could arise to high CO<sub>2</sub> atmospheres. Additionally, because such atmospheres can contain sufficient oxygen (> 5%) for development under otherwise normal conditions, a further form of resistance analogous to that found for fumigants (Champ and Dyte, 1976) can be expected. Bond and Buckland (1979) have demonstrated such a tolerance to CO<sub>2</sub> atmospheres in a strain of *S. granarius* selected in the laboratory. After four selections with 75% CO<sub>2</sub> the LT<sub>50</sub> to 75% CO<sub>2</sub> increased from 1.7 to 4.6 days and for 7 selections with 42% CO<sub>2</sub> the LT<sub>50</sub> to 42% increased from 5.1 to 17.5 days (25°, 100% r.h.). A similar level of tolerance was also found at 60% r.h. to 75% CO<sub>2</sub>. For

relatively short exposures to nitrogen or CO<sub>2</sub>, precedent already exists for a low level of resistance. Strains of Drosophila melanogaster show a range of susceptibility (about 1.5x) to these gases, with increased tolerance associated genetically with resistance to desiccation (Matheson and Parsons, 1973, 1975).

The possibility of the eventual rise of resistance cannot be taken to invalidate the controlled atmosphere technique but must be borne in mind, as in fumigation, so that the technique is not used in situations where it is likely to be incompletely effective thus providing a population of insects selected for tolerance to the control measure. An increase in tolerance may eventually require longer exposure periods to low oxygen atmospheres to achieve complete insect kill and in the case of CO<sub>2</sub> where a 'one-shot' system is used, a system of maintenance of atmosphere may have to be provided. Both eventualities will increase the overall cost of the technique.

### CONCLUSION

It is clear from this review that there are several inconsistencies and gaps in our knowledge of the biological actions of various controlled atmospheres. During the 1970's a large quantity of information on the general subject has been gathered but only a few generalisations are apparent: low humidities accelerate the action of controlled atmospheres; increase in temperature or CO<sub>2</sub> content assists the action of low oxygen mixtures and exposures producing incomplete mortality cause a number of deleterious effects, including delayed development. We still require further work on the basic dose-mortality response to the various gas mixtures, with particular emphasis on tolerant developmental stages and species in order to provide a soundly based exposure schedule for particular conditions and atmospheres. Clarification of the delayed emergence phenomenon, the temperature sensitivity of the action of high CO<sub>2</sub> atmospheres and the influence of gas flow rate on mortality to low oxygen atmospheres is important to the practical use of controlled atmospheres.

It is hoped that this Symposium will provide some of the answers to these questions and give the stimulus necessary to ensure that those problems not yet elucidated will be given the attention they deserve, thus providing a sounder basis for the practical application of the important technique of controlled atmosphere grain storage.

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## III S E S S I O N :

## MICROBIOLOGY OF CONTROLLED ATMOSPHERE STORAGE



## MICROBIOLOGY OF CONTROLLED ATMOSPHERE STORAGE OF GRAINS--AN OVERVIEW

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## INTRODUCTION

Work has been underway for more than 25 years on the use of modified atmospheres to reduce microbiological spoilage of stored grains. Present technology makes such storage feasible, and worldwide food requirements may make it essential. As an overview, this paper will present information first on the basic considerations that must be addressed in controlled atmosphere storage. Next, the influence of both intrinsic and extrinsic factors on the stability of stored grain and associated microbiological activity will be reviewed. Finally, the interactions of these fundamental parameters as they relate to controlling undesirable microorganisms on grain will be assessed.

## BASIC CONSIDERATIONS

The rationale for use of commodity-modified atmosphere, modified atmosphere or controlled atmosphere storage is based on several key considerations. Storage capabilities are increased when spoilage is reduced and when safety is ensured and changes in the grain are minimal. The undesirable activities of biological agents such as insects, mites, rodents, and birds as well as microorganisms must be controlled. Undesirable chemical reactions also must be minimized. Preservation can be further extended through reduced moisture, low temperature, and chemical protectants.

The microbiological advantages of controlled atmosphere storage include the inhibition of aerobic fungi, elimination of mycotoxin production, conservation of desirable quality factors in the grain, and manipulation of economic advantages from extended storage (Christensen, 1978).

The microbiological disadvantages of controlled atmosphere storage include elimination of microorganisms that compete under aerobic conditions, development of populations of certain aerobic microorganisms before the atmosphere is sufficiently modified, requirements for expensive gases and complex technical facilities and capabilities, and generation of adverse quality factors (Christensen, 1978).

The following is a list of many of the major considerations that influence the effectiveness of controlled atmosphere storage: (1) microbial load, (2) type of grain, (3) climate of storage facility, (4) moisture or drying requirement, (5) potential variation in storage conditions, (6) type of controlled atmosphere, and (7) technical capabilities.

A primary and major consideration in the application of controlled atmosphere storage of grain is the types and numbers of microorganisms that play a role in



determining storage stability of grains. The "storage fungi" include *Aspergillus*, *Penicillium*, *Absidia*, *Mucor*, *Rhizopus*, *Chaetomium*, *Scopulariopsis*, *Paecilomyces*, *Neurospora*, *Nigrospora*, *Sporendonema*, (*Wallemia*) (Hyde, 1974; Wallace, 1975; Christensen & Meronuck, 1976). The "field fungi" include *Alternaria*, *Cladosporium*, *Helminthosporium*, *Fusarium*, and numerous other genera. Yeasts that have been considered influential under controlled atmosphere conditions include *Candida* and *Hansenula* (Hyde, 1974; Wallace 1975; Christensen & Meronuck, 1976). Bacteria that must be considered in the evaluation of controlled atmosphere include such genera as *Lactobacillus*, *Clostridium* and *Bacillus*. General groups such as the coliforms are also frequently evaluated. Furthermore, specific functional groups of bacteria that constitute the thermophiles or the psychrotrophs are, in general, strategic in their role in high moisture grains (Hobbs & Greene, 1976; Bothast, 1974).

The principal types of grain that must be considered when one evaluates the microbiology of controlled atmosphere storage include corn (maize), wheat, rye, sorghum, durham, sunflower, rice, barley, millet, oats, and soybeans (Hobbs & Greene, 1976; Christensen & Kaufmann, 1977; Christensen, 1975).

The primary physical parameters, both intrinsic and extrinsic factors, that influence the microbial activity on grain include the moisture or water activity ( $a_w$ ) of the grain, the temperature of storage, the atmosphere surrounding the grain during storage, and duration of the storage period.

#### WATER ACTIVITY

Moisture content or equilibrium relative humidity or water activity ( $a_w$ ) have been key parameters that have been used extensively to predict grain storage stability irrespective of atmosphere. The role of moisture is related to the variations of grains, the limitations of naturally occurring biological substances, the influence of harvesting and early storage, the requirements for viability and product performance considerations, the uniformity within a given unit of grain, the effects of temperature on relative humidity, and the individual microbial responses to various water activity levels.

Christensen and Kaufmann (1977) have previously identified key fungi that are found at various relative humidities and related equilibrium moisture content of common grains. Table 1 restates earlier summaries.



TABLE 1

Equilibrium moisture content of common grains at relative humidities of 65-95%; fungi found at each level (Christensen & Kaufmann, 1977).

% R.H.	Moisture Content	Fungi
65-70	12.5 - 13.5	<i>Aspergillus halophilicus</i>
70-75	14.5 - 15.0	<i>A. restrictus</i> , <i>A. glaucus</i> , <i>Sporendonema</i>
75-80	15.0 - 15.5	Above + <i>A. candidus</i> , <i>A. ochraceus</i> , <i>A. versicolor</i>
80-85	18.0 - 18.5	Above + <i>A. flavus</i> , few species of <i>Penicillium</i>
85-90	19.0 - 20.0	Above + several species of <i>Penicillium</i>
95-100	22.0 - 24.0	All advanced decay fungi, yeasts, and bacteria.

These observations are directly related to the minimum  $a_w$  that supports growth of these various fungi at optimum growth temperatures from 26°C to 30°C (Table 2).

TABLE 2

Minimum water activity ( $a_w$ ) for growth at optimum temperature (Christensen & Kaufmann, 1974).

<i>Aspergillus halophilicus</i>	0.68
<i>A. restrictus</i>	0.70
<i>Sporendonema</i> (Wallemia)	0.70
<i>A. glaucus</i>	0.73
<i>A. candidus</i> , <i>A. ochraceus</i>	0.80
<i>A. flavus</i>	0.85
<i>Penicillium</i> (depending on sp.)	0.80 to 0.90

A recent publication by Northolt (1979) summarized the minimum  $a_w$  levels required for fungal growth and mycotoxin production on several agricultural products. In some cases, the organism was part of the natural flora present on the grain. In other cases, the organism was present in combination with competitive flora on the grain, or it was present as a pure culture. The literature reviewed by Northolt is summarized in part in the adapted information presented in Table 3. The influence of incubation temperature, as well as product and competition from other microorganisms, is readily evident. Direct comparisons are difficult because of the variations in the test parameters. Nevertheless, it appears that reduced competition results in the ability of the organisms to produce mycotoxins at lower  $a_w$  than would otherwise be possible.

TABLE 3

Minimum water activity ( $a_w$ ) for growth and mycotoxin production (Northolt, 1979).

Product	Fungus	°C Temp.	Growth	Mycotoxin
Natural Flora				
Barley	<i>Aspergillus</i> sp.	20	0.80	-
	<i>Penicillium</i> sp.	20	0.87	-
	<i>Aspergillus flavus</i>	19	0.88	-
	<i>Aspergillus flavus</i>	24	-	0.92
Oats	-	25	0.75	-
Sorghum	-	30	0.8	-
Competitive Flora				
Barley	<i>Penicillium viridicatum</i>	12	0.85	0.85
	<i>Aspergillus flavus</i>	35	0.77	-
	<i>Aspergillus flavus</i>	15	0.84	-
Wheat	<i>Aspergillus ochraceus</i>	20	0.75	-
	<i>Aspergillus parasiticus</i>	30	0.70	0.80
	<i>Aspergillus flavus</i>	25	0.85	0.85
Pure Culture				
Corn	<i>Penicillium expansum</i>	19	0.91	-
	<i>Aspergillus ochraceus</i>	19	0.76	-
	<i>Aspergillus ochraceus</i>	25	0.88	-
	<i>Penicillium viridicatum</i>	19	0.85	-
Wheat	<i>Aspergillus flavus</i>	20	-	0.80
Rice	<i>Aspergillus flavus</i>	30	-	0.76
	<i>Aspergillus ochraceus</i>	25	0.86	-

Northolt studied the minimum water activity for growth and mycotoxin production by *A. flavus*, *P. expansum*, *A. ochraceus*, and *A. parasiticus*. A comparison of Northolt's data with those found in the literature shows some dramatic differences in stated minimum water activities. These differences are summarized in Table 4 that was adapted from Northolt (1979).

TABLE 4

Significant differences between minimum  $a_w$  - Literature vs. Northolt (Northolt, 1979).

Product	Fungus	Growth	Mycotoxin
Corn	<i>Aspergillus flavus</i>	-	+0.09
	<i>Penicillium expansum</i>	+0.07	-
	<i>Aspergillus ochraceus</i>	-0.07	-
	<i>Aspergillus ochraceus</i>	+0.05	-
Wheat	<i>Aspergillus ochraceus</i>	-0.08	-
Rice	<i>Aspergillus parasiticus</i>	-0.10	-0.03
	<i>Aspergillus flavus</i>	-	-0.07

+ indicates literature is higher than Northolt

- indicates the reverse

## TEMPERATURE

Storage temperature is a well established parameter that influences microbial activity as well as other characteristics of grain. The ambient temperature may fluctuate with climatic change or it may be partially controlled through construction or mechanical devices. Under specific conditions it may be controlled very precisely for experimental determinations. Reduced temperatures generally reduce chemical reactions, and growth is an example of a response that is slowed by reducing temperatures. Although many chemical reactions continue to accelerate at elevated temperatures, growth above optimum levels generally is somewhat reduced to a maximal level at which growth ceases. Temperature, as an influential parameter, is affected by action of various organisms, including microorganisms that may be metabolically active in association with the grain; or the temperature may be influenced by the climate or by the facility used for storage. The fluctuation or uniformity of the temperature of storage can dramatically influence subsequent response by microorganisms or the grain itself.

Christensen & Kaufmann (1974) reported the minimum, optimum, and maximum temperatures for growth of common storage fungi on grains. Table 5 presents a summary of this information.

TABLE 5

Minimum, optimum, and maximum temperatures (°C) for growth of common storage fungi on grains (Christensen & Kaufmann, 1974).

	<u>Minimum</u>	<u>Optimum</u>	<u>Maximum</u>
<i>Aspergillus restrictus</i>	5-10	30-35	40-45
<i>A. glaucus</i>	0- 5	30-35	40-45
<i>A. candidus</i>	10-15	45-50	50-55
<i>A. flavus</i>	10-15	40-45	45-50
<i>Penicillium</i>	-5- 0	20-25	35-40

Recently Northolt (1979) summarized findings in the literature for a variety of agricultural products. His summary of the literature is adapted in Table 6. It is readily evident that reduction in temperature alone is insufficient to control growth and mycotoxin production by various fungi on grain.

TABLE 6

Minimum temperature (°C) for growth or mycotoxin production on grain (Northolt, 1979).

Product	Fungus	Growth	Mycotoxin
Barley	<i>P. cyclopium</i>	<1	<1
Corn	<i>P. cyclopium</i>	<1	<1
	<i>P. martensii</i>	ND	1
	<i>A. ochraceus</i>	-	10
Wheat	<i>A. ochraceus</i>	-	10
	<i>P. viridicatum</i>	<5	<5
Rice	<i>A. flavus</i>	-	11
	<i>P. cyclopium</i>	<1	<1
Sorghum	<i>P. cyclopium</i>	<1	<1

#### ATMOSPHERE

The storage parameter primary to our efforts here is the atmosphere around the stored grain. Evaluation of the effects of atmosphere on retarding deterioration of various food products has been extensive during the last 25 years. The atmosphere surrounding products of one type or another can be influenced or changed through various means which subsequently lead to the respective identifying titles of controlled atmospheres, modified atmospheres, and commodity-modified atmospheres (Brecht, 1980). In commodity-modified atmosphere, respiration and basic metabolism of the components of the system generally reduce available  $O_2$  and increase  $CO_2$ . In the case of modified atmosphere,  $CO_2$ ,  $O_2$  and  $N_2$  are introduced as an atmosphere over the product at specific concentrations. This is generally a singular event of initial change and should take into consideration anticipated product requirements and subsequent commodity-modified changes. Modified atmosphere is not as dynamic as the commodity-modified atmosphere but is of obvious greater expense. Controlled atmosphere, in its most fundamental definition, is the precise constant maintenance of selected gases such as  $CO_2$ ,  $O_2$ , and  $N_2$  at specific pressures or under partial vacuum. The controlled atmosphere system, as defined in this fashion, is considerably more expensive than the other alternatives and is also technically demanding. This precise response to commodity activity results in a less dynamic system than the others that were described.

Airtight storage is an excellent example of commodity-modified atmosphere storage. It is dynamic and, if functioning as desired, would ideally demonstrate a reduction in  $O_2$  to 0.5-1.0% and if at all possible down to 0.2%  $O_2$ . Simultaneously, increases in  $CO_2$  up to 50% would be observed. Airtight storage is a method where the atmosphere is self-developed and dependent upon the indigenous activity of the organisms and grain system. Airtight storage has utilized a

variety of containers to accomplish appropriate changes in atmosphere, including the traditional pit, tank, bin, and silo (Hyde, 1974).

Atmosphere has a dramatic influence on metabolic activities of fungi. Toxin production by *Penicillium* or *Aspergillus* can be controlled by elevated CO<sub>2</sub> levels. Data presented in Table 7 show that penicillic acid production or aflatoxin production in laboratory media can be inhibited by specific atmospheres containing 60 or 90% CO<sub>2</sub>.

TABLE 7

Atmospheric inhibition of toxin production in laboratory medium.

%	<sup>1</sup> Penicillic Acid (at 20°C or 40°C)	<sup>2</sup> Aflatoxin (at 28°C)
O <sub>2</sub>	20	10
CO <sub>2</sub>	60	90
N <sub>2</sub>	20	-

<sup>1</sup> Lillehoj, Milburn, Ciegler, 1972

<sup>2</sup> Shih & Marth, 1973

The influence of atmosphere on growth or toxin production on grain is also well documented. Studies on corn or wheat have shown that reduced O<sub>2</sub> or increased CO<sub>2</sub> levels will result in the inhibition of growth or toxin production at optimum or suboptimum growth temperatures. (Table 8.)

TABLE 8

Modified atmosphere inhibition of growth or toxin production on grain.

%	<sup>1</sup> Corn	<sup>2</sup> Corn		<sup>3</sup> Corn		<sup>4</sup> Wheat
O <sub>2</sub>	0.5	0.3	0.5	20	20	0 - 4.5
CO <sub>2</sub>	13.5	0.0	13.5	40	60	0 0
N <sub>2</sub>	84.8	99.7	84.8	40	20	100 - 95.5

<sup>1</sup> Wilson & Jay, 1975 (27°C)

<sup>2</sup> Wilson, Huang & Jay, 1975 (27°C)

<sup>3</sup> Lillehoj, Milburn, Ciegler, 1972 (5° & 10° C)

<sup>4</sup> Shejbal, 1979 (22-32° C)

## INTERACTION

In most cases the reports in the literature or the specific observations made in the field have reported temperature or atmosphere or relative humidity ( $a_w$ ) effects on growth or toxin production by fungi on stored grain. Rarely has there been a concerted effort to evaluate the interactions amongst these various storage parameters. Bottomley, Christensen & Geddes (1950) conducted a comprehensive study on corn and, with complete statistical evaluation, determined that: the influence of temperature was statistically significant; the influence of atmosphere was statistically significant; and the influence of humidity was statistically highly significant. When they measured the interaction, they observed that the interaction of temperature and atmosphere was not statistically significant nor was the interaction of temperature and humidity. However, the interaction of atmosphere and humidity was statistically highly significant. Three-way interactions displayed no statistical significance.

The influence of the interaction of atmosphere and temperature on the production of penicillic acid is readily evident in Fig. 1 which has been adapted from Lillehoj, Milburn & Ciegler (1972). As the amount of  $\text{CO}_2$  in the atmosphere was increased, the production of penicillic acid at temperatures below optimum was reduced or totally inhibited. This figure dramatically demonstrates an atmosphere-temperature interaction.

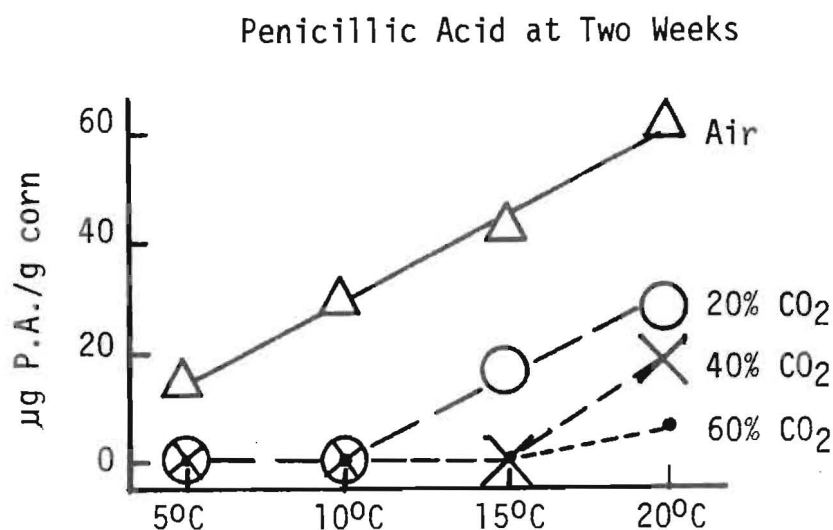


Fig. 1. Influence of temperature and atmosphere on penicillic acid production (Lillehoj, Milburn & Ciegler; 1972).

Interaction between temperature and  $a_w$  on growth or penicillic acid production was also evident in data reported by Northolt, Van Egmond, and Paulsch (1979). Reduction in  $a_w$  not only eliminated penicillic acid production at a variety of growth temperatures, but in general influenced growth directly as a dramatic interaction (Fig. 2).

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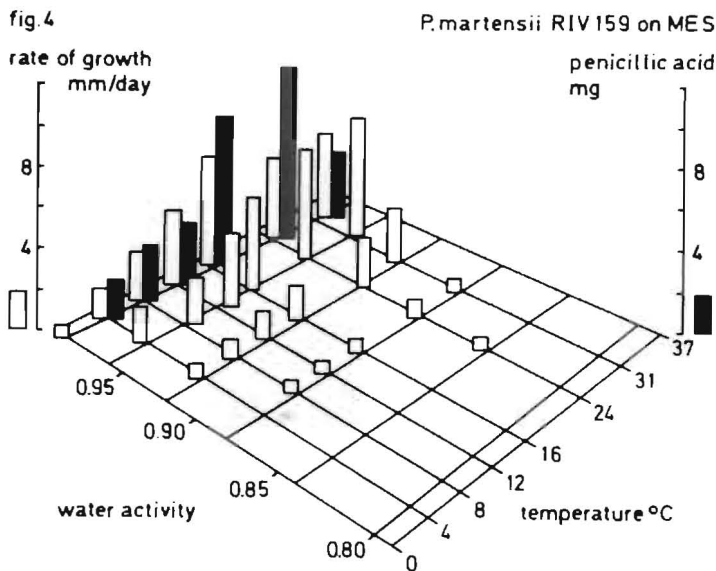


Fig. 2. Interaction of influence of water activity and temperature on growth and toxin production (Northolt, Van Egmond, and Paulsch; 1979).

The interaction of temperature and atmosphere on spore germination by *Penicillium martensii*, the same organism studied in the penicillic acid production, also has been documented by Lillehoj, Milburn, and Ciegler (1972). Key data are reproduced in Fig. 3. These data show that increased levels of  $\text{CO}_2$  dramatically narrow the range of temperatures at which spore germination was observed and reduce the mean percent germination as well.



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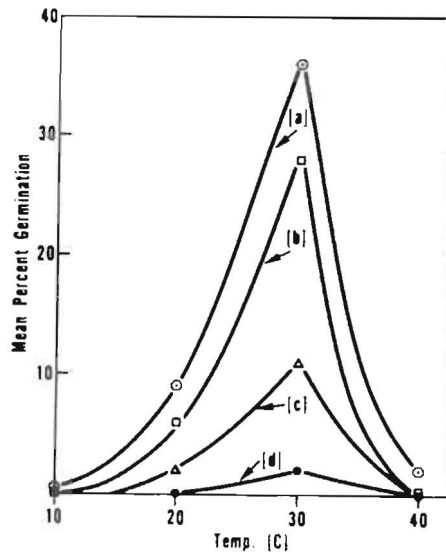


FIG. 2. Mean germination of *P. martensii* spores after 16 hr at 30°C. Gases employed: (a) air; (b) 20% CO<sub>2</sub>, 20% O<sub>2</sub>, 60% N<sub>2</sub>; (c) 40% CO<sub>2</sub>, 20% O<sub>2</sub>, 40% N<sub>2</sub>; (d) 60% CO<sub>2</sub>, 20% O<sub>2</sub>, 20% N<sub>2</sub>.

Fig. 3. Interaction of temperature and atmosphere on spore germination (Lillehoj, Milburn, and Ciegler; 1972).

It should not, therefore, be unexpected that one could observe under appropriate conditions, a three-way interaction among humidity, temperature, and atmosphere. Interrelating the data of Northolt et al. (1979) and Lillehoj et al. (1972) one should be able to speculate that increasing the CO<sub>2</sub> levels associated with *P. martensii* would have an overall interaction and antagonistic effect on the amount of growth, most likely narrowing the range of maximum and minimum temperatures at which growth would be observed, increasing the minimum  $a_w$  at which growth would occur, and reducing the overall amount of growth observed at any level of water activity. Using this combination of existing data as a stimulus, it seems readily evident that there is a need for optimization among these three-way interactions between humidity, temperature and atmosphere.

From the standpoint of the type of grain, the microbiology associated with that grain, the entomology, the energy consumption requirements, the product quality and the overall economics of the situation; an optimal interaction or several interactions could be developed that would lead to a practical, feasible, and effective storage procedure.

As an overview, we have attempted to analyze much of the information that has been available in the literature and to stimulate your imagination in projecting possible interactions from those data. We look forward to the following presentations on oxygen depletion by Pelhate; on the effects of nitrogen storage by Di Maggio; on wet grain storage by Richard-Molard, Cahaghier, and Poisson; and finally, the influence of nitrogen on moist wheat by Serafini, Fabbri, Shejbal, Fanelli, Di Maggio, and Rambelli which should prove enlightening and contribute considerably more data to stimulate technological progress.

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## OXYGEN DEPLETION AS A METHOD IN GRAIN STORAGE : MICROBIOLOGICAL BASIS

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### 1. INTRODUCTION

When compared with the bacteria of the same substrata - and namely grains- the fungi are named aerobes. Now this quality has constituted for nearly a century the biological basis of controlled atmosphere storage (Blanc, 1938 ; Oxley and Hyde, 1957 ; Poisson, 1969 ; Hyde, 1962 ... 1974 ; Masson, 1969). It is true that bacteria greatly influence microbial series in air-tight silos or depleted in oxygen ; yet more or less abundant yeasts generally accompany them and, sometimes also, some filamentous fungi. Therefore one must consider some fungi as facultative aerobes or micro-aerophiles (Bartnicki-Garcia and Nickerson, 1962, Gunner and Alexander, 1964 ; Stotzky and Goos, 1965 ; Trisvyatskii, 1966 ; Tabak and Cook, 1968a ; Curtis, 1969)...

The principle of air-tight storage has a biological basis : in an hermetic container, the metabolic processes of the very seed and the activity of the associated microorganisms reduce oxygen content in the end and accumulate carbon dioxide. Progressively the result of this is a condition first biostatic then inhibiting which could be lethal for spoilage agents and fungi above all (Milner and Geddes, 1945 ; Peterson et al. 1956)... It is then to determine the primary cause of the limiting action : anoxia or carbon dioxide supply ? Many authors have brought answers with shades of meaning in accordance with the diversity of the substrata and their associated flora (Brown, 1922 ; Golding, 1945 ; Geddes and al. 1955 ; Peterson and al. 1956 ; Follstad, 1966 ; Tuite and al. 1967 ; Escoula and Le Bars, 1973 ; Mitchell and Mitchell, 1973 ; Pelhate, 1975, 1976)...

The present work sets forth a few complementary aspects of our own investigations led from the laboratory test to the farm silo by associating the microorganisms behaviour as well as the change of the spontaneously contaminated grain bulk.

### 2. ANAEROBIC GROWTH OF GRAIN FUNGI IN VITRO

#### 2.1 Materials and methods

Some species said to be micro-aerophilic on silages (Pelhate, 1975, 1976, 1977) and classified in 3 ecological categories identified on grains (Pelhate, 1968a, 1979) have been submitted to culture in comparison with more cosmopolitan agents. Inoculated Petri dishes with malt agar media are set in an gas-tight container at 20 and 32°C for 10 days. Anoxia is carried out in 3 conditions :

TABLE 2.1

Growth rates of grain fungi in controlled atmospheres

(% of control in aerobic condition : mycelial growth of filamentous species, budding cells of yeasts)

Species	Atmosphere		Vacuum		CO <sub>2</sub> 10%		CO <sub>2</sub> 100%	
	Temperature (°C)		20	32	20	32	20	32
FIELD FUNGI								
<i>Alternaria tenuissima</i> (NEES ex FR.) WILT.	0	0	0	0	0	0	0	0
<i>Fusarium culmorum</i> (W.G. SMITH) SACC.	0	0	ε	1,2	0	ε	ε	ε
<i>F. poae</i> (PECK) WOLL.	ε	1,6	6,2	7,5	ε	ε	ε	ε
<i>F. sacchari</i> (BUTL.) W.GAMS var. <i>subglutinans</i> (WOLL. et REINK.) NIRENB.	ε	ε	3,6	4,2	ε	ε	ε	ε
INTERMEDIATE FUNGI								
<i>Byssoschlamys nivea</i> WESTL.	0	ε	12,5	16,3	7,5	9,5	9,5	9,5
<i>Cladosporium cladosporioides</i> (FR.) DE VRIES	0	0	0	0	0	0	0	0
<i>Geotrichum candidum</i> LINK	0	0	ε	2,5	ε	1,5	1,5	1,5
<i>Monascus purpureus</i> WENT	0	0	ε	1,2	ε	ε	ε	ε
<i>Mucor circinelloides</i> VAN TIEGH.	ε	ε	6,5	12,6	8,5	3,3	3,3	3,3
<i>f. griseo-cyanus</i> SCHIPPER	0	ε	1,3	4,2	ε	ε	ε	ε
<i>M. racemosus</i> FR.	0	0	2,2	5,3	1,6	4,3	4,3	4,3
<i>Paecilomyces variotii</i> BAINIER	0	0	0	ε	0	ε	ε	ε
<i>Trichoderma koningii</i> Oud.	0	0	0	ε	0	ε	ε	ε
<i>Candida krusei</i> (CAST.) BERKH.	55	40	70	80	25	45	45	45
<i>Hansenula anomala</i> (HANSEN) H. and P. SYDOW	95	90	100	120	75	80	80	80
STORAGE FUNGI								
<i>Aspergillus candidus</i> LINK	0	0	0	ε	0	0	0	0
<i>A. fumigatus</i> FRES.	0	0	0	ε	0	ε	ε	ε
<i>Eurotium herbariorum</i> MALL. and CAIN	0	0	0	ε	0	0	0	0
<i>Penicillium cyclopium</i> WEST.	0	0	0	ε	0	0	0	0
<i>P. roqueforti</i> THOM	0	0	1,6	1,5	0	ε	ε	ε
<i>P. stoloniferum</i> THOM	0	0	0	0	0	0	0	0

-deep vacuum maintained by daily pumping (for a minimal instantaneous pressure of 1 mm mercury)

-carbon dioxide content of 10 % (vol.) in adequate jars ("Gaspak")

-carbon dioxide content of 100 % (vol.) obtained after deep vacuum.

Anoxy tolerance is tested through the radial growth of filamentous fungi or the cell count of yeasts (Pelhate, 1978a).

## 2.2 Results and discussion

The results set forth in table 2.1 show the high fungi inhibition (yeasts excepted) by anoxia. Thus some species grow in no test condition ; they must be considered as obligatory aerobes in comparison with facultative anaerobic yeasts (Tabak and Cooke, 1968a ; Trisvyatskii, 1966). Others poorly grow in deep vacuum (*F. poae* and *F. sacchari*, *M. circinelloides*) and, sometimes, only at higher temperature (*B. nivea* and *M. racemosus*) ; we consider them as micro-aerophiles (Pelhate, 1975, 1978a).

Besides the carbon dioxide incidence in the anoxic condition brings a precision to this classification of species according to two apparently contradictory effects : carbon dioxide enhances the inhibition at the higher content (*F. poae*) or it decreases it at the lower one (*Fusarium* spp., *G. candidum*, *M. racemosus* and *P. roqueforti*) and sometimes even at the higher rate (*B. nivea*, *M. circinelloides* and *P. variotii*). These data are in accordance with previous observations relative to mycoflora of diverse food-stuffs (Bottomley and al. 1950 ; Burmeister and al. 1966 ; Escoula and Le Bars, 1973 ; Mitchell and Mitchell, 1973 ; Watt, 1973 ; Pelhate, 1975, 1976) or to isolated species : *B. nivea* (Yates and al. 1967), *F. sacchari* = *F. moniliiforme* (Tuite, 1961), *G. candidum* (Apelbaum and Barkai-Golan, 1977, *Mucor* sp. (Tuite and al. 1967), *T. koningii* (Walsh and Stewart, 1971) and various species (Golding, 1945 ; Milner and Golding, 1949 ; Durbin, 1955 ; Watt, 1973).

The eventual disparities in estimating specific tolerances can be explained by inevitable technical imperfections (vacuum degrees, gas purity...) or the interplay of manifold factors such as temperature, nature and weight of the substratum, complexity of the floristic series and the resulting specific interactions. The temperature acts directly on growth but also indirectly by diminishing carbon dioxide solubility in the substratum and consequently the inhibiting ability of the latter (Golding, 1945 ; Tuite and al. 1967).

The interplay of the ecological factors will be talked in the following chapter. In summation it appears that fungi are more sensitive to oxygen depletion than to carbon dioxide excess ; and when some species can grow well in anoxia, it is admitted that they utilize the most imperceptible oxygen traces included in the substratum (micro-aerophilic species) or that they modify their metabolism (facultative anaerobes those yeasts) through fermentary processes instead of respiratory ones namely for carbon dioxide (Bartnicki-Garcia and Nickerson, 1962 ; Parr and Smith, 1970) or nitrogen (Tabak and Cook, 1968b). This adaptation may entail original morphosis (Bartnicki-Garcia and Nickerson, 1962 ; Iralu, 1971 ; Escoula and Le Bars, 1973) or sometimes mutant types (Storck and Morill, 1971).

### 3. PRESERVATION TEST OF WET SOYBEAN IN CONTROLLED ATMOSPHERES

#### 3.1 Materials and methods

The very wet grains (moisture content of 30.5 % w.w) are distributed in hermetic flasks : these communicate with a vacuum apparatus and gas containers with carbon dioxide or nitrogen). The atmospheres differ from one another by their composition : confined air, partial vacuum, nitrogen, carbon dioxide and mixtures of these gases ; incubation temperatures were 5, 20 and 30°C. The behaviour of the grain was studied in reference to biological parameters : dynamics of the flora, viability of seeds, or technical ones : soluble proteins, acid number (Thériault, 1978 ; Pelhate and Thériault, 1979).

The present work retains only the first aspect. The microflora spontaneously associated to grains is followed in two ways : on one side, germ counts for bacteria and yeasts superficially grown, on the other, mycelial detection on or in kernels for filamentous fungi. In the first case the germs are suspended by simply steaming grains in sterile solution. The second method is justified by the fact that many species no longer sporulate in controlled atmospheres (Brown, 1922 ; Bartnicki-Garcia and Nickerson, 1962 ; Burmeister and al. 1966 ; Iralu, 1971) ; superficial disinfection of grains before their plating essentially allows detection of internal flora in comparison with total flora.

All these methods have been previously described (Pelhate, 1968a, 1970, 1979).

### 3.2 Results and discussion

In every test condition growth of bacteria and yeasts is obviously fore-most (See Fig.3.1) while the growth of molds is more or less limited (See Fig. 3.2)

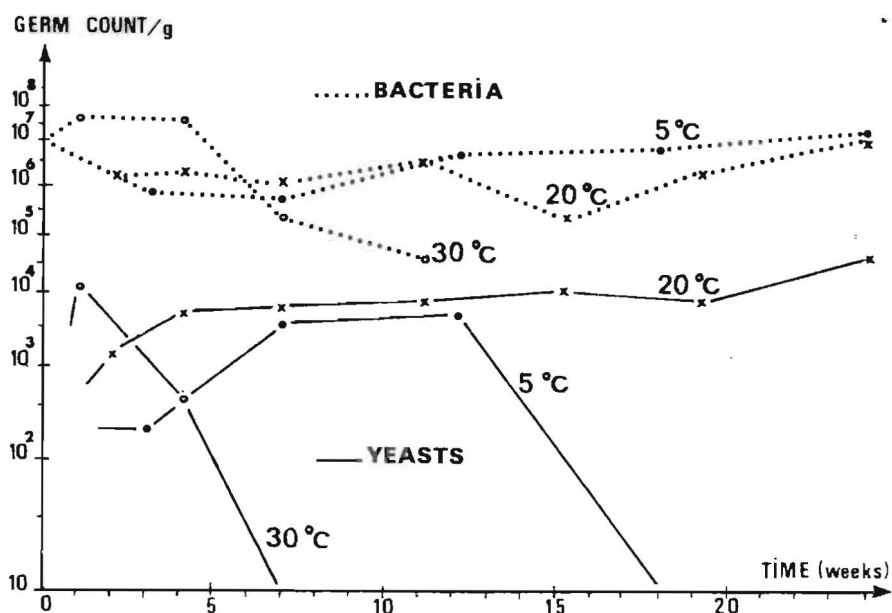


Fig. 3.1. Bacteria and yeasts dynamics on soybean stored in air-tight flasks at 5, 20 and 30°C.

in accordance with the observations of chapter 2. At determined temperature, selective inhibition is more or less identical in spite of the initial diversified test condition (atmosphere composition) ; that can be explained by high grain moisture which triggers off active metabolism of grain-flora complex so that resulting anoxia and carbon dioxide accumulation (See Fig. 3.3) rapidly make ecological environment uniform (See Fig. 3.4).

Hence temperature seems the most prevalent factor in determining of grain behaviour and above all of flora dynamics. This thermal effect is shown according to two trends : on one side, increase of all biological processes (along with



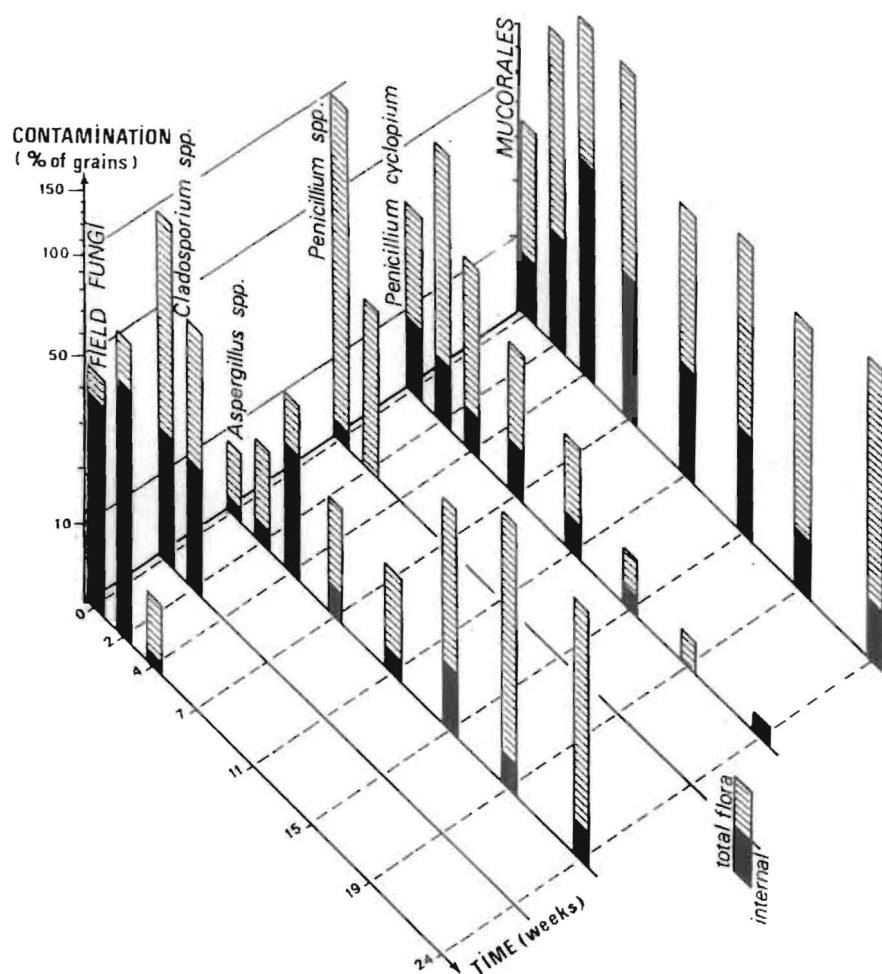


Fig. 3.2. Selective inhibition and dynamics of molds of soybean stored in confined air at 20°C.

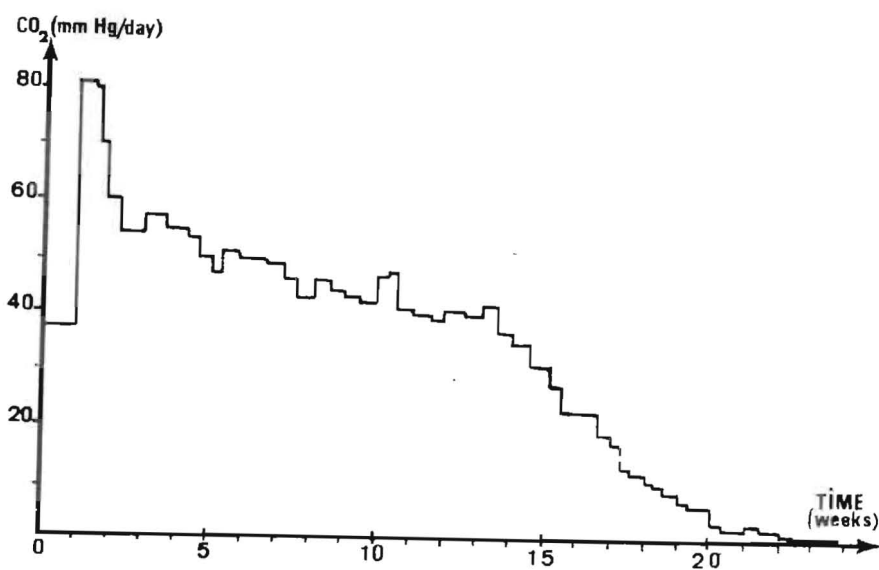


Fig. 3.3. Carbon dioxide production from soybean stored in partial vacuum at 20°C.

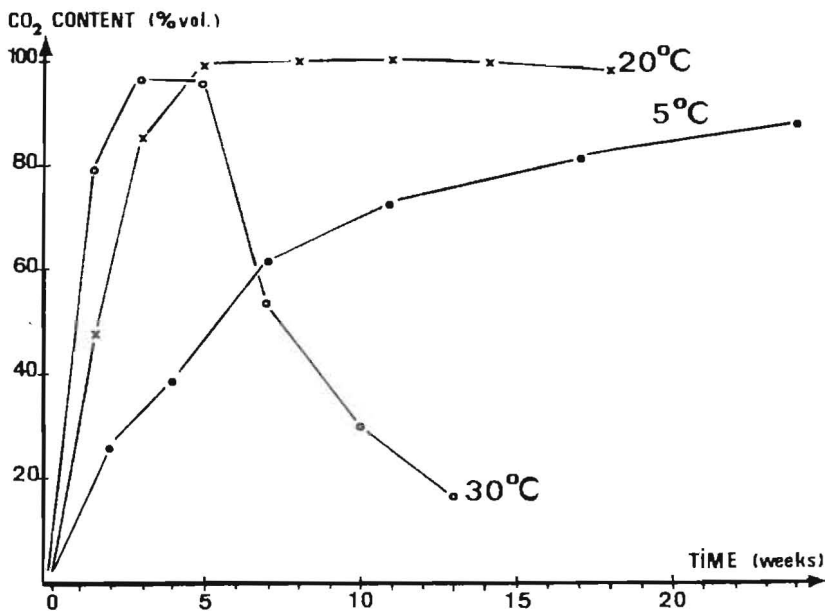


Fig. 3.4. Changes in carbon dioxide content of confined air at 3 temperatures.

respiration of seeds, microorganisms growth) and on the other, determinism of floristic dynamics to the benefit of the thermotolerant and competitive species. Figure 3.5 evinces this succession controlled by the interplay of various ecological factors and particularly temperature :

- at 5°C, all processes are slowed down and namely inhibition of strictly aerobes such as *A. tenuissima* ; limited growth of bacteria and yeasts - the usual characteristics - allows the selective growth of *Penicillium* spp. and *Mucorales* (namely *M. hiemalis*) which will coexist. At the end of storage, *Mucorales* tolerate better an excess of carbon dioxide like *Aspergillus* spp. which survive as traces without growth.
- at 20°C, the decrease of aerobic species is accelerated ; competition between 3 entities, i.e., in addition to bacteria and yeasts, *Aspergillus* spp., *Penicillium* spp. and *Mucorales* ends to the benefit of *Aspergillus* spp. (the most tolerant species to carbon dioxide)
- at 30°C, the advantage is obviously taken to *Aspergillus* spp. (among which *A. repens* = *Eurotium herbariorum*) and *Mucorales* (with *M. circinelloides* f. *griseocyanus*) because of their tolerance respectively to high temperature and carbon dioxide excess.

We can conclude by referring to the complexity of microorganism population dynamics and that of determinism of the spoiling of spontaneously polluted grains stored in air-tight conditions (Carter and Young, 1945 ; Bottomley and al. 1955 ; Tuite and al. 1967 ; Clarke and Hill, 1971). Yet selective inhibition and resultant simplification of microorganism series in conformity with their differential tolerance to oxygen depletion or carbon dioxide excess suggest a promising way of research. Grains always reveal from the harvest a noticeable pollution

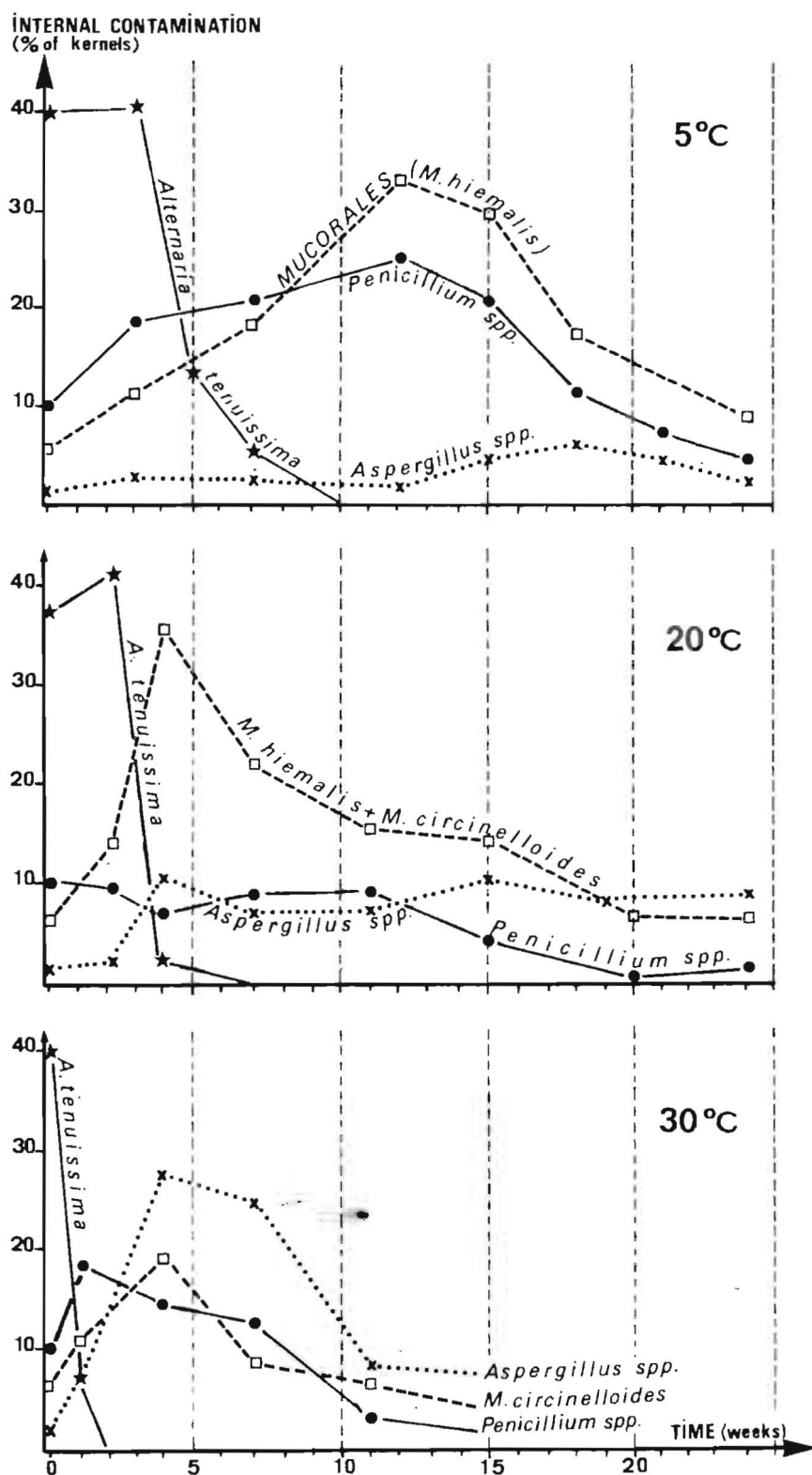


Fig. 3.5. Specific contamination of soybean in air-tight storage at 5, 20 and 30°C.

caused by various agents classified into distinct ecological types (Pelhate, 1968a, 1979) which have an adverse effect or none according to storage conditions (Pelhate, 1967, 1968b). Now wet grains are particularly susceptible to microorganisms and require adequate techniques.

Thus, in the controlled atmosphere methods some arrangements are required : improvement of structures (Mayo and Mc Neal, 1974), decrease of moisture content (Hyde, 1962...) and relatively low temperature maintained to limit biological activities. But in this case, will spontaneous anaerobiosis with its double aspect of oxygen depletion and carbon dioxide accumulation remain efficient enough to avoid the food-stuff decay ? Reducing initial intergranular air and consequently oxygen in the silo is recommended (Glass and al. 1959 ; Tabak and Cooke, 1968b ; Shejbal and al. 1973) ; the silage of wet or rehumidified crushed grain, technique more accessible to the pratician seems valid, at least in an autoconsumption system (Pelhate, 1976, 1978b).

#### 4. PRACTICAL METHOD OF WET CRUSHED GRAIN SILAGE

##### 4.1 Materials and methods

Two types of grains are suited to the silage method : corn (maize) with always a high moisture content at harvest time (up to 35 % w.w. and more), wheat too wet when harvested for safety condition (content moisture more than 15.5 % w.w). To avoid a complementary drying process it is now suggested to realize silage of grain as fodder but after crushing them and if needs be rehumidifying them.

##### 4.2 Results and discussion

###### 4.2.1 Corn

Silage behaviour is dependent on the floristic succession set forth on Fig. 4.1. In peripheral parts of the bulk unavoidable air traces allow abundant growth of mixed specied and consequently a quick and advanced deterioration. In the bulk itself, it is true, high moisture entails an early invasion of anaerobic bacteria (*Lactobacilli*) ; this heating and acidification stage is followed by that of yeasts. Bacteria are only in competition with hygrophilic and carbon dioxide tolerant fungi (*Aspergillus fumigatus* and *Mucorales*). Sometimes thermotolerant *Monascus purpureus* is in competition with yeasts after the heating stage. At the end of the process, *Byssoschlamys nivea* whose performances are remarkable (See chapter 2) can be associated to *Mucorales* ; however fact to a weak aeration caused by opening the silo for consumption *Penicillium roqueforti*, a very common species, generally appears and masters the whole flora ; *Trichoderma* spp. and *Paecilomyces variotii* less common, rarely appear at the final stage.

###### 4.2.2 Wheat

First attempts of wheat silage along the same line were failures as Fig. 4.2

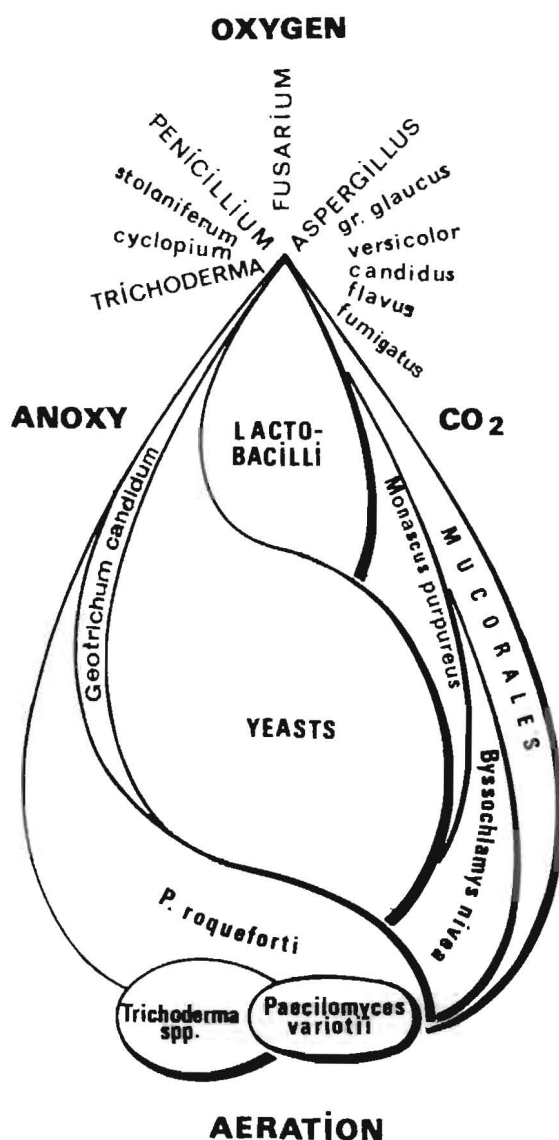


Fig. 4.1. Diagram of microflora dynamics on wet crushed corn silage (35% w.w. average moisture content).

reveals. Three stages can be distinguished in the floristic succession on damp grain principally regulated by specific needs of water (Pelhate, 1968c, 1978c) and water activity of the substratum (See Fig. 4.3).

- In the first stage, xerophilic and primary species as *Aspergillus* (= *Eurotium*) *glaucus* group can grow.
- In the second, according to progressive moisture and temperature gradients, several species actively grow and are in competition with yeasts which are limited to traces. These may be *Aspergillus versicolor* or *A. candidus* then *Penicillium cyclopium*, rarely *P. stoloniferum*.
- In the third, micro-aerophilic and CO<sub>2</sub> tolerant species can grow as among which rarely *A. fumigatus*, sometimes *B. nivea*, typically *P. roqueforti* accompa-

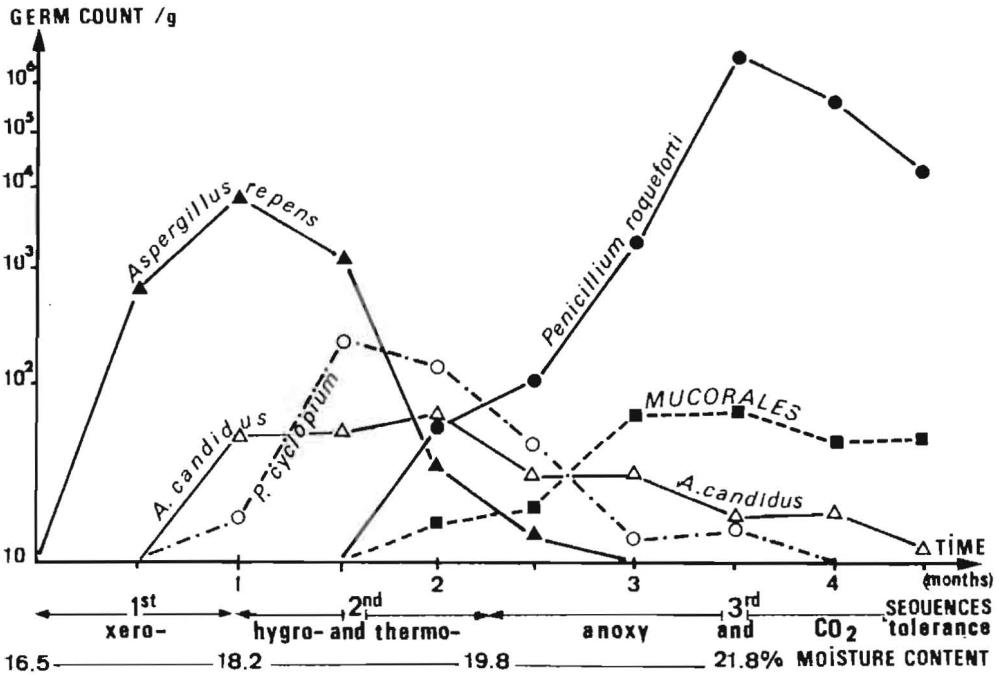


Fig. 4.2. Mold dynamics on damp crushed wheat silage (initial moisture content : 16.5 % w.w.)

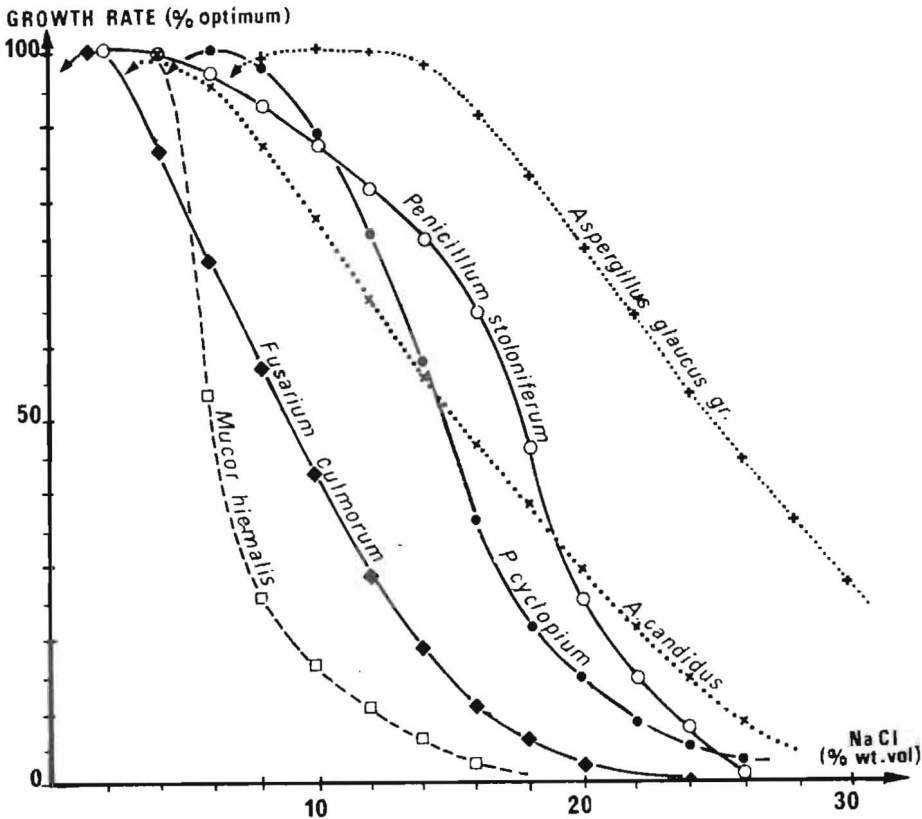


Fig. 4.3. Comparative mold growths in xerophilic condition. Water activity gradient is regulated by NaCl addition to malt agar media.

nied with Mucorales (*Mucor hiemalis*, *M. racemosus*, *M. circinelloides*).

It is therefore important for the two first stages to be avoided so that yeasts and bacteria may develop enough to inhibit spoiler molds. To that beneficial effect we recommend to rehumidify the grain up to 25 % w.w. at least at the time of crushing. It is also advised to store freshly harvested grain devoid of xerophilic storage fungi (Tuite and Christensen, 1957 ; Qasem and Christensen, 1958 ; Pelhate, 1968d). Besides it is important to improve air-tightness of structure to avoid peripheral decay (Nichols and Leaver, 1966).

The dominance of facultative anaerobic yeasts will then ensure a sufficient quality of the food-stuff meant for livestock. It has been established that wet or rehumidified grain along those lines has a better biological value (Mordenti and Zaghini, 1977).

Indeed one must not deny the fact that the food-stuff fresh from the silo had little stability (Mayo and Mc Neal, 1974 ; O'leary, 1978) ; but we say again that the method is devised on farm scale and in autoconsumption system.

## 5. CONCLUSION

In spite of the promising of anaerobiosis principle, practical realizations remain subject to technical difficulties such as more expensive structures and to biological incertainties i.e. pollution hazard, complex dynamics of spoiler microflora.

Yet the method brings actual advantages inherent in changes in biological equilibrium and metabolic processes ; for instance some moulds growing in even partial anoxia environment become unable to produce toxins (Mosely and al. 1971 ; Shih and Marth, 1973 ; Lillehoj and al. 1972 ; Wilson and Jay, 1975). Again air-tight storage of wet grain safeguards technological and biological quality without addition of pesticides and avoids risks of chemical toxicity.

Besides, insects often responsible for primary decay are easily eliminated (Oxley and Wickenden, 1963); in that line, the air-tight storage is justified even for dry grains.

Doubtless the method would have a greater impact if the aims are more clearly defined beforehand namely storage duration, grain utilization.

Last but not least, strictly economic considerations may determine the choice of two alternative methods i.e. complementary drying up or silage in the presence of damp grain.

## SUMMARY

The oxygen content of the intergranular spaces in bulk grain has a considerable effect on the state of the microorganisms since the great majority of these are aerobes (except yeasts, a few moulds and bacterial varieties).

First in vitro experiment with controlled atmosphere, in sealed containers, pointed out that progressive anoxia is most operative condition whereas carbon dioxide in various contents can reduce or enhance the suppressive effect of



oxygen deficiency.

Second assays were carried out with soybean stored in various controlled atmospheres (air-tight condition, partial vacuum or supplied carbon dioxide...) as suitable methods for an oxygen-free condition. Specific and selective action of these modified atmospheres resulted in original and restricted series among the spontaneous microflora ; dynamics of these are obviously dependent on other ecological factors.

According to such a complementary pattern, a practical method of grain storage is suggested as the easiest and cheapest on the farm scale : damp corn or rehumidified wheat grains are crushed before storing into compressed batches.

Key words : *anaerobiosis* ; *anoxo* ; *carbon dioxide* ; *controlled atmospheres* ; *grain storage fungi* ; *moldy grain* ; *wet grain storage*.

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## EFFECT OF NITROGEN STORAGE ON THE FUNGAL CONTAMINATION OF CEREAL GRAINS

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## ABSTRACT

The mycological contamination of soft and hard wheat, malting barley, rice, maize and sunflower seeds at various temperatures and moisture contents, experimentally stored in nitrogen, was studied.

Microbiological analysis showed that the stored grains are preserved as well in anoxia as in air at low moisture contents, not permitting fungal growth. In nitrogen the initial equilibrium of microorganisms is maintained more stable than in aerated grains.

At moisture contents permitting fungal but not yet bacterial growth, the proliferation of moulds in air was extremely high and within 3-4 weeks fungi could be observed macroscopically on the seeds. In nitrogen the development of fungi could be inhibited for several months, depending on the initial microbiological contamination of the grains and the oxygen impurities of the nitrogen used for the substitution of the interstitial atmosphere. The effect of nitrogen storage was shown to influence both mould counts and the selection of some genera of fungi.

## INTRODUCTION

Food preservation research, in particular grain storage, demonstrates that fungi are a major cause of spoilage in stored grains. That fungi recently are recognized as a cause in the seeds of: discoloration, heating, biological decay (as decreasing germination percentage, increase of fatty acids, alteration of sugars, and diminution in weight), and production of mycotoxins (Christensen and Lopez, 1963; Christensen and Kaufmann, 1969; Roberts, 1972; Sinha and Muir, 1973).

From the standpoint of their ecology and the moisture content requirements these fungi can be divided into two groups: field fungi

and storage fungi.

The field fungi invade seeds developing on the plants in the field, or after the seeds have matured and plants are still standing or are cut and swathed. All field fungi require a high moisture content in order to grow, a moisture content in equilibrium with relative humidities of 90-100%.

The storage fungi comprise several species of the genus Aspergillus and Penicillia, they are the major ones involved in the deterioration of stored grains. They have the ability to grow in materials the moisture contents of which are in equilibrium with relative humidities of 70-90%. All of these fungi invade the germ or embryo of the seeds preferentially.

In seed storage the basic requirements and factors influencing all fungal growth are a favourable humidity, temperature, a suitable atmosphere, the length of storage and the condition of the grain, amount of cracked and broken seeds, the nature and distribution of the debris, whether the embryos are alive or dead, the presence, numbers and activities of insects and mites (Christensen and Hodson, 1960; Christensen, 1964; Ayerst, 1969).

We have done intensive research on grain storage, especially under several moisture contents of seeds and different atmospheres. In this paper we report the mycological results of several experiments on grain storage, in air or under nitrogen. The species of seeds studied were soft and durum wheat, paddy, maize, malting barley and sunflower seeds at various moisture contents.

#### MATERIALS AND METHODS

The purpose of mycological examination on the seed is to give enough information about identification of common genera of moulds and the number count of the colonies. The fundamental methods for the mycological analysis of seeds are:

- 1) Samples taken by sterilized steel sampling devices.
- 2) External inspection of seeds under a binocular microscope.
- 3) Total mould counts, obtained by plating an inoculum from an extract of 10 g of grain in 100 ml physiological solution containing an antibiotic, in order to exclude bacterial growth. The values are reported

as number of germs/gramme dry matter (Di Maggio et al., 1976).

4) The internal fungal flora determined by surface - disinfecting 10 g aliquots of unbroken kernels by NaOCl and subsequent aseptical plating of the seeds; the values expressed as percent of the kernels from which fungi grew. For the two mycological examinations nutritive medium used: Mycological Agar (pH = 7 Difco).

5) Isolation and microscopic identification of the common genera of moulds and of some species.

## RESULTS AND DISCUSSION

In this communication we report the mycological results of several typical storage studies on nitrogen atmosphere preservation of grain in laboratory and in pilot-scale storage bins at different moisture contents.

The first experiment was on 300 kg-aliquots of 12% moisture content soft wheat, variety "Conte Marzotto", preserved in silos for 4 + 5 years in air and technical nitrogen ( $< 0.3\%$  oxygen). During this storage the fungi progressively decreased. These results were confirmed by one year preservation periods carried out with soft wheat at 14.5% and with durum wheat at 13% moisture contents both in laboratory silos and in 250 q-lots at ambient temperature (fig. 1).

Twenty three tons of malting barley, national production, 12% moisture content, were preserved for one year in technical nitrogen; total mould counts decreased although some storage fungi (*Penicillia*) appeared towards the end of the trial and internal moulds were frequent in the kernels even at the start of the experiment.

Other results on paddy, maize and sunflower seeds, at moisture contents up to 14.5%, confirmed that there is no difference between storage in air or in nitrogen (Table 1-2).

Storage problems are evident at high moisture contents, higher than 15%, because at the relative humidity in equilibrium with such moisture, moulding and heating of kernels increase.

At the moisture, between 15 and 18%, the beneficial effect of storage in nitrogen is evident also in short periods of time since mould attack is the main cause of fast deterioration.

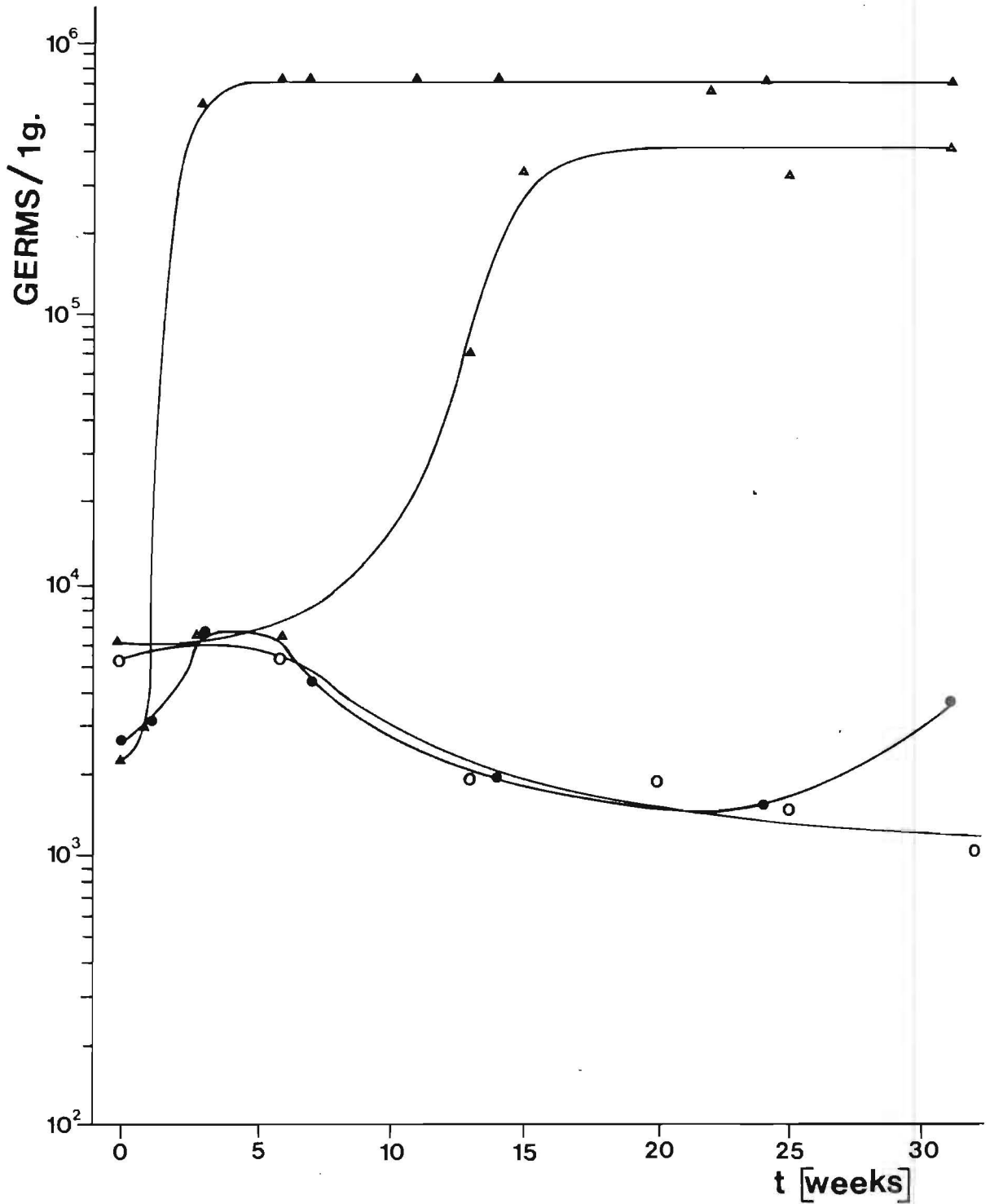


Fig. 1. Evolution of total mould counts in wheat at 14.5% (○-●) and 17% moisture contents (▲-▲), preserved in air (●-▲) and nitrogen (○-▲).



TABLE 1

Mould counts, expressed as germs per g dry matter, of low moisture grains (11%-13%) preserved in air and technical nitrogen.

Grain	Gas	Storage period (weeks)					
		0	5	16	28	40	50
Wheat	N <sub>2</sub>	10 <sup>3</sup>	10 <sup>2</sup>				10 <sup>2</sup>
	Air	6.10 <sup>3</sup>	3.10 <sup>3</sup>				3.10 <sup>3</sup>
Paddy	N <sub>2</sub>	3.10 <sup>4</sup>		5.10 <sup>3</sup>	10 <sup>3</sup>	2.10 <sup>3</sup>	
	Air	2.10 <sup>4</sup>		4.10 <sup>3</sup>	8.10 <sup>3</sup>	10 <sup>4</sup>	
	N <sub>2</sub>	4.10 <sup>3</sup>	9.10 <sup>2</sup>	5.10 <sup>2</sup>	2.10 <sup>2</sup>	5.10 <sup>2</sup>	
	Air	4.10 <sup>3</sup>	10 <sup>3</sup>	3.10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>3</sup>	
Maize	N <sub>2</sub>	6.10 <sup>3</sup>	9.10 <sup>2</sup>	3.10 <sup>2</sup>	5.10 <sup>2</sup>	2.10 <sup>2</sup>	
	Air	6.10 <sup>3</sup>	1.10 <sup>3</sup>	1.10 <sup>3</sup>	2.10 <sup>3</sup>	1.10 <sup>3</sup>	

TABLE 2

Mould counts, expressed as germs per g dry matter, of low moisture content (6%) sunflower seeds stored in air and  $N_2$  at 30°C.

	Storage period (weeks)							
	0	9	11	17	19	21	36	45
Air	$1.10^5$		$7.10^4$		$3.10^4$	$2.10^4$	$2.10^4$	$3.10^3$
$N_2$	$1.10^5$	$8.10^4$		$2.10^4$		$4.10^4$	$3.10^4$	$2.10^3$

In technical nitrogen, containing up to 0.3% oxygen, moulds develop slower than in air but are not inhibited (fig. 1). The better storability of the grains in technical nitrogen is evident especially over long periods of time, but after the lag-phase of fungal development, deterioration cannot be avoided at these residual oxygen concentrations.

In pure nitrogen (less than 0.01% oxygen) fungal proliferation was found to be completely inhibited and total germ counts tended to diminish in time (fig. 2), while internal moulds were very scarce (2-3%).

The same results were obtained for sunflower seeds with 9.5% moisture content, and for maize at 18.2% (Tables 3 and 4).

TABLE 3

Mould counts, expressed as germs per g dry matter, of high moisture (9.5%) sunflower seeds stored in air and  $N_2$  at 25°C.

	Storage period (weeks)			
	0	4	9	13
Air	$2.10^5$	$5.10^4$	$7.10^5$	$5.10^5$
$N_2$	$2.10^5$	$5.10^3$	$4.10^3$	$1.10^3$

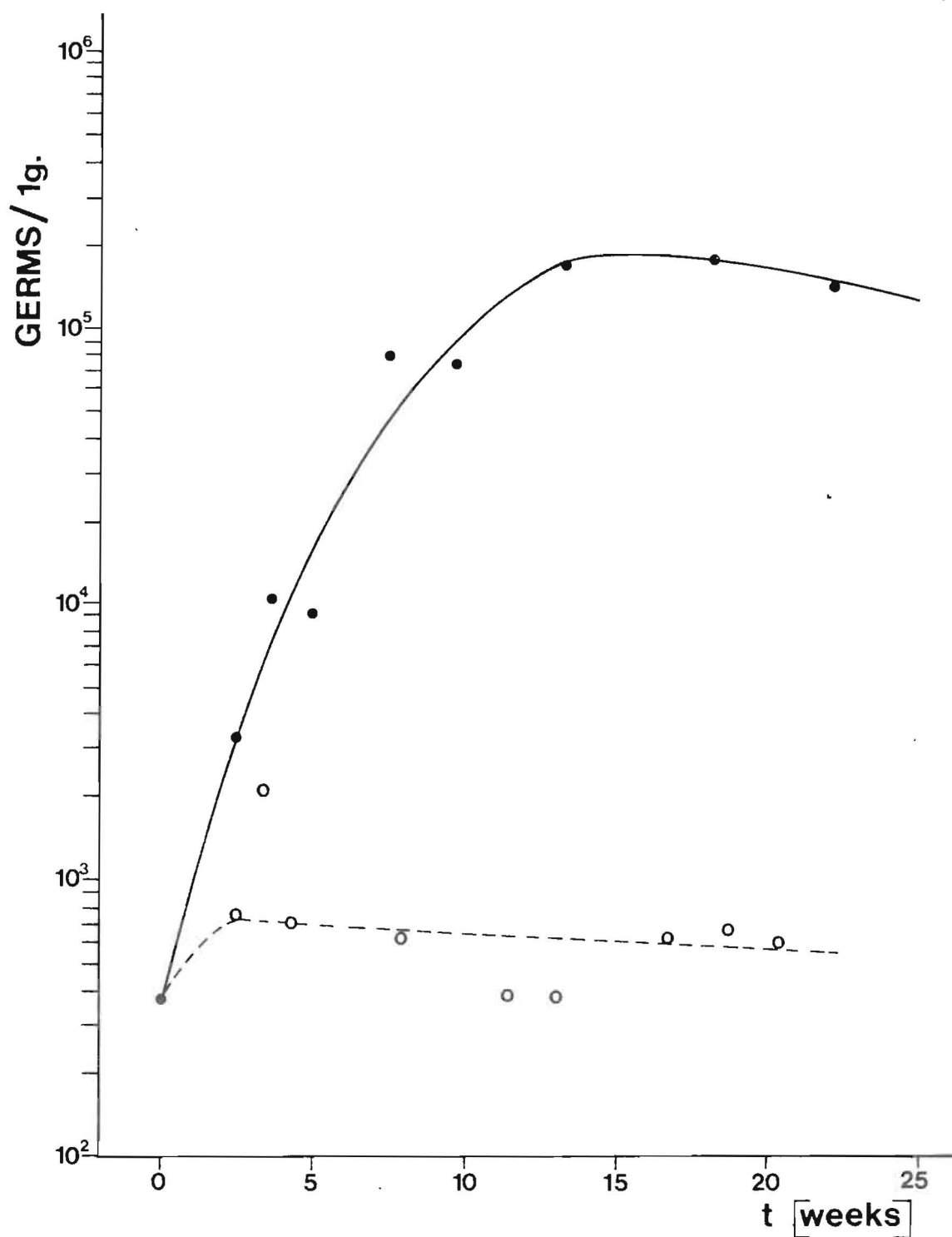


Fig. 2. Evolution of moulds in 18% moisture content wheat, stored in air (●) and pure nitrogen (○).

TABLE 4

Mould counts, expressed as germs per g dry matter, of high moisture (18.2%) maize stored in air and N<sub>2</sub>.

	Storage period (weeks)			
	0	4	11	18
Air	$4.10^2$	$1.10^4$	$1.10^5$	$8.10^5$
N <sub>2</sub>	$4.10^2$	$1.10^2$	$8.10^3$	$1.10^3$

These mycological results are in accordance with the loss of viability of seeds and technological quality, and are strictly correlated to the quality of the seeds at the start of storage.

As to the type of moulds present, the situation was as follows: field fungi (such as: Cladosporium and Alternaria) decreased during storage, while storage moulds (such as: Penicillium and Aspergillus) increased in all the batches. In air Aspergillus and Penicillium growth is faster.

The results of the laboratory and pilot-scale trials were confirmed for wheat and malting barley in two large scale storage trials: 500 t-silos for preservation of wheat and 1500 t for malting barley storage.

In conclusion there can be no doubt on the advantages presented by the storage of grains in nitrogen especially for high moisture content. It permits longer storability of grains with exclusion of temperature and moisture increase, exclusion of off-odours, retarded loss of viability and retarded loss of technological and nutritional quality.

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# INFLUENCE OF NITROGEN ON THE GROWTH OF SOME STORAGE FUNGI ON MOIST WHEAT.

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## ABSTRACT

The storage fungi assayed were: Aspergillus flavus, Aspergillus chevalieri, Penicillium cyclopium, and one strain of yeast. The strains showed different growth curves in air. Growth was tested on wheat sterilized by  $\gamma$ -rays and inoculated by the various strains. Incubation was carried out at 30°C with the cereal at 18 - 19% moisture content, under air and ultrapure nitrogen, in order to study the possible inhibitory effect of nitrogen on mycelial growth, penetration and aflatoxin production.

Susceptibility to oxygen deficiency decreased in the order: A.chevalieri, P.cyclopium, A.flavus, yeast. The inhibiting effect of anoxia was strictly limited to the period of nitrogen application. Growth curves of A.flavus on aerated wheat following the nitrogen treatment were identical to those observed in directly incubated grain. Aflatoxin production in nitrogen was inhibited in respect to the controls in air.

Finally, unsterilized wheat was moistened to 19%, incubated first in air and consecutively in nitrogen. The inhibitory effect of anoxia was confirmed in the overall counts of fungal germs, while it induced a selection of certain mould species.

## INTRODUCTION

The storage and the inalterability of foodstuffs, of seeds in

particular, represent one of the greatest problems of the world economy and development. The fungal microflora is one of the main causes of the food losses on a world scale as it produces discoloration of the seeds, decrease of germinability, loss of weight, fermentation and chemical transformation with possibly the production of mycotoxins.

Modified and controlled atmospheres are one of the most promising methods to prevent and limit the growth of insects, fungi and the production of mycotoxins in seeds stored in silos (Diener and Davis 1969; Epstein et al., 1970; Jay and Pearman, 1973; Bailey and Banks 1974; Wilson and Jay, 1975).

The research carried out on the possibility of storing moist cereals (moisture superior to 14.5%) in nitrogen atmosphere gave good results showing a clear inhibiting or delaying action of nitrogen on the growth of fungal microflora and maintaining moreover a good germinability of the seeds and a low fat acidity (Shejbal and Di Maggio, 1976; Di Maggio et al., 1976; Shejbal, 1978; Di Maggio and Shejbal, 1979).

Not many experiments as regards the problems of storage have been carried out to clarify the behaviour of single fungal species typical of moist stored seeds in conditions of total anoxia. We studied the behaviour of four fungal species (Aspergillus flavus, Aspergillus chevalieri, Penicillium cyclopium and Candida krusei) placed in favourable growth conditions both as regards the seed moisture and as regards the temperature (32°C) and in conditions of total lack of oxygen.

We have analyzed whether, in our experimental conditions, there is an inhibiting effect of nitrogen with a percentage of oxygen inferior to 0.01% on the growth of the assayed fungal species and whether there exists a difference in the efficiency of the treatment on various strains.

Particular attention was directed to A.flavus for its well known pathogenic action as an aflatoxin producer (Heathcote and Hibbert 1978). We have also carried out experiments concerning the duration of



the inhibiting effect of nitrogen after two different periods of exposure (30 and 60 days) of seeds infected with A.flavus and then brought back to air. At last we have examined the influence of nitrogen on the growth of A.flavus in connection with the production of aflatoxins.

#### MATERIALS AND METHODS

The assayed strains were: Aspergillus flavus (ATCC 22548), Aspergillus chevalieri (ATCC 24546), Penicillium cyclopium (ATCC 26162) and Candida krusei isolated from wheat.

The first 3 strains were kept on Czapek-agar culture medium (Difco) at the temperature of 25°C and Candida krusei on Malt-agar (Difco) at the temperature of 25°C. The soft wheat (Manitoba variety) utilized for experiments was sterilized by exposure to  $^{90}\text{Co}$  for 90 min at 6926  $\text{rad min}^{-1}$  using a Gammacell 220 (Atomic Energy Ltd of Canada) (Di Maggio et al., 1974). After sterilization seeds were moistened with sterile distilled water to a value of 18.5% moisture content measured with a thermobalance (Buhler) and were inoculated with  $8 \times 10^6$  conidia of A.flavus, A.chevalieri, P.cyclopium and  $10^5$  of C.krusei.

The seeds were then placed in 250 ml cylindrical glass jars inside which a slow flow of nitrogen (with a percentage of oxygen inferior to 0.01%) from the top to the bottom was maintained. The gas flowed from a cylinder of nitrogen through sterilizing filters, pressure regulators and micrometer valves connected to flowmeters. The gas flow was about 100 ml per day.

In a different set of jars, the nitrogen flow was replaced with an air flow.

The jars were incubated at 32°C in a thermostated incubator, itself containing a nitrogen atmosphere.

The detection of fungal growth was made by the dilution plate method: 10g of ground seeds (by Stomaker) were placed directly in 100 ml of saline solution (NaCl 0.9%). The inoculum was made by plating on Mycological agar (Difco). Incubation was carried out at 25°C for 5 days.

The fungal penetration into the seeds was studied by superficial

sterilization with sodium hypochlorite. Ten grams of whole grains were put in HClO (C.Erba, tit.min. of Cl 8% + 2%) for two minutes. Five washings were then carried out in sterile distilled water. The grains so treated were put on Mycological agar and incubated in a thermostat at 25°C for 5 days.

The samples kept in different air and nitrogen atmospheres were analyzed as regards the production of aflatoxins. The samples were prepared for extraction and quantitative analysis by High Performance Liquid Chromatography (HPLC).

Each wheat sample was homogenized by using a Waring Blendor and extracted with chloroform:methanol (2:1 v/v) for 3 hours. The extracts were filtered through phase separative paper (Whatman, 1PS) and concentrated to 1 ml on a rotatory evaporator.

To purify the aflatoxins from the lipids present in the sample, two thin layer chromatography runs were made: the first with petroleum ether: n-hexane (75:25 v/v) and the second with n-hexane: ethyl-ether: acetic acid (70:30:1.5 v/v); all were made on layers of Stratocrom SIAP, C.Erba.

The aflatoxins do not migrate and are thus totally recovered without any alterations. They were concentrated in a fixed volume of HPLC elution solvent, methanol: distilled water (50:50 v/v) and the analyses were made according to Knutti et al., (1979).

## RESULTS

### Fungal growth in air.

Fig.1 shows the growth curves of the 4 fungal strains on wheat in air. The growth was tested at 4, 7, 10, 14, 18, 21 and 27 days. As can be noted, P.cyclopium is the strain that grows best after a very short adaptation period. A.flavus, A.chevalieri and C.krusei follow at decreasing growth rates.

In all cases a slight decrease of the number of germs can be noted between the inoculum and the first control which is explainable by the normal adaptation of the fungus to the environmental conditions as well as by lack of full viability of the inoculated conidia.

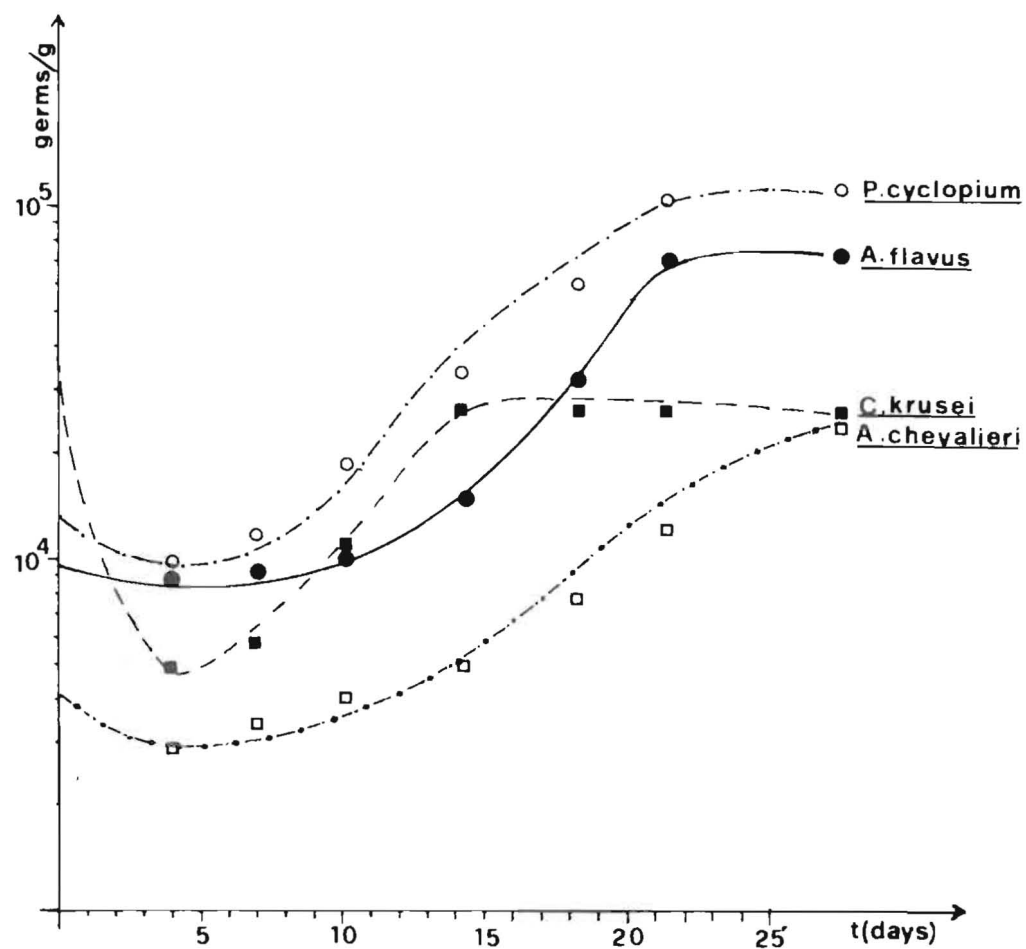


Fig.1. Growth curves on wheat seeds of 4 fungal strains in air.

Table 1 shows that the data relative to the number of germs are confirmed by those of the surface sterilization by which we have determined the fungal penetration inside the seeds.

The greater the growth of the fungus, the higher the percentage of the internally infected seeds.

The moisture content did not vary appreciably during the experiment.

#### Effect of nitrogen on fungal growth.

We studied the possible inhibiting effect of nitrogen on the 4 considered strains as regards fungal growth.

Fig.2 shows the inhibiting effect of nitrogen on the development of the strains after 7, 14 and 21 days. With A.flavus we carried out controls also at 60 and 70 days.

As can be seen, all the strains show a considerable decrease of the number of germs in conditions of complete anoxia, which is however different for the various strains.

P.cyclopium and A.chevalieri are the most sensitive strains to anoxic conditions, as they show at about 21 days an almost total inhibition.

Inhibition is lower for C.krusei and A.flavus. Actually, as can be seen, C.krusei is not inhibited until the 14th day, while it decreases significantly between the 21st and 27th day. This can be explained by the fact that it is possibly microaerophilic and therefore resists longer to conditions of total anoxia. Finally, though inhibited, A.flavus maintains quite high vitality up to 21 days in anoxia and only after 60 days its vitality ceases almost completely.

We wanted also to observe the effect of nitrogen at different stages of the growth curve of A.flavus in air. At 4, 7, 10, 14, 18 days we measured the effect of the exposure to nitrogen for 7 and 14 days. As shown in Fig.3, at 4, 7 and 10 days of growth (corresponding to the adaptation phase of the fungus) the inhibition produced by the nitrogen atmosphere appears only after 14 days and not yet after 7 days exposure to nitrogen.

On the contrary, in the log-growth phase (14th and 18th days of the growth curve) the effect of nitrogen appears already after 7 days of exposure, as the fungus is more sensitive to the conditions of

TABLE 1 Growth of four fungal strains on sterilized wheat in air.

Time (days)	A. flavus		P. cyclopium		A. chevalieri		C. krusei germs/g
	germs/g	infected seeds %	germs/g	infected seeds %	germs/g	infected seeds %	
0	$10 \times 10^3$	0	$14 \times 10^3$	0	$43 \times 10^2$	0	$40 \times 10^3$
4	$9 \times 10^3$	20	$10 \times 10^3$	7	$30 \times 10^2$	7	$5 \times 10^3$
7	$9.5 \times 10^3$	20	$12 \times 10^3$	25	$35 \times 10^2$	16	$7 \times 10^3$
10	$10.5 \times 10^3$	30	$19 \times 10^3$	30	$42 \times 10^2$	20	$10 \times 10^3$
14	$15 \times 10^3$	60	$34 \times 10^3$	44	$52 \times 10^2$	34	$27 \times 10^3$
18	$30 \times 10^3$	100	$60 \times 10^3$	88	$80 \times 10^2$	78	$26.7 \times 10^3$
21	$70 \times 10^3$	100	$102 \times 10^3$	100	$124 \times 10^2$	100	$26.4 \times 10^3$
27	$72 \times 10^3$	100	$114 \times 10^3$	100	$240 \times 10^2$	100	$26 \times 10^3$

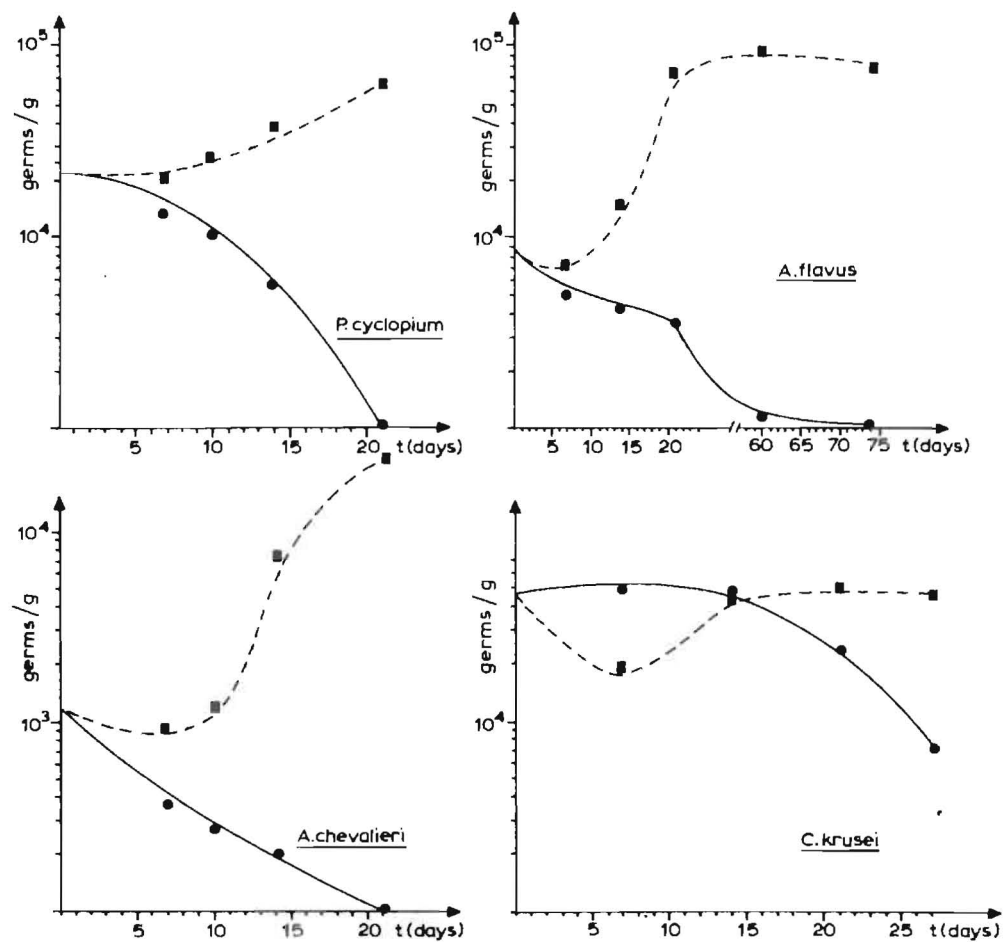


Fig.2. Growth curves on wheat of 4 fungal strains in air and nitrogen.  
(dotted line: growth curve in air, full line: growth curve in nitrogen)

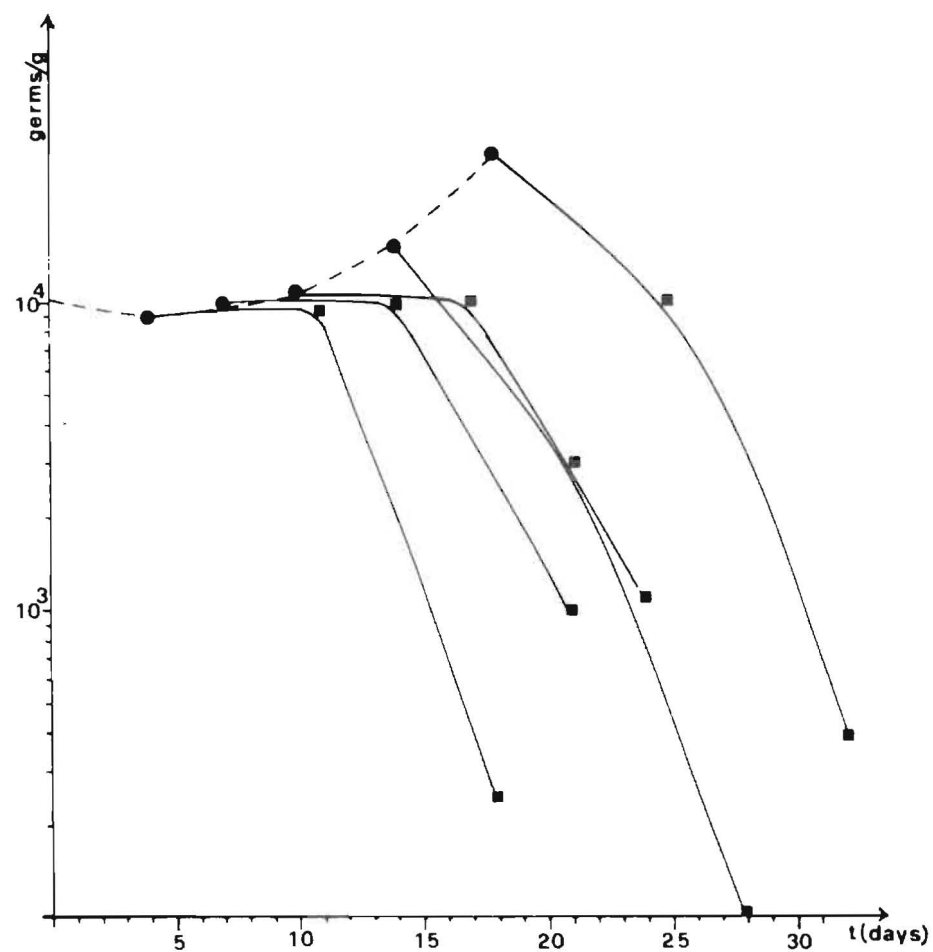


Fig.3. Effect of exposure to nitrogen for 7 and 14 days at different phases of the growth curve of A. flavus in air.



complete anoxia.

Further, we carried out experiments to measure the duration of the inhibiting effect after two different exposure periods to nitrogen on wheat seeds infected with A.flavus, bringing the samples back into an air flow.

The data in Table 2 show that, when the fungus is brought back into air after a 30-days exposure period to nitrogen, its growth appears to be immediately stimulated. After 60 days in nitrogen, the fungus grows more slowly when brought back into air. On the 7th day there is, in fact, a further decrease of the number of germs per gram, while on the 14th day there is a clear increase of fungal growth.

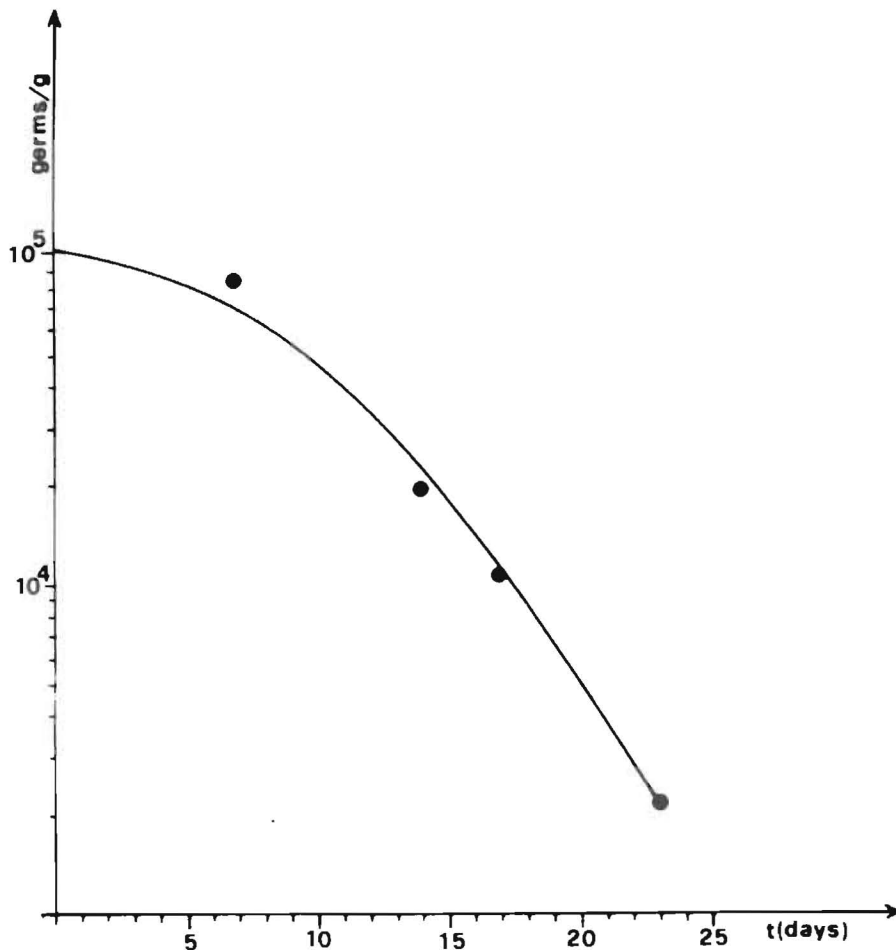
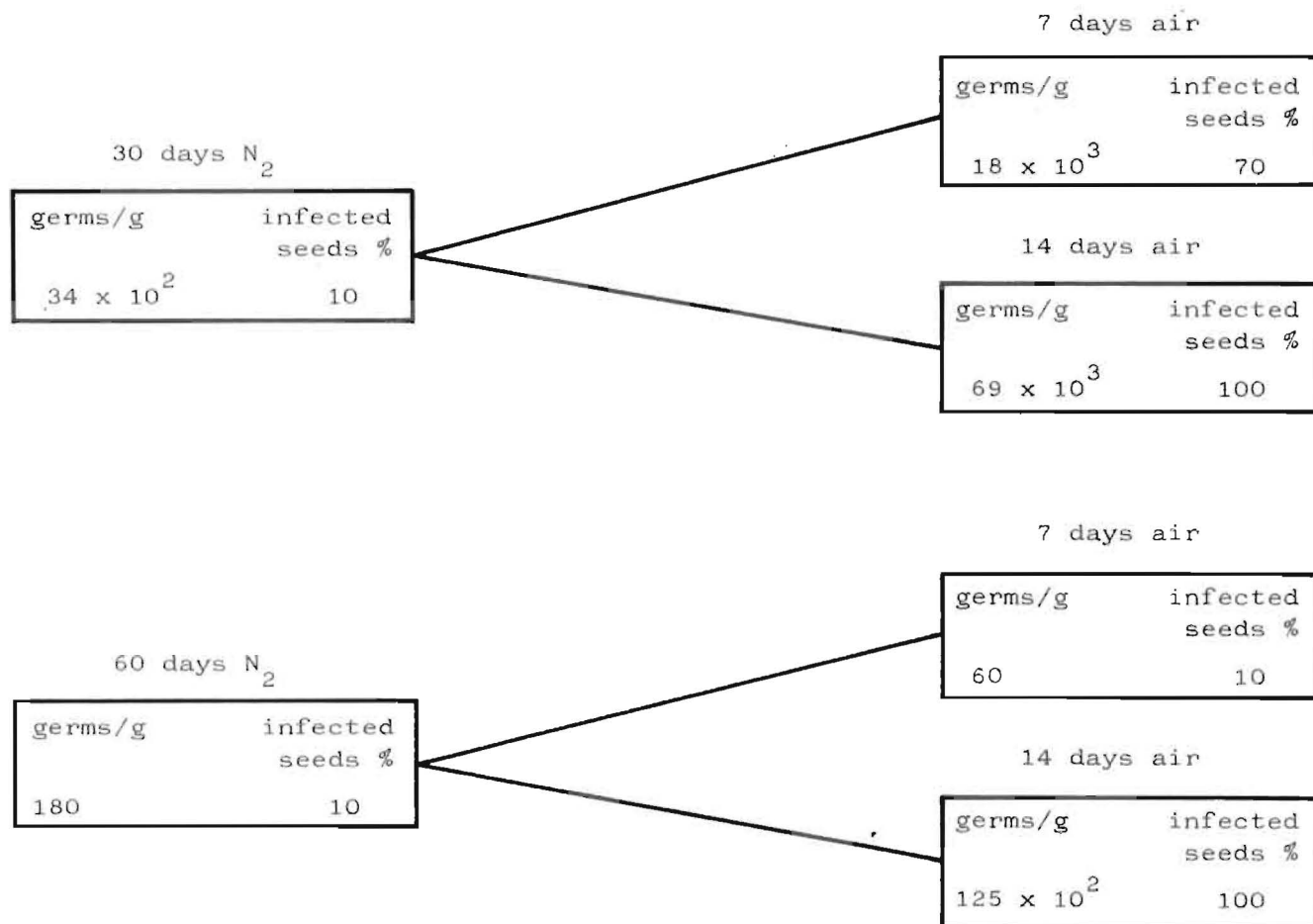


Fig.4. Effect of nitrogen atmosphere on non-sterilized wheat seeds microflora in time.

TABLE 2 Effect of two different exposure times in nitrogen on successive growth in air of A. flavus on wheat.



Finally we carried out an experiment on non-sterilized wheat seeds. Wheat at 13% moisture content, with  $13 \times 10^4$  germs/gram was used. The wheat was moistened to 19% and then kept in air for 10 days. Whereupon it was placed in a nitrogen flow for different periods of time (7, 14, 17, 23 days). The results (Fig.4) showed that nitrogen has an inhibiting effect on fungal growth. Moreover, among the fungal strains found in the seeds, the *Penicillia* and *Aspergilla* result the most sensitive to anoxia while sterile mycelia increase in time.

#### Effect of nitrogen on aflatoxin production.

The determination of aflatoxins produced by *A.flavus* inoculated on wheat seeds shows that the strain used in our experimental conditions produces only aflatoxin  $B_1$  and that the total lack of oxygen inhibits the production of aflatoxin.

Data in Table 3 show that, in our experimental conditions, there is a direct connection between fungal growth and the production of aflatoxin: in fact the production of aflatoxin  $B_1$  increases in connection with fungal growth.

TABLE 3 Growth of *A.flavus* in air and nitrogen on wheat and production of aflatoxin  $B_1$ .

Time (days)	germs/g		aflatoxin $B_1$ ( $\mu$ g/kg)	
	air	$N_2$	air	$N_2$
0	$10.5 \times 10^3$	$10.5 \times 10^3$	-	-
7	$10 \times 10^3$	$7.0 \times 10^3$	8.03	-
14	$18 \times 10^3$	$4.9 \times 10^3$	80.00	-
21	$71 \times 10^3$	$3.7 \times 10^3$	118.04	tr
27	$72 \times 10^3$	$3.5 \times 10^3$	120.00	-
34	$72.5 \times 10^3$	$3.3 \times 10^3$	119.00	-

At the first control (7 days) the number of germs was inferior to those inoculated as a consequence of an adaptation period of the fungus and the lack of viability of some of the inoculating conidia. The inoculated conidia did not contain aflatoxins which explains why the high number of germs present at the beginning of the experiment did not give rise to aflatoxin.

A further confirmation of the nitrogen inhibiting effect on the production of aflatoxin B<sub>1</sub> appears in Fig.5, in which samples were first kept in air for 10 days, with consequent production of aflatoxin B<sub>1</sub> and later submitted to complete anoxia for 7, 14 and 21 days with progressive decrease of aflatoxin B<sub>1</sub>.

The results obtained on the inhibiting effect of nitrogen atmosphere on the production of aflatoxins confirm those presented by other researchers on wheat seeds and peanuts (Wilson and Jay, 1975; Diener and Davis, 1969). On the contrary it was reported that the decrease in respiratory activities stimulates the production of aflatoxins (Shih and Marth, 1974).

#### CONCLUSION

On the basis of the results shown, we can conclude that the conditions of total anoxia prove to be an efficient means of limiting the growth of the tested fungal species growing in favourable conditions of moisture and temperature on stored seeds. This confirms the data of other researchers about the inhibiting effect of a nitrogen atmosphere with low oxygen content both on the field and storage microflora. The inhibiting effect appears to be efficient in a rather short storage period, of about one month, in our experiments.

Inhibition of aflatoxin production was clearly detected and a lowering of the level of the aflatoxin found in infected wheat seeds was observed. This can be a very important factor, considering the high toxicity of these substances produced by A.flavus.

#### ACKNOWLEDGEMENTS:

Thanks are due to Messrs.P. Catani, R. Cavaoli and M. Marini for

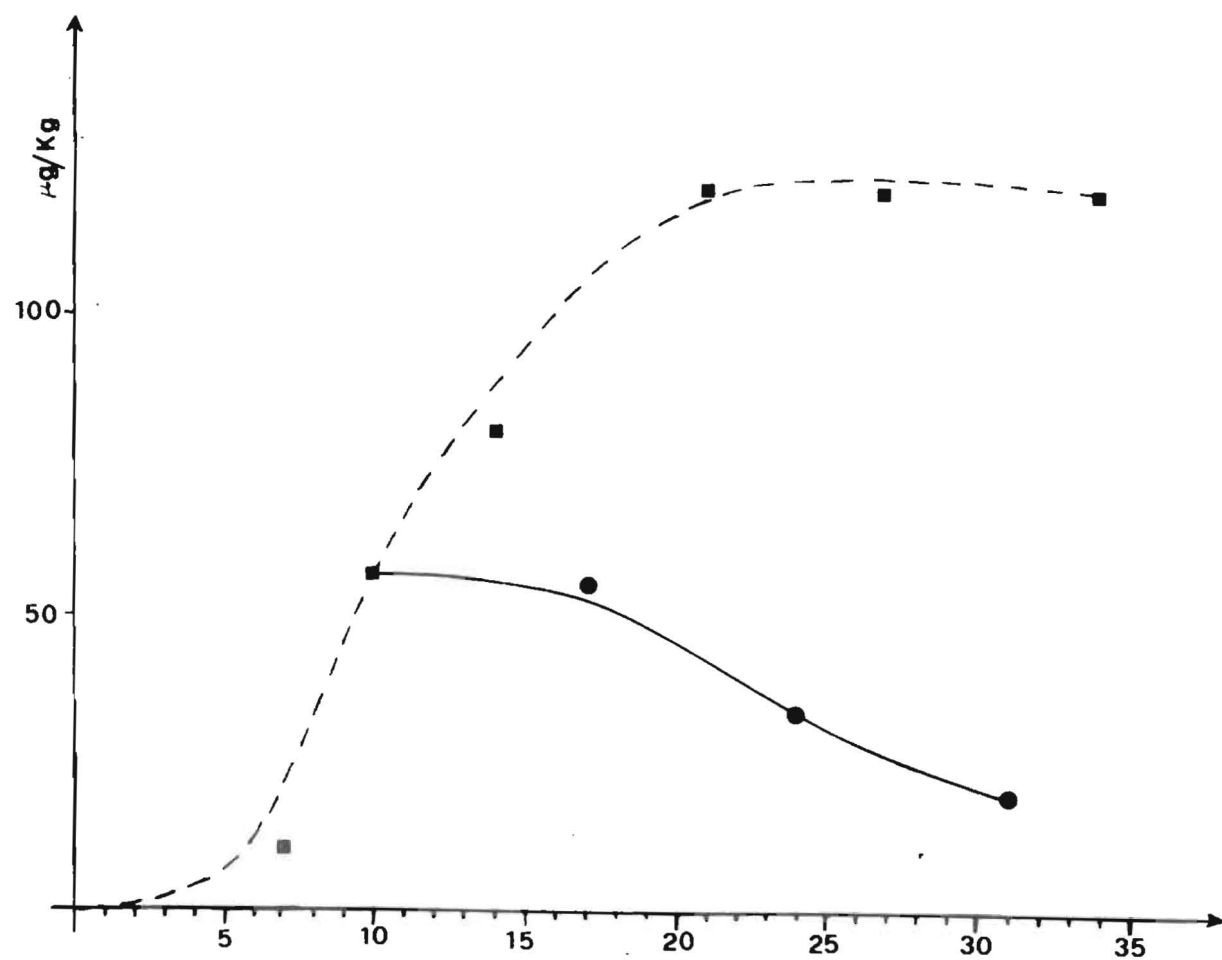


Fig.5. Inhibiting effect of nitrogen on the production of aflatoxin B<sub>1</sub> by a strain of *A. flavus* grown in air for 10 days. (dotted line: in air, full line: in nitrogen)

their help in setting up the gas flow lines and carrying out inspections.

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## WET GRAINS STORAGE UNDER MODIFIED ATMOSPHERES. MICROBIOLOGICAL ASPECTS

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## INTRODUCTION

Grain molds have long been considered as strict aerobic microorganisms but several microbiologists have recently said that under particular conditions fungi may show some metabolic activities and even mycelial growth in oxygen-free atmospheres (GUNNER and ALEXANDER, 1964 ; TABAK and COOKE, 1968 ; BULL and BUSHELL, 1976).

Those recent data could question the feasibility of controlled atmospheres storages from a microbiological point of view.

To investigate further what happens with stored grains, experiments have been carried on with maize at different moisture content levels, in airtight conditions and under nitrogen.

Under anaerobic conditions, molds that are said to be able to grow or to develop metabolic activities, cannot sporulate (TABAK and COOKE, 1968). So, enumeration of conidia by the classical suspension-dilution method cannot be used to demonstrate a possible mold activity. This method has nonetheless been used as a control for anaerobiosis. To some extent, the "Ulster" method (MUSKET and MALONE, 1940) allows to account for mycelial growth but is not really quantitative. That is why the mycological analysis has been complemented with a gas-chromatographic determination of specific volatile compounds which indicate a fungal activity and besides with the high pressure liquid chromatography analysis of fungal ergosterol for mycelial growth examination.

## 1. MATERIALS AND METHODS

1.1 Operating conditions

High moisture grains harvested at 36 p.100 moisture content (M.C.), are used without treatment. Intermediate moisture grains (17 and 23 p.100 M.C.) are heat-dried grains.

In airtight storage experiments, grains are placed into one liter glass containers hermetically closed. For experimental storages under nitrogen, anaerobic jars are used after modification allowing gas introduction. After filling up with grains, air is evacuated and replaced by very high purity nitrogen, three times running and introduced endly with a slight over-pressure.

In each case, anaerobiosis is checked by means of a methylene blue indicator.



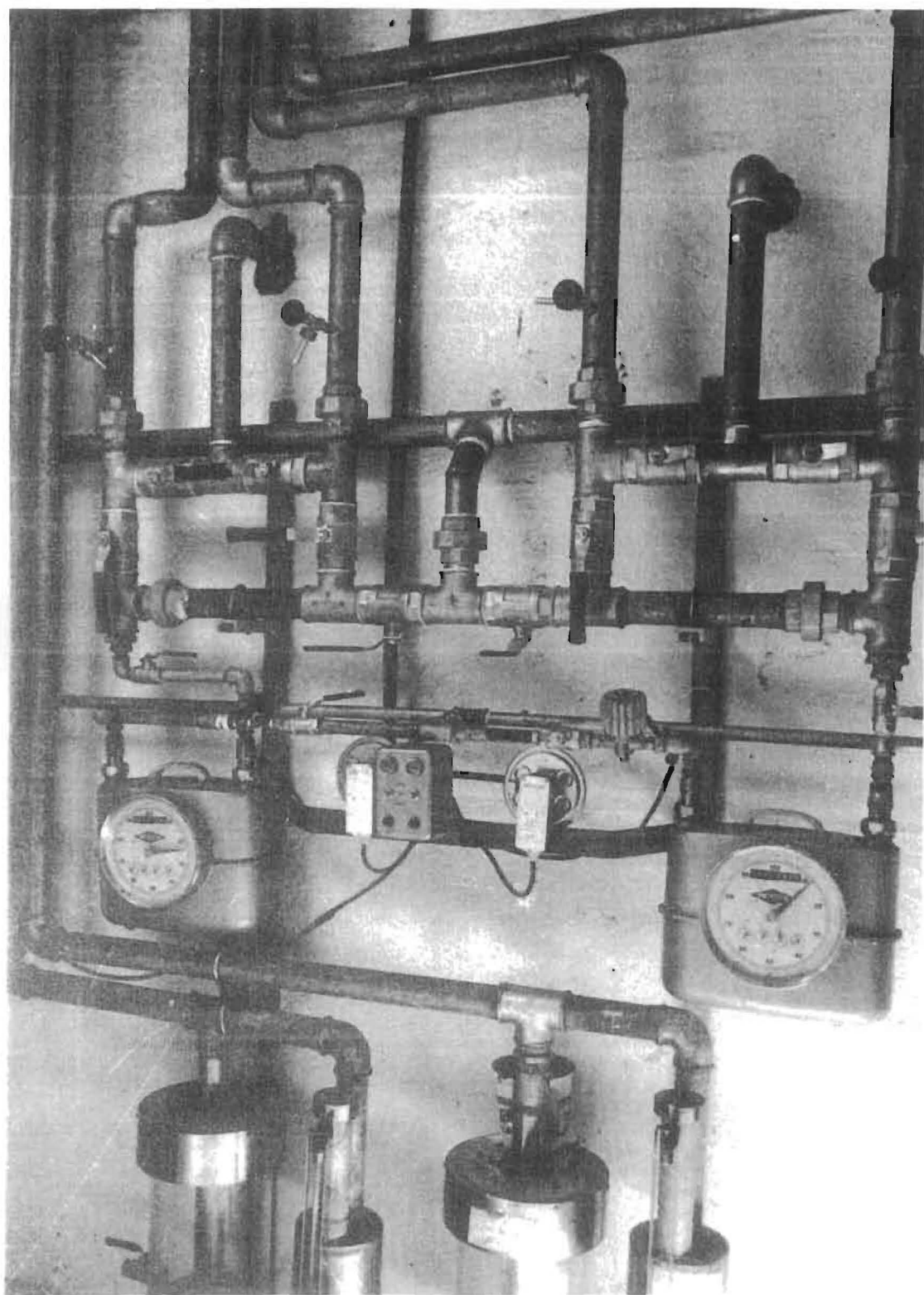


Photo Agenzia Italia, Roma

## 1.2 Methods

### 1.2.1. Enumeration of bacteria

The classical microbiological methods have been employed for enumeration of the following bacteria groups : General mesophilic bacteria (P.C.A.), Enterobacteriaceae (VRBG), Lactobacilli (Rogosa agar), Anaerobes (TGY agar) and especially sporulated Clostridia.

### 1.2.2. Yeasts and molds

As previously indicated, the suspension-dilution method has been used, especially for yeasts counts (CAHAGNIER, 1973). Concerning the Ulster method (malt extract agar) 400 grains are analysed each time, 200 grains being superficially disinfected, 200 grains being not. Disinfected grains lead to aberrant results probably due to an increasing permeability of stored kernels (PELHATE and THERIAULT, 1979). For this reason those results are not considered there.

### 1.2.3. Collection and analysis of volatile organic compounds

The volatiles are desorbed under vacuum, collected in a dry-ice cooled trap and recovered with methylene chloride. The extract is then concentrated by solvent evaporation and analysed by gas-chromatography, using a glass capillary column (50 m length, 0,5 mm i.d.) coated with carbowax 20M. Oven temperature is programmed from 70° to 150°C at a rate of 2°C/min. with helium as carrier-gas (RICHARD-MOLARD et al., 1976).

### 1.2.4. Fungal ergosterol assay

Fungal ergosterol is extracted with methanol from grounded kernels, saponified with KOH, extracted from methanol with petroleum ether, purified and analysed by HPLC using a 5µ-Spherisorb Column (SEITZ and al., 1977).

## 2 AIRTIGHT STORAGES

### 2.1. Microflora evolution

#### 2.1.1. Bacteria

In high-moisture grains (36 p.100 M.C.) the evolution of bacterial microflora is mainly characterised by an active growth of Lactobacilli (fig. 1) during the first 15 days, at 22°C and 30°C. As a consequence, the pH is decreasing from 6 to about 4 in the same time. Enterobacteriaceae are rapidly decreasing and cannot be detected anymore after 2 months. Anaerobic bacteria, others than Lactobacilli, do not grow and sporulated Clostridia practically disappear within three months, probably because of the low pH value.

In grains stored at 23 p.100 M.C., at 22°C and 30°C, all bacterial populations are decreasing slowly (fig. 2) and the pH remains constant. Same results are observed at 17 p.100 moisture content.

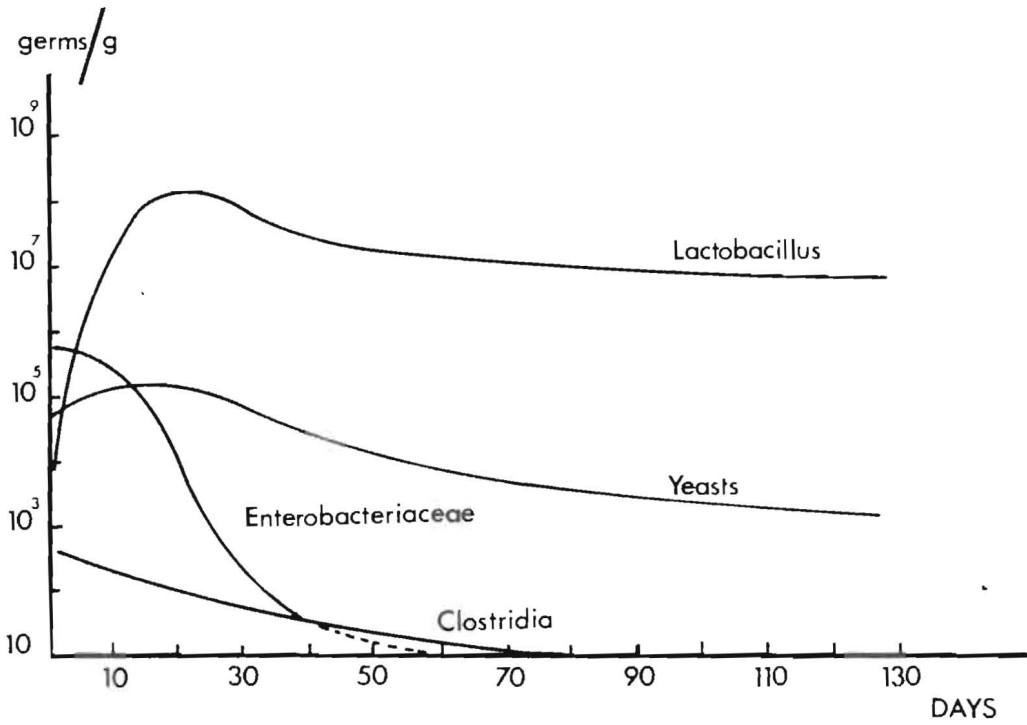


Fig. 1 Airtight stored grains (36 p.100 M.C., 22°C). Evolution of bacteria and yeasts.

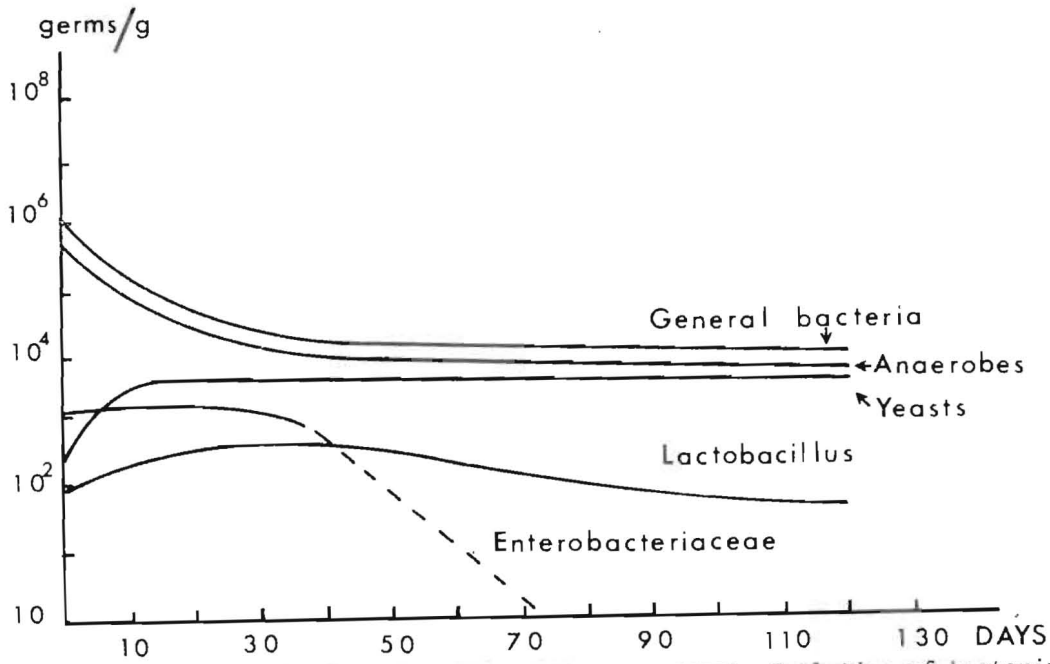


Fig. 2 Airtight stored grains (23 p.100 M.C., 22°C). Evolution of bacteria and yeasts.

2.1.2. Yeasts

In wet grains, yeast counts do not change during about 1 month and then are significantly decreasing. At 23 p.100 M.C., the repartition of yeasts is very heterogeneous and results are more dispersed. Nevertheless the Ulster method results show the population being increasing very slowly during all the experiment, but such an increase does not occur at 17 p.100 M.C.

2.1.3. Molds

On grains stored at 36 p.100 M.C., the initial contamination is about  $2,5.10^4$  germs/g. Two weeks later, it becomes impossible to detect molds by dilution method. Results obtained by Ulster method are resumed in table I.

TABLE I. : Grains airtight stored (36 p.100 M.C., 22°C). Percentage of contaminated grains.

FUNGI	T I M E I N D A Y S					
	0	21	36	65	96	122
FUSARIUM	38	38	0,5	-	-	-
EPICOCCUM	10	2	-	-	-	-
MUCORALES	10	17	.9	-	-	-
PENICILLIUM	20	22	11	-	-	-
TRICHODERMA	3	5	-	-	-	-
YEASTS	60	72	70	67	63	71

Field species like *Fusarium* sp., *Alternaria* sp., *Epicoaccum* sp., are rapidly decreasing in such conditions of humidity and acidity. *Fusarium* species, which are present on 38 p.100 of the grains at the begining disappear within one month. The same evolution is observed with storage species (*Aspergillus* and *Penicillium*) : within two months, the grains seem practically sterile.

The evolution observed at 23 p.100 M.C. is quite different (Table II).

The percentage of grains contaminated by *Fusarium* sp. remain almost constant. Other species are decreasing slowly, except *Penicillia* which seem to be able to grow very slightly.

For the grains stored at 17 p.100 M.C. the decrease of molds population is slower but the growing of *Penicillia* does not occur. No significative difference has been observed between 22°C and 30°C.

TABLE II. : Grains airtight stored (23 p.100 M.C., 22°C) Percentage of contaminated grains.

FUNGI	T I M E I N D A Y S					
	0	7	35	69	96	118
FUSARIUM	38	34	36	36	28	25
EPICOCCUM	10	2	-	-	-	-
CLADOSPORIUM	5	-	-	-	-	-
MUCORALES	10	13	10	10	7	8
PENICILLIUM	-	9	22	46	25	21
TRICHODERMA	3	15	6	-	1	-
YEASTS	60	70	73	78	80	78

## 2.2. Fungal ergosterol

Preliminary assays have shown that a very good correlation does exist between mold growth and ergosterol content of open-stored grains (24 p.100 M.C.). Ergosterol is not detectable in non-moldy grains. Results obtained with grains stored at 36 and 23 p.100 M.C. show that no fungal growth occurs in airtight conditions and confirm the results of mycological analysis (fig. 3). It is interesting to notice that ergosterol determination does not allow to take into account the fungal population decrease, ergosterol remaining present on grains while the percentage of contaminated grains is decreasing.

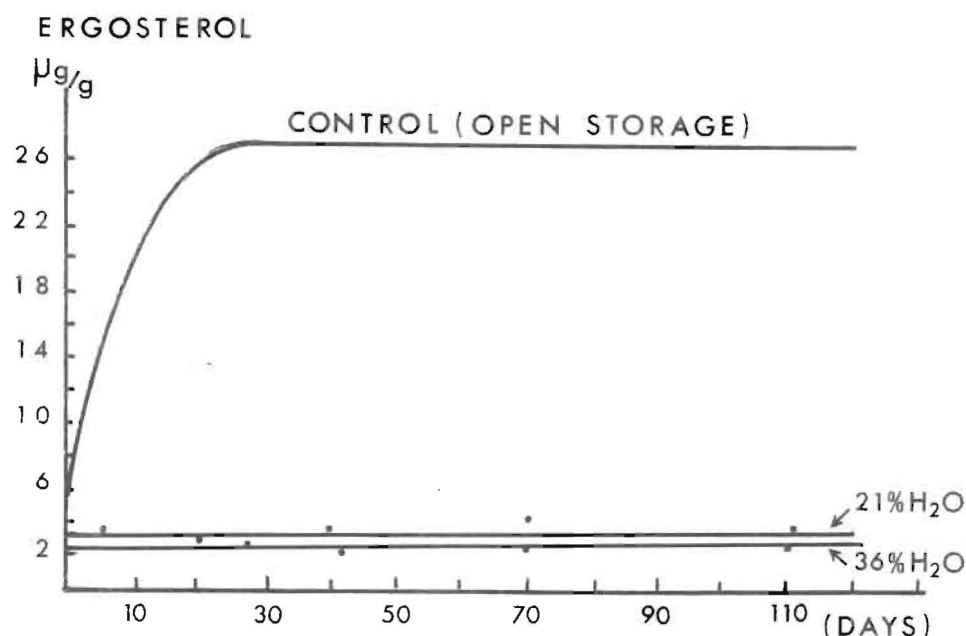


Fig. 3 Fungal ergosterol evolution in grains airtight stored at 21 p.100 and 36 p.100 moisture content.

### 2.3. Volatile organic compounds

Chromatograms shown in the figure 4 are those obtained with grains being stored during 17 days at 22°C.

On wet grains (36 p.100 M.C.), the metabolic activity of yeasts and heterofermentative Lactobacilli is clearly shown and active lactic and alcoholic fermentations occur. Characteristic compounds like ethanol (peak 1), isobutyl and isoamyl alcohols (peaks 2 and 3) and 3-hydroxy-butanone (peak 4) are produced.

But in grains at 23 and 17 p.100 M.C., the metabolic activity remains undetectable and fungal specific compounds such as 1-octene-3-ol or 3-octanone (RICHARD-MOLARD and al., 1976 ; STAWICKI and al., 1973) are not produced.

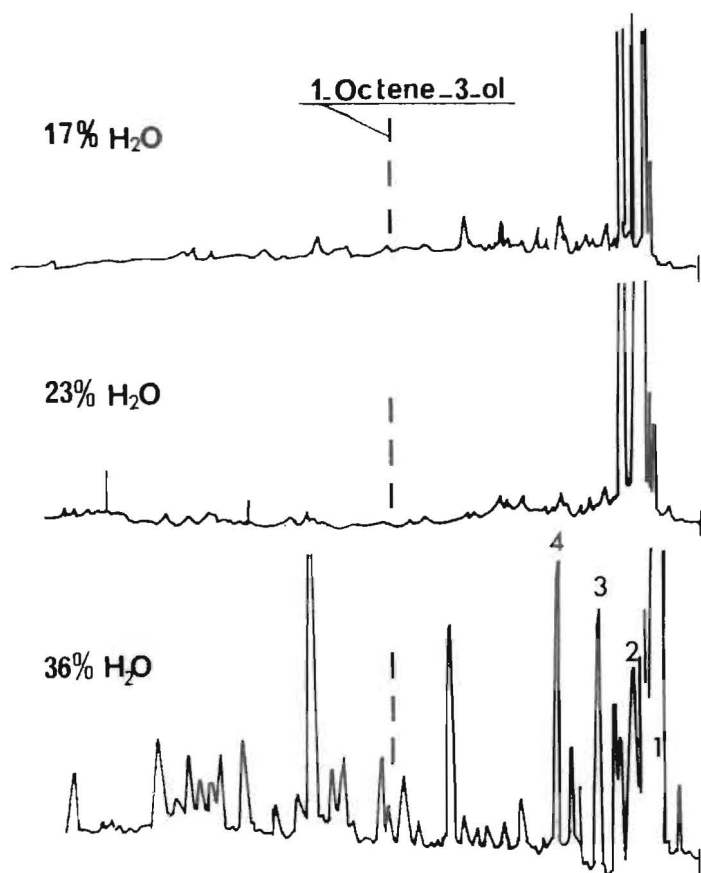


Fig. 4 Volatiles produced in airtight stored grains.

### 3 STORAGE UNDER NITROGEN

In this part of the study, experiments have been carried on at 30°C with grains at 17 and 21 p.100 moisture content.

The results obtained are quite similar to those previously described for airtight stored grain. Therefore it seems not necessary to detail them.

Table III show the molds evolution on grains stored under pure nitrogen.

TABLE III : Grains stored under nitrogen. Percentage of contaminated grains

FUNGI	21 p.100 M.C.			17 p.100 M.C.		
	T I M E (days)			T I M E (days)		
	0	38	164	0	38	164
FUSARIUM	78	60	37	49	57	10
A. FLAVUS	78	-	-	74	66	27
A. NIGER	10	-	-	10	3	1
PENICILLIUM	10	0,5	-	5	-	-
RHIZOPUS	36	-	-	43	18	-

As in airtight stored grain, the initial mycoflora is progressively decreasing, the regression of different species being more rapid at 23 p.100 M.C. than at 17 p.100, *Fusarium* spp. appearing the most resistant to the anaerobiosis. At 23 p.100 M.C. a slight alcoholic fermentation occurs (shown by gas-chromatography) possibly due to yeasts activity.

#### 4 SURVIVAL OF CONIDIA IN AIRTIGHT CONDITIONS

##### 4.1. Experimentals

The pure cultures of storage fungi used in this part of the study were isolated from ensilaged damp grains. In a preliminary step, autoclaved maize grains 30 p.100 M.C., are inoculated with conidia and incubated 8 days at 24°C in air. The moldy grains obtained are then divided in three parts : the first one, used as control, is simply stored in air ; the second part is placed in Gas-Pack anaerobic jars and so stored in strictly anaerobic conditions. A small nylon net-bag is filled with the third part of moldy grains and suspended in a glass container which is then completely filled with freshly harvested damp grain (30 p.100 M.C.) and hermetically closed in order to obtain airtight conditions.

Conidia germinative capacity is checked four month later in the following manner : the total number of conidia produced on grains is determined with the Coulter counter, while conidia that are still able to germinate are numbered on malt agar plates. The germinative capacity is expressed as surviving conidia to total conidia count (Coulter counter) ratio, in percentage.

##### 4.2. Results

As can be seen on fig. 5, conidia survival is drastically reduced for *Aspergillus versicolor* in anaerobiosis or airtight conditions.



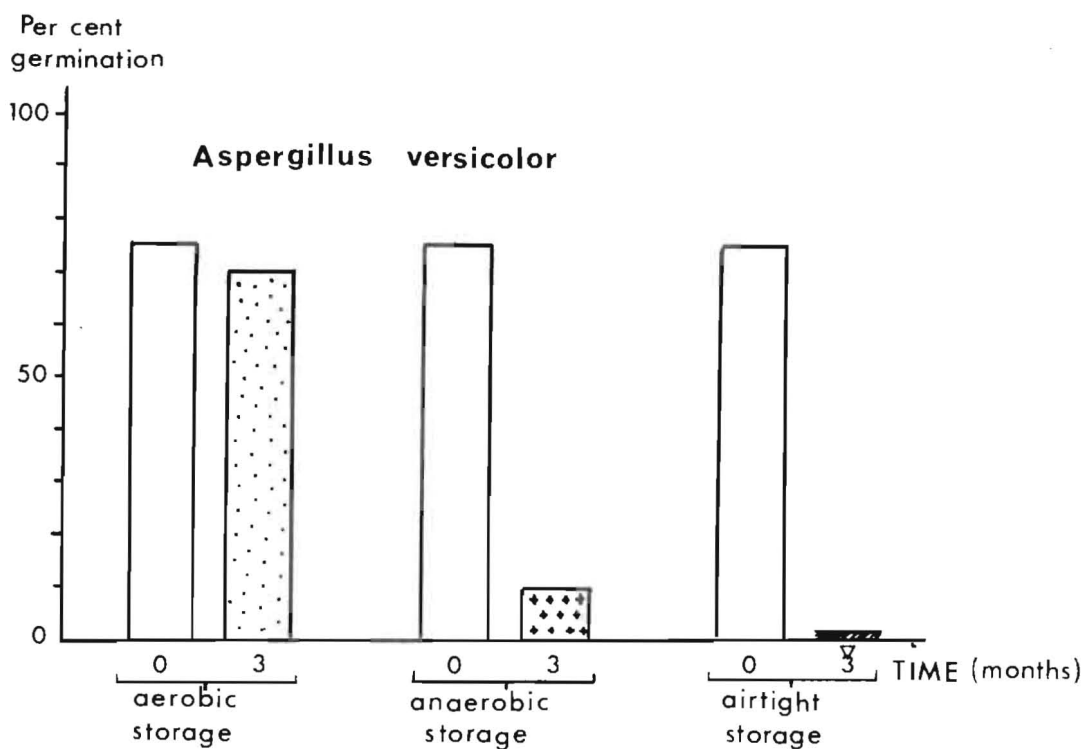


Fig. 5 Germinative capacity of conidia of *Aspergillus versicolor*

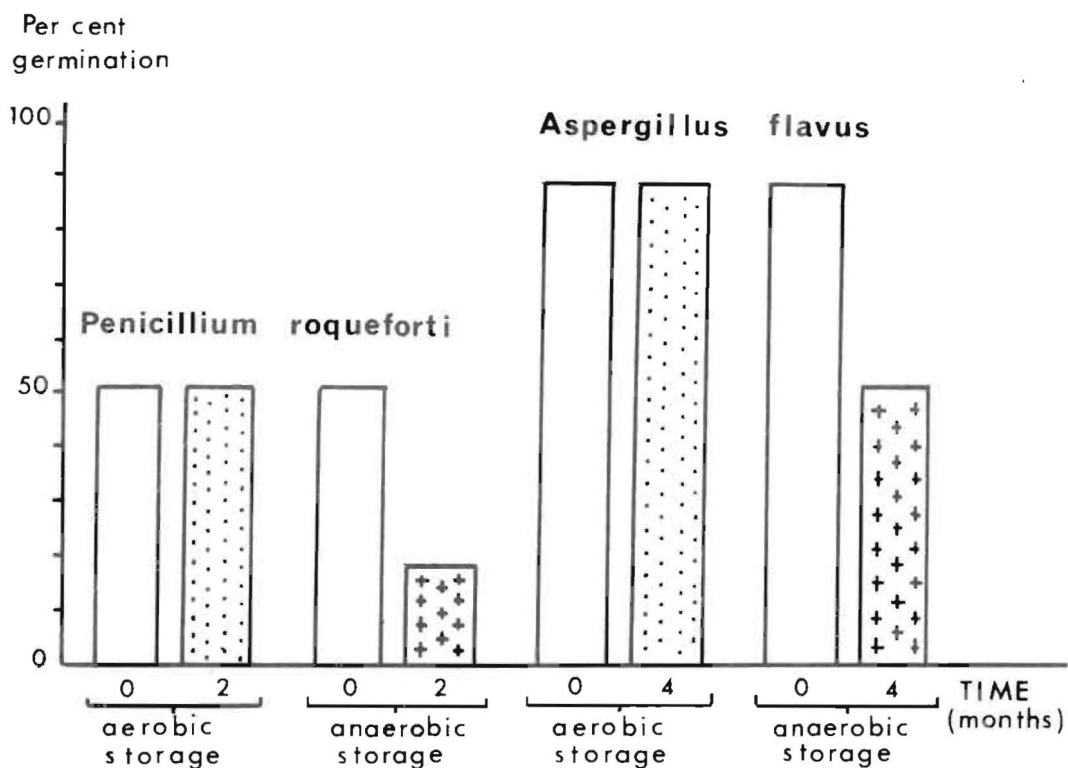


Fig. 6 Germinative capacity of *A. flavus* and *P. roqueforti*.

As a general rule, conidia survival is rather better under strict anaerobiosis than under airtight atmospheres. The behaviour of *A. flavus* and *P. roqueforti* is shown on fig. 6. After four months in anaerobiosis, 50 p.100 of *A. flavus* conidia are still able to germinate but with *P. roqueforti* which is considered as micro-aerophilic, less than 20 p.100 of produced conidia are still able to germinate after only two months.

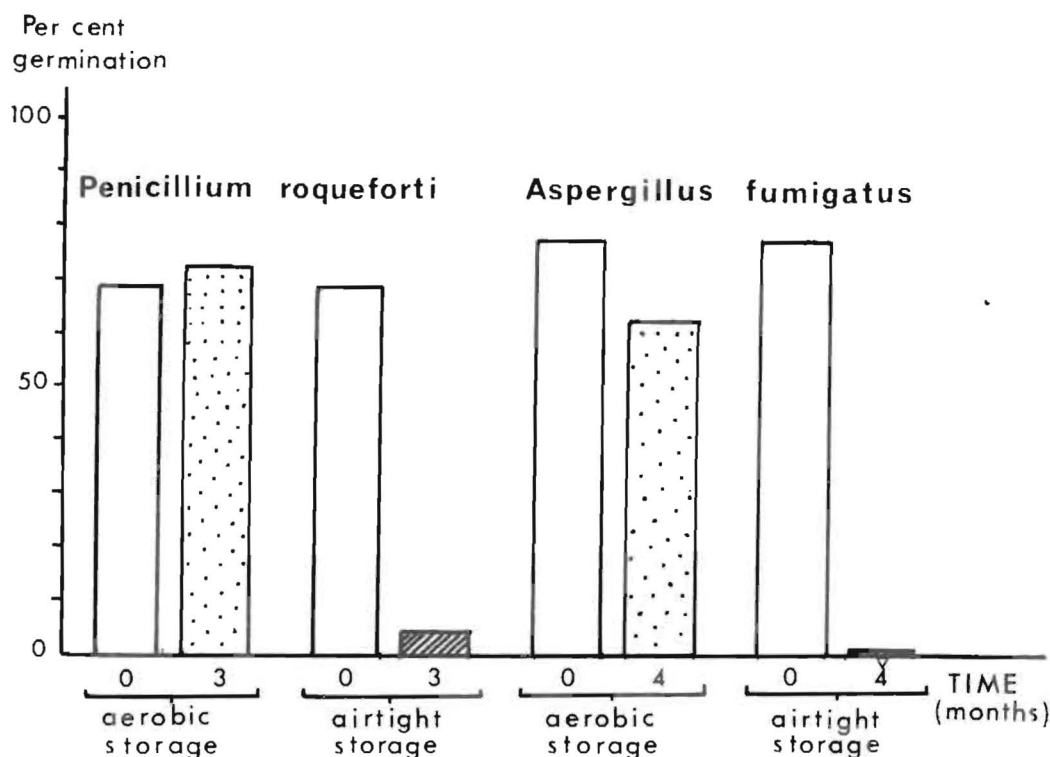


Fig. 7 Germinative capacity of *A. fumigatus* and *P. roqueforti*.

In airtight conditions (fig.7), the decrease of conidia survival is greater for *P. roqueforti* and *A. fumigatus* is shown to be very affected by the lack of oxygen, conidia destruction being nearly complete within four months.

#### CONCLUSION

Results obtained in this study show that no fungal growth or activity can be observed in grains stored under very low oxygen tensions. In all experimented conditions, fungal populations are decreasing, the regression being more rapid when grain moisture content is higher.

Technics of grains storage under modified atmospheres, which are as yet not very wide-spread in France, will certainly undergo an important development in the coming years.

The rapidly increasing cost of energy leads to considering other storing techniques than the usual grain drying. The question is of particular interest in France for maize which is very often harvested at very high moisture content such as 30 p.100 to 40 p.100, or more. Considering the results obtained in this study, it is possible to say that maize which is largely used for animal feeding can be stored under nitrogen or more simply in airtight conditions without microbiological objections. With high-moisture grains, such storage techniques lead to lactic and alcoholic fermentations which modify mainly the organoleptic characteristics of the product, but this seems to be no real problem for use in animal feeding.

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## IV S E S S I O N :

ARTIFICIAL CONTROLLED ATMOSPHERE STORAGE

## STORABILITY OF CEREAL GRAINS AND OIL SEEDS IN NITROGEN.

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## ABSTRACT

The experimental facilities and the research methodology applied in the studies on grain storability in nitrogen with various residual oxygen concentrations are described.

A summary of the results, showing that nitrogen storage offers a large number of advantages over traditional storage techniques, are presented, and the limiting factors for the successful storage of different grains in completely and partially bio-inert atmospheres, especially in regard to moisture content, are described.

## INTRODUCTION

The preservation of quality during long-term storage of grains is a severe problem in many parts of the world. High temperatures and excessive moisture in the stored products or high humidity in the storage ambient permit the proliferation of insects and moulds which cause large losses of qualitative, nutritional and sanitary nature.

Hermetic storage has been used in many countries for a very long time. It permits to exclude exogenous factors which cause grain spoilage and the technique has been extended even to large-scale storage facilities (Hyde et al., 1973).

In order to overcome difficulties linked to the slow natural depletion of oxygen in the interstitial atmosphere in gas-tight stores and to rationalize the technique for industrial storage facilities by excluding excessive carbon dioxide build up and initial spoilage of high moisture grains by fungi, long-term experiments were carried out on the effects of nitrogen on various stored commodities.

In small storage facilities and in simulation experiments the beneficial effect of nitrogen on stored grains was shown (Shejbal, 1978) and a nitrogen fumigation and storage technique was developed which has been successfully operating since several years in large scale storage facilities in Italy (Shejbal, 1979a).

The aim of this paper is to summarize the research approach, the experimental data and the main features of the nitrogen storage technique, indicating its possibilities and limits.

#### MATERIALS AND METHODS

Laboratory storage facilities, micro- and mini-silos, were used for storability tests of soft and durum wheat, barley, maize, rice paddy, peas, oil seeds, nuts etc. In all experiments the evolution of quality changes throughout the storage period was studied, since only in this way it is possible to establish storability limits. This approach necessitates sampling at regular time intervals without disturbing excessively the gas composition in the silos.

The micro-silos (volume 12 l) (Fig. 1) were set up in series in the laboratory and the single silos were unloaded at given storage times, in order to allow the complete analysis of the stored product without disturbing the material to be stored for longer periods of time. This arrangement permits to study especially high moisture grains in which even a short change in the interstitial atmosphere composition, due to sampling, may be detrimental.

Mini-silos were equipped with three sampling points at different heights of the stored product column and were installed either in the laboratory at a constant temperature (Fig. 2) or under an open shed (Fig. 3), thus permitting storage experiments with and without fast temperature fluctuations.

It should be noted that in no case does storage in small containers permit a true simulation of the conditions of a large grain bulk, since heat dissipation from the structure does not allow heating phenomena to occur. Such phenomena are of particular importance in products in which insect or mould proliferation is active or oxidation of fat in oil-seeds may take place. Mini-silos placed at outdoors

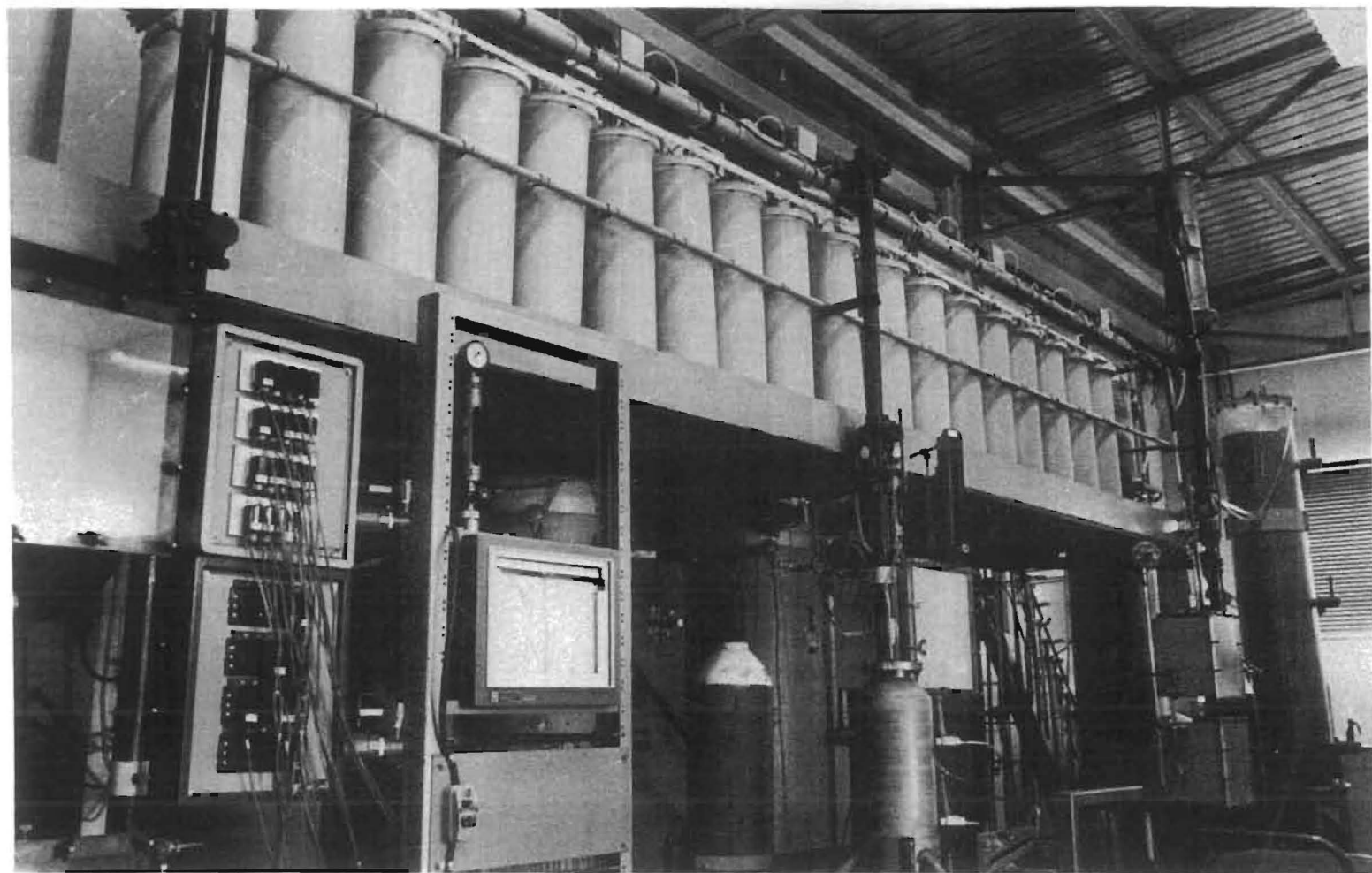


Fig.1. Gas-tight micro-silos (12 l volume, PVC) in laboratory. In foreground temperature and gas flow monitoring equipment.



ambient temperature permit a satisfactory simulation of phenomena occurring in the head-space and upper layers of large storage facilities.

Thermostated mini-silos (Fig. 2) were used for storability tests on dry cereal grains, especially in regard to viability preservation.

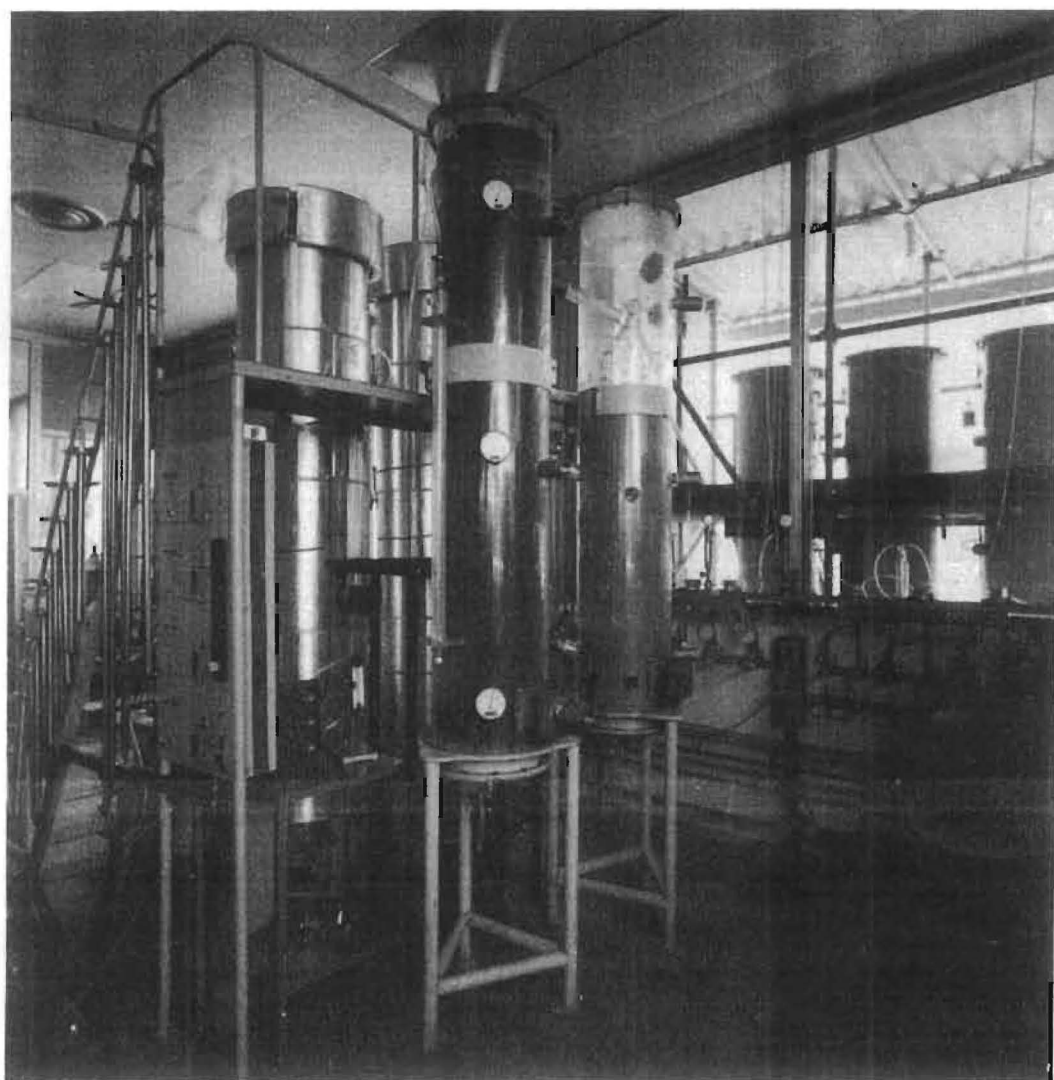


Fig.2. Plexiglass and thermostated steel mini-silos in laboratory. Under the window the automatic pressurization equipment.

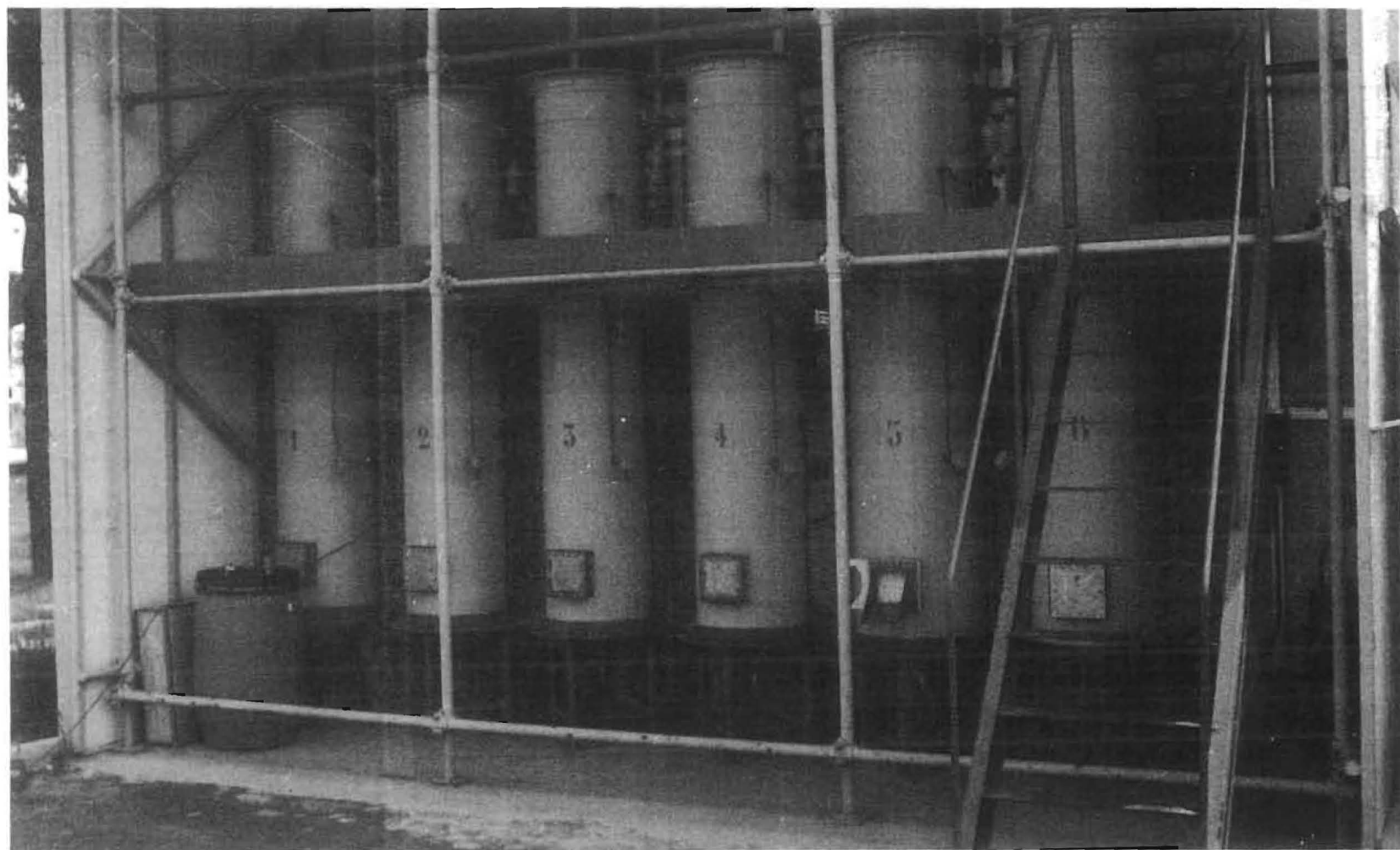


Fig.3. Eternit mini-silos at external ambient temperature. Gas-tightness achieved by epoxy-paints.

For laboratory scale simulation of conditions persisting inside a huge granular stored product bulk, which are nearly adiabatic, an experimental apparatus was designed (Fig. 4), which permits to measure heat production rate of very low intensity in the stored product (Tranchino et al., 1980a). Autocatalytic phenomena taking place in oil seeds were measured in this apparatus.



Fig.4. Adiabatic apparatus for simulation of storage conditions inside a large grain bulk.

Laboratory scale storage experiments were repeated at pilot scale in 27 m<sup>3</sup>-fiber-glass bins (Fig. 5), permitting the assessment of quality changes during storage in various controlled atmospheres also by industrial milling and other processing by the food industry. Scaling up from these bins presents no difficulty.

Gas flows, monitoring of storage conditions, analyses of the interstitial atmosphere composition, as well as biological, chemical, mycological, rheological and nutritional analyses carried out on all tested stored products were described in detail before (Shejbal et al., 1973; Lombardi et al., 1976; Shejbal, 1976; Shejbal, 1979b). The principles of nitrogen preservation of grains developed in the experimental facilities were applied to large-scale gas-tight bins for barley, wheat and sunflower seed storage.

While in the experimental bins purging with nitrogen was always carried out from the top towards the bottom in order to take advantage of the gas density differences, in the large-scale facilities the initial purge is carried out from the bottom towards the top (Fig. 6) in order to avoid excessive pressure in the head-space which might be dangerous for the bin roof. Maintenance of a slight constant positive pressure in the storage facilities ensures the automatic substitution of gas (Fig. 7) lost by leaks or operation of the safety valve when high temperatures in the head space gas are reached due to insolation. The over pressure in the bins also counteracts convective fluxes and diffusion from outside.

The gas-tight bins for malting barley (2200 m<sup>3</sup> each) were built in galvanized steel (Fig. 6) while the silos for wheat (Fig. 8) and sunflower seeds are constituted of a number of medium-sized monolithic fiber glass bins. More details on the large scale facilities are presented elsewhere (Tranchino et al., 1980b).

## RESULTS AND DISCUSSION

The overall picture, obtained in experimental storage trials with cereal grains shows that long term preservation in nitrogen is advantageous at all temperature and moisture content levels (up to 30°C and 19% respectively) as compared to storage in air.



Fig.5. Fiber-glass midi-silos for controlled atmosphere storage trials at pilot scale.

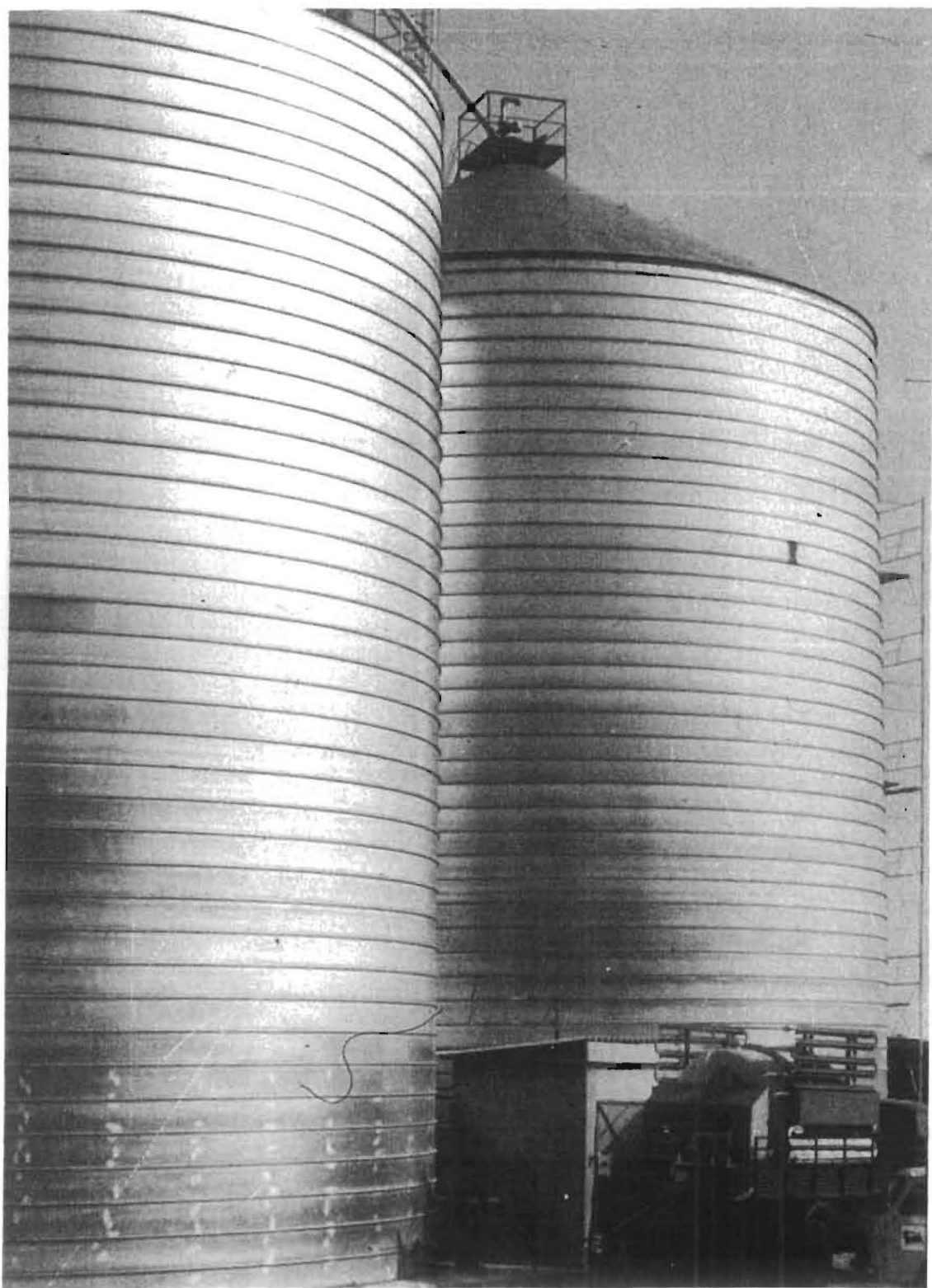


Fig.6. Steel barley storage bins (IVO, Tuscania) during initial purge with gaseous nitrogen from mobile regasification unit.



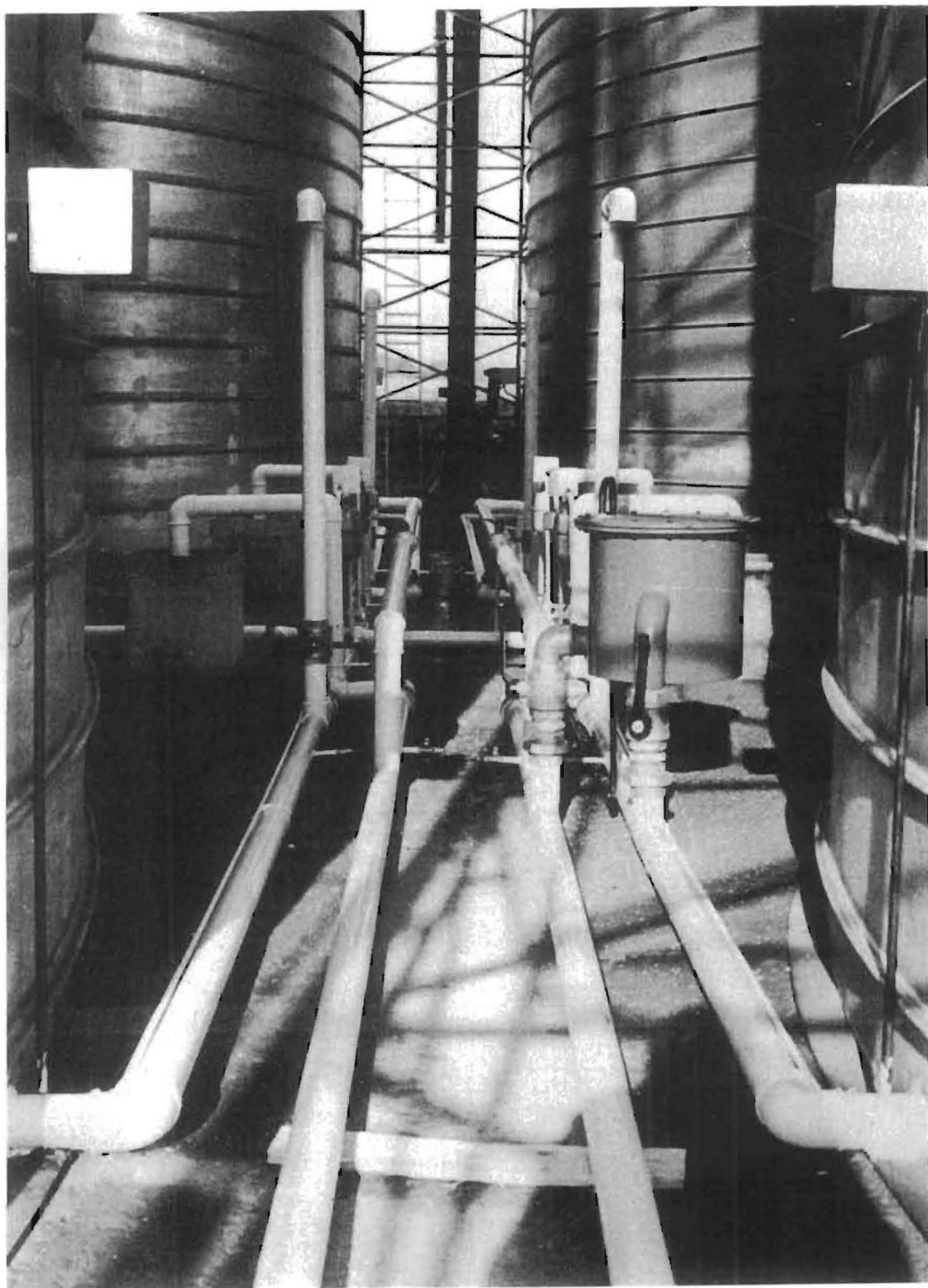


Fig.7. Nitrogen distribution system for initial purge (from bottom) and maintenance of the inert interstitial atmosphere (from the top) of large-scale steel storage bins.





Fig.8. Silo<sub>3</sub> of Società Romana di Macinazione, Rome, for the storage of wheat. The fiber-glass bins (600 m<sup>3</sup> each) are equipped for nitrogen fumigation and storage of grain.

At moisture contents up to 14.5%, the better storability of the grains in technical nitrogen (0.2-0.5% residual oxygen) is evident especially over long periods of time, since mould attack is absent also in the oxygen rich normal atmosphere. Grain quality, i.e. germination energy and capacity, rheological and baking characteristics, as well as the content of nutritional components, is maintained significantly longer than in air at all temperatures studied (10° to 30°C). Germination energy is the most sensitive indicator of grain deterioration (Shejbal and Di Maggio, 1976).

In pure nitrogen (residual oxygen content less than 0.01%) all characteristics, except cereal grain viability, are maintained as well as in technical nitrogen. However, also in this atmosphere, germination energy and capacity are preserved significantly longer than in air.

In Fig. 9 the storage periods are shown in which 90% of the initial viability of soft wheat seeds is preserved, as a function of moisture content and temperature, in pure nitrogen. Such a graphic representation, though correct and easy to understand, has however only a limited value. It may be misleading in practice, since the initial quality and the history of the grains to be preserved under nitrogen are of cardinal importance for successful maintenance of quality in general and viability in particular. Products in which deterioration has already started cannot be safely stored in anoxia. Thus the data in Fig. 9 refer to grain which, at the start of storage in nitrogen, had a germination energy not lower than 90% and a mould count not higher than  $10^4$  germs/g, with storage fungi virtually absent (less than 5% of the total fungal count).

It seems more adequate to indicate the limiting factors of grain storage in nitrogen atmospheres and the rough limits of storability of the various features (Table 1).

At moisture contents between 14.5 and 19%, the beneficial effect of storage in nitrogen is evident also in short periods of time, since mould attack is the main cause of fast deterioration in aerated storage.

In nitrogen containing up to 0.2% oxygen, moulds develop slower

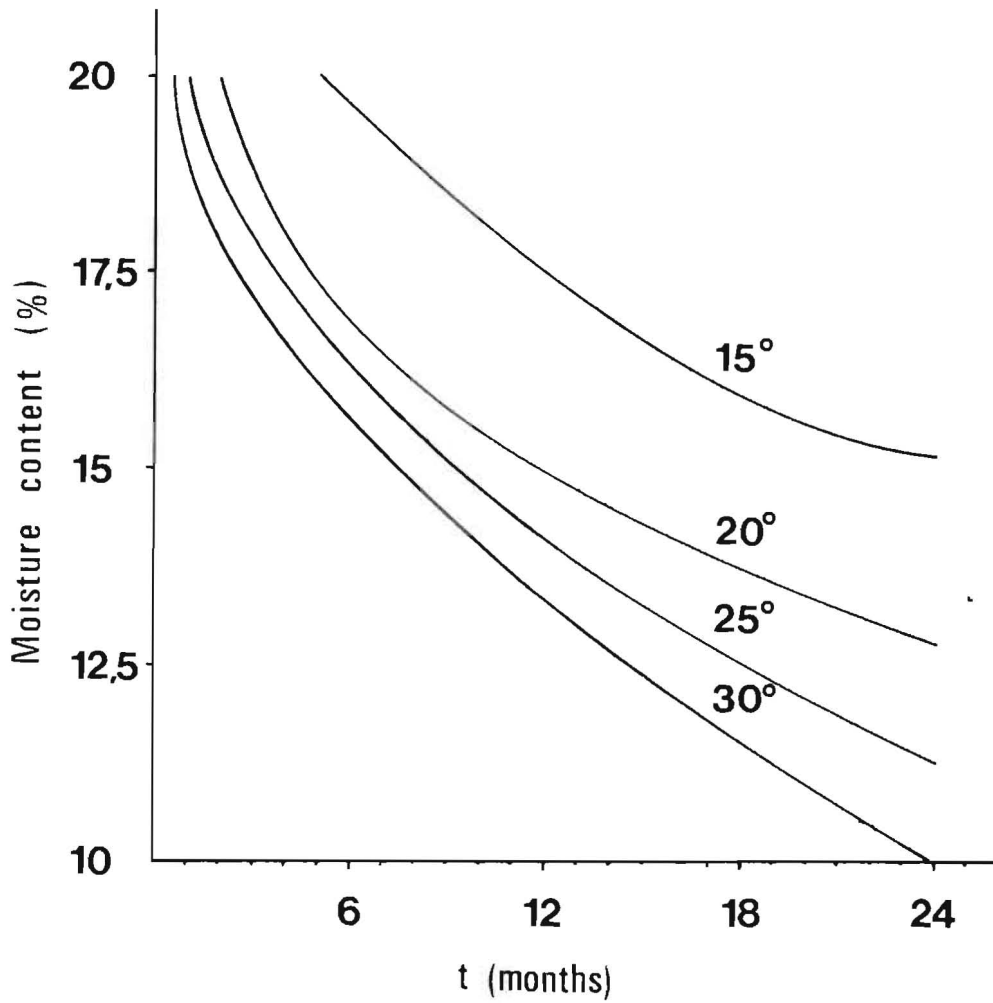


Fig.9. Storage periods in which viability of soft wheat decreases by 10% when preserved in pure nitrogen.

than in air, but are not inhibited. Cereal grains may thus be preserved for significantly longer periods of time than in air, but after the initial lag-phase of fungal development, deterioration cannot be avoided at these residual oxygen concentrations (Di Maggio et al., 1976).

In pure nitrogen, fungal proliferation is completely inhibited and total germ counts tend to diminish in time (Di Maggio 1980). Progressive deterioration of the cereal grains, due to endogenous enzymes, takes place in total anoxia at a slow rate. Thus safe storage of cereal grains at moisture contents, at which, in Mediterranean climatic conditions, it is not possible to maintain grain for more

TABLE 1 CEREAL GRAIN PRESERVATION IN NITROGEN ATMOSPHERES

Moisture content (%)	Temperature (°C)	Residual oxygen concentration (%)	Technol. & nutr. quality preservation (time)	Germination energy preservation (time)	Limiting factor
m.c. $\leq$ 14.5	T $\leq$ 30	0 < O <sub>2</sub> $\leq$ 0.5	years	years	germinability
m.c. $\leq$ 14.5	T $\leq$ 30	O <sub>2</sub> = 0	years	year	germinability
14.5 < m.c. $\leq$ 16	T $\leq$ 25	0.2 $\leq$ O <sub>2</sub> $\leq$ 0.5	year-months	months	mould growth
14.5 < m.c. $\leq$ 16	T $\leq$ 25	O <sub>2</sub> = 0	year-months	months	germinability
16 < m.c. < 19	T < 25	0.1 < O <sub>2</sub> < 0.2	months-weeks	month-weeks	mould growth
16 < m.c. < 19	T < 25	O <sub>2</sub> = 0	months	month-weeks	technological quality

than a few days in the presence of air, can be carried out for several months, depending on the initial quality of the grains and on the actual moisture content. The viability of the grains decreases significantly slower in anoxia than in the presence of air but cannot be arrested (Quaglia et al., 1977). Endogenous degradation of saccharides, resulting in a progressive increase in reducing sugars and a decrease in non-reducing sugars, seems to be independent of the composition of the interstitial atmosphere; it proceeds slower than the loss of viability. The increase of reducing sugars parallels the loss in technological quality of the stored moist grains (Lombardi et al., 1976).

There is an important exception to the behaviour of cereal grains in anoxia (Table 1). In fact, it is not convenient to preserve high moisture paddy (m.c. higher than 16.5%) in anoxic conditions for long periods of time, since anaerobic respiration takes place, causing the development of odours, which may make the product unsuitable for human consumption.

Oil-seed storability is higher in nitrogen atmospheres than in air (Table 2). The quality of the seeds at the start of storage is of fundamental importance, especially as far as fat acidity is concerned. For example, sunflower seeds with fat acidity values not higher than 2 (expressed as mg KOH required to neutralize 1g of oil) may be preserved for long periods of time also at critical moisture content levels but at FAV values of 3 or more, deterioration of the lipids cannot be controlled by anoxia.

The results of the laboratory and pilot-scale trials have been confirmed for wheat and malting barley in the Italian large scale facilities (Tranchino et al., 1980b) and general characteristics of the nitrogen storage method of grain have been determined. In Table 3, the features both of short-term fumigation applications of nitrogen for insect control (see also Banks et al., 1980) and of long-term preservation are summarized.

As can be seen, the substitution of the interstitial atmosphere by inert gas influences positively all the main exogenous product quality deteriorating agents and thus contributes to the stability

TABLE 2 OIL SEED PRESERVATION IN NITROGEN ATMOSPHERES

Moisture content (%)	Temperature (°C)	Residual oxygen concentration (%)	Technol. & nutr. quality preservation (time)	Germination energy preservation (time)	Limiting factor
m.c. $\leq$ 6	T $\leq$ 25	0 < O <sub>2</sub> $\leq$ 0.5	years	years	germinability
m.c. $\leq$ 6	T $\leq$ 25	O <sub>2</sub> = 0	years	year	germinability
6 < m.c. $\leq$ 8	T $\leq$ 20	0.2 $\leq$ O <sub>2</sub> $\leq$ 0.5	year	year	germinability
6 < m.c. $\leq$ 8	T $\leq$ 20	O <sub>2</sub> = 0	year	year	germinability
8 < m.c. < 10	T $\leq$ 20	0.2 $\leq$ O <sub>2</sub> $\leq$ 0.5	months	months	mould growth
8 < m.c. < 10	T $\leq$ 20	O <sub>2</sub> = 0	year	months	technological quality

of the simplified ecosystem in the confined storage environment.

The main advantages and disadvantages of the technique are summarized in Table 4. They refer to highly gas-tight storage structures, mostly of new construction. It is possible to use inert gas for the reduction of oxygen contents in the interstitial atmosphere of grains in less airtight structures when only disinfestation is required. The cost of the gas necessary to maintain an atmosphere lethal to insects will determine, after the initial fast purge, the economic feasibility of such an operation. Biologically non-inert gases, such as carbon dioxide in various mixtures with oxygen and nitrogen, may be more advantageous for short term disinfestations of leaky structures. According to results obtained in parallel experiments to those mentioned above, carbon dioxide can however not be recommended for long term preservation of grains in large bulks for human consumption, since it causes organoleptic changes which may result in an unacceptable depreciation of the stored products, although they may be of a reversible nature. Negative effects of carbon dioxide at high concentrations (above 60%) on quality were observed in all tested grains within weeks or months. Twelve percent moisture content wheat from pilot scale bins was found to be unacceptable at milling after 1 year storage in a mixture of carbon dioxide, nitrogen and oxygen (8: 91.5:0.5 v/v)(Shejbal, 1979a).

As can be seen in Table 4, the apparent disadvantages of the nitrogen fumigation and storage technique are strictly related to the management of the storage facilities. The very fact that the structures have to be gas-tight and filled with inert gas, excludes their use in those places where very frequent grain movement is envisaged and where also the slower disinfestation by anoxia, as compared to chemical pesticides and fumigants, is not acceptable. Where medium and long-term storage is envisaged, the slightly increased investment costs of gas-tight structures are easily outweighed by the advantages and savings achieved by the technique (Tranchino, 1980) and correct management and planning can substantially reduce problems related to partial unloading of storage bins.



TABLE 3 FEATURES OF NITROGEN FUMIGATION AND STORAGE METHOD

Chemical protectants and additives

- excluded both for storage facilities and stored products
  - : toxic residues excluded

Insects and mites

- Perfect penetration of gas through commodity
  - : Immediate inhibition of biological activity
    - = hot spot formation and moisture increase excluded
    - = loss of stored product excluded
    - = infestation excluded
  - : Full kill of all stages of development by 15-20 days treatments
- Development of resistance to long term anoxia exposure excluded
- Simple and safe fumigation treatment

Rodents and birds

- infestation excluded
  - : no loss of stored product

Fungi and yeasts

- proliferation significantly retarded in technical nitrogen, inhibited in pure nitrogen
  - : Storability of grains at critical and medium moisture contents significantly extended due to
    - exclusion of temperature and moisture increase
    - exclusion of off-odours
    - retarded loss of viability
    - retarded loss of technological and nutritional quality
- Contamination by mycotoxins during storage excluded
- Drying may be delayed or avoided
  - : savings on investment costs
  - : savings on operating costs

#### Oxidative reactions

- significantly reduced
  - : fats protected
  - : weight loss reduced
  - : secondary explosions and auto-ignition excluded

#### Viability of caryopses

- Germinative energy and capacity maintained significantly longer than in air
  - : storage of seeds and cereal grains for malt production possible in large storage facilities
- Damaging effect of high temperatures reduced

#### Technological and nutritional parameters of stored products

- preserved for long periods of time without need of additional measures
  - : particularly convenient for medium and long term storage
  - : convenient for safe large-scale buffer and strategic stocks

#### Operating costs

- Storage cost for a one year preservation is in the order of a single fumigation by chemical products or lower
- Operating costs decrease with increase of storage facility capacity

#### Investment costs

- not significantly increased when gas-tightness foreseen at silo construction

TABLE 4 Advantages (+) and disadvantages (-) of nitrogen fumigation and storage technique

- + use of chemical protectants and additives excluded
- + primary and secondary toxic residues excluded
- + full kill of insects at all stages of their development achieved
- + fungi controlled by pure nitrogen
- + quality of grains preserved for longer periods even above critical moisture contents
- + secondary explosions and fire hazards are prevented
- + operating costs are reduced or not increased
- gas-tight storage facilities with slightly increased investment costs needed
- disinfestation operation slower than with toxic chemicals
- partial unloading may increase operating costs and moisture migration

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## EXPERIMENTAL AND COMMERCIAL MODIFIED ATMOSPHERE TREATMENTS OF STORED GRAIN IN AUSTRALIA.

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### ABSTRACT

Modified atmosphere techniques for grain storage have been under study in Australia for over 60 years but until recently have been widely used only during one brief period (1917-1919). Full scale testing of nitrogen-based atmospheres, started in 1972, and has led to development of a process, based on a tanker-delivered liquid nitrogen, similar to that developed by Snamprogetti in Italy. Since, under dry Australian conditions, the quality of grains such as wheat is maintained adequately in storage, the emphasis in the use of nitrogen-based systems has been on insect rather than quality control. More recently the use of CO<sub>2</sub> has been investigated. CO<sub>2</sub>, at present, is the preferred atmosphere for insect control as it is easy to apply and, unlike nitrogen atmospheres, does not require a continuous input of gas after the initial purge if the storage is well sealed. CO<sub>2</sub>-based atmospheres are now in use in Australia for insect control in stored grain. A summary of the unpublished experimental and commercial trials carried out with modified atmospheres in Australia is given. If insect control only is required, it should only be necessary to maintain the modified atmosphere until elimination of insects is achieved. The sealed fabric of the storage should then provide a barrier against reinfestation. This proposition is discussed and examples of storage free of insects for more than four months after brief modified atmosphere treatment are given.

### INTRODUCTION

The technique of modified or controlled atmosphere storage of grain involves alteration of the concentrations of the normal atmospheric gases present in a storage so as to give an artificial atmosphere that is insecticidal and prevents mould growth and quality deterioration of the stored product. Two classes of externally generated modified atmosphere are available: low oxygen atmospheres, (generated by adding nitrogen, or the gas mixture resulting from burning hydrocarbons, to the store) or high CO<sub>2</sub> atmospheres (made by adding carbon dioxide). It should be noted that the technology of use of these atmospheres differs substantially from that of modified atmospheres generated by biological processes within a sealed structure, i.e. hermetic storage. Only externally generated systems are considered in this paper.

There has been a continuing interest in various forms of modified or controlled atmosphere storage of grains in Australia over many years: the first recorded large scale treatments were carried out in 1917. Although these were successful the process was not again used widely until 1979 when two grain storage organisations carried out commercial treatments with carbon dioxide. This use was based on experience gained during the 1970's from various full scale experimental treatments with either carbon dioxide or nitrogen as sources of the modified atmosphere. This paper summarises the details of the unpublished recent commercial and experimental applications of modified atmospheres to large bulks of stored grain (> 300 tonnes) in Australia. Evaluation of such treatments is an important step in the development of the technique into a routine system. The number of sites used in the basic experimental work was limited and application of the technique over a significant number of storages may reasonably expected to reveal problems not encountered previously.

The exposures to modified atmospheres currently believed to give complete insect mortality under Australian conditions are given in Table 1 (Banks and Annis, 1977; Banks, 1979).

TABLE 1

Exposure periods proposed for modified atmosphere disinfestation of grain (< 12% moisture content).

Atmosphere source	Initial target concentration in storage	Final target concentration in storage	Period of exposure within these limits
Carbon dioxide	> 70% CO <sub>2</sub> in air	> 35% CO <sub>2</sub> in air	> 10 days at 20°C
Nitrogen	< 1% O <sub>2</sub> in N <sub>2</sub>	< 1% O <sub>2</sub> in N <sub>2</sub>	> 6 weeks at 20°C <sup>α</sup>

α = Temperature dependent. For other temperatures see Banks and Annis (1977).

The CO<sub>2</sub> dosage regime given in Table 1 is referred to below as the 'target' regime and, if further gas is not added after the initial purging, implies a semilogarithmic gas loss rate of < 6.9% day<sup>-1</sup>.

In Australia, most grains are harvested in a condition regarded as dry by world standards. The receipt of wheat, the major grain crop, into the central bulk handling system is not permitted if the moisture content is > 12%. Though the grain may often be warm in storage (> 30°C) the qualities required for most of its end uses (e.g. baking, animal feed, noodle-making) are adequately retained in air over long periods of storage in the absence of insect pests.

In commercial terms, germination may fall significantly for some commodities, notably malting barley, but most of the grain produced does not need to be viable. Since modified atmosphere storage is generally not required for quality control in Australia, it appears unnecessary to maintain the atmosphere for longer than needed to ensure complete mortality of all insect pests in the storage. A perfectly sealed storage should then, in theory, act as an insect-proof enclosure and prevent re-invasion of the stored grain by insects. However, the standard of sealing currently accepted (Banks and Annis, 1977, 1980), though high does allow for some imperfections in the enclosure. The degree of sealing is assessed by a pressure decay test. This test cannot differentiate between residual leakage resulting from a few large holes in the fabric or an equivalent leak resulting from the combined effect of many small ones. On a 2000 tonne-capacity storage cell, a typical storage unit in Australia, the imperfections may amount to about  $1 \text{ cm}^2$  in total. It is thus possible that there may be a number of imperfections present of a size through which an insect can enter a store. The degree of protection which a store sealed to the required standard affords in practice results from a combination of a number of factors which, because of their random nature, cannot be assessed in the laboratory. These factors include the possible presence of a suitable hole through which an insect may enter, the presence of invading insects and the ability of the insects to locate the hole (see Barrer and Jay, 1980). Some data from commercial practice relevant to the assessment of the protection which can be expected is given below.

#### USE OF MODIFIED ATMOSPHERE TREATMENT OF GRAIN IN AUSTRALIA, 1917-1919

Externally generated atmospheres were used for disinfesting stored bagged grain in Australia during the period 1917-1919. This was possibly the first extensive use of such a system in the world (Winterbottom, 1922). Disruption of world trade by war had led to a substantial holding of grain in bag stacks around Australia. About 60,000 t wheat were treated with a low oxygen atmosphere generated from burning coke in a modified producer gas generator. At that time, carbon dioxide was thought to be the toxic agent to the insects and the generator was tuned to give maximum  $\text{CO}_2$  output, although originally designed to produce carbon monoxide. Fig. 1 shows the arrangement for treatment of a bag stack. We know now (Bailey, 1965) that the lack of oxygen was probably the cause of death, although its action would have been assisted by the  $\text{CO}_2$  present (see Bailey and Banks, 1980). After this highly successful operation, no further large scale use of controlled atmosphere disinfestation has been made for stored grain in Australia until the current experimental and commercial applications.



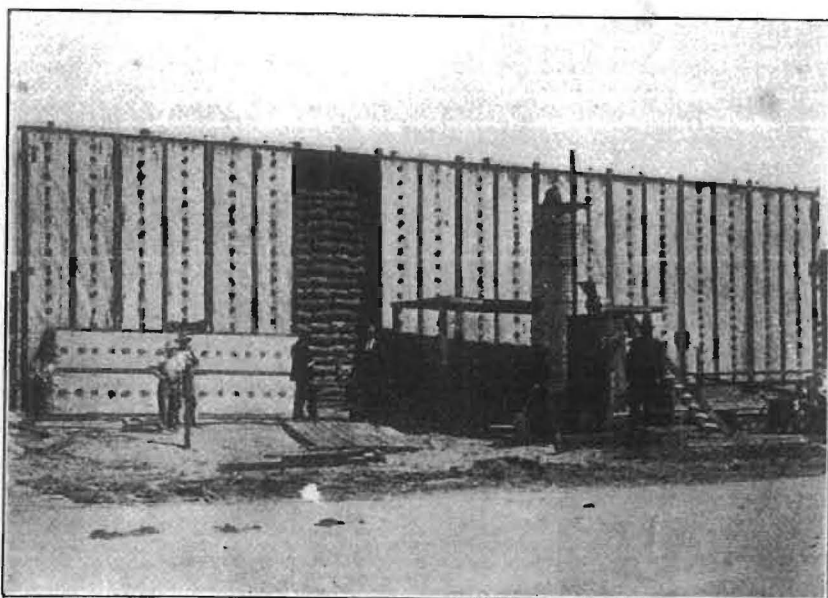


Fig. 1. A bag stack of wheat enclosed in wooden sheeting treated with bituminous materials for modified atmosphere treatment. In Australia during the period 1917-1919. Note the gas generator to the right of the picture (From Winterbottom, 1922).

#### PILOT COMMERCIAL USE OF MODIFIED ATMOSPHERES BASED ON LIQUID NITROGEN

In Australia, experimental trials under full scale storage conditions using tanker-delivered liquid nitrogen as a gas source were begun in 1972. The results of these trials, summarised by Bailey and Banks (1975) and Banks (1979), formed the basis for a publication (Banks and Annis, 1977) setting out how nitrogen atmospheres might be used commercially for treatment of bulk stored grain. The process is similar to that developed by Snamprogetti (Shejbal, 1979) in Italy. Several pilot commercial trials have been carried out using liquid nitrogen. These trials, summarised in Table 2 and 3, were conducted in general according to the procedures set out by Banks and Annis (1977): minor modifications were made as required by the equipment available. In two of these trials, the additional nitrogen required to maintain the low oxygen atmosphere after purging was initially added to the storage using a demand system set to keep a small positive pressure (120 Pa) within the storage. This system was later discarded in favour of a simple continuous gas input system, (Banks and Annis, 1977). The latter used less nitrogen than the former system to maintain the same oxygen level.

The efficiency of generation of the low oxygen atmosphere, can be assessed by comparison against a theoretical gas requirement where the interstitial atmosphere in the bulk is displaced by plug flow and the oxygen content in the headspace is reduced by a mixing process ('E<sub>3</sub>' of Banks (1979)). The efficiencies of purging

TABLE 2.

Pilot commercial usage of modified atmospheres generated in stored bulk grain from liquid nitrogen. Details of the storage and initial addition of nitrogen.

Site, bin no.	Date of trial	Tonnage treated and commodity	Filling ratio	Purge rate ( $\text{m}^3 \text{min}^{-1}$ )	% $\text{O}_2$ at end of purge	Purging efficiency, $E_3$ (%)	Nitrogen used <sup>c</sup> ( $\text{m}^3 \text{t}^{-1}$ )	Carried out by
Tara, 2	1977	1870 wheat	0.96	0.7-0.9	2.0	72	0.81	State Wheat Board, Queensland
Rennie <sup>b</sup> , 2	1977	1890 wheat	0.97	1.0	1.0	55	1.11	Grain Elevators Board, Victoria
Tara, 2	1978	1880 wheat	0.95	0.8-1.5	1.5	65	0.93	State Wheat Board, Queensland
Tara, 3	1978	1870 wheat	0.96	0.8-1.5	2.8	61	0.92	State Wheat Board, Queensland
Macalister, 3	1978	1650 wheat	0.85	1.0	1.0	55	1.11	State Wheat Board, Queensland
Toowoomba, 2	1980	930 barley	0.99	1.2	0.4	65	1.26	Barley Marketing Board, Queensland
Toowoomba, 3	1980	930 barley	0.99	4.8	0.6	79	1.01	Barley Marketing Board, Queensland

<sup>a</sup> = Efficiency,  $E_3$ , as defined in Banks (1979).

<sup>b</sup> = A second bin was also treated at this site and gave similar results.

<sup>c</sup> = Gas usage giving the observed headspace oxygen levels. These figures are not directly comparable with those in Banks and Annis (1977) which are for 1%  $\text{O}_2$  in the headspace.

TABLE 3.

Pilot commercial usage of modified atmospheres generated in stored bulk grain from liquid nitrogen. Details of sealing level of the storage and gas requirement to maintain the low oxygen atmosphere.

Site, bin no.	Date of trial	Pressure decay test, 1500-750 Pa (mins)	Filling ratio	Maximum % O <sub>2</sub> maintained	Observed maintenance rate (m <sup>3</sup> day <sup>-1</sup> )	Predicted <sup>d</sup> maintenance rate (m <sup>3</sup> day <sup>-1</sup> )
Tara, 2	1977	102 <sup>a</sup>	0.96	1.1	30-35	26
Rennie, 2 <sup>b</sup>	1977	6.0	0.97	1.8	27	25
Tara, 2	1978	78 <sup>a</sup>	0.95	2.0	40 <sup>c</sup>	27
Tara, 3	1978	22	0.96	2.0	40 <sup>c</sup>	27
Macalister, 2	1978	19	0.85	> 2.0	> 36	47
Toowoomba, 2	1980	7.8	0.99	1.0	17	20
Toowoomba, 3	1980	7.8	0.99	1.0	17	20

<sup>a</sup> = These values may be too high as they were taken during a period when the headspace was gaining heat.

<sup>b</sup> = A second bin treated at this site gave similar results.

<sup>c</sup> = This rate was used when the oxygen level was already > 2.0% in the bin and gave a slow reduction in oxygen level.

<sup>d</sup> = Derived from Banks and Annis (1977, Table 3) by linear interpolation.

in these trials are given in Table 2 and are, in general, slightly less than obtained under experimental conditions (73-92%; Banks, 1979). It will be noted that even in the case where the purging was least efficient (55%, Rennie, 2), the saving in gas usage, and thus cost of treatment, to be achieved by raising the efficiency to the level found in experimental trials would be small. In this example, an improvement in efficiency from 55 to 75% represents a saving of only  $0.2 \text{ m}^3 \text{ tonne}^{-1}$ . The values of expected nitrogen usage for various filling ratios given by Banks and Annis (1977) were based on results obtained from trials using filling ratios of  $< 0.92$ , and in practice, as the observed efficiencies given in Table 2 suggest, may be slightly low. After purging, the low oxygen atmospheres within the storages were maintained by addition of nitrogen to the headspace. The rates of input required are given in Table 3, together with pressure test values on the filled storages. The rates of gas input required are consistent with those given in Banks and Annis (1977).

At present only one series of treatments using liquid nitrogen as an atmosphere source is being carried out in Australia. These are still in progress. Shejbal (1979) observed that the germination of malting barley could be preserved for a longer period under nitrogen than in air storage. The current trial is designed to verify this observation under Australian conditions. In general, there is little current interest in Australia in use of nitrogen because, under the prevailing storage conditions, carbon dioxide-based systems are at present more attractive, both practically and economically.

## EXPERIMENTAL AND COMMERCIAL APPLICATION OF $\text{CO}_2$ -BASED MODIFIED ATMOSPHERES

### Experimental treatments using carbon-dioxide based systems

Banks (1979) provided details of the experimental trials carried out to that date by CSIRO with carbon dioxide in various types of bulk grain storage and transport vehicles. Wilson et al. (1980) described two pilot commercial treatments in welded steel bins and, in particular, demonstrated the need for gas recirculation during  $\text{CO}_2$  use in sealed structures in order to avoid regions of inadequate  $\text{CO}_2$  concentration in the upper parts of the storage. The Barley Marketing Board, Queensland (unpublished data) treated 950 tonnes of barley with 80%  $\text{CO}_2$  using a purge rate of about  $1 \text{ t h}^{-1}$  in a sealed metal bin (pressure decay time for 1500-750 Pa, 16 mins). An initial  $\text{CO}_2$  level of 79% was achieved with a subsequent decay rate of  $1.8\% \text{ day}^{-1}$  and an overall gas usage of 1.08 tonnes  $\text{CO}_2$  per 1000 tonnes barley. No detrimental effect of the  $\text{CO}_2$  on the barley was observed.

While  $\text{CO}_2$  is now being applied commercially in welded steel storage bins (see below) it is not yet used in other structures in Australia. Until recently, the main limitation to the more widespread use has been the lack of methods for

sealing other structures to an adequate level. This problem has now been overcome (Banks and Annis, 1980). The main constraints will now be financial, as the cost of sealing some structures can be high.

As a demonstration of the feasibility of using  $\text{CO}_2$  in a large shed-type storage, a type commonly found in many grain handling systems, a 16,400 tonne-capacity shed filled with wheat was treated with this gas, after the store had been sealed as described in Banks et al. (1979). The gas was added through a perforated aeration duct which ran longitudinally along the centre of the grain bulk. After purging, the gas was recirculated to maintain a uniform atmosphere within the shed. No further  $\text{CO}_2$  was added. Table 4 gives the general details of two treatments in successive storage seasons.

TABLE 4.

Details of  $\text{CO}_2$  treatment of a large, sealed grain shed (Harden, N.S.W.).

Nominal shed capacity	16,400 t	
Total enclosed volume	33,100 m <sup>3</sup>	
Season	1977-78	1978-79
Load	13,606 t wheat	16,144 t wheat
Pressure decay test (100-50 Pa)	5 mins	5 mins
$\text{CO}_2$ added	33 t	32 t
Rate of $\text{CO}_2$ applied	2.4 t $\text{CO}_2$ per 1000 t wheat	2.0 t $\text{CO}_2$ per 1000 t wheat
Maximum average $\text{CO}_2$	59% v/v	73 % v/v
Average $\text{CO}_2$ at 10 days	38% v/v	43 % v/v
Gas loss rate	4.5% day <sup>-1</sup>	5.2 % day <sup>-1</sup>
Purging efficiency ( $E_1$ ) <sup>a</sup>	73%	92%
Gas addition rate	0.6 t h <sup>-1</sup>	2.8 t h <sup>-1</sup>

<sup>a</sup> Calculated as in Banks (1979), assuming direct displacement of enclosed atmosphere by  $\text{CO}_2$ .

The increased purging efficiency in the second year is largely attributable to slight alterations in the design of the ductwork and increased speed of purging. In the first season the maximum concentration of  $\text{CO}_2$  achieved was lower than the target values (70%). However, an acceptable decay rate (i.e. < 6.9% day<sup>-1</sup>), was attained showing that the shed was adequately sealed. In the second season, the target  $\text{CO}_2$  regime was met. The average  $\text{CO}_2$  levels in the storage over the first 15 days of the trial are shown in Fig. 2. The form of this curve is typical of the decay of  $\text{CO}_2$  concentration which we have observed in various trials. There is an initial rapid decay shortly after purging and then a slower phase in which  $\log [\text{CO}_2]$  is proportional to time. This semi-logarithmic relationship is used below to estimate the initial  $[\text{CO}_2]$  obtained

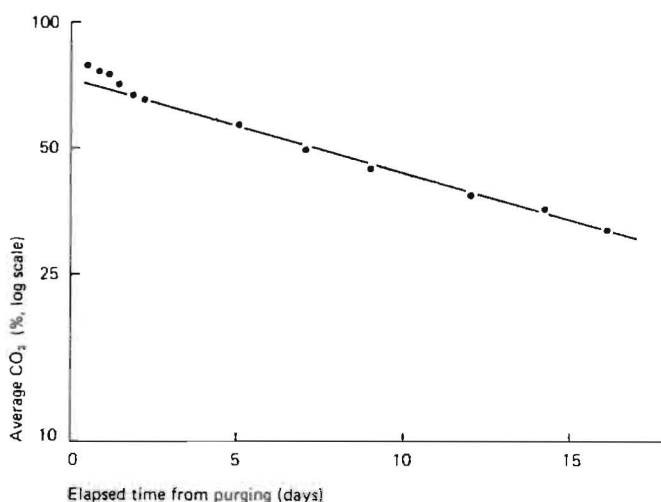


Fig. 2. Decay of average CO<sub>2</sub> concentration with time observed during the treatment of a sealed 16,400-tonne capacity shed as Table 4, 1978-79 season.

but the value calculated is slightly less than the true value because of the initial deviation from the relationship.

#### Commercial treatments with CO<sub>2</sub> in welded steel bins

In an adequately sealed storage, i.e. meeting the gastightness specification given in Banks and Annis (1977, 1980), it has been shown experimentally (Banks, 1979; Wilson et al., 1980 and above) that CO<sub>2</sub> may be applied in a 'one-shot' operation. That is, a rapid initial purge is used to give a high-CO<sub>2</sub> atmosphere within the storage. No further gas need be added. The target regime for insecticidal action (Table 1) can still be met, as gas loss is sufficiently restricted by the sealed enclosure. In contrast, nitrogen-based low oxygen atmospheres require addition of further gas to maintain oxygen levels below the target concentration during the required exposure period. The cost of the additional gas and its storage on site is significant in the overall economics of the process using nitrogen. The lack of need for such additional gas is the main commercial advantage which CO<sub>2</sub> has over nitrogen in the modes of use currently proposed in Australia. The details of commercial usage of CO<sub>2</sub> as a 'one-shot' system are given below. They confirm that the CO<sub>2</sub> application technique demonstrated experimentally (Banks, 1979; Wilson et al., 1980) can be duplicated in routine practice.

Two authorities in Australia concerned with bulk grain storage, the State Wheat Board, Queensland and the Grain Elevators Board, Victoria, have carried out commercial treatments of bulk wheat using CO<sub>2</sub>. The carbon dioxide was supplied to the storage sites as a liquid and applied at the rate of  $0.5-2 \text{ t h}^{-1}$  after vaporisation as described by Wilson et al. (1980). In Victoria, a single

TABLE 5.

Details of commercial treatment of bulk wheat, stored in sealed 2400 m<sup>3</sup> capacity welded steel cells, with CO<sub>2</sub> for the 1979-80 storage season by State Wheat Board, Queensland.

Site, bin no.	Tonnage treated <i>a</i>	Pressure decay test 1500-750 Pa (mins)	CO <sub>2</sub> used (t per 1000 t wheat)	Initial <sup>b</sup> % CO <sub>2</sub>	Decay <sup>b</sup> rate (% day <sup>-1</sup> )	Efficiency of purging (E <sub>1</sub> , %) <sup>c</sup>	Period of storage after CO <sub>2</sub> treatment (months)
Bell, 1	1811	11	1.04	74	5.1	76	12 <sup>e</sup>
Bell, 3	1831	8	1.03	77	8.3	78	11 <sup>ef</sup>
Brigalow, 3	1796	10	1.06	75	4.1	76	3
Brookstead, 2	1809	20	1.10	75	6.3	73	4.5
Cambooya, 3	1870	24	0.98	79	3.7	80	0.7
Macalister, 1 <sup>d</sup>	1800	12	1.04	66	3.4	68	8 <sup>f</sup>
Macalister, 2 <sup>d</sup>	1751	19	1.17	70	3.5	69	7
Macalister, 3 <sup>d</sup>	1831	-	1.07	78	4.5	76	5
Macalister, 4 <sup>d</sup>	1757	7	1.16	82	3.7	87	5
Macalister, 5 <sup>d</sup>	1797	10	1.16	74	2.8	71	5
Meandarra, 1	1644	11	1.18	84	4.1	94	<sup>g</sup>
Meandarra, 2	1866	8	1.05	80	3.4	76	2
Meandarra, 3	1861	7	1.00	62	4.9	62	<sup>g</sup>
Norwin, 1	1861	10	1.20	87	6.0	72	1
Norwin, 2	1874	9	0.96	86	6.9	70	6
Norwin, 3	1888	6	0.98	85	7.4	84	2
Tara, 1	1907	21	0.93	60	1.0	61	6
Ulimaroa, 1	1864	7	1.08	91	9.6	84	5
Ulimaroa, 2	1920	5.5	0.98	68	4.5	64	0.7
Ulimaroa, 3	1842	7	1.05	67	4.6	66	6
Ulimaroa, 4	1874	20	1.06	85	7.7	79	5
Warra, 1	1854	17	1.04	71	3.0	69	1
Warra, 3	1772	34	1.08	62	0.5	65	3.5
Warra, 5	1900	-	0.99	71	5.6	69	4
Warra, 6	1764	25	1.10	66	3.1	68	4 <sup>f</sup>
Warra, 8	1900	24	1.03	38	1.2	35	5 <sup>f</sup>
Yandilla, 1	1786	9	1.04	84	6.1	89	2

*a* = The bulk density of the grain stored varied widely. All bins were full, filling ratio > 0.96.

*b* = Estimated from two readings only taken between 1 and 4 days and 4 and 17 days after purging.

*c* = Efficiency, E<sub>1</sub>, as defined by Banks (1979).

*d* = Purge gas composition variable from 50-100% CO<sub>2</sub> in air.

*e* = Retreated with CO<sub>2</sub> after 7 months as a precautionary measure.

*f* = Insects present on outloading (see text).

*g* = Still in storage.



TABLE 6.

Details of CO<sub>2</sub> usage and tonnage of wheat treated with pure CO<sub>2</sub> (1979) or 85% CO<sub>2</sub> (1980) at two sites by Grain Elevators Board, Victoria.

Site, bin no. (date)	Pressure test <sup>a</sup> for 750-375 Pa (min)	Initial <sup>b</sup> % CO <sub>2</sub>	Decay <sup>b</sup> rate (% day <sup>-1</sup> )	Tonnage treated	Total CO <sub>2</sub> used (t)	CO <sub>2</sub> used (per 1000 t wheat)
Yarrowonga, (1979)	1	69	4.6	11,800	19.73	1.04
	3	89	4.3			
	5	102	6.7			
	7	89	4.7			
	9	97	5.6			
	11	85	4.5			
Rennie, (1979)	1	86	7.9	7,200		
	3	75	5.4			
	4	75	5.6			
	5	85	6.8			
Yarrowonga, (1980)	1	-	-	1,750	14.7	1.13
	3	53	1.5	1,850		
	5	48	0.3	1,790		
	7	65	3.1	1,820		
	9	65	3.2	2,080		
	11	67	3.4	1,870		
	13	59	2.7	1,840		
Rennie, (1980)	1	61	2.6	1,500	7.5	1.06
	3	62	3.7	1,600		
	4	64	3.3	2,000		
	5	56	2.2	2,000		

<sup>a</sup> Interpolated semilogarithmically from observations of the decay rate between 750 and 250 Pa.

<sup>b</sup> Estimated from readings for between 5 and 12 days after purging.

shielded inlet in the wall of the bin, as described by Banks and Annis (1977), was used. The Queensland bins were already equipped with a perforated distribution duct running radially from the floor centre to the bin wall and this was used for gas introduction. In most cases, pure  $\text{CO}_2$  was introduced. Exceptions are given below. The atmosphere within the bin was recirculated as described by Wilson et al. (1980) after  $\text{CO}_2$  introduction.

Tables 5 and 6 give details of the commercial treatments of bulk wheat with  $\text{CO}_2$  carried out for insect control since the beginning of 1979. The State Wheat Board carried out a further 13 treatments, in addition to the 27 summarised in Table 5. The details of these treatments are incomplete, but those available are consistent with the data in Table 5. The treatments given in Table 5 used an average of 1.06 t  $\text{CO}_2$  per 1000 t grain at an average purging efficiency of 74%.

In most of the treatments detailed in Tables 5 and 6, the target  $\text{CO}_2$  regime (Table 1) was exceeded. In a few instances the decay rate exceeded  $6.9\% \text{ day}^{-1}$  but in these cases, e.g. Ulimaroa, 1 (Table 5), the initial  $\text{CO}_2$  level was higher than the minimum acceptable (70%) and the decay rate was such as still to leave  $> 35\% \text{ CO}_2$  after 10 days. In some bins an inadequate initial  $\text{CO}_2$  level was achieved. On some occasions this could be a result of adding an inadequate quantity of  $\text{CO}_2$ , e.g. Meandarra, 3 (Table 5), but appears, on others, to be associated with unusually low loss rates, e.g. Tara, 1 (Table 5). The latter effect also is apparent in the 1980 results shown in Table 6. It should be noted that there is a significant correlation ( $P < 0.01$ ) between calculated initial  $\text{CO}_2$  level and loss rate, suggesting that the very low and high initial  $\text{CO}_2$  levels calculated for some trials may not represent the true values achieved but result from sampling and measurement errors which inevitably occur under the practical constraints of normal commercial practice.

Though the low values for the initial  $\text{CO}_2$  concentrations given in Table 6 may in part be due to inaccuracies in the extrapolation, they may also result from the use of 85%  $\text{CO}_2$  not 100%  $\text{CO}_2$  as a purge gas in the 1980 treatments. If purging is continued, as in these cases, until at least 80%  $\text{CO}_2$  is issuing from the top vent, a considerable quantity of  $\text{CO}_2$  is lost from the bin, resulting in a reduced purging efficiency and thus lower initial  $\text{CO}_2$  levels for an equivalent quantity of 100%  $\text{CO}_2$ . Which of these two effects caused the low initial  $\text{CO}_2$  values cannot be distinguished on the basis of the available data for the treatments.

The results given in Tables 5 and 6 show that the target regime for  $\text{CO}_2$  can be met under routine commercial conditions in full, welded steel bins using about 1.05 tonnes  $\text{CO}_2$  per 1000 tonnes wheat, meeting the current standard for sealing (pressure decay of 1500–750 Pa in  $> 5$  mins in a full bin, see Banks and

Annis, 1980). Further work appears necessary to assess the advantages and disadvantages of purging bins with air-CO<sub>2</sub> mixtures (e.g. 85% CO<sub>2</sub>) rather than with pure CO<sub>2</sub>.

#### Application of CO<sub>2</sub>-based modified atmospheres in concrete cells

Concrete cells are routinely constructed by the State Wheat Board, Queensland to a gastightness standard exceeding that currently considered suitable for modified atmosphere use (see Banks and Annis, 1980). Five of these cells were treated with CO<sub>2</sub>, in the same manner as the welded steel bins discussed above. After the purge was complete the bins were sealed as usual. A substantial negative pressure differential relative to the external atmosphere rapidly formed in the bin, which had to be relieved to prevent structural damage to the bin. In one case a -1500 Pa differential developed over 25 mins. The process continued over several days and is attributed to sorption of CO<sub>2</sub> on the concrete, i.e. carbonation as discussed by Hamada (1968). The details of the treatments in concrete cells are summarised in Table 7. It is notable that, despite a high level of sealing as assessed by the pressure test and a similar usage of gas and efficiency of purging to that obtained in welded steel cells (see Table 5 and 6), the decay rates are much higher and, indeed, are too high to allow the target CO<sub>2</sub> regime to be met unless additional gas is introduced.

Concrete grain storage cells are the only common type of large bulk storage in Australia that has not been treated successfully with 'one-shot' CO<sub>2</sub> atmospheres. The use of CO<sub>2</sub> in concrete cells has been discontinued commercially until the possible effects of the gas on the structure have been fully assessed. It is also necessary to determine if part or all of the sorption process is reversible as this will influence the gas usage in cells that have been treated with CO<sub>2</sub> more than once.

It is notable that CO<sub>2</sub> has been used with some success in the U.S.A. in concrete cells on both maize and peanuts (Jay and Pearman, 1973; Jay et al., 1970). However, further CO<sub>2</sub> was required to maintain adequate CO<sub>2</sub> levels after the initial purge and the rate of addition required was high.

#### INSECTICIDAL EFFICACY OF CO<sub>2</sub> TREATMENTS AND THE DEGREE OF PROTECTION AFFORDED BY A PARTIALLY SEALED STRUCTURE

In the mode of use proposed for CO<sub>2</sub> in Australia, the gas is added rapidly to the sealed enclosure and held there for a period exceeding 10 days. No further gas is added. The laboratory data on the action of air-CO<sub>2</sub> mixtures is incomplete and limited by its very nature to results from treatment of small numbers of insects that must be presumed to be of restricted genetic variability. Though laboratory observations in general support the target regime used, it requires

TABLE 7.

Details of treatment of wheat with CO<sub>2</sub> stored in 3400 m<sup>3</sup> capacity concrete cells by State Wheat Board, Queensland, during 1979.

Site, bin no.	Tonnage treated	Pressure decay test, 1500-750 Pa (mins)	CO <sub>2</sub> used (t per 1000 t wheat)	Initial <sup>a</sup> % CO <sub>2</sub>	Decay <sup>a</sup> rate (% day <sup>-1</sup> )	Efficiency <sup>b</sup> of purging (E <sub>1</sub> , %)
Oakey, 2	2880	6.5	1.05	101	17.3	78
Oakey, 2	2880	6.5	0.93	74	10.0	64
Brookstead, 2	2880	9	1.00	75	19.5	62
Millmerran, 2	3032	20	1.00	98	13.1	68
Meandarra, 1	2787	22	1.15	55	8.9	42
Meandarra, 2	2689	22	1.30	107	31.1	79

<sup>a</sup> = Estimates of initial concentrations of CO<sub>2</sub> and the semilogarithmic decay rate made from two readings only taken from 1 to 2 days and 3 and 17 days after purging.

<sup>b</sup> = Efficiency, E<sub>1</sub>, as defined in Banks (1979).

verification in practice where the numbers and variability of the insects to be controlled are much greater and where the insects are free to move in the grain bulk.

Assessment of the effectiveness of CO<sub>2</sub> treatment under field conditions is a complex problem. In general, adult stored product pest insects are more susceptible than most immature stages to air-CO<sub>2</sub> mixtures. If the latter survive, they may escape detection if inspection is carried out soon after treatment. If adult insects are discovered some time later, it is not possible to distinguish between a control failure in CO<sub>2</sub> use and an infestation derived from insects entering the storage after the end of the treatment. The two problems, the effectiveness of the current target regime and the degree of insect proofing afforded by an enclosure sealed to the current standard for use with modified atmospheres, must thus be considered together.

In the cases discussed below, the grain treated was in a condition (25-30°C, 10-12% m.c.) favourable for rapid multiplication of Rhyzopertha dominica and Tribolium castaneum, the prevalent pest species. Thus, if after a period of some months subsequent to CO<sub>2</sub> treatment insects are still not detectable. It is reasonable to assume that the treatment used gave a very high or complete kill of insects present. Furthermore, since in an unsealed structure, unprotected grain soon becomes infested, absence of infestation after treatment demonstrates that the sealed structure gives significant protection against reinfestation.

The period of storage of the grain in steel bins treated with CO<sub>2</sub> by the State Wheat Board is given in Table 5. The period was determined by operational requirements and not by insect infestation. In most cases, the grain was known to be infested prior to treatment and was stored for more than 3 months after treatment. Except as noted below, no live insects were found when the grain was sampled at a rate of about 0.1 kg t<sup>-1</sup> from the grain stream on outloading. In Victoria, during the 1979 season, with the exceptions given below, no live insects were detected on outloading using a similar sampling technique, although the grain had been held for 7 months after CO<sub>2</sub>-treatment (Table 6) and infestation was present before treatment. (Note the grain treated in 1980 as detailed in Table 6 has not yet been outloaded and no inspections have been carried out). These observations show that, in general, the CO<sub>2</sub> treatment regime used was highly effective and a substantial period of storage (several months) was achieved with grain being free of insects at outloading as judged by Australian commercial standards. Furthermore, reinfestation from external sources was not significant.

### Observations of insect infestation subsequent to CO<sub>2</sub> treatment

Instances of apparent control failures with CO<sub>2</sub> must be considered against this evidence of successful use. There are 6 cases involving 11 lots of grain, summarised below, where infestation has been detected after CO<sub>2</sub> treatment. In those cases where the target regime was met, infestation was detected only after some months of subsequent storage and thus survival of the treatment cannot be distinguished from reinfestation as its source.

Case 1. Bell, 3, Macalister, 1 and Warra, 6. (Kaimkillenbun, 1 (not given in Table 5, data incomplete) apparently similar). Grain harvested at the end of 1978 and treated then with fenitrothion and bioresmethrin (12 and 1 ppm respectively). Dosed with CO<sub>2</sub> as Table 5 one year later. Target CO<sub>2</sub> regime met or almost met (see Table 4). Very few R. dominica detected at outloading when about one-fifth of each bin had been discharged, but not before or subsequently, suggesting that the infestation was at the surface and very localised. Source of infestation: not known. The localised nature of the infestation suggests that it came from external sources shortly before outloading and was not a survival of treatment.

Case 2. Harden. 1977-78 storage season, treated as described in Table 4. Heavy infestation of T. castaneum and R. dominica prior to treatment. Apparently insect-free (probe sampled) 6 weeks after beginning of treatment, but R. dominica with some T. castaneum found after 13 weeks. Suggested source infestation: insect survival from treatment which did not attain the target initial CO<sub>2</sub> level.

Case 3. Harden. 1978-79 storage season, treated as described in Table 4. No insects found by probe sampling prior to treatment. Apparently adequate CO<sub>2</sub> regime. Store unsealed and small quantities of grain removed from time to time from 2 months after CO<sub>2</sub> dosing. Localised but rapidly developing infestation of T. castaneum found 6 months after treatment. Very few R. dominica and C. ferrugineus also present. Suggested source of infestation: insects introduced into store on machinery or personnel or insects flying in after breaking the seal. A very low level of survival from treatment cannot be excluded as source.

Case 4. Rennie, 3 and 4 in 1978-79 storage season. Treated with CO<sub>2</sub> as per Table 6 after 3 months storage. Condensation damage present in bin 3 prior to treatment. After 7 months from treatment, severe condensation on grain surface with many T. castaneum present. Source of infestation: not known. Recirculation duct may have been blocked resulting in low CO<sub>2</sub> levels in upper part of bin.

Case 5. Warra, 8. Grain harvested and treated as Case 6. CO<sub>2</sub> treatment as Table 5. CO<sub>2</sub> level apparently lower after 4 days after treatment (41%) than after 10 days (43%) suggesting poor mixing or recirculation. Many R. dominica present at outloading 5 months after treatment. Suggested source of infestation: insect survival of treatment because of inadequate CO<sub>2</sub> levels in parts of bin.

Case 6. Yarrawonga, 9 and 13. 1978-79 season. 1870 t wheat in each bin treated 2 months after loading with about 1 t CO<sub>2</sub> per 1000 t wheat. CO<sub>2</sub> regime in bin 13 apparently adequate, but in bin 9 inadequate because of leakages. Bin 9 retreated 1 month later after sealing leak, as Table 6, with an adequate CO<sub>2</sub> regime. Inspected by turning the grain through the elevator and sampling after 10 weeks storage after treatment. No insects found. At outloading 7 months after treatment, light crusting present on grain surface and a few *T. castaneum* found. Suggested source of infestation: insects introduced during inspection or inadequate CO<sub>2</sub> levels in upper parts of the bin resulting from blocked recirculation ducts.

In view of the uncertainties inherent in this kind of assessment, cases such as these cannot be taken as firm evidence that the target regime is inadequate, but should be considered in the light of further commercial experience. This should give evidence of whether such apparent control failures are to be expected or whether they result only from incorrect application of the system.

### CONCLUSION

The experimental and commercial trials and treatments reviewed above are part of the general development of various modified atmosphere techniques of grain storage and disinfection for Australian conditions. Techniques are now available for the modification and treatment of most large types of storage with some form of modified atmospheres, although CO<sub>2</sub>-treatment of concrete cells is still not commercially feasible and treatment of a large horizontal shed with nitrogen has not been attempted. In the future, it seems profitable to research on-site generation of various modified atmospheres, giving methods of avoiding the expense of transporting liquified gases to the storage site. A further assessment is required of the period of storage after an inert gas treatment that can be expected before insects reach commercially detectable levels. The technique will also present managerial problems that will need to be resolved. These include the need for a system of transportation of the insecticide-free grain from the storage to its destination which does not allow the grain to become sensibly infested en route, development of suitable remote sampling procedures to detect infestation if it occurs and management of the modified atmosphere treated stocks to ensure that any infestation is promptly eradicated before damage results.

The modified atmosphere systems described above are now at a stage in Australia where they can be regarded as one of the strategies available commercially against insect infestation. Their use will depend on the economics of the systems, which are already competitive with current practices in many situations, and the requirement, if it arises, for treatments that do not leave chemical residues on the grain.



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## METHODS OF APPLYING CARBON DIOXIDE FOR INSECT CONTROL IN STORED GRAIN

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## INTRODUCTION

Since the publication by Jay (1971) on using carbon dioxide ( $\text{CO}_2$ ) to control stored-grain insects, considerable interest has developed worldwide on the use of this technique and on the use of nitrogen ( $\text{N}_2$ ) and on combinations of atmospheric gases to achieve control. This interest has been generated because of the increasing worldwide problem of insect resistance to conventional insecticides and fumigants and also because of the residues associated with the use of these materials. Jay and Pearman (1973) showed that a 4-day  $\text{CO}_2$  treatment of shelled corn having a natural infestation of stored-grain insects gave almost 100% control. Shejbal et al. (1973) showed that similar control could be obtained by using  $\text{N}_2$ , but the time needed to obtain control was 10 days. Banks and Annis (1977) conducted trials with  $\text{N}_2$  in commercial, upright, welded-steel silos. Lethal atmospheres were attained and maintained in these silos for periods up to 30 days.

Both  $\text{CO}_2$  and  $\text{N}_2$  have merit in a residue-free insect-control program. Nitrogen has the advantage of filling 78% of the interstitial spaces initially. However, atmospheric oxygen ( $\text{O}_2$ ) in these interstitial spaces must be reduced to less than 1% to obtain effective insect control, a situation difficult to achieve and maintain in storage facilities that are not gastight. Therefore,  $\text{CO}_2$  is considered by the author to be more efficient than  $\text{N}_2$  in situations where tight sealing is physically impossible or where it is not economically feasible to seal the storage structure to rigid gastight specifications. A  $\text{CO}_2$  concentration of about 60% will give over 95% control of most stored-grain insects after a 4-day exposure at temperatures of  $27^\circ\text{C}$  or higher (Jay, 1971), and the  $\text{CO}_2$  concentration can fluctuate  $\pm 10\%$  and still provide effective control. The low-oxygen  $\text{N}_2$  atmosphere, on the other hand, must be held for 10 or more days at  $27^\circ\text{C}$  or above to be effective against life stages of stored-grain pests. Even so, Shejbal et al. (1973) reported that control of insect eggs was not obtained in a 10-day exposure to 0.5%  $\text{O}_2$  and 99.5%  $\text{N}_2$ . Unpublished laboratory studies by the author have shown that eggs of the red flour beetle, Tribolium castaneum (Herbst), do not hatch in atmospheres above 20%  $\text{CO}_2$  when

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the  $O_2$  level is as high as about 19%. Similarly, AliNiazee and Lindgren (1970) reported that the percentage of egg hatch of I. castaneum and I. confusum Jacquelin duVal was inhibited partially or completely delayed in  $CO_2$  atmospheres, while in similar  $N_2$  atmospheres there was about the same percentage of egg hatch as there was in those eggs exposed to air. The author (unpublished laboratory studies) found that a concentration of about 62%  $CO_2$  and 9%  $O_2$  gave over 90% control of 0- to 25-hour-old eggs of the cowpea weevil, Callosobruchus maculatus (F.), in 2 days' exposure, while concentrations of 99.7%  $N_2$  (balance  $O_2$ ) took more than 3 days to give the same control.

In summary,  $CO_2$  generally kills insects faster than  $N_2$ . It can be used in situations where leakiness may be a problem, and the concentration of 60%  $CO_2$  can be allowed to fluctuate  $\pm 10\%$  (or more, down to a low of 35%) leading to good control. (However, lower overall concentrations will necessitate longer exposure times.) In addition, sorption of  $CO_2$  by grain or oilseeds may make it more effective against species whose immature stages feed inside the kernel. On the other hand,  $CO_2$  is 1 1/2 times as heavy as air and will sink from the top to the bottom of the treated storage facility unless it is tightly sealed. This sinking necessitates either adding  $CO_2$  into the headspace periodically or recirculating the  $CO_2$ . (See Jay et al., 1970, for a description of this method. The method should be modified so that the recirculation fan is placed outside the storage facility to eliminate explosion hazard.)

If the above advantages and disadvantages of using  $CO_2$  have been taken into consideration and there still remains a question of whether to use  $CO_2$  or  $N_2$ , then economic factors enter into the decision. The comparative cost of the two treatments will depend on the availability of the gases, their unit cost (a unit is a ton, pound, cubic foot, cubic meter, etc.), the number of units required for effective insect control, the amount of grain to be treated per year (as the volume of gas used increases, unit costs will be reduced), transportation costs, and rental or purchase costs of vaporization equipment and storage containers (if equipment is purchased, can it be depreciated annually?).

The above advantages, disadvantages, and economic considerations obviously involve a decision-making process for which a flow chart is presented in Figure 1.

This paper presents three methods of applying  $CO_2$  to stored grain. The information presented by Jay (1971) on suggested conditions for using  $CO_2$  should be consulted in conjunction with the material presented here. If a decision is made to use  $N_2$  instead, Banks and Annis (1977) should be studied. However, some techniques described for  $CO_2$  could be used for  $N_2$  with slight modifications. Another method of creating modified atmospheres, the burning of air to reduce its oxygen content, is not considered.

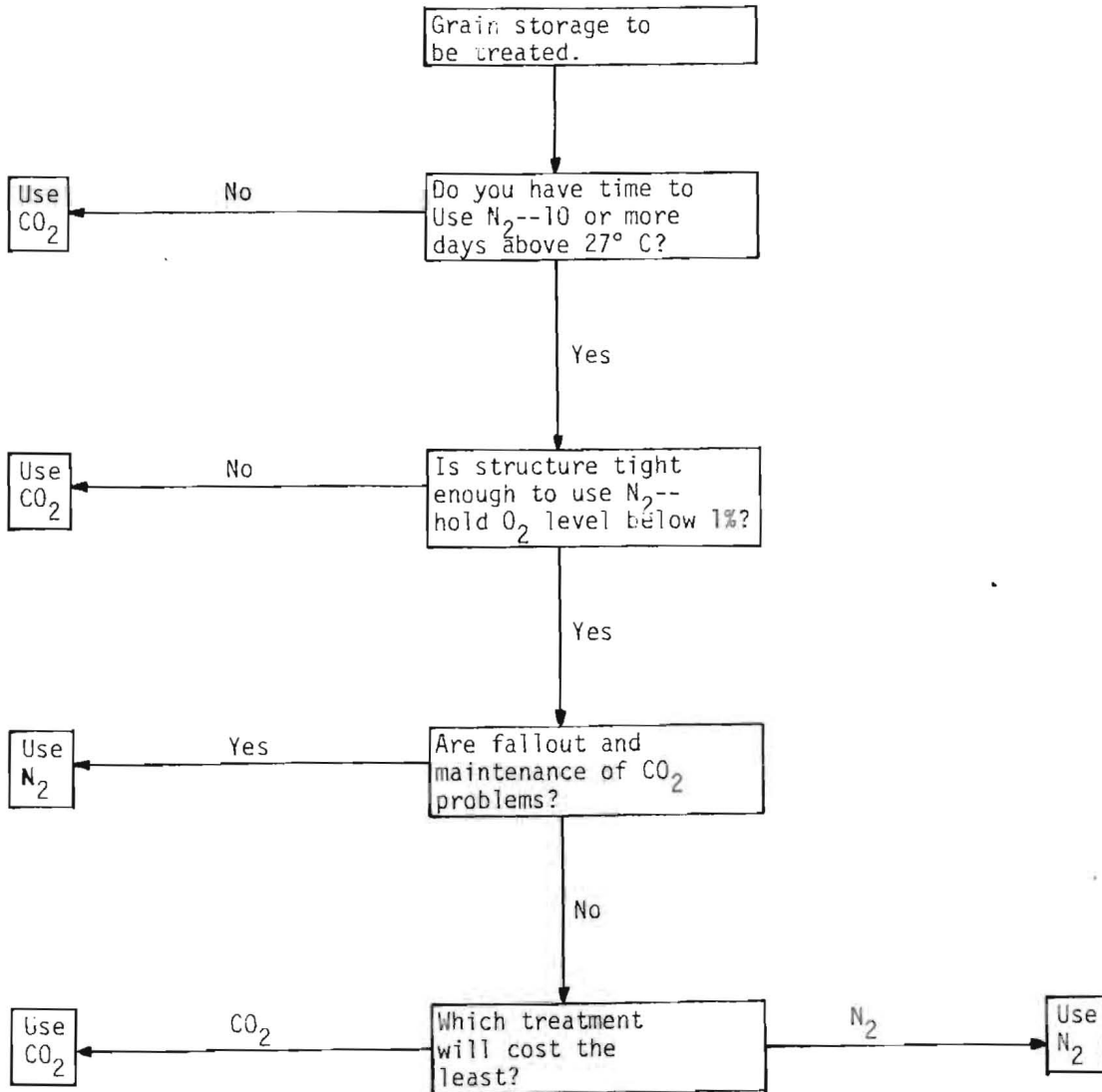


FIGURE 1. Determining which modified atmosphere to use, CO<sub>2</sub> or N<sub>2</sub>.

## METHODS OF APPLYING CO<sub>2</sub>

Since the publication by Jay (1971) became available, the author has conducted several additional field tests. One of these was described by Jay and Pearman (1973) and is summarized here (method 1) for comparative purposes with other application methods. The three methods described attain and maintain a concentration of about 60% CO<sub>2</sub>. The tests were conducted in 1,038-m<sup>3</sup> (36,644-ft<sup>3</sup>) upright concrete silos measuring 24.7 m (81 ft), excluding depth of discharge chute, by 7.3 m (24 ft). The silos each contained about 711 metric tons (28,000 bu) of shelled corn (maize) having an 11% to 16% moisture content. (In some tests the corn was moved into a silo as the CO<sub>2</sub> was being applied.) The equipment used in all tests for applying the CO<sub>2</sub>, including supply tank, vaporizers, and regulators for monitoring and controlling the concentration after the desired concentration was reached, was similar to that described by Jay and Pearman (1973). Deviations will be described in the individual tests.

Method 1: Purge a full silo from the top

This is essentially the method described by Jay and Pearman (1973) and will not be dealt with at length. It involves introduction of gaseous CO<sub>2</sub> into the headspace above the surface of the grain. The CO<sub>2</sub> is forced down into the grain by positive pressure on the headspace of the storage facility. The CO<sub>2</sub> mixes with and displaces a portion of the existing atmosphere and creates a modified atmosphere lethal to any insects present. Its advantages are that it can be used where no other method is available, it requires only one application line, labor requirements are minimal, and costs may be lower than with the other methods. Its disadvantages are that CO<sub>2</sub> is lost in mixing and "blowback," purging time is longer than in method 3, and vaporization requirements are high.

### Method 2: Lift the atmosphere out

In this test and in method 3, a set of air-sampling lines was placed into a silo through each of the two access openings in the flat top prior to filling. These lines differed from those which were probed into the corn in method 1. One of the two openings was about 1.2 m (4 ft) in from the wall, where the corn was discharged into the silo, and the other opening was about 1.2 m (4 ft) from the center of the top. Each set of sampling lines was made up of six 0.6-cm-i.d. (1/4-inch) polyethylene tubes taped together so that six samples could be taken at intervals of about 3 or 6 m (10 or 20 ft) below the surface of the grain. Metal tips having slits to allow air entry were placed on the end of each line. The 12 lines were run to an air-sampling valve mounted outside on the top of the silo. One line was run from this valve to a gas partitioner in a mobile laboratory. This apparatus sampled 12 sites in the silo during each test, and it was similar to that used in method 1.

Prior to filling this silo, a 0.6-m-wide (2-ft) T-shaped pipe made of 2.5-cm-i.d. (1-inch) heavy-duty rubber application hose was lowered into the metal discharge cone at the bottom of the silo. The pipe had a 2.5-cm (1-inch) copper ell on each end. The ells were turned up toward the top of the silo. Fine-mesh screen was soldered over the openings to prevent entry of corn and foreign material. The silo was filled, and the depths of the sampling lines in the corn nearest the wall were determined to be about 1.2, 4.3, 7.3, 13.4, 19.5, and 25.6 m (4, 14, 24, 44, 64, and 84 ft), and near the center, about 0.6, 3.7, 6.7, 12.8, 18.9, and 25.0 m (2, 12, 22, 42, 62, and 82 ft). The difference in depth between the two sets of lines was due to the slope of the grain from the discharge area to the opposite wall. The longest side line and the center lines were in the cone-shaped discharge chute near the T-shaped application pipe.

Gas flow was started into this silo from a full 8,940-lb tank of liquid  $\text{CO}_2$ , using the built-in vaporizer. The access openings in the silo were opened after 0.6 h of application to relieve pressure. After a 1-h application, 100%  $\text{CO}_2$  was found at the lowest (25-m) sampling points, while only small amounts were found at other sampling points. After 4 h, the  $\text{CO}_2$  concentration at about 19 m in the center site had reached 100%, and in the side samples, from 52% to 73%.

Approximately  $84 \text{ m}^3$  (340 lb) of  $\text{CO}_2$  was introduced during the first 4 h of application. At this time the flow rate was increased from about  $21 \text{ m}^3/\text{h}$  (85 lb/h) to about  $46 \text{ m}^3/\text{h}$  (185 lb/h). After 6.75 h,  $\text{CO}_2$  concentrations at the 13-m center and side sites were 95% and 20%, respectively. Samples at depths of 19 and 25 m still contained 100%  $\text{CO}_2$  at this time. After 8.25 h of treatment, the  $\text{CO}_2$  concentration at the 13-m side site was 89%, while samples at 7- and 4-m depths and samples just below the surface contained 2% to 4%  $\text{CO}_2$ . After 11 h, the  $\text{CO}_2$  concentration was 95% at the 7-m center site but was only 2% at the side sampling point at this depth. Carbon dioxide used in the first 12 h was  $454.4 \text{ m}^3$  (1,840 lb). Application was continued at an average rate of  $46.0 \text{ m}^3/\text{h}$  (185 lb/h). After 12 h, the concentration in the 7-m side site had risen to 73%.

After 13.25 h, there was 98%  $\text{CO}_2$  at the 4-m center site, while the side sample at the same depth contained 26%  $\text{CO}_2$ . All samples below these depths contained from 97% to 100%  $\text{CO}_2$ . After 14.5 h, the sample at the 0.6-m center site had a concentration of 93%  $\text{CO}_2$ , and the sample at the 1.2-m side site contained 12%  $\text{CO}_2$ . This low concentration was probably caused by a heavy concentration of foreign material in the area. Approximately  $592.7 \text{ m}^3$  (2,400 lb) of  $\text{CO}_2$  had been used at this time. Flow was terminated, and it is calculated that 2 additional hours at  $46.0 \text{ m}^3/\text{h}$  (185 lb/h) would have been required to

penetrate the heavy concentration of foreign material under the discharge chute at the side.

Ten hours after shutdown all samples at and below 13 m had 80% or more  $\text{CO}_2$ , while samples above this point contained from a trace to 22%. Thirty-two hours after shutdown samples at or below 19 m had 52% to 80%  $\text{CO}_2$ , and samples above this point contained from a trace to 26%.

This silo was not equipped with an aeration fan, a fan shaft, or any other facility for introducing  $\text{CO}_2$  directly from the bottom of the silo. In silos so equipped, there would be no need for the hose and the T-shaped pipe used.

The advantages of this method are low labor requirements,  $\text{CO}_2$  costs comparable to other application techniques, and no loss of  $\text{CO}_2$  in mixing and blowback. On the other hand, this is the slowest of all the methods tested, and there are problems in penetrating areas having a lot of foreign material. Also, the method produces a 100%  $\text{CO}_2$  concentration, and concentrations above 60% seem to result in reduced insect control. (Apparently, a low oxygen,  $\text{O}_2$ , concentration anesthetizes the insects and prevents the venting of  $\text{CO}_2$  and water, which is believed to be partially responsible for death.) However, this could be averted by blending air with the  $\text{CO}_2$ , producing a concentration of about 60%  $\text{CO}_2$  and reducing total  $\text{CO}_2$  used. Finally, two application lines are required, one to purge and one to supplement fallout of the heavier-than-air  $\text{CO}_2$  from the headspace, and vaporization requirements are high.

#### Method 3: Apply $\text{CO}_2$ in the grain stream

This test was conducted with liquid  $\text{CO}_2$  from the same tank previously described. The  $\text{CO}_2$  emerged from the equipment in a semisolid form called "snow" by the  $\text{CO}_2$  industry. However, the snow soon sublimated and produced  $\text{CO}_2$  gas. A  $\text{CO}_2$  "horn" was attached to copper tubing (1.9-cm i.d., or 3/4-inch), which was run from the liquid line on the  $\text{CO}_2$  tank to the top of this silo. The tubing and horn were thoroughly grounded to prevent any sparks around the discharge area. Liquid  $\text{CO}_2$  flow was started into an empty silo at the outer access opening, and in 10 min about 49.4  $\text{m}^3$  (200 lb) had been introduced. Corn flow was then started into the silo and continued, with two brief interruptions of 3 min each, until the silo was filled with about 711 metric tons (28,000 bu) of corn in 2.33 h. At this point about 642  $\text{m}^3$  (2,600 lb) of  $\text{CO}_2$  had been introduced into the silo at a rate of 4.6  $\text{m}^3/\text{min}$  (18.6 lb/min). Carbon dioxide application was continued an additional 0.6 h, except for 10-min interruption. At this time an additional 98.8  $\text{m}^3$  (400 lb) of  $\text{CO}_2$  had been applied, and a small mound of snow had accumulated directly under the horn on the surface of the corn.

During filling, gas samples were taken adjacent to the access door where the  $\text{CO}_2$  blowback from the silo was noticed. Carbon dioxide concentrations were 5.4% to 16.2%, indicating a large loss of gas from the silo.



Three and one-half hours after the start of application (30 min after the snow had accumulated on the top of the corn), a complete series of air samples was taken from this silo. These 12 samples averaged 71% CO<sub>2</sub> and 6% O<sub>2</sub>. The concentration of CO<sub>2</sub> ranged from 82% to 24%, and the O<sub>2</sub> concentration ranged from 2.1% to 11.4%.

Two and one-half hours after the application was stopped, an additional 86.4 m<sup>3</sup> (350 lb) of snow was applied through the top of the silo in 20 min. Twenty minutes later, the CO<sub>2</sub> average was 60% and the O<sub>2</sub> average was 8% in all 12 samples. In this series of samples, the 1-m side sample contained 37% CO<sub>2</sub>, and the 25-m center sample contained 44% CO<sub>2</sub>; the range of CO<sub>2</sub> was 84% to 37%, and the O<sub>2</sub> range was 4% to 11%.

To maintain the CO<sub>2</sub> concentration, gas was reintroduced 6 h after the initial introduction. This was accomplished by running a 1.9-cm-i.d. (3/4-inch) rubber hose from the gasline on the tank into the headspace of this silo. Gas flow was controlled by a CO<sub>2</sub> analyzer equipped with relays that controlled a solenoid valve in the application line. The controller was calibrated to maintain 55% to 60% CO<sub>2</sub> in the silo. This equipment is described in more detail by Jay and Pearman (1973).

Sixteen hours later, a complete series of gas samples was taken from the silo. The CO<sub>2</sub> concentration ranged from 59% to 52%, and the O<sub>2</sub> concentration ranged from 8% to 10%. The test was terminated at this point since experience has shown that once the desired concentration has been attained, the CO<sub>2</sub> analyzer and associated equipment will maintain the concentration within the silo at the concentration lethal to most stored-grain and oilseed insects.

This method is fast, and vaporization equipment requirements are low. The disadvantages include danger of explosion caused by improperly grounded application equipment, a potential need for two application lines, excessive loss of CO<sub>2</sub> from blowback, and high labor requirements (constant attention during application is required).

## DISCUSSION

The decision on which application technique to use will have to be based on several factors (Fig. 2). Table 1 presents a breakdown of the CO<sub>2</sub> costs for each method. The costs are based on the quantities of liquid CO<sub>2</sub> used in each test at the most recent prices the author could obtain and do not include the expense of renting storage tanks and vaporization equipment. At the lowest quoted price for CO<sub>2</sub>, the costs ranged from \$0.0175/bu (\$490 for 28,000 bu) for method 3. Since each method has its advantages and disadvantages and, in some cases, only one or two of the three methods could be used, the difference in cost between the three methods may be considered minimal.

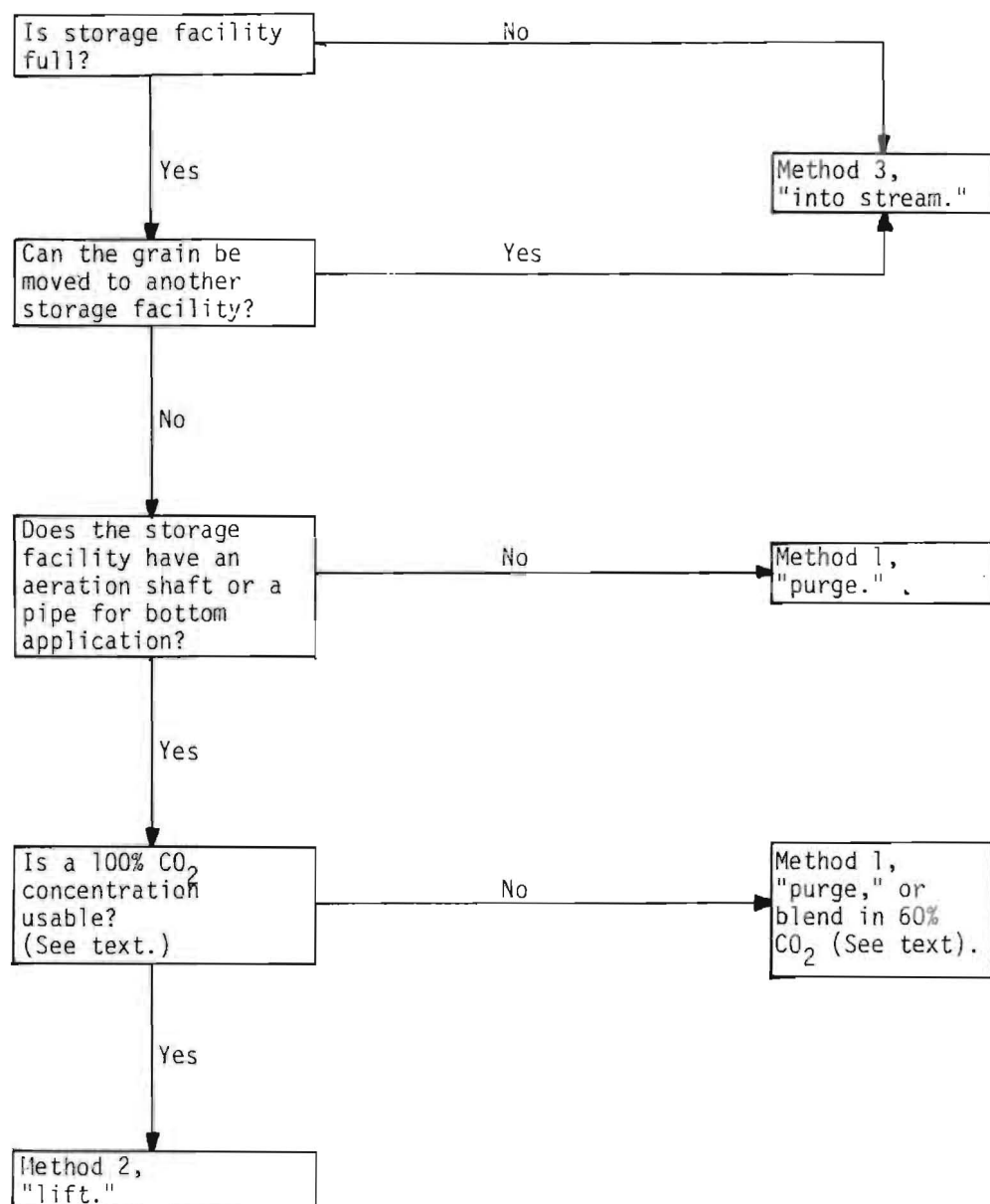


FIGURE 2. Selecting a method for applying CO<sub>2</sub>.

TABLE 1

Characteristics of three CO<sub>2</sub> application methods [CO<sub>2</sub> applied to 711 metric tons (28,000 bu) of corn].

Characteristic	Application method		
	Method 1, "purge"	Method 2, "lift"	Method 3, "into stream"
Time to attain lethal concentration (h)	8	16.5	3
Quantity to reach lethal concentration:			
Cubic meters <sup>1/</sup>	625	715	827
Pounds	2,530	2,900	3,350
Quantity to maintain lethal concentration for 96 h (including purge time):			
Cubic meters per hour	17.8	<u>2</u> /19.3	.19.3
Pounds per hour	72	<u>2</u> /78	78
Total CO <sub>2</sub> to treat:			
Cubic meters	2,332	2,568	2,679
Pounds	9,445	10,400	10,850
Cost per bushel (\$):			
CO <sub>2</sub> at \$0.052/lb <sup>3/</sup>	0.0175	0.0193	0.0202
CO <sub>2</sub> at \$0.078/lb <sup>4/</sup>	0.0263	0.0290	0.0290
CO <sub>2</sub> at \$0.090/lb <sup>5/</sup>	0.0304	0.034	0.0349
Cost per metric ton (\$), CO <sub>2</sub> at \$0.052/lb <sup>3/</sup>	0.691	0.761	0.794

1/ Calculated from 8.72 ft<sup>3</sup> CO<sub>2</sub> gas produced from 1 lb CO<sub>2</sub> liquid at -17.8° C and 305.5 lb/in<sup>2</sup> absolute.

2/ Calculated from "into stream" application, method 3.

3/ U.S. price, 1978, yearly usage of 100-500 tons CO<sub>2</sub>.

4/ U.S. price, 1978, yearly usage of 50-100 tons CO<sub>2</sub>.

5/ U.S. price, 1978, yearly usage of 0-50 tons CO<sub>2</sub>.

These studies were conducted at only one storage facility, in upright concrete silos containing corn. Little effort was made to correct the obviously high leakage. Anyone using this information to conduct further field tests should consider all potential leaks. In upright concrete silos any cracks in walls are potential areas for gas leaks. The largest losses will occur around the discharge spouts at the bottom of the silos.

Each facility to be treated with modified atmospheres will have varying factors of volume, type and amount of grain, leak rate, temperature, vaporization equipment (if required), and other factors. Therefore, this information should be used as a guide and not as a representative indicator as to how a treatment will work in a given situation. Additional field studies are needed.

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## ADVANCES IN GRAIN STORAGE IN A CO<sub>2</sub> ATMOSPHERE IN JAPAN.

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### INTRODUCTION

The reduction of post-harvest losses is now a common food strategy throughout the world. A considerable amount of work has been carried out by several groups in each country on loss assessment and reduction studies. Storage must be one of the most important stages for the reduction of post-harvest losses.

In Japan rice is the major staple food grain and one of the few self-sufficient foods. The storage and distribution system controlled by the government is well established for the annual consumption of rice and post-harvest losses are assumed to be kept at an acceptable level. Reserves of surplus rice for food security purposes, however, are still hindered by storage problems. The Japanese people usually cook polished rice grains in water without adding seasoning, so the eating quality of the cooked rice directly reflects the properties of the grain. The Japanese are very sensitive to the taste of cooked rice, so that even the very slight changes in the physical and chemical nature of rice after it has been stored for a year are detected and cause dislike. The loss of quality or acceptability in rice during storage is serious because rice is stored in ambient temperature warehouses where it is hot and humid in summer. In addition to this, the rice has been transported and stored non-hermetically in small bags made of jute or kraft paper. The longer the storage period, the greater the quality loss. Grain storage in a CO<sub>2</sub> atmosphere has been advancing step by step since 1967 in Japan. Few scientific studies on controlled atmosphere storage of grains have been reported, so advances in grain storage in a CO<sub>2</sub> atmosphere can be summarized in the results obtained from the experiments of the author's group.

### GRAIN STORAGE IN A CO<sub>2</sub> ATMOSPHERE

It is favorable for the safe storage of grain to eliminate the oxygen from the structures where grains are stored, in addition to controlling the stored temperature and the moisture content of the grains. Although oxygen concentration in small pouches of dried foods can be reduced easily by evacuation or the use of oxygen scavenger, it does not seem to be practical to apply these techniques to grain storage. In the hermetic storage of grains, it is assumed that the oxygen will be exhausted and that CO<sub>2</sub> will be produced within a few days due to the respiration both of the grains and of contaminated organisms. The rate at which the oxygen is

exhausted depends mainly on the moisture content of the grain. The higher the moisture content of the grain, the higher the rate of exhaustion. During the period of oxygen exhaustion, however, chemical changes inevitably take place affecting the quality of the grain. Grain storage in an inert gas atmosphere seeks to avoid or lower the changes which must be caused by the presence of oxygen. Fundamentally, what is involved is the retardation or suppression of the respiration of the grain itself, the insects, microorganisms and so on. Normally, the gases used for this purpose are either carbon dioxide or nitrogen, both readily available commercial products. Results obtained in the controlled atmosphere storage of various fruit and in the gas storage of processed foods show some differences with regard to these gases. The authors are interested in the following two distinctive features of  $\text{CO}_2$ .

(1) Carbon dioxide is the end product of the aerobic combustion of organic materials in living organisms. Thus, the accumulation of  $\text{CO}_2$  beyond a certain critical concentration acts as a signal that can slow down the rate of the reactions by which it was formed, a type of control known as "feed-back inhibition". In fact, inhibition of succinate dehydrogenase is well known in connection with the  $\text{CO}_2$  disease of some fruit. This enzyme is one of the members in the tricarboxylic acid cycle, which takes place in the mitochondria and consists of a cycle series of reactions in respiration. The authors also reported the inhibition of catalase by higher partial pressure of  $\text{CO}_2$ . This enzyme is often noticed as a good index of viability of the grain. In addition, it is proved that the activity of ribulose-1,5-diphosphate carboxylase, which is the key enzyme in the Calvin cycle, is controlled by  $\text{CO}_2$  and  $\text{O}_2$ , similar to the manner of hemoglobin in animal blood. The reversible nature of the inhibition by  $\text{CO}_2$  has been reported in the study on the activity and metabolism of goat sperms stored in physiological liquid under various partial pressures of  $\text{CO}_2$ . These biological results indicate the distinctive feature of  $\text{CO}_2$  in the metabolism of stored grains.

(2) Another distinctive feature of the action of  $\text{CO}_2$  is its sterilisation effect on the growth of fungi and other microorganisms. Even when there is a high level of oxygen remaining in the vessel, a higher concentration of  $\text{CO}_2$  (more than 80-90%) effectively prevents the fungal growth, while  $\text{N}_2$  cannot do so. This is a favorable characteristic for the practical use of  $\text{CO}_2$  in the prolonged storage of grain, because the risk of contamination of oxygen into a stored vessel cannot be eliminated.

## RESULTS OF SEVERAL EXPERIMENTS

### 1. Fundamental experiments on a laboratory scale (Mitsuda and Yasumatsu, 1955, Mitsuda et al., 1958, 1969, Mitsuda, 1969a, 1969b)

On the basis of hints obtained from studies on the respiration of cold-blooded animals in hibernation, which were conducted in the laboratory at Kyoto University

around 1952, the authors introduced the hermetic storage of cereal grain in naturally cold places. This is based on lowering the respiration in grain by using both  $\text{CO}_2$  and low temperatures, thus retaining the original freshness of the grain even after prolonged storage. The most desirable temperature for maintaining rice grain quality is around  $13^{\circ}\text{C}$ . The less the variation in temperature throughout the year, the better will quality be retained. Appropriate places satisfying these conditions are spaces under the water of lakes, ponds or man-made pools at the seacoast or spaces under the ground. Laboratory-scale storage experiments were carried out from 1967 to 1969. The results obtained in these experiments are:

- (1) Packaging materials for underwater or underground storage of grains must be:
  - i) Resistant to structural attack by water, ii) Moistureproof,
  - iii) Resistant to physical shock, and iv) Airproof.

A flexible bag made of plastic laminated films with a biaxially-oriented polyamide film (nylon type), a polyvinylidene chloride film and a polyethylene film, was found to meet all of the above requirements very successfully.

(2) The moistureproof quality of the bags was confirmed in storage experiments which were carried out in water baths, ponds and man-made pools.

(3) Carbon dioxide was used for grain storage and the airproof quality of the bags was confirmed. An interesting phenomenon was also found that grain in flexible bags containing  $\text{CO}_2$  was tightly packed as if packed in vacuo.

(4) Various factors for large-scale storage in water were examined and suitable forms of storage unit and equipment were designed. The annual data of the weather conditions in Lake Biwa were collected and examined in order to select a storage place in the lake.

## 2. Underwater and underground storage experiments (Mitsuda and Kawai, 1970, Mitsuda et al., 1971a, 1971b, 1972, 1973).

An experiment in underwater storage of cereal grains was carried out from 1969 to 1972. Paddy, brown and polished rice were packaged in the plastic bags with  $\text{CO}_2$  and stored for 3 years in the water of Lake Biwa. Wheat, barley and soybeans were stored in the same way as the rice grains. In parallel with the underwater storage experiment, the underground storage of brown rice was also carried out at an abandoned mine on Shikoku Island. These experiments were carried out to investigate the possibility of storing grain on a practical-scale in naturally cold places, i.e. with a temperature below  $13^{\circ}\text{C}$ . Results obtained are summarized as follows:

(1) The waterproof quality of the laminated film in such grain storage conditions proved to be highly satisfactory over a relatively long period.

(2) Underwater storage prolonged the storage life of the grains. The original freshness of the grains even after prolonged underwater storage could be shown by the fact that considerably higher biological activities such as germinative capacity, and catalase and peroxidase activities were detected for the grains stored in the



water than those kept in atmospheric conditions. In particular the germinative capacity of the paddy rice retained its original value even after a storage period of 3 years. Changes in the contents of vitamin B, reducing sugar, water-soluble nitrogen, and other substances were also found to be less for the rice stored underwater. Palatability of the grains stored underwater was found to be satisfactory by panel tests. The strength of stale-flavor as determined by the amount of volatile carbonyl compounds, rheological characteristics as measured by texturometric indices and cooking qualities as revealed on boiling, showed that the deterioration proceeded at a considerably lower rate for the grains stored in the water than for those stored in atmospheric storage.

(3) As to the atmosphere within the bags of laminated film, the bag containing CO<sub>2</sub> gas was found to be more effective than that containing air in preventing changes in chemical composition, in lowering the diminution of peroxidase activity, and in retarding the development of stale-flavor.

(4) A total system on a 10 thousand metric ton-scale has been drafted for underwater storage. The cost for this system amounts to about \$34.00 or less per ton of rice, as calculated by the supporting committee for grain storage research at the Research Institute for Production Development in March, 1970. The committee was composed of five companies in Japan; UNITIKA Ltd., Sumitomo Chemical Co., Sekisui Chemical Co., Ltd., Asahi-Dow Co., Ltd., and Taiyo Kogyo Co., Ltd. The cost appeared fairly low compared with that for temperature and humidity controlled storage systems.

(5) Various kinds of plastic laminated films were compared with each other as to their abilities to maintain grain qualities. Experimental results suggested that the composition of the laminated film could be simplified in accordance with the storage conditions. The usefulness of polyvinyl chloride bottles was also tested in underground storage.

### 3. Transportation and storage experiments in ambient temperatures (Mitsuda et al., 1974).

The rice stored in low-temperature warehouses (below 20°C) or in country elevators (12-13°C) located in granary areas had often suffered significant deterioration when polished in a large-scale central mill and transported from there to urban areas. This kind of trouble was particularly noticeable in the rainy season or summer. The biggest cause of this trouble was the packaging method. Open packages such as straw, jute or kraft paper could not prevent the permeation of vapor and/or gases in the atmosphere. Polyethylene bags were also unsuitable in the same way because they contained a few tiny holes to add flexibility and prevent slipping in loading. Another problem was the storage of rice in polished form. In Japan, storage of rice in polished form had been avoided because the rice deteriorated much more rapidly than brown or paddy rice. The recent development of a large-scale central polishing system was, however, increasing the necessity to store and

transport rice in polished form and thus causing deterioration of the qualities of rice as consumed. The need for new methods of storing and transporting the polished rice was the major impetus for this experiment. A transportation and storage experiment of grain packed by the CEM (Carbon dioxide Exchange Method) technique was carried out from August 1971 to March 1973 in order to develop a new system for transportation and storage of grains with the least loss of quality. Two varieties of japonica rice which had been stored in a low-temperature warehouse (below 20°C) and in a country elevator (12-13°C) were polished at a large-scale central polishing factory in Iwate Prefecture, one of Japan's granary areas. About one metric ton of polished rice was packed by the CEM and the conventional methods using kraft paper bags and polyethylene bags. The rice was transported from Iwate to an urban area in a 5 ton container by rail and the journey took 5 days during August, the hottest month in Japan. The ambient temperature in the container fluctuated between 20°C and 47°C during transportation. Biochemical and palatability changes were examined in the rice just after arrival at destination and after 3 and 8 months storage at a rice dealer's. Some of the rice was stored for another 11 months and then examined again. Sample analysis included:

moisture content, water-soluble acidity, free fatty acid content, TBA value, amylography, cooking qualities, microbiological tests, gas chromatography of volatile carbonyls from cooked rice, texture evaluation of cooked rice by using a texturometer, and sensory analysis of cooked rice by 24 panels.

The results obtained in these analyses demonstrated that rice packed by the CEM technique could be maintained in better condition than that packed by conventional methods, particularly in terms of the analyses of water-soluble acidity, free fatty acid content, amylography and gas chromatography of volatile carbonyls from cooked rice. The development of mould and insects was perfectly retarded in the skin-packages in the CEM method without using fumigation, but this was not possible in the rice stored in conventional bags.

A new type fully automatic packaging machine was constructed by the Japan Steel Works, Co., Ltd. which made it possible to carry out the large-scale packaging and transportation test in January 1973 needed to obtain the basic data for the practical feasibility of CEM. These experiments were performed in cooperation with agricultural government offices, agricultural auxiliary organisations and some private companies. In this experiment, about 3,500 packages of polished rice weighing 5kg each were prepared by using the packaging machine at Niigata Prefecture, also one of Japan's granary areas. The packages were palletised and transported by train from Niigata to Osaka, the second biggest city of Japan. Polishing conditions, the amount of CO<sub>2</sub> gas required for the process with the packaging machine, the temperature changes of the rice, the correlation between breakage incidents and the thickness of bags and so on were all observed. Opinions of retailer on the process were also gathered. Examination of all the packages carried out after their arrival at Osaka revealed

that only two packages were broken and only 1.4% of them had suffered pin-holing and failed to remain airtight. The data obtained in this experiment together with the relatively low cost suggested that this technique could be suitable for use on an industrial scale.

#### 4. Development of CEM skin-packages in Japan (Mitsuda et al., 1975, Mitsuda, 1979).

After accumulating the basic data for the practical feasibility of the CEM technique in cooperation with agricultural government offices, the technique has been adopted by the industry for the preservation of polished rice since July 1973. The merits of CEM skin-packaging are:

- (1) The packages are easy to produce;
- (2) Permeation of moisture and gas is almost completely prevented;
- (3) Deterioration caused by aerobic microorganisms, insects and oxygen is effectively prevented;
- (4) There is no breakage or slipping of bags in loading;
- (5) Packages are reshapable even after sealing;
- (6) This technique can be widely used in food packaging and safe storage because it eliminates the need for chemical food additives.

The skin-packaging of polished rice has also been accepted by various types of consumer. For example, many Japanese who live abroad as well as people engaged in deep sea fishing and mountain-climbing have reported the successful retention of flavor in rice stored in CEM skin-packages. CEM skin-packages have also made it possible to develop a new type of polished rice which retains the germ portion, because the rapid deterioration of this type of polished rice is effectively prevented by this packaging and storage method. At present, CEM skin-packages are produced at more than 30 polishing factories in Japan. Financial support for promoting the CEM packaging of polished rice is offered by the National Rice Distribution Association to rice retailers under the direction of the government.

#### 5. The seed storage experiment (Mitsuda et al., 1979).

A long-term storage experiment was carried out from 1972 to 1977 with the cooperation of the Chiba-Ken Foundation Seed and Stock Farm in order to develop a convenient system for long-term storage in place of the annual reproduction of seed grain. Paddy rice from the harvest of 1971 with moisture contents of 5.9, 8.7, 11.3, 14.0 and 16.3% respectively were packed in CEM skin-packages and stored at temperatures of 0, 10, 20, 30°C and ambient temperatures respectively.

Table 1. shows one of the results obtained by germination tests of the storage of paddy rice in a CO<sub>2</sub> atmosphere. Significant viability reduction was observed in the paddy rice stored with higher moisture content and higher storage temperatures. Compared with the data obtained from non-airtight storage, the rate of viability reduction was found to be 2 to 4 times slower in the high moisture

content of rice packed by CEM technique. Actual germination ability in the field was proved to agree well with germination capacity in Petri dishes in the laboratory. In addition to this, growth rate in the field and various characteristics of plant and frequency of abnormal growth were also examined on the Farm. Results obtained showed any abnormality in growth of paddy rice stored in a CO<sub>2</sub> atmosphere for 5 years. Peanuts and wheat have also been tested in the same way.

TABLE 1.

Storage periods before the germination of paddy rice decrease below 90% at various moisture contents and temperatures

Moisture content (wet basis)	Storage temperature				
	30°C	20°C	10°C	0°C	ambient temperature <sup>a)</sup>
16.3 %	1.6	6.9	5.8	32.6	3.8 months
14.0	3.1	11.1	49.4	>60	6.1
11.3	16.0	52.9	>60	>60	37.6
8.7	37.8	>60	>60	>60	>60
5.9	>60	>60	>60	>60	>60

a) 4.0-34.2°C.

#### 6. Experiments on grain storage in a CO<sub>2</sub> atmosphere in steel cans.

With the discovery of the usefulness of CEM skin-packages for safe storage of rice, needs and feasibilities for famine reserves of rice have been one of the serious concerns in local governments and governmental offices. The development of storage systems on larger scales of one metric ton or more in steel cans and containers has been required, in addition to the system using small bags of CEM skin-packages. To meet these social requirements, a project team was organized on May, 1976 for the development of a new system of CO<sub>2</sub> gas storage on larger scales with the cooperation of the university, companies and corporations under the leadership of the author's group. The Nippon Steel Corporation, one of the project members and the biggest company in Japan, had been conducting some basic experiments on grain storage in its Fundamental Research Laboratory since 1971 in order to find wider application of the use of steel cans. In this project team, the artificial gas storage using CO<sub>2</sub> gas had been adopted in a steel can storage system. Experimental results obtained for the CO<sub>2</sub> gas storage of grain in steel cans can be summarized as follows:

(1) Grain quality analysis: Polished rice has been packed in more than 40 drum cans 200 l in volume and stored at ambient temperature (below 30°C) for 2 years in a CO<sub>2</sub> atmosphere. The chemical and sensory evaluation tests of the stored grain have been carried out mainly by the Japan Grain Inspection Association and the quality of the stored polished rice has proved to be of a satisfactory level for

conventional consumers' use. These results using steel cans have confirmed the previous data obtained using CEM skin-packages.

(2) The characteristics of  $\text{CO}_2$  gas adsorption by canned grain: The characteristics of  $\text{CO}_2$  gas adsorption by the canned grain proved to be in satisfactory agreement with the results obtained by Warburg's manometry in our laboratory. These results, some of which were shown in Figs. 1 to 3, provided the fundamental knowledge for the development of automatic filling equipment and a pressure-controlling method for storage cans.

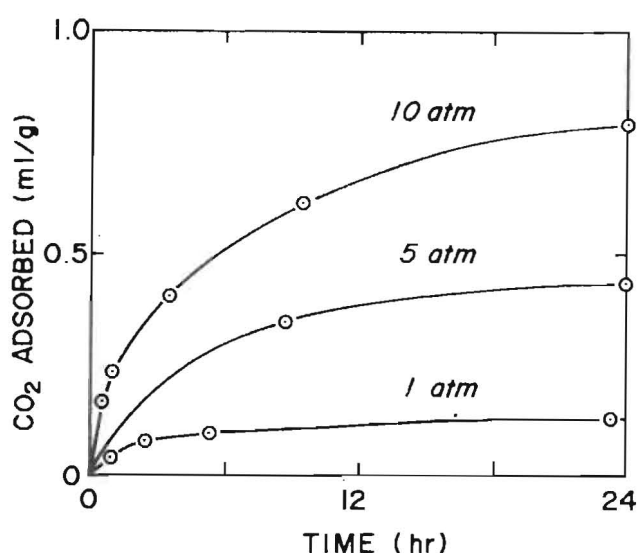


Fig. 1.  $\text{CO}_2$  adsorption rate in various pressures.

A small autoclave 0.8 liter in volume with a pressure gauge was used as a test vessel in the experiment at various pressures. The amount of  $\text{CO}_2$  gas adsorbed by grains was determined by the titration method and calculated with the amount of acid required to neutralize the sodium hydroxide solution in which the desorbed  $\text{CO}_2$  gas was trapped by the grains in a  $\text{N}_2$  gas stream. Although the adsorption rate did not increase even at higher  $\text{CO}_2$  pressures, the amount of  $\text{CO}_2$  gas adsorbed by grains increased with the increase of  $\text{CO}_2$  gas pressure. The amount of  $\text{CO}_2$  gas adsorbed by grains and  $\text{CO}_2$  gas pressure agree with the sorption isotherm of Freundlich as in the case of various partial pressures of  $\text{CO}_2$  gas determined previously (Mitsuda et al., 1973b).

(3) The development of counter-current type filling equipment: In order to get effective purge of intergranular air and maximum adsorption of  $\text{CO}_2$  gas, filling equipment of counter-current type was newly developed in the Nippon Steel Corporation. It has a filling capacity of 150-450kg grain per hour into steel cans. Operation of this equipment was stable and smooth for the production of grain cans

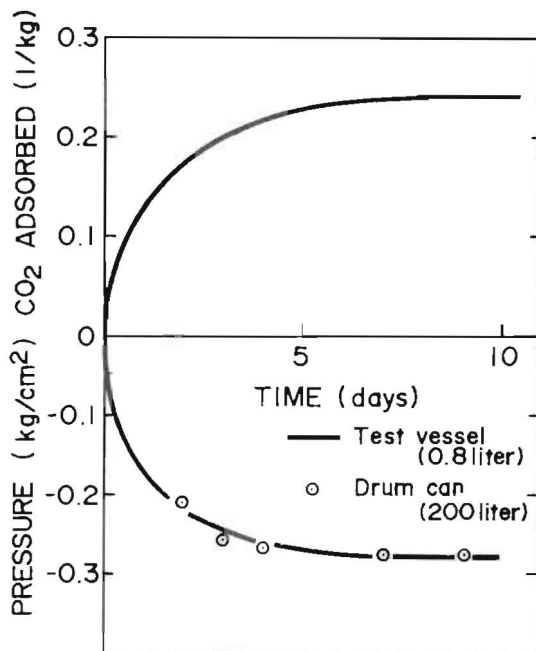


Fig. 2(a). Adsorption equilibrium in the test vessel and drum can.

Changes in the amount of CO<sub>2</sub> gas adsorbed by grains and changes in the inner pressure of the test vessel were determined correspondingly. Adsorption equilibrium was obtained after 7 days. Changes in the inner pressure of the drum can also followed the same curve obtained with the test vessel.

Initial high-pressure treatment was carried out by pressing the grain to 10 atm with CO<sub>2</sub> gas for several minutes as shown in Fig. 2(b). Changes in the inner pressures followed the same procedure after the inner pressure had been reduced to atmospheric pressure. When the rice grain had been pressed to 10 atm and reduced to atmospheric pressure, temporary increase of inner pressure was observed as shown in the above three curves, while gradual decrease was observed without an initial high-pressure treatment as shown in the lowest curve. This behavior may be caused by two opposite factors of increasing and decreasing the pressure. The relationship between equilibrated pressure and the required time for initial high-pressure treatment was plotted in Fig. 2(c). This indicates that the equilibrated pressure in the vessel can be set at positive when the high-pressure treatment is carried out for more than 60 min. (as shown in Fig. 2(c)).

with an adequate pressure of CO<sub>2</sub> gas. This equipment has made it possible even at present to start making large-scale famine reserves of polished rice in steel cans and containers below the one-metric ton unit.

For the practical application of these systems to the prolonged storage of rice in Japan, further studies are also necessary concerning prolonged experiments on the strict determination of shelf life for canned rice, selection of suitable varieties of rice for prolonged storage, and finding the suitable conditions for the storage of rice in brown and paddy forms.

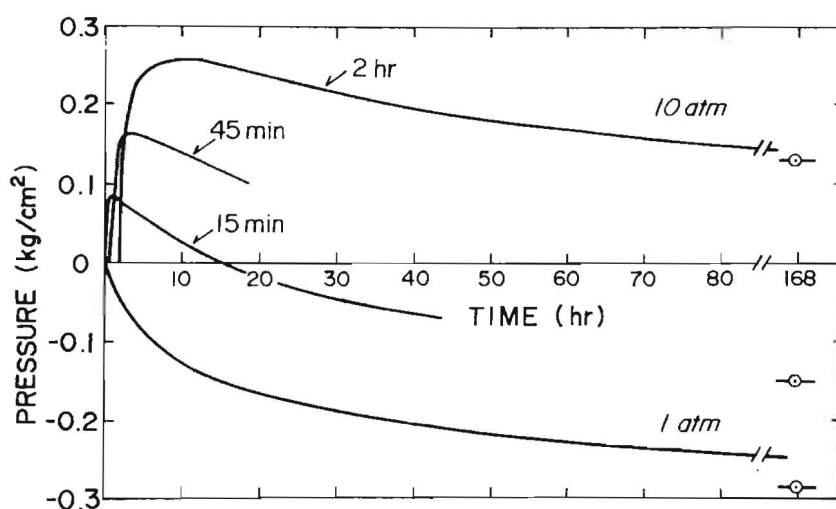


Fig. 2(b). Changes in the inner pressures of a test vessel with or without initial high-pressure treatment.

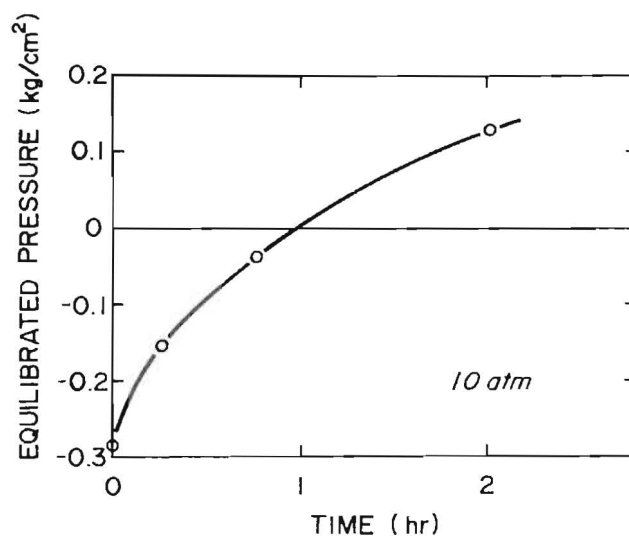


Fig. 2(c). The relationship between equilibrated pressure and the required time of initial high-pressure treatment.



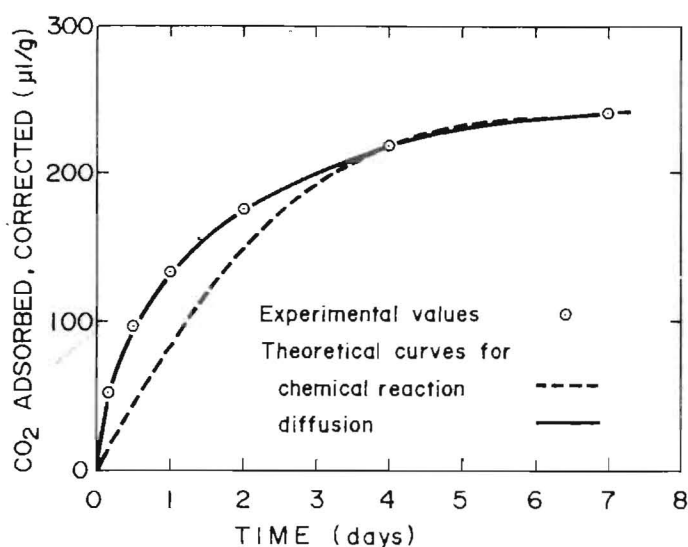


Fig. 3. Rate-limiting curves of CO<sub>2</sub> adsorption by grains.  
Equation for the rate-limiting curves were as follows:

$$1 - (1-f)^{1/3} = \theta/\theta_B \quad (\text{reaction rate-limiting})$$

$$3 [1 - (1-f)^{2/3}] - 2f = \theta/\theta_B \quad (\text{diffusion rate-limiting})$$

Experimental values were proved to agree well with the theoretical curve for diffusion rate-limiting into the granule.

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## CHARACTERISTICS OF CARBON DIOXIDE GAS ADSORPTION BY GRAIN AND ITS COMPONENTS.

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## INTRODUCTION

During experiments of grain storage in a  $\text{CO}_2$  atmosphere, grains in flexible laminated plastic film bags originally containing  $\text{CO}_2$  gas were found to be tightly packed as if packed in vacuo (Mitsuda et al., 1971). This led to the discovery of an interesting adsorption phenomenon of  $\text{CO}_2$  gas by grains at a low moisture content and to the development of a technique for skin-packaging, the Carbon dioxide Exchange Method (CEM) (Mitsuda et al., 1972a). Various experiments have been carried out to examine the mechanism of this adsorption phenomenon (Mitsuda et al., 1972b, 1973, 1975, 1977). The purpose of this paper is to summarize the experimental results on the characteristics of  $\text{CO}_2$  gas adsorption by grain and its components for the practical use of  $\text{CO}_2$  gas in artificial controlled atmosphere storage of grains.

## CHARACTERISTICS OF CARBON DIOXIDE GAS ADSORPTION BY GRAIN

Equilibrium. Figure 1 shows the time course for  $\text{CO}_2$  gas adsorption and desorption by grains at  $25^\circ\text{C}$ . The velocity of adsorption varies with the species of grain. Fifty to sixty percent of the maximum adsorption takes place within the first 6 hours in the case of rice and red beans and in about 4 hours for peanuts seeds. The graph also shows that equilibrium is obtained after about 24 hours. Desorption curves show that the  $\text{CO}_2$  gas adsorption by grains is almost completely reversed when the grain is allowed to stand in air. The velocity of desorption is larger than that of adsorption. The long time needed to reach adsorption equilibrium and the variation of the rate with the species of grain suggested that diffusion of  $\text{CO}_2$  gas into the grain kernel is important in this phenomenon.

Temperature dependence. The amount of  $\text{CO}_2$  gas adsorbed by grains varies depending on temperature, increasing at a lower temperature. The velocity of adsorption is not affected as much by temperature.

Moisture dependence. The maximum adsorption of  $\text{CO}_2$  gas was measured at moisture contents of rice ranging from 0 to 16.3% to investigate the relationship between the moisture content and the amount of  $\text{CO}_2$  gas adsorbed by rice grain. The  $\text{CO}_2$  gas adsorption was found to depend on the moisture content of rice as shown in Fig. 2. Adsorption increases almost linearly in brown rice but decreases almost linearly in paddy rice as the moisture content of the grains increases. Hull and

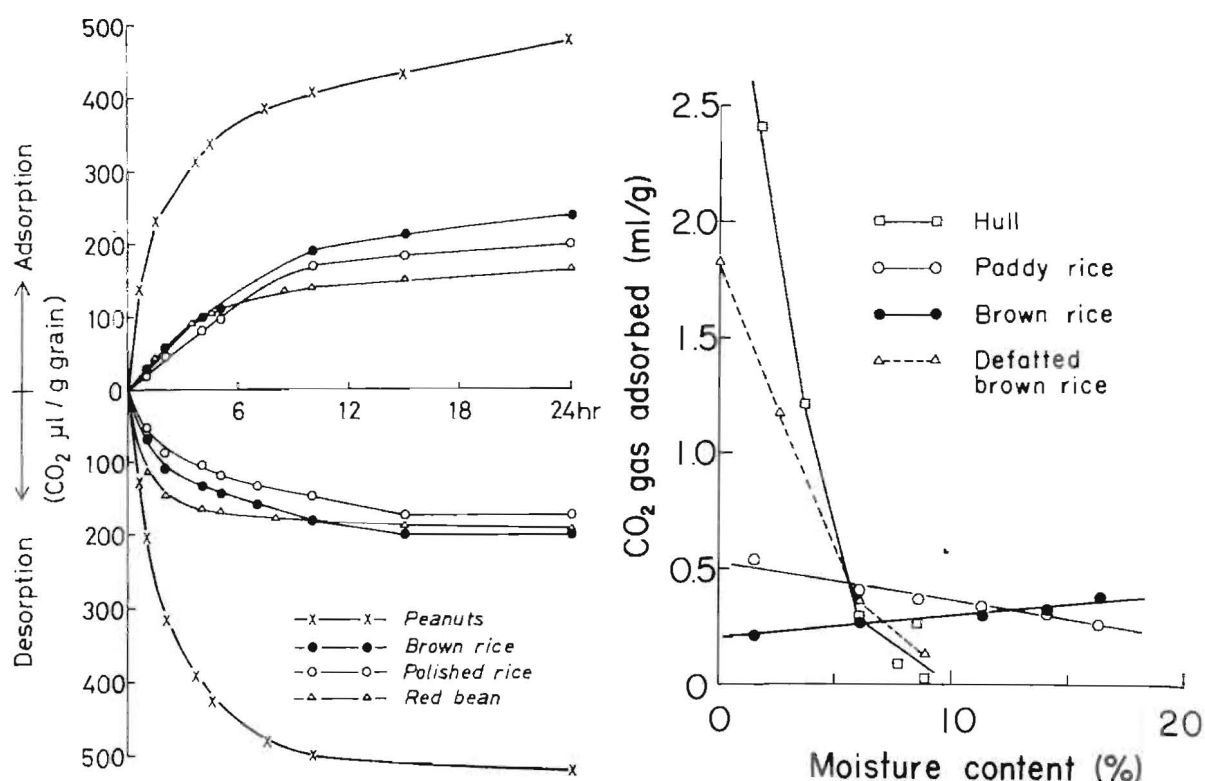


Fig. 1 (left). Time course of  $\text{CO}_2$  gas adsorption and desorption by grains (at  $25^\circ\text{C}$ ). One gram of grains was put into a vessel of a Warburg's manometer and was shaken in an atmosphere of  $\text{CO}_2$  gas at a constant temperature bath adjusted to  $25^\circ\text{C}$ . The amount of  $\text{CO}_2$  gas adsorbed was measured with the lapse of time. To study desorption one gram of grains was placed in an atmosphere of  $\text{CO}_2$  gas for more than 48 hrs to adsorb the gas until fully saturated. This grain was transferred quickly into the vessel and then shaken in air.

Fig. 2 (right). Relationship between the moisture content and  $\text{CO}_2$  gas adsorbed by rice grain. Defatted brown rice was prepared as follows: Grains were dehydrated and defatted by a gradual ethanol-aceton or ethanol-hexane schedule. Samples were then heated at  $105^\circ\text{C}$  for 24 hr to remove the solvent remained in the kernel. Grain samples with various moisture contents were prepared by equilibrating them for 3 to 14 days in desiccators with saturated salt solution. The amount of  $\text{CO}_2$  gas adsorbed by the grains was measured in the same way as described in Fig. 1.

defatted brown rice adsorb remarkable amounts of  $\text{CO}_2$  gas at the lower moisture content. The maximum solubility level of  $\text{CO}_2$  gas in water contained in grains, calculated by Bunsen's absorption coefficients, is found to be lower than one-half of the amount of  $\text{CO}_2$  gas adsorbed. This suggests that the  $\text{CO}_2$  gas adsorption phenomenon in grains is not merely caused by dissolution of gas into the water.

Kinds of grains. Many kinds of cereal grains and pulses showed a similar adsorption phenomenon. Table 1 shows the adsorption amount of CO<sub>2</sub> gas by each of these grains at 20°C for 3 hr. Wheat, corn, peanuts, soybeans, red beans, sesame seeds, and their flours show the same adsorption phenomenon as rice, but the velocity of adsorption and the maximum amount of CO<sub>2</sub> gas adsorbed vary with the kinds of grains. Peanuts, soybeans, and sesame seeds are good adsorbents, and wheat, rice and flours seem to be relatively poor ones. Compared with conventional adsorbents, such as silica gel and activated charcoal, these grains are all poor adsorbents.

TABLE 1.

The amount of CO<sub>2</sub> gas adsorbed by various grains.

Kinds of grains	Adsorption amount at 20°C for 3 hr	Kinds of grains	Adsorption amount at 20°C for 3 hr
Paddy rice	86 ml/kg	Peanuts	560 ml/kg
Brown rice	90	Soybean flour	440
Polished rice	70	Soybean flour	216
Rice flour	60	Red beans	64
Wheat	75	Coffee beans	123
Wheat flour	60	Sesame seeds	230
Corn	170	Black tea	115

Grain components. As shown in Table 2, an inverse relationship was obtained between fat content of brown rice and the amount of CO<sub>2</sub> gas adsorbed. Carbohydrates such as sugars, cellulose and starch in purified forms failed to adsorb the CO<sub>2</sub> gas. A clear relationship was not observed between the protein content of grains and the amount of CO<sub>2</sub> gas adsorbed, although many kinds of pulses of high protein content were found to be good adsorbents.

TABLE 2.

Relationship between the fat content of brown rice and the amount of CO<sub>2</sub> gas adsorbed.

	Fat content <sup>a)</sup>	CO <sub>2</sub> gas adsorbed <sup>b)</sup>
Raw brown rice	2.67 %	128 µl/g
Defatted brown rice with: Ethanol	2.51	129
Ether	2.16	185
Chloroform-methanol (2:1)	1.35	568
Ethanol-acetone schedule	0.20	1758

a) Determined by Soxhlet methods with ether.

b) Determined by the Warburg's manometry at 25°C.

All figures are shown on a dry basis.

Porosity. The porosity of the grain kernel was proved to influence the CO<sub>2</sub> gas adsorption phenomenon. A high pressure mercury penetration porosimeter was used for the determination of pore-volume and pore-size distribution of rice kernel in the range of 42 - 300 Å. A relatively larger size of pores was found in paddy compared with those of brown and polished rice. Defatting and/or heat-drying treatment to rice kernel, however, produced abundant pores in their intergranular spaces of starch. Pore volume between the range of 42 Å and 12 μ in diameter was estimated to be 264, 134, 73.7, 31.2 and 21.3 μl/g (on dry basis) for hull, defatted brown rice, paddy, brown and polished rice respectively.

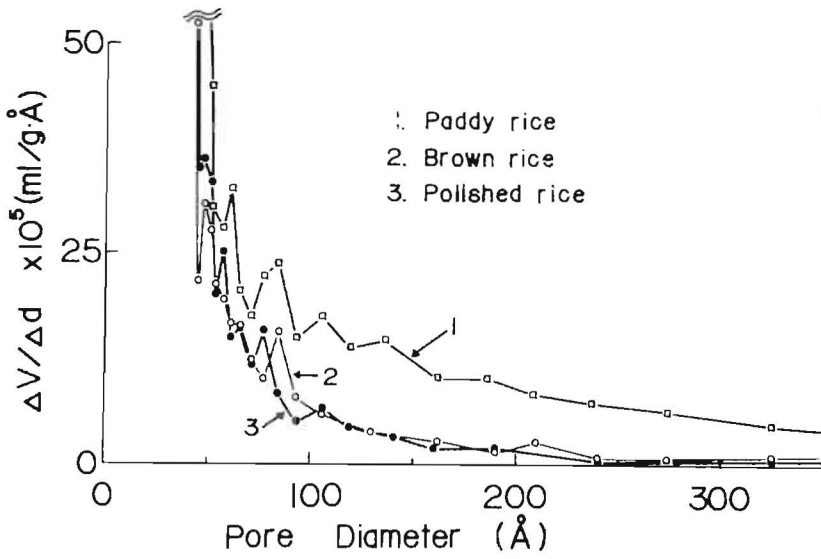


Fig. 3. Pore-size distribution of rice grain.

TABLE 3.  
Comparison of pore-volumes of rice grain and hull.

	Hull	Paddy	Brown	Polished
Total pore volume (42 Å - 12 μ) (μl/g)	264	73.7	31.2	21.3
Micro-pore volume (42 Å - 354 Å) (μl/g)	136	38.1	15.4	11.0

Sorption isotherm. According to the classic sorption isotherm of Freundlich, a linear relation is obtained when the logarithms of pressure (P) and sorption volume (V) are plotted. This relation may be written:  
 $\log V = \log k + \frac{n}{n} \log P$ , where k and  $\frac{n}{n}$  are constants.

Experimental results obtained with the adsorption of  $\text{CO}_2$  by brown rice at various partial pressures of  $\text{CO}_2$  were found to agree with the isotherm as shown in Fig. 4. An almost linear relation between  $\log V$  and  $\log P_{\text{CO}_2}$  was obtained in the higher partial pressure of the  $\text{CO}_2$  ( $P_{\text{CO}_2}$ ) region but not in the lower one. The constant  $\underline{n}$  was calculated as about 1.5.

$$V = \frac{(C_0 - C_e) v}{100 - C_e}$$

$V$ : volume of  $\text{CO}_2$  gas adsorbed by brown rice (ml)

$C_0$ : original  $\text{CO}_2$  gas composition (%)

$C_e$ : equilibrated  $\text{CO}_2$  gas composition (%)

$v$ : original mixed gas volume (ml)

$$\log V = \log k + \underline{n} \log P_{\text{CO}_2}$$

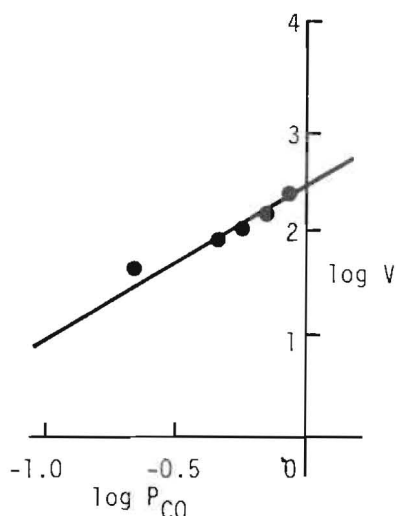


Fig. 4. Relation between the amount of  $\text{CO}_2$  gas adsorbed and the partial pressure of  $\text{CO}_2$  (at  $25^\circ\text{C}$ ). Brown rice was packed in plastic film bags under different compositions of  $\text{N}_2$  and  $\text{CO}_2$  gas. After the adsorption equilibrium was obtained at  $25^\circ\text{C}$  under atmospheric pressure, the composition of headspace gases was analyzed by gas chromatography and compared with the original composition. The amount of  $\text{CO}_2$  gas adsorbed by brown rice was calculated using the equation shown in this figure.

Results obtained in these experiments can be summarized as follows:

- (1) Solubility of  $\text{CO}_2$  gas into the moisture and lipids of the grain is assumed to have a minor effect on the  $\text{CO}_2$  adsorption phenomenon. No direct relationship is also presented between any special components of grain and the amount of  $\text{CO}_2$  gas adsorbed.
- (2) Diffusion of  $\text{CO}_2$  gas into the grain is important in this phenomenon. This adsorption phenomenon is very similar to that observed in sorption of gases by charcoal and silica gel, which sorb gases into their many pores.

#### THE $\text{CO}_2$ GAS-PROTEIN INTERACTION IN A GAS-SOLID PHASE.

In the course of investigations to elucidate the mechanism involved in the  $\text{CO}_2$  gas adsorption phenomenon by grains, various proteins in solid form were found to be capable of adsorbing  $\text{CO}_2$  gas. The characteristics of  $\text{CO}_2$  gas adsorption by highly purified protein has been investigated by Warburg manometry and the B.E.T. surface area analyzer.



Adsorption of CO<sub>2</sub> gas by proteins. Carbon dioxide gas was adsorbed by casein at more than 500  $\mu\text{l/g/24hr}$  while ethylene and oxygen were less than 200 and 50  $\mu\text{l/g/24hr}$  respectively. Approximate equilibrium was obtained after 24 hours. Helium, nitrogen, hydrogen and air were also examined but none of them was adsorbed considerably by casein under the same conditions in the case of CO<sub>2</sub> gas. The amount of CO<sub>2</sub> gas adsorbed by proteins is shown in Table 4. Various proteins were found to adsorb CO<sub>2</sub> gas when they were placed in the high partial pressure of the gas concerned. Casein, gelatin and raw silk were revealed to be the better adsorbents as compared with the others.

TABLE 4.

Adsorption of CO<sub>2</sub> gas by various proteins in dried state.

Proteins	CO <sub>2</sub> gas adsorbed <sup>a)</sup>
Casein	662 $\mu\text{l/g}$
Gelatin	532
Egg albumin	267
Glutamine	231
Hemoglobin	182
Zein	150
Soybean flour	220
Whole milk	200
Bleached Chlorella algae	163
Hydrocarbon-assimilating yeast	115
Gluten	111
Rice bran	109
Raw silk	690
Fibroin, from silk	110
Chinese white rabbit hair	106

a) Determined by Warburg manometry at 25°C for 24 hr.  
All figures are shown on a wet basis.

Moisture dependence. In Fig. 5, the amount of CO<sub>2</sub> gas adsorbed by casein and gelatin are plotted against their moisture content. The lower the moisture is, the greater the adsorption amount of CO<sub>2</sub> increases.

Temperature dependence. The amount of CO<sub>2</sub> gas adsorbed by zein varies depending on temperature, as shown in Fig. 6, increasing at a lower temperature. Other proteins also show a similar tendency as shown in Fig. 7. The temperature dependence of CO<sub>2</sub> adsorption by protein is close to that of physical adsorption. Although large amounts of CO<sub>2</sub> gas were adsorbed by histamin, tyramine, L-lysine and L-arginine, temperature dependence of the adsorption is somewhat different from that of protein, such as casein and gelatin.

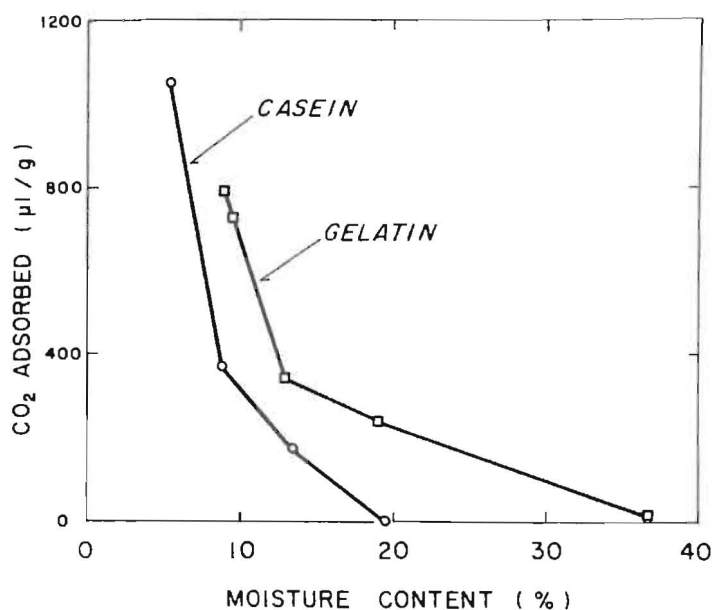


Fig. 5. Effect of moisture content on the amount of CO<sub>2</sub> gas adsorbed by casein and gelatin. Protein samples at various moisture contents were prepared by equilibrating them for 3 to 14 day in desiccators with saturated solutions of LiCl, K<sub>2</sub>CO<sub>3</sub>, Mg(NO<sub>3</sub>)<sub>2</sub>, NaCl and KNO<sub>3</sub> at relative humidities between 11 and 88%.

Reversibility of CO<sub>2</sub> gas adsorption by protein and others. Reversibility of the CO<sub>2</sub> gas adsorption by protein, protein hydrolyzates, amines and amino acids was examined. More than 90% of CO<sub>2</sub> gas which had been adsorbed by casein and gelatin desorbed when they were put into an atmosphere of low partial pressure of CO<sub>2</sub> but only a few percentage desorbed in the case of amino acid and histamine. Interaction between CO<sub>2</sub> gas and amines and amino acids seemed to be "chemical reaction" or "chemisorption" rather than "physical adsorption".

Effect of particle size on CO<sub>2</sub> adsorption by bacto-peptone and casein. Bacto-peptone, one of the protein hydrolyzates for a microbial culture medium, showed an interesting result on CO<sub>2</sub> adsorption. This was one of the samples which fairly adsorbed CO<sub>2</sub> gas when they were tested in larger particles above 32 mesh. Gas adsorption increased more than 20 times when the sample was ground. The less the peptone particle size, the more the amount of CO<sub>2</sub> gas adsorbed. This result suggested that the gas diffusion process in the particles was the rate-limiting factor in CO<sub>2</sub> gas adsorption by proteins. This was confirmed by the determination of a diffusion coefficient of CO<sub>2</sub> gas in casein particles of three different sizes. By applying the Boyd equation, the degree of the adsorption ( $V_t/V_{eq}$ ) was found to be in proportion to a square root of time in the region below one-half of  $V_t/V_{eq}$  as shown in Fig. 9. The diffusion coefficient obtained in these samples was around  $10^{-9}$  cm<sup>2</sup>/sec. This value was in the proper range of the CO<sub>2</sub> gas diffusion coefficient in the solid, but lower in liquid and in gas ( $10^{-5}$  -  $1$  cm<sup>2</sup>/sec).

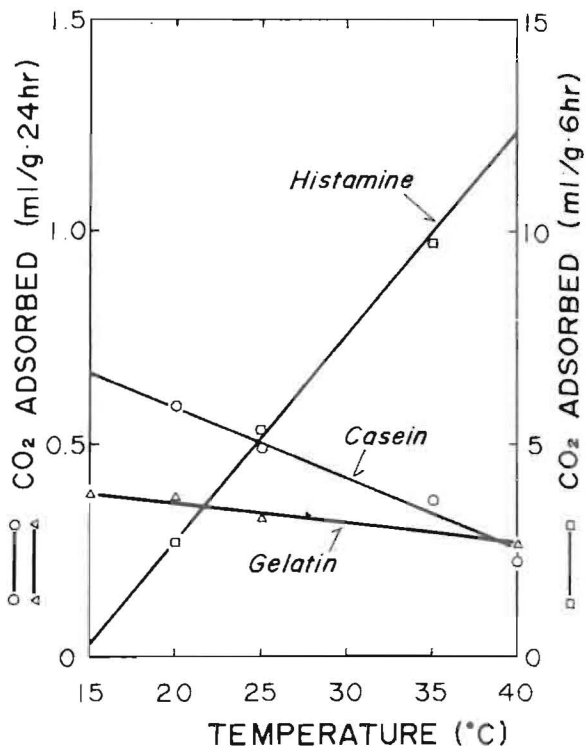
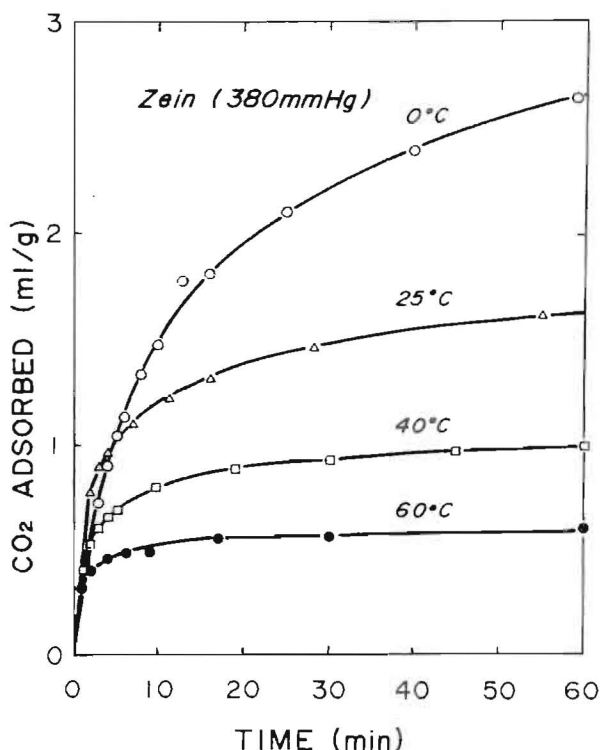


Fig. 6 (left). Effect of temperature on CO<sub>2</sub> gas adsorption by zein. The amount of CO<sub>2</sub> gas adsorbed was measured with the lapse of time by the B.E.T. surface area analyzer at one-half of atmospheric pressure.

Fig. 7 (right). Temperature dependence of CO<sub>2</sub> gas adsorption by casein, gelatin and histamine. The amount of CO<sub>2</sub> gas adsorbed was measured in the same way described in Fig. 1.

Adsorption isotherms. Fig. 10 indicates the adsorption isotherms for N<sub>2</sub> and CO<sub>2</sub> in casein determined by the B.E.T. method. A typical polymolecule adsorption curve (BET type) was obtained with N<sub>2</sub> gas adsorption in -196°C. The carbon dioxide gas adsorption curve, however, showed an adsorption curve of the Henry type in which the amount of gas adsorbed was in proportion to the pressure. Because the experimental pressure (76 cmHg) at 25°C is situated in the lower region of the saturated vapor pressure (4,830 cmHg) of CO<sub>2</sub> gas, experimental results can also be assumed to be in the early part of BET, Langmuir, Freundlich and other types of isotherms.

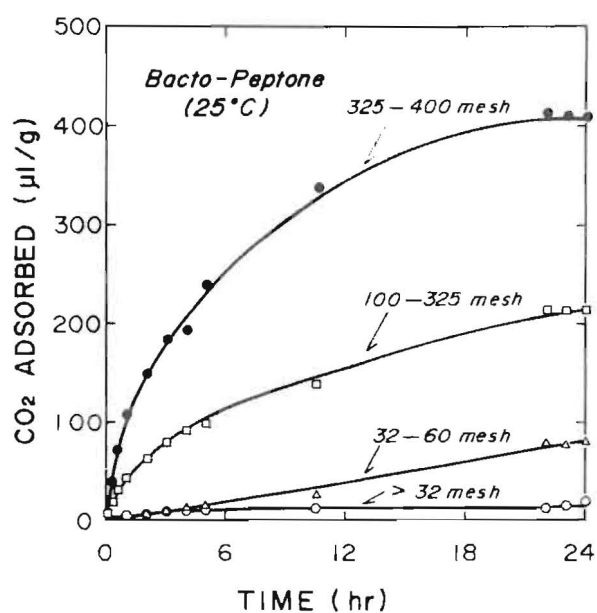


Fig. 8. Effect of particle size on CO<sub>2</sub> adsorption by bacto-peptone.

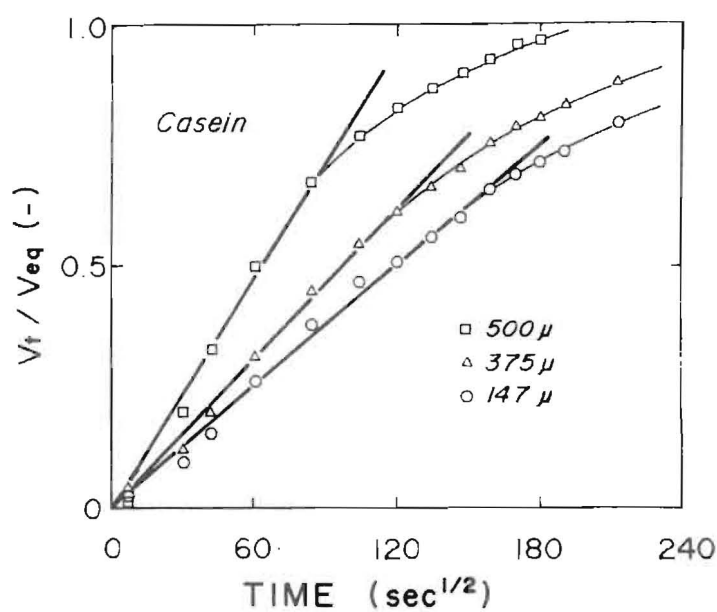


Fig. 9. Effect of particle size on CO<sub>2</sub> adsorption by casein.

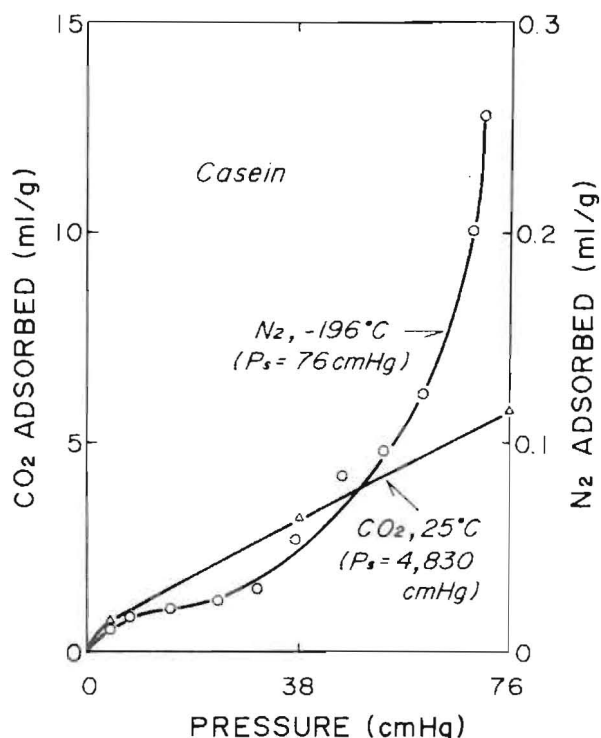


Fig. 10. Adsorption isotherms for  $N_2$  and  $CO_2$  in casein. The amount of gas adsorbed was measured by the B.E.T. surface analyzer in the condition described in this figure.

#### Particular functional groups involved in $CO_2$ gas adsorption by protein.

In order to identify the functional groups which really contribute to  $CO_2$  gas adsorption by proteins,  $\epsilon$ -amino groups of lysine residues of egg albumin were chemically modified with trinitrobenzene sulfonic acid to various degrees. Experimental results showed that about 60% of the total amount of  $CO_2$  gas adsorbed by solid egg albumin diminished by completely modification of the  $\epsilon$ -amino groups of lysine residues. This directly provides evidence that the  $\epsilon$ -amino group of lysine is capable of adsorbing the  $CO_2$  gas in protein in the same manner as in free amino acid. As to the remaining 40% of the total amount of  $CO_2$  gas adsorbed by egg albumin, the guanidinium group of arginine residues and  $\alpha$ -amino group in protein were shown to take part in it by the following experiments. Correlation coefficients found between the amount of  $CO_2$  and each of the variables, the lysine content, the arginine content and the total of lysine and arginine contents of protein and its hydrolyzates were 0.76, 0.54 and 0.85 respectively. Chemical modification of the guanidinium groups of arginine residues of lysozyme by 1,2-cyclohexanedione

also showed the decrease of the amount of CO<sub>2</sub> gas adsorbed by lysozyme. The ability of CO<sub>2</sub> gas adsorption by  $\alpha$ -amino group of amino acids and oligopeptides was found to be developed by the elongation of the peptide chain of glycine and other amino acids, by the removal of the  $\alpha$ -carboxyl group of histidine and tyrosine to corresponding amines and by the esterification of  $\alpha$ -carboxyl group of leucine with *p*-nitrophenol. These results clearly indicate that CO<sub>2</sub> binding sites in protein in the gas-solid phase system are  $\epsilon$ -amino,  $\alpha$ -amino and guanidinium groups. Table 5 gives the amounts of CO<sub>2</sub> gas adsorbed by a series of oligopeptides and collated with the  $pK_a$  value for their  $\alpha$ -amino groups reported in the literature.

TABLE 5.

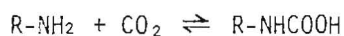
Effect of peptide chain length on the CO<sub>2</sub> gas adsorption by amino group.

	$pK_a$ values ( $\alpha$ -NH <sub>2</sub> )( $\epsilon$ -NH <sub>2</sub> )		CO <sub>2</sub> /NH <sub>2</sub> (mmoles/mole)
Gly	9.78		0.024
Gly-Gly	8.25		0.111
Gly-Gly-Gly	8.09		0.465
Gly-Gly-Gly-Gly	-		1.47
Polyglycine	7.4		-
Lys	9.18	10.79	3.97
Lys-Gly	-		4.49
Lys-Glu-Ala	-		6.50
$\gamma$ -Glu-Cys-Gly	8.75		1.03

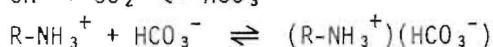
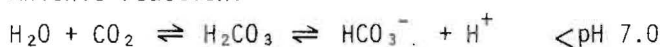
Differences between the CO<sub>2</sub> gas adsorption by proteins and by amino acids and amines.

The reasons why similar functional groups of proteins and amino acids were able to show different reactivity to CO<sub>2</sub> gas, such temperature dependence, moisture dependence and reversibility were examined. Differences between the CO<sub>2</sub> gas adsorption by proteins and by amino acids and amines can be best explained with the following two different interactions between the amino group and CO<sub>2</sub>:

1. Carbamate formation.



2. Anionic reaction.



The  $pK_a$  values of amino groups in protein are lower than those in amino acids. In the solid state of protein, therefore, amino groups must be present in the unprotonated

form ( $\text{R-NH}_2$ ) and form carbamate salts with  $\text{CO}_2$ . This interaction is weak and reversible as is well known in the interaction of hemoglobin and  $\text{CO}_2$  in blood. Characteristics of  $\text{CO}_2$  gas adsorption by protein are assumed to be caused by the carbamate formation. On the other hand, amino groups in free amino acids and amines seem to be present in the protonized form ( $\text{R-NH}_3^+$ ) even in a solid state for their higher  $\text{pK}_a$  values. This amino group in protonized form may react with the bicarbonate ion which is formed from  $\text{CO}_2$  and water via carbonic acid. An experiment on the  $\text{CO}_2$  gas adsorption by the basic amino acids such as lysine and arginine in dried, and moistened form revealed that the amount of  $\text{CO}_2$  gas adsorbed by these free amino acids increased remarkably with their increased moisture content, while an inverse relationship was observed in protein as shown in Fig. 5.

### CONCLUSION

The  $\text{CO}_2$  gas adsorption phenomenon observed in grain storage in a  $\text{CO}_2$  atmosphere is assumed to be caused by a mechanism combined with a diffusion process in the grain kernel and a carbamate formation of  $\text{CO}_2$  gas with functional groups of protein which are exposed in the internal surface of the grain kernel. The reversible interaction of  $\text{CO}_2$  gas with functional groups of protein is assumed to contribute to retaining grain qualities during the storage.

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# APPLICATION OF ARTIFICIAL CONTROLLED ATMOSPHERES TO GRAIN STORAGE IN THE TROPICS: CASE STUDY OF NIGERIA

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## ABSTRACT

Nigeria produces about 10 million tons of grains annually which need to be stored between one harvest and the other over a period of 8-10 months. Storage methods range from local cribs used by the peasant to warehouse and silo structures used by large-scale grain storage establishments such as the National Grains Board and Agro-allied industries. Insect infestation is controlled only by chemical application. A lot of problems are encountered with silo storage methods and chemical control of insects under Nigerian conditions. This paper examines the application of artificial controlled atmospheres to grain storage in the tropics against the background of existing storage practices and problems in Nigeria.

A research project is being carried out in collaboration between ASSORENI/SNAMPROGETTI and NSPRI, in order to introduce the Nitrogen Storage Technique for Grains into Nigerian practices. The first two of the project's three research phases are described.

In phase I maize was stored for 10 weeks in minisilos filled with nitrogen. The insect infestation, fungal deterioration, seed viability, chemical and nutritional changes in the maize were measured during

the experiment and results showed that the inert gas storage method maintained the quality of the maize much better than storage in air. Another trial storing cowpeas, a grain legume, in the minisilos, is in progress.

Phase II of the project, involving four pilot-scale silos (3 ton-capacity), fully equipped with automatic physical measurement devices and filled with maize, has also commenced. Preliminary results are presented.

## INTRODUCTION

Grain forms a substantial part of the staple food in the tropics. Most tropical countries produce far less grain than they require locally and therefore have to spend their hard earned foreign exchange for importing grain. Nigeria produces about 10 million tons of grains annually mainly guinea corn, millet, maize and rice; the Maize and Produce Board in Kenya handles about 5 million bags of maize annually; while the National Agricultural Marketing Board in Zambia handles about 7 million bags of maize annually.

The methods at present used in Nigeria for storage of grains vary according to the level of storage. At the village level or the peasant farmer's level, a very effective structure called "CRIB" has been developed. It serves both as a natural drier and a container. The next level of grain storage is the medium scale level. The conventional warehouse or 'godown' has been recommended for use at this level. Warehouses and silos are used for large scale storage. However some problems are being encountered with the use of silos and therefore warehouses are more favoured.

In all these structures, enormous losses occur during storage. F.A.O. estimated an annual loss of 10% in both farm and store (Anon, 1947) and a loss as high as 24.7% has been reported by Adesuyi and Shode (1977) in farm level storage. The major causes of grain storage losses have been identified as insects, moulds and rodents. Birds can be a major factor in some cases. As a result of these losses, it has been realised that any major effort to increase food production must be supported by effective storage facilities. In realisation of this

fact, the seventh special session of the United Nations General Assembly in 1975 passed a resolution that every effort should be made to achieve a 50 percent reduction in post-harvest food losses by the year 1985. This resolution resulted in the Reduction of post-harvest food losses programme of the F.A.O. in 1977.

Chemicals are at present being used extensively to reduce post-harvest grain losses. There are however many problems and risks associated with the use of chemicals, both to consumers and operators. These are mainly:

- (i) Danger to storage personnel
- (ii) Danger to consumers
- (iii) Taint and damage to seed viability
- (iv) Problem of insect resistance to chemicals in use.

Therefore the need for a safer alternative is evident.

Hermetic storage, in which oxygen deficient atmospheres are produced by the aerobic respiratory processes of the grain, its microflora and insects to a level that inhibits the further existence or development of aerobic organisms, has long been known. However, the rate at which this process occurs is very critical because the longer it takes, the more damage is done by the organisms causing deterioration. In cases of dry grain storage with low insect infestation, the process of self-sterilisation is so slow that it may not be practicable.

To accelerate the depletion of oxygen to a level suitable for the elimination of all the developmental stages of storage insects, and suppression of microorganisms, it is convenient to introduce an inert gas such as nitrogen to replace the oxygen-rich interstitial atmosphere in storage bins (Shejbal, 1979). Successful trials in large-scale facilities using nitrogen gas for the commercial storage of wheat and barley for short and long term periods (Shejbal, 1978) confirmed research carried out in Italy for several years in laboratory and pilot scale silos (Shejbal, 1979). It is known that the use of nitrogen for grain preservation could eliminate some of the major problems of grain storage, if applicable in Nigerian climatic conditions, such as:

- Toxicity of insecticides both to consumers and operators.

- Insect infestation and reinfestation.
- Insect resistance.
- Taint and poor palatability.
- Loss of food reserve through metabolic activities of the stored produce.

Furthermore it could:

- Increase the possibility of storing wet grain until it can be dried.
- Retain viability of stored produce for a long period.
- Improve the palatability of stored produce.

Use of controlled atmosphere would therefore be of value in Nigeria in control of both insect and mould growth especially in the main maize crop which is harvested during the rainy season at moisture contents of 22-25% (Adesuyi, 1968) without assurance of sunshine to dry it.

A collaborative work between ASSORENI (Snamprogetti) and NSPRI was therefore initiated to evaluate this technique and for transferring the technology to Nigeria. The project is divided into three phases, lasting for a total of 30 months. They are as follows:

- 1st phase: Small-scale trials utilizing two minisilos, each with a capacity of  $0.6 \text{ m}^3$ .
- 2nd phase: Experimental pilot-scale trials utilizing four silos, each with a capacity of  $4 \text{ m}^3$ .
- 3rd phase: Large-scale field trials utilizing two silos, each with a capacity of 40 to 50 tons.

The following is a report on the first trial in the first phase using the minisilos and on preliminary data of the second phase.

#### MATERIALS AND METHODS

Two airtight stainless steel mini-silos, useful volume  $0.65 \text{ m}^3$ , situated in complete shade at the NSPRI, Ibadan are used in the trials of phase 1°.

The minisilos (Fig.1) are equipped with 3 sampling points for gas and grain analyses SA1 - SA3 and SB1 - SB3 for the silos A and B respectively. Thermistors are located in the central axis of the grain column. The silos are connected with the nitrogen distribution system

silos was nearly zero. A rapid purge of the silos was carried out in order to substitute the interstitial atmosphere with nitrogen, whereupon a slight constant overpressure was maintained during the whole storage period. For this a pre-set pressure gauge which automatically cuts off the supply of gas into the silos by a solenoid switch when the pressure build-up within the silo attains the set pressure (100 mm w.g.), is incorporated into the gas distribution system and located on the instrument pannel. The initial purge with ultrapure nitrogen was carried out for 14 hours at a rate of 50 litres per hour within one day after loading.

As controls in air, maize of the same quality was maintained in two metal drums, CA and CB (180 l volume each) situated in proximity of the mini-silos.

The concentrations of carbon dioxide and oxygen within the silos were checked once every week, and after every purge following introduction or removal of test insect cages. The temperature of stored grain, pressure within each silo, ambient temperature and humidity were recorded thrice daily.

During loading the maize was sampled in both the silos and controls for insect infestation, insect damage, biochemical and microbiological analysis and sensory evaluation. Samplings were repeated at 4 and 10 weeks and similarly analysed.

Viability tests of stored maize were carried out using fifty seeds per replicate, germinated in sterilized soil in the laboratory (Fig.2).

For trials in phase 2 of the collaborative work, four pilot-scale gastight steel silos, useful volume  $4 \text{ m}^3$  each, were built and erected in Ibadan. The alignment of the silos is in a North-South direction in an unshaded area in order to expose the silos to full and direct sunshine.

As can be seen in Fig.3, each silo is protected in a different way against solar radiation: one of the silos is fully insulated with 5 cm rock-wool and galvanized steel sheets, another is insulated similarly only on the roof, while the third is not insulated at all and the last is shaded on the top with a thatch made of palm leaves.

Each silo is equipped with 50 thermistors dislocated in such a way



Fig.2. Viability tests of stored maize in sterilized soil.

that the temperature in the axial and radial sense can be measured at the surface of the bins and at various depths in the stored product mass. The temperature readings are recorded automatically at preset time intervals.

The nitrogen gas flows, pressure maintenance devices and pressure relief valves are similar to those of phase 1, the overpressure maintained in the bins is 50 mm w.g. and gas consumption is measured by gas meters (Fig.4). The four bins were loaded with grade I yellow maize, mean moisture content 12.9%.

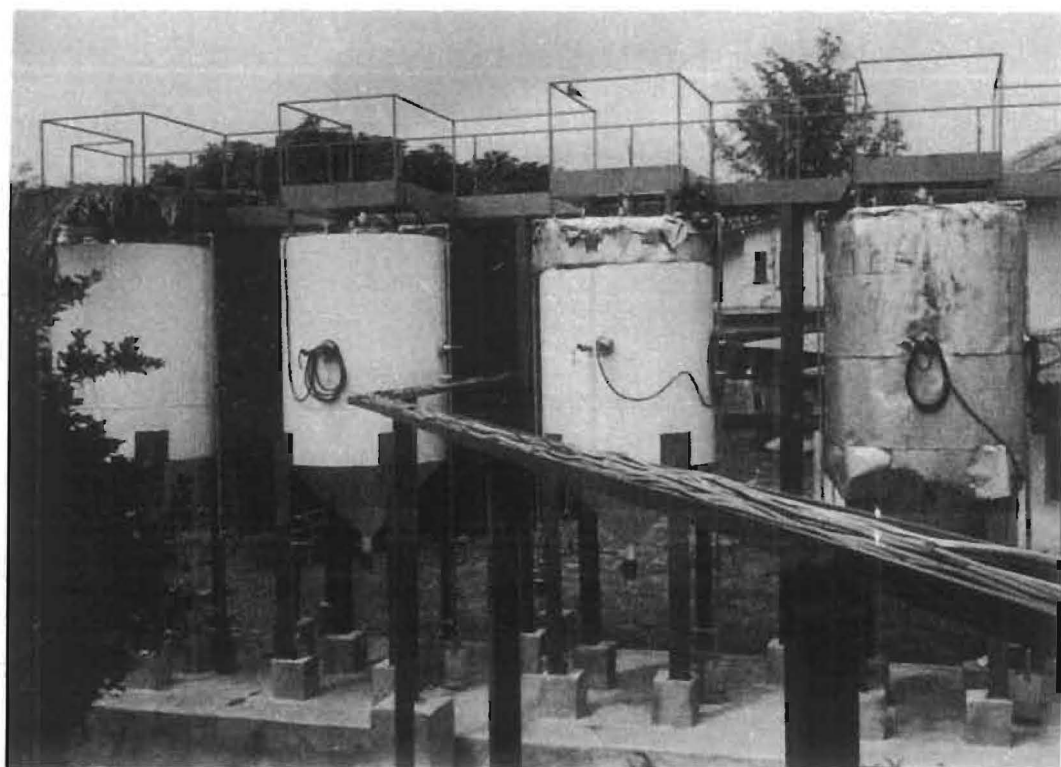


Fig.3. Pilot-scale metal storage bins differently protected against direct solar radiation. Sampling and temperature measurement points are visible on the 4 m<sup>3</sup> -silos. In the foreground, wires conducting to the automatic recorder of temperature at 50 distinct points in each bin.





Fig.4. Pilot-scale metal storage bins with automatic interstitial nitrogen pressure maintenance device, gas meters and pressure releaf valves.

## RESULTS

### Phase 1.

Temperature measurements in silo A gave an average of  $27.2^{\circ}\text{C}$  with a range of  $26.5^{\circ}\text{C}$  to  $27.8^{\circ}\text{C}$ ; silo B an average of  $26.6^{\circ}\text{C}$  with a range of  $26.1$  to  $27.5^{\circ}\text{C}$ , the ambient relative humidity ranged between 72.0 and 80.9% with an average of 76.3%. The oxygen content of the interstitial atmosphere in the silos was maintained at 0.05% - 0.1% by purges after grain or insect samplings.

The initial moisture content of the experimental maize was 12.7%.

The moisture content level was maintained at about 13% in the minisilos but rose steadily to 16.5% in control A and to 17.2% in control B (see table 1).

The results of the entomology experiments are presented in another paper (Williams et al., 1980). It was found that the egg and the adult stages of Sitophilus zeamais (maize weevil) were more susceptible to nitrogen than the larval and pupal stages. The duration of exposure required to kill the egg, larval, pupal and adult stages of S.zeamais were 4.0, 7.5, 8.0 and 3.0 days respectively while 6.0 days exposure was required to obtain a full kill of larvae of Trogoderma granarium (Khapra beetle).

Tables 2 and 3 show that the initial damage to the maize was between 1.8 and 2.2% and the insect pest population was fairly high with Sitophilus zeamais being the most predominant species. However, it can be seen from the same tables that after 10 weeks storage in nitrogen, the percentage damage remained the same with a sharp contrast in the control where the damage has increased by about 2,100%; secondly, no insect pest was found even after incubation in maize stored in nitrogen, whereas the population in the control had increased by 765%.

Mouldiness of the maize as determined by dilution plating analysis was  $9.9 \times 10^5$  colonies per gram of maize in the initial samples. While this amount of mouldiness increased substantially to an average of  $2.5 \times 10^7$  in the controls, maize stored in nitrogen maintained about the same level of mouldiness as the initial samples. At some points, a decrease to an average of  $3.9 \times 10^4$  colonies per gram was obtained in one of the silos (table 4).

The most predominant species were Aspergillus flavus Link; Fusarium moniliforme Sheld and Aspergillus niger V. Tieghem. Others of rather less importance are Aspergillus tamarii Kita, Paecilomyces varioti Bainier, Rhizopus arrhizus Frischer and Penicillium sp. All these moulds are commonly isolated from grains and other crops during storage in Nigeria. Table 5 shows the average abundance of the different mould species in the maize stored in nitrogen and the controls.

Results of chemical analyses, viability and sensory evaluations are summarized in Table 6. As can be seen, proximate parameters remained

TABLE 1

MOISTURE CONTENT OF MAIZE STORED IN NITROGEN AND IN AIR FOR 4 AND 10 WEEKS. PRE-LOADING MOISTURE CONTENT WAS 12.7%.

Sample	Percent moisture content of maize during storage for weeks	
	4	10
SA 1	12.6	12.7
SA 2	12.7	-
SA 3	12.9	12.7
AVERAGE	12.8	12.7
SB 1	13.1	13.0
SB 2	13.0	-
SB 3	12.9	13.1
AVERAGE	13.00	13.1
CA	15.0	16.5
CB	15.1	17.2
AVERAGE	15.1	16.9

## NOTE

SA 1 = Silo A Point 1 (bottom)

SA 2 = Silo A Point 2 (middle)

SA 3 = Silo A Point 3 (top)

idem for silo B

CA = Control drum A (mean sample at 20 cm depth)

idem for drum B.

TABLE 2

INSECT DAMAGE ASSESSMENT ON 500 GRAIN SAMPLES FROM MAIZE STORED IN NITROGEN AND IN AIR.

Treatment	Damage %	
	0 week	10 weeks
Mini Silo A	2.2	2.0
Mini Silo B	1.8	1.6
Controls	1.8	38.0

TABLE 3

INSECT SPECIES AND POPULATION INFESTING MAIZE STORED IN NITROGEN AND IN AIR. COUNTS FROM 1KG SAMPLES.

Period	Location		Sitophilus zeamais	Cryptolestes sp.	Carpophilus sp.	Tribolium castaneum	Moth	Lasioderma serricorne	Total
0 week	Mini silo	Top	10	0	5	9	2	1	27
		Middle	6	0	3	6	0	3	18
		Bottom	8	1	6	8	0	6	29
	Control		9	0	8	4	0	2	23
10 weeks	Mini silo	Top	0	0	0	0	0	0	0
		Middle	0	0	0	0	0	0	0
		Bottom	0	0	0	0	0	0	0
	Control		154	4	1	6	3	8	176

TABLE 4

AVERAGE MOULD COUNTS FROM MAIZE STORED IN NITROGEN AND IN AIR.

Sample	Average number of mould colonies isolated per gram of maize after storage for 4 and 10 weeks. The initial count was $9.9 \times 10^5$ .	
	4	10
SA 1	$2.8 \times 10^5$	$5.0 \times 10^4$
SA 2	$3.9 \times 10^5$	-
SA 3	$8.4 \times 10^5$	$2.6 \times 10^4$
AVERAGE	$5.0 \times 10^5$	$3.9 \times 10^4$
SB 1	$2.4 \times 10^5$	$4.4 \times 10^4$
SB 2	$1.5 \times 10^5$	-
SB 3	$1.6 \times 10^5$	$1.8 \times 10^5$
AVERAGE	$1.9 \times 10^5$	$1.1 \times 10^5$
CA	$5.8 \times 10^6$	$2.2 \times 10^7$
CB	$4.0 \times 10^6$	$2.8 \times 10^7$
AVERAGE	$4.0 \times 10^6$	$2.5 \times 10^7$

Note: For symbol explanation see Table 1.

constant during storage (crude protein, oil content, total ash), while total hexose sugars, total reducing sugars, fructose, glucose, sucrose and starch content remained unaltered in nitrogen but considerable changes of these carbohydrate components were observed for maize stored in the control drums. An increase in the free fatty acid content of oil (very high also at start of experiment) was observed in all samples, particularly marked in the maize stored in air.

The viability tests showed a slight loss for maize stored in nitrogen while there was a substantial reduction in the viability of the controls.

There was no adverse effect on the palatability of maize stored in nitrogen for ten weeks while the control had very poor palatability. The sensory evaluation was carried out on pap (ogi) made from the flour with boiling water. The corn flour was obtained by steeping whole grains in water for 48 hours. Sulphur dioxide was added to the water to prevent fermentation. The corn was wet-milled and sieved.

TABLE 5

AVERAGE ABUNDANCE OF DIFFERENT MOULD SPECIES IN MAIZE STORED IN NITROGEN AND THE CONTROL

Sample	Storage Time (weeks)	Average abundance of the different mould species given as no. of mould colonies per gram in maize						
		A. flavus	A. niger	A. tamarii	F. moniliforme	P. varioti	Penicillium sp	R. arrhizus
O (initial)	0	$5.1 \times 10^5$	*p	$2.1 \times 10^5$	$3.3 \times 10^5$	p	p	p
SA		$3.6 \times 10^5$	$0.4 \times 10^4$	p	$0.9 \times 10^5$	p	$1.3 \times 10^5$	p
SB	4	$6.1 \times 10^4$	$2.6 \times 10^4$	$1.7 \times 10^4$	$4.0 \times 10^4$	p	$6.0 \times 10^3$	-
C		$2.2 \times 10^6$	$1.5 \times 10^6$	$1.7 \times 10^4$	$1.8 \times 10^6$	-	p	p
SA		$4.5 \times 10^4$	p	p	p	p	$6.0 \times 10^3$	-
SB	10	$10.7 \times 10^4$	$4.9 \times 10^3$	-	-	$1.9 \times 10^4$	-	-
C		$2.6 \times 10^7$	$2.9 \times 10^6$	$3.1 \times 10^4$	$1.8 \times 10^4$	$1.1 \times 10^6$	$3.9 \times 10^6$	$1.3 \times 10^3$

\*p (present) means that the colonies are few and irregular.

TABLE 6

CHANGES IN CHEMICAL AND ORGANOLEPTIC FEATURES OF MAIZE STORED IN NITROGEN AND IN AIR FOR 10 WEEKS.

	nitrogen	air
crude protein	unchanged	unchanged
oil content	unchanged	unchanged
ash	unchanged	unchanged
sucrose	unchanged	decreased
glucose	unchanged	increased
fructose	unchanged	increased
starch	unchanged	decreased
free fatty acids	increased	strongly increased
viability	slightly decreased	strongly decreased
palatability	no adverse change	very poor



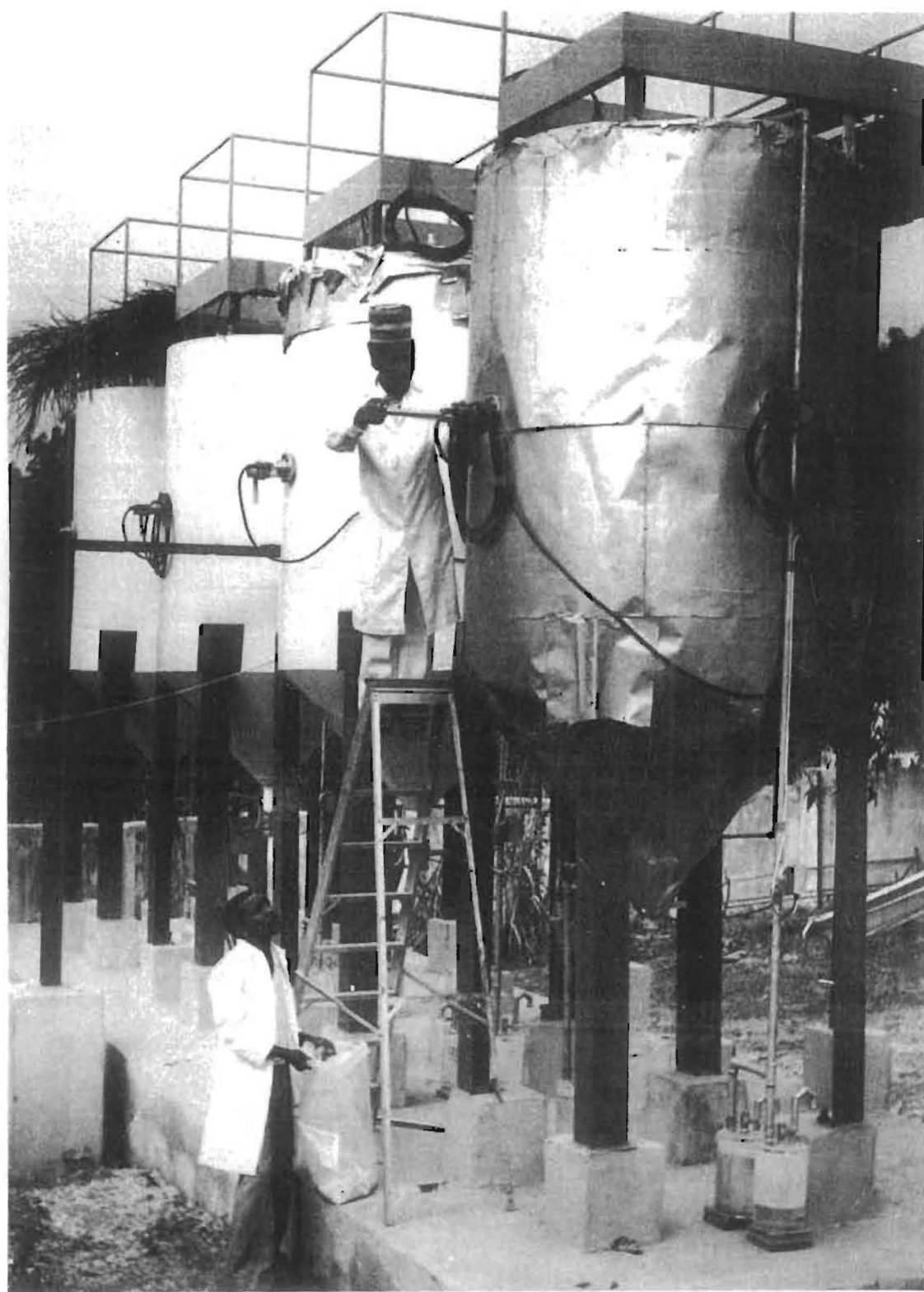


Fig.5. Sampling of pilot-scale nitrogen grain storage bins.

### Conclusion regarding the first storage trial in phase 1.

Nitrogen used as inert gas for storage of grains has been found effective in preserving the quality and reducing the substantial losses to which maize is prone under the conditions of the experiment.

As a result of the encouraging trend, the minisilos have now been filled with cowpeas (Vigna sp.). Cowpea is a very important vegetable protein in Nigeria. It is highly susceptible to insect damage especially by Callosobruchus maculatus which infests it from the field before harvest and continues its destruction in the store. Two varieties of cowpeas, the white and brown, are being tried. The set-up is similar to the trial described above.

### Phase 2.

The aims of the first stage of phase 2 of the collaborative research being two-fold:

a) to find the most effective method of adapting metal silos for nitrogen storage of grains to give good storage results in the humid tropics;

b) to find out the effectiveness of using nitrogen gas for controlling deterioration of stored grains under the different treatments to be applied in a),

temperature fluctuations, moisture migration, nitrogen consumption and changes in quality parameters of the stored maize were studied.

As can be seen in Fig.6, diurnal temperature fluctuations of the exposed metal surfaces of the roofs of the unshaded bins are very strong. The largest temperature fluctuation in the headspace gas is observed in the unprotected bin, while in the completely insulated bin and in that with an insulated roof, a certain attenuation of the phenomenon is achieved. The best protection is however obtained by simple shading of the top of the bin (Fig.7); both the roof surface and headspace temperatures show significantly lesser and slower fluctuations. Also the temperature difference between the metal and the gas is small at all periods of the day.

By sampling of the maize on the surface and by direct observation it was found that condensation had not occurred in any of the bins du-

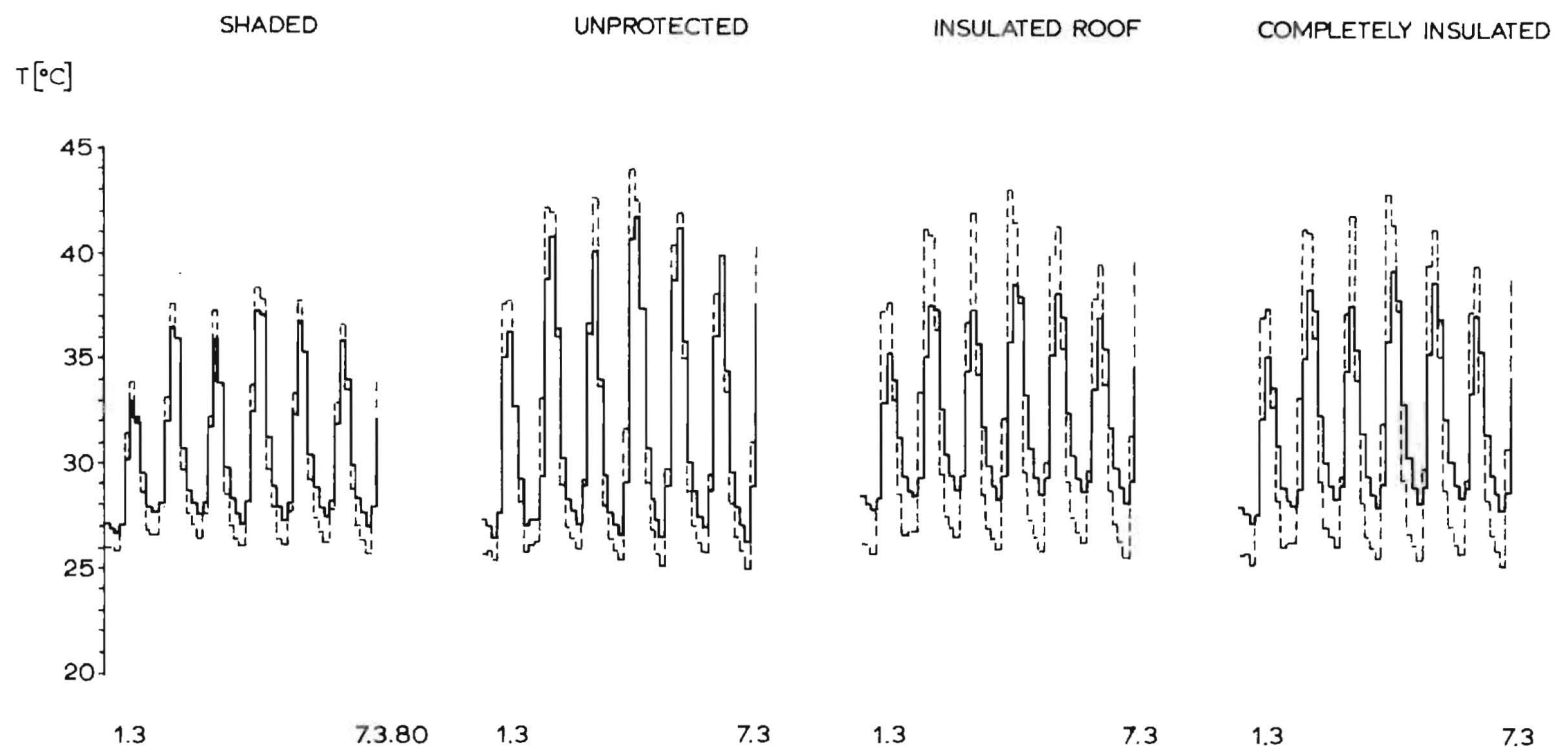


Fig.6. Diurnal temperature fluctuations of metal roof (broken line), in head-space gas (broad full line) and at the depth of 30 cm in stored product (thin full line) in 4 pilot-scale airtight storage bins differently protected against direct sunshine. The graph shows temperature readings recorded every 3 hours for one week (1st to 7th March 1980) during a nitrogen storage trial with dry maize.

All the quality parameters assessed in the experiments of phase 1 are being followed in this phase. Results during the first 4 months indicate that no changes have occurred in any of the silos, where a zero concentration of oxygen is maintained.

#### Conclusions regarding the first storage trial in phase 2.

Storage of dry maize in airtight metal bins in a nitrogen atmosphere is possible in the humid tropical climate. Exogenous agents, normally causing grain deterioration, are suppressed by complete anoxia. This is easily achieved by maintaining a slight overpressure inside the storage facilities by nitrogen. Heat production in the grain mass being reduced to a minimum, no temperature gradients build up and moisture migration does not occur.

Temperature fluctuations in the stored cereal grain are minimal and where grain is in direct contact with the gastight silo wall, no condensation occurs at ambient temperature excursions typical for the humid tropics.

Large thermal fluctuation in the headspace of metal bins occur due to effects of direct sunshine. At low moisture contents of the stored product in the described experiment (13% m.c. maize) the rate of temperature drop and the minimal ambient temperature reached were found to be such that the dew point was not reached on the roof of an unprotected metal bin. Simple shading of the roof was shown to give better protection against temperature fluctuations in the headspace and on the metal surface than elaborate and costly thermal insulation, thus indicating that higher moisture grain may be stored in a nitrogen atmosphere in the described climatic conditions, without danger of condensation. Experiments are being undertaken to establish the moisture content limits for safe long-term storage of cereal grains in the humid tropics.

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# ADIABATIC STORAGE TRIALS AS AN EXPERIMENTAL TOOL FOR PREDICTING STORABILITY IN ARTIFICIAL CONTROLLED ATMOSPHERES.

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## ABSTRACT

An experimental apparatus designed to follow autocatalytic phenomena which cause production of even very low quantities of heat (down to approx 0.07 W) is described. Such phenomena may occur inside industrial grain or oil seed storage bins, causing very damaging hot spots.

The results obtained with the described instrumentation thus permit to predict the behaviour of the stored products and their storability in various practical situations.

The behaviour of sunflower seeds at various moisture contents, initial temperatures and interstitial gas composition was studied in the apparatus. The existence of autocatalytic phenomena was observed. They increased in function of the level of the initial moisture content of grain, of the initial temperature and the oxygen content in the interstitial atmospheres.

Even in nearly complete anoxia (residual oxygen lower than 0.1% vol) heat development in the sunflower seed mass was observed. However the induction time was found to be significantly longer in this condition as compared to the corresponding tests in air.

## INTRODUCTION

The argument faced in the present work is to study and to simulate the thermal behaviour of grains stored in a silo.

It is well known that the axial part of a silo the grain is in

quasi-adiabatic conditions owing to the low thermal conductivity of the surrounding grains. The heat generated by the grain, even at very low rate, can increase the temperature in a sensible manner. At this increased temperature the heat generation rate increases so that an autocatalytic process can be activated, able to produce hot spots in the bulk of grain.

In order to study the conditions of hot spot generation, an experimental apparatus has been designed able to measure heat production rate of very low intensity.

The induction time and the heat production of sunflower seeds at various moisture contents, initial temperatures and interstitial gas compositions has been measured with the apparatus. These values are compared to the heat lost by the bulk of the seeds at steady state: only if heat production is equal to heat loss a steady state condition is possible. On this basis a simple storability criterium may be considered to predict the possibility of hot spot generation.

#### EXPERIMENTAL APPARATUS

The experimental apparatus is a microsilos with a double PVC wall with an air interspace. (Fig. 1).

The internal diameter is 20 cm, the height is 50 cm, the capacity is 5 kg of sunflower seeds.

The microsilos is immersed in a thermostatic stirred water bath having a controlled and homogeneous temperature: the difference in temperature at different positions and/or different time is less than  $0.1^{\circ}\text{C}$ . The bath has an external 3 cm thick rock wool thermal insulation and is heated by four electrical radiators of 85 W and  $0.3\text{ m}^2$  of surface each. An air flow is supplied at the bottom of the bath through a perforated tube in order to increase the heat exchange between radiator-water and water-microsilos and to homogenize the temperature.

Two temperature probes (calibrated resistances) are placed at the same height on the wall of the microsilos: the first ( $T_1$ ) on the internal wall in contact with the stored product, the second ( $T_2$ ) on the external wall, in contact with the bath. When the internal wall

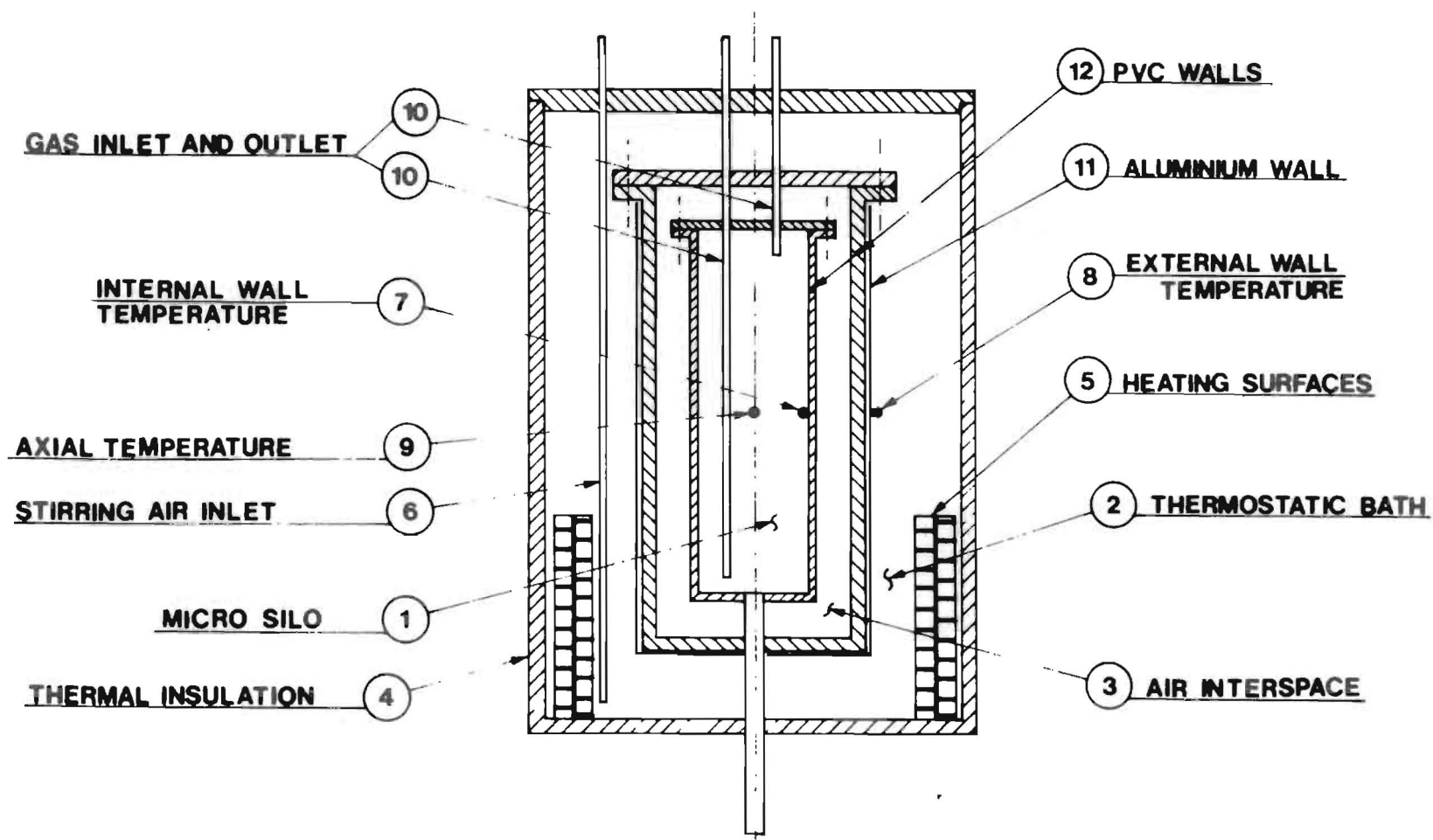


Fig.1. Experimental apparatus for adiabatic storage tests.



temperature  $T_1$  is greater than  $T_2$ , the temperature control system actions the electrical heating until equalization of the two temperatures is reached.

A third temperature probe is placed in the centre of the microsilos in order to measure the bulk temperature.

Three identical instruments with the above mentioned characteristics have been built in order to test at the same time the same product in different experimental conditions.

#### CALIBRATION OF THE APPARATUS

The principle of the set up is to maintain at every time the temperature of the bath at the same value as the temperature of the stored product, in order to achieve adiabatic conditions.

To approach these conditions we tried to minimize the heat loss by the product ( $Q_1$ ) and the heat gained by the product ( $Q_2$ ). The heat lost by the product is:

$$Q_1 = \alpha S(T_1 - T_2)$$

where  $S$  is the microsilos wall area and  $\alpha$  is the thermal conductivity through the walls. We tried to minimize  $\alpha$  by the two PVC walls with air interspace, and to minimize  $(T_1 - T_2)$  by the temperature control. Nevertheless  $\alpha$  and  $(T_1 - T_2)$  are not equal to zero so that  $Q_1$  is equal to the heat conducted through the microsilos walls with a temperature gradient less or equal to the sensibility of the temperature control. The sensibility is equal to  $0.1^\circ\text{C}$ . The value of  $Q_1$  was measured by calibration tests performed with electrical heating sources of known values.

The heat gained by the product is:

$$Q_2 = \alpha S(T_2 - T_1)$$

During the operation of the apparatus the thermal control system heats the bath until the temperature  $T_2$  becomes equal to  $T_1$ . But the heating of the bath may generate temperature gradients so that at the time when heating stops some parts of the bath may have a temperature greater than  $T_2 = T_1$ . The water of these parts mixes with the bulk and a mean temperature  $T_2' = T_2 + \Delta T$  greater than the product temperature  $T_1$  may build up in the bath (overshot), and heat is gained

by the product.

The above mentioned reduction of the thermal conductivity to minimize  $Q_1$ , contributes to minimize  $Q_2$  too. To reduce  $\Delta T$  the bath is homogenized by air bubbling; besides heat exchange is enhanced by the large area of the heating surface. In this way we obtained values of  $\Delta T$  smaller than  $0.1^\circ\text{C}$  in the bath.

$Q_1$  and  $Q_2$  were measured by calibration tests performed by operating the apparatus with simulation of heat generation by a  $0.82\ \Omega$  electrical resistance supplied by direct current of measured intensity. The resistance was placed in a 5 kg wheat bulk in the microsilos, having a very low moisture content (8%) in order to be sure that the heat generated by the product is zero.

The product temperature was measured as function of time at a 0.03, 0.07 and 0.20 W electrical power supply (Fig.2). The temperature of the product ( $T_3 \approx T_1$ ) is constant at the lowest power supplied (0.03 W), it is a straight line function of the time at the other values of the power supplied. This behaviour is in agreement with the hypothesis of quasi - adiabatic conditions with a constant power supply. The heat adsorbed by the product was calculated by means of the measured temperature increase using a value of  $0.5\ \text{Kcal/kg}^\circ\text{C}$  as specific heat of the grain. The values of the heat absorbed by the grain are 0, 0.04 and 0.23W, when the values of the heat supply are respectively 0.03, 0.07 and 0.20 W.

It appears that the sensibility of the thermal control system is about 0.03 W. In other words at a very low heat production rate the system is not perfectly adiabatic and loses about 0.03 W ( $Q_1 = 0.03\ \text{W}$ ), at high values of heat production rate the tested product gains from the surrounding about 0.03 W ( $Q_2 = 0.03\ \text{W}$ ).

In conclusion the experimental apparatus appears to be able to study and to measure heat production rates in the field of 0.07 - 0.20 W with a  $\pm 0.03\ \text{W}$  error.

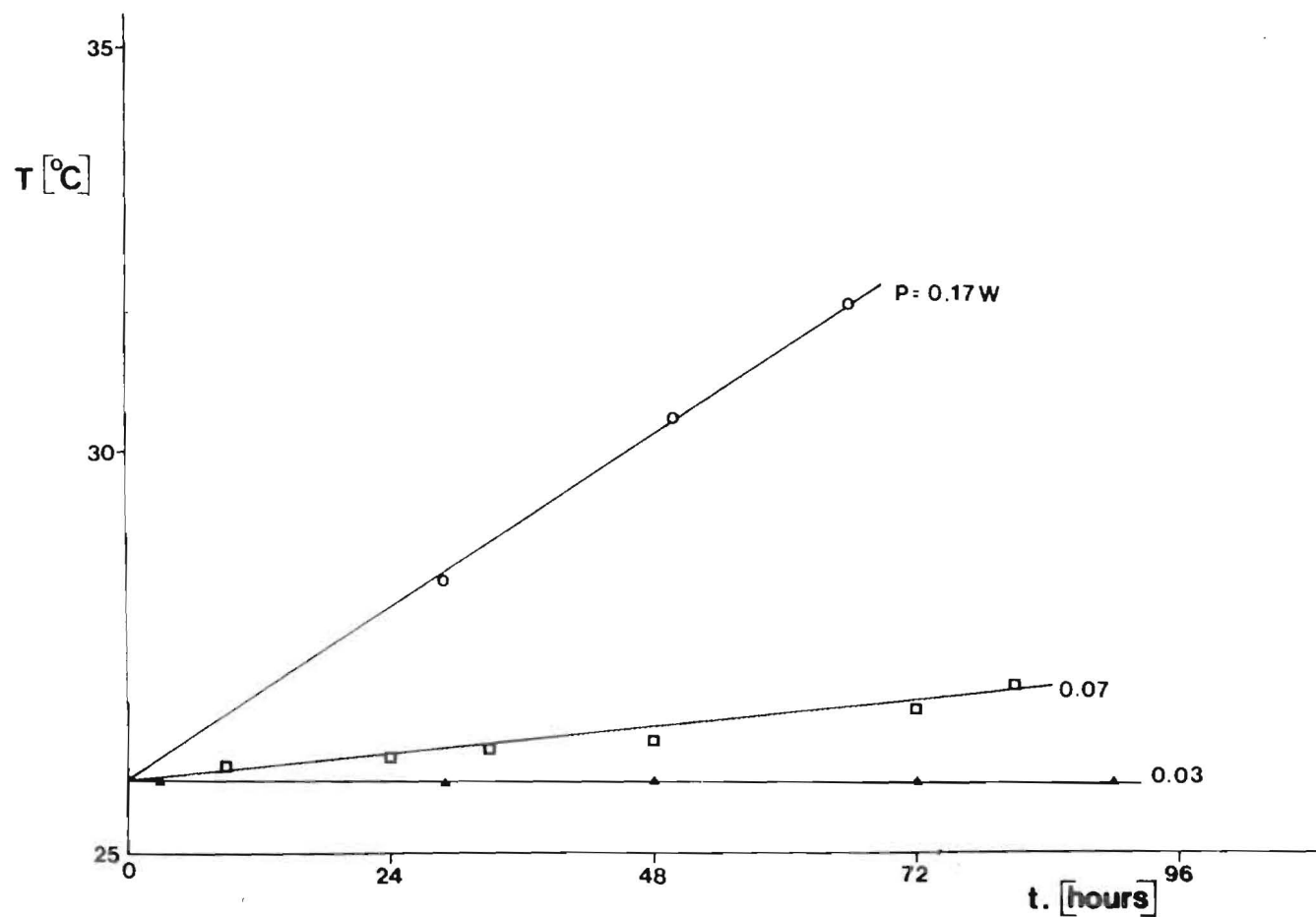


Fig.2. Calibration tests. Product temperature versus time at different values of the external power supplied ( $P$ ).

## EXPERIMENTAL RESULTS

The apparatus was used to study the behaviour of third grade sunflower seeds.

The moisture content of some lots of the seeds was increased by a slow addition of water in order to have three lots of products to be tested with 8.4, 9.3 and 12.6% moisture content. The time necessary for moistening was about 24 hours.

Two different preservation systems were tested:

(1) Controlled atmosphere (ACT): the interstitial atmosphere of the seeds was pure nitrogen (the residual oxygen content was 0.05% vol) the microsilos were purged with UPP nitrogen and maintained at a slight positive pressure for the whole test time.

(2) Confined atmosphere (ACF): the interstitial atmosphere of the seeds was nitrogen with a decreasing oxygen content in function of the time owing to the activity of the seeds contained in the gas tight microsilos.

Such behaviour is shown in fig. 3 where the decreasing oxygen content is shown simultaneously with the increasing temperature.

In fig. 4 the results of the ACF tests at 26°C and 21°C initial temperature are shown: the seed temperature versus time sharply increases after an induction time  $t_i$  that increases with decreasing moisture content of the seed. The time  $t_i$  is approx. 0 at 12.3% moisture content, approx. 2 days for 9.3% m.c., approx. 5 days for 8.4% m.c., at 26°C initial temperature.

At 21°C a very different behaviour appears and the seed at 9.3% m.c. does not show any increase in temperature for the whole test time (about 22 days).

In fig. 5 the results of the ACT tests at 26°C initial temperature are shown: the seed temperature increases in function of time in about the same way as in the ACF tests, but the induction times are much longer:  $t_i$  is greater than the test duration (100 days) at 8.4% m.c.; it is approx. 25 days at 9.3% m.c., and approx. 15 days at 12.6% m.c.

The comparison between ACF and ACT the preservation technologies is shown in fig.6 for sunflower seeds 9.3% m.c.

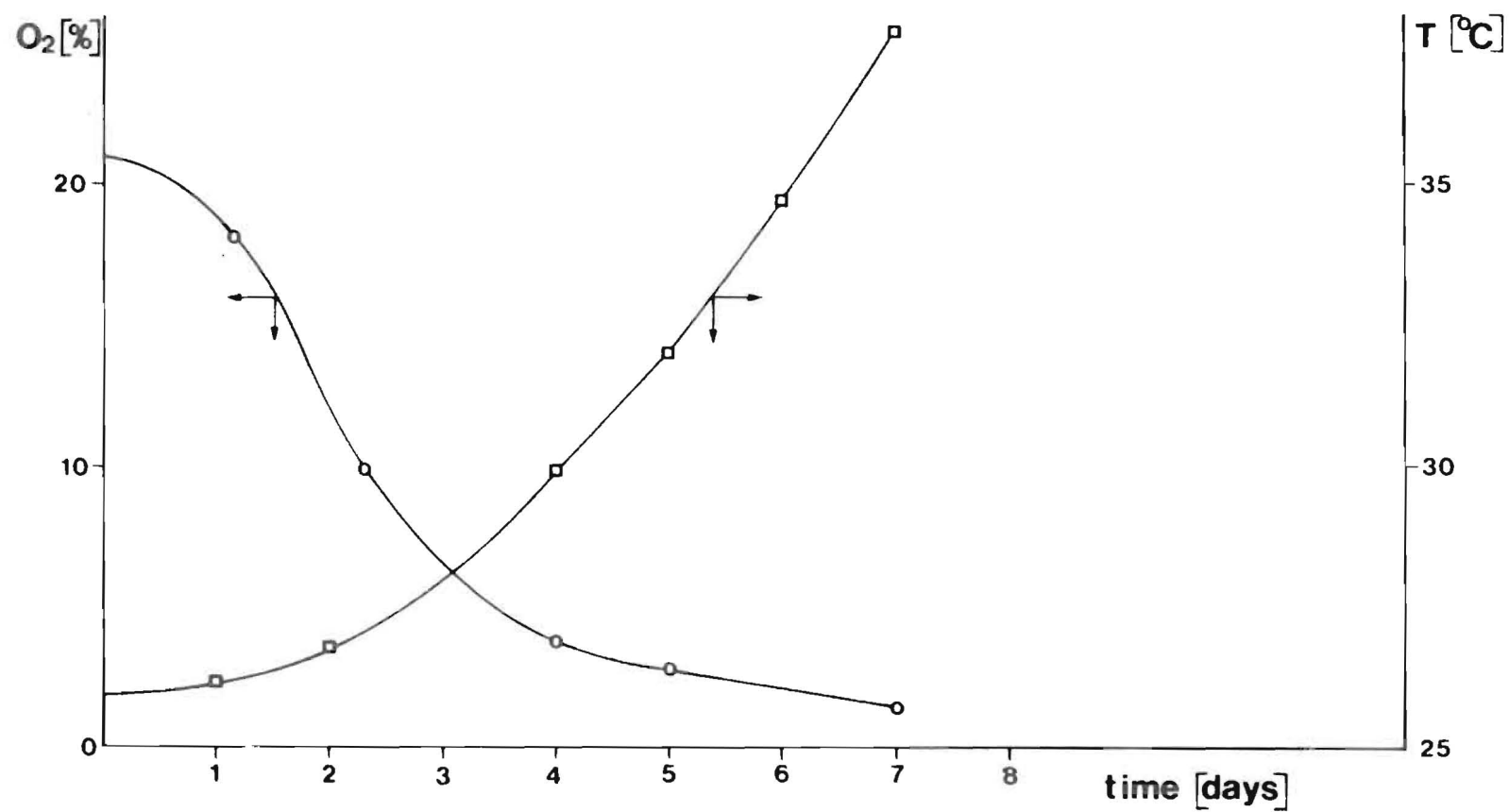


Fig.3. Confined Atmosphere tests. Temperature and oxygen content versus time for sunflower seeds at 9.3% moisture content.

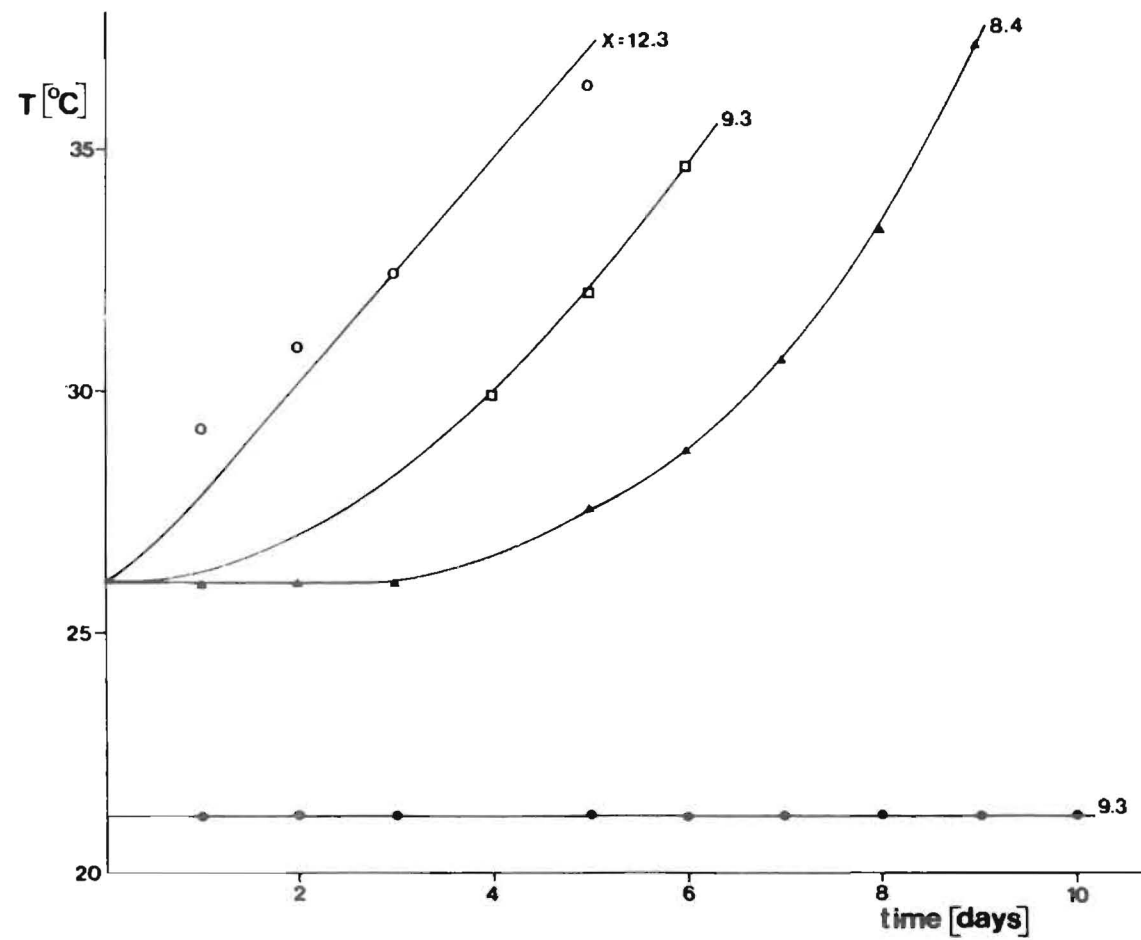


Fig.4. Confined Atmosphere tests. Temperature versus time for sunflower seeds at different moisture contents ( $X$ ).

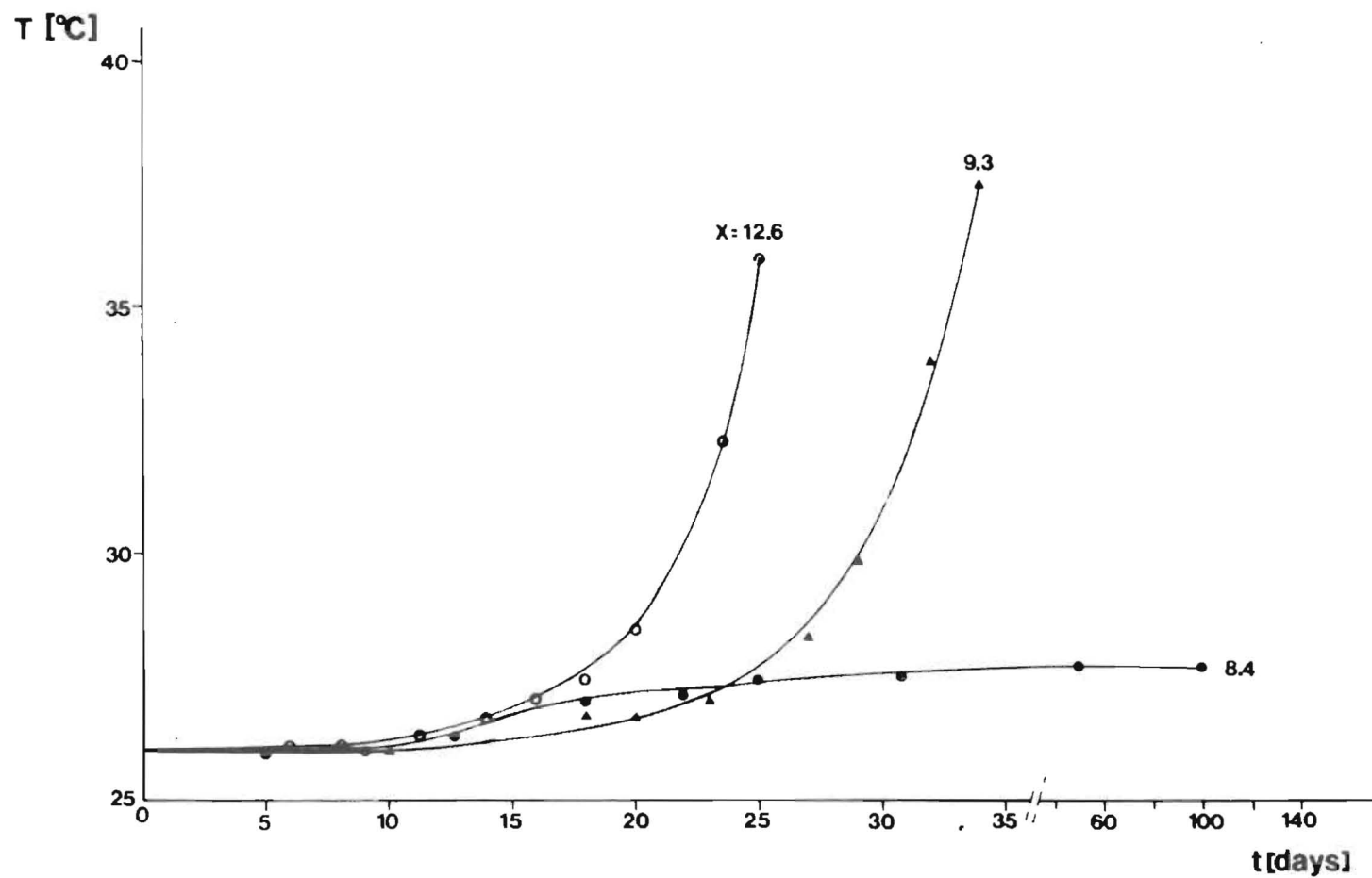


Fig.5. Controlled Atmosphere tests. Temperature versus time for sunflower seeds at different moisture contents ( $X$ ).

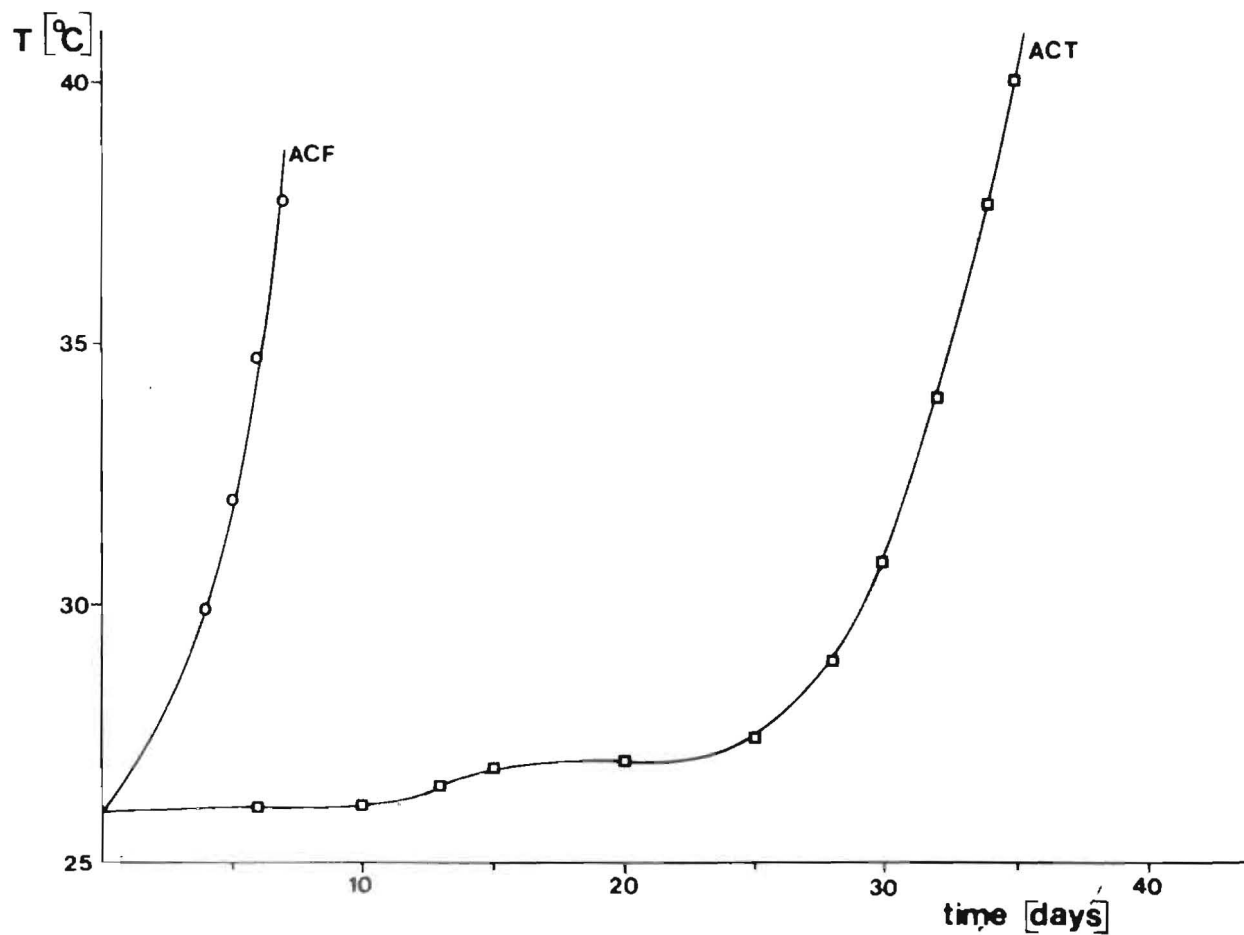


Fig.6. Comparison between confined (ACF) and Controlled (ACT) atmosphere preservation of sunflower seeds at 9.3% moisture content.



## DISCUSSION OF THE RESULTS

The results obtained allow to calculate the heat generation rate  $S_g$  at the tested conditions.

In adiabatic conditions:

$$S_g = C_p \frac{dT}{dt} \quad (1)$$

where  $S_g$  is measured as cal/(h x kg) and  $C_p$  is the specific heat of sunflower seeds assumed to be equal to 500 cal/kg °C.

The temperature  $T$  and the gradient  $dT/dt$  measured during the described tests change in function of time. The gradient values may be assumed to be function of temperature alone, so that  $S_g$ , as function of  $T$  may be calculate. The calculated values of  $S_g$  for 9.3% m.c. sunflower seed preserved in ACF and ACT are shown in figure 7. These values, together with the induction times are the fundamental parameters required to predict storability of a product in a given condition.

An exact prediction may be possible only with a detailed and complicated model. But if we need only a rough storability prediction we can utilize a simple model, here described, that allows anyhow to evaluate the influence of the more important variables:

- product characteristics, summarized by the  $S_g = f(T)$  function
- silo diameter ( $2R$ )
- ambient temperature ( $T_o$ )
- preservation technology (ACF or ACT).

The model examines whether the system may attain a steady state condition. If it is not possible, the system has the trend to generate hot spots in oposite case no heating will occur.

The steady state conditions for a cylindrical homogeneous system having a thermal conductivity  $K$  and a heat generation rate  $S_g$  are represented by the following temperature distribution (Bird et al. 1960):

$$T - T_o = \frac{S_g R^2}{4 K} \left[ 1 - \left( \frac{r}{R} \right)^2 \right] \quad (2)$$

where the sunflower seed thermal conductivity  $K$  is assumed to be equal to 1.44 cal/cm.h.°C,  $T$  is the temperature at a given distance  $r$  from the axis of the cylinder,  $T_o$  is the external temperature corresponding to the cylinder radius  $R$ ,  $S_g$  is the heat generation rate at each particular temperature  $T$ . The maximum temperature occurs at the axis of the cylinder ( $r = 0$ ):

$$T_{\max} = T_o + \frac{S_g R^2}{4 K} \quad (3)$$

At steady state the heat generation rate is equal to the heat dissipation rate in every position and every time. So that from equation (3) we can calculate the steady state heat dissipation rate ( $S_d = S_g$ ) as function of the maximum temperature  $T = T_{\max}$

$$S_d = \frac{4 K}{R^2} (T_{\max} - T_o) \quad (4)$$

In other words steady state conditions are possible if the heat dissipation rate, indicated by equation (4), may equalize the heat generation rate measured in the present work for sunflower seed and having the temperature dependence shown in figure 7:

$$S_g = f(T_{\max}) \quad (5)$$

From a graphic point of view steady state is possible if the straight line represented by equation (4) intercepts the curve represented by equation (5).

This analysis is performed in figure 7 for 9.3% m.c. sunflower seed for the ACF and ACT preservation technologies. The diameter of the silo is assumed equal to 1 m; the external surrounding temperature is assumed to be equal to 23, 25 and 27°C for the three conditions

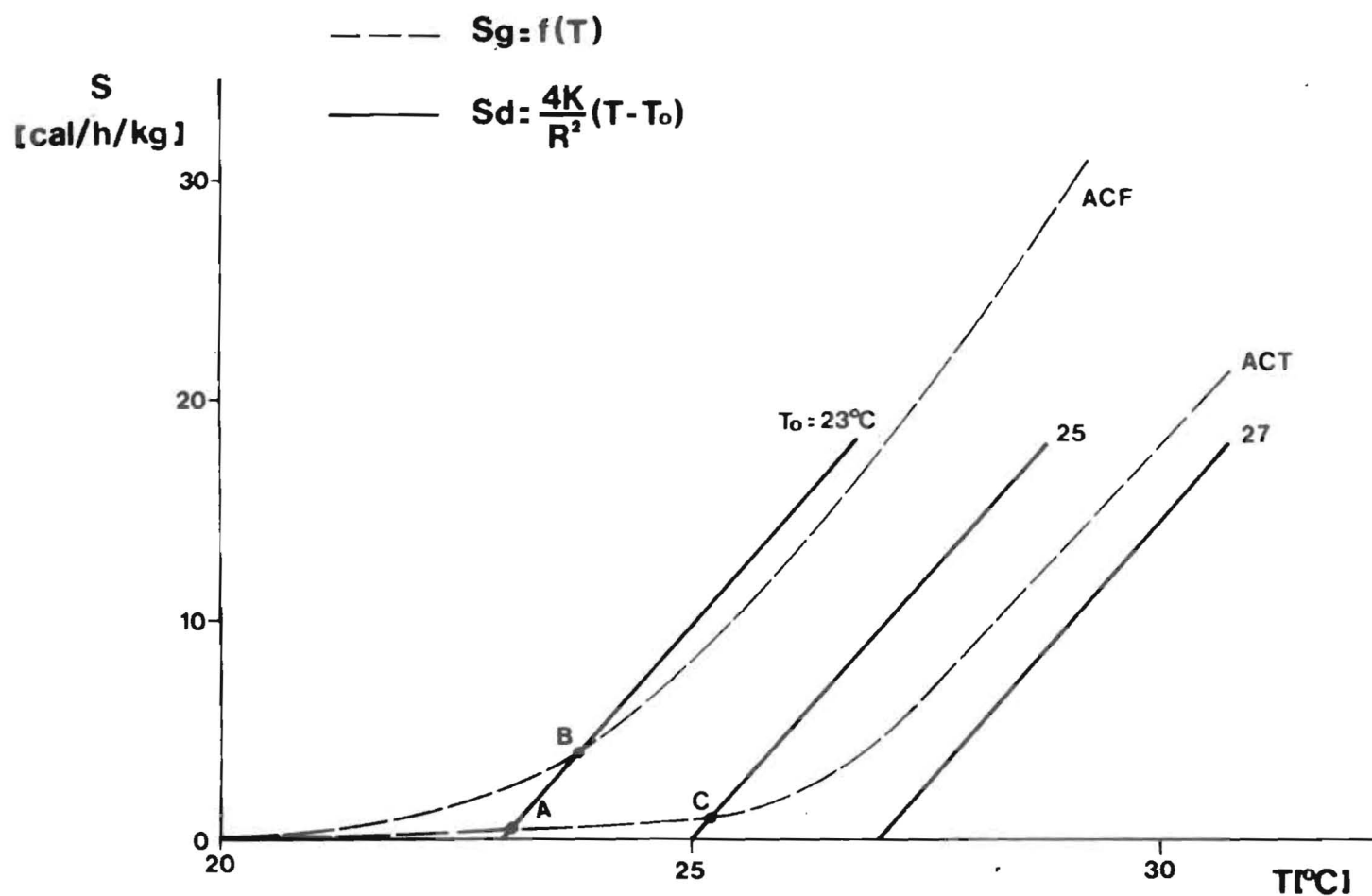


Fig.7. Heat generation rate ( $S_g$ ) and heat dissipation rate ( $S_d$ ), function of the product temperature of sunflower seeds at 9.3% moisture content at different surrounding temperatures  $T_o$  and 1 m silo diameter.

examined.

When  $T_o$  is equal to  $23^{\circ}\text{C}$  the straight line (4) intercepts the ACF and ACT curves (5) in the A and B points. The system has the trend to attain steady state condition for both the ACF and ACT technologies with the maximum temperatures equal to the coordinates of the A and B points.

When  $T_o$  is equal to  $25^{\circ}\text{C}$  the straight line (4) intercepts the ACT curve only (point C). It means that in controlled atmosphere the system has the trend to attain a steady state, in confined atmosphere it has not.

At last when  $T_o$  is equal to  $27^{\circ}\text{C}$  it is impossible to equalize the heat generation rate to the heat dissipation rate.

This analysis is performed again in figure 8 with a constant external surrounding temperature  $T_o = 25^{\circ}\text{C}$  and three values of the silo diameter: 0.6, 1 and 2 m.

The system appears stable (steady state possible) for both ACF and ACT conditions at 0.6 m diameter (points A' and B'); it is stable in ACT conditions only at 1 m silo diameter (point C); it is unstable in both cases when the silo diameter is greater than about 2 m.

The extension of this analysis allows to determine the trend of the system as function of the silo diameter and the external surrounding temperature for 9.3% m.c. sunflower seeds (figure 9).

Silos of low diameter allow to preserve the product at high external surrounding temperature without any trend to hot spot formation. Increasing the silo diameter, the maximum safe surrounding temperature decreases. Furthermore it appears that the ACT preservation technique extends the conditions for the safe preservation of the product in comparison with the ACF preservation system.

The above described model considers only the existence of a steady state, but it does not evaluate the way and the velocity of the system development. Therefore a system does not generate any hot spot, although it is in conditions of hot spot trend, if the preservation time is less than the induction time.

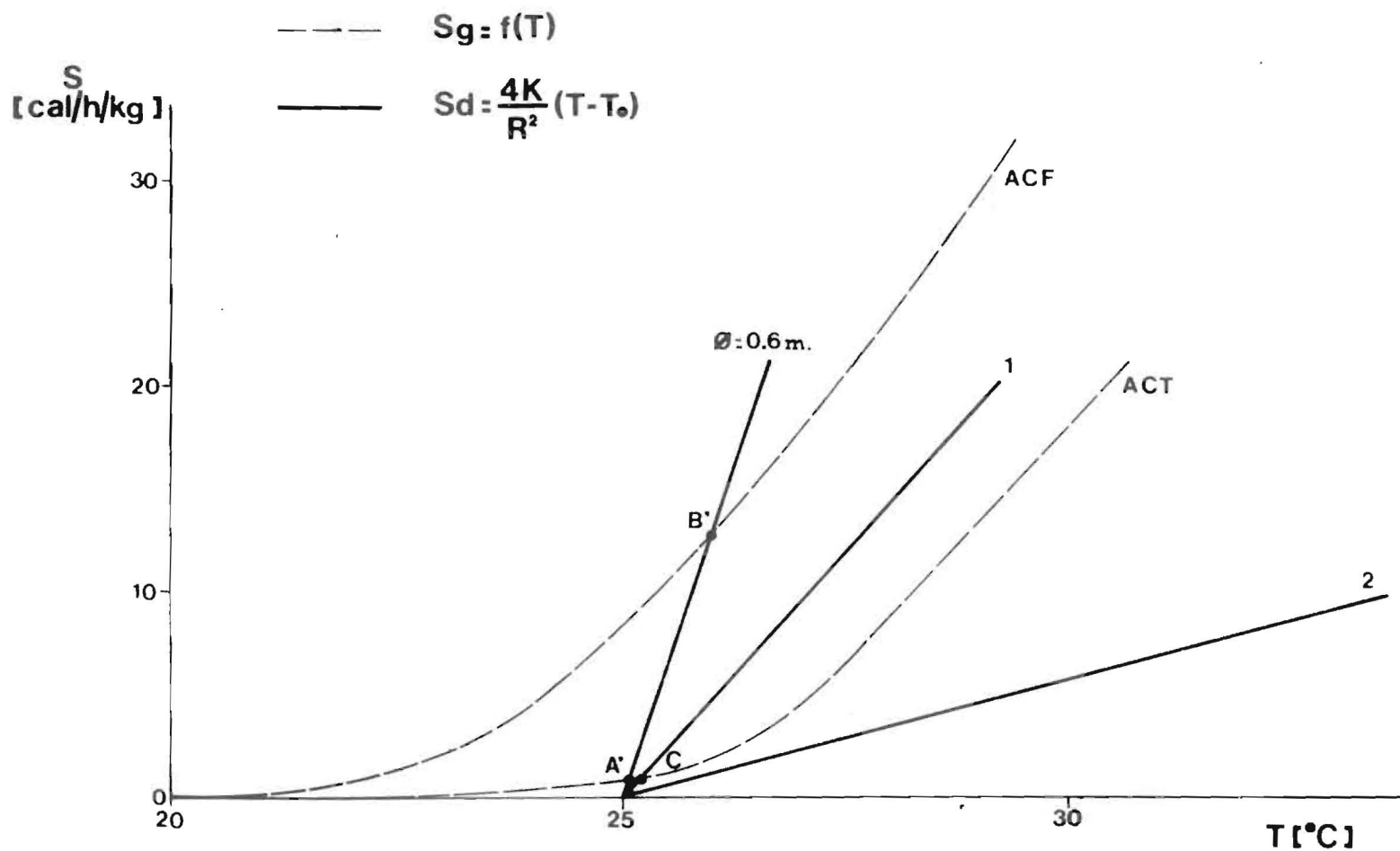


Fig.8. Heat generation rate ( $S_g$ ) and heat dissipation rate ( $S_d$ ), function of the product temperature of sunflower seeds<sup>g</sup> at 9.3% moisture content at different silo diameter and 25°C surrounding temperature.

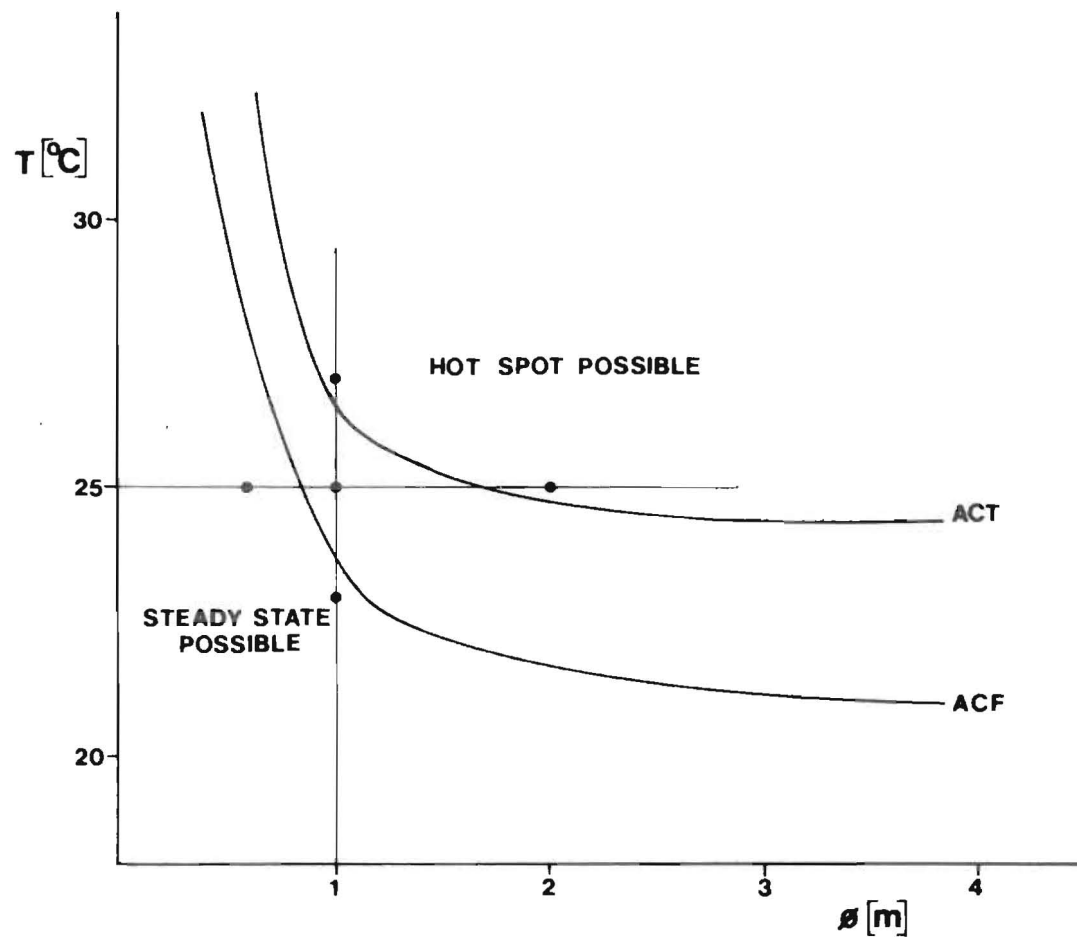


Fig.9. Trend of 9.3% moisture content sunflower seeds as function of silo diameter  $\varnothing$  and surrounding temperature  $T$  for confined and controlled atmosphere conditions.

## CONCLUSIONS

The results obtained in the present work allow to draw the following conclusions:

- The above described apparatus is suitable to measure the fundamental parameters required to predict the storability of a product from the point of view of hot spot generation.
- These parameters are: the induction time  $t_i$  and the heat generation rate  $S_g$ .
- For sunflower seeds decreasing values of  $t_i$  and increasing values of  $S_g$  were measured with increasing moisture content and temperature of the seeds. Even in nearly complete anoxia (residual oxygen content about 0.05%) heat development in the sunflower seed was observed. However the induction time was found to be significantly longer, and the heat generation rate lower than the corresponding values in confined atmosphere conditions.
- A simple storability criterion was presented. It considers the trend of the system, analyzing the heat generation rate and the heat dissipation rate as function of temperature. This criterion allows to understand the hot spot formation in large silos and/or at high surrounding temperatures, while in the same conditions hot spots do not build up at lower silo diameters and/or lower surrounding temperatures. This criterion must be used with precaution because of its simplifying assumptions; but of course it appears more correct than the direct extension of results obtained with laboratory silos of very low diameters.

## ACKNOWLEDGEMENTS

Thanks are due to Mr. G. Leonardi and Mr. M. Marini for the effective collaboration in carrying out both the project and construction of the apparatus and the experiments themselves.

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## V S E S S I O N :

PRESERVATION OF QUALITY IN CONTROLLED ATMOSPHERES



ring the first 4 months of the nitrogen storage trial (January to May 1980). The moisture content in the upper layer of the bins remained practically unaltered during the storage period (13.05 - 13.55%).

The initial purge of the storage bins by pure nitrogen reduced the oxygen content in the grain mass to less than 0.5%. Due to the high temperature of the stored maize, the residual oxygen was consumed within hours by the stored product and a condition of complete anoxia was maintained throughout the storage by an over-pressure of 50 mm w.g. ensured by an automatic supply of pure nitrogen from the top.



Fig.7. Experimental shaded steel bin at NSPRI, Ibadan. Simple shading of the roof of an airtight metal bin, leaving enough space between the thatch of palm leaves and the top of the storage structure to ensure free air circulation, permits safe storage of grain in a nitrogen atmosphere.

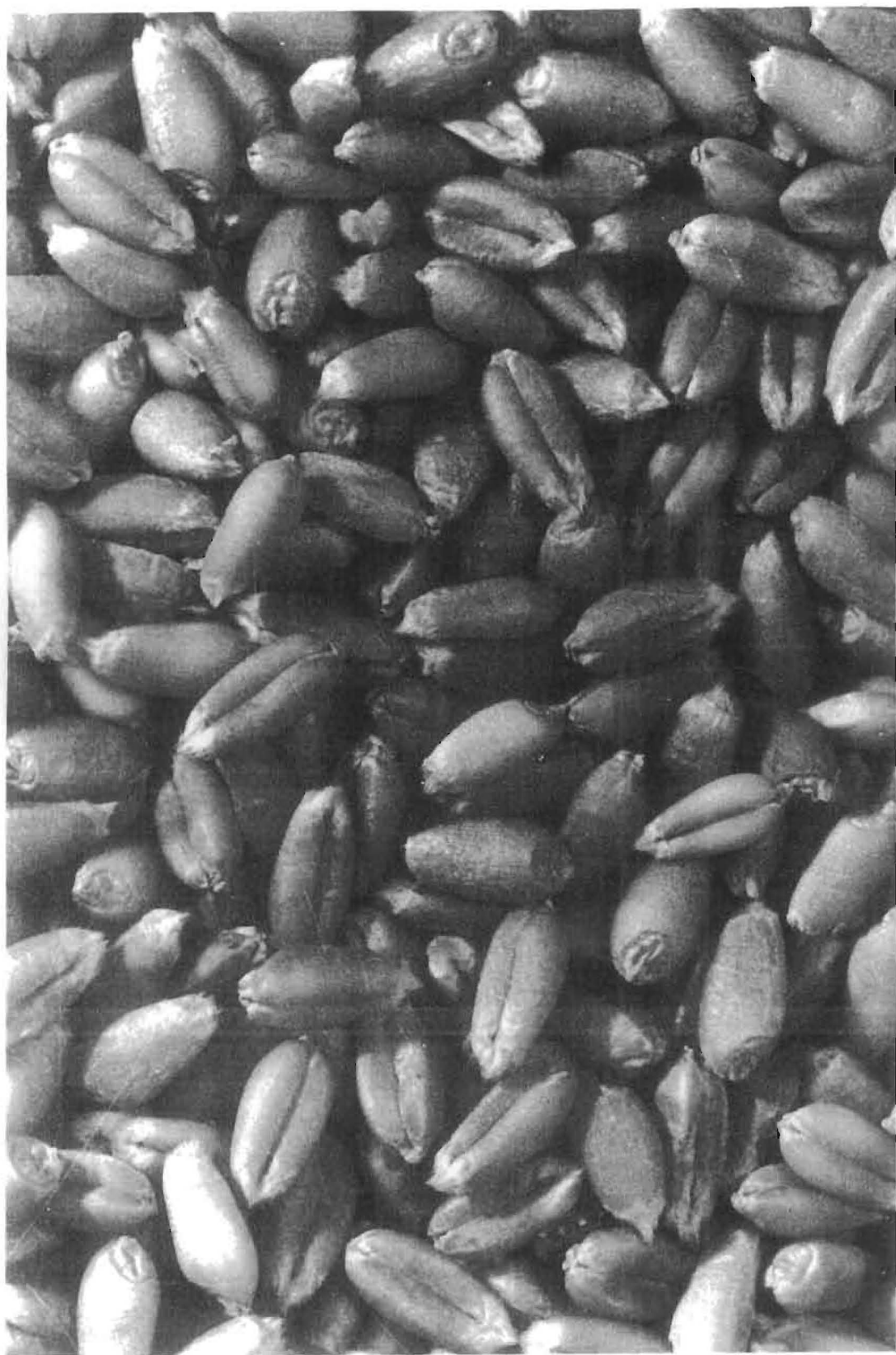


Photo Agenzia Italia, Roma

## CHANGES IN QUALITY OF WHEAT DURING 18 YEARS STORAGE

BY S.W. PIXTON

### 1. INTRODUCTION

Rapid deterioration in the quality of dry grain is usually caused by insect, mite or rodent attack but even in the absence of these pests quality declines slowly in storage. This investigation was started in 1957 to examine the changes that take place when grain is held in large quantities for a long period in the British Isles. It was assumed that grain deliberately kept on a national scale for several years would be stored in specially constructed containers that could be made airtight or be maintained at a low temperature and that the grain would be pest free. In such containers the grain would be protected from insect and rodent attack and if initially too dry to be vulnerable to fungi would remain so.

Development of fungi and mites can be minimised by keeping the equilibrium relative humidity (e.r.h.) of the grain below 65% but many species of stored product insects can do considerable damage to even drier grain. Whilst all thrive at 70% e.r.h., many can develop at 40% e.r.h. Control of insects is usually achieved by fumigation or with insecticides. Not only are such treatments expensive but they may need to be repeated if the grain remains long in storage and so give rise to objectionable residues or undesirable chemical changes in the grain. Insect development, however, is restricted by low temperature and, in well made storage structures, by the depletion of oxygen. Bailey (1955, 1956, 1957, 1965) showed that certain insects common in storage are killed when the oxygen concentration falls below 2% by volume and concluded that this depletion of oxygen rather than the increase in carbon dioxide was the important factor controlling insect populations. Spratt (1975), however, demonstrated not only that oxygen is used up more quickly when carbon dioxide is present but that adults die at higher oxygen concentrations. Thus more effective control can be expected when the reduction of oxygen is accompanied by a corresponding increase in carbon dioxide. Burges and Burrell (1964) showed that in dry grain at a temperature of 5°C those insects and mites that are not killed are inactive and cause no heating.

Although it is difficult to remove oxygen completely it is commercially practicable to keep concentration low in carefully built storage structures. Also if there is sufficient advantage in storing grain at low temperature

economical methods for doing this could be developed. Handling charges form an expensive fixed charge to which storage for long periods, that requires little, therefore adds little. The real cost of long term safety, therefore, is the extra cost of cooling or keeping a low oxygen concentration.

This experiment was set up to investigate the effects of coolness and low oxygen concentration, singly and in combination, on the keeping quality of dry wheat stored for human food and for seed. The experimental design, the wheat characteristics examined, and the results obtained after eighteen years storage are described in this paper.

## 2. MATERIALS AND METHODS

### 2.1 Type of wheat used

Two very different types of wheat were stored, a hard Canadian breadmaking wheat No 1 Northern Manitoba, and a soft English wheat, Cappelle, which was suitable for the production of biscuit flour. In 1957 when this experiment was started these were the types of wheats usually stored in the U.K. The Canadian wheat, of the 1956 harvest, had a moisture content of 11.9% on receipt but the Cappelle wheat, harvested in 1957, received at approximately 15%, had to be dried and was eventually used at 12.6%. Both wheats were thoroughly cleaned and aspirated before loading.

### 2.2 Storage conditions

The wheats were stored in four welded steel cylindrical bins approximately 3 m high and 2 m diameter. These were almost airtight but no attempt was made to ensure perfect sealing. Each bin was divided by wood and hardboard into two semi-cylindrical chambers each of one tonne capacity. One lot of each of Canadian and of English wheat was put separately into each bin (see Fig. 1). To prevent the development of moisture gradients a gentle circulation of the atmosphere in each bin was provided.

Two bins were provided with a continuous supply of fresh air pumped through at a rate equivalent to one complete change of atmosphere approximately every eight days. Before it reached the bins the air was passed through a column of dry wheat (initially at 9% moisture content) which was changed periodically. This arrangement ensured that the moisture contents of the wheats were not much changed and that there was no metabolic loss of oxygen.

In the other two bins the oxygen concentration was reduced to 1%-2% by volume by flushing with nitrogen. The oxygen concentration was measured at intervals and maintained at the correct level by further flushing as required. A slight positive pressure was maintained in the bins to prevent oxygen entering the bins from the atmosphere.

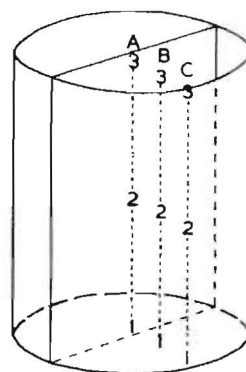
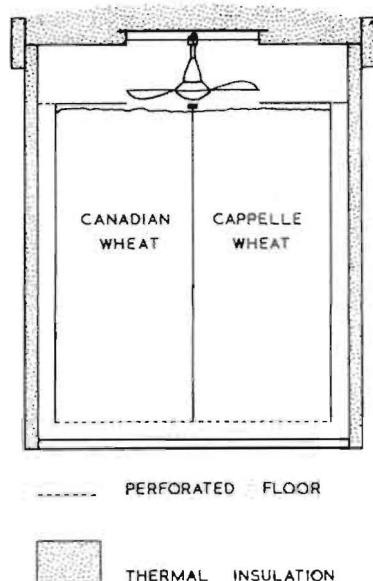


Fig. 1. Section of a storage bin

Fig. 2. Sampling points within each bin

A cold room maintained continuously at  $4.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  was built to enclose two of the bins one with low oxygen atmosphere and one with normal oxygen atmosphere. The other two bins were exposed to outdoor ambient temperatures, but were thoroughly insulated to simulate conditions within large bulks. All four bins were under the cover of a Dutch barn walled in on three sides.

Temperatures in the centre and at the outer edge of each of the eight wheat bulks, of the cold room and of the ambient air were measured by thermocouples connected to a slow chart recorder. Provision was also made in each bin for gas exchange and for gas sampling.

The four storage conditions are summarised in Table 1.

TABLE 1

Summary of the storage conditions used

Oxygen concentration by volume	Temperature of grain
< 2%	
Atmospheric	$4.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$
< 2%	Ambient
Atmospheric	(Annual cycle $2^{\circ}\text{C} - 20^{\circ}\text{C}$ )

### 2.3 Tests carried out on the wheats

In order to be able to detect changes, some perhaps very small, which might take place over a long period it was necessary to characterise the wheats in their original condition as closely as possible, and to use for this purpose tests which could be precisely repeated from recorded description alone. The following determinations were made on each type of wheat initially and at intervals of two years.

Moisture content: oven drying,  $113^{\circ}\text{C}$ . 4 hr. (Oxley et al, 1960)

Protein: a) N x 5.7 (Kjeldhal), 13.5% moisture basis

b) N soluble in  $\text{K}_2\text{SO}_4$ , 13.5% moisture basis

Fat: a) Total, by acid hydrolysis, % dry weight basis

b) Free fatty acid, mg KOH/100g grain, dry weight basis

Carbohydrate: a) Non reducing sugars as sucrose, mg/10g grain dry weight basis

b) Reducing sugars as maltose, mg/10g grain dry weight basis

Milling: a) Extraction, % total products

b) Colour grade number

Dough tests a) Resiliency

b) Extensibility

c) Water absorption, l/Kg

)  
)  
) --- by extensometer  
)

Baking tests: pup loaves with 0, 10, 20, 30 ppm potassium bromate added

Amylograph maximum viscosity

Titrateable acidity of flour, %  $\text{KH}_2\text{PO}_4$

Vitamin B<sub>1</sub> assay

Viability: a) Germinative energy

b) Germinative capacity, %

Microflora: a brief examination for living micro-organisms, as described later.

### 2.4 Sampling

An average sample of each variety of wheat was taken at the start of the experiment by bulking 200 g samples from each 50 Kg of wheat as it was loaded

into the bins in rotation. Subsequently at 2-yearly intervals 1 Kg samples were drawn from each kind of wheat in each bin from nine sampling positions (Fig. 2) by using a vacuum spear. The moisture content of each sample was determined separately and then all other tests listed were made on the thoroughly mixed bulked samples for each wheat from each bin.

## 2.5 Microflora

A large number of small samples of both types of wheat were collected as the bins were being filled. These were bulked and divided into 10 g portions which were placed in sterile test-tubes. Some of these sub-samples were examined immediately for micro-organisms and the rest distributed randomly between the four bins where they were pushed into the surface of the wheat and so encountered the same environment as the grain bulks. The examination procedure was to culture from a number of whole grains from each sub-sample and to make dilution plate counts of fungi and bacteria. A detailed account of the methods used and of the results obtained after 10 yr storage is given by Pixton et al, (1975).

The main experiment was terminated after 18 years when the bins were required for another experiment.

## 3 RESULTS AND DISCUSSION

### 3.1 General observations on the wheats

The wheats were examined for signs of mould and for abnormal smell or taste. None was ever detected; the wheats were always bright and free running.

### 3.2 Chemical analyses (Fig.3)

The content of crude protein, initially 12.9% for Canadian and 8.9% for English wheat, and of salt-soluble protein, initially 2.4% for Canadian and 1.9% for English wheat, remained unchanged under all conditions of storage. Values for total fat, initially 2.9% and 2.4% for Canadian and English wheat respectively, increased by about 0.5%. This small increase can be attributed to metabolism of carbohydrate during the 18 yr storage period.

Free fatty acid values increased steadily in both types of wheat. The rate of increase in the warmer bins was approximately twice that in the cooler bins.

There was a slight reduction in total sugars after 8 yrs storage, then little change of either maltose or sucrose for 8 yrs, then a reduction of both in the next 2 yrs especially in the sucrose. Since both types of wheat, in all four bins, were similarly affected this must be a genuine effect of ageing.

The titratable acidity of the individual flours milled from the wheats (not given in Fig.3), expressed as per cent  $\text{KH}_2\text{PO}_4$  (Kent-Jones and Amos, 1957) after 18 yrs storage ranged from 0.38% to 0.4% for Canadian flour and from 0.27% to 0.31% for English flour. A figure of about 0.40% is "normal" for freshly milled flour. At 0.60% and above marked deterioration of the baking quality occurs.

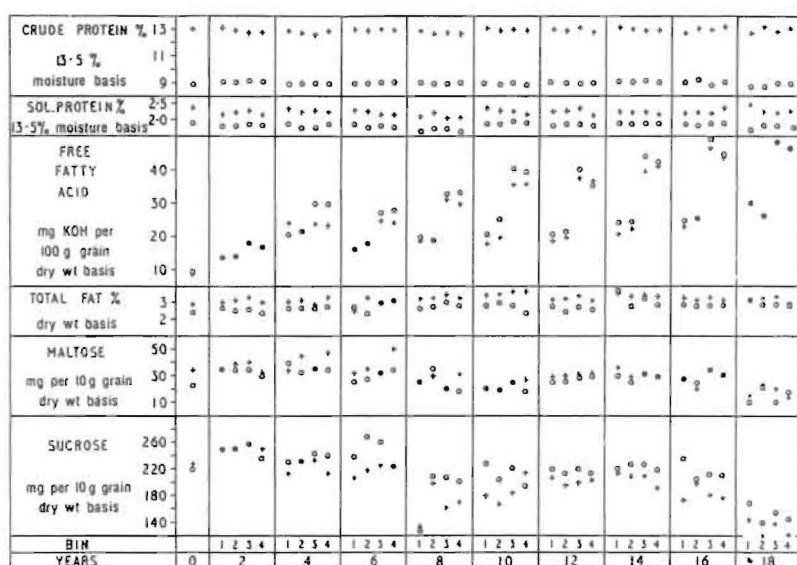


Fig. 3 Results of chemical analyses obtained at 2-yearly intervals up to a total storage period of 18 years.

+ Canadian wheat: O English wheat: ● result the same for both varieties.

The vitamin B<sub>1</sub> content remained unchanged throughout ranging from 3.6 to 3.7 µg/g (1.15 - 1.18 i.u./g) for the Canadian wheat and from 3.7 to 4.0 µg/g (1.18 - 1.28 i.u./g) for the English wheat.

### 3.3 Milling and physical dough tests (Fig.4)

The wheats were milled on a Buhler mill with constant setting, flour yield being calculated as a percentage of total products. This procedure takes into account moisture and product loss in milling and enabled a comparison to be made of yields and colour numbers at different sampling times. The colour number gives an indication of the contamination of the flour by fine bran particles. The lower the colour number the whiter the flour and the lower is the ash content. A low number would be obtained if the endosperm separated easily from the bran during milling which would also lead to a higher yield. Starch cells becoming more friable with age would have the same effect.

Over the total storage period yields varied irregularly with the highest yields in the last two years of storage. There was no correlation between flour yield and colour number, but it is interesting to note that the lowest numbers were obtained in the last four years of storage when the yields were the highest.

Water absorption, which is the amount of water a dough will take and hold during fermentation, remained fairly constant, for both types of wheat, throughout the total storage period at about 0.55 l/Kg for Canadian wheat and



0.46 l/Kg for the English wheat.

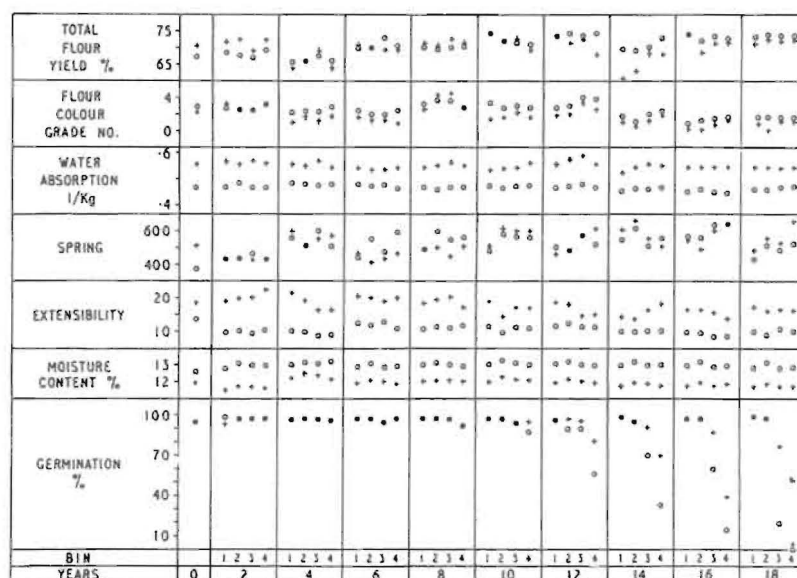


Fig.4. Results of flour yield, colour number, physical dough tests, moisture content determination and germination tests obtained at 2-yearly intervals up to a total storage period of 18 yrs.  
+ Canadian wheat: ○ English wheat; ● result the same for both varieties.

Spring (resiliency), as the name implies, measures the elastic properties of the gluten, i.e., its ability to recover after stretching. Extensibility measures the capacity of the dough to stretch without breaking. As the storage period increased the dough from both the Canadian and English wheats became "shorter" in that spring increased and correspondingly extensibility decreased. This is a well known ageing phenomenon and was more marked in the wheats stored in the warmer conditions especially those in normal air. However in the last two years spring showed a tendency to decrease indicating that at the end of the storage period the gluten was softening, presumably because of enzymic action.

The amylograph maximum viscosity (not given in Fig.4) gives a measure of the activity of the enzyme amylase in the flour which is essential to the baking process in the production of carbon dioxide in fermentation. The maximum viscosity, particularly for the Canadian wheats tended to increase with time. For both types of wheat the viscosity was higher in the warmer storage conditions. This first became apparent after 10 yrs storage, at about the same time that a decrease in total sugars occurred. We do not know if the amylase activity decreases or if, during storage, the starch becomes less susceptible to amylase attack.

### 3.4 Baking tests

Throughout the storage period the flour milled from the Canadian wheat responded to the addition of potassium bromate, the optimum response being obtained with a treatment rate of 20 ppm. The "bromate response" is the amount of potassium bromate required to obtain optimum improvement in a dough. Generally, the higher the response the stronger is the flour. The volume of a loaf made with Canadian flour with 20 ppm potassium bromate added was initially 715 cm<sup>3</sup> when the wheat was first stored. After the wheat had been stored at low temperature for 18 yrs the loaf volume was 815 cm<sup>3</sup> and if storage was at ambient temperature the volume was 760 cm<sup>3</sup>. Over the last two years of storage there was a decrease in loaf volume. This supports the contention made earlier that the gluten was softening. English wheat, primarily a biscuit wheat had very little bromate response, initially the loaf volume being 575 cm<sup>3</sup> rising to 640 cm<sup>3</sup> at the end of storage.

In the baking process the quantity of carbon dioxide produced during fermentation depends on the level of alpha amylase and damaged starch cells present in the flour as well as on the level of reducing sugars. Fungal alpha amylase was added to both the Canadian and English wheat flours, at the rate of 224 ppm, to correct a deficiency of natural amylase which had decreased after the first 8 yrs of storage. Good loaves were always obtained from the Canadian wheats those stored at low temperature producing marginally the best loaves.

### 3.5 Moisture content (Fig. 4)

The overall mean moisture content of both types of wheat in all four bins rose by about 0.5% during the first two years and thereafter remained virtually constant over the entire storage period. The difference between the moisture content of the two wheats, in the same bin, after several years of storage varied from 1.1% to 1.3%. This is an expression of the different moisture content/e.r.h. relationship of the two wheats.

Throughout the period of storage the moisture content of both types of wheat at low temperature tended to be higher at the top and side than the bottom and centre of the bulk. By contrast, in the warmer bins the moisture content at the bottom and centre tended to be higher than the side and top and the differences were slightly greater. These slight vertical and horizontal gradients were probably caused by temperature and consequent vapour pressure gradients that accompany seasonal changes of climate.

### 3.6 Viability (Fig. 4)

Under ambient conditions the germinative capacity fell during storage. The Canadian wheat began to lose both energy and capacity gradually between 10 and 12 yrs and more steeply between 14 and 18 yrs when it had declined to 4%. This process started two years earlier in the English wheat which fell to zero germination after 18 yrs storage. At low oxygen tension the loss in capacity

started a little later, after about 12 yrs storage. It was the more marked in the English wheat in which the capacity fell to 19% after 18 yrs compared with 77% for the Canadian wheat.

At low temperature, however, the germinative energy and capacity of the wheats were maintained over the entire 18 yrs of storage in the bins. For this reason about 50 Kg of the wheats stored at low temperature and normal oxygen tension were kept in a closed metal container in a room at 5°C, and now, after 22 yrs, the germinative capacity for the Canadian wheat is still 95% and that of the English wheat 96%.

### 3.7 Microflora

No growth of micro-organisms took place in samples of the wheats stored up to 10 yrs in tubes, dryness being the main controlling factor. However, many micro-organisms were viable on the wheats at the end of this period. Low oxygen tension had little or no effect on the survival of micro-organisms but, with the exception of Aspergillus that survived almost as well at ambient temperature as at low temperature, all diminished much more quickly at ambient temperature. This decline in fungal viability paralleled the decline in grain viability which was also greater under ambient conditions. If there was a slight increase in the moisture content of the wheats, growth of xerophilic fungi such as the Aspergillus restrictus and A. glaucus groups, could take place at ambient temperature. A greater increase in moisture content would be necessary before fungal growth could take place in the low temperature conditions.

## 4. CONCLUSIONS

No change has taken place in the germinative energy or capacity of the wheats stored at 5°C for 22 yrs. Both varieties of wheat stored at ambient temperature and oxygen tension have little or no viability after 18 yrs storage. Low oxygen tension in the ambient temperature conditions appears to have supported viability for a longer period, but both wheats showed a reduced energy.

Free fatty acid values throughout have continued to show an increase. The rate of increase, and the final level of free fatty acid of the wheats in the warmer bins was about twice that found in the cold bins. In the last two years both reducing and non-reducing sugars have decreased again in both types of wheat in all bins.

Whilst water absorption has remained steady, resiliency increased with time at the expense of extensibility, but, in the last two years of storage resiliency of the Canadian doughs decreased indicating that the gluten is softening possibly due to enzymic action. This is also reflected by a fall in loaf volume.

Although moisture changes were unimportant in this experiment with dry grain, they could be serious in grain with a higher initial moisture content or where

there are violent climatic fluctuations.

With the exception of Aspergillus which survived almost as well at ambient temperature as at low temperature all micro-organisms diminished much more quickly at ambient temperature. There was no development of fungi, dryness being the controlling factor.

Clean dry wheat, providing it is protected from atmospheric moisture, from rapid changes of temperature and from insect infestation will still produce good loaves after 18 yrs storage in spite of the fact that germinative energy and capacity are greatly reduced. It is necessary, however, to add alpha amylase to the flour in the baking process to compensate for loss of the natural enzyme. Low temperature prevents a decline in viability but otherwise has only a marginal effect on loaf quality. Low oxygen tension, whilst giving some support to viability at ambient temperature, had no effect on loaf quality.

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## FUNCTIONAL AND END-USE PROPERTIES OF VARIOUS COMMODITIES STORED IN A LOW OXYGEN ATMOSPHERE

CHARLES L. STOREY

### ABSTRACT

Germination, milling, and breadmaking characteristics of wheat were unaffected by exposure to a generated or combustion atmosphere composed of less than 1% oxygen, 9.0-9.5% carbon dioxide and the balance principally nitrogen for periods of 1/2 to 6 months. Similarly, germination, milling and cooking properties of long- and medium-grain rice exposed as rough, brown and milled rice were unchanged after 6-months of treatment. No adverse effect on the quality of malt produced from barley stored in the atmosphere was observed and no significant reduction was obtained in the germination of the barley. Almonds and raisins stored in the low oxygen atmosphere were equal or superior in flavor, quality, and acceptability to samples stored in normal atmospheres for periods as long as one year.

### INTRODUCTION

In U.S. agriculture, modified atmospheres of various compositions have been used to prevent the spoilage of foods and to create storage environments where it is possible to maintain the keeping qualities and growth characteristics of several types of fruits, feeds, vegetables and ornamental plants. Examples are the controlled atmosphere (CA) storage of apples to retain marketable conditions; the storage of animal feeds to maintain vitamin content and palatability; and the "blanketing" of food products such as macaroni, pickles, dried milk and citrus products during processing and transportation. Nitrogen and carbon dioxide are listed in the U.S. Federal Register under Title 21 - Food and Drugs as multiple purpose food substances generally recognized as safe when used under good manufacturing practices. The combustion gas produced by exothermic inert atmosphere generators has also been recognized by the U.S. Federal Food and Drug Administration as a secondary direct food additive when used to displace  $O_2$  in the processing, storage or packaging of beverage products and other foods (CFR, Title 21, Chapter 1, Part 173.350).

Previous studies with low oxygen atmospheres produced by exothermic inert atmosphere generators emphasized their use as a direct residue-free substitute for conventional chemical fumigation of grain. Equally important is their potential use in providing a modified storage environment for the continuous protection of bulk stored grain and other agricultural products.

Studies were developed to document the effects of long term storage in the generated "combustion" atmosphere on quality of grain, grain products and other bulk stored food commodities. Data presented here were obtained from the following sources: Storey et al., 1977; Guadagni et al., 1978a and 1978b; Storey and B. Webb, USDA Regional Rice Quality Laboratory, Beaumont, Texas (unpublished data; and Storey and K. Finney, USDA Grain Quality and End-Use Properties Unit, U.S. Grain Marketing Research Laboratory (unpublished data).

## MATERIALS AND METHODS

### Controlled atmosphere generator

A laboratory scale exothermic modified atmosphere generator built by Gas Atmospheres, Inc. of Port Washington, WI was used in the tests. The generator produces 2.83 M<sup>3</sup>/hr of modified atmosphere composed of less than 1% O<sub>2</sub>, 9-9.5% CO<sub>2</sub>, 86-89% N<sub>2</sub>, 1% Ar, and 1.5% or less each of H<sub>2</sub> and CO. Oxygen levels in the modified atmosphere were measured periodically throughout each test period with a paramagnetic oxygen analyzer. Although some minor variations occurred during day-to-day operations, the concentrations of O<sub>2</sub> in the modified atmosphere flowing through the various commodities was within a range of 0.25 - 0.5%.

### Commodity treatments

#### (Cereal Grains)

Hard red winter wheat, two types of rice (long grain-Lebonnet and medium grain - Brazos) in the form of rough, brown, and milled rice and two malting barley cultivars (six-rowed type-Larker and two-rowed type-Klages) were subdivided into lots of 55 kg (wheat), 2,000 g (rice) and 1,000 g (barley) that were either placed in plastic containers 28 cm x 28 cm x 90 cm (wheat) or 1.9 liter glass jars (rice and barley) and stored under a flowing (50 to 100 cc/min) generated atmosphere or in a normal atmosphere. Wheat was treated at a temperature of 27 ± 1°C and 50 ± 5% relative humidity. Rice and barley were treated at temperatures of 18 ± 1 or 20.5 ± 1 and 27 ± 1°C and 50 ± 5% relative humidity. Treatment periods were 1/2, 1, 2, 3, and 6 months for wheat and 1, 3, and 6 months for rice and barley. A control sample of each commodity was held in a sealed container stored at 4°C during the test period. Samples removed from generated atmosphere or normal air storage during the test period were held at 4°C until processed and analyzed.

#### (Commercial Malts)

Two commercial malts (brewer's and distiller's) were stored in lots of 425 g in 0.95 liter glass jars under generated or normal atmospheres at 15 ± 5% and 65 ± 5% relative humidity and 27 ± 1°C. Samples were removed after storage for 3, 6, and 12 months and held with control samples at 4°C until analyzed.

## (Almonds)

Nonpareil inshell almonds and almond meats were subdivided into 2,000 gram lots and stored in 3.8 and 1.9 liter glass jars under a flowing (50 cc/min) generated or normal atmosphere at  $50 \pm 5\%$  relative humidity and  $18 \pm 1$  and  $27 \pm 1^\circ\text{C}$ . Sample lots were removed after storage for 1, 3, 6, 9, and 12 months.

Nonpareil inshell almonds were also subdivided into 223 kg lots and stored in metal silos (45 cm in diameter by 4.27 m high) under flowing (500 cc/min) generated atmosphere, flowing normal air, and static air. Samples (33 kg) for quality evaluation were drawn from the silos after storage for 1, 3, 6, 9, and 12 months. Untreated samples and samples removed from the jars and the silos during the test period were held at  $4^\circ\text{C}$  until analyzed for flavor stability.

## (Raisins)

Field run Thompson variety raisins were subdivided into 1362 gram lots and stored in 1.9 liter glass jars under flowing (50 cc/min) generated or normal atmosphere and static air at  $50 \pm 5\%$  relative humidity and  $18.5 \pm 1$  and  $27 \pm 1^\circ\text{C}$ . Sample lots from each treatment were removed after storage periods of 1, 3, 6, 9, and 12 months. Untreated raisins and raisins removed from the jars during the test period were held at  $4^\circ\text{C}$  until submitted for flavor sensory evaluation.

Analytical methods

## (Wheat)

Milling, flour analysis, and breadmaking characteristics of the treated wheat were determined by the Grain Quality and End-Use Properties Unit of the U.S. Grain Marketing Research Laboratory. Germination tests were conducted by the Kansas Board of Agriculture, State Seed Laboratory. Ash and protein were determined as described by American Association of Cereal Chemists (1962). The baking procedures of Shogren et al (1969) for 10 g flour samples were used in the breadmaking studies.

## (Rice)

Tests to measure the effect of storage of the rice in the generated atmosphere were conducted by the USDA Regional Rice Quality Laboratory, Beaumont, Texas. Physiocochemical Properties generally recognized as tests of cooking quality, including amylograph peak and setback viscosities, alkali spreading values, and water uptake values were determined for each rice sample. Milling yields of rough and brown rice and germination of rough rice were also determined. Tests for parboil-canning stability were used to evaluate the suitability of the treated rice for use in heat processed foods such as soup (Webb and Adair 1970).

## (Barley and Commercial Malt)

The barleys were malted as described by Dickson et al. (1968) and the malts were analyzed by the USDA Barley and Malt Laboratory, Madison, Wisconsin according to the methods of the American Society of Brewing chemists (1958).



## (Almonds and Raisins)

All sensory evaluations of flavor stability of the treated almonds and raisins were performed by laboratory personnel of the USDA Western Regional Research Center, Berkeley, California. The shelled almonds and raisins were placed in coded paper cups and presented to a panel of judges for ranking in order of least off-flavor. Separate tests were also conducted to determine the relative likeability or acceptability of the samples from different treatments (ASTM, 1968).

## RESULTS

Cereal Grains

Storage for 1/2 to six months at temperatures ranging from  $18 \pm 1$  to  $27 \pm 1^\circ\text{C}$ , a relative humidity of 50%, and flowing atmospheres containing less than 1% oxygen that were produced by the exothermic inert atmosphere generator had no consistent or significant adverse or beneficial effect on the functional and end-use properties of dry stored wheat, rice or barley. Presentation of data is therefore simplified by giving only the results for the longest exposure period (6 months) at the highest temperature ( $27 \pm 1^\circ\text{C}$ ).

Germination of each grain stored in the generated atmosphere was consistent with the viability of grains stored in normal air at the same temperature or under cold storage ( $4^\circ\text{C}$  Table 1).

TABLE 1.

Germination of wheat, rice, and barley stored for 6-months at  $27 \pm 1^\circ\text{C}$  and  $50 \pm 5\%$  relative humidity in normal air or in atmospheres produced by an exothermic inert atmosphere generator (composition  $<1.0\%$   $\text{O}_2$ , and  $9.0\text{--}9.5\%$   $\text{CO}_2$ , the balance principally  $\text{N}_2$ ) or at low temperature in normal air.

Commodity	Generated atmosphere	Normal air	Cold Storage ( $4^\circ\text{C}$ )
Wheat (HRW) <sup>1/</sup>	90	91	93
Rough rice (long grain)	90	91	92
Rough rice (medium grain)	92	91	93
Barley (six-rowed)	95	96	95
Barley (two-rowed)	98	98	97

<sup>1/</sup> HRW = hard red winter wheat

Milling characteristics, flour analysis and breadmaking properties of wheat stored in the generated atmosphere were virtually identical to those of wheat stored in normal air or cold storage (Table 2). The reduction in loaf volume was somewhat less for bread made from wheat held in cold storage than from wheat stored in the generated atmosphere or normal air.



TABLE 2.

Milling characteristics, flour analysis, and bread making properties of wheat stored for 6 months at  $27 \pm 1^\circ\text{C}$  and 50% relative humidity in normal air or in atmospheres produced by an exothermic inert atmosphere generator (composition  $<1.0\%$   $\text{O}_2$ , and 9.0-9.5%  $\text{CO}_2$ , the balance principally  $\text{N}_2$ ) or at low temperatures in normal air.

Factor	Cold storage ( $4^\circ\text{C}$ )	Generated atmosphere	Normal air
Milling characteristics <sup>1/</sup>			
Ash (%)	1.66	1.65	1.65
Protein (%)	11.3	11.0	11.0
Flour yield (%)	74.8	74.1	73.9
Chemical analysis of flour <sup>1/</sup>			
Ash (%)	0.43	0.40	0.40
Protein (%)	10.3	9.9	10.1
Characteristics of bread <sup>2/</sup>			
Mixing time <sup>3/</sup> (min)	3 5/8	3 1/2	3 1/2
Baking absorption (%)	65.9	67.5	66.8
Loaf volume <sup>4/</sup>	816	805	806

1/ Chemical data expressed on a 14% moisture basis.

2/ Crumb grains and crumb colors were satisfactory for all treatments.

3/ Corrected to 12.0% protein.

4/ Corrected to 10.0% protein.

Milling yields, amylographic viscosity and physiochemical (quality) characteristics of long grain and medium grain rice varieties stored as rough rice, brown rice and milled rice in the low oxygen generated atmosphere were also consistent with results obtained from rice stored in normal air or cold storage. Table 3 shows data obtained for rice milled after storage for 6 months as rough rice.

The storage of six-rowed and two-rowed barley under the generated atmosphere had no significant effects on the quality of malt produced from the stored barleys (Table 4). Some slight improvement in increased diastatic power and alpha-amylase occurred in the two-rowed barley stored in air compared to barley stored in the generated atmosphere, but these changes are not considered to be of practical importance.

#### Commercial malts

There were no significant changes in any of the malts stored for three months at 15 or 65% R.H. nor in malts stored for 6 or 12 months at 15% relative humidity. Malts stored for 6 and 12 months at 65% relative humidity had increased moisture content to such an extent that the final moisture level probably had a greater effect on malting characteristics than the composition of the air under which they were stored. Significant decreases in extract, wort protein, diastatic power and alpha-amylase and increases in fine - coarse grind extract and wort color occurred in each malt stored at high relative humidity. Under these conditions, storage in the generated atmosphere did not provide any advantage or disadvantage over storage under normal air.

TABLE 3.

Milling yields, amylographic viscosity, and physicochemical (quality) characteristics of long grain (Lebonnet) and medium grain (Brazos) rice varieties stored as rough rice for 6 months at  $27^{\circ} \pm 1^{\circ}\text{C}$  and 50% R.H. in normal air or in atmosphere produced by an exothermic inert atmosphere generator (composition  $<1.0\%$   $\text{O}_2$ , and 9.0-9.5%  $\text{CO}_2$ , the balance principally  $\text{N}_2$ ) or at low temperatures in normal air.

Factor	Long Grain Rice			Medium Grain Rice		
	Cold Storage (4°C)	Gen. atms.	Air	Cold Storage (4°C)	Gen. atms.	Air
Test weight 1 bushel	45.6	45.4	45.3	46.2	46.5	46.4
Milling yield whole kernel %	66.4	65.8	66.2	66.1	66.3	66.2
Amylographic viscosity						
Peak B.U.	740	760	785	690	663	670
After 10 min at 95°C B.U. <sup>1/</sup>	415	465	470	355	345	345
Breakdown B.U. <sup>1/</sup>	325	295	315	335	320	325
Milled rice analysis						
Alkali spread value 2% KOH	4.4	4.3	4.8	6.9	6.8	6.9
Swelling No. at 77°C	80	79	76	203	190	223
Swelling No. at 95°C	508	492	478	523	513	495
Amylose content %	24.9	24.9	24.3	18.3	18.4	18.6
Brown rice protein %	7.56	7.57	7.33	8.11	8.17	7.89
Parboil-canning stability						
Solids loss	16.1	16.2	14.6	26.1	26.0	25.9

<sup>1/</sup> Barbender Units

TABLE 4.

Composition and malting characteristics of two malting barley varieties stored for 6 months at  $27 \pm 1^{\circ}\text{C}$  and 50% relative humidity in normal air or in atmospheres produced by an exothermic inert atmosphere generator (composition  $<1.0\%$   $\text{O}_2$ , and 9.0-9.5%  $\text{CO}_2$ , the balance principally  $\text{N}_2$ ) or at low temperatures in normal air.

Factor	6-rowed barley LARKER			2-rowed barley KLAGES		
	Cold Storage 4°C	Gen. atms.	Air	Cold Storage 4°C	Gen. atms.	Air
Moisture	12.2	12.2	11.9	11.9	12.0	11.7
Fine grind extract (%)	78.1	78.3	78.9	80.4	80.3	80.7
Fine coarse graind extract (%)	1.9	1.7	1.7	1.7	2.1	1.9
Wort color (SRM) <sup>a</sup>	1.9	1.9	1.9	1.2	1.2	1.3
Malt-protein (%)	13.44	13.56	13.25	13.50	13.69	13.69
Wort-protein (%)	5.19	5.20	5.42	4.87	4.81	4.97
Wort/malt protein (%)	39.1	39.5	41.4	37.0	36.6	38.1
Diastatic power (°L)	188	190	187	160	161	166
Alpha-amylase (20°C)	42.7	40.9	41.8	51.3	56.4	58.7

<sup>a</sup>Standard reference method.

### Almonds

Sensory comparisons of flavor changes indicated that storage in the low oxygen generated atmosphere caused less off-flavor development than storage in normal atmosphere for both nut meats and inshell almonds. Higher storage temperature increased off-flavor development in meats stored in normal air, but had no significant effect on those stored in the generated atmosphere.

### Raisins

Raisins stored under the low oxygen generated atmosphere for up to 1 year were equal or superior in flavor quality and acceptability to similar samples stored under flowing or static normal air.

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## PRESERVATION OF CHEMICAL PARAMETERS IN CEREAL GRAINS STORED IN NITROGEN

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## ABSTRACT

Soft wheat and maize at various moisture contents were stored in experimental and pilot scale silos in air and technical or pure nitrogen. Samples of cereal grains and wheat flour were chemically analyzed throughout the storage periods for the main quality parameters.

The results have shown that preservation in nitrogen, especially at higher moisture contents, has a protective effect, permitting satisfactory storage for longer periods than by traditional preservation methods.

## INTRODUCTION

During storage, wheat is subject to changes that are not only due to biotic factors, but also to physico-chemical phenomena independent from the first, although often related to them.

Among the chemical components of wheat, the lipids are the most susceptible to alterations during maturation and storage of the product itself or of the flour (MacMurray and Morrison, 1970).

Storage of cereals and of their derived products implies lipid changes due to oxidation and hydrolysis processes (Moran et al., 1954) resulting in the increase of the acidity degree. Oxidation effects the double bonds of the fatty acids in the lipid molecule; the unsaturated fatty acid content thus determines the storage alterations reflected by the physical, organoleptic and technological characteristics of cereals and their derived products.

The incidence of these phenomena depends first of all on storage conditions and particularly on the moisture content (Cucudet et al., 1954) and temperature (Glass et al., 1959) of storage.

Since this subject has a great importance, we thought it necessary to pay particular attention to the investigation of the possible wheat lipid changes at different moisture contents during storage in nitrogen atmosphere.

## MATERIALS AND METHODS

Soft wheat of different varieties and Goliath maize were tested at natural and artificially raised moisture contents for their storability in nitrogen versus oxygen containing atmospheres (Shejbal, 1979).

Samples of wheat and its flour, and maize, were analyzed throughout the storage periods, with special regard to:

1. Viability
  - 1.1 Germination energy
  - 1.2 Germination capacity
2. Determination of proteins
  - 2.1 Total proteins
  - 2.2 Gluten quantity
  - 2.3 Gluten quality
3. Determination of sugars (according to AAC methods)
  - 3.1 Reducing sugars
  - 3.2 Non reducing sugars
4. Lipid characterization (according to AACC methods and Carnovale and Quaglia, 1973)
  - 4.1 IR spectra of acetone extracts
  - 4.2 Acidity index
  - 4.3 Fatty acid composition of ether extract
  - 4.4 Determination of tocopherol content.

## RESULTS AND DISCUSSION

### Long-term storage of dry soft wheat

Two samples of soft wheat, "Conte Marzotto" variety, at an initial moisture content of 10.5%, were stored in minisilos in air and in tech-

nical nitrogen for 5 years. Storage temperature was 18°C in winter and 32°C in summer.

During the second year the wheat moisture content was increased to 12%, a level closer to that currently present in national wheat.

Table 1 shows that germination capacity remained high during the first 3 years in both samples; during the fourth year, and particularly in the last months of the experiment, germination capacity decreased significantly in air stored wheat, but remained high in nitrogen stored wheat.

Gluten quality was reduced in both wheat stored in air and nitrogen, but air storage had a greater adverse effect.

These results show that wheat stored in partial absence of oxygen remains unaltered during a far longer period of time than that required in Italy for commercialization.

Because of the encouraging results obtained in minisilos we considered it necessary to repeat the experiment under the same conditions on pilot scale.

#### Dry soft wheat storage in a pilot scale plant

Table 2 shows the chemical and biological data of wheat "Conte Marzotto" at a low moisture content and derived flour at trial start and after 58 weeks. It seems that during the storage period no significant change occurred either in the wheat or its flour. Only the acidity degree expressed by the fatty acid index (FAV) showed a slight increase.

Table 3 shows analogous results for flour demonstrating that I.R. absorption of -COOH groups has the same trend as FAV in the whole wheat.

These results confirm the possibility of employing such a storage technique for dry soft wheat.

Table 1 - Storage of soft wheat "Conte Marzotto" in air and in nitrogen

Parameters	Time (years)						
	0	3		4		5	
		air	nitrogen	air	nitrogen	air	nitrogen
Moisture content (%)	10.5	12.2	11.9	12.5	12.3	12.7	12.3
Germinative capacity (%)	96	93	93	75	87	4	87
Gluten (% d.m.)	11.3	10.1	10.2	10.1	9.9	9.4	9.3
Gluten quality	excellent	good	excellent	short	good	short	good

Table 2 - Storage of soft wheat "Conte Marzotto" on pilot scale in technical nitrogen for 58 weeks

Parameters	Time "0"	58 weeks
Moisture content (%)	10.5	10.5
Total proteins (Nx5.70) (% d.m.)	14.0	13.4
Gluten (% d.m.)	10.1	10.1
Reducing sugars (mg maltose/10 g)	26	38.3
Non reducing sugars (mg sucrose/10g)	185	215
Germinative capacity (%)	99	99
FAV units	23.0	25.2

Table 3 - Chemical features of flour obtained by industrial milling of soft wheat "Conte Marzotto" stored in nitrogen for 58 weeks

Parameters	Time "0"	58 weeks
Moisture content (%)	13.7	13.9
Total proteins (Nx5.70) (% d.m.)	13.1	13.1
Reducing sugars (mg maltose/10 g)	21	15.1
Non reducing sugars (mg sucrose/10 g)	137	152.7
Gluten (% d.m.)	10.4	10.1
A (-COOH) U.A.	0.42	0.48



In order to evaluate the possibility of applying nitrogen atmospheres to the storage of wheat with a higher moisture content, tests were carried out on soft wheat with a moisture content above 14.5%, a value at which serious storage problems, even for short periods, arise in Italy.

#### Soft wheat storage at critical moisture content

Soft wheat "Cappelle" variety, at 14.5% moisture content was stored in mini-silos in air and in technical nitrogen, with 0.2% of oxygen, for 32 weeks. The temperature was 18-20°C during the first 20 weeks, and at trial end, in the summer, it reached 26°C.

Germination capacity of wheat stored in air and in nitrogen did not show any noticeable variation during the experiment, only a slight increase of the acidity degree, with a FAV enhancement could be demonstrated (table 4).

Table 4 - Storage of soft wheat "Cappelle" at critical moisture content in air and in technical nitrogen for 32 weeks

Parameters	Time "0"	Air	Nitrogen
Moisture content (%)	14.5	14.5	14.3
Total proteins (Nx5.70) (% d.m.)	12.4	12.0	12.4
Germinative capacity (%)	93	91	93
FAV units	26	33	32

Results reported in table 5 on chemical parameters in the flour obtained from the wheat, do not show any significant difference in gluten and sugar between flours obtained from the samples stored in air and in nitrogen.

Flour from air stored wheat seems to indicate that during storage a slight lipo-oxidative process has taken place, whereas no such phenomenon appears to have affected the nitrogen stored wheat. In fact this is shown by the data reported in table 6, where the increase of the acidity degree during 32 weeks of storage, as revealed by I.R. absorp-

Table 5 - Chemical features of soft wheat "Cappelle" flour at critical moisture content stored in air and in technical nitrogen for 32 weeks

Parameters	Air	Nitrogen
Gluten (% d.m.)	8.7	8.8
Gluten quality	very good	very good
Reducing sugars (mg maltose/10 g)	23	27
Non reducing sugars (mg sucrose/10 g)	160	172

Table 6 - Free fatty acids percent composition of flour obtained from wheat at the beginning (time "0") and after 8 months storage in air and in nitrogen

Analysis	Time "0"	After 8 months storage	
		dry wheat in air	dry wheat in nitrogen
Palmitic acid (16:0)	23.9	23.5	24.3
Stearic acid (18:0)	1.4	tr	tr
Oleic acid (18:1)	8.8	10.9	8.8
Linoleic acid (18:2)	61.3	52.1	62.9
Linolenic acid (18:3)	4.5	10.1	3.7
Arachidonic acid (20:4)	tr	3.2	0.3
$R = \frac{\text{unsaturated}}{\text{saturated}}$	3.0	3.2	3.1
A (-COOH) U.A.	0.22	0.32	0.30

tion of -COOH moiety, appears to be higher in air stored wheat. In this same wheat, linoleic acid decreases in air, proving thus that an oxidative process occurs. This does not take place in the nitrogen stored wheat, where the fatty acid composition remains almost the same as that of flour derived from wheat milled at the start of the trial.

#### Soft wheat storage at overcritical moisture content

Since soft wheat storage at overcritical moisture content might be of great practical value, we decided to proceed to 3 test series with artificially moistened wheat.

Our experiment was carried out on different wheat varieties, i.e. Marzotto and Cappelle as an example of Italian and French medium force wheat, and Red Spring as an example of force wheat. Samples were moistened, by repeated additions of distilled water and successive mixing, to a level of 17.4%, 18.0% and 17.5% respectively.

Each wheat sample was stored in minisilos, in air and in technical nitrogen, for 32 weeks.

Germination capacity of moistened wheat decreases more rapidly in air than in nitrogen: after 10 weeks storage in air it reaches 55%, and 70% if stored in nitrogen. At the end of the experiment it reached 0 level for all the samples (table 7).

Also the fat acidity value (FAV) shows a greater increase when wheat is stored for 10 weeks in air than in nitrogen, this difference is confirmed at trial end (table 7).

No change in protein content was demonstrated.

Gluten quantity of flour from wheat stored in air proved to be less than that of the wheat stored in nitrogen, although both suffered a quality deterioration. Reducing sugars increased more in air than in nitrogen stored wheat, while the non-reducing sugars experienced a reduction from their initial value of 232, 270, 288 mg sucrose 10 gr dry weight for Cappelle, Manitoba, Marzotto respectively (table 8).

Table 7 - Storage of three varieties soft wheat at over critical humidity in air and in technical nitrogen for 32 weeks

Parameters	Cappelle			Red spring			Marzotto		
	Time 0	Air	Nitrogen	Time 0	Air	Nitrogen	Time 0	Air	Nitrogen
Moisture content (%)	17.4	18.0	17.0	18.0	18.0	17.8	17.5	17.5	17.1
Total proteins (Nx5.70) (% d.m.)	12.4	12.3	12.0	12.4	12.2	12.3	12.0	11.9	12.0
Germinative capacity (%)	94	0	0	88	0	0	88	0	0
FAV units	30	47	42	37	91	46	32	36	42

Table 8 - Chemical features of flour from three varieties of soft wheat at over-critical moisture content, stored in air and in nitrogen for 32 weeks

Parameters	Cappelle		Red spring		Marzotto	
	air	nitrogen	air	nitrogen	air	nitrogen
Gluten (% d.m.)	6.7	7.3	10.7	10.6	6.6	7.1
Gluten quality	short	short	short	short	short	short
Reducing sugars (mg maltose/10 g)	64	48	148	148	108	80
Non reducing sugars (mg sucrose/10 g)	123	142	92	90	150	188

Table 9 shows the free fatty acid composition of the flour lipidic extract. The unsaturated saturated fatty acid ratio indicates that this index does not change with time in the nitrogen stored moist wheat, but it appears to become twice as high in the air stored wheat, where hydrolysis takes place.

I.R. analysis of the acetonic extract provides a further evidence by showing that especially the air stored wheat exhibits an absorption change in the area characteristic for the  $-COOH$  moiety (table 9).

Table 9 - Free fatty acids percent composition of flour obtained from "Cappelle" at over critical humidity stored in air and in technical nitrogen for 32 weeks

Analysis	Time "0"	Air	Nitrogen
Palmitic acid (16:0)	23.9	15.3	26.0
Stearic acid (18:0)	1.4	tr	tr
Oleic acid (18:1)	8.8	9.3	10.5
Linoleic acid (18:2)	61.3	70.6	60.0
Linolenic acid (18:3)	4.5	4.7	2.8
Arachidonic acid (20:4)	tr	tr	0.7
$R = \frac{\text{unsaturated}}{\text{saturated}}$	3.0	5.5	2.8
A ( $-COOH$ ) (U.A.)	0.22	0.71	0.36

#### Wet harvested maize storage

Maize at 18-19% moisture content was stored in nitrogen and air at external ambient temperatures in mini-silos from autumn to spring and in microsilos at  $21 \pm 1^\circ C$  for over 20 weeks.

As can be seen in fig.1, at ambient external temperatures, the absence of oxygen did not permit any substantial increase in fat acidity, neither in a pressurized silo (P) nor under nitrogen flow (F), due to the virtual absence of moulds. At the same time, however, some carbohydrate transformation took place, shown by the decrease in nonreducing

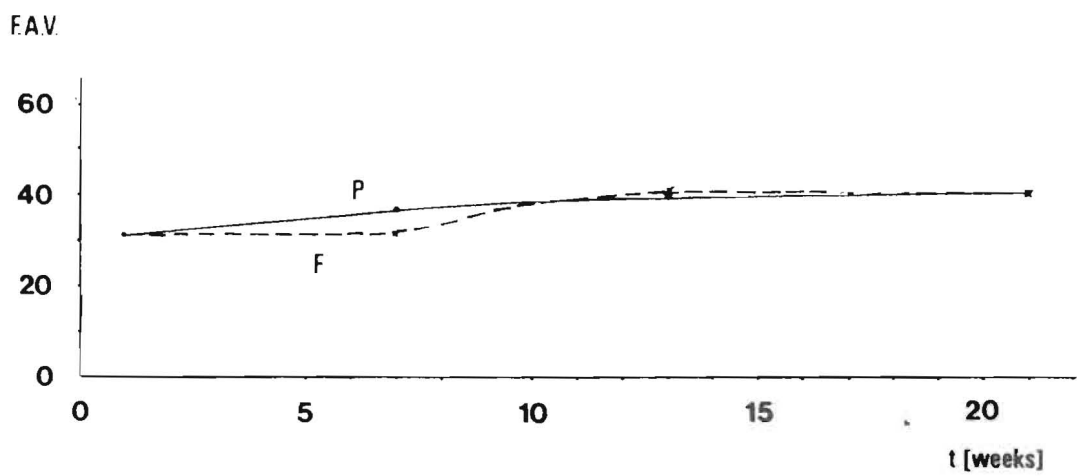


Fig.1. Evolution of fat acidity in wet harvested maize stored at ambient temperature in nitrogen flow (F) and in a pressurized mini-silo (P).

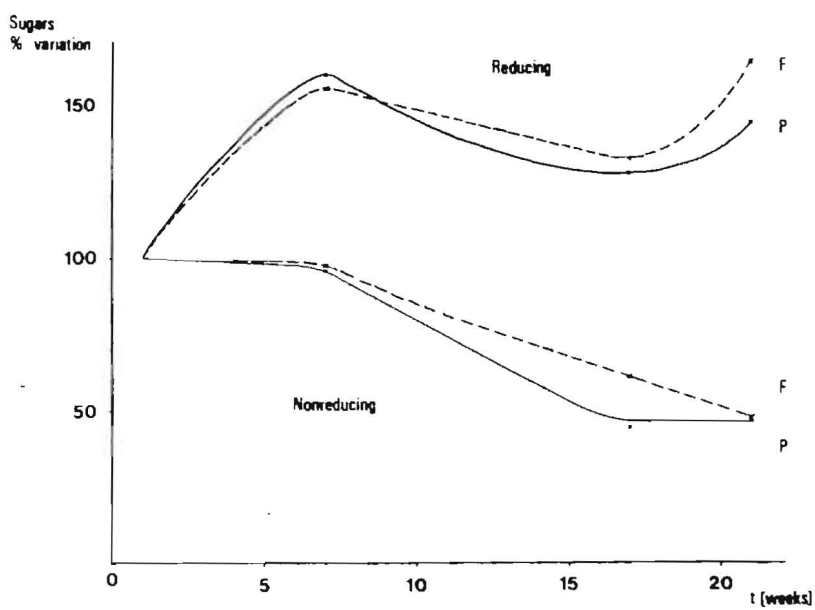


Fig.2. Evolution of reducing and nonreducing sugar content in wet harvested maize stored at ambient temperature in nitrogen flow (F) and in a pressurized mini-silo (P).

sugars, due to the moisture content dependent activation of the respective enzymes (fig.2).

At a constant temperature of 21°C, the effect on fat acidity was similar as in the preceding experiment, as far as nitrogen preservation was concerned. In pressurized airtight microsilos in air, a high concentration of carbon dioxide was soon reached, moulds (except *Candida*) were largely inhibited and therefore, here too, fat acidity did not increase (fig.3).

On the contrary, in microsilos, in which some air was added regularly (because of a leak of the pressurized silos), moulds proliferated freely and fat acidity increased accordingly.

As can be seen in fig.4, the effect of the various gas conditions on carbohydrates is not very much pronounced, the enzymes involved in their transformation not being oxygen dependent. The decrease in reducing sugars is due to them being consumed by proliferating moulds.

The separation of starch from proteins was not affected by storage in anaerobic conditions, while it was largely impaired in air storage.

It is, of course, impossible to maintain viability for long periods of time at moisture contents as high as 18-19%. In fact, it decreased rapidly already during the first four weeks of storage even in nitrogen, although slower than in air.

The results show that the limiting factor of moist maize preservation in controlled atmospheres is the presence, even of small amounts, of oxygen, which permit mould growth.

## CONCLUSION

The storage experiments on maize and various wheat varieties with different moisture contents and for different storage periods have proved the advantages of technical nitrogen, particularly when moisture content is overcritical. Nitrogen atmosphere allows prolonged storage without need of the common disinfesting drugs or other protective treatments. Storage is however limited in time by mould proliferation, the development rate of which is slowed down, although not totally inhibited, when oxygen is present at 0.2 to 0.5% as is the case of technical nitrogen.



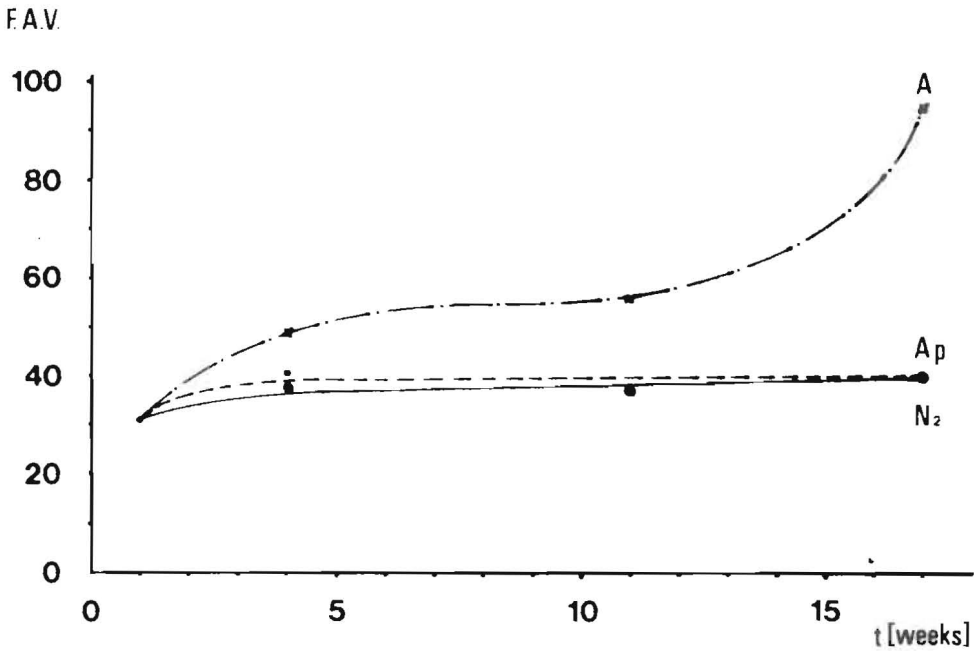


Fig.3. Evolution of fat acidity in wet harvested maize stored at 21°C in micro-silos under nitrogen (N<sub>2</sub>), confined atmosphere (A<sub>p</sub>) and with regular additions of air (A).

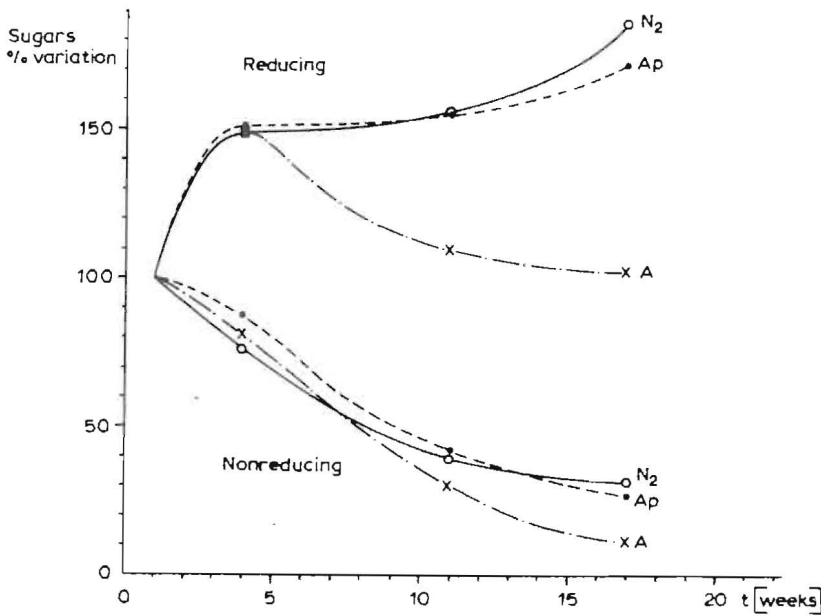


Fig.4. Evolution of reducing and nonreducing sugar content in wet harvested maize stored at 21°C in micro-silos under nitrogen (N<sub>2</sub>), confined atmosphere (A<sub>p</sub>) and with regular additions of air (A).

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# CHANGES IN THE RHEOLOGICAL CHARACTERISTICS AND BAKING QUALITY OF WHEAT AT DIFFERENT MOISTURE CONTENTS STORED UNDER NITROGEN.

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## ABSTRACT

Soft wheat stored at various levels of moisture content in experimental and pilot scale silos under nitrogen and air was analysed as to its rheological and baking properties.

It could be shown that preservation in partial or total anoxia of the grains never induced any impairment of the technological quality of the flour obtained either by experimental or industrial milling and analysed by farinogram, extensogram, Chopin alveogram and bread-making tests. At medium and high moisture contents a protective effect of the nitrogen atmosphere was observed.

Similar data were obtained for durum wheat stored at pilot scale in technical nitrogen.

## INTRODUCTION

Evaluation of the technological quality of the material to be stored is essential for any investigation on the preservation of cereals in an inert atmosphere. Our purpose was to study the rheological characteristics of flour obtained from soft wheat stored at different humidity degrees and of durum wheat semolina.

## MATERIALS AND METHODS

Soft wheat of different varieties and durum wheat were tested at natural or artificially increased moisture contents.

Parameters taken into account for the rheological study were those generally used in comprehensive investigations on doughs and thus apt to evidenciate the dough structure:

- Farinogramme (ICC Standard 115)
- Brabender extensogramme (ICC Standard 114)
- Alveogramme (ICC Standard draft 121)
- Hagberg-Perten method (ICC Standard 107)
- Baking test (Lombardi et al. 1976).

Each one of these parameters allows to evaluate different quality features of doughs; we thus judge it necessary to employ all of them.

The semolina analyses were: moisture content, proteins, gluten, farinogramme. On wheat itself a germination test and fungus count were also carried out.

Experimental milling of wheat samples was carried out with a Bühler MCK 201 mill, which, in its reduced diagramme (3 breakage cylinders and 3 re-milling cylindres), conforms perfectly to industrial mills. Industrial milling was carried out by the Pietro Agostinelli Mill, Rome.

## RESULTS AND DISCUSSION

### - Long term storage of dry soft wheat

Our experiments were carried out on wheat, variety "Marzotto", stored in nitrogen,atmosphere for 5 years at 10.5% initial moisture content (increased up to 12% during the second year).

After 5 years, flour of the sample stored in nitrogen was compared to that of the same wheat stored under normal air ventilation.

Comparison of the parameters (table 1) shows that:

- the degree of softening of both samples is identical
- extensigraphic indexes exhibit, on the contrary, an evident difference: increased resistance and decreased extensibility of dough from nitrogen atmosphere stored wheat, which gives an R/E ratio of particular significance (0.95)
- alveographic data also indicate that the nitrogen stored sample has a more marked tenacity
- the Hagberg-Perten index gave identical values for both samples.

Table 1 - Rheological parameters of soft wheat "C. Marzotto" flour stored in air and in nitrogen for 5 years

Parameters	After 5 years storage	
	in air	in nitrogen
FARINOGRAMME		
Resistance (sec)	5'45"	4'30"
Degree of softening (B.U.)	95	95
EXTENSOGAMME		
Resistance (R) (cm)	8.8	13.5
Extensibility (E) (cm)	18.3	14.1
R/E	0.47	0.95
ALVEOGRAMME		
Stability (P) (mm)	62.7	109.5
Extensibility (L) (mm)	95.0	85.0
Deformation work (W) (erg $10^3$ )	184.4	286.4
HAGBERG-PERTEN INDEX	392	392

The baking test has resulted in bread of normal smell and normal colour, almost undistinguishable for both samples, the only difference being the porosity (the alveoli form was more regular in the bread obtained from the sample stored in nitrogen).

Our results show that nitrogen storage had no negative effect on dough, but on the contrary improved it by increasing the R/E ratio.

#### - Dry soft wheat storage in a pilot scale plant

Wheat samples of variety "Marzotto", stored in silos with nitrogen atmosphere for 58 weeks, were analysed. The sufficient amount of stored wheat (22 tons) enabled us to carry out an industrial milling and thus to compare the data with those obtained by experimental milling.

By comparing time 0 and time 58 weeks (table 2) the results are:

- no difference in farinographic indexes
- elasticity increase in the nitrogen stored sample with resistance decrease further confirmed by a reduced tenacity according to the

Table 2 - Rheological parameters of flour obtained by industrial milling of soft wheat "C. Marzotto" stored in nitrogen for 58 weeks

Parameters	Time "0"	After 58 weeks
FARINOGRAMME		
Resistance (sec)	6'15"	6'30"
Degree of softening (B.U.)	80	80
EXTENSOGAMME		
Resistance (R) (cm)	8.5	6.8
Extensibility (E) (cm)	14.7	16.4
R/E	0.58	0.41
ALVEOGRAMME		
Stability (P) (mm)	91.7	68.5
Extensibility (L) (mm)	55.0	95.0
Deformation work (W) (erg $10^3$ )	176.4	187.0
HAGBERG-PERTEN INDEX	386	354

Table 3 - Rheological parameters of soft wheat "Cappelle" flour at critical moisture content at trial's start (Time "0") and after 32 weeks storage in air and in technical nitrogen

Parameters	Time "0"	After 32 weeks storage	
		in air	in nitrogen
FARINOGRAMME			
Resistance (sec)	3'00"	2'10"	1'55"
Degree of softening (B.U.)	110	110	105
EXTENSOGAMME			
Resistance (R) (cm)	7.9	5.5	6.8
Extensibility (E) (cm)	15.6	19.7	17.7
R/E	0.50	0.28	0.39
ALVEOGRAMME			
Stability (P) (mm)	38.6	40.9	39.9
Extensibility (L) (mm)	98.0	125	88.0
Deformation work (W) (erg $10^3$ )	124.0	149.3	113.2
HAGBERG-PERTEN INDEX	215	174	195

alveogramme.

The baking test shows no noteworthy differences.

Nitrogen storage for one years does not affect the dough structure obtained from flour of wheat stored in this way.

- Storage of soft wheat at critical moisture content

This study concerns a French soft wheat "Cappelle" variety, stored in two different silos in air and in technical nitrogen atmosphere, at critical moisture contents (i.e. at 14.5%).

The comparison (table 3) shows:

- an essential uniformity between the degree of softening
- a higher R/E ratio of the sample stored in nitrogen with respect to the one stored in air, although both ratios are lower than the "0" time level
- reduced alveographic extensibility (L) of the nitrogen sample, while the stability value (P) is almost constant.

- Soft wheat storage at overcritical moisture content

In this experiment the same analyses were repeated on samples of different varieties stored under the above conditions (see "critical moisture content"), their moisture content having been increased artificially to the level of 17.4% to 18.0%.

The comparison (table 4) shows:

- the degree of softening indicates a better stability of the samples stored in nitrogen, except for the "Marzotto" variety
- extensigraphic data show a general increase of resistance and a decrease of extensibility with storage, which results in a higher R/E ratio. This trend is confirmed by the alveographic data on tenacity and extensibility.

An increase of the deformation work is further to be pointed out (this phenomenon being more marked in the samples stored in nitrogen atmosphere). Obviously it is higher in hard wheat and results from the higher stability of doughs obtained from stored wheat flour.

In summary, the test at overcritical moisture content revealed

Table 4 - Rheological parameters of three varieties soft wheat flours at over critical humidity at trial's start (time "0") and after 32 weeks storage in air and in technical nitrogen

	Cappelle			Red spring			Marzotto		
	O	A	N	O	A	N	O	A	N
FARINOGRAMME									
Resistance (sec)	3'00	1'10"	1'45"	5'40"	2'05"	2'25"	3'15"	1'40"	2'00"
Degree of softening (B.U.)	110	105	75	70 ,	40	30	100	60	75
EXTENSOGAMME									
Resistance (R) (cm)	7.9	<u>3.6</u>	10.3	4.8	15.7	14.6	6.6	10.7	11.7
Extensibility (E) (cm)	15.6	10.4	14.3	21.4	14.5	13.0	16.3	8.5	9.2
R/E	0.50	0.35	0.73	0.22	1.08	1.12	0.40	1.26	1.27
ALVEOGRAMME									
Stability (P) (mm)	38.6	70.9	56.2	113.6	145.0	130.0	73.4	110.2	114.5
Extensibility (L) (mm)	98.0	38.0	74.0	76.0	97.0	87.0	39.0	39.5	36.0
Deformation work (W) (erg 10 <sup>3</sup> )	124.0	140.0	159.6	311.2	401.8	410.3	142.0	141.6	171.0
HAGBERG-PERTEN INDEX	215	229	201	303	406	396	389	401	413



that samples stored in air are subject to important negative changes, while the samples stored in nitrogen were affected in a lesser negative way.

- Storage of durum wheat

Durum wheat storage in nitrogen (Shejbal, 1976) was also tested. The trial, carried out on a pilot scale, concerned Italian wheat of undetermined variety, harvested in Puglia, and artificially moistened.

As can be seen in table 5 its results that:

- germination capacity is reduced in air (a massive infestation of the aerated sample stored took place)
- chemical physical parameters of semolina obtained from the samples stored in air and in nitrogen are constant
- mould counts in all samples did not increase.

Table 5 - Characteristics of durum wheat and semolina at the beginning of the experiment (time "0") and after 24 weeks of storage in air and technical nitrogen

Analysis	Time "0"	After 24 weeks	
		in air	in nitrogen
H <sub>2</sub> O (%)	13.5	13.7	13.2
Germinative capacity (%)	93	70	92
Gluten (% d.m.)	10.3	10.4	10.4
Total proteins (% d.m.) (Nx5.70)	11.6	11.4	11.5
Degree of softening (B.U.)	70	50	40

## CONCLUSION

The total results of the rheological tests indicate that soft and durum wheat storage in a nitrogen atmosphere does not cause any adverse effect on the technological quality of the product, so that the use of nitrogen can be considered beneficial especially since the samples stored at high moisture contents are protected by anoxia in respect to the controls preserved in air.

## ACKNOWLEDGEMENTS

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PRESERVATION OF CHEMICAL AND ORGANOLEPTIC PARAMETERS IN DIFFERENT  
VARIETIES OF HAZELNUTS IN NITROGEN AND IN AIR.

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ABSTRACT

The paper describes storage experiments carried out with three varieties of shelled hazelnuts (cv. Roman, Piedmontese and Akcakoca). The object of the work was the evaluation, based on chemical and organoleptic analyses, of the storability in nitrogen as compared with traditional methods.

Storage was carried out at room temperature (approx. 22°C) in pure nitrogen in microsilos and in an apparatus simulating a silo of twenty meters height. Controls in air were kept in bags at - 20°, + 4°, + 35°C and at room temperature. Moisture content remained nearly constant in hazelnuts stored under nitrogen, while it decreased in the control samples. Lipolytic activity was higher in the nuts preserved in nitrogen because of the higher moisture content as shown by fat acidity analyses. On the other hand the anoxic environment slowed down the oxidative activity and preserved better organoleptic properties.

Fat, sugar and protein contents did not change during storage. After one year of storage, hazelnuts preserved in nitrogen rated significantly better in organoleptic tests than those in air at room temperature and slightly better or equal to those at low temperature.

Storability in nitrogen of the three cultivars studied decreased in the order: Piedmontese - Roman - Akcakoca. High mechanical pressure had an adverse influence on storability.

## INTRODUCTION

In the course of recent years serious deteriorations occurred repeatedly during the storage of hazelnuts used by confectionary industries. On the basis of the result of storability studies (Radtke and Heiss, 1971; Barthel et al., 1974; Hadorn et al., 1977; Hadorn et al., 1978;), it has been decided that the best way for storing this kind of commodity is to keep the warehouse at controlled temperature and relative humidity. The temperature must be kept between three and six degrees centigrades and the relative humidity between fifty and sixty percent. Yet the increasing cost of energy makes this kind of storage more and more expensive.

Following the good results obtained with nitrogen atmospheres for cereal storage (Shejbal, 1976; Shejbal, 1979), we decided to apply the same technique to hazelnuts. The aim of this work was to evaluate the storability in nitrogen as compared to traditional methods. For future storage of shelled hazelnuts in large silos we had to know if the nuts at the bottom would be damaged by the mechanical pressure caused by the column of nuts. We also had to deal with the problem of a larger interstitial atmosphere in commodities other than cereals.

The results of this research can be used as a model for the behaviour of other high fat content seeds and dried fruits.

## MATERIAL AND METHODS

Three varieties of hazelnuts were chosen: two Italian ones (Piedmontese and Roman) and a Turkish one (Akçakoca). A sample of each varieties of shelled hazelnuts was stored under different experimental conditions as described in Table 1.

The experimental preservation in nitrogen atmosphere of the three varieties of hazelnuts was carried out in cylindrical air-tight laboratory microsilos in stainless steel (useful volume 0.1 cubic meter). The microsilo and gas flow are shown schematically in Figure 1.

The interstitial atmosphere of the nuts was replaced by a rapid purge (100 liters per hour) from the top with a volume of nitrogen corresponding to the double of the silo volume. The oxygen - poor atmosphere was maintained by an automatic system of pressostats and

TABLE 1

Experimental storage conditions of three varieties of hazelnuts.

Storage Condition	Temperature	Rel. Humidity	% O <sub>2</sub> in Interst. Atmosphere
Control (C)	- 20°C	---	21
Low temperature and humidity (cool, air)	3 - 6 °C	50 - 60%	21
Ambient temperature, low humidity (amb., air)	18 - 25°C	50 - 65%	21
High temperature, low humidity (hot, air)	35°C	30 - 40%	21
Ambient temperature, nitrogen atmosphere (amb., N <sub>2</sub> )	18 - 25°C	60 - 70%	max 0.5
Ambient temperature, nitrogen atmosphere, mechanical pressure (amb., N <sub>2</sub> , P)	18 - 25°C	40 - 50%	max 1.0

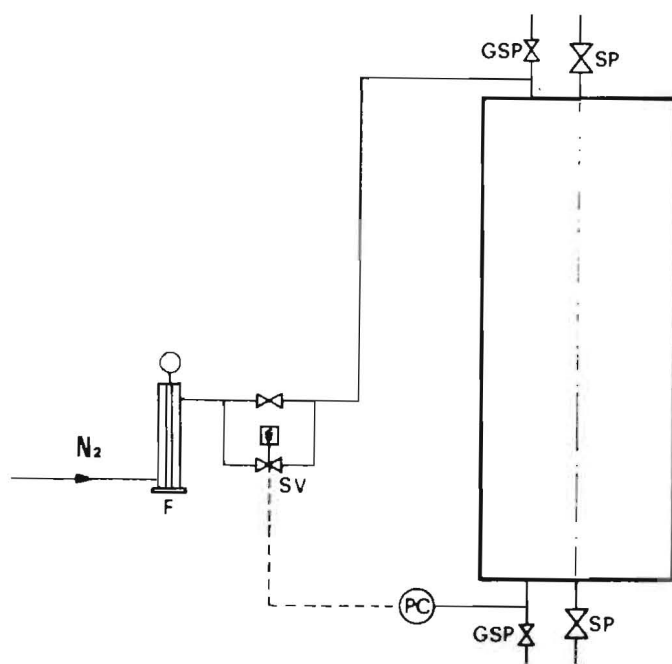


Fig.1. Cylindrical air-tight microsilos and gas flow sheets. F = Flowmeter; GSP = Gas sampling point; PC = Pressure control; SP = Sampling point; SV = Solenoid valve.

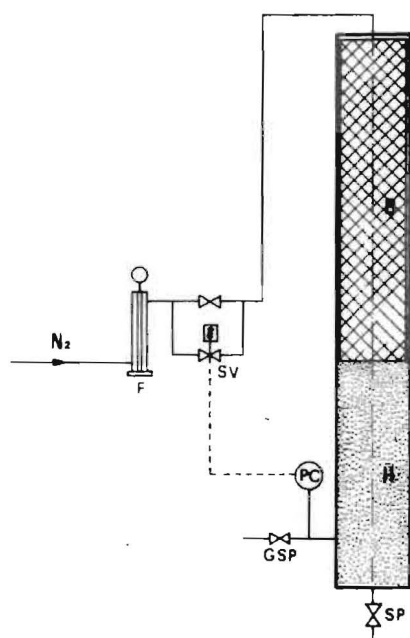


Fig.2. Apparatus simulating a silo of twenty meters height. B = Iron bar; F = Flowmeter; GSP = Gas sampling point; H = Hazelnuts; PC = Pressure control; SP = Sampling point; SV = Solenoid valve.

electric valves that bled in nitrogen whenever the internal over - pressure dropped under 200 millimeters of water (Shejbal, 1978).

The apparatus, simulating a silo of twenty meters height (Fig. 2), was a stainless steel tube with an internal iron bar which pressed the nuts at the bottom of the tube at the same mechanical pressure as at the bottom of a big silo. In this apparatus (useful volume 4 liters) Akcakoca hazelnuts were stored.

All the 10kg-samples in air were kept in cotton bags at the various experimental temperatures. The length of the storage trials was twelve months for the Roman and Piedmontese varieties and nine months for the Akcakoca variety. Temperature and relative humidity were recorded continuously and every fortnight analysis of the interstitial atmosphere composition in the microsilos was carried out.

Every three months samples were taken from each experimental storage condition and tested for (Hadorn et al., 1978):

Moisture (oven method)

Moisture (Karl Fischer)

Protein (Kjeldahl)

Fat

Surface fat

Ash

Alkalinity of ash

Glucose

Fructose

Sucrose

pH of a 10% suspension

Total acidity

Free fatty acids in the extracted oil

Induction period of oil at 110°C

UV - differential graph E<sup>1%</sup><sub>232</sub>

Fatty acid distribution

Enzymatic activity of a ground - up average sample

Enzymatic activity on the cut surface of individual hazelnuts

Microbiological and mycological examination

## Visual assessment

## Organoleptic test

The organoleptic pannel test classification should be interpreted as follows:

- 4 Equal to excellent
- 3 Equal to good
- 2 Equal to deteriorated, but acceptable
- 1 Equal to deteriorated, unacceptable
- 0 Equal to bad

Rating 2 is considered as the critical point underneath which it is not possible to use the nuts.

In all the graphes we report the absolute values found in the samples, taken every three months.

## RESULTS AND DISCUSSION

The moisture content remains nearly constant in hazelnuts stored under nitrogen, while it decreases in low and ambient temperature air storage (Fig.3). This is an important fact for the activity of the

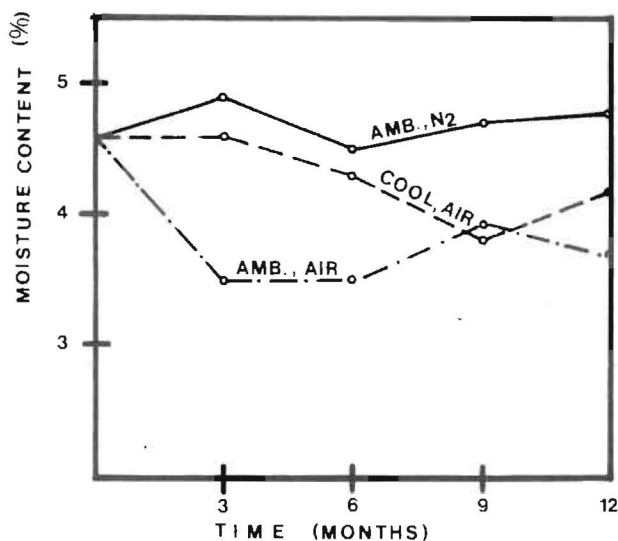


Fig.3. Moisture content of Piedmontese hazelnuts during storage under various conditions.



endogenous lipase in fats. The solubility of the lipolytic enzymes is the first step in the hydrolyzation of lipids; therefore at the higher moisture contents, the enzyme activity is higher. This is obvious from the results of the analysis of the fat acidity (expressed as percent of oleic acid).

The fat acidity of the hazelnuts preserved under nitrogen was significantly higher than of the other samples (Fig. 4). The control after twelve months remains almost constant. The hazelnuts in cool storage changed very little, followed by those kept at high temperature (because of the low humidity) and by those in ambient temperature.

The same trend was found in the Piedmontese and Akcakoca nuts. Yet we should note that the fat acidity of the nuts stored under nitrogen with mechanical pressure was significantly higher (Fig. 5), although the moisture content, due to repeated purges, was low (3.6%). In fact technical difficulties were encountered in maintaining an oxygen-poor atmosphere in this apparatus for a long time.

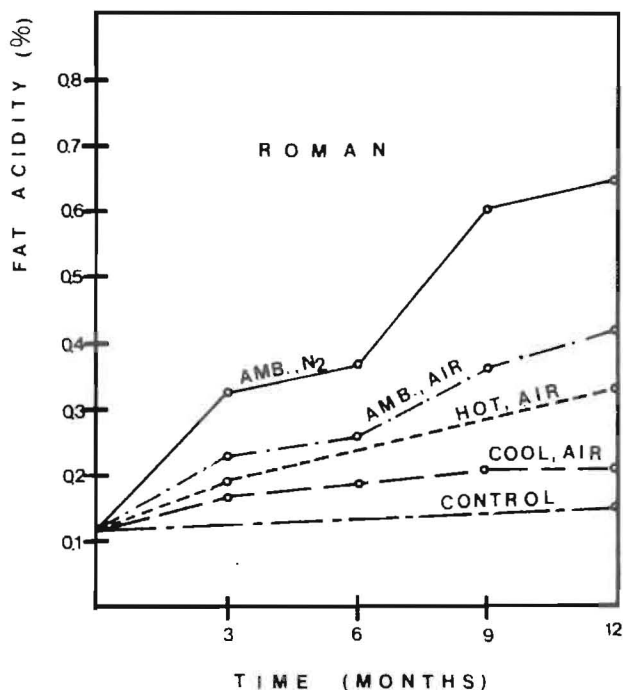


Fig.4. Increase of fat acidity in Roman hazelnuts during storage under various conditions.

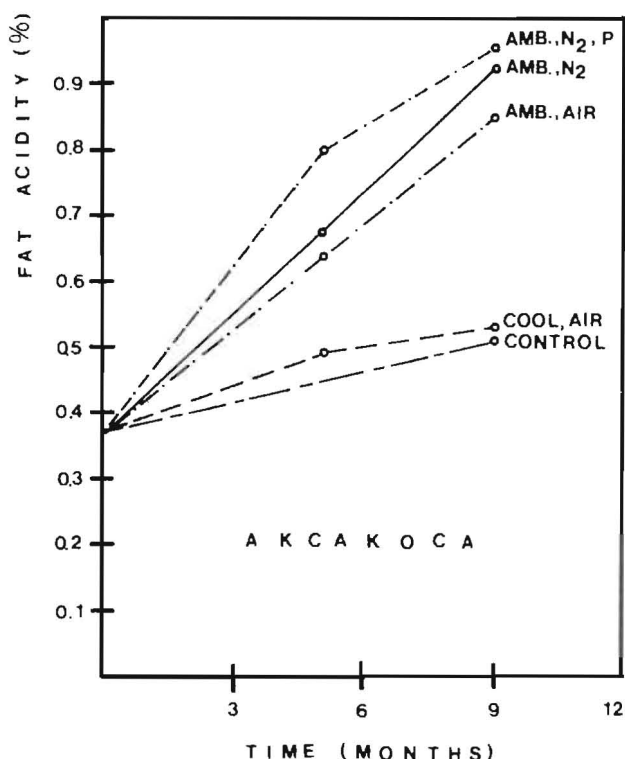


Fig.5. Increase of fat acidity in Akcakoca hazelnuts.

The fat acidity of Roman and Piedmontese hazelnuts never exceeded 0.7% which is considered as the critical point for the industrial use of the nuts. On the contrary the Akcakoca surpassed this critical point. The high values of the fat acidity did not always correspond to deterioration of quality.

Analysis of the intermediate compounds of the peroxidation, catalysed by the lipoxygenase, was carried out in order to detect the oxidative degradation of lipids. The spectrophotometric analysis of extracted oil showed, by the numbers of conjugated double bonds, the amount of the hydroperoxy fatty acids. These organic acids (Galliard, 1975) are toxic to the seeds and are converted into a set of more stable compounds, as aldehydes, with characteristic flavour properties.

The different steps (Yamamoto et al., 1980 a; Yamamoto et al., 1980 b) of the enzymatic reactions that lead to these volatile com-

pounds are: lipids + lipase ( $H_2O$ )  $\longrightarrow$  fatty acids (linoleic acid) + + lipoxygenase ( $O_2$ )  $\longrightarrow$  hydroperoxy fatty acids  $\longrightarrow$  aldehydes. In the oxygen-poor atmosphere this lipid degradation pathway is slowed down immediately after the free acids are formed.

The extinction values, at 232 nm, of the isooctane solution of hazelnut oil from nuts stored in nitrogen atmosphere are comparable to those from cool storage (Fig. 6). Neither reveals any remarkable change.

The spoilage is speeded up in the presence of oxygen as well as at high temperature. The trend is the same for Roman hazelnuts.

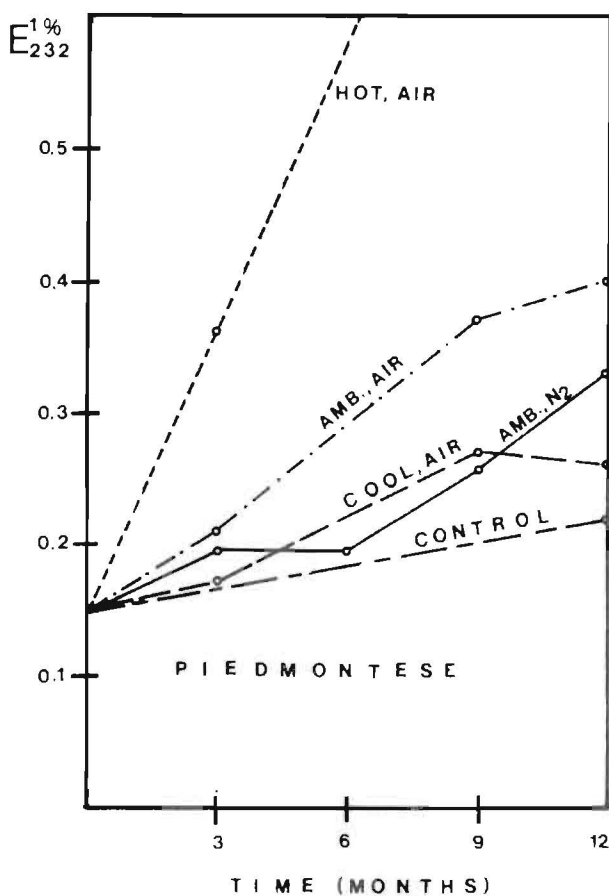


Fig.6. Increase of diene-extinction value in Piedmontese hazelnuts in the course of storage.

The graph in Figure 7 refers to the Akcakoca hazelnuts and confirms what said before. There is no difference between the nitrogen atmosphere and cool storage. The nuts under mechanical pressure rate slightly worse.

The organoleptic tests give a further confirmation of the substantial equivalence of the storage under nitrogen as compared to the traditional methods at low temperature.

Considering the histogram (Fig. 8), which refers to the organoleptic test of the Piedmontese nuts, it seems that the results of the various types of storage do not differ, even if in air storage at am-

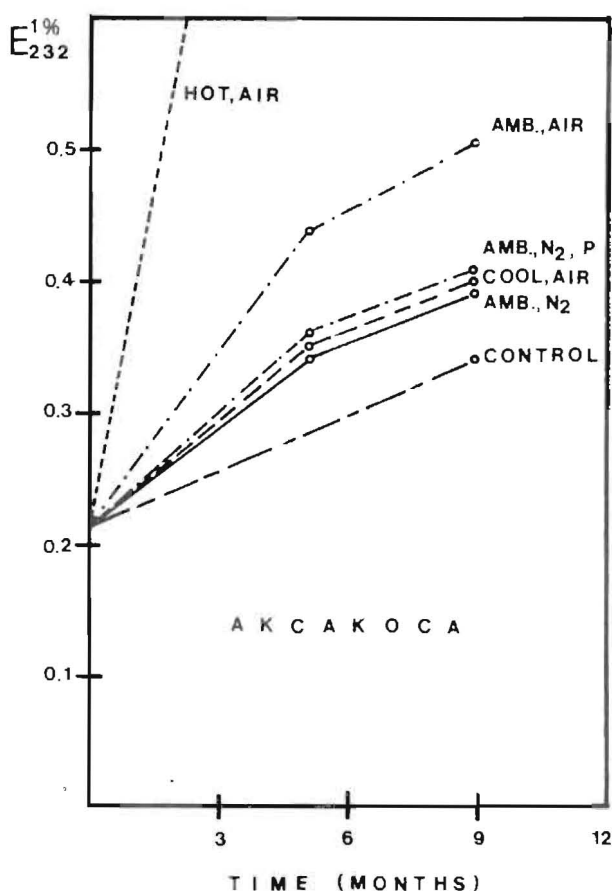


Fig.7. Increase of diene-extinction value in Akcakoca hazelnuts.

bient temperature the critical value (2.00) is reached.

This is due to the excellent quality of Piedmontese hazelnuts that makes the spoilage hardly noticeable.

In Roman nuts (Fig. 9) the general picture is clearer. After one year of storage in nitrogen atmosphere the same or slightly better rating is reached as in cool storage, while the nuts stored in air at ambient temperature are clearly worse.

The Akcakoca nuts (Fig. 10) completely confirm this trend.

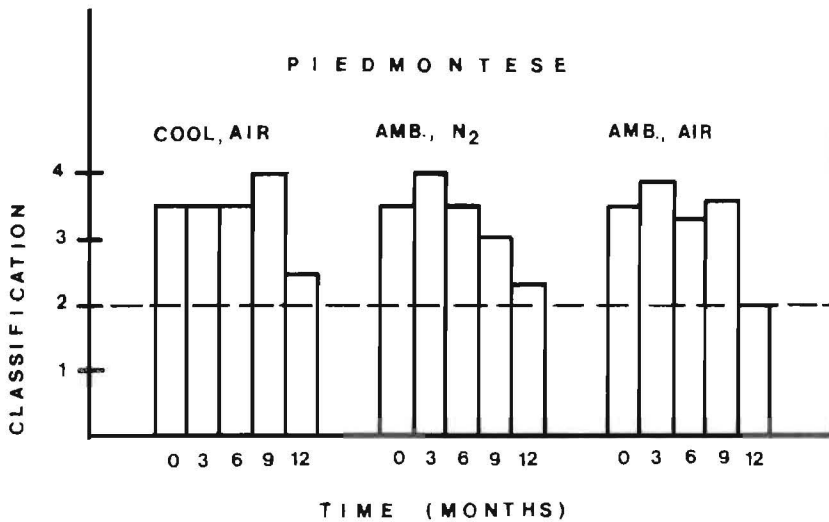


Fig.8. Organoleptic examination of Piedmontese hazelnuts during storage under various conditions.

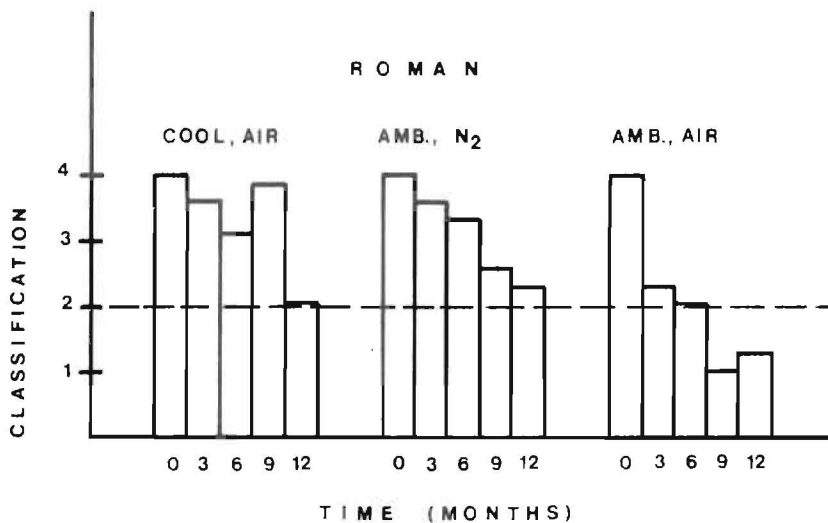


Fig.9. Organoleptic examination of Roman hazelnuts.

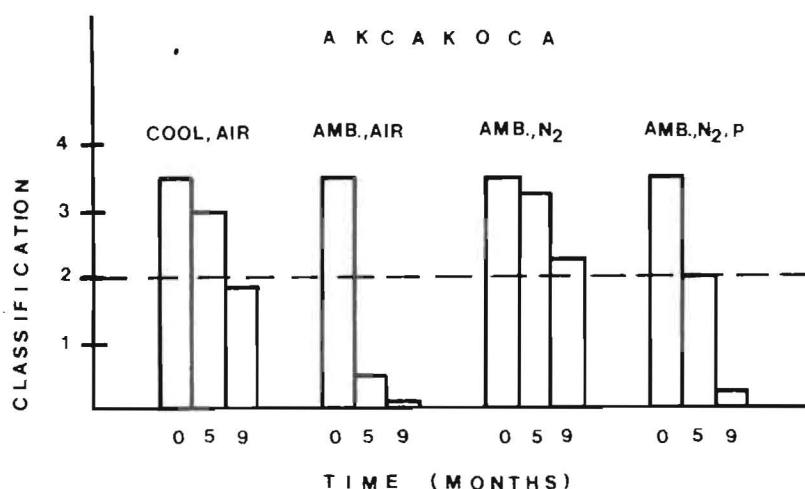


Fig.10. Organoleptic examination of Akcakoca hazelnuts.

## CONCLUSIONS

All the observed data, summarized in Tables 2, 3 and 4, show that nitrogen storage is better or equal to cool storage.

Yet we have to observe the results of the storage under mechanical pressure more carefully. It seems that, in some way, mechanical pressure speeds up deterioration as is clearly shown by the results of the organoleptic tests. Yet it should be remember that in this simulation technical troubles with the airtightness of the apparatus were encountered and all the experiments were carried out at a high oxygen content (about 1%). Therefore mechanical pressure has probably an adverse influence on storability but, due to the technical problems, it was not possible to establish the exact importance of this phenomenon.

Furthermore it can be said that storability of the three varieties of hazelnuts, in this experiment, decreases in the order: Piedmontese - Roman - Akcakoca. However it should not be overlooked that the Roman and Piedmontese hazelnuts were superior in quality and were stored immediately after the harvest. On the contrary the Akcakoca nuts had been shipped from Turkey and were kept for a long time at customs. So the deterioration process had already started at the beginning of our storage. This contributed to the worse results with the Akcakoca variety.

TABLE 2

Composition and characteristic values of Piedmontese hazelnuts at the beginning and the end of storage.

Analyses	Time (months)			
	0	12		
		Storage condition		
		cool air	amb. air	amb.N <sub>2</sub>
Moisture by drying-oven method (%)	4.6	4.2	3.7	4.8
Protein, N x 5.3, (%)	12.4	12.7	12.7	12.2
Fat (%)	68.7	68.7	68.5	68.5
Surface fat (%)	2.1	0.3	—	0.6
Ash (%)	2.0	2.2	2.1	2.2
Glucose, enzymatic (%)	0.03	0.01	0.02	0.03
Fructose, enzymatic (%)	0.03	0.01	0.07	0.03
Sucrose, enzymatic (%)	4.0	4.2	4.3	4.2
pH - value of 10% suspension	6.7	6.7	6.7	6.4
ffa (% oleic acid)	0.2	0.2	0.4	0.4
Induction period of oil at 110°C (hr)	13.50	12.50	12.75	12.50
uv - differential graph E <sub>232</sub> <sup>1%</sup>	0.15	0.26	0.40	0.33
Fatty acid distribution (%)				
(total = 100%) C <sub>16</sub>	6.0	5.5	5.5	5.5
C <sub>18</sub>	2.5	2.2	2.3	2.4
C <sub>18:1</sub>	82.0	84.1	83.9	84.3
C <sub>18:2</sub>	8.8	7.7	7.8	7.3
Organoleptic test	3.50	2.50	2.00	2.25

TABLE 3

Composition and characteristic values at the beginning and the end of storage of Roman hazelnuts.

Analyses	Time (months)			
	0	12		
		Storage condition		
		cool air	amb. air	amb. N <sub>2</sub>
Moisture by drying-oven method (%)	4.8	4.5	3.9	5.3
Protein, N x 5.3, (%)	12.7	12.9	12.9	12.5
Fat (%)	65.6	65.6	65.9	65.6
Surface fat (%)	6.8	1.2	3.6	2.7
Ash (%)	2.3	2.3	2.3	2.3
Glucose, enzymatic (%)	0.03	0.02	0.01	0.04
Fructose, enzymatic (%)	0.03	0.02	0.04	0.04
Sucrose, enzymatic (%)	4.7	4.3	4.9	4.9
pH - value of 10% suspension	6.6	6.5	6.5	6.3
ffa (% oleic acid)	0.1	0.2	0.4	0.6
Induction period of oil at 110°C (hr)	10.5	9.5	9.5	8.5
uv - differential graph E <sub>232</sub> <sup>1%</sup>	0.26	0.33	0.43	0.24
Fatty acid distribution (%)				
(total = 100%)				
C <sub>16</sub>	5.4	5.0	5.0	5.1
C <sub>18</sub>	2.2	1.9	2.0	1.9
C <sub>18:1</sub>	79.1	81.4	81.4	81.0
C <sub>18:2</sub>	12.5	11.2	11.1	11.6
Organoleptic test	4.00	2.00	1.25	2.25



TABLE 4

Composition and characteristic values at the beginning and the end of storage of Akcakoca hazelnuts.

Analyses	Time (months)				
	0	9			
		Storage condition			
		cool air	amb.air	amb.N <sub>2</sub>	amb.N <sub>2</sub> P
Moisture by drying-over method (%)	4.0	4.6	3.2	4.3	3.6
Protein, N x 5.3, (%)	14.2	13.9	14.3	14.2	13.9
Fat (%)	68.5	67.4	67.5	67.6	67.2
Surface fat (%)	1.2	3.4	1.2	0.9	1.1
Ash (%)	2.0	2.1	1.9	1.9	1.9
Glucose, enzymatic (%)	0.02	0.04	0.03	0.03	0.02
Fructose, enzymatic (%)	0.02	0.06	0.03	0.03	0.02
Sucrose, enzymatic (%)	3.3	3.6	3.9	3.6	3.7
pH - value of 10% suspension	6.5	6.2	6.1	6.1	6.1
ffa (% oleic acid)	0.4	0.5	0.8	0.9	1.0
Induction period of oil at 110°C (hr)	11.75	8.25	9.75	9.25	9.25
uv - differential graph E <sub>232</sub> <sup>1%</sup>	0.22	0.40	0.50	0.39	0.41
Fatty acid distribution (%)					
(total = 100%)					
	C <sub>16</sub>	4.9	4.4	4.4	4.4
	C <sub>18</sub>	2.2	1.9	1.9	1.8
	C <sub>18:1</sub>	82.3	84.3	84.2	84.0
	C <sub>18:2</sub>	10.0	9.0	9.1	9.4
Organoleptic test		3.50	1.75	0.00	0.25

## ACKNOWLEDGEMENTS

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# ENVIRONMENTAL INFLUENCE OF INERT GAS ON THE HERMETIC STORAGE OF UNPOLISHED RICE

SHOJI YANAI and TAKASUKE ISHITANI

## INTRODUCTION

The quality of cereals stored under anaerobic conditions is estimated differently by a variety of investigators. From the experience in the hermetic storage of Japanese-produced rice in air and carbon dioxide gas, Kondo et al (1934) have reported on the excellent quality of hermetic storage for preserving the quality of rice. Based upon the hermetic storage test of unhusked rice in air and nitrogen carbon dioxide and oxygen gas, Roberts (1961) has pointed out that changes in the germination rate of unhusked rice under anaerobic conditions are dependent upon the water content and temperature, and that the nitrogen-filled package resulted in a better preservation of germination rate than that of the air-filled package.

The superiority of the carbon-dioxide-filled rice package in the preservation of quality has recently been reported by Mitsuda et al (1972, 1973). These motivated the present investigative work on the advantages of the nitrogen- and carbon-dioxide-filled hermetic rice packages over the air-containing packages.

## EXPERIMENTAL METHODS

### 1. Specimens and Inoculation

Two species of moist-land nonglutinous rice produced in Niigata, Senshuraku with a water content of 16.7% and Koshiji-Wase with a water content of 15.5%, were used in the experiments.

The water content of specimens was regulated using a thermo-hygrostat by means of the weight method. The humidification and dehumidification conditions were set at 15°C and RH 95% and at 15°C and RH 30%, respectively. Specimens I of Senshuraku was divided into three groups, where the water content was regulated at 15.5, 16.7 and 17.2%, respectively. The water content of Specimens II of Koshiji-Wase was regulated at 15.5, 16.7 and 17.2%. In addition, Specimens III of Koshiji-Wase with a water content of 16.6 and 18.1% was prepared.

All specimens were inoculated with two groups of *Aspergillus glaucus* and *Aspergillus restrictus* that had been isolated from Japanese produced rice.

The inoculation procedure was as follows: initially prepare strains plate-cultured individually on a Koji Agar medium, let spores of mold fungi fall into a specimen by turning a culture dish upside down and then mix the spores completely with the specimen to attain uniform inoculation.

## 2. Storage Methods

Specimens I: Under a water content condition, 450g of specimens per package were hermetically packed with a triple-layer (PET·Al·PE) laminate film comprising 12 $\mu$  polyester, 9 $\mu$  aluminum foil and 60 $\mu$  polyethylene or with 70 $\mu$  low density polyethylene (PE) and stored at 10°C and 20-25°C for two years. Part of specimen packages were filled with nitrogen gas.

Specimens II: As with specimens I, 450g of specimens per package were put in PET·Al·PE pouches under a water content condition and stored at 25 to 28°C for eight months in carbon-dioxide- or nitrogen-filled package or in an air-containing-filled package.

Specimens III: Specimens were put in small-sized stainless-steel-made pressurized testing containers, manufactured by Nitto Autoclave Co., Ltd. These containers were pressurized to 10 Kg/cm<sup>2</sup> using cylinders of compressed carbon dioxide, nitrogen and air. As the controls, 50 Kg/cm<sup>2</sup> -pressurized air packages in the above-mentioned containers and non-pressurized hermetic packages in PET·Al·PE and PE pouches were prepared. All test and control specimens were measured after three-months' storage at 25°C.

In filling of nitrogen and carbon dioxide gases, each gas was poured repeatedly into a pouch or container in a desiccator so as to completely replace the air between rice grains prior to sealing.

## 3. Measured Items and Measuring Methods

The measured items for specimens are water content, germination rate, reducing sugar content, fatty acidity, palatability, composition of gases in pouches and mold count. The level of yeast and bacteria were determined by measurement of the number of colonies that had grown at 30°C in three to five days by a plate culture using Koji Agar and standard Agar media. Rosenthal's chromium sulfate method was used to culture anaerobic bacteria.

## EXPERIMENTAL RESULT AND DISCUSSIONS

### 1. Preservation Effect of Air-containing and Nitrogen-filled Package Rice

Changes in rice quality and microorganism count observed when unpolished rice with water content of 14.5 to 16.6% in air-containing and nitrogen-filled packages was stored for one to two years, are summarized in Tables 1 and 2.

The value of water content in all specimens, except those packed with PE, has suffered little change during the period of storage.

Concentration change of the oxygen contained in PET·Al·PE pouches for the air-containing package division depends upon the water content of the specimens. In case of low temperature (10°C) storage, the oxygen concentration fell to about 10% in a year, and to about 1.0 to 2.0% in two years. For 20 to 25°C storage, naturally, reduction in oxygen concentration became more rapid and the accumulated

Table 1. Changes in Characteristics of Brown Rice during Hermetic Storage for 1 Year under Different Atmosphere

Initial moisture content (%)	Storage condition													
	20°C - 25°C						10°C							
	Air		N <sup>2</sup>		Air		N <sup>2</sup>		PE					
	14.6	16.6	14.5	16.6	14.5	15.8	16.6	14.5	15.8	16.6	14.5	15.8	16.6	
Moisture content (%)	14.6	16.8	14.6	16.8	14.7	15.9	16.8	14.7	15.9	16.8	14.9	16.1	17.0	
Gas composition in pouches	O <sub>2</sub> (%)	1.4	1.6	0	0.2	12.9	10.7	10.4	0.3	0	-	20.9	20.9	20.9
	CO <sub>2</sub> (%)	2.1	22.1	0.4	13.5	0.4	0.6	1.1	0.1	0.2	-	0.01	0.01	0.02
Germination (%)		56	0	51	0	97	98	92	98	98	95	94	94	
Reducing sugars a)		238	655	246	679	223	231	241	224	228	257	233	242	236
Fat acidity b)		43.0	62.4	55.0	60.5	28.6	28.5	30.4	28.8	32.5	-	29.2	27.0	30.3
Mold count /g c)		4.5x10 <sup>3</sup>	1.1x10 <sup>3</sup>	1.4x10 <sup>3</sup>	1.2x10 <sup>3</sup>	2.1x10 <sup>3</sup>	3.7x10 <sup>3</sup>	1.2x10 <sup>3</sup>	2.2x10 <sup>3</sup>	4.2x10 <sup>3</sup>	8.5x10 <sup>3</sup>	1.6x10 <sup>3</sup>	3.3x10 <sup>3</sup>	8x10 <sup>3</sup>
Bacteria count /g d)		2.3x10 <sup>5</sup>	4.5x10 <sup>4</sup>	6.4x10 <sup>5</sup>	3.9x10 <sup>5</sup>	5.7x10 <sup>6</sup>	7.9x10 <sup>6</sup>	5.9x10 <sup>6</sup>	7.1x10 <sup>6</sup>	8.9x10 <sup>6</sup>	5.1x10 <sup>6</sup>	6.1x10 <sup>6</sup>	6.7x10 <sup>6</sup>	7.7x10 <sup>6</sup>

PE: Low density polyethylene pouches

a: Initial reducing sugar 221mg·glucose per 100g dry rice

b: Initial fat acidity 16.8mg·KOH per 100g dry rice

c: Initial mold count 2.5 - 8.3 x 10<sup>2</sup>d: Initial bacteria count 3.0 - 3.2 x 10<sup>7</sup>

Table 2. Changes in Characteristics of Brown Rice during Hermetic Storage for 2 Years under Different Atmosphere

Initial moisture content (%)	Storage condition													
	20° - 25°C						10°C							
	Air		N <sub>2</sub>		Air		N <sub>2</sub>		PE					
	14.5	16.6	14.5	16.6	14.5	15.8	16.6	14.5	15.8	16.6	14.5	15.8	16.6	
Moisture content (%)	14.6	16.8	14.6	16.6	14.6	16.1	16.7	14.6	16.1	16.8	15.2	16.5	17.2	
Gas com- position pouches	O <sub>2</sub> (%)	1.2	0.7	0	0	1.8	1.4	1.0	0.1	0.3	0	20.6	20.7	19.9
	CO <sub>2</sub> (%)	3.5	28.1	1.0	16.5	1.1	1.2	2.4	0.1	0.3	0.6	0.1	0.1	0.2
Germination (%)		0	0	0	0	96	98	74	98	96	92	92	86	42
Reducing sugars		328	849	305	856	243	256	285	252	247	290	250	259	325
Fat acidity		68.7	95.4	83.6	104.7	31.6	33.5	35.6	38.3	47.9	53.4	24.8	29.6	38.9
Bacteria count /g		1.1x10 <sup>3</sup>	1.7x10 <sup>2</sup>	1.2x10 <sup>4</sup>	1.6x10 <sup>3</sup>	1.6x10 <sup>6</sup>	1.9x10 <sup>6</sup>	9.9x10 <sup>6</sup>	4.5x10 <sup>6</sup>	4.9x10 <sup>6</sup>	3.5x10 <sup>6</sup>	1.6x10 <sup>7</sup>	1.8x10 <sup>6</sup>	9.8x10 <sup>5</sup>
Organoleptic evaluation (over-all)		-1.75	-3.50	-1.55	-3.27	-0.50	-0.56	-0.05	0.00	0.5	0.277	-0.50	-0.125	-0.055

carbon dioxide gas increased. Also in the nitrogen-filled division, the greater accumulation of carbon dioxide gas was observed for specimens with the higher water content. These tendencies, consistent with those reported by Glass et al. (1959) for wheat, indicate that metabolic action under anaerobic conditions are appreciable also in unpolished rice and become more active with the increasing water content.

Change in the germination rate of the specimens stored at 20 to 25°C, shows little difference between the air-containing and nitrogen-filled packages. In specimens stored for a year in both packages, the germination rate was reduced to about 50% for low water content and reached to 0% for 16.6% water content. On the contrary, in specimens stored at 10°C for a year, the germination rate, though showing no difference between the two above-mentioned packages for low water content was higher in the nitrogen-filled package than in the air-containing package for high water content. The germination rate of specimens stored in the nitrogen-filled package hardly reduced even for two-years' storage. As may be understood also from an extreme reduction in the germination rate of PE - packed rice (cf. Table 2), this indicates that the oxygen-free condition is favorable to the preservation of germination rate of unpolished rice.

Change in fatty acidity, independent of the initial water content and storage temperature of specimens, tended to be greater for the nitrogen-filled package than for the air-containing package in all cases.

Change in reducing sugar content showed little difference between these two packages and gradually increased with the lapse of storage period.

The initial count of microorganisms in 1g of unpolished rice specimens was  $2.5$  to  $2.8 \times 10^2$  for mold fungi,  $2.7$  to  $4.7 \times 10^3$  for yeast,  $3.0$  to  $3.2 \times 10^7$  for aerobes (chiefly Chromogenic *Pseudomonas*) (Iizuka et al., 1963),  $5.3$  to  $5.8 \times 10^6$  for anaerobes. As shown in Fig.1, however, after one-year's storage at 20 to 25°C, the yeast count decreased most rapidly, next in order are the anaerobe and aerobe counts, and the mold count hardly fell. With a higher initial water content, a decrease in the micro-organism count became more rapid. Specimens stored in air-containing packages showed a significantly decreasing tendency of the microorganism count in comparison with those stored in nitrogen-filled packages. In specimens stored at a low temperature of 10°C, as shown in Fig.2, a decrease in microorganism count was small and reached to no more than the order of 1 micro-organism, after two-years' storage.

As pointed out in the previous report (Yanai et al., 1978), the preservation of PE-packed rice with a high water content is restricted to 20 days or so on account of the multiplying mold fungi at room temperature. On the contrary, for rice packed hermetically with PET·Al·PE pouches hardly permeable to gas, no increase in mold count was observed even after two-years' storage and therefore

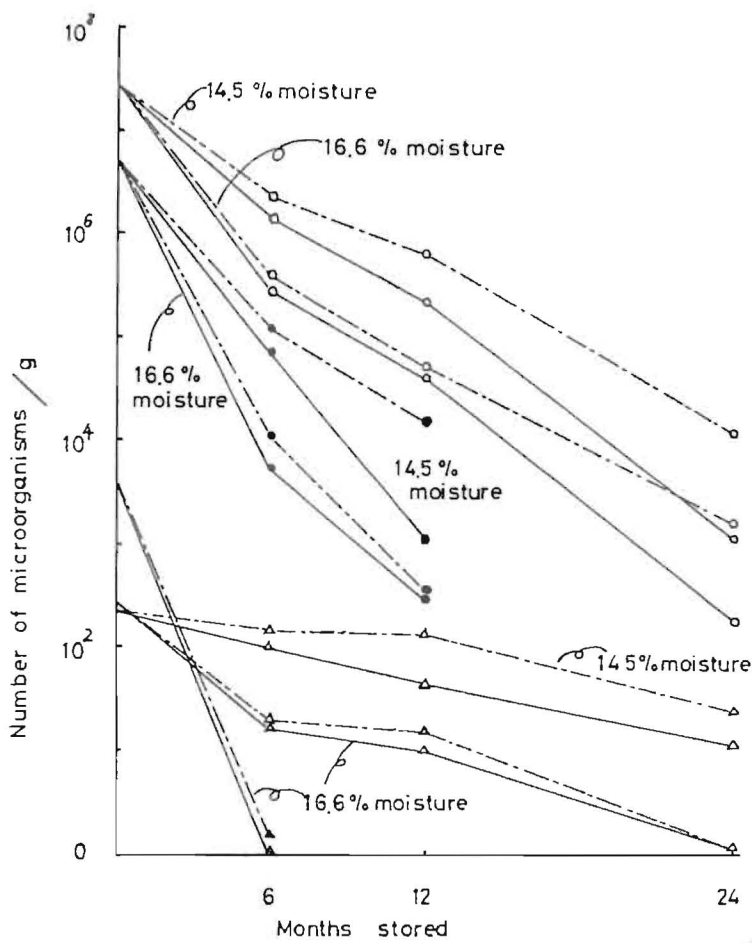


Fig.1. Number of viable microorganisms on brown rice during hermetic storage for 2 years at 25~28°C

— Air seal	○—○ Aerobic bact.
- - - N <sub>2</sub> seal	●—● Anaerobic bact.
	△—△ Storage fungi
	▲—▲ Yeasts

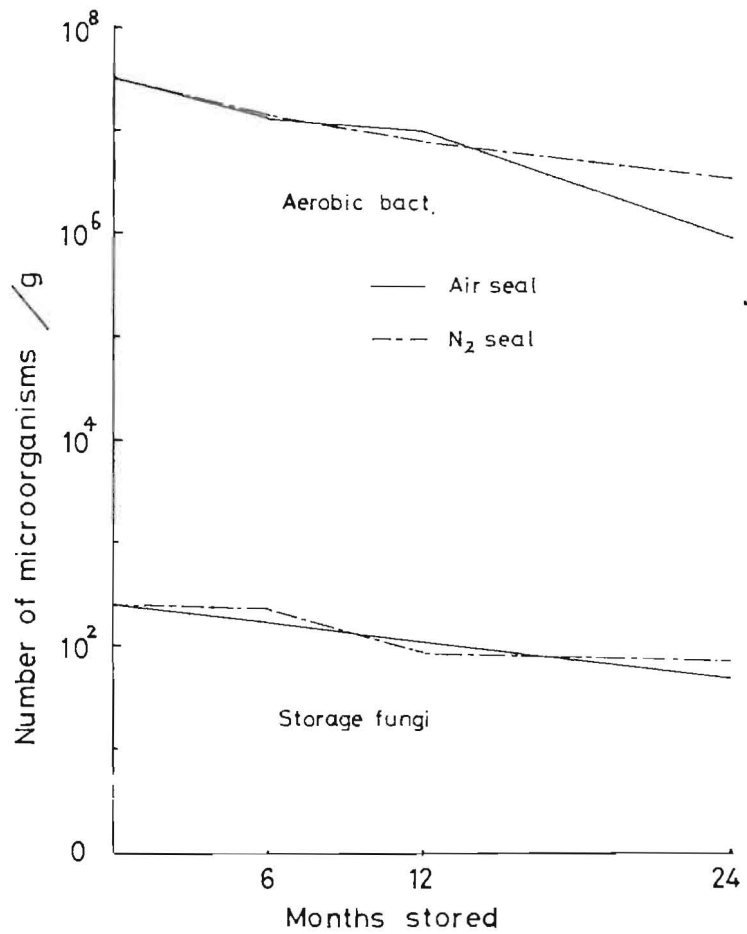


Fig. 2. Number of viable microorganisms on brown rice during hermetic storage for 2 years at 16.6 % moistur and 10°C



there may be no necessity to consider the damage from mold fungi.

Palatability tests gave no appreciable palatability -preserving effect of nitrogen gas in specimens stored at 20 to 25°C, whereas a good appreciation of this effect was obtained in those stored at 10°C with a significant difference from those stored in an air-containing package being detected at the 10 percent level of significance.

It was stated above that a decrease in the aerobic count of specimen rice is closely related to the storage temperature and water content and the number of viable microorganisms is greater for the nitrogen-filled package than for the air-containing package. Fig.3 indicates a statistically significant correlation between a decrease in bacteria count and palatability. Specimens showing a decrease in bacteria count such as those packed with PE, even if showing a low value of both reducing sugar content and fatty acidity, obtained no good appreciation of palatability (cf. Table 2).

This indicates that environmental conditions including a decrease in bacteria count are inappropriate for preserving the quality of unpolished rice. This comment can be supported by the parallelism observed between a fall in the germination rate of unpolished rice and a decrease in bacteria count(Iizuka,1961).

## 2. Preservation Effect of Carbon Dioxide Gas.

Changes in rice quality and microorganism count observed when unpolished rice packed with PET·Al·PE pouches was stored at 10°C and at 25 to 28°C for eight months, are summarized in Table 3.

The value of the water content in all specimens has undergone little variation during the period of storage. As with Experiment 1, a change in the germination rate of a specimen with a water content of 15.5% exhibited little difference between the air-containing and nitrogen-filled packages. However, specimens in a carbon-dioxide-filled package displayed a significant decreasing tendency concerning the germination rate. A drastic reduction in all specimens with a high water content could afford no comparison between different water contents.

Fatty acidity in all specimens gradually increased during storage without detectable difference between different filling gases: by a factor of 3.2 to 3.3 for those with a high water content stored at 25 to 28°C; and, by a factor of 1.2 to 1.3 for those stored at 10°C. Change in reducing sugar, as in fatty acidity, exhibited no difference between different filling gases, with the exception of a slightly greater tendency to increase for specimens with a water content of 17.2% in a carbon-dioxide-filled package.

The microorganism counts have suffered changes similar to those shown in Experiment 1 (cf. Table 1).

To summarize: values measured in specimens, though varying with the storage

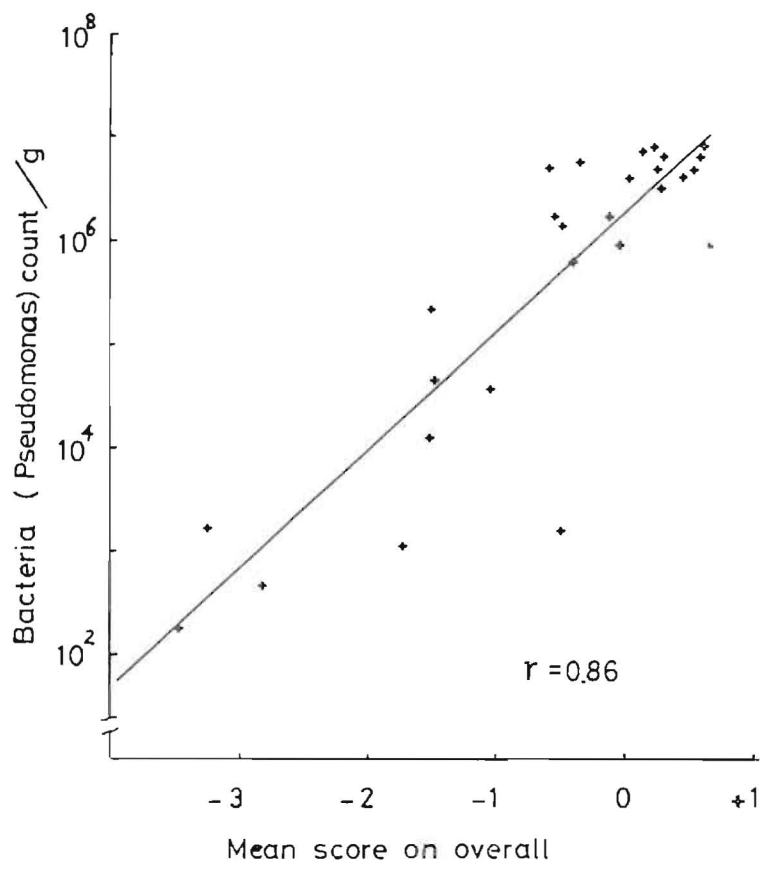


Fig.3. Correlation of means of sensory score on overall and bacteria count of brown rice during hermetic storag for 1~2 years under different atmosphere

Table 3. Changes in Characteristics of Brown Rice during Hermetic Storage for 8 Months under Different Atmosphere

Initial moisture content	Storage condition											
	25-28°C									10°C		
	15.5%			16.7%			17.2%			17.2%		
	Air	N <sub>2</sub>	CO <sub>2</sub>	Air	N <sub>2</sub>	CO <sub>2</sub>	Air	N <sub>2</sub>	CO <sub>2</sub>	Air	N <sub>2</sub>	CO <sub>2</sub>
Moisture content (%)	15.7	15.6	15.7	16.7	16.8	16.8	17.2	17.2	17.3	17.2	17.3	17.3
Gas composition O <sub>2</sub> (%)	1.1	0.1	0.3	0.8	0.1	0.2	0.7	0.2	0.2	19.6	1.4	0.1
in pouches CO <sub>2</sub> (%)	9.4	3.6	92.6	40.8	31.4	96.4	51.1	36.9	96.4	2.1	8.9	97.1
Germination (%)	11	12	2	4	1	0	1	0	0	99	100	100
Reducing sugars a)	313	292	328	479	483	495	578	492	608	262	281	277
Fat acidity b)	59.7	63.3	60.7	62.0	62.5	59.5	62.1	60.2	60.0	38.6	40.1	376
Mold count /g c)	4.4x10 <sup>2</sup>	6.3x10 <sup>2</sup>	4.5x10 <sup>2</sup>	3.7x10 <sup>2</sup>	5.1x10 <sup>2</sup>	4.2x10 <sup>2</sup>	2.1x10 <sup>2</sup>	2.1x10 <sup>2</sup>	2.5x10 <sup>2</sup>	2.2x10 <sup>2</sup>	1.9x10 <sup>2</sup>	1.7x10 <sup>2</sup>
Bacteria count/gd)	2.3x10 <sup>4</sup>	1.7x10 <sup>4</sup>	1.4x10 <sup>4</sup>	1.6x10 <sup>3</sup>	2.6x10 <sup>3</sup>	1.4x10 <sup>3</sup>	2.0x10 <sup>3</sup>	2.5x10 <sup>3</sup>	2.0x10 <sup>3</sup>	4.5x10 <sup>3</sup>	5.8x10 <sup>3</sup>	5.4x10 <sup>3</sup>

a: Initial reducing sugar 226mg·glucose per 100g dry rice

b: Initial fat acidity 18.2mg·KOH per 100g dry rice

c: Initial mold count 7.2 - 8.6 x 10<sup>3</sup>/gd: Initial bacteria count 1.2 - 2.4 x 10<sup>7</sup>/g

Table 4. Changes in Gas Composition in a Container and Moisture Contents of Brown Rice during Hermetic Storage for 3 Months at 25°C under Various Conditions

Storage condition		Moisture content (%)		Oxygen (%)	Carbon dioxide (%)
		Initial	Final		
50 kg/cm <sup>2</sup>	Air	16.6	16.7	19.38	0.47
		18.1	18.2		
10 kg/cm <sup>2</sup>	Air	16.6	16.8	19.73	1.30
		18.1	18.2		
10 kg/cm <sup>2</sup>	N <sub>2</sub>	16.6	16.8	0.40	1.20
		18.1	18.3		
10 kg/cm <sup>2</sup>	CO <sub>2</sub>	16.6	16.9	2.04	89.95
		18.1	18.4		
PET·AI·PE	Air	16.6	16.7	1.18	19.01
		18.1	18.3	0	51.32
PE	Air	16.6	16.1	18.1	0.7
		18.1	17.6	-	-

temperature and water content, showed an appreciable difference between different filling gases, and therefore carbon dioxide gas is not considered to exhibit any particularly excellent effect concerning the preservation of the quality of unpolished rice.

### 3. Preservation Effect of Pressurized Inertia Gases

Table 4 shows changes in the gas component within containers and water content of specimens observed when unpolished rice with an initial water content of 16.6 to 18.1% was stored at 25°C for three months under atmospheric or elevated pressure.

The oxygen concentration between rice grains stored in an air-containing package under atmospheric pressure fell to 0 to 1.18%, whereas the carbon dioxide concentration rose to 19 to 51%. On the other hand, with the pressurization storage, the oxygen and carbon dioxide concentrations showed little change and assumed values approximate to those in air. This indicates that the pressurization storage depresses respiration of unpolished rice (and microorganisms within). Fig.4 shows another great influence of the pressurization storage system upon the germination rate of unpolished rice. The influence of pressurized gases upon the germination rate decreases in the order of carbon dioxide gas, air and nitrogen gas. Increasing the water content of unpolished rice enhances the effectiveness of pressurized gases in reducing the germination rate. In unpolished rice packed with PE pouches, the resultant germination rate was lower than that for the pressure-applying and nitrogen-filling storage methods in spite of the advantageous factor that the water content decreased during storage. This is attributable to the presence of oxygen gas or, as discussed later, to the multiplication of mold fungi.

Fig.5 shows changes in fatty acidity observed before and after the period of storage. Except in PE-packed rice specimens showing a reduction in water content during storage, the fatty acidity in specimens with a water content of 16.6% increased by a factor of 2.0 to 2.5 during storage without any wide variation with the individual specimens. In specimens with a water content of 18.1%, the fatty acidity increased significantly for 50 kg/cm<sup>2</sup>-pressurized air-containing and 10 kg/cm<sup>2</sup>-pressurized carbon-dioxide-filled packages, whereas it increased slightly without any great mutual difference for 10 kg/cm<sup>2</sup>-pressurized air-containing, 10 kg/cm<sup>2</sup>-pressurized nitrogen-filled and PET·Al·PE pouch packages. As shown in Fig.6, the reducing sugar content, as with the fatty acidity, increased rapidly for 50 kg/cm<sup>2</sup>-pressurized air-containing and 10 kg/cm<sup>2</sup>-pressurized carbon-dioxide-filled packages with a significant contrast to pressurized nitrogen-filled and PET·Al·PE pouch packages.

Change in the mold count of specimens during storage is shown in Fig. 7. The mold count increased significantly in PE-packed specimens, whereas it fell below the initial count in pressurized and non-pressurized air-containing hermetic specimens. Especially in 50 kg/cm<sup>2</sup>-pressurized air-containing specimens, the ratio of viable mold fungi was 0.6 to 1.0%. With higher water content, the ratio

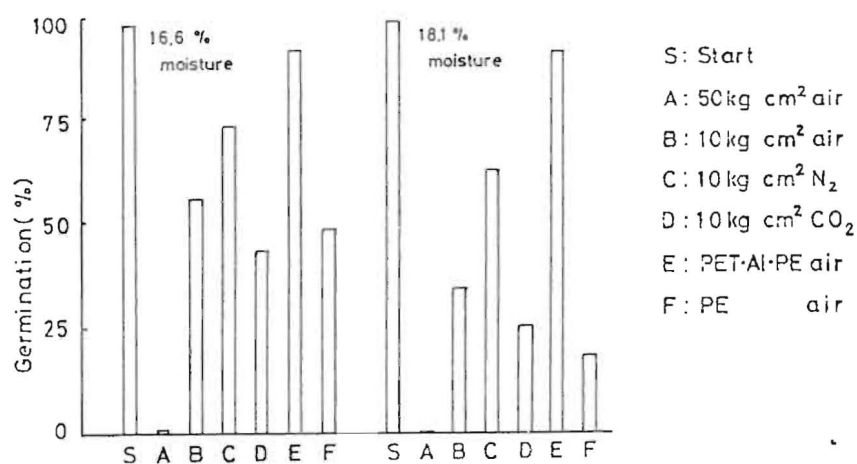


Fig. 4. Germination percentage of brown rice during hermetic storage for 3 months at 25°C under various conditions

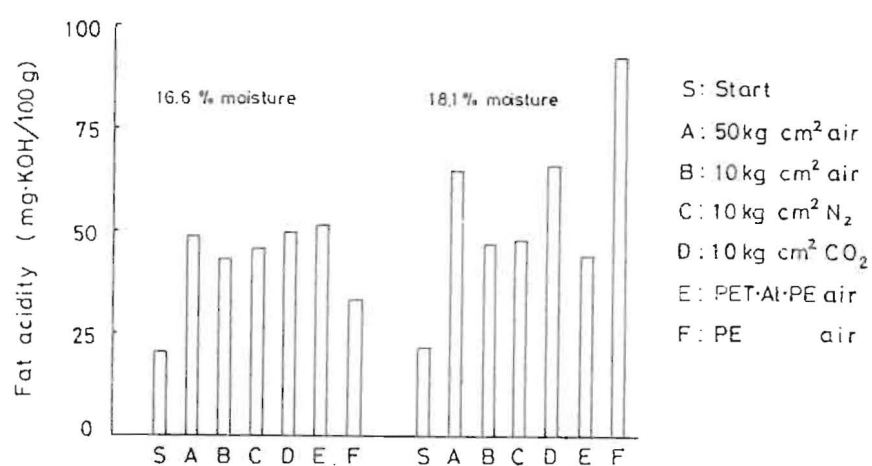


Fig. 5. Fat acidity of brown rice during hermetic storage for 3 months at 25°C under various conditions

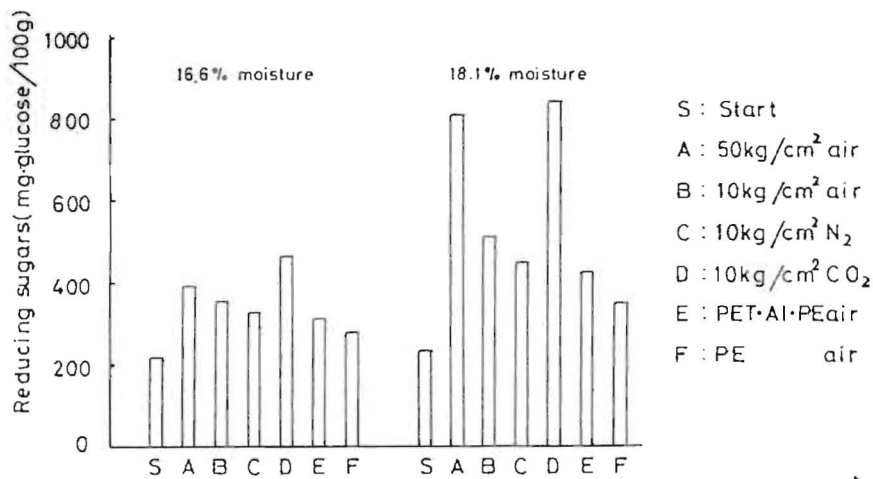


Fig. 6. Reducing sugars of brown rice during hermetic storage for 3 months at 25°C under various conditions

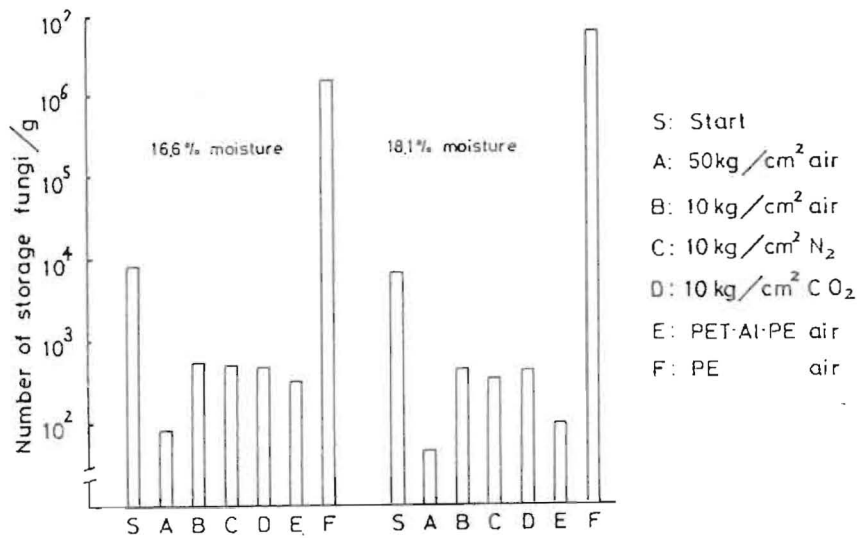


Fig. 7. Number of storage fungi of brown rice during hermetic storage for 3 months at 25°C under various conditions

of viable mold fungi had a tendency to lower. A decrease in mold count observed also in  $10 \text{ kg/cm}^2$ -pressurized specimens hardly depended upon the water content of specimens and the kind of filling gases. The ratio of viable mold fungi in these specimens was about 4.0 to 7.0%. This value was higher than that observed in non-pressurized air-containing specimens.

To summarize: Measurements of the germination rate and reducing-sugar content in unpolished rice stored at elevated pressure, showed negative results for the preservation of the rice quality in comparison with the storage at atmospheric pressure. Upon palatability test, the pressurization storage was estimated to be inferior in glutinosity and taste to the non-pressurized storage. Rice specimens with a high water content stored in a pressurized carbon-dioxide-filled package displayed a strong reduction in rice quality in comparison with those stored in pressurized nitrogen-filled and air-containing packages. In case of storage in a carbon-dioxide-filled package, the chemical influence of gas molecules is an important consideration as well as the physical and direct effect of pressurization.

The results obtained in Experiments 1, 2, and 3 can lead to the following conclusion: For the gas-filling rice storage at ordinary temperatures, there is no great difference between nitrogen and carbon dioxide gases in their influence exerted upon the rice quality. Any hermetical packing material that can be used for a gas-filled package does not seem to greatly differ in preserving the rice quality from a simple air-containing package.

In contrast to this, under low-temperature conditions, the clearly-seen effectiveness of the inert-gas-filling storage method in preserving the rice quality was established upon carrying out the palatability test, though not in the chemical measured quantities, such as reducing the sugar content and fatty acidity. The filling of inert gases is considered to make possible the long-term storage even of unpolished rice with a high water content because of effectively preventing an appreciable reduction in palatability.

By way of conclusion, the authors would like to express their deep gratitude to Mr. N. Ishima, of their institute, for his collaboration concerning the palatability tests.

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## BEHAVIOUR OF RAPESEEDS AND SUNFLOWER SEEDS STORED IN AIRTIGHT CONDITIONS. EVOLUTION OF MICROFLORA AND FAT ACIDITY

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### INTRODUCTION

Fairly often rapeseeds and sunflower are harvested in France at moisture content higher than 9-10 p.100 (wet weight) ; so they cannot be stored just as they are, without becoming rapidly damaged. This situation is mainly due to the evolution of agricultural and harvesting techniques in connexion with climatic and economic factors such as the lateripeness of sunflower or the necessity to reduce the losses in the field for rapeseed.

During the last ten years, prestorage techniques allowing to wait before drying, without excessive seed degradations have been experimented as well in laboratories as on pilot or industrial scale. Among these techniques, the storage of oil-seeds in airtight conditions seems of particular interest today because of the saving of energy which might be realised.

It seems therefore interesting to recall the behaviour of oil-seeds stored in airtight conditions at different temperature and moisture content levels, the main criteria under examination being the evolution of microflora and the increase of fat acidity. Most of the example given are taken from different laboratory experiments carried on rapeseed and sunflower seeds stored in glass container hermetically closed.

Microbiological analysis which have been restricted to general bacteria and mold counts, are performed in the following manner with a known quantity of seeds, between 50 and 100 g, a suspension is done in a sterile physiological solution. Enumeration of microorganisms is then realised by the classical dilution method using suitable culture mediums. The fat acidity is checked according the french norm (AFNOR NF-V-03-903) on the fat extracted from ground seeds by soxhlet extraction. Results are expressed as fat acidity value (FAV), corresponding to the quantity of potassium hydroxyde necessary to neutralize the acidity of one gram of fat.

# 1. Influence of storage atmosphere on the microflora evolution

On rape seeds stored under aerated conditions at 12, 15 and 18 P.100 moisture content (22°C), molds are actively growing and sporulating (fig. 1) (Poisson et al., 1971a) and the level of contamination is about  $10^7$  germs/g within 50 days at 15 and 18 p.100 M.C.

With seeds at 12 p.100 M.C., mold growth is slower but damages become evident within two months.

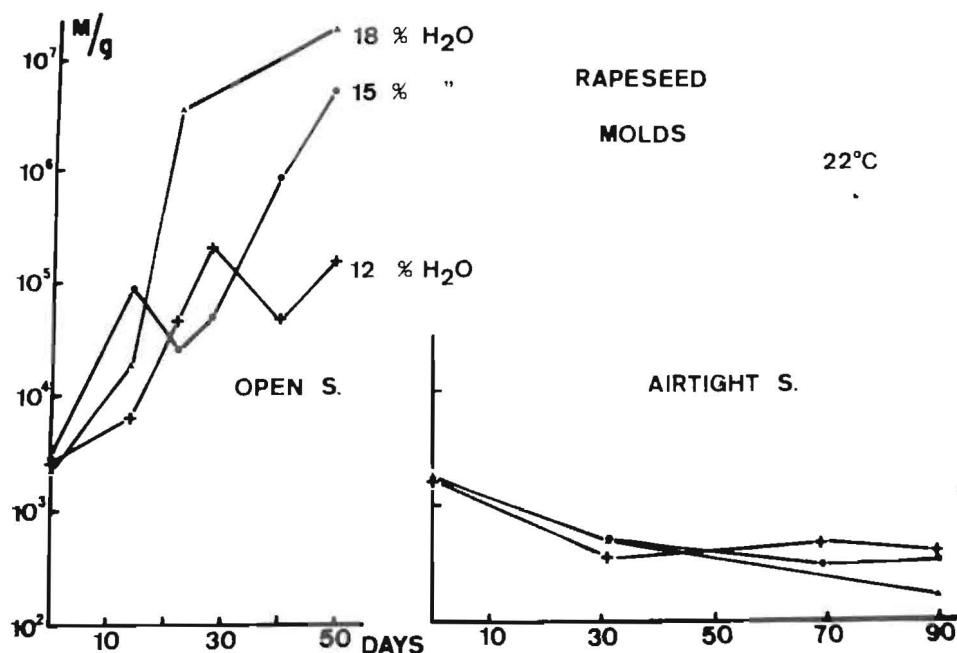


Fig.1. Evolution of molds in rapeseeds stored in air or under airtight conditions.

With rapeseeds at the same moisture content but stored in airtight conditions, no mold growth can be observed during three months. Sometimes a very short period of mold development occur in the first days of storage but, as a general rule, the mold population is decreasing because of the lack of oxygen. Under such conditions, bacteria also undergo a regression on seeds stored at 22 p.100 M.C. or less. Microbial evolutions on seeds at higher moisture content have not been investigated.

On fig. 2 (Guilbot and Poisson, 1966), are shown regression curves for molds and bacteria observed on whole sunflower seeds stored in airtight conditions.

As previously observed with rapeseeds, the decrease of microbial populations is more rapid on seeds with higher moisture content, and this evolution is accelerated by an increase in temperature.

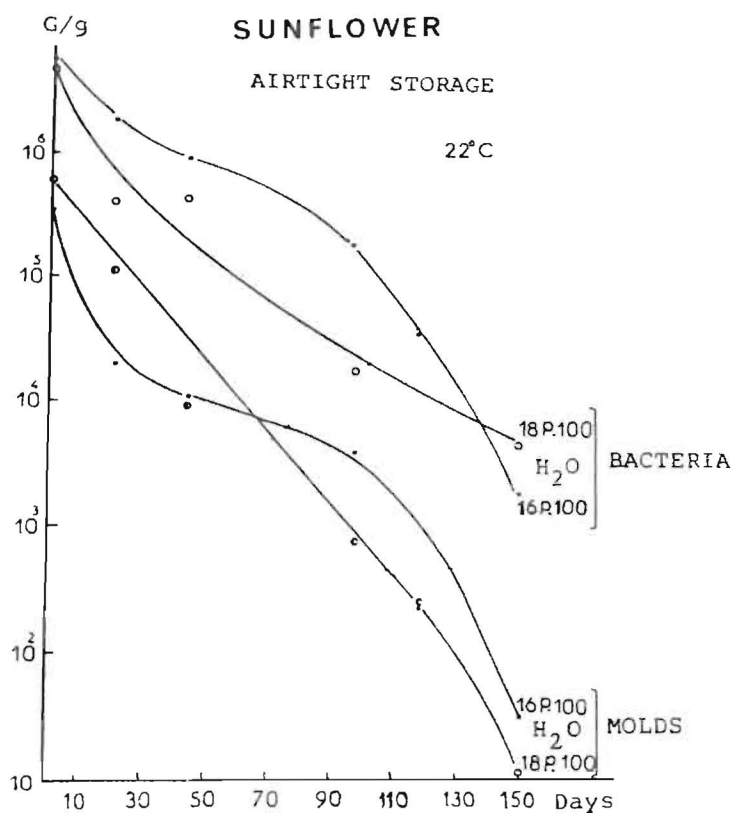


Fig. 2. Evolution of bacteria and molds in sunflower seeds stored in airtight conditions.

## 2. Effect of storage atmosphere on fat acidity evolution

The fat acidity of oil-seeds is increasing when they are open-stored but also under airtight conditions. Nevertheless there is a great difference in the intensity of fatty acids liberation, depending on the type of storage. This is true for whole seeds and also for by-products such as oil-cakes.

Fig. 3 (Poisson, 1971b) gives an example of the evolution of fat acidity of rapeseed-cakes, stored at 15 p.100 M.C., in open and airtight conditions ; in spite of the low residual fat content of the cakes, the greater increase in acid number under aerated conditions is clearly shown and can be correlated with the important growth of molds in the product, during the first three months at 22°C.

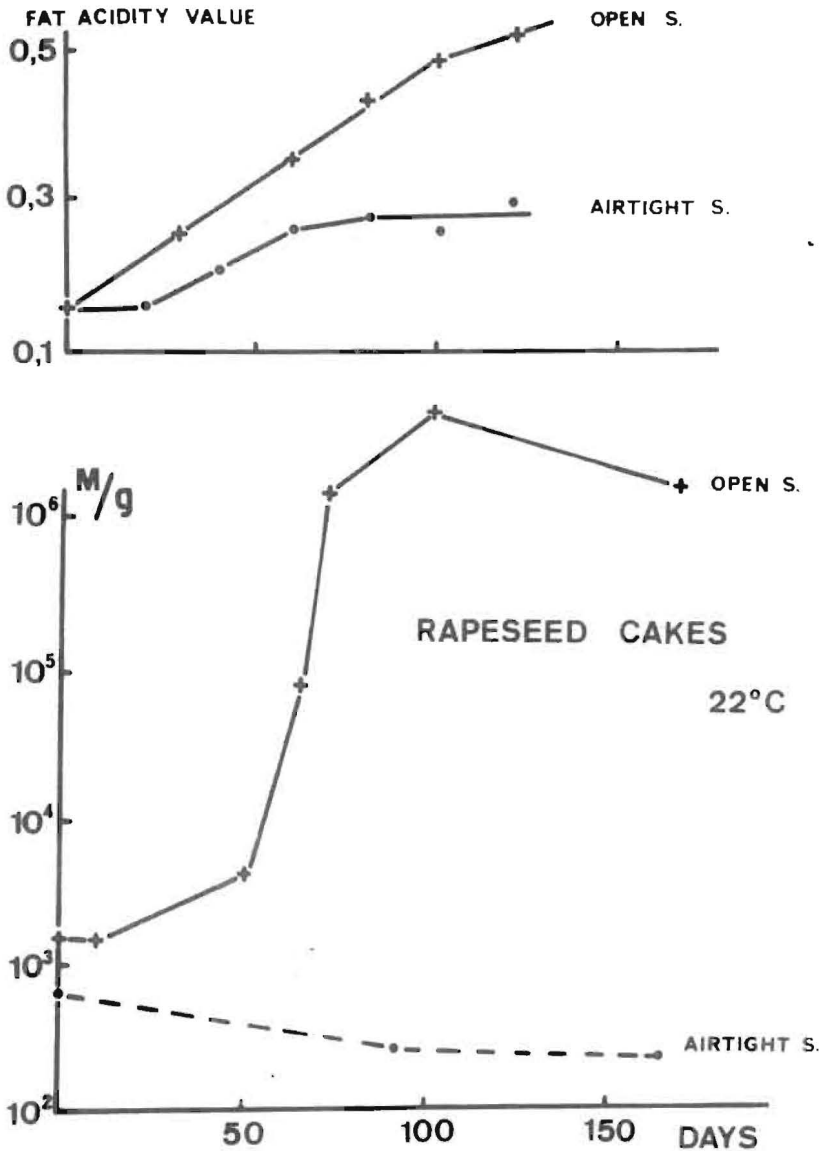


Fig. 3. Fat acidity increase and mold evolution in rapeseed-cakes stored at 15 p.100 moisture content.

There is some evidence that microorganisms, and especially molds, are mainly responsible for the acidification of the oil under aerated conditions : laboratory experiments carried on artificially decontaminated sunflower seeds showed no significant increase of acidity, even at water activities as high as 0,90 (Guenot, 1977).

Considering now broken seeds of sunflower, stored at a moisture content of 6,2 p.100, an interesting behaviour can be observed.

At this moisture content, the kernels, that is to say the seeds without protective shells, are known to be at a water activity of about 0,75 which allows only a very slow growth of storage molds *Aspergillus* and *Penicillium*, under aerated conditions. Nevertheless, after two months in open storage the fat acidity is significantly increasing probably due to molds (fig. 4) (Poisson et al., 1972). A lipasic activity of the seed itself might occur but it is very difficult to distinguish molds activity from the possible activity of the seed. In addition, auto-oxidation of fat may occur under such storage conditions.

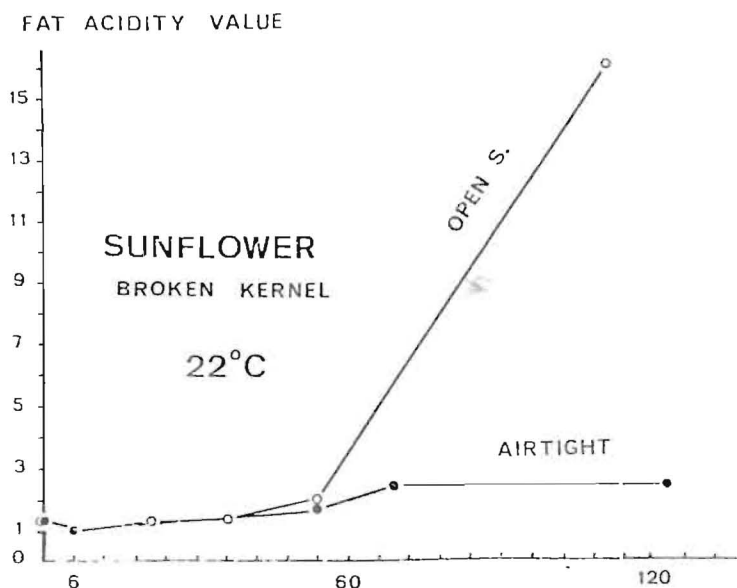


Fig. 4. Evolution of fat acidity of broken kernels under open and airtight conditions.

It is therefore very interesting to notice that in airtight conditions at the same moisture content, the degeneration of oil is strongly repressed and that no increase in fat acidity can be observed.

This protective action of airtight storage is of special interest because it is well known that, even at low moisture contents, broken oil-seeds are very susceptible to rapid storage damage, probably due to a greater diffusibility of enzymes and oil. With storage under oxygen-free atmosphere, the technological value of oil can be practically preserved during at least four months.

## 2.1. Influence of moisture content on the acidification

The acidification of stored seeds is clearly dependent on the humidity and even a slight difference in moisture content can lead to very different behaviours during airtight storage. This is shown in fig. 5 (Cahagnier et al., 1964) with rapeseeds airtight stored at 11,8 and 14,2 p.100 initial moisture content during one year. After the first six months the fat acidity value remains unchanged in both samples, but after 10 or 11 months the acidification is rapidly increasing in the wet seeds whereas it is still slight in the seeds at 11 p.100 M.C.

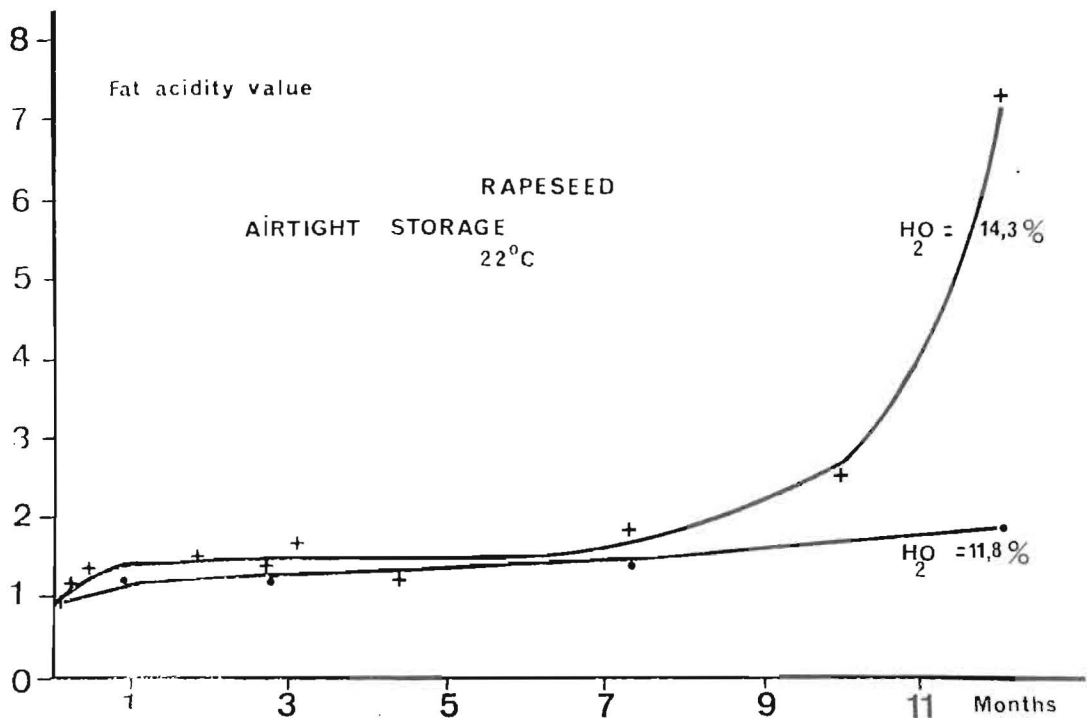


Fig. 5. Influence of moisture content on the fat acidity of rapeseeds airtight stored.

As a consequence, it can be emphasized that the relationship between the acidification rate and moisture content is not linear. These data are in accordance with others (Jouin et al., 1963-1965) obtained on rapeseeds airtight stored at moisture contents between 6,5 p.100 and 25 p.100, with a low initial acidity (0,5 - 1). Provided that the moisture content remains below 15 p.100, there is only a very slight increase of the fat acidity during the first months of storage since the initial value is increasing approximatively twofold after six months.

## 2.2. Influence of temperature on acidification

With regard to the influence of temperature on fatty acids liberation in oil-seeds during storage, it can be said that the rate of acidification is greater at higher temperature, but the fat acidity evolution is always slower in airtight conditions than in open storage. An example is given in the fig. 6 (Poisson et al., 1972) which show

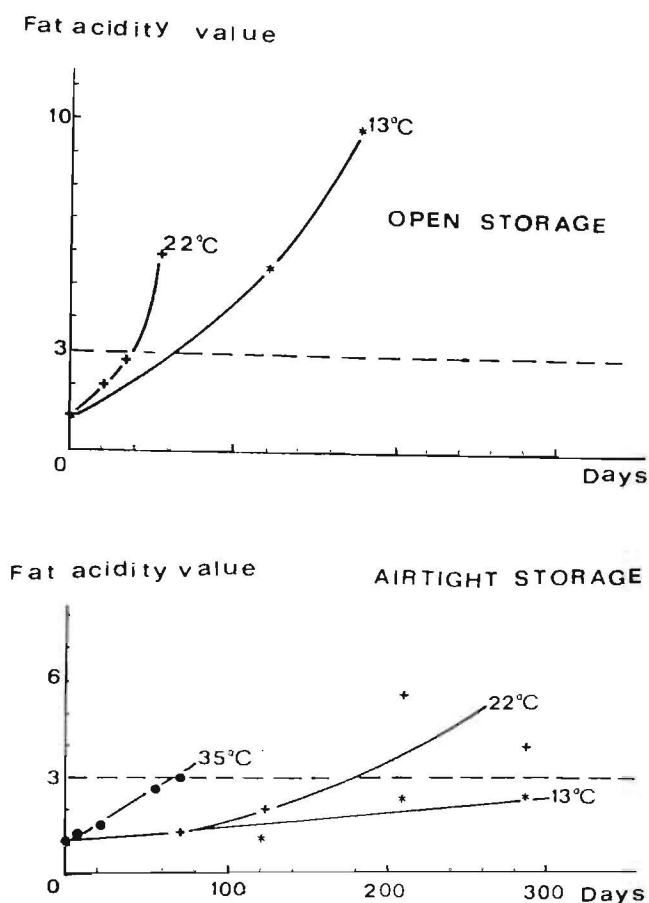


Fig. 6. Sunflower kernels. Evolution of fat acidity

the evolution observed with sunflower at 6,2 p.100 moisture content : during open storage, the acid number reach the limit value of 3 within about two months, whereas this value is not reached under airtight conditions after ten months.

### 3. Organoleptic qualities of airtight stored seeds

If airtight storage of oil-seeds is ensuring a satisfactory preservation against moulding and fat acidification during significantly longer periods of time than storage under aerated conditions, it must be pointed out that at critical high moisture content and temperature, the seeds undergo a lot of modifications and especially the formation of off-odors, and over-pressure in the containers. Such modifications may be due to some microbial fermentations but also to deviated metabolisms of the seed itself.

Fortunately off-odors in the stored seeds seem to have no repercussion on oil quality, but it may not be the same for products like oil-cakes which are used in animal feeding.

In each case of off-odors formation it has been observed that the organoleptic modifications occur a long time before the beginning of fat acidity increase ; so off-odors detection could be a criterion much more sensitive than acidification for storage damages in airtight conditions of the seeds.

### 4. Preservation diagrams

Taking into account the most widely used criteria for seed damage assessing, i.e. microorganisms enumeration, fat acidity and germinative capacity for storage in air and fat acidity and off-odors formation for airtight storage, it has been possible to draw up comparative diagrams for rapeseed and sunflower seed preservation (Masson, 1969). This is shown in fig. 7 on which the upper curves represent the limits for airtight stored seeds and the lowers correspond to open storage. Sunflower seeds, for instance, can be securely stored in airtight conditions at 10 p.100 moisture content during more than six months whereas in open storage they will be damaged within less than six weeks.



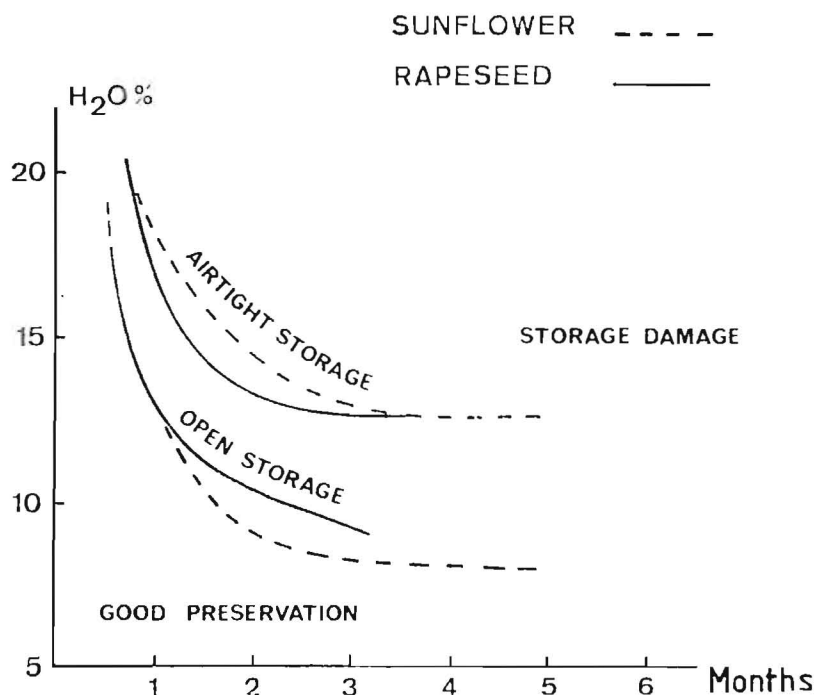


Fig. 7. Preservation diagram for sunflower and rapeseed (fat acidity value below 1,5).

## CONCLUSION

As previously indicated, whatever the moisture content of seeds could be, the period of preservation is always longer under airtight condition than in open storage. The higher the moisture content is, the smaller is the difference but for rapeseeds as well as for sunflower seeds with moisture content from 7-8 to 13-15 p.100 M.C., airtight storage is of real practical interest especially if the seeds are split or broken.

With high moisture seeds, airtight storage leads to off-odors formation due to microbial fermentations. Such organoleptic modifications might be objectionable for oil-cakes used in animal feeding and further investigations are needed in this direction.

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## VI S E S S I O N :

FACILITIES FOR ARTIFICIAL CONTROLLED ATMOSPHERE  
STORAGE AND ECONOMIC ASPECTS

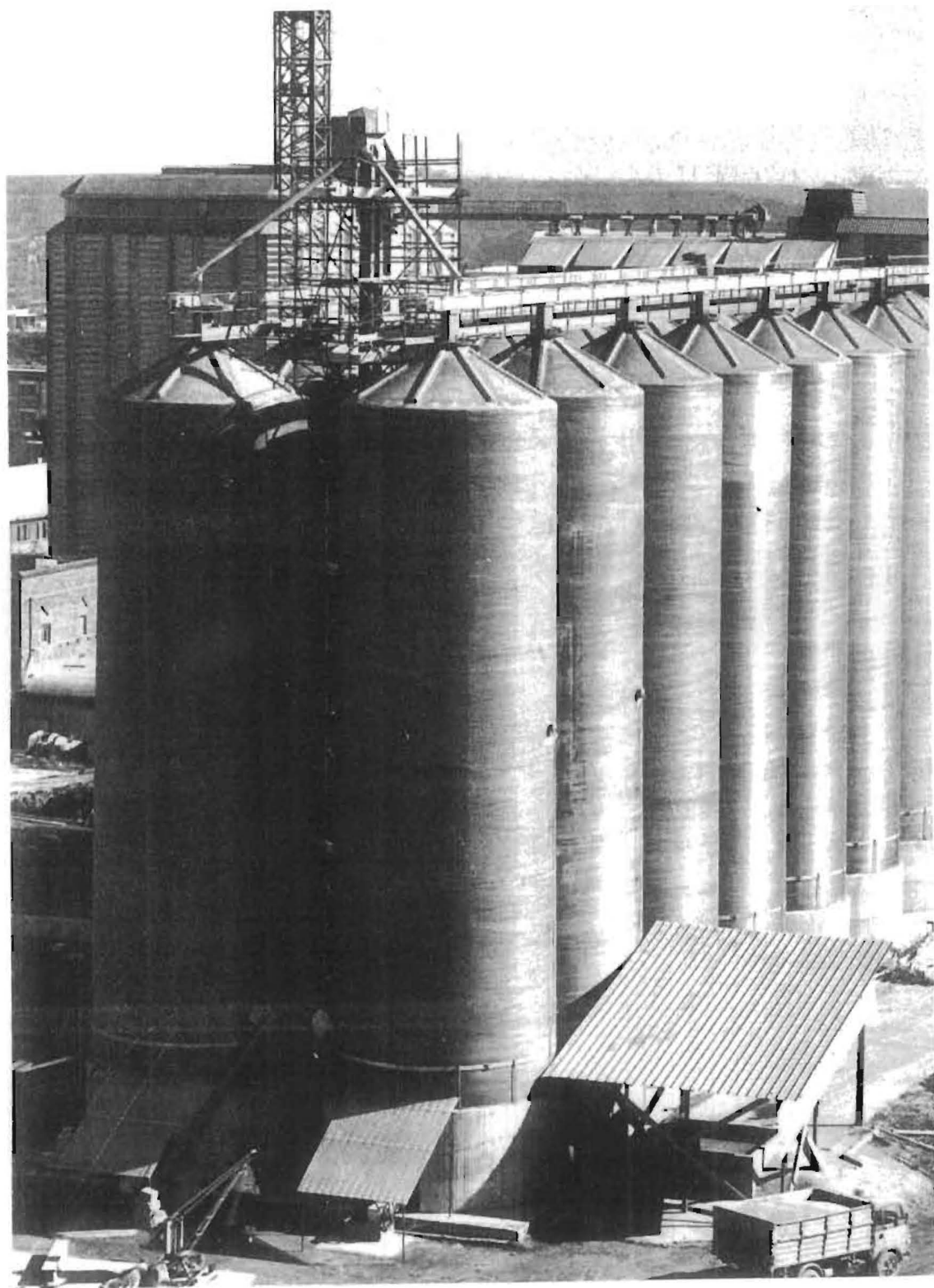


Photo Agenzia Italia, Roma

# SIMULATION MODEL OF TWO-DIMENSIONAL HEAT TRANSFER IN CONTROLLED-ATMOSPHERE GRAIN BINS

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## ABSTRACT

A finite-difference model was developed to simulate conductive heat transfer in both the radial and vertical directions in free-standing, controlled-atmosphere cylindrical bins of stored grain. Temperatures throughout a grain bulk can be predicted during the storage period using input data of initial grain temperatures, mean daily temperatures, solar radiation, average monthly wind velocities and thermal properties of the grain, bin structure and soil. Heat generation in the grain bulk was assumed negligible but it could be readily incorporated into the model.

To verify the model, predicted temperatures were compared with measured temperatures in 46 t of rapeseed stored for 41 months and 52 t of barley stored for 23 months. The standard errors of estimate for the temperatures at the centres of the bins were 1.3°C for rapeseed and 1.8°C for barley. Inclusion of a submodel of natural convection currents in the grain bulk did not result in more accurate predictions of temperature and computer time was increased by a factor of about 25 over that for two-dimensional conduction alone.

Temperatures of grain in controlled-atmosphere bins predicted using both one- and two-dimensional heat transfer models were compared. The differences between the centre temperatures predicted by the two models were less than 0.2°C for diameter-to-height ratios of 0.5 or less while the differences increased to over 2.0°C for diameter-to-height ratios of greater than 1.2.



Following the finite difference method of Yaciuk et al. (1975), the heat balance for any interior spatial element,  $m, n$ , is (symbols are defined in Key to Symbols):

$$\begin{aligned}
 & k_{m,n+} \left[ \left( n \Delta r + \frac{\Delta r}{2} \right) \Delta \theta \Delta z \right] \left[ \frac{T_{m,n+1} - T_{m,n}}{\Delta r} \right] \Delta t + k_{m,n-} \left[ \left( n \Delta r - \frac{\Delta r}{2} \right) \Delta \theta \Delta z \right] \left[ \frac{T_{m,n-1} - T_{m,n}}{\Delta r} \right] \Delta t \\
 & + k_{m+,n} \left[ n (\Delta r)^2 \Delta \theta \right] \left[ \frac{T_{m+1,n} - T_{m,n}}{\Delta z} \right] \Delta t + k_{m-,n} \left[ n (\Delta r)^2 \Delta \theta \right] \left[ \frac{T_{m-1,n} - T_{m,n}}{\Delta z} \right] \Delta t \\
 & = n (\Delta r)^2 \Delta \theta \Delta z c_{m,n} \rho_{m,n} \left( T'_{m,n} - T_{m,n} \right) \quad (1)
 \end{aligned}$$

If dimensionless moduli  $U_{m,n-}$ ,  $U_{m+,n}$  and  $U_{m-,n}$  are defined in equations similar to that for  $U_{m,n+}$ :

$$U_{m,n+} = \frac{c_{m,n} \rho_{m,n} (\Delta r)^2}{k_{m,n+} \Delta t} \quad (2)$$

$$\text{and } E = \frac{(\Delta r)^2}{(\Delta z)^2} \quad (3)$$

then the predicted temperature at the end of the time increment  $t + \Delta t$  is:

$$\begin{aligned}
 T'_{m,n} = & \left[ \frac{2n+1}{2n U_{m,n+}} \right] T_{m,n+1} + \left[ \frac{2n-1}{2n U_{m,n-}} \right] T_{m,n-1} + \left[ \frac{E}{U_{m+,n}} \right] T_{m+1,n} + \left[ \frac{E}{U_{m-,n}} \right] T_{m-1,n} \\
 & + \left[ 1 - \frac{2n+1}{2n U_{m,n+}} - \frac{2n-1}{2n U_{m,n-}} - \frac{E}{U_{m+,n}} - \frac{E}{U_{m-,n}} \right] T_{m,n} \quad (4)
 \end{aligned}$$

If the five spatial elements,  $m, n-1$ ;  $m, n+1$ ;  $m, n$ ;  $m-1, n$ ; and  $m+1, n$  have equal thermal properties, Eq. (4) reduces to:

$$T'_{m,n} = \left[ \frac{2n+1}{2n U} \right] T_{m,n+1} + \left[ \frac{2n-1}{2n U} \right] T_{m,n-1} + \frac{E}{U} [T_{m+1,n} + T_{m-1,n}] + \left[ 1 - \frac{2(E+1)}{U} \right] T_{m,n} \quad (5)$$

$$\text{where } U = \frac{c \rho (\Delta r)^2}{k \Delta t} \quad (6)$$

The equation for the predicted temperature of a centre element;  $m, 0$ ; with no heat flow across the centre axis can be developed using a procedure similar to that used above and to that used by Yaciuk et al. (1975):

$$T'_{m,0} = \frac{4}{U_{m,0+}} T_{m,1} + \frac{E}{U_{m+,0}} T_{m+1,0} + \frac{E}{U_{m-,0}} T_{m-1,0} + \left[ 1 - \frac{4}{U_{m,0+}} - \frac{E}{U_{m+,0}} - \frac{E}{U_{m-,0}} \right] T_{m,0} \quad (7)$$

For a bin with constant thermal properties:

$$T'_{m,0} = \frac{4}{U} T_{m,1} + \frac{E}{U} [T_{m+1,0} + T_{m-1,0}] + \left[1 - \frac{2(E+2)}{U}\right] T_{m,0} \quad (8)$$

## 2.2 Heat transfer at top surface of bulk

For a spatial element at the top of the grain,  $M,n$ , the heat balance equation is:

$$\begin{aligned} k_{M,n+} \left[ \left( n\Delta r + \frac{\Delta r}{2} \right) \Delta \theta \frac{\Delta z}{2} \right] \left[ \frac{T_{M,n+1} - T_{M,n}}{\Delta r} \right] \Delta t + k_{m,n-} \left[ \left( n\Delta r - \frac{\Delta r}{2} \right) \Delta \theta \frac{\Delta z}{2} \right] \left[ \frac{T_{M,n-1} - T_{M,n}}{\Delta r} \right] \Delta t \\ + \bar{h}_b \left[ n(\Delta r)^2 \Delta \theta \right] \left[ T_b - T_{M,n} \right] \Delta t + k_{M-,n} \left[ n(\Delta r)^2 \Delta \theta \right] \left[ \frac{T_{M-1,n} - T_{M,n}}{\Delta z} \right] \Delta t \\ = \frac{n(\Delta r)^2 \Delta \theta \Delta z}{2} c_{M,n} \rho_{M,n} (T'_{M,n} - T_{M,n}) \end{aligned} \quad (9)$$

With the dimensionless Biot number defined as:

$$B_T = \frac{\bar{h}_b \Delta r}{k} \quad (10)$$

the predicted temperature of the element, for a bin of constant thermal properties is:

$$\begin{aligned} T'_{M,n} = \left[ \frac{2B_T \Delta r}{U \Delta z} \right] T_b + \left[ \frac{2n+1}{2nU} \right] T_{M,n+1} + \left[ \frac{2n-1}{2nU} \right] T_{M,n-1} + \frac{2E}{U} T_{M-1,n} \\ + \left[ 1 - \frac{2(E+1)}{U} - \frac{2B_T \Delta r}{U \Delta z} \right] T_{M,n} \end{aligned} \quad (11)$$

## 2.3 Heat transfer at bottom surface of bulk

For the bottom layer of grain, soil temperature must be used for  $T_{-1,n}$  in Eq. (1), along with appropriate thermal properties for grain, concrete and soil. The method of calculating mean thermal properties for any particular element is given by Yaciuk et al. (1975). Soil temperatures were predicted by a model developed by Singh and Schulte (1977) from equations presented by Merva (1975). The model was modified for the soil temperatures under a bin by assuming that the soil temperature profile in the horizontal direction under a bin approximates the soil temperature profile in the vertical direction.

## 2.4 Heat transfer at wall surface of bulk

By solving the heat balance equation the predicted temperature of an exterior surface element at the wall is:



$$T'_{m,N} = \left[ \frac{8NB_W}{(4N-1)U_{m,N-}} \right] T_a + \left[ \frac{8N-4}{(4N-1)U_{m,N-}} \right] T_{m,N-1} + \frac{E}{U_{m+,N}} T_{m+1,N} + \frac{E}{U_{m-,N}} T_{m-1,N} \\ + \left[ \frac{8N \Delta t}{(4N-1)\Delta r c_{m,N} \rho_{m,N}} \right] q_r + \left[ 1 - \frac{8NB_W + 8N-4}{(4N-1)U_{m,N-}} - \frac{E}{U_{m+,N}} - \frac{E}{U_{m-,N}} \right] T_{m,N} \quad (12)$$

where the dimensionless Biot number for the exterior wall surface is:

$$B_W = \frac{\bar{h}_c \Delta r}{k_{m,N-}} \quad (13)$$

The convective heat transfer coefficient,  $\bar{h}_c$ , for the exterior wall surface was calculated by the method presented by Yaciuk et al. (1975).

For a bin with constant thermal properties, Eq. (12) becomes:

$$T'_{m,N} = \left[ \frac{8NB_W}{(4N-1)U} \right] T_a + \left[ \frac{8N-4}{(4N-1)U} \right] T_{m,N-1} + \frac{E}{U} [T_{m+1,N} + T_{m-1,N}] \\ + \left[ \frac{8N \Delta t}{(4N-1)\Delta r c_p} \right] q_r \left[ 1 - \frac{8NB_W + 8N-4}{(4N-2)U} - \frac{2E}{U} \right] T_{m,N} \quad (14)$$

The equations for the temperatures of the four remaining elements 0,0; M,0; M,N; and 0,N are developed according to the procedures presented above.

## 2.5 Radiant heat transfer at wall surface of bulk

In Eqs. (12) and (14), the net radiant heat flow to the wall surface,  $q_r$ , is calculated by:

$$q_r = q_e + q_s + q_f + q_d - q_o \quad (15)$$

where:

$$q_e = \sigma \alpha F_{be} T_a^4 \quad (16)$$

$$q_s = \sigma \alpha F_{bs} T_s^4 \quad (17)$$

$$q_o = \sigma \alpha T_{m,N}^4 \quad (18)$$

and the solar radiation components  $q_f$  and  $q_d$  were calculated for Winnipeg according to the procedure explained in the next paragraph. The long-wave absorptivity and emissivity of the bin wall surface were set equal. The two shape factors,  $F_{be}$  and  $F_{bs}$ , were each set equal to 0.5. The effective sky temperature,  $T_s$ , was estimated to be 210 K (Kreith 1973).

The cylindrical bin wall was divided into 20 vertical strips with an angular width of 18°. For each hour of daylight during the period July 1957 to

December 1975 for which measured values of radiation on a horizontal surface,  $H$ , at Winnipeg were available the solar radiation on each of these vertical bin wall strips,  $H_v$ , was calculated using the equation of Jones and Kemp (1977):

$$H_v = \frac{1}{4} \left( 2R_b H + 2R_b H_b + H_d + \gamma H \right) \quad (19)$$

The beam,  $H_b$ , and diffuse,  $H_d$ , components of the measured radiation were estimated using the relationships given for Winnipeg by Ruth and Chant (1976). The hourly radiation values were summed for each day and then fitted to the equation:

$$H_{vs} = a_1 H_o + \frac{a_2 H_s}{H_o} + a_3 \quad (20)$$

The extraterrestrial radiation,  $H_o$ , was calculated by the method given by Duffie and Beckman (1974). For the average total radiation striking all sides of a cylindrical bin at Winnipeg the coefficients for the equation are:  $a_1 = 0.1152$ ,  $a_2 = 15960$ , and  $a_3 = -3133$  ( $R = 0.97$ ). This daily value of  $H_{vs}$  and the short-wave (solar) absorptivity of the bin wall material, were used to calculate the instantaneous rate of direct and diffuse radiation on the bin wall element ( $q_f + q_d$ ) in Eq. (15). (For the average total radiation falling on the southern 55% of a bin the coefficients are:  $a_1 = 0.0472$ ,  $a_2 = 23170$ , and  $a_3 = -2472$  ( $R = 0.97$ )).

The above calculation of solar radiation is directly applicable to Winnipeg only. A similar calculation procedure could be used for locations where the radiation on a horizontal surface has been measured and the relationship between diffuse and beam radiation has been developed. For locations not having measured radiation data the following equation given by Duffie and Beckman (1974) can be used:

$$H_{av} = H_o \left( b_1 + b_2 \frac{S}{S} \right) \quad (21)$$

Duffie and Beckman (1974) give values for the coefficients,  $b_1$  and  $b_2$  for a number of locations and climate types around the world. For locations similar to Winnipeg the coefficients (Table I) based on measured sunshine hours and radiation at Winnipeg for 1972 to 1975 could be used.

## 2.6 Coefficients used in model

To obtain stable solutions with the finite difference model the space and time increments were chosen so that the coefficients of Eqs. (5), (8), (11), and (14) were greater than zero. Thirty-six temperature nodes, six vertically and six horizontally, were used to simulate the temperatures in a bin. A time increment of 6 h was used. The temperature at the end of each finite time increment

TABLE I

Coefficients based on measured data at Winnipeg, Canada for 1972 to 1975 that can be used to predict solar radiation on a horizontal surface

Month	$b_1$	$b_2$	R
January	0.367	0.431	0.92
February	0.384	0.451	0.92
March	0.334	0.552	0.94
April	0.250	0.578	0.94
May	0.200	0.596	0.97
June	0.198	0.575	0.96
July	0.213	0.544	0.96
August	0.201	0.548	0.95
September	0.215	0.595	0.96
October	0.231	0.522	0.95
November	0.287	0.477	0.91
December	0.350	0.419	0.88

was calculated for every spatial increment using the temperatures calculated for the end of the previous time increment.

For ambient air temperatures the daily averages of the measured maximum and minimum temperatures at the Winnipeg International Airport were used. The thermal properties of rapeseed were assumed to be: specific heat,  $1700 \text{ J kg}^{-1}\text{K}^{-1}$ ; thermal conductivity,  $0.12 \text{ W m}^{-1}\text{K}^{-1}$ ; and bulk density,  $700 \text{ kg m}^{-3}$  (Moysey et al. 1977, Timbers 1975). The thermal properties of barley were assumed to be: specific heat,  $1560 \text{ J kg}^{-1}\text{K}^{-1}$ ; thermal conductivity,  $0.15 \text{ W m}^{-1}\text{K}^{-1}$ ; and bulk density,  $670 \text{ kg m}^{-3}$  (Disney 1954).

### 3. MODEL OF CONVECTION CURRENTS IN GRAIN BULK

During periods of temperature variation throughout a grain bulk, convective air currents can develop carrying heat and moisture through the grain (Muir 1973). An attempt was made to model these convective currents as a refinement of the temperature prediction model. The driving force causing air movement was assumed to be the differences in force of gravity on equal volumes of air of different densities. The air density in each spatial element was calculated according to its predicted temperature. For simplification, the air was assumed to move vertically within the columns of spatial elements (Fig. 1) and to move horizontally only in the bottom row of spatial elements. The total pressure of the air at the bottom of each column was calculated by summing the forces of gravity on air masses in each element of the column. The net pressure acting on the bottom of any column was assumed to be equal to the difference between the pressure of the column itself and the average pressure of all other columns. The velocity of the air entering or leaving the bottom of the column was found from the relationship between pressure and velocity for the grain (ASAE 1979). The aeration

model for near-equilibrium conditions (Thompson 1972) was used to simulate heat and moisture transfer between the grain and circulating air. The air entering each element was assumed to be in equilibrium with the upstream element. Air entering the top of a column was assumed to be 5°C above the outside ambient temperature and to have a constant relative humidity of 60%.

#### 4. EXPERIMENTAL BINS

To develop and validate the model no temperature data were readily available for controlled-atmosphere bins. Therefore, temperatures measured by Sinha and Wallace (1977) in two cylindrical galvanized-steel bins with 5.56-m diameters located 30 km south of Winnipeg, Canada were used. Although these bins were not of the controlled-atmosphere type the joints in the wall sheets and between the wall and concrete floor were sealed against moisture movement. The presence of high CO<sub>2</sub> levels in the rapeseed bin (Sinha and Wallace 1977) indicates that air movement through the walls was small. It was assumed that the natural air movement through the bin structure was small enough that it had negligible effects on the grain temperatures. As is the case in controlled-atmosphere bins of dry grain it was assumed that the low level of biological activity in the bins generated a negligible amount of heat.

One bin contained 45.7 t of rapeseed to a depth of 2.7 m. The other bin contained 51.9 t of barley to a depth of 3.2 m. Temperatures were measured at the bin centre and at eight locations equidistant around three concentric circles at each of four depths in the rapeseed bin and five depths in the barley bin. Because the simulation model assumes no heat flow in a circumferential direction the eight temperatures on a concentric circle were averaged for each depth and radius combination. Because the wall temperatures were measured several centimetres from the wall these were not used. For each measurement time there were therefore 12 values for rapeseed and 15 values for barley. For the bin of rapeseed, temperature measurements for the period 13 August 1974 to 31 January 1978 (41 months) were used. For the bin of barley, temperature records for 13 August 1974 to 12 July 1976 (23 months) were used.

#### 5. RESULTS AND DISCUSSION

##### 5.1 Development of model

Several simulations were run for the rapeseed bin using different thermal properties. The best agreement between simulated and measured temperatures was obtained with a convective heat transfer coefficient at the top grain surface of  $1.0 \text{ W m}^{-2} \text{ K}^{-1}$ , a temperature for the air above the grain surface of 5°C above outside ambient temperature, and 0.28 and 0.89 for long-wave and short-wave emissivities of the galvanized steel wall. These emissivity values are the ones given by Kreith (1973) for dirty galvanized iron. The model developed using the

rapeseed bin data was then verified by predicting temperatures in the barley bin using the thermal properties of barley instead of rapeseed.

## 5.2 Predicted temperatures compared with measured temperatures

In comparing simulated and measured temperatures (Fig. 2) the standard error of estimate for the two-dimensional model was 2.0°C overall for both rapeseed and barley bins. The standard errors of estimate at the centre of the bin were 1.3°C for rapeseed and 1.8°C for barley. The maximum differences between simulated and measured results at any time during the 41 months of storage were 9.3°C at the surface, and 2.6°C at the centre of the rapeseed bin. The maximum differences were 7.0°C at 20 cm below the surface and 4.0°C at the centre of the barley bin. Increasing the number of space increments and decreasing the time interval would increase the accuracy but at the expense of increased computer time.

## 5.3 Convection model

Inclusion of a model of the convection currents in a grain bulk did not result in more accurate predictions of temperature. Apparently, the original assumption that convective heat transfer in the grain bulk is negligible is valid. The amount of moisture migration predicted by the model was greater than that measured and computer time was increased by a factor of about 25 over two-dimensional conduction alone.

## 5.4 Comparison of one- and two-dimensional models

The temperatures for the centre of grain bins predicted by one- and two-dimensional models were compared over a period of 14 to 16 mo. Grain depths were varied from 1 to 8 m in a 4-m diameter bin and 6 to 24 m in a 12-m diameter bin. For the one-dimensional model 11 temperature nodes were used. For the two-dimensional model 6 vertical and 6 horizontal temperature nodes were used. For both models the time increment was 1 h.

The differences between the centre temperatures predicted by the two models were negligible ( $\leq 0.2^\circ\text{C}$ ) for diameter-to-height ratios of 0.5 or less and larger ( $\geq 2.0^\circ\text{C}$ ) for ratios greater than 1.2 (Fig. 3). The primary advantages of the two-dimensional heat transfer simulation model over the one-dimensional model are its increased accuracy and increased number of locations at which temperatures are predicted. However, computer time for the two-dimensional model is increased in approximate proportion to the increase in number of temperature nodes. For most simulations the accuracy of the two-dimensional model is probably only required for bins having a diameter-to-height ratio of greater than 1.2. The two-dimensional model is also required for bins where a vertical temperature gradient is caused by heat generation or forced air movement through the grain bulk.

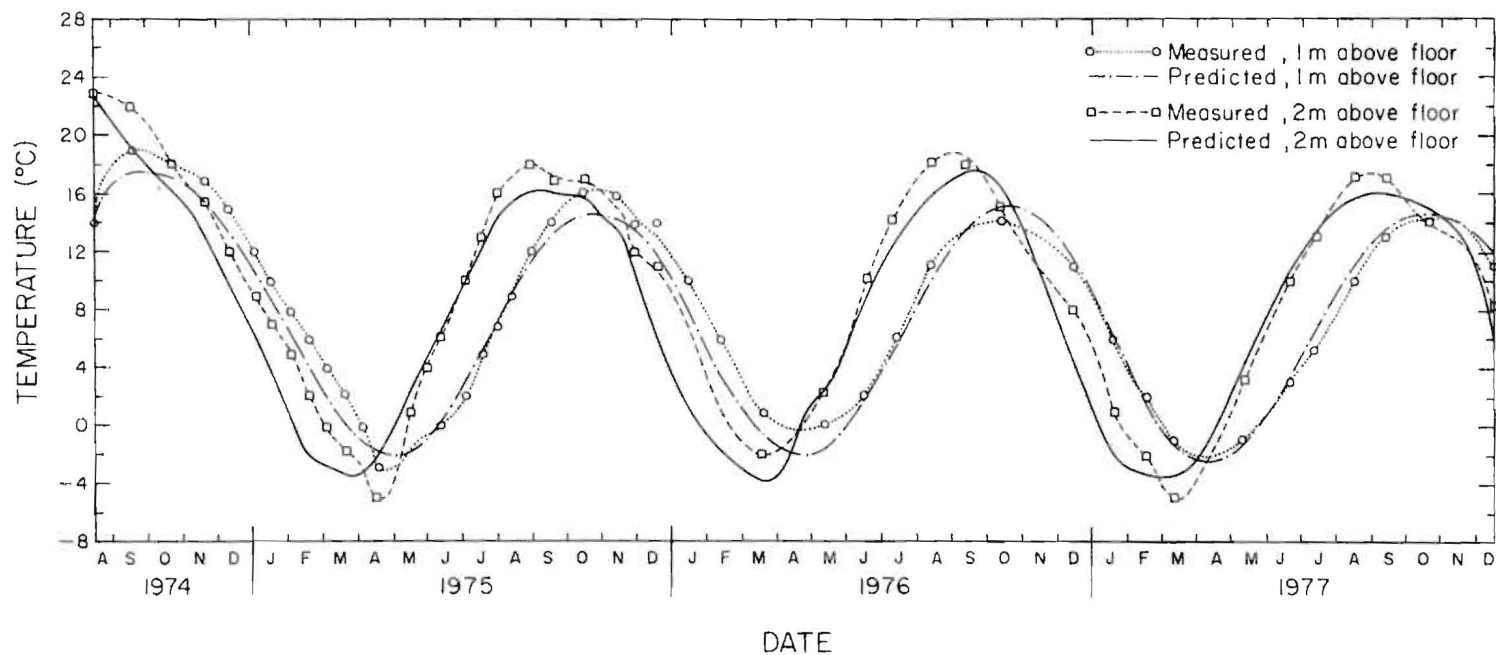


Fig. 2. Predicted and measured temperatures along the centre axis of a 5.56-m diameter cylindrical bin containing rapeseed of 2.7-m depth located 30 km south of Winnipeg, Canada.

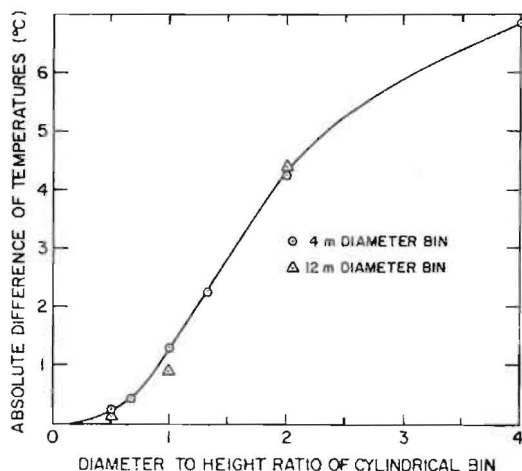


Fig. 3. Average absolute differences between temperatures at the centre of cylindrical bins predicted using one- and two-dimensional heat transfer models.

### 5.5 Ecological significance of bin temperature simulation

Relatively accurate prediction and confirmation of rapeseed and barley temperatures at different depths of commercial bins is of considerable ecological significance. In temperate climates, regulation of the numbers of insect and mite pests of stored grain is primarily dependent on temperature and secondarily on moisture content of the stored grain and oilseeds; other variables, such as food quality, species genotype and dockage also affect their multiplication (Sinha and Wallace 1973). Regulation of fungal populations, however, depends primarily on moisture content and secondarily on temperature (Wallace et al. 1976). Cereals and oilseeds stored in bulk on western Canadian farms retain their moisture content within a 1-3% range for several years. Distribution of the moisture content within this range, however, is uneven. Temperature fluctuations within the same bin occur over a larger range, sometimes as large as 40°C. Species of stored grain fauna and microflora exploit microenvironments with different combinations of temperature and moisture. Prior knowledge of such areas favorable for rapid multiplication of pest organisms should be an integral part of stored grain and oilseed protection strategy. For example, if bins have pockets with temperatures (over 30°C) favorable for development of the rusty grain beetle, *Cryptolestes ferrugineus* (Steph.), turning (Muir et al. 1977) or ventilating such grain in cold weather while the insect population is still low could prevent serious insect infestations.

#### KEY TO SYMBOLS

$a_1, a_2, a_3$  coefficients

$b_1, b_2$  coefficients in Eq. (18) for a given location

$B_T$  Biot number for top grain surface

$B_W$	Biot number for exterior wall surface
$C_{m,n}$	specific heat of element $m,n$ ; $J\ kg^{-1}K^{-1}$
$F_{be}$	radiation shape factor for bin-to-earth
$F_{bs}$	radiation shape factor for bin-to-sky
$\bar{h}_b$	convective heat transfer coefficient at top surface of bulk, $Wm^{-2}K^{-1}$
$\bar{h}_c$	convective heat transfer coefficient at exterior wall surface, $Wm^{-2}K^{-1}$
$H$	measured total radiation on a horizontal surface ( $H_b + H_d$ ), $W/m^2$
$H_{av}$	average radiation on a horizontal surface for a given period of time, $J/m^2$
$H_b$	beam radiation on a horizontal surface, $W/m^2$
$H_d$	diffuse radiation on a horizontal surface, $W/m^2$
$H_o$	extraterrestrial radiation for the given location and time period, $J/m^2$
$H_s$	daily total radiation on a horizontal surface, $J/m^2$
$H_v$	radiation on a vertical surface, $W/m^2$
$H_{vs}$	daily radiation on a vertical surface, $J/m^2$
$k_{m,n+}$	mean thermal conductivity between nodal points $m,n$ and $n,n+1$ ; $[Wm^{-1}K^{-1}]$
$k_{m,n-}$	mean thermal conductivity between $m,n$ and $m,n-1$ ; $[Wm^{-1}K^{-1}]$
$k_{m+,n}$	mean thermal conductivity between $m,n$ and $m+1,n$ ; $[Wm^{-1}K^{-1}]$
$k_{m-,n}$	mean thermal conductivity between $m,n$ and $m-1,n$ ; $[Wm^{-1}K^{-1}]$
$m$	number of spatial element in vertical direction (Fig. 1)
$M$	number of spatial element at top surface of bulk (Fig. 1)
$n$	number of spatial element in radial direction (Fig. 1)
$N$	number of spatial element at wall surface (Fig. 1)
$q_d$	direct solar radiation, $W/m^2$
$q_e$	earth-to-bin radiation, $W/m^2$
$q_f$	diffuse solar radiation, $W/m^2$
$q_r$	net radiation, $W/m^2$
$q_o$	bin-to-surroundings radiation, $W/m^2$
$q_s$	sky-to-bin radiation, $W/m^2$
$r$	radial distance, $m$
$R$	correlation coefficient
$R_b$	cosine of angle of incidence divided by cosine of zenith angle (Duffie and Beckman 1974)
$s$	observed daily hours of bright sunshine for the given period of time, $h$
$S$	calculated maximum possible hours of bright sunshine for the given period of time, $h$
$t$	time, $s$
$T_a$	temperature of ambient air surrounding bin, $K$
$T_b$	temperature of air above the grain surface, $K$
$T_{m,n}$	temperature of element $m,n$ at time $t$ , $K$
$T'_{m,n}$	temperature of element $m,n$ at time $t + \Delta t$ , $K$
$T_s$	effective temperature of the sky, $K$



U	dimensionless modulus
z	vertical distance, m
$\alpha$	long-wave absorptivity of bin wall material
$\gamma$	ground reflectance (0.7 for snow cover, 0.2 for no snow)
$\Delta$	finite increment
$\epsilon$	long-wave emissivity of bin wall material
$\theta$	included angle of bin sector, rad
$\rho_{m,n}$	density of element m,n, kg m <sup>-3</sup>
$\sigma$	Stefan-Boltzmann constant, $5.67 \times 10^{-8} \text{ W m}^{-2}\text{K}^{-4}$

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## WATER VAPOUR AND HEAT TRANSFERS IN GRAINS SILOS AND THEIR CONSEQUENCES ON STORAGE

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The preservation of grain quality during storage in aerated elevator and airtight or confined silos is chiefly depending on the temperature and the water content of the grains (Sinha, 1973). In experimental studies these parameters are generally kept constant (Pixton, Griffiths, 1971 ; Yaciuk and al., 1975). But even if this is possible in experimental conditions, it is not at all the case in practice. Very often some important gradients of temperature and water distribution occur in elevators, the consequence of which are moisture and heat transfers through bulk grains. Water content and temperature can increase in some particular points, inducing the development of molds and starting the alteration process.

In order to discuss these phenomena, it is previously necessary to describe the equilibrium state of water in grains. Then we will report the results of our studies on water and heat transfers in aerated conditions, discussing finally about some suggestions concerning airtight conditions.

### 1. THE EQUILIBRIUM STATE OF WATER IN GRAINS

#### 1.1. Water adsorption in grains

Due to their high polarity, water molecules can easily form hydrogen bonds with polar groups of macromolecules like starch and proteins. The amount of water adsorbed is a function of the number and accessibility of these polar sites (Guilbot and al., 1961, 1979 ; Eagland, 1975).

When a thermodynamical equilibrium is reached it is common to define in the product the "Activity of Water" ( $A_w$ ). This concept is closely correlated to the chemical potential of water (Morin, 1979), but it is easy to demonstrate that  $A_w$  is, in practice, with a very small error, equal to the relative humidity (R.H.) of intergranular air.

The affinity between cereal products and water (sometime called hygroscopicity) could be macroscopically depicted by sorption-desorption isotherms. Typical curves are shown in Figure 1, in which the  $A_w$  of the product (or the R.H. of air in equilibrium) is shown on the X-axis and the moisture content of the product is plotted on the Y-axis.

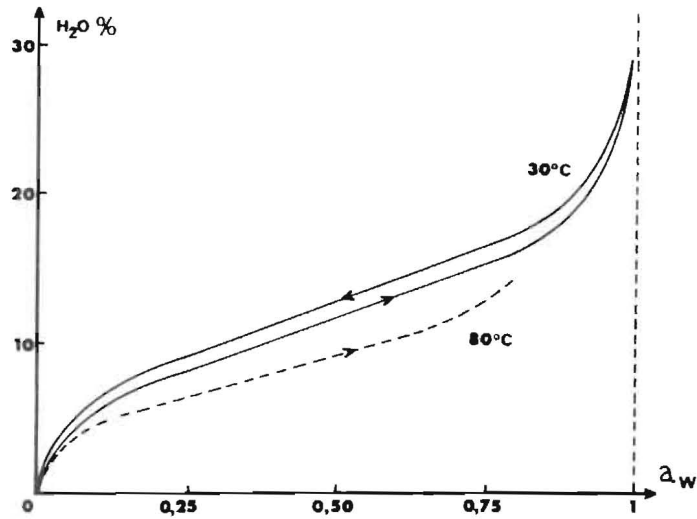


Fig. 1. Sorption (S) and desorption (D) isotherms curves of maize, for different temperatures.

One must emphasize that every point on these curves corresponds to a thermodynamical equilibrium state between product and surrounding atmosphere.

Sorption curves, the shape of which is generally sigmoidal for biological products, is mainly characterized by two properties :

- the desorption curve (obtained by a dehydration process of wet product) is higher than adsorption curve (humidification process of dry product) ; this fact generally called hysteresis, has no clear explanation ;
- the curve, often called "isotherm", is depending of temperature : higher the temperature, lower is the moisture content for the same  $A_w$ .

Adsorption and desorption are respectively exo- and endothermic processes (corresponding to the energy of formation and rupture of hydrogen bonds).

## 1.2. Functionnal availability of water, and its role in storage technology

Very interesting for storage studies is the interpretation of sorption curves in terms of mobility and functionnal availability of water. The concept of "solvent water", has been established by Guilbot and Lindenberg (1960) and more recently by Duprat (1975). These authors have demonstrated that water fraction comprised between the sorption curve and its extrapolated linear part, shows marked solvent properties ; this water has a sufficient mobility to play the role of a diffusion medium for biochemical reactions. The fraction of water

below the linear part, strongly bound, is un-available, and has no solvent properties at all.

It has been shown that many reactions require a minimum quantity of "solvent water" to start and their speeds increase as a function of the amount of available "solvent water", e.g., enzymatic reactions (Loncin and al., 1968 ; Drapron, 1972), denaturation of proteins (Multon and Guilbot, 1975), carbohydrate gelification (Doublier and al., 1975), growth of microorganisms (Scott, 1957). Figure 2 shows the  $A_w$  threshold of the main types of alterations causes occurring in cereals.

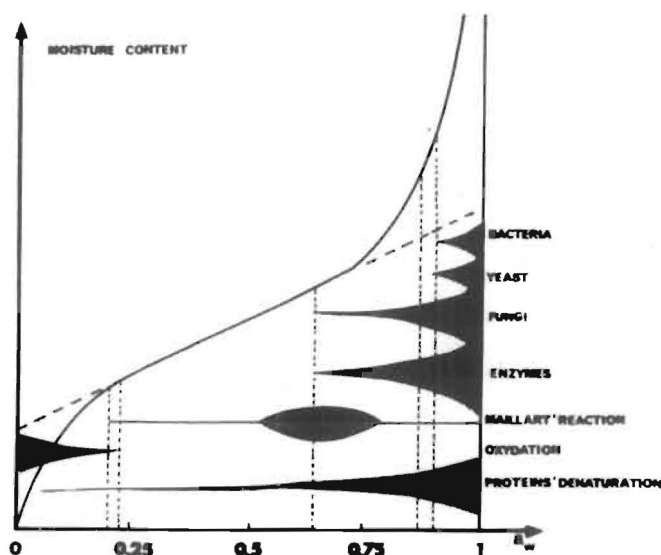


Fig. 2.  $A_w$  threshold above or below which the biochemical reactions of alterations may occur in natural products.

Of course all the parameters characterizing the surrounding medium interact with  $A_w$  and it is advised to express faint differences in the principles shown on fig. 2 with respect to time, temperature and oxygen composition of atmosphere. For example, even if the grain is very wet, in lack of oxygen, fungi cannot grow, enzymatic oxydations (and particularly grains and bacterial respiration) cannot occur. This is the reason of the success of airtight storage.

On the other hand a lot of physical properties of grains and their components are also functions of the quantity of available water, and of  $A_w$ . Particularly important are : rheological behavior of wheat grains ; thermal characteristics, heat capacity and heat transfer coefficient, heat of hydration.

After this short recall on state of water in equilibrium with grains, let us now discuss on the consequences of non equilibrium conditions.

## 2. WATER AND HEAT TRANSFER IN GRAINS

Two different non equilibrium conditions can occur separately or simultaneously in bulk grains : water activity gradient, and or temperature gradient. The resulting phenomena - water and heat transfer - are very complicated and associate all together convection and conduction, desorption and condensation with corresponding heat exchanges.

As it has been recently shown by Troude (1979) in a general review, most authors have tried to modellize these phenomenon, and to describe them with appropriate equations. But these mathematical representations are unable to explain the mechanisms themselves. Following a different way, we have studied separately water and heat transfer in bulk grains, trying to explain them with regard to sorption properties.

### 2.1. Water activity gradients between maize grains

Generally speaking when a lot of grains is homogeneous and stabilized, the distribution of the moisture content of individual kernels is gaussian, with a variation coefficient comprised between 2 and 5 % ; the mean value of the distribution is equal to the moisture content measured by ordinary oven methods (Beullier and Multon, 1976).

If two parcels of grains, the moisture contents of which are different, are mixed at the same temperature, there is between the grains a "water activity" gradient, associated to a difference of water vapour pressure in atmosphere in close contact with the grains ; it induces a water molecules transfer which go on, until "Activity of Water" would be the same in all grains (at that time, the moisture content of grains will be the same, in the limit of hysteresis effect).

We have studied these transfers in a confined and airtight atmosphere by measuring the change, as a function of time, of the distribution of moisture content among individual kernels (Beullier and Multon, 1976).

The figure 3 shows that in case of wet and dry grains just mixed (time zero), the two populations are well separated on the frequency histogram ; after 1 to 3 days, the distribution is yet bimodal, and after 5 to 6 days, there is only one homogeneous gaussian population, where it is not possible to distinguish the original population from the mixture.

Therefore, 5 or 6 days are necessary to equilibrate the  $A_w$  between grains and a mechanical shaking of the grains does not quicken the process.

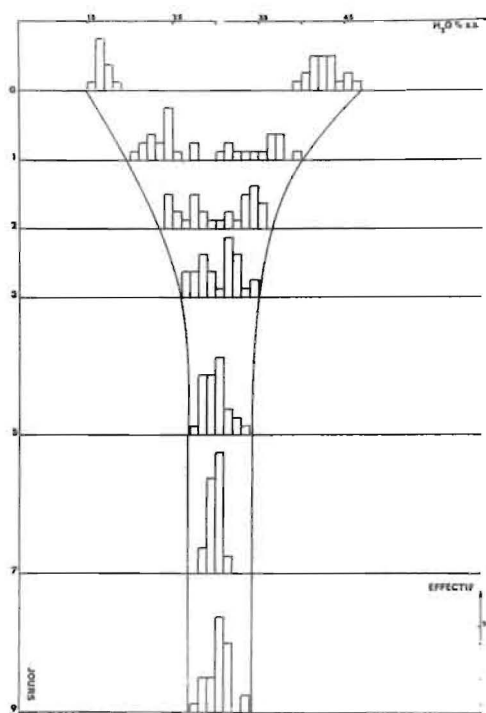


Fig. 3. Change, as a function of time, of frequency distribution of moisture content of individual kernels in a mixture of wet and dry grains.

Similarly, in case of addition of liquid water into bulk grains, a minimum of 5 days is required in order to homogenize the sample.

Thus transfers of water vapour in grains, without temperature gradients, are very slow; when equilibrium is reached, a small dispersion of moisture content remains always and indefinitely between grains, due to hysteresis effect.

## 2.2. Temperature gradient

More often, cereals in bulk are subjected to a temperature gradient, for example :

- one part of a silo is heated by sun, the other part is in the shade ;
- in hot countries, at night, the grain in peripheric part of the silo is cooler than grain in the middle of the cell ; it is the opposite during the day ;
- in a boat, grains in contact with the side and bottom of the hull are cooler than in the center.

In bulk grains, a gradient of temperature generates several phenomena :

- a heat transfer starts from hotter part towards cooler part

- a water transfer (mass transfer) takes place from hotter grains towards cooler grains, according to sorption equilibrium curves.

Therefore in certain places, temperature and or moisture content of grain can increase. If the availability of water becomes sufficient, bacteria and molds can multiply : their respiration (or fermentation at a lesser degree) produces water and heat, increasing again the availability of water. With such exploding process, alteration of grains can develop very quickly.

In order to have a better view of these phenomena, we have set up a pilot elevator in which a constant heat gradient was created; heat and water transfers were followed with appropriate sensors, as a function of time, during 80 days (Multon and al., 1980).

The cylindric metal elevator under a non heated shed, diameter 3.28 m, height 5 m, and capacity 35 metric tons, was equipped with 48 thermocouples and 6 hygrometric probes, connected with recorders. The elevator was filled with maize.

A spherical electric radiator, 22.25 cm diameter, located at the geometric center of the elevator was maintained at 60°C to simulate the effects of a localized heating in the grain. The gradient temperature was about 48°C between the surface of the radiator (60°C) and the walls of the elevator (12°C).

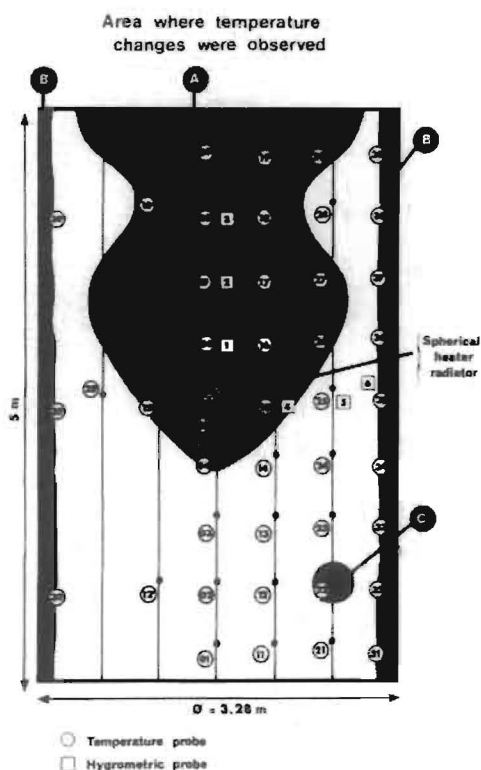


Fig. 4. Zones of thermal changes in artificial heating with radiator.



Changes of intergranular air temperature and relative humidity are very slow and were observed only in a zone (fig. 4) located above the radiator, never below.

This zone showed a curious shape of an inverted amphora, the bottom of which is the radiator ; the top is the upper board of the elevator.

The grain layers within a few centimeters of the inner surfaces (zone B on fig.4) reflected the variations of outside temperature. But, in sectors other than A, B and a very limited adventitious point C, no variation in the grain temperature was noted.

At first temperature changes take place only above the radiator. Therefore, the heat transfer is induced, not by conduction or radiation, but by natural convection of intergranular air.

Considering the temperature sensors located vertically with regard to the radiator, we observe on fig. 5 that the increase of temperature starts much earlier near the radiator, but unexpectedly, nearer is the radiator and lower are the rate of increase and the maximum temperature reached (40°C for the nearest sensor, 53°C for the farthest one).

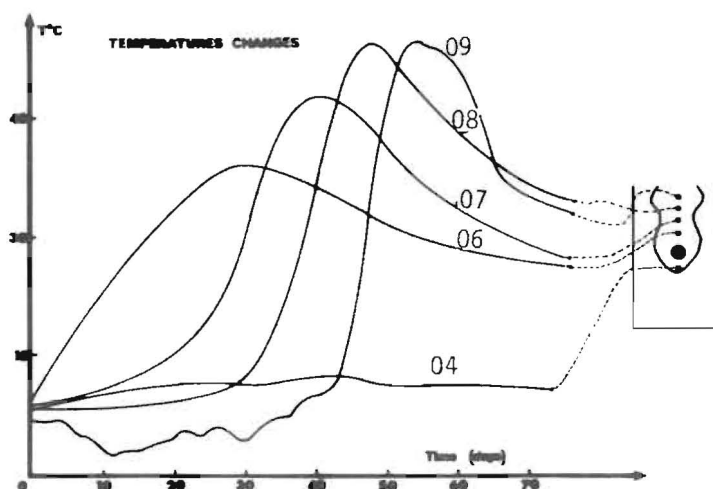


Fig. 5. Temperature changes as a function of time in artificial heating trial. (The place of sensor in the elevator, corresponding to each curve, is shown at right).

The connected changes in air relative humidity are shown on fig. 6. The three probes located horizontally show no change, except at the point in contact with the wall, which reflected the variations of the outside temperature.

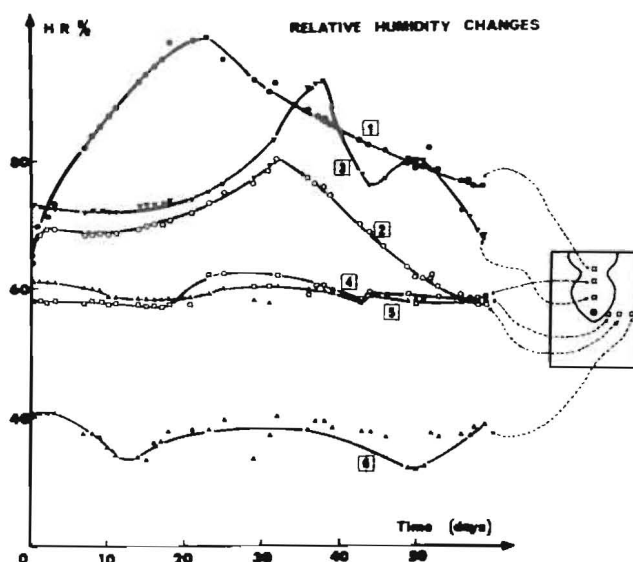


Fig. 6. Intergranular relative humidity changes as a function of time in artificial heating trial. (The place of sensor in the elevator, corresponding to each curve, is shown at right).

Comparison between the simultaneous changes of temperature and R.H. shows that the arrival of the wave front of water vapour (R.H. increase) occurs about 10 days before the arrival of temperature front.

Samples taken during unloading of the elevator showed an increase of the moisture content of grains on the upper part, and indicated a high fungi growth, chiefly *A. fumigatus*, *flavus*, and *Mucors*.

In order to explain these complicated phenomena, both physical and biological, we may suggest the following hypothesis :

- When the radiator started to heat, the temperature of surrounding grains increases, inducing a water vapour desorption from grains ; then a convective phenomena takes place, the wet and hot air going up through kernels.
- Higher, in contact with colder layers of grains, the temperature of air decreases and its R.H. increases, as shown by probes (fig. 6) : then these grains adsorb water vapour and their moisture contents increase.
- Consecutively and gradually, the slight amount of heat liberated by adsorption and the flow of heat coming from the radiator increase the intergranular air temperature, as shown by temperature probes (fig.5) ;  $A_w$  in grains is increased.
- Wet and warm conditions being thus created, respiration of grains and micro-organisms increases quickly, producing a high peak of temperature.
- The elevation of temperature induces a desorption, and again water vapour and heat go up ; vital phenomena are stopped ; temperature and R.H. decrease : the temperature tends to stabilize near  $35^{\circ}\text{C}$ , probably as a result of equilibrium

between heat flow coming from radiator and heat loss due to the convection.

These phenomenon, described on a point, spreads step by step, with an increasing intensity, from the radiator up to the top of the silo, where water vapour gathers : then moisture content of upper layers of grains increases (15 to 17 %).

Moisture increase and oxygenation near to the surface explain the development of molds, causing a great damage into grains.

Some other experiments were made with a "pocket" of very wet grains, instead of radiator, in order to simulate a natural heating. The behavior was quite different, even if there was also a convective migration toward the top of elevator.

### CONCLUSION

Water and heat transfers described in aerated conditions are, of course, much more complicated when there are simultaneous gradients of water activity and temperature. In airtight conditions of storage, phenomenon are certainly quite different.

Concerning water vapour transfer, anaerobic storage changes probably nothing : according to Dalton's law, in a mixture of gas, each of them acts like if he was alone ; under vacuum, diffusion of water vapour would be accelerated. Concerning heat transfer, on the opposite, one can think that convection flow and heat exchanges could be decreased by vacuum.

After these first experimental approaches, a lot of work remains to do, concerning horizontal gradients, alternative gradients and airtight conditions. We are right now starting a new experiment with a more sophisticated device, with which we hope to improve our knowledge of these transfers.

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## PROBLEMS AND THEIR SOLUTIONS FOUND IN THE CONSTRUCTION OF CONCRETE BINS FOR HERMETIC STORAGE.

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### ABSTRACT

Concrete double-wall bins were designed and constructed to study the hermetic storage of grain. These bins have a sandwich of bitumin and PVC sheeting between the walls and have two openings fitted with rubber gaskets for filling and discharge of the grain. When construction was completed, the bins were tested for leaks by purging them with carbon dioxide ( $\text{CO}_2$ ). Heavy initial losses of this gas were observed and attempts were made to further seal the bins. The final approach was to reseal the cracks in the bins and then to paint the inside with two coats of CIBA-GEIGY LTD.'s Araldite epoxy resin. A layer of glass wool cloth was placed between these two coats of epoxy. The opening covers were also caulked with a 1:1 mixture of Araldite and zinc oxide after they were secured in place. These modifications stopped the high  $\text{CO}_2$  losses and resulted in the bins being satisfactory for hermetic storage.

### INTRODUCTION

The majority of grain is currently stored in Egypt in open air shounas which subjects it to attack by various biological factors. Rainfall and infiltration of soil water also can damage the grain. Total annual losses in storage can amount to 24 million Egyptian pounds.

Tremendous damage and resultant losses also occur when jute bags used for grain storage are exposed for a long time to the sun, especially during the summer. Rodents and birds also severely damage the jute bags. A lot of grain is scattered in situ and during transportation due to these damaged bags.

During storage grain has to be treated with protectants to keep the infestation away for short periods. When the insect infestation starts, fumigation of infested stocks is essential. The chemical and mechanical means for rodent control is also essential whenever populations become apparent. The use of chemicals for grain intended for human and animal consumption is not desirable for the protection of such materials. Despite the fact that all chemicals are used within tolerances, treated grains are generally considered contaminated and toxic effects may occur at a later time.

Hermetic storage has several advantages when compared with other systems of storage. The most important advantages is its efficiency in pest control without the use of toxic chemicals. A joint project between the U.S. Department of Agriculture, SEA, AR, Savannah, Georgia, U.S.A, and Cairo, Egypt was financed under PL 480 in 1975 to study the hermetic storage of grain under Egyptian conditions. This project was aimed at:

1. Protection of stored grain with the least possible use of toxic chemicals.
2. Avoidance of rodent and bird attack.
3. Saving the majority of jute bags.

The experimental bins were constructed according to specifications and were tested for gas-tightness by purging them with  $\text{CO}_2$ . A considerable amount of this  $\text{CO}_2$  escaped in a very short time. Therefore, this paper described the detection of the leaks and the modifications made to try to overcome leaks.

#### SPECIFICATION OF THE EXPERIMENTAL BINS

The experimental bins were constructed according to the following specifications:

1. Cylindrical in shape, with a top cone about 1/2 meter high from the top end to the cylinder.
2. Double walls, the inner of concrete and the outer of bricks.
3. A thick layer of polyvinyl chloride sheets were fixed on the outer surface of the inner wall with bitumin (sandwich).
4. Inner walls were smoothed by a concrete-mortar mix.
5. All aperture covers were strengthened to prevent twisting due to weather conditions.
6. Strong and flexible rubber gaskets were fixed firmly around the apertures.

These specifications are shown in figures 1 to 5.

#### TESTS CONDUCTED TO EVALUATE THE HERMETIC SEALING OF BINS

After the bins had been accepted from the contractor, several trials were conducted to insure that the bins could be hermetically sealed and fit for the purpose of this study. The experimental bins were filled with wheat. They were purged with  $\text{CO}_2$  through the lower sampling aperture by using copper tubing connected to a  $\text{CO}_2$  cylinder. All other apertures were tightly closed except the release valve at the top. Each experimental bin was purged with a certain amount of  $\text{CO}_2$  by weight, and both openings were then closed. After each test, the wheat was discharged from the bin and bins were refilled with wheat before the next test. Samples of air inside the bins were drawn for  $\text{CO}_2$  concentration measurement from two points, i.e. from near the bottom of the bin and from near the upper surface of wheat, through sampling tubes especially fixed for this purpose. Trials were conducted as follows:

##### Trial n° 1

Bin numbers 3, 4 and 5 which had PVC liners and bin number 1 (control, no PVC liner) were used for the first trial and were completely filled with wheat. After purging with about 5 kgs of  $\text{CO}_2$ , both openings were tightly closed and the  $\text{CO}_2$  concentrations were estimated after different intervals. The results are illustrated in Table 1. Data in this table clearly indicate that the  $\text{CO}_2$  escaped from these

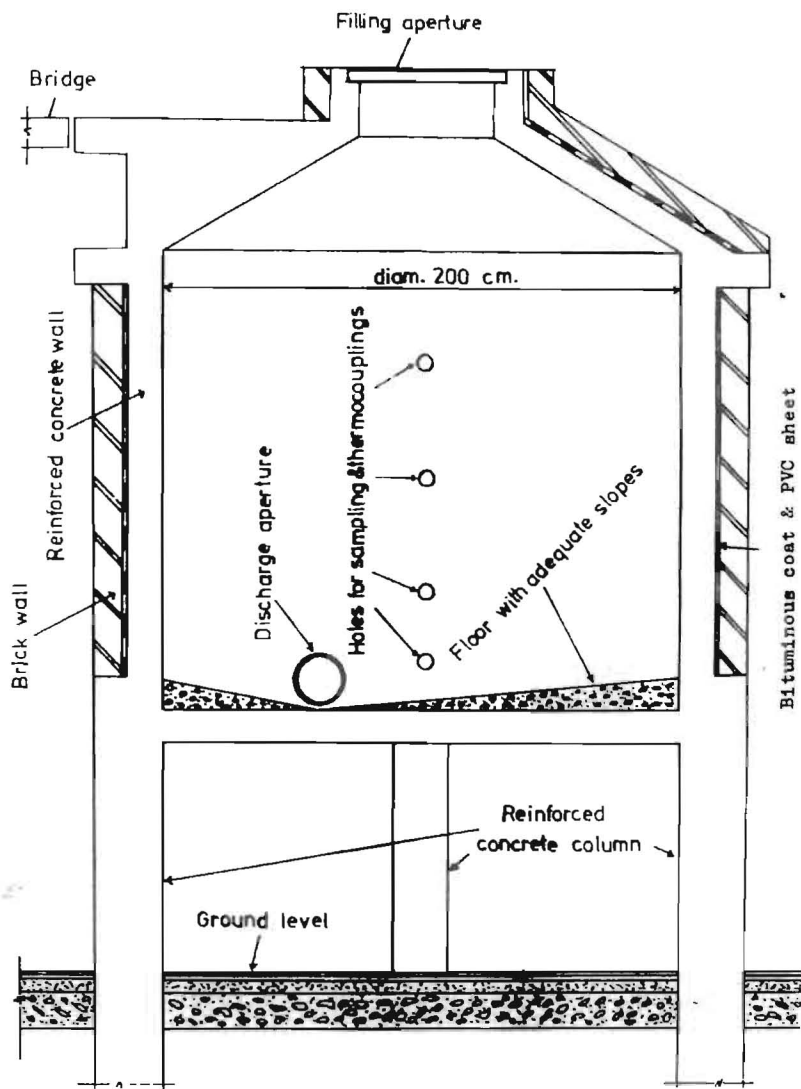


Fig.1. A detailed figure of an experimental bin showing the double wall and the PVC sheeting in between; also, the filling and the discharging apertures and the sampling holes.



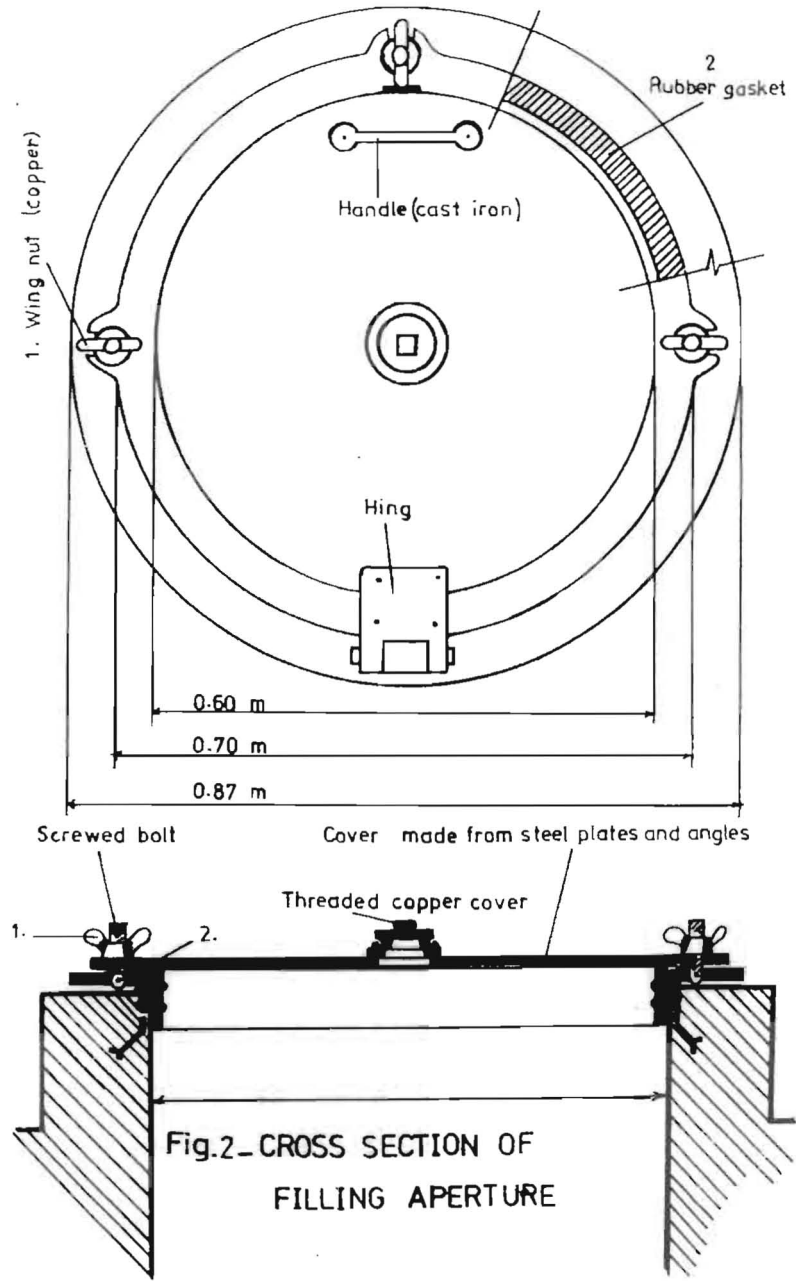


Fig.2-CROSS SECTION OF  
FILLING APERTURE

Fig.2. A cross section of the filling aperture and the steel cover.

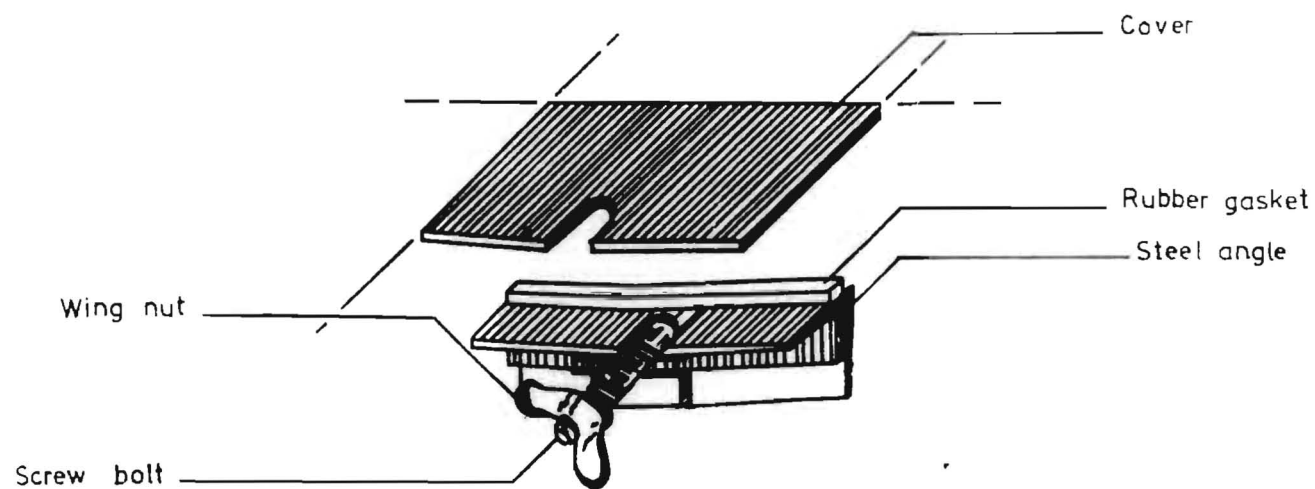


Fig.3. Isometric for the cover of the filling aperture.

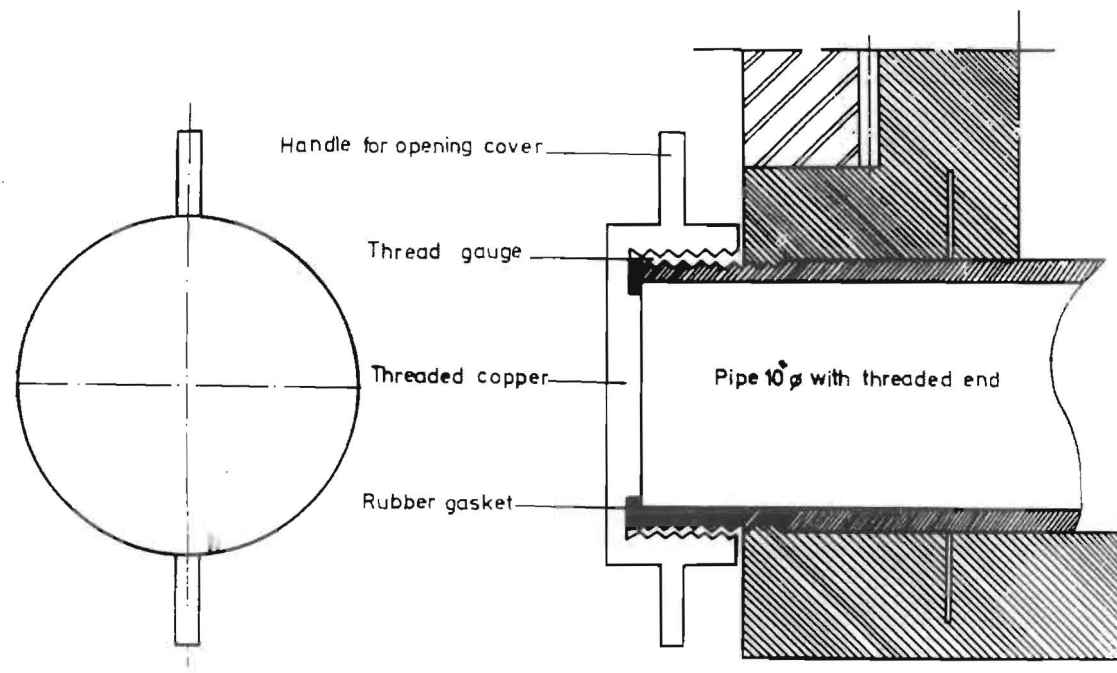


Fig.4. A cross section of the emptying aperture.

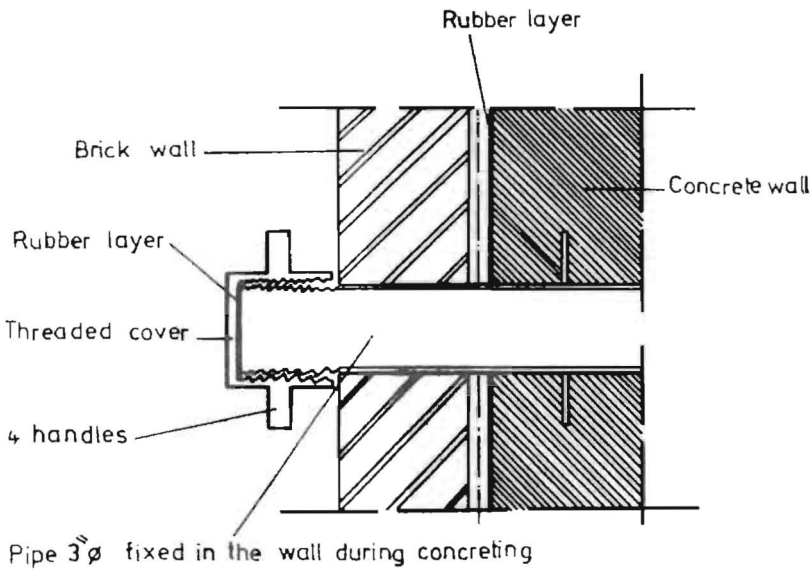


Fig.5. A sampling pipe.

TABLE NO. 1 PERCENTAGE OF CO<sub>2</sub> V/V IN THE PURGED BINS

NO.OF BIN	TYPE OF CONSTRUCTION	LOCATION OF SAMPLE	% AGE OF CO <sub>2</sub> - HOURS AFTER TREATMENT						
			0	1	2	3	4	5	
1	Without	Up	25	-	6	4	4	0.2	
	PVC	Down	25	-	20	15	10	1.6	
	Sheeting	Mean	25	-	13	9.5	7	0.9	
3		Up	4	3	0.4	0.4	0.4	0.0	
	With	Down	73	40	20	14	10	0.2	
	PVC	Mean	38.5	21.5	10.2	7.2	5.2	0.1	
4	With	Up	24	6.0	4.0	1.8	1.6	0.4	
	PVC	Down	78	61.0	42.0	32.0	26	0.8	
	Sheeting	Mean	51	33.5	23	16.9	13.8	0.6	
5	With	Up	52	20.6	16.4	10	8	1.4	
	PVC	Down	88	83	68	54	43	0.4	
	Sheeting	Mean	70	51.8	42.2	32	25.5	0.9	

bins as well as from the control bin, probably through the inner concrete walls and then to the outside atmosphere from any weak point which was not sealed or from the gaskets of the upper and lower apertures.

Bins having PVC liners had higher initial concentrations in these tests than did Bin n° 1 (control, no liner) but all bins had about the same mean CO<sub>2</sub> concentration after 5 hours. These results caused us to further study the construction of the bins.

Trial n° 2

We were advised by civil engineers and chemists working in the field of the construction of stores for inflammable materials that the inner surface of the bins should be painted with araldite epoxy resin paint (a CIBA-GEIGY product) to get better maintenance of the concentrations.

We chose bin n° 4 for these tests. This bin was emptied of wheat and the inside surfaces of the walls and floor were painted twice using "Topcoat 020" paint. This paint is made up of two compounds, i.e.:

Topcoat 020 resin (white in color)

Topcoat 020 hardener (green in color).

The mixing ratio is 100 parts of resin and 60 parts of hardener by weight.

After painting, the bin was again filled with wheat and the different apertures were tightly closed with a mixture of Chinese zinc, fine sand and cement.  $\text{CO}_2$  was then purged in, and concentrations were found to be as shown in Table 2. It is clear from this table that there was still a large (44.8%) loss of the  $\text{CO}_2$  concentration within the first 20 hours. This loss could mainly be attributed to sorption of  $\text{CO}_2$  by wheat grains or to leakage or movement of the gas in the intergranular spaces. The loss occurring during the following 6 days was also high indicating that the araldite coating was not effective. The percentage of loss of  $\text{CO}_2$  per day during this period was about 13.9%.

These problems led us to additional studies to make the bins tighter.

Trial n° 3

A civil engineer conducted a close inspection of the internal walls of the bins. He detected many very fine deep cracks in the walls of the bins and concluded that in the concrete the sand particle size was too large and the percentage of sand was too high. He then suggested that a partial removal of the inner concrete surfaces of the bins was essential. This step should be followed by filling in the

TABLE NO. 2 CO<sub>2</sub> CONCENTRATIONS (V/V) IN BIN NUMBER

## 4 AFTER BEING PAINTED WITH ARALDITE

TIME AFTER CO <sub>2</sub> PURGING	CO <sub>2</sub> CONCENTRATION %			% LOSS IN CO <sub>2</sub> CONC. % DAY
	UP	DOWN	MEAN	
1 hr.	68	88	78	)
				)
2 hrs.	68	80	74	)
				)
3 hrs.	54	70	62	)
				)
20 hrs.	32	54	43	)
				44.8 )
				)
7 days	68	7.5	7.1	) 13.9

cracks with mortar, then applying a new layer of concrete. The suggested last steps were (1) paint the inner surfaces of the walls with araldite epoxy resin, (2) apply a layer of glass wool fixed with araldite and (3) repaint the walls again with araldite. Tests were first conducted on bin n° 4 after removal of the old paint coat and following the steps outlined above.

Table 3 presents results of CO<sub>2</sub> concentration analysis in bin n° 4 after the modifications, refilling with grain, and repurging with CO<sub>2</sub>. These data indicate that the bin was holding CO<sub>2</sub> better than before the changes. The loss in CO<sub>2</sub> concentration during the first day was about 18.4% and the gradual decrease in CO<sub>2</sub> concentration during the following 6 days was about 2.35% per day. These figures correspond to losses of about 44.8% and 13.9% before making these modifications (Table 2). However, losses were still high and further steps were needed to make the bins more gas-tight.

TABLE NO.3 CO<sub>2</sub> CONCENTRATIONS IN BIN NO. 4

AFTER IT WAS REPAIRED AND PAINTED					LOSS IN CO <sub>2</sub> CONC./DAY
TIME OF SAMPLING AFTER PURGING WITH CO <sub>2</sub>	% DOWN	CO <sub>2</sub> UP	MEAN		
1 hr.	54	12	33	)	
				)	
3 hrs.	52	16	34	)	
				)	
5 hrs.	48	20	34	)	
				)	
7 hrs.	44.4	23.6	34	)	
				)	
22 hrs.	33.2	25.4	29.3	)	
				)	
25 hrs.	29	24.8	26.9	)	18.48 )
					)
6 days	25.3	21.6	23.4		)
					)
7 days	23.6	22.6	23.1		)
					)
11 days	22	20.6	21.3		) 2.35



Trial n° 4

Several other bins were repaired in the manner described for bin n° 4. Every possible point which might be responsible for CO<sub>2</sub> escape was caulked with a paste composed of Chinese zinc powder (used in wall painting) mixed with araldite HY and araldite impregnation DY at the rate of one part of each of both araldites (H and G). This mixture was then added to the zinc powder in small amounts until it reached the paste stage. This paste was plastered all around the upper and lower opening covers and wherever other CO<sub>2</sub> leaks were detected. These other leaks were found by using the soap bubbling method for gas leak detection. This paste has to cure for 48 hours before it gets hard and effective. After conducting this step, the wheat-filled bins were purged with CO<sub>2</sub> and the resultant concentrations are shown in Table 4. It is clear from this data that a significant drop in CO<sub>2</sub> concentration occurred during the time elapsed between the first two determinations in all bins. This time was as short as a few hours in bin 3 and 4, which may indicate that the high initial drop in CO<sub>2</sub> concentrations was due to the sorption of the gas by the wheat kernels.

The concentration of CO<sub>2</sub> after the second determination remained nearly constant or decreased very steadily and slightly as shown in Table 5. Data in this table indicate that the percentage of decrease ranged between 0 and 0.4% per day. This decrease is considered to be an acceptable level of decrease for hermetic storage and thus these bins under these conditions meet the requirements of the field trials. After the bins became fit for field trials, bins 3, 4 and 5 were left for longer periods of storage and CO<sub>2</sub> concentration was determined at monthly intervals as presented in Table 6. This table shows that there was a CO<sub>2</sub> loss in the three purged bins. The rate of CO<sub>2</sub> depletion was 0.47%, 0.35% and 0.17% per day during the different periods of storage which were 4, 6 and 12 months, respectively. The CO<sub>2</sub> loss in the 3 bins is mainly attributed to two main factors, i.e. CO<sub>2</sub> absorption by wheat kernels or to leaks. In this case the depletion due to absorption may be most prominent, otherwise CO<sub>2</sub> would have escaped gradually.

TABLE NO. 4      AVERAGE PERCENT CO<sub>2</sub> CONCENTRATION (V/V) IN THE  
INTERGRANULAR SPACES IN THE BINS AFTER DIFFERENT PERIODS  
OF PURGING (AVERAGE OF TWO LOCATIONS; I.E., NEAR THE TOP  
AND NEAR THE BOTTOM)

T.E.	BIN 3	T.E.	BIN 4	T.E.	BIN 5	T.E.	BIN 6	T.E.	BIN 7	T.E.	BIN 8
1h	67.6	1h	79.9	1h	53.1	1h	75.4	1h	69.6	1h	69.4
2h	57.2	5h	70.3	2d	50	5d	64.8	2d	59.4	7d	49
3d	58.4	6d	70.4	3d	48.5	6d	64.4	3d	59.2	8d	49.4
7d	57.5	8d	69.4	7d	49.1	8d	64.2	7d	59.4	10d	50.6
8d	56.3	12d	69.5	8d	49.2	12d	62.0	8d	60.3	14d	49.8
		13d	66.3			13d	62.6			15d	50.2

T.E.      equals    TIME ELAPSED AFTER PURGING CO<sub>2</sub>

H          equals    HOURS

D          equals    DAYS

TABLE NO. 5 PERCENT CO<sub>2</sub> DEPLETION DURING MEASUREMENT PERIOD 6 OR MORE DAYS AFTER THE SECOND DETERMINATION AND PERCENT DEPLETION PER DAY DURING THESE PERIODS.

BIN NO.	3	4	5	6	7	8
-----						
TIME ELAPSED BETWEEN SECOND AND LAST DETERMINATION OF CO <sub>2</sub> IN DAYS	7	12	6	8	6	8
-----						
% OF DROP IN CO <sub>2</sub> CONCENTRATION	1.6	5.7	1.6	3.4	0.0	0.0
-----						
% OF DROP IN CO <sub>2</sub> CONC./ DAY	0.2	0.47	0.26	0.42	0.0	0.0
-----						

It can be seen from these studies that a considerable concentrations of CO<sub>2</sub> lethal to all insects could be purged in and maintained in the intergranular spaces for periods of up to one year, as long as the bins are made relatively gas-tight.

#### SUMMARY OF RESULTS

Satisfactory results in construction could be obtained with very close supervision. It is essential that the construction materials be up to standards, i.e., the sand used must be fine and when mixed with the cement, the standard ratio should be followed.

TABLE NO. 6 THE DEPLETION IN THE CONCENTRATION OF CO<sub>2</sub> FROM BINS  
DESIGNED FOR HERMETIC STORAGE OF GRAIN DURING STORAGE  
PERIODS OF UP TO 12 MONTHS

PERIOD OF STORAGE (MONTHS)	<u>BIN. NO. 3</u>		<u>BIN NO. 4</u>		<u>BIN NO. 5</u>	
	CO <sub>2</sub> CONCEN- TRATION %	% OF DEP- LE- TION	CO <sub>2</sub> CONCEN- TRATION %	% OF DEP- LE- TION	CO <sub>2</sub> CONCEN- TRATION %	% OF DEP- LE- TION
initial*	67.6	-	79.9	-	53.1	-
1	52.7	22.04	64.8	18.90	48.9	7.91
2	39.6	41.42	54.6	31.67	44.4	16.38
3	32.3	52.22	49.8	37.67	39.3	25.99
4	29.5	56.36	41.0	48.69	33.5	36.91
5			38.0	52.44	32.0	39.74
6			30.0	62.45	26.0	51.04
7					27.2	48.78
8					24.2	54.43
9					26.0	51.04
10					24.0	54.80
11					-	-
12					21.0	60.45
CO <sub>2</sub> DEP- LETION PER DAY DURING THE PE- RIOD	0.47%		0.35%		0.17%	

\* DIRECTLY AFTER PURGING WITH CO<sub>2</sub>

This study also showed that:

1. The construction of a double wall bin with PVC fixed as a sandwich for making them gas-tight was not successful. This problem was solved by painting the inner surfaces with araldited epoxy resin two times and placing a layer of glass sheets between the coats of paint.
2. Consideration should be given to the reduction of the number of apertures to a minimum. A better design for the aperture covers is highly recommended, otherwise caulking the edges of the covers with a paste of zinc-oxide and araldite 1:1 should be considered.
3. Rubber gaskets are not satisfactory for hermetic sealing of apertures especially if they are exposed directly to weather conditions.

## REQUIREMENTS FOR THE INTEGRATION OF LARGE-SCALE HERMETIC STORAGE FACILITIES WITH CONVENTIONAL SYSTEMS

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### 1. INTRODUCTION

Large-scale hermetic structures can be satisfactorily used for the long-term preservation of food grain, and may be built partially or totally underground to simplify construction and to reduce costs. While in such structures it is convenient to handle grain in bulk, in the tropical countries where the technology could be employed, grain is generally held in bags. There is need therefore to suitably integrate handling systems to avoid congestion and delays in transport of the grain.

Quality requirements for grain intended for long-term air-tight storage are more stringent than for short term, conventionally stored produce and must be taken into consideration when selecting appropriate sites for hermetic bins. Locating in areas of surplus production will ensure that fresh, good quality grain will be received with the minimum of delay.

When planning for a large scale hermetic food reserve, it is important to have clear policy guidelines for smooth operation. A clear decision must be made on the role of the food reserve and priorities on maintaining its status in relation to food stocks in conventional stores.

Experience has shown that a large scale grain famine reserve must not be isolated from the general handling and marketing system. It must as far as possible be an economic enterprise and be able to receive produce directly from farms, as well as, in the absence of famine, to dispose grain stored for long periods directly into the normal marketing outlets.

### 2. AIR-TIGHT BULK STORAGE IN THE TROPICS

Grain stored in bulk in an air-tight structure requires to be handled in a different way from conventional silos.

## 2.1 Moisture migration and condensation

By definition, an air-tight structure does not permit ventilation, and grain in such containers should be thoroughly dry below 12.5%. To prevent moisture migration, the grain should be cooled to ambient temperatures before loading. Under tropical conditions, there exist fairly wide differences between day and night temperatures. The effects of these temperature variations are greater on semi-underground structures and less on fully underground silos. Moisture condenses on the roof and drops on the surface layer of the grain enhancing fungal growth. These problems cannot be entirely solved economically but with adequate precautions can be made negligible.

## 2.2 High temperatures and grain heating

Day temperatures in the tropics are generally above 20°C and are often in the region of 25 - 30°C. Although grain itself is a fairly poor conductor of heat, the inter-granular atmosphere will respond more rapidly to high external temperatures.

External temperatures, notwithstanding, severe grain heating can occur when the grain bulk is not free from dust and thrash that accumulates during mechanical harvesting and handling. In temperate countries, low external temperatures have a delaying effect on internal heating when grain is contaminated with extraneous matter. In the tropics, grain intended for long term air-tight storage must be thoroughly cleaned to avoid serious internal heating of the grain bulk.

## 2.3 Grain Handling

There is no difference in principle in loading and unloading partially and completely underground hermetic silos. These operations are however not as conveniently undertaken as in fully above ground silos. Semi-underground silos are easier to unload than fully underground structures.

The type of conveying system used will have a direct effect on the capital costs. Mechanised belt conveying systems (overhead or underground) are expensive and may not be conveniently designed for use with air-tight silos.

Conveying equipment should be simple and require little maintenance and should handle the grain with the minimum amount of damage. Handling equipment should have sufficient capacity to meet annual requirements of loading and unloading a pre-determined proportion of the storage capacity. In periods of shortage, these should be anticipated a few weeks in advance since the normal site capacity will be unable to handle emergency requirements.

It is obviously uneconomic to have a large quantity of surplus equipment on stand-by for periods of crisis. Suitable integration of famine storage with

conventional systems will ensure that surplus handling capacity on site will be profitably used when there is sufficient grain in the conventional system.

## 2.4 Monitoring and quality control

Provision has to be made on site for recording the produce by weight over a weigh-bridge and maintaining an accurate check of site records by weighing grain into and out of the hermetic silos. This will ensure that an accurate assessment is made of the various losses and an economic evaluation of possible remedies can be made.

Grain quality can be monitored rapidly and on a routine basis by fitting each bin for remote sensing of temperature, moisture and oxygen. Provision should also be made for taking grain samples. Constant monitoring of the produce will enable a check to be maintained on the quality as well as take preventative measures to avoid serious mishaps.

## 3. OPERATIONAL PROCEDURES AT THE "CYPRUS" BINS IN KENYA

### 3.1 Handling on arrival

Farm produced grain normally arrives in bags and is thoroughly inspected before acceptance. The accepted bags and produce are passed over a weigh-bridge and emptied into the intake pit of the cleaning and drying plant. Since the grain is expected to remain in the bin for at least 3 years, only fresh good quality produce is accepted.

### 3.2 Cleaning and drying

Dust and dirt are removed from the grain before it passes into the drier where the moisture content is brought down to 12.5%. The dry grain is fed into a temporary "dry bin" (part of drying plant itself) and later discharged into a bulk carrier for delivery (via the weigh-bridge) to the site of the hermetic bin being filled.

### 3.3 Filling

The bulk carrier feeds the grain into the intake hopper of the loader and the grain is elevated by a chain and flight conveyor with an average capacity of 65 tonnes per hour. A bin can be filled within 3-4 days but generally takes longer because of delays in supply and handling. However, no filling is expected to take longer than 3 weeks. At the later stages of filling a spinner is used to obtain a complete trimming of the bin. (This is necessary because the top of the "Cyprus" bin is nearly horizontal, less than the angle of repose of the grain). The bin is then sealed for at least 6 months to attain hermetic conditions and control infestation.



### 3.4 Emptying

A bin to be emptied is first properly ventilated for at least 2 hours by opening the top and side hatches. Wet and rotten grain below the top hatch, at the ring beam, and at the bottom is removed separately, weighed and destroyed. The damp and mouldy grain next to the rotten grain is also removed separately, dried and aerated in the cleaning plant, bagged and retained for inspection if suitable for animal feed.

The unloader is a separate machine from the loader. Unloading is done by lowering the (Redler) Boom Conveyor through the side hatch. The grain is discharged directly into a bulk carrier and conveyed via the weigh-bridge to the cleaning and bagging plant.

## 4. OPERATIONAL DIFFICULTIES AND POSSIBLE IMPROVEMENTS

In addition to the structural problems which have been satisfactorily solved, the normal operation of the hermetic storage sites has been subject to a set of handling problems arising from inadequate pre-planning and design of sites for suitable integration with conventional handling systems. As a result the facilities on site were not sufficiently equipped to receive, clean and dry grain rapidly during the 4-8 week peak delivery period after the harvest.

This resulted in considerable delays especially in the early filling operations in 1972 (Figure 1). The time lapse between beginning and concluding a filling is indicated by the open block in front of the black strip which is the length of time the produce remained in the bin. The open block at the end of the black strip is the period during which emptying was initiated and completed for a particular bin. An arrowhead at the end of an emptying period indicates that the contents were transferred to another bin for a further period of storage. Transfers were made when heating problems were especially severe.

The filling periods were considerably reduced during the second time the bins were filled in 1975. This was because more efficient equipment was installed on site and handling rates were improved (Table 1). The cleaning rate is dependant on the rate at which the produce passes through the drying plant. Drying capacity will have to be increased by at least 4 times.

## 5. OPERATIONAL COSTS

The bins require constant maintenance for sealing cracks and for preservation of the bituminous membrane on the dome. There are several site installations including the cleaning and drying plant, laboratories, garage and general utility buildings. The special loading and unloading equipment, bulk-carriers, tractors and other vehicles also need maintenance. The roads and ground surface near the bins must be constantly repaired to avoid damage to the underground structure.

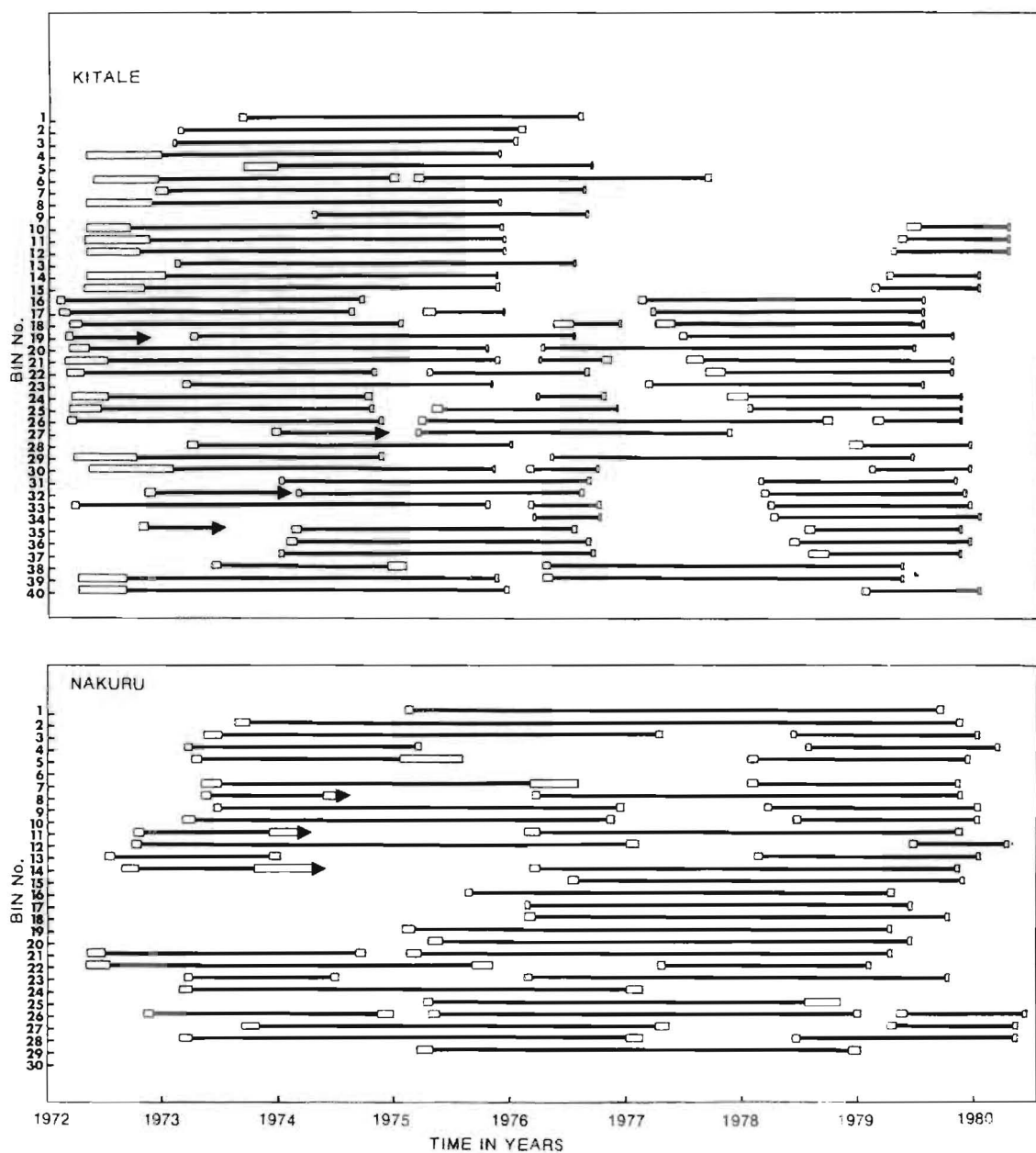


Fig. 1. Filling and emptying operations over the years 1972-1980 at the Kitale and Nakuru sites.

NOTE: The figure illustrates records for maize storage only. However, at Nakuru, wheat was stored on several occasions and this partly accounts for the long periods shown "empty" for certain bins. Bins 6, and 30 remained unused at Nakuru due to defects in construction.

TABLE 1

Handling capacity at the Kitale and Nakuru sites and future requirement.

Operation	Handling capacity (tons per hour)		
	Initial	Current	Required
1. Cleaning	15-20	15-20	30-40
2. Drying (to 12.5%)			
from 13.5%	8-10	8-10	30-40
from 14.5%	4-5	4-5	15-20
from 15.5%	2-2.5	2-2.5	10-15
3. Loading (filling)	5-15	60-70	60-70
4. Unloading (emptying)	5-15	40-50	80-100
5. Bagging	20-24	20-24	80-100

During operation there are various costs including pest control, fuel and labour hire charges. There is a minimum technical and maintenance force under permanent employment.

These costs have generally averaged in the region of 60,000 U.S. dollars per annum and gives an average cost of 0.6 U.S. dollars per ton per year of storage. These costs compare very favourably with conventional storage where annual pest control costs alone are in excess of 0.3 U.S. dollars per ton per year (pest control in the hermetic bins costs less than 0.00133 U.S. dollars per ton per year).

## 6. INTEGRATION OF HERMETIC STORAGE FACILITIES WITH CONVENTIONAL SYSTEMS

In addition to the uncertainties of rainfall and other climatic factors, many countries in the tropics often face difficulties in obtaining essential agricultural inputs like fuel and fertilisers. Establishing sufficient food reserves for use in periods of shortage is a wise course of action. These reserves should be essentially long term i.e. of 3-5 years duration.

Experience in Kenya has shown that the handling of such reserves requires careful planning and should as far as possible fit in with the normal handling procedures for conventionally stored produce.

### 6.1 Siting of hermetic storage facilities

Since it is essential that fresh, good quality grain be used, the hermetic structures should be sited in an area of surplus production. Farmers will be encouraged to deliver directly and avoid delays, normally caused by the use of intermediaries, and there will be lower transport costs. In constructing hermetic structures partly or fully underground, account should be taken of the physical structure of the soil and depth of the water table.

## 6.2 Cleaning, drying and handling equipment

Grain intended for long term storage must be dry and free from dust and thrash. In planning for capacities of equipment needed for such work consideration must be given to long term projections and the possibilities of extending the use of the facilities. For example, drying facilities originally built for the "Cyprus" bins were later found to be inadequate to cope with a more general demand from the farming community for drying facilities to enable early delivery for conventional storage. Additional facilities were constructed in 1975 several miles away from the Kitale site and it was not possible to satisfactorily integrate these facilities for use by the "Cyprus" bins site during peak delivery periods. Additional drying facilities for conventional storage have since been constructed at Kitale and will be available for drying grain for the "Cyprus" bins in 1981.

## 6.3 Stock control

When hermetic storage is planned for 3 to 4 years, there should be a proper schedule to turn-over 1/3rd or 1/4th of the stock every 3 or 4 years. This will reduce the quantities being handled each year and also ensure that the work load and operations on site are spread out through the year. Every year, grain that has been stored for 3 or 4 years may be released into the conventional marketing system for milling. In this way, some of the grain which has acquired a certain amount of mustiness from long term storage can be satisfactorily blended with fresh grain in the milling process.

Each year, emptying operations should begin several months in advance of the next harvest and the empty structures suitably repaired and serviced for immediate use. Many delays in receiving and filling bins occur because of the insufficient time given for undertaking maintenance work.

## 6.4 Interacting systems

This paper gives attention to the aspects that need to be taken into consideration when a country is planning to improve its food security by preserving a fraction of its national requirements on a long term basis. Although hermetic storage is a low-cost and technologically effective system, there are other alternative technologies.

Whichever technology is adopted in a given country, the concept of a long term famine reserve will remain. Whatever conventional handling systems are already operating, there will be a need to provide for an adequate interaction with the famine reserve system.

The main areas of interaction are illustrated in Figure 2. Grain is often handled in bags in tropical countries because produce can be conveniently delivered in this way by large numbers of small scale producers, or merchants

and other intermediaries. Conventional systems are therefore geared to handling produce in bags. Since long term famine reserves are more conveniently handled in bulk, some additional handling will be required.

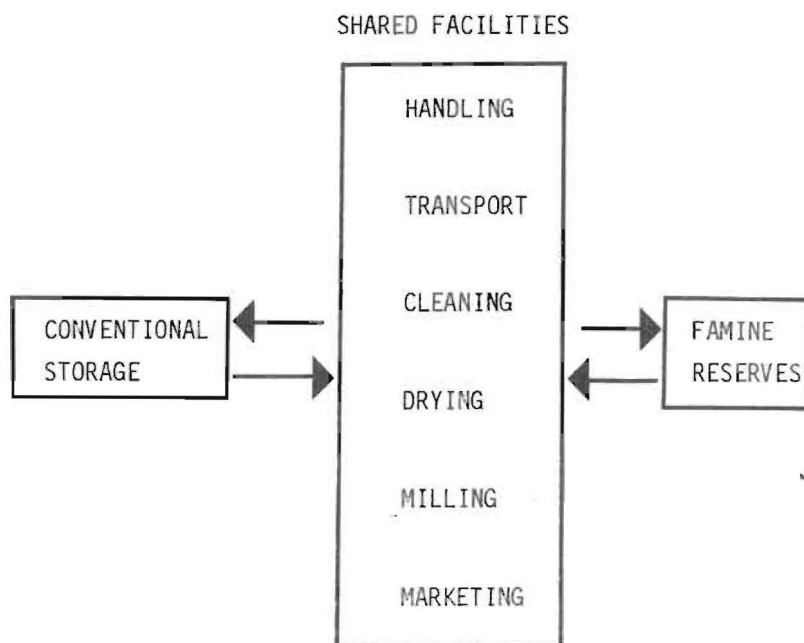


Figure 2. Common facilities for conventional storage and hermetic famine reserves.

For rapid acceptance and, more important, for rapid despatch in times of crisis, adequate provisions for transport must be made. Rail transport can often be provided for at relatively low cost and wagons for handling in bag or bulk can be made available for transport of grain as well as other commodities. Road transport is more convenient in bags but in periods of crisis rapid turn-round of trucks carrying bags may not be easily achieved.

Cleaning and drying facilities are essential for long term food storage and are increasingly in demand for conventional bag storage. Such facilities may therefore be conveniently shared.

After the grain has completed a specified period of storage there is need to release it to the consumer. The grain has to be milled and then marketed. When grain has been stored for a long period of time it will acquire a certain amount of mustiness and other off-odours. Under tropical conditions these odours are generally unavoidable but when mixed with fresh produce during milling are completely undetectable. Adequate liaison should therefore be maintained with milling and marketing organisations to obtain consumer acceptance of produce after long term storage.

## 7. PLANNING AND ECONOMIC ASPECTS

Total construction costs for the "Cyprus" bins in Kenya were given as 14 U.S. dollars per ton capacity in 1966-67. These were found to be extremely favourable in comparison with costs for conventional bag stores at that time. In 1975 the construction costs for modern conventional bag stores for the Maize Board were put as 22 U.S. dollars per ton capacity. With current world inflationary trends these will have risen considerably and hermetic storage with its low constructional costs should be extremely competitive.

Hermetic storage is not an alternative to more conventional forms of bag and bulk storage. It is a long term option and has a role in preserving food stocks for security in the event of famine. This aspect must be taken into account besides other purely economic costs when considering future investments.

A famine reserve must not be held in isolation. It must be operated as an economic enterprise during normal years and must be integrated with the existing grain industry. Thus, it may be located in a grain surplus area and operated on a site adjacent to conventional stores and a milling complex. This will enable sufficient "over-capacity" to be economically utilised during non-famine periods and to ensure that sufficient capacity exists for handling during periods of crisis.

Plans for long term famine reserves should therefore take into consideration long range construction programmes for the grain industry. The economies of scale can be successfully employed, especially in the use of common facilities, and overall costs for both, long term and conventional storage, considerably reduced.

## CONTROLLED ATMOSPHERE GRAIN STORAGE IN CHINA

CARL RÄNNFELT

### INTRODUCTION

During October–November 1979, FAO conducted a UNDP financed study tour/workshop, hosted by the Chinese Government, on Storage Structure Design for 20 participants from different Asian countries. The information in this article is based on observations made during the study tour.

### 1. BACKGROUND

The annual grain harvest in China is about 320 million tons and it is estimated that about 80 million tons are kept in central stores.

About 11 million tons are kept in locally built silos with clay/straw walls (see Fig. 1). Traditional warehouses (see Fig. 2), where the grain is normally stored in bulk, and outdoor stacks (usually with about 200 tons of grain each 2,000 bags x 100 kgs/bag, and covered by 5–7 layers of reed mats) are commonly used facilities at central stores.

The grains stored are wheat (in the North) and paddy (in the Centre and South). Near big consumer centres, milled rice for consumption is stored in bags.

The areas referred to in this article are located between 40°N (Beijing) and 30°N (Shanghai), with a humid continental to humid sub-tropical climate.

### 2. FACILITIES FOR CONTROLLED ATMOSPHERE STORAGE

Special structures for controlled atmosphere storage are rarely used in China except for low temperature stores and underground stores. Means of controlling the atmosphere for stored grain are usually applied in the normal standard warehouses and in the clay/straw walled silos.

### 3. NATURAL COOLING

In the North, natural cooling is used. If grain is not cool when put into storage, it is later ventilated naturally by means of doors and ventilation openings or, if necessary, taken out of the warehouse or silo for cooling and then brought back in again. Small suction fans are used in some stores to cool the grain. In some cases, the grain is covered with plastic (see Fig. 3) and a layer of sand after cooling to prevent reheating when the warm season arrives.

It should be noted that most warehouses have a ceiling limiting the heat exchange between the interior and the ambient air.

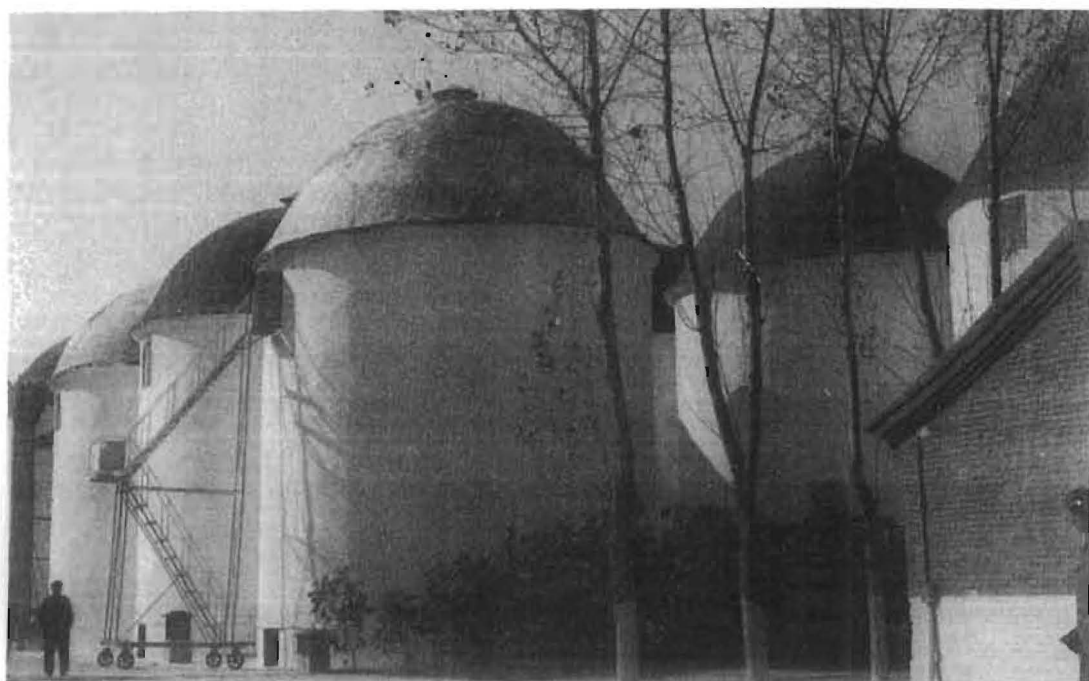


Fig. 1. Clay-straw silo near Beijing  $\varnothing$  8 m, height 8.5 m, holding capacity about 250 tons of wheat.



Fig. 2. Traditional warehouse in brick near Wuxi. In this warehouse, ventilation openings are mechanically adjusted by the small motor seen to the right.





Fig. 3. Interior of clay/straw walled silo with the grain covered with polyethylene (to prevent air exchange and prevent the grain from warming up).

Temperature control is a normal routine in most grain stores. When the ambient temperature is below  $15^{\circ}\text{C}$ , readings are made every month and when above  $15^{\circ}\text{C}$  every week.

Due to the low winter temperatures in the North, grain temperatures can be kept below  $15^{\circ}\text{C}$ . The water content of stored grain is of the order of 13-14%.

#### 4. LOW TEMPERATURE STORES

In the Centre and South, low temperature stores are used for milled rice in bags. Traditional warehouses have been insulated ( $K$  value = 0.5) and sealed, and cooling units installed (see Fig. 4). The photograph shows a  $1,000\text{ m}^2$  store in Shanghai for about 2,000 tons of bagged milled rice. A 22 kW cooling unit is installed and is reported to work for about 800 hours per year, or 3-6 hours per day depending on the season. The temperature in the store is kept at  $15^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and the relative humidity at about 50%. The air distribution unit in the low temperature store is shown in Fig. 5.

#### 5. NITROGEN ATMOSPHERE STORAGE

Low temperature stores are as yet comparatively few but storage in nitrogen atmosphere is quite common. In fact, it is assumed that about one-third of the grain kept in the Shanghai area is stored in a nitrogen atmosphere.

Arrangements are simple and can be applied in any normal warehouse. Stacks of about 100 tons are covered with polyethylene (0.23 mm) and sealed (see Fig. 6). Air is evacuated and replaced by nitrogen. This method is considered suitable only in the temperature range  $+5^{\circ}\text{C} - +30^{\circ}\text{C}$ . Even if low temperature stores are considered technically better, nitrogen atmosphere storage is going to be used a lot because of the simplicity of the method.

#### 6. UNDERGROUND STORAGE

Underground storage is used to a fair extent in Henan Province where topography and soil conditions are suitable for the construction of this type of storage.

Underground bins are usually bell shaped with a diameter of 8-18 m and a depth of 8-12 m. The bins are entirely covered and therefore maintain a low, nearly constant temperature. Usually grain with about 13% moisture content is put into these bins at a temperature of  $10^{\circ}\text{C}$  or lower. This type of storage is reported to cost only 40% of the cost of normal storage and in some cases grain has been kept for twelve years in such underground bins without deterioration.

#### 7. CONCLUSIONS

It was impressive to see in China how, with very simple means and low cost methods, controlled atmosphere storage was applied in normal warehouses. Extremely good management and hygiene and minute control of the grain reduced losses to

an absolute minimum. Chemicals were used sparingly and only a small part of the stored grain was treated or fumigated.

Development of storage structures and methods is empirical but long experience on large quantities of grain has proved the merit of the techniques in use.

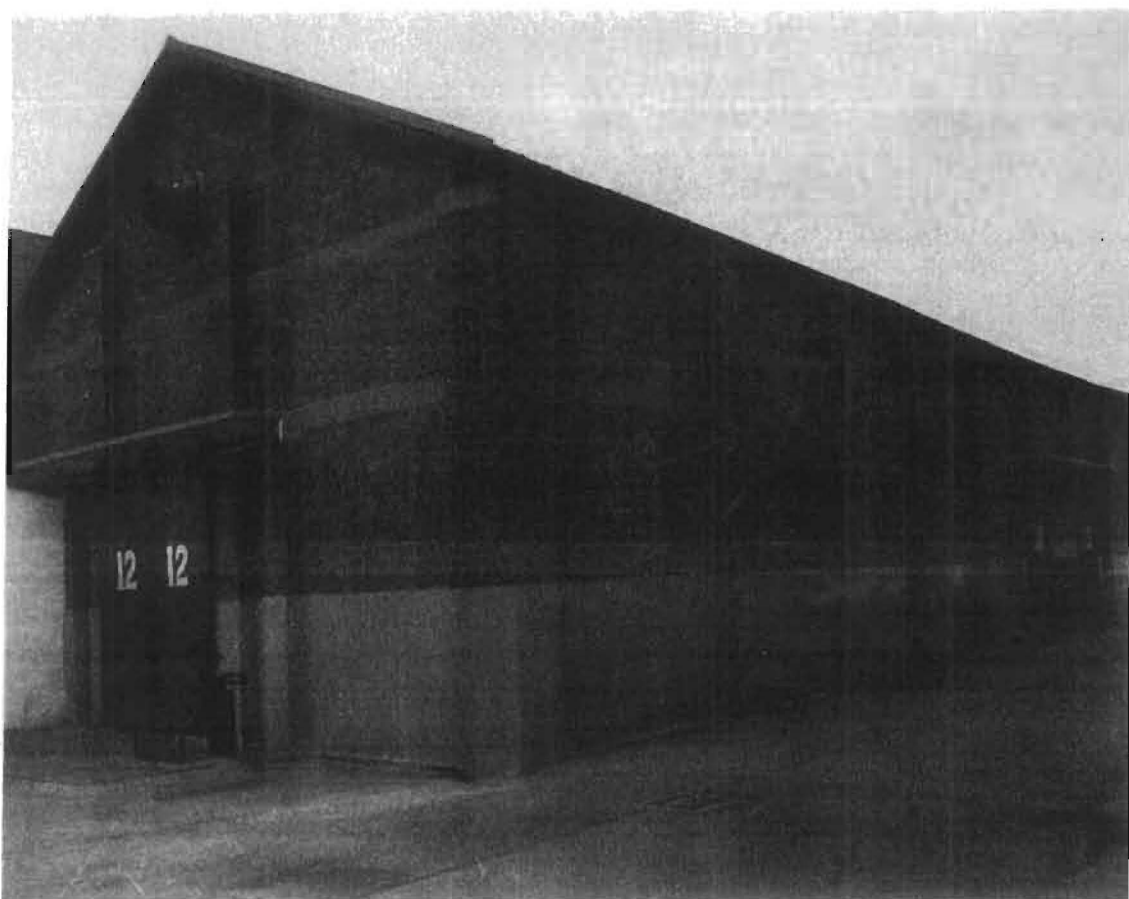


Fig. 4. Traditional warehouse, Shanghai,  $1,000 \text{ m}^2$ , which was remodelled in 1975 for low temperature storage of milled rice in bags.

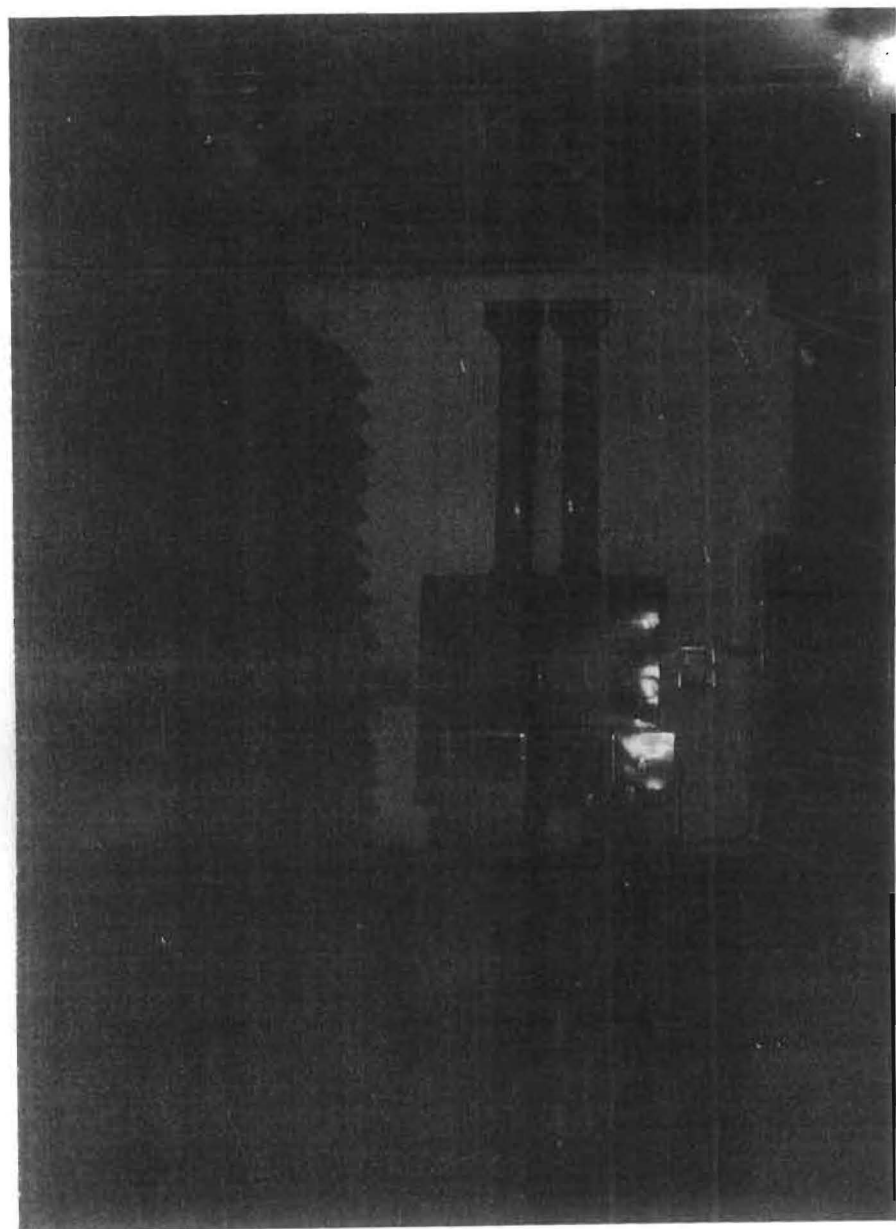


Fig. 5. Interior of warehouse. Air distribution unit. Milled rice stored. 3.6 m high. Temperature  $+15^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .



Fig. 6. Stack of bags, 13 bags high (= 2.5 m) being covered with polyethylene for nitrogen atmosphere storage.

# THE FIRST ITALIAN LARGE SCALE FACILITIES FOR THE STORAGE OF CEREAL GRAINS IN NITROGEN.

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## ABSTRACT

The main characteristics of the first industrial storage plants for cereal grains, operating according to the Snamprogetti preservation technology in nitrogen, are described. In particular the differences in technical solutions in function of the various preservation necessities are put into evidence.

The storage plants operate with excellent results since three years. The data accumulated during their operation permit to draw the following conclusions:

- the nitrogen preservation method can be applied also to industrial plants of large dimensions, obtaining in all parts of the bins conditions adequate for the satisfactory preservation of cereal grains;
- the investment costs for the instrumentation needed for the preservation in nitrogen do not cause significant variations in the overall investment costs of the storage plants;
- the nitrogen consumption during the preservation depends on the particular technical solutions adopted, yet they are in any case in the order of 2 - 5 SCM/ton.

## INTRODUCTION

The first Italian large scale facilities for the storage of grains in nitrogen, operating since three years are:

- (1) IVO, Tuscania: storage complex with 4 silos, 2,200 m<sup>3</sup> each.

The silos have a flat base and a conical roof; the construction material is folded galvanized steel for the cylinder and welded steel for the roof and the floor. The silo diameter is 13 m, the height of the cylinder is 15.3 m, the total height is 19 m.

(2) S.R.M., Roma: storage complex with silos of  $600\text{ m}^3$  each. The storage complex is composed of 26 fiber glass silos, 6 m in diameter and 20 m height. The silos are based on conical concrete bases coated with reinforced polyester painting connected to the cylinder.

These two complexes have very different characteristics either on the basis of preservation necessities (1 year preservation in the IVO plant, few months in the SRM plant) or on the basis of the product to be preserved (malting barley, and wheat for human consumption for the two plants respectively).

In order to build these two plants many technical and economic questions had to be solved, concerning:

- 1 - silo gastightness
- 2 - nitrogen supply system
- 3 - plant safety
- 4 - interstitial atmosphere substitution
- 5 - maintaining of the controlled atmosphere.

These questions are briefly discussed in the following, and the experimental results obtained during the operation of the plants are reported.

#### SILO GASTIGHTNESS

To maintain the controlled atmosphere inside the silo, a good gastightness of the silo itself is necessary in order to avoid oxygen penetration from outside and/or large nitrogen consumption.

Many different industrial silos can satisfy this requirement: welded steel bins, folded galvanized steel bins, fiber glass bins, coated concrete silos.

The test for gastightness of the silo is normally performed before the loading, in order to seal the eventually discovered discontinuity in the structure. But the test is very difficult to be performed with an empty silo particularly for thin wall and/or metal silos, because

the surrounding temperature changes produce substantially pressure variations in the silo and incoherent results of the test. Only a rough test may be performed with an empty silo.

More accurate results may be obtained performing the test after the grain loading because the gas temperature inside the silo changes much less, owing to the large heat capacity of the grain.

In any case some temperature variation in the head space occurs, so that for very gastight structures exact results may be obtained only with a 3 - 4 days pressostatic test.

The test is performed maintaining a slight positive pressure inside the silo for the whole test time with a pressostat which supplies measured quantities of gas when the pressure drops under set value  $P_s$ . With a filling ratio of 95%, the pressure variations related to the surrounding temperature variation (less than 30°C) are lower than 50 mm water column. So that the silo safety valve does not operate (the operating pressure of the valve is about 100 mm w.g.). The gas inside the silo is discharged only through the discontinuities of the structure and the measured quantity  $Q$  of gas supplied to the silo to maintain the pressure over the  $P$  set is equal to gas loss from the silo at the medium pressure between  $P_{set}$  and  $P_{max}$ , attained during the test.

This quantity  $Q$  is a meaningful gastight parameter as it equals to the nitrogen consumption for the operation of the controlled atmosphere silo.

The measured values of  $Q$  for the two plants are reported in table 1. The values change from one silo to the others so that the minimum and the maximum of these values are reported.

To compare these values with published data of gastightness, we have calculated, from the measured values of  $Q$ , the usual gastight parameters:

- the constant flow rate  $F$  necessary to maintain the pressure inside the silo at a given constant value, in particular a 250 Pa. (about 25 mm w.g.), at constant internal temperature;
- the time  $T$  necessary for a pressure decay from 250 to 125 Pa. at constant internal temperature.



TABLE 1 SUMMARY OF GASTIGHTNESS TESTS:

Plant	Structure of the bin	Capacity of the bin (m <sup>3</sup> )	Filling ratio	Measured gas consumption giving P medium = 45 mm w.g. (m <sup>3</sup> /day)	Calculated time for a decay from 250 to 125 Pa. (h)	Calculated flow rate giving a constant pressure P = 250 Pa. (l/s)
IVO	folded steel, welded steel floor and roof	2260	0.95	4 - 6	5.8 - 8.6	0.03 - 0.05
SRM	fiber-glass painted concrete floor	600	0.92	4 - 8	1.1 - 2.2	0.03 - 0.06

Experimental values of these parameters are not presented because in these very gastight structures it was impossible to obtain reproducible results owing to temperature variations.

#### NITROGEN SUPPLY SYSTEM

The nitrogen of the required purity for controlled atmosphere application may be supplied in different ways:

- liquid nitrogen supplied by a Commercial Company that also leases the storage and vaporization facilities;
- gaseous nitrogen production from an autonomous plant joined to the grain storage complex. Different kinds of nitrogen production plants may be utilized: combustion, molecular sieve, air liquefaction plants.

Yet the relatively low cost of the liquid nitrogen supplied by commercial companies in Italy (about 15  $\text{¢}/\text{m}^3$  for large nitrogen consumption in 1978) makes the utilization of autonomous nitrogen production plants economic only for large complexes of not less than 50,000 ton capacity. Therefore the nitrogen necessary for the operation of the two plants has been supplied by commercial Italian companies.

The difference in the nitrogen flow rate and consumption during the purge and maintenance operations suggests the utilization of different facilities for the two operations:

- a mobile large liquid nitrogen storage tank, having a very efficient vaporization facility, is used for purging the air in the silo;
- a static small liquid nitrogen storage tank is using for maintaining the controlled atmosphere inside the silo.

#### PLANT SAFETY

A pressure relief valve must be fitted to the roof of the silo in order to prevent excessive pressure build up or reduction, which may cause structural damage to the silo.

The two plants utilize a two plate valve operating at 100 mm w.g. A hydraulic seal on the nitrogen supply line prevents any excessive pressure build up during the purge of the air in the silo.

The hazard due to the lack of oxygen inside the silo is prevented excluding the possibility for men to get into the structures during

the controlled atmosphere operation.

At unloading of the silos the oxygen content in the internal atmosphere must be tested and ventilated if necessary before the personal entry of a person is allowed.

#### SUBSTITUTION OF THE INTERSTITIAL ATMOSPHERE

The substitution of the interstitial atmosphere for a grain bulk stored in a large silo and/or with a low height/diameter ratio presents some difficulties from the efficiency and nitrogen consumption points of view.

Better conditions should be attained by a plug flow of the supplied nitrogen. Actually strong diffusion phenomena occur between the nitrogen front and the air inside the bulk of the grains and still more in the headspace.

Moreover the convective currents, caused by the temperature gradients inside the bulk of the grains and by the temperature differences at the walls, carry the nitrogen along preferential directions enhancing the diffusion phenomena.

For the two plants the purge was performed supplying gaseous nitrogen to the bottom of the silo at a constant flow rate. We measured the oxygen content in the atmosphere different points in the silo and at different times during the purging operation.

These results have been utilized to test the purging model that assumes complete displacement of the gas in the load space and perfect mixing in the headspace of the storage bin (Banks, 1979).

According to this model to oxygen concentration  $(C)_x$  in the load space at a distance  $x$  from the nitrogen inlet must present a discontinuous reduction from 21% to 0% at nitrogen front arrival time

$$(t_a)_x = \frac{n(V_B)x}{W}$$

that is:

$$\begin{array}{ll} (C)_x = 21\% & \text{when } t < (t_a)_x \\ (C)_x = 0\% & \text{when } t > (t_a)_x \end{array}$$

where  $(V_B)_x$  is the volume of the stored product, from the nitrogen inlet to the section  $x$  of the silo,  $n$  is the product porosity,  $W$  is the nitrogen flow rate.

At the outlet of the silo the oxygen concentration  $C$  predicted by the model is an exponential function of time beginning from the nitrogen front arrival time to the border of the load space:

$$t_a = \frac{n V_B}{W}$$

that is:

$$C = 21\%$$

$$\text{when } t < t_a$$

$$C = 21 \exp \left[ -\frac{t - t_a}{\tau} \right]$$

$$\text{when } t > t_a$$

$$\text{where } \tau = \frac{V_{HS}}{W}$$

and where  $V_B$  is the total volume of the load space and  $V_{HS}$  is the volume of headspace.

The comparison between theoretical and experimental results is shown in figures 1 and 2 for two tests performed at the SRM plant at different purging flow rates. The indicated values of the velocity refer to the cross section of the bin.

A shift of the experimental results from the theoretical values in the load space appears: large axial and radial diffusion phenomena appear in the middle section of the silo. The nitrogen rises quicker along the axis of the silo and along the wall facing the sun than along the other wall.

Convective fluxes may be responsible for this behaviour since the temperature of the wheat was greater than the ambient temperature.

On the contrary at the outlet section of the silo a quite good fitting of the experimental results was obtained. The fitting is in any case better at the higher purging velocity.

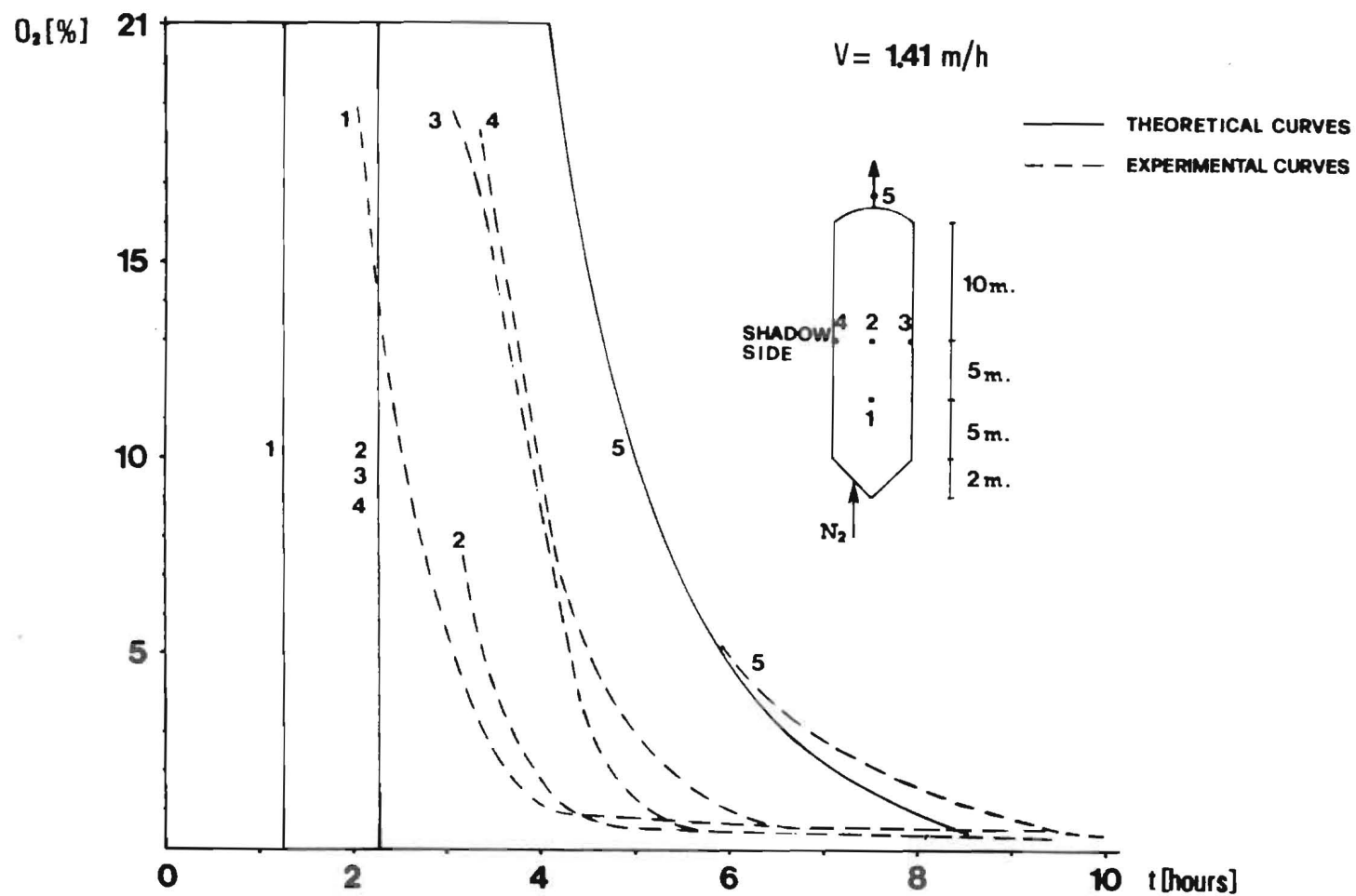


Fig.1. Comparison between theoretical and experimental results of a purging test in a SRM plant fiberglass bin at gas velocity of 1.41 m/h.

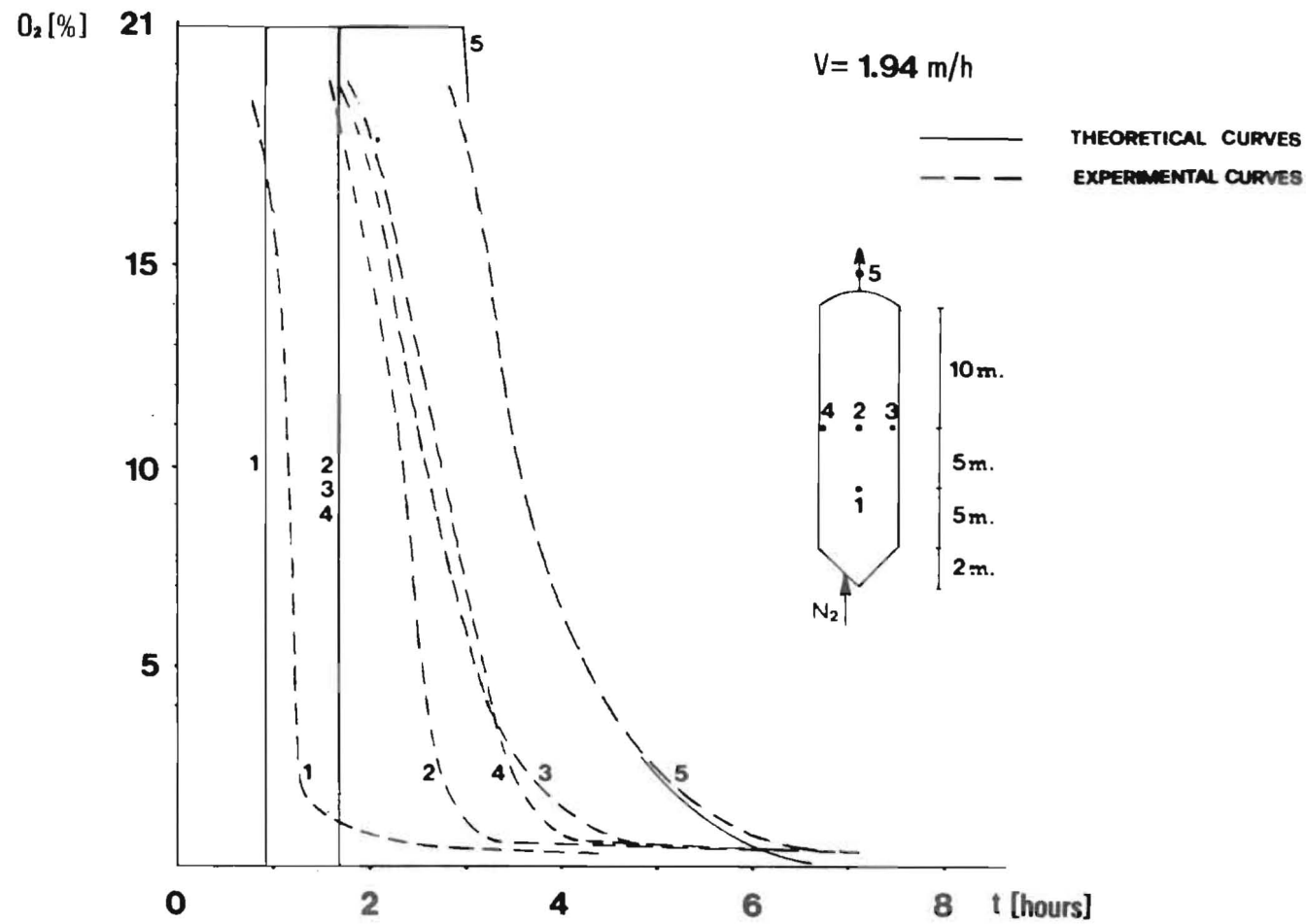


Fig.2. Comparison between theoretical and experimental results of a purging test in a SRM plant fiberglass bin at gas velocity of 1.94 m/h.

To summarize the results obtained during the purge operation in the two plants, we can consider the efficiency of purging  $E$  defined as the ratio of the nitrogen consumption predicted by the model and the experimental one  $V_G$ :

$$E = 100 \frac{n V_B + V_{HS} \ln \frac{21}{C}}{V_G}$$

In table 2 the  $E$  values obtained during the tests performed at the two plants, to a residual oxygen concentration of 0.5% vol. at the end of purging, are reported.

A clear increase in the efficiency with an increasing purge flow rate appears. Moreover, at the same flow rate, a greater efficiency occurs in the test performed at the SRM plant in comparison to the IVO plant. The greater ratio of the height to the diameter of the silos (3.3 versus 1.3) may be responsible for the greater efficiency in the SRM test.

#### CONTROLLED ATMOSPHERE MAINTENANCE

After purging the silo it is necessary to maintain a constant value of the controlled atmosphere composition. Oxygen may penetrate inside the silo owing to convection and/or molecular diffusion.

In order to prevent this phenomenon in the two plants, a slight positive pressure was maintained in the silo. In this way convective fluxes from outside are present and diffusion is hindered by the overall flow rate from inside to outside. To reduce nitrogen consumption, the pressure of the pressostat was set at the lowest value capable to avoid any increase in the oxygen content: about 20 mm w.g.

The pressure variation inside a silo of the IVO plant is reported in fig. 3. The pressure increases during the morning from the set value,  $P = 20$  mm w.g., attaining a maximum value of about 70 mm w.g., which is less than the operating pressure of the safety valve.

TABLE 2 SUMMARY OF PURGING TESTS:

Plant	Filling ratio	Average purge flow rate (m <sup>3</sup> /h)	Equivalent Velocity in empty silo (m/h)	Final head space oxygen content (% vol)	Efficiency (%)
IVO	0.95	22	0.17	0.5	33
IVO	0.95	66	0.50	0.5	50
IVO	0.95	133	1.00	0.5	67
IVO	0.95	200	1.50	0.5	83
SRM	0.92	40	1.41	0.5	91
SRM	0.92	55	1.94	0.5	94



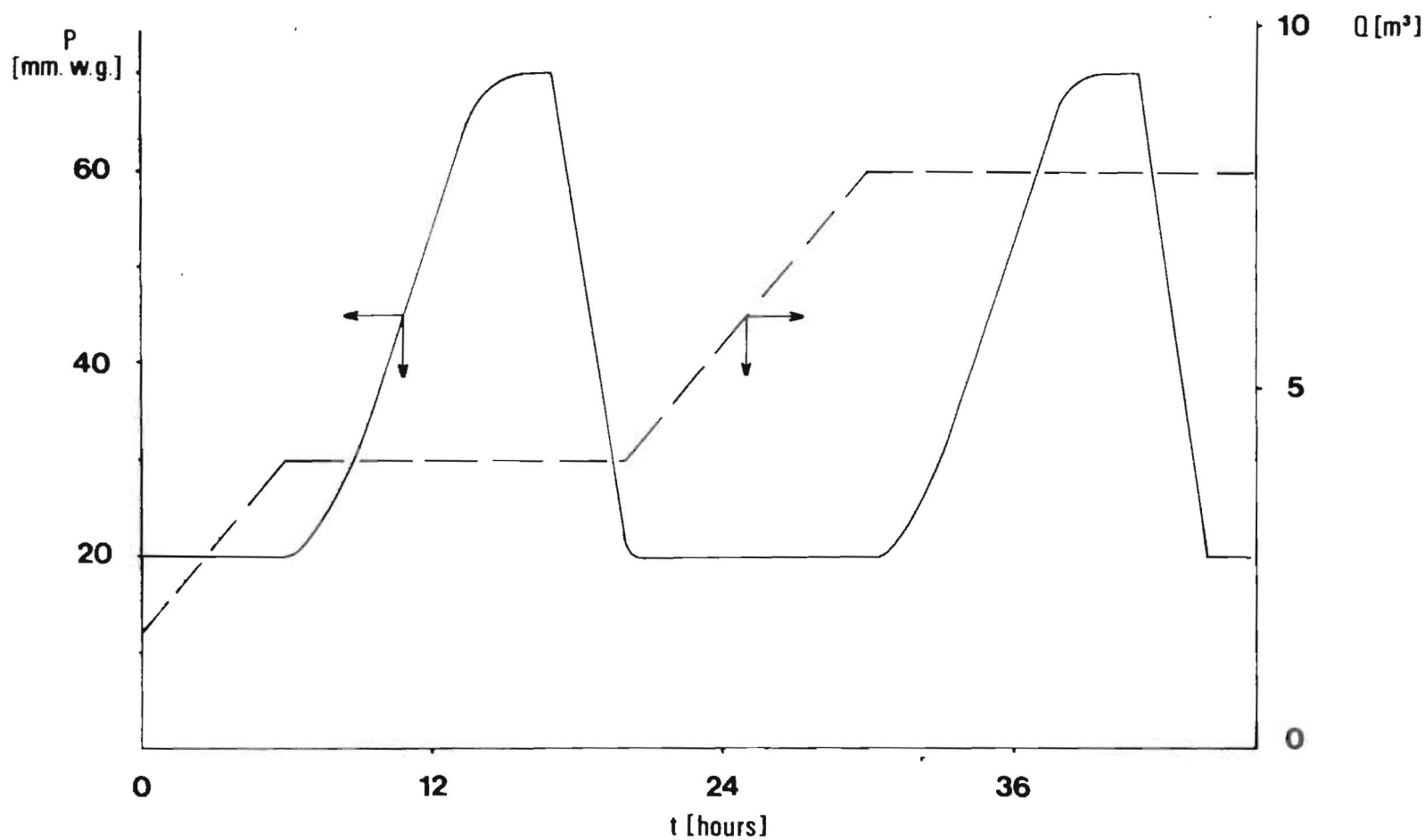


Fig.3. Diurnal changes in pressure in an IVO plant metal bin and nitrogen consumption for the maintenance of  $P_{\min} = 20$  mm-w.g.

This maximum value is in agreement with the prediction that may be done knowing the filling ratio and the headspace temperature variation:

$$P_{\max} = P_{\text{set}} \left[ 1 + \frac{V_{\text{HS}}}{V_{\text{HS}} + V_{\text{B}}} \frac{\Delta T}{T} \right]$$

where  $T$  is the initial headspace temperature ( $^{\circ}\text{K}$ ).

It appears that for a given headspace temperature variation, such a limit value of the filling ratio exists that below it  $P_{\max}$  becomes greater than the operation pressure of the safety valve and very large nitrogen consumption for the maintenance builds up.

In fig. 3 the maintenance nitrogen consumption is also reported: during the morning the pressure is greater than  $P_{\text{set}}$  and the nitrogen consumption is zero;

in the night a nitrogen consumption equal to the gas loss during the whole day occurs. This consumption was  $4 \text{ m}^3/\text{day}$  for the tested silo.

## CONCLUSIONS

The data accumulated during the operation of the first industrial plants for the storage of cereal grains in nitrogen, allow to draw the following conclusion:

- the nitrogen preservation method can be applied also to industrial plants of large dimensions, obtaining in all parts of the bin condition adequate for the satisfactory preservation of the cereal grains;
- the total nitrogen consumptions measured during the operation of the IVO plant, at near to optimum purge velocity, are about 0.9 and  $2 \text{ m}^3/\text{ton}$  for 1 month and 1 year preservation time respectively (table 3);

TABLE 3: SUMMARY OF NITROGEN CONSUMPTIONS

Plant	Load (ton)	Purge		Maintenance		Total consumptions	
		Flow rate (m <sup>3</sup> /h)	Nitrogen consumptions (m <sup>3</sup> )	Consumptions for 1 month (m <sup>3</sup> )	Consumptions for 1 year (m <sup>3</sup> )	for 1 month m <sup>3</sup> /ton	for 1 year m <sup>3</sup> /ton
IVO	1600	200	1356	120 ÷ 180	1460 ÷ 2190	0.92 ÷ 0.96	1.76 ÷ 2.22
SRM	440	55	366	120 ÷ 240	1460 ÷ 2920	1.10 ÷ 1.38	4.15 ÷ 7.47

- the total nitrogen consumptions measured at the SRM plant are 1.2 and 5.8 m<sup>3</sup>/ton for 1 month and 1 year preservation time respectively (table 3).

The high value of the surface to volume ratio for the SRM silos is responsible for higher nitrogen consumption.

#### ACKNOWLEDGEMENTS

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## CONVERSION OF EXISTING GRAIN STORAGE STRUCTURES FOR MODIFIED ATMOSPHERE USE

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### ABSTRACT

It is uneconomic to provide a complete system of new sealed structures in an existing bulk handling system. Since storages must be sealed if modified atmospheres are to be used, methods must be developed for the sealing of existing structures. A pressure decay time in a storage of 300-10,000 tonnes capacity of five minutes for an excess pressure drop of 2500-1500 Pa or 1500-750 Pa or 500-250 Pa denotes that the structure is suitably sealed. In Australia, all types of bulk grain storage in use, bolted metal shed or bin, concrete cell and welded metal bin, have been sealed experimentally to an adequate standard for use for modified atmospheres. Generalised procedures are given for sealing of these types of storage and the mechanical modification required for application, distribution and removal of modified atmospheres are detailed. Further work is required to define the optimum commercial sealing process for concrete cells and bolted metal structures.

### INTRODUCTION

If the various techniques of modified atmosphere storage either for insect, mould or quality control are to be applicable soon to a significant part of the grain storage industry methods must be developed for making the necessary modifications to existing storages. The existing system represents an enormous capital investment and it is not economically feasible to replace more than a very small proportion of it with structures specifically built to take some form of modified atmosphere treatment. In established bulk handling systems, the rate of replacement of existing structures and construction of new ones is low and thus cannot be expected to contribute a significant quantity of suitable storages quickly.

The provision of a sealed enclosure with correct fitments is crucial to the success of a modified atmosphere treatment. Gas usage is restricted and the possibility of insect survival reduced (see below). The aim of this paper is to show that despite the apparent difficulty of the task, sealing of large existing storages (> 300 tonne capacity) is not impossible, but can be carried out easily and with simple techniques. The variety of the storage structures in use and individual variations in design within a general type necessitate that an intelligent approach be made to the problem of sealing and does not allow a general prescription for success to be given. Nevertheless there are some generalisations, given below, which can be made as a guide to persons wishing to carry out modification of particular storage types. However, before giving a

description of the sealing operations required it is necessary to set a target level of gastightness, which, when achieved, shows that the storage is suitable for use with modified atmosphere.

#### GASTIGHTNESS SPECIFICATION

It is not practically feasible to convert existing structures to completely leak-free enclosures. Some leakage is inevitable, but the magnitude of the leakage tolerable is of concern here. It can be shown (Banks and Desmarchelier, 1979; Banks et al., 1975) that there is a level of sealing at which the gas losses caused by wind and the 'stack' effect are small compared with those resulting from changes in temperature and barometric pressure. There is little benefit in achieving a higher level of sealing than this. If gas loss from temperature and barometric pressure variation were prevented by sealing only dangerous pressure differentials could be generated across the fabric of the structure. In current practice, excessive pressure differentials are relieved by allowing gas interchange through a safety valve and so gas loss still occurs. Methods other than sealing, such as the provision of a heat reflectant roof, must be used to minimise gas loss caused by temperature changes.

Mathematical analysis (Banks and Annis, unpublished data; see Banks et al., 1975 for a similar analysis for freight containers) of the factors causing gas loss from structures and the influence of leak size on the magnitude of the loss, leads to a specification of the 'gastightness' of structures suitable for controlled atmosphere use in terms of the time taken for an applied pressure to decay. In Australia, for structures of 300 to 10,000 tonnes capacity, a decay time of 5 minutes for an applied excess pressure drop of either 2500 to 1500 Pa, 1500 to 750 Pa or 500 to 250 Pa in a full storage is regarded as satisfactory and has been found to be so in practice (Banks et al., 1980). The test range is chosen so that it is the highest usable without unduly stressing the storage fabric of the store. Above 10,000 tonnes capacity, pressure testing is difficult to carry out satisfactorily and requires very stable atmospheric conditions. Below 300 tonnes capacity the standard is difficult to meet in practice and thus must be relaxed despite the consequent higher leakage rate and higher rate of maintenance gas requirement on a per tonne basis. Methods for pressure testing of storages are given in Banks and Annis (1977).

The gastightness specification given above is a design standard, which will give the optimum gas usage performance for modified atmospheres. If a structure fails to meet this standard, it does not mean that it is unsuitable for use with modified atmospheres, but only that leakage may be increased under certain meteorological conditions, notably during high winds, with the result that a higher rate of gas input may be required to maintain the correct atmosphere. The possibility that localised regions may develop where the gas concentrations

are inadequate for insect or mould control is also increased. In contrast to use of nitrogen and other low oxygen atmospheres, if  $\text{CO}_2$  is used for insect control, only a single application of the gas is necessary, if the storage meets the gastightness specification (Banks, 1979; Wilson et al., 1980). The absence of the need for a continuous input of gas to maintain the correct atmosphere is a significant advantage of  $\text{CO}_2$ -based modified atmospheres over low-oxygen ones. However if the specification is not met, additional gas may be required and this advantage is eliminated. A management decision must be made whether additional sealing effort is desirable or whether an increased gas cost and possibility of a control failure can be tolerated.

### SEALING OF DIFFERENT TYPES OF STORAGES

There are three general types of storage. These are classified by their mode of construction and material used: welded metal bins, concrete cells and storages made of bolted sheet metal. Each general type has some common features which usually require attention (see Fig. 1) though individual structures may have other particular problems to be overcome before a satisfactory seal can be effected. Additionally most storages have various access points through the

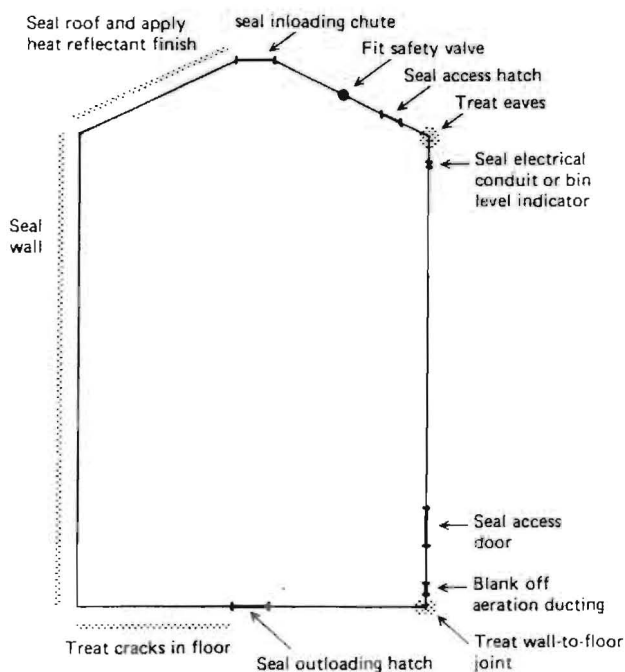


Fig. 1. Diagram of a generalised storage showing areas usually requiring treatment in an existing structure in order to provide a sealed enclosure suitable for modified atmospheres.

fabric of the building, here called 'penetrations', which may require sealing or modification before modified atmosphere systems can be used. A general description of the sealing techniques\* used successfully in Australia for various structural types and penetrations is given below.

### Welded Metal Bins

Detailed procedures for the treatment of welded metal bins have already been given in Banks and Annis (1977).

Welded metal bins are usually relatively easy to seal to the required standard. Many bins are set on a concrete ring beam with a concrete floor. In larger bins the floor itself does not usually require sealing, although the cracks should be filled with a flexible filler (e.g. bitumen emulsion, thiocol or silicone rubber). The junction of the steel wall and concrete ring beam often leaks and must be treated. The region is subject to substantial movement from both loading stresses and thermal expansion and should be treated with a resilient sealer such as a thiocol rubber. A bitumen emulsion sealer, overcoated with a PVC protective film, has also been found successful here (A.D. Wilson, personal communication). In smaller bins (e.g. 300 tonnes capacity) where sealing is more critical, the floor itself may need treatment with a sealer. Materials such as those used to surface industrial floors are suitable. The eave join will require sealing if it is not continuously welded and can be treated with sealant systems suitable for joins in bolted metal structures (see below).

Using these techniques, and after treating the various penetrations (see below), welded steel bins are routinely sealed under commercial conditions in Australia to the level of gastightness specified for modified atmosphere use. Pressure test results from some of these operations have been given in Banks (1979) and Wilson et al. (1980).

### Concrete bins

It is possible to design and construct cylindrical concrete cells to achieve the require standard for modified atmosphere use (see Fig. 2). However most concrete cells are not constructed to be gastight and usually have cracks in the fabric formed during the curing of the concrete and from subsequent movement caused by outloading stresses on the walls. There may also be regions of the fabric which are porous to gases. Even in well-constructed bins this cracking

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\* The classes of sealant compounds, not individual proprietry products, are given in the descriptions below as the availability will depend on where the storage to be sealed is located. Because of the importance of the skill of the applier rather than the exact properties of the compound used, many different brands of the same material may be successful in a given situation.



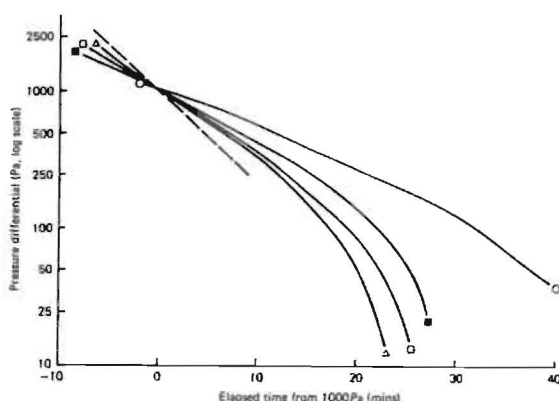


Fig. 2. Pressure decay curves for four 2000 tonne capacity concrete cells, constructed to be gastight and without any sealing treatment for the walls after construction (Gladstone Terminal, Queensland. After five years continuous use. Curves for bins full of wheat, filling ratio 0.95). Dashed line shows the acceptable pressure decay standard.

is often sufficient after operation of the storage for a few seasons to prevent the bin from meeting the pressure test specification.

In the absence of substantial porosity of the fabric, it, in theory, should be possible to treat the individual cracks and thus produce a sealed structure. However we have not found this to be a consistently effective method of giving an overall seal. The network of cracks can be very extensive and it is not possible to tell which cracks are the cause of the leakage and which are only superficial. Treatment of all visible cracks is usually impractical, but on one occasion treatment of the larger externally visible cracks on a concrete cell was found to give a substantial improvement in gastightness (Cooperative Bulk Handling Pty Ltd., unpublished results). This approach has been unsuccessful in other cases (see Fig. 3). In cases where cracks must be sealed it is important to use a resilient material such as a polysulphide rubber as the use of rigid materials may only aggravate cracking (Theimer, 1975).

As an alternative to treatment of individual imperfections, a sealant system can be applied over the whole bin surface. This approach has been used in the past for the sealing of grain storages in various parts of the world (e.g. Japan (Shimizu Construction, 1976); Switzerland (Burns Brown and Heseltine, 1951); USSR (Sergeev et al., 1969)). Currently candidate sealant systems for concrete cells are being evaluated by CSIRO Division of Entomology and the Australian Wheat Board's Coordinating Committee on Silo Sealants under full scale conditions (2000 tonne capacity cells). Full scale testing is being carried out as the

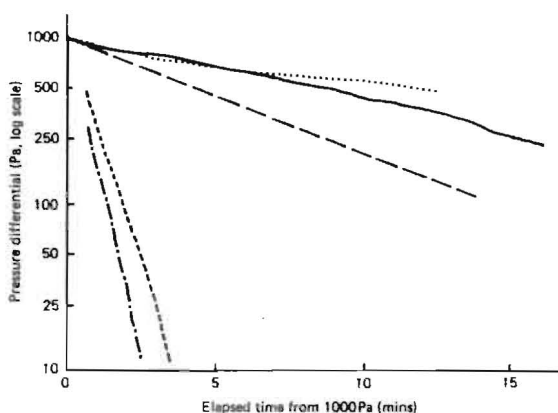


Fig. 3. Pressure decay curves for an 1300 tonne capacity bin, constructed 1935, before treatment (—·—), after coating the walls and roof with an acrylic emulsion (Siloseal) (·····) and use for one season (——) and for a similar and adjacent bin after filling of wall and roof cracks > 1.5 mm across only (-----). (Sydney Terminal, New South Wales) (Curves for bins full of wheat, filling ratio 0.95). Acceptable decay standard (——) also shown.

range of problems which a suitable sealant system must overcome either have not been adequately defined or are not reproducible in the laboratory. In particular, the appropriate combination of flexibility, extensibility and abrasion resistance is not known. (Criteria used for the selection of promising materials is given in Appendix 1). Also less easily quantifiable factors such as ease of application and durability in use are important to the overall success of a system. At present, two internally-applied coating systems, a styrene-acrylic and a polychloroprene-based material show particular promise as cheap, effective sealants. Both are applied as water-based emulsions. Fig. 3 shows the improvement in pressure test results after applying the styrene-acrylic system. The other system gave similar results.

The wall-to-roof join in a capped concrete cell is often designed without any form of seal between the bin wall and the roof slab. This region can leak significantly and must be carefully sealed. It is subject to substantial thermal and mechanical movement and thus requires a flexible sealant system. A flexible cornice made from a woven fibreglass mat, fixed around the inside of the join and coated with a thick flexible sealer such as a styrene-acrylic emulsion, has been used successfully here.

In many concrete cells the roof does not rest directly on the cylindrical wall. These 'open-topped' cells require the construction of some form of capping to close off the cell. Various forms of closure based either on membrane systems or sheet polystyrene-metal sandwich materials supported on normal roof trusses

are under test at present in Australia. Any such system is likely to appear expensive if viewed simply as a component of the costs of conversion of a storage to controlled atmosphere use, but the cost may be partly offset by the other advantages it provides, notably dust control.

#### Bolted Metal Structures (Bins and Sheds)

Many grain storage structures are made of sheet metal bolted or riveted together and fixed to a metal or wooden framework. They are often provided with open eaves and ventilators on the roof ridge. Tobacco stores, which are often constructed similarly, have been sealed routinely in the United States using a PVC coating system for the retention of fumigations (Roop, 1949; Anon., 1972). A similar technique presumably could be used for large grain storages and has been successfully demonstrated in Australia on a small farm storage bin (2 tonnes capacity) (Banks and Annis, unpublished data). It does not seem necessary to treat the complete structure with the sealant, as was done with the tobacco stores but only the lap joints, boltheads and other leak prone regions. A cheaper cosmetic and protective coating could then be applied over the whole storage.

Recently a shed-type storage of 16,400 tonnes capacity was sealed in Australia as a demonstration of the feasibility of sealing such large structures (Banks et al., 1979). The shed was constructed from corrugated galvanised iron sheeting, supported on a portal frame with A-frame wall supports and before treatment had ventilated eaves and roof ventilators. The eaves and ventilators were covered in with galvanised iron sheet riveted to the structure. All joins were then coated with a thick styrene-acrylic emulsion, reinforced where movement was expected with woven fibreglass tape embedded in the sealant. Silicone rubber sealant was used in some movement-prone joins. Larger holes and leaks, such as where the corrugated roof met the flat ridge capping, were filled with polyurethane foam, formed in situ.

After sealing, the roof and end gables were coated with a white acrylic paint as a heat-reflectant and protective treatment. Application of a heat-reflectant treatment to the roof is a necessary part of the modification of storages with large roof areas and headspaces for modified atmosphere use. Temperature changes are a major cause of gas loss in such structures and the treatment reduces the magnitude of the diurnal temperature variation in the headspace and thus the quantity of gas loss from this source. Pressure test results obtained over three seasons are given in Fig. 4. The sealed structure was successfully treated with CO<sub>2</sub> for insect control (Banks et al., 1980).

Other sealant systems (e.g. some polyurethanes) appear suitable for bolted metal structures and require testing. It is not yet certain which system will give the best results. However, as with the sealing of concrete cells, a major factor in the success of a sealing project is the skill of the applier and his

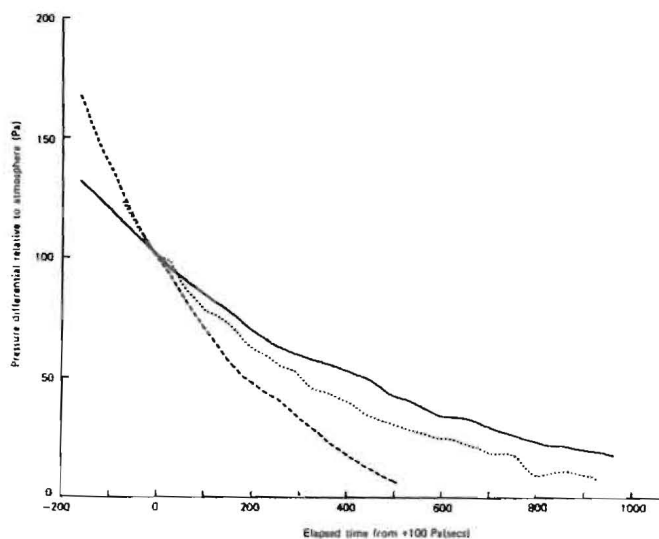


Fig. 4. Pressure decay curves for a 16,400 tonne capacity shed-type storage sealed as described (see text). Tests after sealing (—), after one seasons use (.....) and after two seasons use (-----). Minor maintenance only carried out between seasons. (Curves for shed filled to capacity with wheat, filling ratio 0.58).

awareness of how the sealing system must be applied rather than the particular treatment used.

Sealing methods for farm-scale storages (< 300 tonnes capacity) are detailed in Williams et al. (1980).

#### SEALING OF PENETRATIONS

Grain storages usually have a number of fitments in the structure fabric which must be sealed in order to provide an enclosure of adequate gastightness. Correct treatment of these penetrations is very important as they are often major sources of leakage. These fitments include access doors, in- and outloading chutes, electrical conduit and aeration fans and ductwork. In many cases, they may be sealed suitably by ad hoc methods. Although expensive, silicone rubber building sealants and polyurethane foam can be very useful materials for this, providing a removable, but durable seal. In our experience, cheaper methods, such as taping or sealing with butyl mastics as recommended by Jay (1971), can be unsatisfactory in practice and jeopardise the overall success of a sealing operation. Taping with PVC tape can, however, be used to reinforce sealed joints treated with silicone rubber.

Some penetrations, particularly outloading valves, because of their design and accessibility, are often very difficult to seal satisfactorily and may require structural modification or even complete replacement in order to ensure

that a good seal can be obtained reliably. Gastight valves of the type used in recirculatory fumigation facilities are suitable for modified atmosphere use.

Doorways into large storage sheds may also present problems for sealing. Spraying of the regions which may leak with a strippable PVC film has been recommended (Roop, 1949). Doors may also be sealed by judicious use of foam polyurethane or of silicone rubber sealants, reinforced with fibreglass or fabric open mesh tape where appropriate. In some cases, it may be better to replace the doors completely with a specifically designed gastight system rather than to attempt to seal the existing ones.

### MECHANICAL REQUIREMENTS

If modified atmospheres are to be applied to a storage not specifically designed for their use, some mechanical modifications are required in addition to general sealing. These have been set out by Jay (1971) for  $\text{CO}_2$  and Banks and Annis (1977) for modified atmospheres generally. The modifications are to allow the initial introduction of the gas to make the required atmosphere, to provide a means of maintaining the correct atmospheric composition, to prevent structural damage to the sealed structure from excessive pressure differentials across the enclosure walls and sometimes to provide a means of replacing the modified atmosphere with normal air.

#### Gas Handling and Distribution Systems

Either nitrogen or  $\text{CO}_2$  can be introduced efficiently into cylindrical grain storages through inlet systems fitted into the base of the bin wall without additional ductwork. The dimensions of the introduction pipework are dependent on the back pressure which can be tolerated by the gas production system. In Australia, 8 cm diameter pipework is normally used, which can conveniently handle the purge rates currently applied (e.g.  $6 \text{ m}^3 \text{ min}^{-1}$ ). Precautions must be taken to ensure the end of the introduction pipe does not become blocked with grain. A simple shielded inlet system suitable for this has been described (Banks and Annis, 1977). Using a single inlet of this type, satisfactory purging efficiencies have been obtained even in large squat cylindrical storages (73% for nitrogen; 79% for  $\text{CO}_2$ , see Bordertown II and IV trials and definitions in Banks (1979)). Since high purging efficiencies can be achieved in cylindrical bins with simple introduction systems, there is little scope for improvement using more complex systems incorporating ductwork within the storage. However, existing ductwork such as fitted for aeration can be used conveniently for gas introduction and may provide a slight improvement in purging efficiency.

Even in horizontal storages, with large length to height ratios, relatively high gas usage efficiencies can be obtained with  $\text{CO}_2$  introduced from a single

point. An efficiency of 73% was obtained in a 16,400-tonne capacity shed with gas introduced at one end of the storage, improving to 92% using a longitudinal perforated introduction duct (Banks et al., 1980). The high efficiency achieved with  $\text{CO}_2$  can be attributed to its density which causes the gas to layer horizontally even in large structures. No studies have been carried out on the introduction of nitrogen into large horizontal storages, but presumably, because nitrogen is similar in density to air and thus, unlike  $\text{CO}_2$ , has little tendency to layer when introduced, some introduction ductwork will be necessary in order to achieve adequate purging efficiencies with this gas.

With modified atmospheres which are to be maintained indefinitely, a system of external pipework is required to bring additional gas into the storage in order to compensate for leakage and keep the modified atmospheric composition correct. For gases lighter than air, i.e. nitrogen, the additional gas is introduced into the top of the storage; for those heavier, at the base (Banks and Annis, 1977). Two systems are available for maintenance of a modified atmosphere: a demand system, where gas is introduced when the pressure or gas concentration in the storage falls below a set level (Jay and Pearman, 1973; Shejbal, 1979) and a continuous system (Shejbal and Di Maggio, 1976; Banks and Annis, 1977), where gas is fed in at a constant rate. On this basis of available data there appears to be little difference in gas consumption using the two processes (Banks, 1979), but they have not been rigorously compared. In either case, if sealing has been adequate, the gas flows required are likely to be small and thus narrow bore pipework (e.g. 1 cm diam.) will be suitable. A steel 2000-tonne bin sealed to the standard given above requires about  $25 \text{ l min}^{-1}$  of nitrogen (Banks and Annis, 1977).

For 'one-shot'  $\text{CO}_2$ -based modified atmosphere systems, where no maintenance gas is used, it is necessary to keep the internal gases mixed in order to prevent formation of regions of inadequate  $\text{CO}_2$  concentration for insect control (Wilson et al., 1980). This can be accomplished by recirculating the storage atmosphere from the base into the headspace via external pipework using a small sealed blower. A recirculation rate of about 0.1 volumes per day has been found to be adequate for bins and a shed (Banks and Annis, unpublished data). Recirculation can also be used to maintain a low-oxygen atmosphere with a propane-fired catalytic converter. Navarro et al. (1979) used a recirculation rate through the converter of about  $300 \text{ l min}^{-1}$  for this on a 1200-tonne bin. At such a rate only narrow pipework (e.g. 3 cm diam.) would be required.

### Pressure relief valves

Pressure relief valves are required in sealed storages to prevent damaging pressures from developing in storages as a result of temperature and barometric pressure variation, wind effects or excessive rates of gas introduction in the absence of adequate venting. Simple systems suitable for sheds (Banks et al., 1979) and grain storage bins (Banks and Annis, 1977) have been described. However, both systems would benefit from further design work to provide cheap, rapidly acting systems. The pressures at which such relief valves should operate is dependent on the design of the storage and the pressure differential it can tolerate. In many cases and particularly for sheds, this differential can be small, e.g. 250 Pa.

### Removal of the modified atmosphere

A means of venting the atmosphere must be provided to enable personnel to enter a storage treated with a controlled atmosphere for outloading or inspection. In small storages, natural ventilation may be sufficiently rapid for this when access hatches and other penetrations are opened. In large storages forced ventilation is required if it is necessary to enter the storage within a few days after unsealing. Sealable ventilation fans can be fitted to ventilate the workspace when required. A fan capable of an extraction rate of  $500 \text{ m}^3 \text{ min}^{-1}$  was fitted in each gable end of a 16,400 tonne sealed storage treated with  $\text{CO}_2$  was found adequate. This allowed access within 24 hours. Such large extraction fans are also useful for dust control within the sealed storage during grain movement. In the absence of some form of ventilation in large stores, the air may become dust-laden within the store making working conditions there unpleasant and creating a possible dust-explosion risk.

### CONCLUSION

At present in Australia only welded steel bins, treated as set out above, are in commercial use with modified atmospheres. However the techniques for the conversion of both concrete cells and shed-type storages to take modified atmosphere treatments have been demonstrated experimentally. The cost of commercial sealing of large horizontal sheds (> 15,000-tonnes capacity) to an adequate standard of gastightness for controlled atmospheres will vary with method of construction but will probably lie between \$A2-5 per tonne capacity, while the treatment of capped concrete cells is expected to be about \$A10 per tonne capacity for a 2000-tonne cell. Where a choice is to be made, this cost difference is likely to favour conversion of horizontal storages. The sealing of several more large horizontal storages in Australia is currently under consideration.



With the conversion of a significant number of existing facilities to a sealed condition, it will be possible to assess the problems and potential of the various forms of modified atmosphere storage under the operational constraints of the industry and not at a restricted experimental level as hitherto. The experience gained in carrying out the modifications should be of great assistance in optimising sealing strategies and thus, as sealing is a major cost component of modified atmosphere storage, reducing the overall cost of the process and giving a viable system for use with existing structures.

#### ACKNOWLEDGMENTS

This work was carried out with the financial support of the Australian Wheat Board. The experimental sealing of a concrete cell at Sydney terminal was carried out and financed in part by Gardner Bros. Pty., Sydney. All sealing trials reported here have been carried out on storages owned by the various State Bulk Handling Authorities and we are grateful for their assistance in these projects.

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## APPENDIX I.

### Criteria uses for the selection of candidate sealants for internal application to concrete cells

- (1) The sealant system should be able to bridge cracks up to 1.5 mm across so to create a continuous film which will not fail when opened to 2 mm during outloading.
- (2) It must be sufficiently plastic or elastic not to crack when imperfections in the structure open under pressures from the filling or emptying of the bin. A 100% extension on 0.3 mm crack should be withstood without failure.
- (3) Because of high application costs, the system should be effective using 3 or less coats.
- (4) Good adhesion to old concrete is required. A primer may be necessary. The coating must be able to withstand  $\pm 2500$  Pa on a 1 mm wide crack, 30 cm long without loss of adhesion.
- (5) The sealing system must be easily repairable if damaged.
- (6) The coating must be suitable for use with foodstuffs. Data should be available to show no adverse effects on direct contact with the sealant.
- (7) The coating must not creep or perish.
- (8) The coating should have a significant degree of abrasion resistance. A different material is acceptable in high wear areas.
- (9) It is essential that the material is stable to gaseous methyl bromide or phosphine fumigants and is unaffected by high CO<sub>2</sub> levels (e.g. 60%).
- (10) U.V. radiation resistance for the coating is not required.
- (11) The coating must at least be stable between 0-42°C.
- (12) The total system should have at least a 10 year life but it is acceptable that easily accessible areas such as the outloading cone may be repaired prior to this.

## PNEUMATICALLY FORMED REINFORCED CONCRETE DOMES FOR GRAIN STORAGE FACILITIES, BUILT WITH BINISHELLS TECHNOLOGY

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### INTRODUCTION

The purpose of these notes is to explain in summary the Binishells patented technology for the pneumatic formation of reinforced concrete domes and some of the main advantages offered by such Binishells structures for the realization of facilities for artificial controlled atmosphere storages of grain.

Our experiences are as designers and builders of reinforced concrete structures and we wish to put them at all the silos experts disposal (International Public Bodies, scientists, designers, consultants, builders and utilizers) who may contribute to the solution of problems concerning the storage and the preservation of cereals in the world.

We do hope that from these information on a technology of construction, collaborations with the experts of different activities may come out.

In fact, instead of suggesting solutions with products and/or equipment already defined, we believe it is great more useful to propose technologies which may be employed with the collaboration of experts and by using the local resources and manpower existing in each country of utilization.

In the areas which are typical of a developing agricultural economy, above all, everybody knows which are the negative economical and social repercussions, deriving from the imposition of outside system and products, which do not involve either the local technicians or workers and then cause instinctive refusals, as the well-known lack of maintenance, etc.

The Binishells technology allows the construction of dome-shaped structures of different sizes, up to 36 - 40 m in diameter (that is a capacity of cu.m 7.500 - 10.000 each) by using local resources (unskilled manpower and conventional materials), reducing the importations to the minimum and offering many advantages, such as :

- . building rapidity, i.e.: a complex of 100 domes of 32 m dia., with a total capacity of about cu.m 550.000 is built in Pakistan in 200 days;

cost economy : up to a saving of 40+60% in comparison with concrete storages built with conventional systems. Further considerable advantages are obtained by making such structures gas-tight.

#### BINISHELLS PATENTED TECHNOLOGY

Some information on the technology are given here below.

This new building system is based on the pneumatic formation of reinforced concrete structures, i.e. upon the use of the pneumatic dynamic form which lifts all the building materials from the ground, e.g.: fluid concrete, steel reinforcement, etc.

The main special equipment and the different phases of the process are illustrated below.

#### The Foundation

The foundation consists of a small circular continuous beam (e.g., for a 36 m diameter dome, the section is 60 cm by 85 cm) which is built in a standardized way. The load on the ground is very low, by virtue of the dome's lightness and monolithicity. Before casting, a special tubular form (4 - fig.1) for the anchoring of the internal pneumatic form is inserted in the foundation. This form is inflated before casting and, subsequently, after the concrete has set, deflated and removed (1 - fig.2) to leave an eye-let for insertion of the tubular anchorage connected with the edge of the internal pneumatic form.

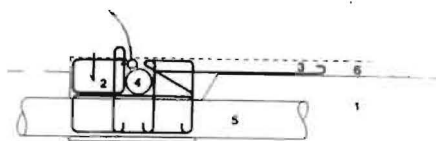


Fig. 1

#### Internal Pneumatic Form as an uplifting system

The pneumatic form consists of a membrane in nylon reinforced neoprene, with tubular anchorage at the edge (2 - fig.2). It is anchored to the foundations by the above illustrated system and allows both air tightness and mechanical resistance.

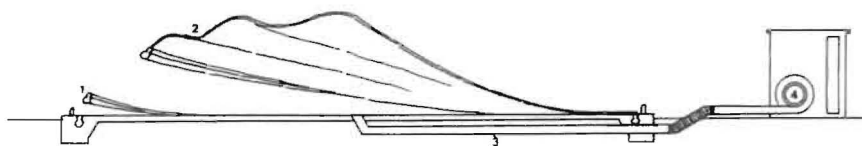


Fig. 2

#### Pumping Station

The pumping station consists of one or more low pressure electric blowers.

Air inlet and outlet are made through pipes in P.V.C. or other materials (5 - fig.1), under the floor. The shape of the structure is stabilized by air circulation, i.e. by pumping air into the pneumatic form and letting out small quantities to avoid any movement of the structure (1 - fig.7).

The internal pressure needed to lift the membrane and construction materials is of few hundredths of an atmosphere (from 0,03 to 0,05 Atm.).

### Steel Reinforcement

Steel reinforcement consists of a mesh of spirals (4 - fig.3) plus additional reinforcing bars (5 - fig.3). The mesh is made of steel spirals hooked to an external bar anchored to the foundations (3 - fig.3).

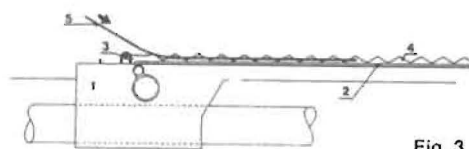


Fig. 3

These spirals are manufactured at close pitch; therefore they take up little space and can be easily packed and carried to the job site. Each structure requires 10-15 types of spiral lengths (fig.4).

Reinforcement consists of straight steel bars, varying from 5 or 8 mm in diameter. This is placed inside the spirals and left free to slide (fig.4, dark lines).

Placing is easily carried out directly on the membrane at ground level, following a standard drawing and carrying out the same operations for any size of structure. During uplifting the spirals stretch and the bars settle and slide, maintaining the required position with the laps needed to ensure the continuity of the reinforcement.

The spirals have several functions, the most important of which are : - to prevent the concrete from sliding during the uplifting - to guarantee concrete thickness - to uplift and settle the reinforcing bars - to control the uplifting and the shape.

The shape of the structure is defined both by the type of pneumatic form and spirals. Laying of reinforcement takes 1-2 days for structures having dimensions

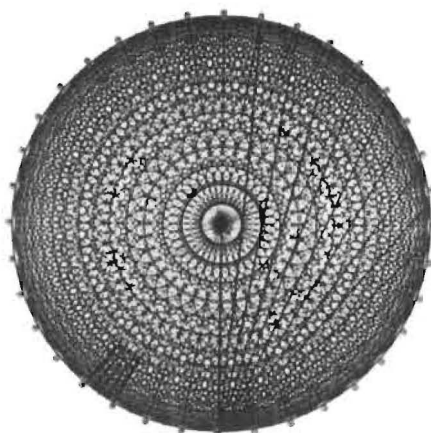


Fig. 4

varying from 12 or 36 m in diameter, with a team of 6-8 workers.

Uplifting of Concrete and its Characteristics

Concrete consists of a regular mix with a high workability and is placed on the pneumatic form at ground level. The characteristics of the mix are usually:

Sand	60%	Water/cement ratio	0,50
Gravel (max 12-15 mm)	40%	Abram's slump	16+18 cm
Cement	400 Kgs/cu.m		

Retarders and plasticizer additives, easily available and in such doses as not to alter strenght of the concrete, are used in order to allow the placing, uplifting and vibration of the concrete.

Placing is usually carried out with concrete truck mixers. Concrete is placed on the membrane at ground level and spread by conventional methods (fig.5).

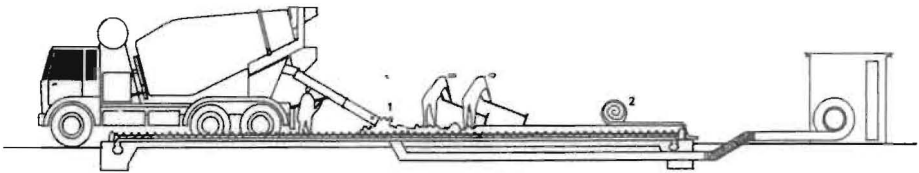


Fig.5

During uplifting as the surface increases, the thickness both of the spirals and of the concrete decreases.

However, the whole system is calculated in order that the final shape assures the correct concrete thickness for the complete covering of the steel reinforcement.

As already explained, the concrete sliding during the uplifting, is prevented by both the spirals and P.V.C. sheet (external membrane).

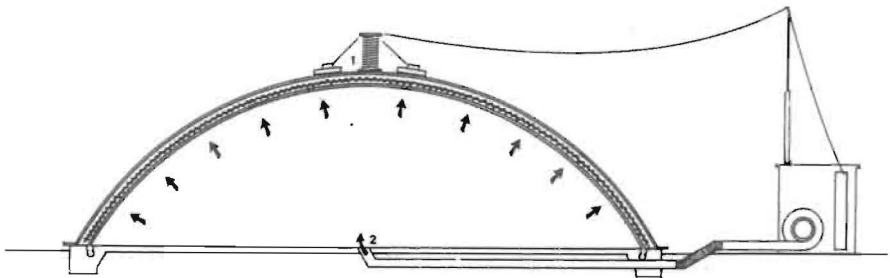


Fig. 6

### External Membrane (= P.V.C. sheet)

Before uplifting, a P.V.C. sheet is laid on the concrete and anchored to the foundations in a simple and quick way (2 - fig.5).

During the uplifting, the P.V.C. sheet is put under stress, compressing the concrete (fig.6).

Its functions are mainly: - to protect the concrete both from rain and strong evaporation due to the sun - to help hold the concrete - to allow vibration.

### Vibration

The vibration equipment is placed in the centre of the structure before the uplifting and lifted with the other materials (1 fig.6). Vibration is carried out when uplifting is completed, by means of high frequency vibrators fixed on rolling carts (3 fig.8). These rolling carts, anchored to a cylinder placed at the top of the structure, are pulled downwards, drawing paths which are similar to helicoids.

Each vibrator makes a path partially overlapping the one made by the previous vibrator, so obtaining a total tamping and vibration of the surface.

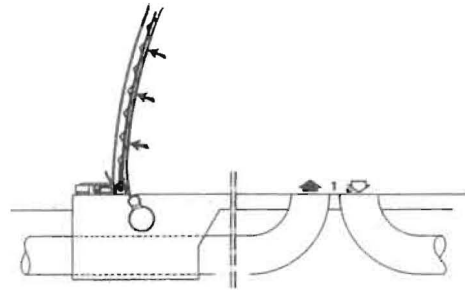


Fig. 7

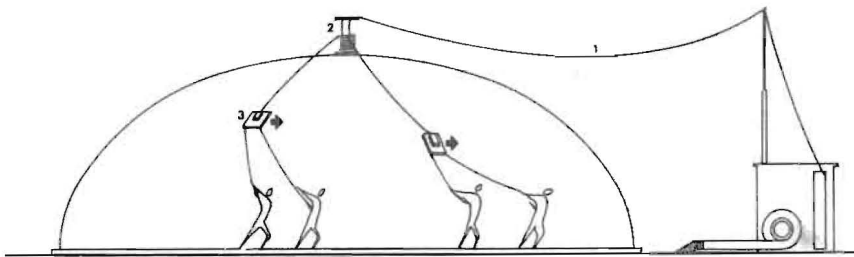


Fig. 8

### Setting of the Structure and Deflation of the Membrane

After stabilization and vibration, the concrete of the structure sets and hardens between two highly waterproof membranes, which allow a gradual evaporation of water and protect it against atmospheric agents (fig.7).

The device used, the controlled evaporation of water, the acceleration of setting due to both its own heat and sun irradiation, allow the elimination of any shrinkage.

and the obtaining of a perfect monolithic structure.

Usually, the membrane is deflated after 1-3 days, according to the size of the dome and to atmospheric conditions.

Before deflation, tests are carried out on the concrete and hence deflation of the membrane is carried out without risk.

Sometimes, it happens that, due to particular atmospheric conditions and to strong sun irradiation, setting is remarkably accelerated.

After an adequate period of time, an opening is cut in the structure to enter and remove the membrane for re-uses (3 - fig.9).

#### Opening in the Structure

Cuts are made with normal rotary saws or with other simple tools. Before making the cuts, if so required by calculation, reinforcement may be added in reinforced concrete or steel members (4 - fig.9).

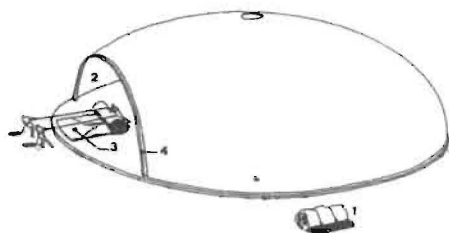


Fig. 9

#### Transportation and Re-uses of Equipment

All equipment are easily carried on one lorry and there is the possibility of a quick move, with remarkable savings (1 - fig. 9).

Equipment investments are small and their amortization has a low incidence because of their many re-uses.

#### Insulation and Finishings

Binishells domes are reinforced concrete monolithic structures, and their finishings, i.e. thermal and acoustic insulation, are carried out with materials which are normally available on the market.

#### Building a dome time

The usual time for the construction of a dome is hereunder outlined :

- . laying of the internal membrane and equipment, etc.: 1 day,
- . laying of the steel reinforcement, etc.: 1+3 days (according to size),
- . cast of concrete, uplifting and vibration, etc.: 0,5 days,
- . set and hardening of concrete and deflating of membrane, etc.: 1,5+2,5 days.

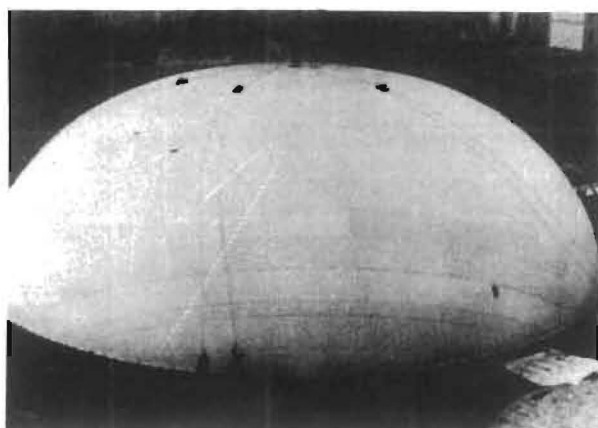
A mass production can be organized by a contemporary employ of more than one pneumatic form. For example: a production of 4 domes per week (100 domes in 200 days) can be realized with 4 membranes and three teams of workers.



Casting of concrete at ground upon the internal membrane and steel reinforcing, already laid, for a structure of 36 m dia.

Centre: steel reinforcing not covered yet by concrete - Right: folded P.V.C. sheet - Left: truck mixer and concrete pump.

Starting the inflation :  
P.V.C. sheet not yet under tension.  
Equipment for vibration is placed on top.



Inflation is completed and P.V.C. sheet is under tension. Vibration is carried out by means of vibrators which are driven one after the other, around the dome surface, from top to base.

The whole work here illustrated lasts from three to six hours.



## ADVANTAGES OF BINISHELLS STRUCTURES FOR STORAGE FACILITIES

The main advantages are summarized here below.

### Complete utilization of local resources :

infact, materials and unskilled labour to be found on-the-spot are utilized. Moreover, the construction can be carried out by local organizations and through licence agreements.

### Economy in the costs is due to :

- reduced quantity of labour and materials required for the construction (due also to the reduced thickness of the structures: 4-8 cm);
- small dimensions of the foundations, which have a continuous circular shape, and limited depth (the load on the ground is limited to 0,6-0,8 Kgs/cm<sup>2</sup>.);
- simplicity of the equipment, their easy transportability and large re-use in subsequent utilizations;
- reduction of the transport costs and investments for building equipment;
- elimination of maintenance costs ;
- reduction of overheads due to the rapidity and simplicity of execution.

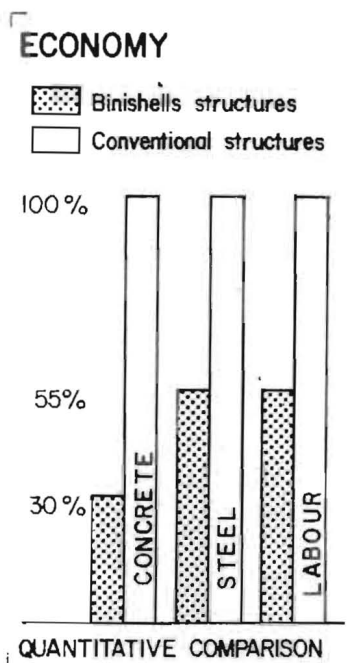
### Rapidity and simplicity of execution :

A 36 m diameter structure having a capacity of 7.450 cu.m is built in 6-8 days by using simple equipment and by a reduced team of workers.

The simplicity of the foundations, moreover, eliminates unforeseen events and ground consolidation.

### Long durability, high resistance and minimum maintenance :

The structures are monolithic, in reinforced concrete, and completed with a conventional and economical external waterproofing and do not require any maintenance. Moreover, the Binishells structures have an enormous natural resistance to all stresses, such as: external loads, earthquakes, typhoons, pressure waves, etc. They can also be covered with earth and camouflaged.



Possibility of utilizing the structures both for bulk and bags storage:

This advantage is shown in two aspects:

- simple warehouses for bags (domes with one or two doors) can be built at the beginning, and subsequently completed with fixed or mobile economic systems for filling and discharging which can be added in a simple way; then changing of a Binishells warehouse for bags into a bulk storage is very simple ;
- if the structures are equipped with the mechanization from the beginning, the same plant can be utilized both for bulk and for bags so that different situations which may occur along the year, can be easily faced.

Possibility of building gas-tight structures :

Such operation simply requires the application of a special paint on the inner surface of the dome. Also such application can be done from the beginning or subsequently. The Binishells structures are monolithic, without any crackings, joints, etc. and thus allow an easy application of the paint and a safe tightness in the future.

The paint employed by us was hardly tested by Snamprogetti and found completely suitable.

POSSIBLE TYPE OF STORAGE FACILITIES

Among the large range of possibilities of utilization of the Binishells structures, some of the most significant solutions are listed here below.

With regard to building (B), we can have the following classification :

- B.1. Flat bottom with domes at ground level :
  - B.1.1. with entry door - B.1.2. without entry door ;
- B.2 Flat bottom with dome built on a cylinder (to increase capacity) :
  - B.2.1. with entry door - B.2.2. without entry door ;
- B.3. Inclined bottom :
  - B.3.1. with only one cone - B.3.2. with multiple hoppers.

With regard to shape (S) of structures, we can have :

- S.1. shape with ellipsoidal section (of height H and base diameter D, with  $H=1/3 D$ ),
- S.2. shape with parabolic section (of height H and base diameter D, with  $H=1/3 D$ ),
- S.3. other shapes (square base, etc.)

With regard to dimensions (D), we can have bins of any dimensions :

As an example, here below we state dimensions of the ellipsoidal shape standard

structures with relevant capacities for the flat bottom solution.

D (m)	H (m)	V (cu.m)
15	5.00	600
20	6.30	1,360
25	8.00	2,580
32	10.20	5,450
36	11.00	7,450
40	12.00	10,050

With regard to combination (C) of several bins there is the possibility of a large range of compositions placing several similar or different domes side by side, i.e.:

C.1. with N° 1 bin - C.2. with N° 2 bins

C.3. with N° 3 bins - C.4. with N° 4 bins .....

... C.n. with N° "n" bins.

With regard to equipment and mechanization (M) we can have :

M.1.-Simple equipment for: doors, ventilation and/or lighting, but without filling and discharging equipment.

M.2.-Mobile or semifixed simple mechanization :

M.2.1. filling and discharging through screw conveyors in mobile elements assemblable one to the other.

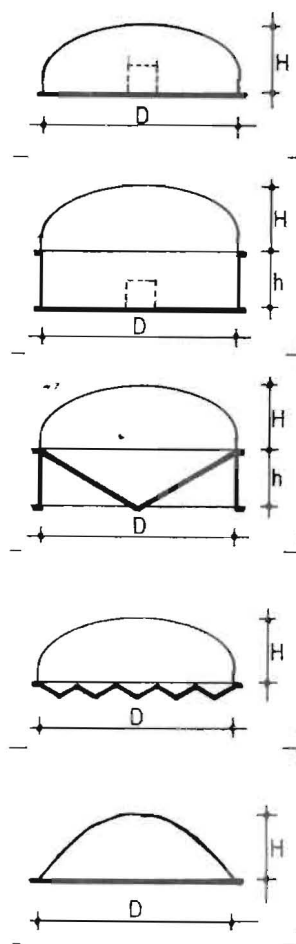
M.2.2.-filling through chain conveyors fixed to the structure - discharging through screw conveyors in mobile elements assemblable one to the other.

M.2.3.-filling through chain conveyors fixed to the structure - discharging through belt or chain conveyors in adit.

M.2.4.-filling through chain conveyors fixed to the structure - discharging through belt or chain conveyors in adit with turning extractor conveying the material in the discharging hoppers.

M.3.-High Mechanization

M.3.1.-filling through centralized mechanization with a distribution made through pipes which allow a natural gravitational fall - discharging through belt or chain conveyors running in adit with turning extractors conveying the material



in the discharging hoppers.

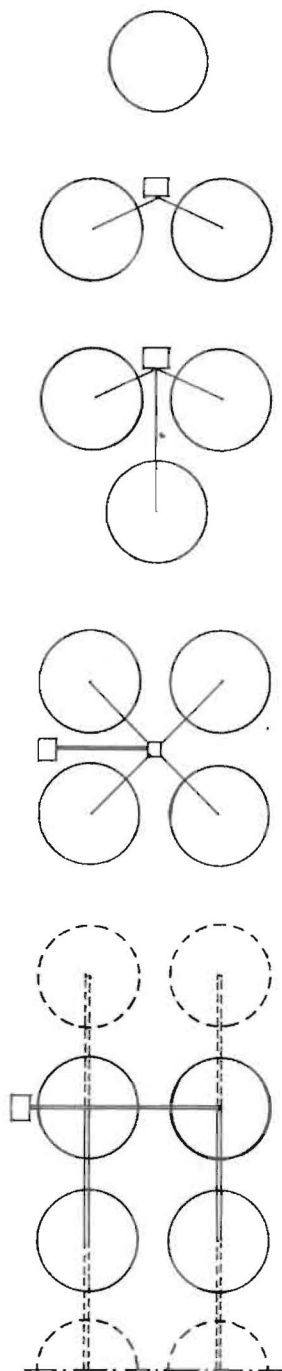
M.3.2.-filling through centralized mechanization with a distribution made through horizontal chain conveyors - discharging through belt or chain conveyors running in adit, with turning extractors conveying the material in the discharging hoppers.

With regard to use of controlled atmosphere (C.A.) :

it can be adopted both for the use in bulk and in bags for any kind of Binishells structures.

#### GAS-TIGHT COATING

"Gas-tight coating" (IDEA-SEBINO) is a transparent varnish especially elaborated for cement gas-tightness. This varnish is an one-component product forming a very hard, elastic film, highly resistant to abrasion and to effect of most chemicals, which finds its application on porous bases (concrete in particular), efficiently permeates those bases and insures a perfect gas-tightness. It is applied preferably with a brush or a roller on a dry and dust-free surface, with normal precautionary rules regarding varnishes. It may be applied in one or more coats, with interval of 12 hours as a minimum and of 24 hours as a maximum . The film becomes hard according to DIN standard 53.157 of about 165" for a thickness of 40 microns. The abrasion is of 5 mg, according to the Taber CS 10 abrasion table for a pressure of 1000 g. The film thus formed, once hardened, is characterized by its high degree of transparence as well as its high elasticity, and its slight yellowing when exposed to light. At the end of 14 days after applying the final coat, under a temperature of 20° C and an ambient relative humidity of 50%, the maximum chemical resistance of the varnish is obtained.



## ECONOMIC ASPECTS OF NITROGEN STORAGE OF GRAINS.

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### ABSTRACT

In this paper the economics of the nitrogen preservation technique of grains is compared with other storage methods, such as turning of the cereal grain bulk, disinfestation-fumigation, ventilation and refrigeration.

A complete analysis of storage center components is carried out. However, since the main objective of the work is the comparison between the various storage technologies, those cost items which can be considered equal in the different techniques or are independent of these are not quantified (general costs, personell etc.).

The economic evaluations carried out on the basis of direct industrial experience and literature data show that:

- nitrogen storage of grains is competitive in respect to other storage systems;
- the problem of gastightness of the silos, when treated at the moment of the silo construction, can be solved with no substantial increase of costs;
- the operating cost determined by nitrogen consumptions depends on the mode of supply of the gas and on the size of the storage plant. In any case this cost is in the order of the cost of chemical disinfections or lower for storage complexes of large dimensions (over 50,000 tons).

### INTRODUCTION

The scope of this work is to furnish evaluation elements on the application of the nitrogen technology for grain preservation in comparison with the traditional systems.

A homogeneous comparison between the different technologies is very difficult because grain preservation plants have design and construction characteristics which are always very different and can significantly influence the unit operating costs.

In order to perform the study, it was necessary to accept simplifying hypotheses to fix a common basis for the comparison. Two analyses were performed:

a) Analysis of the costs concerning the preservation process only

In this case, only the costs of the specific equipment used and of the utilities consumption for the application of the preservation process have been considered.

b) Full analysis of all costs of a storage center

This analysis is more correct since the choice of the preservation technology may have an influence over the magnitude and the distribution of all the costs that form the final preservation cost.

However this analysis is limited to the definite realization considered.

The results of the two types of analyses allow to obtain a picture, complete enough, of the economic aspects of the nitrogen preservation technology.

These evaluations should be considered preliminary and the costs data only indicative values since they were worked out on the basis of data coming from different, non homogeneous sources.

#### ANALYSIS OF THE COSTS CONCERNING THE PRESERVATION PROCESS ONLY

In this analysis we consider only the costs of the specific equipment used for the application of the preservation technology and the costs relevant to the utilities consumption for the operation of these facilities.

All the other costs:

- the cost of the silos
- the cost of the auxiliaries, roads, land etc.
- the cost of the grain conveying mechanization
- the cost of maintenance, handling etc.

are considered to be constant and unaffected by the preservation technology.

This is only a hypothesis because additional costs for the gastightness of the silo are required for the controlled atmosphere preservation technology.

Nevertheless gastight silos (galvanized seamed sheet silos, dome shaped reinforced concrete silos etc.) may cost less than non gastight (concrete vertical silos, vitrified sheet-steel etc.).

On the other hand the operating simplicity connected with the use of the nitrogen technology allows considerable reductions in the labour costs. In fact some typical operations of the traditional systems are completely excluded, such as the frequent controls of the stored product, its turning and, above all, the treatments with chemical disinfectants that require the assistance of specialized personnel.

The other preservation technologies considered for the comparison are:

- turning of the product and chemical disinfestation
- ventilation and chemical disinfestation
- refrigeration.

Through the above mentioned hypothesis, the comparison is not homogeneous if the efficiencies of the different technologies are not taken into account.

It is not possible to compare on the same basis "preventive" and very efficient technologies like controlled atmosphere or refrigeration and "curative" and/or less efficient technologies like turning, ventilation and chemical disinfestation.

A common basis may be found with one of the two following assumptions:

- the initial characteristics of the preserved product being equal (moisture content equal to the critical value for example), we can evaluate an average loss of product relevant to each preservation technology;
- the results of the preservation being equal (zero loss for example), we can evaluate the savings permitted by the more efficient technolo-

gies that allow to preserve poor quality products and/or with higher moisture content.

1. Comparison at the initial characteristics of the preserved product being equal.

In table 1 the investment cost and the running cost are summarized for each preservation technology:

- for turning the investment cost refers to the temperature control and chemical distribution facilities; the running costs refer to the electrical energy consumption for turning and to the chemical consumption;
- for ventilation the investment cost refers to the fans, distribution pipes, temperature control and application of chemicals; the running costs refer to the electrical energy consumption for ventilation and to the chemical consumed;
- for refrigeration the investment cost refers to the cooling plant, fans and distribution pipes; the running cost refers to the electrical energy consumption;
- for controlled atmosphere technology it is assumed to supply the plant with liquid nitrogen, so that the investment refers to the gas distribution and control plant; the running cost refers to the nitrogen consumption.

For all the investment, cost depreciation is estimated at a rate of 17% per year. In table 1 the average loss of product that can be estimated for each preservation technology is also indicated. These values arise from the existing literature data and from direct experience acquired.

Losses are indicated in certain ranges, since they are statistical values. Furthermore, all other conditions being equal, they depend on variable basic data, namely:

- on product physical characteristics (fragility and pulverizability, presence of impurities in the interstices, etc.);
- on the evaluation of quality losses: they differ in connection with the different utilization of the product, with the basic value as marketable goods, etc.



TABLE 1 SAVINGS ALLOWED BY THE NITROGEN TECHNOLOGY IN COMPARISON WITH OTHER PRESERVATION SYSTEMS (ITALY - 1978)

	Transfer of products and chemical di- sinfestation	Ventilation and chemical disinfestation	Refrigeration	Nitrogen technique
Investment cost for preservation installations (\$/ton)	1.0	2.0	6.0	5.0
- ammortization (\$/ton)	0.2	0.3	1.0	0.8
- operating cost (\$/ton)	0.5	0.5	0.3	0.3
- product losses { (%)	1.0 + 2.0	0.5 + 1.0	0	0
{ (\$/ton)	<u>1.5 + 3.0</u>	<u>0.7 + 1.5</u>	<u>0</u>	<u>0</u>
Total cost (\$/ton)	2.2 + 3.7	1.5 + 2.3	1.3	1.1
Savings allowed by nitrogen technology in comparison with the others (\$/ton)	1.1 + 2.6	0.4 + 1.2	0.2	-

For the evaluation of such losses an average value of 150 \$/ton has been assumed for the preserved product.

It is then possible to calculate an overall cost of preservation for each technology examined.

From a comparison of these costs it appears that the most efficient and economical are those preservation systems which, on the basis of the results of the more detailed calculations, involve higher investment costs. Particularly the nitrogen preservation system appears to be most economic, since it allows a saving in the order of 1 \$/ton/year as compared to all other technologies and of 0.2 \$/ton/year as compared to the refrigeration technology. These evaluations refer to temperate zones. It is clear that the savings become much higher in tropical countries, where losses with traditional storage techniques are higher due to the climatic conditions.

## 2. Comparison at the result of preservation being equal.

Reference is made, hereinafter, to the case of maize preservation for a period of time of six months in a 27,000 ton storage complex in 1978 in U.S.A.

The informative principle of this analysis is that, while by the preservation system based on ventilation it is possible to preserve in the area concerned, without any losses, a product having 15.5% maximum moisture, with the nitrogen preservation system, it is possible to preserve, for the same period of time of six months, a product having an initial moisture content of 19%.

This possibility involves a saving in the cost for drying which is expressed in the following as a lower cost of the maize at the moment of harvest and purchase.

The cost of maize at 15.5% moisture is, in U.S.A., about 2.00 \$/Bu (1 Bu = 0.027 ton); maize with higher moisture is penalized by a 4 c/Bu discount for each unit of percent exceeding 15.5. Thus the cost (C in \$/Bu) of the maize with X moisture higher than 15.5 is:

$$C = 2 - 0.04 (X - 15.5)$$

The comparison of the costs for preserving maize having 15.5% moisture by means of ventilation and of the costs for preserving maize having a moisture varying from 17 to 19% by means of the treatment with nitrogen, is made in table 2 on these bases.

The initial cost of the maize and the preservation costs are referred to the dry weight of the product in order to avoid considerations on the quantity of water associated with the maize.

The basic data for the evaluation are the same as in point 1. In this case too, it is assumed that liquid nitrogen will supply the plant.

From the comparison of the costs of maize dry weight at the end of the preservation period it appears that it is more convenient to preserve maize having 19% moisture by means of nitrogen than to preserve maize having 15.5% moisture by means of ventilation since it is possible to have a saving of about 1.4 \$/ton.

The application of nitrogen technology to products having lower moisture results in smaller advantages and, in the border-line case, for maize having 17% moisture, we have approximately the same final cost as in the case of ventilation of maize having 15.5% moisture.

#### FULL ANALYSIS OF ALL COSTS OF A STORAGE CENTER

This analysis is extracted from a study, supported by the Italian Ministry of Foreign Affairs, evaluating the possibility of the introduction of the nitrogen technology into a tropical country.

For the nitrogen technology application two different plants are considered:

- Dome-shaped reinforced concrete silos with proper internal coating adequate to attain the required gastightness. The storage complex considered is made by 12 bins with a total capacity of 50,000 tons of bulk cereal. An on spot nitrogen production plant is designed in order to supply the required quantity of the gas. The equipment for the loading and the unloading of the cereals from the bins is very simple and is made by fixed and mobile screw conveyors.
- Storage complex as described above. The alternative is in the equipment for the loading and unloading of the cereal, that is made with

TABLE 2 SAVINGS ALLOWED BY THE NITROGEN TECHNOLOGY FOR MOIST MAIZE PRESERVATION IN A  
27,000 TON STORAGE COMPLEX (USA - 1978)

	Traditional storage technique		Nitrogen technique	
Maize Moisture Content (%)	15.5	17	18	19
Initial cost of Maize (\$/Bu)	2	1.94	1.90	1.86
(\$/ton)	74.07	71.85	70.37	68.89
Initial cost of dry matter (\$/ton d.m.)	87.66	86.57	85.82	85.05
Cost of the installations for the preservation				
- ammortization (\$/ton)	0.11	0.80	0.80	0.80
- operating cost (\$/ton)	<u>0.01</u>	<u>0.28</u>	<u>0.28</u>	<u>0.28</u>
- total	0.12	1.08	1.08	1.08
- total referred to dry matter (\$/ton d.m.)	0.14	1.30	1.32	1.33
Final cost of dry matter (\$/ton d.m.)	87.80	87.87	87.14	86.38
Savings referred to dry matter (\$/ton d.m.)		- 0.07	0.66	1.42
Savings in 6 months (\$)		- 1,570	14,610	31,050

a fixed plant.

For the traditional preservation technology (turning of the product, chemical disinfestation etc.) we consider 5 different storage complexes representative of the most common types of silos utilized in tropical countries (Balwanth Reddy, 1976):

- Conventional Godowns (C.G.)

These are horizontal structures utilized for bag storage of grains having 5000 tons capacity, with a floor dimension of 124 m. x 21.7 m. and 5.6 m. high brick walls with three compartments and longitudinal platform on one side. Road and rail sidings are normally provided for receipt and despatch of grains.

- Flat Bulk Storage Godowns (B.G.)

These are flat godowns suitable for handling grains in bulk. Their shape is similar to the conventional godowns.

- Reinforced Concrete Circular Bins 23.5 m. diam. and 10.5 m. high (C.B.)

These bins are lower in height than typical silos, they have a flat bottom and dome shaped top.

- Reinforced Concrete Vertical Silos (V.S.)

These silos are tall structures, 30 to 40 m. high and 8 - 11 m. in diameter. They have high capacity loading and unloading equipment, drying and aeration facilities, capability to mix grains and fumigation on site.

- Port silos (P.S.)

The structural shape of these silos is similar to R.C.C. Silos. They are installed in batteries close to the berth. They have additional pneumatic unloaders, of up to 800 tons per hour capacity.

## 1. Investment costs

The investment costs for each of the described plants are reported in table 3.

The items considered are:

### - Land

The requirement of land for storage varies according to designs. Thus; bulk storage, vertical structures, such as silos and bins would require less land, compared to the conventional flat storage facilities.

Our estimation of the element of land cost is based on its requirement for different designs and sizes of depots; and its cost in different regions.

The cost of land would vary from location to location, depending upon the nature of land, proximity to road, rail or main cities or due to its strategic importance.

### - Approach Road

The initial cost of construction of internal roads is affected by the extension of the storage complex and by the construction materials (concrete, bitumen etc.).

### - Railway Siding

Similar to approach roads, the length of railway siding varies according to the extension and location of the depot from the main line.

### - Ancillaries (Office, Compound Wall, Foreign Amenities, etc.)

In a storage depot, in addition to the main structure, administrative complexes, water supply and drainage systems, canteen block, lavatory and sanitary arrangements, compound walls/fencing, quarters for watchman, etc., are provided, depending on the size and staff strength.

### - Equipment

It includes the loading and unloading facilities costs. For the nitrogen technology it includes the cost of nitrogen production and of the distribution plant.

TABLE 3: SUMMARY OF INITIAL CAPITAL INVESTMENT COST PER TON OF STORED PRODUCT-1979 (US \$/ton)

TRADITIONAL PRESERVATION TECHNOLOGY						NITROGEN TECHNOLOGY	
Item	C.G. Conventional godowns unit of 5,000 ton capacity	B.G. Flat godown for bulk storage of 10,000 tonnes unit	C.B. R.C.C. circular bins of 20,000 tonnes	V.S R.C.C. silos of 20,000 tonnes	P.S. Port silos of 50,000 tonnes	D.S.1. Dome- -shaped R.C.silos of 50,000 tonnes	D.S.2. Dome- -shaped R.C.silos of 50,000 tonnes
Land	1.66	1.66	1.57	0.56	4.62	0.70	0.70
Civil works (35 years for C.G. and 50 years for silos)	36.96	46.20	46.20	75.77	140.46	27.49	29.91
Railway siding (15 years)	4.81	4.81	4.81	3.14	2.96	4.81	3.14
Approach Roads (10 years)	2.22	1.85	1.85	3.14	2.96	1.85	3.14
Ancillaries (35 years)	4.71	4.71	4.71	4.99	4.62	4.71	4.71
Equipments (10 years)	<u>0.73</u>	<u>16.63</u>	<u>18.48</u>	<u>64.68</u>	<u>161.90</u>	<u>14.33</u>	<u>33.71</u>
Total capital US \$/ton	<u>51.09</u>	<u>75.86</u>	<u>77.62</u>	<u>152.28</u>	<u>317.52</u>	<u>53.89</u>	<u>75.31</u>

### Civil Works

The initial costs of construction of godowns and silos are included.

This includes also foundation roofing, floor finish, etc. From the values indicated (Balwanth Reddy, 1976), it is possible to draw the costs of silos and godowns working according to the conventional preservation technology (table 3). Table 3 also shows the costs of airtight silos to be utilized for the application of the nitrogen technology.

The overall investment costs obtained indicate that the investment costs for storage according to the nitrogen technology are equivalent or even lower than those referring to units functioning according to conventional technology.

### 2. Running Costs

It is understood that these are inclusive of material and labour. They can be divided into fixed costs, depending on the capacity of the storage unit, but not on the quantity of cereal grains treated, and variable costs, which are proportional to said quantity.

The fixed costs are (table 4):

#### - Depot Establishment

The cost relevant to the permanent staff engaged in managing the depot. For storage units working according to the nitrogen technology, the costs for personnel are considered equal to the values relating to conventional preservation, even though in this case a lesser operational activity is required for the preservation of the cereal grains.

#### - Head Office and Other Overheads

It regards the cost of head office and regional office staff engaged in import, procurement, transportation, distribution operations and other activities.

#### - Maintenance and Repairs Cost

The annual maintenance and repair are evaluated as a percentage of the present value of capital cost.



TABLE 4 SUMMARY OF ANNUAL CURRENT COSTS.PRICES PER TON HANDLED AT 100% UTILIZATION, ONE ANNUAL TURNOVER - 1979 (US \$/ton)

TRADITIONAL PRESERVATION TECHNOLOGY						NITROGEN TECHNOLOGY	
Item/ton	C.G. conventional godowns	B.G. bulk godowns	C.B. circular bins	V.S. silos	P.S. port silos	D.S.1.	D.S.2.
1. Maintenance of buildings	0.54	0.62	0.62	0.91	1.58	0.42	0.44
2. Maintenance of equipments	0.03	0.67	0.75	2.61	6.63	0.58	1.36
3. Local taxes	0.14	0.22	0.23	0.49	1.05	0.16	0.25
4. Depot esta- blishment	4.07	2.01	2.01	3.14	3.14	3.14	3.14
5. Head office oversheads	0.96	0.96	0.96	0.96	0.96	0.96	0.96
6. Handling	2.09	1.04	1.04	1.04	1.04	1.27	1.04
7. Grain loss	3.70	1.85	1.85	1.85	1.85	--	--
8. Energy	0.03	0.24	0.24	0.48	0.48	0.48	0.48
9. Preservation	0.44	0.44	0.44	0.44	0.44	0.35	0.35
10. Dunnage	0.35	--	--	--	--	--	--
11. Insurance	0.55	0.55	0.55	0.55	0.55	0.55	0.55
12. Gunny	<u>10.16</u>	<u>5.08</u>	<u>5.08</u>	<u>5.08</u>	<u>5.08</u>	<u>5.08</u>	<u>5.08</u>
Total cost US \$/ton	23.06	13.70	13.77	17.55	22.71	13.00	13.65

For simplicity, we propose to take the maintenance charges as 1 per cent for civil works and 2.5 per cent on equipment on a linear scale.

- Local Taxes

The taxes on assets including the corporation tax on land and buildings, is taken at the usual rate of 25% of the present value of capital cost.

- Loading In and Out Charges

Also known as handling charges in the godown or silo, the operations involved are:

- a) Unloading from truck and stacking to the required height, in the case of conventional godowns;
- b) Unloading from the wagon and stacking;
- c) Destacking and loading into trucks/wagons;
- d) Weighing and standardisation charges.

Weighing and standardisation operations are not involved in every case and are required only for about 10 per cent of the grains handled.

Handling in and out is basically a labour-intensive operation and it should be confined to conventional godowns. Because of the existing systems of arrival and despatch in bags, even silos incur certain handling cost.

- Storage Loss

Storage loss is dependent on the initial conditions of the grains, pests, type, duration of storage and preservation methods. We have estimated the annual grain loss in conventional godowns for bag storage as 2% and the corresponding figure in bulk silo/bins as 1 percent in case of conventional preservation.

Nitrogen technology providing the preventive protection of the cereal, allows to obtain no product loss. The cost of grain loss per ton of grain is calculated on the basis of 180 \$/ton cereal cost.

- Preservation Cost

It includes the cost of chemicals used for prophylactic and curative treatment.

In the case of nitrogen technology the protection treatment is achieved by nitrogen, coming from the gas production plant. The treatment cost indicated corresponds to the cost of the utilities consumed by the plant for 1 year preservation assuming the following unit costs for the utilities:

- electric power	3.5 ¢/Kwh
- GPL	15.4 ¢/kg
- cooling water	1.8 ¢/cm

- Cost of Dunnage

This is required only in the case of conventional godowns and CAP storage to provide protection against surface dampness.

- Cost of Gunny

At present a large part of the distribution of foodgrains in tropical countries is in bags. The purchases are normally in bags. From the procurement stage onwards, since the present handling, weighing and transportation systems are in bags, gunnies are utilized both for silo and conventional storages. In the case of silos, the bags are slit open to pour grains into hoppers of elevators used for filling the bins.

The bulk discharge is rebagged before handling and loading into trucks or wagons. Therefore, the use of gunny is unavoidable in both systems of storage at present.

- Energy Cost

It refers to the energy required for the operation of conveyers, bucket elevators, operation of the locomotives and generators.

The same costs are considered irrespective of the preservation technology. Actually, the costs of energy are higher in the case of conventional preservation, where the cereal is turned to aerate and cool, than in the case of preservation according to nitrogen technology, where the energy costs only relate to the loading and unloading of the product.

- Insurance Cost

Grains are insured against fire, theft, etc., and the prevailing insurance charge is 0,55 \$/ton. The same incidence has been considered for the case of nitrogen technology even though it probably allows a certain saving due to the reduced fire hazards it involves.

3. Analysis of Preservation Costs

It appears that the investment and running costs for storage units working according to the nitrogen technology are equivalent to or even lower than those referring to units working according to conventional technologies.

For a more reliable comparison, however, it is necessary to take into account the different duration of the various components of the storage unit.

All the costs can therefore be referred to a common base of duration (50 years) summing up the discounted present values, at 10% interest, of the replacement costs of components with life shorter than 50 years (table 5). Furthermore, the various running costs per year that will be incurred in the various cases shall also be considered. In order to calculate an overall cost, it is necessary to calculate the discounted present value, at 10% interest, of the subsequent yearly payments anticipated for the running of the plant for the base duration of 50 years (table 5).

The summation of the present values of the investment and running costs results in the local preservation costs for the various cases considered, as referred to the yearly preservation cycle and to 100% utilization of the storage capacity. These costs are referred to a common base and allow immediate comparison. It therefore appears that nitrogen technology involves overall preservation costs lower than those involved by the conventional technologies even where the latter is applied to very economical silos such as the conventional and the flat godowns.

An equivalent but more convenient expression for preservation costs is possible by expressing them as costs per ton per year. This is obtained by estimating the capital costs as a uniform annual series on

TABLE 5

SUMMARY OF PRESENT VALUES, AT 10% INTEREST, OF CAPITAL COST AND CURRENT COST FOR ONE ANNUAL TURNOVER AND 100% UTILIZATION OF STORAGE CAPACITY - 1979 (US \$/ton).

TRADITIONAL PRESERVATION TECHNOLOGY						NITROGEN TECHNOLOGY	
Item	C.G.	B.G.	C.B.	V.S.	P.S.	D.S.1.	D.S.2.
P.V. of capital cost	54.98	89.42	91.61	194.94	419.60	65.33	98.93
P.V. of annual cost	<u>251.70</u>	<u>149.54</u>	<u>150.30</u>	<u>191.55</u>	<u>247.88</u>	<u>141.89</u>	<u>148.99</u>
Total present value	306.68	238.96	241.91	386.49	667.48	207.22	247.92

TABLE 6 SUMMARY OF ANNUAL TOTAL COSTS PER TON AT 10% INTEREST, FOR ONE TURNOVER AND 100% UTILIZATION OF STORAGE CAPACITY - 1979 (US \$/ton)

TRADITIONAL PRESERVATION TECHNOLOGY						NITROGEN TECHNOLOGY	
Item	C.G.	B.G.	C.B.	V.S.	P.S.	D.S.1.	D.S.2.
Annuity figures based on present value of capital cost	5.55	9.02	9.24	19.66	42.32	6.59	9.98
Annual current cost	23.06	13.70	13.77	17.55	22.71	13.00	13.65
	<u>28.61</u>	<u>22.72</u>	<u>23.01</u>	<u>37.21</u>	<u>65.03</u>	<u>19.59</u>	<u>23.63</u>

the basis of the present value estimates of investment costs of table 5 at 10% interest and 50 years, and adding to this the running costs recurring every year. Table 6 provides the total cost per ton handled in different designs, with the implicit assumption that there is only one turnover with capacity utilization at 100 per cent.

The overall preservation costs, even where they are expressed as yearly costs, are lower with the nitrogen technology than with the conventional technology.

The saving in the yearly costs allowed by the nitrogen technology as applied to storage units D.S.1, compared to conventional preservation costs with the most economical type of unit (B.G.), is about 3.13 \$ per ton per year corresponding to about 156,500 \$ per year for a 50,000 ton storage unit.

## CONCLUSIONS

The results of this study show that the nitrogen technology for the preservation of grains is competitive, from an economic standpoint, as compared to the other technologies used.

In the cases examined, the investigation showed that a saving of about 1 \$/ton year is obtained by treating the cereal according to the nitrogen technology.

These economical advantages arise from 1) the reduction in quantitative and qualitative losses of product, 2) the widening of the range of products to be stored (high moisture maize, for example), 3) the possibility of using very large and cheap structures that cannot be used with traditional preservation technologies.

The increasing costs of energy sources necessary for grain drying and that for chemicals for grain disinfestation permit to predict more and more economical advantages arising from the nitrogen technology.

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## SYSTEMS OF SUPPLY OF NITROGEN FOR THE STORAGE OF GRAINS IN CONTROLLED ATMOSPHERE

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### INTRODUCTION

In order to create and maintain a low-oxygen atmosphere in storage rooms for grains and other commodities a supply of nitrogen gas is needed to substitute the oxygen-enriched normal atmosphere. Nitrogen can be supplied in liquid state in pressure bottles or in case of big quantities -like normally needed for storage purposes- in tanks which are delivered by nitrogen producing factories through a distribution network more or less developed in the various countries. The cost of industrially produced nitrogen varies very much accordingly to the distances from the production factories or from main distribution-centers and depends upon the conditions of the road -or other communication-infrastructures. In Italy for instance selling price varies in a proportion of 1 : 4 and more from central distribution points like Rome, Milan etc. and distant periferic areas. In addition frequently there are difficulties in the supply, distribution and returning of the devices. Handling of the orders may create other kinds of organisatory troubles, especially in periods of strikes, emergencies in the transportation and other trying circumstances. Research of scientists and of the industry focused therefore increasingly on the development of relatively small equipments able to produce locally, directly on the storage premises, the required nitrogen avoiding the described inconveniences.

### SYSTEMS OF LOCALLY PRODUCED NITROGEN

The first method of production of nitrogen was cryogenic separation of air. Incoming air is liquefied and its components are separated by distillation or rectification using their differing vapour pressure. It is a method which requires rather high electric supply (0.8 kWh/ $\text{Nm}^3 \text{N}_2$ ) and very high capital investment (see table 1). Operating



cost is therefore rather high for small users and this kind of equipment is mainly recommended for large-scale facilities.

For purposes of creating approximately inert atmospheres the required nitrogen can be produced also by combustion of hydrocarbons, such as propane, butane, methane or similars. The obtained gas is relatively impure containing especially  $\text{CO}_2$  and  $\text{H}_2\text{O}$  which must be removed by means of additional downstream molecular-sieve adsorbers, like zeolite molecular sieves, after passing a cooling system. The adsorption of the impurities takes place in an equipment consisting of two containers, one of which is alternatively operating in the adsorption phase at 3 + 7 bar of pressure and at room temperature. The second is working in the regeneration phase. Regeneration is obtained by creating with a vacuum-pump a depression of about 100 Torr contemporaneously flushing with the produced gas mixture. An example of combustion consuming oxygen and leaving the inert compounds like  $\text{N}_2$  in the atmosphere is given by the following equations:



Even with the described complex procedure the total operation cost is nevertheless low and, considering the produced unit of gas nitrogen, more or less independent from the size of the equipment.

Servicing and maintenance with this kind of equipment is relatively complicated. The investment cost is also very reduced and only approximately one third or even less of the other described systems.

A recent interesting further development is the pressure-swing adsorption. It is based on multiple beds of molecular-sieve coke utilising the differing diffusion rates of oxygen and nitrogen molecules within the extremely small pores of the coke. Simultaneously with the oxygen are adsorbed also the water vapour and the carbon dioxide contained in the air, which simplifies the whole operation not requiring additional purification like the combustion system. For continuous operation a set of two adsorbers is provided, alternatively operating in adsorption and regeneration. In the adsorption (loading) phase oxygen is predominantly adsorbed while nitrogen

passes through and leaves the unit. Optimal pressure in this phase is about 3 + 4 bar. After the loading phase the coke sieve is desorbed by depressurisation at 70 Torr. Loading and regeneration phases are relatively short in duration (about 60 s each). A big advantage of this system, is the simplicity of the operation and of the servicing. The degree of purity goes up to 99.9% by volume and can be regulated through regulation of the air-flow rate, of the pressure and of the periods for the loading and regeneration phases. The higher the purity, the lower the product-gas quantities. These can be increased, with the same other conditions, by increasing operating pressure. Energy consumption is proportional to purity, being e.g. 0.4 kWh / m<sup>3</sup> for 99% nitrogen and 0.6 kWh for 99.9% purity. Generally can be stated that the pressure-swing adsorber system (PSA-system) has low operational cost but on the other hand a rather high cost of investment, considerably higher than the equipments based on the combustion of hydrocarbon and subsequent purification.

All the three described systems are able to produce an atmosphere with a very high nitrogen content from 99 to 99.9%, keeping impurities (H<sub>2</sub>O, CO<sub>2</sub>, CO etc.) at extremely low levels. They are also adapted for usage in technologies where such requirements are demanded. Higher investment and operating cost have to be accepted in such cases.

It must be added that there are also quite a number of experiences (U.S.A., Australia, Israel) with less sophisticated applications for the treatment and preservation of grains, with acceptable results as regards disinfestation and quality. These technologies allow to make use of very simple equipments based also on the burning of hydrocarbons (natural gas, propane or similar) or other combustion materials, like ethanol or other alcohols. This signifies that in all localities there will be availability of the required fuel. Likewise no particularly perfect gas-tightness is needed, cutting down investment- and maintenance-cost and increasing the feasibility of transforming existing conventional structures. There are types of simple burners which even don't require any electric energy and cooling water, with the effect of a remarkable reduction of the

Table 1 : Comparison of different systems of  $N_2$  -production  
for small-volume consumption

System	Prod. $Nm^3$ /h	Investment cost Lit/ $Nm^3$ $N_2$ / h	Electric supply kWh / $Nm^3$ $N_2$	Water $m^3$ / $Nm^3$ $N_2$	Fuel kg/ $Nm^3$ $N_2$	Operating cost Lit/ $Nm^3$ (without assisting manpower)
Cryogenic separation	100	3.100.000	0.7 + 0.8	0.08	-	240
	50	4.500.000				
Hydrocarbon burner	100	600.000				
(with zeolite mole- cular sieve)	50	880.000	0.3 + 0.4	0.06	0.12	70
Simple burner	100	300.000	-	-	0.12	39
	50	500.000				
Pressure-swing ad- sorber	100	2.300.000	0.4 + 0.5	0.07	-	77
	50	3.400.000				

operating cost. Production capacity of the burners can reach 100-120  $\text{Nm}^3 \text{N}_2 / \text{h}$  and even more.

### ECONOMICS

At this point some economic figures might be useful in order to compare the competitiveness of the described systems. In table 1 (next page) a comparison of different systems of local nitrogen production is given, with indication of investment cost, operating cost and of the consumption of some utilities. Prices are based on the Italian market situation and given in Lire (e.g. electricity 85 Lit/kWh, water 50 Lit / $\text{m}^3$ , propane-fuel 325 Lit/kg).

To calculate the cost of locally produced nitrogen some preliminary assumptions have to be made :

- (1) Depreciation can be fixed from 15 to 19% of the initial cost, with an average of 18%.
- (2) Maintenance is supposed to be from 4 to 6% of the initial cost varying accordingly to the requirements of the various types of machine.
- (3) Sizing of the capacity of the equipment has to be calculated in order to cover the consumption in the purging- and in the maintenance- phase.

For purging a ratio of  $1 + 1.5 \text{ m}^3$  of gas per  $\text{m}^3$  of storage volume was found in most experiences in the field. Variation is determined also by the flow-rate of the gas, being inversely proportional to the velocity of flow. For the calculation of the maintenance gas the amount of the losses of the structure have to be known. These are extremely varying accordingly to the type of structure, accuracy of execution of the gas-seal, age of the silo or storage and various other factors. Average values found in field operations are ranging from 10 to 30 l per  $\text{m}^3$  in 24 hours.

With such premisses we can calculate the nitrogen consumption for a full year operation in a fair gastight silo applying locally produced nitrogen as follows.

Table 2 : (see next page)

Table 2 : Nitrogen consumption in  $\text{m}^3$  in the storage of grains

Storage capacity t	Purging phase (ratio 1 : 1.25)	Maintenance phase	
		(average losses 20 day	$\text{l/m}^3$ /day) year
1.000	1.663	27	9.855
5.000	8.312	133	48.545
10.000	16.625	266	97.090
20.000	33.375	532	194.180
50.000	83.250	1.332	486.180

For the choice of the capacity of the nitrogen-producing equipment the following productions per day and per year of the most common types available corresponding to the hourly production have to be considered.

Table 3 : Production capacity  $\text{Nm}^3 \text{N}_2$

1 hour	24 hours (1 day)	8760 hours (1 year)
15	360	131.400
25	600	219.000
50	1.200	438.000
100	2.400	876.000

As a general rule capacity has to cover daily requirements for maintenance and must give the possibility to do the purging in a reasonable time.

Examining the figures in table 2 and 3 we can ascertain:

- Even the equipments with the smallest nitrogen production are able to cover the requirements of maintenance up to 10.000 t of storage capacity. Middle size machines can cope with storage capacities up to 50.000 t. In good airtight conditions of the storage the

Table 4 : Cost of locally produced nitrogen

Storage capacity t	Total consumption $N_2 / m^3$	Cost Lit / $m^3$ *			
		Cryogen.sep.	Simple burner	Hydroc.burner with purific.	Pressure-swing adsorber
1.000	11.518	4.729-6.491	559-664	938-1285	3.436-4.678
5.000	56.857	1.149-1.506	145-166	245-316	763-1.009
10.000	113.715	694-873	91-102	157-193	420-543
20.000	227.555	467-556	65-70	114-131	248-310
50.000	569.430	330-366	49-52	87-94	135-170

\* The first number referres to a production of  $50 m^3 / h$ , the second to a  $100 m^3 / h$  production

smallest units are always sufficient.

- In order to accomplish the purging in an acceptable time it is advisable to choose machines with high nitrogen production (50, 100 or more  $\text{m}^3$  per hour).

In table 4 we tried to calculate some production costs for the nitrogen, assuming different storage capacities and basing the calculation on the previously indicated premisses. Considering that the price of liquid nitrogen in Italy ranges in average from 200 to 400 Lire at a certain distance from the main distribution points and accordingly also to the delivered quantities it can be easily derived that even with an all the year around operation the use of nitrogen production equipments is not economical at very small storage capacities around 1000 + 2000 t. Comparing the various systems, cryogenic separation is scarcely competitive even in case of relatively large facilities. The pressure-swing adsorption in such cases begins to be economical with capacities of 20.000 t and up, whereas hydrocarbon burners, especially the simple types without purification are employable also at relatively low capacities (3000 + 5000 t). If a perfect gastightness enables to reduce the consumption of nitrogen and if the annual consumption is reduced on account of the shorter periods of storage (e.g. only short storage after disinfection) only the simple burner system is left over. Some examples are given in table 5.

Table 5 : Cost of nitrogen in case of reduced storage time and losses (Lit /  $\text{m}^3$ )\*

Time of operation	Losses/ day 1/ $\text{m}^3$	Total $\text{N}_2$ $\text{m}^3$	Hydrocarbon with purif.	Simple burner	PSA
1 month	10	2.06	312-555	184-330	1023-1970
	20	2.46	273-476	161-283	869-1662
3 months	10	2.85	245-420	144-249	761-1445
	20	4.05	193-317	113-187	558-1040
5 months	10	4.05	193-317	113-187	558-1040
	20	6.45	148-225	85-132	380-682

\*Based on an equipment with a production capacity of  $50 \text{ m}^3/\text{h}$ .

The first number is referring to 10.000 t, the second to 20.000 t of storage capacity.

### CONCLUSIONS

Supply of nitrogen for the disinfection and storage of grains is very often more practical and also economical if produced directly at the storage facilities with proper equipment instead of the delivery of commercial liquid nitrogen. This applies particularly to localities far-away from distribution centers, especially if difficulties in transportation, like insufficient roads, shortage in tanks, strikes, organizatory or other inconveniencies are given.

Among the various systems hydrocarbon burners are the most economical, whereas pressure-swing adsorbers, which are simpler in servicing and operating, fit in very well in large facilities, especially with long-term storage. One of the simplest and doubtless the cheapest system is the use of simple burners without purification. Their field of application is limited to technologies where a certain level of  $\text{O}_2$  (1-2%) and of  $\text{CO}_2$  (up to 13-14%) is accepted. On the other hand such a technology simplifies also the construction of the storage structures or the transformation of existing facilities not requiring a very high airtightness. Maintenance of the structures is for the same reasons less demanding. This facilitates the application of the nitrogen method of disinfection and storage also in developping countries where most circumstances and conditions are favourable for its application.

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# SEAL-O-SILO SYSTEM (METHOD FOR RESTORING AIRTIGHTNESS OF REINFORCED CONCRETE SILOS USED FOR STORAGE OF CEREALS)

H. TAKADA, T. YOSHIDA, M. NONAKA

## 1. INTRODUCTION

This system, called Seal-O-Silo, was developed for the purpose of repairing the airtightness of reinforced concrete silos. Advantages of the Seal-O-Silo System are as follows.

### High reliability

This method eliminates omissions caused by oversight in conventional methods. As materials and equipments used in this method are standarized, the complete and lasting airtightness can easily be realized.

### Rapidity of repair

While repair work by conventional methods takes at least 30 days per silo, this method takes only 10 to 14 days.

### High degree of safety

The degree of safety during repair work is extremely high since the silo interior requires little human labor due to the use of specially designed equipment.

## 2. THE NEED FOR IMPROVEMENT OF SILO AIRTIGHTNESS

### 2.1 STANDARDS FOR AIRTIGHTNESS IN JAPAN

Existing silos in Japan, used for imported food and feed cereals, are estimated to have the total capacity about four million tons. About 75% of these silos are made of reinforced concrete.

All imported cereals must be fumigated upon entry to Japan, under the Plant Quarantine Law. Fumigation is done in silos which can be sealed, so as to contain the fumigant (usually, methyl bromide). The Ministry of Agriculture and Forestry, which is in charge of administration of this law, inspects silos and authorizes their use for fumigation. Silos are required to show a loss, after 48 hours, of no more than 30% of the concentration of fumigant, introduced into the silo at 10 mg per cubic meter of volume (the silos which pass are designated as "Grade A" silos).

An expedient means of determining whether silos are satisfactorily airtight is to raise the internal pressure above normal and measure the drop in pressure over time. In practice, this is done by raising the pressure to more than 500 mmAq, and observing pressure after 20 minutes have passed from the time when the level of 500 mmAq has been attained. If pressure is 200 mmAq or more, the silo passes inspection.

## 2.2 CAUSES OF CRACKS

Reinforced concrete silos often develop cracks after having been used for a long period, and leak fumigant gas to the extent that they no longer pass inspection standards. The following are reasons for this deterioration of performance.

- ① Problems of design, such as failure to properly foresee pressure imparted to silo walls when the silo is filled and emptied.
- ② Problems arising during construction, such as poor or faulty positioning of R-bars or poor placement of concrete.
- ③ Changes in functioning of equipment used to fill and empty silos; changes in the equipment used. Both can impart pressures to silo walls in excess of those anticipated at the design stage.
- ④ Contraction of concrete due to drying. Over time, some of the water originally used to make concrete is lost.
- ⑤ Deterioration due to the influence of carbon dioxide; some stored grains will generate sufficient carbon dioxide as a product of respiration to promote neutralization of concrete.
- ⑥ Uneven sinking of silos, such as may occur when insufficient study was made prior to constructing a number of silos at one site.
- ⑦ Damage caused by earthquakes and other temporary phenomena.

## 2.3 DISTRIBUTION PATTERNS OF WALL CRACKS

Distribution pattern of actual silo wall cracks is shown in Fig.1.

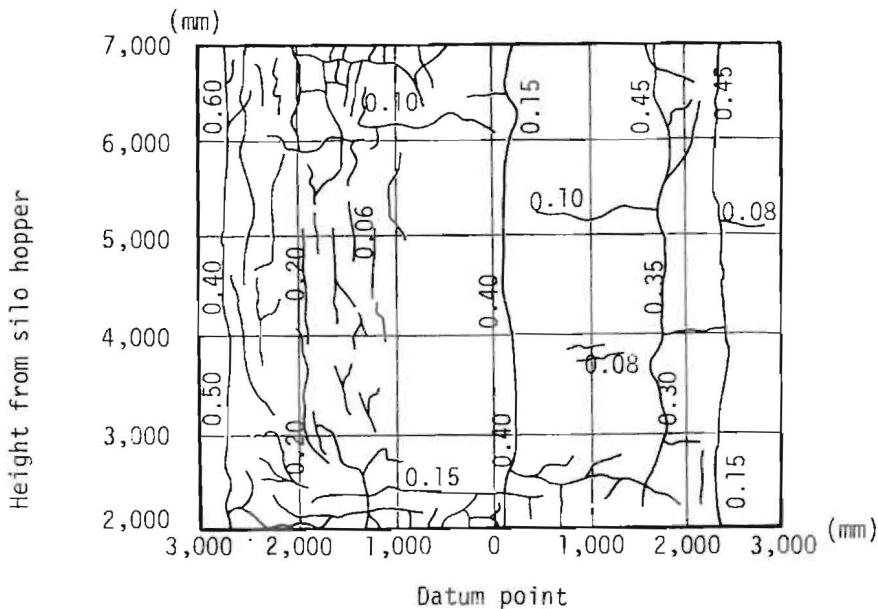
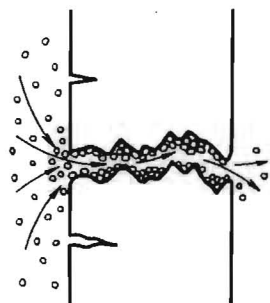


Fig.1. Distribution pattern of wall cracks on part of reinforced concrete silo which was constructed at 1967.

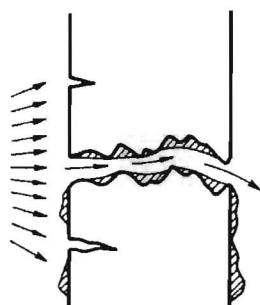
Exterior wall is 220mm at thickness.

### 3. THE SEAL-O-SILO SYSTEM

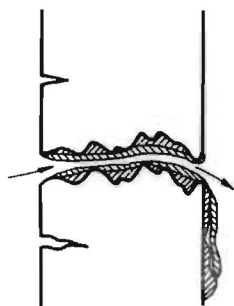
The principle of the Seal-O-Silos System is that particles of sealant will be forced into cracks and pinholes by the combination of a differential in atmospheric pressure (a higher pressure inside the silo) and micro-turbulence of the atmosphere as it moves through cracks and pinholes toward the low-pressure exterior. Sealing process of cracks in this system are as follows.



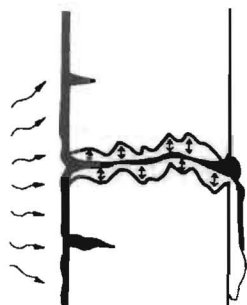
First air is introduced into a silo, to raise the atmospheric pressure inside the silo and enlarge the cracks in its wall. Air will begin to leak out of the cracks and pinholes. The sealant in fine powder form (Bintight-P) is sprayed into the interior atmosphere, and, borne by air currents, gradually enters cracks and pinholes. During this process, micro-turbulence are generated inside the cracks and the pinholes, causing the fine powder to be deposited in the cracks and the pinholes.



Next a liquid sealant (Bintight-L1) is sprayed. It enters cracks and pinholes and reacts with the powder sealant deposited there. With the combined effects of capillarity and air pressure, formation of a paste gradually forced deeper into the cracks and pinholes.



After the above procedure is repeated for several cycles (SOS cycles), the airtightness of the silo improves considerably. But in this state moisture is present in the cracks and pinholes so that as the silo dries and the moisture evaporates, the airtightness level will decrease. To counter this decrease, several cycles of the same procedure are repeated on the following day.



Cracks in the wall are always in a dynamic state due to the deposition and discharge of grains, the repeated drying and dampening of the silo and so on. To insure complete airtightness amid these dynamic changes, SOS System includes a finishing process in which a special sealant (Bintight-L2) is forced into the cracks and pinholes by the same procedure as above. After this procedure, sealant expanded and restoration completed.

Fig.2. The left side of wall is inside to silobin, in which the atmospheric pressure raised.

### 3.1 COMPARISON OF SEAL-O-SILO SYSTEM AND CONVENTIONAL METHOD

When cereals is stored after restoration, the silo which is repaired with Seal-O-Silo System hardly develops cracks again. But the silo which is repaired in conventional method sometimes develops cracks again. The principle is shown as follows.

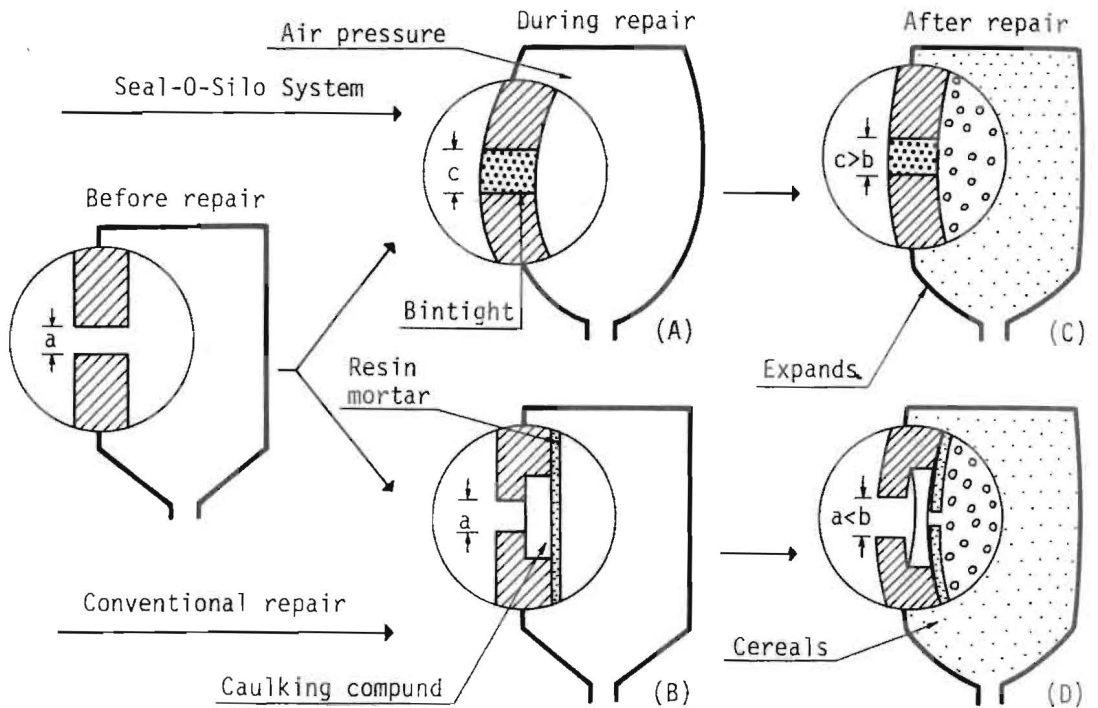


Fig.3. The dynamic changes of the silo wall and cracks.

(A) The repair work is carried out after raising the air pressure to expand the cracks. As a result, the crack width " $c$ " is greater than the width " $a$ " before repair.

(B) The silo interior is repaired at normal pressure by cutting cracks into a U-shape or a V-shape, filling the cut with caulking compound and coating over it with resin mortar. As a result, the crack width remains the same as the width " $a$ ".

(C) When cereals is stored after restoration, the silo wall expands by a small amount. But since the wall has been repaired by increasing the internal air pressure in such a way that the crack width " $b$ " after the deposition of the grain will be smaller than the width " $c$ " during repair, the crack can withstand this expansion.

(D) When the wall has been repaired at normal pressure, it cannot always withstand the expansion caused by cereals storage so that the caulking compound becomes stretched and the resin mortar sometimes re-opens. Also, the resin mortar can wear out or peel off after a long period of repeated deposition and discharge of cereals.

#### 4. MATERIALS USED IN THE SEAL-O-SILO SYSTEM

##### 4.1 GENERAL CHARACTERISTICS

Three kinds of materials, Bintight-P, Bintight L-1, and Bintight L-2, were developed for Seal-O-Silo System. General characteristics of each materials are as follows.

##### 4.1.1 Characteristics of materials are shown in TABLE, Fig 4, Fig 5 and Fig 6

TABLE-1 Characteristics of Materials

B-P	Specific gravity	Powder characteristics		Lubricant	Strength of compression (kg/cm <sup>2</sup> )		Expandibility & Toxicity
		Ratio of surface area to weight (cm <sup>2</sup> /g)	Particle size (μ)		1day	2days	
	3.12	6,000 or more	10	Yes	469	744	Fig.3, TABLE.2
B-L1	Specific gravity	Coefficient of viscosity (cp)	Surface tension (dyne/cm)	Activity	pH	Color	Penetration & Toxicity
	1.0	1.0 or less	40.0 or less	Anionic	6	None	Fig.4, TABLE.2
B-L2	Specific gravity	Avr. particle size (μ)	Coefficient of viscosity (cp)	Solids	pH	Major ingredient	Penetration & Toxicity
	1.0	1.0 or less	10--100	20.0%	9	SBR	Fig.5, TABLE.2

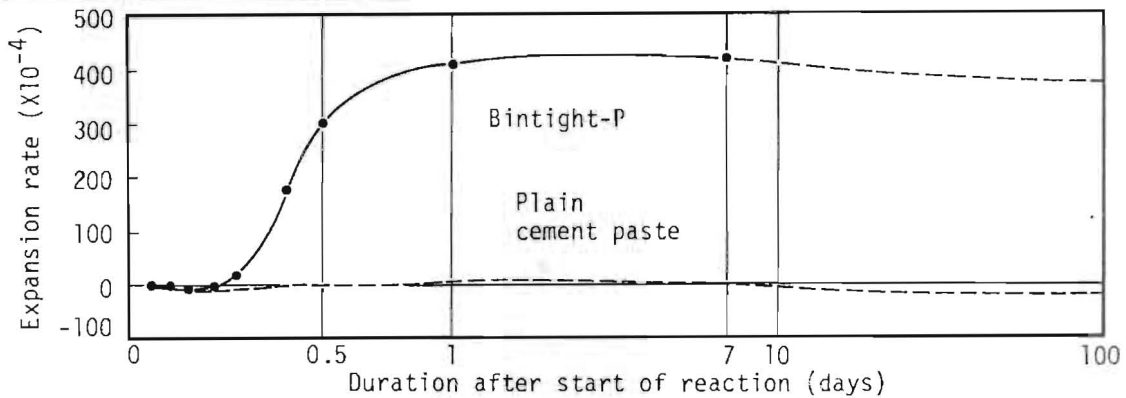


Fig. 4 Initial expansion of Bintight-P

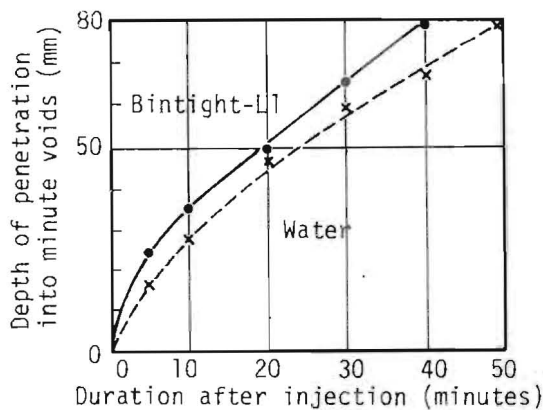


Fig. 5 Speed of penetration of Bintight-L1

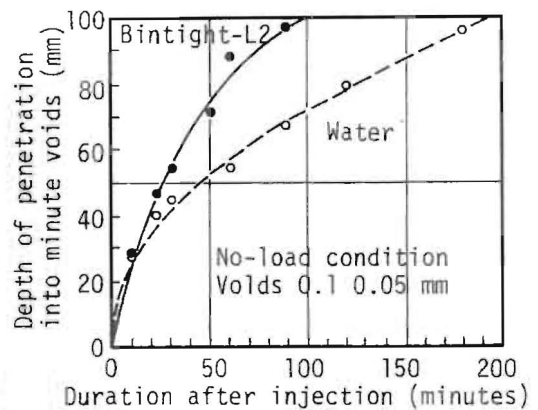


Fig. 6 Bintight-L2

4.1.2 Nontoxicity of materials

Nontoxicity of materials is shown in TABLE 2.

TABLE 2 Results of water quality test

	Water standard (Japan)	Bintight-P	Bintight-L1	Bintight-L2
Order	Not out of ordinary	None	None	None
Color	5 degrees or less	0	0	0
Turbidity	2 degrees or less	0	0	0
pH	5.8--8.6 ppm	8.6	5.8	6.0
Na <sup>++</sup>	10 ppm	1.68	0.04	6.40
SO <sub>4</sub> <sup>--</sup>	200 ppm	5.4	5.4	2.0
NH <sub>4</sub> -N	Not detected as same time	Not detected		
NH <sub>2</sub> -N		Not detected		
NH <sub>3</sub> -N		Not detected		
Free residual chlorime	0.1 or less ppm	1.5	2.0	---

4.2 RESTORATION OF AIRTIGHTNESS

As a silo is to be made more airtight by use of the Seal-O-Silo System the thickness of the silo wall, interior process pressure, number of SOS cycles, width of cracks, and other factors must be thoroughly studied.

4.2.1 Wall thickness, and interior process pressure

The relationship between the area of paste which will penetrate into cracks and the thickness of the wall and interior process pressure is shown in Fig. 7

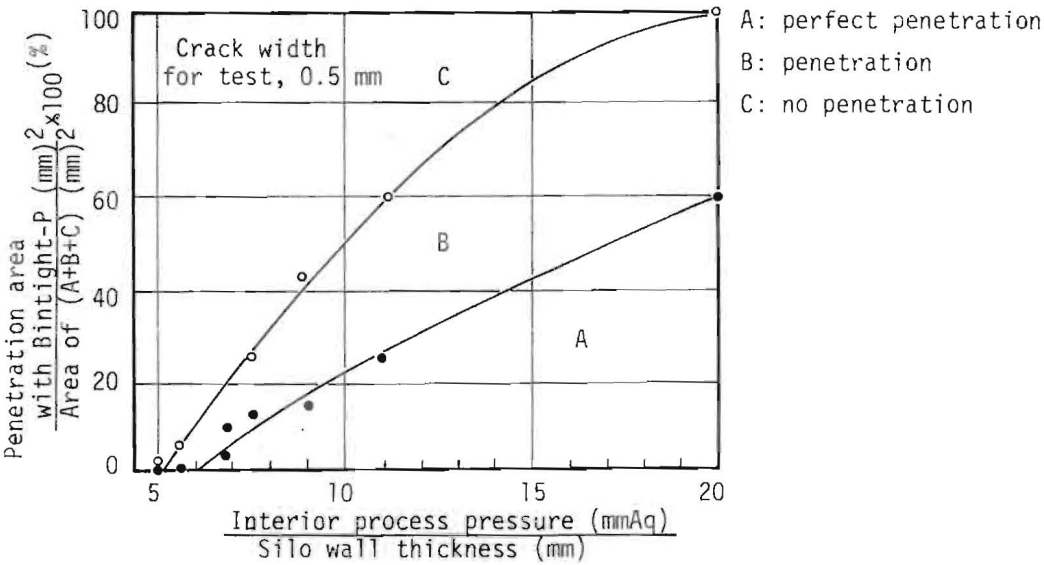


Fig. 7 Injection results of Bintight-P

#### 4.2.2 Number of SOS cycles

The relationship between the airtightness of a silo and the number of SOS cycles is shown in Fig. 8

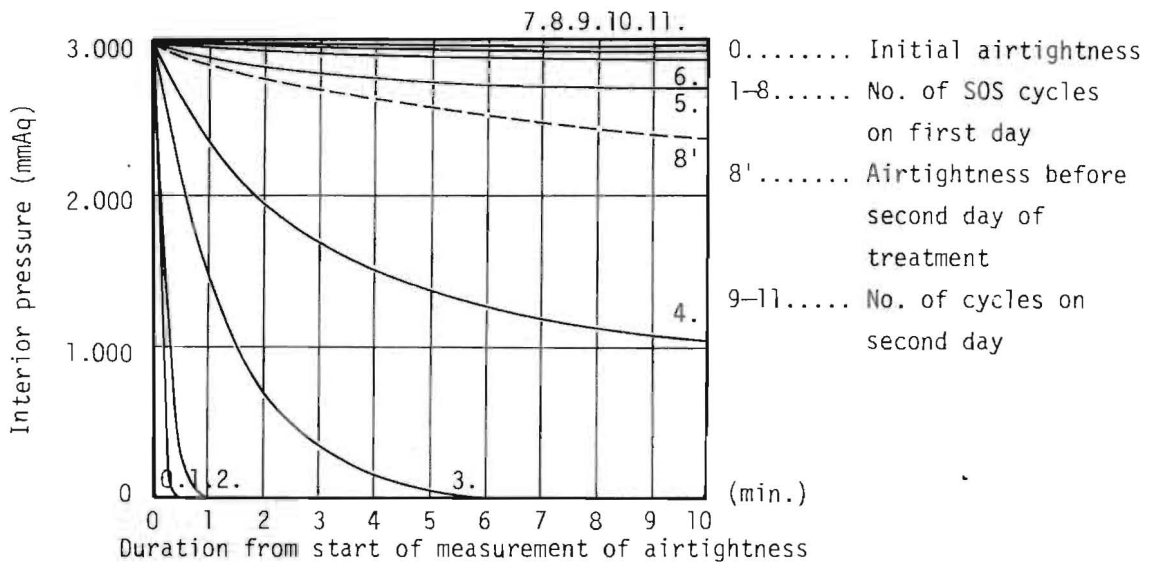


Fig. 8 Improvement of airtightness according to numbers of treatment cycle

#### 4.2.3 Crack width (Kennedy, T.B., ASCE paper, 1958)

When cracks or pinholes are to be filled with a powder, the narrower of crack the smaller must be diameter of the powder particles. When the ratio of crack width to particle diameter approaches unity, crack mouths will tend to become clogged. For particles to penetrate into a crack, it is necessary that the ratio is 3-5 or higher.

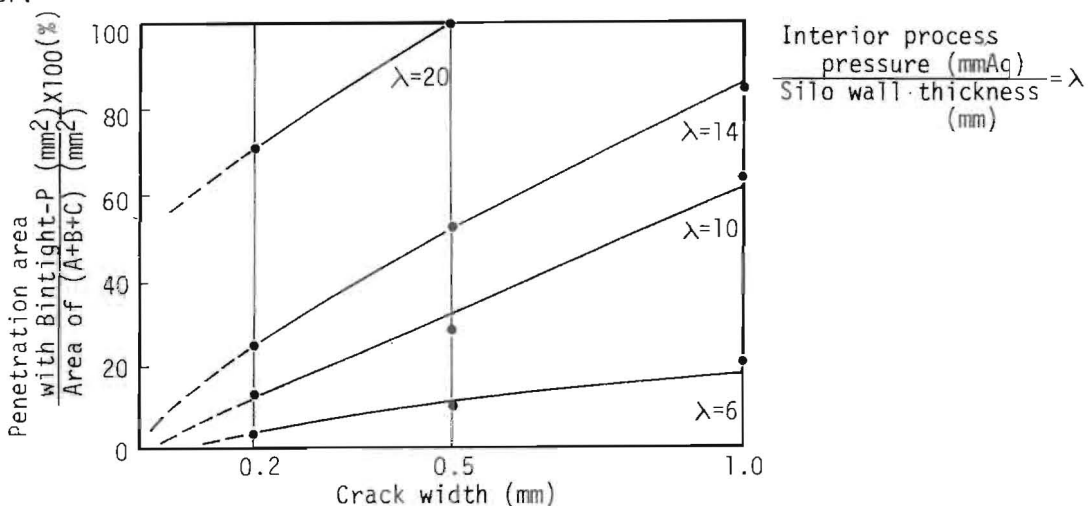


Fig. 9 Effects of crack width to injection effectiveness of Bintight-P



## 5. SEAL-O-SILO SYSTEM EQUIPMENTS

Seal-o-Silo System consists of the air supplying unit, the liquid controlling, supplying unit, and the movement controller of the top slab. These equipments are designed for easy assemble and simple ones. The diagram of Seal-o-Silo System is shown in Fig. 10, Fig. 11 and Fig. 12.

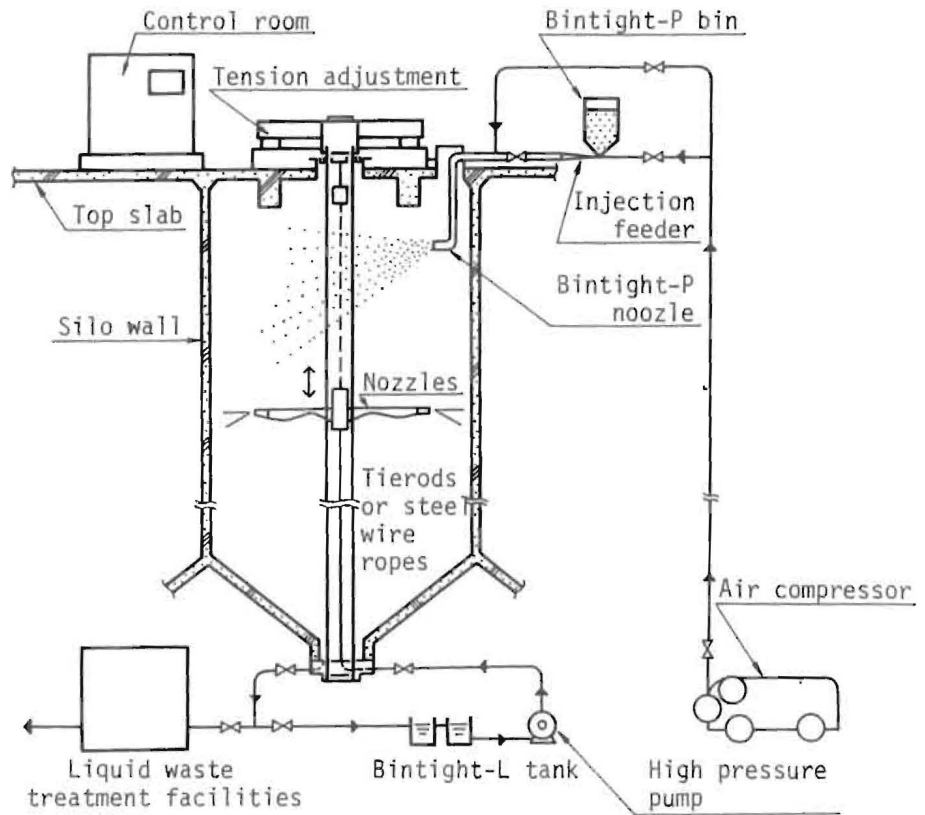


Fig.10 Diagram of SOS equipments

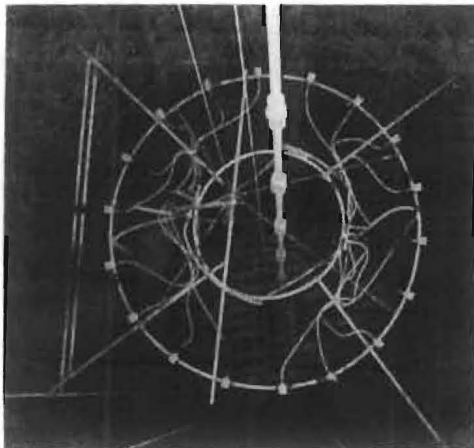


Fig. 11 Nozzles (interior of silo)



Fig. 12 Controllers on the top slab

## 6. A EXAMPLE REPAIR WORK:

### 6.1 BASIC DATA ON THE IMPROVED SILO

Structure ; Reinforced concrete silo.

Use ; Storage of imported cereals.

(mostly soybeans, maize, rape)

Date constructed ; 1961-1967.

Constructor ; Shimizu C.C.(SCC), Mitsui  
C.C.(MCC). Toda C.C.(TCC)

Scale ; See Fig. 13. (\*-Nonfumigant silo)

Date repair worked ; Dec. 1976-Dec. 1977.

Number of repaired work bins ; 40 bins.

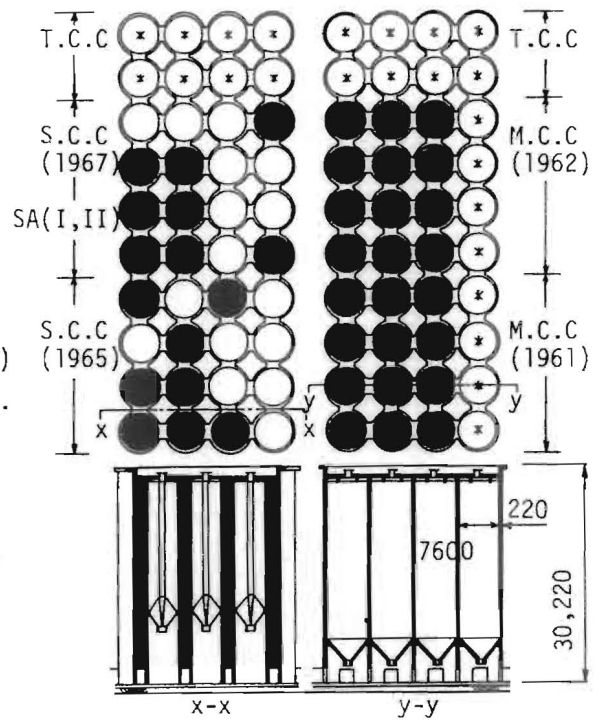


Fig. 13 Improved silos plan

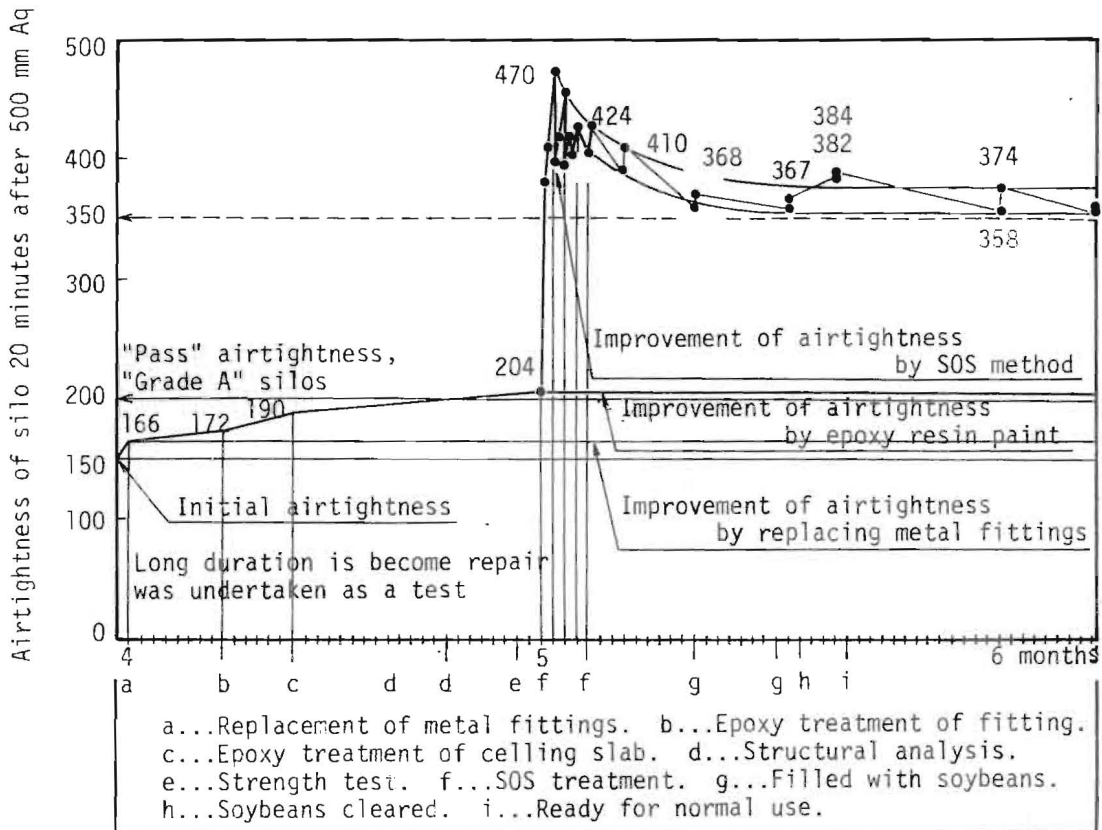


Fig. 14 Improvement of airtightness

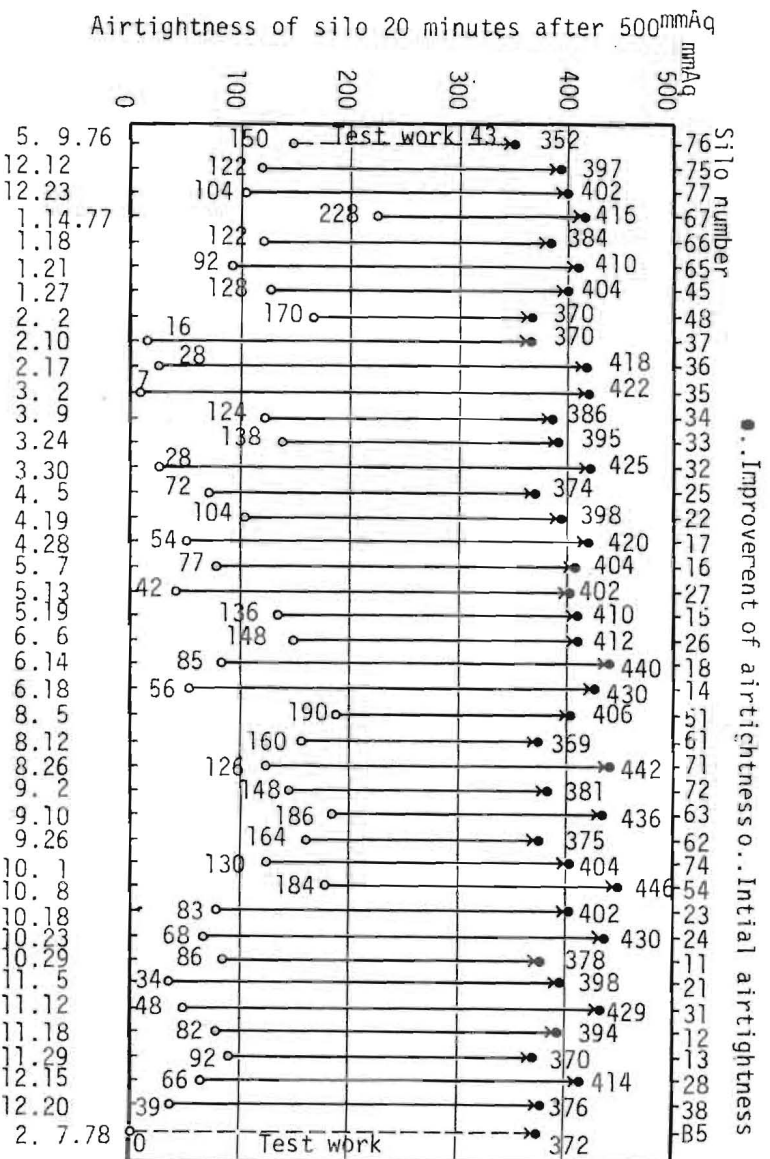


Fig. 15 Relationship between the level of initial airtightness and the level of improvement of airtightness.

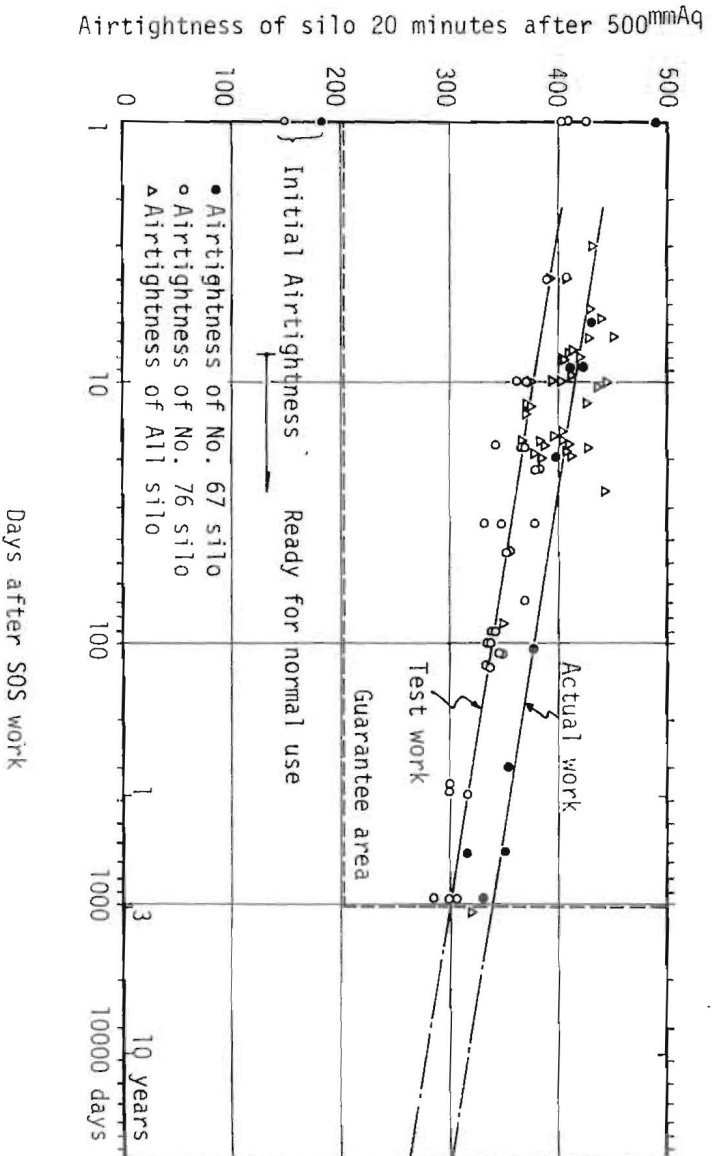


Fig. 16 Airtightness after repair work.

### 6.3 THE CONTENT OF IMPROVEMENT OF AIRTIGHTNESS

Airtightness of silo is improved by replacement of metal fittings, painting with epoxy resin, and SOS treatment.

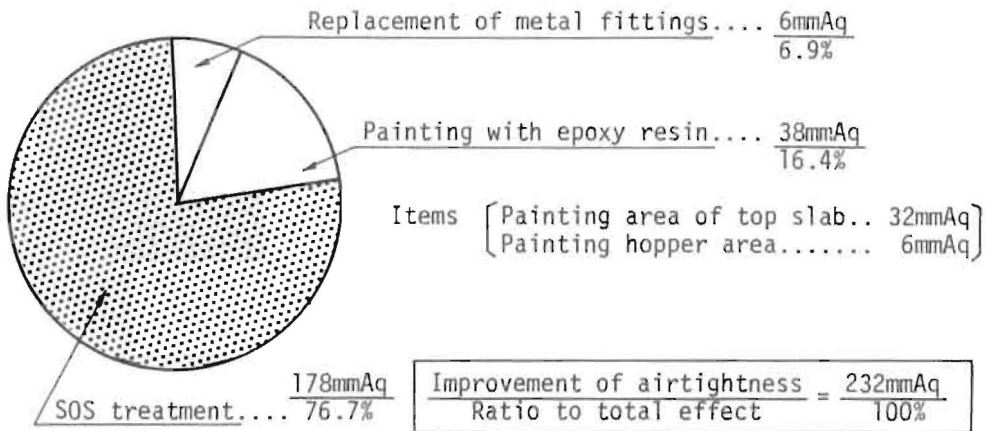


Fig. 17 The content of improvement of airtightness

### 6.4 CONFIRMATION THAT BINTIGHT-P HAD PENETRATED INTO CRACKS

During the Seal-O-Silo System, Bintight-P and Bintight-L1 had been applied in paste form to cracks, so as to penetrate toward the outside, (see Fig. 19) and after the completion of processing core borings were made in the silo wall to ascertain whether the compounds had fully penetrated the cracks. This was confirmed, verifying the value of the Seal-O-Silo System.

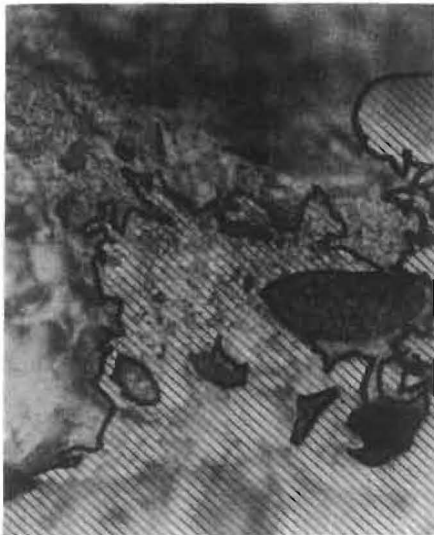


Fig. 18 Crack filled with Bintight-P, verified by a core boring.

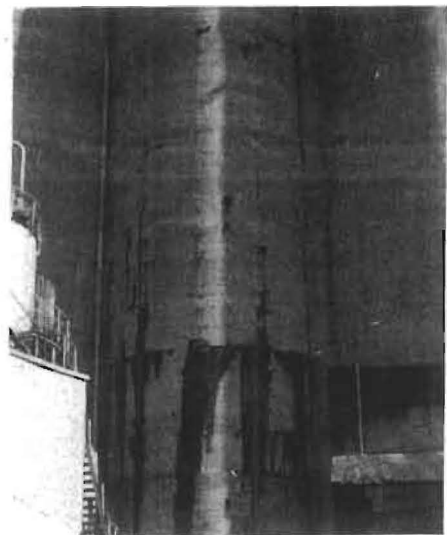


Fig. 19 Bintight-P penetrated through a crack of a silo wall.

## 7 CONCLUSION

The airtightness of grain silos is essential in the countries where fumigation for all imported cereals in silos is regulated.

The airtightness (gastightness) of storage silos is also important in the case of storing grains with gas for a long time.

Seal-o-Silo System, a system for improving airtightness of silos, can repair old silos in Japan. Their airtightness can well satisfy the Japanese standard.

Also, for the purpose of holding gastightness to high degree for a long time, this system is expected to be applicable.

In spite of further proceeding with researches and development of silo airtightness restoration, it is important to exchange internationally many informations about storage of grains.

We believe firmly that Seal-o-Silo System greatly contribute to food and feed problems.

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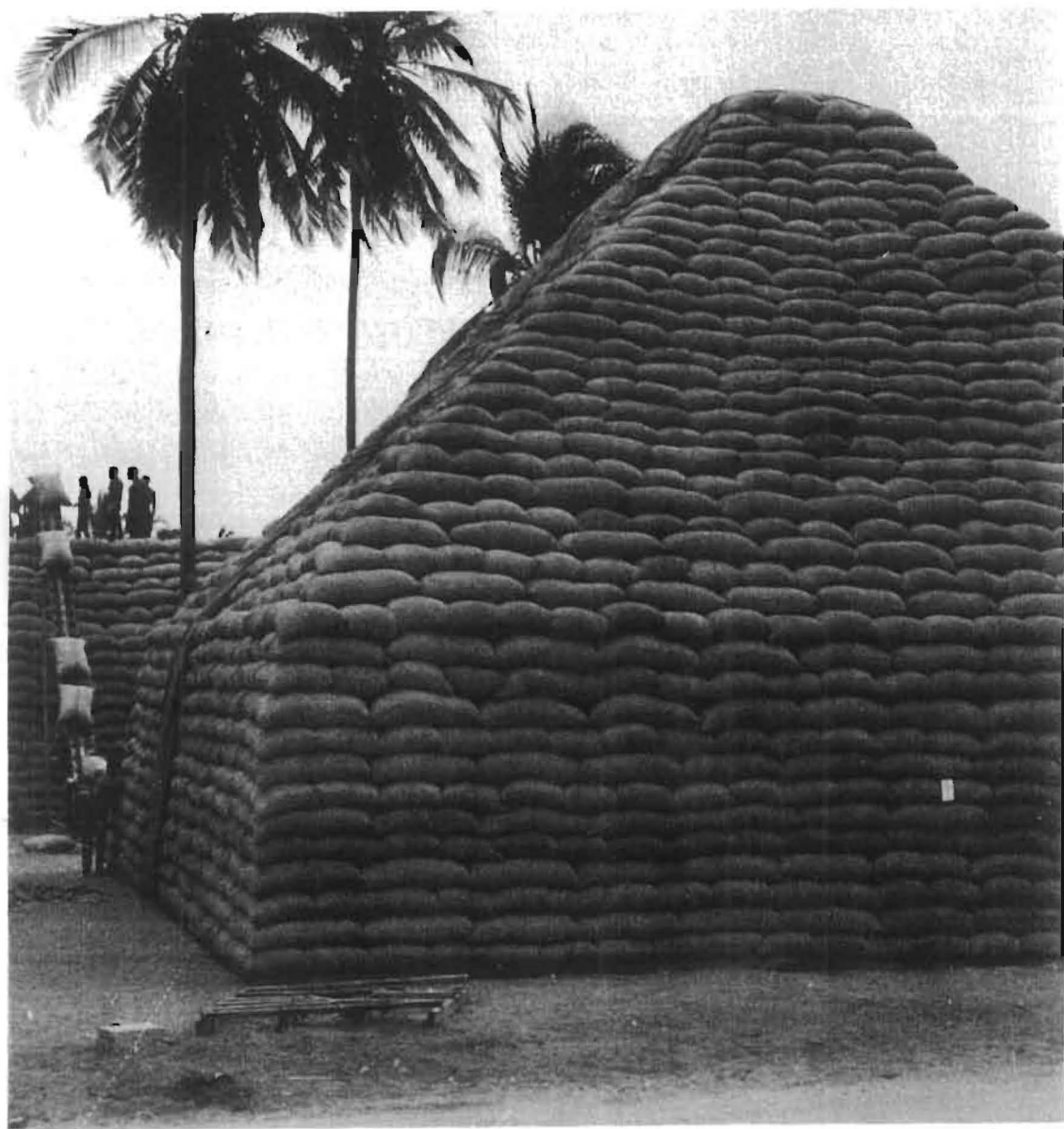
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VII S E S S I O N :

CONTROLLED ATMOSPHERE STORAGE OF GRAINS  
IN THE PRESENT AND FUTURE (A ROUND TABLE DISCUSSION)

Moderator: Dr. J. Shejbal



F.A.O. Photo, 1967

MODERATOR

Ladies and gentlemen, this is the last Session of the International Symposium on Controlled Atmosphere Storage of Grains. It will have two parts. First, the wrap-up rapporteurs of the preceding six Sessions will tell you about their impressions and conclusions from the papers and discussions which we heard during the respective Sessions and about their own ideas about the trends and problems for the future.

Then, a general discussion will be held, which will be recorded, and later transcribed for publication in the Book of Proceedings of this Symposium. This round table discussion will have no precise sequence and anyone will be free to comment on whatever aspect of our general subject.

It would be desirable if we could talk about as many problems which remain open as possible and indicate what future fundamental and applied research work in the field of controlled atmosphere storage of grains we think should be addressed.



Wrap-up report of I Session:

#### NATURAL AIRTIGHT STORAGE

by Mr. N.J. BURRELL

In the First Session we heard from Dr. Girish and Dr. Kamel about the many types of traditional underground or semi-underground structures that have been used for centuries in different parts of the world and are still used, particularly in India, Africa and the Arab countries but also in Europe.

We heard how the shape, depth, size, materials and methods of construction are mainly governed by the local soil or rock conditions, climate and presence or absence of water in the soil. Thus some structures could safely be built several metres below ground level, others were partly below and partly above, and others were mainly above ground.

We also heard that little scientific data is available to demonstrate the success or otherwise of these traditional stores and information on gas levels, insect activities or mortalities, moisture contents, temperatures, mould growths and other spoilage, is lacking.-

However, some careful studies have been made in India by Dr. Girish and Dr. Kamel has informed us of the plans to study some of the numerous different storage structures that are common in the Arab countries.

It seems, however, that although detailed scientific investigations have only recently begun on traditional stores, and perhaps these structures can be replaced or improved, it should not be taken for granted that such changes are bound to be beneficial as these have been exposed to some sort of evolutionary process by successive generations of users who, no doubt, have introduced numerous alterations over the years to suit local conditions.

But now that methods and staff are becoming available for measuring the physical and biological factors within these stores, we can now get a much better idea of what happens in these structures to make

them satisfactory. In the long term, it is possible that some traditional methods may outlast any immediate improvements that seem appropriate at present. There seems to be a clear need for caution to allow the old methods and new methods to continue side by side, until the new ones have been shown to be superior and that they will provide a lasting solution to the present problems of wastage. If we compare the traditional storage methods with new possibilities it may be possible to forecast the appropriate advances that can be made.

For example, we know which modern materials are impermeable to gases and could be used to fabricate completely air-tight containers, whereas we have very little information on how rapidly oxygen diffuses through soils and other materials covering a traditional underground bin.

But we can only guess at the full effect of a layer of earth over the top of a traditional grain pit. Obviously the layer provides shade; it prevents rapid temperature changes; almost certainly it prevents or greatly restricts condensation; it provides a thick barrier to insects and may even act as an abrasive to the protective outer layer of grain insects and might thereby cause their deaths by desiccation. If the pit is a deep one, it will probably be exposed to cooler conditions than a modern bin built above ground and, if any condensation takes place in an earth pit, the condensation may be absorbed by the pit, or the pit lining, before mould growth can take place.

Suppose we react in the obvious way and consider replacing traditional pits by new surface bins made 100% airtight; what new problems will we face?

Clearly such bins will be exposed to higher temperatures, greater temperature variations, solar radiation and radiation cooling and because a thin plastic or metal surface is prone to sudden temperature changes, condensation is far more likely to occur even if the bin is shaded or thinly covered with thermal lagging.

It should not be forgotten, also, that sealed containers that are opened frequently can expect to become leaky as gaskets or plastics degrade with temperature, time or ultra violet radiation, and once the gaskets give way, then access to the container by insects may be

easier than if it were below ground.

Perhaps we should therefore bury the new structures or cover them with a thick layer of earth, but we know that plastics can be attacked by termites, other insects and rodents so that plastics may be unsuitable, but are metals any better? How long can they survive burial in the soil?

The basic difficulty seems to be, to avoid replacing old problems with new ones. Perhaps it would be better to consider building structures from materials known to last many years. Perhaps concrete structures rendered gas proof with bitumen would be suitable or perhaps glass fibre materials have advantages over traditional subterranean stores?

With reference to many papers produced for this Symposium, one possibility seems to be to provide air-tight containers which might be flushed out with gases and no doubt there are possibilities for longer storage. But is it economically viable to supply millions of containers to individuals farmers and families living at a subsistence level and also provide reservoirs of gases to enable them to flush oxygen from the food store periodically after the store has been opened to obtain food for day to day consumption?

Several of the containers described by Dr. Girish and Dr. Kamel were provided with linings of dried stems, leaves, dung and other plant material. The object of the lining seems partly to keep the food product clean, away from the earthen, brick, concrete or stone walls and also to absorb moisture entering through the walls. In several instances reported by Dr. Girish and Dr. Kamel it seemed likely that oxygen may have been preferentially absorbed from the bin by microbiological breakdown of this liner. Dr. De Lima also reported oxygen levels in bins near 20% for 2 or 3 years, but also reported a sudden drop in oxygen when condensation and mould growth occurred. In my own paper I mentioned 2 tests where we had purposely used damp biological materials to remove oxygen.

Is it not conceivable that perhaps the most economic method of removing oxygen from sealed air-tight or semi-air-tight stores on small farms and in family stores is to make use of this process? Perhaps

this can be done by purposely placing damp biological material in the container or fermenting beers or wines inside the store for a suitable period until sufficient oxygen is removed to kill the pests present.

## Wrap-up report of II Session:

## ENTOMOLOGY OF CONTROLLED ATMOSPHERE STORAGE

by Mr. C.L. STOREY

Papers presented in Session II, Entomology of Controlled Atmosphere Storage, were in general agreement on the following aspects of insect control by modified atmospheres.

First, it was agreed that alternatives to pesticide treatments are needed. Reasons cited included health and safety factors such as chemical residues, toxic action of fumigants, and future regulatory requirements affecting fumigant availability.

Second, support for the development of modified atmosphere control as a specific method of insect disinfestation was expressed, and data was provided documenting the lethal and sublethal effects of various atmospheres against several species of the principal stored product insects.

Third, it was generally agreed that the degree of mortality achieved with various modified atmospheres is a product of the introduction of such factors as gas composition, time, temperature, humidity, insect species and stage/age of development. Specifically, we concurred that treatment at higher temperatures increases the effectiveness of modified atmosphere and decreases the time of exposure required to achieve high mortality.

Similarly, treatments at lower humidity are more effective than those at higher humidity. Furthermore, data presented suggest that internally developing species are more tolerant than externally developing species, mature larvae and pupal stages are generally the most tolerant stages of development and early instar larvae and adults the most susceptible. The value of  $\text{CO}_2$  in assisting the action of low oxygen mixtures was recognized although opinions differed as to the nature of its effect.

Finally, there was a tentative agreement that conflicting data on the relative susceptibility of various insect species and the speed

of action for their control may be caused by miscomparisons of atmospheres of different composition.

Some fundamental differences were evident in both the data presented in the papers and in the discussions that followed. Specific differences in the basic dose-mortality response of various stored product insects to atmospheres of similar composition of  $O_2$ ,  $N_2$  and  $CO_2$  were identified and remain unresolved. The most pronounced difference of opinion concerned which atmosphere composition offers the most effective control and which method of application is best suited for the treatment of bulk stored commodities. Such differences of opinion should not be viewed as detrimental, but should serve as a stimulus to encourage further development of modified atmosphere technology.

Future entomological priorities suggested are to:

- Establish treatment schedules for specific gas concentrations and environmental conditions;
- Further explore the integration of modified atmosphere treatment with traditional storage management procedures including aeration, cooling and chemical treatments;
- Expand studies on the mode of action of modified atmospheres, the long term effects of sublethal exposures on population development, and the possible development of insect resistance.

Wrap-up report of III Session:

## MICROBIOLOGY OF CONTROLLED ATMOSPHERE STORAGE

by Dr. E. DE LAS CASAS

The contamination of grain by micro-organisms begins in the field:  
1) through the roots; 2) through the surface of the plant;  
3) through incorrect treatment of the grain during harvest, threshing, cleaning, drying, storage, management and transport; 4) through lack of sanitation.

The papers presented in this Symposium on Controlled Atmosphere Storage described one of the various types of technologies used to minimize the growth and effect of the micro-organisms of the grain. These technologies may include drying, cooling, chemical treatment, ventilation, etc.

In the overview, presented as a basis for the discussion in the Microbiological Session, the advantages and disadvantages of the modified or controlled atmosphere storage were noted, as were the basic considerations that influence the usefulness and effectiveness of this technique. Controlled atmosphere permits the storing of grain with a somewhat higher than normal moisture content (m.c.), thus diminishing the hazards of increased deterioration of grain. However, some possible disadvantages seem less well understood. For instance, under aerobic conditions, through the presence of certain species of Lactobacillus it is possible to estimate the freshness of the grain or to forecast the approximate length of storability of the product. However, fungi and other micro-organisms may act on this bacteria in an antagonistic manner. No information was presented to judge if this useful freshness guide is available for utilization in modified or controlled atmospheres, nor were data offered about any changes relative to the above phenomenon.

Among the major basic considerations mentioned, which influence the effectiveness of controlled atmosphere, were "microbiological load", i.e., the amount of original microbial infection. Before the

mechanism leading to the accumulation of this microbiological load in the mass of grain can be understood, the effect and implications of stress factors on the grain, such as insects, other arthropods, small vertebrates, physical damage, etc. need to be known.

The overview discussed briefly the interactions among physical and biological parameters. Grain in storage constitutes an ecosystem created by man. Abiotic and biotic factors in this ecosystem rarely act individually and, as the storage period is prolonged, several previously unobserved ecological associations and interactions were reported within the controlled atmosphere grain storage ecosystem. Antagonistic interactions and/or the production of certain substances by micro-organisms may affect micro-organism growth. The need to understand these interactions is quite evident in the research on controlled atmosphere grain storage. The studies seem to indicate that fungi are more sensitive to oxygen depletion than to an excess of carbon dioxide.

Because of the complexities of the micro-organism population dynamics, the selected inhibition, the resulting sequence of species development, differential response to oxygen depletion and to carbon dioxide excess, it is obvious that more research needs to be carried out toward a better understanding of these biological processes.

Other topics presented and discussed at this Symposium included, inter alia:

- the effect of air-tight storage on micro-organisms in maize;
- the effect of inert atmospheres, especially nitrogen, on seed quality due to fungi inhibition (particularly as influenced by m.c.);
- the growth inhibition of fungi as a result of the total lack of oxygen;
- the question of aflatoxins; and,
- the problems of maintaining seed viability in controlled atmosphere conditions in the presence or absence of micro-organisms.

In the general discussion following the Session's papers, it was observed that the subject matter of the papers seems to indicate a great interest in the use of high moisture grain for animal feeds.



The question was raised as to whether economic comparisons of controlled atmosphere storage have been made with alternative techniques, such as the application of organic acids. One research group indicated this had not been done since the price of propionic acid has risen, due to petroleum costs, to the point that this acid's use is no longer economical.

By alluding to mechanical damage to insects caused by inert materials, a discussant noted that very few comparable studies have been reported on causing damage to micro-organisms (which cause trouble to man and animals) which has a significant detrimental effect on any of their various essential characteristics. He also called attention to situations in which micro-organisms may appear to be dead but are actually viable. If researchers do not exercise self-caution in this regard, they may unintentionally draw erroneous conclusions.

A significant conclusion from some papers was the demonstration of the inhibitory effect of pure nitrogen on fungi. Also the promising results showing inhibition of the production of at least one mycotoxin were interesting. The inhibition of some micro-organisms under carbon dioxide, helping to allow a longer storability at higher than normal m.c., thus preventing losses in nutritional qualities, was also demonstrated.

Differences in physical variables, sampling and evaluation of mycoflora, as well as different emphases on species of storage and/or field fungi, make it somewhat difficult to compare the results of the papers as a group. However, in spite of all the promising results and technology with gases or with anaerobic principles, practical realization generally remains subject to the following: economy; technical difficulties such as the need for more expensive structures; and, several biological unknowns such as the confusing and complex dynamics of the mycoflora which causes the grain to spoil and deteriorate.

We still need to find the optimum method for practical use of controlled atmosphere storage which is of cost/benefit value, and is easy to install, maintain and use, in order to reduce microbiological problems. We need to be more specific on the research on mycotoxins. Perhaps some mycotoxins that affect animals will not affect humans.

We need to know more about the inhibition of mycotoxins other than aflatoxin, e.g., Fusarium with its mycotoxin T-2, growing on extremely high m.c. grain.

Finally, the need should be stressed to stimulate research on control of micro-organisms in modified and controlled atmosphere storage on a practical scale in more of the developing countries.

Wrap-up report of IV Session:

#### ARTIFICIAL CONTROLLED ATMOSPHERE STORAGE

by Dr. H.J. BANKS

In this Session we heard 5 papers on the practical application of modified atmospheres and two were theoretical ones on topics closely related to the practical problems which may be encountered. Many of the papers were by several authors but are referred to below only by the name of the person who presented it.

Three of these papers described successful commercial use of forms of controlled atmosphere storage. Yamamoto gave details of the CEM technique, a system of packaging rice under  $\text{CO}_2$ , used commercially in Japan; Shejbal gave examples of the preservation of wheat and barley under nitrogen in Italy; while Banks presented details of commercial use of  $\text{CO}_2$  for the disinfestation of stored wheat in Australia.

The practical applications described are the cumulation of and even possibly the end of the line for the research effort which many of us here have made in the studies of the effects of various atmospheres in the storage environment. Collation and comparison of the results of commercial treatments are important as, under full-scale practical constraints (as noted both by Shejbal and Banks) many problems become apparent which either were not anticipated or by their very nature were not amenable to study in the laboratory.

The range of papers presented on the practical applications reflects in part the different philosophies of approach which are required under the different economic and storage conditions prevailing in the different countries. However, I believe that the main reason for the different approaches has been the degree of sealing attained in the enclosures used for the studies. In Shejbal, Adesuyi and Yamamoto's papers, efforts have been made to provide virtually gas-tight enclosures. In all cases, they were purpose-built systems, not modifications of existing ones. In the first two cases, the approach has been to provide indefinitely a positive pressure differential across

the enclosure walls by addition of nitrogen whenever the internal pressure fell below a set value. The addition was required to compensate for gas loss from temperature fluctuations combined perhaps with a slight loss from leakage. Since temperature changes cause gas loss because of the rigidity of the enclosure, use of some compensating device such as a "breather bag" might be appropriate.

In Yamamoto's work, the high  $\text{CO}_2$  atmosphere is retained for a long period by the gas impermeable membrane enclosing the commodity. Since the internal atmosphere is at reduced pressure, temperature variations do not cause gas loss. In the treatment described by Banks the enclosures used were more leaky than those used by Shejbal, Adesuyi or Yamamoto though still of a relatively high standard. A constant over pressure could not be maintained within these enclosures when nitrogen was used without excessive gas usage. A constant bleed-in instead was used to maintain the low oxygen atmosphere. It was still possible to use  $\text{CO}_2$  without additional gas after purging, because of its more rapid biological action and lower sensitivity to leakage before it ceases to be insecticidal. In the treatments described by Jay a yet lower standard of sealing was used and it was even necessary to add further gas after purging in order to maintain a satisfactory level of  $\text{CO}_2$ .

We also note a graduation in the exposure times used in the various studies. Yamamoto, Shejbal and Adesuyi used indefinite exposure times to obtain complete insect control within the enclosure: more than 10 days for high- $\text{CO}_2$  atmospheres and several weeks for nitrogen-based ones. Jay used only a few days exposure to  $\text{CO}_2$ /air mixtures. These differences are largely in response to the reasons for which the modified atmosphere treatment was applied. In the first three cases, both insect control and quality preservation are required because of the high moisture content or instability of the commodity stored. In the cases described by Banks, insect control only was required as moulding and quality deterioration was not a problem because of the dryness of the commodity. Since the modified atmosphere was not maintained indefinitely, complete insect kill was required, otherwise there would be a resurgence of infestation during subsequent storage.

In Jay's work the treatment was applied only to control but not necessarily eliminate an existing infestation.

A graduation in gas usage is also apparant. Studies with very gas-tight enclosures either used no (Yamamoto) or little (Shejbal, Adesuyi), additional gas. With structures of a lower gas-tightness no more gas was required for high-CO<sub>2</sub> atmospheres, but about three times as much additional nitrogen was needed to maintain the low oxygen ones, while for still more leaky structures, Jay used a high rate of CO<sub>2</sub> addition to maintain the high-CO<sub>2</sub> atmosphere. The overall CO<sub>2</sub> usage in the latter case was several times that used in the treatments detailed by Banks.

The studies provide an excellent example of the choice facing grain storage authorities:

- Seal well and use little gas; or,
- Seal less well and use more gas and, in extreme cases, even then complete insect control may not be achieved.

There was some discussion on which was the best mode of purging grain storages with nitrogen or CO<sub>2</sub>. Jay found in general that application of CO<sub>2</sub> as a snow directly into the grain stream entering a bin was the preferable method, largely because of the speed with which it could be applied and the lack of need for a vaporiser. The method took about 15% more gas than used by purging from an introduction point at the base of the bin.

The most efficient process used involved application of vaporised CO<sub>2</sub> into the bin headspace. It was also considerably faster but it was not made clear why this was so. The application rate into the base of the bin was between one-tenth and one-twentieth of that used in commercial Australian treatments summarised by Banks and using a similar mode of CO<sub>2</sub> introduction. Both Shejbal and Banks gave examples of nitrogen application to the base of storages. Shejbal said that application into the headspace had been attempted but it was inconvenient and gave rise to excessive pressures within the storage.

The extension of nitrogen-based storage techniques into the humid tropics as detailed by Adesuyi for pilot scale trials is an important step if it can be successfully demonstrated technically and economi-

cally. In the trials four sealed bins were used, each with a different treatment designed to test if insulation or shading affected moisture migration in the headspace. As may be predicted from model studies, shading of the roof substantially reduced the diurnal headspace temperature variation compared with an unshaded bin. Insulation of the headspace or even the whole bin had little effect. However, the metal protecting the insulation appeared to be unpainted galvanised iron. This may have become very hot compared with the white paint of the control bin, thus negating the insulating effect.

The theoretical studies presented augment the practical ones. In Yamamoto's second paper the reason for the success of  $\text{CO}_2$  packaging in gas-impermeable films is clearly demonstrated.  $\text{CO}_2$  was reversibly sorbed by commodity proteins creating a partial vacuum within the package. The magnitude of  $\text{CO}_2$  sorption on wheat, 0.14 tons  $\text{CO}_2$ /1000 tons wheat, is similar to that, 0.2 tons  $\text{CO}_2$ /1000 tons wheat, found by Banks in practice.

Tranchino's paper gave an elegant demonstration of the heat generation by sunflower seeds stored at above the "safe" moisture level and the beneficial influence of a nitrogen or hermetic atmosphere on delaying the onset of this process.

Overall I believe this session gave a diverse but coherent picture of the practical approaches which may be made to controlled atmosphere grain storage. Many of the problems remaining are economic or managerial and not ones which can be solved by research studies alone. It is to be hoped that others will accept the challenge of integration of the controlled atmosphere technique in one or more forms. A true assessment of the advantages and drawbacks of the techniques can only be made from commercial practice. However, before adopting the method a choice must be made between the various approaches to its use. The flow scheme given below, similar in form to those given by Jay, summarises the results of the various papers in this section to assist in this choice.

Decision is  
taken to use  
CA technique

↓  
Is the structure  
well sealed?

No

Use CO<sub>2</sub>, but expect  
a high gas usage and  
possible insect  
survival (1)

↓ Yes

Is quality  
preservation or  
mould control  
necessary?

No

Choose between a N<sub>2</sub> or  
CO<sub>2</sub> - based fumigation  
on economic grounds.  
N<sub>2</sub> is probably prefera-  
ble in untreated  
concrete cells.

↓ Yes

Apply low oxygen  
atmospheres (2)  
continuously

#### NOTES

- 1) Incomplete kill is undesirable as it could lead to selection for insect resistance to the technique.
- 2) The literature suggests either low oxygen or high-CO<sub>2</sub> atmospheres may be effective in such cases, but only nitrogen atmospheres have been comprehensively studied.

Wrap-up report of V Session:

## PRESERVATION OF QUALITY IN CONTROLLED ATMOSPHERES

by Mr. S.W. PIXTON

In Session V seven papers were presented dealing with preservation of quality in food stored in controlled atmospheres. All dealt with human foods including wheat, rice, malt, dried fruit, shelled hazelnuts and almonds. High moisture rapeseed and sunflower seed were also considered.

The main reason for manipulating the storage environment is to control, or prevent attack by, insects, mites and fungi. This approach to storage is becoming increasingly important.

Control of insects, which make a large contribution to food losses throughout the world, is usually achieved by fumigation or with insecticides. Not only are such treatments expensive, but they may well need to be repeated if grain remains long in storage and so give rise to objectionable residues or undesirable chemical changes in the food. Control is needed most in the developing countries where losses have the greatest impact and where the natural environment is so suitable for both insect and mould development.

It is effect on the pests, including fungi, of a modified atmosphere that receives first priority and often the effect of the treatment on the product being protected receives less attention. If the product is destined for human consumption, or is seed, then the essential quality factors must remain unimpaired. I was pleased to find that a lot of careful, detailed quality assessment work has been, and is being done on the basic foods stored under different environmental conditions.

My impressions from the papers given, and from the discussions were that temperature had the greatest effect, low temperature of storage, generally, being beneficial. But even at ambient temperatures in atmospheres completely free of oxygen, or almost so, the various important parameters of the products were maintained for considerable



periods even at moisture contents much higher than normal. Germination was held, baking factors remained satisfactory, and although FFA and FAV increased, no objectionable taints developed. In fact it was pointed out that almonds, hazelnuts and raisins stored in a low oxygen atmosphere were superior in flavour, quality and acceptability compared with samples stored in an atmosphere of ambient oxygen tension for periods up to one year. Rice packaged in an atmosphere of very low oxygen and stored at a low temperature for two years was superior in palatability to a similar sample packaged in an atmosphere of normal oxygen.

The overall conclusion is that storage in a low oxygen atmosphere, preferably, but not necessarily, at low temperature produced no consistent adverse effects, or particularly beneficial effects, in the functional and end use properties of dry food products.

There are difficulties. No one system of storage is suitable for all purposes, or circumstances. In the developed countries the introduction of a new system could well be decided if products are susceptible to deterioration in air, and unacceptable to the market, then, introduce a new system such as storage in a nitrogen atmosphere; in the developing countries, where the benefits of a new proven system could be most marked, then, introduce the new system alongside the old.

Whatever the system of storage, and procedures that modify the storage atmosphere are attractive for a variety of reasons, it must not adversely affect the quality of the product. That, together with reducing losses, is paramount. The thorough work on quality assessment reported in this session has made a valuable contribution to the knowledge of product storage behaviour.

More needs to be done and higher moisture grain and feed stored for animal feeding should also be included, as well as the relatively high protein oilseeds. Here different criteria would apply but they would be equally important. We also need to know more about the effect of nitrogen, for example, in packeted instant foods in relation to maintenance of quality and increased shelf life. These are just

some of the areas that need consideration.

I would like to take this opportunity of thanking Assoreni, and particularly Dr. Shejbal, for organising this Symposium.

Wrap-up report of VI Session:

## FACILITIES FOR ARTIFICIAL CONTROLLED ATMOSPHERE STORAGE AND ECONOMIC ASPECTS

by Mr. G.G. CORBETT

The papers and discussion in Session VI may be dealt with under four subject headings: structures; equipment for producing the controlled atmosphere; economic aspects; and the integration of controlled atmosphere (CA) facilities in complete storage systems.

It is clear that the key point regarding structures is the degree of gas-tightness. In Australian work, a decay time of five minutes for an applied excess pressure drop of either 2,500 to 1,500 Pa, 1,500 to 750 Pa or 500 to 250 Pa in a full storage is regarded as satisfactory. (I noticed, however, that the pressure attained in the bolted metal shed, which Dr. Banks and his co-workers successfully sealed, was only 100 Pa). A Japanese standard decay test of 500 - 200 mm. water gauge in twenty minutes was quoted. In the Italian work with nitrogen atmospheres, gas-tightness was expressed as the nitrogen flow rate necessary to maintain a slight positive pressure (45 mm. w.g.). These flow rates were calculated to be equivalent to a pressure decay from 250 to 150 Pa in a time period measured in hours, reflecting the much greater gas-tightness of the structures used. Finally, in the work in Egypt on a small concrete bin, the gas-tightness was discussed in terms of the daily percentage depletion of carbon dioxide. Clearly there is a need for some uniformity in methods of measuring and expressing gas-tightness.

Many different structures for CA storage were described and discussed. Dr. Kamel's paper on the small concrete bin demonstrated the difficulty of sealing this material and described the methods and materials which were finally successful. The same difficulties were apparent in the much larger semi-underground ('Cyprus') bins, though here the leakage was concentrated at the joints between the above ground dome and the underground cone. There was much discussion of

the use of plastic films, both for above ground silos, heaps or stacks of bagged grain and as liners for pits. There seems to be a need for the compilation and publication of information on the relevant properties of plastics available for these purposes. Conventional welded metal silos present no gas-tightness problems and have been successfully used for CA storage in Australia, France and elsewhere. Conventional concrete cells, on the other hand, seem to be difficult and expensive to seal. It is good to note that there is an active Australian Committee on Silo Sealants working on this problem. Australian work has shown that it is possible to seal bolted metal silos and sheds to a standard allowing successful application of carbon dioxide "fumigation", but such sealing, particularly of sheds, requires considerable expertise. Finally, we heard of two newer forms of silo construction - fibreglass and folded metal - which have been successfully used for nitrogen atmosphere storage in Italy, and of a concrete dome structure formed by a novel technique which offers possibilities for both bag and bulk CA storage. A technique for sealing these domes was described, but to date it has been tested only on a small structure of 4 m. diameter.

Further important points on the design and operation of CA structures were underlined in this session. One was the need for careful attention to apertures (or penetrations in Australian terminology). Even if floor, walls and roof are perfectly sealed, degradation by time or temperature of aperture sealing will reduce gastightness to an unacceptable level. The second point was the desirability of a high filling ratio, to minimise temperature and pressure variations in the head-space. To the same end, exposed surfaces should be highly reflective, to reduce solar radiation effects. Especially in highly gas-tight structures, a pressure relief valve to deal with both positive and negative pressures is essential. Further studies are necessary on heat and moisture transfer within enclosed structures. Two such studies were reported on open structures, but as one of the authors concluded: "In airtight conditions of storage, phenomena are certainly quite different".

On the equipment to produce controlled atmospheres, we heard in

this and earlier Sessions of machines to produce high nitrogen or carbon dioxide gas mixtures, apart of course from the use of these gases in liquid form, which is the primary form used to date in industrial applications of CA grain storage. For nitrogen, there appear to be two practicable and economic alternatives to the use of liquid nitrogen in locations far from production/distribution centres - the catalytic burner and the pressure-swing adsorption system. Similarly for carbon dioxide there are two types of gas producer, the exothermic generator and the catalytic converter already in commercial use, though to a small extent only, for CA grain storage. The expansion of such storage should therefore not be limited to areas served by a liquid gas supply, though the economics of local gas production will require close examination on a case-by-case basis.

Only one paper was presented giving a comparison, on both a capital and operational cost basis, of CA storage (using liquid nitrogen) with conventional storage using chemical pest control. This comparison showed substantial savings for the CA storage system, a large part of such savings coming from the elimination of grain losses assumed to be 1-3%. As the author of this paper pointed out, economic comparisons between different forms of CA storage, and between CA and conventional storage, are somewhat complicated and depend on many factors such as the product involved, length of storage period, size and location of storage installation, types of storage structures compared, and so on. It would nevertheless be interesting to compare, for the same structure, the cost of obtaining pest control by nitrogen and by carbon dioxide CA storage and by chemical fumigation.

Finally, two papers in this Session stressed the importance of careful planning, particularly in developing countries, to integrate CA storage into existing storage systems. Both these papers dealt with the 70 Cyprus bins in Kenya, which are the only CA storage structures considered in depth in this Session that have been in continuous use for the last 10 - 15 years. The many problems, both with the structures themselves and with their integration into a marketing system involving procurement, drying, transport and short and long term storage, might have been avoided, or at least reduced, by more careful

planning - or, as was suggested in the discussion, by first constructing a small number of bins on a trial basis. In the present state of the art, this cautious approach, at least in developing countries, might well be the overall recommendation from this session to policy-makers contemplating investment in CA grain storage.

## GENERAL DISCUSSION

Dr. SHUYLER

I was pleased to note that Mr. Burrell included the mention of the problem of rodents in connection with traditional modified atmosphere storages. I think that this is one aspect that was missing almost entirely from the papers and I am aware of the fact that, for example, earlier reports from Somalia indicate that this can be a very, very severe problem not only in reducing the controlled atmosphere nature of the store but, of course, in producing additional losses.

Dr. GIRISH

I have three suggestions for your consideration. Data are lacking on the effect of modified atmospheres on mites. Of course, India is not a country with particular problems with mites, but this is one of my suggestions.

Second, data are really lacking on the effect of modified atmospheres on brown rice, paddy and maize. To some extent data exist on wheat. Dr. Adesuyi will endorse my view that data are also lacking on the oilseeds, so there is a need to have more studies on the effect of modified atmospheres, particularly on these commodities.

My third submission to the Chairman and learned gathering is: You see, for the use of modified atmospheres we cannot discard our existing storage structures. I wonder how to modify or how to renovate the existing storage structures to the use of the modified environment. We have heard an excellent paper from Dr. Banks. This aspect, in which developing countries are perhaps most interested, of how to use their existing storage structures for this new technology is very important.

Then my last request to the learned audience and our Chairman is, how can we improve this technology, particularly at farm level storage? Sixty to seventy percent of the food grains in developing countries is stored at farm level. How can this new technology be appropriately applied, with some modifications, in the existing structures

which are prevalent at farm level?

Mr. BAILEY

There is a little information on the effects of 99.5% CO<sub>2</sub> on Tyrophagus putrescentiae. Eggs are the most tolerant stage requiring six days for complete mortality (25°C). Information on Acarus siro indicates that adults are killed by four days exposure at 20°C to either pure nitrogen or pure carbon dioxide. In general we are very short of good data on the effects of CA on mites.

Dr. GIRISH

This is well known that even in insects the susceptibility varies from species to species. What I submitted is, that data are very lacking. Can one conclude from one or two papers?

MODERATOR

If I may comment on research in relation to its constraints in this kind of work: it is limited by time, by possibilities and the number of variables. I think more model work should be carried out. A simplification is what we really need. If we get good models in collaboration with physiologists, we may get results faster for applications.

Dr. YAMAMOTO

We have the data of brown and paddy rice in the under water and underground storages. Also the varieties we use are japonica not the indica type.

MODERATOR

Well, of course, rice is different from country to country. We have carried out work on rice which was not presented during the Symposium. We feel that rice is just as well preserved as other cereal grains up to a moisture content of about 16.5%. Above that there is a problem with anaerobic respiration which may cause off-flavours.



Mr. BURRELL

In the tests which I described on effects of airtight storage on insects, we did not count the numbers of mites present at the beginning of our tests but we knew that there were many present. At the end of our experiments in a butyl rubber silo, in which we had a heavy infestation of insects, we concentrated on the insects but we looked at the mite survivors and there were some A.siro, some Cheyletus eruditus and some Glycyphagus destructor, very few but there were some.

MODERATOR

In that experiment of yours the oxygen concentration reached what level, do you remember?..

Mr. BURRELL

The oxygen concentration in that particular bin came down to 0.3% for a short period but most of the time it was between 4 and 5%. The observed mites were all adults. We did not look at other stages.

MODERATOR

May I refer to the problem of oilseeds. As a matter of fact, during the Symposium only the work done in Nantes, France and in our, the Assoreni Laboratories, referred to oilseeds. I again feel that this is a subject where work is needed in model experiments.

Dr. NAVARRO

Storage of oilseeds is a problem due to heating problems existing during storage. Normally cottonseed or soybeans may be initially stored at low moisture contents, but during storage, especially under tropical or sub-tropical conditions, moisture migration takes place and then heating may be very significant. It is a point that workers planning research on wheat in controlled atmospheres should take, into consideration, for designing more experiments to see whether there are the possibilities to maintain these seeds and to prevent any heating problems in these products.

Dr. ADESUYI

I would like to make some comments on what we have discussed so far in this Symposium. There is no doubt that there is an urgent need for good storage techniques in the developing countries and controlled atmosphere storage is one of the possibilities that can be adapted or developed to get answers for some of the problems. Controlled atmosphere, in my mind, is more feasible in large scale storage rather than on the small scale. Many of the developing countries have plans to establish, or they have established, famine reserves in large scale storage, and for inter-seasonal stabilization of prices. I see the use of controlled atmosphere for storage of grains and other products being feasible in these large scale proposals.

Second, I am in agreement with Dr. Banks in his suggestion; or his implication, that the ball is now in the court of the commercial companies to select whatever technique that they think suitable for implementing the use of controlled atmospheres for grain storage. In saying this, I also would like to state that the companies should bear in mind that whatever technology they select must be one that works. I am talking from past experiences. Some technologies have been sold to developing countries that never worked and of course this has made some of the authorities develop fits even when they only hear the technologies named. I will not blame them for this. Money is a big problem in these areas. Also I am in agreement with Dr. Girish in his suggestion that more work should be done in trying to adapt existing stores for the use of controlled atmospheres. For example, in Nigeria we have a lot of stores of varying capacities and it would be very useful if we could know ways by which to adapt these stores to fit into the new technique of controlled atmospheres.

Lastly, Dr. Girish also mentioned that about 60 to 70% of the grains available in developing countries are stored by local farmers. I put it at about 90% in Nigeria; so these are the people to whom we are to direct more attention. I would also like more work to be done on the adaptation of small containers like drums, or the containers mentioned by Dr. Kamel in Yemen, to improve them so that they could be used for airtight storage either with or without gas.

Dr. BANKS

I think that you raised a very important point, Dr. Adesuyi, in saying that countries must have technology that works. I think that the problem is that it is not just technology that works that is needed, it's technology that works in the hands of those who use it, and this has a very special bearing on the adaptation of existing structures. I have no doubt that given an existing structure almost anywhere in the world, there is a technology available to make it gas-tight to a standard where you could use some form of controlled atmosphere in it. The problem is not to do that process, but how to manage that store, and to somehow arrange for the enthusiasm of the managers and the people using it to continue to use it satisfactorily. I think this is a problem related to how the technology really is to be sold, from the people who developed it, be they industrial companies or scientists, all the way through into the practical situation. I don't know any real solution to this problem, except that I think that one of the things is that the scientist ought to work directly with the managers in the pilot development of any process. It is quite easy, for instance, to seal a godown; whether it's economic, is another problem. And having got it to a stage where it might be economic, it is yet another problem to make it work under the local conditions and I believe only with the utmost cooperation of management to develop the available local expertise, will it ever work.

MODERATOR

I think this speaks, in a certain sense, for the use of new structures. It also speaks for highly automatic new structures, which is, in fact, what we are aiming at in the Assoreni work. We want the store to work automatically from loading and the initial purge, down to unloading, without really ever having to touch it. It is automatic. And when you just control the consumption of your gas, you know what you have, that's it: quite fool-proof.

Mr. STOREY

I think it is interesting that questions raised by the representatives of two so-called developing nations, are no different from the questions raised by myself.

At the risk of sounding like the Ugly American: I have heard about all I need to hear about saving grain in under-developed countries. I think, instead, we need to devote a lot more attention in trying to preserve it in the producing countries. I don't believe there is anyone here that is not going to be touched in one form or another by a US grain-based commodity, at least in the foreseeable future. I recognize that in a developing country, when you are out of food, you starve. And this is a little like being 6% unemployed. If you are one of the six percent, you are a hundred percent unemployed! If we look at where the grain is being stored that you will probably consume in the foreseeable future, it is sitting on individual farms in the United States. We presently have almost 60% of our grain supply situated on individual farms, still owned by the farmers. And there is nothing in the present technology that has been discussed that would lend itself to the protection of this grain.

So I refer myself to the same question raised by the two previous gentlemen: "What are we going to do about all the grain that is kept at the individual farm?".

Prof. ZANON

From this meeting it has come out very clearly, there is no doubt, there are real advantages in the use of these modified or controlled atmospheres. Where the doubt still remains is, which method will be most convenient and the most used; because there are some differences in opinions. For instance we heard about high  $\text{CO}_2$ : 35, 40 or even 100%  $\text{CO}_2$ . There are people in favour of very, very pure nitrogen, such as a purity of 99.9% with 0.1% oxygen; or at maximum, they say, 0.5% oxygen.

Well, I want to put another question to this plenum: what about other experiences or opinions? What about a third possibility or alternative, which will be very easy to create in very economic struc-

tures? That alternative would be a very low oxygen content atmosphere, say 1%, combined with a certain amount of  $\text{CO}_2$ . And this question is asked, because it would be very easy to achieve with simple burners, which cost little, and which can be placed even at the farm or any small unit. You can create such an atmosphere, which will be, for instance, with 1% or 2%  $\text{O}_2$  and say 13%  $\text{CO}_2$ . Which is present in such a combustion as is attained with such burners.

The second question is, would it be possible to prolong the purging time, so that cheaper machines could be used for producing nitrogen on the spot? So what would be, in your opinion, the maximum purging time to give the results we are expecting from such a method?

#### MODERATOR

I think that low oxygen concentrations of approximately 1% for disinfestation are quite all right. And in that case the time necessary for fumigation is quite short.

Yet I do not believe that controlled atmosphere generation by burners could be used on farms or in very poorly controlled conditions. I think we would run into very big legal problems. Imagine what would happen if we just put a car next to the shed where we keep our grain and push in whatever comes out of the car exhaust pipe. Everyone would say - but you are putting in hydrocarbons! And, of course, if somebody does not use a burner well enough, he may run into the same problem. So I am not quite convinced that this would, from a legislative point of view, be permitted.

May I ask for a comment on this by Mr. Storey, who, I think, has heard some voices of this kind in the United States. What is the situation for the permission of the use of burners in these situations?

#### Mr. STOREY

There are two aspects to this. We have recently submitted a petition to the Environmental Protection Agency, proposing an exemption from the requirement of tolerance for controlled modified atmospheres containing either less than 2% oxygen or more than 60% carbon dioxide

for control of stored product insect pests as a post-harvest use on all agricultural commodities. This proposal includes exemption from the tolerance requirement with respect to the modification of the environment produced by the so-called burner atmospheres. I have been informed by EPA that we will probably be granted this petition sometime this summer. So, as far as using the material as a pesticide, we probably are going to be given approval.

The next question is, are there going to be unresolved compounds in this atmosphere, that will somehow cause the material to be prohibited? I have heard many reports, but I have seen no documented material concerning the presence of unsaturated hydrocarbons or any other carcinogenic material in this atmosphere. Obviously, there could well be; if you get into certain burning situations, this could be a problem. I still believe the technology is available to take out anything that is present that would be of a harmful nature, if you want to do it. The question is then does this still leave it within an economic range to make it useful? I agree with Dr. Shejbal, that probably the use of this technique on farms is impractical, not so much from the technical standpoint but rather the economic one. There simply isn't sufficient margin presently in the marketing of grain to allow the farmer to spend that much money. If we made it worthwhile to market grain free of insects or, if we legislate against the marketing of grain that contains insects, then many of these things, that are not now feasible, could become so.

#### MODERATOR

May I repeat Prof. Zanon's question: "What will happen if we prolong the purging time?" I suppose this is an economic question and maybe Ing. Tranchino could comment on that?

#### Ing. TRANCHINO

I want to comment the questions by prof. Zanon. We have found in our trials that decreasing the nitrogen flow rate, the efficiency of the purge decreases, so that a higher nitrogen consumption arises.

In some cases, decreasing the flow rate to 1/4, the nitrogen

consumption becomes higher than 2 times, and the time of the purge higher than 8 times. Our results are in agreement with the others that Dr. Banks has found in his trials.

The possibility to control the nitrogen flow rate allows to reduce the nitrogen consumption for the maintenance of the controlled atmosphere too. If the nitrogen can be supplied to the silo at the time of need only (when the pressure decreases) it is possible to reduce its consumption to the gas leakage from the silo only. This is possible with liquid nitrogen supply, it is not with a gaseous nitrogen production plant.

The economical comparison between different nitrogen supply systems must take into account these considerations, evaluating the cost of the total nitrogen consumption on the basis of the cost of one cubic meter of nitrogen is not the full information for the comparison.

We have performed this analysis and we have found that increasing the capacity of the storage complex, the cost of nitrogen consumption with a gaseous production plant decreases and becomes lower than the value for liquid nitrogen supply at a critical capacity that is in the range of 20,000 - 50,000 t., depending on the market prices.

#### Prof. ZANON

One thing I really wanted to know in order to calculate the economics is, what is the maximum time permitted for doing this purging operation? Then all the other considerations can be made, and we have a lot of experience on that, too. Partially I agree with Ing. Tranchino, but if I can I wish to learn from the people who are here, and have experience in the field, what they consider the maximum time allowed to do the purging operation, in order to have all the advantages we expect, considering also the economic side of this operation. We don't have to search for perfection but we have to search, let's say, for a compromise which gives us enough results at a reasonably low cost. I think that's a very general rule.



MODERATOR

I wonder whether the participants would be of my opinion, that it is impossible to give a simple answer to such a complicated question. We have so many different situations that it would just be too complicated to generalize, except if all the work were done by researchers. We would have to have certain standards, as was called for in one of the Rapporteur's papers, also.

Dr. BANKS

I think, Prof. Zanon, I can answer your question in part. I would like to support Ing. Tranchino's comments that in vertical storages it is necessary to purge as fast as possible and neither his results nor those in Australia have found an upper limit to this in terms of rate of gas put in, in order to attain the maximum efficiency of purging. Thus, if you have a slower rate of purging, you can expect to use somewhat more gas. And the balance is there: if you purge more slowly you will lose more gas. And this may be up to 50% more gas over the kind of purging rates that we have used. This applies to nitrogen. But there is a problem, because nitrogen is less dense than air; this exaggerates the mixing of the front as it passes through the grain. If you are using burner gas, you have maybe 12% CO<sub>2</sub> present and under such conditions you are putting a more dense gas into the bottom of the silo and it may be that you get a better purging efficiency using this more dense gas in the same way that you do with high carbon dioxide atmospheres.

So while the answer appears to be that you should put it in as fast as possible, the answer is complicated because the quantity of CO<sub>2</sub> present may allow a better efficiency at lower rates.

Dr. NAVARRO

From the point of view of insect control, what is the maximum limit of oxygen permitted in a gas-tight structure when applying controlled atmospheres? There is well documented work on this subject when we deal with hermetic storage. In this storage, normally, in field conditions, the oxygen concentration does not drop to 0 or



0.1%. What we normally get in field conditions is 1 - 4% oxygen concentration, whereas that of carbon dioxide lies between 8 to 14 - 15%. In these conditions all documented reports indicate that good control of insects was achieved.

Indeed when we carefully examine the results obtained in laboratory conditions, we can see that most of the insects can tolerate a certain low level of oxygen concentration, provided the carbon dioxide level is suitable for their existence. These reports are well documented and Prof. Calderon presented some of the results in this respect.

Dr. SHUYLER

With respect to the comment of Dr. Navarro, when we were in China, we had reported to us, I believe in at least three places, that insect control appeared to be complete with concentrations of oxygen no lower than 8%. I merely add this, so that it's in the information of the Symposium.

Prof. CALDERON

I should like to ask you to comment on the fact that although in this Symposium we obtained much information about the subject we are dealing with, I am afraid that we omitted a precious bit of information which was gained in the past, may be thirty or forty years ago, on this technique. I refer to the underground silos of Argentina and, after that, those constructed in Uruguay. I know that, from reading the first reports by these people, there is undoubtedly a lot of know-how and experience which has been gained in these places. It is a pity that we do not know much about the advantages and technical difficulties which have resulted from this long and, I think, very laborious work. I should also like to point out that in the Sixties the Belgian erected new silos in Brazzaville for the same purpose and I wonder whether there is some experience gained from these silos. Also, the silos erected in Venezuela, air-tight and built specially for this purpose, would be of interest. When I was there, they were already abandoned for this use, but undoubtedly there was some reason

for that. But, there must also be some experience and maybe there were problems which might be overcome. So, I think that we should in the future, or maybe now, if there is someone who could comment on this, learn a lesson from these past experiences. This would give us more information than we have.

#### MODERATOR

I must say that I invited several people from Argentina: from the Junta de Granos and the Ministry of Public Works but unfortunately they were unable to attend. I went to Argentina about two years ago and I talked to the people on the spot. What the Junta de Granos said was, that they would never build these underground structures again. The main reason for this is that the water level in Argentina is generally so high that the lower part of these underground structures suffered a very important leakage of water from the outside and much grain was lost, especially by quality deterioration. These underground structures were built mostly during World War II, because that was the period when grain could not be exported from Argentina to other parts of the world. So, the structures built were very large and, thus, another huge problem was caused by the fact that unloading was so difficult. This is the reason why in the present programmes in Argentina only above ground, mostly horizontal structures, are envisaged. Unfortunately, they do not have enough money to build structures which would be sufficiently gas-tight to be used for controlled atmosphere storage.

Is there any other comment on the question raised by Dr. Calderon, please?

If not, Mr. Storey wanted to ask a question.

#### Mr. STOREY

I wish to raise a question which, I believe was not directly addressed at this meeting. As you may know, we have a rather unique method of remodeling our elevators (silos) in the United States: we simply raise a little dust, then ignite it and we start over! As a result of these experiences, we are quite concerned about breakage of

grain. My question: I should like to know whether anyone has made studies concerning the long-term storage of grain and its subsequent effect on breakage when handling?

#### MODERATOR

Is there anybody who can answer this?...

Just to come back to explosions. I should like to stress that secondary explosions in elevator (silo) bins could be avoided if grain was maintained in an inert atmosphere.

#### Mr. STOREY

I have seen a few elevators after the explosions and I doubt very seriously whether it would make a great deal of difference; Because the initial explosion opens the roof on almost all the cells. The secondary explosion then occurs from dust filtering into the top of the cells. I am not sure whether a cell sealed in a low oxygen environment would have any greater effect than simply a sealed cell, so that the passage of the front would not have access to the cell itself. In the elevator at Galveston for example, the explosion went up an air shaft from the rail area, down a long pipe that was used for feeding grain into a steel storage structure and the top blew off that as if a can opener had been used. But those cells that were blocked off did not explode. So, I am not sure whether simply blanket-ing the atmosphere with the low oxygen environment would be of benefit. It would certainly not hurt, but whether it would actually stop the secondary explosion or not?...

#### Mr. WILSON

Prof. Zanon, I think I have an answer which would satisfy your request to know the time of purging. In 1976 I purged two 1,900 t. cells with nitrogen and the total time of purging was 10 hours. We purged 2,200 m<sup>3</sup> and then carried out maintenance with the gas for 4 weeks. I have studied the economics of that and then the following year, I did the identical experiment on the same cells using CO<sub>2</sub> and I did the costings there. Without wanting to get deeply involved in

the cost differentials, I think I would like to say that costs are funny things. They are likely to be favourable for one gas one year and possibly for another in another year. But our situation, of course, is that we can get  $\text{CO}_2$  cheaply and our total purge time for  $\text{CO}_2$  was 1 ton of  $\text{CO}_2$  per 1,000 t of wheat. So therefore the purging time in the 1,900 t. bin was 1.9 hours, compared to 10 hours with  $\text{N}_2$  to get the oxygen level down to, I think, 1.8% and then we had to maintain it for the 4 weeks. Therein we incurred extra costs.

#### MODERATOR

I wonder whether we could treat another subject which I think is extremely important: the safety of the controlled atmosphere storage in comparison with chemical products; fumigants, etc., and their residues. In developed countries these products are used a lot and we know that no fumigant exists that would not leave residues. But, is it not true that, in developing countries, either on the farms or in certain other conditions where not enough control is exercised regarding the use of fumigants, abuses may easily occur? We know that there are countries that now have small losses (India is an example of this) because they widely apply so much of these pesticides.

I think that it is an important thing that we have come together to talk about controlled atmosphere storage also in order to reduce the problems and the dangers deriving from these chemicals. Are any of you of my opinion?

#### Mr. RIPP

Yes, definitely!

My first comments are on two points that Mr. Storey made. He mentioned that American grain is going to touch most countries. I am sure that this is correct. I would just like to make the point: if you get short of grain at all, we can send you a few shiploads.

Second, on the grain dust explosions. You raised a query about whether the explosion would in fact penetrate the slab over the top of the concrete cell. I believe the answer is that if the explosion is in the early stage, where the progress of the flame is limited by

ignition of particle to particle, this would not penetrate the slab of the concrete cell. But if it has developed far enough, so that the shock wave becomes the cause of transmission, this travels at thousands of meters per second as opposed to several meters per second in the burning system. That would certainly penetrate the concrete slab on the cell. Whether the cell would actually explode would depend on whether the dust within the cell was forced into the cells atmosphere by the vibrations of the preceding explosion(s).

As to the pesticide question: This is a major problem in Australia. Our climate is such that conditions for insect development are ideal throughout the year. This led us to search for and use pesticides to control insects. Because 90 to 95% of our production is exported and of course we must live up not only to our own standards but to the standards of overseas countries and they have a dislike for chemical residues, regardless of Codex Alimentarius Commission figures. And we have had that problem for quite some time. There appears to be a rapidly growing objection to chemical residues overseas, from an Australian point of view. We must get to a stage where we can handle grain insect free without pesticide residues. We are faced by our own standards of shipping under a nil tolerance for insects, so that if in one of our storage units of 3,000 t one insect is found, that storage is rejected in total. These are serious standards and I believe personally that there must be some reduction in these particular standards to enable achievement of no pesticide residues and also the development of controlled atmosphere in its place. Because there are always insects where grain is being handled, however minor, and the handling of grain from a controlled atmosphere storage to a ship, however short the conveying equipment is, it could still be subject to the presence of an odd insect in that transmission or transport. Similarly, in ships themselves. Ships too can have the presence of insect infestations and on arrival in overseas parts, grain may be rejected because of this. The presence of pesticide is an extremely major problem as far as we are concerned.

If I may lead on from that to the problem that has been raised about sealing of current storages, which is close to the hearts of

everybody. The difficulty of sealing is not so great. I can say that from experience. The difficulty is in facing up to the problems. We have gone through several years of acclimatising ourselves to the fact that we have to do something. Therefore we face the problem. And on the basis of Bank's work and the Silo Sealant Committee's work, that was mentioned by one of the speakers, we are now facing up to the sealing problem. We are in the process, at the moment, of demonstrating, to farmers, the methods of sealing the storages that they are using. And this is in the area of 600 bushel capacity in quite small. We are also sealing 2,000 t vertical concrete cells. We are also in the process of sealing a 20,000 t - warehouse style storage and a 286,000 t warehouse-style storage. The costs that were mentioned by Mr. Corbett were taken from the proceedings and therefore commenced at A.\$ 10 per ton upwards. I should like to add to the record, that we also have costs of sealing down below A.\$ 1 per ton. Partly from economy of scale in the 286,000 t storage that is a shearing-shed type (I like that because we also have a few shearing sheds although some of the by products of shearing sheds are not compatible with grain handling) and it is perforated and thus can and will be sealed. We'll have problems, no doubt, but when we'll come to those problems, we'll overcome them.

The cost of provision of the gases to be used has been mentioned. I would only make the point that (I believe it has been made in several places), that all costs will be relative to the availability of the gases at the time, the situation, the type of storage and so many other variables. In one instance, and once again we have a facility of 900,000 t capacity in various types of structures, that is less than 3 km from a production plant that has a by-product of  $\text{CO}_2$  up to 20,000 t a year. We are going to pipe onto that supply, therefore we can also cope with the long-term problem of continued charging. We can continually charge to overcome sealing quality, as far as needed. But only in this particular instance and then when we go 50 miles away from there we are back to the costs of transport, liquification, vaporisation, etc.

My questions during this Symposium have been changed completely

to enthusiasm to get back and get into the application of controlled atmosphere. I particularly appreciate the points that were made by the Rapporteurs on the multidisciplinary approach that is most necessary and the integration of planning to introduce the system. The Symposium has been invaluable in the collection of knowledge and clarifying the concept of controlled atmosphere storage. The coverage has been total, from old to new, all types and all situations. But the value of this Symposium will be in the demonstration of commercial application of this system. Therefore I would hope that this Symposium is followed by a similar Symposium:

- a) on the application of controlled atmospheres to existing storages of all types and in all countries
- b) on the design of new storages, once again of all types and all situations. Including the total integration of all disciplines I believe to be most necessary, and all constraints faced by handling food. Thank you.

#### MODERATOR

I must say that it must be a real satisfaction for Mr. Bailey, who is the person who first undertook the entomological work on controlled atmosphere storage in Australia, to see that in Australia there is so much enthusiasm and so much is done in the application of the system.

#### Dr. SHUYLER

I wish to return to the subject of pesticides and particularly in respect to developing countries. First of all, with respect to the abuse of pesticides in these countries, we consider that use in storage is extremely small at present. For example, there are only very few countries in which there is use, in the post-harvest system, of pesticides at the farm level. At the central level, yes, there is use. There, however, we find that frequently there is the situation of under-use of pesticides because of the lack of resources. This may be due either to lack of money resources or of timeliness of shipment deliveries.



Where we do have a concern is where there are food security reserves held in place: in some cases we are suggesting at least 1 1/2 years before turnover and hoping to be able to achieve two years. Experience so far indicates that some of the countries involved will probably be planning to fumigate that grain at least every 3 months. This certainly could lead to a build-up in residues which has not been studied, because there have been no prior needs to study this kind of problem. In fact, the need for such frequent re-fumigation seems to arise mostly from cross-infestations from nearby stores which are not involved in the security food reserves. We are, of course, in that particular situation, also concerned about the subsequent development of resistance to the fumigants, which has been demonstrated by Dr. Champ in his world-wide survey and in the resulting work with Mr. Dyte, to be a very real potential problem. With regard to the recommended residue limits themselves, I think we must keep in mind that the standards used by FAO will be those which are derived from the FAO/WHO "Joint Meeting on Pesticide Residues", as it is frequently referred to, earlier mentioned as being a meeting of experts chosen by FAO and WHO, respectively. These standards may be different from those which are used in individual developed or developing countries.

Dr. CALVERLEY

I should like to confirm in some respects what Dr. Shuyler has said. In our view the presence of insecticide residues as an aspect of safety is not a serious consideration in small-scale storage in the tropics. What however, we are concerned about is the limited misuse of insecticides, leading to dangerous residues. We have in fact come across reports of organochlorides being used for storage of grain in South America simply because they happened to be available.

I think that in the question of safety and the protection of grains against insects and other organisms, we need to put the problem into perspective. Our view is that the greatest danger arises not from the use of insecticides but from inadequate drying and the consequences of mould and other organism growth on stored grain. We have



already established that serious consideration should be given to the possibility of a correlation between consumption of aflatoxin contaminated products and carcinogenesis. So I think that we have to put the whole problem into perspective and, on the whole, in terms of safety, I think that the future of controlled atmospheres is in the limitation of mould growth and the prevention of aflatoxin development in small farm stores.

Prof. CALDERON

I should like to comment on something of a general nature. In fact I would like to propose a recommendation coming from this meeting. Since there is a general consensus that storage structures have to be more tight, I would not say air-tight, but more sealed than they are, not only for the purging with nitrogen or  $\text{CO}_2$ , etc., but also for simple fumigation. In fact we know that in some places, and I saw it myself in countries in Central America, newly-built metal storage structures have free space between the top and the walls of the structure, which makes them impossible to seal in any way. Let's make a recommendation that in the future, building of storage structures, by donor organizations and in general, should see to it that storage structures for every use should be more airtight or should be made so as to be sealed for the one purpose of fumigation or controlled atmosphere storage.

Dr. BANKS

Dr. Calderon, in response to this, I think we should bear in mind a recommendation that has been around for very many years, that ventilation should be controllable. And controllable means that if you wish to have rapid ventilation, you may have rapid ventilation; if you wish to have slow ventilation, you may have slow ventilation; and, if you wish to have no ventilation, that is, if you have an air-tight structure, you may have that, too. And your recommendation does not take into account the very frequent occasions when ventilation is very necessary. Under such circumstances, I feel that perhaps one could more correctly endorse the recommendation that has been

around for very many years for controllable ventilation and to make a specific plea for that to include the cases where this is completely gas-tight. May I have your comment?

Prof. CALDERON

Yes, I understand your point, Dr. Banks, but I don't think that there is any interference. I did not mean air-tight structures only. I thought that every structure to be erected in the future should be better sealed. I did not say that it should be entirely air-tight so that ventilation would be impossible. For controlled ventilation two or three openings could be enough to permit its perfect functioning. In fact, in our Institute, the bins which were constructed so they could be made air-tight are also possible to be ventilated. So I did not mean especially air-tight storages as you, maybe, understood.

MODERATOR

I wonder whether we can really expect to have recommendations as a result of a Symposium of a scientific nature. But, of course, these comments will be included in the final discussion which will be published.

My wish is that international organizations, like FAO, and others, would take controlled atmosphere storage into some more consideration when they are asked, by interested countries: "How should we store grains?". They are the experts. I think, so far, we are still in a situation in which technology transfer between developed and developing countries occurs often by applying what exists all over the place in our countries and then in the developing countries they have to go through many of our problems, before they can change to something better. I do think that this Symposium has shown that controlled atmosphere storage, as a general concept, is a better way for preservation and fumigation. And, I don't think that we should allow everybody to go through all the problems just because insecticides should be sold and certain types of bins should be sold and so on. Maybe this is a little provocative, I intend it to be. I wonder whether people from international organizations would want to comment on this?

Will they, in the future, say: "Well, there was a Symposium during which it was shown that we have reached a certain degree of world-wide applicability of controlled atmospheres. So take it into consideration!"?

Mr. NICHOLAS

We shall certainly take it into consideration, when you prove to us that you have got something that works. And works at the level at which we are aiming at: the small farmer, at this stage. I have no doubt that there is much technical expertise and much feasibility about it. But the feasibility really is important to us when applied to the particular circumstances for which help is required. Thank you.

MODERATOR

I am quite aware that, of course, FAO is concerned mostly with the small farm and village storage. But I know, and everybody knows, that there are also large storage facilities being installed and maybe what we have heard here could at least, at this stage, be taken into consideration for those.

I should like to turn your attention to a problem on which there has not been much comment so far, that is the microbiological aspect of controlled atmosphere storage.

We shall have to understand more of the microbiological situation. But I am not quite sure whether the wrap-up Rapporteur's affirmation is correct that, for this, the best way to go is to understand the whole ecosystem in every single aspect - a very complex situation -, or whether we should rather try to simplify. In that case, we might make single, more simple, model experiments, as were reported in the microbiology session, which would at least indicate what we can expect from certain mould species.

Dr. DI MAGGIO

I would like to answer the observation by Dr. De Las Casas on the lack of bacterial examinations of samples in controlled atmosphere storage carried out by us. We have examined grain with a moisture

content of 18 to 19%. At this moisture content bacterial growth is low during storage. Therefore we studied only mould proliferation which is the major cause of grain deterioration as has been shown by Christensen and Kaufmann. The fungal proliferation depends strictly on the presence of oxygen.

Dr. DE LAS CASAS

When I said "the ecosystem" I was referring to the biological interactions that are present in the ecosystem. And you just pointed out that we have to have model, pilot studies in order to understand that. Certainly it is difficult to understand the ecosystem but we have to deal with the ecosystem as a whole. We cannot work with individual components only, we have to relate them to the whole and I am sure you are aware of plenty of work that has been done on the relationships between insects and moulds. Insects need the moulds, the moulds need the insects. Now it has been shown that mycotoxins have a detrimental effect on the fecundity of the insects. We know that some pathogenic bacteria can be carried by the insects for a long, long time and be excreted in the system and contaminate the grain. This is the type of inter-relationship which I was referring to.

MODERATOR

Yes, this of course refers to the ecosystem as it is before controlled atmosphere storage.

May I just come back to your observation on the Salmonella. I remember your paper in which you did not confirm a significant relationship between Sitophilus granarius and Salmonella. Am I right?

Dr. DE LAS CASAS

Right. But one of the other insects, the lesser meal worm, can be a carrier of Salmonella and transmit it to turkeys.

MODERATOR

We may perhaps get some more comments on the possibilities of controlling microorganisms and especially moulds. We have not talked

much about very high moisture content grains. What can we expect from these high moisture contents?

Prof. BUSTA

I am not sure that we have sufficient information, whether it be model systems or general systems, on specific commodities and high moisture. I agree with your comment, Dr. Shejbal, that we need to use model systems to define the activity of a specific mould and a specific condition. I agree that we need to study high moistures which permit the growth of moulds. I also would suggest that we then integrate in a modeling approach the moisture content (though I would prefer "water activity" or "equilibrium relative humidity" that would go across commodities with an easier conversion) in relation to the inert atmospheres of one type or another and their control of specific moulds.

In relation to the production of mycotoxins in general, that is, aflatoxin and other mycotoxins, all the evidence that was presented here, I believe, indicated that if one controls the growth of mould, one controls the mycotoxin production. To my knowledge there is no evidence that mycotoxins are produced by any dormant yet metabolizing mould system. So I think that we can concentrate on controlling the growth of the moulds.

I do not think that it has been considered adequately, by those individuals concerned with the fumigation approach, that in high moisture systems if the insects are controlled with fumigation using controlled atmosphere, one might not be controlling the moulds. And then the moulds in fact would be the major concern, rather than the insects.

For specific levels, I think it has been demonstrated that the anaerobic systems down at the 0.1 to 0.5% oxygen content will, in fact retard the mould growth or inhibit it adequately even in high moisture systems, even at moisture contents, or water activities, considerably above what one would consider even feasible in grain.

In addition, I think that the approach of using moist grain for feed is a very feasible approach, utilizing naturally produced organ-

ic acids, which then appear to be controlling the mould in addition to the controlled atmosphere.

#### MODERATOR

I was struck by the fact, in the mycology Session, that whenever oxygen content was reduced sufficiently, there was a decrease in mould counts. That was a general feature which went through all the papers that were presented. As soon as there was some residual oxygen, things changed and were more complicated.

I would just like to make a comment on one of our experiments, also referring to what Dr. Banks said, that it is very important to carry out large scale tests. We saw in some of our metal bins condensation at the roof. And we had water dripping onto the barley stored there, because the head-space was too big. The moisture content of the upper layer of barley rose from 13.5% to 19%. But we observed a decrease in mould counts in that layer throughout the whole storage period of nine months. Of course this refers to a situation in which we really did not have any oxygen present. And the fact that moulds did not grow, as such, demonstrates we did not have any oxygen leaking in. This was extremely important for us, because it means that even if moisture migration or condensation occurs, and the storage is sealed well enough, we can deal with critical moisture contents. It would be interesting to see whether, if one of the Cyprus bins could be really sealed, similar results could be obtained in that situation, in order to compare experiments on a large scale.

#### Prof. ZANON

It seems that lowering oxygen to very low levels and/or increasing to high percentages carbon dioxide there is a certain reduction in mould growth and especially by the production of mycotoxins in these fungi. The importance of mycotoxins in grains for human and animal nutrition was emphasized in the last years by numerous findings in England and Denmark; especially after rainy growing seasons barley resulted highly mould-infected and, with the traditional way of storage, rich in mycotoxins, especially aflatoxin. Consequently meat and

bacon of pigs fed with such products showed high contamination and as a result heavy intoxications occurred also in humans consuming these victuals. In one only season, after due examination, over 10,000 pigs had to be sequestered from the market in order to avoid negative consequences for consumers.

In 1978/1979, in co-operation with H.K. Frank and H. Hansen of the Bundesanstalt für Ernährung at Karlsruhe (Germany), a series of trials was carried out in order to clarify some of the mentioned aspects. Wheat and barley were put in gas-tight plastic containers of 120 liter capacity filling them half full. The water content of the grains was 16.8%. The following four different compositions of atmosphere were compared:

- 12 - 14% CO<sub>2</sub>, 1 + 2% O<sub>2</sub>, the remainder N<sub>2</sub>
- 20% CO<sub>2</sub>, the remainder N<sub>2</sub>
- 8% CO<sub>2</sub>, 2% O<sub>2</sub>, the remainder N<sub>2</sub>
- control in air.

The period of storage was 9 months.

In spite of the low oxygen and the increased CO<sub>2</sub> - content also the samples from controlled atmosphere showed some moulds (especially of the species Aspergillus, Penicillium and to a low extent Mucor). The samples were tested with the thin-layer chromatography method in order to ascertain eventual presence of mycotoxins. Whereas all samples taken from the control in air had more than 50 ppb aflatoxin, no mycotoxin at all could be determined in the samples from controlled atmosphere, which means that the eventual content is lower than 1 ppb. According to regulations of the European Community, for humans a tolerance of 5 ppb mycotoxin and for lactant animals, 10 ppb is fixed, which is five, respectively ten times the determinable amount.

These remarkable results for human nutrition will be further-investigated by studies concerning the influence of low oxygen and increased CO<sub>2</sub> atmospheres in retarding or avoiding the production of mycotoxins after exposing the stored product to normal air, respectively to determine the course of the eventual delaying action.



Dr. SHUYLER

I think it is obvious from the comment I made earlier, that I do think that the use of controlled atmospheres with respect to strategic food reserves, or what we in particular refer to as food security stocks, is an area of the post-harvest system in which these techniques are likely to be found useful at a relatively early stage. I cannot help but note also, in respect to Dr. De Las Casas' comments much earlier, that I would be concerned not with the effect of the controlled atmosphere on the food, but rather that we may at the time the food enters the food security stock and enters into a controlled atmosphere storage, already be too late. That is, the sanitation will have been so lacking in the harvesting, the gathering, the threshing, the drying, and the transport stages, that there already will have been contamination and infestation by by moulds (and in some cases even by bacteria) and isects. This hazard is increased because, in some countries, there is even an incentive to farmers, not intended but nevertheless present, to add water to the grain in order that they can sell more weight of grain to the government. This, together with the previous lack of sanitation and the frequent high content of foreign matter, may mean that food security stocks, at the time of the need to rotate grain with material that is unacceptable according to the standards of good health and nutrition we wish to promote, even though we have used controlled atmosphere storage. I think that this is perhaps a message that needs to come across.

Dr. BANKS

If I may make a brief comment on Prof. Busta's remarks. He implied that some people here had been wishing to use the fumigation approach under conditions of high moisture storage. I don't think any of the papers actually did recommend that. I think there is a very definite distinction between those cases where you are disinfesting a dry, storable commodity and those cases where you are applying a controlled atmosphere to control mould for quality preservation and the prevention of mycotoxins.



Prof. CALDERON

Again a general remark. From this meeting, as far as I can see, there are two very difficult problems which were not discussed enough and which are still remaining quite unsolved.

The first one is the sealing of structures and making them air-tight; the costs concerned, the methods etc.

The second one is the problem of moisture migration. I think that moisture migration in a large scale hermetic storage, a sealed storage, is a major problem, especially in tropical countries. We heard something about Cyprus bins and also other cases, but I would like to think that from this Symposium we could get a stimulus for research on this problem. I think it concerns more engineers than biologists. The solution of this problem will make life easier for hermetic storage.

MODERATOR

May I ask Dr. Muir whether he thinks that we can expect much data from modeling of heat transfer and convection currents in gas-tight storage structures? Your models so far do not really refer to gas-tight structures.

It is so difficult to carry out direct measurements in some countries. We have the experience of Nigeria, to where we shipped all the equipment, which is working beautifully, but it is very expensive to get all the material there and to do the work on the spot.

Dr. MUIR

First of all, the model we have developed is applicable to hermetic or sealed storages. The heat transfer and convection currents in a sealed storage would be the same as in the bins at which we were looking.

We should like to be able to model moisture migration. I feel that it is a very important problem. But so far we have not had the idea of just how to measure and get the suitable model. I, of course, look more at Canadian conditions, and moisture migration under Canadian conditions is a seasonal problem. In tropical conditions it appears

to be a diurnal, a night and day problem. But I think once we could come up with a model, it is going to help considerably.

Dr. ALABI

I find this Symposium very, very useful, and succeeding in bringing, so many eminent scientists from all over the world to come and discuss all these problems is really very important. I wish to say, however, that I would not think that this should be a once and for all Symposium. Maybe, periodically, it would be good to get people together to review what is available. This would be very helpful.

One other comment which I wish to make is that, looking around, it would appear to me as if the attendance had been a little bit restrictive. Restrictive in the sense that only countries which are involved in this type of work are present. I think that developing countries will benefit a great deal from this type of work and I am wondering how to make them aware of what has been discussed and what is available. The Proceedings will be printed, but how will the people who did not participate in this Symposium know that they are available?

Developing countries, as I said, would benefit a great deal because these techniques which are now available, controlling pests, etc., in grain with the use of inert gas, nitrogen, and other gases, would, I am sure, be very beneficial. I know that it will cost a lot of money, which maybe we'll not be able to afford, but it would be useful if we could get some of the organizations, maybe FAO would be very useful in this, to put the donor countries in touch with the developing countries, so that all of them would be made aware of what possibilities there are for the use of controlled atmospheres in preserving grains.

I think FAO cannot say: "We shall only take part in this if it fits in with small farmers", because, as you mentioned, Mr. Chairman, there are several places where they are trying to have these bulk storages and I think it might fit in with their programmes for the prevention of food losses. After all, it is in the developing countries that most of the losses occur. If it is possible to reduce the

loss by 50%, it will make a lot of difference in developing countries where they do not have enough food.

So these are a few thoughts with which I would like to leave with us. This technique should be made available to other developing countries. Since they might not have the resources, we shall have to rely also on the developed countries who have always been helping, the donor countries, so that they can make it possible to have these things carried out in developing countries.

#### Ing. PULEO

For some time now, RBS International has been using a method of constructing "made to measure" cylindrical silos called the "Lipp System" which has wholly satisfied its design objectives, dictated not only by economic factors and reasons of speed and quality, but also by the wish to satisfy any and every specific market demand.

In recent years the validity of this method has been confirmed further by the introduction of new cereal storage techniques based on the use of low pressure gas silos, for which new procedures and materials were needed. With regard to the materials used, silos have been built in concrete and steel as well as with resins. Steel has, however, remained the most economical material for building silos. The Lipp system makes the use of steel even more economical because it reduces the construction time, transport and labour costs. Besides black steel, pre-painted galvanised steel can be used with considerable qualitative advantages over the other systems for constructing gas-sealed silos, which cannot use these materials all.

Due also to the extraordinarily widespread presence of Extraction de Smet - Belgium, by now the construction process has been used the world over; it consists of building silos measuring 3-25 meters in diameter and with a maximum height of 35 meters on site, using automatic patented machines and huge coils of one or other type of metal. The result is a silo with smooth, straight, extremely thick inside walls, and helical ribbing on the outside for further strengthening.

Part of the money saved on assembly, transport and storage is used

to thicken the shell for greater strength against the stresses of continual loading and unloading, thus guaranteeing greater durability and highly economical operation. The ability to use aluminium; electroplated, rust-proof, pre-painted galvanised; or, black steel equally easily, ensures that the silos are suitable for any type of product, whether liquid or solid, and renders them fully resistant to external atmospheric agents.

After a lengthy process of technical and economic studies this new construction method was adopted for the first large industrial-scale plant for the storage of 6,000 tons of barley in a controlled nitrogen atmosphere. The barley storage plant was constructed by I.V.O. in Tuscania (Italy). Capable of storing up to 6,000 tons of barley it consists of 4 silos, 13 meters diameter, a shell height of 15.30 meters, and a total height of roughly 19.00 meters.

The edges of the galvanised steel plates were coated with a butyl-ic rubber-based adhesive before helical seaming; as a consequence the rubber is trapped on the inside of the seams sealing the silo without making contact with its contents. The bottom endplate and cone-shaped roof made up of steel plate panels were sealed by welding them to the shell. The bottom endplate is made with eight 7 mm. thick plate panels which form a circular ring on the outside and an ortogonal structure on the inside, and with seven 2 mm. thick rectangular and trapezoidal plate panels. The bottom endplate was assembled on site by welding directly on the foundation bed.

The shell was constructed in galvanised steel plates between 2 and 4 mm thick. The 4-mm thick plate was reinforced by vertical IPE 120 stiffeners to a height of 2.50 m, welded to the shell and bottom endplate ring. The upper edge of the shell is reinforced by a circular section welded to the shell.

The roof - with its 30° slope - is made of forty-eight 2 mm. thick steel plate panels reinforced along the edges by cold-bent ribbing, and a conical disk in 2.5 mm. thick plate, reinforced by means of 48 welded ribs. The roof was assembled on the ground by airtight welding and then lifted and installed onto the shell using a crane truck. Again the welding is air-tight.

Stresses were calculated on the basis of the calculations published in "Berechnung von Lipp-Silos" in May 1975, with loads and tension modified in accordance with current Italian regulations. A mini-computer was used for calculations with a special programme for cylindrical silos prepared by RBS International. This programme carries out stress analysis under various load conditions (e.g. loading and unloading of material, overloading due to snow and wind) and automatically establishes the dimensions of the shell by continuously checking strength and stability in relation to height.

After 3 years in use these silos have incontrovertibly demonstrated the ideal nature of the Lipp construction method for controlled atmosphere storage of grains.

Mr. WILSON

I would like to make a few comments on the very informative discussion of Dr. Muir. With this in mind I want to make a recommendation to the Symposium, that we look more into the natural convection currents in grain.

The reason for saying that is that I could not really apply Dr. Muir's technique to our experiences which surely must also relate to the developing countries' experiences. For example, I've had rice loads come in at a temperature of 27°C and then, immediately, other loads at up to 40°C. Of course that is related to the fact that some farmers have harvested during night time, while others harvested during the day. I have also taken a tremendous number of temperature readings in the cells and I found that the upper temperatures are much higher than in the Canadian and American experiences. What we don't know enough about is the convection currents in concrete cells, where the winds on one side create a pressure differential inside. Once we know more about this information and learn how to manipulate the convection currents cheaply by stimulating them, not by expensive equipment but just by normal ventilation, we then can relate that to our covered storages. (In my particular part of Australia, we have got just under one quarter of a million tonnes of grain in stores completely covered by soil in 49 different sites). And then we can look

at our convection current knowledge in this non-hermetic sealed storage versus hermetically sealed storages.

I would also like to say that, in Australia, we are, on the farm level, getting mini-sized hermetically sealed storages that are going to be covered by plastic, and so on. I am sure this information will go to the developing countries, but I would like the research people to look far, far deeper into the mode of action of air currents in grain.

Dr. BANKS

I would like to refer to the comments of the Rapporteur of the 6th Session, Mr. Corbett, who called for the discussion of sealing standards. I think this is a very important aspect and one to which we should pay a lot of attention. I have already referred to it in the paper. But let me first say that it is a very complex subject and the reason why in my paper there were several different standards given, is that it very much depends on what structure you are testing and what pressure you can apply. In very big structures, it is not possible to apply very high pressure because of structural problems. In a concrete cell it is often possible to apply very high pressure and the higher the pressure that you apply, the easier it is to make an accurate assessment of your pressure decay test. This is why you are given a number of different figures. These are all mathematically related and it is possible to cast a standard in terms of a formula. But if you do this, this is not readily available to people who are not equipped with electronic calculators; so, it is normally given in a number of very simple standards.

The standard in Japan appears higher than the one I gave you. The standard in Japan, as I understand it, is 5000 Pa. to 2500 Pa. in 20 minutes. This compares with a 5 minute time that we request in Australia. The difference is largely because the Japanese standard is based on an empty bin and ours is based on a full bin. This, in fact, means that the times are different for the same standard of sealing. And this complicates the matter very considerably. You have to make a correction for how much grain you have in your cell in

order to be able to compare one standard with the next. In fact, the Japanese standard is very similar to our standard if you take into account first the difference in filling and secondly the fact that the decay test is not a strictly logarithmic decay. Thus you may get a slower fall at higher pressures than you get at lower pressures. Under these conditions you can get a standard and you can write the standard in terms of the level at which you can expect wind and the chimney effects to have a negligible effect on gas loss. This can be analyzed mathematically and you end up with a standard which we have in Australia, that, shall we say, 2 inches to 1 inch water gauge, which is 500 to 250 Pa., a decay time of 5 minutes. If you exceed this decay time, you have a better sealed structure but you don't substantially alter your gas loss rate, because the gas loss rate at higher sealing is concerned only with the temperature and barometric pressure fluctuations.

So, it is possible to make a standard, but it is also necessary to take into account what you are going to do. We have a real balance between those people who wish to have an extremely high standard of sealing and maintain a pressure in a structure; those who wish to maintain a gas within a structure for as long as possible, without adding appreciable gas; and those who are prepared to add a lot of gas irrespective of the leakage. These three objectives must in turn be related to the economics.

So it is extremely difficult to produce a uniform standard. You can produce a number of theoretical standards that will fit given structures.

One other problem is that in the process of actually doing these kinds of tests, it becomes increasingly difficult to get an accurate answer for the large structures. A comment was made earlier that the pressure test I quoted for one very large structure (16,000 t.) was in fact only taken up to 150 Pa., whereas our standard starts at 500 Pa. This was for structural reasons. But it is also a fact that large structures become increasingly difficult to test by this process.

I think we have to look at alternative methods of testing very



large structures. This is still very much in its infancy, but one way you can do it is by filling it with a tracer gas and observing the gas loss rate. The standard that we have chosen is equivalent to a gas loss rate of about 5% per day. Now this is confusing term because other people have talked about gas loss rate in terms of percent per day. Our phraseology does not mean that you go from, say, 50% to 45%  $\text{CO}_2$ , although you have then lost 5%. This is a term involved in the logarithmic decay rate. There is a very substantial confusion between these two process and we perhaps ought to have an alternative term to look at the standards. But, in general, I would like to support the call for some form of uniform standard, though we will have to qualify it in terms of what we finally are going to do with the structure.

#### MODERATOR

There is another question which is quite interesting, that of flexible films and sheets and covered stacks, etc., which Mr. Corbett raised. May I just refer to a person who is not here, M. Le Du (France), who has used these structures for years with very good results with wet harvested maize for short-term preservation before drying. This is, in fact, a question which was not mentioned much during the Symposium: that controlled atmosphere storage, not for disinfection, but for short-term maintenance of wet harvested grains has potential. Not much work has been carried out on this. Of course, one would not expect this to be carried out in normal storage structures, but rather in improvised, inflatable containers.

#### Dr. ADESUYI

I really do not want to be an agent of doom about all these plastic films. But, some years back we did carry out some trials with plastic sheets, which you can inflate and it forms a dome shape. We stored cocoa in this. After stacking the cocoa, it deflates, so that it just forms a cover. We did not use that for more than about 4 weeks, because the condensation was so high on the top that we had to terminate the experiment. So, I think we have to be very careful under what conditions we use these plastic films for storage. I must add



that this was carried out under the humid tropical conditions. Maybe in the drier North it might work.

Mr. BAILEY

We are continually being reminded of the storage problems of the developing countries. I would like to suggest that we may be quite close to an answer to most of their problems. I refer to the Cyprus bins built in Kenya. These were designed to reduce losses without the use of chemical pesticides and in this they have, in fact, been very successful - reducing losses to about 10% of those occurring in conventional storage in Kenya. Certainly, there are problems with these bins, such as water penetration in the below-ground cone and trouble with the air-tight seal at the joint of the roof to the ring beam.

I believe that if these problems were looked at again they could be solved without great expense when building new structures of this type. Also perhaps the discharge arrangements could be improved.

I suggest that, if this was done, the bins could be of great value to many developing countries.

MODERATOR

The very large roof of the Cyprus bins is what worries me most in these structures. In the long years in which these structures were operating now, it seems to have been impossible to seal them well enough to obtain low oxygen concentration which would control moulds. In many countries a very large roof may mean quite a lot of condensation, except if the structures could be filled to leave practically no head space, and problems with fungi would be inevitable with oxygen concentrations of the order described by Dr. De Lima in his papers. It is quite difficult to separate well the moulded fraction of the grain from the well preserved bulk, even if it represents only a small fraction of the total mass. Especially this is true in Kenya because of unloading difficulties. It seems a pity to me that the concept of controlled atmosphere storage, which can permit a no-loss preservation, should not prevail in achieving this full success, only because of structural difficulties.

Dr. CALVERLEY

I find a good deal of sympathy with Mr. Bailey's suggestion. I think that there must be some compatibility here between the biological requirements and the civil engineering requirements.

We have talked about the economy of controlled atmosphere storage. We really have not talked about the economy of grain storage in general. In particular when we look at the costs of maintaining reserve stocks, this is a very expensive operation. Not only in terms of capital involvement in the grain but also in the civil engineering and the mechanical engineering aspects required in the plant. This of course is one of the great attributes of holes in the ground.

The great attraction, even today, of the Cyprus bin, that as a structure, which is all weather proof and can generally be operated under all weather conditions, is that, as a civil engineering structure, it is an extremely cheap structure. We have in fact persevered with the development of this design in a modest sort of way and we feel that, at limits of 5,000 or 10,000 t. per cell, specifically designed for long-term storage, where you have a throughput only once every 3, 4, 5 or 6 years, that these do provide a very cheap capital expenditure.

I concur with your concern about the large surface area which is exposed. But I think that, from the studies that we have done on Cyprus bins, a great deal of that difficulty could be resolved, if, during the design and the construction stage, adequate insulation and reduced insolation were built into the design. In the case of the bins in Kenya, as Dr. De Lima pointed out, we looked at the difficulties to which you have referred, and the loss that occurred, the troubles that were caused by the high moisture content on the top of the bins, and it was decided that this was of such small magnitude that on no account was any remedial construction work justified.

So, I think there is a question here of further discussion to achieve compatibility between the biological requirements and those of civil engineering and mechanical engineering all in relation to costs.

Dr. DE LIMA

The hermetic bins in Kenya have been used routinely to store famine reserves for nine years without serious difficulties. Any structure will have maintenance problems, but this does not mean that the design or principle is at fault. Another speaker has mentioned that hermetic structures are no longer being used as such in Zaire and Argentina, but there are several reasons for this including change of circumstances and inadequate planning. Likewise, in many countries, there are numerous other examples of inappropriate technology.

The hermetic structure requires middle-level technology and can be constructed in developing countries with local materials and expertise. The use of artificially controlled atmospheres requires a new technology and infrastructure not readily available in developing countries.

MODERATOR

In the introduction of the nitrogen storage technique we are in the stage in which you were many years ago, when you built the seventy bins in Kenya. We are just going to build two bins this year in Nigeria and we are going to try them, trying to find out what can really be done directly on the spot. I consider this the only way of real technology transfer from a developed to a developing country; to do it together with the people on the spot, as we are doing with the Nigerian Stored Products Research Institute. I think credit should be given to NSPRI to have gone into this experience with us for the benefit of more than just Nigeria - for many other tropical countries.

But, there is a big difference between the climate of Kenya and the climate of, for example, southern Nigeria. I think that this is one of the reasons why the Cyprus bins in Kenya have all this condensation; so much heat is lost during the night due to such a big temperature fluctuation.

Dr. SHUYLER

Just a brief comment, that we here in this Symposium are admittedly lacking in many of the areas of knowledge that we might wish to

include. I make this comment in connection with the idea of rehabilitation or improvement of existing facilities and specifically in connection with the problems which you have noted in Argentina and the hermetic-type storage which is semi-underground. There have been developments in technology regarding "water-proofing" (this may be a bit of an exaggerated term) of structural elements from moisture contained in soil, which are in use, in the UK and the USA that I know of (maybe right here in Italy, too, and I haven't learnt of it), that are so much improved in terms of reduced cost, ease of use, that it would be improper, for instance, for us to even think of comparing them with any former technology. But there is no one here that really knows enough about it, including myself.

Mr. NICHOLAS

Mr. Chairman, thank you for giving me this opportunity to express the delight of FAO that ENI is taking an interest in saving and preserving food. After all, it is an integral part of saving energy, which seems to occupy everybody in these days.

As co-sponsors of this Symposium, we will continue to take an interest in the conclusions on the work of controlled atmosphere storage of crops. We hope that this Symposium will be a step forward to practical action in the application of this promising technology.

I would like to take this opportunity to correct the wrong impression that seems to have been created by my very brief intervention earlier on. Because, after all, FAO are co-sponsors of this meeting and this is an indication that we think there are great possibilities.

My colleague earlier referred to the aspects of food security, where very large structures are built and where the possibilities of using this type of technology exist. Nevertheless, when you get to a situation where you hope that the very large numbers of small farmers, who actually store grain on their farms, need help, and if you listened carefully to what Mr. Bailey said about the Cyprus bins and what Dr. Calverley about the cost relationship between the biological feasibility of storing grain and the engineering and the economic aspects of it, I think you will agree with me, that FAO would be quite

wrong to use the small farmers of developing countries as a basis for experiment. The rush introduction of unproven technology (and somebody very rightly said what is proven is what works in the hands of the end users, not what works in the laboratory or in a controlled field experiment), is something I am sure that nobody would like us to use whatever farmers as a means of experimenting.

I would like to take this opportunity to say, therefore, that where the technology is proven, whether in food security's large-scale facilities or on the small scale of farmers, we will continue to take an interest and we will try to apply it, where it is appropriate and where it is feasible.

#### MODERATOR

Thank you all very much for this interesting and rich discussion and your participation in the Symposium.

Dr. Nencini, Vice-Director of the Basic Research Laboratory of Assoreni, will now officially close the Symposium.

#### Dr. NENCINI

Let me say just a few final words on behalf of Assoreni and our President, Prof. Cernia, who, unfortunately, could not attend the final hours of this meeting.

We were very pleased to see such a world-wide interest and participation in this Symposium. We have been working in this field for quite a few years, and we know that the problems which we had to tackle were not only in strictly technical areas. There is a particular problem of the inter-disciplinary approach, which is needed strongly in such a type of research, and which has been mentioned and stressed in these days here, too. Furthermore, technology transfer is a problem not only from one country to another, but also from one kind of mentality to another. What I mean here is the industrial approach to agricultural problems. I hope and I really believe that with the help of our co-sponsors, FAO in particular, and of all the participants in this meeting, which has given us so many new hints and ideas, and a deeper understanding of the problems, it will be possible to go

further in the practical application of this new technology.

I hope that you enjoyed your stay here. I think that Dr. Shejbal has done his best to make you feel at home, and, thanks to his high level relations in the Vatican, he was able to permit the visit to the Pope's Gardens, which we all enjoyed very much, I think. Unfortunately his relations were not high enough to guarantee good weather conditions. And this was a fault in our organization, I hope that you have not found many others.

Well, I wish to thank you all very heartily - Chairmen, Speakers, the Rapporteurs - and I wish you all a good trip back to your countries. Good-bye.

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