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 12μ polyester, 9μ aluminum foil and 60μ polyethylene or with 70μ 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^2 using cylinders of compressed carbon dioxide, nitrogen and air. As the controls, 50 Kg/cm^2 -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 priro 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

	Storage condition												
	20°C - 25°C				10°C								
Initial moisture content (%)	Air		N^2		Air			N^2			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
Moisutre 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_2 (%)	1.4	1.6	0	0.2	12.9	10.7	10.4	0.3	0	-	20.9	20.9	20.9
Gas composition in pouches CO_2 (%)	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	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)	4.5×10	1.1×10	1.4×10^3	1.2×10	2.1×10^3	3.7×10^2	1.2×10^3	2.2×10^3	4.2×10^3	8.5×10	1.6×10^3	3.3×10^2	8×10
Bacteria count /g d)	2.3×10^5	4.5×10^6	6.4×10^5	3.9×10^6	5.7×10^6	7.9×10^6	5.9×10^6	7.1×10^6	8.9×10^6	5.1×10^6	6.1×10^6	6.7×10^6	7.7×10^6

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 $2.5 - 8.3 \times 10^2$
d: Initial bacteria count $3.0 - 3.2 \times 10^7$

Table 2. Changes in Characteristics of Brown Rice during Hermetic Storage for 2 Years under Different Atmosphere

	Storage condition												
	20° - 25°C				10°C								
Initial moisture content (%)	Air		N_2		Air			N_2			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 composition pouches O_2 (%)	1.2	0.7	0	0	1.8	1.4	1.0	0.1	0.3	0	20.6	20.7	19.9
Gas composition pouches CO_2 (%)	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	1.1×10^3	1.7×10^2	1.2×10^4	1.6×10^3	1.6×10^6	1.9×10^6	9.9×10^5	4.5×10^6	4.9×10^6	3.5×10^6	1.6×10^3	1.8×10^6	9.8×10^5
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 x 10^2 for mold fungi, 2.7 to 4.7 x 10^3 for yeast, 3.0 to 3.2 x 10^7 for aerobes (chiefly Chromogenic Pseudomonas)(Iizuka et al., 1963), 5.3 to 5.8 x 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

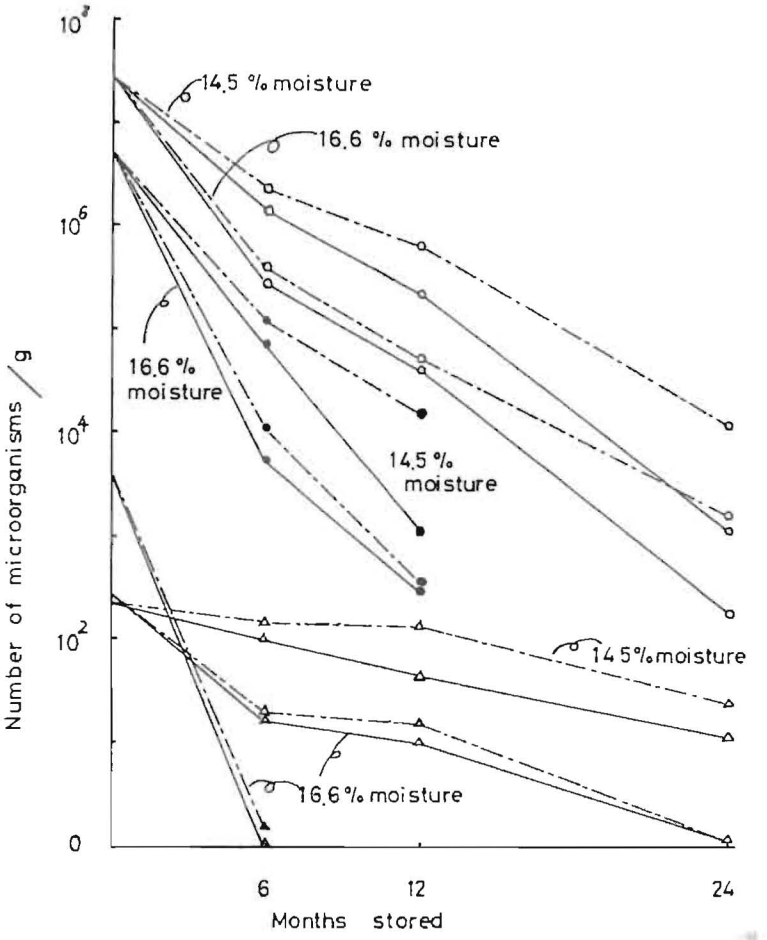

Fig. 1. Number of viable microorganisms on brown rice during hermetic storage for 2 years at 25~28°C

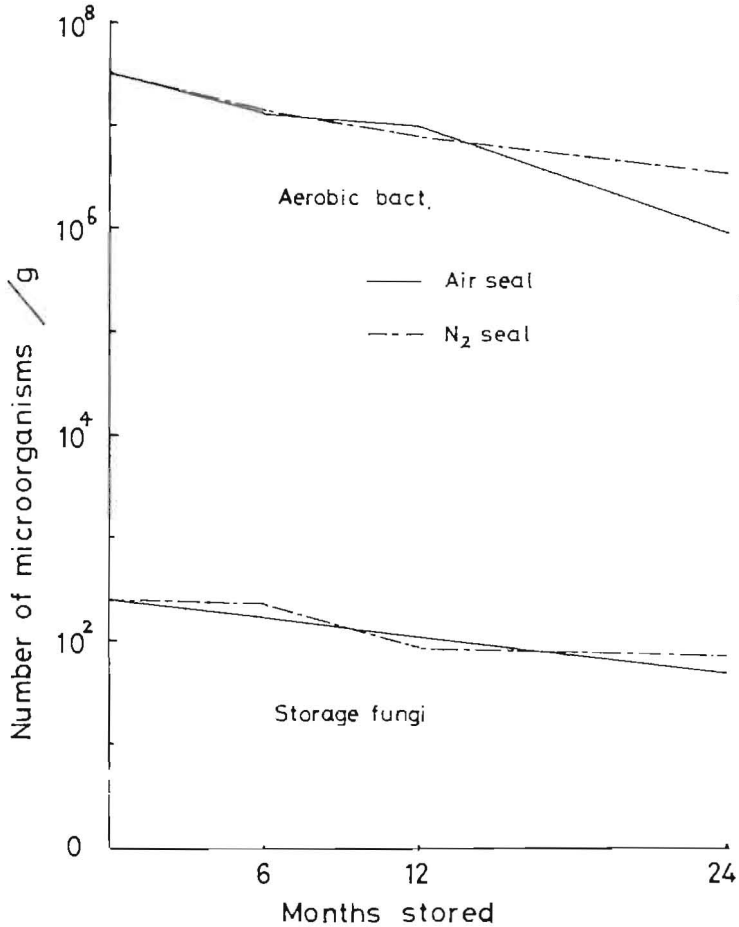

Fig. 2. Number of viable microorganisms on brown rice during hermetic storage for 2 years at 16.6 % moistur and 10°C

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 aerobe 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 under gone 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

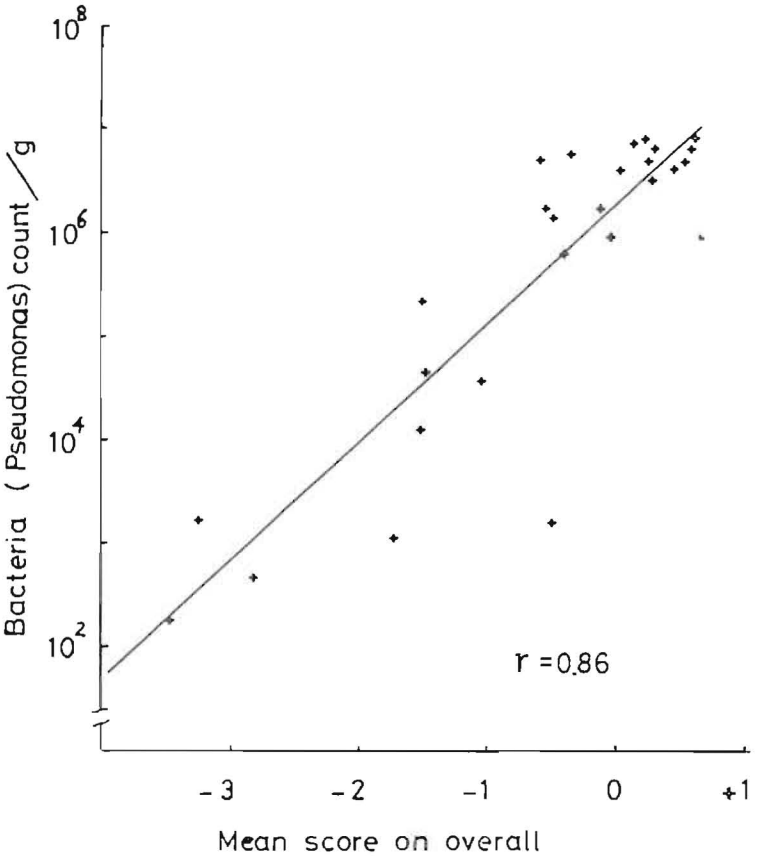

Fig. 3. Correlation of means of sensory score on overall and bacteria count of brown rice during hermetic storag for 1~2 years under different atmosphere

Table 3. Changes in Characteristics of Brown Rice during Hermetic Storage for 8 Months under Different Atmosphere

Storage condition												
	25-28°C									10°C		
Initial moisture content	15.5%			16.7%			17.2%			17.2%		
	Air	N_2	CO_2	Air	N_2	CO_2	Air	N_2	CO_2	Air	N_2	CO_2
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 in pouches O_2(%)	1.1	0.1	0.3	0.8	0.1	0.2	0.7	0.2	0.2	19.6	1.4	0.1
CO_2(%)	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)	4.4×10^2	6.3×10^2	4.5×10^2	3.7×10^2	5.1×10^2	4.2×10^2	2.1×10^2	2.1×10^2	2.5×10^2	2.2×10^3	1.9×10^3	3.7×10^3
Bacteria count/g d)	2.3×10^4	1.7×10^4	1.4×10^4	1.6×10^3	2.6×10^3	1.4×10^3	2.0×10^3	2.5×10^3	2.0×10^3	4.5×10^4	5.8×10^4	5.4×10^4

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 10^3/g
d: Initial bacteria count 1.2 - 2.4 x 10^7/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^2	Air	16.6 18.1	16.7 18.2	19.38	0.47
10 kg/cm^2	Air	16.6 18.1	16.8 18.2	19.73	1.30
10 kg/cm^2	N_2	16.6 18.1	16.8 18.3	0.40	1.20
10 kg/cm^2	CO_2	16.6 18.1	16.9 18.4	2.04	89.95
PET·AI·PE	Air	16.6 18.1	16.7 18.3	1.18 0	19.01 51.32
PE	Air	16.6 18.1	16.1 17.6	18.1 -	0.7 -

temperature and water content, showed an appreciable difference between different filling gases, and threrfore 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^2-pressurized air-containing and 10 kg/cm^2-pressurized carbon-dioxide-filled packages, whereas it increased slightly without any great mutual difference for 10 kg/cm^2-pressurized air-containing, 10 kg/cm^2-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^2-pressurized air-containing and 10 kg/cm^2-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^2-pressurized air-containing specimens, the ratio of viable mold fungi was 0.6 to 1.0%. With higher water content, the ratio

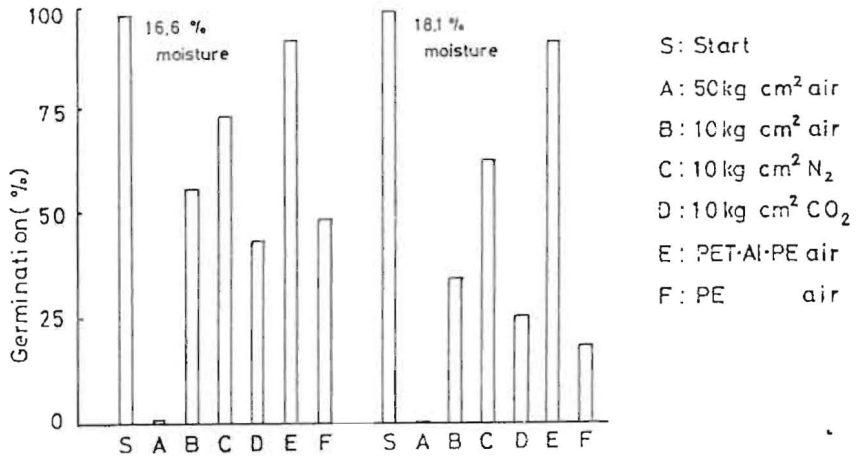

Fig. 4. Germination percentage of brown rice during hermetic storage for 3 months at 25°C under various conditions

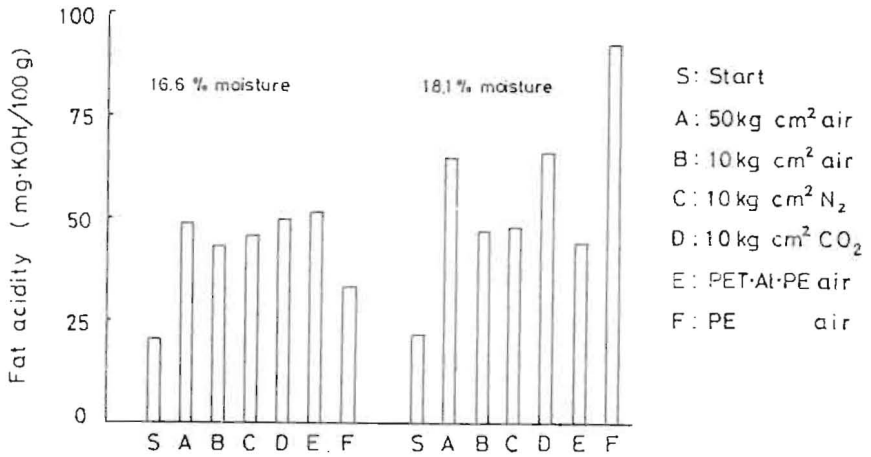

Fig. 5. Fat acidity of brown rice during hermetic storage for 3 months at 25°C under various conditions

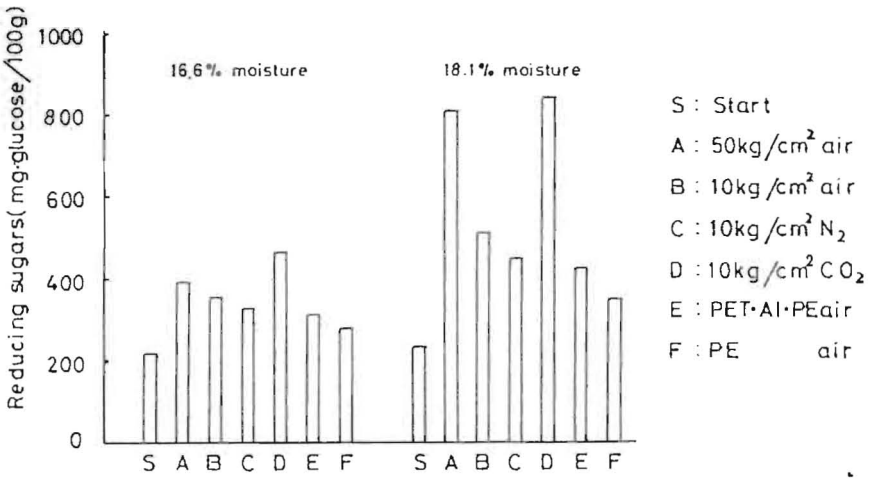

Fig.6. Reducing sugars of brown rice during hermetic storage for 3 months at 25°C under various conditions

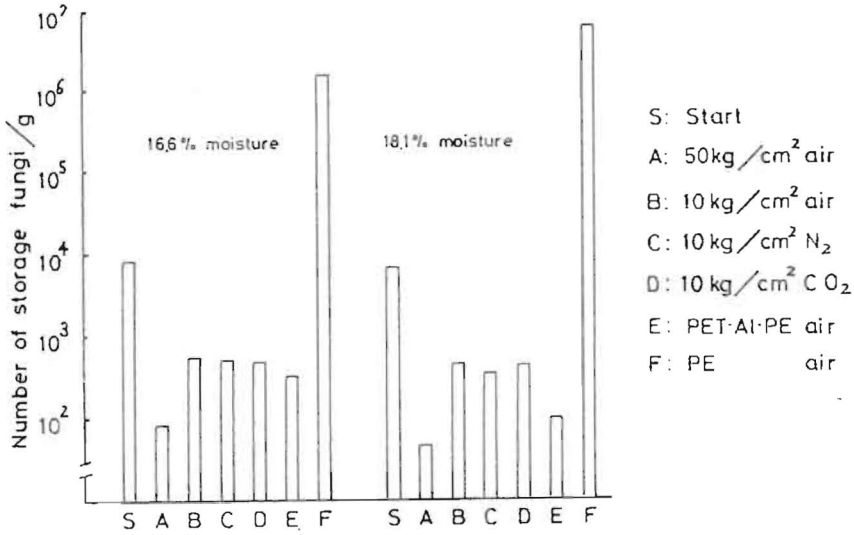

Fig. 7. Number of storage fungi of brown rice during hermetic storage for 3 months at 25°C under various conditions

of viable mold fungi had a tendency to lower. A decrease in mold count observed also in 10 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 appriciable 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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