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Hermetic and modified atmosphere storage of shelled peanuts to prevent free fatty acid and aflatoxin formation

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Abstract: The development of free fatty acids (FFA), molds and aflatoxins formation was studied on peanuts with 7.0% and 8.0% moisture contents stored under hermetically sealed conditions or in an atmosphere of 99% carbon dioxide and in comparison to aerated storage at 30 ± 1°C for 3 months. The laboratory trials were carried out using 0.95l capacity mason jars containing clean peanuts or peanuts with 3% broken peanuts at the two moisture content levels. In addition, semi commercial trials were carried out using 20kg of sound peanuts stored in hermetic SGBIIZ (GrainPro Inc.) bags at the same two moisture contents and the controls containing 7kg of sound peanuts were stored in ordinary PE bags. Each trial was replicated 3 times. Moisture content, FFA (% of oleic acid), Colony Forming Units (CFU) of molds and B1, B2 G1 and G2 aflatoxins were checked on the outset of storage and after 3 months. Respiration rates based on the oxygen consumption of the microflora and the peanuts and the carbon dioxide evolved were monitored periodically. The fastest respiration rate (oxygen depletion) obtained in the jars was with 8% moisture content peanuts containing 3% broken nuts after 18 days while at 7% moisture content sound peanuts reduced its oxygen concentration to a level of 1% after 84 days. In the SGBIIZ hermetic bags, at the 8% moisture content, the oxygen was depleted after 44 days compared to the laboratory tests in jars which the lowest oxygen was obtained after 28 days. In all trials, aflatoxins level was below the threshold limit (≤ 0.3µg/kg). In peanuts stored under hermetic conditions the CFU formation (≥ $1.3*10^4$) and FFA rise (≥ 0.57) were suppressed compared to the aerated samples. Best quality preservation for peanut storage with low CFU (≤ $9.7*10^1$) and low FFA (≤ 0.77) was obtained under controlled atmosphere of 99% carbon dioxide for both moisture contents in peanuts containing 3% broken.

Key words: peanut storage, hermetic, carbon dioxide atmosphere, moisture content, shelled peanuts, FFA, aflatoxins, CFU, oxygen depleted atmosphere

Introduction

Peanut seeds may be stored either in-shell or shelled. A general belief is that seeds are better preserved when stored in-shell than after shelling. However, the former method has two disadvantages. The first is that in-shell peanuts occupy a far greater storage volume. The second is that a larger percentage of nuts are damaged mechanically during shelling since the in-shell peanuts are stored at low moisture content (m.c.) to prevent degradation during storage. The higher the level of broken nuts, the lower is the germination percentage of the seeds (Gavrielit-Gelmond, 1971).

The two factors known to influence the preservation of peanut seed are temperature and relative humidity (r.h.) (Haferkamp *et al*., 1953; Roberts, 1972; Smith, 1982). Molds that affect the germination power of seed are also influenced by the ambient humidity and temperatures in storage (Christensen, 1972). However, literature on the comparative preservation of shelled and in-shell peanut seed is limited. According to Gavrielit-Gelmond (1971), to preserve peanut seed for one year at 21°C, m.c. of 5% or less is necessary. Boswell

et al. (1940) reported on peanut seed preservation at different temperatures and relative humidities. Navarro *et al.* (1989) reported that significant differences between in-shell and shelled peanuts for cv. Hanoch, but not for the cv. Congo. The calculated moisture content required to maintain 90% germination for shelled seeds stored for six months at 15°C was 8% for Hanoch and 7.9% for Congo. To conserve the same germination level for 6 months at 26°C, the calculated moisture contents were 7.1% for Hanoch and for Congo.

Aflatoxin contamination of peanuts has been of worldwide concern since the 1960's. The aflatoxins are secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus*. Research has shown that these fungi can infect peanuts and produce aflatoxins before digging, in the windrow, after harvest, and in storage (Wilson and Flowers, 1978).

Modified atmospheres have shown promise in controlling the *Aspergillus flavus* group and the subsequent aflatoxin production in stored peanuts. Sanders *et al*. (1968) reported that little aflatoxin was found on inoculated peanuts maintained in an atmosphere of 60% CO_2, 20% O_2, and 20% N_2, as compared with 206pg of aflatoxin per g of peanuts stored in normal air at 25°C and 90% relative humidity. The high CO_2 atmosphere is similar to that recommended by Jay (1971) for control of insects in stored products. Landers *et al*. (1967) reported little aflatoxin production on peanuts with a 28.9% moisture content held in an atmosphere of 99% N_2 and 1% O_2. Pattee and Sessoms (1967) reported that increases in fat acidity were highly correlated with visible *A. flavus* growth, and that fat acidity reached 60mg of KOH per 100g of peanut nuts before aflatoxins were detected. However, Landers *et al*. (1967) found aflatoxins in peanuts with fat acidity lower than 60mg of KOH per 100g of nuts (Wilson and Jay, 1975).

The present work intended to study the effects of storage of peanuts with 7.0% and 8.0% moisture contents under aerobic and hermetically sealed conditions on the development of FFA's and aflatoxin formation in the nuts.

Material and methods

Storage conditions

Freshly harvested peanuts of cv. Hanoch medium size (#38) were purchased locally from Tnuvot Sade, Beer Sheva, Israel. At their purchase, the peanuts were at about 5 to 6% moisture content (wet basis) and they were supplied while they were shelled at the packing house of Tnuvot Sade. The moisture content of each lot was raised by spraying with calculated amounts of water. These amounts were intended to give moisture contents of approximately 7.0% and 8.0%. To raise the moisture content, a rapid method of spraying atomized distilled water directly on the nuts was used. The total quantity of water needed to attain given moisture content was calculated and applied in successive small aliquots each of which was divided equally between all the nuts in the sample. For this treatment the nuts were spread out in a single layer on a polyethylene liner. The upper surface of each layer was sprayed so that it is thoroughly wetted but none of the water ran off on to the liner. When the water had been absorbed, the nuts were turned and the other side is sprayed in the same way. Once again time was allowed for the added water to be absorbed. This procedure was repeated until the calculated amount of water had been added and absorbed. After moisture adjustment these samples were allowed to equilibrate at least 10 days at 4 ± 1°C.

Jar size tests

To assess the effect of dockage on the respiration rate of the shelled peanuts, for aerated and hermetic conditions an additional set of peanuts that contained 3% of broken nuts was tested.

This set of test was achieved by adding to the clean peanuts, mechanically crashed peanuts (particle size less than 4mm) of the same moisture level. Then, the sound peanuts were placed in hermetic mason jars of 0.95l capacity (each jar accommodated about 0.7kg of peanuts) for aerobic, hermetic, or under 99% carbon dioxide atmosphere at 30 ± 1°C for 3 months. In addition, the set of hermetic and aerobic were tested with 3% broken nuts and stored under same conditions. Each treatment was exposed to the above conditions at two moisture contents. Each trial was replicated 3 times. Accordingly, there were 3 jars for each set of moisture content nuts, for a total of 30 jars for the tested storage period.

Bag size tests

To test the effect of hermetic conditions in SGBIIZ (GrainPro Inc.), bags provided with inlet and outlet sampling ports were filled with 20kg of sound peanuts at the two m.c. In addition, ordinary PE bags containing 7kg of sound peanuts stored in aerated non-sealed plastic bags served as control samples. The peanuts were stored at controlled temperature conditions 30 ± 1°C.

The wholesomeness of the bags was checked by visual inspection that showed some tears and punctures caused to the bags. It was assumed that the damage to the bag liner was caused during the handling and transporting with the peanuts inside that made each bag a heavy load to carry. To ensure better gas tightness, on day 27 after the start of the trials with the bags, all possible visual damage was repaired using a specially designed adhesive tape (used in the refrigeration industry) and then a pressure test in the range of 10 to 20mm water gage was performed.

Test methods

FFA content: At the outset of storage as well as following 3 months of storage the FFA content of the peanuts was tested according to the AOCS Test Methods (2008). This method determines the free fatty acids in the oil removed from the seed by petroleum ether extraction at room temperature. (AOCS Official Method Ab 5-49) (Ayresm, 1983).

Aflatoxin determination: At the outset of storage as well as following 3 months of storage the aflatoxin content of the peanuts was tested according to the AOCS Test Method Aj 6-95 (Firestone, 2009). This method specifies a screening procedure based on an enzyme-linked immunosorbent screening assay (ELISA) for the detection of aflatoxins B1, B2, G1 and G2. The method is based on an international collaborative study conducted jointly by AOAC and IUPAC.

Moisture content: The moisture content of the nuts at the outset of the storage and following 3 months of storage was determined by electronic sensors that measures water activity (Rotronic, UK).

In addition, samples were double checked for their moisture content by drying the nuts and calculating the amount of water on a wet basis. This method determines the moisture by distillation with an immiscible solvent (AOCS Official Method Ca 2a-45) (Firestone, 2009).

Monitoring concentrations of oxygen and carbon dioxide inside the jars: The respiration rate was determined based on the oxygen consumption and the carbon dioxide evolved from the peanuts. The oxygen concentration of the interstitial air space of ~ 760g peanuts was periodically measured in a respirometer that consisted of 0.95l mason jars with two 1/16" i.d. copper tubes soldered to the lid. An electrolytic sensor type oxygen monitor (Emproco Ltd., HGA11-PB, Israel) equipped with an internal pump delivering approx. 500ml/min gas sample

flow was used for monitoring oxygen levels in the jars. The "HGA11-PB" was equipped with inlet and outlet gas ports that enabled gas circulation by a closed loop gas flow system using PVC flexible 1/16" i.d. tubes connected with the two copper tubes soldered to the jar lid. The copper tubes had different lengths from inside the jars, one ended at approx. 40mm and the other at approx. 180mm from the center of the lid reaching at appox. 15mm from the bottom serving as gas inlet into the jar. The top end of each copper tube was equipped with T-type two-way valves from outside the jars situated between each flexible and copper tube. One port of the valve was directly connected to the copper tube, the second port to the oxygen monitor and the third port to the atmosphere. Depending on the position of the valve, gas flow was conveyed either from the jars into the oxygen monitor or from the monitor to the atmosphere.

Results

Table 1 shows the moisture content (%), FFA (% oleic acid), aflatoxin (µg/kg) values and CFU (Colony Forming Units) for molds at the beginning of the trials for the targeted 7 and 8% moisture contents and after 90 days of storage. All results are the average of 3 replicates.

Typical peanuts aflatoxin were checked; B1, B2, G1 & G2. At all replicates the initial level of the aflatoxins and after 90 days of storage were below the threshold limit (<0.3µg/kg).

The m.c. obtained in Table 1 were lower than the expected. There was a significant increase at the general count of the moulds. The appearance of the nuts was of green-yellow rotten nuts at the control. Table 1 clearly indicates the suppression of moulds development in the hermetic and in 99% carbon dioxide atmosphere. The lowest CFU of moulds count obtained were for the samples contained 3% dockage stored under hermetically sealed conditions in 99% carbon dioxide atmosphere for both moisture contents (less than $9.7*10^{1}$).

In all aerated control samples of the 8% m.c. the FFA level jumped to a level in which the nuts could not be marketable (≥ 2.57%). The aerated 7% m.c. samples in this trial were dry and only the samples containing 3% broken nuts increased its FFA content to an unmarketable degree (1.50 ± 0.12).

The lowest FFA level that was obtained was for the 7% m.c. hermetically stored samples with 3% broken nuts under 99% carbon dioxide atmosphere (0.43 ± 0.07). Although the 8% m.c. stored under 99% carbon dioxide atmosphere FFA level was low as well (0.77 ± 0.03).

Excluding the samples of the 8% m.c. with broken nuts that were stored under hermetic conditions all of the hermetically stored nuts under all treatments did not rise their FFA content significantly.

Figure 1 shows the decline in oxygen inside the mason jars at the 7 and 8% moisture content in hermetically sealed jars containing sound peanuts and 3% broken peanuts. The difference in the respiration rate of the two tests clearly indicates the enhancing effect of the presence of broken peanuts in the jars. Respiration of the 8% m.c. sound peanuts reached lowest oxygen concentration after 28 days while of the peanuts containing 3% broken reached after 18 days. Respiration of the 7% m.c. of the peanuts containing 3% broken reached lowest oxygen concentration after 68 days while sound peanuts did reduced its oxygen concentration and remained at a level of 1%.

Table 1. Moisture content (%), FFA (% oleic acid), Aflatoxin (µg/kg) values and CFU (Colony Forming Units) for molds at the beginning of the trials for the targeted 7 and 8% moisture contents and after 90 days of storage at 30°C.

Moisture Content (%)	Tested parameters	Initial	After 90 days				
			Hermetic sound peanuts	Hermetic with 3% broken peanuts	CO_2 with 3% broken peanuts	Control	Control with 3% broken peanuts
7	% Moisture Content	5.97 ± 0.03	6.80 ± 0.20	7.20 ± 0.21	6.60 ± 0.40	6.33 ± 0.53	6.60 ± 0.26
	FFA (% oleic acid)	0.36 ± 0.01	0.63 ± 0.53	0.70 ± 0.17	0.43 ± 0.07	0.57 ± 0.03	1.50 ± 0.12
	Aflatoxin (µg/kg)	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
	CFU molds	$3*10^2$	$1.8*10^3 \pm 1.2*10^3$	$1.7*10^3 \pm 7*10^2$	$9.7*10^1 \pm 28$	$1.3*10^4 \pm 9*10^3$	$4*10^4 \pm 3*10^3$
8	% Moisture Content	7.53 ± 0.07	6.87 ± 0.15	6.37 ± 0.2	7.10 ± 0.32	6.63 ± 0.19	7.30 ± 0.17
	FFA (% oleic acid)	0.42 ± 0.09	0.67 ± 0.17	2.13 ± 0.07	0.77 ± 0.03	2.57 ± 0.47	4.00 ± 0.42
	Aflatoxin (µg/kg)	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
	CFU molds	$3.1*10^2$	$8.4*10^3 \pm 5*10^3$	$6.3*10^1 \pm 18$	$1.2*10^1 \pm 4$	$7.6*10^5 \pm 5*10^5$	$7.5*10^5 \pm 2*10^5$

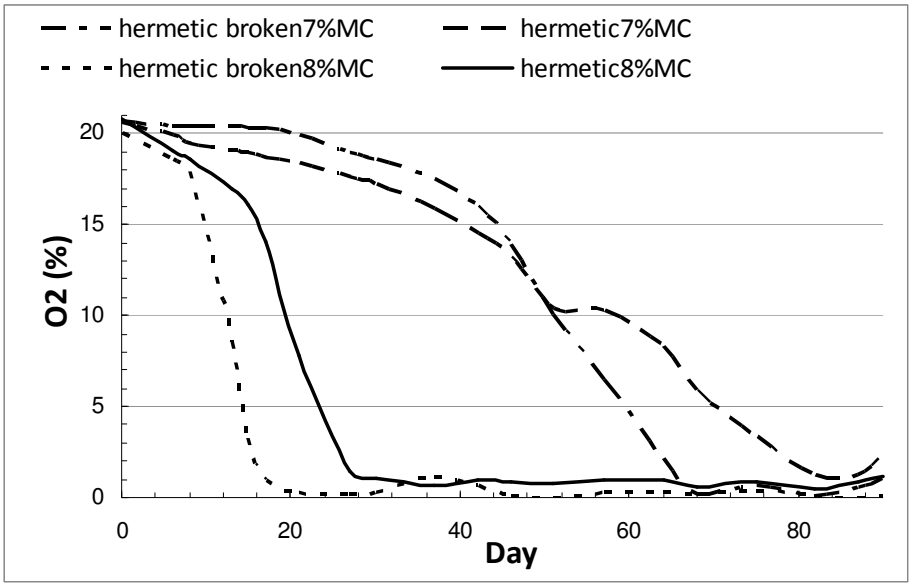

Figure 1. The decline in oxygen inside the mason jars at the 7 and 8% moisture content with or without broken nuts (3%) both hermetically stored at 30°C. Results are average of 3 replicates.

Figure 2 shows the increase in carbon dioxide inside the mason jars hermetically stored at 30°C with the 8 % moisture content sound peanuts that reached maximum level of 25% while there was a parallel increase of the carbon dioxide with the peanuts containing 3% broken nuts which reached the 31.67%. The changes in carbon dioxide level of the 7% m.c. sound peanuts and the peanuts containing 3% broken nuts both reached 16% during the same storage period. In contrast to the depletion of the oxygen and the increase of the carbon dioxide at the aerated samples of the two tested moisture contents stored at 30°C as shown in Figure 3, there were only slight and non significant changes during the 90 days of storage both for the sound and broken nuts. The oxygen remained at a level of 20% and the carbon dioxide remained at a level of approximately 3%.

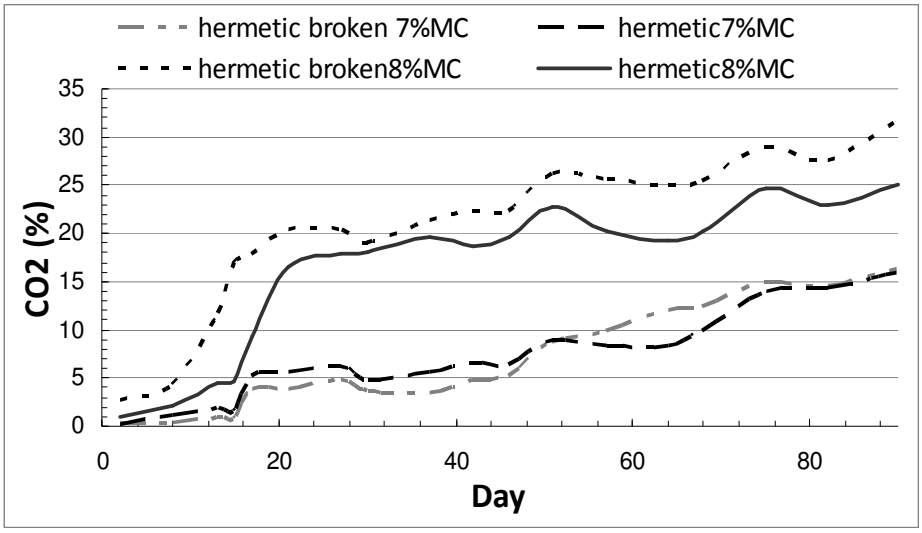

Figure 2. The increase in carbon dioxide inside the mason jars at the 7 and 8% moisture content with or without broken nuts (3%) both hermetically stored at 30°C. Results are average of 3 replicates.

Table 2 shows the moisture content (%), FFA (% oleic acid), aflatoxin (µg/kg) values and CFU for molds at the beginning of the trials for the targeted 7 and 8 % moisture contents and after 90 days of storage within the SGBIIZ (GrainPro Inc.). All results are the average of 3 replicates. At all replicates the initial level of the aflatoxins and also after 90 days of storage were below the detectable threshold limit (< 0.3µg/kg). Thus the oxygen consumption was insufficient and dropped at one replicate of the 8% m.c. after 44 days and in another bag of the 7% m.c. the oxygen remained high (above 15%) even after 90 days of storage (data not shown). The CFU of the aerated bags were higher than the hermetically sealed SBGIIZ and did not stop the increase in CFU for both 7 and 8% m.c.'s ($7.6*10^4 \pm 3.8*10^4$ and $9.2*10^4 \pm 2.7*10^4$, respectively).

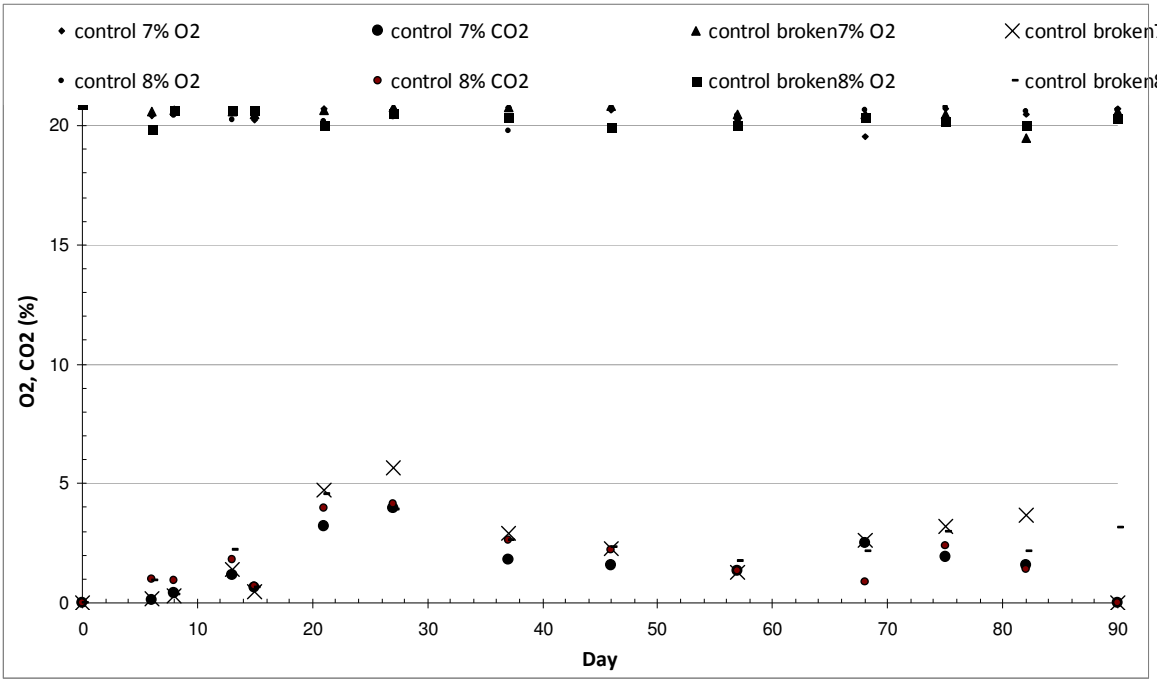

Figure 3. The decline in oxygen and the increase in carbon dioxide inside the mason jars at the 7 and 8% moisture content with or without broken nuts (3%) at the control stored at 30°C. Results are average of 3 replicates.

Table 2. Moisture content (%), FFA (% oleic acid), Aflatoxin (µg/kg) values and CFU (Colony Forming Units) for molds at the beginning of the trials for the targeted 7 and 8% moisture contents and after 90 days of storage within the SGBIIZ (GrainPro Inc.) at 30°C.

Moisture Content (%)	**Treatment**	**Initial**	**After 90 days**	
			Hermetic storage	**Control at 30^0C**
7	**% Moisture Content**	5.97 ± 0.03	6.63 ± 0.34	6.53 ± 0.35
	FFA (% oleic acid)	0.36 ± 0.01	0.75 ± 0.09	1.10 ± 0.1
	Aflatoin (µg/kg	< 0.3	< 0.3	< 0.3
	CFU molds	$3*10^2$	$7.6*10^4 \pm 3.8*10^4$	$2.4*10^5 \pm 1.4*10^5$
8	**% Moisture Content**	7.53 ± 0.07	6.23 ± 0.22	6.13 ± 0.34
	FFA (% oleic acid)	0.42 ± 0.09	1.35 ± 0.24	1.56 ± 0.23
	Aflatoin (µg/kg)	< 0.3	< 0.3	< 0.3
	CFU molds	$3.1*10^2$	$9.2*10^4 \pm 2.7*10^4$	$1.4*10^6 \pm 2*10^5$

Figure 4 shows changes in the gas composition of hermetically sealed SGBIIZ that contained sound peanuts over 90 days of storage. In accordance, the carbon dioxide concentrations which increased to 20 and 30% for the 7 and 8% m.c., respectively.

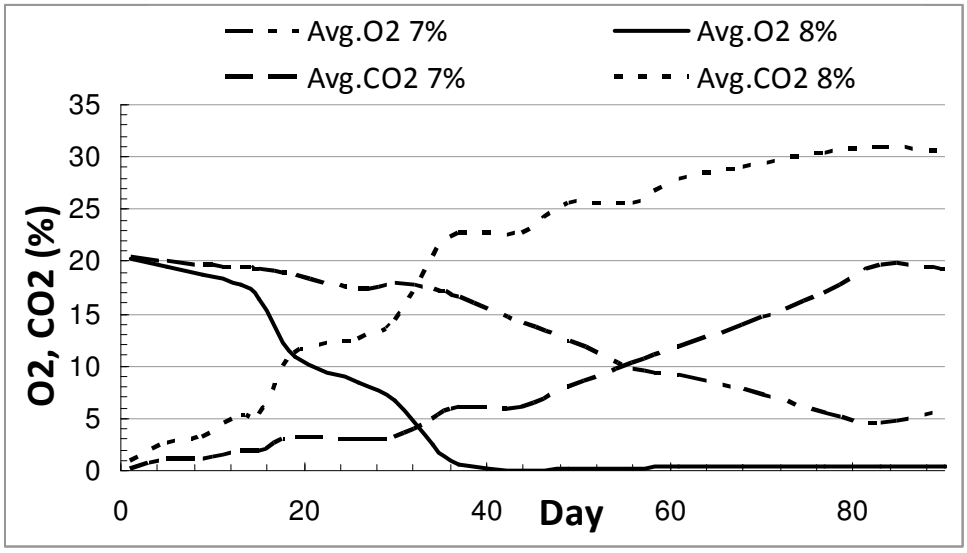

Figure 4. The decline in oxygen and the increase in carbon dioxide inside the SGBIIZ at the 7 and 8% moisture content both hermetically stored at 30°C. Results are average of 3 replicates.

Discussion

FDA enforces a ruling that 20 parts per billion (μg/kg) is the maximum aflatoxin permitted in all foods and animal foods, including peanut butter and other peanut products. In this study, in contrast to the aflatoxins level there was a significant increase at the general count of the moulds expressed as CFU.

The low m.c. obtained within the aerated 7% m.c. samples in this trial were dry thus did not increase significantly the FFA content. Free fatty acids (FFA) are not only a function of peanut maturity, but also of the degree of damage to the nuts. Sound mature peanuts generally have FFA content less than 0.5% as was obtained in the beginning of this trial. Storing dry nuts even in hot and humid conditions allow preserve their quality. The 8% m.c. samples released some of its moist to the micro-environment inside the jars. The reason for this loss might be because of the hot air that was blowing during the trial. The reason for the increase in FFA content in the 8% m.c. with broken nuts stored under hermetic conditions might be because of a leakage of oxygen into the jar allowing microflora's enzymes to produce more free fatty acids. It is interesting to notice the parallel behavior of the 8% m.c. samples compared to the light increase of the 7% m.c. reaching at the end of the storage period the same level of carbon dioxide concentration (Fig. 2). High rate of oxygen consumption within the broken nuts of the 8% m.c. lead to suppression of CFU of molds on these samples.

The same order of magnitude was achieved when the broken (3%) nuts stored under hermetic conditions with 99% carbon dioxide atmosphere both within 8 and 7% m.c.'s (Table 1). Low consumption rate of oxygen in the samples either with or without broken nuts of the 7% m.c. (Fig. 1) is in accordance to the high CFU of molds obtained. CFU of moulds at

the aerated samples of the 8% m.c. increased to an unedible level (10^5 CFU) with FFA content of more than 2.57% while during the hermetic storage it remained low (Table 1). It is interesting to mention that not only the development of the moulds under 99% carbon dioxide atmosphere was suppressed, but there was a decrease compared to the beginning of the trial, which was higher in two orders of magnitude ($< 3.1*10^2$).

Storing peanuts hermetically in the SGBIIZ did not suppressed formation of the CFU. There was a marked and significant difference between the hermetic bags to the hermetic jars containing CO_2. Changes in the gas concentration in the bags were found slightly different than the laboratory tests in jars containing sound peanuts of similar m.c. These differences were particularly significant for 8% m.c peanuts. The oxygen depleted after 44 days compared to the laboratory tests which indicated the depletion after 28 days. In accordance to the slow depletion of oxygen, within the laboratory tests the oxygen did not deplete completely and remained at a level of approximately 1% after 90 days of storage.

Enriching the peanuts environment with CO_2 suppressed the development of micro flora and lipase activity resulting in high quality peanuts with low FFA content.

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