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The effect of moisture level on high-moisture maize (*Zea mays* L.) under hermetic storage conditions—*in vitro* studies

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Abstract

Under humid and warm conditions harvested grains are susceptible to molding and rapid deterioration. Therefore, they should be dried to safe moisture levels that inhibit the activity of microorganisms. Drying to these moisture levels is not economical for farmers in developing countries. Preservation of grains at intermediate moisture levels under hermetic storage conditions could be feasible and economical in warm and moist climates.

The purpose of the current study was to examine the effect of various moisture contents (m.c.) on the quality of maize grains in self-regulated modified atmospheres during hermetic storage.

Maize at 14, 16, 18, 20 and 22% m.c. was initially conditioned for 28 days in tightly wrapped plastic bags and then stored in sealed containers at 30 °C for up to 75 days. Carbon dioxide produced within the containers replaced the oxygen. As the m.c. increased the time for O_2 depletion shortened, from 600 h at 14% m.c. to 12 h at 22%. The maize at 20 and 22% m.c. exhibited the highest dry matter (DM) losses, the lowest germination rates and the highest yeast and bacteria counts. The major fermentation product in the hermetically sealed maize was ethanol (0–5 g kg^{-1} DM), along with lower concentrations of acetic acid (0–1 g kg^{-1} DM).

The results obtained from the *in vitro* experiments indicate that maize at the tested moisture levels can be stored satisfactorily under sealed conditions in which self-regulated atmospheres provide protection against microflora damage. Further large-scale trials will be needed to evaluate the economic feasibility of storing high-moisture maize.

Keywords: High-moisture maize; Hermetic storage; Self-regulated atmospheres

1. Introduction

Worldwide there is increasing demand for high-quality and safe food, free of chemical and physical contaminants and pathogens. Grain growers and users must maintain and protect their harvested grain from insect and microbial damage (Sinha, 1995).

Under dry conditions, grains (paddy rice, maize, etc.), can be stored for extended periods provided that there is no insect infestation or microbial activity. Under humid storage conditions, however, the grains may deteriorate rapidly, resulting in qualitative and quantitative losses, and this deterioration is accelerated at higher temperatures. Qualitative losses include appearance changes, nutritional degradation, loss of germination capacity, presence of insect fragments and mold contamination (Sinha and Muir, 1973). Some of these are difficult to detect visually (Lacey et al., 1980).

In tropical developing countries a large proportion of the crop is harvested under humid and warm climatic conditions, and most small farmers lack equipment for drying grains (Mendoza et al., 1982). Consequently the crop is stored while still relatively moist and warm, which results in rapid deterioration of the grains, mainly because of growth of molds. Even when the crop can be (sun) dried after harvest, exposure to high relative humidity during open storage may result in resumption of moisture uptake

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by the stored grain, with resulting enhanced deterioration (Landers and Davis, 1986). Post-harvest losses of food grains, caused by insect infestation and mold activity, have been conservatively estimated at 10–15% (Grolleaud, 2002).

Molds growing on grains present a second threat, through production of mycotoxins, the secondary metabolites produced by fungi that grow on a wide range of agricultural commodities including cereals and oilseeds (Epstein et al., 1970). Mycotoxins pose a serious health risk to both humans and animals (Van Rosenburg, 1977; Vedman, 2004).

Maize is an important crop that is used worldwide as human food, as a raw material for starch and ethanol production, and as animal feed. Because of its relatively high moisture and starch contents maize is very susceptible to molding.

To prevent molding and rotting in tropical and subtropical regions, grains should be dried immediately after harvest, and cooled or treated with antifungal chemicals such as propionic acid. However, humid conditions and economic constraints prevent rapid drying to safe moisture levels (14% and below). On the other hand, it is easier to process the maize grains at higher moisture contents (m.c.). Therefore, a technology that enables safe maize storage at intermediate m.c. (around 18%) is desirable.

For animal feeding purposes, it is possible to ensile high-moisture maize at 25–28% m.c. with and without microbial and chemical additives. During the ensiling fermentation of high-moisture maize, lactic acid bacteria produce organic acids—mainly lactic and acetic acids—which decrease the pH to 4.0–4.5. However, if not treated against molds with suitable antifungal agents, such silages spoil quickly upon aerobic exposure (Wardinski et al., 1993; Dawson et al., 1998; Taylor and Kung, 2002).

Under tropical conditions grains can be sun-dried to intermediate moisture levels of about 18% relatively easily. A feasible technology to store such intermediate moisture grains, including maize, is known as self-regulated modified atmosphere (Richard-Molard et al., 1980, 1987). In this technology, storage of various grains at intermediate moisture levels (15–18%) in sealed containers results in depletion of oxygen and enrichment in CO_2, as a result of respiration of the grains, insects and microorganisms (Navarro and Donahaye, 2005; Navarro et al., 1990, 1993, 1999). In addition, the limited microbiological activity may result in production of volatile fatty acids (VFAs). Both the anaerobic conditions and the VFA inhibit fungal development (Moon, 1983; Weinberg et al., 1993). This technology is environmentally friendly and does not involve the use of antifungal chemicals. The ecosystem that forms in the sealed containers with the self-regulated atmosphere, and the inter-relationship between grain respiration and microbial activity has not yet been fully explored.

The purpose of the present study was to examine the effect of m.c. on the quality of maize grains in self-regulated modified atmospheres during hermetic storage.

2. Materials and methods

2.1. Preparation of the maize samples

Maize grains at about 14% m.c. were brought to the laboratory from a local feed center. After removing impurities and broken kernels by screening, the maize was divided into five batches, which were then moistened to the targets of 14, 16, 18, 20 and 22% m.c., respectively. This was done by spraying calculated amounts of distilled water over the grains, which were spread in a thin layer in a 30×40 cm plastic tub. The grains were thoroughly hand-mixed after wetting, taking care not to leave any water in the tub. The moistened maize samples were tightly wrapped in plastic bags (8 kg per bag) and stored for 4 weeks at 5 ± 1 °C, for conditioning. During that time each bag was shaken for a few minutes every day.

2.2. Experimental

The maize from the same moisture treatment was removed from the bags and thoroughly mixed. Then maize at each m.c. was placed in 1-L glass jars, about 500 g per jar. The jars were sealed with a screw-cap gas-tight lid and special clamps. Twelve jars were prepared for each m.c., three of which were sampled for analysis after 15, 35, 55 and 75 days. To enable gas sampling, a hole was drilled in the lid and fitted with a silicon-rubber septum. The exact volume of each jar was pre-determined by measuring the volume of distilled water that filled it. The sealed jars were stored at 30 ± 1 °C.

2.3. Analytical procedure

The germination percentages of the grains at each m.c. were determined after storage on damp filter paper for 10 days at 18 °C. The m.c. of the maize samples was determined by forced-air oven drying at 105 °C for 24 h. Equilibrium relative humidity (e.r.h.) was determined at 25 °C with a Novasina MS1 Hygro Measuring System (Defensor®, Pfaffikon, Switzerland). The pH was measured in a 10-fold aqueous extraction of a 20-g sample, with a Delta 230 pH meter (Mettler, Schwenzebach, Switzerland). Ethanol and VFA were determined in aqueous extracts by means of a gas chromatograph equipped with a semi-capillary FFAP (nitroterephthalic acid-modified polyethylene glycol) column (Hewlett-Packard, Waldborn, Germany), over a temperature range of 40–230 °C. Losses were evaluated according to weight loss, and expressed as gas loss ($g\,kg^{-1}$). Ethanol in the headspace was determined with a gas chromatograph according to Davis and Chace (1969).

Atmospheric gas composition in the headspaces was determined by withdrawing gas samples with a 3-ml gas-tight syringe. The concentrations of O_2, N_2 and CO_2 were determined with an SRI 8610c gas chromatograph (SRI Instruments Inc., Las Vegas, NV, USA) equipped with a thermal conductivity detector. The gas chromatograph had two columns, one packed with Poropak Q for CO_2 determination and the other with Molecular Sieve 5a for O_2 and N_2 determination. The columns were maintained at 40 °C and the detector temperature was 200 °C. The percentages of the gases were computed with Peak-Simple software for Windows.

2.4. Microbiological analysis

Microbiological evaluation included enumeration of total aerobic bacteria in plate count agar (Scharlau Microbiology, Barcelona, Spain), and yeasts and molds on spread-plate malt extract agar (Difco, Detroit, MI, USA) acidified with lactic acid to pH 4.0. The plates were incubated for 3 days at 30 °C.

2.5. Statistical analysis

The statistical analysis included analysis of variance and Duncan's multiple range test, which were applied to the results by using the GLM procedure of SAS (1982).

3. Results

At the beginning of the hermetic storage period the m.c. of the maize in the 14%, 16%, 18%, 20% and 22% moisture categories was 13.7±0.1, 16.1±0.0, 18.4±0.1, 20.4±0.1 and 22.8±0.2% m.c., respectively. The m.c. increased, by 8–17 g kg^{-1} during hermetic storage, because of respiration activity. The mean e.r.h. over the experimental period, in the various treatments, which resulted in initial target m.c.s of 14%, 16%, 18%, 20% and 22%, was 77.5±0.3, 85.2±0.3, 89.2±0.3, 91.5±0.4 and 92.5±1.2%, respectively.

Figs. 1–5 show the change in atmospheric gas contents within the sealed containers of maize at each m.c. The higher the m.c., the shorter the time it took for the O_2 to be consumed and replaced with CO_2 during the aerobic respiration. Most of the O_2, in the containers with 14, 16, 18, 20 and 22% m.c. was consumed after 600, 120, 48, 24 and 12 h, respectively. This indicates that the respiration rate increased with increasing maize m.c. In the maize with 14–16% m.c. CO_2 replaced only the O_2, and at first the N_2 percentage remained constant. However, for the higher m.c. maize, as more CO_2 was produced, the percentage of N_2 decreased in the sealed containers.

After the aerobic respiration phase the anaerobic respiration continued to produce CO_2. The levels of anaerobic respiration were measured after a plateau of CO_2 level was reached and up to 1776 h (74 days) (Fig. 6).

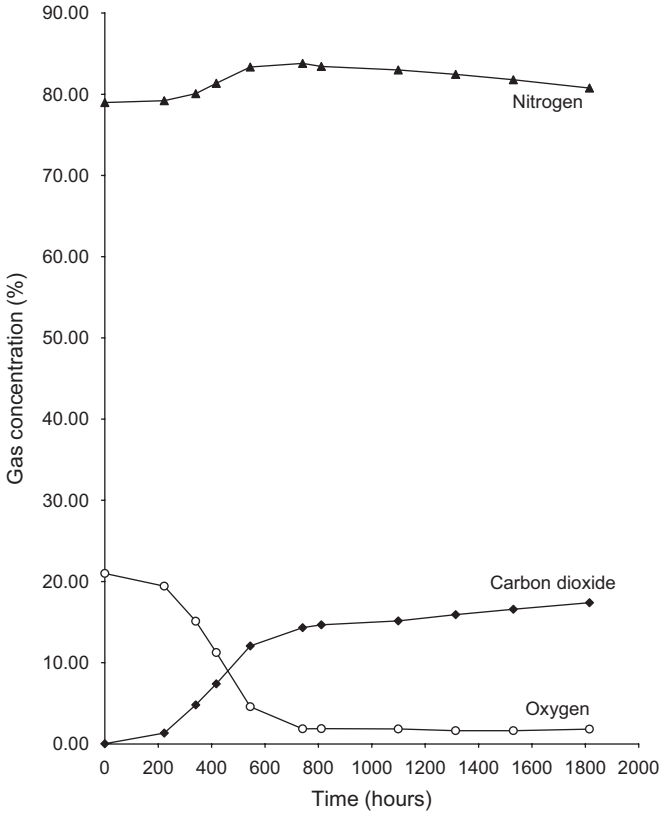

Fig. 1. Gas changes in corn at 14% moisture content during hermetic storage.

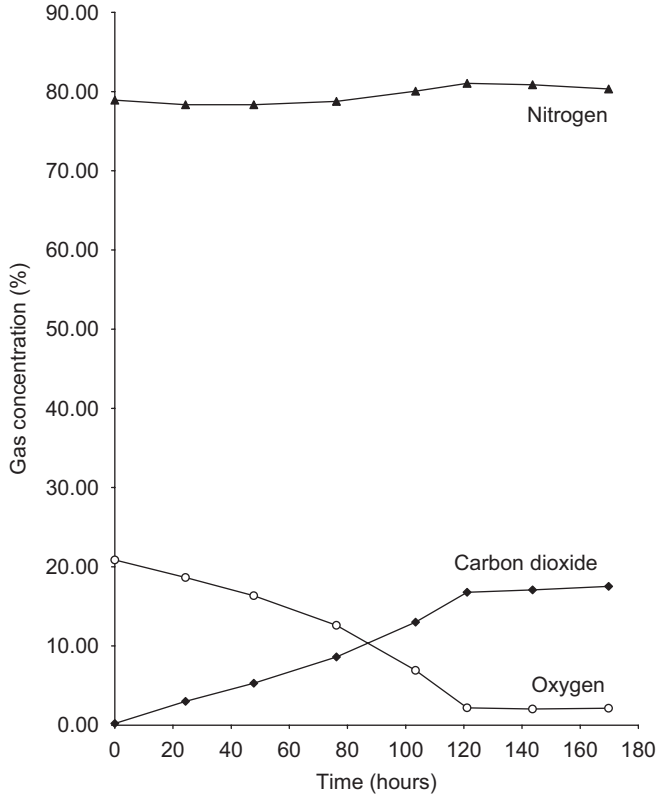

Fig. 2. Gas changes in corn at 16% moisture content during hermetic storage.

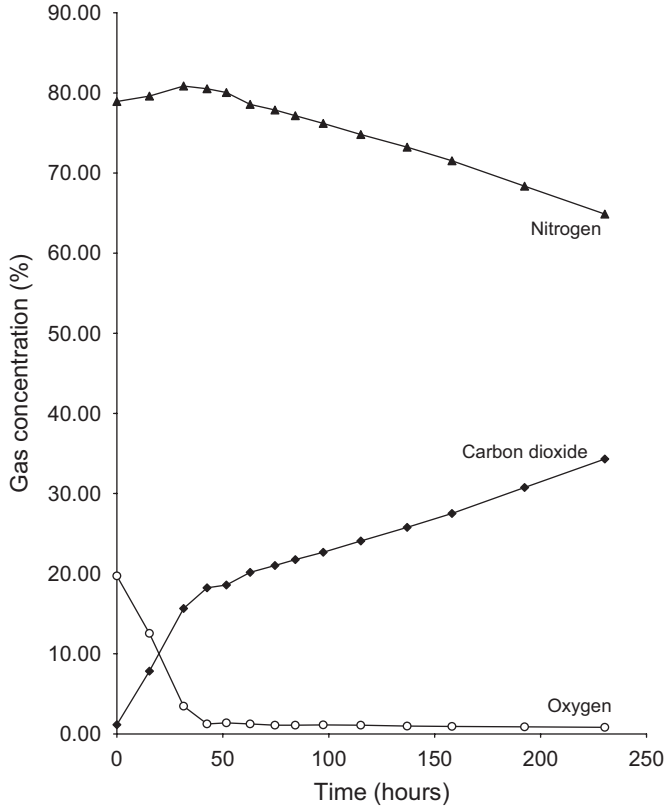

Fig. 3. Gas changes in corn at 18% moisture content during hermetic storage.

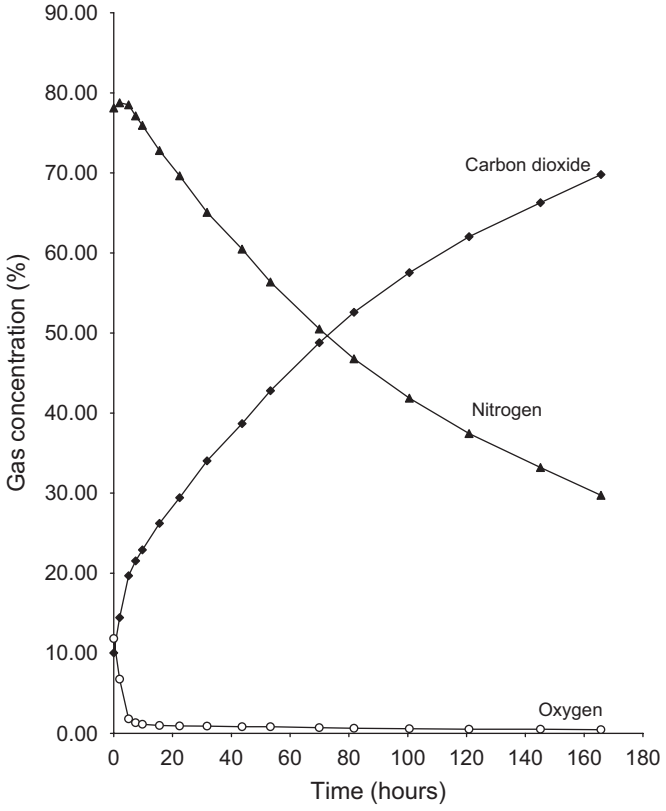

Fig. 5. Gas changes in corn at 22% moisture content during hermetic storage.

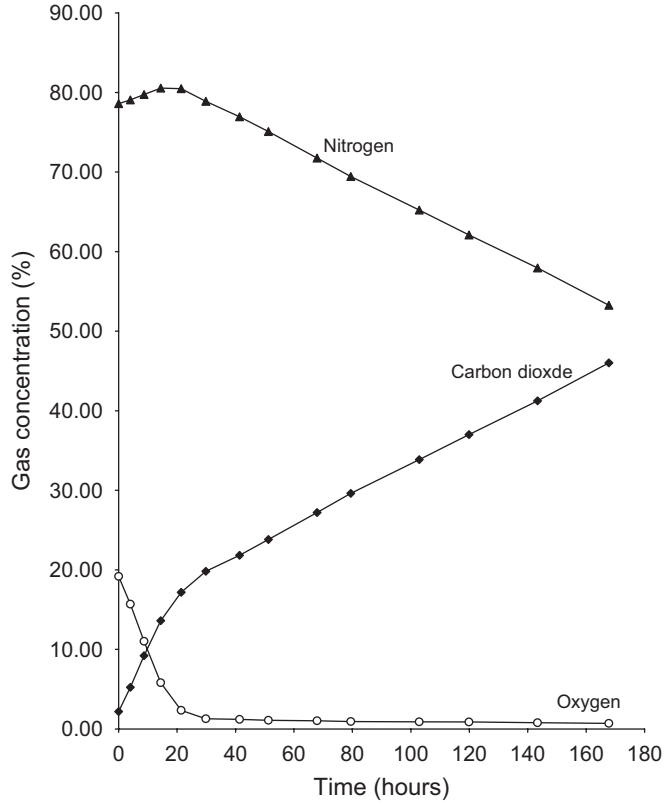

Fig. 4. Gas changes in corn at 20% moisture content during hermetic storage.

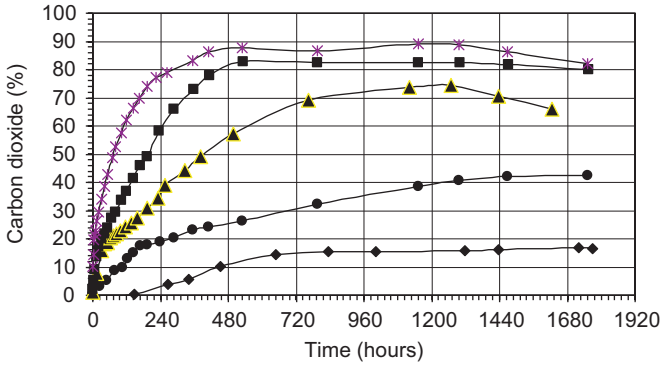

Fig. 6. Levels of CO_2 at moisture contents of 14%, 16%, 18%, 20% and 22% and 30 °C throughout the tested hermetic storage period (♦, m.c. 14%; ●, m.c. 16%; ▲, m.c. 18%; ■, m.c. 20%; and –✳–, m.c. 22%).

During the same period, the levels of O_2 were monitored to ensure that anaerobic conditions were maintained (Fig. 7).

The pH of the maize in the various moisture treatments was around 6.0 and it did not change much during the hermetic storage, except for that with 22% m.c., in which it decreased from 5.8 on day 0 to 5.5 on day 75.

Table 1 summarizes the dry matter (DM) losses and germination percentages of the maize stored in the self-regulated atmospheres in the sealed containers. The germination percentage decreased during the storage period, and decreased as the m.c. increased. With 18%

m.c. and above the germination percentage decreased to zero after 35 days of storage. Dry matter losses increased with increasing m.c. of the maize.

The major volatile products found in the maize were ethanol and acetic acid (Table 2). The highest concentrations were found in the maize with the higher m.c. (20 and 22%). Ethanol concentrations increased during storage, whereas those of acetic acid remained constant or decreased slightly. Propionic and butyric acids were detected at low concentrations ($<0.3\,g\,kg^{-1}$ DM). Ethanol was also detected in the headspace of the sealed containers, and its concentration followed the same trend as its content in the maize (Table 3). These findings might indicate yeast activity.

Tables 4–6 summarize the results of the microbiological analyses. No visible molds could be detected in any of the treatments. At 14–18% m.c. the numbers of molds, yeasts and bacteria in the various treatments were similar and tended to decrease during storage. The populations of these microorganisms were within the safe limits regarding freedom from substantial spoilage ($<\log_{10}/g=4.0$). At 20 and 22% m.c. the numbers of yeasts and bacteria were higher, tended to increase during storage, and reached

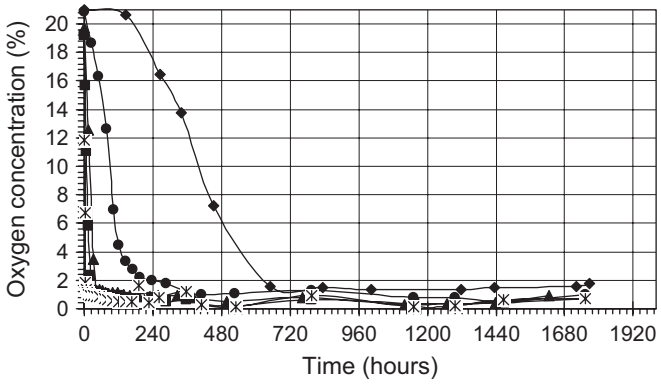

Fig. 7. Levels of O_2 at moisture contents of 14%, 16%, 18%, 20% and 22% and 30 °C throughout the tested hermetic storage period (♦, m.c. 14%; ●, m.c. 16%; ▲, m.c. 18%; ■, m.c. 20%; and –✳–, m.c. 22%).

Table 3
Ethanol content ($mg\,kg^{-1}$ of air) in the headspace of moist maize in hermetic storage

Time (days)	Moisture (%)				
	14	16	18	20	22
55	37	1124	4630	6929	7689
75	148	1279	4496	5048	6571

Table 1
Percentage germination (G) and percentage dry matter losses (DML) during hermetic storage of maize at various moisture contents

Time (days)	Moisture (%)									
	14		16		18		20		22	
	G	DML	G	DML	G	DML	G	DML	G	DML
0	84.3	–	82.8	–	76.0	–	75.0	–	28.6	–
15	79.9^{a}	0.02^{c}	78.1^{a}	0.03^{c}	68.3^{a}	0.11^{c}	27.7^{b}	0.28^{b}	10.3^{c}	0.51^{a}
35	81.7^{a}	0.01^{d}	79.7^{a}	0.05^{d}	3.0^{b}	0.22^{c}	0.0^{b}	0.44^{b}	0.0^{b}	0.59^{a}
55	72.3^{a}	0.03^{e}	61.0^{b}	0.11^{d}	0.0^{c}	0.37^{c}	0.0^{c}	0.51^{b}	0.0^{c}	0.71^{a}
75	58.3^{a}	0.02^{e}	21.0^{b}	0.15^{d}	0.0^{c}	0.41^{c}	0.0^{c}	0.59^{b}	0.0^{c}	0.74^{a}

For each measured parameter, within a row, means followed by different letters are significantly different ($P<0.05$).

Table 2
Ethanol (Et) and acetic acid (HAc) contents ($g\,kg^{-1}$ DM) in the maize under hermetic storage

Time (days)	Moisture (%)									
	14		16		18		20		22	
	Et	HAc	Et	HAc	Et	HAc	Et	HAc	Et	HAc
0	0	0.5	0	0.7	0	0.7	0	0.4	0	1.0
15	0^{d}	0.2	0^{d}	0.3	0.7^{c}	0.4	1.5^{b}	0.5	2.5^{a}	0.5
35	0^{d}	0.5	$0.3^{c,d}$	0.6	1.3^{c}	0.7	2.8^{b}	0.4	3.7^{a}	0.8
55	0.1^{d}	0.5	0^{d}	0.3	2.0^{c}	0.5	2.8^{b}	0.4	4.1^{a}	0.6
75	0^{d}	0.4	$0.9^{c,d}$	0.4	$2.0^{b,c}$	0.3	$3.8^{a,b}$	0.4	5.0^{a}	0.5

For ethanol, within a row, means followed by different letters are significantly different ($P<0.05$).
Propionic and butyric acids were detected at low concentrations ($<0.3\,g\,kg^{-1}$ DM) in some samples, with no consistent pattern.

Table 4
Mold numbers ($\log_{10}$ (CFU g^{-1})) in maize under hermetic storage

Time (days)	Moisture (%)				
	14	16	18	20	22
0	3.2*	4.9	4.7	4.7	6.8
15	3.3[b]	3.1[b]	3.7[b]	3.7[b]	5.2[a]
35	2.7	1.9	2.3	0.7	2.1
55	2.5	1.0	2.4	NF	NF
75	1.9[a,b]	1.6[a,b]	2.2[a]	NF[b]	NF[b]

Within a row, means followed by different letters are significantly different ($P<0.05$).
NF, not found (below the detectable level, $\log_{10}$ (CFU g^{-1}) <2.0).
*For day 0 there was one sample only for each moisture level and they indicate mold growth during the equilibration phase at 5 °C.

Table 5
Yeast numbers ($\log_{10}$ CFU g^{-1}) in maize under hermetic storage

Time (days)	Moisture (%)				
	14	16	18	20	22
0	2.7	NF	NF	3.5	5.4
15	2.8[b]	2.9[b]	2.7[b]	3.5[b]	5.6[a]
35	1.5[b]	2.4[b]	2.4[b]	4.7[a]	5.3[a]
55	1.3[c]	1.3[c]	3.1[b,c]	4.9[a,b]	6.5[a]
75	NF[d]	1.1[d]	3.9[c]	5.2[b]	6.4[a]

Within a row, means followed by different letters are significantly different ($P<0.05$) (for day 0 there was one sample only).
NF, not found (below the detectable level, $\log_{10}$ (CFU g^{-1}) <2.0).

Table 6
Bacteria numbers ($\log_{10}$ CFU g^{-1}) in maize under hermetic storage

Time (days)	Moisture (%)				
	14	16	18	20	22
0	2.9	4.4	3.8	4.5	NF
15	1.6	2.1	1.7	3.7	2.7
35	3.7[b]	2.7[b]	2.8[b]	3.8[b]	5.9[a]
55	3.1[c]	3.5[b,c]	2.1[c]	5.2[a,b]	6.1[a]
75	3.0[b,c]	3.2[b,c]	2.0[c]	4.8[a,b]	6.2[a]

Within a row, means followed by different letters are significantly different ($P<0.05$) (for day 0 there was one sample only).
NF, not found (below the detectable level, $\log_{10}$ CFU g^{-1} <2.0).

population levels ($>\log_{10}/g=6.0$) usually associated with spoilage of vegetable food commodities.

4. Discussion

In developing countries there is a strong need for technologies that would enable long-term storage of grains under warm and humid ambient conditions. The current trend is to avoid preservative chemicals that might be hazardous to humans or animals, and to use environmentally friendly technologies. The major spoilage factors in grains include fungi, bacteria and insects. Donahaye et al. (2001) studied the quality preservation of moist paddy stored under hermetic conditions in the laboratory with promising results. The current experiments were designed to test the efficacy of hermetic storage of maize grains at a range of moisture levels, focusing on fungal control. The m.c. range was obtained under laboratory conditions by adding calculated amounts of water to the grain. This method of conditioning the maize samples was adopted because of technical difficulties in obtaining samples of the desired m.c. from farms. Such conditioning was practiced in studies to determine m.c. and e.r.h. of cereal grains including maize varieties by Pixton and Warburton (1971) and other workers (Bottomley et al., 1952; Gough and King, 1980; Lacey et al., 1980).

In hermetic storage of grains, self-regulated atmospheres are generated and this technology complies with the requirements mentioned above. The anaerobic conditions that are generated within sealed containers inhibit aerobic yeasts, molds and insects, which are major spoilers of grains (Hyde et al., 1973; Magan and Lacey, 1984). In these studies, the anaerobic conditions were achieved by the respiration of living organisms in the sealed containers. In contrast to this concept, Serafini et al. (1980) and Shejbal (1980) studied the possibilities of obtaining O_2 depleted atmospheres by purging test jars or experimental bins using nitrogen for the preservation of high-moisture grain. In experiments carried out by Shejbal (1980) rigid structures consisting of gas-tight metal bins were used. These types of metal bins are more suitable for purging with nitrogen, since the alteration of the headspace atmosphere by self-regulated atmospheres requires more respiratory metabolism than the smaller volume in flexible structures. In studies using high-moisture paddy, the effectiveness of sealed plastic structures, where the headspace is minimized, was reported by Navarro et al. (1997). When the objective is to control insects, it appears that anaerobic conditions provide an excellent solution to prevent insect development and thereby prevent insect damage (Navarro and Caliboso, 1996).

Germination capacity was negatively impacted as the m.c. was increased (Table 1). Similar results were obtained by Moreno et al. (1988) who studied the influence of hermetic storage on the behavior of maize seed germination. The sharp decline in germination percentage within a short time discourages hermetic storage of maize for more than 35 days at above 16% m.c.

The respiration of grain samples under aerobic and anaerobic conditions resulted in dry matter losses (Table 1). These dry matter losses reached levels of 0.41%, 0.59% and 0.74% at 18, 20 and 22% m.c., respectively. These levels are comparable with acceptable dry matter losses experienced in the drying processes. Kuppinger et al. (1977) compared germination, dry matter loss, and spore counts of microorganisms under aerated drying conditions to evaluate the quality of maize.

Germination was not found to be a good quality indicator because of high rates even after molding was already visible. Dry matter loss varied from 0.6% to 3.0% for maize dried from 35% m.c., while the dry matter loss of pre-dried maize was only 0.04%.

The maximum permissible storage time in low temperature drying systems depends on grain type and physical condition, m.c. and temperature. In these aerobic systems, Saul and Lind (1958) and later Steele et al. (1969) monitored CO_2 production to indicate the amount of dry matter loss due to microbial growth in shelled maize. From these studies, the shelf-life was established based on dry matter loss of less than 1%. Steele et al. (1969) calculated the permissible storage time for field-shelled maize at various temperatures and m.c. based on 0.5% dry matter loss. Thompson (1972) incorporated these data into a deterioration index and adopted a dry matter loss of 0.5% as the major constraint in establishing minimum airflow requirements for drying grain with unheated air. In the present study, the measured dry matter losses (Table 1) were within the acceptable limits for drying grain and provide evidence for the adoption of 0.5% or 1.0% dry matter loss as parameters for a storability index.

Heavy-duty flexible PVC liners were developed for the manufacture of storage cubes, which are very efficacious for on-site hermetic storage of intermediate m.c. grains and beans (Navarro et al., 1990, 1999, 2002). These are hermetic storage structures named Volcani Cubes known also as GrainPro Cocoons™ (Navarro and Donahaye, 2005). The present results in a laboratory-scale model system to simulate conditions in these facilities indicate that some respiration and microbiological activity take place in intermediate m.c. maize stored in hermetically sealed containers. At 14% m.c. almost no biological activity took place, and the grains retained their quality, as expected in dry storage of grains.

In all the moisture treatments self-regulated atmospheres were generated that were characterized by depletion of O_2 and build-up of CO_2. The higher the m.c. the faster the build-up of CO_2 in the sealed container, accompanied by an increase in pressure (Figs. 1–5). From gas exchange of O_2 and CO_2 in Figs. 1 and 2, it appears that at 14 and 16% m.c. the respiration was aerobic and no excess of pressure was observed. Thus, the CO_2 concentration did not exceed 20% by volume. Above 18% m.c., a gradual increase in CO_2 concentrations was observed that exceeded the volume of consumed O_2 (Figs. 3–5), indicating that anaerobic respiration occurred (Hyde et al., 1973; Zettler and Navarro, 2001). The jars used in these experiments were equipped with septa through which periodic gas samples were taken. During the gas sampling, pressure build-up was observed, particularly at 18–22% m.c. Each gas sampling released some excess of pressure, since to ensure representative gas sampling in the gas-tight syringe, the first gas sample was released to the atmosphere. If the entire gas content had been retained in the jars, the pressure would have been much higher than the ambient. This was evident from the levels of CO_2 reaching 74%, 83% and 89% at 18, 20 and 22% m.c., respectively (Fig. 6). In a completely sealed structure, it is assumed that N_2 as an inert gas would not take part in the aerobic or anaerobic respiratory metabolism. Therefore, the amount of N_2 present would remain as in the original atmosphere. However, due to losses during sampling, the actual amount as well as the proportion of N_2 in the mixture will decrease, while for CO_2 both the amount and proportion will increase because of anaerobic repiration. Hence, the percentages of N_2 and CO_2 measured change continually and very high levels of CO_2 are recorded.

The expected increase in pressure has an important implication; any gas-tight container with high m.c. maize must be equipped with a pressure release valve. In small experimental jars, the lack of a pressure release valve has no extreme importance as it may in large rigid or flexible structures. Since large structures, in principle, are not designed to withstand such pressures they are apt to explode at weak joints.

At the high 20 and 22% m.c. levels, large numbers of yeast and molds were found during the initial stages of storage, and these led to higher dry matter losses (Table 1). The starting hypothesis of this study was that in intermediate m.c. maize under sealed storage conditions, limited microbial activity would result in production of VFAs, which inhibit the yeasts and molds that are the major spoilage microorganisms in such commodities (Moon, 1983; Weinberg et al., 1993). However, ethanol was found in higher concentrations than VFAs, and this might indicate yeast activity (Table 2). Acetic acid was found in concentrations that were probably too low to inhibit yeasts and molds. From studies on silage fermentation it is apparent that VFA concentrations that inhibit fungi are at least $10\,g\,kg^{-1}$ DM (Weinberg et al., 1993). Even in the higher m.c. maize not enough VFAs were produced, because the water activity was too low to support the microbial activities that usually produce them, such as those of heterofermentative lactic acid bacteria (Troller and Stinson, 1981) or enterobacteria (Frazier and Westhoff, 1978). The lack of sufficient VFAs enabled yeasts to develop in substantial numbers in the maize with the highest m.c. (20% and 22%) during the early stages of storage. After 35 days of storage no molds were detected, even at the highest m.c., probably because the O_2 was depleted very rapidly in these treatments. Molds found in the lower m.c. maize (14–18%) could have used the O_2 which was available for a longer time in these treatments (Figs. 1–5). In this regard it should be mentioned that in silage, molds survived in atmospheres with O_2 concentrations as low as 1.0% (Lisker et al., 1989). Therefore, for maize to be used for feed or for the extraction of starch, a complete analysis of mycotoxins, particularly of aflatoxin and fumonisins would be necessary. Above 25% m.c., maize may undergo lactic acid fermentation and ensiling, which results in pH decrease (Wardinski et al., 1993; Dawson et al., 1998; Taylor and Kung, 2002). Such

storage, however, is suitable for holding maize for animal feeding purposes only.

Results of the current work may serve as a model for the development of the technology of storing high m.c. maize for ethanol production, where the presence of mycotoxins might not be a critical issue. However, since after harvest maize must be preserved, the conventional technology of preservation is drying, which is a costly operation, and adding moisture just before use. The concept proposed in this work is to avoid the drying process by storing the maize at high m.c. until it is used for feed or processed for the production of starch or ethanol. Further experiments are warranted with pilot and commercial scale hermetically sealed plastic structures in order to determine the maximal safe m.c. for this technology under field conditions, and to determine its economical feasibility.

5. Conclusions

In the laboratory-scale experiments, intermediate and high m.c. maize at 16–22%, was stored without spoilage in hermetically sealed jars. Prevention of deterioration of the maize under such conditions is apparently due to the self-regulated anaerobic atmospheres generated under sealed conditions, and not due to VFAs which were present at very low levels. Additional chemical analysis such as determination of mycotoxin levels would need to be carried out before this technology is implemented for storing high m.c. maize for animal feed, human food and starch extraction. Although at 20 and 22% m.c., yeast and bacteria counts, and dry matter losses were significantly higher than at lower m.c., results are sufficiently encouraging to justify further commercial scale trials on storage of maize for ethanol production and they should be accompanied by economical feasibility studies.

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References

Bottomley, R.A., Christensen, C.M., Geddes, W.F., 1952. Grain storage studies. X. The influence of aeration, time and moisture content on fatty acids, non-reducing sugars and mold flora of stored yellow corn. Cereal Chemistry 29, 53–64.

Davis, P., Chace, W.G., 1969. Determination of alcohol in citrus juice by gas chromatograph analysis of headspace. Hortscience 4, 117–119.

Dawson, T.E., Rust, S.R., Yokoyama, M.T., 1998. Improved fermentation and aerobic stability of ensiled high moisture maize with the use of *Propionibacterium acidipropionici*. Journal of Dairy Science 81, 1015–1021.

Donahaye, J., Navarro, S., Andales, S., del Mundo, A., Caliboso, F., Sabio, G., Felix, A., 2001. Quality preservation of moist paddy under hermetic conditions, In: Donahaye, E.J., Navarro, S., Leesch, J.G. (Eds.), Proceedings of an International Conference on Controlled Atmosphere and Fumigation in Stored Products, 29 October–3 November 2000, Fresno, CA. Executive Printing Services, Clovis, CA, USA, pp. 209–225.

Epstein, E., Steinberg, M.P., Nelson, A.I., Wei, L.S., 1970. Aflatoxin production as affected by environmental conditions. Journal of Food Science 35, 389–391.

Frazier, W.C., Westhoff, D.C., 1978. Food borne infections and bacterial intoxications. In: Frazier, W.C., Westhoff, D.C. (Eds.), Food Microbiology. McGraw-Hill, New York, pp. 419–453.

Gough, M.C., King, P.E., 1980. Moisture content/relative humidity equilibria of some tropical cereal grains. Tropical Stored Products Information 39, 13–17.

Grolleaud, M., 2002. Post harvest losses; discovering the full story. FAO Corporate Document Repository, FAO Agro Industries and Post-harvest Management Service (AGSI). 〈http://www.fao.org/DORCEP/004/AC301E/AC301E00.htm〉.

Hyde, M.B., Baker, A.A., Ross, A.C., Lopez, C.O., 1973. Airtight grain storage. FAO Agricultural Services Bulletin 17, 71pp.

Kuppinger, H.V., Muller, H.M., Muhlbauer, W., 1977. Die beluftung-strocknung vonerntefrischem und vorgetrocknetem kornermais unter thermodynamischem undmikrobiologischem aspekt. Grundlage Landtechnik 27, 119–132.

Lacey, J., Hill, S.T., Edwards, M.A., 1980. Micro-organisms in stored grains: their enumeration and significance. Tropical Stored Products Information 39, 19–32.

Landers, K.E., Davis, N.D., 1986. Influence of atmospheric gases on aflatoxin production by *Aspergillus flavus* in peanuts. Phytopathology 57, 1086–1090.

Lisker, N., Taari, E., Ashbell, G., Henis, Y., 1989. Chemical and microbiological changes occurring in wheat silage maintained under modified atmospheres. Journal of the Science of Food and Agriculture 47, 125–127.

Magan, N., Lacey, J., 1984. Effects of gas composition and water activity on growth of field and storage fungi and their interactions. Transactions of the British Mycological Society 82, 305–314.

Mendoza, E., Rigor, A.C., Mordido Jr., C.C., Marajas, A.A., 1982. Grain quality deterioration in on-farm level of operation. In: Teter, N.C., Frio, A.S. (Eds.), Progress in Grain Protection. Proceedings of the Fifth Annual Grains Post-harvest Workshop, 19–21 January 1982, Chiang Mai, Thailand, pp. 107–117.

Moon, N.J., 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. Journal of Applied Bacteriology 55, 453–460.

Moreno, E., Benavides, C., Ramirez, J., 1988. The influence of hermetic storage on the behaviour of maize seed germination. Seed Science and Technology 16, 427–434.

Navarro, S., Caliboso, F.M., 1996. Application of modified atmospheres under plastic covers for prevention of losses in stored grain. Final Report submitted to US Agency for International Development, CDR Project no. C7-053, August 1990–November 1995, 32pp.

Navarro, S., Donahaye, E.J., 2005. Innovative environmentally friendly technologies to maintain quality of durable agricultural products. In: Ben Yehoshua, S. (Ed.), Environmentally Friendly Technologies for Agricultural Produce Quality. CRC Press, Boca Raton, FL, USA, pp. 204–260.

Navarro, S., Donahaye, E.J., Rindner, M., Azrieli, A., 1990. Airtight storage of grain in plastic structures. Hassadeh Quarterly 1, 85–88 (in Hebrew).

Navarro, S., Varnava, A., Donahaye, E.J., Preservation of grain in hermetically sealed plastic liners with particular reference to storage of barley in Cyprus. In: Navarro, S., Donahaye, E.J. (Eds.), Proceedings of an International Conference on Controlled Atmosphere and Fumigation in Grain Storages, 11–13 June 1992, Winnipeg, Canada, 1993. Caspit Press Ltd., Jerusalem, Israel, pp. 223–234.

Navarro, S., Caliboso, F.M., Sabio, G.C., Donahaye, E.J., 1997. Quality conservation of paddy stored under gastight seal outdoors in the Philippines. In: Donahaye, E.J., Navarro, S., Varnava, A. (Eds.),

Proceedings of an International Conference on Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996. Print Co. Ltd., Nicosia, Cyprus, pp. 159–168.

Navarro, S., Donahaye, E.J., Ferizeli, G.A., Rindner, M., Azrieli, A., 1999. Sealed granary for use by small scale farmers. In: Zuxun, J., Quan, L., Yongsheng, L., Xiachung, T., Lianghua, G. (Eds.), Proceedings of the Seventh International Working Conference on Stored Products Protection, 14–19 October 1998, Beijing, PR China, vol. 1, Sichuan Publishing House of Science and Technology, Chengdu, Sichuan Province, PR China, pp. 434–443.

Navarro, S., Donahaye, E.J., Rindner, M., Azrieli, A., Dias, R., 2002. Seed storage in the tropics under gastight sealed conditions. In: Sukprakaran, C., Ruay-Aree, S., Srzednicki, G., Longstaff, B., McGlasson, B., Hocking, A., Graver, J., van, S., Wongkobrat, A., Visarathanonth, P. (Eds.), Proceedings of the 20th ASEAN/2nd APEC Seminar on Post Harvest Technology, 11–14 September 2001, Chiang Mai, Thailand. Funny Publishing Co. Ltd, Bangkok, Thailand, pp. 180–186.

Pixton, S.W., Warburton, S., 1971. Moisture content/relative humidity equilibrium of some cereal grains at different temperatures. Journal of Stored Products Research 6, 283–293.

Richard-Molard, F., Cahagnier, D.B., Poisson, J., 1980. Wet grains storage under modified atmospheres. Microbiological aspects. In: Shejbal, J. (Ed.), Controlled Atmosphere Storage of Grains. Elsevier, Amsterdam, pp. 173–182.

Richard-Molard, F., Diawara, D.B., Cahagnier, B., 1987. Susceptibility of cereal microflora to oxygen deficiency and carbon dioxide concentration. In: Donahaye, E.J., Navarro, S. (Eds.), Proceedings of the Fourth International Working Conference on Stored-Products Protection, 21–26 September 1986. Tel Aviv, Israel, Maor-Wallach Press, Caspit, Jerusalem, Israel, pp. 85–92.

SAS, 1982. User's Guide. Statistical Analysis Systems, Cary, NC.

Saul, R.A., Lind, E.E., 1958. Maximum time for safe drying of grain with unheated air. Transactions of the ASAE 2, 29–33.

Serafini, M., Fabbri, A.A., Shejbal, J., Fanelli, C., Di Maggio, D., Rambelli, A., 1980. Influence of nitrogen on the growth of some storage fungi on moist wheat. In: Shejbal, J. (Ed.), Controlled Atmosphere Storage of Grains. Elsevier, Amsterdam, pp. 157–171.

Shejbal, J., 1980. Storability of cereal grain and oil seeds in nitrogen. In: Shejbal, J. (Ed.), Controlled Atmosphere Storage of Grains. Elsevier, Amsterdam, pp. 185–205.

Sinha, R.N., 1995. The stored-grain ecosystem. In: Jayas, D.S., White, N.D., Muir, W.E. (Eds.), Stored Grain Ecosystems. Marcel Dekker, New York, pp. 1–32.

Sinha, R.N., Muir, W.E., 1973. Grain Storage: Part of a System. The Avi Publ. Co. Inc., Westport, CT.

Steele, J.L., Saul, R.A., Hukill, W.V., 1969. Deterioration of shelled corn as measured by carbon dioxide production. Transactions of the ASAE 12, 685–689.

Taylor, C.C., Kung, L., 2002. The effect of *Lactobacillus buchneri* 40788 on the fermentation and aerobic stability of high moisture maize in laboratory silos. Journal of Dairy Science 85, 1526–1532.

Thompson, T.L., 1972. Temporary storage of high-moisture shelled corn using continuous aeration. Transactions of the ASAE 15, 333–337.

Troller, J.A., Stinson, J.V., 1981. Moisture requirement for growth and metabolite production of lactic acid bacteria. Applied and Environmental Microbiology 42, 682–687.

Van Rosenburg, S.J., 1977. Role of epidemiology in the elucidation of mycotoxins health risks. In: Rodricks, J.V., Hesseltine, C.W., Mehlman, M.A. (Eds.), Mycotoxins in Human and Animal Health. Pathotox Publishers, Park Forest South, IL, USA, pp. 699–711.

Vedman, B., 2004. Mycotoxins in the animal production chain. In: Proceedings of the 2nd World Mycotoxin Forum, Nordwijk, the Netherlands, February 2004. European Mycotoxin Awareness Network, pp. 275–280.

Wardinski, F.A., Rust, S.R., Yokoyama, M.T., 1993. Effect of microbial inoculation of high moisture maize on fermentation characteristics, aerobic stability and cattle performance. Journal of Animal Science 71, 2246–2252.

Weinberg, Z.G., Ashbell, G., Hen, Y., Azrieli, A., 1993. The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. Journal of Applied Bacteriology 75, 512–518.

Zettler, J.L., Navarro, S., 2001. Effect of modified atmospheres on microflora and respiration of California prunes. In: Donahaye, E.J., Navarro, S., Leesch, J.G. (Eds.), Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, 29 October–3 November 2000, Fresno, CA. Executive Printing Services, Clovis, CA, USA, pp. 169–177.