

## **The biological and physical aspects of hermetic storage: A critical review**

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### **Abstract**

Recent reports on hermetic storage reveal the gaps in our knowledge on this modest looking technology. This presentation discusses the contribution of each of the biological components that cause the change of the atmospheric composition and the importance of the physical aspects of permeation to oxygen and gas-tightness of the tested enclosures. The manufacture of flexible liners that conform to prerequisite specifications of durability to climate, gas permeability, and physical properties, enabled the development of the storage systems based on the hermetic principle. Hermetic storage takes advantage of sufficiently sealed structures that enable insects and other aerobic organisms in the commodity or the commodity itself to generate the modified atmosphere by reducing oxygen (O<sub>2</sub>) and increasing carbon dioxide (CO<sub>2</sub>) concentrations through respiratory metabolism. The successful application of the technology depends on biological and physical factors that should be considered in the application of the technology. The biological aspects includes responses of storage insects, microflora and the commodity itself to altered atmospheric gas compositions containing low O<sub>2</sub> or high CO<sub>2</sub>, temperature and r.h. in dependence of exposure period of time. The physical aspects include the response of the structure that makes the storage hermetic to air ingress rate and its durability to weather conditions. Although the maximum air ingress rate equivalent to 0.05%O<sub>2</sub>/day is targeted, very often this poses a serious challenge in the application of the technology. Such target O<sub>2</sub> ingress rate is dependent on the liner permeability and the level of gas-tightness achieved. This low O<sub>2</sub> ingress level, is difficult to obtain in rigid structures, it requires additional special equipment of a breathing bag and pressure relief valve, but is achievable in practice using flexible liners. Therefore, the hermetic storage method has emerged as an acceptable technology applied using flexible structures and less considered as applicable in rigid structures.

Keywords: modified atmospheres, hermetic storage, carbon dioxide, stored-product insects, respiratory metabolism

### **1. Introduction**

There is increasing interest in application of hermetic storage for durable agricultural products (Murdock and Lowenberg-DeBoer, 2014). Although hermetic storage has been in use for several thousand years preserving grains in airtight pots or containers (Adler et al., 2000), in modern times, storage size has increased from small family storages to large bulks representing many producers or a portion of a country's total production. In the 1960s and 70s, large aboveground hermetic storage in some African and Asian countries was discredited because of severe condensation problems, particularly in metal structures (Navarro et al., 1994). Semi-underground storage has been used successfully in Argentina, Kenya, and Cyprus; Australia and Israel have successfully used bunker storage systems from the 1980s.

With recent improvements in materials and construction of flexible, nonporous bags and liners, a variety of size options offer protection for products from 25 to 1,000 kg up to 10,000 to 15,000 tonnes (Navarro, 2010). Commodities including cereals, oilseed grains, pulses,

cocoa, and coffee can be stored safely for many months, maintaining high quality and limiting molds and mycotoxins. Plastic structures suitable for long-term storage systems, as well as intermediate storage of grain in bags or in bulk have been developed and applied. Storage systems based on the hermetic principle include the following:

1. Bunker storage in gastight liners for conservation of large bulks of 10,000 to 15,000 tonnes capacity) (Navarro et al., 1984, 1994);
2. Flexible gastight silos supported by a weld-mesh frame of 50 to 1,000 tonnes capacity for storage of grain in bulk or in bags (Calderon et al., 1989; Navarro et al., 1990, 1998a);
3. Gastight liners for enclosing stacks of 5 to 1,000 tonnes capacity, called storage cubes or Cocoon<sup>TM</sup>, and designed for storage at the farmer-cooperative and small trader level or larger commercial and strategic storage facilities (Donahaye et al., 1991). These structures are in current use for bagged storage of cereals;
4. Silo bags of 200 tonnes capacity for on-farm grain storage directly in the field. This technique was originally used for grain silage, and involves storing dry grain in sealed plastic bags. The silo-bag is 60 m long and 2.74 m diameter (Bartosik, 2012), it has a cover made of three layers (white outside and black inside) with 235  $\mu\text{m}$  of thickness (Cardoso et al., 2008);
5. A highly efficient method of using air-tight bags for masses of 25 kg to 1,000 kg of product is commercially available. This development is based on hermetic storage bags called SuperGrainbags<sup>TM</sup>, designed to hold 50 kg of paddy or corn. SuperGrainbags<sup>TM</sup> serve as gastight liners for outer bags made of either polypropylene or jute.
6. The problem of applying present-day technology to provide hermetic storage for subsistence farmers lies in the need to provide an easily sealable low-cost container. The most recent attempt to address this problem has been through the construction of a small granary for use by small scale farmers, suitable to store up to 1,000 kg, termed GrainSafe (Navarro et al., 1998b). This granary was equipped with an upper collapsible sleeve for loading and a lower collapsible sleeve for unloading. The hermetic flexible bag was inserted into a rigid sheath surrounding the vertical sides of the hermetic bag.
7. Purdue Improved Cowpea Storage (PICS) bags (designed for storage of 50 kg or 100 kg cowpea) consist of three plastic bags: two 80-mm high-density polyethylene (HDPE) bags, one surrounded by the second; both are enclosed by a third bag made of woven polypropylene (Murdock and Baoua, 2014).
8. Metal silos, also hermetically sealed but physically stronger than flexible liners, have been heavily promoted in Central America (Hellin and Kanampiu, 2008) and their feasibility is currently being explored in Sub-Saharan Africa (Tefera et al., 2011).

These structures enabled the application of modern MA technology. Since this technology is relatively the newest and the terminology used is less elaborated, it creates much confusion of what we mean by hermetic storage of grain. Therefore, it seems reasonable that this type of storage has been referred to a type of Modified Atmosphere (MA) that can be applied for the protection of grain also termed as “sealed storage” or “air-tight storage” or “sacrificial sealed storage” (Navarro, 2006). This method takes advantage of sufficiently sealed structures that enable insects and other aerobic organisms in the commodity or the commodity itself to generate the MA by reducing oxygen ( $\text{O}_2$ ) and increasing carbon dioxide ( $\text{CO}_2$ )

concentrations through respiratory metabolism. Respiration of the living organisms in storage (insects, fungi, and grain) consume oxygen (O<sub>2</sub>), reducing it from near 21% in air to 1 to 2% while production of carbon dioxide (CO<sub>2</sub>) rises from an ambient 0.035% to near 20% or higher according to the level of moisture content. This environment kills insect and mite pests and prevents aerobic fungi from growing. Elevated CO<sub>2</sub> and depleted O<sub>2</sub> levels will generally maintain stored grain quality for long periods. Grain with excessive moisture may be invaded by lactate-forming bacteria and yeasts.

Flexible or rigid structures with higher O<sub>2</sub> ingress rates can be used to protect the grain from rain or increase of moisture provided the grain is dry and without any infestation. The question is whether these structures should be considered under the term of “hermetic storage” or just simply “sealed storage” without the expectations that they will develop a biogenerated atmosphere to protect the grain and use fumigation to control the insects.

Regarding the possibility of using hermetic storage for the quality preservation of organic products, we need to consider the level of quality required from the specific product. For most cereal grains, preventing development of insects or microflora at an economic threshold is acceptable. However, for some products like oil seeds, nuts and pulses, our investigations have demonstrated that use of carbon dioxide or nitrogen for a Modified Atmosphere or a Controlled Atmosphere is most desired.

This report refers to parameters required to describe the degree of gas tightness of the flexible structures that can be considered as hermetic storages like Cocoons, GrainSafe, Super GrainBags, Silobags, PICS or facilities alike.

## **2. Effects of hermetic storage on grain insects**

One of the biological agents that may be present in the grain bulk is insects. In addition to insects the stored product fauna contains also mite pests. Insects and mites are aerobic organisms that require oxygen for their development. Reduction of the oxygen tension in the grain bulk might be possible either by the natural consumption of oxygen by the biological agents or by artificially producing a low oxygen atmosphere to control insects and mites.

Several hermetic storage works were reported recently, one relates on the use of PICS in comparison to poly propylene bags reported by Njoroge et al., (2014). They were able to monitor oxygen and carbon dioxide conditions prevailing in PICS bags over the six months of storage of maize. Oxygen concentration dropped steadily from 19.23 to 7.18% and 7.82% within three months in PICS bag with maize infested artificially and PICS bag infested naturally, respectively. Carbon dioxide concentration, on the other hand, increased gradually to 13.17% and 13.93%, respectively, within the same period. Thereafter, oxygen and carbon concentrations, on average, stabilized at 6.66% and 13.84%, respectively, in subsequent months. Njoroge et al. (2014) implied that there was no difference in the progression of oxygen and carbon dioxide profiles in PICS although the latter had been infested with *Prostephanus truncatus* (Horn). Evidently, oxygen depletion and carbon dioxide build-up did not reach extreme levels probably because of monthly opening of bags during sampling. Elsewhere, Ognakossan et al. (2013) reported that oxygen levels could be modified by the respiration and metabolism of insects, fungi and the grain itself to a level of 1 to 2% or below. Other researchers, however, also reported failure of extreme drop in oxygen concentration. Moreno-Martinez et al. (2000), for instance, observed gradual decrease in oxygen that eventually stabilized at about 8.4% within 30 days in clean grain stored without insect infestation and fungal infection under hermetic conditions.

Similar trials were carried out by Mutungi et al. (2014) on storage of mung bean and pigeon pea in hermetic triple-layer bags to determine losses caused by *Callosobruchus maculatus*

(F.). Oxygen and carbon dioxide concentrations in the PICS bags over the six-month period in both pulse types, oxygen levels dropped rapidly within the first two months.

Murdock and Baoua (2014) described the various features of the PICS technology by detailing the background of the development, mode of action, and need for future investigations. Murdock and Baoua (2014) speculated that there are at least sixteen different factors contribute to the effectiveness of PICS bags. The degree of contribution of each may be substantial or very small. Accordingly PICS bags stop bruchid populations from expanding and thereby prevent destructive losses. These triple bags seem to be more effective than might be expected based solely on the oxygen barriers presented by the two HDPE liners. Murdock and Baoua (2014) attributed the effectiveness of PICS to the triple-layer, and to the factors that contribute to that effectiveness which remain to be investigated in depth. Although Murdock and Baoua (2014) agree that some of their hypotheses are speculative, and yet should lend themselves to experimental tests.

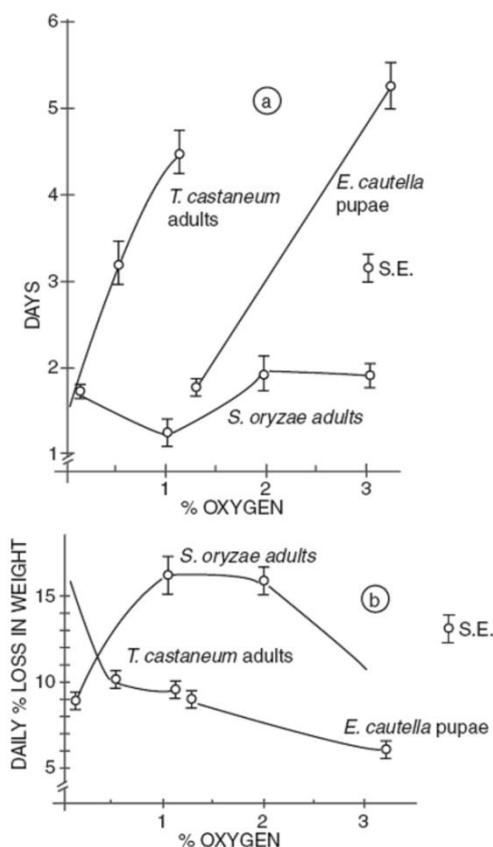
Baoua et al. (2014) tested the triple bag hermetic technology for post-harvest preservation of Bambara groundnut. They conducted experiments to evaluate the performance of hermetic triple bagging using PICS bags for storage of Bambara groundnut (*Vigna subterranea* (L.) Verdc.). One set of experiments used grain heavily infested by *C. maculatus* while a second set began with a low level infestation. Two to five days after the beginning of the experiments, oxygen level inside the bags averaged about 21% in the controls but decreased significantly in PICS bags, reaching 10% with the heavily infested grain but falling only slightly in the lightly infested grain.

Baoua et al. (2013) compared side by side the GrainPro and PICS bags for postharvest preservation of cowpea grain in Niger. They reported that when bruchid-infested cowpea was stored for more than four months in PICS bags or in commercially-available GrainPro SuperGrainBags, preservation of the grain was equally good in both types of bags. In both bag types oxygen (O<sub>2</sub>) levels dropped rapidly during the first 24 h after closure, eventually reaching levels of 1-3%. Over the four-plus months of the experiment damage levels did not significantly increase in either type of bag while control grain kept in a conventional woven plastic bag suffered severe damage. Most of the insects found in both GrainPro and PICS bags at the end of the experiment were dead. Surprisingly, in spite of the report that the single layer SuperGrainBags showed more bruchid holes than did triple-layer PICS bags, the O<sub>2</sub> level was lower and the CO<sub>2</sub> level was markedly higher in the SuperGrainBags than in the PICS bags.

Murdock et al. (2012) investigated the causes of bruchids death during hermetic storage of cowpea. They observed that when cowpea grain was stored in airtight containers, destructive populations of the cowpea bruchid (*C. maculatus*) don't develop even though the grain put into the store was already infested with sufficient *C. maculatus* to destroy the entire store within a few months. A great deal of data previously available on insect response to modified atmospheres and all in depth detailed laboratory and field studies carried out by previous workers were not cited by any of the above mentioned authors. It would seem that the behavior of bruchid beetles is significantly different than other stored product insect species. In addition one gets the impression that the effectiveness of hermetic storage for stored products has started with the investigations related to bruchids that attack pulses. It is therefore, important to mention the pioneering work of Bailey (1955, 1956, 1957) who observed that suppressed storage insect development was at about 5% O<sub>2</sub>, but the exposure time required to kill the insects was very long. Navarro (1978) showed significant differences in mortality of adults of *Tribolium castaneum* (Herbst) in nitrogen between 0.1 and 1.0 % O<sub>2</sub>. Mortality data with *Tribolium confusum* J. du Val in nitrogen (Jay and Pearman, 1971;

Shejbal et al., 1973; Tunc and Navarro, 1983) showed a critical oxygen level at 0.9% and that >1.4% O<sub>2</sub> was found to be ineffective.

Nitrogen is commonly used to produce a low oxygen atmosphere that causes anoxia. Nitrogen causes a progressive hypoxia or anoxia and generally the lower the oxygen level the higher is the mortality. For effective control, the O<sub>2</sub> level should be <3%, and preferably <1%, if a rapid kill is required (Banks and Annis, 1990; Fleurat Lessard, 1990; Navarro, 1978; Adler et al., 2000) (Fig. 1).



**Figure 1** Relationship between oxygen concentration and the time required for 95% mortality (a), and the effect on daily percent loss in weight (b) of three stored-product insects at 54% RH and 26°C. (Redrawn, from Navarro (1978))

Adults of the grain mite, *Acarus siro* L. were exposed to various O<sub>2</sub> atmospheres in nitrogen. The exposure time to obtain complete mortality at 2% O<sub>2</sub> was 48 h and 72 h at temperatures of 26 and 15°C, respectively (Navarro et al., 1985).

The important role of low O<sub>2</sub> concentration rather than high CO<sub>2</sub> in causing mortality of stored-product insects in hermetic storage was demonstrated by Bailey (1965). Only later was the importance of the synergistic effect of concomitant O<sub>2</sub> depletion and CO<sub>2</sub> accumulation for insect control clearly demonstrated (Calderon and Navarro, 1979, 1980; Donahaye et al., 1996). These synergistic and combined effects are essential for successful insect control, as shown by studies of the effects of incomplete air-tightness upon insect populations (Oxley and Wickenden, 1963; Burrell, 1968). To demonstrate the effects of incomplete air tightness Navarro et al. (1994) modeled a fixed O<sub>2</sub> ingress rate equivalent to about 0.24%/day for a structure with a volume of 10 m<sup>3</sup>. Accordingly a cyclic change in concentrations is obtained as a result of O<sub>2</sub> ingress and the ability of insects to survive at low O<sub>2</sub> levels. These theoretical

cyclic changes in O<sub>2</sub> concentrations are also observed in different laboratory and field studies (Navarro et al., 1984, 1994).

### 3. Effects of combinations of low oxygen and high carbon dioxide

Researchers have been interested in increasing the efficacy of MA on insects by attempting to combine very low oxygen in combination with very high carbon dioxide concentrations. However, increasing the carbon dioxide concentration in the normal atmosphere reduces proportionally the partial pressure of the oxygen available to insects. Gas burners or fossil fuel burners also have the capability to generate a combination low in oxygen and high in carbon dioxide. For example, a typical propane burner would produce an atmosphere of 0.5% oxygen, 13.5% carbon dioxide, 1% nitrogen and 1% argon. Therefore, unless a mixture of nitrogen and carbon dioxide or a gas burner atmosphere is used, the simplest way to achieve a low oxygen and high carbon dioxide atmosphere is by using carbon dioxide in air (Storey, 1975).

In the case of hypoxia (2 to 5% O<sub>2</sub>), when a small proportion of CO<sub>2</sub> (5 to 40% CO<sub>2</sub>) is added to the initial mixture of N<sub>2</sub>/O<sub>2</sub>, the mortality rate increases considerably (Calderon and Navarro, 1979). When CO<sub>2</sub> is added to low O<sub>2</sub> atmospheres, there is a synergistic effect, which is obvious from the significant interaction between the concentrations of these two gases (Calderon and Navarro, 1980).

Krishnamurthy et al. (1986) tested the response of adults of *Sitophilus granarius* (L.), *T. castaneum*, *Oryzaephilus surinamensis* (L.), *Cryptolestes ferrugineus* (Stephens) and *Rhyzopertha dominica* (F.) exposed to simulated combusted atmospheres containing low O<sub>2</sub> (0.5-2.6%) and increased CO<sub>2</sub> (10-30%) with the balance N<sub>2</sub> at 20°C and 70% r.h. The mixtures containing 1-1.6% O<sub>2</sub> killed all the insects within 7 days if they also contained greater than or equal 10% CO<sub>2</sub>. At 2.0-2.6% O<sub>2</sub> *C. ferrugineus* was more tolerant of CO<sub>2</sub> than the other insects tested, whereas at 0.5% O<sub>2</sub> *S. granarius* was the most tolerant species, needing 8-10 days for complete kill.

Conyers and Bell (2007) carried out laboratory tests on five species of stored-product beetles, *C. ferrugineus*, *O. surinamensis*, *S. granarius*, *S. oryzae* and *T. castaneum* to MAs based on simulated burner gas, four different O<sub>2</sub> levels, 3, 4, 5 and 6%, with CO<sub>2</sub> levels of 9.5, 8.5, 7.5 and 6.5%, respectively at 20 and 25°C, 75 and 85% r.h. After exposure to the MAs for 28 d an assessment was made of the mortality of adults, the number of adults from progeny produced under the MAs and, for the simulated burner gas, the number of adults from progeny produced in a 28-d period after exposure to the MA. The O<sub>2</sub> content preventing population growth varied with species and temperature. For simulated burner gas or N<sub>2</sub> it was about 4% for *O. surinamensis*, *S. granarius* and *S. oryzae*, and about 3% for *C. ferrugineus* and *T. castaneum* at 25°C. At 20°C it was about 3% for all species tested. When CO<sub>2</sub> was increased to 10 or 20%, reducing O<sub>2</sub> to 5% was sufficient to eliminate emergence of *S. granarius* at 20°C, but a few individuals emerged at 25°C. For *C. ferrugineus* there was a 95% reduction with 5% O<sub>2</sub> plus 20% CO<sub>2</sub> at 20°C, but not at 25°C.

### 4. Effect of air relative humidity on desiccation and insect mortality in hermetic storage

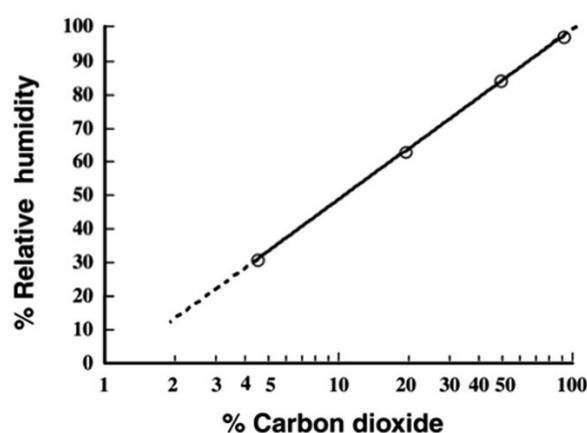
Murdock et al. (2012) have reported that the cause of death of cowpea bruchids under hermetic storage being desiccation resulting from an inadequate supply of water. According to Murdock et al. (2012) when water supply is blocked by lack of oxygen, the insects gradually desiccate and die as they continue to respire, albeit slowly. Although the statement that the reason of death under hermetic storage by desiccation seems corroborated by other

investigators, the relation of lack of oxygen under the relative humidity that prevails in grain storage and desiccations has not been considered by Murdock et al. (2012).

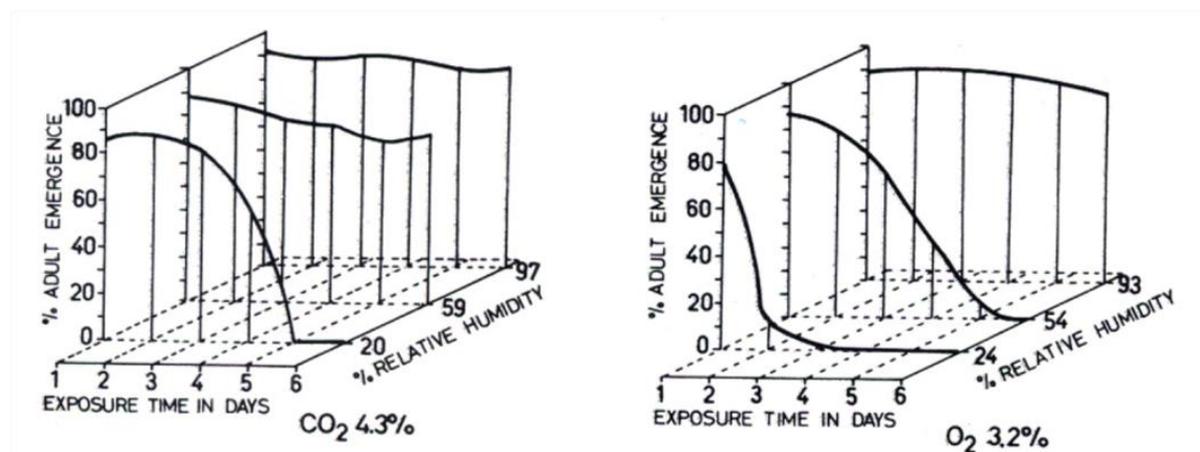
Conversely, previous works have elaborated the cause of death as a complex metabolic response in relation to the relative humidity of the storage environment. Although there are evidences that low humidity environments are more effective in causing mortality (Navarro and Calderon, 1973, 1974; Navarro, 1975) and at low humidity the lethal effects are attributed to desiccation rather than the toxic action of the MAs (Navarro, 1978), the inability of insects to recover after treatment was also attributed to the lack of sufficient triglycerides as substrates for energy metabolism (Friedlander and Navarro, 1978, 1979; Navarro and Friedlander, 1974; Donahay, 1991).

Laboratory studies have shown that lowering the relative humidity increases the effectiveness of modified atmospheres. Jay et al. (1971), working with adults of *T. confusum*, *T. castaneum*, and *O. surinamensis*, found that decreasing the relative humidity in atmospheres containing 99% N<sub>2</sub> (balance O<sub>2</sub>) from 68% r.h. to 9% r.h. gave an increase in mortality of from 3 to 98.5% in 24 h exposure of the red flour beetle. The two other insects showed a similar response to reduced relative humidity. These three species also exhibited a similar response to mixtures of CO<sub>2</sub> in air at lowered relative humidities.

Desiccation plays a large role in the mortality of stored product insects when exposed to some modified atmospheres. Jay and Cuff (1981) showed that when larvae, pupa, and adults of the red flour beetle were exposed to varying concentrations of CO<sub>2</sub> or O<sub>2</sub>, weight loss was much higher in some of the atmospheres than in others or in air. Navarro and Calderon (1974) showed a linear relationship of the combined effect of CO<sub>2</sub> and relative humidity in producing a lethal environment for *Ephestia cautella* (Wlk.) pupae (Fig. 2). Navarro and Calderon (1980) demonstrated also the strong dependence of low concentrations of CO<sub>2</sub> and O<sub>2</sub> on causing mortality in *E. cautella* pupae (Fig. 3). Other laboratory studies have shown that the susceptibility of different species or strains of the same species vary considerably when insects are exposed to the same concentrations of modified atmospheres (Jay and Pearman, 1971).



**Figure 2** The combined effect of carbon dioxide and relative humidity on the time needed to produce 95% mortality values for *Ephestia cautella* pupae after four days of exposure. (Redrawn, from Navarro and Calderon (1974))



**Figure 3** Effect of 4.3% carbon dioxide and 3.2% oxygen on adult emergence from *Ephestia cautella* pupae exposed to different relative humidities at 26°C. (Redrawn, from Navarro and Calderon (1980))

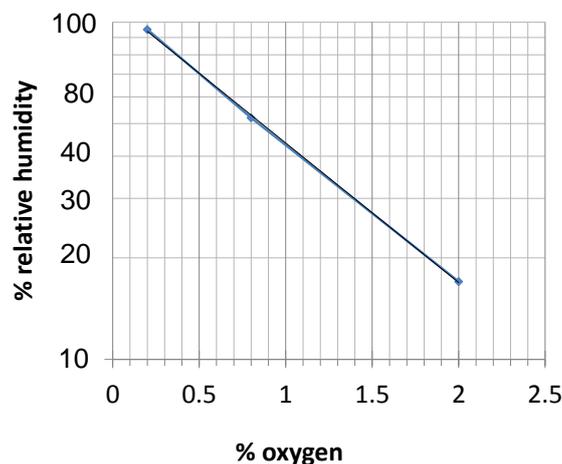
The tested relative humidity in their work (Chiappini et al., 2009) was sufficiently low (<18%) to result in complete mortality after 120 h at 3% O<sub>2</sub> and 29°C. Whereas, Calderon and Navarro (1979) demonstrated that after 120 h to 2% O<sub>2</sub> and 30°C at 57% r.h. mortality of *T. castaneum* adults was only 50%. To prevent commodity deterioration due to high humidity that favor microflora activity, the ambient relative humidity of storage should be below 65% and preferably in the range of 50 to 60% r.h. that is considered safe storage (Navarro and Donahaye, 2005). Storage insects keep their water reserves by opening their spiracles for a short duration open to permit expelling carbon dioxide and intake of oxygen. In this way storage insects survive the low humidity ambient by maintaining a balance between the metabolic water they are able to produce and the loss of water. However, when the insect storage environment is depleted of oxygen (at about 2-3%) (Navarro, 1975, 1978; Navarro and Calderon, 1980) (Fig. 4) or at slightly elevated carbon dioxide of 4.3% (Navarro and Calderon, 1980) at a low relative humidity environment, insect's water deficit is significantly increased. The role of the relative humidity in the storage environment in causing water loss was detailed by Navarro (1975, 1978), and Navarro and Calderon (1980). This water loss is accompanied by the inability of storage insects to compensate for the water loss and thereby, they die due to desiccation. In the presence of sufficient humidity in the environment, insects survive until anoxia or hypercarbia becomes the dominant factor in causing insect mortality (Navarro, 2006) (Fig. 4).

### 5. Effects of temperature and hermetic storage on insects

At temperatures from 20 to 30°C, most species and developmental stages show >95% mortality in <10 days at both 0 and 1.0% O<sub>2</sub> (Annis, 1987). *Trogoderma granarium* Everts larvae (12 d at 0% O<sub>2</sub>), *S. oryzae* pupae (20 days at 0% O<sub>2</sub>; >14 d at 1% O<sub>2</sub>), and *S. granarius* adults (16 days at 1% O<sub>2</sub> are the only exceptions so far found (Annis, 1987).

The effect of temperature on the length of time necessary to obtain good control with modified atmospheres is as important as with conventional fumigants. Jay (1971) states that to obtain good control; "the temperature of the grain should be above 21°C during the application of CO<sub>2</sub>". Navarro and Calderon (1980) compared the effect of temperature on exposure time required to produce mortality of adults of three storage insects to MAs. Chiappini et al. (2009) aimed to evaluate the time necessary to obtain total mortality of insects treated in controlled atmospheres with O<sub>2</sub> percentages higher than those normally used in

practice. They estimated the possible positive influence of a temperature increase in order to compensate for the effects of the reduced anoxia by exposing *T. confusum* adults to various range of O<sub>2</sub> percentages (1 to 10%) and temperatures (23 to 40°C). Their results showed that total mortality could be achieved within a week, even in quite moderate conditions of temperature (29–37°C) and lowered O<sub>2</sub> percentage (5–8%). They demonstrated that the total mortality achieved in the various exposure periods was due to the effect of the two parameters (temperature and O<sub>2</sub> percentage) together.



**Figure 4** The combined effect of oxygen and relative humidity producing 95% mortality for *Tribolium castaneum* (Herbst) adults after 4 days exposure at 26°C. (Redrawn, from Navarro (1975))

Donahaye et al. (1994) reported on responses of, larval, pupal, and adult stages of the Nitidulid beetles *Carpophilus hemipterus* (L.) and *Urophorus humeralis* (F.) exposed to simulated burner-gas concentrations at three temperatures of 26, 30, and 35°C. Comparison of exposure times showed that the effect of temperature on treatment efficacy was most pronounced at the 1% O<sub>2</sub> level where for the three stages of both species tested, values of LT<sub>50</sub> at 26°C were about half those at 35°C. However, at 3% O<sub>2</sub> and 35°C, LT<sub>50</sub> levels were only marginally reduced (Donahaye et al. 1994).

Soderstrom et al. (1992) examined the influence of temperature over the range of 38 to 42°C on the influence of hypoxia and hypercarbia on *T. castaneum* adults for 60 h exposures. Although the different experimental conditions make comparison difficult, their results clearly indicate that raised temperatures could be used to reduce treatment duration. Donahaye et al. (1996) exposed egg, larvae, pupae and adults of *T. castaneum* to three low oxygen concentrations at 26, 30 and 35°C. At all levels of O<sub>2</sub> (1, 2 and 3%) in typical respiration atmospheres in hermetic conditions (similar to burner gas atmospheres) the LT<sub>99</sub> values at 35°C were significantly lower than at 26°C. Work on all four development stages of *E. cautella* showed the strong influence of temperature on mortality values when the insects were exposed to CO<sub>2</sub> concentrations varying from 60 to 90% in air (Navarro et al., 2002, 2003).

Downes et al. (2008) studied the effect of changing environmental conditions on the metabolic heat rate (MHR) of *S. oryzae* and *T. castaneum* with the attempt to demonstrate that heat treatments for disinfestation performed in a hypoxic or hypercarbic CA may be effective at lower temperatures than required in air. The MHR of adult *S. oryzae* was changed little over a wide range of oxygen concentrations, the value in air was close to that in pure oxygen and was only reduced by about 20 and 50% at oxygen concentrations of 5 and 2%, respectively. Heat treatment at 45°C was more effective when the CA was 5% O<sub>2</sub>+60%

CO<sub>2</sub>+N<sub>2</sub>, or 60% CO<sub>2</sub>+N<sub>2</sub>, than in air, but larval *T. confusum* still survived in the former CA, indicating there were differences in the response between species and/or stage.

Mitcham (2007) reviewed the response of insects to high temperature during exposure to controlled atmospheres. The author noted that, in general, the higher the temperature, the faster mortality is achieved under a given atmosphere.

## **6. Contribution of microflora in generating a lethal atmosphere**

Significant biological agents that may develop in grain are molds, yeasts and bacteria named under the general name of microflora. Molds are mostly aerobic organisms that require oxygen for their development. Reduction of the oxygen tension in a bag containing grain may be possible by the natural consumption of oxygen by the microflora.

Elepano and Navarro (2008) studied hermetic storage of high moisture corn (maize) under tropical conditions. They used shelled corn of 26% m.c. stored in a Cocoon™ under hermetic conditions for 96 days to demonstrate the effectiveness of maintaining its quality prior to subsequent drying or processing. The Cocoon™ was made of a plastic liner and capable of holding 11 tonnes of corn stored in standard bags. Corn was sampled before and after storage for determination of m.c., starch, ethanol, aflatoxin, and germination rate. The initial corn temperature in the Cocoon™ reached 45°C and then equilibrated with that of the ambient at 30°C after the first week of hermetic storage. The initial oxygen concentration dropped within one day and remained at an average of 0.54% throughout the storage period. Average m.c. at the end of storage increased to 29%. Corn in the control bags deteriorated after three days and temperature increased to 55°C. The high moisture corn in the Cocoon™ initially had 59 ppb of aflatoxin due to a logistical delay of about 3 days for acquiring the corn from several farmers. Aflatoxin level increased to 90 ppb after one week of storage and remained at that level for 96 days. No presence of insects was observed in the corn samples stored in the Cocoon™. Feeding trials indicated that the corn from hermetic storage was palatable to cows and swine. Results of the study indicate that wet corn can be safely stored for extended periods of time without significant increase in aflatoxin, and without significant changes in starch and ethanol content. These studies indicate that if sufficient gastightness is achievable, a lethal concentration of modified atmosphere can control insect populations and prevent toxigenic fungi development under hermetic conditions.

## **7. Response of the structure that makes the storage hermetic to air ingress rate**

Sanon et al. (2011) studied the effect of triple-bagging of cowpeas within high density polyethylene bags to control the cowpea beetle *C. maculatus*. In the laboratory bruchids numbers and seed damage were significantly reduced when storing cowpeas within 2 layers High Density Polyethylene (HDPE) bags of at least 80 mm wall thicknesses. This thickness considerably reduced oxygen concentration in the bag after 5 days of storage and inhibited insect development.

Baoua et al. (2012) tested the performance of triple bagging hermetic technology for postharvest storage of cowpea grain in Niger. In these PICS bags the O<sub>2</sub> levels within the bags initially fell to about 3% while the CO<sub>2</sub> rose to nearly 5%. After five months of storage, new and used 50 kg bags and new 100 kg bags preserved the grain equally well. There were greatly reduced numbers of adults and larvae in the PICS bags versus the controls, which consisted of grain stored in single layer woven bags.

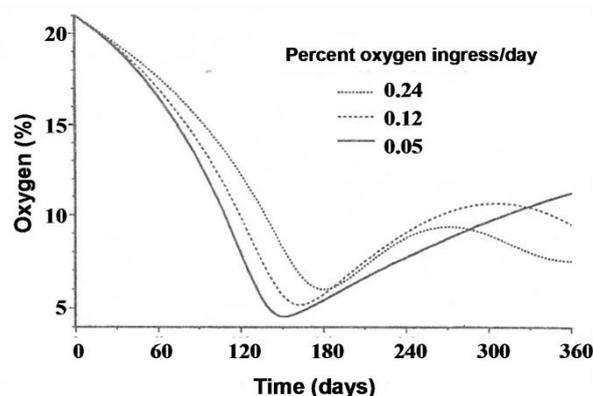
Baoua et al. (2013) in their comparison of side by side the GrainPro and PICS bags for postharvest preservation of cowpea grain in Niger they discovered that the single layer SuperGrain™ bags showed more bruchid holes than did triple-layer PICS bags. According to

them PICS bags had no holes penetrating through both of the inner high density polyethylene (HDPE) layers, meaning that an intact O<sub>2</sub> barrier remained in place even after the inner HDPE layer was holed.

Njoroge et al. (2014) were able to monitor oxygen and carbon dioxide conditions prevailing in PICS bags containing maize infested with *P. truncatus*. Although oxygen concentration dropped steadily and carbon dioxide concentration, increased gradually the nature of the liners to permeability and gas tightness level were not reported. The key to successful hermetic storage is air tightness and control of condensation. In modern times, storage size has increased from small family storages to large bulks representing many producers or a portion of a country's total production.

Since it is difficult to maintain complete gas tightness without any O<sub>2</sub> ingress into the large commercial structures, some tolerances that would permit quality preservation of the grain during hermetic storage were established. We need to consider that the main cause of deterioration of dry grain is insects. While the main cause of deterioration of moist grain is microflora. The grain responds differently in the ecosystem of storage when it is at intermediate moisture but close to the critical level where fungi is the dominant microflora. While at higher moisture levels the dominant microflora are mostly yeasts and bacteria. Therefore, hermetic storage may be addressed to dry grain or moist grain storage.

For the application of hermetic storage to dry grain, an ingress rate of 0.05%O<sub>2</sub>/day is sufficient to arrest the theoretical weight loss, caused by insects or microflora, at a level of 0.018% over one year storage period (Navarro et al., 1994). For dry grain storage, this level is critical since even at short storage periods of 3 to 6 months at this ingress rate, the possibility of a residual surviving insect population is eliminated at an economical threshold. For higher O<sub>2</sub> ingress rates, at temperature that permits activity of biological agents, the weight loss continues to rise in proportion to the O<sub>2</sub> ingress rate and insect damage might be very significant and cannot be arrested. Ingress rates of up to 0.15%O<sub>2</sub>/day can be tolerated (Fig. 5). However, for moist grain, at higher O<sub>2</sub> ingress rates than 0.15%/day, permits grain deterioration that might lead to development of mycotoxins (Weinberg et al., 2008). This low O<sub>2</sub> ingress level, is difficult to obtain in rigid structures, but is achievable in practice using flexible liners. It could serve as a guideline for the sealing specifications of structures appropriate to the hermetic storage method.



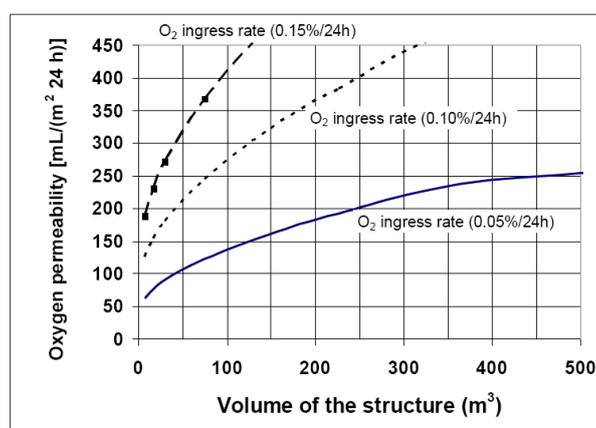
**Figure 5** Calculated oxygen concentrations in a 10-m<sup>3</sup> grain mass containing a fixed level of initial infestation of 2 insects/kg, having an oxygen intake of 157 µL per insect per day, using a sealed liner at different oxygen ingress rates. (adapted, from Navarro et al. (1994))

Experience shows that hermetic storage works best for large structures. This is obvious from the lower surface area/volume ratio in large bulks compared with small bulks. The factor of O<sub>2</sub> ingress rate, in practice is a goal difficult to achieve. Therefore, depending on the commercially available membrane permeability, engineers should aim at designing hermetic structures of sufficiently large dimensions. To emphasize the importance of the size of the structure in hermetic storage, calculations were made assuming a permeability level of 200 mL O<sub>2</sub>/(m<sup>2</sup> 24 h) for structures of different dimensions ranging from 1 to 1,000 m<sup>3</sup> (Navarro et al., 1994). The calculations demonstrate the importance that low-permeability liners must be preferred for hermetic storage of small scale storages like individual bags.

The air-tightness of the bags can be negatively affected by improper sealing of the end of bag and perforations in the plastic cover. The use of pressure test (or pressure drop test) is one of the methods to evaluate the level of air-tightness of a storage structure (Navarro, 1998). Membranes of plastic permit gas permeation and gas exchange. Pressure tests, are not capable of measuring the degree of permeability losses.

Navarro (2012) analyzed O<sub>2</sub> ingress levels that are achievable in practice using flexible liners. The O<sub>2</sub> ingress levels shown in Figure 6 could serve as a guideline for O<sub>2</sub> permeability specifications of flexible liners appropriate to the hermetic storage method. For small volumes, such as bag size hermetic storage structures, a low permeability to O<sub>2</sub> is essential and for large volumes higher permeability levels can be tolerated. To exemplify such tolerances Figure 6 was prepared that clearly shows the importance of selecting extremely low O<sub>2</sub> permeability liners when using small hermetic storage units. According to Fig. 6 hermetic storage structures with capacities similar to bag size (about 100 L) would require liners of a permeability level of <50 mL O<sub>2</sub>/(m<sup>2</sup> day) for ingress rate of 0.05% O<sub>2</sub>/day.

The O<sub>2</sub> ingress levels shown in Figure 6, could serve as a guideline for the sealing specifications of structures appropriate to the hermetic storage method. Flexible structures with higher O<sub>2</sub> ingress rates than 0.15% O<sub>2</sub>/day, may be used to protect the grain from rain or increase of moisture, provided the grain is dry and without any infestation. The question is whether these structures should be considered under the term of “hermetic storage” or just simply “sealed storage” without the expectations that they will develop a biogenerated atmosphere to protect the grain and use fumigation to control the insects.



**Figure 6** Oxygen permeability requirements [mL/(m<sup>2</sup> 24h)] of liners in relation to various storage capacities (m<sup>3</sup>) and oxygen ingress rates (%/24h) for successful application of hermetic storage of dry grain. (adapted, from Navarro (2012))

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