Spring 2014

Quality Changes in Hermetically Stored Corn Caused by Fungi and Sitophilus Zeamais

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By Nda-Agyima K. Addae-Mensah

Entitled
QUALITY CHANGES IN HERMETICALLY STORED CORN CAUSED BY FUNGI AND SITOPHILUS ZEAMAIS

For the degree of Master of Science in Agricultural and Biological Engineering

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QUALITY CHANGES IN HERMETICALLY STORED CORN CAUSED BY FUNGI AND SITOPHILUS ZEAMAIS

A Thesis

Submitted to the Faculty

of

Purdue University

by

Nda-Agyima K Addae-Mensah

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science in Agricultural and Biological Engineering

May 2014

Purdue University

West Lafayette, Indiana

This thesis is dedicated to the women in my life, my mom, Dr. E. G. Addae-Mensah, M.D, my wife Dr. E. Addae-Mensah, PhD and my twin sister Dr. A. Boateng, M.D. Without their support and encouragement I could not have come this far.

ACKNOWLEDGEMENTS

My utmost gratitude goes to the Almighty God for seeing me through another milestone.in my academic pursuit which is only a stepping stone for greater things to come. His guidance and faith in me carried me through many obstacles throughout my time at Purdue. I am very grateful to my mom, wife and sister who have continued to provide me inspiration, encouragement, advice and prayers throughout my stay at Purdue. God richly bless you. I would also like to thank my father A. Addae-Mensah, PhD whose belief in me was very critical to completing this hurdle in my life. I would also like to thank my son Patrick for allowing me to work for hours in the laboratory without seeing him. I can certainly not leave out my brother S. Addae-Mensah, J.D who continues to support me.

I would also like to thank Dr. Linda Mason who not only made me use her lab and introduced me to the world of stored grain insects. I am truly grateful for her superior mentorship and her supportive approach to resolving research difficulties. I will like to thank Dr. K. Ileleji for his criticisms, engagements and allowing me to use his laboratory to carry out research. I will like to extend my sincere gratitude to Dr. R. Stroshine who gave me words of encouragements, provided suggestions and also made his research laboratory available to me. Also thanks go to Dr. C. Woloshuk who introduced me to a lot of fungi and mycotxin materials and also allowed me to use his laboratory. I also

would like to thank Judith Santini from the department of Agronomy, Dr. B. Craig, who provided me with over twenty hours of statistical consultation and training for my research. My thanks also goes to my uncle Dr. I. Addae-Mensah, PhD for providing me with multiple approaches to conduct my research and also providing me with meteorological data.

I will also like to thank Mr. Hipp, Purdue University Travel grant program and also Dr. B. Engel, head of the department of Agricultural and Biological Engineering for the financial contributions they made to my successful completion at Purdue University. Finally I would give thanks to everyone in my research group including for all the support they have given me while during my time at Purdue.

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ABSTRACT

Addae-Mensah, Nda-Agyima K.. M.S.A.B.E., Purdue University, May 2014. Quality Changes in Hermetic Stored Corn caused by Fungi and Sitophilus zeamais (Motschulsky). Major Professors: Klein Ileleji and Linda Mason.

Hermetic storage has been shown to be effective in controlling insect pests and maintaining grain quality of dry grains at 13% moisture and below. However, the feasibility and use of hermetic storage for grains at intermediate mid-moisture levels under the influence of the sub-Saharan African weather conditions is relatively unknown. Hermetic storage experiments were conducted on grade 1 "6297 and 6333 VT RIB" hybrid corn under controlled temperature conditions at 10°C and 25°C at target approximate moisture content levels of 11, 15, 18 and 21% wet basis for a total storage period of 6 months. Corn quality was evaluated by using mold counts, aflatoxin levels, free fatty acids, germination, gas composition, and nutritional composition. The results showed that hermetic storage is effective at suppressing mold growth, minimizing aflatoxins levels and maintaining the nutritional content of corn stored at 11 and 15% MC w.b. Non-hermetic storage was better at preserving germination. Mold count, aflatoxin, and free fatty acids generally increased with temperature, moisture content and storage time.

Germination had an inverse relationship between these variables for both hermetic and non-hermetic storage. Nutritional content such as protein, starch and oil decreased

with storage time across storage types but remained unchanged in hermetic storage. Mold growth was accompanied by a high temperature rise due the difference between the storage medium and ambient conditions. This difference was highly pronounced at high initial moisture contents (21%) of corn stored at 25°C. Hermetic storage was superior to non-hermetic storage in attaining insect mortality and suppressing insect damage before and after emergence. Hermetic storage was also superior to non-hermetic storage in suppressing insect emergence. However, this relationship was also modulated by the moisture content of the corn.

CHAPTER 1. INTRODUCTION

This MS thesis research focuses on the changes in quality (seed germination, mold growth, fat acidity, aflatoxin contamination, chemical composition, insect damage and insect pest population) in hermetic (air-tight) stored corn under three different moisture content levels (11, 15, 18 and 21%) stored at a temperate (10^{o}C) and sub-tropical (25^{o}C) condition. The thesis is in partial fulfillment for an MS (thesis option) in the Agricultural and Biological Engineering at Purdue University, West Lafayette, Indiana. Drying corn (maize), a major staple in sub-Saharan Africa, from harvest moisture (about 30%) to safe storage moistures (below 13%), is one of the primary challenges that cause huge post-harvest losses (PHL) of smallholder farmers in the humid tropics such as Ghana. Additionally, smallholders access to supply lucrative value-added markets with corn are closed because of the high levels of aflatoxin caused by storage fungi prevalent in the region. The study investigated corn quality changes due to mold (fungi) and the effect of insect infestation (*Sitophilus zeamais*) in hermetically stored corn. The moisture levels investigated were low moisture (11%) and intermediate moisture (15%, 18% and 21%) corn stored hermetically for up to 6 months to study the effect of fungi deterioration on quality (germination, fat acidity, mold count, aflatoxin and chemical composition). The second study investigated *Sitophilus zeamais* mortality and

progeny suppression in hermetically stored corn at 11%, 15%, 18% and 21% and the effect of *Sitophilus zeamais* on insect damaged kernels.

1.1 Problem Statement

In the tropics especially across the sub-Saharan African (SSA), corn is regarded as a common staple crop used mainly for human consumption and livestock feed with only a small fraction stored for seed. Most often corn is only stored in small quantities of several bags (100 kg per bag) from an average farm size of 2 ha per household. Due to high humidity conditions and limited sunshine hours during rainy season which coincides with time of harvest, most smallholders are not able to dry down their corn to safe moisture content levels of 13% mc or below, especially south of the Sahelian region. Corn harvested at high moisture levels (~30% mc) is most likely dried to between 15% to 21% moisture using open air sun drying; the primary means of drying in this region. Because of the warm temperatures, which can reach as high at 30^{o}C, corn stored at these intermediate moisture levels in the humid tropics cannot be stored for long before spoilage sets in. Successful results from the Purdue Improved Cowpea Storage (PICS) bags in suppressing Bruchids infestation and damage in cowpea (Murdock et al. 2012; Baoua et al. 2012) and economic and financial benefits (Jones et al. 2011), there has been a surge in interest in the use of hermetic bags for other types of grain. PICS is a triple bag hermetic storage system consisting of an outer woven polyethylene bag and two inner 80µ high-density polyethylene bags that has been rigorously tested for cowpea storage in SSA. A study by Jones et al. (2011) have also showed that high profits with the PICS bags were evident in regions such as Ghana, Tanzania and Malawi to name a few and that

these regions showed increasing potential for their use. While it is advisable to use hermetic technology for storing corn only after it has been effectively dried down to 13% mc or below, there are limited studies to indicate that corn can be safely stored hermetically at the intermediate moisture levels achieved by farmers under the warm weather of the humid tropics. Achieving low moisture levels below 13% with open-air drying during the harvest season is often not possible in the humid tropics.

Challenges with the quality of stored corn are not only manifested in the formation of mold but can also cause the production of aflatoxins, reduction in grade and subsequent value, insect infestation and germination. Mold development in hermetic storage depends on a number of factors including the length of storage time, moisture content levels, storage temperature, insect infestation and the presence of broken corn and foreign material. Understanding how these factors and their interaction affect quality changes in hermetically stored corn is the major focus of this research (See Figure 1.1).

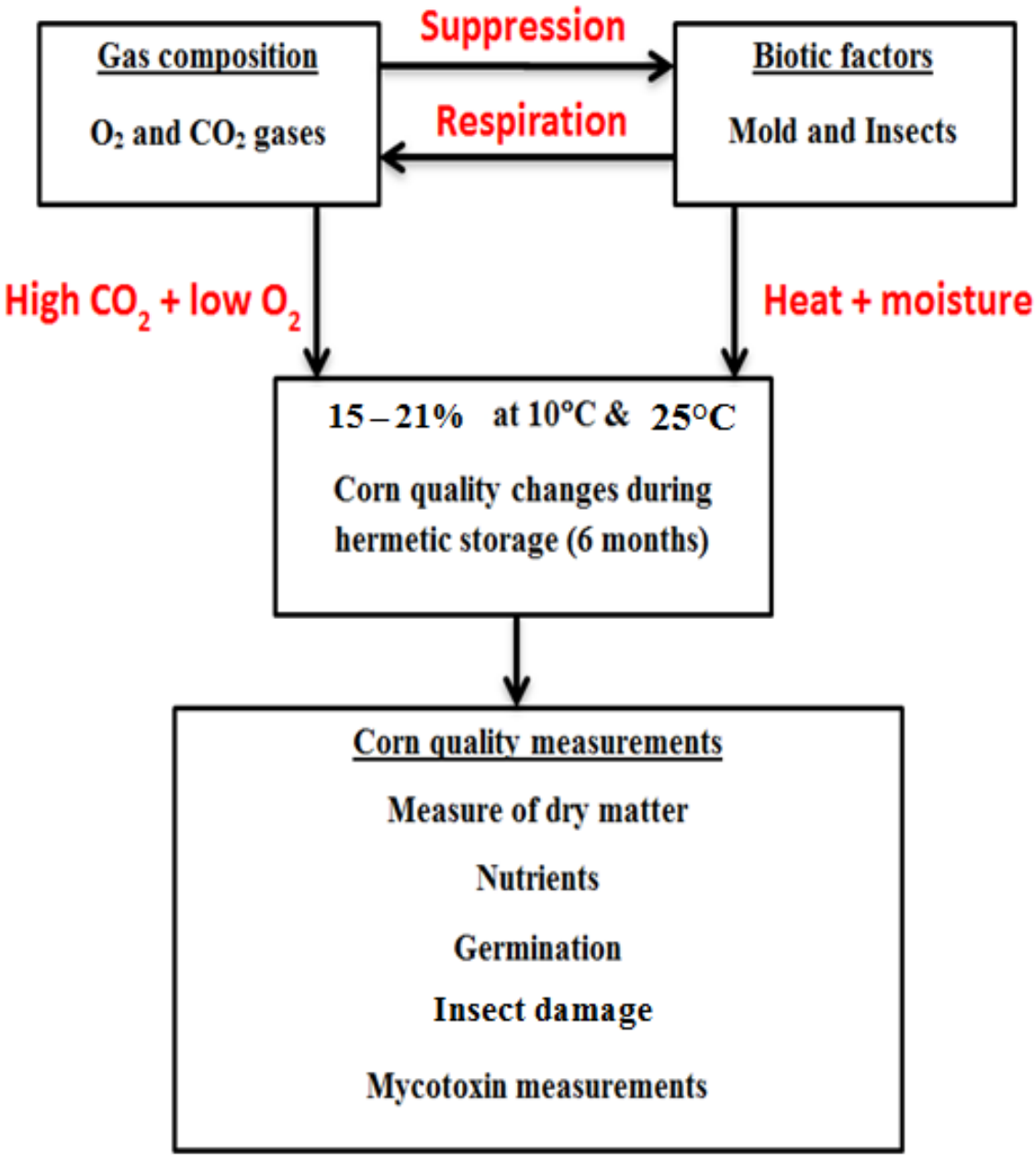

Figure 1:1 Schematic diagram of research problem

1.2 Research hypothesis and goals

Grains, especially maize and sorghum grown in the major season in the humid tropics of SSA are normally harvested in humid rainy weather conditions when there is

limited sunshine hours for drying. Most grain harvested at 30% mc or more cannot be properly dried to safe moisture levels below 13.5% mc, and thus may go into storage at intermediate moisture levels between 15 and 21%. The literature shows that intermediate to high moisture levels of corn stored under aerobic conditions have a higher invasion by mold compared to corn stored under hermetic conditions (Moreno-Martinez et al., 2000). Additionally, the rate of mold growth tends to decrease over storage time under hermetic conditions (Weinberg et al., 2008).

This research work seeks to address gaps in knowledge regarding corn quality changes (loss) such as mold growth and aflatoxin development in hermetic storage at moisture contents ranging from 15 to 21% mc stored under cool and warm conditions for various storage times. What we do know is that hermetic storage has been successful in storing cowpeas at moisture levels of 13% and below and is also very effective in suppressing insect infestation in areas across the sahel region (areas encompassing the Sahara desert). (Navarro et al.1994; Yakubu et al. 2010). However there is limited knowledge for hermetically stored corn at intermediate moisture levels (11% to 21%) at both cool and warm temperatures with the warm temperature being of particular interest. It is hypothesized that the rate of mold growth, levels of aflatoxins and insect damage will be lower when corn is stored hermetically at moistures of 11%, 15%, 18% and 21% at 25^{o}C than when stored aerobically.

1.3 Specific objectives

Specific objectives of this study were to:

1. Investigate the quality changes due to fungi deterioration of corn stored hermetically at 11%, 15%, 18% and 21% moisture levels under 10^{o}C and 25^{o}C indicated by kernel germination, mold count, fat acidity, aflatoxin contamination and corn chemical composition
2. Investigate *Sitophilus zeamais* (maize weevil) control in corn stored hermetically at 11%, 15%, 18% and 21% under 25^{o}C indicated by mortality, progeny development and insect damaged kernels.
3. Develop an Extension publication for use in training agricultural extension agents based on results from Objective 1 and 2

1.4 Thesis outline

The research thesis comprises of six chapters. The introduction and literature review are Chapters 1 and 2, respectively. Chapters 3, 4 and 5 consist of Objectives 1, 2 and 3, respectively. Chapter 6 is the conclusion and recommendations for future work.

1.5 References

Baoua, I. B., V. Margam, L. Amadou, L.L. Murdock (2012). Performance of tripple bagging technology for postharvest storage of cowpea grain in Niger. Journal of Stored Products Research 10/2012; 51:81-85.

Murdock, L. L., Magam, V., Baoua, I., Baffe, S., Shade, R. E. (2012). Death by desiccation: Effects of hermetic storage on cowpea bruchids Journal of Stored Products Research 49:166-170

Navarro, S., Donahaye, E., & Fishman, S. (1994, April). The future of hermetic storage of dry grains in tropical and subtropical climates. In *Proceedings of 6th International Working Conference on Stored Product Protection. CAB International, Wallingford, UK* (pp. 130-138).

Jones, M., Alexander, C., & Lowenberg-DeBoer, J. (2011). An initial investigation of the potential for hermetic purdue improved crop storage (PICS) bags to improve incomes for maize producers in sub-saharan africa (p. 55).Working paper 11-3.

Yakubu, A., Bern, C., Coats, J., & Bailey, T. (2010). Non-chemical on-farm hermetic maize storage in east Africa. *Julius-Kühn-Archiv*(425), S. 338

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CHAPTER 2. LITERATURE REVIEW

2.1 The corn crop

Corn (Zea mays L.), a food crop introduced in western and eastern Africa in the 16th and 17th century by the Portuguese is widely consumed on the African continent (FAO, 1994). Corn is the most widely consumed staple food in sub-Saharan Africa (SSA). It serves about 50% of Sub-Saharan Africans (IITA, 2010) with over 1.2 billion people in Africa consuming this crop as food. Other applications of the crop include use as animal feed, processed food, flour, and sweeteners. In SSA, the grain, leaves, stalk, tassel and cob of corn have economic values for food and non-food consumption (IITA, 2007). The corn crop is processed into many dishes depending on location and ethnicity, and accounts for 30-50% of the daily food consumed by people in low-income families in SSA (FAO, 2001). Corn is highly regarded due to its nutritional value. It contains vitamin A, C, E, protein, calcium and phosphorus (FAO, 1994).

2.1.1 Physical and thermal properties of corn

In handling and processing of grains having a good understanding of the physical and chemical properties is a necessity. Table 2.1 and 2.2 show these properties for corn. The reason why the physical and thermal properties should be understood because, for

example the bulk density determines volume of the storage medium required to store a desired amount of grain and the rate at which grain flows in grain dryers. Thousand-kernel weight is directly related to the drying rate for grains. The porosity which is the percentage of the volume of grain influences the resistance of a grain bulk to airflow. The specific heat which is defined as the energy required by a mass unit of grain to increase in temperature by 1°C determines the energy required to reach a desired temperature at a specified evaporation rate. The thermal conductivity of grains helps us determine the resistance of the conduction of thermal energy within the kernels. The higher the conductivity the lower the thermal gradients during the drying process. The repose angle informs us on how best grains can be stored in flat storage medium at a uniform depth and the specific surface area, defined as the surface area per unit volume of grain enables us determine to what extent moisture and energy is exchanged with atmospheric air during drying (Brooker et al. 1992).

Table 2:1 Physical and thermal properties of corn adapted from Brooker et al. 1992

Property	Corn (English units)	Corn (S.I units)
Bulk density	46.5 Ib/ft^3	745 kg/m^3
1000-kernel weight & porosity	0.72 & 40 (Ib) (%)	325 (g) & 40 (%)
Specific heat	0.48 (Btu/Ib °F)	2.01 (kJ/kg. °C)
Thermal conductivity	0.092 (Btu/hr ft °F)	0.159 W/m°C
Repose angle	35° (deg)	35° (deg)
Specific surface area	238 (ft^2/ft^3)	784 m^2/m^3

2.1.2 Chemical composition of corn

The chemical composition of corn such as protein, fat, starch and fiber are determined by proximate analysis (NIR) technology. These parameters change when corn quality changes over a period of time. This could be a reference point for any changes in composition that occur as a result of deterioration or invasion by pests during storage.

Table 2:2 Chemical composition of corn

Nutritional content	Chemical composition of corn (%)
Protein (%)	9.8
Fat (%)	4.9
Starch (%)	63.6
Fiber (%)	2.0

2.2 Importance of storage to global food security

Low moisture corn that is free of insects and microbial activity can be stored for extended periods of time. Corn is prone to post harvest losses during storage. When corn is stored for long periods of time and variables such as moisture and temperature are not well controlled its quality progressively decreases over time. In effect, the eventual quality of stored corn can be explained by the complex interactions between the physical, sanitary, and intrinsic variables of quality, and their relationships within the eco-system (Multon, 1988; Tipples, 1995). Under warm and humid climatic conditions in regions

such as SSA (see Figure 2.1), corn can deteriorate rapidly. High temperature and moisture are the main driving forces of this deterioration.

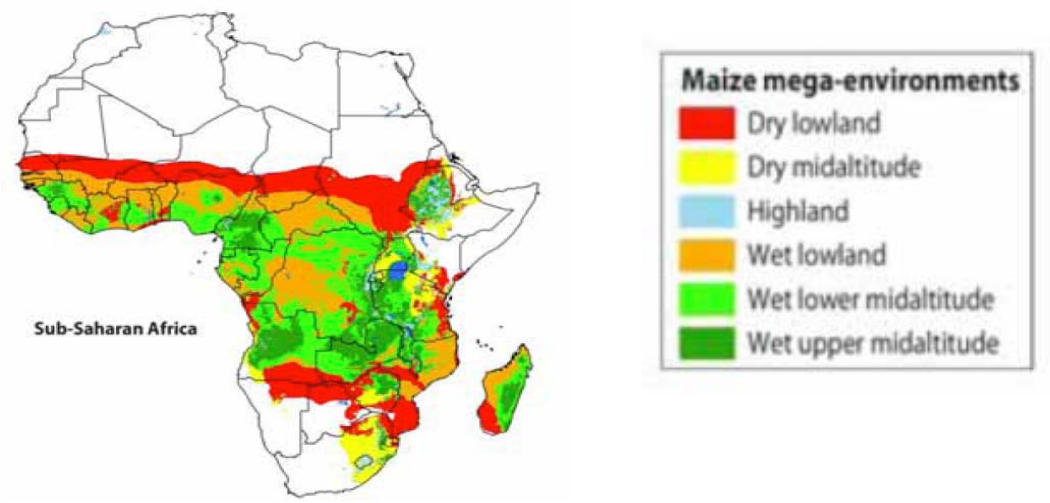

Figure 2:1 Climatic conditions in SSA, (Adapted from CIMMYT, 2011)

Grain is in constant demand throughout the year and thus needs to be stored beyond the harvest time and until the next harvest. Grain production increase should be accompanied by storage and preservation practices to ensure an uninterrupted supply of grain to meet increasing demands (Chakraverty et al, 2003). The main objective of grain storage is to preserve quality, including nutritive value, and keep the grain in good condition for marketing and processing thereby reducing product and financial losses. Storage of food grains, a component of postharvest operations, is an ongoing challenge for both industrialized and developing countries (Chakraverty et al, 2003).

Alarmingly, most developing countries in Africa produce less corn than its population needs, and approximately 28% of the African corn supply is imported from other countries. According to Godfray et al. (2010), as the world population increases

within the next 40 years, there is an expected steady increase in demand for food, with the increase projected to reach 70 - 100% by 2050.

According to a report by FAOSTAT (2010), corn production increased by only 2.3% in SSA, compared to 6.0% in Asia and 5.0% in Latin America. Figure 2.2 shows corn production data since 1961. One interesting trend is that while production for coarse grains and root crops plateaued over the past decade, the production of main grains such as corn has seen a steady increase in production, mainly due to an increase in demand. The reasons for this demand may stem from the increased use of corn for food, feed and biofuels. As the demand for main grains such as corn increases globally, the world's poorest (low income earners) especially those from developing countries are most vulnerable to negative effects of market and availability fluctuations, price hikes and a global or trade crisis. Put simply, developing countries need to increase production and minimize waste from on-farm handling, storage and shipment in order to meet the needs of their population and store grain for unforeseen times of global or trade crisis, thus minimizing their reliance on grain imports.

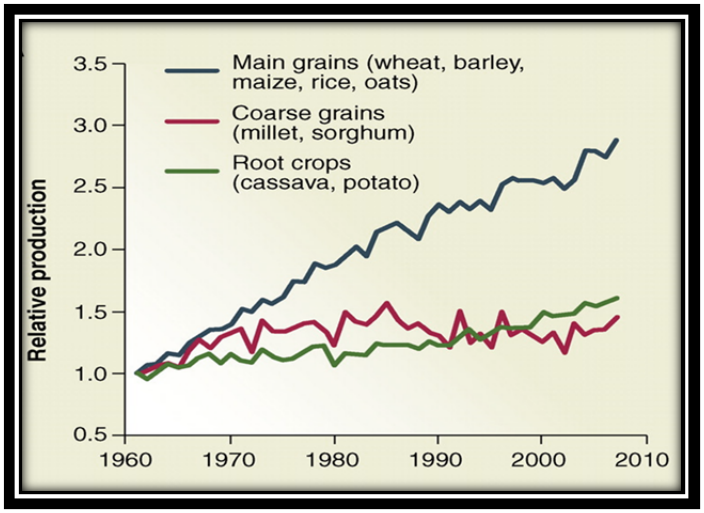

Figure 2:2 World grain productions from 1961 to 2010 (Adapted from Godfray et al. 2010).

2.3 Post-harvest handling along the value chain in SSA

The post-harvest and marketing system is a link of related activities and steps from time of harvest to the consumer (Rembold et al., 2011). The steps in the supply chain of corn are (1) harvesting after field drying, (2) drying (3) shelling, (4) winnowing, (5) transporting to store, (6) storage, (7) transport to market and (8) market storage. Figure 2.3 illustrates the post-harvest food chain in SSA. In most African rural farming communities, the post-harvest handling of corn involves a number of stages. First, the corn is allowed to dry to some extent in the field before harvest. After harvest, the corn is left in piles on the field until transportation is available. The corn is then transported to threshing facilities or personal households. Husks are removed and it is threshed and later spread out to dry on mats or tarps. During drying (a critical operation right before storage), the corn is stirred and turned over to facilitate moisture removal. One method by which moisture content of the corn is checked subjectively is by biting kernels between

the teeth. Kernels which shatter when bitten are considered dried enough while those which break neatly into two halves is considered still wet. Other methods of determining the corn to be 'safe' for storage include feeling a handful on the palm for dampness or shaking a few on the palm to discern sound variation between dry and wet grains. Even though estimating the moisture content by biting and by feel has been a common practice by small holder farmers in SSA, anecdotal evidence suggest that these farmers cannot accurately predict grain moisture using these subjective methods.

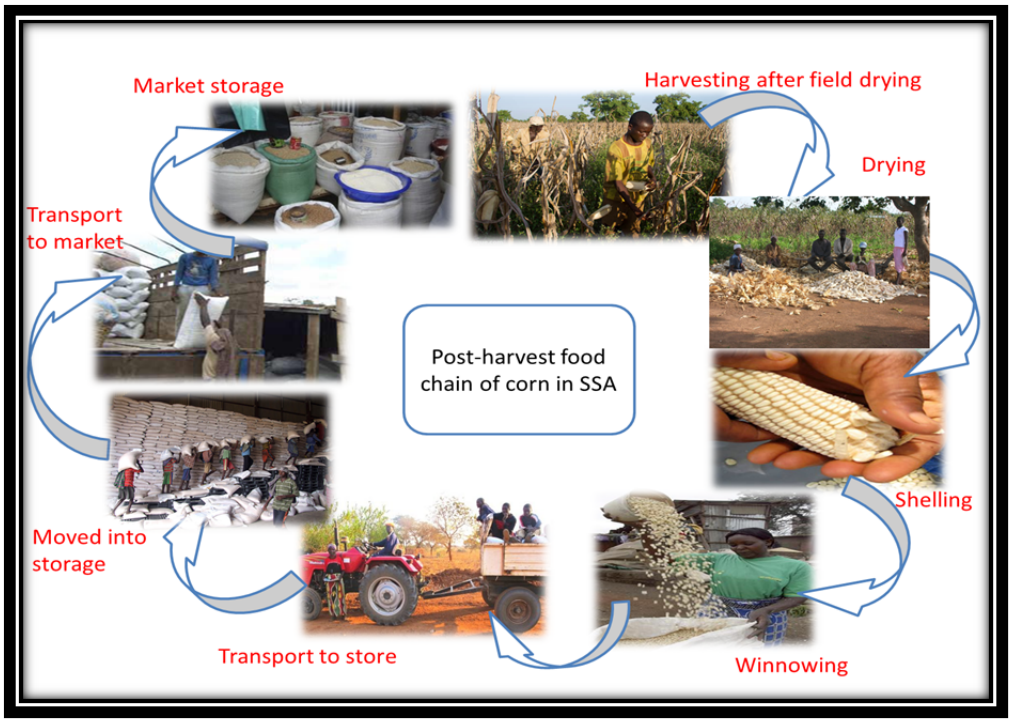

Figure 2:3 Post-harvest food chain of corn in SSA

2.4 Post-harvest losses (PHL) in the humid tropics of SSA

The aim for the post-harvest system is to ensure that the harvested product reaches the consumer meeting the negotiated nutritional and product safety expectations (Hodges, 2011). Table 2.3 gives a profile of corn losses in eastern and southern Africa.

Table 2:3 Profile loss of corn (humid climate) in Eastern and Southern Africa Estimates are weighted averages (Adapted from Hodges, 2011)

Post-harvest activity	Loss profile (% weight losses)
Harvesting/ field drying	6.4
Drying	4
Shelling/threshing	1.2
Winnowing	-
Transport to store	2.3
Storage	5.3
Transport to market	1
Market storage	4

In addition to low production, the table above shows that developing countries lose a majority of their yield to poor handling, storage and infrastructure, with a total weight loss of 24.2%. PHL could also be physical, nutritional, monetary or economic. PHL ranged between 16-23% in SSA from 2003 and 2008 (Rembold et al., 2011). This high percentage of post-harvest loss in SSA resulting from handling and transportation may be due to 1) suboptimal knowledge of the proper procedures for handling after

harvest 2) lack of investment and knowledge in storage practices that minimize spoilage during storage 3) lack of infrastructure/machinery for transportation and trade.

Other causes of losses include harvesting methods, handling procedures, drying techniques and poor storage structures all of which exacerbate mold contamination, insect damage and infestation. PHL losses contribute to higher food prices and a decline in food security. PHL could be reduced by increasing the efficiency of the supply chain by intervening with the right tools to address the various challenges.

PHL can be quantitative or qualitative. Qualitative losses may include aflatoxin contaminated grain and loss in nutritional value. When grains are harvested at high moisture they are prone to deterioration by fungi. Some other factors that influence deterioration along the value chain include mechanical damage, high temperatures and rainfall during harvest, insect damage and microorganism contamination. Because farmers primarily depend on open-air sun drying, frequent rainfalls and high humidity during harvest in the humid tropics provide insufficient sunshine hours necessary to dry grain to safe moisture levels. The estimated average sunshine hours in the month of August between 1981 and 2010 in Kumasi, the capital of the Ashanti region located in the middle belt of Ghana was 2.6 (Ghana Meteorological service, 2011). This makes solar drying to a safe moisture level below 13.5% a challenge with open air drying alone. Thus, it is likely that the criterion farmers use to determine when the corn is at a high moisture may not be 13% or below, hence it is likely that corn will be placed in storage at unsafe moisture levels above 15% is highly inevitable. This is suggestive of an inefficient system of production and storage that is non-sustainable. To reduce the risk of income loss, farmers often hastily market their grain to millers, wholesalers, retailers and then

consumers because of the potential loss that would be incurred in storage due to spoilage. Therefore, excess grain gluts in the market are very typical at harvest time reducing prices, while grain deficits develop late in the season where grain prices could easily quadruple. Therefore, a sufficient long-term storage method is important to increase income and food security in farming households in SSA. Figure 2.4 gives an illustration of one of many ways dried corn is transferred from small-scale farmers to consumers.

Figure 2:4 Food chain of corn in SSA (Hodges, 2011)

Storing corn that may not be completely dry for an extended period of time increases the likelihood of spoilage. If corn with mold is not discarded appropriately but is either knowingly or unknowingly consumed, because of a lack of alternative food source for poor consumers, this may lead to an increased predisposition for health issues.

As the demand for locally grown corn increases, small-scale farmers in SSA may aspire to harvest and store larger quantities of corn for longer periods of time. In order to limit the progression of quality change in corn stored in sacks, distributors or small-scale farmers would need to acquire adequate knowledge and resources to allow them to use the best available methods of treating and storing of their corn.

2.5 Grain drying

In grain drying systems, moisture moves from a point of higher vapor pressure in the product to be dried to a point of lower vapor pressure in the drying medium, usually - air at a rate dependent on the difference in water vapor pressure between the two points. The vapor pressure of the air depends on its relative humidity and temperature. Table 2.4 shows the moisture distribution in a kernel of freshly harvested corn. Vapor pressure within a kernel depends on the temperature and moisture content and to a lesser degree on composition.

Table 2:4 Moisture distribution in a freshly harvested corn kernel (Brooker etl al, 1992).

Kernel part	MC (% w.b.)
Whole kernel	36.0
Germ	48.2
Endosperm	30.7
Pericarp	52.6

When air passes through grain it either absorbs or releases moisture. Kernels will absorb moisture from the air when their equilibrium relative humidity is less than the relative humidity of the air. Grain will lose moisture to the air in the reverse situation, that is, when the equilibrium relative humidity of the grain is greater than the relative humidity of the air. When dry air passes over wet or high moisture grain, air absorbs the moisture from the kernels increasing the humidity ratio while decreasing the air's drying potential. No further drying occurs when the air relative humidity reaches the equilibrium relative humidity (ERH) of the grain. Equilibrium moisture content (EMC) is the

moisture content when grain comes to equilibrium with air under constant environmental conditions (Loewer et al., 1994). If the air is not in contact with the kernels until it reaches their ERH, the air will not attain its maximum possible relative humidity. If the air does attain equilibrium its relative humidity will be the same as the grain's ERH, and no further drying will occur as the air continuous to travel through the grain mass. Drying rate can increase for a given airflow if moisture is removed from the air before it is passed through the grain or if the air's temperature is increased.

2.5.1 Grain drying in SSA

In tropical countries, high temperatures and high relative humidity together with high cost of drying systems often leaves small-scale farmers with no feasible alternative but to use the sun to dry their harvested corn. Open air solar drying by spreading grain on a mat, plastic or tarp on thin layers (see Figure 2.5) is the predominant drying method used by small-scale farmers in SSA. Albeit inexpensive, this form of drying is slow and dependent on the prevailing environmental conditions compared to drying using mechanical means. High humidity and few days of sunshine during the harvest season in the humid tropics of SSA make it very difficult to bring down the corn moisture content below the critical of 13% in a timely manner. Furthermore, if the farmer is not very attentive to the weather, rain could re-wet the kernels and prolong the drying process. Under these prevailing conditions, small-scale farmers face the problem of drying corn to acceptable moisture levels that prevent the growth of molds and other micro-organisms during storage. The presence of excessive moisture above the critical levels of 13% in a hot humid environment makes corn prone to spoilage by fungi, which in turn could lead

to aflatoxin contamination. Furthermore, there is also danger of contamination during solar drying of shelled corn. Birds often fly above the drying mats with the intention of feeding on the corn and in the process release their droppings on the mat contaminating the corn. Rodents may also contaminate the corn with their droppings while attempting to feed off the corn. These activities also increase the possibility of mold growth in corn while contaminating it with foreign materials. Additionally, contamination from dust generated by passing vehicles is common for grain dried along the side of the road.

Figure 2:5 On-the-tarp drying of corn in SSA

2.5.2 Post-harvest storage and storage in SSA

Corn storage is one of the most important steps in the post-harvest food supply chain. High grain quality must be maintained to achieve higher market value, permit safe human consumption and provide seed with good germination qualities for planting. Storage should provide protection to prevent loss of the crop from unfavorable weather conditions and to sustain the cultivation of corn for subsequent seasons. Corn is in

demand on a regular basis over several seasons, and the storage of harvested corn is required to vary from months to several seasons. Small-scale farmers store corn in varying amounts and for varying time periods and for several different purposes such as food consumption, periodic planting and generating income during lean seasons. What drives costs of grain storage are market value, storage costs, labor, insect control, cost of maintenance, and storage losses (FAO, 1994). The moisture content of grain in storage plays a major role in defining the quality of grain.

2.5.3 Challenges in drying and storing corn in SSA

Consuming contaminated corn with molds poses health risks to humans. Some molds produce mycotoxins, a secondary metabolite. Aflatoxin, a highly toxic mycotoxin, suppresses the immune system. It can cause chronic damage to the liver and kidney, and the digestive and nervous systems. Aflatoxins can also interfere with the reproductive system of humans. Aflatoxin contaminated corn results in high storage and economic losses and is one measure used in marketing to determine grain quality. Typically, major grain grading standards require that grain sold not exceed a certain aflatoxin threshold levels. In the U.S., the United States Food and Drug Administration, Department of Agriculture (USDA), Federal Grain inspection Service (FGIS) threshold level for aflatoxin in corn is 20 ppb (CAST, 2003), the threshold for Ghana is 15 ppb (Ghana Grain Standards Board, 2003) and for the European Union (EU) it is 4 ppb (ready-to-eat) and 10 ppb (if further processed) (www.usda.gov). In Europe he recommended safe limit for fungi in processed food is 103-104 colony-forming units (cfu) per gram. The UN World Food Program (WFP) Purchase-for-Progress (P4P) allowable limit for aflatoxin

threshold is 10 ppb analyzed with the ELISA technique (Kang'ethe, 2011). A challenge faced by the WFP in purchasing grains from SSA is that farmers are not able to meet their high quality standards, especially with regards to aflatoxin levels. Hence, storing corn with high moisture content not only exposes consumers to severe health risk from aflatoxin contamination, it also results in suboptimal revenue from high storage losses.

2.6 Stored Grain Ecosystem

Abiotic conditions that influence mold growth and mycotoxin production during storage are water activity, temperature and gas composition (Magan & Olsen, 2004). The critical moisture content for safe storage is the moisture that produces a water activity (a_w) of about 0.65. Below this water activity most stored grain fungi cannot grow. In the stored corn eco-system quality and nutritive changes occur because of the interactions between physical, chemical and biological factors (Chulze, 2010). Figure 2.6 illustrates the interactions between environmental and biological factors that influence quality change of grains in the storage ecosystem.

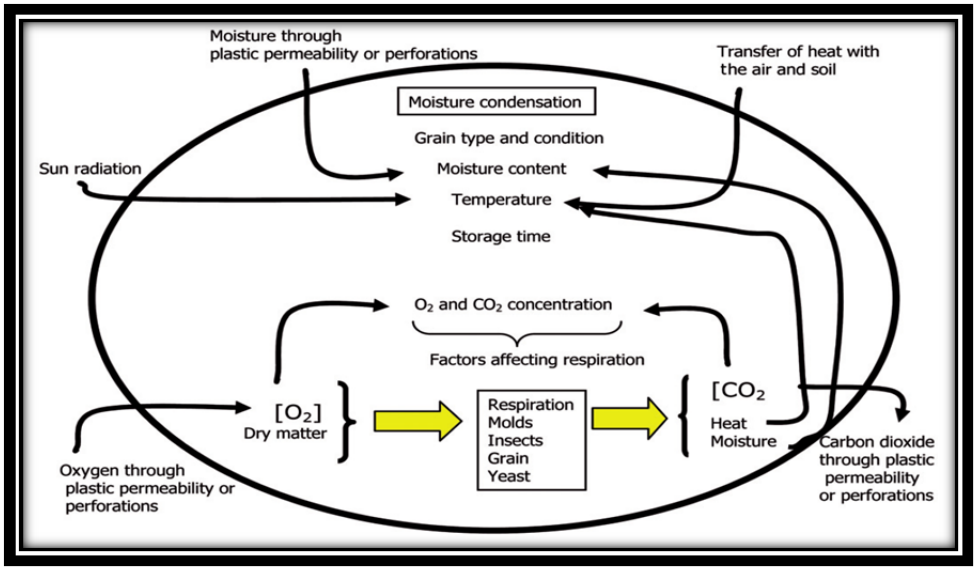

Figure 2:6 Major factors affecting the respiration of grains and microorganisms (Adapted from Cardoso et al. 2008)

2.7 Respiratory quotient

Aerobic respiration needs oxygen to release carbon dioxide, water and energy (in the form of heat). Anaerobic respiration needs carbon dioxide, and releases less energy. The respiratory quotient (R.Q) is defined as carbon dioxide produced divided by oxygen consumed. For normal respiration of carbohydrates the R.Q. is equal to 1 (Huxoll, 1961). However for fats the R.Q. is 0.7. Moisture content is related to respiration. Molds begin to grow at a water activity (a_w) of $\geq$ 0.65. Increase in mold growth is a major contributor to increased respiration within the storage ecosystem (Milner and Geddes, 1954).

2.8 Ecological system of grain storage

Factors influencing the conservation of corn quality during storage interact in a complex way. The components that make up the ecological system and interact with each other include: 1) Stored grain (principal biotic factor), the storage structure (abiotic

factor), the storage atmosphere (abiotic – external & internal factors), grain temperature (abiotic – external & internal factors), humidity of the air in the structure (abiotic – external & internal factors), presence of foreign matter (abiotic internal factor), presence of microorganisms (biotic – external & internal factors) and presence of insects (biotic – external & internal factors) (Calderon, 1981; Navarro, 2001).

The physical properties of corn are influenced by abiotic and biotic factors in grain storage. The proliferation of mold is affected by moisture content and temperature (Wilson and Desmarchelier, 1994). High moisture content grains are prone to mold and insect pest development during storage. Corn, which is very hygroscopic in nature, consists of water and dry matter. Water in grain can be categorized into three parts: absorbed water, adsorbed water and chemically bound water. In an airtight space, the moisture in the grain produces a vapor pressure less than the saturated vapor pressure of free water. An equilibrium is maintained between the moisture in the grain and the moisture in the air.

2.9 Moisture content

Moisture content is a major factor that influences grain spoilage during storage. Water activity (a_w) resulting from the moisture content of the grain influences microbial growth, germination, insect feeding behavior, and grain weight. To attain a state of equilibrium, moisture moves from the air into the grain or from the grain into the air depending on which has the higher vapor pressure. If placed in an enclosed container at constant temperature, in a state of equilibrium, the rate of water movement out of the grain equals the rate of water movement into the grain. The moisture content of the grain

at this point is the equilibrium moisture content (EMC) and the relative humidity of the air is the equilibrium relative humidity (ERH). The EMC is very important when drying grain while the ERH, also known as the water activity (a_w) is important for grain storage (Loewer et al. 1994).

In a storage environment, moisture content influences several quality attributes of stored grains. Over time, the moisture content of stored grains interacts with biotic factors, environmental factors and gas composition in a systematic way to eventually affect fungal development and survival, insect mortality and progeny, germinability and in some cases grain damage (Loewer et al. 1994). Several studies have evaluated one or two combinations of these interactions and the eventual effect on some aspects of the quality of stored corn. This section will discuss existing research findings on the effect of moisture content, water activity on fungal development and survival, insect mortality and progeny, germinability and grain spoilage. The relationship between moisture content, relative humidity and temperature is illustrated in Figure 2.7. This figure shows that for a specific relative humidity, as temperature increases the moisture level required for maintaining a state of equilibrium decreases. Several studies have indicated that when temperature is held constant, there is an approximate increase in EMC of 1% MC to every 5% change in relative humidity (Ross et al, 1973; Moreno et al, 1988).

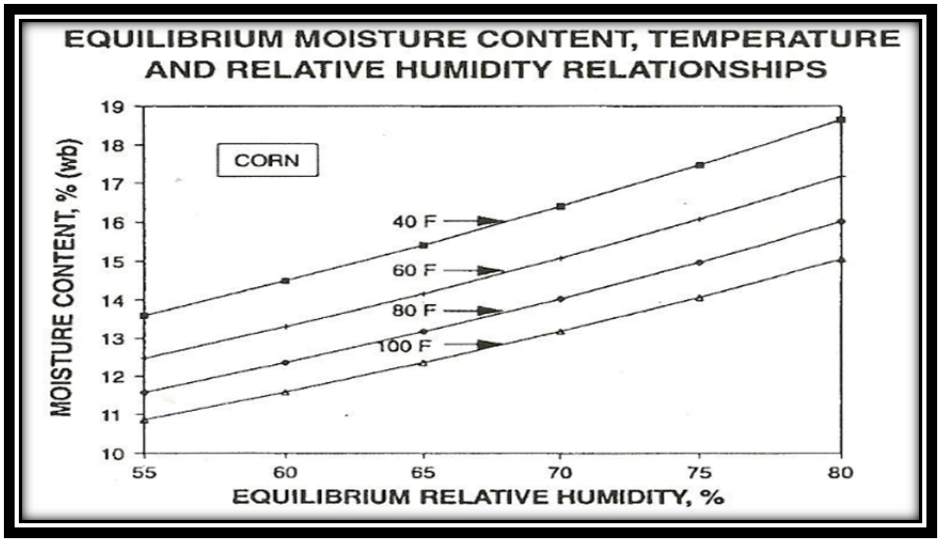

Figure 2:7 Equilibrium moisture content, temperature and relative humidity relationships (Adapted from Ross et al., 1973)

These factors are often associated with temperature difference either within the grain mass or between the grain mass and a portion (roof, wall) of the bin in which the grain is stored.

Movement of moisture in stored grain could occur by: 1) diffusion of moisture due to vapor gradients, 2) translocation of moisture due to convection currents, 3) condensation forming on the inside roof surface and 4) water vapor exchange with atmospheric air at the grain surface (Navarro, 2001). Moisture migration is often driven by temperature differences within a given grain bulk. Under non-isothermal conditions, convection currents resulting from temperature gradients drive moisture movement in the grain.

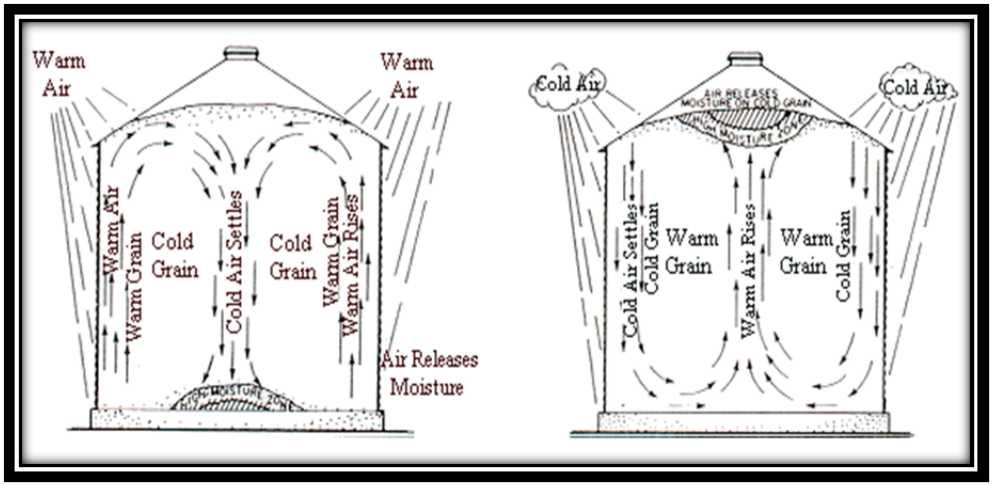

Figure 2:8 Air currents in grain storage bins for summer and winter conditions (Adapted from Hilborn, 1976)

When grain is harvested and placed in storage during warmer temperatures especially in late summer in northern latitudes the grain mass begins to lose heat as colder temperatures approach (especially in the winter). The grain next to the bin wall starts to cool much faster than grain in the center of the bin which is still warm. Convention currents are created as cold air by the bin wall is forced downwards and a recirculation pattern develops (see Figure 2.8). The warm air in the center of the bin rises because it is less dense and carries moisture from the grain with it. The warm air moving up releases moisture when it comes into contact with the cold grain at the surface of the grain mass. This leads to condensation further creating conditions favorable for the proliferation of mold growth and aflatoxin production. In tropical and sub-tropical countries of sub-Saharan Africa (SSA), the reverse of this usually occurs (Navarro, 2001).

Moisture content is a major factor that influences grain spoilage during storage. The relative humidity of the air and the moisture content of the grain influence microbial growth. Moisture moves from the air into the grain and from the grain into the air. If

placed in an enclosed container at constant temperature, a state of equilibrium will develop in which the rate of water movement from the grain equals the rate of water movement into the grain. The moisture content of the grain at this point is the equilibrium moisture content (EMC) and the relative humidity of the air is the equilibrium relative humidity (ERH).

The EMC is important when drying grain while the ERH is equally important for grain storage (Loewer et al. 1994). This information can be used to determine safe storage. For example, at 40°C corn with moisture content at or below 12% is considered safe for storage, whereas corn with moisture content that exceed 13% is considered unsafe for storage.

2.10 Microorganisms in stored grains

In the tropics and sub-tropics, the development of microflora is highly likely in corn storage since it is a challenge, especially for small scale farmers, to keep corn dry and at safe moisture levels. Spores of microflora are usually present in corn storage. Microflora consist mainly of actinomycetes, fungi and bacteria. When grain moisture levels are low, naturally occurring mold spores stay in a dormant state and remain inactive until the environment is suitable for them to proliferate. Microbial activity and susceptibility are related to the ERH. Microflora will multiply when ERH is at least 65%. At moisture conditions above safe levels, grain deterioration from microflora development may increase at an exponential rate.

Fungi which can survive in dryer conditions compared to bacteria can cause huge problems in storage since they can overcome lower levels of moisture under high stress

levels. Fungi are non-synthetic, eukaryotic organisms that thrive on dead organic material (saprophytic) and living material (parasitic). Fungi like to feed on matter containing nutrients. They reproduce either asexually, sexually via spores or by a combination of the two. Spores are fungal reproductive cells and are a single cell about 3 – 30 μm in diameter. They settle on a substrate, germinate and project a germ tube. The germ tube develops into a thread-like filament known as hypha, this hypha develops and branches into other hyphae usually seen as a white mass of filaments called mycelium. Hyphae can produce chemicals to repel other fungi growing on food substrates (Meronuck and Stuckey, 1988; Mills, 1991; Richard-Molard, 1988). Fungal spores are not affected by ultraviolet rays of sunlight. This is why solar drying does not influence levels of aflatoxin. Conditions favorable for fungal growth are 70% RH and a pH of 5.0 with optimum temperatures between 20°C and 35°C (Christiansen and Kaufman, 1969).

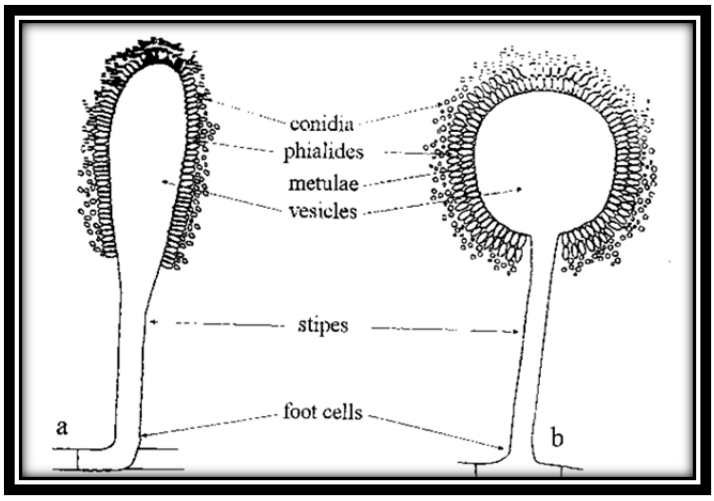

Figure 2:9 Schematic diagram of Aspergillus spp. (Adapted from Klich, 2002)

Fungi are classified as true or fungi-like organisms. The genera *Aspergillus* and *Penicillium*, which have nonmotile spores, are classified as true spores and under the

subgroup “Deuteromycetes”. Under microscopic evaluation, *Aspergillus spp* exhibit distinct features. These fungi develop asexually by budding conidiophores borne on conidia. Figure 2.9 shows a schematic diagram of *Aspergillus spp.* Eurotium species, sexual states of Aspergillus *flavus* can infect corn pre and post- harvest. Optimum conditions for its growth include 30-35°C, 16-17% MC and 85% RH (Lacey and Magan; 1991). *A. flavus* produces a potent carcinogen called aflatoxin which has been shown to cause liver cancer in humans (Christensen, et al., 1977). Aflatoxin content can increase if drying and storage is not properly performed (Marin et al., 2004).

2.10.1 Effect of moisture on fungi

The major factors that influence the growth and reproduction of microorganisms include moisture content, temperature, initial infestation, gas composition, pH, grain condition, storage time and foreign material (Loewer et al. 1994). Notwithstanding that these factors interact in a complex manner, this section will focus on the effect of moisture content/water activity, temperature and gas composition on microbial growth and survival.

When moisture levels are low, microbial growth tends to be at a minimum in stored grains. Each type of mold species has its own moisture preference. When the moisture of corn is at 13% MC or below fungal growth is minimal. When the moisture content of corn is above 13% MC and the temperature is above 25°C the environment is conducive for mold growth. As the moisture increases mold grows faster. Water activity resulting from the relative humidity of the air and the moisture content of the stored grains interact with other variables such as temperature and gas composition to influence microbial

growth. The equilibrium relative humidity below 65% is considered "safe" from the growth of microorganisms on corn when the equilibrium moisture content is 13.1% at a temperature of 26.7°C. Microorganisms absorb moisture and nutrients when the relative humidity is above the critical level. The growth and reproduction stall when the relative humidity falls below the critical level. The minimum, optimal and maximum relative humidity required for microbial growth is dependent on the species of the organism. For example, Navarro (2001) reported that the optimal relative humidity for the development of Aspergillus spp.is 80% (i.e. $a_w = 0.8$).

2.10.2 Effect of temperature on fungi

Most storage fungi require high temperatures for their growth. Common storage fungi grow within a range of 17°C to 44°C, with optimum growth in the range of 29°C to 32°C. The influence of temperature on growth of different types of microorganisms is illustrated using data from Navarro (2001) shown in Table 2.5. Storage microflora may be categorized into three groups: Psychrophilic, Mesophilic and Thermophilic. Most storage fungi are mesophilic (Navarro, 2001). The optimum growth temperature for mesophile fungi is between 20°C and 40°C and for thermophiles it is between 50 and 60°C.

Table 2:5 Range of temperature requirements of three groups of storage microorganisms

	Range		
Group of Microflora	Minimum (°C)	Optimum (°C)	Maximum (°C)
Psychrophilic	(-8) -0	10 -20	25 -30
Mesophilic	5 -25	20 -40	40 - 45
Thermophilic	25 -40	50 -60	70 - 80

Growth of fungi and temperature (After Navarro, 2001)

An increase in grain temperature increases the production of moisture due to increase in respiration rate of the grain and microorganisms. The rate of respiration is doubled with a 10°C rise in temperature, within the range of fungal survival and development (Navarro, 2001).

2.10.3 Effect of gas composition on fungi

Mold species involved in the deterioration of stored corn are aerobes but they can grow under limited oxygen levels and significant levels of CO_2. Their tolerance for low O_2 and high CO_2 is influenced by the presence of water. When the moisture levels of corn are increased their tolerance level for low O_2 decreases and fungal development increases. Pilot studies conducted by Addae-Mensah and Ileleji (2012) showed that after 14 days of storage, corn stored at 22% MC had notably more visible mold growth compared to corn stored at 14% MC.

Modified atmosphere influences the suppression of mold growth in stored corn (Dixon and Kell., 1989; Magan and Lacey, 1988). Microorganisms need oxygen to

survive and reproduce. Microorganisms can be classified as aerobic, anaerobic, facultative anaerobic and microaerophillic (Loewer et al. 1994). Aerobic microorganisms require oxygen to develop, while anaerobic microorganisms can develop without the presence of oxygen. The chemical reaction for anaerobic respiration of these organisms is shown.

$$\mathbf{C_6H_{12}C_6 = 2C_2H_5OH + 2CO_2 + 22(kcal/mole)} \qquad (1)$$

Anaerobic respiration

When given limited oxygen supply, some microorganisms may continue to develop by partially decomposing carbon dioxide, producing lactic acids, acetic acids and alcohols. This process is commonly known as fermentation. Heat produced in this reaction is much less than in aerobic respiration. In ensiling, the process of fermentation takes place in hermetic environments at relatively high moisture levels (Rodriguez et al 2002). Facultative anaerobic microorganisms grow either in the presence or absence of oxygen while microaerophillic microorganisms grow in very low levels of oxygen (Loewer et al. 1994).

According to Magan and Lacey (1984), oxygen levels below 0.14% suppress the development of most fungi species. Carbon dioxide levels above 50% result in complete inhibition of development of most fungal species. In most bio-generated modified atmospheres, decrease in oxygen results in a simultaneous increase in carbon dioxide. Several studies have evaluated the effect of high carbon dioxide and low oxygen level on fungi development. Studies by (Magan and Lacey 1984) have shown that when carbon dioxide levels exceed 50% mycelia growth is suppressed. However it is the combinations of gases that determine the increase or decrease of mold growth. Species such as

(P.roqueforti) can grow in environments with 80% and above CO_2 levels. However O_2 should be at least 4% for this to occur. The consensus is that carbon dioxide levels that exceed 35% will suppress fungi growth in low oxygen environments. When ambient levels of oxygen are present, Magan and Alfred. (2007) showed that levels of carbon dioxide exceeding 60-80% can still result in suppression of fungi. Banks (1981) also stated that low oxygen and high carbon dioxide levels were the main variables responsible for inhibiting fungal development in high moisture corn.

A recent study by Weinberg et al. (2008) illustrates the importance of carbon dioxide in suppressing mold growth. Although their data showed that all oxygen was depleted after 15 days of storage this did not result in an immediate suppression of fungi. In fact, they reported further that complete suppression of fungi was attained after 55 days of storage, this time period also coincides with the time it took to attain a carbon dioxide level of 80%. Moreno et al. (2000) also reported that even though complete oxygen depletion was attained after 3 days of storage, complete suppression of fungi did not occur immediately, complete death of fungi was attained after 30 days of storage. Even though Moreno et al. (2000) did not report carbon dioxide levels, it is likely that the delay between the day of complete oxygen depletion and complete fungi suppression was due to the time needed for accumulation of carbon dioxide to a level that was high enough to be toxic to the fungi.

2.11 Aflatoxins in grain storage

Under unsafe storage conditions fungi produce the metabolites that produce mycotoxins (Shotwell, 1977). Under certain environmental conditions, *A. flavus* fungi in grain can produce aflatoxin, a carcinogen. Mycotoxins include ochratoxin, zearalenone and trichothecenes. Aflatoxins were discovered in animals which fed on grains and seeds (White et al., 2003). Common aflatoxins include B_1, B_2, G_1, G_2 and M_1. This fungus can proliferate both in the field and during storage. Aflatoxins can occur on a wide variety of commodities such as nuts, oilseeds, grain and processed food. Aflatoxins are most frequently produced by *A. flavus* and *A. fumigatus*. Insects act as vectors by transporting fungal spores on their bodies and contaminating grain when they move from a location to another (Lynch & Wilson, 1991).

Storage pests such as the *Sitophillus zeamais* (Motschulsky) and S*athartus quadricollis* contribute to the production of aflatoxins (Lamboni and Hell, 2009) by disseminating spores of A. flavus in the field and stored products. When insects feed on the corn they break or dislodge the pericarp making the corn more susceptible to the invasion of storage fungi such as *A. flavus* (Tuite et al.1985; Wicklow, 1988). Metabolic activity by insects increases the relative humidity creating more favorable conditions for *A. flavus* growth. Sinha & Sinha, (1992) found more *A. flavus* fungi and greater aflatoxin production in insect-damaged maize samples from different localities in India than with corn samples without insects.

Aflatoxins are hepatocarcinogens and contribute to the prevalence of hepatocellular cancer in Africa (White et al., 2003). Aflatoxins which could be mutagenic,

teratogenic and immunosuppressive affect the liver when consumed in high doses and may cause deaths and chronic cancer (CAST, 2003). Aflatoxins suppress synthesis of protein, nucleic acid and damage the liver by mobilizing liver fat (White et al., 2003). Salmonellosis in corn was known to have high concentrations of aflatoxins (CAST, 2003). In Africa a strong relationship between dietary aflatoxin and liver cancer was found (White et al., 2003). Young children can also be exposed to aflatoxins which can stunt their growth (Gong et al., 2004; Williams et al, 2004). In the US, the FDA has assigned action levels for aflatoxin concentrations depending on use. Guidelines set by the U.S. Food and Drug Administration in 1978, regulate the permissible amounts of contamination in corn before the grain must be disregarded. For human consumption it is 20 ppb, for mature and non-lactating livestock it is 20 – 100 ppb for finishing swine it is 200 ppb and for finishing cattle it is 300 ppb (White et al, 2003; Munkvold , 2003).

2.12 Fatty acids

Grain damaged by microflora undergoes chemical changes which result in increase in free fatty acids (FFA), an increase in reducing sugars, a decrease in non-reducing sugars and an increase in respiration due to breakdown of oil by fungal related lipases (Bottomley et al, 1950). FFA measurement is an efficient measure of grain deterioration mainly due to mold growth. Biochemical deterioration of grain fats or oils is either oxidative or hydrolytic.

Grain lipids are categorized as either polar or non-polar compounds based on their solubility in solvent systems (Christensen, 1982). Fatty acids are major components of all types of lipids which are non-polar lipids. Lipids occur as components of intercellular

membranes, spherosomes, and starch and protein bodies in cereal grains. Fats in grain are readily broken down by lipases into free fatty acids and glycerol during grain storage especially when moisture content and temperature are high and hence favorable for grain deterioration. This process is facilitated by mold growth due to the high lypolytic activity of the molds (Goodman and Christensen, 1952). Therefore, fatty acids can be used as a measure of grain deterioration. Fat acidity value is quantified by titration and defined as milligrams of pottassium hydroxide required to neutralize the free fatty acids in 100 g of corn (AACC, 2009). In sum, fatty acid production is a result of enzymatic hydrolysis of the fats by lipases or by chemical saponification (Pomeranz, 1985).The lipid content of corn ranges from 5.2 to 6.0% (Cooper & Morrison, 1978). Goodman and Christensen (1952) found that some fungi are able to grow on corn oil and convert a portion of it into fatty acids as food. A study by Baker et al. (1957) used fat acidity tests as an indicator of grain deterioration and found an increase in fatty acids with fungal colonization. A study by Dirks et al. (1995) showed that fungal lipase activity increases when corn deteriorates. Fungal lipase causes fatty acid levels to increase. Ileleji et al. (2003) studied the relative storability of high-oil and BT corn hybrids and compared them to conventional hybrids. They studied the effects of oil content on the production of fatty acids and found that high-oil corn hybrids had higher fat acidity values than their paired normal-oil corn hybrids and changes in fat acidity values were higher post storage than pre storage. The aforementioned studies show that fat acidity tests can be used as a reliable indicator of grain deterioration.

2.13 Stored grain insects

Stored grain insect pests consist of 250 species, 40 families of beetles and 70 species of moths (Halstead, 1986; Cox and Bell, 1991). They are categorized as *Primary insect pests* and *Secondary insect pests*. Primary insects develop and feed primarily inside the grain kernel while secondary insects develop outside the kernels and feed mainly on fines. The maize weevil (*Sitophilus zeamais* Motschulsky.), a primary, cosmopolitan insect commonly found in SSA and belonging to the Family Curculionidae, is a small 3 mm in length brown, with four patches on its elytra. The female bores a hole in the grain, deposits her egg in the hole and then plugs it up with a gelatinous secretion. The larva feed and pupates on the grain. The new adults that emerge within the grain feed on the corn tissue on their way out of the grain onto the surface where the life cycle would have completed. The female then mates and lays about 350 eggs within a lifespan of 5 months. The temperature range for a complete life cycle is 14°C - 34°C and development is complete within 25 days at 29°C and 70% RH (Navarro, 1991). The heat and moisture produced by the larvae and pupae often contribute to the development of hotspots.

Insects lower corn quality by feeding on corn kernels and contaminating the grain with their waste, cast skins, webbing and body parts. Insect pests incur substantial damage to whole kernels, consume broken kernels and fines, raising grain temperature and moisture content which may cause fungal growth and heating in grain. Fungal growth results in increase in carbon dioxide, heat (i.e. hot spots up to 55°C for *A. flavus*) and moisture. Insect damaged kernels or number of live insects may reduce marketability of grains especially when it is significant enough to reduce food grade.

Certain variables within the storage environment influence the species composition of insect population. Insects in stored corn need certain environmental conditions that favor their development.. Insect development is highly influenced by temperature and grain moisture content (Mason and Strait, 1997). Insects have a maximum, minimum and optimal temperature range for their development, oviposition, insect progeny and survival. Put together, insect mortality is affected by temperature, intrinsic growth rate and exposure time (Fields, 1992). Egg production is affected by insect species, temperature, ambient humidity, grain moisture and diet (Navarro, 2001). Moisture content, water activity, and gas composition do not act in isolation, but rather have a combined effect on insect survival and progeny, especially in a hermetic storage environment. The section below discusses the effect of each factor and their combined effect on insect mortality and progeny.

Maize weevil causes quantitative losses primarily by feeding. Insects contaminate the grain with their excreta containing uric acid, fragments of dead insects and webbing. Insects spread storage fungi and their activity leads to the generation of heat in the local areas. The hotspots cause moisture and temperature gradients in the grain mass favoring mold growth leading to grain deterioration. Levels of insect infestation is a commonly used quality assessments (Chakraverty et al., 2003).

2.13.1 Effect of moisture content on insect mortality

Insect feed on grain to enable them produce enough moisture for their survival. The higher the moisture contents of grain the higher the rate of increase in insect pests.

Moisture requirements vary with insect species. Moisture and temperature in hermetic storage varies because of the metabolic water produced, grain contamination from their fecal matter and atmospheric composition.

However, when gas composition in the storage environment is modified, the moisture content modulates insect survival and progeny. Insects have been shown to modify their feeding behavior by closing their spiracles to increase their tolerance to hypoxia environments Navarro (1978) reported that when exposed to a low oxygen environment, adult *S. orzyae* (Coleoptera: Curculionidae) increased their tolerance to the toxic environment by closing their spiracles.

2.13.2 The effect of relative humidity on insect mortality and progeny

When oxygen levels decrease mortality of adult insects occurs. The effect of low oxygen is however modulated by relative humidity. Calderon and Navarro (1980) showed that when pupae of *Ephesia cautella* were exposed to low, moderate and high humidity, the time needed for complete suppression of adult emergence was directly related to the relative humidity. At a high humidity (i.e. 97%) 80 to 95% of the pupae emerged as adults. At the lowest humidity (i.e. 24%) emergence of adult insects was completely suppressed within 4 days of exposure. The data by Calderon and Navarro (1980) is consistent with established trends that adult insects survive in an ideal humidity that is equal to or greater than 60% (Howe, 1965). Relative humidity modulates the effect of carbon dioxide on insect mortality. Calderon and Navarro (1979) measured relationships between carbon dioxide and relative humidity at a fixed temperature and exposure time. In general, increase in relative humidity required a directly proportional increase in

carbon dioxide to attain 95% mortality of insects. In general, the ERH is directly proportional to the moisture content of the grain, therefore as moisture content increases, ERH increases and insect mortality decreases.

2.13.3 The effect of temperature on insect mortality and progeny

The ideal temperature for insect survival is approximately 25°C to 32 °C. Insect development decreases at lower temperatures. Oviposition of most insects has been shown to halt at 15 °C and insect mortality can be attained at temperatures below 5°C. Extreme high temperatures (i.e. > 45 - 50 °C) also results in insect mortality. Insect life cycle lasts approximately 25 days, but it can be affected by temperature and gas composition (IRRI, 2009).

Grains stored in low oxygen environments (i.e. < 1% 0_2) with temperatures between 20 and 30 °C results in insect mortality of 95%. Exposing *S. zeamais* to -10°C for 7 days will attain a complete kill (Ileleji et al, 2007). The effect of temperature on the exact time needed to attain complete mortality (i.e. LT_{99}) is modulated by the species of insects (Annis and Graver, 1987). Yakubu et al. (2010) investigated the influence of temperatures and moisture contents on the rate of suppression of insects and insect mortality in hermetic storage of corn and concluded that a complete kill of the maize weevil can be achieved using this technology. However, the hermetic environment enclosure was highly dependent on the moisture content, temperatures within the enclosure, insect count and the size. Chiappini et al (2009) evaluated the effect of temperature on insect mortality in a low oxygen environment (5-8%). They reported that

LT_{99} could be attained within 7 days at 20°C to 37 °C. Finkelman et al. (2003) evaluated the effect of temperature and carbon dioxide on insect mortality at four different developmental stages (Eggs, larvae, pupae and adults) of *Ephesia cautella.* Navarro et al. (1985) also reported that at 2% oxygen concentration levels, 48 hours was needed to obtain complete insect mortality at 26 °C compared to 72 hours at 15 °C. In general, one can conclude that higher temperatures results in faster rates of mortality for all insect stages when exposed to modified atmospheres with low oxygen and high carbon dioxide. Furthermore, complete mortality of adult insects could be attained in a shorter amount of time compared to eggs, larvae and pupae. Below 45 °C, Finkelman et al (2003) showed that adults and pupae appear to be the most susceptible to the negative effect of increase in temperature.

2.13.4 Effect of gas composition on insect mortality

When oxygen is low and carbon dioxide is high, low grain moisture content and inter-granular humidity contribute to high insect mortality due to death by desiccation (Murdock, 2012). Different species of insects show different carbon dioxide requirements for complete kill of their eggs, larvae, pupae or adults. Navarro and Donahaye (2005) suggest that a minimum requirement of 35% CO_2 over a 14 day period is sufficient for attaining complete insect mortality of the red flour beetle. Complete kill of the majority of stored-product insects can be attained in 10 to 48 hours in 100% carbon dioxide (Kashi, 1981). In a bio-generated atmosphere, oxygen, carbon dioxide and nitrogen change in tandem. Therefore an increase in carbon dioxide is also suggestive of

a decrease in oxygen and a consequent change in nitrogen. Ideally, the most lethal is a synergistic environment with low oxygen and high carbon dioxide. Some studies seem to suggest that high nitrogen behaves in like manner with high carbon dioxide (e.g. Donahaye, 1990). However the effect of nitrogen on insect mortality, fungi development and overall corn quality changes in general is beyond the scope of this proposed project. Even though it will not be addressed in detail in this proposal, it is acknowledged that nitrogen does change with resultant change in oxygen and carbon dioxide and it affects biotic variables within the hermetic storage environment. The efficacy of oxygen versus carbon dioxide is also related to the type of insect species. According to Banks and Annis (1990) high carbon dioxide levels (i.e. > 60%) is most effective at killing internal feeding insects such as *S. zeamais,* whereas low oxygen (hypoxia) environments are most effective at killing external-feeding insects.

Moreno et al. (2000) reported that oxygen concentration was directly related to insect mortality. Finkelmann et al. (2003) showed that at a given temperature, increase in carbon dioxide results in a measurable decrease in the time needed to attain complete mortality of insects, especially when carbon dioxide concentrations exceed 80%. Adults and pupae appear to be the most sensitive to high carbon dioxide levels.

2.14 Germination of stored grains

Corn seeds are of vital importance for human nutrition in SSA where it is a staple food. Small-scale farmers use organic farming methods, which often require them to save some of their harvest for planting. Therefore, good seed quality parameters such as germinability should be preserved for the immediate and subsequent planting seasons.

The association of official seed analysts defines seed germination (see Figure 2.10) as "the emergence and development from the seed embryo of those essential structures which, are indicative of the ability to produce a normal plant under favorable conditions" (Copeland and McDonald, 1985; 2001). Germination is a quality index measure of stored grain viability.

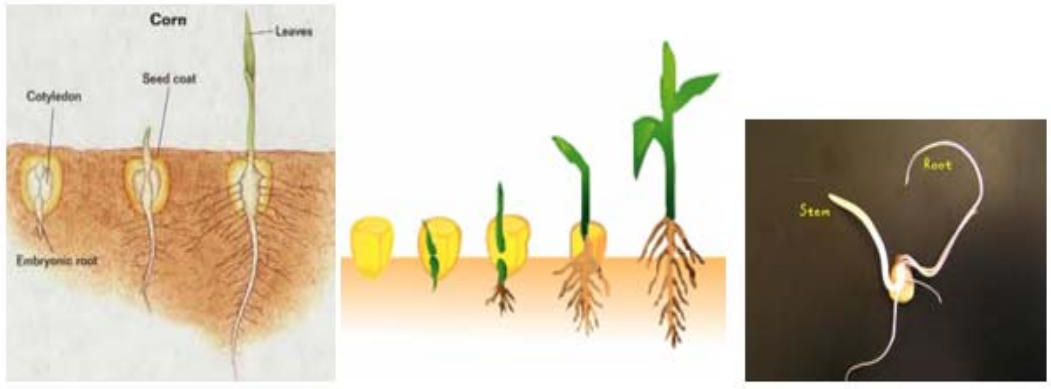

Figure 2:10 Stages of germination for corn seed diagram of Aspergillus spp. (Adapted from Klich, 2002)

Seed quality and vigor is maintained when proper storage is carried out. Seed viability is the ability of the seeds to survive, grow and multiply. In order for a seed to remain viable the embryo must be alive. Even though germination and seed vigor are used interchangeably, the official definition of seed vigor is "the sum total of those properties of the seed that determine the level of activity and performance of the seed during germination and seedling emergence" (ISTA, 2006). Seed quality comprises of the viability of the seed, germination percentage and seed strength. According to guidelines by ISTA (2006), seed vigor assessment involves direct (e.g. stress tests to evaluate germination percentage or seedling emergence) and indirect tests such as conductivity.

How well a seed grain tolerates stress tests provides an indication of its vigor. The cold test is a stress test that simulates unfavorable conditions for germination, and it is often used to assess vigor of corn seeds. Seed viability, seed dormancy and environmental effects influence germination percent. Germination percentage could be defined as the ratio of live seeds (seed that will germinate) relative to the total seeds in storage with the same storage conditions.

The germination process can be classified into three phases 1) water imbibition, 2) lag phase and 3) radicle emergence. The imbibition phase is also related to a slow increase in respiration until it reaches a plateau, which is usually followed by a sharp increase in respiration with radicle emergence (Christiansen, 1982). The metabolic activity that ensues from imbibition requires oxygen, and it is modulated by temperature. Immersing dry grains into water results in a period of rapid water imbibition followed by slower imbibition until the seeds are fully hydrated. When immersed in water, seeds leak metabolites such as organic compounds and inorganic salts, amino acids and proteins. The more vigorous seeds leak less of these metabolites compared to less vigorous seeds (Mathews and Bradnok, 1968; Copeland and McDonald, 2001). Electrical conductivity measurements can thus be used to quantify metabolite leakage and hence assess the vigor of a seed grain such as corn. Less vigorous seeds will lose the most amounts of metabolites and this can often manifest in death or damage to the seed's embryo (ISTA, 2006). The tetrazolium test is often used to assess seed vigor. It is based on the principle that seeds with living embryo will be brightly stained. This test involves removal of the seed coat, creation of an incision to expose the embryo and then immersed in a triphenyl tetrazolium chloride (TTC) solution, followed by a period of incubation. Examination of

the seeds after the incubation period will show the most vigorous seeds as the ones with the entire embryo stained (ISTA, 2006). Some of the tests outlined here will be used in assessment of germination in the proposed study and will be discussed in more detail in the methods section of this document.

In essence, water, oxygen and temperature are the main factors that enable germination of a seed grain. However, even when these three conditions are favorable for a particular seed product, some seeds may fail to germinate. Such seeds can be classified as dormant. Put another way, seed dormancy can be explained as the seeds inability to germinate under environmental conditions suitable for germination. Favorable conditions for germination and absence of dormancy determine seed viability. Hell et al. (2000) reported that corn seed quality is not only verified by the condition of grain. It can also be evaluated by the seeds ability to be planted the following season.

Factors affecting seed viability after storage are: temperature, water, light intensity and gas composition. The resilience of grains and seeds are highly influenced by seed maturity, environmental and moisture conditions (Christensen and Kaufman, 1969). The lower the moisture content and temperature the longer the allowable safe storage time for preservation of seed germination (Copeland and McDonald, 1985). The viability of most seeds are inversely related to storage time (Guberac et al, 2003; Pascual et al, 2006).

2.14.1 The effect of moisture content and temperature on germination

The seed life of grain is related to initial moisture content and its interaction with storage temperature. Temperature is a factor that depends on moisture level and storage

time. Germination decreased after corn seeds where stored at -10°C, 2.5°C and 10°C respectively (Gilbert et al., 1997). Table 2.6 shows that the storage life of seed is doubled for every 5°C drop in temperature within a 5°C - 25°C range and 1% drop in seed moisture.

Table 2:6 Maximum storage time (weeks) for corn w.r.t. germination (Adapted from Gilbert et al., 1997)

Temperature (°C)	Moisture Content (%)						
	12%	13%	14%	15%	15.5%	16%	18%
25	26	14	9	5	3.5	2	0.5
20	40	22	14	9	5.5	3	1.5
15	70	34	22	14	8.5	5	2.5
10	120	60	38	24	14.0	8	3.5
5	200	100	52	39	22.0	15	6.0

2.14.2 The effect of gas composition on germination

Germination is influenced by oxygen and carbon dioxide levels. Annis and Banks (1990) evaluated the relationship between germination and gas composition. Increase in carbon dioxide had the greatest effect on germination. The most lethal effect on germination is a combined increase in carbon dioxide and decrease or absence of oxygen. Storage time for maintaining viable seeds is much reduced in such situations and further shortened as temperature and moisture content increases.

2.14.3 The Effect of fungi on germination

The viability of seeds is affected by microflora. Grains infected by mold can no longer be used for planting purposes. High humidity and temperatures do not favor seed viability. Storage fungi invade the embryo of corn kernels when conditions are favorable. The embryo or germ may be totally infected by mold without visible evidence when moisture content is above safe levels (Christensen, 1982). The corn kernel is first weakened by the mold which later kills the germ. When mold attacks the grain, it kills off the living tissues of the embryo where it mostly functions as a parasite (Christensen, 1982). A small dose of isolates of *A. flavus* on grain will result in no seedling emergence. After the embryo and germ dies, discoloration with brown color surfaces (Christensen, 1982).

2.15 Grain damage

Kernels that are damaged can adversely affect grain quality and increase grain deterioration. Kernels that have sustained damage to the pericarp in the vicinity of either the crown, tip cap or germ pericarp have an increased susceptibility to invasion by microorganisms. The greater the sustained damage to a corn kernel the lower the recommended moisture level needed for safe storage.

Stroshine and Yang (1990) used Thompson model in a computer simulation which they used to examine the effects of physical damage and hybrids, on dry matter loss during drying of 22% m.c shelled corn. Multipliers such as theirs were used to correct allowable storage time for differences in grain damage and hybrid resistance to storage mold. Dry matter loss of the hybrids resistance to mold growth was consistently 30 to 40%

lower than DMLs for susceptible hybrids. They used the equation initially developed by Steele (1967), computed the damage multiplier from the equation, and then fitted data to an exponential equation having a coefficient of determination of 0.67.

2.16 Grain-Grading systems

Grade standards for grain were developed to facilitate marketing, identify economic factors important for end users and reflect storability (Hill, 1988). A grain grading system significantly influences the efficiency of storage. Grading systems ease trade in markets to meet buyer requirements and forces producers to meet the desired quality. Variation in physical appearance of grain, moisture content, fungal infection or discoloration of the grain affects the quality, price and storage life of grains. Corn quality is a major factor that affects the price for end users. The quality of corn produced by most farmers varies significantly due to differences in soils, climate, insects, and management practices with respect to harvesting, drying and storage.

Grain grades were established to allow buyers and sellers to settle on a common price by the guidance of rules that are commonly accepted and understood. The main principle is that end use of corn that drives the factors and tests are most important for measuring quality. The grading system is categorized into two: fair average quality system and the numeric system (CGC, 1993). The average quality system represents the average quality of the grain; shipments of grain are checked by third parties at their final destination (White and Johnson, 2003).

Sellers and buyers in the Mediterranean regions and Europe in the late 1800s normally met with the grain consignment and bargained until they had an agreement

(Commission et al., 1983). This usually had some drawbacks with regards to corn quality because the origin of the corn was only based on word communication. Regulations of grain quality started as a form of penalty or a fee. That is, when the quality is not as expected a penalty is normally collected. In 1856, the Chicago Board of Trade began establishing a grade for wheat and extended into southern Russia at the beginning of the 19th century. Hill (1990) reported that the U.S. Grain Standards act passed in 1916 authorized the U.S. Department of Agriculture (USDA) to develop standards, grading etc. to ensure quality of the grains. Grain Inspection, Packers and Stockyards Administration (GIPSA), a sub section of the USDA controlled the development, inspection and enforcement of the U.S. grain grades.

In the numerical grading system, corn grades are rated on a scale from U.S. Grade No. 1 to the poorest quality called sample grade. A tabulation of the different grades and their requirements are shown in Table 2.7.

Table 2:7 US Grades: Grades and Grade Requirements for Corn

		Maximum limits		
		Damaged Kernels		
U.S. Grade No.	Minimum Test Weight (Ib/bu)	Heat-Damaged kernels (%)	Total Damaged (%)	BCFM (%)
1	56.0	0.1	3.0	2.0
2	54.0	0.2	5.0	3.0
3	52.0	0.5	7.0	4.0
4	49.0	1.0	10.0	5.0
5	46.0	3.0	15.0	7.0
Sample[b]				

Adapted from the Official United States Standards for Grain (USDA-GIPSA, 2004)

In the US grain grading system there are four factors of measurement: test weight, heat-damaged kernels, total damaged kernels, and broken corn and foreign material (BCFM). A grade is lowered when any of the factor requirements are not met. The U.S. Federal grain standards define limits on fine material, heat damage, damage and test weight.

Other countries also have their grading system by which grain is traded. Like in the U.S., the EU countries and other developed countries such as Russia and Japan have developed grading systems for use in the trade. In Africa, grade systems have been developed by the standard organizations of individual African nations, some more

developed than others. However, these are not necessarily used by small producers (See Table 2.8 for Ghana grade standard).

In Ghana, located in western part of Africa, corn is graded according to the presence of other grains, degree of physical defect, foreign matter and fifth. The grade factors and safe moisture requirements are shown in Table 2.8 & 2.9.

Table 2:8 Ghana Grain standards (Corn)

Grade factors	Grade 1	Grade 2	Grade 3
Disease	0.5	0.5	0.5
Discolored	2.0	3.0	3.0
Broken / Chipped	5.0	6.0	10.0
Insect damaged	3.0	5.0	7.0
Stained	0.0	0.5	0.5
Germinated	0.0	0.5	0.5
Shriveled	1.0	2.0	3.0
Other grains	0.5	0.5	1.0
Total defective	11.0	16.0	22.5
Inorganic	0.0	1.0	2.0
Organic	1.0	2.0	5.0
Filth	0.5	0.5	1.0

(Adapted from Ghana Standards Board, 2003)

Table 2:9 Maximum moisture content storage at 70% RH and 27°C

Grain type	Safe moisture levels of grain (%)
Wheat	13.5
Maize	13.5
Paddy rice	15
Sorghum	13.5
Millet	16

Source: (Adapted from Brooker et al. 1992)

2.17 References

AACC. 2009. *AACC Approved Methods*. 9th ed. Vol. I. 02–03A: Fat acidity — Rapid method, for corn. St. Paul, Minn.: AACC.International, St. Paul, MN, U.S.A.

Addae-Mensah, N. K., & Ileleji, K. E. (2012). Corn Quality Changes in hermetic storage. *Lab Scale Pilot Studies*.

Annis, P., & Graver, J. S. (1987). Sealed stacks as a component of an integrated commodity management system: a potential strategy for continued bag-stack storage in the ASEAN region. *Evans, DE, and Wellisch. PK, ed., Grain storage in ASEAN: some research and development options. CSIRO Division of Entomology Report* (42).

Baker, D., Neustadt, M. H. & Zeleny, L. (1957) Application of fat acidity test as index of grain deterioration. *Cereal Chemistry.* 34, 226-233.

Banks, H. J., & Annis, P. C. (1990). Comparative advantages of high CO2 and low O2 types of controlled atmospheres for grain storage. *Food preservation by modified atmospheres*, 93-122.

Banks, H. (1981). Effects of controlled atmosphere storage on grain quality: a review. *Food Technol. Aust, 33*(7), 335-340.

Bottomley, R., Christensen, C. M., & Geddes, W. (1950). Grain storage studies DC. The influence of various temperatures, humidities, and oxygen concentrations on mold growth and biochemical changes in stored yellow Corn. *Cereal Chemistry, 27*(4), 271-296.

Brooker, D. B., Bakker-Arkema, F. W., & Hall, C. W. (1992). *Drying and storage of grains and oilseeds*: Van Nostrand Reinhold, New York .

Calderon, M. (1981). The ecosystem approach for apprehending the extent of postharvest grain losses. *Phytoparasitica, 9*(2), 157-167.

Calderon, M., & Navarro, S. (1979). Increased toxicity of low oxygen atmospheres supplemented with carbon dioxide on *Tribolium castaneum adults. Entomologia Experimentalis et Applicata, 25*(1), 39-44.

CAST. (2003). Mycotoxins: Risks in Plant and Animal Systems. Task Force Rep. 38. Council for Agricultural Science and Technology, Ames, IA.

Chakraverty, A. (2003). *Handbook of postharvest technology: cereals, fruits, vegetables, tea, and spices* (Vol. 93): CRC Press.

Chiappini, E., Molinari, P., & Cravedi, P. (2009). Mortality of *Tribolium confusum* J. du Val (Coleoptera: Tenebrionidae) in controlled atmospheres at different oxygen percentages. *Journal of Stored Products Research, 45*(1), 10-13.

Christensen, C., & Sauer, D. (1982). Storage of cereal grains and their products. American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Christensen, C. M., Mirocha, C. J., & Meronuck, R. A. (1977). *Molds, mycotoxins and mycotoxicoses* (Vol. 142): Agricultural Experiment Station Miscellaneous report 142. University of Minnesota, St. Paul, MN.

Christensen, C., & Kaufman, H. (1969). Mycotoxins and grain quality. *Grain storage: the role of fungi in quality loss. University of Minnesota, Press Minneapolis*, 76-93.

CGC. (1993). Quality control of Canadian grain. In: Grains and Oilseeds Handling, Marketing, Processing. 4th ed. Vol. 1. Winnepeg, MB, Edited by edited by Amalendu Chakraverty, Arun S. Mujumdar, Hosahalli S. Ramaswamy. Canadian International Grain Institute, pp. 289 – 308.

Chulze, S. (2010). Strategies to reduce mycotoxin levels in maize during storage: a review. *Food Additives and Contaminants, 27*(5), 651-657.

Canadian Grain Commission, & Commission canadienne des grains. (1983). The History and Evolution of the Western Canadian Wheat Grading and Handling System. Canadian Grain Commission.

Cooper, N. R., & Morrison, D. C. (1978). Binding and activation of the first component of human complement by the lipid A region of lipopolysaccharides. *The Journal of Immunology, 120*(6), 1862-1868.

Copeland, L. O., & McDonald, M. B. (2001). Copeland, L. O., & McDonald, M. B. (2001). Seed germination. In *Principles of Seed Science and Technology* (pp. 72-123). Springer US.

Copeland, L. 0, and McDonald, M. B. 1985. *Principles of Seed Science and Technology. 2nd ed. Burgess Publication Co., Minneapolis, MN*, 91-95.

Cox, P., and Bell, C. (1991). Biology and ecology of moth pests of stored foods. *Ecology and management of food-industry pests. FDA Technical Bulletin, 4*, 181-193.

Dirks, B. M., Boyer, P. D. Geddes, W. F. (1955). Some properties of fungal lipases and their significance in stored grain. *Cereal Chem, 32*, 356.

Dixon, N. M., and Kell, D. B. (1989). The inhibition by CO2 of the growth and metabolism of micro-organisms. *Journal of Applied Microbiology, 67*(2), 109-136.

Donahaye, E. (1990). Laboratory selection of resistance by the red flour beetle, Tribolium castaneum (Herbst), to an atmosphere of low oxygen concentration. *Phytoparasitica, 18*(3), 189-202.

FAO. (1994.). *Grain Storage Techniques, Evolution and Trends in developing countries*. FAO, Rome, Italy. 1994, 1-23, 25-39, 41-65, AND 165- 187

FAO. (2001). *Food and Agricultural Organization of the United Nations.* FAO, Rome, Italy.

FAOSTAT, http://faostat.fao.org/default.aspx (2010)

Fields, P. G. (1992). The control of stored-product insects and mites with extreme temperatures. *Journal of Stored Products Research, 28*(2), 89-118.

Finkelman, S., Navarro, S., Rindner, M., Dias, R., & Hezy, T. (2003). Practical aspects to the use of vacuum as a method to control storage insects. In *Proc. Annu. Int. Res. Conf. on Methyl Bromide Alternatives and Emission Reductions.(San Diego, CA.) GL Obenauf, Ed. Methyl Bromide Alternatives Outreach, Fresno, CA* (pp. 74-1).

Ghana Meteorological Service. (1981 – 2011). Annual Summary of Observations in Ghana. Ghana Meteorological Service, Accra, Ghana

Gilbert, J., Tekauz, A., & Woods, S. (1997). Effect of storage on viability of Fusarium head blight-affected spring wheat seed. *Plant disease, 81*(2), 159-162.

Godfray, H. C. J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C. (2010). Food Security: The Challenge of Feeding 9 Billion People. *Journal of the American Association for the Advancement of Science,12 February 2010: Vol. 327 no. 5967 pp. 812-818 DOI: 0.1126/science.1185383*

Goodman, J., & Christensen, C. M. (1952). Grain storage studies. XI. Lipolytic activity of fungi isolated from stored corn. *Cereal Chem, 29*, 299-308.

Gong, Y., Hounsa, A., Egal, S., Turner, P. C., Sutcliffe, A. E., Hall, A. J., . . . Wild, C. P. (2004). Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. *Environmental health perspectives, 112*(13), 1334.

Guberac, V., Maric, S., Lalic, A., Drezner, G., & Zdunic, Z. (2003). Hermetically sealed storage of cereal seeds and its influence on vigour and germination. *Journal of Agronomy and Crop Science, 189*(1), 54-56.

Halstead, D. (1986). Keys for the identification of beetles associated with stored products. I—Introduction and key to families. *Journal of Stored Products Research, 22*(4), 163-203.

Hell, K., Cardwell, K., Sétamou, M., & Poehling, H. (2000). Maize storage practices and their influence on aflatoxin contamination in stored grains in four agroecological zones in Bénin, West-Africa. *J. Stored Prod. Research, 36*, 365-382.

Hill, L. D. (1990). *Grain Grades and Standards: Historical issues shaping the future*. University of Illinois Press.

Hill, L. D. (1988). Grain Grades: They Lack Economic Rational. *Choices*, *3*(1).

Hodges, R. (2011). Missing Food: The case of Postharvest Grain Losses in Sub-Saharan Africa The International Bank for Reconstruction and Development / The World Bank. Report No. 60371-AFR

Howe, R. (1965). The biology of the two common storage species of *Oryzaephilus* (Coleoptera, Cucujidae). *Annals of Applied Biology, 44*(2), 341-355.

Huxoll, C. C. 1961. Some environmental factors influencing deterioration of high moisture shelled corn in hermetic storages. MSc Thesis. West Lafayette, Indiana: Purdue University.

IITA (2007). International Institute of Tropical Agriculture, Ibadan, Nigeria. Retrieved on June 23rd, 2012 at http://www.iita.org/web/iita/publications

IITA (2010). International Institute of Tropical Agriculture, Ibadan, Nigeria. Retrieved on June 23rd, 2012 at http://www.iita.org/web/iita/publications

Ileleji, K. E., Maier, D. E., & Woloshuk, C. (2007) Evaluation of different temperature management strategies for suppression of Sytophillus zeamais (Motschulsky) in stored maize. *Journal of Stored Products Research 43*(4), 480-488.

Ileleji, K., Wilcke, W., & Morey, R. (2003). Relative storability of high-oil and Bt corn hybrids compared to conventional hybrids. *Transactions of the ASAE, 46*(2), 407-414.

ISTA (2006) *International Rules for Seed Testing*. Edition 2006. International Seed Testing Association, Switzerland.

Kang'ethe, W. G. (2004) Agricultural development and food security in sub-Saharan Africa (SSA). *Food and Agriculture Organization (FAO). Rome. Italy*.

Kashi, K. P. (1981). Controlling pests in stored grain with carbon dioxide. *Span; progress in agriculture*.

Klich, M. A., & Center, U. S. A. R. S. S. R. R. (2002). *Identification of common Aspergillus species*: Centraalbureau voor Schimmelcultures Utrecht.

Lamboni, Y., & Hell, K. (2009). Propagation of mycotoxigenic fungi in maize stores by post-harvest insects. *International Journal of Tropical Insect Science, 29*(1), 31-39.

Loewer, O. J., BRIDGES, T., & BUCKLIN, R. On-farm drying and storage systems. 1994. et al. *American Society of Agricultural Engineers*.

Lynch. R.E. and Wilson. D.M. (l991) Enhanced infection of peanut, Arachis hypogaea L.., seeds with *Aspergillus flavus* group fungi due to external scarification of peanut pods by lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller). Peanut Sci. 18, 110-116

Magan, N., Aldred, D. 2007. Why do fungi produce mycotoxins? In "Food Mycology: a multifaceted approach to fungi and food". Eds. J.Dijksterhuis and R. Samson. CRC Press,Boca Raton, FL, USA.

Magan, N., & Olsen, M. (2004). *Mycotoxins in food: Detection and control*: Woodhead Publishing.

Magan, N., & Lacey, J. (1988). Ecological determinants of mould growth in stored grain. *International Journal of Food Microbiology, 7*(3), 245-256.

Marín, S., Velluti, A., Ramos, A. J., & Sanchis, V. (2004). Effect of essential oils on zearalenone and deoxynivalenol production by *Fusarium graminearum in* non-sterilized maize grain. *Food microbiology, 21*(3), 313-318.

Mason, L. J., & Strait, C. A. (1998). Stored product integrated pest management with extreme temperatures (pp. 141-177): Westview Press, Boulder.

Matthews, S., & Bradnock, W. (1968). Relationship between seed exudation and field emergence in peas and French beans. *Hort. Res, 8*, 89-93.

Milner, M., & Geddes, W. (1954). Respiration and heating. *Storage of cereal grains and their products, 152-220*.

Moreno-Martinez, E., Jiménez, S., & Vázquez, M. E. (2000). Effect of *Sitophilus zeamais* and *Aspergillus chevalieri* on the oxygen level in maize stored hermetically. *Journal of Stored Products Research, 36*(1), 25-36.

Moreno, E., Benavides, C., & Ramirez, J. (1988). The influence of hermetic storage on the behavior of maize seed germination *Seed Science and Technology, 16*, 427-434.

Multon, J. (1988). Interactions between water and the constituents of grains, seeds and by-products. In JL Multon, AM Reimbert, D Marsh, AJ Eydt, eds, Preservation and Storage of Grains, Seeds and Their By-Products. Lavoisier Publishing, New York, pp 89–159

Munkvold, G. P. (2003). Epidemiology of Fusarium diseases and their mycotoxins in maize ears. *European Journal of Plant Pathology, 109*(7), 705-713.

Meronuck, R. A. and Stuckey, R.E.., (1988). The significance of fungi in cereal grains, pp. 8- 20, Proc. Stored Grain Managrement: Insects, Molds, Engineering, and Economics, Cooperative Extension Service, Division of Agriculture, Oklahoma State University, Circular E- 872.

Mills, J. T. (1991). Ecology and control of organisms decomposing stored foods, pp 33-56, in Ecology and Management of Food-Industry Pests, (Gorham, R. J., Ed.), Association of Official Analytical Chemists, Arlington, VA.

Murdock, L. L., Magam, V., Baoua, I., Baffe, S., Shade, R. E. (2012). Death by desiccation: Effects of hermetic storage on cowpea bruchids *Journal of Stored Products Research 49:166-170*

Navarro, S., & Donahaye, J. (2005). Innovative environmentally friendly technologies to maintain quality of durable agricultural produce. *Environmentally Friendly Technologies for Agricultural Produce Quality, CRC Press, Taylor & Francis Group, Boca Raton, FL*, 205-262.

Navarro, S. (2001). *The mechanics and physics of modern grain aeration management*: *CRC Press, Taylor & Francis Group, Boca Raton, FL,* 672 pp.

Navarro, S., & Calderon, M. (1980). Integrated approach to the use of controlled atmospheres for insect control in grain storage. *Amsterdam, Elsevier*, 73-78.

Navarro, S. (1978). The effects of low oxygen tensions on three stored-product insect pests. *Phytoparasitica, 6*(2), 51-58.

Pascual, B., San Bautista, A., López-Galarza, S., Alagarda, J., & Maroto, J. (2006). Germination behaviour after storage of caper seeds. *Seed Science and Technology, 34*(1), 151-159.

Pomeranz, Y. (1985). Functional properties of food components. *Functional properties of food components.* New York: Academic Press, Inc.

Rembold, F., Hodges, R., Bernard, M, Knipschild H, Leo, O. (2011). An innovative framework to analyse and compute quantitative postharvest losses for cereals under different farming and environmental conditions in East and Southern Africa . The African Postharvest Losses Information System (APHLIS) (pp. 1–73).

Richard-Molard, D. (1988). General characteristics of the microflora of grains and seeds and the principal resulting spoilages. In: Multon, J.L. (Ed.), Preservation and Storage of Grains, Seeds, and their By-products. Lavoisier Publishing, Inc., New York, NY, pp. 226–243.

Rodríguez, J., Bartosik, R., Malinarich, H., Exilart, J., Nolasco, M., & Balcarce, E. E. A. I. (2002). Grain storage in plastic bags: the silobag system. *EEA INTA Soyabean final Report Balcarce, Argentina*.

Ross, I. J., Hamilton, H. E., & White, G. M. (1973). Principles of Grain Storage. In L. O. J.:, B. T. C.; & B. R. A. (Eds.), *On-Farm Drying and Storage Systems* (pp. 171-185). University of Kentucky , Lexington: Cooperative Extension Publication AEN - 20.International Working Conference on Stored-Products Protection, Tel Aviv, Israel, Maor-Wallach Press, Caspit, Jerusalem, Israel.

Shotwell, O. (1977). Aflatoxin in corn. *Journal of the American Oil Chemists' Society, 54*(3), 216-224.

Sinha, K. K., & Sinha, A. K. (1992). Impact of stored grain pests on seed deterioration and aflatoxin contamination in maize. *Journal of Stored Products Research, 28*(3), 211-219.

Steele, J. L. 1967. Deterioration of damaged shelled corn as measured by carbon dioxide production. PhD thesis. Ames, Iowa: Iowa State University.

Stroshine, R., & Yang, X. (1990). Effects of hybrid and grain damage on estimated dry matter loss for high-moisture shelled corn. *Transactions of the ASAE, 33*(4), 1291-1298.

Tipples, K. H. (1995). Quality and nutritional changes in stored grain. *Stored-grain ecosystems*, 189-202.

Tuite, J., Koh-Knox, C., Stroshine, R., Cantone, F. A., and Bauman, L. F. 1985. Effect of physical damage to corn kernels on the development of *Penicillium* spp. and *Aspergillus glaucus* in storage.*Phytopathology* 75:1137.

Wicklow, D.T., Horn B. W., Shotwell O.L. and Hesseltine C. W. and Caldwell R.W (1988). Fungal interferance with Arspergillus flavus infectio{Sinha, 1992 #591}n and aflatoxin contamination of maize grown in a controlled environment. *Phytopathology* 78, 68-74.

Weinberg, Z., Yan, Y., Chen, Y., Finkelman, S., Ashbell, G., & Navarro, S. (2008). The effect of moisture level on high-moisture maize (Zea mays L.) under hermetic storage conditions--in vitro studies. *Journal of Stored Products Research, 44*(2), 136-144.

White, P. J., & Johnson, L. A. (2003). *Corn: chemistry and technology* (No. Ed. 2). American Association of Cereal Chemists.

Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., & Aggarwal, D. (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *The American Journal of Clinical nutrition, 80*(5), 1106.

Wilson, S., & Desmarchelier, J. (1994). Aeration according to seed wet-bulb temperature. *Journal of Stored Products Research, 30*(1), 45-60.

Yakubu, A., Bern, C., Coats, J., & Bailey, T. (2010). Non-chemical on-farm hermetic maize storage in east Africa. *Julius-Kühn-Archiv*(425), S. 338.

CHAPTER 3. QUALITY CHANGES CAUSED BY FUNGI IN HERMETIC STORED CORN AT MID-MOISTURE LEVELS UNDER COOL AND WARM CONDITIONS

3.1 Introduction

Small-scale farmers in sub-Saharan Africa own about 2 ha of land on average as compared to much larger portions (≥ 100 ha) owned by fellow farmers in developed countries (MOFA, 2011). In the sub-Saharan African (SSA) region the average corn yields between the year 2005 and 2008 was about 1.4 t/ha (Smale et al. 2011).

About 21 years ago, land used for the cultivation of corn increased annually at a steady rate of 1.09 %, while in 2009 land for corn production was increasing at a steady rate of 2.66% (FAOSTAT, 2011).

Price fluctuations largely depend on the harvest season, with low prices usually occurring right after harvest. These price fluctuations vary across the sub-region of Africa. They are influenced by factors such as storage costs which eventually affect corn production (Jones, 2011). When losses are incurred storage cost increases so when storage is done properly it improves farmer's profits.

Small scale farmers from the leading producing areas of corn (maize) in regions of Ghana near Kumasi (6°43'N 1°36'W) such as Ejura Sekyeredumase, Nkoranza, Kintanpo, Afram Plains and Techiman have been challenged by the ability

to dry down corn to safe moisture levels of 13% or below. The primary reason is that the use of sun drying, which is the common practice during the major harvest season (July to August) is inefficient in drying corn because of the limited daily sunshine hours (i.e. $\leq$ 2.6 hrs.) and high ambient relative humidity (about 80% on average) influenced by the major rainy season during this period (Ghana Meteorological Service, 2011).

Anecdotal evidence indicates that corn harvested above 30% moisture is normally dried to moistures between 15 and 21% using open-air drying, but not as low as 13% or below for long-term safe storage under humid conditions. Because farmers typically have no access to moisture meters to measure grain moisture content, they determine grain moisture using subjective means such as crushing between their teeth or shaking a handful of kernels in their palm. This means that there is high probability that poorly dried grain goes into storage and becomes moldy with time in storage.

Storing insufficiently dried corn at mid-moisture range (15 - 21%) in hermetic or non-hermetic storage presents conditions that are favorable for the proliferation of xerophilic toxin produced by storage mold. One of the common toxins produced by molds known to contaminate stored corn in parts of Africa is aflatoxin (Hell et al., 2011). Aflatoxin is a secondary metabolite produced by *aspergillus flavus* and *aspergillus parasiticus*. Aflatoxin is highly toxic and suppresses the immune system (Ellis et al., 1991). It can cause chronic damage to the liver and kidney, and the digestive and nervous systems. Aflatoxins can also interfere with the reproductive system of humans. According to Farombi (2006), in certain parts of Africa liver

cancer (Hepatocellular carcinoma) caused by aflatoxins contributed to 70% of cancer related deaths. Consuming corn products that are contaminated with aflatoxin (often not discernible to the naked eye) can cause adverse health effects to adults and infants.

Significant research has been published on the effectiveness of hermetic storage in controlling stored pests (Navarro et al., 1994; Moreno-Martinez et al., 2000; Emekci et al., 2001; Quezada et al., 2006; De Carli et al., 2010; Murdock et al., 2012). Hermetic storage has been used commercially for storage of corn, rice, cocoa, coffee and sunflower seeds (Villers et al., 2010). The system has also been found to be successful in storing cowpea that is grown in the Sahel region as reported by Murdock et al. (2012) where the grain is harvested sufficiently dry and suitable for long periods of storage. In general hermetic storage is preferred for storing sufficiently dry grain than wet grain (Banks et al., 1981; Moreno et al., 1988). The rate of mold growth tends to decrease over storage time under hermetic conditions compared to non-hermetic storage (Dixon and Kell, 1989; Magan and Lacey, 1988).

When there is limited oxygen supply in hermetic storage some microorganisms may continue to develop by partially decomposing carbon dioxide, producing lactic acids, acetic acids and alcohols known as fermentation (Sauer et al., 1992; Weinberg et al., 2008). There is a gap in knowledge particularly on the efficacy of hermetic storage on mold suppression at mid-moisture levels between 15% – 21% Villers et al. (2010), as well as aflatoxin contamination. The objective of this study was to investigate the quality changes due to fungi deterioration of corn stored hermetically at 11%, 15%, 18% and 21% moisture levels under 10^{o}C and 25^{o}C indicated by kernel

germination, mold count, fat acidity, aflatoxin contamination and corn chemical composition.

3.2 Materials and Methods

3.2.1 Experimental design

Quality changes were measured by monitoring changes in germination, mold growth, aflatoxin levels, fat acidity, and nutritional content before and after storage for 2, 4 and 6 month periods. Oxygen, carbon dioxide and the grain temperature of the storage environment in the one liter jars used were monitored and used to explain changes in corn quality. This study used a repeated measure factorial design to evaluate the effect of temperature, moisture content, storage type (hermetic vs. non-hermetic-aerobic) and storage time on corn quality measures such as mold count, aflaxtoxin levels, seed germination, fat acidity and nutritional content. Corn was stored at four moisture levels (11% as control, 15, 18, and 21%) under two temperature conditions (10 and 25°C) for three storage periods (2, 4 and 6 months). During the storage tests, temperature was measured every two hours, while oxygen and carbon dioxide were measured weekly. All destructive and nondestructive quality tests were performed at the end of the storage period on pooled samples of two replications per treatment/storage condition and period.

3.2.2 Sample source and preparation

Shelled corn used for the experiment was of U.S. grade no. 1 yellow dent corn of hybrid "6297 and 6333 VT RIB". The grain was purchased from a seed supply company, Ceres Solution in Lafayette, Indiana, US at approximately 12% moisture content. In order to describe the storability of the grain, characterize product yield and grain quality, sub-samples were taken to Titus Grain Inspection Service (Tipton, Indiana), a local licensed grain grader for grade classification. Corn was kept in cold storage (-20°C) in January of 2013 to suppress or stop any mold from proliferating, as well as eliminate any prior insect infestation should there be any. Prior to preparation of the samples for the experiment, the samples were removed from cold storage and allowed to thaw for 24-h in a 0 - 4°C cooler. The samples were then cleaned over a 4.76-mm (12/64-in.) round–hole sieve to remove fines and foreign material leaving small broken and chipped kernels. Sub-samples were divided using a boerner divider (Seedburo Equipment Co., Chicago, IL, USA) in order to obtain a representation of the samples.

An estimate of moisture levels in the bags holding the shelled corn was conducted using a moisture meter, Grain Analyzer Computer (GAC) 2100 (Dickey-John Corporation, Auburn, IL) and the moisture content was found to be 12.5 $\pm$ 0.7%. Later, samples were measured using the air-oven standard (ASABE Standards, 2000) and the moisture content was about 11% (10.8 $\pm$ 0.1%). To conduct the storage tests, corn was rewet to target mid-moisture levels of 15% (14.6 $\pm$ 0.1%), 18% (17.8 $\pm$ 0.0%) and 21% (21.2 $\pm$ 0.2%) using the methodology according to Ileleji et al. (2003). The values in parenthesis were the exact final moistures achieved after rewetting.

Corn was conditioned by mixing in a mechanical roller for two hours using calculated amounts of distilled water and stored in plastic bags in a 0 - 4°C refrigerator for 72 hours to complete moisture equilibrium. The final moisture after rewetting was determined upon the equilibration period using the air-oven standard method ASABE S352.2 (ASABE Standards, 2000).

Initial quality assessment was conducted before the samples were placed in storage. The samples before storage tests had a percent germination of 99%, aflatoxin levels were below detection level (0 – 2.5 ppb) using QuickScan QuickTox Kit , mold counts were below 2 CFUs by plating , and fat acidity value of 20 by AACC Standard Method 02–03A procedures. The nutritional content determined by using INFRATEC 1229 NIR transmittance equipment (Foss Tecator AB, Höganäs, Sweden) were 60.4%, 8.6% and 3.1% for starch, protein and oil, respectively.

3.2.3 Storage tests

Shelled-corn samples (650 g each) were conditioned to the target moisture levels and were placed in 1L glass jars (height of 0.23 m and diameter of 0.1 m) that were hermetically sealed and not hermetically sealed (aerobic conditions). Two replications per treatment were stored for 2, 4 and 6 months periods, after which they were removed from storage for analyses. The corn samples in the jars occupied approximately 70-80% by volume of the jars with some amount of headspace present. Two tiny holes were drilled in the lids and covered with septum to allow for continuous temperature monitoring using a thermocouple and intermittent gas sampling at regular time intervals as shown in Figure 3.1. Temperature in the grain

was monitored every hour at the center of the grain mass using thermocouples connected to 2625 Hydra data loggers (Fluke Corporation, Everett, WA 98203) and HOBO data loggers were used to monitor temperature conditions in the temperature chambers (Percival Scientific Inc., Boone, IA). Carbon dioxide and oxygen were measured using the Mocon PAC Check® Model 325 headspace analyzer (Mocon, Minneapolis, MN, USA) on air pulled with a hypodermic needle positioned a few millimeters below the bottom of the jar. The analyzer which uses an automatic internal sampling pump measures levels of oxygen and carbon dioxide concentration of the air within the grain mass through an infrared and electro-chemical cell. The analyzer was used 30 min after self-calibration against ambient conditions and the filters were replaced every two weeks. Calibration of the Mocon device was validated every week throughout the entire period of the experiment. Gas composition was measured every 7 days from the beginning to the end of the storage tests for all moisture treatments and storage periods. Since 4 ml sample of air was drawn for every reading taken the storage jars were allowed to establish air equilibration with the atmosphere after every sample reading.

Each of the storage jars containing corn was classified as an experimental unit. Jars were placed in separate temperature chambers (Percival Scientific Inc., Boone, IA) set to 10°C and 25°C to stimulate a comparison between temperate and tropical climates respectively. The experimental units (jars) were placed in the environmental chamber after the center of the grain bulk in the jars had warmed up from thawing under 0-4^{o}C in the cooler to room temperatures (20°C to 25°C). For the hermetic jars, airtight lids were placed over the jars to seal air-tight while Whatman filter paper and

mesh wire fabric were placed on the non-hermetic jars. The non-hermetically sealed jars were used as the control. The filter paper and mesh allowed the exchange of air creating aerobic storage conditions, and were removed only after the storage tests were completed.

Half of the storage jars in each storage chamber were then placed in constructed Styrofoam insulation which was done to investigate whether mold growth would be accompanied by a marked temperature rise (hot spot). Because the jars were relatively small, the insulation prevented heat loss from the jar. Although relative humidity data was not measured in the jars, moisture content and weight of dry matter was measured before and after storage. Because of the relatively small headspace volume in the jars compared to the quantity of corn (20-30%), it is accurate to assume that the air in the jar headspace came to equilibrium with the corn moisture. At the end of the experiment, grain samples from both replications were separately tested for quality indicated by kernel germination, mold count, fat acidity, aflatoxin contamination and corn chemical composition, and the average determined.

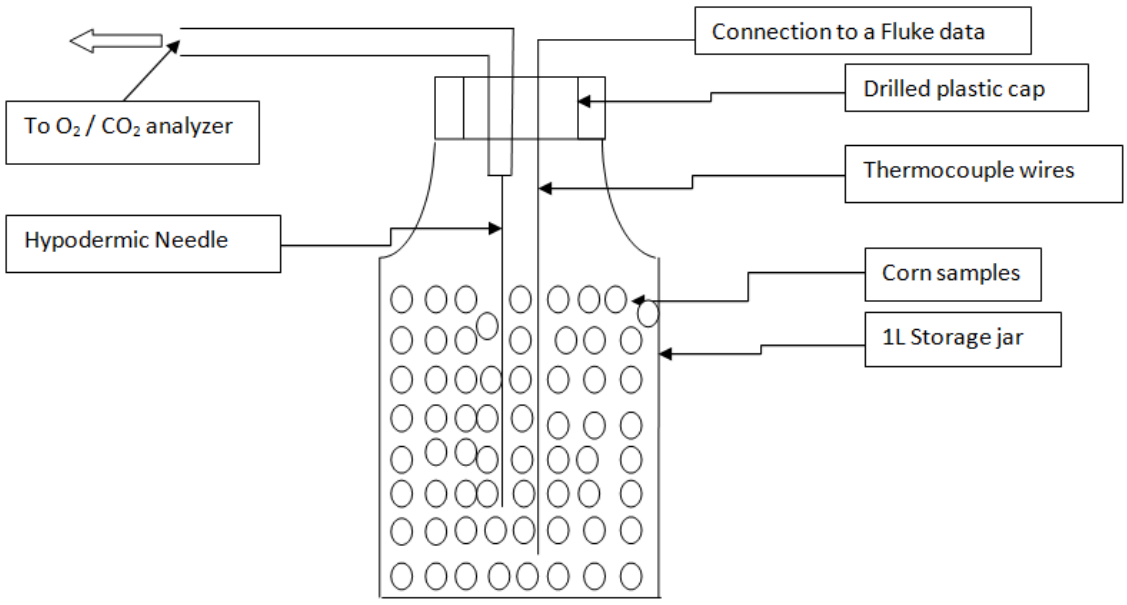

Figure 3:1 Experimental set-up of storage jar

3.2.4 Microbiological analysis

After the storage tests for the respective storage periods, the kernels were removed from storage and dried down using a forced-air oven at 28°C for microbiological analyses. Microbiological analyses were conducted using two methods: (1) kernel plating to isolate and identify the type and kind of fungi that was growing on the kernels and (2) serial dilution to determine the total mold count by counting the colony forming units (CFUs).

Each replication per storage treatment was divided into 3 sub-samples using a boerner divider to ensure equal representation of mold samples. About 30 g of the samples were poured into a 250 ml container; 50 ml of 0.05% sterilized Triton-X-100 solution was added to the mixture and shaken for 1 min. The mixture was then decanted with the solution poured into 50 ml corning tubes and placed in a freezer at -20°C to be used for serial dilution. Kernels were then plated by surface sterilizing

with 100 ml of sodium hypochlorite (NaClO), and shaken for 1 – 2 min. to remove most of the external contaminants. Afterward, the bleach was poured off and about 200 ml of distilled water was added and stirred for about 1 min to wash off the bleach. 50 corn kernels was plated on mal salt agar (MSA) media and incubated at 30°C for a period of 5 days. Malt salt agar was used because of its ability to isolate storage fungi, its high osmotic concentration to isolate highly osmophillic fungi mainly Aspergillius spp., its inhibition of most bacteria and its ability to discourage growth of non-storage fungi. The MSA medium contained 2% malt extract, 6% sodium chloride and 2% agar (Moreno-Martinez et al., 2000). The medium was autoclaved for 15-20 minutes because of the acidic nature of the medium. Ten kernels were plated in each petri dish and incubated in a 30 $\pm$ 1°C chamber. After 5-7 days, morphological identification of fungi associated with kernels was done by scraping mycelia advancing from margins of the grains with a pick. The mycelia were mounted on slides for microscopic examination. Identification of the isolates was based on color of shooting spores, morphology of mycelia and the arrangement of conidia structures (Tuite et al., 1985; Pitt and Hocking, 1997; Klich, 2002). Mold counts were performed before and after storage. The total mold count was performed by serially diluting the contents of the 50 ml corning tubes on the MSA media to 1:100 dilution factors. 100 µl of each dilution were plated. After 5-7 days of incubation, colonies were counted giving CFU values at 30 $\pm$ 1°C.

3.2.5 Aflatoxins Analyses

Aflatoxin analysis was conducted for corn samples before and after the storage. Aflatoxin was determined using the QuickTox Kit for QuickScan (Envirologix Inc., Portland, ME). About 20 g of corn was grinded and sieved using # 20 screen size. 2 ml/g of 50% ethanol in water was used to extract aflatoxins. The samples were shaken by hand for 2 min and allowed to settle into separate layers. 100 µl corn sample extract was mixed with 100 µl dilution buffer (standard aflatoxin-enzyme solution). After it was stirred and test strips where placed into the vials. After 5 minutes, part of the strips was placed into the Quick Scan reader for aflatoxin determination in parts per billion (ppb).

3.2.6 Fat Acidity

Fat acidity tests were conducted on kernels before (0 month) and after (2, 4 and 6 months) storage tests. Fat acidity was determined according to AACC Standard Method 02–03A for determining the fat acidity in corn (AACC, 2009). The procedure was carried out by extracting free fatty acids from 20-g of ground corn samples using 50 ml purified grade toluene and titrated the extract with CO_2 –free standard solution of 0.0178N KOH. Three titrations (three replicates) from 20-g corn samples each were conducted. Fat acidity values obtained for each 20-g ground corn sample were averaged and represented as milligrams of KOH required to neutralize free fatty acids from 100-g corn.

3.2.7 Germination

Germination tests were conducted on corn samples before and after storage according to methods by Moog (2006). Seed germination was determined by placing 200 kernels in trays. De-ionized water was used to moisten the kernels lined with paper towels and wrapped with plastic linings to minimize evaporation. Kernels that germinated were counted and germination percentage calculated by determining the ratio of germinated kernels to the total number of kernels multiplied by a 100.

3.2.8 Nutritional content (Chemical composition)

A Infratec^{TM} 1229 (Foss Tecator AB, Höganäs, Sweden) grain analyzer was used to determine the chemical composition of kernels before and after storage tests. The analyzer measured the starch, protein, oil on an 'as is' basis. It is important to note that the results obtained using this device was "AS IS" values for the constituents.

3.2.9 Statistics

Analysis of Variance (ANOVA) and multiple linear regression was performed to evaluate the effect of: initial moisture content, storage time, temperature and storage type on different measures of corn quality: 1) mold counts (CFUs), 2) aflatoxin, 3) germination (%), 4) fat acidity and 5) chemical composition (%).

3.3 Results

This study evaluated quality changes in hermetically stored corn at mid-moisture levels (15-21%) under tropical storage conditions. This section discusses the results on

the effect of moisture content, temperature, storage time and gas composition on the major quality measures: mold, aflatoxin and germination, fat acidity, and nutritional content (chemical composition).

3.3.1 Moisture content

Table 3.1 shows the mean and standard deviation of two replications of moisture content before and after 2, 4 and 6 months of storage, respectively. The initial moisture content after rewetting corn to the target moistures of 11%, 15%, 18% and 21% were 10.8%, 14.6%, 17.8% and 21.2%, respectively. For consistency and to avoid confusion, the target moistures were used in reporting the data.

Table 3:1 Moisture content (M.C.) (Mean (%) ± (SD)) over storage periods at temperatures of 10 and 25°C

Storage time (months)	10°C		25°C	
	Hermetic	Non-hermetic	Hermetic	Non-hermetic
11% initial M.C.				
0	10.80 ± 0.00	10.80 ± 0.00	10.80 ± 0.00	10.80 ± 0.00
2	11.44 ± 0.33	10.47 ± 0.16	11.61 ± 0.20	10.88 ± 0.61
4	11.43 ± 0.54	11.99 ± 0.59	11.70 ± 0.08	11.54 ± 0.66
6	10.95 ± 1.37	13.04 ± 1.04	11.58 ± 0.15	11.83 ± 1.34
15% initial M.C.				
0	14.60 ± 0.00	14.60 ± 0.00	14.60 ± 0.00	14.60 ± 0.00
2	16.88 ± 0.14	15.55 ± 0.21	16.56 ± 0.13	12.82 ± 0.42
4	16.68 ± 0.10	16.03 ± 0.06	17.81 ± 0.38	12.76 ± 0.11
6	16.79 ± 0.17	16.69 ± 0.05	19.34 ± 0.43	11.68 ± 0.40
18% initial M.C.				
0	17.80 ± 0.00	17.80 ± 0.00	17.80 ± 0.00	17.80 ± 0.00
2	18.93 ± 0.02	17.27 ± 0.02	18.87 ± 0.23	15.67 ± 0.71
4	19.07 ± 0.08	17.43 ± 0.12	20.86 ± 1.27	14.15 ± 0.12
6	19.49 ± 0.12	18.03 ± 0.01	21.00 ± 1.51	13.28 ± 0.08
21% initial M.C.				
0	21.20 ± 0.00	21.20 ± 0.00	21.20 ± 0.00	21.20 ± 0.00
2	23.39 ± 0.31	21.12 ± 0.57	22.51 ± 0.34	35.42 ± 5.42
4	22.26 ± 0.52	30.68 ± 0.98	24.68 ± 0.37	38.20 ± 15.02
6	24.80 ± 1.82	41.15 ± 7.01	24.95 ± 1.61	46.78 ± 6.96

Post storage moisture content for hermetically stored corn increased. For non-hermetic storage, moisture content after storage slightly increased for 10°C for all moistures and at 25°C moisture levels decreased for 15 and 18% mc except 11 and 21% mc.

3.3.2 Temperature profiles during storage

Temperature was measured hourly in both non-insulated and insulated jars to monitor

the rise in temperature caused by mold growth for the different initial moisture contents of corn stored at 10°C and 25°C. Because the jars were small with about 650 g of corn, we insulated one of the replicate jars to prevent rapid heat dissipation from within the small corn (grain) mass, so as to capture any heat rise due to hotspot development from molding corn. Temperature data points were plotted using 24 h averages. Figure 3.2 and 3.3 depicts temperature data measured in both non-insulated and insulated jars at different initial moisture content levels. The spikes in the chamber temperature data were due to the opening and closing of the chamber for data collection. These figures show that at 10°C, there was a small but significant difference statistically between the chamber temperature and that of the insulated and non-insulated jars for all the moisture contents.

3.3.3 Gas composition during storage

Fig 3.4 and 3.5 shows production of carbon dioxide and oxygen within the hermetic and non-hermetic jars at each initial moisture level period from the beginning (i.e. day zero) to the end of 6 months of storage. The general trend was as expected, the higher the initial moisture content the higher the rate of respiration and consequently the higher the amount of carbon dioxide produced and oxygen depleted.

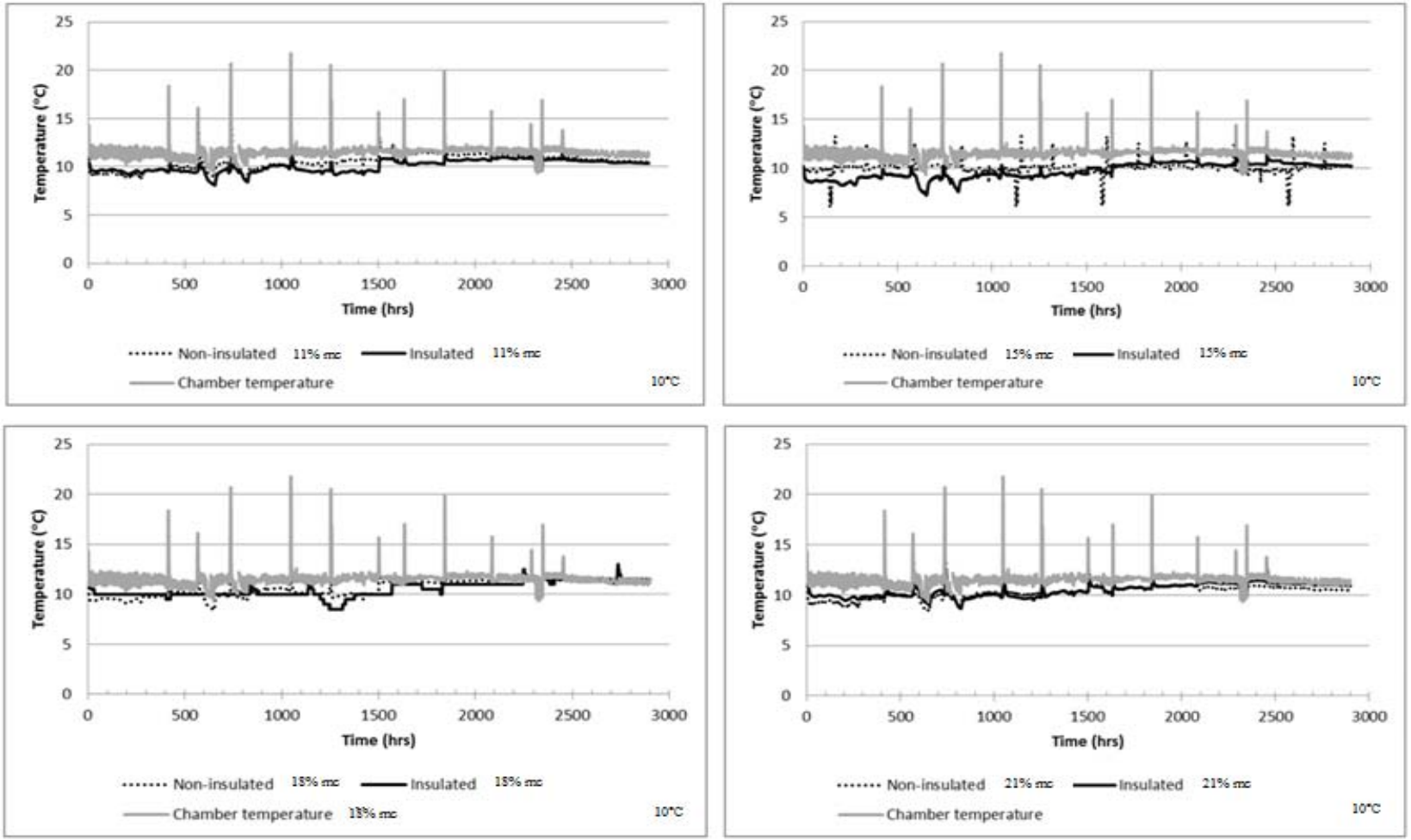

Figure 3:2 Response of temperature to mold across storage time for 11 % MC (top left panel), 15 % MC (top right panel), 18% MC (bottom left panel) and 21% MC (bottom right panel) at 10°C for both insulated and non-insulated jars

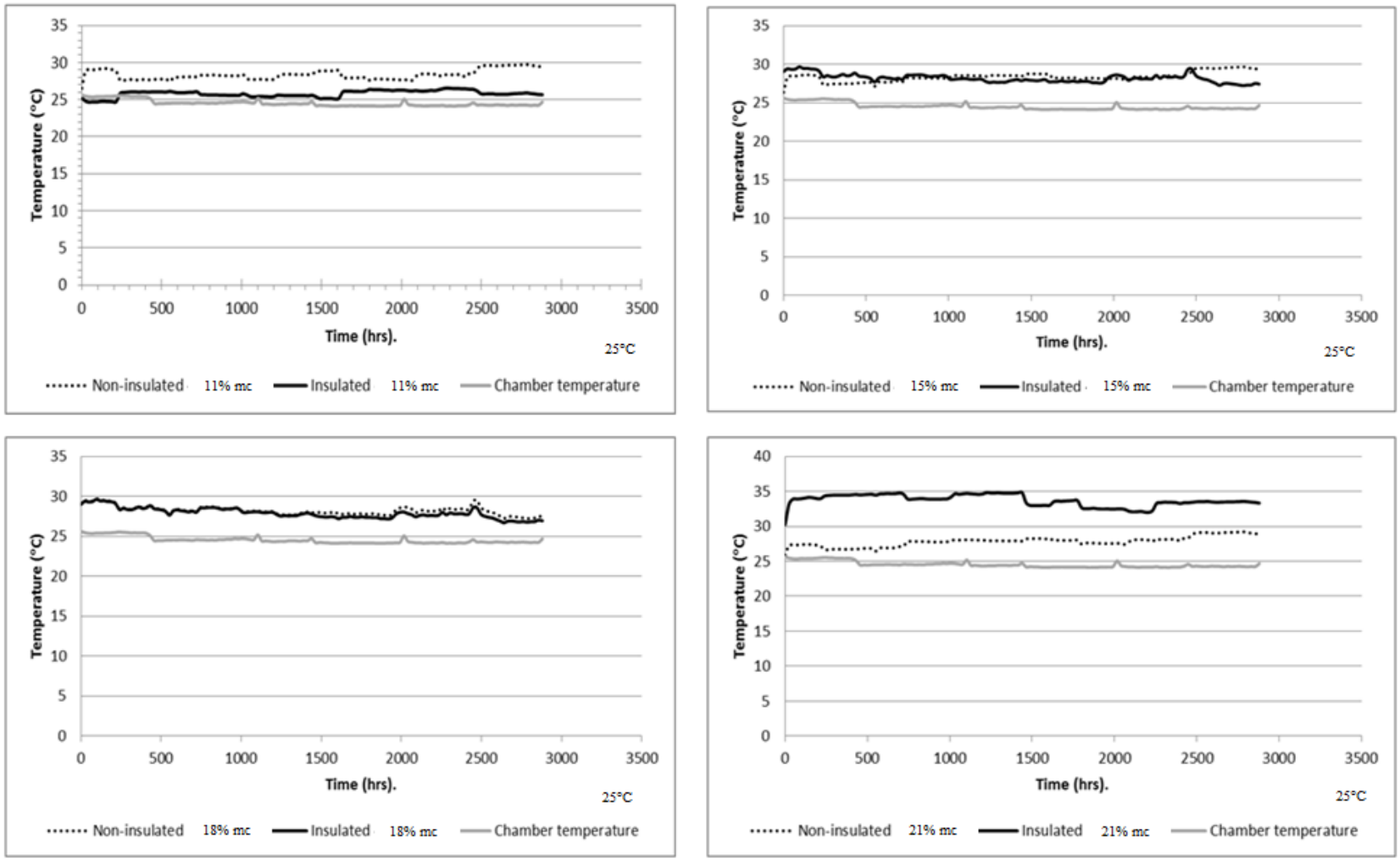

Figure 3:3 Response of temperature to mold across storage time for 11 % MC (top left panel), 15 % MC (top right panel), 18% MC (bottom left panel) and 21% MC (bottom right panel) at 25°C for both insulated and non-insulated jars

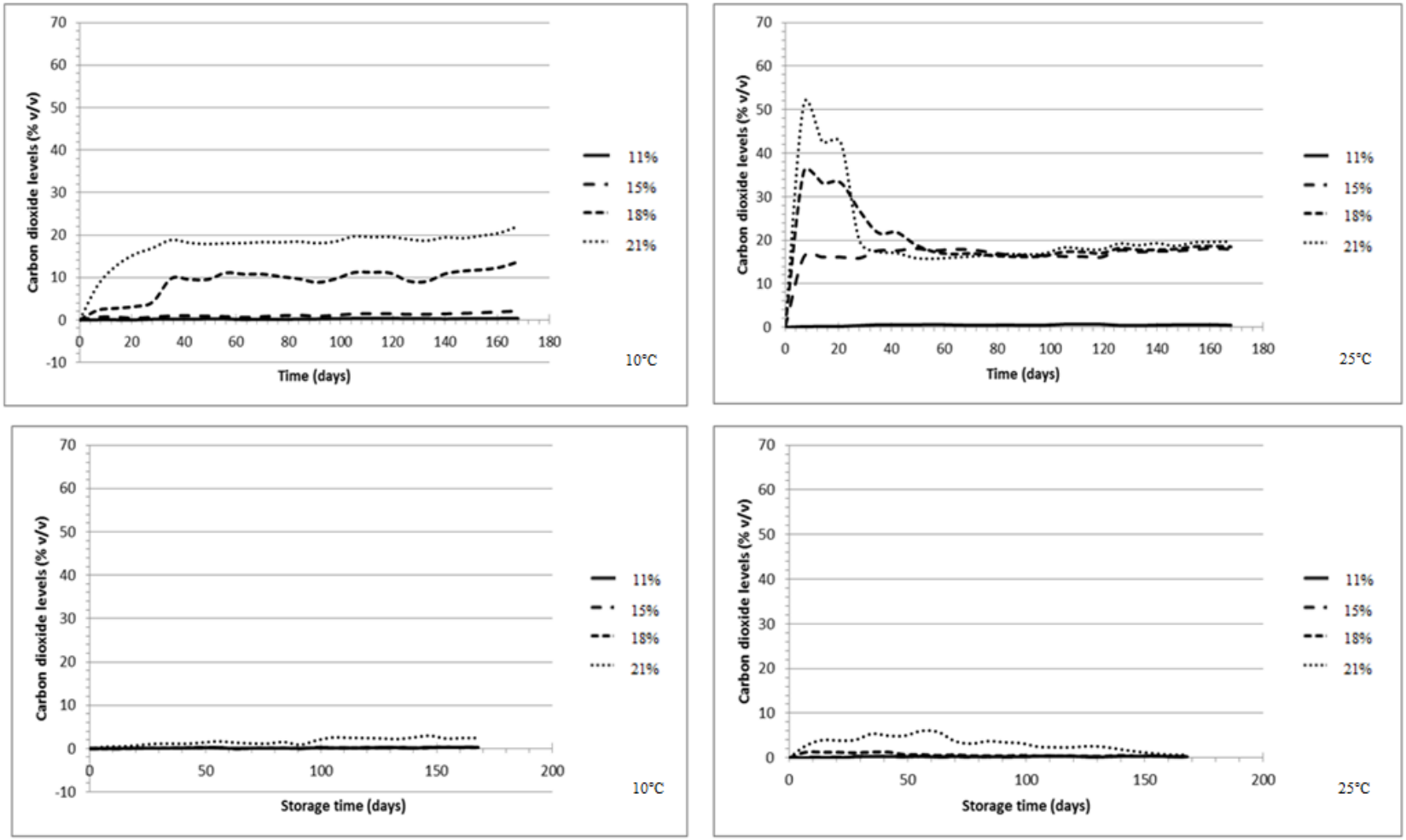

Figure 3:4 Carbon dioxide levels across storage time for hermetic (top panels) and non-hermetic (bottom panels) at 10°C (left panels) and 25°C (right panels).

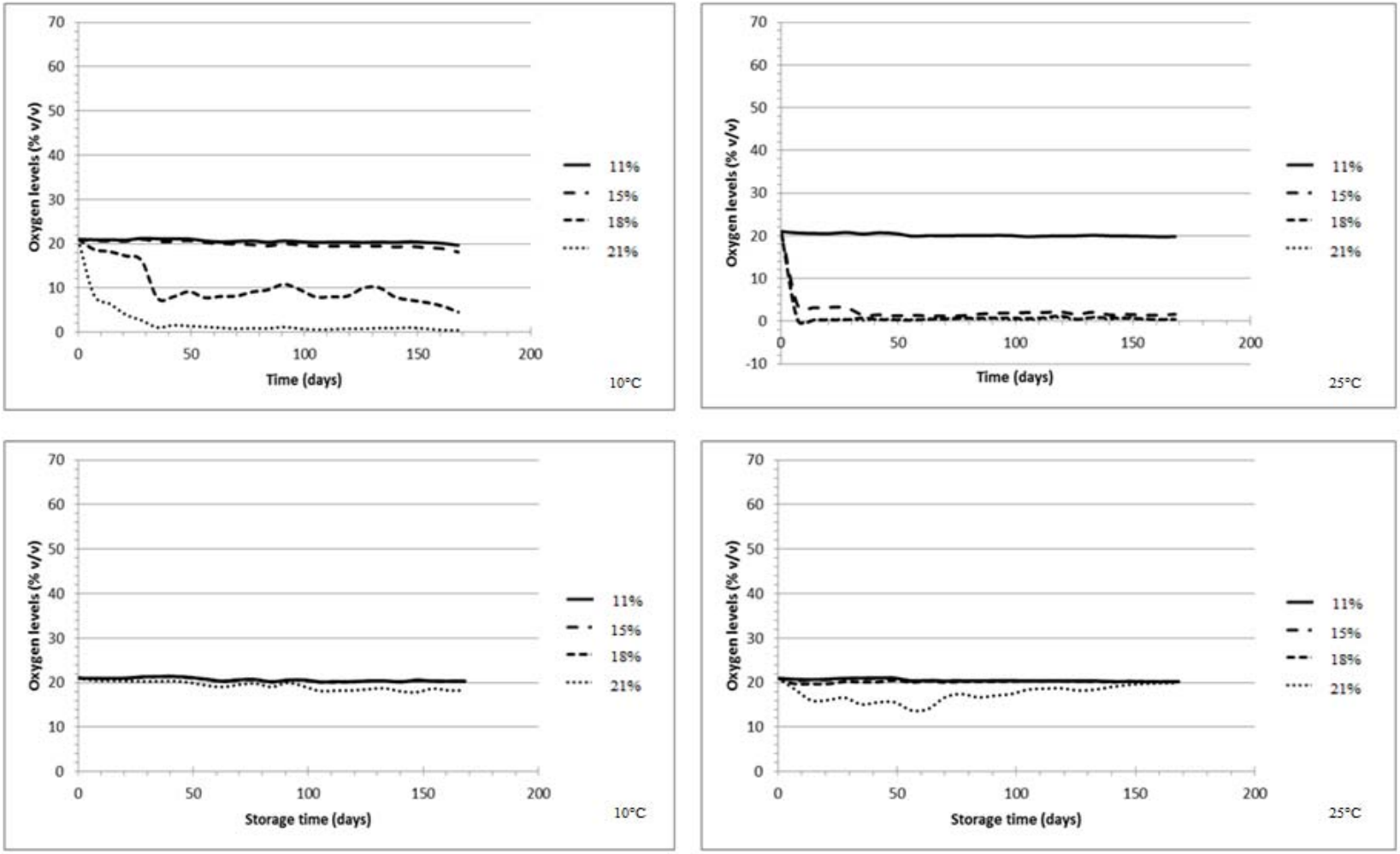

Figure 3:5 Oxygen levels across storage time for hermetic (top panels) and non-hermetic (bottom panels) at 10°C (left panels) and 25°C (right panels).

3.3.4 Mold count after storage

Mold counts across all four moisture levels for corn stored hermetically and non-hermetically at 10 and 25°C are shown in Fig 3.6. In general, mold count (CFUs) increased with increase in initial moisture content, temperature and storage time for both storage types. For moisture effects, mold counts increased with moisture content. For temperature effects, mold counts were higher at 25^{o}C compared to 10^{o}C. For storage time effects, mold counts increased with storage time. Mold counts were also higher at month 2 compared to month 4 and month 6. Mold counts were higher at month 4 compared to month 6. This trend of increasing mold count with increase in storage time is the opposite of the expected effect in a truly hermetic environment. It is likely that mold increase may be attributed to air leakage or reintroducing ambient air to replace the 4ml used for gas composition measurements. As stated in the methods section, 4ml of ambient air (i.e. 20% oxygen and 0% carbon dioxide) was introduced every 7 days to replace the gas used for measurements. This was done to minimize creating a vacuum. However, this reintroduction of ambient air every 7 days may likely be the reason for the trend of increasing mold with increase in storage time. A post-hoc study was conducted to evaluate whether the 4ml of ambient air replacement was sufficient to encourage the proliferation of aerobic mold. Two samples were used, one that was sealed during the entire storage period and the other with a septum hole and opening to allow air replacement. The study showed that introducing 4ml or more every 7 days in the hermetically sealed jar was enough to keep fungi still growing on the stored corn.

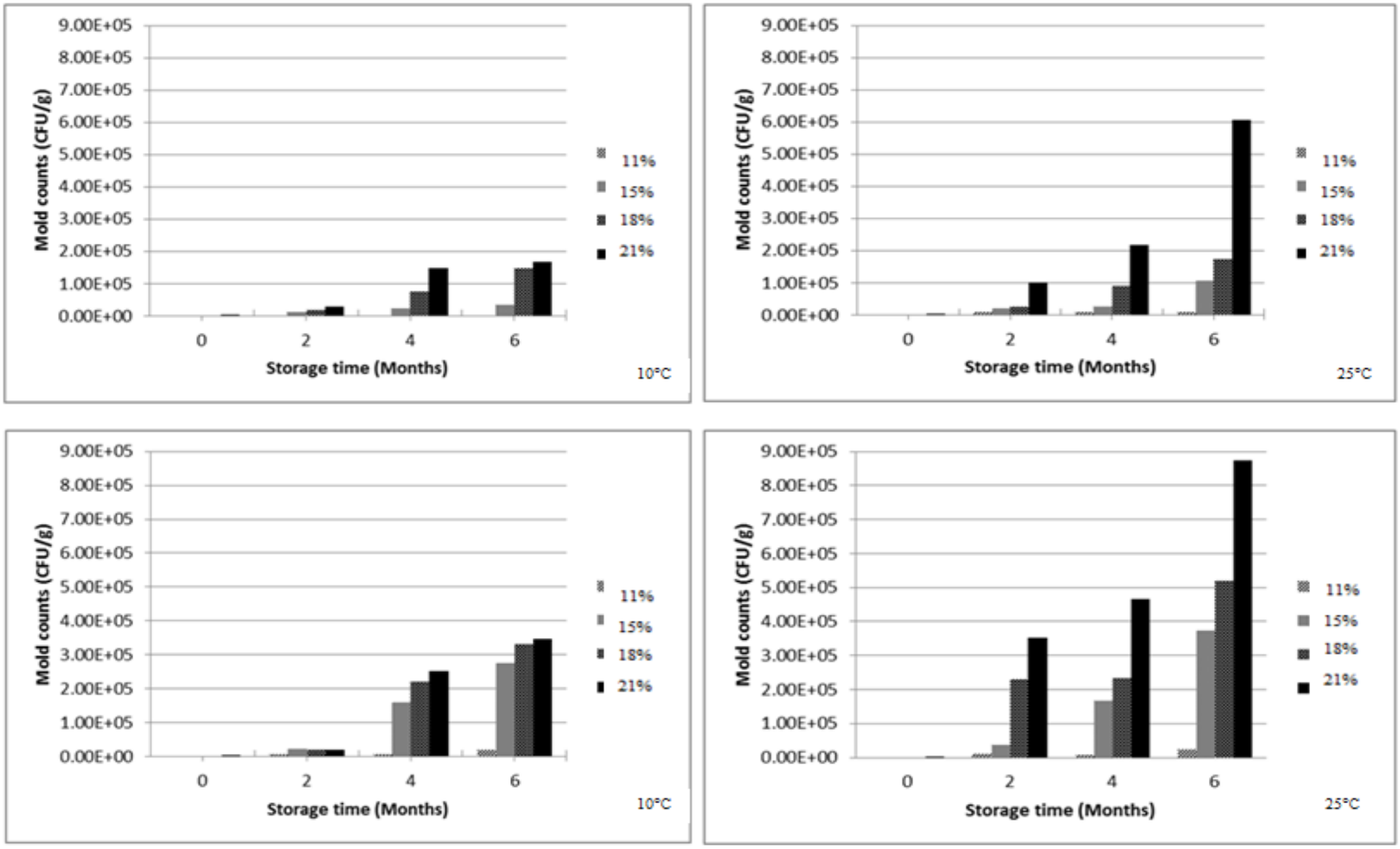

Figure 3:6 Mold count (CFUs/g) for different moisture contents levels across storage time for hermetic (top panels) and non-hermetic (bottom panels) at 10°C (left panels) and 25°C (right panels).

3.3.5 Visible characterization of kernels to molding during storage

After six months of hermetic storage, 11% initial moisture samples stored at 25°C hermetically and non-hermetically had no visible signs of mold, no off-odor, no noticeable smell, characteristics of the corn was free flowing. However, samples at 15% moisture content had some visible mold while non-hermetically stored corn contained more traces of mold growth than corn stored hermetically. For 18% moisture, odor of fermentation was present confirming possible occurrence of anaerobic respiration and mold in the hermetically stored corn. There was a musty odor showing greenish and dark coloration in the non-hermetically stored corn at 18%. At 21% moisture, hermetically stored samples showed the strongest signs of fermentation from odor with high pressure build-up. At 10°C corn stored hermetically there were no visible signs of mold especially for corn stored at lower moistures.

3.3.6 Aflatoxin concentration after storage

Table 3.2 shows that most of the aflatoxins (ppb) contamination levels measured for the storage periods were below the limits of detection (LOD). In general, aflatoxin levels increased with increase in temperature, moisture content and time.

Table 3:2 Aflatoxin levels (ppb) over storage periods at temperatures of 10 and 25°C

Storage time (months)	10°C		25°C	
	Hermetic	Non-hermetic	Hermetic	Non-hermetic
11% initial M.C.				
0	*LOD	*LOD	*LOD	*LOD
2	* LOD	*LOD	* LOD	*LOD
4	* LOD	*LOD	* LOD	*LOD
6	* LOD	*LOD	* LOD	*LOD
15% initial M.C.				
0	*LOD	*LOD	*LOD	*LOD
2	* LOD	*LOD	* LOD	*LOD
4	*LOD	*LOD	* LOD	*LOD
6	*LOD	*LOD	* LOD	*LOD
18% initial M.C.				
0	*LOD	*LOD	*LOD	*LOD
2	*LOD	*LOD	* LOD	172.5 $\pm$ 10.61
4	*LOD	*LOD	* LOD	> 180
6	*LOD	*LOD	* LOD	> 180
21% initial M.C.				
0	*LOD	*LOD	*LOD	*LOD
2	*LOD	*LOD	7.23 $\pm$ 1.10	> 180
4	*LOD	*LOD	50.50 $\pm$ 6.36	> 180
6	*LOD	*LOD	66.50 $\pm$ 4.95	> 180

* Limit of detection (LOD) indicates Aflatoxin levels (ppb) below the detection limit (0 ppb).

3.3.7 Germination after storage

Moisture content, temperature and storage type critically affected germination. Table 3.3 shows seed germination percentage over the total storage period at 10°C and 25°C temperatures for corn stored hermetically and non-hermetically.

Table 3:3 Seed germination (Mean (%) ± (SD)) over storage periods at temperatures of 10°C and 25°C

Storage time (months)	10°C		25°C	
	Hermetic	Non-hermetic	Hermetic	Non-hermetic
11% initial M.C.				
0	99.00 ± 1.00	99.00 ± 1.00	99.00 ± 1.00	99.00 ± 1.00
2	95.00 ± 1.41	97.50 ± 0.71	96.00 ± 1.41	96.25 ± 3.18
4	92.25 ± 1.06	95.00 ± 1.41	91.00 ± 2.12	94.50 ± 3.54
6	90.25 ± 1.77	77.50 ± 1.41	89.55 ± 2.05	62.00 ± 1.41
15% initial M.C.				
0	99.00 ± 1.00	99.00 ± 1.00	99.00 ± 1.00	99.00 ± 1.00
2	89.00 ± 1.41	95.75 ± 0.35	37.00 ± 4.24	78.25 ± 1.06
4	67.00 ± 1.41	92.50 ± 2.12	0.50 ± 0.71	64.25 ± 1.06
6	64.75 ± 3.18	53.00 ± 4.24	0.00 ± 0.00	42.75 ± 2.47
18% initial M.C.				
0	99.00 ± 1.00	99.00 ± 1.00	99.00 ± 1.00	99.00 ± 1.00
2	59.50 ± 10.61	75.75 ± 1.06	0.00 ± 0.00	23.75 ± 3.89
4	29.25 ± 1.06	60.25 ± 1.06	0.00 ± 0.00	22.75 ± 3.89
6	25.50 ± 4.95	34.50 ± 0.71	0.00 ± 0.00	0.00 ± 0.00
21% initial M.C.				
0	99.00 ± 1.00	99.00 ± 1.00	99.00 ± 1.00	99.00 ± 1.00
2	6.25 ± 1.77	19.25 ± 1.77	0.00 ± 0.00	11.00 ± 4.24
4	4.75 ± 1.77	5.00 ± 1.41	0.00 ± 0.00	0.00 ± 0.00
6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Seed germination decreased with increase in initial moisture content irrespective of storage type and temperature. Hermetic storage was less favorable for preserving germination. Germination decreased with increase in moisture content, temperature and storage time. With respect to the relationship between germination and gas composition, seed germination decreased with increase in carbon dioxide production and oxygen depletion. Germination was lower in hermetic compared to non-hermetic storage.

3.3.8 Fat acidity after storage

Moisture content, temperature and storage type critically affected fat acidity values. Fat acidity data is shown in Table 3.4. In general, free fatty acids increased with increase in initial moisture content of the stored corn and storage time. Fat acidity values were lower in hermetic storage than in non-hermetic storage. Overall, fat acidity increased significantly with storage time. Across all conditions, the mean levels of fat acidity were for month 0, 2, 4 and 6 respectively. In summary, fat acidity was only significantly higher after month 4 and month 6 of storage.

Table 3:4 Fat acidity value (Mean (%) ± (SD)) over storage periods at temperature s of 10°C and 25°C

Storage time (months)	10°C		25°C	
	Hermetic	Non-hermetic	Hermetic	Non-hermetic
11% initial M.C.				
0	26.6 ± 1.72	26.6 ± 1.72	26.6 ± 1.72	26.6 ± 1.72
2	60.3 ± 0.10	65.4 ± 0.17	83.9 ± 1.72	87.6 ± 2.25
4	71.2 ± 1.30	105.2 ± 6.19	182.4 ± 0.65	183.2 ± 1.95
6	75.7 ± 2.60	143.5 ± 2.34	288.4 ± 2.34	349.4 ± 1.95
15% initial M.C.				
0	26.6 ± 1.72	26.6 ± 1.72	26.6 ± 1.72	26.6 ± 1.72
2	67.8 ± 1.72	122.5 ± 1.12	274.2 ± 1.12	312.7 ± 19.73
4	108.2 ± 3.61	164.8 ± 3.24	305.6 ± 3.90	340.8 ± 6.49
6	229.6 ± 2.83	236.0 ± 5.84	339.3 ± 2.97	385.0 ± 2.83
18% initial M.C.				
0	26.6 ± 1.72	26.6 ± 1.72	26.6 ± 1.72	26.6 ± 1.72
2	187.3 ± 1.30	215.7 ± 4.05	314.6 ± 0.00	361.1 ± 8.51
4	215.0 ± 6.26	285.0 ± 1.72	331.1 ± 5.07	374.5 ± 6.49
6	295.5 ± 1.95	328.1 ± 5.84	387.3 ± 5.19	415.7 ± 1.95
21% initial M.C.				
0	26.6 ± 1.72	26.6 ± 1.72	26.6 ± 1.72	26.6 ± 1.72
2	264.8 ± 2.34	269.7 ± 0.00	344.6 ± 13.0	363.3 ± 6.49
4	282.2 ± 2.25	301.2 ± 6.83	360.3 ± 1.30	404.5 ± 0.00
6	356.6 ± 5.19	380.9 ± 1.95	402.3 ± 2.25	471.9 ± 2.25

3.3.9 Nutritional content after storage

Table 3.5 shows nutritional data for protein, starch and oil content. Across all conditions, protein levels decreased slightly with time.

Table 3:5 Nutritional content (%) (‘AS IS’) value over storage periods at temperatures of 10 and 25°C

		Starch content (%)				Protein content (%)				Oil content (%)			
	Storage time	10°C		25°C		10°C		25°C		10°C		25°C	
	(months)	H	NH	H	NH	H	NH	H	NH	H	NH	H	NH
11% IMC	0	60.4	60.4	60.4	60.4	8.6	8.6	8.6	8.6	3.1	3.1	3.1	3.1
	2	60.7	60.7	60.5	60.8	8.7	8.5	8.4	8	3.2	3.2	3.3	3.4
	4	61	60.6	60.7	61.1	8.1	8.2	8.5	8	3.1	3.3	3.1	3.2
	6	60.3	60.2	60.7	60.8	8.3	8.8	8.3	8.3	3.5	3.4	3.3	3.2
15% IMC	0	60.4	60.4	60.4	60.4	8.6	8.6	8.6	8.6	3.1	3.1	3.1	3.1
	2	60.8	60.5	60.4	60.1	8.3	8.2	8.4	8.7	3.4	3.4	3.5	3.5
	4	60.5	61.2	60.8	60.1	8.2	8.3	8.5	9.6	3.5	3.3	3.4	5.2
	6	60.2	60.5	57.6	60.9	8.6	8.2	9.2	8.1	3.6	3.4	3.6	3.2
18% IMC	0	60.4	60.4	60.4	60.4	8.6	8.6	8.6	8.6	3.1	3.1	3.1	3.1
	2	61	60.6	60.8	60.9	8.4	5.2	8.2	8.1	3.3	3.3	3.4	3.3
	4	65.4	60.4	61.8	61	7.2	8.2	8.6	8.1	5.6	3.6	3.4	3.2
	6	60.8	60.8	61.2	61.8	8.3	8.1	8.2	8.3	3.2	3.6	2.9	2.7
21% IMC	0	60.4	60.4	60.4	60.4	8.6	8.6	8.6	8.6	3.1	3.1	3.1	3.1
	2	60.9	62.1	60.6	54.8	8.9	7.9	8.5	11.8	3.7	3.6	3.5	3.5
	4	60.3	58.3	61.1	47.8	9.1	10	8.7	13.8	3.8	5.1	4.1	3.9
	6	60.1	60.1	61.2	62	8.3	8.8	8.2	8.5	3	5.3	3.3	3.2

*’H’ means hermetic and ‘NH’ means non-hermetic

3.3.10 Statistical analysis

Four-way ANOVA was used to evaluate the effect of temperature, storage time, moisture content and storage type on germination. The main effect was significant for temperature [F (1, 36) = 86.22, p = .0007], storage type [F (1, 36= 24.11, p = 0.0080], moisture content [F (2, 36) = 126.37, p = .0002], and storage time [F (1, 36) = 23.84, p = .0060]. The two-way interaction between temperature and moisture [F (2, 36) = 17.97, p = .01] and temperature and storage type [F 2, 36) = 10.17, p = .0270] were significant. Fisher's protected least significant difference (LSD) was used to determine which of the groups were different and represents the smallest significance between the two groups (means). The data in Table 3.6 includes all the factors that were significant and their respective LSD values. LSD mean separation tests were determined using 4-way interaction. The range of means was greater than 0.5, population skewed and the sample size moderate so the data was arcsin transformed. The mean germination at 10°C (M = 1.0489, SE = 0.029) was higher than at 25°C (M = 0.6639, SE = 0.029). The mean germination in non-hermetic (M = 0.9582, SE = 0.029) was higher than in hermetic (M = 0.7546, SE = 0.029). The mean germination for 11%MC (M = 1.2698, SE = 0.036) was higher than at 15%MC (M = 0.8360, SE = 0.036) and 18% MC (M = 0.4633, SE = 0.036). 21% MC was dropped in the statistical analysis for germination because the mean and standard deviation in the descriptive statistics were all equal to zero. The mean germination at 2 months (M = 1.0248, SE = 0.036) was higher than at 4 months (M = 0.8694, SE = 0.036) and 6 months (M = 0.6749, SE = 0.036). All other four, three and two-way interactions were not statistically significant. In summary, germination was

significantly lower at 25^{o}C when compared to 10^{o}C; in hermetic compared to non-hermetic storage, for higher MCs and also for longer storage times.

Four-way ANOVA was used to evaluate the effect of temperature, storage time, moisture content and storage type on mold. For mold counts, the main effect was significant for temperature [F (1, 48) = 127.79, p < .0001], storage type [F (1.48= 75.59, p = 0.0001], moisture content [F (2, 48) = 1278.01, p < .0001], and storage time [F (1.48) = 118.28, p < .0001]. The two-way interaction between temperature and moisture [F (3, 48) = 10.32, p = .0088]; moisture and storage type [F (2,36) = 17.09, p = .0024], temperature and time [F (2,48) = 6.40, p = .0326] ; storage type and storage time type [F (2,48) = 6.51, p = .0314], and moisture and storage time type [F (2,48) = 10.37, p = .0059], were significant. All other four, three and two-way interactions were not statistically significant. In summary, mold was significantly higher at 25°C when compared to 10^{o}C; in non-hermetic compared to hermetic storage, for higher MCs and also for longer storage times. The mean mold at 10°C (M = 6.1700, SE = 0.222) was lower than at 25°C (M = 9.7167, SE = 0.222). The mean mold in non-hermetic (M = 9.3073, SE = 0.222) was higher than in hermetic (M = 6.5795, SE = 0.222). The mean mold for 11%MC (M = 2.6071, SE = 0.314) was lower than at 15%MC (M = 7.1090, SE = 0.314), 18% MC (M = 9.5524, SE = 0.314) and 21%MC (M = 12.5050, SE = 0.314). The mean mold at 2 months (M = 4.9390, SE = 0.272) was lower than at 4 months (M = 8.0445, SE = 0.272) and 6 months (M = 10.8466, SE = 0.272).

Four-way ANOVA was used to evaluate the effect of temperature, storage time, moisture content and storage type on fat acidity. For mold counts, the main effect was significant for temperature [F (1, 6) = 1794.09, p < .0001], storage type [F (1, 6) = 157.75,

$p < 0.0001$], moisture content [$F (3, 6) = 1074.53$, $p < .0001$], and storage time [$F (1, 6) = 490.41$, $p < .0001$]. The two-way interaction between temperature and moisture [$F (3, 6) = 46.32$, $p = .0002$]; and moisture and storage time [$F (6, 6) = 6.44$, $p = .0196$] were significant. The three-way interaction between temperature, moisture and time [$F (6, 6) = 38.48$, $p = .0002$], and moisture, time and storage type [$F (6, 6) = 4.71$, $p = 0.0405$] were also significant. All other four, three and two-way interactions were not statistically significant. In summary, fat acidity was significantly higher at 25°C when compared to 10^{o}C; in non-hermetic compared to hermetic storage, for higher MCs and also for longer storage times. The mean fat acidity at 10°C (M = 201.330, SE = 1.97) was lower than at 25°C (M = 319.321, SE = 1.97). The mean fat acidity in non-hermetic (M = 277.819, SE = 1.97) was higher than in hermetic (M = 242.831, SE = 1.97). The mean fat acidity for 11%MC (M = 141.341, SE = 2.79) was lower than at 15%MC (M = 240.543, SE = 2.79), 18% MC (M = 309.240, SE = 2.79) and 21%MC (M = 350.177, SE = 2.79). The mean fat acidity at 2 months (M = 212.208, SE = 2.41) was lower than at 4 months (M = 250.952, SE = 2.41) and 6 months (M = 317.816, SE = 2.41).

Four-way ANOVA was used to evaluate the effect of temperature, storage time, moisture content and storage type on oil, protein and starch. The main effect of moisture on protein was the only significant independent variable [$F (3, 48) = 6.74$, $p = .0238$]. All other main effects and four, three and two-way interactions were not statistically significant for oil, protein and starch content. In summary, protein was significantly higher for higher MCs. The mean protein for 11%MC (M = 8.3417, SE = 0.207), 15%MC (M = 8.5250, SE = 0.207) and 18% MC (M = 8.1583, SE = 0.207) were statistically significant however at 21%MC (M = 9.3750, SE = 0.207) it was not statistically significant.

The data in table 3.6 shows that temperature, storage, moisture and time affected germination, mold growth, gas composition, fat acidity and protein.

Table 3:6 Analysis of variance results showing the factors and their interactions related to their dependent variables

Dependent variable	*Factors*	*df*	*F-value*	Pr < F	*Sig.*	*LSD*
ArcsinGermination	Temp	1	86.22	0.0007	**	0.1151
	Storage	1	24.11	0.008	**	0.1151
	Moisture	2	126.37	0.0002	**	0.141
	Temp * Moisture	2	17.97	0.01	*	0.199
	Storage * Moisture	2	10.17	0.027	*	0.199
	Time	2	23.84	0.006	**	0.141
$\sqrt{Mold}$	Temp	1	127.79	<.0001	**	0.7677
	Storage	1	75.59	0.0001	**	0.7677
	Moisture	3	178.01	<.0001	**	1.0857
	Temp * Moisture	3	10.32	0.0088	**	1.5355
	Storage * Moisture	3	17.09	0.0024	**	1.5355
	Time	2	118.28	<.0001	**	0.9403
	Temp * Time	2	6.4	0.0326	*	0.7226
	Storage * Time	2	6.51	0.0314	*	1.3297
	Moisture * Time	6	10.37	0.0059	**	1.8805
$\log CO2$	Temp	1	27.48	0.0019	**	0.1323
	Storage	1	370.85	<.0001	**	0.1323
	Moisture	3	91.22	<.0001	**	0.1871
	Temp * Moisture	3	6.34	0.0273	*	0.2645
	Storage * Moisture	3	31.15	0.0005	**	0.2645
O2	Temp	1	59.98	0.0002	**	1.039
	Storage	1	666.92	<.0001	**	1.039
	Temp * Storage	1	45.67	0.0005	**	1.4693
	Moisture	3	118.21	<.0001	**	1.4693
	Temp * Moisture	3	19.62	0.0017	**	2.0779
	Storage * Moisture	3	85.74	<.0001	**	2.0779
	Temp * Storage * Moisture	3	21.67	0.0013	**	2.9387
Fat Acidity	Temp	1	1794.09	<.0001	**	6.8163
	Storage	1	157.75	<.0001	**	6.8163
	Moisture	3	1074.53	<.0001	**	9.6396
	Temp * Moisture	3	46.32	0.0002	**	13.633
	Time	2	490.41	<.0001	**	8.3482
	Moisture * Time	6	6.44	0.0196	*	16.7
	Temp * Moisture * Time	6	38.48	0.0002	**	23.61
	Storage * Moisture * Time	6	4.71	0.0405	*	23.61
Protein	Moisture	3	6.74	0.0238	*	0.7169

3.3.11 Linear regression analysis

Multiple linear regressions was used to assess the predictive role of moisture content, time in storage and the microclimate conditions (temperature, oxygen and carbon dioxide) on mold growth, aflatoxin levels and germination under hermetic conditions.

Carbon dioxide, oxygen, moisture content, storage time, temperature and storage type were subjected to stepwise multiple regression analysis to evaluate the predictive strength of these variables on corn quality measures. A separate regression model was evaluated for each of the three dependent variables (i.e. mold, aflatoxin and germination) for only hermetic conditions. All regression values are shown in Table 3.7.

For mold count, carbon dioxide, storage time, temperature, oxygen and moisture content were significant predictors in hermetically stored corn. The stepwise regression model developed to predict mold count in hermetic storage conditions yielded:

$$\boldsymbol{y = -287.08 + 1.554x_1 + 0.408x_2 + 0.251x_3 + 1.242x_4 + 0.237x_5 + 72.006}$$

where y = mold count (CFUs), x_1 is carbon dioxide levels, and x_2 is the storage time, and x_3 is the temperature, x_4 is oxygen levels, and x_5 is moisture content. This regression model explained about 73.10% of the variance.

For aflatoxin, moisture content and temperature were the only significant predictors in hermetically stored corn. The stepwise regression model developed to predict aflatoxin levels in hermetic storage conditions yielded:

$$\boldsymbol{y = -27.694 + 0.417x_1 + 0.244x_2 + 7.245}$$

where y = aflatoxin levels (ppb), x_1 is moisture content and x_2 is temperature . Albeit significant, this was a weak model, only explaining 22.2% of the variance. The weakness

of the model may be attributed to the partial categorization method used to assess aflatoxin contamination post storage.

For germination, oxygen, storage time, and moisture content were the only significant predictors in hermetically stored corn. The stepwise regression developed to predict seed germination in hermetic storage conditions yielded:

$$\boldsymbol{y = 26.630 + 0.862x_1 - 0.126x_2 - 0.078x_3 + 8.153}$$

where y = seed germination (%), x_1 is oxygen levels, x_2 is the storage time and x_3 is the moisture content. The results showed an adjusted R^2 of 0.968. This was an excellent model, with oxygen and storage time explaining about 96.8% of the variance.

Table 3:7 Summary of stepwise multiple linear regression analyses. The predictor variables were carbon dioxide, oxygen, moisture content, temperature and storage time. The dependent variables were mold, aflatoxin and germination.

	Mold growth model			Aflatoxin model			Germination model		
Variable	*B*	*SEB*	β	*B*	*SEB*	β	*B*	*SEB*	β
Carbon dioxide	12.007	3.887	1.554**	-	-	-	-	-	-
Oxygen	8.786	3.369	1.242*	-	-	-	4.071	0.178	0.86***
Storage time	11.830	2.310	.408***	-	-	-	-2.445	0.511	-0.126***
Moisture content	3.424	1.559	.237*	1.334	0.356	0.417***	-0.758	.329	-0.078*
Temperature	2.168	0.606	.251**	0.466	0.213	0.244*	-	-	-
R^2		0.731			0.222			0.968	
F-value		35.320			9.984			627.47	

* P<.05, ** P<.01 and *** P<.001. The - symbol indicates variable was not entered into the stepwise regression model.

'B' are the constants

'SEB' are the error terms or noise

'β' are the effects or regression coefficients

3.4 Discussion

This study evaluated quality changes in hermetic and non-hermetically stored corn. For hermetic storage, the results showed that quality of corn deteriorates with increase in moisture content. For non-hermetic storage, the quality of corn generally decreased with storage time for all MC. Moisture content and temperature are critical factors for maintaining quality during storage, especially when they reached unsafe levels and make conditions favorable for corn deterioration. In the present study, the presence of microorganisms augmented the rate of depletion of oxygen and increased the carbon dioxide levels. (Foster et al., 1955; Hyde and Oxley, 1960; Richard-Molard et al., 1988; Adhikarinayake *et al*., 2006). For hermetically stored corn at 18 and 21% MC carbon dioxide levels initially increased and then decreased after 25 days and then plateaued at approximately 20% for the remainder of the storage period. We believe this modification of carbon dioxide level influenced levels of nitrogen and maintained equilibrium in a manner similar to that reported by Weinberg et al. (2008). The initial decrease in carbon dioxiode levels may be attributed to corn absorbing a considerable amount of the initially emitted carbon dioxide in the interstatial air due to the porosity of the kernels. The amount of carbon dioxide absorbed depends on temperature and moisture content (Mitsuda et al., 1973). Studies of some investiagtors have suggested that when gas composition levels of 1-2% oxygen and 15-40% carbon dioxide are reached anaerobic activity increases (Gonen and Calderon, 1968; Burrell et al, 1978; Clarke and Hill, 1981). On the contrary, lower moisture level (ie. 11%, 15%) treatments stored hermetically at 10°C did not experience any anaerobic respiration since oxygen levels were above 0%.

One of the replicated jars was insulated with Styrofoam to prevent rapid heat dissipation from within the small corn (grain) mass, so as to capture any heat rise due to heat produced by molding corn. This was done for all four moisture levels. Trends of temperature in the insulated and non-insulated jars were expected for a 21% initial moisture however lower initial moistures at 11% was not expected because temperatures in the non-insulated jars was higher than in the insulated jars.

We expected that for the respective moisture contents the temperatures between the insulated and non-insulated jars may have been more pronounced than what was actually recorded because at the lowest moisture content the temperature in the insulated jars was lower than in the non-insulated jars. Probably the jars were not equilibrated exactly at 25°C (temperatures could be lower, 20-23°C-room temperature in laboratory) before they were insulated. The lower temperature which was possibly at room conditions surrounding the jars were trapped by the insulation covering the jars but the temperatures correspondingly increased due to respiration and this was what affected the temperatures across all moisture levels.

The optimum temperature range for mold growth is 25°C – 30°C (Norholt et al., 1977). When compared to identical MC and temperature conditions, mold count was lower in the hermetic jars compared to the non-hermetic jars, which suggests suppression of all aerobic mold proliferation due to the bio-generated modified atmosphere.

Visual examination of the mold in hermetic storage combined with the presence of ethanol for hermetically stored corn at 18 and 21% suggested that aerobic molds were present. Mold growth increased with storage time in hermetic storage although it was lower than non-hermetic storage. We believe there was possible introduction of ambient

air (oxygen) during gas sampling. The mold in the jars was determined to be aerobic mold after observing the jars as shown in appendix 1. Each jar stored after 6 months had 24+ ml of air introduced in total, 4 months after storage had 16+ ml and 2 months had 8+ ml of air replaced in the jars. This could be enough to proliferate mold growth. When the oxygen data at 25°C for hermetic storage was observed, the corresponding mold data was expected to be less than in 10°C since the oxygen was depleted faster at 25°C. The increase in aflatoxin at 21% moisture over time (2, 4 and 6 months) can be explained from the mold data increasing with time.

Weinberg et al (2008) quantified mold species in hermetically stored corn and they reported proliferation of anearobic mold at similar MC conditions. On the contrary, characteristics of the mold in the non-hermetic condition were consistent with aerobic molds such as *Aspergillus*. A few other studies have also reported that aerobic mold is suppressed in hermetic compared to non-hermetic storage (Clarke and Hill, 1981; Moreno et al., 1988; Tabak and Cooke, 1968; Quezada et al., 2006).

Consistent with a previous report by Moreno-Martinez et al. (2000), our current findings show that corn hermetically stored between 15% and 18% moisture content had negligible mold invasion after 2 to 6 months of storage compared to non-hermetic storage.

Atmospheric gases affect aflatoxin biosynthesis (Ellis et al., 1991). Aflatoxin levels were highly suppressed in hermetic storage (Table 2) except at 21% mc.. All moisture content treatments of corn stored both hermetically and non-hermetically at 10°C were below limits of detection. However in hermetic storage at 25°C corn at mid moistures were below limits of detection (0 – 2.5 ppb) when stored after only 2 months. After 4 to 6 months of storage, aflaxtoxin was present at levels above the criterion of 15

ppb (Ghana Grain Standards Board, 2003) for 18 and21% MC in non-hermetic storage and at 21% in hermetic storage which was not expected. This may have been due to the way the gas was sampled.

From our mold identification and microscopic evaluation studies aflatoxins produced were by A.flavus since aflatoxins, a secondary metabolite can be produced by A.flavus (Woloshuk and Shim, 2013; Klich, 2007). Previous studies have shown that aflatoxin production will be suppressed at carbon dioxide levels of at least 50% (Giorni et al., 2008; Taniwaki et al., 2009; Wilson and Jay, 1975).

In hermetic storage, seed germination of mid moisture grain fell rapidly with increase in moisture content and temperature. The mold produced negatively affected seed germination. Biological activity of grain and mold respiration were the major factors that caused the rapid decline. Researchers such as Weinberg et al. (2008); Guberac et al. (2003) have shown that germination fall to zero in a few weeks at 22% mc and above.

The resilience of grains and seeds are highly influenced by moisture conditions (Christensen and Kaufman, 1969). The lower the moisture content the longer the allowable safe storage time for preservation of the seed (Copeland and McDonald, 1985). Our results showed that germination decreased with higher moisture content regardless of temperature and storage type. Several studies have reported similar trends (Costa et al.,2010; Guberac et al., 2003; Moreno et al.,2000; Moreno et al., 1988; Weinberg et al., 2008).

Fat acidity levels correlated and had similar trends to mold counts indicating that it can be used as good indicator of grain deterioration. There was an increase in moisture content with increase in fat acidity. Increase in free fatty acids during storage is

comparable to results reported by Singh et al. (2000), Megahad and El kinawy (2002) and Nagi et al. (2012). The high fat acidity values may have been due to experimental bias that was consistent across all the tests.

Nutritional content did not change appreciably in hermetic storage compared to non-hermetic storage. Similar results correlated with studies conducted by Reed et al. (2007). Slight changes in starch and protein content were observed in non-hermetic storage. Protein content results in Table 5 generally remained unchanged in hermetic storage. The combined effect of moisture content, temperature and gas composition influenced the post storage quality of corn. High levels of carbon dioxide and low levels of oxygen suppressed the rate of mold growth in hermetic storage compared to non-hermetic storage. These findings are consistent with previous research studies (Oxley and Wickenden (1963); Richard-Molard et al., 1988; Weinberg et al., 2008).

Stepwise multiple linear regressions were used to evaluate the predictive strength of moisture content, temperature, storage time, carbon dioxide and oxygen on the dependent variables: mold growth, aflatoxin levels, and seed germination in hermetically stored corn. Mold was significantly predicted by carbon dioxide, storage time, temperature, oxygen and moisture content. Aflatoxin level was significantly predicted by moisture content and temperature. Seed germination was significantly predicted by oxygen level, storage time and moisture content.

3.5 Conclusion

In summary, hermetic storage was effective at suppressing mold growth for corn at 11% and 15%, and may show promise as a cost-effective and accessible system of suppressing

the growth rate of mycotoxins and aerobic mold under tropical conditions. For hermetic storage to be successful the moisture level of the grain being stored is a critical factor; the lower the moisture content the better for hermetic storage. Future work may extend hermetic storage to grain stored in larger quantities based on sub-tropical conditions and small holder farmers' needs.

3.6 References

AACC. 2009. *AACC Approved Methods*. 9th ed. Vol. I. 02–03A: Fat acidity — Rapid method, for corn. St. Paul, Minn.: AACC.International, St. Paul, MN, U.S.A.

Adhikarinayake, T. B., Palipane, K. B., & Müller, J. (2006). Quality change and mass loss of paddy during airtight storage in a ferro-cement bin in Sri Lanka. *Journal of Stored Products Research*, *42*(3), 377-390.

ASABE 2000 "ASABE S352.2 Moisture measurement --- Unground grain and seeds," in ASABE Standards 2000. St Joseph, MI: American Society of Agricultural Engineers, 2000, pp. 563.

Banks, H. (1981). Effects of controlled atmosphere storage on grain quality: a review. *Food Technol. Aust, 33*(7), 335-340.

Burrell, N. J., Armitage, D. M., & Hill, S. T. (1978). The effect of cooling damp barley before airtight storage. *Journal of Stored Products Research*, *14*(1), 53-59.

Christensen, C., & Kaufman, H. (1969). Mycotoxins and grain quality. *Grain storage: the role of fungi in quality loss. University of Minnesota, Press Minneapolis*, 76-93.

Clarke, J. H., & Hill, S. T. (1981). Mycofloras of moist barley during sealed storage in farm and laboratory silos. *Transactions of the British Mycological Society*, *77*(3), 557-565.

Copeland, L. O, and McDonald MB. (1985). *Principles of Seed Science and Technology. 2nd ed. Burgess Publication Co., Minneapolis, MN*, 91-95.

Costa, A. R., Faroni, L. R. D. A., Alencar, E. R., Carvalho, M. C. S. and Ferreira, L. G. (2010) Quality of corn grain stored in silo bags. *Revista Ciema Agronamica, 41*(2), 200-207.

De Carli, M., Bresolin, B., Noreña, C. P. Z., Lorini, I., & Brandelli, A. (2010). Efficacy of modified atmosphere packaging to control Sitophilus spp. in organic maize grain. *Brazilian Archives of Biology and Technology, 53*(6), 1469-1476.

Dixon, N. M., & Kell, D. B. (1989). The inhibition by CO_2 of the growth and metabolism of micro-organisms. *Journal of Applied Microbiology*, *67*(2), 109-136.

Ellis, W. O., Smith, J. P., Simpson, B. K., Oldham, J. H., & Scott, P. M. (1991). Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection, and methods of control. *Critical Reviews in Food Science & Nutrition*, *30*(4), 403-439.

Emekci, M., Navarro, S., Donahaye, J. E., Rindner, M., & Azrieli, A. (2001). Respiration of stored product pests in hermetic conditions. In *Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products. Executive Printing Services, Clovis, California, USA* (pp. 26-35).

Farombi, E. O. 2006. Aflatoxin contamination of foods in the developing countries: Implications for hepatocellular carcinoma and chemo preventive strategies. *African Journal of Biotechnology* (Kenya) 5:1-14

Foster, G. H., Kaler, H. A., & Whistler, R. L. (1955). Grain Storage, Effects on Corn of Storage in Airtight Bins. *Journal of Agricultural and Food Chemistry*, *3*(8), 682-686.

Ghana Meteorological Service. (1981 – 2011). Annual Summary of Observations in Ghana. Ghana Meteorological Service, Accra, Ghana

Giorni, P., Battilani, P., Pietri, A., & Magan, N. (2008). Effect of a_w and CO_2 level on Aspergillus flavus growth and aflatoxin production in high moisture maize post-harvest. *International Journal of Food Microbiology, 122*(1-2), 109-113.

Gonen, M., & Calderon, M. (1968). Changes in the microfloral composition of moist sorghum stored under hermetic conditions. *Trop. Sci*, *10*(2), 107-114.

Guberac, V., Maric, S., Lalic, A., Drezner, G., & Zdunic, Z. (2003). Hermetically sealed storage of cereal seeds and its influence on vigour and germination. *Journal of Agronomy and Crop Science, 189*(1), 54-56.

Hell, K., & Mutegi, C. (2011). Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. *African Journal of Microbiology Research*, *5*(5), 459-466.

Hyde, M. B., & Oxley, T. A. (1960). Experiments on the airtight storage of damp grain. *Annals of Applied Biology*, *48*(4), 687-710.

Ileleji, K., Wilcke, W., & Morey, R. (2003). Relative storability of high-oil and Bt corn hybrids compared to conventional hybrids. *Transactions of the ASABE, 46*(2), 407-414.

Klich, M. A., (2002). Identification of common Aspergillus species: Centraalbureau voor Schimmelcultures Utrecht, The Netherlands.

Klich, M. A. (2007). Aspergillus flavus: the major producer of aflatoxin. *Molecular Plant Pathology*, *8*(6), 713-722.

Magan, N., & Lacey, J. (1988). Ecological determinants of mould growth in stored grain. *International Journal of Food Microbiology, 7*(3), 245-256.

Megahad, O. A., & El Kinawy, O. S. (2002). Studies on the extraction of wheat germ oil by commercial hexane. *Grasas y aceites*, *53*(4), 414-418.

Mitsuda, H., Kawai, f., Kuga, M., and Yamamoto, A. (1973). Mechanics of carbon dioxide gas adsorption by grains and its application to skin packaging. *Journal of Nutritional Science 19*, 71-83.

Moog, D. P. (2006). Assessing the susceptibility of shelled corn to invasion by storage fungi. Ph.D. thesis, Purdue University, West Lafayette, IN.

Moreno, E., Benavides, C., & Ramirez, J. (1988). The influence of hermetic storage on the behavior of maize seed germination *Seed Science and Technology, 16*, 427-434.

Moreno-Martinez, E., Jiménez, S., & Vázquez, M. E. (2000). Effect of Sitophilus zeamais and Aspergillus chevalieri on the oxygen level in maize stored hermetically. *Journal of Stored Products Research, 36*(1), 25-36.

Murdock, L. L., Magam, V., Baoua, I., Baffe, S., Shade, R. E. (2012). Death by desiccation: Effects of hermetic storage on cowpea bruchids *Journal of Stored Products Research* 49:166-170

Nagi, H.P.S., Kaur, J., Dar, B.N & Sharma, S. (2012). Effect of Storage period and packaging on the shelf life of cereal bran incorporated biscuits. *American Journal of Food Technology* 7**:** 301 – 310.

Navarro, S., Donahaye, E., & Fishman, S. (1994, April). The future of hermetic storage of dry grains in tropical and subtropical climates. In *Proceedings of 6th International Working Conference on Stored Product Protection. CAB International, Wallingford, UK* (pp. 130-138).

Norholt, M. D., & Paulsch, W. E. (1977). Differences between Aspergillus flavus strains in growth and aflatoxin B1 production in relation to water activity and temperature [Food contamination]. *Journal of Food Protection*, *40*. 778, 1977

Oxley, T., & Wickenden, G. (1963). The effect of restricted air supply on some insects which infest grain. *Annals of Applied Biology, 51*(2), 313-324.

Pitt, J. I., and Hocking, A.D. (1997). Fungi and food spoilage. Blackie Academic and Professional, imprint of Chapman & Hall, 2- 6 Boundary Row, London SE1 8HN, UK.

Quezada, M. Y., Moreno, J., Vázquez, M. E., Mendoza, M., Méndez-Albores, A., & Moreno-Martínez, E. (2006). Hermetic storage system preventing the proliferation of Prostephanus truncatus. Horn and storage fungi in maize with different moisture contents. *Postharvest Biology and Technology, 39*(3), 321-326.

Reed, C., Doyungan, S., Ioerger, B., & Getchell, A. (2007). Response of storage molds to different initial moisture contents of maize (corn) stored at 25 C, and effect on respiration rate and nutrient composition. *Journal of Stored Products Research*, *43*(4), 443-458.

Richard-Molard, D. (1988). General characteristics of the microflora of grains and seeds and the principal resulting spoilages. In: Multon, J.L. (Ed.), Preservation and Storage of Grains, Seeds, and their By-products. Lavoisier Publishing, Inc., New York, NY, pp. 226–243.

Sauer, D. (1992). *Storage of cereal grains and their products*: American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Singh R., Singh G. and Chauhan G.S. (2000). Development of soy-fortified biscuits and shelf life studies. *Journal of Food Science and Technology* 37**:**300 -303.

Tabak, H. H., & Cooke, W. B. (1968). The effects of gaseous environments on the growth and metabolism of fungi. *The Botanical Review*, *34*(2), 126-252.

Taniwaki, M., Hocking, A., Pitt, J., & Fleet, G. (2009). Growth and mycotoxin production by food spoilage fungi under high carbon dioxide and low oxygen atmospheres. *International Journal of Food Microbiology, 132*(2-3), 100-108.

Tuite, J., Koh-Knox, C., Stroshine, R., Cantone, F. A., and Bauman, L. F. 1985. Effect of physical damage to corn kernels on the development of *Penicillium* spp. and *Aspergillus glaucus* in storage.Phytopathology 75:1137.

Villers, P., Navarro, S., De Bruin, T. (2010). *New Applications of Hermetic Storage for Grain Storage and Transport*. Unabridged version of Paper presented at the 10th International Working Conference on Stored Product Protection (IWCSPP), Estorial, Portugal, June 2010.

Weinberg, Z., Yan, Y., Chen, Y., Finkelman, S., Ashbell, G., & Navarro, S. (2008). The effect of moisture level on high-moisture maize (Zea mays L.) under hermetic storage conditions--in vitro studies. *Journal of Stored Products Research, 44*(2), 136-144.

Wilson, D. M., & Jay, E. (1975). Influence of modified atmosphere storage on aflatoxin production in high moisture corn. *Applied microbiology, 29*(2), 224-228.

Woloshuk, C. P., & Shim, W. B. (2013). Aflatoxins, fumonisins, and trichothecenes: a convergence of knowledge. *FEMS microbiology reviews*, *37*(1), 94-109.

CHAPTER 4. QUALITY CHANGES CAUSED BY *SITOPHILUS ZEAMAIS* (MOTSCHULSKY) IN HERMETICALLY STORED CORN AT MID-MOISTURE LEVELS UNDER TROPICAL CONDITIONS

4.1 Introduction

Maize is the main staple food in sub-Saharan Africa. In the sub-Saharan African continent the annual production is about 29 million tons (Christopher *et al*., 1996). About 75% of this total production is consumed as human food. The quality of corn can be categorized into these three main categories: physical (example broken kernels, heat damage) sanitary (example insect fragments, mycotoxins) and intrinsic (example feed value and starch content). Certain properties of corn quality can be influenced by the genetic characteristics and their interactions with environmental conditions during the growing season. Other management factors that influence grain (corn) quality include time of harvest, storage time, system of harvesting and handling, post-harvest treatment, storage practice, transportation method (Brooker et al. 1992), microbial activity, and insects.

Small scale sub-Saharan African maize farmers are challenged by insect infestation, which leads to a substantial amount of post-harvest loss (Rembold et al., 2011). In sub-Saharan Africa (SSA), one important insect that attack stored maize is the maize weevil *Sitophilus zeamais* Motschulsky (Vowotor et al., 2005). Use of traditional storage enclosures such as jute bags encourages infestation and proliferation

of insects. Alzouma (1981) reported that insect infestation resulted in a value loss of $20 million USD. Approximately, three decades later, farmers still attribute post-harvest loss to insect infestation (Moreno-Martinez et al., 2000). For example in the Kenyan highlands total losses due to insects was 57%. Grisley (1997) reported that losses caused by insects were higher than those caused by diseases. In Zimbabwe, grain damage of 92 % in stored maize was reported due to insect pests (FAO, 1994). One of the several factors responsible for these losses includes grain damage by insects and weight loss (FAO, 1994). The maize weevil causes quantitative losses primarily by way of feeding while damage is caused by developing larvae that hatch from the eggs, which completes its lifecycle in the kernel before emerging out as an adult.

Minimizing postharvest losses of maize due to storage insects such as maize weevil, is important for financial reasons as well as for maintaining food security worldwide (FAO, 1994). Simple technologies can be used for safely storing corn without insecticides. Hermetic storage creates a biological modified atmosphere containing high carbon dioxide and low oxygen levels which is toxic to insects. Significant research has been published on the effectiveness of hermetic storage in controlling stored pests (Navarro et al., 1994; Moreno-Martinez et al., 2000; Emekci et al., 2001; Quezada et al., 2006; De Carli et al., 2010; Murdock et al., 2012). Hermetic storage, such as the triple layer Purdue Improved Cowpea Storage (PICS) bags have been used in west and central African countries in the Sahelian region (Baoua et al., 2013) and provides a form of hermetic storage that is practical and useful under Sahelian conditions. Storing grains hermetically increases availability of high quality, insecticide-free cowpeas long after the harvest season (Murdock et al., 2012). Evidence from recent studies has shown that this

hermetic storage technology has been successful in storing dry cowpeas (i.e. ≤11% MC) without the destruction by cowpea weevils, *Callosobruchus maculatus* (F.) (Boua et al., 2013). The success of hermetic storage has also been shown using hermetic jars to attain complete kill of maize weevils and also suppress progeny in low moisture maize (Moreno-Martinez et al., 2000). Numerous investigators have also studied hermetic storage of corn using maize weevils. However, minimal work has been done on investigating the quality of corn with respect to insect damage when corn is immediately harvested during the rainy season in sub-tropical African countries below the Sahelian region. Small holder farmers in these regions often harvest their corn during the rainy season. Limited sunshine hours coupled with high humidity often inhibits them from drying their corn down to safe moisture levels prior to storage. The wet harvested corn may also have been attacked by insects when placed in storage causing damage and weight loss reducing marketability and affecting food grade. For example, the USDA Federal Grain Inspection Service (FGIS) has a rejection threshold of 2 live weevils per kg of corn or 5 or more live beetles per kg of corn (GIPSA, U., 2004). This study investigated investigated *Sitophilus zeamais* (maize weevil) control in corn stored hermetically at 11%, 15%, 18% and 21% under 25^{o}C indicated by mortality, progeny development and insect damaged kernels.

4.2 Materials and methods

4.2.1 Sample source and preparation

Shelled corn used for this experiment was U.S. grade No. 1 yellow dent corn of hybrid "6297 and 6333 VT RIB". The grain was purchased from a seed supply company in Lafayette, Indiana, US at approximately 12% moisture content. Corn was kept in cold storage (-22°C) to kill any insect pests before it was used in the experiments. Prior to preparation of the samples for the experiment, the samples were removed from cold storage and allowed to thaw for 24-h in a 0 - 4°C cooler. Samples were cleaned over an inclined sieve 3 times to remove any dead insects. The samples were also cleaned over a 4.76-mm (12/64-in.) round–hole sieve to remove fines and foreign material leaving small broken and chipped kernels. Sub-samples were divided using a boerner divider (Seedburo Equipment Co., Chicago, IL, USA) in order to obtain a representation of the samples prior to moisture content measurements.

4.2.2 Experimental design

Storage atmosphere changes were measured by monitoring changes in gas composition, carbon dioxide and oxygen. This study used a repeated measures factorial design to evaluate the effect of initial moisture content (11% as the control, 15%, 18% and 21% mc), storage type (hermetic and non-hermetic) and storage time (15 and 30 days) on corn quality measures such as insect damaged kernels, insect mortality, and insect emergence (F1 progeny). All quality tests were performed at the end of the storage period for three replications per treatment/storage condition and period. The experiment was conducted at 25°C. Insect emergence was determined after 30 d of incubation on samples

stored after 15 and 30 days for both storage types. Shelled-corn samples (650 g) were conditioned to target moisture levels and were placed in 1L Mason jars that were hermetically sealed. Three replications per treatment were stored at 15 and 30 d periods, after which they were removed from storage for analyses. Prior to the start of the experiment, two tiny needle size holes were drilled in the lids and covered with septum to allow for continuous gas sampling at regular time intervals. Temperature in the grain was not monitored during the entire storage period. Carbon dioxide and oxygen were measured daily using the Mocon PAC Check® Model 325 headspace analyzer (Mocon, Minneapolis, MN, USA) on air pulled with a hypodermic needle positioned a few millimeters below the bottom of the jar. A 4 ml sample of air was drawn from the jars for every reading taken and no air was introduced after measurements were taken. The analyzer was used 30 min after self-calibration against ambient conditions and the filters were replaced every two weeks. Calibration of the Mocon device was validated every week throughout the entire period of the experiment by allowing the unit to sit at ambient conditions for at least 30 min to enable the device to calibrate itself against ambient conditions. Each of the storage jars containing corn was classified as an experimental unit. Jars were placed in a temperature chamber (Percival Scientific Inc., Boone, IA) set to 25°C to mimic tropical conditions. Airtight lids were placed over the hermetic jars to seal air-tight while Whatman filter paper and mesh wire fabric were placed on the non-hermetic jars. The non-hermetically sealed jars were the control treatments. The screened covers were used to allow the exchange of atmospheric gases and were removed only after the storage tests were completed.

4.2.3 Maize weevil colonies

Maize weevils, *Sitophilus zeamais* (Motschulsky) were from a colony obtained from the Stored Products Laboratory at the Department of Entomology, Purdue University on corn kernels kept in an environmental chamber at 25°C. Sixty-eight randomly selected 3-4 week old adult maize weevils were placed in each experimental jar. A total of 48 experimental units were used in the experiment. Four moisture levels (11%, 15%, 18% and 21%), one temperature condition (25°C), two storage conditions (hermetic vs non-hermetic condition), two storage periods (15 and 30 days) with three replications (4 x 1 x 2 x 2 x 3 = 48 experimental units). The ratio of the number of insects to grain used was 0.11 (68 insects to 650-g of corn).

4.2.4 Moisture content

Samples were obtained before and after storage. Fifteen gram grain samples were used to determine the moisture content of each sample. Moisture was measured using the air-oven standard ASABE S352.2 (ASABE Standards, 2000), 103°C for 72 hours. Corn at 11% MC wb was used as a control against 15%, 18% and 21% MC corn.

4.2.5 Insect mortality

Insect mortality was determined by counting the number of dead insects after 15 and 30 days of storage for both storage types. Percent insect mortality was evaluated by determining the number of dead weevils divided by the total number of maize weevils (both dead and alive) and then multiplied by 100% to obtain the percent insect mortality.

4.2.6 Insect damage

Grain samples were assessed for insect damage before, during and after the storage period of 15 and 30 days for both storage types. The insect damaged kernels were determined using USDA-FGIS standards on 250-g of per jar. Kernels were considered insect damaged when the kernels and pieces of kernels possessed insect-bored holes or feeding damage. Kernels may also be considered insect damaged when whole or broken, weevil-bored or which have evidence of boring. Broken kernels were also observed for insect boring and tunneling. The percent insect damaged kernels were evaluated by determining the number of damaged kernels divided by the total number of kernels in 250-g of corn and then multiplied by 100% to obtain the percent insect damaged kernels.

4.2.7 Insect progeny production

Insect progeny production was evaluated by counting the total number of insects present and subtracting the initial number (68) after 45 (for 15 days of storage plus 30 days of incubation) and 60 (for 30 days of storage plus 30 days of incubation) days after the start of the experiment. At the end of the storage period (15 and 30 days), after insect mortality count, all initial insects (dead or alive) were removed and sieved from the corn before insect emergence started. Every three days, the jar contents were sieved and emerged progeny were counted until the 30^{th} day to determine F1 progeny numbers.

4.2.8 Statistical analysis

Analysis of Variance (ANOVA) was performed to evaluate the effect of: initial moisture content, storage time, insect infestation and storage type on insect mortality, insect damage kernels before and after emergence and insect emergence.

4.3 Results

4.3.1 Gas composition

Oxygen decreased over time in all insect-infested hermetic jars across all four moisture levels (Figure 4 1). Complete oxygen depletion was attained within the first 5 days for 18% and 21% moistures. Oxygen levels without insects decreased to approximately zero within the first 25 days of hermetic storage for similar moisture levels. Thus, an additional 20 day was required to deplete to the same amount of oxygen in non-infested corn compared to infested corn. At lower moistures (11% and 15% M.C.) oxygen levels only reduced to about 3% after 30 days of storage while oxygen levels without insects remained stable within 20%.

Carbon dioxide levels increased, as expected, in each of the jars across all moisture levels. Carbon dioxide increased the most at higher moistures (18% and 21%) but plateaued at 20% (Figure. 4.2). Some insects died after 15 d. Carbon dioxide at lower moistures in the insect-infested jars increased at a slower rate and remained below 20% after 30 d. These levels were similar to the carbon dioxide levels obtained in the hermetic jars without insects.

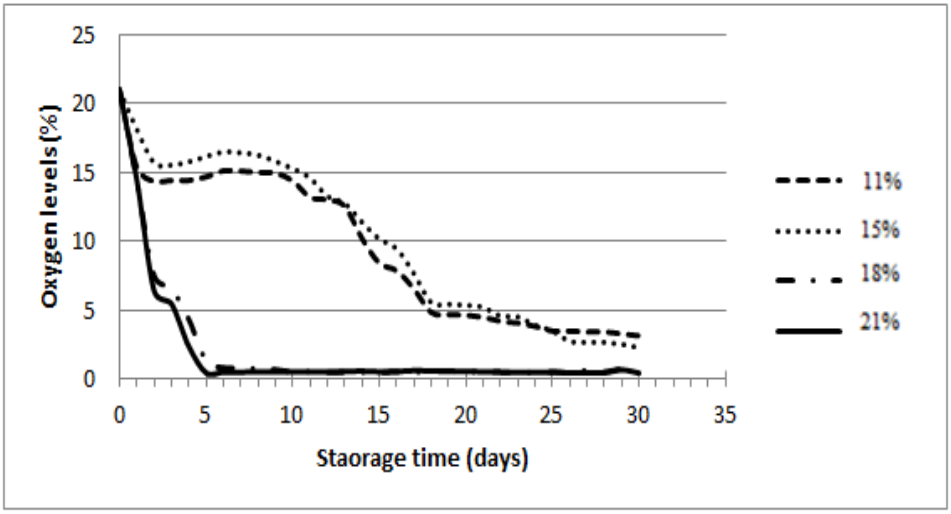

Figure 4:1 Oxygen levels after 30 day of storage under hermetic storage

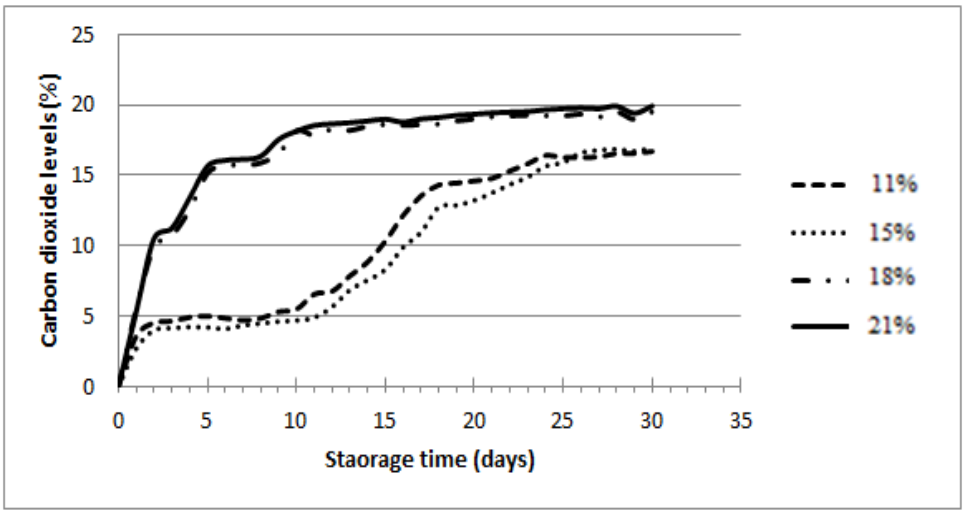

Figure 4:2 Carbon dioxide levels after 30 days of storage under hermetic storage

4.3.2 Insect mortality

Moisture content and storage type affected insect mortality. The results show the percent insect mortality after 15 and 30 days of hermetic storage (Table 4.1). For hermetically stored corn, after 15 days of storage, corn at 21% mc attained complete kill of insects. Hermetic corn stored at 18% mc attained over 60% mortality and corn stored at 11 and 15% mc attained less than 50% mortality after 15 d of storage. After 30 days of hermetic storage all moisture contents achieved 80 % or higher levels of mortality. Thus the higher the moisture content the higher the insect mortality which can be attributed to the fast rate of oxygen depletion with increase in moisture levels. Insect mortality after 15 and 30 days of non-hermetic storage was below 50% irrespective of the initial moisture content (Table 4.1).

4.3.3 Insect emergence

Insect emergence data after 15 and 30 days of hermetic and non-hermetic storage plus an additional 30 days of incubation showed that the average number of maize weevils was lower in hermetic storage compared to non-hermetic storage (Table 2.2). The average number of maize weevils increased with storage time after 30 days of incubation across all four moisture levels regardless of storage conditions.

4.3.4 Insect damage

For hermetically stored corn, insect damaged kernels fell below 6% of kernels across all moistures after 15 and 30 days of storage (at the end of the storage period but before F1 emergence). The insect damaged kernels were higher at lower moistures (11%

and 15%). This was expected based on the lower mortality attained at these moisture levels. For non-hermetically stored corn, the damage caused by maize weevils was much higher after 15 and 30 days of storage (at the end of the storage period but before emergence) compared to hermetically stored corn. Corn at moistures of 15% and 18% had the highest number of kernels damaged by insects. Corresponding insect damage percentage increased after the 30 day incubation for both storage types and across all four moistures (Table 4.1).

4.3.5 Statistical analysis

The main effect was significant for storage time, moisture content and storage type. Two-way interactions (Time * Moisture), (Time * Storage) and (Moisture * Storage) were all statistically significant at an alpha level of 0.05. The three-way interaction (Time * Moisture * Storage) was also statistically significant. Fisher's protected least significant difference (LSD) multiple comparison procedure was used to make direct comparisons between two means (or groups). Means and standard deviations for insect mortality are shown in Table 4.1. ANOVA results, including the LSD values are shown in Table 4.3. In summary, the mean insect mortality at 15 days was lower than at 30 days. The mean insect mortality in non-hermetic was lower than in hermetic.

The main effect was significant for storage time, moisture content and storage type. Two-way interactions (Time * Moisture), (Time * Storage) and (Moisture * Storage) were all statistically significant at an alpha level of 0.05. The three-way interaction (Time * Moisture * Storage) was also statistically significant. Fisher's protected least significant difference (LSD) multiple comparison procedure was used to make direct comparisons

between two means (or groups). . Means and standard deviations for insect mortality are shown in Table 4.1. ANOVA results, including the LSD values are shown in Table 4.3. In summary, the mean insect damage (before emergence) at 15 days was lower than at 30 day. The mean insect damage for non-hermetic was higher than in hermetic storage. The mean insect damage (before emergence) was statistically significant at 11% MC and 21% MC but not statistically significant for 15% MC and 18% MC.

The main effect was significant for storage time, moisture content and storage type. Two-way interactions (Time * Moisture), (Time * Storage) and (Moisture * Storage) were all statistically significant at an alpha level of 0.05. Fisher's protected least significant difference (LSD) multiple comparison procedure was used to make direct comparisons between two means (or groups). Means and standard deviations for insect mortality are shown in Table 1. ANOVA results, including the LSD values are shown in Table 3. In summary, the mean insect damage (after emergence) at 15 days was lower than at 30 days. The mean insect damage (after emergence) for non-hermetic was higher than the mean insect damage for hermetic storage. The mean insect damage (after emergence) for was lowest at 11% MC (M = 0.4432, SE = 0.0129), compared to 15% MC (M = 0.5380, SE = 0.0129) and 18% MC (M = 0.6176, SE = 0.0129).

The main effect was significant for storage time, moisture content and storage type. Two-way interactions (Time * Moisture) and (Moisture * Storage) were both statistically significant at an alpha level of 0.05. The three-way interaction (Time * Moisture * Storage) was also statistically significant. Fisher's protected least significant difference (LSD) multiple comparison procedure was used to make direct comparisons between two means (or groups). . Means and standard deviations for insect emergence are shown in Table 4.2.

ANOVA results, including the LSD values are shown in Table 4.3. In summary, the mean emerged insects at 15 days (M = 2.0029, SE = 0.0126) was lower than at 30 days (M = 2.2796, SE = 0.0126). The mean emerged insects for non-hermetic was higher than for hermetic. The mean emerged insects for 21% mc was lower than 18% mc, 11% mc and 15% mc. The difference in damage levels from what the farmer sees and the consumer sees after it is sent to the market could be huge. Insect damage was higher at 11% MC and 15% MC than at 18% MC...

Table 4:1 Insect mortality and total emergence (mean ± SD) at various moisture levels over storage periods at 25°C

Moisture content	Storage time (days)	Insect mortality (%)		Insect damage before F1 emergence (%)		Insect damage after F1 emergence (%)	
		H	NH	H	NH	H	NH
11% MC	15	37.90 ± 0.18	13.05 ± 1.33	2.37 ± 0.25	5.47 ± 0.76	6.37 ± 0.80	24.67 ± 4.51
	30	88.19 ± 1.42	25.33 ± 2.52	3.60 ± 0.75	9.33 ± 0.67	9.23 ± 1.40	40.67 ± 4.51
15% MC	15	44.22 ± 2.34	21.57 ± 2.05	4.50 ± 0.66	12.53 ± 2.25	9.30 ± 1.23	36.33 ± 4.16
	30	92.82 ± 2.34	35.10 ± 2.01	5.03 ± 0.96	20.29 ± 2.74	10.00 ± 2.82	59.00 ± 5.57
18% MC	15	67.30 ± 1.82	28.29 ± 1.69	0.63 ± 0.25	18.23 ± 3.66	3.60 ± 1.22	72.00 ± 4.58
	30	98.91 ± 0.96	38.35 ± 1.58	0.47 ± 0.15	30.50 ± 6.38	2.50 ± 1.31	80.00 ± 7.21
21% MC	15	100.00 ± 0.00	0	0.50 ± 0.44	0.83 ± 0.21	1.03 ± 0.25	2.67 ± 1.53
	30	100.00 ± 0.00	0	0.57 ± 0.21	11.83 ± 2.64	1.57 ± 0.60	16.00 ± 2.00

Table 4:2 Total emergence (mean ± SD) at various moisture levels over storage periods at 25°C

Moisture content	Storage time (days)	Total Emergence after 30 days	
		H	NH
11% MC	15	34.33 ± 11.02	298.00 ± 32.00
	30	130.33 ± 18.56	564.00 ± 77.35
15% MC	15	141.00 ± 15.13	633.67 ± 24.01
	30	193.67 ± 13.61	683.33 ± 75.27
18% MC	15	19.33 ± 4.73	558.67 ± 32.62
	30	13.00 ± 2.00	784.33 ± 43.25
21% MC	15	9.67 ± 1.15	119.00 ± 2.65
	30	27.00 ± 2.00	664.33 ± 53.31

Table 4:3 Analysis of variance results for insect mortality, damaged kernels and emergence

Dependent variable	*Factors*	*df*	*F-value*	Pr > F	*Sig.*	*LSD*
ArcsinMortality	Time	1	1189.45	<.0001	**	0.0162
	Moisture	3	539.35	<.0001	**	0.0229
	Time * Moisture	3	79.94	<.0001	**	0.0324
	Storage	1	5955.75	<.0001	**	0.0162
	Time * Storage	1	304.14	<.0001	**	0.0229
	Moisture * Storage	3	158.95	<.0001	**	0.0324
	Time * Moisture * Storage	3	71.81	<.0001	**	0.0459
ArcsinDamaged kernels (before)	Time	1	82.23	<.0001	**	0.0176
	Moisture	3	79.1	<.0001	**	0.0249
	Time * Moisture	3	4.54	0.0092	**	0.0353
	Storage	1	650.63	<.0001	**	0.0176
	Time * Storage	1	60.28	<.0001	**	0.0249
	Moisture * Storage	3	75.13	<.0001	**	0.0353
	Time * Moisture * Storage	3	6.89	0.001	**	0.0499
LogTotal Insects emerged	Time	1	241.9	<.0001	**	0.0362
	Moisture	3	276.9	<.0001	**	0.0512
	Time * Moisture	3	64.73	<.0001	**	0.0725
	Storage	1	3590.97	<.0001	**	0.0362
	Moisture * Storage	3	167.45	<.0001	**	0.0725
	Time * Moisture * Storage	3	19.12	<.0001	**	0.1025
ArcsinDamaged kernels (after)	Time	1	60.4	<.0001	**	0.0264
	Moisture	3	197.05	<.0001	**	0.0373
	Time * Moisture	3	3.3	0.0327	*	0.0527
	Storage	1	1254.2	<.0001	**	0.0264
	Time * Storage	1	45.76	<.0001	**	0.0373
	Moisture * Storage	3	142.91	<.0001	**	0.0527

4.4 Discussion

The current study evaluated the effectiveness of hermetic storage in attaining control and/or elimination of insects and suppressing insect damage and progeny for maize stored at mid-moisture levels. The results suggest that when all the oxygen is depleted and carbon dioxide levels are at a maximum in an hermetic environment, leads to high insect mortality, low insect damage and low F1emergence.

Oxygen levels decreased while carbon dioxide levels increased by the end of the storage period for the insect-infested hermetic jars for all four moisture levels. There was a large separation noted between oxygen and carbon dioxide levels from insects and corn at 11% and 15% moistures and insects and corn at 18% and 21% moistures. At 11% and 15% MC corn oxygen depletion was not as fast as corn at 18% and 21% because of the lower respiration rate of the corn stored at the lower MC levels. Weinberg et al. (2008) also obtained similar findings.

Carbon dioxide levels increased rapidly for corn at 18% and 21% while corn at 11% and 15% increased gradually over the storage period. The results from the gas composition data are in line with those of Oxley and Wickenden (1963). They reported that all insects exposed to 2% of oxygen or lower will die. Their gas composition levels in both storage types influenced insect mortality, insect damage and insect emergence. Navarro (2012) reported that high carbon dioxide (> 60%) and low oxygen (< 8%) are highly effective at expediting the rate of insect mortality.

The current data also allowed us to compare the effectiveness of hermetic storage on the mortality of insects. Hermetic storage achieved a 100% mortality at the end of both 15 and 30 days for corn stored at 21% while no insect died for the same conditions

when stored non-hermetically. At 21% moisture corn in non-hermetic storage insects mostly died due to the proliferation of mold. For corn stored at 11% MC for a period of 30 days there was approximately 63% increase in insect mortality when corn was hermetically stored than when it was non-hermetically stored.

In corn storage, high insect mortality is desired for controlling insect pests and 100% mortality was achieved at moisture levels of 21% MC in hermetic storage. This percentage varied by 30% across all moisture levels. We can conclude that at 21% MC oxygen levels depleted so fast that insects could no longer survive within that environment. However, it must also be noted that mold will grow at this moisture level. The distinguished change in the insect mortality data between the two storage types further highlights the robustness of the hermetic systems. It should also be noted that it is the simultaneous effect of both oxygen and carbon dioxide that affects insect mortality (Calderon and Navarro, 1980).

These results could further contribute to the reduction of insecticide use by small scale farmers. Our results show that the hermetic storage technology has a similar effect on maize weevils when they feed on corn and has been shown in the research conducted by Purdue University using Purdue Improved Cowpea Storage (PICS) bags where cowpea grain and cowpea weevils were used (Moreno et al., 1994; Murdock et al., 2012). Other investigators showed that 100% of insects can be obtained at mid moisture levels (Villers et al. 2010). This is percent kill of insects is dependent on the number of insects at the beginning of storage.

For each storage period, storage type and moisture level insect damage (before emergence) and some interactions were also statistically different between the levels

tested. After 15 and 30 days of storage the damage caused by these maize weevils was comparatively lower in hermetic storage versus non-hermetic storage. While insect damaged levels remained below 1% in hermetic storage of corn stored at 18% MC and 21% MC it increased up to approximately 5% for corn stored at 11% MC and 15% MC. The insect damage data revealed that damage for corn stored at 11% MC after 15 days of storage was lower than when it was after 30 days in hermetic storage. These 68 insects feed on the grain and lay their eggs in the grain kernel. Annis (1897) reported that different life stages of the maize weevils have higher tolerance than the adult weevils.

After the 30 days of storage the eggs laid by the female adults will have completed one life cycle and turned into adults. Therefore after 30 days the additional damage noted is a result of the feeding damage by the F1 generation. These moisture conditions of corn hardly respired as fast so there was enough oxygen for the maize weevils to survive to actually lay eggs. However a similar scenario cannot be said about storage of corn at 18% mc and 21% mc which recorded damage levels below 1% in hermetic storage since at those moisture levels oxygen depletion and carbon dioxide accumulation was drastic enough to allow minimal time to not only to lay eggs or develop well but to also fight for survival. For the non-hermetic storage case the scenario was different as there was an abundance of ambient air. There was an increase in damage levels between storage times across moisture levels.

The post-emergence insect damage levels increased correspondingly from the pre-emergence insect damage levels measured immediately after storage as it relates to increasing moisture content.

4.5 Conclusion

In summary, hermetic storage was superior to non-hermetic storage in attaining insect mortality. Hermetic storage was also superior to non-hermetic storage in suppressing insect emergence. However, this relationship was also modulated by the moisture content of the corn as the wetter the corn the higher the respiration rate, and thus faster the attainment of lethal levels of CO_2. Further research work could investigate longer term studies to see if those that emerge are able to live long enough to reproduce or if it levels out..

4.6 References

Alzouma, I. (1981). Observations on the Ecology of Bruchidius Atrolineatus Pic. and Callosobruchus Maculatus F (Coleoptera, Bruchidae) in Niger. In *The Ecology of Bruchids Attacking Legumes (Pulses)* (pp. 205-213). Springer Netherlands.

Baoua, I. B., Amadou, L., & Murdock, L. L. (2013). Triple bagging for cowpea storage in rural Niger: Questions farmers ask. *Journal of Stored Products Research*, *52*, 86-92.

Brooker, D. B., Bakker-Arkema, F. W., & Hall, C. W. (1992). *Drying and storage of grains and oilseeds*: Van Nostrand Reinhold, New York .

Calderon, M., Navarro, S., 1980. Synergistic effect of CO2 and O2 mixtures on two stored grain insect pests: In: Shejbal,J. (Ed.), Controlled Atmosphere Storage of Grains, Proceedings of an International Symposium, Rome, 1980, pp. 79–84.

Carli, M. D., Bresolin, B., Noreña, C. P. Z., Lorini, I., & Brandelli, A. (2010). Efficacy of modified atmosphere packaging to control Sitophilus spp. in organic maize grain. *Brazilian Archives of Biology and Technology, 53*(6), 1469-1476.

Emekci, M., Navarro, S., Donahaye, J. E., Rindner, M. I. R. I. A. M., & Azrieli, A. (2001, `October). Respiration of stored product pests in hermetic conditions. In *Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products* (Vol. 29, pp. 26-35). Clovis, CA: Executive Printing Services.

GIPSA, U. (2004). Grain Inspection Handbook, Book II, Grain Grading Procedures. *US Department of Agriculture, Marketing and Regulatory Programs, Grain Inspection, Packers and Stockyards Administration, Federal Grain Inspection Service, Washington, DC*.

Grisley, W. (1997). Crop-pest yield loss: A diagnostic study in the Kenya highlands*. *International Journal of Pest Management*, *43*(2), 137-142.

FAO. (1994.). *Grain Storage Techniques, Evolution and Trends in Developing Countries*. FAO, Rome, Italy. 1994, 1-23, 25-39, 41-65, AND 165- 187

Moreno-Martinez, E., Jiménez, S., & Vázquez, M. E. (2000). Effect of Sitophilus zeamais and Aspergillus chevalieri on the oxygen level in maize stored hermetically. *Journal of Stored Products Research, 36*(1), 25-36.

Murdock, L. L., Magam, V., Baoua, I., Baffe, S. and Shade, R. E. (2012). Death by desiccation: Effects of hermetic storage on cowpea bruchids *Journal of Stored Products Research 49:166-170*

Navarro, S. (2012). The use of modified and controlled atmospheres for the disinfestation of stored products. *Journal of Pest Science*, *85*(3), 301-322.

Navarro, S., Donahaye, E., & Fishman, S. (1994, April). The future of hermetic storage of dry grains in tropical and subtropical climates. In *Proceedings of 6th International Working Conference on Stored Product Protection. CAB International, Wallingford, UK* (pp. 130-138).

Oxley, T., & Wickenden, G. (1963). The effect of restricted air supply on some insects which infest grain. *Annals of Applied Biology, 51*(2), 313-324.

Quezada, M. Y., Moreno, J., Vázquez, M. E., Mendoza, M., Méndez-Albores, A., & Moreno-Martínez, E. (2006). Hermetic storage system preventing the proliferation of corn and storage fungi in maize with different moisture contents. *Postharvest Biology and Technology, 39*(3), 321-326.

Rembold, F., Hodges, R., Bernard, M, Knipschild H, Leo, O. (2011). An innovative framework to analyse and compute quantitative postharvest losses for cereals under different farming and environmental conditions in East and Southern Africa . The African Postharvest Losses Information System (APHLIS) (pp. 1–73).

Villers, P., Navarro, S., & De Bruin, T. (2010). New applications of hermetic storage for grain storage and transport. *Julius-Kühn-Archiv*, (425), S-446.

Vowotor, K. A., Meikle, W. G., Ayertey, J. N., & Markham, R. H. (2005). Distribution of and association between the larger grain borer (Prostephanus truncates) (Coleoptera: Bostrichidae) and the maize weevil (Sitophilus zeamais) Motschulsky (Coleoptera: Curculionidae) in maize stores. *Journal of stored products research*, *41*(5), 498-512.

Weinberg, Z., Yan, Y., Chen, Y., Finkelman, S., Ashbell, G., & Navarro, S. (2008). The effect of moisture level on high-moisture maize (Zea mays L.) under hermetic storage conditions--in vitro studies. *Journal of Stored Products Research, 44*(2), 136-144.

Yakubu, A., Bern, C., Coats, J., & Bailey, T. (2010). Non-chemical on-farm hermetic maize storage in east Africa. *Julius-Kühn-Archiv*(425), S. 338

CHAPTER 5. USE OF SMALLHOLDER HERMETIC SYSTEMS FOR CORN STORAGE DURING THE MAJOR HARVEST PERIOD IN THE HUMID TROPICAL ZONE, ASHANTI AND BRONG-AHAFO REGIONS OF GHANA

5.1 Applications and strategies

Major grain (corn) producing areas in Ghana are the Brong Ahafo and Ashanti region. Storage of grain in this region is hampered by the inability to dry down corn to safe moisture levels. The primary method, solar drying, is inefficient in drying corn because of too little sunshine and high humidity during the majority of the harvest season. Corn harvested very wet is normally dried to mid-moistures of 15 to 21% using primarily ambient air. Thus, corn may be put into storage at unsafe moisture contents resulting in molds, aflatoxin and maize weevils infestation, which reduces marketability and increases health risks.

Hermetic storage is an airtight storage mechanism that uses naturally bio-generated modified air with buildup of carbon dioxide and reduction of oxygen by the respiration of grains and microorganisms, to create a toxic atmosphere that eventually kills storage insects and molds. It minimizes biological organism damage and effectively increases the marketability of the stored grain. The effectiveness of the hermetic storage technology with regards to mold growth is pronounced when grain are stored at safe moisture levels.

This chapter explores the advantages and disadvantages of storing hermetically with regards to quality loss at different moisture levels.

In deciding the most suitable initial moisture levels of corn before stored hermetically the final grain use must be determined. (1) Storing for seed (germination purposes), (2) Storing for consumption especially nursing mothers and infants and (3) Storing to retain the nutritional composition

Results suggested that hermetic storage was superior to non-hermetic (i.e open air) storage for killing storage weevils, suppressing weevil emergence, and reducing total damage and reducing mold and aflatoxin development for corn stored up to 18% MC. Hermetic storage was only suitable for 60 days for corn at 18% and 21% MC. For seed germination, open-air/ non-hermetic storage was more superior at preserving germination.

In tropical and humid climate conditions, corn at 21% MC can be stored hermetically for only a period of 2 months at 25°C after which it will no longer be considered suitable for human consumption. Moistures less than or equal to 18% can be stored successfully for a period of 6 months. Corn is only suitable for germination purposes for a period of 2 months in hermetic storage when stored at 11% MC or below.

<u>Moisture level: 11% at 25°C</u>:

Hermetic storage:

- The best moisture level of corn stored hermetically for seed, *mold growth was minimal (the difference between storage types was less than 25% (no aflatoxin) (RECOMMENDED) and so can be consumed, insect damage was 2-3 times lower than in traditional storage.

Non-hermetic storage:

- Storing for seed was much more successful compared to hermetically stored corn, *mold growth was just a little higher (no aflatoxin) but can also be consumed, insect damage was 2-3 times higher than in hermetic storage

Moisture level: 15% at 25°C:

Hermetic storage:

- Seed quality was greatly reduced compared to 11% mc, *mold growth was higher than at 11% mc but lower compared to traditional storage at 15%, (no aflatoxin) (RECOMMENDED), insect damage was 3-4 times lower than in traditional storage after 1 month of storage

Non-hermetic storage:

- Seed quality reduced but better compared to hermetically stored corn at this moisture level so seed quality was better in non-hermetic storage, *mold growth doubled compared to hermetic storage at 15%, (no aflatoxin), insect damage was 3-4 times higher than in hermetic storage

Moisture level: 18% at 25°C:

Hermetic storage:

- The seed can no longer germinate after 2 months of storage, *after 6 months of storage mold growth was greater compared to 11% and 15%, (no aflatoxins), insect damage was negligible after 1 month of storage probably due to high mold count resulting in the death of insects. (NOT RECOMMENDED)

Non-hermetic storage:

- Germination is greatly reduced after 2 months of storage, *mold growth more than doubled after 6 months of storage when compared to hermetic storage, (aflatoxin levels were above acceptable levels), 30% of the grains were damaged by insects (NOT RECOMMENDED).

Moisture level: 21% at 25°C:

Hermetic storage:

- The seed can no longer germinate after 2 months of storage, *mold growth increased compared to 18%, insect damage was negligible after 1 month of storage due to death related to high mold content. (NOT RECOMMENDED)

Non-hermetic storage:

Germination is minimal after 2 months of storage,* mold growth was more than four times hermetically stored corn, 15% of the grains were damaged by insects (lower than at 18% largely due to mold growth) (NOT RECOMMENDED).

*NB: It should be noted that there was some amount of air-exchange between the storage container and the environment

5.2 Recommendations for use of hermetic storage

Hermetic storage technology has proved successful in storing corn that is grown in the Sahel region of Africa where the grain is harvested sufficiently dry and suitable for

extended periods of storage. The success of hermetic storage is dependent on the initial moisture content of corn and its storage temperature.

1. If hermetically storing corn for seed, dry the corn down before storing them hermetically.
2. Seed corn should be separated from the corn used for consumption. The corn that is the driest should be used for seed if this is not possible, store seed corn non-hermetically in order to maintain the integrity of the germ.
3. Small holder farmers should use corn at moistures of 21% or above first if immediately needed for consumption (by nursing mothers and children.. If the corn is not needed immediately, it is important to store at for about 7 days to kill insects and dry down to moisture content below 15 %mc before storage.
4. Do not reuse hermetic bags or containers that stored corn at high moistures if there was some leakage of air due to the possibility of aflatoxin
5. The limit of moisture by which corn should be stored hermetically for consumption should not exceed 15%mc.
6. Store high moisture corn hermetically for the first 7 days to kill insects before drying down to minimize mold growth

5.3 References

Annis, P. C., & Banks, H. J. (1993). Is Hermetic Storage of Grains Feasible in Modern Agricultural Systems. *Pest Control & Sustainable Agriculture*, 479-482.

Banks, H. (1981). Effects of controlled atmosphere storage on grain quality: a review. *Food Technol. Aust, 33*(7), 335-340.

Ben-Yehoshua, Shimshon. 2005. Environmentally Friendly Technologies for Agricultural produce Quality. Chapter 8. InnovativeEnvironmentally Friendly Technologies to Maintain Quality of Durable Agriculture Produce.

Kays, S. (1997). Stress in harvested products. *Postharvest Physiology in Perishable Plant Products. SJ Kays (ed.)*, 335-408.

Kennedy, L., & Devereau, A. (1994). Observations on large scale outdoor maize storage in jute and woven polypropylene sacks in Zimbabwe.[abstract] Natural Resources Institute (NRI), University of Greenwich, UK, 290-295.

McFarlane, J. (1988). Storage methods in relation to post-harvest losses in cereals. *Insect Science and its Application, 9*(6), 747-754.

Multon, J. (1988). Interactions between water and the constituents of grains, seeds and by-products. In JL Multon, AM Reimbert, D Marsh, AJ Eydt, eds, Preservation and Storage of Grains, Seeds and Their By-Products. Lavoisier Publishing, New York, pp 89–159

White, N., & Jayas, D. (1991). Factors affecting the deterioration of stored flaxseed including the potential of insect infestation. *Canadian Journal of Plant Science, 71*(2), 327-335.

Villers, P., Navarro, S., & De Bruin, T. (2010). New applications of hermetic storage for grain storage and transport. *Julius-Kühn-Archiv*, (425), S-446.

CHAPTER 6. FUTURE RESEARCH

6.1 Future work

Future work for corn stored at moistures in the range of 15 to 21% and 25^{o}C or above need to be conducted on the field with PIC bags, GrainPro Superbags or other type of hermetic storage technology. For this technology to be trusted by small scale farmers in sub-Saharan Africa, larger scale trials are needed to test the system at a scale closer to those used by small scale farmers since these trials were carried out in small jars (1 l of size). Also, it would be good to explore the idea of how to utilize high moisture corn, say from 18 to 21% to eliminate intial harvest infestation in the first 2 months of storage before finally drying down to safe moistures of 13% and below.

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APPENDICES

Appendix A Storage environment

Figure A1- Storage jars

Appendix B Statistical data

Table B1: Statistical tables on transformed data results presented on back transformed means (chapter 3)

Germination		
Temp	N	Mean (BT)
10	36	75.15a
25	36	37.97b
Storage	N	Mean (BT)
Control	36	66.94a
Hermetic	36	46.92b
Moisture	N	Mean (BT)
10.8	24	91.21a
14.6	24	55.06b
17.8	24	19.97c
Time	N	Mean (BT)
2	24	73.04a
4	24	58.36b
6	24	39.04c

Fat acidity		
Temp	N	Mean (BT)
10	24	201.330b
25	24	319.321a
Storage	N	Mean (BT)
Control	24	277.819a
Hermetic	24	242.831b
Moisture	N	Mean (BT)
21.2	12	350.177a
17.8	12	309.240b
14.6	12	240.543c
10.8	12	141.341d
Time	N	Mean (BT)
6	16	317.816a
4	16	250.952b
2	16	212.208c

Mold		
Temp	N	Mean (BT)
10	48	6.17b
25	48	9.72a
Storage	N	Mean (BT)
Control	48	9.31a
Hermetic	48	6.57b
Moisture	N	Mean (BT)
21.2	24	12.51a
17.8	24	9.55b
14.6	24	7.11c
10.8	24	2.61d
Time	N	Mean (BT)
6	32	10.85a
4	32	8.05b
2	32	4.94c

CO_2		
Temp	N	Mean (BT)
10	48	1.06b
25	48	2.03a
Storage	N	Mean (BT)
Control	48	0.44b
Hermetic	48	4.85a
Moisture	N	Mean (BT)
21.2	24	5.77a
17.8	24	2.099b
14.6	24	1.126c

O_2		
Temp	N	Mean (BT)
10	48	16.02a
25	48	12.73b
Storage	N	Mean (BT)
Control	48	19.86a
Hermetic	48	8.89b
Moisture	N	Mean (BT)
10.8	24	20.20a
14.6	24	15.56b
17.8	24	12.27c
21.2	24	9.46d

Protein		
Moisture	N	Mean (BT)
21.2	12	9.375a
17.8	12	8.525b
14.6	12	8.34b
10.8	12	8.16b

Tables B2: Statistical tables of the transformed data (results are presented on back transformed means) (chapter 4)

Insect Mortality		
Time	N	Mean (BT)
15	24	45.77b
30	24	72.4a
Storage	N	Mean (BT)
Control	24	29.4b
Hermetic	24	86.03a
Moisture	N	Mean (BT)
11	12	41.01d
15	12	50.1c
18	12	63.2b
21	12	81.1a

Damaged kernels (before)		
Time	N	Mean (BT)
15	24	4.15b
30	24	7.83a
Storage	N	Mean (BT)
Control	24	12.07a
Hermetic	24	1.78b
Moisture	N	Mean (BT)
11	12	4.87b
15	12	9.7a
18	12	8.31a
21	12	2.1c

Damaged kernels (after)		
Time	N	Mean (BT)
15	24	15.1b
30	24	22.96a
Storage	N	Mean (BT)
Control	24	39.4a
Hermetic	24	4.77b
Moisture	N	Mean (BT)
11	12	18.39c
15	12	26.26b
18	12	33.53a
21	12	3.9d

Insect emergence		
Time	N	Mean (BT)
15	24	100.68b
30	24	190.36a
Storage	N	Mean (BT)
Control	24	472.25a
Hermetic	24	40.58b
Moisture	N	Mean (BT)
11	12	163.39b
15	12	328.93a
18	12	101.62c
21	12	67.25d