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Spoilage Characteristics of Pea under Adverse Storage Conditions

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Abstract

Field pea is the most produced and exported pulse crop in Canada. Field pea exported to countries with tropical climates is at particular risk due to rapid deterioration. It is therefore important to develop practical strategies for safe storage of feed pea. Knowledge on spoilage characteristics of pea stored in adverse storage conditions is important in the transportation and storage of this export commodity. This study was conducted to examine the conditions that lead to quality losses in storage and transport of pea. Tropical and subtropical conditions were simulated in airtight chambers. Relative humidities (RH) of 60, 70, 80 and 90% and temperatures of 10, 20, 30 and 40°C were examined. Both whole sound pea and feed-grade pea were observed for changes in moisture content (MC) and mold appearance at different time intervals. The amount of produced carbon dioxide (CO_2) was measured in airtight chambers to control the condition existing in sealed airtight chambers. Also all components of feed-grade pea were exposed to RH of 90% and temperature of 40°C in separate airtight chambers to find the effect of each part on mold appearance. Molds were identified after appearance on the samples. Mold-free days for both feed pea and whole sound pea were modeled at temperatures of 10, 20, 30 and 40°C and RH of 80 and 90%.

Introduction

Field pea (*Pisum sativum* L.) is an annual cool-season legume crop that is grown on over 25 million acres worldwide. Field pea or "dry pea" is marketed as a dry, shelled product for either human food or livestock feed (Anonymous, 2003a).

Early in the 20th century, Ontario and then Manitoba led in the Canadian pea production. Since the mid 1980's, Saskatchewan has produced the majority of Canadian field pea (70%) with significant acreage in Alberta (25%) and Manitoba. Ontario is no longer a large-scale producer (Agriculture and Agri-Food Canada, 2004).

Three major grades are generally used for field pea other than green pea in descending order of quality: a) No. 1 Canada; b) No. 2 Canada; and c) No. 3 Canada. The maximum limits of yellow field pea used for human consumption are less than 0.05% FM (ergot) for all grades: 3, 5, and 10% total damage (splits or broken, shriveled, heated, insect damage, or other damage), and 1, 2, and 3% content of pea of other colors, for No. 1 Canada, No. 2 Canada, and No. 3 Canada, respectively (Canadian Grain Commission, 2003).

In the export market, feed pea is classified as mixture of green and yellow peas with various specifications on moisture, FM, and percentage of pulses other than green and yellow peas. Mixtures of pea falling below No. 3 Canada are graded as feed pea. The maximum allowable level of FM is 6% for feed pea. However, it is not uncommon for Canadian marketing companies to ship feed pea with 8% or more FM. The quality of feed pea is affected significantly by FM level and composition. At a given storage temperature and RH, FM may adsorb moisture differently from the pea. Generally, the FM adsorb more moisture, thereby increasing the susceptibility of feed pea to microbial development and quality deterioration. Apart from accumulating adsorbed moisture, FM may also block natural or forced airflow in storage, thus creating an environment conductive to localized mold development (Booth et al., 2001).

Canada is a temperate country, so there is usually no concern for feed pea storage, but as this commodity is exported to tropical countries the potential for product spoilage in transit or at shipment destinations is relatively high, due to exposure to high RH and variable temperature conditions. Moisture condensation is also likely to occur due to the contact of cold material (pea) with warm air. These adverse conditions influence the water uptake of pea. Biochemical reactions and the activity of microorganisms are also influenced by the RH and temperature of the surrounding air. These environmental conditions affect mold growth and the stability of pea during storage and transport.

The main objective of this research project is to determine safe storage conditions for Canadian produced and exported feed pea when stored under high humidity and temperature conditions such as those prevailing in tropical and subtropical regions of the world. Tropical storage conditions were simulated by storing samples of both whole sound and feed-grade pea at temperatures of 10, 20, 30 and 40ºC and RH of 60, 70, 80, and 90%. The specific objectives are:

a) to determine the number of mold-free days for clean pea and typical feed pea subjected to the aforementioned storage conditions;
b) to determine the effect of feed pea components on molding;
c) to identify genus of molds and examine their potential toxicity; and,
d) to model mold development under adverse storage conditions.

Review of Literature

Canadian pea exports to tropical and subtropical countries account for about 60% of the total exported pea. It is important to know what happens to feed-grade pea when it reaches the export destination with humid and warm conditions.

Effect of Moisture and Temperature on Fungal Growth

Moisture and temperature are the most important factors in storage of grains and grain products because they influence the rate of deterioration during storage. Moisture migration in storage results from temperature gradients within the grain bulk. The higher the moisture content (MC) of the grain and the greater the temperature difference within the grains, the more rapid the moisture transfer will be (Bala, 1997; Anderson and Alcock, 1954; Christensen, 1982).

Most rapid deterioration occurs under conditions of high temperature and moisture. Pulses harvested at MC at or above 15% require careful management during storage. In general, drier seed can be more safely stored. At moisture levels of 8-11% in the seed, there is no risk of damage if the seeds are placed in cold storage. Reducing MC and temperature increases the longevity of the seed (Kosolofski et al., 1998).

Fungi are the major cause of deterioration and decay in stored grains. Invasion by storage fungi may increase the equilibrium moisture content (EMC) at the RH that permits their growth. For this reason, RH rather than the MC is suggested to be used as a measure of the grain's liability to attack by storage fungi. RH decreases with increasing temperature at constant MC. The major storage fungi comprise only of a few species of *Aspergillus* that grow in nearly dry condition and several species of *Penicillium* that grow mainly in grains of high moisture content stored at low temperatures. A variety of other fungi may grow in high moisture grain before drying or in grains that become wet during storage. *Rhizopus, Mucor*, and *Nigrospora* are among the most common of these (Christensen, 1982; Bala, 1997; Justice and Bass, 1978; Brooker et al., 1992; Sauer, 1992).

The physical condition, viability, and MC of the seed and the ambient temperature, and RH of the storage area largely determine fungal activity. Consequently, the fungal population reflects the kind and efficiency of the postharvest handling, conditioning, and storage environment of the seed lot (Justice and Bass, 1978).

Effect of Storage on Quality Factors of Field Pea

Gorecki et al. (1985) investigated the proteins of pea seeds stored at 50 and 90% RH after 7 months. After 7 months at 90% RH, there was a marked deterioration in seeds and germination was below 20%; germinating seeds produced smaller seedlings. Seeds stored at 50% RH were not affected. Deterioration of seeds caused a change in proteins of seeds with decreased vigor and viability.

Powell and Matthews (1978) studied samples of 13 seed lots from six cultivars of pea drawn from commercial warehouses followed by storage of up to two years. With increased time in storage, a decline in seed vigor was indicated by an increase in the leaching of electrolytes from the seeds and reduced vital staining, although the viability was still maintained at a level above the minimum standard (80%).

Study on breakability and size of field pea by Cassells and Green (1982) showed that delay in harvest affects postharvest breakage to a greater degree than seed MC.

Seed coat durability of field pea as affected by seed MC and temperature was studied by Ehiwe et al. (1987). Results of this study showed that at all temperature levels tested (-40, -25, -10, 6,

24 and 40ºC), seed coat breakage increased linearly with decrease in MC from 18.3% to 6.3%. In most cases, breakage increased with a decrease in temperature. It was recommended that pea should not be handled at MCs below 14% or temperatures below -25ºC.

Bennett-Lartey (1991) studied the moisture adsorption rate and the longevity of pea, sunflower and groundnut seeds using a rapid deterioration test and a storage test lasting 35 days. It was identified that RH, temperature, O_2 and CO_2 content affected seed viability during storage, where RH and temperature were the most important factors influencing seed longevity. RH affected seed quality by influencing the seed MC and the growth and reproduction of fungi and insects.

Vertucci and Roos (1990) concluded that the MC at which physical and physiological changes were observed differed among different seed species, and correlated with the lipid content of the seed. Seeds with higher lipid contents had lower thresholds of respiration and lower MC for optimum storage.

A study by Vertucci et al. (1994) on the optimum moisture contents for storage of pea seeds illustrated that there is an optimum water content for seed storage and that, it increased with decrease in temperature. The optimum MC increased as temperature decreased. The rate of deterioration decreased as temperature decreased for each MC studied. Seeds aged much faster when stored under light rather than in the dark. Significant deterioration was progressive with time, suggesting that aging caused it.

Mills and Woods (1994) studied the deterioration in seed quality of initially sound field pea (*Pisum sativum L. 'Titan'*) and white bean (*Phaseolus vulgaris L. 'Seafarer'*) during storage for 147 days at temperature-moisture levels typical of storage condition in Manitoba. In this study, the time required for development of off-odors and visible mold, fat acidity value (FAV), conductivity (seed electrolyte leakage), germination, occurrence of particular fungi and their association with off-odors and seed quality were assessed. Spoilage increased as temperature and moisture increased, as a result of changes in off-odors, FAV, conductivity, and germination levels. No off-odors were detected after 147 days of storage for pea initially stored at 22ºC and 14.5% MC or for bean stored at 22ºC and 14.2% MC. Among the quality parameters used, the presence of off-odors was most closely related to the onset of quality deterioration in pea and bean, because off-odors were produced by both mycological and biochemical processes during deterioration. In pea, visible molds followed the same pattern as off-odors at moderate moisture and temperature. Under cooler temperature and lower MC conditions however, mold developed slowly; under warmer and drier conditions, mold was not visible. The most important storage fungi associated with pea and bean quality deterioration are *Penicillium* and *Eurotium* species. Other postharvest fungi isolated included *A. flavus* Link and *Rhizopus arrhizus* Fischer. In pea, *A. candidus* Link *ex Fr.* and to a lesser extent *A. ochraceus* Wilhelm and *A. wentii* Wehmer additionally occurred. The preharvest fungi *Cladosporium cladosporioides* (Fres.) de Vries and *Alternaria alternata* Keissler and bacteria occurred on both seed types (Mills and Woods, 1994).

Mills et al. (1995) examined the factors affecting the cooking, physical, chemical, and biological characteristics of field pea and white bean. Water uptake increased as storage temperature and initial MC increased in pea. Phytic acid content decreased with an increase in initial MC.

Booth et al. (2001) showed that storage conditions at temperatures of 30ºC and above combined with RH of 80% or higher, limited the storage life of whole sound pea to less than 100 mold-free days. This was also true at RH of 85-90% or higher, at all temperatures (10, 20, 30 and 40ºC) studied. Experiments with feed pea mixtures, although limited in duration, showed similar trends, with increased hygroscopicity of the damaged pea and foreign material-contaminated samples making them even more susceptible to molding. McKay et al. (2003) showed that pea at 18% moisture could be stored for 20 weeks at 20ºC, but only for 4 weeks at 25ºC.

Materials and Methods

Material

A 15 kg sample of No. 1 Canada 'Mozart' field pea was obtained from Walker Seeds Ltd. of Tisdale, SK. The seeds were sieved and hand-sorted for quality. Quality factos such as smoothness, roundness, and an intact seed coat were used to sort pea. Only good quality whole pea was used in this experiment. This is referred in the experiment as clean pea.

The sample of feed pea was mixed by hand using whole yellow and green peas and pea screenings also obtained from Walker Seeds Ltd. According to the analysis done by Booth et al. (2001), a feed-grade pea sample contains 54.1% whole yellow pea, 2.0% foreign materials, 0.9% cracked seed coats (yellow), and 5.1% whole pea of other color (green), 0.4% cracked seed coats (green), 13.8% splits (yellow), 4.8% splits (green), 7.3% shriveled (yellow), 4.8% shriveled (green), 3.0% other damage (yellow), 0.1% other damage (green), 1.9% small (yellow) and 1.8% small (green). Other damaged yellow and green peas are referred to any damage other than splits, insect damage, heated or shriveled. In other words, any discoloration or physical damage on the face of the cotyledon is referred as other damaged pea (Canadian Grain Commission, 2003). Typical feed-grade pea samples were assembled to reflect these ratios. For the storage studies, 10 g of sample was spread in one layer on the petri dishes; eight petri dishes were placed in each airtight chamber; four on each shelf.

Experimental plan

The variability in the composition and quality of feed pea makes it difficult to determine how individual variables such as temperature, RH, and FM affect mold development. Therefore, tests were conducted on both pea of a common commercial variety of No. 1 Canada to eliminate as many variables as possible and typical feed-grade pea as well as feed pea components to isolate the variables that impact mold development. The results from No. 1 Canada were used as a standard against the other sample. This data was then used to determine the effect of temperature and RH on both MC and mold growth.

In this study, both whole sound and feed-grade peas were stored under temperatures of 10, 20, 30, and 40°C and humidities of 60, 70, 80, and 90%, each in duplicate in airtight chambers. The experimental plan for this study is shown in Table 1. In preliminary experimental trials, all components of feed-grade pea were exposed to the temperature of 40ºC and RH of 90% in airtight chambers, in order to determine the effect of each component on mold appearance.

Experimental equipment

Forty-eight airtight cylindrical chambers were designed and built to provide controlled environment for pea storage. They were made of PVC pipe, with clear acrylic end plates and two shelves to hold the petri dishes. The interior dimensions were 190 mm$\times$205 mm. The two round shelves were joined together with a bolt placed on a stand constructed of a PVC pipe end cap. The cap had a large hole drilled out of the top and six small holes drilled around the outer edge in order for the air to circulate easily throughout the container. The top support tray was made of acrylic or pexiglass in order to increase visibility through to the second shelf of samples. Figure 1 shows the components of the airtight chamber. The top of the support trays had fishing line tied to it, as well as a fishing leader. The leader was used to suspend the shelves from the bottom of the scale in order to measure weight without disrupting the samples, nor the storage environment. A glass dish containing the solution to control the humidity levels was placed at the bottom of the test chamber. Eight 60 mm$\times$15 mm plastic petri dishes were placed on the shelves in each airtight chamber. The clear acrylic end plate was fastened with five wing nuts, two rubber washers were used, one at the bottom and the other, at the top of the chamber, to

help provide airtight condition. Rubber stoppers were used to close the access holes on the top of the acrylic plate in order to provide airtight condition inside the chamber.

Four controlled environment cabinets (Conviron Plant Growth Chamber PGR15, Controlled Environment Ltd., Winnipeg, MB) at the Phytotron facilities of the College of Agriculture, University of Saskatchewan were used for each temperature setting for storage and moisture adsorption test in static environment. After a day of temperature stabilization, 16 airtight chambers were placed inside the cabinets at 10 and 20°C and 8 airtight chambers inside the cabinets at 30 and 40°C. As the number of airtight chambers was less than the required, 8 chambers were located in each Conviron chamber, four at each RH of 80 and 90%. It was apparent that these samples will become molded in less than 2 months and then the experiment could carry on by changing the RH inside the airtight chambers to 60 and 70%. This allowed for the testing of four humidity levels at each temperature in duplicate for both whole pea and feed-grade pea.

Maintenance of relative humidity inside the airtight chambers

A glass dish containing 200 ml saturated salt solution or dilute sulphuric acid solution was placed at the bottom of each test chamber in order to control RH levels inside the containers during storage studies in the static environment. The sulphuric acid concentrations and the salts that were used are provided in Table 2. RH of 60, 70, 80 and 90% were created by saturated salt solutions in airtight chambers at temperatures of 10, 20 and 30°C, while the same range of humidity was provided by dilute sulphuric acid solutions at 40°C in airtight chambers. The four RH levels at each temperature for both whole and feed-grade peas were tested in duplicate. RH of the chamber was stabilized for approximately one week before placing samples inside the chambers. The solutions controlling the humidity were checked weekly. The RH within the airtight chambers was measured once a week using a Vaisala HM 34 solid-state humidity and temperature sensor (Vaisala Inc., Woburn, MA). A hole with a rubber stopper was located on the top of each chamber for the insertion of the humidity and temperature sensor.

Weighing of the samples inside the chambers

Weight of the samples was recorded once a week in order to measure their MC. This was done by removing a rubber stopper at the top of the lid and running a fishing leader to a hook at the bottom of a scale (Mettler PL1200, Mettler Instrumental, CH-8606, Greifensee, Zurich, Switzerland). The weights were taken this way to minimize the disruption of the samples and environment in the container.

Measurement of CO_2 inside the chamber

It was thought that if the containers were completely sealed to the outside environment, the CO_2 content of air in the chamber would increase resulting in mold growth retardation or inhibition on the samples. Therefore, CO_2 content was tested every two to three weeks by removing some of the container air using a syringe from the hole with the rubber stopper, limiting the disturbance of the samples. Gas samples were analyzed for CO_2 using a gas chromatograph at the Department of Soil Science of the College of Agriculture, University of Saskatchewan. CO_2 concentration was high in contaminated samples, showing molds' respiration. The amount of CO_2 (ppm) inside the test chambers was almost the same as the ambient CO_2 during the experiment at all the temperatures and RH of 60 and 70% and also at 10°C and 80% RH. The CO_2 concentration inside other samples varied within the range of normal CO_2 variation in the air.

Moisture content measurement

The initial MC of the whole pea, feed-grade pea, and all components of feed-grade pea was determined using standard S352.2 of the American Society of Agricultural Engineers (ASAE, 2003) and standard 44-15A of the American Association of Cereal Chemists (AACC, 1995). The MC of pea at which molds started to appear was calculated based upon the weight of the samples and its initial MC, and it was also determined by AACC standard 44-15A methods.

Fungi identification

Samples were examined for mold appearance every day and as soon as mold was observed, the contaminated samples were removed from the chambers. The contaminated petri dishes of samples were photographed and then sent for mold identification to Discovery Seed Labs, Ltd. of Saskatoon, SK. A rough identification of the fungi that grew from the material was made according to cultural appearance and by microscopic examination of their sporulating structures. In addition, a crude estimate of the extent of fungal growth from the material was made.

Data analysis and processing

Data were imported to a Microsoft Excel (Microsoft Corp., Redmond, WA) worksheet. A model was identified to fit the mold-free days data, in order to predict mold development in storage after specified time. This model is similar to the model suggested by Khoshtaghaza et al. (1999) to estimate the number of days until the development of visible mold growth in alfalfa cubes:

$$Y = 10^{(a-b\,T-c\,RH)} \quad (1)$$

where:

Y = storage time (d)

T = temperature (°C)

RH = relative humidity (%)

a, b, c = constants

Equation 1 is valid for tested ranges of 16 to 39°C and 70 to 85% RH up to 90 days for alfalfa cube storage. A spoilage index (SI) was also indicated by Khoshtaghaza et al. (1999) as follows:

$$SI = \sum_{i=1}^{n} \left(\frac{\Delta t}{Y}\right)_i \quad (2)$$

where:

Δt = time interval (d)

In equation 2, Y is the storage time (d) to molding calculated from equation 1 using temperature, and RH at time t. Δt is the time interval during which the temperature and humidity are constant. Index i represents each data set at time t; n represents the total number of data (t = n Δt). When SI ≥ 1, the model indicated that cubes in the container were moldy. There may be a number of uncertainties in these calculations. SI index in this study is calculated only as a demonstration on how it can be used to determine the onset of mold development for pea samples during storage and transport. For SI index to be accurate another storage study has to be conducted for data verification, wherein RH and temperature will be measured at close intervals, i.e., every day or every half a day.

The Solver from Microsoft Excel was used to identify mold-free days model for both clean and feed-grade peas at temperatures of 10, 20, 30, and 40°C and RH of 80 and 90%. The same program was used to identify a spoilage index for both feed pea and clean pea.

Results and Discussion

Fungi Identification

The majority of fungi isolated were species of *Aspergillus* and *Penicillium*. Fungi were not identified to species level in this experiment because it was a highly specialized and time-consuming task. The classification scheme used for the *Aspergillus* and *Penicillium* spp. in this work was based on microscopic and cultural characteristics; upon this classification, they were numbered into putative (mentioned or believed as a formal category) species. In some cases, it was not even possible to classify *Aspergillus* and *Penicillium* into putative species because the growth of fast-growing fungi, such as *Rhizopus* and *Mucor*, in the petri dishes had interfered so much with the growth of the former fungi. These cases were reported as, for example, *Aspergillus* #?. The single species of *Rhizopus, Mucor* and *Cladosporium* found were also not identified to species because of the lack of sporulation. The three fungi that were identified to species level are either common saprophytes on dead plant tissues (*Alternaria alternata, Fusarium equiseti*) or a known foliar pathogen of pea (*Ascochyta pinodes*).

Aspergillus, *Penicillium*, *Rhizopus, Mucor* and *Nigrospora* are mainly storage fungi, whereas, *Alternaria alternata, Fusarium equiseti, Cladosporium* sp. *and Ascochyta pinodes* are field fungi. Field fungi may cause molding of plant products in storage, but they invade the seeds while the plant is still growing in the field. All of field fungi have high water requirements for growth. The damage caused by field fungi is done before harvest and does not continue to increase during storage. With a few exceptions, storage fungi develop only after the product is in the storage. Storage fungi are common in materials exposed constantly to RH of 65-90%, where free water is not available (Sauer, 1992).

Seeds that had been stored at higher temperatures and RH levels were molded mainly by storage fungi. Figures 2 to 4 show close up views of the fungi that appeared on both clean and feed-grade pea. Table 3 summarizes the fungi observed at each temperature and RH. Only species of *Aspergillus* and *Penicillium* that are storage fungi were identified on clean pea and no field fungi was observed on clean pea at all the temperatures tested.

Mold-free days

The number of days based on visual inspection the pea samples surface was mold-free, MC of pea at the end of storage or at the time of mold appearance, RH and RH standard deviation are listed in Tables 4 to 6. Mold growth occurred above 16.2% MC for feed pea components (small pea at 40°C, 90% RH). Mold appeared on feed-grade pea above 13.5% MC at high temperature (40°C, 80% RH) and MC above 15% at lower storage temperature (20°C, 80% RH). For clean pea, mold appearance occured above 14% MC at high temperature (40°C, 80% RH) and above 14.8% at lower storage temperature (20°C, 80% RH). As fungi like elevated temperature and RH, shorter mold-free days of storage resulted from storage at higher temperature and higher RH. Mold was not detected visually on both feed-grade and clean peas at temperatures of 10, 20, 30, and 40°C and RH of 60 and 70% for the whole experiment duration, 216 days at 10 and 20°C, 162 and 156 days at 30°C and RH of 60 and 70%, respectively; and, 174 days at 40°C. Due to the problem with RH and changing the salt solution, the number of mold-free days for pea at 10°C and 60% was 163 days. We could speculate that storage duration at this condition would also be 216 days or longer.

As the preliminary testing on feed pea components at 40°C and 90% RH shows, other damaged pea was the first component that was molded, i.e. after 6 days of storage, followed by foreign material and split pea that both molded after 9 days of storage. The same result was concluded from the daily visual observation. Other damaged pea or the foreign materials were the first components that became moldy inside the petri dishes at any storage condition.

Equation 1 was fitted to the number of mold-free days (d) as a function of temperature and RH for both feed-grade and clean peas. For clean pea:

$$Y = e^{(12.35 - 0.055\,T - 8.69\,RH)} \quad (3)$$

with R^2 of 0.91 and MSE of 140.73.

For feed-grade pea:

$$Y = e^{(7.10 - 0.05\,T - 2.71\,RH)} \quad (4)$$

with R^2 of 0.81 and MSE of 163.67.

Equations 3 and 4 are valid for temperature range of 10 to 40°C and RH range of 80 to 90% up to 105 days of storage for feed-grade pea and up to 126 days of storage for clean pea. A similar equation was used by Khoshtaghaza et al. (1999) for alfalfa cubes during transit and storage. The number of mold-free days obtained by equation 3 for clean pea was compared to the number of mold-free days for alfalfa cube in similar condition of temperature and RH. Clean pea had a higher number of mold-free days in identical storage condition. This could be explained by the fact that alfalfa cube is a porous product, composed of small particles, which are compressed to form a cube, whereas clean pea is a seed with a seed coat protecting the endosperm and has less capacity to adsorb moisture.

The spoilage index (SI) was calculated similar to the SI indicated by Khoshtaghaza et al. (1999), using equation 2. This equation could simply be expressed as:

$$SI = \frac{Y_{exp}}{Y_{Cal}} \quad (5)$$

where:

Y_{exp} = experimental storage time at which the samples went molded (d)

Y_{cal} = calculated storage time from equation 3/4 (d)

The values for spoilage index for both feed and clean pea are presented in Tables 7 and 8 respectively. SI is indicative of pea quality during transport and storage. Good quality pea has a low SI and spoiled and molded pea has SI ≥ 1.

As Tables 7 and 8 show, the probability of pea to go molded is 1.0 when SI ≥ 1. For example, the probability of clean pea to go molded after 43 d of storage at 20°C and 81.4% RH is only 0.66, whereas clean pea will surely go molded (probability = 1.0) after 79 days of storage at 20°C and 79.4% RH. These values are only correct for temperatures of 10, 20, 30, and 40°C and RH of 80 and 90%. There are a number of uncertainties in these calculations. It was expected for all the spoiled and moldy samples to have a SI ≥ 1, whereas in some cases in this study when mold appeared on the samples, SI was lower than one. RH and temperature

information should be taken every hour for SI to be more accurate. Tables 7 and 8 demonstrate how the mold-free days equation (equations 3 and 4) can be used to determine the SI of a batch of stored pea as long as the storage condition (temperature and RH) are monitored, e.g. every 1 h.

Conclusions

The storage stability of pea is similar to other agricultural products; they can be kept safe at low temperature and low humidity. Particularly, this research showed that maintaining temperatures and RH in the airtight chambers below 20°C and 70% extended the storage life of both clean and feed pea to more than 216 days (maximum number of days tested at these conditions). Maintaining temperature and RH below 40°C and 70% extended the shelf life of both feed pea and clean pea to more than 174 days (maximum number of days tested at these conditions). Therefore, during transport and storage, temperature and RH should not exceed the mentioned values for both whole sound pea and feed-grade pea.

A preliminary test was done on the effect of feed pea components on molding. As a result of this experiment, other damaged pea (any discoloration or physical damage on the face of the cotyledon), foreign materials and split pea were identified as the most putative components that accelerates molding.

Fungi that appeared on the pea samples were identified. In whole sound pea at all temperatures and RH, only species of *Aspergillus* and *Penicillium* (storage fungi) were observed. On feed-grade pea, aside from storage fungi such as *Aspergillus* and *Penicillium spp., Rhizopus* and *Mucor spp*. were observed, field fungi such as, *Alternaria alternata, Cladosporium spp.*, *Fusarium equiseti* and *Ascochyta pinodes and* bacterium were also identified, suggesting that foreign materials in feed-grade pea pose greater risk of microbial development during adverse storage conditions.

Models were developed based on the mold-free days for both feed-grade and whole sound peas showing how many days pea can be stored mold-free by knowing the temperature and RH of the storage environment. Also, a spoilage index (SI) was calculated that can be used to show the onset of mold growth during transport and storage. When the SI ≥ 1 the samples already became molded.

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Table 1. Experimental plan for storage and moisture adsorption study in static and dynamic environment.

Variable	Level	Number
Type of pea	Whole sound, Feed-grade	2
Temperature	10, 20, 30, 40°C	4
Relative humidity	60, 70, 80, 90%	4
No. of treatments		32
No. of replicates		2
No. of runs/ experiment		64

Table 2. Saturated salt and dilute sulfuric acid solutions used in the experiments (Bala, 1997; Rahman, 1995; Booth et al., 2001).

	Target RH			
Temperature	60%	70%	80%	90%
10°C	NaBr	$CH_3CO_2Li.2H_2O$	K_2CrO_4	$Sr(No_3)_2$&H_2SO_4
20°C	NaBr	$SrCl_2$	$(NH_4)_2SO_4$	KNO_3
30°C	NaBr	$SrCl_2$	$(NH_4)_2SO_4$	KNO_3
40°C	H_2SO_4 (25.9%)	H_2SO_4 (21.4%)	H_2SO_4 (16.9%)	H_2SO_4 (12.5%)

Table 3. Type of molds observed on both feed pea and clean pea after spoilage.

Temperature (°C)	RH (%)	Type of pea	Mold species
10	80	Clean	*Aspergillus*
10	80	Feed	*Aspergillus*, *Penicillium*, *Alternaria alternata*
20	80	Clean	*Aspergillus*
20	80	Feed	*Aspergillus*, *Penicillium*, Bacterium *Cladosporium, Alternaria alternata, Ascochyta pinodes*, unknown fungus
20	90	Clean	*Aspergillus, Penicillium*
20	90	Feed	*Aspergillus, Penicillium, Rhizopus, Alternaria alternata, Mucor Fusarium equiseti,* unknown fungus
30	80	Feed	*Aspergillus, Penicillium, Rhizopus Alternaria alternata*
30	80	Clean	*Aspergillus, Penicillium*
30	90	Feed	*Aspergillus, Penicillium, Rhizopus Alternaria alternata, Mucor*
30	90	Clean	*Aspergillus, Penicillium*
40	80	Clean	*Aspergillus*
40	80	Feed	*Aspergillus,* Bacterium
40	90	Clean	*Aspergillus*
40	90	Feed	*Aspergillus, Penicillium, Rhizopus*

Table 4. Relative humidity and number of mold-free days for feed pea components.

Feed pea Components	Relative humidity (%)		Mold-free days	MC at mold onset (% w.b.)
	Mean	Standard dev.		
Other color	86.4	5.70	12	21.0
	85.6	5.40	14	20.2
Shriveled	90.0	3.39	12	20.5
	87.9	4.59	15	19.6
Cracked seed coat	88.2	2.96	15	19.6
	87.9	3.82	15	19.4
Split	85.4	5.70	9	20.1
	86.4	4.70	9	21.2
Small	84.9	2.70	15	16.2
	85.9	4.56	15	16.3
Other damage	88.5	1.34	6	21.7
	87.5	1.27	6	22.0
Foreign material	85.7	5.47	9	18.1
	88.0	5.52	9	18.9

Table 5. Relative humidity of storage chambers and number of mold-free days for whole sound pea.

Temperature	Relative humidity (%)		Mold-free	MC at mold onset (% w.b.)
(°C)	Mean	Standard dev.	days	Termination of storage
10	60.1	1.60	163*	12.4
	60.3	1.10	163*	12.5
	71.0	2.52	212*	14.9
	70.7	3.11	212*	14.5
	80.9	3.07	119	20.3
	80.6	3.37	119	21.1
	81.0	5.09	125	20.5
	83.9	3.27	104	23.2
20	58.8	2.10	216*	11.8
	57.7	2.50	216*	11.5
	70.5	2.90	216*	14.4
	71.6	2.10	216*	14.8
	79.4	5.19	76	15.9
	76.6	4.79	90	14.8
	81.4	5.04	42	15.7
	82.5	5.39	35	17.4
30	57.9	1.00	162*	11.1
	58.0	1.90	162*	11.0
	70.3	2.60	156*	13.4
	70.1	2.20	156*	13.3
	77.7	2.57	53	14.6
	77.2	3.02	53	14.5
	83.1	4.04	31	20.8
	82.8	4.34	31	20.9
40	62.4	2.90	174*	10.8
	61.0	0.80	174*	10.8
	69.7	1.80	174*	12.5
	72.1	1.60	174*	12.8
	77.5	4.30	53	14.1
	77.2	4.36	53	14.0
	86.3	5.30	18	21.4
	82.9	5.83	21	16.5

* Samples were not molded at this time.

Table 6. Relative humidity of storage chambers and number of mold-free days for feed-grade pea.

Temperature (°C)	Relative humidity (%)		Mold-free days	MC at mold onset (% w.b.) termination of storage
	Mean	Standard dev.		
10	60.6	1.30	163*	12.5
	60.8	1.20	163*	12.6
	70.3	2.86	213*	14.6
	70.1	2.77	213*	14.8
	78.2	5.34	64	16.9
	80.2	3.81	71	17.4
	80.5	4.98	105	21.5
	80.7	5.07	105	22.4
20	58.6	2.20	216*	11.9
	60.3	2.70	216*	12.1
	71.7	2.90	216*	14.5
	71.5	2.10	216*	14.6
	75.4	8.92	56	15.5
	74.2	5.02	63	15.0
	81.6	5.66	35	16.9
	82.4	6.16	35	17.3
30	57.9	2.20	162*	11.2
	58.1	2.10	162*	11.1
	70.6	2.10	156*	13.6
	70.1	2.50	156*	13.4
	76.9	3.22	34	14.0
	76.7	3.27	34	14.0
	84.7	3.57	25	20.5
	82.4	3.83	25	19.8
40	60.7	0.80	174*	10.8
	60.7	2.40	174*	10.7
	71.5	0.50	174*	12.2
	71.4	0.80	174*	12.7
	76.7	4.26	34	13.5
	77.1	7.38	27	14.6
	84.9	3.45	14	18.2
	87.8	0.60	14	20.7

* Samples were not molded at this time.

Table 7. Spoilage index values for clean pea.

Temperature (°C)	Mean RH (%)	Storage duration (d)	Spoilage index
10	80.9%	120	1.01
10	80.6%	120	0.99
10	81.0%	126	1.07
10	83.9%	105	1.15
20	79.4%	79	1.01
20	76.6%	93	0.94
20	81.4%	43	0.66
20	82.5%	36	0.61
30	77.7%	54	1.04
30	77.2%	54	0.99
30	83.1%	32	0.98
30	82.8%	32	0.96
40	77.5%	40	1.31
40	77.2%	54	1.72
40	86.3%	19	1.34
40	82.9%	22	1.15

Table 8. Spoilage index values for feed-grade pea.

Temperature (°C)	Mean RH (%)	Storage duration (d)	Spoilage index
10	80.5	105	1.28
10	80.7	105	1.28
10	78.2	64	0.73
10	80.2	71	0.86
20	81.6	35	0.73
20	82.4	35	0.74
20	75.4	56	0.98
20	74.2	63	1.07
30	84.7	25	0.94
30	82.4	25	0.88
30	76.9	34	1.03
30	76.7	34	1.03
40	84.9	14	0.88
40	87.8	14	0.95
40	76.7	34	1.71
40	77.1	27	1.37

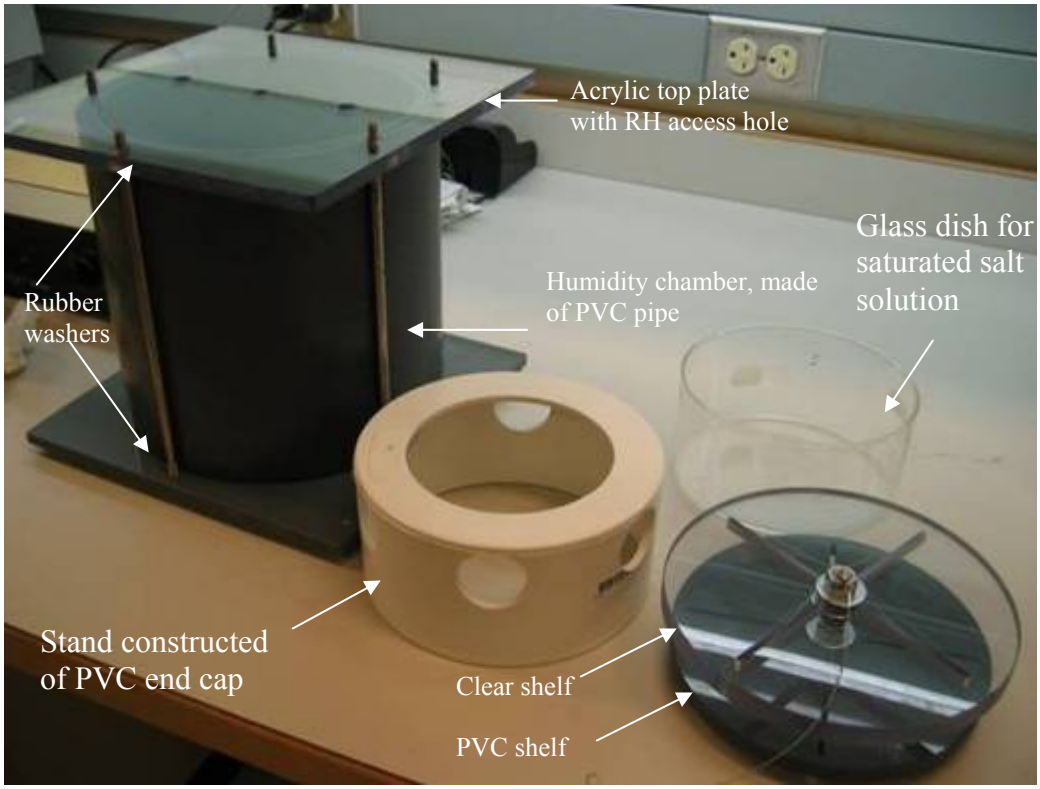

Figure 1. Different components of airtight chambers.

Figure 2. Photograph of the *Aspergillus* appeared on clean pea at 40°C and 90% RH.

Figure 3. Photograph of the fungi that appeared on feed pea at 40°C and 90% RH.

Figure 4. Photograph of the fungi that appeared on feed pea at 10°C and 90% RH.