MODIFIED ATMOSPHERE PACKAGING AND POSTHARVEST QUALITY OF PIGEON PEA

by

Karla Michelle Deza Durand

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Approved by:

Dr. Carol Harper, Ph.D. Chairman Graduate Committee	Date
Dr. Annette Wszelaki, Ph.D. Member Graduate Committee	Date
Dr. Lynette E. Orellana, Ph.D. Member Graduate Committee	Date
Dr. Francisco M. Monroig, Ph.D. Graduate Studies Representative	Date
Dr. Edna Negrón de Bravo, Ph.D. Chairperson of the Department, CITA	Date

Abstract

Pigeon peas are the most important leguminous crop in Puerto Rico, as they are the most highly consumed. Puerto Ricans prefer to consume fresh pigeon peas rather than canned or frozen peas; however, their shelf-life is shorter than processed pigeon peas, so the use of modified atmosphere packaging (MAP) can play an important role in extending their shelf-life. Therefore, the purpose of this research was to develop a MAP system for fresh pigeon peas to increase their shelf-life and also provide knowledge of the postharvest quality. Pigeon peas were stored at 0, 5, 10, 15 and 20 °C, in order to characterize their postharvest quality. Changes in respiration rate, mass loss, titratable acidity (TA), pH, solid soluble content (SSC), texture and color were assessed as quality indicators. Two Cryovac films (PD 941 and PD 961) were evaluated at 0 and 20 °C. The treatments were MAP 1 (PD 941 air headspace), MAP 2 (PD 941 2% O_2 + 5% CO_2 + 93% N_2 headspace), MAP 3 (PD 961 with 6 hole punctures of 1mm in diameter) and MAP 4 (PD 961 air headspace). Measurements of %CO_2 were performed at 0, 7 and 21 days. Mass loss, TA, pH, SSC, color, texture and microbiological analysis were assessed at 7, 14 and 21 days. Storing pigeon peas at 15 and 20 °C was detrimental to their overall quality, as evidenced by higher respiration rates, greater mass losses, yellowing and decay. In addition, at 0, 5, and 10 °C, there were observed minor changes in all indicators of quality, however, chilling injury was found at 0 and 5 °C, therefore, 10 °C was the optimum temperature for storage. Furthermore, MAP 1 had minor changes in all indicators of quality reaching a shelf-life of 14 days at 0 °C, which represents an increase of 100% in comparison to air storage at 0 °C in addition to an enhanced green color of the seed. Finally, under temperature abuse of 20 °C no off-odor or extensive decay was observed using the film PD941.

Resumen

El gandul es la leguminosa más importante de Puerto Rico. Los puertorriqueños prefieren consumir el gandul fresco y no así el enlatado o congelado, sin embargo el tiempo de vida útil del gandul fresco es más corto que el procesado, por lo que el uso de un empaque en atmósfera modificada podría extender la vida útil de este. El propósito del presente estudio fue desarrollar un empaque en atmósfera modificada (MAP) para el gandul fresco e incrementar su tiempo de vida útil, además, de proveer conocimientos acerca de su calidad postcosecha. Los gandules fueron almacenados a 0, 5, 10, 15 y 20 °C con la finalidad de caracterizar la calidad postcosecha. Los cambios en la tasa de respiración, pérdida de masa, acidez titulable (AT), pH, contenido de sólidos solubles (CSS), textura y color fueron utilizados como indicadores de calidad. Dos películas plásticas de la compañía Cryovac (PD 941 y PD 961) fueron evaluadas a 0 y 20 °C. Los tratamientos fueron los siguientes: MAP 1 (PD 941 con aire en el volumen libre), MAP 2 (PD 941 con 2% O_2 + 5% CO_2 + 93% N_2 en el volumen libre), MAP 3 (PD 961 con 6 orificios de 1mm de diámetro en la superficie de la bolsa y aire en el volumen libre) y MAP4 (PD 961 con aire en el volumen libre). El contenido de CO_2 en las bolsas fue analizado a los 0, 7, 14 y 21 días. La pérdida de masa, AT, pH, CSS, textura, color, olor objetable y análisis microbiológicos fueron evaluados a los 7, 14 y 21 días de almacenamiento. Almacenar los gandules a 15 y 20 °C la calidad del gandul se redujo debido a la alta tasa de respiración, mayor pérdida de masa y avanzada senescencia. A 0, 5 y 10 °C, los cambios en los indicadores de calidad fueron menores, sin embargo a 0 y 5 °C se observo daño. La temperatura óptima de almacenamiento fue 10 °C. Por otro lado, MAP 1 mantuvo la calidad del gandul por 14 días a 0 °C, lo cual represento un incremento del 100% de la vida útil del gandul en comparación al almacenamiento al aire a 0 °C,

además de realzar el color verde de la semilla. Finalmente, bajo temperatura de abuso a 20 °C no se detecto olor objetable o extensiva pudrición en el gandul usando el film PD 941.

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Introduction

Pigeon peas [*Cajanus cajan* (L.) Millsp] are the most important leguminous crop in Puerto Rico, which represents an important contribution to the total consumption of vegetables on the island and ranks 4th in importance among edible legumes in worldwide production (Morton, 1976). Per capita consumption of fresh and frozen pigeon peas were 1.8 pounds during 1999/2000 (Department of Agriculture of the Commonwealth of Puerto Rico, 2004). Pigeon peas occupy an important place in human nutrition as a source of dietary proteins, carbohydrates and minerals as a staple crop in several countries (Singh, 1980). Immature seeds of pigeon peas are reported to contain per 100 g, 117 calories, 69.5% moisture, 7.2 g protein, 0.6 g fat, 21.3 g total carbohydrate, 3.3 g fiber and 1.4 g ash, which are important nutritional attributes for the consumer (Singh *et al.*, 1993). In Puerto Rico, the fresh immature seeds are very popular and sell for twice the price of the mature dried seed because they are tastier, more tender and cook in less time (Morton, 1976). However, they are currently distributed to traditional markets and supermarkets and sold without any special care in packaging, refrigeration and sanitation.

While pigeon peas have economic and nutritional importance in Puerto Rico, no research has been previously reported about the postharvest physiology and use of modified atmosphere packaging on this commodity. Much research has focused on pigeon pea pre- harvest factors (Bidlack *et al.*, 2001, Khattra *et al.*, 2000; Durairaj and Ganapathy, 2000; Echavez and Bosques, 1998) for the canning and drying markets.

Postharvest physiology is commonly defined as the study of living, respiring plant tissue that has been separated from the parent plant (Shewfelt, 1986). After harvest, the commodity is still living as it continues to perform metabolic reactions in order to maintain its physiological system and this causes a reduction of its quality and shelf-life. In order to extend the shelf life of the commodity, it is necessary to retard certain deteriorative processes. One such method to extend fresh vegetable shelf-life is the use of modified atmosphere packaging (MAP) systems (Mazza and Jayas, 2001; Shewfelt, 1986). MAP is defined as the enclosure of food products in materials that create a gas barrier so that the gaseous environment changes from ambient conditions (78% N_2, 21% O_2, and 0.03% CO_2) (Church and Parsons, 1995). The benefits of MAP in fresh vegetables includes a reduction in chlorophyll breakdown, an improvement in texture, reduction in some physiological disorders caused by ethylene production and chilling injury, and a reduction in microbial activity. The shelf-life of fresh pigeon peas is shorter than processed pigeon peas (Rakotonirainy *et al.*, 2001). For this reason, packaging could help to maintain the taste and general appearance of the fresh commodity.

MAP can be used to expand the distribution and marketing of pigeon peas, also, which is potentially useful for growers and consumers. Therefore, the objectives of this research were:

- To characterize the postharvest physiology of fresh pigeon peas.
- To develop a modified atmosphere packaging system for fresh pigeon peas.
- To extend the shelf-life of fresh pigeon peas.

Literature review

Description of pigeon peas

The pigeon pea pod is green or green and purple and contains around 2-9 seeds per pod depending on the variety (Nene *et al.,* 1981; Morton, 1976) (Fig 1). The seeds are green, when immature, but through maturation the seed changes to white, yellow, brown, and sometimes black, which is objectionable to consumers (Kathra *et al.,* 2000; Morton, 1976).

Figure 1. Pigeon pea plant and seeds

Pigeon peas are photoperiod-sensitive plants. The flowering is controlled by the length of the night, for this reason, short days (long nights) decrease time of flowering (less than 12 hours), which affects the time of cultivation and harvesting (Alemañy 2001; Morton, 1976). Short day pigeon pea plants have two genotypes: determinate and indeterminate. Determinate pigeon pea plants are characterized by the growth of the pods at the end of the branches until reach a determinate high; in contrast the pods grow on the whole plant in indeterminate genotypes (Hernandez, 2002) (Table 1). In general, short day varieties may flower between 90-320 days after planting, and harvest can be made after 50 to 60 days after induction (Valenzuela and Smith, 2002; Acosta *et al.,* 1992).

Table1. Different cultivars of pigeon peas produced in Puerto Rico

<table>
<tr><td colspan="3">Photoperiod</td></tr>
<tr><td colspan="2">Sensitive</td><td>No-sensitive</td></tr>
<tr><td colspan="2">Short days</td><td>Determinate</td></tr>
<tr><td>Determinate</td><td>Indeterminate</td><td rowspan="2">I-8-3-1 (Lazaro)
I-8-2
II-5-6
I-13
I-58-3</td></tr>
<tr><td>Cortada
PR-147
2-B-bushy
Line 84</td><td>16-1
Pinto
Kaki
Blanco de Yauco
Guerrero</td></tr>
</table>

The planting of indeterminate genotypes can be made until October, but is preferably from June to August, with harvesting between December and March, and they are mainly used for the fresh market (Hernandez, 2002; Acosta *et al.,* 1992; Morton, 1976). Meanwhile, determinate genotype mainly used for the canning industry and planting is recommended in June, July and August for manual harvest, and between August and November for mechanical

harvesting (Hernandez, 2002). Long day cultivars (short nights) are not produced in Puerto Rico because the hours of daylight are not enough to induce flowering (Hernandez, 2002; Morton, 1976).

Postharvest quality

Postharvest quality of commodities can be defined as the study of physiological changes that occur in the commodity after harvest. These changes deplete the commodity's own resources in order to maintain the metabolic reactions, causing an irreversible decline in quality (Maguire *et al.,* 2004; Shewfelt, 1986). Quality can be defined as "the composite of physical, chemical and sensory characteristics that differentiate individual items of the product and have significance in determining the degree of acceptability of the item to the buyer" (Shewfelt, 1994; Watada, 1986). No quality characteristics for fresh pigeon peas have been reported. However, the tenderness, green color and sweet taste can be the most important.

After harvest, many physiological changes occur, so the respiration rate can give an overall perspective of commodity metabolism (Maguire *et al.,* 2004; Kader and Salveit, 2003a). Aerobic respiration is the central process in living cells that release energy through the utilization of organic compounds. This energy is trapped in the biological form of adenosine triphosphate (ATP), which is used to drive energy to catabolic and anabolic reactions inside the cell (Kays, 1991; Wills *et al.,* 1982).

While the commodity is attached to the parent plant, it obtains all the energy it needs from the balance between utilization of carbon compounds (respiration) and acquisition

(photosynthesis). However, once the commodity is harvested this balance is changed and the source of organic compounds comes from the reserves of the commodity, which are depleted by the respiration process causing a decrease in the quality of the commodity (Maguire *et al.,* 2004; Kays, 1991).

The respiration process under aerobic conditions involves a series of oxidation-reduction reactions, where glucose is the main metabolite used to maintain an adequate supply of ATP. However, other substrates like proteins and organic acid are used when glucose is exhausted (Kays, 1991; Wills *et al.,* 1982). The complete oxidation of glucose involves three main reactions: glycolysis or the Embeden-Meyerhof- Parnas (EMP) pathway, the tricarboxilic acid cycle (TCA) or Krebs cycle and the electron transport system, the presence of oxygen is essential in the two last processes (Kader and Saltveit, 2003a; Kays, 1991; Wills *et al.*, 1982).

In the glycolytic pathway, the glucose obtained from starch breakdown is phosphorylated by the enzyme starch phosphorylase to form glucose-1-phosphate and converted to glucose -6-phosphate by the enzyme hexose isomerase. Free glucose is formed from glucose 6- phosphate by the enzyme hexokinase. Subsequently, glucose 6- phosphate is isomerizaded by phosphohexose isomerase to fructose 6-phosphate and then converted to fructose 1, 6-biphosphate by the enzyme phosphofructokinase (Kays, 1991; Wills *et al.*, 1982).

Fructose 1, 6-biphosphate is broken down in dihydroxyacetonephosphate and 3-phosphoglyceraldehyde by an aldolase reaction. Both compounds are in equilibrium in order to obtain two 3-phosphoglyceraldehyde molecules. The 3-phosphoglyceraldehyde molecules are

oxidized forming two 1, 3-diphosphoglyceryc acid and two NADH. The 1, 3-diphosphoglyceric acid then progresses through a series of enzymatic steps (deshydrogenase, phosphoglycerate kinase, mutase, enolase and pyruvate kinase) to finally obtain two ATP, two water molecules and two pyruvates by each molecule of glucose (Brandis-Heep, 2000; Kays, 1991; Wills *et al.*, 1982) (Fig. 2).

The second step is the tricarboxylic acid (TCA) or Krebs cycle (Fig. 3). The first step is when pyruvate moves by diffusion from cytoplasm to mitochondria where it is oxidized to acetyl coenzyme A by pyruvate dehydrogenase and then combined with oxaloacetic acid to form citric acid, which moves through a series of steps in order to obtain oxaloacetic acid to begin the cycle again (Kader and Salveit, 2003; Wills *et al.,* 1982). In this process, for each pyruvate molecule three carbon dioxide (CO_2) molecules and four electron pairs in the form of NADH molecules are liberated in the conversion of pyruvic acid to acetyl coenzyme A, isocitric acid to α-ketoglutaric acid, and α-ketoglutaric acid to succinic acid and the step of malic acid to oxaloacetic acid to complete the four molecules of NADH. Besides, one molecule of $FADH_2$ is produced in the conversion of succinic acid to fumaric acid and one single molecule of ATP to succinil CoA to succinic acid (Kays, 1991; Wills *et al.,* 1982).

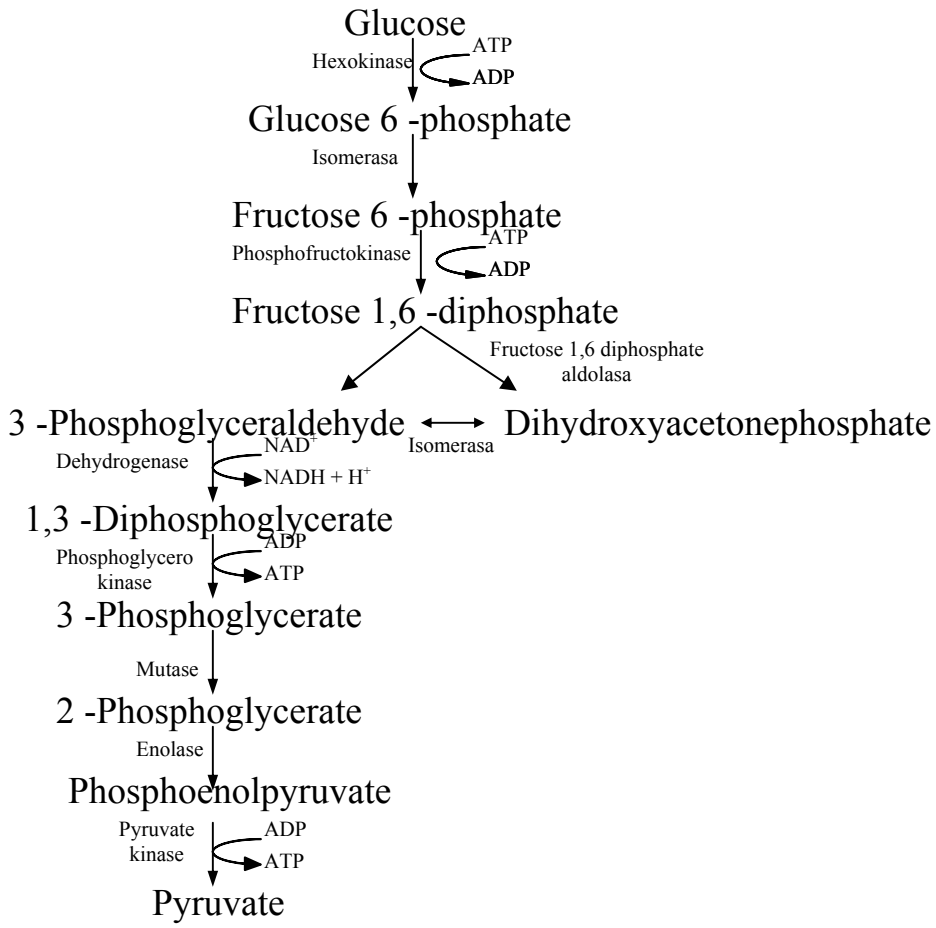


Figure 2. The glycolytic pathway for aerobic oxidation (Modified from: Kays, 1991 and Wills *et al.,* 1982).

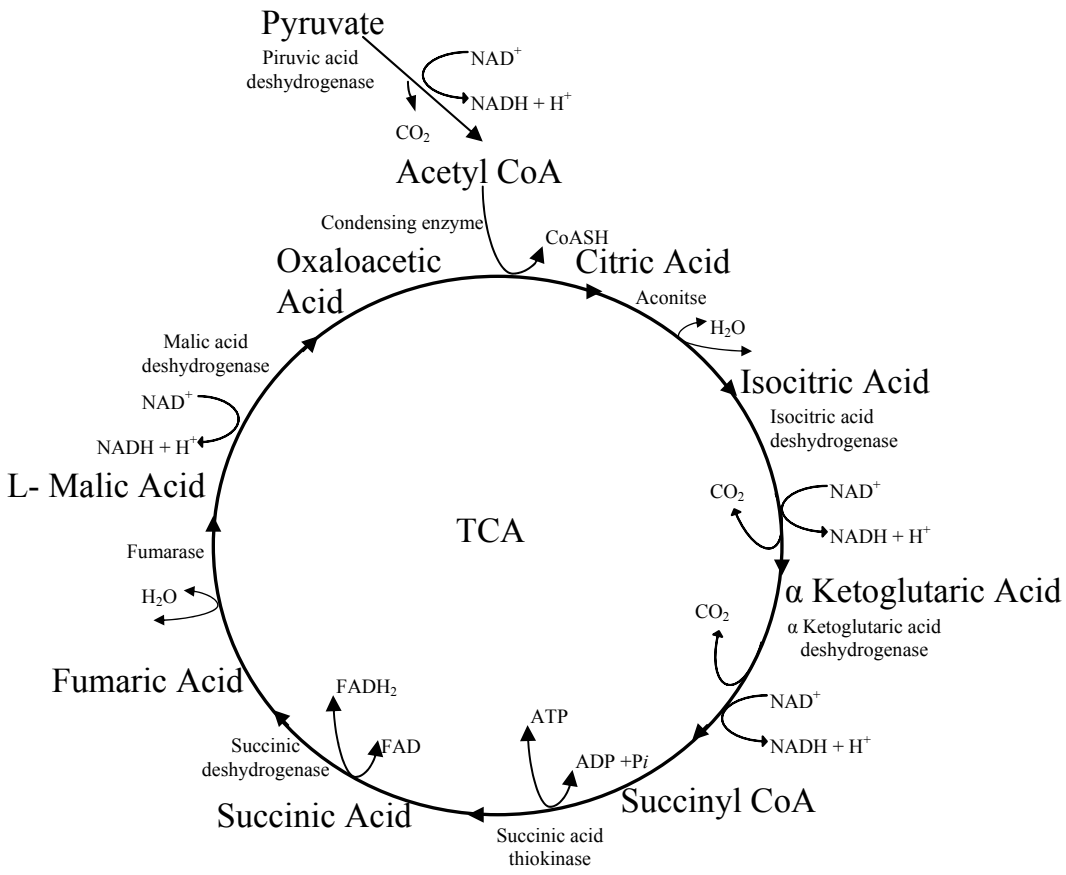


Figure 3. The tricarboxilic acid cycle (Modified from: Kays, 1991 and Wills *et a.l,* 1982).

The third pathway, the electron transport system occurs in the cristae of the mitochondria and involves the oxidation of $FADH_2$ and NADH obtained in the TCA and glycolisis. In this pathway the electrons are removed in a series of reactions to combine with oxygen to form water and the energy release is trapped and conserved in a biological form of ATP. The overall reaction can be written as:

$$C_6H_{12}O_6 + 6\ O_2 + 38\ ADP + 38\ Pi \rightarrow 6\ CO_2 + 6\ H_2O + 38\ ATP + 686\ Kcal$$ (Kader and Salveit, 2003a; Wills *et al.,* 1982)

In the absence of oxygen the commodities can initiate anaerobic respiration, where glycolisis is the only source of ATP production. Here the pyruvate is decarboxylated to forms lactic acid and acetaldehyde with a release of one molecule of CO_2. Subsequently, acetaldehyde form alcohol by the action of the enzyme alcohol dehydrogenase. The final equation can be written as follows:

$$C_6H_{12}O_6 + 2ATP + 2\ ADP + 2\ Pi \rightarrow 2\ CO_2 + 2\ ethanol + 4\ ATP$$ (Kader and Salveit; 2003a, Wills *et al.,* 1982)

Anaerobic respiration is detrimental to the quality of the commodity due to the fact that more glucose must be oxidized to maintain the requirements of the cell, resulting in the production of off-odors and off-flavors.

The release of CO_2 and consumption of O_2 in the respiration process makes it possible to measure the respiration rate as a function of one of these metabolites during maturation, ripening and senescent periods to obtain a respiratory pattern (Wills *et al.,* 1982). Commodities can be divided in two respiratory patterns: climacteric and non-climacteric. Non-climacteric commodities showed a steady decline in respiration after harvest such as green bean and peas, in contrast, climacteric commodities increase the respiration rate during ripening until a peak is reached after which there is a subsequent decline in respiration. Tomatoes, watermelons, and chili peppers present climacteric ripening (Maguire *et al.,* 2004; Kader and Salveit; 2003; Wills *et al.,* 1982). No pattern of respiration has been reported for pigeon peas.

The respiration rate can be affected by several intrinsic and environmental factors. Church and Parsons (1995) indicated that the respiration rate depends on product type (fruit or

vegetable), variety and stage of maturity. Wills *et al.* (1982) mentioned that peas have a high respiration rate (260 ml CO_2 kg^{-1} h^{-1} at 5 °C) because they are harvested at an immature stage. Wounding also stimulates the respiratory rate (Kays, 1991). Tewfik and Scott (1954) found that a mechanically shelled pea increases in CO_2 production. Batrash *et al.* (1993) reported that broccoli florets had an increase of 40% in respiration rate compared to intact heads during storage in air at 4 °C. Also, the higher respiration rate reached by broccoli florets was affected by the increase in the surface area to volume ratio, which was twice that of the intact heads.

Environmental factors, such as temperature also influence the metabolic rate of the commodity. Temperature is the most important environmental factor affecting metabolic reactions. When the temperature increases all enzymatic reactions increase, resulting in a significant increase in the respiration rate. Tewfik and Scott (1954) observed that the respiration rate of peas increases with an increase of temperature, with 38.7, 55.1, 72.1 and 295.5 mg kg^{-1} h^{-1} at 0, 3.3, 6.7, and 22.2 ºC, respectively. In contrast, a decrease in temperature gives a reduction in respiration rate and metabolism (Kays, 1991; Wills *et al.,* 1982). However, not all metabolic reactions have the same patterns. Kays (1991) mentioned that a decrease in temperature causes a decrease in respiration rate of potatoes; however, the conversion from starch to sugar increases significantly, which is undesirable in potatoes.

Nonetheless, not all the effects of reducing temperature are beneficial. Chilling injury is a common physiological disorder that arises during storage between 0 and 10 ºC in tropical and subtropical crops (Serrano *et al.,* 1997).

The chilling mechanism is explained as the changes in the membrane lipids of the cell, which become from flexible liquid crystalline into a solid gel structure at critical low temperature (Wang, 1982; Wills *et al.,* 1982). This change affects the enzymatic activity of the membrane, and the synthesis and degradation of proteins causing a metabolic disruption of the cell, which is developed under low temperature and usually observed once the sensitive commodity is returned to non-chilling temperature (Kays, 1991; Wills *et al.,* 1982). The physical change of the membrane causes secondary responses or symptoms that are irreversible and results in quality loss and death of the commodity (Kays and Paull, 2004; Wills *et al.,* 1982). The symptoms depend on the temperature, the duration of exposure, plant part, cultivar, maturity at harvest, and also appear to increase after transferring to non-chilling temperatures (Kays and Paull, 2004; Medlicott *et al.,* 1990, Morris, 1982; Wang, 1982). As the time of exposure increases, the sensitivity of the commodity to the chilling temperature increases. Secondary effects are frequently observed, such as loss of membrane integrity, leakage of solutes, like sugars, amino acids and minerals salt, which also can be used to improve the bacterial and fungal growth (Kays and Paull, 2004; Wills *et al.,* 1982). Other symptoms include uneven and abnormal senescence, an increase in water loss, increasing CO_2 and ethylene production, surface pitting and internal browning (Serrano *et al.*, 1997; Wills *et al.,* 1982; Eaks and Morris, 1956). Pitting of the skin is often due to collapse of the cell; take a form of surface discoloration, meanwhile, browning usually appears around the strand, probably by the action of polyphenol oxidase released from the disruption of the vacuole, as well known in apples and pineapple (Wills *et al.,* 1982).

Some techniques have been proposed to reduce chilling injury in different fruits and vegetables. These techniques include conditioning at near chilling temperatures before chilling,

intermittent warming, pre treatments with calcium, chemical treatments and controlled or modified atmosphere (Morris 1982; Wang, 1982). Still, the best way to avoid chilling injury is storing the sensitive commodity above the threshold of chilling temperature (Morris, 1982).

Temperature conditioning consists of exposing the sensitive commodity to temperatures slightly above the critical chilling temperature before transfer to this temperature. Sweet peppers stored at 10 °C for 5 to 10 days had reduced chilling injury at 0 °C (McColloch, 1962). In addition, the interruption of chilling temperature storage in sensitive commodities with one or more short periods of warm temperature is defined as the technique of intermittent warming (Wang, 1982). This warming allows the tissues to metabolize the toxic substance accumulated during chilling storage (Wang, 1982). Controlled and modified atmospheres can be reduced chilling injury symptoms and also extended the shelf-life of the commodity. Avocados variety "Fuch" and "Waldin" stored with a gas mixture of 2% O_2 and 10% CO_2 at 7 °C for 3-4 weeks avoid chilling injury at ripening at 21 °C. Serrano *et al.* (1997) mentioned that chilling injury symptoms in mature green peppers were reduced considerably and increased their shelf-life using a modified atmosphere packaging with 16.1% O_2 and 4.5% CO_2 at 2 °C. Skog (1996) found that conditioned "Fantasia" nectarines at 20 °C with 5% CO_2 for 2 days to subsequently transferred to 0.5 °C under 5% O_2 and 12% CO_2 delays the appearance of chilling injury from 17 to 38 days of storage.

On the other hand, many chemical treatments are used to reduce chilling injury. Growth regulators such as jasmonic acid, methyl jasmonate and methyl salicylic acid, which are found in a wide range of plant, induced a mechanism to protect the commodity from chilling injury

(Gonzales *et al.,* 2001). Likewise, diphenylamine is an antioxidant commercially used to control scald in chilling injury apples (Smock, 1961). Tasneem (2004) indicated that methyl jasmonate at 1 and 7 °C and diphenylamine at 4 and 7 °C were successful to reduce chilling injury in mangoes cv. Kent.

Quality attributes of fresh vegetables

Many compositional changes can occur during the ripening of vegetables that influence their appearance, texture and flavor. Some changes can be desirable, while others can be detrimental to the quality of the commodity (Maguire *et al.*, 2004; Kader, 1986). Moreover the selection of each quality attribute needs to be selected and evaluated depending on the commodity, the intended use for the consumer and the salability of the vegetable, where a single unacceptable attribute can cause the commodity to be unusable though other attributes are still acceptable (Shewfelt, 1986).

Appearance comprises external appeal of the commodity. Color is the most visible change that occurs during storage of many vegetables and it can serve as an indicator of ripeness and absence of disease or insect injury to the consumer (Maguire *et al.*, 2004; Shewfelt, 1994, Wills *et al.*, 1982). The green color of fresh vegetables is due to chlorophylls, which are tetrapyrrole pigments in which the porphyrin ring is in the dihydro form and the central metal atom is magnesium. There are two chlorophylls, “a” and “b”, which occur together in a ratio of about: 1:25 (deMan, 1999). Shewfelt (2003) indicated that spinach, broccoli and green beans have a chlorophyll content of >200, 50-100 and 1-10 mg/g fresh weight, respectively.

Yellowing and browning are the most common defects in appearance in commodities (Shewfelt, 1994). During advanced senescence, chlorophylls located in the chloroplast, are oxidized enzymatically unmasking the yellow xanthophylls that coexist with chlorophyll, causing appearance of yellowing (Abott, 2004). Gnanasekharan *et al*. (1992) indicated that tomato storage above 30 °C developed yellowing due to disappearance of chlorophyll and the inhibition of lycopene synthesis caused by ethylene production. In addition, browning can occur by degradation of chlorophyll to brown pheophytin, or by mechanical damage that unites polyphenoloxidase and phenolic compounds to form brown pigment (Wills *et al.*, 1982). Yellowing and browning can be inhibited using low temperature storage, atmospheric modification, chemicals and careful handling (Shewfelt, 1994).

Another defect in appearance is the shriveling caused by the transpiration process, which is the evaporation of water on the produce surface by the heat of respiration (Maguire *et al.*, 2004; Wills *et al.*, 1982). Besides shriveling, transpiration can cause loss of color and saleable weight (Maguire *et al.,* 2004). Prussia *et al.* (1990) reported that the color loss in southern peas is affected by moisture loss in addition to higher temperatures of storage.

Moisture loss can also contributed with changes in texture. Bourne (1978) defined texture as the group of physical characteristics that result from the structural elements of the food, are sensed by sensation of touch in the hand or in the mouth, are related to the deformation, disintegration and flow of food under force, and is measured as a function of force, distance and time. The texture can be quantified by sensory evaluation or instrumental analysis. Usually the

texture in commodities is expressed as hardness, defined as the peak force during the first compression cycle. The puncture test is usually used to measure hardness and consists of measuring the force required to push the probe or punch into a food to a depth that causes irreversible deformation (Bourne, 1980). In addition, hardness of commodities is a function of structure, physiology and biochemical characteristics of the tissue and needs to be considered when measuring texture (Abott, 2004; deMan, 1999). Abott (2004) mentioned that toughening in asparagus, broccoli and pineapple is a result of cell wall lignification during maturation, also Viña and Chaves (2003) concluded that texture in fresh cut celery increased during 7-14 days at 0 and 10 °C of storage caused by lignifications of fibers and xylem vessels. However, during fruit ripening, cell wall changes in starch to non-starch polysaccharides cause a softening in fleshy fruits (Yashoda *et al.,* 2006).

The flavor of vegetables is composed of aroma and taste. Aroma, due to volatile compounds is detected before and during mastication (Sims and Golaszeweski, 2003; Shewfelt, 1994). Major volatiles included esters, terpenes, aldehydes and alcohols (Shewfelt, 1994). Non - climacteric vegetables do not synthesize compounds that are as aromatic as climacteric fruits, but these compounds are still important in purchasing decisions (Wills *et al.*, 1982). Otherwise, taste of vegetables is a result of combination of sweet, sour, salty and bitter sensations. Sugars, primarily glucose, sucrose and fructose contribute to sweetness (Shewfelt, 1994). Wills *et al*. (1982) indicated that peas are sweeter at an immature stage; however, with advancing maturity, sugar is converted to starch with the loss of sweetness. The perceptions of sourness and saltiness are due to the presence of organic acids such as citric, malic and acetic, and sodium and potassium, respectively. The bitterness in vegetables is related to phenolic compounds and is

considered a defect in carrots (Sims and Golaszeweski, 2003). In addition, flavors of vegetables are influenced by temperature and time of storage. Viñas and Chaves (2003) indicated that glucose loss was around 22% and 50% of the initial value at 0 and 10 °C respectively, after 30 days of storage.

Basic principles of modified atmosphere packaging

Modified atmosphere packaging (MAP) system is defined as the enclosure of food products in materials that create a gas barrier so that the gaseous environment changes from ambient conditions (78% N_2, 21% O_2, 0.03% CO_2 and traces of noble gases) (Fig. 4) (Church and Parsons, 1995).

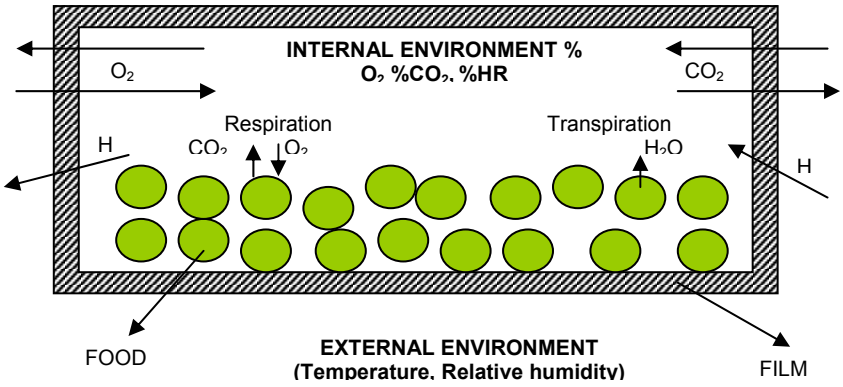


Figure 4. Modified Atmosphere Packaging (MAP) System.

The goal of MAP in fresh commodities is to create an atmosphere in the package with enough low O_2 and high CO_2 concentrations (relative to air) to maintain the quality of produce at a specific cold temperature (Sanz *et al.*; 1999, Zagory, 1998). MAP can be created actively by flushing a mixture of gases inside the package or passively by the respiring commodity. In active MAP, the desirable atmosphere can be achieved by flushing out the air with a mixture of O_2,

CO_2 and N_2 inside the package immediately reaching the steady state. Usually nitrogen is used as the balance or filler gas to provide a precise concentration of other gases in the package, or to prevent package collapse caused by absorption of CO_2 (Al-Ati and Hotchkiss, 2002; Church and Parson, 1995). However, in a passive MAP, the optimal atmosphere is developed by the respiration process of the commodity working together with the permeability of the packaging, which causes a reduction in O_2, while increasing the CO_2 concentration. After a period of adjustment, the steady state is established inside the package (Jayathunge and Illeperuma, 2005; Al-Ati and Hotchkiss, 2002).

Since O_2 and CO_2 permeate through plastic, permeability of the film package is important in designing a MAP system (Jayathunge and Illaperuma, 2005; Talasila *et al.*, 1995). If the respiration rate of the commodity and film permeability characteristics are properly matched, the selected film must allow O_2 to enter at a rate hat matches the consumption by the commodity. Similarly, CO_2 must be vented out of the package at a rate that will offset its evolution (Rakotonirainy *et al.*, 2001). However, if a film of excessive gas permeability is used there will be no atmospheric modification. Conversely, if a film of insufficient permeability is used, an atmosphere of O_2 content of less than 2% v/v will cause quality losses due to anaerobiosis (Church and Parsons, 1995).

The principal characteristic for a film in MAP are listed as follow: (Kader *et al.,* 1992).

- Required permeability for the different gases.
- Non-reactive with produce.
- Good transparency and gloss.
- Thermal and ozone resistance.
- Light weight.
- Commercially suitability.

- Resistance to puncture
- Ease of handling.
- Low temperature heat sealability.
- Ease of printing for labeling purpose.
- Nontoxic.
- Weatherability.

In order to choose the correct plastic film for MAP, the permeability ratio (P_{CO_2}/ P_{O_2}) or β is very useful because help to choose the most appropriate film for a recommended modified atmosphere (Zagory, 2000; Mannapperuma and Singh, 1994). The permeability ratio required by the product is calculated using the desired partial pressure of O_2 and CO_2 inside the package and the respiratory quotient, this ratio can be matched with the permeability ratio of the film (β) at a specific temperature of storage. Even though, several limitations for design of packaging exist due to the lack of information about film permeability and respiration rate of the commodity at different storage temperatures. Exama *et al*. (1993) indicated that at 4 °C most commercial films have the desired O_2 and CO_2 permeability for produce with low or medium respiration rates, however, for produce with high respiration rates, the combination of polymeric and perforated films must obtain an adequate flux of O_2 and CO_2 (Fig. 5).

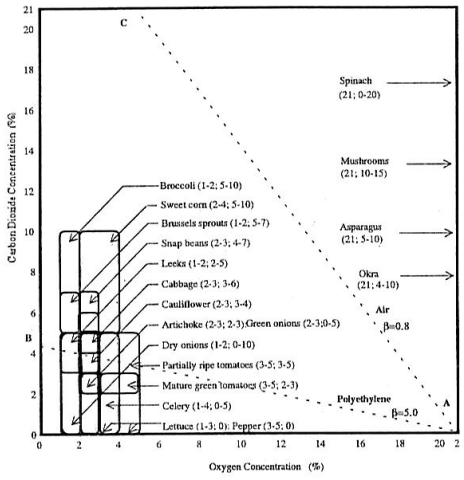


Figure 5. Recommended MAP of fresh vegetables and permselectivity for polyethylene films (A-B line) (Mannapperuma and Singh, 1994).

Different films are used for MAP systems. Low density polyethylene (LDPE) films are the most common in the industry (Zagory, 2000), due to the wide range of β values (2~5) that make them suitable for commodities which require low levels of O_2 and CO_2, such as celery, carrots and cabbage (Mannappperuma and Singh, 1994; Exama *et al*., 1993). In addition, other films are available in the market like ethyl cellulose, polyethylne terephalate (PET), polyamide (PA), high density polyethylene (HDPE), polyvinil chloride (PVC) and Saran, which have a β value of 2.4, 3.4, 4.7, 4.8, 6.1 and 10.2 respectively at 4 °C (Exema *et al*., 1993).

Effects of modified atmospheres on the quality of the commodity

MAP has been successful in the marketing of fresh produce by working together with low temperatures in order to maintain freshness, ensure safety and extend shelf-life. However, no MAP has been developed for pigeon peas and its quality effects investigated.

Changes in the composition of the gaseous atmosphere of the commodity can result in significant changes in the respiratory process. Low oxygen and high carbon dioxide concentrations have an effect on respiratory pathways (Kays, 1991). A decreasing O_2 content around fresh vegetables reduces the respiration rate in proportion to the O_2 concentration, which is important in postharvest handling in order to delay senescence in comparison with storage in air (Kays, 1991; Kader, 1986; Wills *et al.*, 1982). The respiration rate is decreased by low O_2 due to a reduction in the activity of polyphenol oxidase, ascorbic acid oxidase and glycolic acid oxidase (Kader, 1986). Kays (1991), Kader (1986) and Wills *et al*. (1982) observed that respiration rate had a significant reduction below 10 % O_2, with an optimal value between 2-5%

of O_2; however, this range depends on gas diffusion characteristics of the tissues of the specific commodity and the storage temperature. As the temperature is reduced the required concentration of O_2 by the respiration process is also decreasing (Wills *et al.*, 1982). Lebermann (1968a) mentioned that the respiration rate of broccoli decreased 50 % when O_2 content was reduced from 21 % to 1 % at 23.8 °C, also Kader (1986) mentioned that the respiration rate of fresh vegetables in an atmosphere with 3 % O_2 was proportionally reduced between 10-46 % at 0 °C and 20-60 % at 10 or 20 °C.

Extremely low O_2 levels (<2%), however, can cause anaerobic respiration. At this point the tricarboxylic acid cycle (TCA) is blocked. Pyruvic acid is no longer oxidized, but it is accumulated and is decarboxylated to form CO_2 and acetaldehyde, which is then reduced to ethanol, resulting in a production of off-odor, off-flavor and tissue breakdown (Kays, 1991; Kader, 1986). Under aerobic conditions, CO_2 has a significant effect in decreasing the respiratory rate when the storage substrate of the commodity is not depleted (Kader, 1986). However, a high level of CO_2 about 15 % results in toxic levels of succinate in apples (Kays, 1991) and ethanol and acetaldehyde production in black currants (Kader, 1986).

In fact, the combined effects of reduced O_2 and elevated CO_2 levels on the respiration rate are greater than either component alone (Kader, 1986). Lebermann (1968a) found that respiratory activity of broccoli was reduced 59 % in an atmosphere of 2 % O_2 and 20 % CO_2 in comparison with samples stored in air at 7.2 °C, however, an atmosphere with 2% O_2 the respiration rate was reduced only 28 %.

Loss of chlorophyll is slowed down in vegetables kept in modified atmospheres. Lebermann (1968b) found that chlorophyll retention of broccoli had a maximum value of 41.5 mg/100 g fresh weight when it was stored in an atmosphere with 2 % O_2 and 20 % CO_2 at 7.2 °C for 16 days. In addition, Singh *et al.* (1972) indicated that chlorophyll retention of lettuce was significantly higher in atmospheres with 2.5 % O_2 and 2.5 % CO_2 packaged in polyethylene bags at 1.7 °C for 60 days of storage. Furtheremore, Ontai *et al*. (1992) mentioned that sugar peas maintained greenness for at last three weeks in an atmosphere with 21 % O_2 and high CO_2, also Pariasca *et al*. (2000) found that snow pea pods packaged in polymethyl pentene polymeric films in atmospheres of 5 % CO_2 and 5 % O_2 showed a better external quality of the pod at 5 °C for 21 days of storage.

The texture of vegetables can also be influenced by modified atmospheres. Lebermann (1968b) found that the texture of cooked broccoli stalks decreased with an increased level of CO_2 (0, 5, 10 and 20 %) regardless the O_2 content (21, 5, and 2%) at 7.2 °C for 16 days of storage. Kader (1986) mentioned that toughening of asparagus spears is retarded by atmospheres with 12 ± 2 % CO_2 at 4 °C. This tenderization seems to be related to CO_2 mediated increase in pH of the tissue (Kader, 1986; Lebermman, 1968b) since O_2 had little effect on tenderness of asparagus or broccoli. However, Ontai *et al.* (1992) indicated that sugar pea pods stored under low O_2 were more turgid than when stored in 21 % O_2.

Modified atmospheres can improve the retention of flavor, but it depends on the commodity (Wills *et al.*, 1982). Elevated CO_2 can reduce the rate of sugar to starch conversion, which is undesirable in peas and sweet corn, and also improves the retention of organic acids in

tomato but accelerates loss of acids in asparagus (Kader, 1986; Wills *et al.*, 1982). Pariasca *et al*. (2000) found that snow pea pod storage in an atmosphere of 5 % O_2 and 5 % CO_2 had a higher content of total sugar and had a better taste and absence of off-flavor.

Effects of modified atmospheres on microorganisms

The common microflora of vegetables such as *Erwinia herbicola*, *E. carotovora*, *Flavobactrium*, *Xanthomonas*, *Enterobacter agglomerans*, *Pseudomonas spp.*, *Lactobacillus spp*., and yeasts and molds contribute to the decline of commodity quality (FDA/CFSAN, 2001; Jay, 1996), however, MAP in combination with low storage temperature is an effective way to reduce the growth of spoilage microflora and foodborne pathogens, due to increasing the solubility of CO_2 in the liquid phase surrounding the food. No research has been reported about the effects of MAP in pigeon pea's microorganism.

CO_2 is a bacterial and fungal growth inhibitor. Although it is unknown how CO_2 affects the microorganism, Farber (1991) summarized several theories in four main effects as follows: alteration of cell membrane function, direct inhibition of enzymes or decreased the rate of reactions, changes in pH caused by penetration of the bacterial membrane and direct changes of physico-chemical properties of protein. However, CO_2 has an overall effect on microorganisms increasing the duration of lag phase, but as soon as bacteria move from the lag to log phase of growth, the inhibitory effect is reduced (Jay, 1996; Church and Parson, 1995).

In addition, the effect of CO_2 is influenced by the microorganism type. Gram negative bacteria are more sensitive than gram positive, where pseudomonas are inhibited with 10-20% CO_2, the growth of lactic acid bacteria can be enhanced by CO_2 content (FDA/CFSAN, 2001; Jay, 1996). Conversely, molds are strictly aerobic microorganisms and their growth is inhibited by CO_2 concentrations as low as 10%, however, yeast growth is more resistant to CO_2 concentration (Al-Ati and Hotchkiss, 2002; FDA/CFSAN, 2001).

An appropriate level of O_2 content can also reduce the growth of spoilage microorganisms (aerobic bacteria) (Church and Parson, 1995) and inhibit the growth of strictly anaerobic bacteria such as *Clostridium botulinum* (Farber, 1991).

In summary, much research is needed in pigeon pea about the postharvest quality characteristics at different storage temperatures to define the appropriate storage handling to reduce the quality losses and studies in changes of environmental composition to maintain optimal levels of quality, increase the shelf-life of the commodity under realistic handling systems.

Materials and Methods

Plant material

Pigeon peas (cvs. “Lazaro”, “Combinada” and “Pinto”) were analyzed in this study. The pigeon pea crop was harvested when 10% dry and sorted on the basis of maturity (green pod and full shell) and freedom from defects (disease and physical damage). “Lazaro” was obtained from the Agriculture Experimental Station of the University of Puerto Rico in Isabela during May 2005, and “Combinada” and “Pinto” came from independent growers, harvested in October 2005 and March 2006, respectively. The study was carried out in the laboratories of Food Science and Technology, Agriculture and Biosystem Engineering, Chemistry and Postharvest Physiology at the University of Puerto Rico, Mayagüez.

Postharvest quality

Respiration rate determination

Five hundred grams of fresh pigeon peas were weighed and placed in 1 gal. glass jars and hermetically sealed and stored at different temperatures: 0, 5, 10, 15, 20 ºC, with three replicates per temperature. The respiration rate was measured each day for 7 days. A 10 ml syringe was inserted into the septum of each jar and the sample inside of the syringe was analyzed using a gas analyzer (Servomex Food Package Analyzer Serie 14000, Sugar Land, Texas, USA) to measure the CO_2. Carbon dioxide standards were used for calibration of the equipment. The respiration

rate was calculated as milligrams of CO_2 per kilogram per hour on a fresh weight basis using a conversion factor, as in the following equation (Kader, 1992):

$$R\ CO_2 = [CO_2\ ml/\ weight\ (kg) * time\ (h)\ ] * Conversion\ factor$$

CO_2 (ml) = Head space (ml) * change in CO_2 in the jar (%)

Quality changes

Data were taken at the beginning of the experiment (initial) and after 7 days and 7 days plus 2 days at room temperature in March 2005 and October 2006, after 15 days and at 15 days plus 2 days at room temperature in March 2006 and at the end of the shelf-life in October 2005.

Mass loss

At the end of 7 days, all treatments were weighed using a balance (OHAUS, Pine Brook, New Jersey, USA). The mass loss was calculated from the difference between the initial and final weight and expressed as a percentage of the initial weight, using the following equation:

$$\text{Mass loss} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Where: W1= initial weight, W2=final weight

Color measurement

Color analysis was done on 30 pigeon peas pods and seeds randomly selected within each of the 3 replicates for each treatment. Colorimetric measurements were recorded using a colorimeter HunterLab - MiniScan XE (Hunter Associate Laboratory, Inc., Reston, Virginia, USA) calibrated with white and black standard tile (X=79.8, Y=84.6, Z=90.4). Values of "L", "a" and "b" were measured at the center of the pod and seed (one on each side) using a black carton to reduce the aperture of the colorimeter to 0.8 mm in diameter. "L" represents the lightness index ranging from 0 for black to 100 to white and parameters "a" represents green to red (- to +) and "b" blue to yellow (- to +). A validation of this methodology was done using the Royal Horticulture Society Colour Chart from a range of colors (Appendix 1). Additionally, the pods and seeds were evaluated subjectively using a 5-point scale for yellowing, shriveling, and decay (Tables 3, 4 and 5). A color photograph was used to score the individual pods in general appearance for each defect.

Table 2. Rating scale for yellowing.

Score	Visual quality description	Defects
1	None	Completely green
2	Slight	Recognition of color change
3	Moderate	Slight yellowing
4	Severe	Obvious yellowing
5	Extremely severe	Complete yellowing

Modified from Kader *et al.,* (1973).

Table 3. Rating scale for shriveling.

Score	Visual quality description	Defects
1	None	None
2	Slight	Recognition of shriveling
3	Moderate	Slight shriveling
4	Severe	Obvious shriveling
5	Extremely severe	Complete shriveling

Modified from Kader *et al.,* (1973).

Table 4. Rating scale for mold growth.

Score	Visual quality description	Defects
1	None	None
2	Slight	Slightly objectionable, may impair salability
3	Moderate	Objectionable, definitely impair salability
4	Severe	Salvageable, but normally not salable
5	Extremely severe	Not usable

Modified from Kader *et al.,* (1973).

Hardness

The texture was expressed as hardness, which is the maximum peak force during the first compression cycle. The hardness was measured in 30 pigeon pea seeds from each treatment. Texture measurements were performed using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) with a 2 mm diameter cylinder probe. The instrument was calibrated with the following settings:

Mode: Measure force in compression

Option: Return to start

Pre-test speed: 2.5 mm/s

Test speed: 2.5 mm/s

Post-test speed: 2.5 mm/s

Distance: 3.5 mm

Trigger type: Auto-5g

Titratable acidity

Titratable acidity was determined using 10.0 g of pigeon pea seeds homogenized in distilled water in a ratio of 1/10 w/v. From each replicate three samples were analyzed, nine replicates for each treatment. The samples were titrated with NaOH 0.1N (Sigma Aldrich, St. Louis, Missouri, USA) using a pH meter (Acummet AR15, Fisher Scientific, Hampton, New Hampshire, USA). The total acidity was expressed in terms of malic acid (67 g/equ g), using the following equation:

$$\% \text{ Total acidity} = \{(V \text{ sample} - V \text{ blank}) * N \text{ NaOH (equ g/L)} * 67 \text{ g/equ g}\} / \text{Sample weight}) * 100$$

pH analysis

The pH measurement was performed using a portable pH meter (Acummet AR15, Fisher Scientific, Hampton, New Hampshire, USA) previously calibrated with buffer solutions of pH 4 and 7. The electrode was placed inside the homogenized sample prepared for titratable acidity evaluation. The pH measurement was done for nine replicates per treatment and the value was registered once it had stabilized.

Soluble solids content

The juice of pigeon pea seeds was used to determine the soluble solids content. A garlic press was utilized to extract the juice. Nine replicates for each treatment were done. The analysis was carried out using a refractometer (Abb-3L, Bausch & Lomb, Rochester, New York, USA) at 20 ºC. The values were expressed in percentage of total soluble solids (%).

Modified atmosphere packaging of pigeon peas

Fresh pigeon peas of variety "Combinada" were sorted and defective pods were discarded. One hundred and sixty- five grams (± 1.0 g) of pigeon peas were weighted and packaged in pre-formed bags manufactured by the Cryovac Company: PD 941 and PD 961. The treatments were as follows: MAP 1 (PD 941 air headspace), MAP 2 (PD 941 2% O_2 + 5% CO_2 + 93% N_2 headspace), MAP 3 (PD 961 with 6 hole punctures of 1mm in diameter) and MAP 4 (PD 961 air headspace), as it shown in Table 6. The sealed package size was 18 cm x 28 cm. All treatments were made in triplicate and stored at 0 °C for 7, 14 and 21 days and at 20 °C for 7 days. The technical data sheet of the films were provided by the manufacturer (Appendix 2).

Table 5. Characteristics of the modified atmosphere packagings used in the experiment.

MAP	Bag Material	Headspace	Transmission CO_2	Transmission O_2	W.V. Transmission	Type of MAP
MAP 1	PD 941	Air	31,000	16,544	5.0	Passive
MAP 2	PD 941	Gas mix	31,000	16,544	5.0	Active
MAP 3	PD 961 + 6 holes	Air	Unknown	Unknown	Unknown	Passive
MAP 4	PD 961	Air	21, 000	7, 000	0.90-1.10	Passive

Transmission rate CO_2 and O_2 ($cc/m^2/24$ h), at 23 °C, 1 atm.
W. V. Transmission= Water Vapor Transmission (g/100 sq. in./24 h) at 23 °C, 100% RH.

Gas analysis

Measurements of CO_2 concentration were performed at 0, 7 and 21 days at 0 °C and at 7 days at 20 °C using a gas analyzer (Servomex Food Package Analyzer Series, 1400 Sugar Land, Texas, USA). A 10ml syringe was inserted inside the package to take the sample. The concentration was expressed in percentage (%) of CO_2.

Quality evaluations

Quality evaluations were taken at 7, 14 and 21 days at 0 °C and at 7 days at 20 °C. Changes in mass loss, color, hardness, titratable acidity, ph, and soluble solids content were assessed as quality indicators. The procedures were similar to the postharvest analysis described above. The off-odor was also evaluated with a subjectively 5-point scale, 1= None, 2=Slight, 3= Moderate, 4= Severe and 5= Extremely severe.

Microbiological Analysis

Twenty-five grams of pigeon pea seed of each replicate was homogenized with 225 ml of butterfly phosphate in a stomacher bag for 60 minutes in a blender (Seward, Laboratory Blender Stomacher 400®). An aliquot of 10 ml of the sample was transferred to 90 ml of diluents to prepare decimal dilutions of 10^{-2}, 10^{-3} and 10^{-4}.

Lactobacillus spp.

Lactobacillus spp. was cultured in MRS agar (Difco™, Becton, Dickinson and Company, Sparks, Maryland, USA). An aliquot of 0.1 ml of the dilutions was pipetted into a petri dish and cultured using the pour plate technique in duplicate. Plates were placed into an anaerobic jar with an oxygen scavenger or Gas Pack (BD BBL™ Gas Pack™ Plus Anaerobic System Envelops with Palladium Catalyst, Becton, Dickinson and Company Sparks, Maryland, USA) and dry anaerobic indicator strip (Becton, Dickinson and Company Sparks, Maryland, USA). Microbial counts

were done after incubation of the anaerobic jar for 48 hours at 35 ± 2 ºC (Isotemp Incubator, Fisher Scientific, Hampton, New Hampshire, USA).

Fungi count

A stock solution of antibiotic was prepared by dissolving 0.1 g of streptomycin sulfate in 40 ml of 95% ethanol. This solution was added to 960 ml of potato dextrose agar (Difco™, Becton, Dickinson and Company, Sparks, Maryland, USA) after autoclaving and cooling to 55 ºC. The medium was poured into petri dishes by duplicate and spread with 0.1 ml of the appropriate dilutions. Petri dish microbial counts were done after incubation for 5 days at 25 ºC (Isotemp Incubator, Fisher Scientific, Hampton, New Hampshire, USA).

Statistical Analysis

A completely randomized design (CRD) was used in the postharvest experiments. In order to determine differences between experiments (October 2005 and March 2006) at 7 days of storage and at 7 days plus 2 days at room temperature, a split plot arrangement was used. If no significant interaction was found between experiments (October 2005 and March 2006) and temperatures of storage (0, 5, 10, 15 and 20 °C), data of both experiments could be joined and analyzed as a CRD, in contrast, if the interaction was significant the data could be analyzed separately as a CRD. In addition, a split split plot arrangement was used to analyze the respiration rate of pigeon peas in May and October 2005 and March 2006, similarly, if no significant interaction was found between experiments, temperatures of storage and days of

analyses, then the data of the three experiments could be joined and analyzed as a CRD. If the interaction was significant, the data could be analyzed separately as a CRD. Moreover, the data at 15 days of storage, 15 days plus 2 days at room temperature and at the end of the shelf life was analyzed as a CRD.

The experimental design of modified atmosphere packaging test was a completely randomized design (CRD) with factorial arrangement. The first experiment was a factorial 4 x 3, in which the factors were atmospheres and time of storage. The second experiment was a factorial 4 x 2, in which the factors were atmospheres and temperature of storage.

The data were subjected to ANOVA tests. The difference between means was determined by Tukey's multiple range test at a 95% confidence interval. The statistical analysis was performed with Infostat student version (2002).

Results and Discussions

Postharvest quality

Respiration rate

A significant interaction ($p \leq 0.05$) was found between the experiment, temperature of storage and days, therefore, the data of the three experiments were analyzed separately as a CRD. The respiration rate of pigeon peas increased with increasing storage temperature, 1.7, 3.6, 5.5 and 9.1 times the average that of the rate of 0 °C for 5, 10, 15 and 20 °C, respectively (Fig.6). Significant differences were found between all temperatures in the three experiments ($p \leq 0.05$). In May 2005, the respiration rate reached an average value of 40.8, 63.8, 99.9, 150.2, and 267.7 mg CO_2/kg h at 0, 5, 10, 15 and 20 °C, respectively. Meanwhile, in October 2005 the rate of respiration showed lower values than the first with 17.3, 29.9, 75.6, 122.0 and 214.7 mg CO_2/kg h at 0, 5, 10, 15 and 20 °C, respectively. Finally, in March 2006 the carbon dioxide production was 23.8, 50.1, 92.9, 142.1 and 203.2 mg CO_2/kg h at 0, 5, 10 15 and 20 °C respectively.

Probably, the changes in respiration rate caused by increasing temperature can be attributed to an increase of enzymatic activity in pigeon peas. This is accordance with Wills *et al.* (1982) and Kays (1991) who mentioned that increases in temperature affect the rate of enzymatic reactions that control many pathways in the respiration process of the cell. Therefore, an increasing temperature can shorten the shelf-life of pigeon peas because of the faster metabolic reactions depleting the storage substrate; in contrast pigeon peas stored at low temperatures can

experience an extended shelf-life by slowing the metabolic reactions. Pigeon peas achieved a maximum shelf-life of 30, 23, 13, 9, and 7 days at 0, 5, 10, 15 and 20 °C, respectively.

Likewise, the changes in respiration rate values between the experiments (May 2005, October 2005 and March 2006) can be attributed to the differences in carbon dioxide production between cultivars and the state of maturity. Due to the availability, the "Lazaro" variety was used in May 2005, "Combinada" in October 2005 and "Pinto" in March 2006. The maturity state of "Combinada" was slightly higher than "Lazaro" and "Pinto". Kays (1991) indicated that is common to find differences in respiratory rates between cultivars of the same plant, and also by the maturity state. There are no reported respiration rates for pigeon peas, however, our data is close to the range of respiration rates of peas with the pod stored for 7 days by Tewfik and Scott (1954). In addition, the high respiration rate of pigeon pea is characteristic of immature vegetables, because young cells are more active than old cells and tend to have higher respiration rates (Mohammed and Brecht, 2003; Kays, 1991; Wills *et al.*, 1982).

The highest respiration rate was observed after zero days of storage. In May 2005, the highest carbon dioxide production was observed at zero days of storage in all temperatures ($p \leq 0.05$) with the highest respiration rate at 20 °C at 354.92 mg CO_2/kg h and the lowest at 0 °C at 118.59 mg CO_2/kg, which was not significantly different than the respiration rate at 5 °C ($p \geq 0.05$). Subsequently, the respiration rate decreases throughout the storage time. The same pattern was observed in March 2006; however, in October 2005 the carbon dioxide production did not show the rapid fall after zero days of storage, in contrast, it was maintained more or less constant with the exception of 20 °C, due to the higher metabolic rates at this temperature,

probably due to the maturity state. According to the results, this rapid fall of carbon dioxide production is typical of a non-climacteric pattern of respiration; therefore, pigeon peas can be classified as a non-climateric vegetable. This is in agreement with Kader and Salveit (2003a), who mentioned that a rapid decrease in respiration rate after harvest is characteristic of immature fruit-type vegetables such as peas, green beans, and cucumbers. Wills *et al*. (1982) indicated that sudden peaks observed in non-climacteric vegetables can be attributed to sprouting or wounds, caused by fungal attack. Both characteristics were apparent in pigeon peas stored at 20 °C for 7 days of storage in the three experiments.

Mass loss

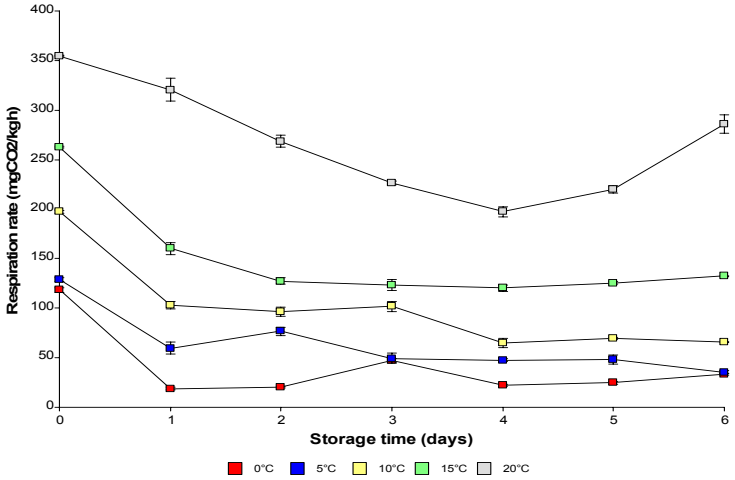
In general, high temperatures increased the percentage of mass losses in pigeon peas after 7 days of storage (Fig. 7). Mass losses were significantly higher ($p \leq 0.05$) at 15 and 20 °C than at 0, 5 and 10 °C in May and October 2005, with average values of 7.0, 7.9, 8.7, 21.1 and 19.6 % in May 2005 and 2.3, 2.5, 2.6, 5.6 and 11.3 % in October 2005 at 0, 5, 10, 15 and 20 °C, respectively. In addition, in March 2006, significantly higher values of mass loss were observed at 20 °C with average values of 20.1 %. However, at 15 °C the mass loss was significantly lower with 3.9 % average values and did not show significant differences from mass losses at 0 and 10 °C.

Perhaps mass losses can be attributed to transpiration processes, which are affected by the differences in the driving force. At high temperatures, the relative humidity (RH) decreased to values of 47 and 51 % at 20 and 15 °C, respectively, but the relative humidity of pigeon pea is

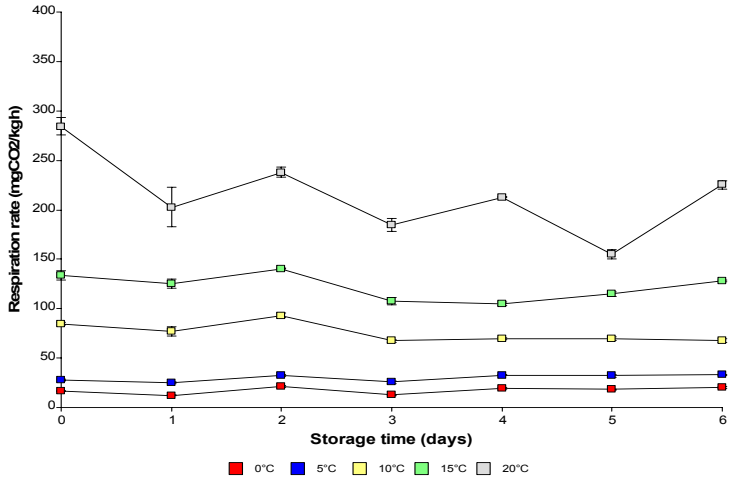
96%. The magnitude of this difference could be the driving force that increased the water loss, as occurred in May 2005. Maguire *et al.* (2004) mentioned that mass losses > 10 % are detrimental to salability of vegetables, for this reason storing pigeon peas at higher temperatures with low RH is not beneficial. Therefore, in the experiment of October 2005 the air was humidified at 15 and 20 °C to reach a relative humidity between 65-76% which caused a decrease in mass loss. However, the quality of pigeon peas decreased due to fungal growth. This result agrees with Wills *et al.* (1982) and Ben-Yehosua and Rodov (2003) who mentioned that the humidification of the air is a good method to reduce mass loss; however, the mold growth can be enhanced. Likewise, in March 2006, the air was humidified at 15 °C resulting in a low mass loss due to saturation of the cool chamber.

In contrast, at low temperature, such as at 0, 5 and 10 °C, mass losses were reduced to around one third the values at 20 °C in May 2005 and one fifth the value in October 2005, due to higher relative humidity in the air. Also, in October 2005 the values were low due to the humidification of the air, although in March 2006 the air was humidified at 5 °C and the mass loss increased in comparison with October. This may be due to the differences in cultivar and maturity state, and also it could be attributed to chilling injury that was observed at 0 and 5 °C which also caused higher mass loss at 0 and 5 °C in March 2006. Wills *et al.* (1982) mentioned that high water loss is a symptom of chilling injury. Ben-Yehosua and Rodov (2003) mentioned that carbon loss caused by the respiration process can be an important source of weight loss when moisture loss is low. Likewise at 10 °C in March 2006 the cold chamber was saturated and that caused low values. Mold growth was not enhanced with the humidification of the system at low temperatures after 7 days of storage.

May 2005



October 2005



March 2006

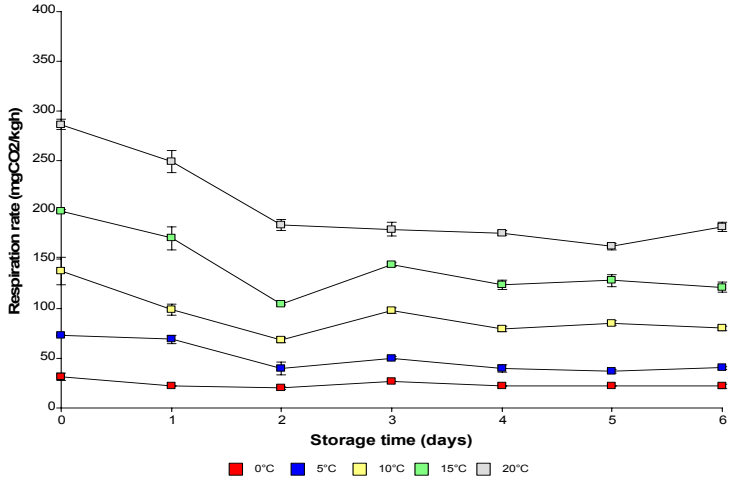
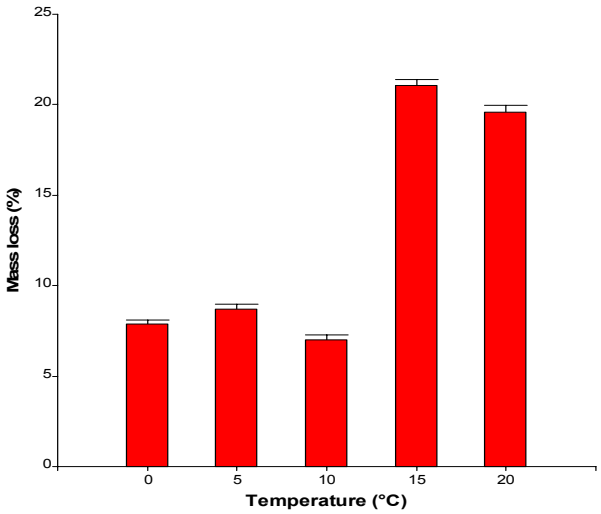
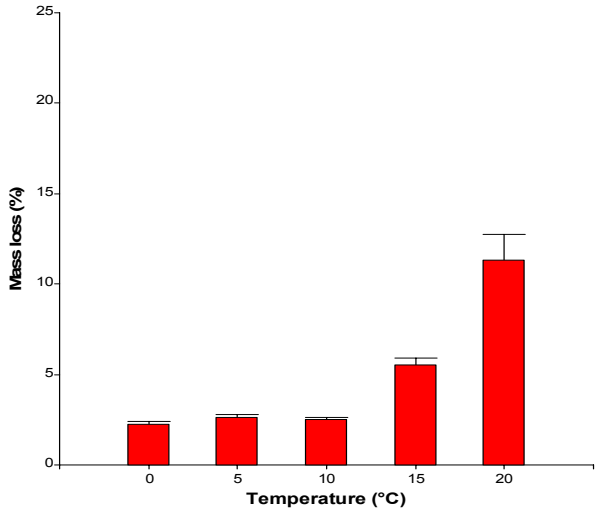


Figure 6. Changes in respiration rate (mg CO_2/kg h) of pigeon peas stored for 7 days. For points not showing errors values, errors is hidden by the symbols for the data points.

May 2005



October 2005



March 2006

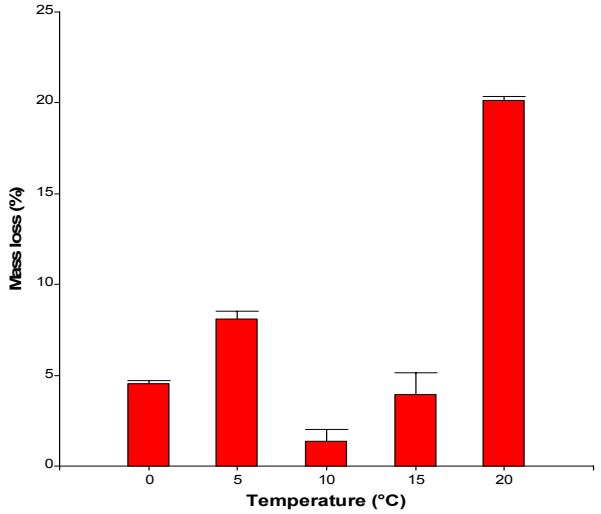


Figure 7. Mass losses of pigeon peas at five temperatures after 7 days of storage.

Titratable acidity, soluble solids content and pH

No significant interaction ($p \geq 0.05$) was found between the titratable acidity (TA) of pigeon pea after 7 days and 7 days plus 2 days at room temperature (7 + 2) between the experiments of October 2005 and March 2006, consequently, those were grouped together and analyzed as CRD. Similarly, no significant interaction ($p \geq 0.05$) was observed in the soluble solids content (SSC) of pigeon pea after 7 days of storage (Tables 6 and 7).

Table 6. Changes in titratable acidity (TA), pH and soluble solids content (SSC) of pigeon peas for each temperature tested in October 2005.

Storage (days)	Temp. (°C)	Seed		
		TA (%)	pH	SSC (%)
0	Initial	0.12	6.66	9.20
7	0	0.09 ab	6.76 c	15.56 a
	5	0.09 ab	6.76 c	16.03 ab
	10	0.08 a	6.65 bc	16.36 ab
	15	0.07 a	6.55 b	18.51 bc
	20	0.11 b	6.31 a	19.79 c
7 + 2	0	0.10 a	6.55 c	24.38 a
	5	0.08 a	6.37 b	24.42 a
	10	0.07 a	6.33 b	23.64 a
	15	0.08 a	6.25 a	27.17 a
End shelf-life				
30	0	0.12 b	6.48 c	14.92 a
23	5	0.10 a	6.43 b	15.64 a
13	10	0.11 b	6.34 a	16.19 a
9	15	0.12 b	6.34 a	16.67 a

Data in blue font are composite of October 2005 and March 2006.
Means followed by different letter within each time point are significantly different ($p \leq 0.05$).
Pigeon peas cvs. "Combinada" were analyzed in this study.

Table 7. Changes in titratable acidity (TA), pH and soluble solids content (SSC) of pigeon peas for each temperature tested in March 2006.

Storage (days)	Temp. (°C)	Seed		
		TA (%)	pH	SSC (%)
0	Initial	0.09	6.45	15.5
7	0	0.09 ab	6.51 b	15.56 a
	5	0.09 ab	6.40 ab	16.03 ab
	10	0.08 a	6.41 ab	16.36 ab
	15	0.07 a	6.45 b	18.51 bc
	20	0.11 b	6.29 a	19.79 c
7 + 2	0	0.10 a	6.53 c	33.35 c
	5	0.08 a	6.37 a	30.47 b
	10	0.07 a	6.44 b	27.18 a
	15	0.08 a	6.44 b	26.49 a
15	0	0.06 a	6.43 b	19.78 b
	5	0.07 a	6.22 a	18.40 a
15 + 2	0	0.05 a	6.12 a	20.84 a
	5	0.09 b	6.23 b	21.67 a

Data in blue font are composite of October 2005 and March 2006.
Means followed by different letter within each time point are significantly different ($p \leq 0.05$).
Pigeon peas cvs. "Pinto" were analyzed in this study.

The average of TA, pH and SSC for fresh pigeon pea was shown in Table 6 and 7. Variety "Pinto" has lower values of TA and pH than "Combinada", however, the percentage of SSC was higher in "Pinto". This result can be attributed to the differences between varieties.

Significantly higher percentages of TA were found at 20 °C than at 10 and 15 °C ($p \leq 0.05$), but no significant differences were observed with 0 and 5 °C ($p \geq 0.05$) after 7 days of storage. In addition, after 7 days of storage, a significantly lower pH value was reached at 20 °C than at 0, 5, 10 and 15 °C in October 2005 ($p \leq 0.05$), and also in March 2006, the value of pH was significantly lower at 20 °C than at 0 and 15 °C ($p \leq 0.05$). Perhaps, this reduction in pH at 20 °C is related to the increase of acidity at the same temperature, probably caused by fermentation processes due to catabolic degradation and microorganism attack, which was observed during the

experiments. Some authors, such as Aguayo-Gimenez (2003), mention that when fruit is over mature or deteriorated the TA increased and caused a reduction in pH and this result can be an indicator of an activation of pathway similar to the fermentation process of bacteria. Laminkara *et al.* (2000) observed increases of TA and decreases of pH in fresh cut cantaloupe stored at 20 °C, resulting from increases of lactic acid produced by lactic acid bacteria. Moreover, a tendency of increasing pH with high TA values at 0 °C probably could be a symptom of chilling injury. Wills *et al.* (1982) mentioned that chilling injury causes a release of amino acid in the commodities. The amino acids could suffer decarboxylation and release the amino group raising the pH. Alemañy (2001) mentioned that pH of pigeon peas seed stored at 5 °C can be attributed to release of amino group from decarboxylation of amino acids.

Once pigeon peas were transferred to room temperature for 2 days after 7 days of storage no significant differences in TA were observed between temperatures, the values remained similar to those at 7 days of storage ($p \geq 0.05$). In addition, significantly higher values of pH were obtained at 0 °C October 2005 and March 2006 than other treatments once pigeon peas were transferred to room temperature after 7 days of storage ($p \leq 0.05$). Probably, this trend of increasing pH from 0 °C once transferred to room temperature can be attributed to chilling injury that may have caused a release of amino acids and subsequently decarboxylation due to the catabolic process that liberates amino groups. This result is according Fife and Frampton (1935), who indicated that the increase of pH in living tissues was due to a release of ammonia from organic nitrogen compounds.

In addition, pigeon peas stored for 15 days in March 2006 did not show significant differences between 0 and 5 °C, but significantly higher pH was found at 0 than at 5 °C ($p \leq 0.05$). However, once they were removed to room temperature for 2 days (15 + 2), significantly higher TA was obtained at 5 than at 0 °C, likewise, the pH was significantly higher at 5 than at 0 °C ($p \leq 0.05$). It is believed that this result is caused by chilling injury symptoms presented at 0 and 5 °C.

A pattern of increasing the TA at the end of the shelf-life was observed under all temperatures in comparison to 7 days of storage, where a significantly lower value was obtained at 5 °C than at 0, 10 and 15 °C ($p \leq 0.05$), with no significant differences among them. In contrast, the pH showed a trend of decreasing in comparison with 7 days of storage and the increase of storage temperature. Significantly higher pH values were found at 0 and 5 °C than at 15 and 10 °C ($p \leq 0.05$). No significant differences in pH were found between 10 and 15 °C ($p \geq 0.05$). It seems that the increase in TA and decrease in pH probably is related to senescence process. Organic acids tend to decrease with the storage time due to respiration processes (Kays and Paull, 2004), however, this was not show by our results. It is believed that the fermentation process due to catabolic degradation and microorganism attack can cause this behavior, as explained before, especially at 10 and 15 °C, which showed extensive decay. However, at 0 and 5 °C, these changes may be more related to chilling injury, therefore, no decay was observed. Although one of the chilling injury symptoms is the increase of decay, at 0 and 5 °C this behavior was not observed may be due to low temperature, which causes the growth rate of microorganisms to decrease (Krist *et al.,* 2000).

After 7 days of storage, significantly higher values of SSC were attained at 20 °C than at 0, 5 and 10 °C ($p \leq 0.05$), with the exception of 15 °C (Fig. 3). In addition, after transfer to room temperature the percentages of SSC were higher than percentages obtained at 7 days of storage, with no significant differences between temperatures in October 2005 ($p \geq 0.05$). However, in March 2006, significantly higher percentages of SSC were observed at 0 and 5 °C than at 10 and 15 °C. In addition, at 15 days of storage in March 2006, higher percentages of SSC were found at 0 than at 5 °C, in contrast after transfer to room temperature no significant differences were observed between temperatures ($p \geq 0.05$). Finally, at the end of the shelf-life the percentages of SSC in all storage temperatures did not show significant differences between temperatures ($p \geq 0.05$). Probably the increased SSC with increasing temperature at 7 days of storage can be caused by starch degradation to glucose, in order to maintain the higher respiratory demand at 15 and 20 °C. Starch reserves are low in immature commodities, such as pigeon peas; therefore, the depletion is fast and detrimental to their quality. Kays (1991) mentioned that increased sugar content in leaves is due to catabolism of carbohydrate caused by increasing respiration.

Moreover, high sugar content in pigeon peas is a desirable quality characteristic. Maintaining them at low temperatures such as 0, 5 and 10 °C can retard the rate of sugar to starch conversion, where percentages of SSC of 9 to 16 %, preserve the sweetness of pigeon peas, more than 16% could be related to the senescence process Wills *et al*. (1982) mentioned that peas stored at low temperatures slow down the rate of conversion from sugar to starch. Nevertheless, at 0 and 5 °C pigeon peas suffered chilling injury that may have caused a release of sugar content as indicated by Wills *et al.* (1982), as was observed at 7 days.

On the other hand, once pigeon peas were transferred to room temperature after 7 days and 15 days storage the percentage of SSC increased extensively probably due to the rising respiration rate and water loss. Kays and Paull (2004) mentioned that respiration rate increases after removing the commodities from low temperatures to room temperature, increasing the sugar content due to carbohydrate catabolism. Also, water loss can increase the relative percentage of SSC causing the concentration of soluble solids to increase due to dehydration of the commodity. In addition, the inconsistent differences at the end of the shelf-life can be attributed to similar catabolism characteristics in the senescence process.

Color

Significant interactions were found in the "L", "a" and "b" values of the pod and seed at 7 days of storage and at 7 days plus 2 days at room temperature in the experiments of October 2005 and March 2006 at different storage temperature, with the exception of the "L" value of the seed at 7 days of storage and "L" value of the pod at 7 days plus 2 days at room temperature, as shown in Tables 8, 9, 10 and 11 in blue font. Color measurement of initial quality of pigeon peas in March 2006 indicated the pod and seed was brighter (higher "L" value) and greener (lower "a" value) than October 2005, which indicated that pigeon peas analyzed in October 2005 had a slightly higher maturity than March 2006. In addition, is important to mention that the color of the pod varieties used in these experiment was composed of both green and purple colors (chlorophyll and anthocyanin pigments) (Morton, 1976). This can change the color values, depending on how much green or purple in each reading.

Table 8. Changes in color measurement of pigeon pea pods and seeds for each temperature tested in October 2005.

Storage (days)	Temp. (°C)	Pod			Seed		
		L	a	b	L	a	b
0	Initial	37.66	0.29	7.99	42.56	-2.87	13.22
7	0	35.16 a	0.16 a	8.11 a	38.90 a	-3.30 ab	13.30 a
	5	35.54 ab	0.33 a	7.92 a	38.80 a	-3.38 a	13.68 a
	10	35.73 b	0.46 a	8.18 a	40.77 b	-2.70 bc	14.39 b
	15	35.30 ab	0.89 b	7.88 a	40.76 b	-2.23 c	14.41 b
	20	35.60 ab	1.46 c	8.09 a	41.73 b	-0.04 d	13.13 a
7 + 2	0	34.34 a	0.87 a	7.51 a	39.76 ab	-2.03 a	12.43 a
	5	34.60 a	0.77 a	8.00 a	40.80 bc	-1.57 ab	12.62 a
	10	34.77 a	0.90 a	8.02 a	42.23 c	-1.06 b	13.65 b
	15	34.64 a	1.21 a	7.60 a	38.14 a	-1.59 ab	13.22 ab
End shelf-life							
30	0	35.35 b	0.68 a	7.39 a	40.03 a	-2.72 a	12.23 a
23	5	35.39 b	1.37 b	7.44 a	40.46 a	-2.40 a	12.70 a
13	10	35.15 ab	1.07 ab	7.33 a	40.45 a	-1.85 ab	13.04 a
9	15	34.62 a	0.99 ab	7.29 a	39.98 a	-1.21 b	12.83 a

Data in blue font are composite of October 2005 and March 2006.
Means followed by different letter within each time point are significantly different ($p \leq 0.05$).
Pigeon peas cvs. "Combinada" and "Pinto" were analyzed in October 2005 and March 2006, respectively.

Table 9. Changes in color measurements of pigeon pea pods and seeds for each temperature tested in March 2006.

Storage (days)	Temp. (°C)	Pod			Seed		
		L	a	b	L	a	b
0	Initial	33.96	-0.18	8.15	37.93	-3.77	13.42
7	0	34.61 ab	-0.92 a	9.12 d	38.90 a	-2.46 a	10.81 a
	5	34.47 ab	-0.39 b	8.89 cd	38.80 a	-2.56 a	10.75 a
	10	34.61 ab	0.26 c	7.63 ab	40.77 b	-3.17 a	12.88 b
	15	35.03 b	0.52 c	8.00 bc	40.76 b	-2.49 a	13.10 b
	20	34.04 a	2.23 d	6.92 a	41.73 b	0.07 b	12.82 b
7 + 2	0	34.34 a	1.26 ab	6.97 a	37.81 a	-1.03 a	10.84 a
	5	34.60 a	1.80 b	7.32 ab	38.57 ab	-1.14 a	11.31 a
	10	34.77 a	1.00 a	8.10 b	39.50 b	-1.32 a	12.75 b
	15	34.64 a	1.38 ab	8.21 b	---	---	---
15	0	34.26 a	0.37 a	6.46 a	37.91 a	-2.73 a	10.45 a
	5	34.15 a	1.55 b	6.43 a	38.54 b	-2.61 a	11.47 b
15 + 2	0	32.07 a	1.46 a	6.02 a	36.67 a	-1.46 a	10.01 b
	5	32.09 a	1.99 b	6.36 a	36.49 a	-0.45 b	9.13 a

Data in blue font are composite of October 2005 and March 2006.
Means followed by different letter within each time point are significantly different ($p \leq 0.05$).
Pigeon peas cvs. "Combinada" and "Pinto" were analyzed in October 2005 and March 2006, respectively.
----: No data

Table 10. Changes in color measurement of pigeon pea pods and seeds for each temperature tested in comparison with the initial color of the pigeon peas in October 2005.

Storage (days)	Temp. (°C)	Pod			Seed		
		L	a	b	L	a	b
0	Initial	37.66 c	0.29 a	7.99 a	42.56 c	-2.87	13.20 a
7	0	35.16 a	0.16 a	8.11 a	38.90 a	-3.30 ab	13.30 a
	5	35.54 ab	0.33 a	7.92 a	38.80 a	-3.38 a	13.68 a
	10	35.73 b	0.46 a	8.18 a	40.77 b	-2.70 bc	14.39 b
	15	35.30 ab	0.89 b	7.88 a	40.76 b	-2.23 c	14.41 b
	20	35.60 ab	1.46 c	8.09 a	41.73 bc	-0.04 d	13.13 a
0	Initial	37.66 b	0.29 a	7.99 a	42.56 c	-2.87 a	13.22 ab
7 + 2	0	34.34 a	0.87 b	7.51 a	39.76 ab	-2.03 b	12.43 a
	5	34.60 a	0.77 ab	8.00 a	40.80 bc	-1.57 bc	12.62 ab
	10	34.77 a	0.90 b	8.02 a	42.23 c	-1.06 c	13.65 b
	15	34.64 a	1.21 b	7.60 a	38.14 a	-1.59 bc	13.22 ab
End shelf life							
0	Initial	37.66 c	0.29 a	7.99	42.56 b	-2.86 a	13.22 b
30	0	35.35 b	0.68 ab	7.39 a	40.03 a	-2.72 ab	12.23 a
23	5	35.39 b	1.37 c	7.44 a	40.46 a	-2.40 ab	12.70 ab
13	10	35.15 ab	1.07 bc	7.33 a	40.45 a	-1.85 bc	13.04 ab
9	15	34.62 a	0.99 bc	7.29 a	39.98 a	-1.21 c	12.83 ab

Data in blue font are composite of October 2005 and March 2006. Means followed by different letter within each time point are significantly different ($p \leq 0.05$). Pigeon peas cvs. "Combinada" and "Pinto" were analyzed in October 2005 and March 2006, respectively.

Table 11. Changes in color measurement of pigeon pea pods and seeds for each temperature tested in comparison with the initial color of the pigeon peas in March 2006.

Storage (days)	Temp. (°C)	Pod			Seed		
		L	a	b	L	a	b
0	Initial	33.96 a	-0.18 bc	8.15 bcd	37.93 a	-3.77 a	13.42 b
7	0	34.61 ab	-0.92 a	9.12 d	38.90 a	-2.46 a	10.81 a
	5	34.47 ab	-0.39 b	8.89 cd	38.80 a	-2.56 bc	10.75 a
	10	34.61 ab	0.26 cd	7.63 ab	40.77 b	-3.17 ab	12.88 b
	15	35.03 b	0.52 d	8.00 bc	40.76 b	-2.49 bc	13.10 b
	20	34.04 a	2.23 e	6.92 a	41.73 b	0.07 d	12.82 b
0	Initial	33.96 a	-0.18 a	8.15 b	37.93 a	-3.77 a	13.42 b
7 + 2	0	34.34 ab	1.26 bc	6.97 a	37.81 a	-1.03 b	10.84 a
	5	34.60 b	1.80 c	7.32 ab	38.57 ab	-1.14 b	11.31 a
	10	34.77 b	1.00 bc	8.10 b	39.50 b	-1.32 b	12.75 b
	15	34.64 b	1.38 ab	8.21 b	---	---	---
0	Initial	33.96 a	-0.18 a	8.15 b	37.93 a	-3.77 a	13.42 c
15	0	34.26 a	0.37 b	6.46 a	37.91 a	-2.73 b	10.45 a
	5	34.15 a	1.55 c	6.43 a	38.54 b	-2.61 b	11.47 b
0	Initial	33.96 b	-0.18 a	8.15 b	37.93 b	-3.77 a	13.42 c
15 d + 2	0	32.07 a	1.46 b	6.02 a	36.67 a	-1.46 b	10.01 c
	5	32.09 ab	1.99 c	6.36 a	36.49 a	-0.45 c	9.13 b

Data in blue font are composite of October 2005 and March 2006. Means followed by different letter within each time point are significantly different ($p \leq 0.05$). Pigeon peas cvs. "Combinada" and "Pinto" were analyzed in October 2005 and March 2006, respectively. ----: No data.

At 7 days of storage, at 15 and 20 °C the pod of the pigeon pea became significantly more red (positive “a” value) than at the other temperatures in October 2005 and March 2006 ($p \leq 0.05$). In comparison with the initial “a” value of the pod, no significant differences were observed with 0, 5 and 10 °C in October 2005 and with 0 and 10 °C in March 2006 (Table 10). The “b” value of the pod did not differ significantly in October 2005 between treatments and also with the initial “b” value. In March 2006, by contrast, the “b” value was significantly higher at 0 °C than the other temperatures, with the exception of 5 °C and the initial “b” value ($p \leq 0.05$).Likewise, the initial “b value did not differ significantly with 10 and 15 °C in March 2006 ($p \geq 0.05$) (Table 11). In addition, the differences in “L” value of the pod among the treatments were smaller, yet, in comparison with the initial value, there was a tendency toward darkening (lower “L” value) than the other treatments in October 2005 ($p \leq 0.05$) and significant lightening (higher “L” value) than 15 °C in March 2006. No significant differences were found with the other temperatures ($p \geq 0.05$).

The “L” value of the seed after 7 days of storage became significantly lighter at 10, 15 and 20 °C (higher “L” value) than at 0 and 5 °C ($p \leq 0.05$). Once this was compared with the initial “L” value of the seed in October 2005 significantly lighter “L” values were achieved than the other storage temperatures, with an exception at 20 °C, in contrast, in the March 2006 study did not show significant differences with 0 and 5 °C ($p \geq 0.05$). Also, the “a” value became less green (less negative) with the increase of temperature. At 20 °C in October 2005 and March 2006 ($p \leq 0.05$), the “a” value was significantly lower. Also in March 2006, the seed had a slightly red color at 20 °C (positive “a” value), indicating the severe deterioration of its quality. Also in comparison with the initial “a” value of the seed in October 2005, no significant differences were

observed with 0, 5 and 10 °C ($p \geq 0.05$) and in March 2006 with 10 °C ($p \geq 0.05$). Likewise, the "b" value was significantly higher with increasing temperature with significant differences at 10 and 15 °C in October 2005 and 10, 15 and 20 °C in March 2006. In addition, once the values were compared with the initial "b" value of the seed in October 2005 no significant differences were observed at 0, 5 and 20 °C; in contrast, in March 2006 no significant differences were found at 10, 15 and 20 °C ($p \geq 0.05$).

Color changes of the pod and seed are one indicator of reduced quality as the storage temperature increases, especially the "a" value which became slightly red in the pod. This is probably due to the degradation of chlorophyll, which is more rapid at higher temperatures. Shewfelt (2003) mentioned that chlorophyll is degraded during senescence showing lighter yellow pigments. It seems that the "a" value of the pigeon pea pod and seed remain similar to fresh pigeon peaa at 0, 5 and 10 °C at 7 days of storage, probably due to the decrease of the metabolic activity at low temperatures. This is in agreement with Wills *et al.* (1982), who mentioned that low storage temperatures decrease the rate of deterioration in non-climacteric commodities, such as peas, which retarded the degradation of chlorophyll. However, the increase in the "a" value of the seed at 0 and 5 °C could be attributed to chilling injury damage.

In general, once the pigeon peas were transferred to room temperature, the "a" value of the pod was more positive than at 7 days of storage. In March 2006, the "a" value of the pod was significantly higher at 5 than 10 °C; however, in October 2005 the "a" value did not differ significantly between treatments. Likewise, the "L" value of the pod did not differ significantly between treatments in October 2005 and March 2006. Similarly, the "b" value of the pod did not

show significant changes between treatments in October 2005, however, in March 2006, a tendency toward higher “b” values (more yellowing) with the increasing of temperature was observed at 10 and 15 °C. When compared to the initial “a” value of the pod no significant differences were found with pods at 5 °C after 7 days of storage plus 2 days at room temperature in October 2005 ($p \geq 0.05$). However, in March 2006 the initial “a” value of the pod was significantly greener than the treatments ($p \leq 0.05$). Moreover, pigeon peas at room temperature became significantly darker (less “L” value) in comparison with the fresh pigeon pea pod in October 2005, regardless of the previous storage temperature ($p \leq 0.05$), in contrast, in March 2006 the initial “L” value of the pod did not show significant difference with pods earlier stored at 0 °C ($p \geq 0.05$). In October 2005, the initial “b” value showed no significant difference with pigeon pea pods at room temperature; however, in March 2006 the initial “b” value did not show differences with pods at 5, 10 and 15 °C after 7 days plus 2 days at room temperature ($p \geq 0.05$).

Moreover, the “L” values of the seeds after 7 days of storage plus 2 days at room temperature were significantly higher at 10 °C than at 0 and 15 °C, with no significant difference with 5 °C in October 2005 and also in March 2006 the same pattern was observed, a significantly higher “L” value was found at 10 °C than at 0 °C, with no significant difference with 5 °C. Moreover, the “a” value of the seed became significantly more negative at 0 than at 10 °C, with no significance difference with 5 and 15 °C in October 2005. However, in March 2006 the “a” value remained similar between temperatures. The “b” value was significantly higher at 10 °C than at 0 and 5 °C, with no significant difference with 15 °C in October 2005. Also in March 2006, the 10 °C treatment had significantly higher “b” values than at 0 and 5 °C. In addition, pigeon pea seeds after 7 days plus 2 days at room temperature were significantly less greener

(less negative "a" value) than the fresh seeds in October 2005 and March 2006 ($p \leq 0.05$). Moreover, seeds previously stored at 5 and 10 °C did not show significant differences with the initial "L" value in October 2005 and with at 0 and 5 °C in March 2006 ($p \geq 0.05$). Also, pigeon pea seeds after 7 days plus 2 days at room temperature had no significant differences with the initial "b" value in October 2005, in contrast, the "b" value of fresh seeds was not significantly different from seeds previous stored at 10 °C in March 2006 ($p \geq 0.05$).

This result could indicate that the color of pigeon peas diminishes once they are transferred to room temperature after all previous storage temperatures, due to more rapid senescence and loss of moisture. Moreover, the changes at 0 and 5 °C can be affected by chilling injury, which was obvious in the pods and seeds with more reddish and greener color, respectively. Mekwatanakam (1998) mentioned that green bean pods became greener after 7 days of storage at 5 °C can be an indicator of chlorophyll breakdown, and also Prussia *et al.* (1990) reported that the color loss in southern peas is affected by moisture loss in addition to higher temperatures of storage.

After 15 days of storage in March 2006, the "L" and "b" value of pigeon pea pods stored at 0 and 5 °C were not significantly different, but the "a" value was significantly higher at 5 than at 0 °C (pods became more reddish). Once the pigeon peas were transferred to room temperature, the same pattern was observed, as above. In addition, the seeds after 15 days of storage showed significantly different "L" and "b" values at 5 than at 0 °C. Likewise at room temperature, the seeds did not differ significantly in "L" value between temperatures, but the "a" and "b" value at 0 °C was significantly higher than at 5 °C. Moreover, the initial "a" value of the seeds and pods

were significantly greener (negative “a” value) than at 0 and 5 °C after 15 days and 15 days plus 2 days at room temperature. Also, the same behavior was observed with the initial “b” value of the pods and seeds, which was significantly more positive than at 0 and 5 °C at 15 days an once they were transferred to room temperature ($p \leq 0.05$). The initial “L” value of pigeon pea pods and seeds did not show significant differences with pods and seeds stored under 0 and 5 °C for 15 days. After 15 days plus 2 days at room temperature, pigeon pea pods at 5 °C did not show significant differences with the initial “L” value ($p \geq 0.05$). In contrast, the seeds were significantly less bright at 0 and 5 °C in comparison with the initial “L” value ($p \leq 0.05$).

The results indicated that pigeon pea pods and seeds stored at 0 and 5 °C decreased their quality after 15 days and the quality degradation was more severe once they were transferred to room temperature. It appeared that once chilling injury had occurred, continued cold storage exacerbated chilling injury symptoms. This is in according with Smith *et al.* (2003), who mentioned that chilling damage often depends on temperature and storage time. Also, once they were transferred to room temperature the water loss and respiration rate could increase as a part of chilling symptoms, accelerating the loss of chlorophyll and senescence (Wills *et al.,* 1982).

At the end of the shelf-life in October 2005, the “L” value of the pod was significantly darker at 15 °C than at 0 and 5 °C, with exception of 10 °C. Likewise, the “a” value of the pod was significant lower at 0 than at 5 °C (color become more reddish). In addition, the “L” value of the seed did not significantly differ between the treatments. In contrast, the “a” value of the seed was significantly higher at 0 and 5 °C than 15 °C, but no differences were found with 10 °C. The “b” value of the pods and seeds did not show significant differences between temperatures.

When compared to the initial “a” value of the pods and seed, no significance differences were found with 0°C in the pod and also with 0 and 5 °C in the seed ($p \geq 0.05$). The pods and seeds at the end of the shelf-life were significantly darker than the initial the initial “L” value of the pods and seeds, regardless of the storage temperature (≤ 0.05). In contrast, the “b” value of pigeon pea pods at the end of the shelf-life under different storage temperatures did not show differences with the initial “b” value of the pod. Moreover, pigeon pea seeds at 5, 10 and 15 °C had no differences from the initial “b” value of the seed ($p \leq 0.05$).

Probably, at the end of shelf-life the pattern of decreasing “a” values of the seed under all temperatures (less negative) and increasing in the pod (more positive) is an indicator of chlorophyll degradation that is observed in advanced senescence. However, at 0 and 5 °C the changes were also related to chilling injury damage, with increasing storage time, showing a greener pod at 0 °C, and extensive yellow spot on the pod at 5 °C, respectively that could be related to pheophytin. Likewise, the higher “a” value of the seeds at 0 and 5 °C could be also attributed to chilling injury. This injury was not too severe, therefore, in comparison with the initial “a” values of the seeds no significant differences were found, with a reduction in green color was around 5 and 16 % at 0 and 5 °C, meanwhile at 10 and 15 °C, the reduction was around 35 and 58% in green color at the end of the shelf-life. It could be possible to use this disadvantage to improve the color of the seed of pigeon pea and increase the shelf-life without the pod; if no other sensory properties are affected. Similarly, Morris (1982) indicated that the color of oranges improved with chilling.

Visual quality

In general, as the time and temperature of storage increased, the visual quality of the pigeon pea was reduced, as found in other crops (Kays, 1991; Wills *et al*., 1982). Significant interactions were found for yellowing, shriveling and decay of the pods and seeds after 7 days of storage and in the seeds after 7 days plus 2 days at room temperature in the experiments of October 2005 and March 2006 between storage temperatures. However, the yellowing, shriveling and decay of the pods at 7 days plus 2 days at room temperature did not show any interaction, and therefore, the data were combined and analyzed as shown in Tables 12, 13, 14 and 15 in blue font.

Table 12. Visual quality of pigeon pea pods and seeds for each temperature tested in October 2005.

Storage (days)	Temp. (°C)	Pod			Seed		
		Yellowing	Shriveling	Decay	Yellowing	Shriveling	Decay
0	Initial	2.0	1.0	1.0	1.3	1.0	1.0
7	0	2.0 a	1.0 a	1.0 a	1.4 a	1.0 a	1.0 a
	5	3.1 b	1.0 a	1.0 a	2.1 b	1.0 a	1.0 a
	10	3.1 b	2.0 b	1.0 a	2.6 c	1.1 a	1.0 a
	15	3.4 c	2.3 c	2.4 b	2.7 c	1.2 a	1.0 a
	20	3.7 d	3.6 d	3.5 c	3.7 d	1.7 b	1.9 b
7 + 2	0	3.1 a	4.0 b	1.0 a	2.9 a	2.4 a	1.1 a
	5	3.4 a	4.0 b	1.0 a	3.2 ab	2.5 a	1.1 a
	10	3.3 a	3.7 a	1.9 b	3.8 c	3.9 b	1.0 a
	15	4.0 b	4.2 b	3.6 c	3.7 bc	2.2 a	1.0 a
End shelf-life							
30	0	1.5 a	1.9 a	1.3 a	2.3 a	1.7 b	1.0 a
23	5	3.7 b	3.0 b	1.1 a	3.4 b	1.1 a	1.0 a
13	10	3.5 b	3.0 b	3.3 b	-----	-----	-----
9	15	3.5 b	3.0 b	3.7 b	3.6 b	1.5 b	1.5 b

Subjective rate: 1= None, 2=Slight, 3= Moderate, 4= Severe and 5= Extremely severe. Data in blue font are composite of October 2005 and March 2006. Means followed by different letter within each time point are significantly different ($p \leq 0.05$). Pigeon peas cvs. "Combinada" and "Pinto" were analyzed in October 2005 and March 2006, respectively. -----: No data.

Table 13. Visual quality of pigeon pea pods and seeds for each temperature tested in March 2006

Storage (days)	Temp. (°C)	Pod			Seed		
		Yellowing	Shriveling	Decay	Yellowing	Shriveling	Decay
0	Initial	1.1	1.0	1.0	1.0	1.0	1.0
7	0	1.3 a	1.3 a	1.0 a	1.6 a	1.1 a	1.0 a
	5	1.7 a	1.1 a	1.0 a	1.8 a	1.1 a	1.0 a
	10	2.3 a	1.9 b	1.6 ab	2.4 b	1.3 a	1.0 a
	15	3.3 b	1.9 b	2.0 b	2.6 b	1.2 a	1.0 a
	20	4.0 d	2.7 c	3.7 c	3.9 c	2.4 b	1.7 b
7 + 2	0	3.1 a	4.0 b	1.0 a	2.8 a	2.0 b	1.0
	5	3.4 a	4.0 b	1.0 a	3.0 a	2.0 b	1.0
	10	3.3 a	3.7 a	1.9 b	3.2 a	1.7 a	1.0
	15	4.0 b	4.2 b	3.6 c	4.2 b	2.2 b	1.0
15	0	2.2 a	1.6 a	1.0 a	-----	-----	-----
	5	4.0 b	1.8 a	2.2 b	2.2	1.4	1.0
15 + 2	0	4.2 a	3.9 a	3.8 a	2.3 a	2.3 a	2.8 a
	5	4.8 b	4.1 a	4.6 b	3.4 b	3.6 b	3.1 a

Subjective rate: 1= None, 2=Slight, 3= Moderate, 4= Severe and 5= Extremely severe. Data in blue font are composite of October 2005 and March 2006. Means followed by different letter within each time point are significantly different ($p \leq 0.05$). Pigeon peas cvs. "Combinada" and "Pinto" were analyzed in October 2005 and March 2006, respectively.-----: No data.

Table 14. Visual quality of pigeon pea pods and seeds for each temperature tested in comparison with the initial quality of the pigeon pea in October 2005.

Storage (days)	Temp. (°C)	Pod			Seed		
		Yellowing	Shriveling	Decay	Yellowing	Shriveling	Decay
0	Initial	2.0 a	1.0 a	1.0 a	1.1 a	1.0 a	1.0 a
7	0	2.0 a	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a
	5	3.1 b	1.0 a	1.0 a	2.1 b	1.0 a	1.0 a
	10	3.1 bc	2.0 b	1.0 a	2.6 c	1.1 a	1.0 a
	15	3.4 cd	2.3 c	2.4 b	2.7 c	1.2 a	1.0 a
	20	3.7 d	3.6 d	3.5 c	3.7 d	1.7 b	1.9 b
0	Initial	2.0 a	1.0 a	1.0 a	1.1 a	1.0 a	1.0 a
7 + 2	0	3.1 b	4.0 c	1.0 ab	2.9 b	2.4 b	1.1 a
	5	3.4 b	4.0 c	1.0 b	3.2 bc	2.5 b	1.1 a
	10	3.3 b	3.7 b	1.9 c	3.8 c	3.9 c	1.0 a
	15	4.0 c	4.2 c	3.6 d	3.7 c	2.2 b	1.0 a
End shelf life							
0	Initial	2.0 a	1.0 a	1.0 a	1.1 a	1.0 a	1.0 a
30	0	1.5 a	1.9 b	1.3 a	2.3 b	1.7 b	1.0 a
23	5	3.7 b	3.0 c	1.1 a	3.4 b	1.1 a	1.0 a
13	10	3.5 b	3.0 c	3.3 b	----	----	----
9	15	3.5 b	3.0 c	3.7 b	3.6 c	1.5 b	1.5 b

Subjective rate: 1= None, 2=Slight, 3= Moderate, 4= Severe and 5= Extremely severe. Data in blue font are composite of October 2005 and March 2006. Means followed by different letter within each time point are significantly different ($p \leq 0.05$). Pigeon peas cvs. "Combinada" and "Pinto" were analyzed in October 2005 and March 2006, respectively.-----: No data.

Table 15. Visual quality of pigeon pea pods and seeds for each temperature in comparison with the initial quality of the pigeon pea tested in March 2006.

Storage (days)	Temp. (°C)	Pod			Seed		
		Yellowing	Shriveling	Decay	Yellowing	Shriveling	Decay
0	Initial	1.2 a	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a
7	0	1.3 ab	1.3 a	1.0 a	1.6 b	1.1 a	1.0 a
	5	1.7 b	1.1 a	1.0 a	1.8 b	1.1 a	1.0 a
	10	2.3 c	1.9 b	1.6 ab	2.4 c	1.3 a	1.0 a
	15	3.3 d	1.9 b	2.0 b	2.6 c	1.2 a	1.0 a
	20	4.0 e	2.7 c	3.7 c	3.9 d	2.4 b	1.7 b
0	Initial	1.2 a	1.0 a	1.0 ab	1.0 a	1.0 a	1.0
7 + 2	0	3.1 b	4.0 c	1.0 ab	2.8 b	2.0 c	1.0
	5	3.4 b	4.0 c	1.0 b	3.0 b	2.0 c	1.0
	10	3.3 b	3.7 b	1.9 c	3.2 b	1.7 b	1.0
	15	4.0 c	4.2 c	3.6 d	4.2 c	2.2 c	1.0
0	Initial	1.2 a	1.0 a	1.0 a	1.0 a	1.0 a	1.0
15	0	2.2 b	1.6 b	1.0 a	-----	-----	-----
	5	4.0 c	1.8 c	2.2 b	2.2 b	1.4 b	1.0
0	Initial	1.2 a	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a
15 + 2	0	4.2 b	3.9 b	3.8 b	2.3 b	2.3 b	2.8 b
	5	4.8 c	4.1 b	4.6 c	3.4 c	3.6 c	3.1 b

Subjective rate: 1= None, 2=Slight, 3= Moderate, 4= Severe and 5= Extremely severe. Data in blue font are composite of October 2005 and March 2006. Means followed by different letter within each time point are significantly different ($p \leq 0.05$). Pigeon peas cvs. "Combinada" and "Pinto" were analyzed in October 2005 and March 2006, respectively.-----: No data.

Tables 12 and 13 show the initial quality data of pigeon pea used in the experiments of October 2005 and March 2006. A slight yellowing in the pods and seeds was observed in October 2005, but no yellowing was found in the pigeon peas used in March 2006, and also no decay or shriveling was observed in either experiment. These results indicate that the pigeon peas used in March 2006 were of optimal quality and maturity, although, pigeon peas harvested in October 2005 were slightly more mature than March 2006.

After 7 days of storage, pigeon pea quality was consistently reduced with progressive increases in storage temperature. In October 2005, the pods and seeds showed significantly less yellowing at 0 °C compared to the other temperatures ($p \leq 0.05$) and also, no shriveling or decay

was observed at 0 and 5 °C in the pods and at 10 °C in the seed. The same trend was observed in March 2006 with little change in yellowing, shriveling and decay at 0, 5 and 10 °C. However, chilling injury symptoms, such as yellow spots were found in the pods of pigeon peas stored at 5 °C. In addition, the quality of seeds and the pods were significantly diminished at 20 °C compared to 0, 5, 10 and 15 °C ($p<0.05$) in October 2005 and March 2006, and showed extensive fungal growth on the seed, germination and off-odor, making them unacceptable for sale (Fig. 8 and 10). When compared with the yellowing of pigeon pea pods and seeds after 7 days of storage significant changes was found at 0 °C in comparison with the initial quality in October 2005 (Table 14). In March 2006, no differences in yellowing were observed between pods stored at 0 °C and fresh, however, the yellowing of the seeds at 7 days of storage was significantly higher than the fresh ($p\leq0.05$) (Table 15). Moreover, little change in shriveling of the pod was observed between at 0 and 5 °C and the initial quality score in October 2005 and March 2006 ($p\geq0.05$). Likewise, slight changes in shriveling of the seeds were observed at 0, 5, 10 and 15 °C and fresh in October 2005 and March 2006 ($p\geq0.05$). No development of decay in the pod and seed was observed at 0, 5 and 10 °C similar to the fresh pigeon pea pod and seed at 0, 5, 10 and 15 °C ($p\geq0.05$).

Perhaps the rapid diminishing of the quality of pigeon peas at high storage temperature was a result of advanced senescence. Mohammed and Brecht (2003) and Wills *et al.* (1982) found that the green color of vegetables is rapidly degraded by the senescence process, unmasking yellowing colors due to xanthophyll synthesis. Kader (1992) stated that resistance to fungal attack decreases with senescence and at temperatures between 20-25 °C. Our results indicated that at low temperatures, such as 0, 5 and 10°C, the quality of pigeon peas was

maintained, and at 0 °C was similar to fresh pigeon peas, probably due to the reduction of the metabolic activity. However, chilling injury symptoms, like yellow spot on the pod at 5 °C, decrease the quality of pigeon pea.

Once the pigeon peas were transferred to room temperature, their quality was further diminished by shriveling and yellowing. After 7 days of storage plus 2 days at room temperature, significantly higher yellowing scores and decay of the pod were observed at 15 °C than at the other temperatures ($p \leq 0.05$) with moderate to severe scores. Shriveling of the pod increased in at all temperatures, with no significant differences at 0, 5 and 15 °C with severe to extremely severe scores. Similarly, significantly higher values of yellowing of the seed were observed at 7 days of storage plus 2 days at room temperature at 10 °C with no significant differences at 15 °C in October 2005 and at 15 °C in March 2006. Also, significantly higher values of shriveling were found at 10 °C than at the other temperatures ($p \leq 0.05$) in October 2005, with moderate to severe scores. In contrast, in March 2006, significantly less shriveling was observed at 10 °C. Moreover, no decay in the seed was found at any temperature in either experiment. In addition, pigeon pea pods and seeds were significantly more yellow and shriveled at 7 days plus 2 days at room temperature in comparison to fresh pigeon peas in October 2005 and March 2006 ($p \leq 0.05$). However, at 0 °C no differences in decay were found in comparison with fresh pigeon pea pods in October 2005 and March 2006 ($p \geq 0.05$) (Fig. 9).

As is expected, the quality of pigeon peas was reduced once they were removed to room temperature, probably due to rapid senescence of the commodity caused by an increase of metabolic activity and water loss, which limited the salability of the pigeon pea in all

temperatures. Although, the water loss in the seed was lower than in the pod, with slight to moderate scores, these scores were detrimental for the seeds due to the loss of brightness and slightly shrunken appearance. This behavior could be attributed to the barrier of the pod that reduced the air movement over the surface of the seeds preventing shriveling and also protecting them from fungal growth. Jay (1996) mentioned that the pod is a natural barrier of the vegetables to avoid fungal growth, that develops once the pod or skin is injured or after bacterial attack. In addition, Kays and Paull (2004) indicated that sometimes chilling injury symptoms are obvious once the commodity is removed from the chilling temperature to room temperature, which was observed at 0 °C because of the rapidly diminishing quality to severe scores after 7 days of storage. However one of the chilling injury symptoms is the enhancement of bacterial and fungal attack due to the release of metabolites, such as amino acids and sugars (Wills *et al.,* 1982), but this behavior was not observed at 0 °C in the pod. Probably the water activity of the pod could be reduced from the release of water due to a higher water loss, that is also a usually symptom of chilling injury, this assumption was supported from experimental observation.

After 15 days of storage in March 2006, the yellowing score of the pod was significantly more severe than at 5 than at 0 °C with scores of slight to moderate. In addition, when compared to initial pod yellowing, at 0 and 5 °C decrease the quality, especially at 5 °C , with severe scores ($p \leq 0.05$). Likewise, pigeon peas at 5 °C had a slight decay development, which was significantly different from fresh pigeon pea pods ($p \leq 0.05$). As is expected, at 15 days the seeds stored at 5 °C were significantly more yellowing and shriveling than fresh seeds, with slight to moderate scores ($p \leq 0.05$). This result showed that the salability of pigeon peas at 5 °C was limited especially by pod quality due to chilling injury damage (yellow spot). Wills *et al.* (1982) mentioned that the

longer the amount of time that the commodity is exposed to chilling temperatures the more severe the symptoms. Once the pigeon peas were transferred to room temperature after 15 days of storage, the quality of the pod and seed was reduced significantly in both 0 and 5 °C. Severe to extremely severe scores for shriveling and decay of the pod were given at 0 and 5 °C with no significant differences among them. However, the yellowing of the pod was significantly more severe at 5 than at 0 °C ($p \leq 0.05$) and also, the yellowing and shriveling score of the seeds was significantly higher at 5 than at 0 °C with moderate to severe scores. Likewise, the yellowing, shriveling and decay of pigeon pea pods and seeds were significantly more severe in comparison with fresh pods and seeds ($p \leq 0.05$) This behavior was mostly caused by chilling injury, which develops more severe symptoms when transferred to room temperature.

In general, at the end of the shelf-life in October 2005, the quality of the pigeon peas was limited mainly by yellowing and decay. Significantly less yellowing and shriveling of the pod was attained at 0 °C than at 5, 10 and 15 °C ($p \leq 0.05$). In addition, the decay of the pod was significantly lower at 0 and 5 °C than at 10 and 15 °C, with moderate to severe scores at higher temperatures (Fig. 11). Moreover, the yellowing of the seeds was significantly less at 0 °C than at 5, 10 and 15 °C and also shriveling and decay received lower values at 0 °C than the other temperatures. When compared to the initial quality, the pigeon pea pods at 0 °C had a lower yellowing score, similar to fresh pods; however, yellowing of the seeds at the end of the shelf-life was significantly greater than fresh seeds, regardless of the storage temperature, even though, at 0 °C the seeds had a slight score. In addition, the shriveling of the seeds at 5 °C was not significantly different from fresh seeds ($p \leq 0.05$). In contrast, the shriveling of the pods at the end of the shelf life was significantly higher than the initial value under all storage temperatures

($p \geq 0.05$). Likewise, the pods and seeds stored at 0 and 5 °C did not have significant differences in decay with fresh pigeon pea pods and seeds ($p \geq 0.05$). At low temperatures, such as 0 °C, the quality of pigeon peas were most like fresh pigeon pea, due to the reduction in the metabolic activity and also limited fungal and bacterial growth. However, the chilling injury symptoms observed on the pod at 0 °C (greener pod) due to pheophytin, may decrease the acceptability of pigeon peas with the pod in the market, even though the time of storage is increased and the seed quality scores remained high.

This suggested that shelled pigeon peas could potentially be sold for a longer period of time. In addition, the results indicated that the best potential storage temperature for pigeon peas is 10 °C, which provided better quality results, with no chilling injury symptoms, and mold growth and senescence were not as extensive as in the 15 and 20 °C.

Texture

In general, the hardness of pigeon pea seed progressively increased with increasing of temperature and storage. No significant interaction was found between hardness of pigeon pea in October 2005 or March 2006 and different temperatures and different temperatures after 7 days of storage ($p \leq 0.05$), therefore, the data was combined and analyzed together, as shown in Fig.12. A significant interaction was found between hardness of pigeon pea in October 2005 and March 2006 and different temperatures after 7 days plus two days at room temperature ($p \geq 0.05$).

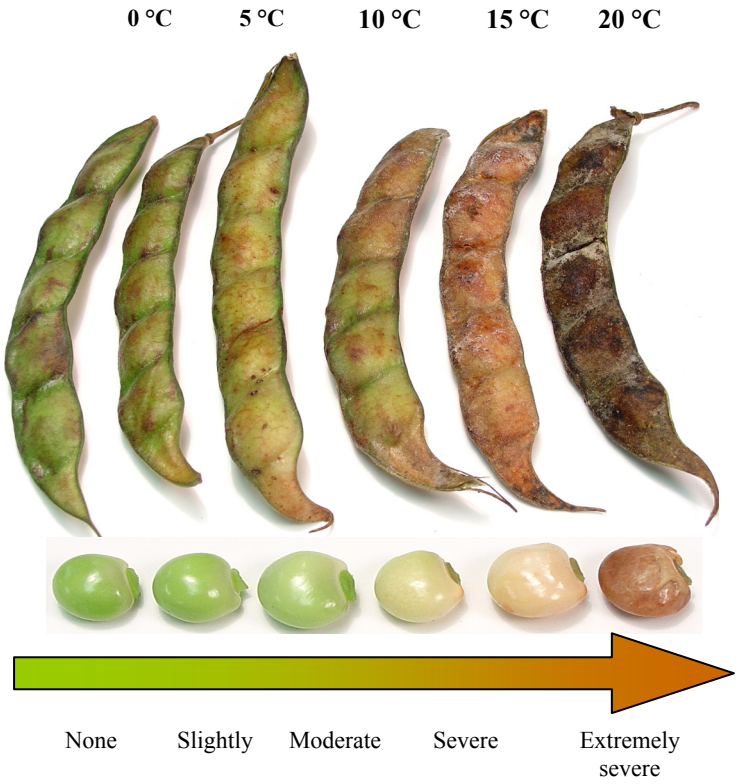


Figure 8. Changes in color of pigeon peas found at 7 days of storage.

Figure 9. Changes in color and shriveling of pigeon peas after 7 days plus 2 days at room temperature.

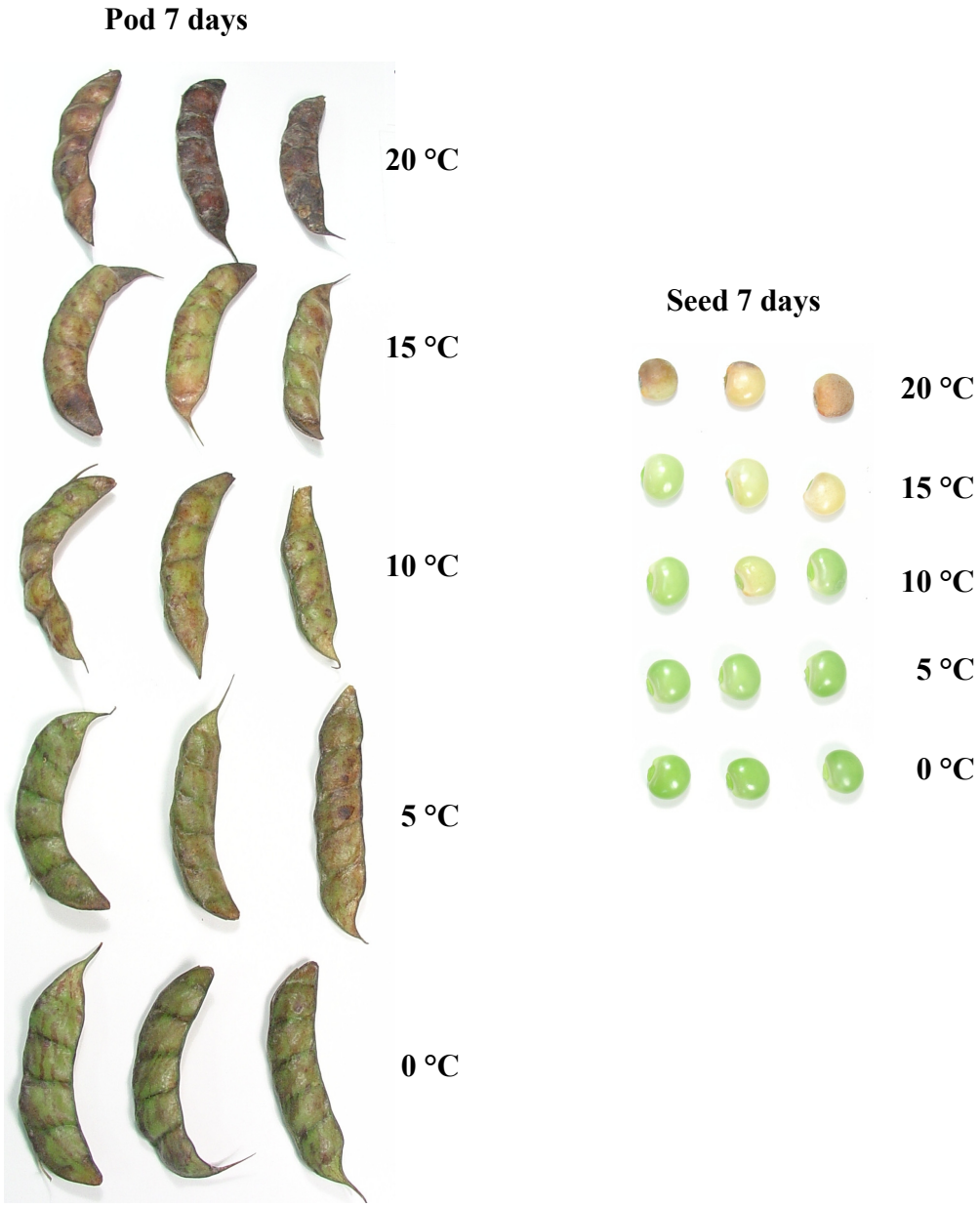


Figure 10. Changes in color of pigeon peas after 7 days of storage at five temperatures: 0, 5, 10, 15 and 20 °C.

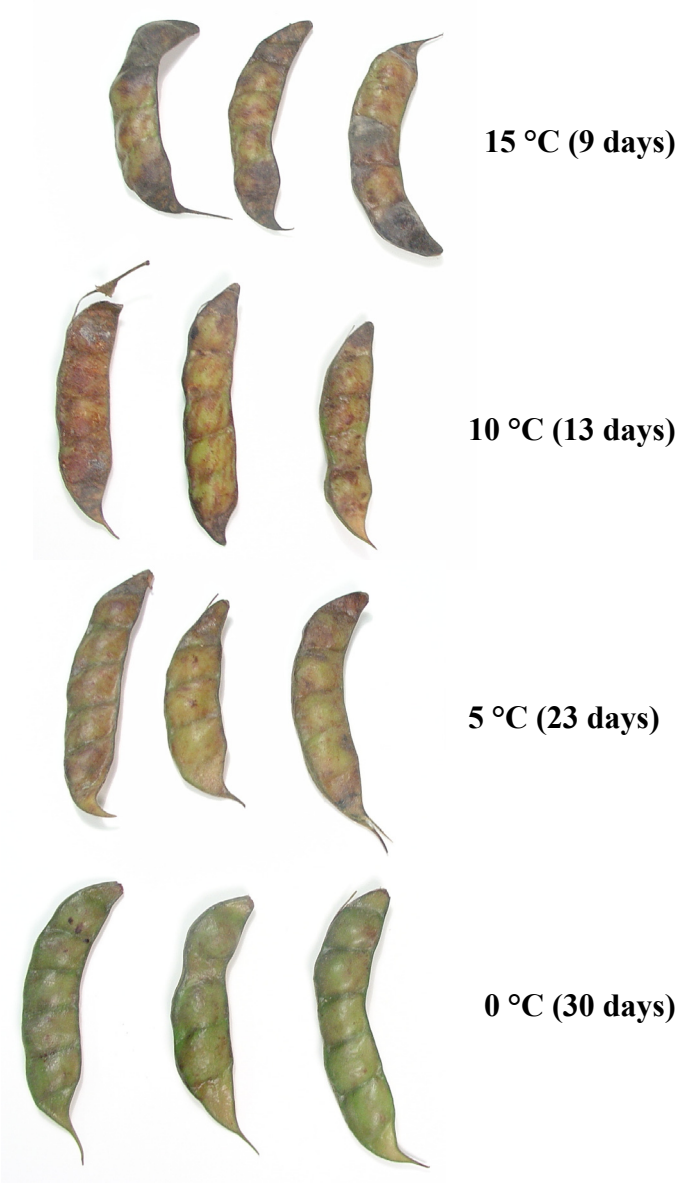


Figure 11. Changes in color of pigeon peas pod at their maximum seed shelf-life

Significantly higher values for texture were observed in pigeon peas stored at 15 and 20 °C, than at 0, 5 and 10 °C, with 950.67 and 1031.92 g of force, respectively. No significant differences were observed between 0, 5 and 10 °C. The increased hardness was directly correlated to increase in temperature. Smith *et al.* (2003) indicated that lignifcation of the tissue of immature vegetables, such as peas, is associated with storage temperature mediated respiration rate, therefore, low temperature limited the tissue toughening, and the inverse effect was observed at high storage temperature. Similarly, Freeman and Sistrunk (1978) reported that shear values of snap bean pods increased with the temperature of storage.

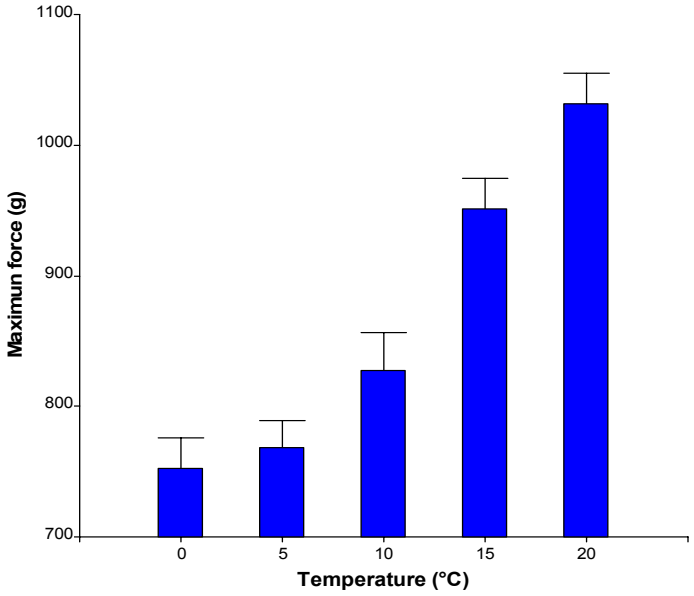


Figure 12. Variation in texture of pigeon peas stored at five different temperatures for 7 days.

Once the pigeon peas were transferred to room temperature for 2 days after 7 days of storage, the hardness increased regardless of the previous storage temperature (Table 16), with values between 1000 to 1140 g force approximately, with no significant differences among 0, 5, 10 and 15 °C in the experiment of October 2005 and March 2006. The increase of hardness in all

treatments was probably caused by increases in the respiration rate at room temperature, which promoted lignification or increases of insoluble pectins (Smith *et al.*, 2003; Kays and Paull, 2004).

Table 16. Changes in texture of pigeon peas for each temperature tested in October 2005 and March 2006

Storage (days)	Temp. (°C)	Maximum Force (g)	
		October 2005	March 2006
7 + 2	0	1022.1 a	1053.1 a
	5	1069.7 a	1104.7 a
	10	1050.1 a	1138.8 a
	15	1092.8 a	------
End shelf-life			
30	0	905.3 a	------
23	5	821.3 a	------
13	10	900.2 a	------
9	15	894.6 a	------
15	0	------	867.7 a
	5	------	891.9 a
15 + 2	0	------	1116.4 a
	5	------	1083.2 a

Means followed by different letter within each time point are significantly different ($p \leq 0.05$).
Pigeon peas cvs. "Combinada" and "Pinto" were analyzed in October 2005 and March 2006, respectively.
-----: No data.

The differences in the driving force were also likely due to water loss and dehydratation of the seed. Trail *et al.* (1992) found that the shear value of green beans pods was due to the loss of water and increase of insoluble pectins, which were more evident at 29 °C than at 2 °C. It is also appears that at 0 and 5 °C, no chilling injury symptoms related to texture were evident, therefore, no significant differences were observed between other treatments. After 15 days of storage in March 2006, no significant differences were observed between 0 and 5 °C and also when they were transferred for 2 days at room temperature. However, the hardness of pigeon peas stored at 0 and 5 °C increased in comparison with 15 days of storage. As expected, the

hardness of pigeon peas increased at the end of shelf-life regardless the storage temperature ($p \geq 0.05$) (Table 16). This trend could be attributed to the increased lignification in the cell wall as the time of storage increased until they reach their maximum shelf-life, which was dependent on storage temperature. Smith *et al.* (2003) indicated that storage temperatures often interact with storage duration to affect the shelf-life of vegetables.

Shelf-life

On basis of the evaluation of a range of quality indices, the shelf-life of pigeon peas was reduced at high storage temperatures, such as 15 and 20 °C. Due to higher respiration rates, greater decay and changes in color, pigeon peas at 15 and 20 °C attained an average shelf-life of 9 and 7 days, respectively. In addition, at low temperatures the presence of chilling injury caused serious damage to the pods at 0 and 5 °C, though decay was not observed. The shelf-life was less than 7 days at both temperatures. The optimum storage temperature was at 10 °C with a maximum shelf-life of 13 days for the seeds, but 10 days was recommended in order to avoid fungal growth on the pod that was observed at 13 days (Table 17).

Table 17. Comparison of the most important characteristics in pigeon peas in the postharvest experiment.

Temperature (°C)	Respiration rate average between varieties (mg CO_2/kg h)	Overall visual quality	Texture	SSC	Chilling injury	Shelf-life pod (days)	Shelf-life seed (days)
0	27.3	Good	Tender	Good	Yes	7	30*
5	47.9	Good	Tender	Good	Yes	7	23*
10	89.5	Good	Tender	Good	No	13	13
15	140.1	Bad	Hard	Sene.	No	9	9
20	228.5	Bad	Hard	Sene.	No	7	7

Sene. = Senescence.
* = Possible shelf-life of the seed, if no other sensory properties are affected.

Modified Atmosphere Packaging

Gas composition

Significant interactions were observed between days and film packaging at 0 °C (Fig. 13). After day 7 of storage, a significantly higher value (6.1 %) of CO_2 was reached in MAP 4 ($p \leq 0.05$), with no significant differences at 14 and 21 days of storage at 5.1% CO_2 in equilibrium. Meanwhile, MAP 2 and MAP 1 did not show significant differences at 7, 14 and 21 days at 2.3% of CO_2 in equilibrium. MAP 3 had significantly lower concentrations of CO_2 with no significant differences at 7, 14 and 21 days of storage at 1.2% CO_2 in equilibrium ($p \leq 0.05$).

A significant interaction was observed between temperature and film packaging at 20 °C (Fig. 14). Significantly higher values of CO_2 content were reached at 20 °C in MAP 4 and MAP 3 with 12.4 and 10.1% of CO_2, respectively, than MAP 1 and MAP 2 with 6.0 and 4.8%, respectively, with significant differences among them ($p \leq 0.05$). Low values of $\%CO_2$ were achieved inside films packaged and stored at 0 °C with no differences among them, with the exception of MAP 4 at 0 °C, which also did not show significant differences with MAP 1 at 20 °C.

Perhaps, the differences in the concentration of CO_2 in different films packages in both temperatures can be attributed to the permeability of carbon dioxide and thickness of the film. Barron *et al.* (2002) and Al-Ati and Hotchkiss (2002) similarly indicated that the variances of $\%CO_2$ inside the packages is determined by permeability of CO_2 through the film and thickness. MAP 4 caused an accumulation of CO_2 to injurious levels (5%) for peas, as recommended by

Kader (1992). However, once the packages were transferred to 20 °C, the CO_2 film permeability changes in MAP 3 and MAP 4 were not enough to compensate for the increased respiration rate of the pigeon peas due to the detection of off-odor. However, MAP 1 and MAP 2 maintained a relative increase of % CO_2 with no off-odor.

Perhaps the pigeon peas need a film with higher O_2 and CO_2 permeability, which would allow during temperature abuse, the increased supply of O_2 and at the same time, more quickly release the CO_2 production to avoid anaerobic conditions. For instance, MAP 1 and MAP 2 with a high O_2 and CO_2 permeability provided better conditions for pigeon pea storage at 0 and 20 °C. Presence of no off-odors and the better quality of pigeon peas support this fact. In contrast MAP 4, had a relative low O_2 and low CO_2 permeability, in comparison with MAP 1 and MAP 2, and for MAP 3 these values are unknown. From experimental observation, MAP 3 had a very low CO_2 content at 0 °C and high at 20 °C, which probably means that CO_2 released faster than O_2 enter, reaching an atmosphere with low O_2 and CO_2 due to the holes. During temperature abuse, this caused a greater reduction in O_2 and increase in CO_2, although the CO_2 transmission achieved with the holes was not enough to release the CO_2 produced by the high respiration rate. This assumption was supported experimentally by the fact off-odor was detected at 0 and 20 °C. This is in agreement with Zagory (1998), who mentioned that holes in the package result in an equilibrium atmosphere with low O_2 and CO_2. Mekwatanakam (1998) found that PD 941 film was more effective than PD 961 in maintaining overall quality in green beans at 5 °C. In addition, Charles *et al.* (2005) mentioned that at 20 °C the O_2 demand is high and a film with high O_2 and CO_2 permeability is better to maintain the respiration under aerobic conditions and

prevent the accumulation of CO_2 above injurious levels. Kader (1992) indicated that the limit of tolerance of CO_2 could be temperature dependent for each commodity.

The steady state was rapidly reached in MAP 1, MAP 2 and MAP 3 at 0 °C, due to film permeability (Kader, 1992). The diffusion of CO_2 out of the bag was equal to CO_2 production before 7 days of storage. Talasila *et al.* (1995) stated that if the time to reach the steady state is close to the shelf-life of the commodity, MAP benefits will be fulfilled. MAP 4 reached the steady state at 14 days of storage at 0 °C, however, the shelf life of the pigeon peas at this temperature less than 7 days in air at 0 °C. In addition, at 20 °C it is not possible to observe the steady state because of the amount of the storage time.

Therefore, according to our results MAP 1 and MAP 2 (PD 941) are the best film packages. However, MAP 1 (air headspace) is recommended because this package developed a passive atmosphere similar to MAP 2 (active atmosphere) after 7 days of storage. Using an active atmosphere could increase the cost of production to growers. Also, MAP 1 could be considered beneficial for the preservation of pigeon peas at 20 °C.

Mass loss

In general, the mass loss was lowest at 0 °C with a tendency to increase as the storage time increased in MAP 1, MAP 2 and MAP 4. MAP 3 showed a pattern to decrease at 21 days of storage. Likewise, significantly lower mass losses were found in all film packages at 7 days of storage with 0.46% mass loss. MAP 1 had significantly lower mass loss than other treatments with a mass loss of 0.40% ($p \leq 0.05$) (Fig. 15). As expected the mass loss increased significantly

once the temperature of storage increased to 20 °C ($p \leq 0.05$) with 2.09% mass loss, regardless of the film type. No significant interaction was found between temperature and film type ($p \geq 0.05$) (Fig. 16). The different packaging materials were effective in reducing mass loss when compared to pigeon peas stored in air at 0 and 20 °C. The most effective was MAP 1 at 0 °C. MAP 2 has the same water vapor transmission of MAP 1, however, its mass loss was high, which was not expected. This behavior could be attributed to water stress. MAP 3 showed a contradictory decreasing pattern that could represent water vapor condensation because of unknown water vapor transmission of this bag. This result would agree with Trail *et al.* (1992), who indicated that snap bean pods had a maximum mass loss of 2.6% after 16 days of storage at 10 °C due to the polylefilm packaging.

Initial quality of pigeon peas

The variety "Combinada" was used for the MAP experiment, which was analyzed upon arrival to the laboratory as shown in Table 18.

Table 18. Quality of pigeon peas upon their arrival in the laboratory.

Quality Attributes	Average values
pH	6.55
TA (%)	0.12
SSC (%)	10.93
Pod Color	
L	35.01
a	0.05
b	7.04
Seed Color	
L	37.98
a	-2.93
b	13.25
Texture (g)	707.67

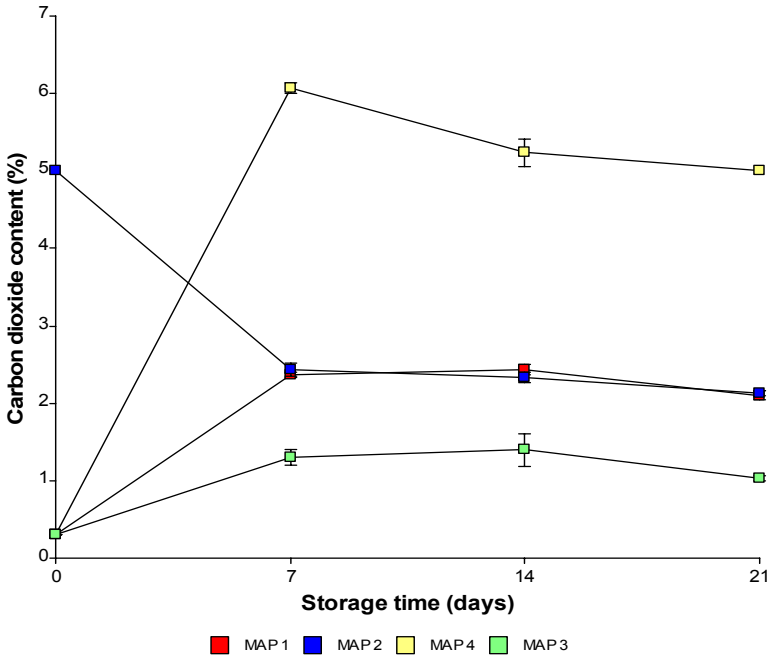
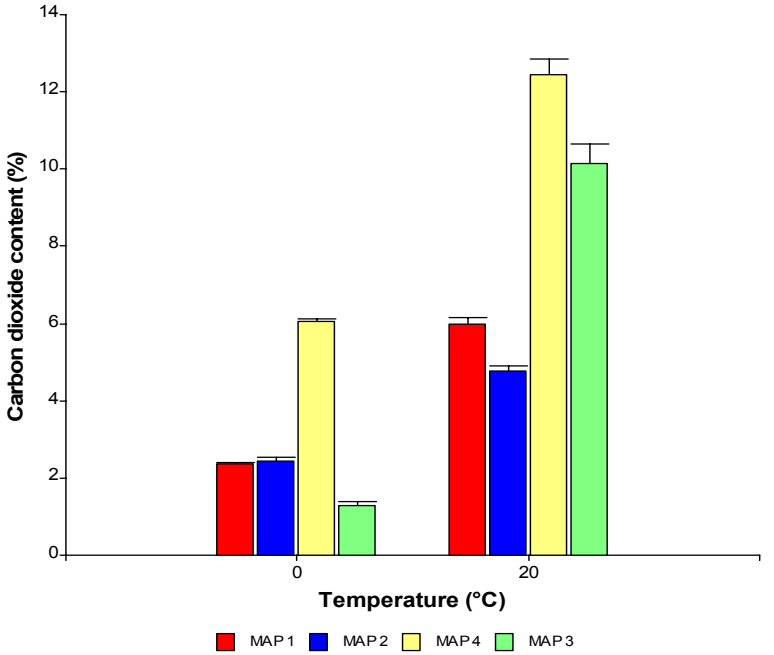
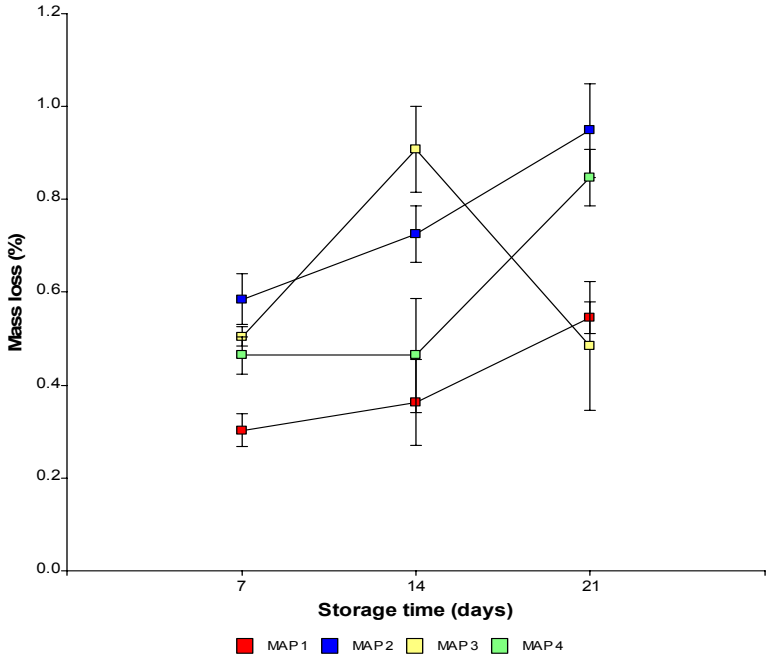


Figure 13. CO_2 concentration of pigeon peas packaged in different films at 0°C for 7, 14 and 21 days.



MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 14. CO_2 concentration of pigeon peas packaged in different films at 20 °C for 7 days.



MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure. 15. Changes in mass loss of pigeon peas packaged in different films at 0°C for 7, 14 and 21 days.

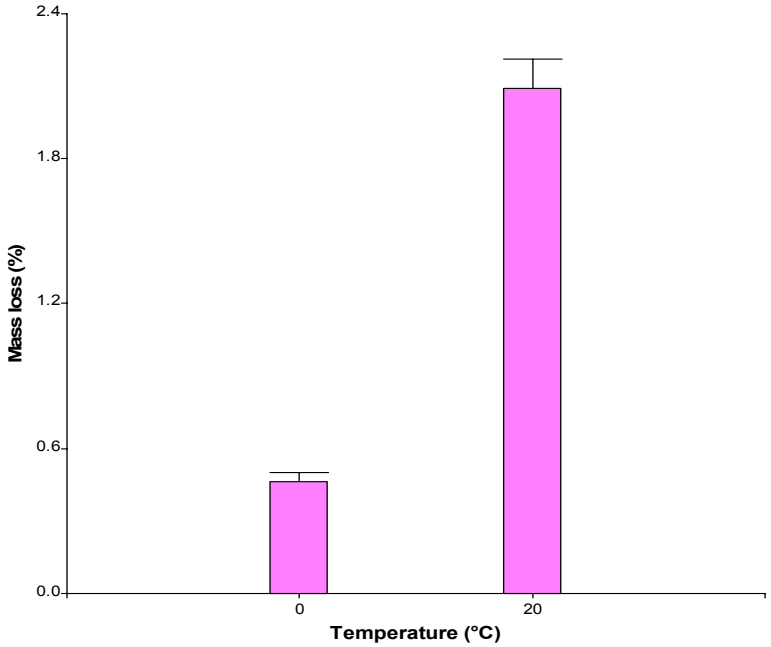


Figure. 16. Changes in mass loss of pigeon peas packaged in different films at 20 °C for 7 days.

Color

A significant interaction of pod "L" value was found between days and film packaging at 0 °C (Fig.17A). A continuous decrease in "L" values of the pod (color became darker) was observed in MAP 2 at 14 and 21 days of storage when compared to the other treatments ($p \leq 0.05$), with the exception of MAP 3 at 7 days of storage. MAP 1 showed "L" values more or less constant with no significant differences at 7, 14 and 21 days of storage. Also MAP 4 did not show significant differences at 7 and 14 days ($p \geq 0.05$). A significantly higher seed "L" value (color became lighter) was reached in MAP 3 compared to the other treatments, regardless of the time of storage ($p \leq 0.05$) (Fig. 17B). In addition, a significantly lower mean "L" value was observed after 21 days of storage than after 14 days ($p \leq 0.05$). No interaction was observed between days and film types at 0 °C in the pigeon pea seeds.

A significant decrease in "a" value (color became greener) of pigeon pea pods were observed after 14 days of storage under all film types ($p \leq 0.05$) and then subsequently increased (became slightly red) at 21 days of storage (Fig. 18A). Similarly to the pod, the "a" values of the seeds decreased significantly after 14 days of storage under all films tested (became greener) except for MAP 3. At 21 days of storage, the "a" value increased under all film types with no differences among them ($p \leq 0.05$). In addition, MAP 3 had a significantly lower mean "a" value (less green) of the seed than the other film packages ($p \leq 0.05$) (Fig.18B). he "b" values of the pods and seeds showed an inverse trend compared to the "a" values (Fig. 19A and 19B). The "b" values of the seeds increased (became more yellow) after 14 days of storage with no differences for film types. In addition, a significant increase of "b" values of the pods was reached in MAP 3

and MAP 2 after 14 days than after 7 days of storage ($p \leq 0.05$) (more yellow). Meanwhile, the 'b" values of MAP 1 did not show significant differences between 7 and 14 days of storage. In contrast, MAP 4 showed a subsequent decrease in "b" value of the pod after 7 days of storage. Probably, the decrease in "a", "L" and "b" values of the pod after 14 days of storage can be attributed to the earlier chlorophyll breakdown that caused a greener color of the pod, which increased in magnitude after 21 days of storage and then attained a darker green brown color, which is characteristic of pheophytin. This is in agreement with Mekwatanakam (1998), who mentioned that the bean pod became greener after 7 days of storage due to chlorophyll breakdown, and also deMan (1999), who indicated that pheophytin is formed by degradation of lipoproteins bound to chlorophyll and then showed an olive-brown color.

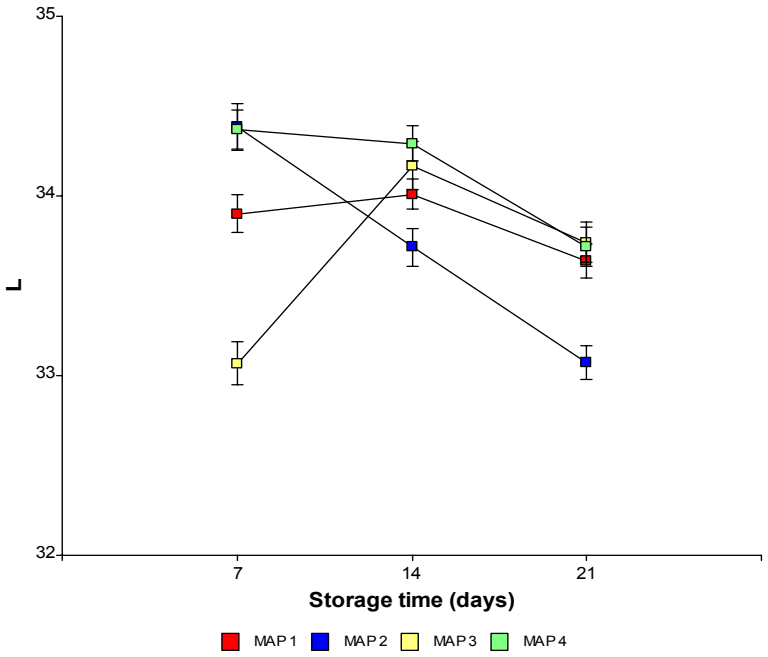
In addition, it seems that CO_2 content in MAP 4 diminished the magnitude of chlorophyll breakdown in the pigeon pea pods. However, after 14 days, became positive, similar to the other film packages. This is in accordance with Wills *et al.* (1982) who mentioned that high CO_2 content can diminish the chlorophyll breakdown. Maybe higher values of CO_2 can reduce the chlorophyll breakdown of pigeon pea at low temperature but the presence of off-odor could increase, as happened in MAP 4. The shelf-life of pigeon pea at 0 °C was extended to 14 days with MAP1 and MAP 2, instead of less than 7 days in air at 0 °C.

A significant interaction of "L" values of the pod was observed between temperature and atmosphere, as is shown in Figs. 20A and 20B. At 0 °C, MAP 3 showed a significantly lower "L" value of the pod than the rest of the treatments, with the exception of MAP 1 at 0 and 20 °C. At 20 °C the "L" value showed a significantly higher mean under all film types (color became

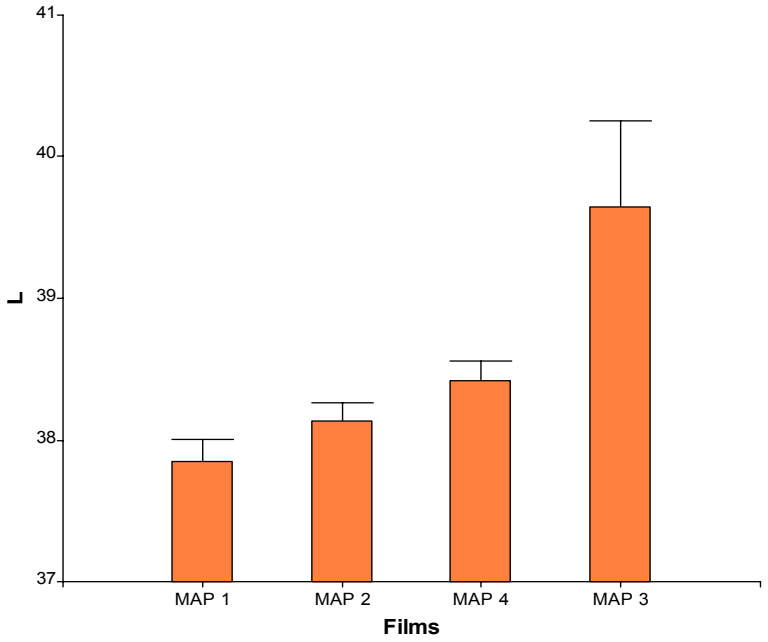
lighter). In addition, the "L" value of the seed increased significantly (the color became lighter) as the temperature of storage increased to 20 °C, regardless of the film type. No interaction was observed between atmosphere and temperature in the seed.

On the other hand, the "a" value of the pod increased significantly at 20 °C in MAP 3 and MAP 4, however, no significant differences were observed between 0 and 20 °C of storage in MAP 1 and MAP 2 (Fig. 21A). Similarly to pod behavior, the "a" value of the seed was significantly higher at 20 °C in MAP 3 than the rest of the treatments (less green) (Fig. 21B). Moreover, the "b" value of the pod decreased significantly at 20 °C in MAP 1, MAP 2 and MAP 4 than at 0 °C. No significant differences were found between MAP 3 at 0 and 20 °C (Figs. 22A and 22B). Perhaps, the increasing "a" value of the pod and the trend to decrease "a" value of the seed at 20 °C in the film packages can be attributed to high % CO_2 inside this bag, which reduce the degradation of chlorophyll in the pod in comparison with the storage of pigeon peas in air at 20 °C, and enhanced the color of the seed, with exception of MAP 3. Lebermann (1968a) and Groeschel *et al.* (1966) also found that an increase of CO_2 is important to avoid chlorophyll degradation. However, increases in MAP 4 and MAP 3 were accompanied by extensive off-odor and decay. In this particular case, color was not an indicator of pigeon pea quality. No off-odor was found in MAP 2 and MAP 1. Both packages can also work under temperature abuse. Other color values, such as "L" and "b" increased at 20 °C, but not as expected, because the changes were not as extreme as observed in the postharvest experiments, potentially due to the high CO_2.

(A)



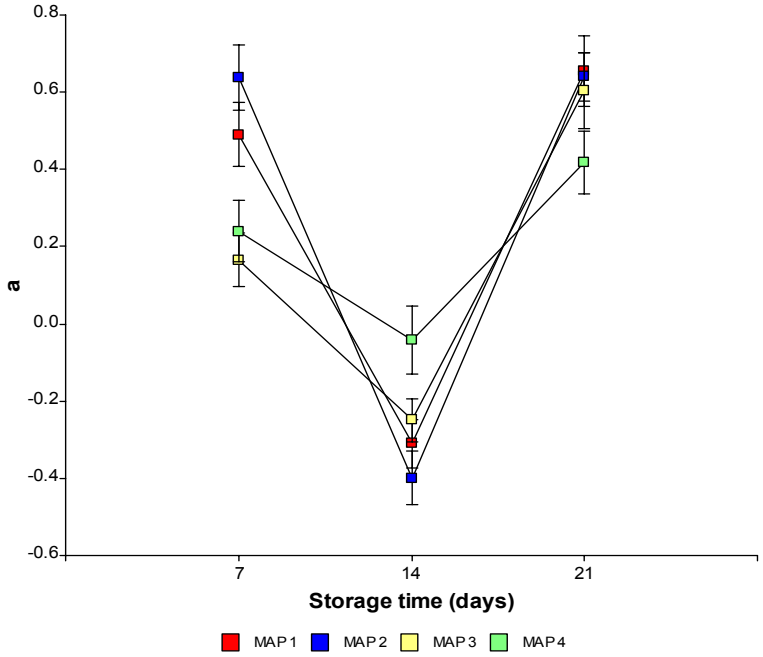
(B)



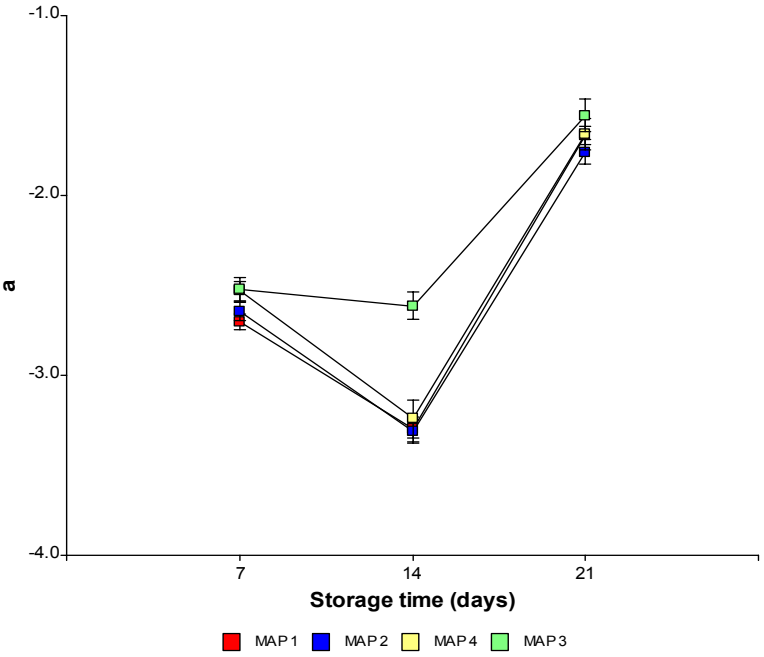
MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 17. Changes in "L" value of pigeon peas packaged in different film types at 0 °C for 21 days of storage (A) pod and (B) seed.

(A)



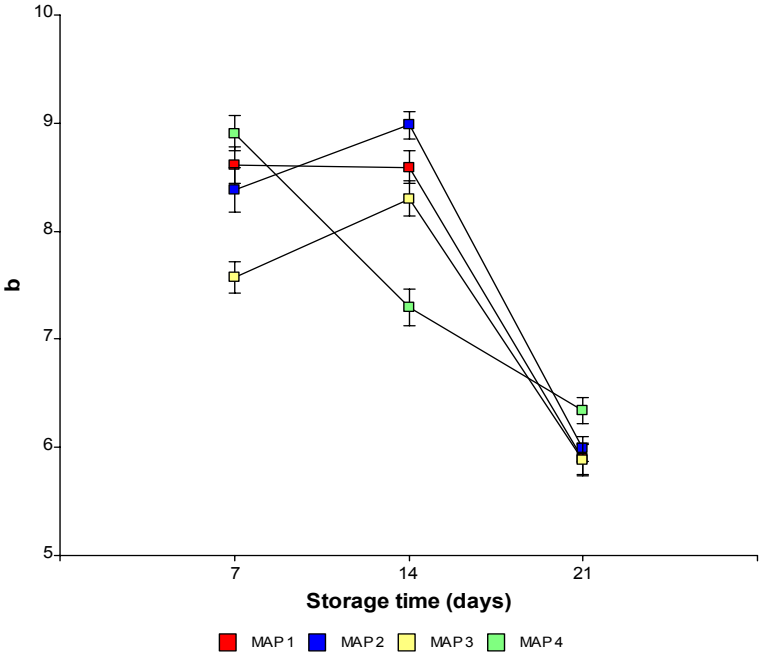
(B)



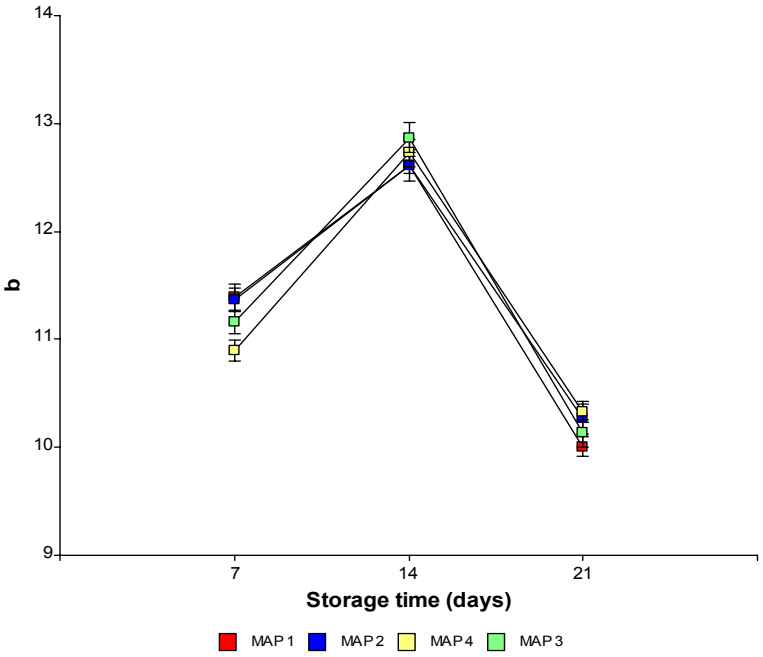
MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 18. Change in "a" value of pigeon peas packaged in different film types at 0 °C for 21 days of storage (A) pod and (B) seed.

(A)



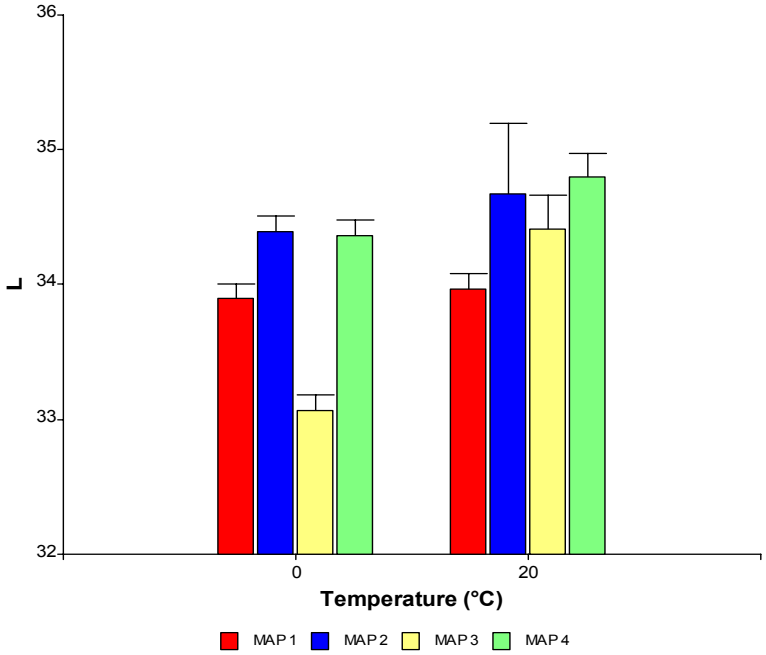
(B)



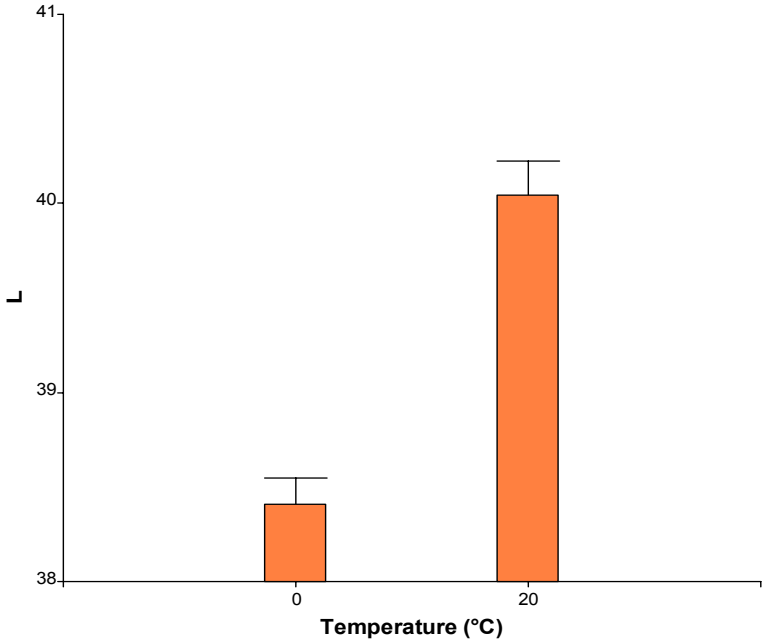
MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 19. Changes in "b" value of pigeon peas packaged in different film types at 0 °C for 21 days of storage (A) pod and (B) seed.

(A)



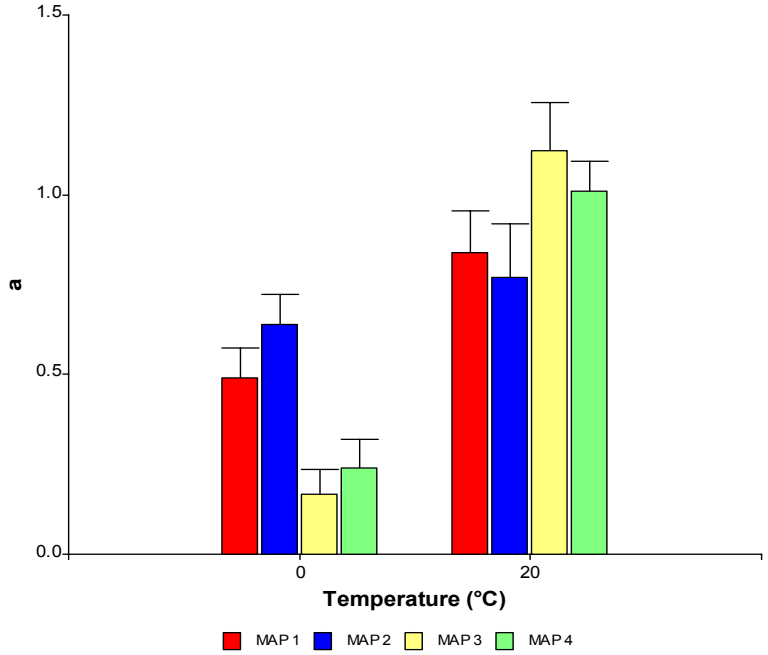
(B)



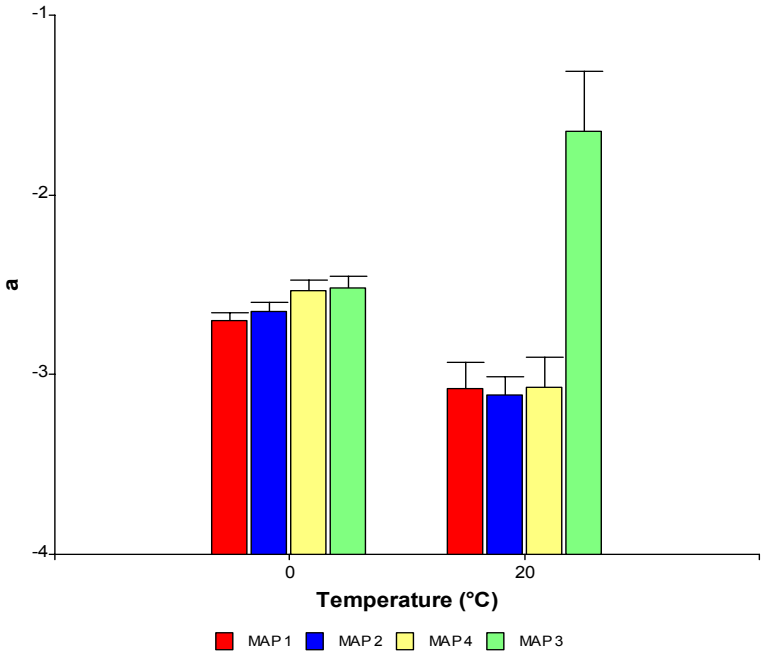
MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 20. Changes in "L" value of pigeon peas packaged in different film types at 0 and 20 °C for 7 days of storage (A) pod and (B) seed.

(A)



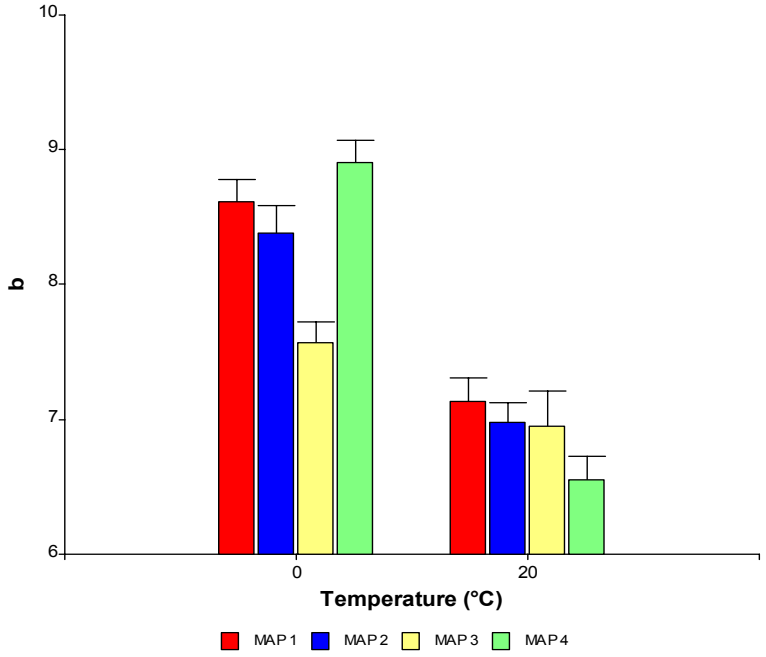
(B)



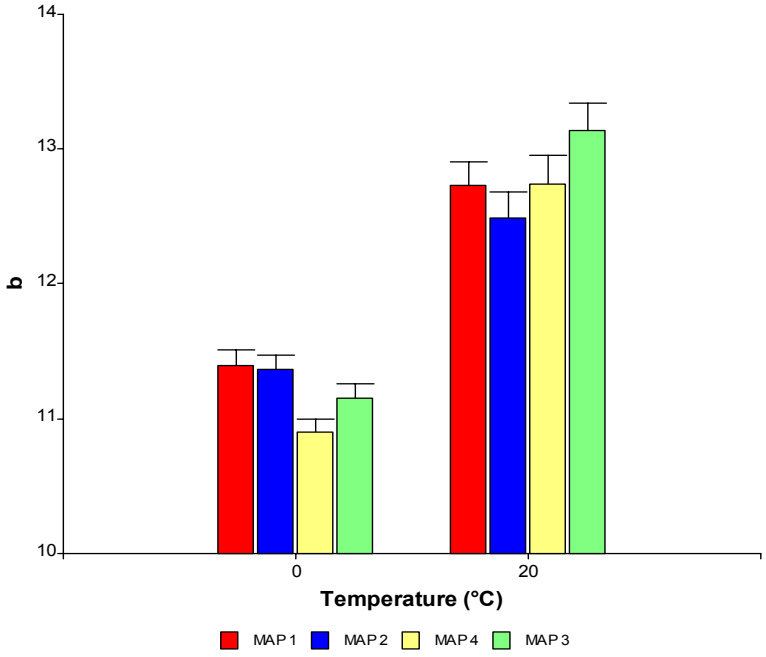
MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 21. Changes in "a" value of pigeon peas packaged in different film types at 0 and 20 °C for 7 days of storage (A) pod and (B) seed.

(A)



(B)



MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 22. Changes in "b" value of pigeon peas packaged in different film types at 0 and 20 °C for 7 days of storage (A) pod and (B) seed.

Visual quality and off-odor

The yellowing of pigeon pea pods was significantly higher after 21 days of storage under all film packages and also rapidly increased in MAP 2 after 14 days of storage, with slight to moderate score ($p \leq 0.05$). Similarly, the yellowing of the seed increased with the storage time and showed significantly higher values after 21 days of storage with slight to moderate scores regardless of the film type.

In addition, the shriveling value of the pods increased after 21 days under all film packages, with no significant differences among them and with MAP 4 after 14 days of storage, with scores between slight to moderate ($p \geq 0.05$) (Table 19). Likewise, the shriveling of the seed was significantly higher in MAP 3 and MAP 1 after 14 days of storage than the rest of the treatments. Also, no mycelial growth was observed in the pods and seeds of pigeon peas under all film types stored at 0 °C. This indicated that pigeon pea pods and seeds mostly retained their fresh appearance at 0 °C, which denotes only slight product shriveling, hardly detectable by visual inspection, especially in the seed. This was probably due to the low respiration rate and protective role of the film packaging, and also at this temperature the mold growth could be inhibited depending on the type of mold (Sommer, 1992).

At 20 °C storage, the yellowing value of the pod significantly increased regardless of the film type with moderate to severe scores. No interactions were found between film packages and temperatures of storage. In contrast, significant interactions were observed in the yellowing of the seed in MAP 3 at 20 °C with moderate to severe scores. Similarly to pod yellowing, the

yellowing of the seed increased significantly with an increase in temperature. Probably the increase of yellowing in the pods was due to the increase of temperature that accelerates the senescence of pigeon peas. A much greater loss of chlorophyll (close to severe) was observed in the air stored samples at this temperature, as was shown in the postharvest results. It seems that the content of CO_2 inside the bags was responsible for the changes. This concurs with the findings of Shewfelt (2003), who indicated that yellow pigments appear with senescence and also, Groeschel *et al.* (1966), who mentioned that chlorophyll shows a greater loss in air stored samples than in modified atmospheres.

MAP 1 at 20 °C showed significantly lower pod shriveling than the rest of the treatments, with none to slight score ($p \leq 0.05$) (Table 20). This behavior can be attributed to the protective role of the film packaging. In contrast, the shriveling of the seed did not show significant differences among the film types at 20 °C of storage, with none to slight scores, due to the protective role of the pod. Decay at 20 °C was significantly higher in MAP 3 and MAP 4 than in MAP 2 and MAP 1 ($p \leq 0.05$). In addition, a similar tendency was found in seed decay with significant higher values in MAP 4 and MAP 3, with none to slight scores ($p \leq 0.05$).

Off-odor had a significant interaction between treatments and time at 0 °C of storage. MAP 1 and MAP 2 had no detected off -odor at 7 and 14 days of storage, the off-odor was recognized after 21 days of storage, with moderate scores. In contrast, MAP 3 and MAP 4 rapidly develop off-odor, with significantly moderate scores at 7 and 14 days of storage, with significantly severe off-odor at 21 days of storage at 0 °C ($p \leq 0.05$).

Likewise, significant interaction was found between temperature and the film types. At 20 °C the off-odor was significantly higher in MAP 3 and MAP 4, with severe score ($p \leq 0.05$). However, in MAP 1 and MAP 2 the slightly recognition of off-odor had no significant different with MAP 1 and MAP 2 at 0 °C ($p \geq 0.05$).

Table 19. Visual quality and off-odor changes in pigeon peas packaged in different film types at 0 °C for 7, 14 and 21 days of storage.

	Pod		Seed		Off-odor
	Yellowing	Shriveling	Yellowing	Shriveling	
MAP (A)					
MAP 1	2.6 a	2.2 a	2.4 ab	1.2 b	1.4 a
MAP 2	2.9 b	2.0 a	2.2 a	1.0 a	1.7 a
MAP 3	2.8 ab	2.2 a	2.6 b	1.1 b	3.3 b
MAP 4	2.7 ab	2.2 a	2.5 b	1.0 a	3.1 b
Storage time (B)					
7 days	2.4 a	1.9 a	2.1 a	1.0 a	2.0 a
14 days	2.6 b	2.1 b	2.3 b	1.2 b	2.0 a
21 days	3.3 c	2.4 c	2.9 c	1.0 a	3.0 b
Significance					
A	*	*	*	*	*
B	*	*	*	*	*
AxB	*	*	n.s	*	*

* $p \leq 0.05$, n.s = non significant Means with the same letter are not statistically significant $p \geq 0.05$.

Table 20. Visual quality and off-odor changes in pigeon peas packaged in different film types at 0 and 20 °C for 7 days of storage.

	Pod			Seed			Off-odor
	Yellowing	Shriveling	Decay	Yellowing	Shriveling	Decay	
MAP (A)							
MAP 1	2.8 a	1.6 a	1.9 a	2.2 ab	1.9 a	1.0 a	1.3 a
MAP 2	2.9 a	2.0 bc	1.6 a	2.1 a	1.9 a	1.0 a	1.3 a
MAP 3	3.0 a	2.2 c	2.8 b	2.9 c	2.1 a	1.4 ab	3.0 b
MAP 4	2.9 a	2.0 bc	3.3 b	2.4 b	2.0 a	1.5 b	3.0 b
Temperature (B)							
0 °C	2.4 a	1.9 a	-----	2.1 a	-----	-----	1.5 a
20 °C	3.4 b	2.0 b	-----	2.7 b	-----	-----	2.6 b
Significance							
A	n.s	*	*	*	n.s	*	*
B	*	*	-----	*	*	-----	*
AxB	n.s	*	-----	*	*	-----	*

* $p \leq 0.05$, n.s = non significant Means with the same letter are not statistically significant $p \geq 0.05$.

Microbiological analysis

Significant differences in fungal growth were found between treatments at 0 °C (Fig. 23). MAP 2 had a significantly higher fungal count than MAP 4 and MAP 3, with 2.56, 1.68 and 1.69 log CFU/g ($p \leq 0.05$) respectively. No significant differences were found among MAP 2 and MAP 1 ($p \geq 0.05$). Additionally, a significant increase in fungal growth was found at 14 days of storage regardless of the treatment ($p \leq 0.05$). No significant interaction was observed ($p \geq 0.05$). Furthermore, the simple effect of *Lactobacillus* growth in MAP 4, MAP 3 and MAP 2 treatments stored at 0 °C, did not show significant differences between 7, 14 and 21 days of storage. However, MAP 1 showed a significant increase at 21 days of storage compared to 7 and 14 days of storage with 3.41 log CFU/g ($p \leq 0.05$). Also, regardless of storage time, MAP 3 showed a significantly higher *Lactobacillus* growth than MAP 4 and MAP 2 ($p \leq 0.05$), with the exception of MAP 1 ($p \geq 0.05$) (Fig. 24).

Probably, the reduced fungal and *Lactobacillus* counts found at 0 °C were due to the low storage temperature and maybe had little or no effect on quality losses. This is in accordance with Sommer (1992) and Krist *et al.* (2000), who indicated that as the temperature decreased from the optimal temperature the global growth of the microorganism is reducing because of the lag phase extended as a result of slow germination. Probably, in MAP 4 the effect of low temperature and high CO_2 had an additionally effect to extent the lag phase with a subsequent reduction of the log phase, resulting in the reduction of fungal growth. This result is in agreement with Al-Ati and Hotchkiss (2002) who mentioned that molds are obligate aerobic microorganisms and their growth is inhibited by CO_2 concentrations as low as 10%. Also, the

CO_2 concentration inside this bag was not enough to stimulate *Lactobacillus* growth at 0 °C. Batt (2000) similarly indicated that *Lactobacillus* growth is sometimes enhanced by 5% CO_2.

In the case of MAP 3 the fungal growth was reduced, in contrast the *Lactobacillus* growth increased. The reduction of fungal growth could not be attributed to the CO_2 content because of the concentration was low (1.2 %), maybe it was more related to low content of O_2. Probably low O_2 concentration restricted mold growth, allowing the increase of *Lactobacillus* growth. This statement is supported by the belief that MAP 3 had a low O_2 permeability.

In contrast, in MAP 2 and MAP 1 the fungal growth was higher, maybe due to the higher O_2 permeability of the bag and also the CO_2 content was not high enough for reducing fungal growth. Likewise, the differences in *Lactobacillus* growth pattern between both treatments could be attributed to the initial flushing of MAP 2 with 2 % O_2 that could stimulate *Lactobacillus* growth until the new equilibrium of O_2 was reached.

On the other hand, at 20 °C pigeon peas packaged in MAP 1 showed significantly lower fungal growth than MAP 2 with 2.00 log CFU/g and 3.40 log CFU/g, respectively ($p \leq 0.05$). As expected, fungal growth increased with an increase in temperature and reached 3.57 log CFU/g at 20 °C (Fig. 25). Similarly, *Lactobacillus* growth was significantly increased at 20 °C with 3.42 log CFU/g, regardless of the film type. No significant differences between film types were found regardless of the storage time ($p \geq 0.05$). In addition, no significant interactions were observed between temperature and atmosphere for *Lactobacillus* and fungal growth ($p \geq 0.05$) (Fig. 26).

As expected, fungal and *Lactobacillus* growth increased with increasing temperature possibly due to the increase of the metabolic rate of the microorganism. Krist *et al*. (2000) also indicated that the growth rate of microorganisms increase with an increase in temperature. The fungal and *Lactobacillus* growth were not so high, especially in MAP 3 and MAP 4, which had high CO_2 and presented off-odors. This could be because the pod is a natural barrier, which provided some protection to the seed from fungal and *Lactobacillus* attack, because extensive decay was observed on the pod surface. Sommer (1992) mentioned that fruit skins give protection against fungal attack, which also depends on favorable environmental conditions. Also, it could be possible that there was a competitive interaction with other spoilage microorganisms. It is believed that at high temperatures, CO_2 decreases its inhibitory action for gram negative bacteria such as *Erwinia*, which is the cause of the white soft rot characteristic of lima beans (Jay, 1996), and gives a soft and watery appearance to the pod and produces off-odors. Hao and Brackett (1994) found *Erwinia carotovora*, *Xanthomonas campestris* and *Pseudomonas fluorescens* in bell pepper at 25 °C in modified atmosphere packaging of 10% CO_2, 5%O_2 and 85%N_2.

In general, in MAP 3 the reduction of O_2 and the increase of CO_2 content could reduce the fungal count and prevent the *Lactobacillus* growth at 0 °C, but also this treatment adversely influenced the development of off-odor, caused by injurious CO_2 levels. For this reason, MAP 1 could be the best option that retards the growth of *Lactobacillus* after 21 days of storage (beyond its shelf-life) with little fungal growth and with no compromise of quality at 0 °C. Once it is transferred to temperature abuse, low fungal growth was found in the seed and also only slight decay was observed on the pod.

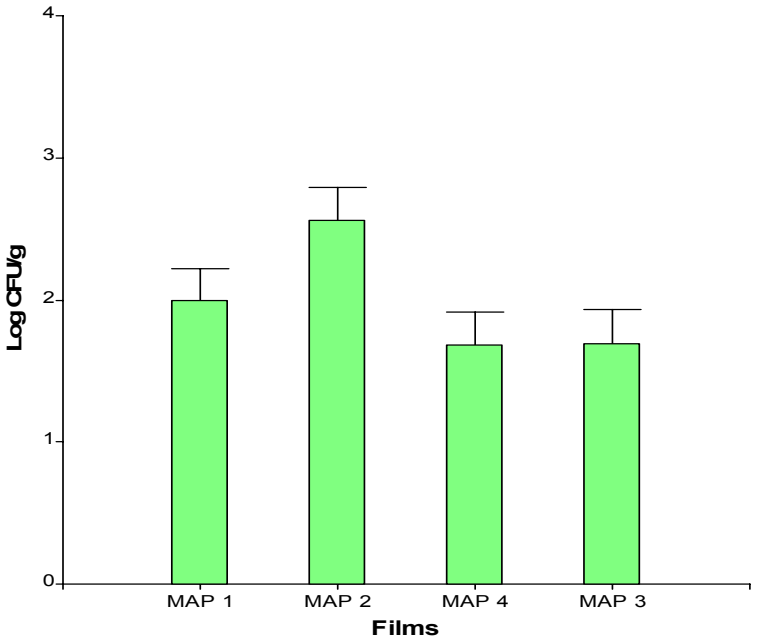
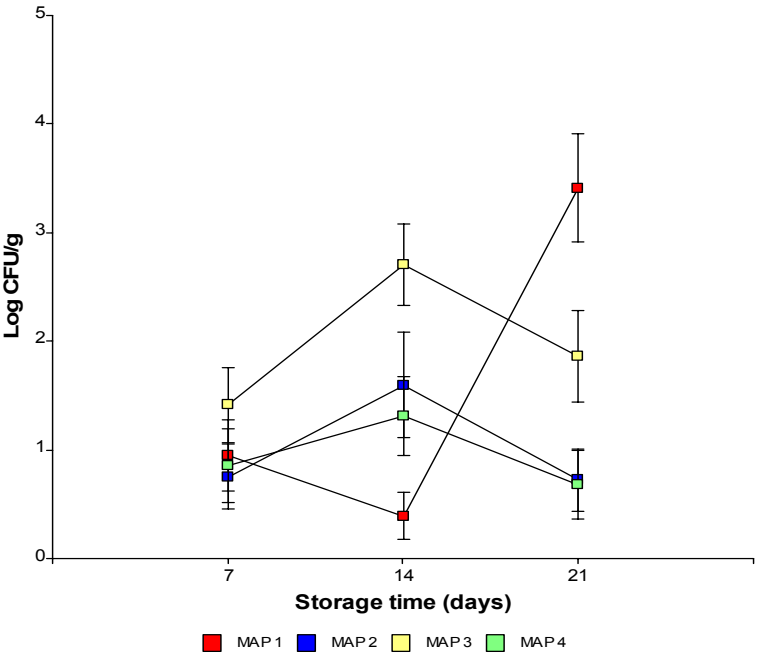


Figure 23. Changes in fungal growth for different film types during storage of 7, 14 and 21 days at 0 °C.



MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 24. Changes in *Lactobacillus* growth for different film types during storage at 7, 14 and 21 days at 0 °C.

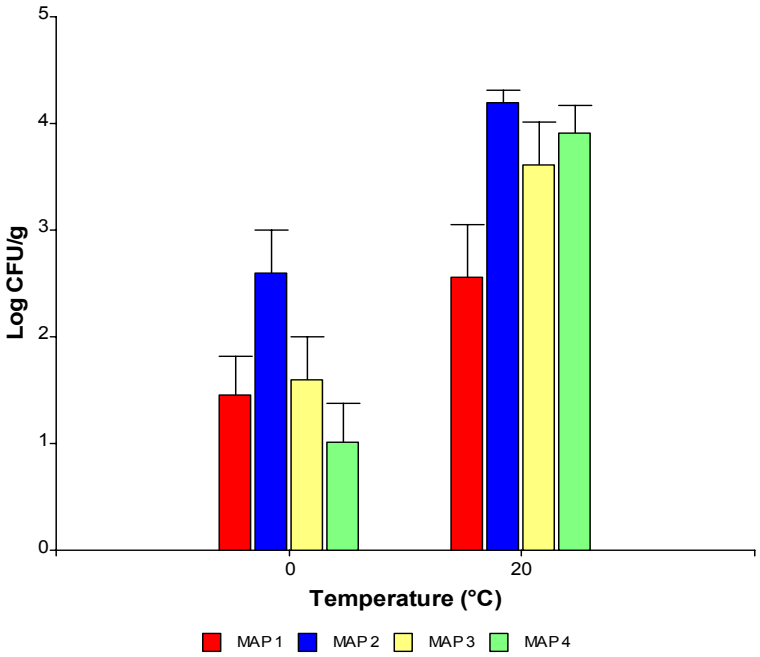
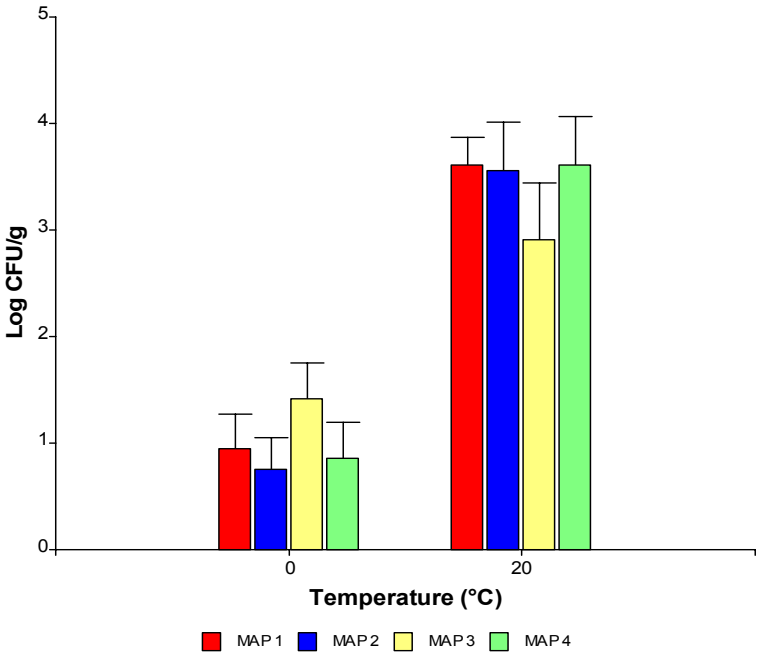


Figure 25. Changes in fungal growth for different film types stored at 0 and 20 °C for 7 days.



MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 26. Changes in *Lactobacillus* growth for different film types stored at 0 and 20 °C for 7 days.

Titratable acidity, soluble solids content and pH

Significant interactions were found for titratable acidity (TA) and pH of pigeon peas among film types and days at 0 °C, however only the film type had an effect in solid soluble content (SSC) (Figs. 27, 29 and 31). The TA of MAP 4 at 0 °C was significantly higher at 7 days than at 14 and 21 days of storage with no significant differences among them, with 0.19, 0.17 and 0.15 %, respectively. Likewise, this film presented a significantly higher percentage of SSC than other film types with 13.22 % at 0 °C, regardless of the storage time.

The % TA in MAP 1 and MAP 2 was not significantly different at 7, 14 and 21 days; however, there was a trend to increasing TA after 21 days in both film types at 0 °C. MAP 1, after 21 days of storage, had a significantly lower pH, with 6.33, than MAP 2 at 14 and 21 days of storage at 0 °C. This suggested that increases in TA and the lower pH observed after 21 days in MAP 1 probably was caused by *Lactobacillus* growth, which was high after 21 days. It could have produced lactic acid that would cause a decrease in pH and also an increase in TA, even though this was not significant. Aguayo-Gimenez (2003) found a similar occurrence in yellow melon stored at 5 °C for 14 days packaged in PPO + citric acid, which demonstrated high TA and reduction in pH caused by microorganisms like lactic acid bacteria.

The simple effect in MAP 3 at 0 °C showed a significantly higher TA at 7 days of storage than at 14 and 21 days with 0.18%, similar to MAP 4, and no significant differences in pH and SSC during the time of storage. However, the decrease in TA can be attributed to the respiratory process; therefore, no significant increase in SSC was observed. This result is in accord with Kays and Paull (2004), who mentioned that TA decreased with the storage time due to the

respiratory process. It seems that the chemical changes were not detrimental in MAP 3, MAP 2 and MAP 1 at 0 °C, even with the low pH reached after 21 days of storage. Likewise, the percentage of SSC in those films was similar to that of fresh pigeon peas, which is an important characteristic in the quality of pigeon peas.

At 20 °C a significant decrease in the percentages of TA was observed ($p \leq 0.05$). MAP 2 and MAP 3 at 20 °C had significantly lower TA than MAP 3 and MAP 4 at 0 °C (Fig. 28). Moreover, at 20 °C the pH of pigeon peas in MAP 3 was significantly lower than in MAP 2 at 20 °C, with 6.44 and 6.56, respectively. No significant differences were found with other treatments (Fig. 30). No significant interactions and main effects were observed in SSC of pigeon peas packaged at 0 and 20 °C ($p \geq 0.05$). Probably the decrease in TA at 20 °C in MAP 3 can be attributed to a decrease of organic acids caused by an increase in the respiration rate at 20 °C (Kays and Paull, 2004). However, in MAP 3, the decrease in TA was accompanied with a decrease rather than an increase in pH as in MAP 2. Perhaps, as was found in peas, the increase of internal CO_2 (10.1 % at 20 °C) was balanced with a decrease in malic acid and pH (Wager, 1974).

Texture

No significant main effects were found between film types ($p \geq 0.05$), however, a tendency toward increased hardness in MAP 4 was observed with 925.28 g of force. This is in agreement with Groeschel *et al.* (1966) who mentioned that the texture of green beans in atmospheres of 0 - 10 % CO_2 with 2 % O_2 stored at 7 °C remained nearly constant for two weeks.

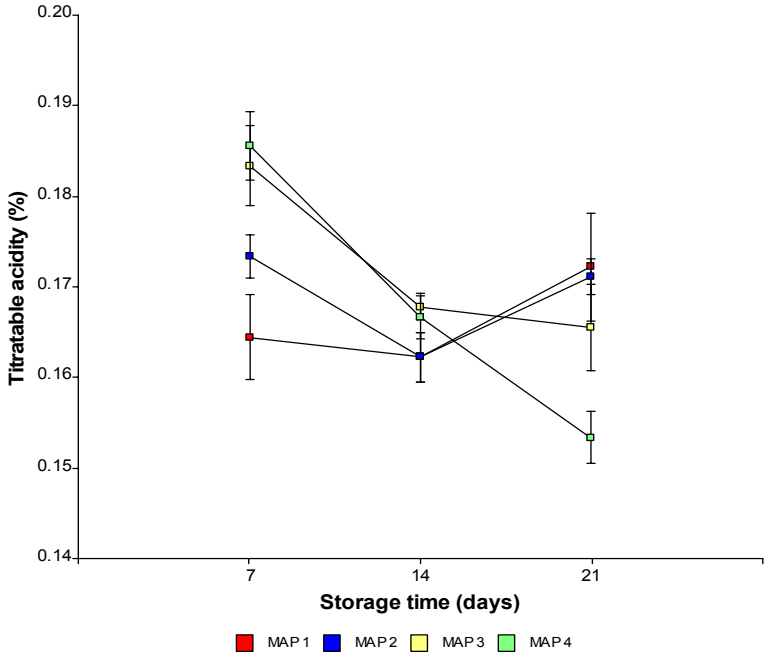
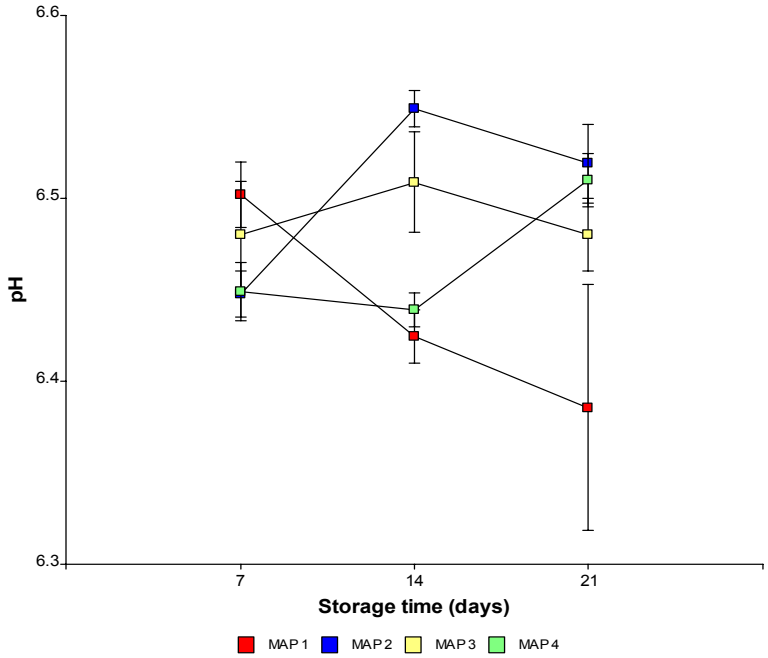
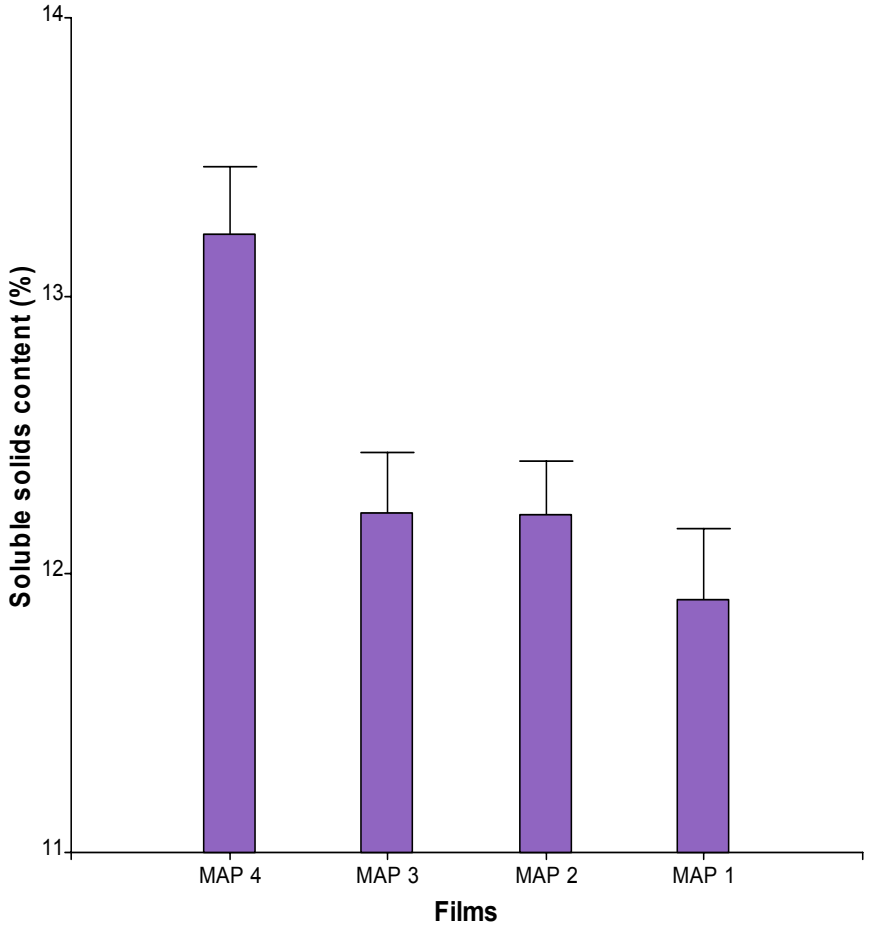


Figure 27. Changes in TA of pigeon pea packaged in different film types at 0 °C during 21 days.



MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 28. Changes in pH of pigeon pea packaged in different film types at 0 °C during 21 days.



MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 29. Changes in SSC of pigeon peas packaged in different film types at 0 °C.

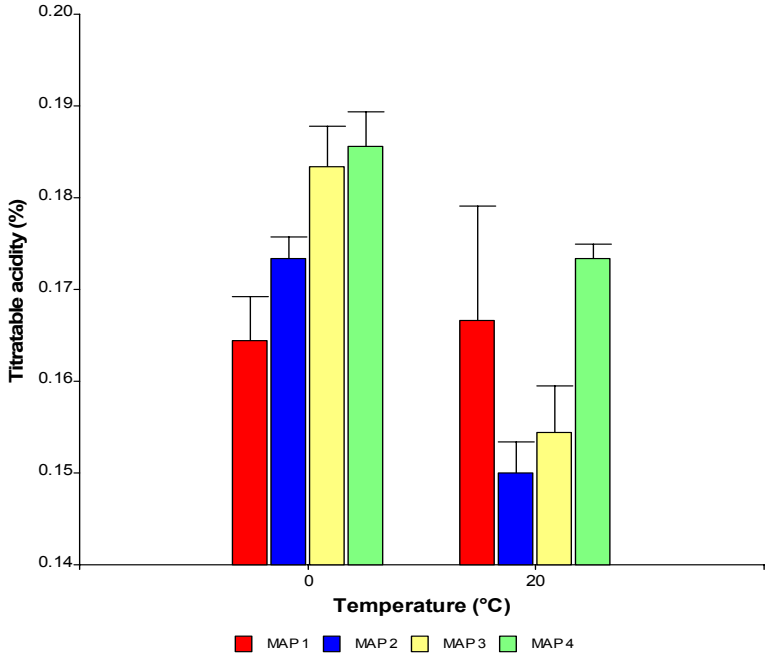
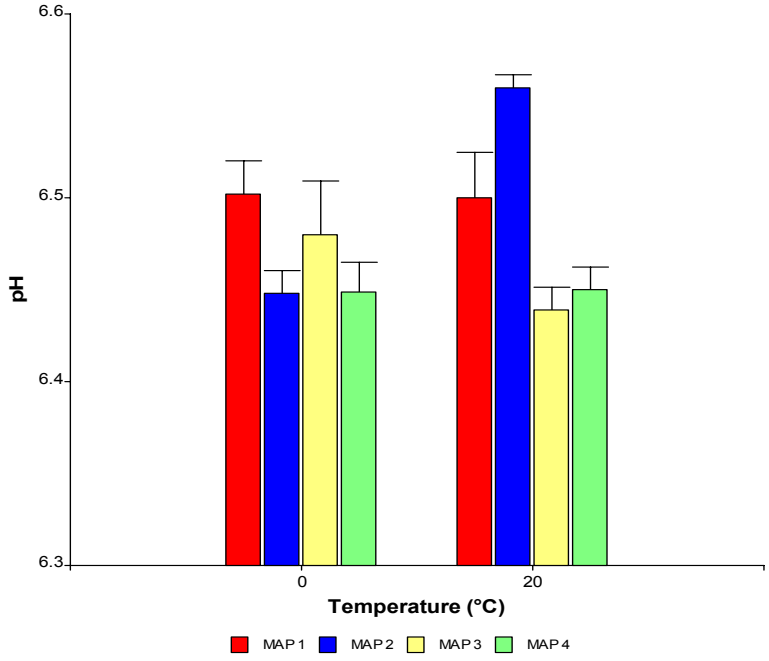


Figure 30. Changes in TA of pigeon pea packaged in different film types at 0 and 20 °C for 7 days.

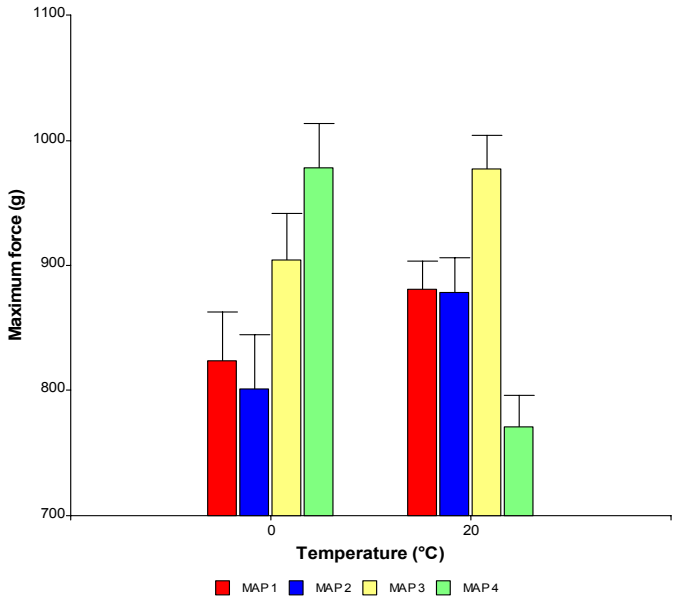


MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 31. Changes in pH of pigeon pea packaged in different film types at 0 and 20 °C for 7 days.

The effect of modified atmosphere packaging on vegetables depends on the commodity (Weichmann, 1986) in combination with the temperature of storage. Buescher and Henderson (1977) indicated the same tendency for green beans stored in air with 0-30% CO_2. However, other authors mentioned that MAP might be reduce the toughening in vegetables, such as asparagus, in atmospheres with 12 ± 2% CO_2 at 4 °C (Kader, 1986). In our experiment a tendency towards increased toughening with high CO_2 content at low temperatures was found. No significant interaction was observed between the film type and storage time ($p \geq 0.05$). Significant interactions were found between temperature of storage and film type ($p \leq 0.05$). All film types showed a pattern towards increased hardness at 20 °C with the exception of MAP 4, with significantly lower values of MAP 4 at 0 °C and MAP 3 at 20 °C (Fig. 32).

Probably this hardness of pigeon pea seeds was caused by lignification and accumulation of pectic substances on the cell wall, which was also accompanied with an increase in respiration and accumulation of CO_2 content between 4.8-10.1% CO_2 reached at 20 °C of storage (Smith *et al.*, 2003). However, it seems that a level of 12.4% CO_2, as found in MAP 4 decreased the hardness of the seed, maybe this concentration caused an increase of spoilage microorganism, such as *Erwinia* or *Pseudomonas* that increased their pectinase and off-odors (Jay, 1996). This result also agrees with Hao and Brackett (1994), who indicated that a MAP of 10% CO_2, 5% O_2 and 85% N_2 did not significantly reduce the pectinase activity of *Erwinia carotovora*, *Xanthomonas campestris* and *Pseudomonas fluorescens* in bell pepper at 25 °C of storage.



MAP 1 (PD 941 air) MAP 2 (PD 941 gas mix) MAP 3 (PD 961+ 6 holes) MAP 4 (PD 961 air)

Figure 32. Variation in texture of pigeon peas packaged in different film types at 0 and 20 °C for 7 days.

Shelf-life

An atmosphere of 2.3 % CO_2 at 0 °C, as in MAP 1 (PD 941 air headspace) was successful in extending the postharvest life of pigeon peas from less than 7 days in air storage to 14 days at 0 °C. Under temperature abuse, this film reached 6.0% CO_2 at 20 °C with no development of off-odors. The pigeon peas were also greener and tender with little decay after 7 days of storage than the other treatments as it shown in Table 21.

Table 21. Comparison of the most important characteristics in the MAP experiments.

MAP	Perm. CO_2	Perm. O_2	Overall quality at 0 °C	Off-Odor	Texture	Reduce chilling injury	Overall quality at 20 °C	Cost of production	Shelf-life (days)
MAP 1	31,000	16,500	Best	None	Tender	Yes	Best	Low	14
MAP 2	31,000	16,500	Good	None	Tender	Yes	Good	*High	14
MAP 3	Unknown	Unknown	Moderate	Severe	Tender	Yes	Bad	Low	7
MAP 4	21,000	7,000	Moderate	Severe	Hard	Yes	Bad	Low	7

*High cost of production due to the gas mixture, in comparison with the other bags.

Conclusions

1. Pigeon peas are classified as a highly perishable vegetable with a non-climacteric pattern of respiration.

2. At high storage temperatures such as 15 and 20 °C, the shelf-life of pigeon peas is limited by their high respiration rate, yellowing, decay and toughening of the seed. They reached a shelf-life of 9 and 7 days at 15 and 20 °C, respectively.

3. At 0 and 5 °C, the metabolic activity of pigeon peas was reduced and showed a good overall quality. However, the shelf-life was limited by the color of the pod due to chilling injury. At both temperatures, the shelf-life was less than 7 days based on pod color.

4. Pigeon peas stored in air at 10 °C retained the best overall quality with 13 days as their maximum shelf-life, however, 10 days are recommended.

5. Pigeon peas packaged in MAP 1 (PD 941 air headspace) at 0 °C with a 2.3% CO_2 retained the best overall quality. Their soluble solids content was similar to that of fresh pigeon peas. They did not experience excessive chlorophyll breakdown until 14 days of storage, in fact, they demonstrated enhanced coloring of the seed and also showed a low hardness during the storage time. The shelf-life of pigeon peas increased by 100 % (14 days) in comparison with storage in air at 0 °C.

6. MAP 1 also could be considered beneficial for the preservation of pigeon peas at 20 °C.

7. Pigeon peas packaged in MAP 4 (PD 961 air headspace) at 0 °C showed a high content of soluble solids, which was associated with the high CO_2. However, off-odors were detected in this package after 7 days of storage and severe off-odors occurred after 14 days of storage. The tendency towards increased hardness of the seed and increased yellowing reduced the shelf-life to 7 days.

8. Pigeon peas packaged in MAP 3 (PD 961 + 6 holes) at 0 °C had a shelf-life of only 7 days due to the extensive development of off-odors and high yellowing scores of the seeds.

9. Once pigeon peas were transferred to room temperature, their overall quality diminished, due to advanced senescence and water loss.

10. Soluble solids content (SSC), “a” values and texture are the most important parameters of quality in pigeon peas.

11. At 0 °C storage, fungal and *Lactobacillus* growth are not a quality concern.

Recommendations

1. To develop a modified atmosphere packaging system with PD941 at 10 °C.

2. To develop a sensory analysis with pigeon peas packaged in MAP 1.

3. To develop a modified atmosphere packaging system with pigeon peas seeds at 0 and 10 °C.

References

Abbott, J. 2004. Textural quality assessment for fresh fruits and vegetables. In: Shahidi, F., A. Spainer, Ch. Ho and T. Braggins (eds.). Quality of fresh and processed foods, pp. 265-276. Kluwer Academic / Plenum Publishers, New York, NY.

Acosta, N., A. Armstrong, L. Almodovar, A. Morales, J. Reyes, E. Gonzales, E. Schroder, J. A. Negron and A. Bosques. 1992. Conjunto Tecnológico para la producción de gandules. Est. Exp. Agr. Univ. de Puerto Rico, PR, Pub. 116.

Aguayo-Giménez, E. 2003. Innovaciones tecnológicas en la conservación de melón y tomate procesado en fresco. Tesis Doctoral. Universidad Politécnica de Cartagena, Cartagena, España., 399 pp.

Al-Ati, T. and J. Hotchkiss. 2002. Application of packaging and modified atmosphere to fresh cut fruits and vegetables. In: Lamikanra, O (ed.) Fresh-cut fruits and vegetables: science, technology and market. CRC Press. Boca Raton. FL.

Alemañy, A. 2001. Determinación de los factores que contribuyen al enegrecimiento del gandul refrigerado desgranado a maquina. Tesis M.Sc. Universidad de Puerto Rico, Mayagüez, P. R., 77 pp.

Barron, C., P. Varoquaux, S. Guilbert, N. Gontard and B. Gouble. 2002. Modified atmosphere packaging of cultivated mushroom (*Agaricus bisporus* L.) with hydrophilic films. J. Food Sci. 67: 251 – 255.

Batrash, S., J. Makhlouf, F. Castaigne and C. Willemot. 1993. Optimal controlled atmosphere conditions fro storage of Broccoli Florets. J. Food Sci. 58 (2): 338 – 342.

Batt, C. 2000. *Lactobacillus*. In: Robinson, R., C. Batt and P. Patel (eds.). Encyclopedia of food microbiology, pp. 1134- 1144. Academic Press. San Diego, CA.

Ben-Yehoshua, S. and V. Rodov. 2003. Transpiration and water stress. In: Bartz, J and J. Brecht (eds.). Postharvst physiology and pathology of vegetables. Second edition. Revised and expanded. Marcel Dekker, Inc. New York, NY.

Bidlack, J.E., S.C. Rao, and D.H. Demezas. 2001. Nodulation, nitrogenase activity, and dry weight of chickpea and pigeon pea cultivars using different *Bradyrhizobium* strains. J. Plant Nutr. 24:549-560.

Bourne, M. 1978. Texture profile analysis. Food Technol 32:62-66.

Bourne, M. 1980. Texture evaluations of horticultural crops. HortScience 15: 51 - 57.

Brandis- Heep, A. 2000. Metabolic pathways / Release of energy aerobic. In: Robinson, R., C. Batt and P. Patel (eds.). Encyclopedia of food microbiology, pp. 1272 - 1279. Academic Press. San Diego, CA.

Buescher, R. W. and J. Henderson. 1977. Reducing discoloration and quality deterioration in snap beans (*Phaseolus vulgaris*) by atmosphere enriched with CO_2. Acta Hort. 62: 55 - 60.

Charles, F., J. Sanchez and N. Gontard. 2005. Modeling of active modified atmosperepackaging of endives exposed to several postharvest temperatures. J. Food Sci. 70: E443 – E449.

Church, I., and A. Parsons. 1995. Modified atmosphere packaging technology: Review. J. Sci. Food Agric. 67:143-152.

deMan, J. 1999. Principles of food chemistry. Third edition. Aspen Publisher, Inc. Gaithersburg, Maryland, 520pp.

Departamento de agricultura del estado libre asociado de Puerto Rico. 2004. Estadísticas de consumo. http://www.agricultura.gobierno.pr.

Durairaj, C. and N. Ganapathy. 2000. Yield losses caused by blister beetle, *Mylabris* sp. (Coleoptera:Meloidae) in pigeon pea and its economic injury level in cowpea. Trop. Agric. 77:133-136.

Eaks, I. and L. L. Morris. 1956. Respiration of cucumber fruits associated with physiological injury at chilling temperatures. Plant Physiol. 33: 308-314.

Echavez , R. and A. Bosques. 1998. Reaction of new long-day pigeon pea genotypes to rust (*Uredo cajani*). J. Agric. Univ. Puerto Rico 82: 201-208.

Exama , A., J. Arul, R.W. Lencki, L.Z. Leee and C. Toupin. 1993. Suitable of plastic films for modified atmosphere packaging of fruits and vegetables. J. Food Sci. 58: 1365 - 1370

Faber, J. M. 1991. Microbiological aspects of modified atmosphere packaging technology- A review. J. Food. Prot. 54: 58-70.

FDA / CFSAN. 2001. Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh- cut produce. In: Analysis and Evaluation of Preventive Control Measure for the Control and Reduction/Elimination of Microbial Hazard on Fresh and Fresh – Cut Produce. http://www.fda.gov.

Fife, J.M., and V.L. Frampton. 1935. The effect of carbon dioxide upon the pH and certain nitrogen fractions of the sugar beet plant. J. Biol. Chem. 109: 643.

Freeman, D.W. and W.A. Sistrunk. 1978. Effects of postharvest storage on the quality of canned snap beans. J. Food Sci. 43: 211 - 214.

Gnanasekharan, V., R. Shewfelt and M. Chinnan. 1992. Detection of color changes in green vegetables. J. Food Sci. 57: 149 – 154.

Gonzales-Aguilar, G. A., J. G. Buta and C. Y.Wang. 2001. Methyl jasmonate reduces chilling injury symptoms and enhances color development of 'Kent' mangoes. J. Sci. Food Agri. 81: 1244 – 1249.

Groeschel, E. C., A. I. Nelson, and M.P. Steinberg. 1966. Changes in color and other characteristics in green beans stored in controlled refrigerated atmospheres. J. Food Sci. 3: 488-496.

Hao, Y. Y. and R.E. Brackett. 1994. Pectinase activity of vegetable spoilage bacteria in modified atmosphere. J. Food Sci. 59: 175 – 177.

Harker, F. R., J. Redgwell and I. C. Hallet. 1997. Texture of fresh fruit. Hort. Rev. 20: 1221 – 224.

Hernandez, K. 2002. Efectos de biorreguladores en la florecida de cultivares de gandul (*Cajanus cajan* (L) Huth) sensitivos e insensitivos al fotoperiodo. Tesis M.S. Universidad de Puerto Rico, Mayagüez, P.R., 86 pp.

Jay, J. 1996. Modern food microbiology. 5th Edition. 661 pp, Chapman and Hall, New York, NY.

Jayathunge, L. and Ch. Illeperuma. 2005. Extension of postharvest life of oyster mushroom by modified atmosphere packaging technique. J. Food Sci. 70: E573 – E578.

Kader, A. 1986. Biochemical and physiological basis for effect of controlled and modified atmospheres on fruits and vegetables. Food Technol 40: 99 – 105.

Kader, A. 1992. Modified atmosphere during transport and storage. In: A.A. Kader (eds) Postharvest technology of horticulture crops, pp 85 – 93. University of California Division of Agriculture and Natural Resources, Oakland, CA.

Kader, A. and M. E. Saltveit. 2003a. Respiration and gas exchange. In: Bartz, J and J. Brecht (eds.). Postharvst physiology and pathology of vegetables. Second edition. Revised and expanded. Marcel Dekker, Inc. New York, NY.

Kader, A. and M. Saltveit. 2003b. Atmosphere modification. In: Bartz, J and J. Brecht (eds.). Postharvst physiology and pathology of vegetables. Second edition. Revised and expanded. Marcel Dekker, Inc. New York, NY.

Kader, A., W. J. Lipton, and L.L. Morris. 1973. System for scoring quality of harvested lettuce. HortScience 8: 408-409.

Kays, J. S. 1991.Postharvest physiology of perishable plant products. 532 pp, Van Nostrand and Reinhold. New York, NY.

Kays, J. S. and R.E. Paull. 2004. Postharvest biology. 568 pp, Exon Press, Athens, GA.

Khattra S., A. Kaur, and G. Sing. 2000. Visual indicators of physiological maturity in pigeon pea [*Cajanus cajan* (L.) Millsp.]. Trop. Agric. 77:126-129.

Krist, K., T. Ross and D. S. Nichols. 2000. Ecology of bacteria and fungi in foods. In: Robinson, R., C. Batt and P. Patel (eds.). Encyclopedia of food microbiology, pp. 539 - 563. Academic Press. San Diego, CA.

Lamikanra, O., J.C. Chen, D. Banks and P.A. Hunter. 2000. Biochemical and microbial changes during the storge of minimally processed cantaloupe. J. Agric. Food. Chem. 48: 5995 – 5961.

Lebermann, A. 1968a. Post- harvest changes of broccoli stored in modified atmosphere. 1. Respiration of shoots and color of flower heads. Food Technol 22: 487 - 518.

Lebermann, A. 1968b. Post- harvest changes of broccoli stored in modified atmosphere. 2. Acidity and its influence on texture and chlorophyll retention of the stalks. Food Technol 22: 487 – 518.

Maguire, K., H. T. Sabarez and D. J. Tanner. 2004. Postharvest preservation and storage. In: Hui, Y., S. Ghazala, D. Graham, K. D. Murrell and N. Wai-Kit (eds.). Handbook of vegetable preservation and processing, pp. 39-65. Marcel Dekker, Inc. New York, NY.

Mannapperuma, J. and P. Singh. 1994. Modeling of gas exchange in polymeric packages of fresh fruits and vegetables. In: Singh, P. and F. Oliveira (eds.). Minimal processing of foods and process optimization. An interface, 437 – 457 pp. CRC Press Inc. Boca Raton, FL.

Mazza G., and D. Jayas. 2001. Controlled and modified atmosphere storage In: Eskin R., and D. Robinson (eds). Food Shelf Stability. Chemical, Biochemical and Microbiological Changes. pp. 149-167, CRC Press. Boca Raton. FL.

McColloch, L. P. 1962. Chilling injury and alternaria rot of bell peppers. U. S. Dep. Agr. Mktg. Res. Rpt. 536.

Medlicott, A. P., J. M. Sigrist and O.Sy. 1990. Ripening of mangoes following low temperature storage. HortScience 115: 430 - 434.

Mekwatanakam, W. 1998. Modified atmosphere packaging of vegetables. Thesis PhD Oregon State University, Oregon, USA. 178 pp.

Mohammed, M. and J. Brecht. 2003. Inmature fruit vegetables. In: Bartz, J and J. Brecht (eds.). Postharvst physiology and pathology of vegetables. Second edition. Revised and expanded. Marcel Dekker, Inc. New York, NY.

Morris, L.L. 1982. Chilling injury of horticulture crops: An overview. HortScience 17 (2): 161 – 162.

Morton, J.F. 1976. The pigeon pea (*Cajanus cajan* Millsp.), a high-protein, tropical legume. HortScience 11:11-19.

Nene, Y.L., J. Kannaiyan and M. V. Reddy. 1981. Pigeonpea diseases resisitence-screening techniques. ICRISAT. 9: 1-14.

Ontai L., R. Paull, and M. Saltveit Jr. 1992. Controlled atmosphere storage of sugar peas. HortScience 27:39-41.

Pariasca, J., T. Miyazaki, H. Hisaka, H. Nakagawa and T. Sato. 2000. Effect of modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage on the quality of snow pea pods (*Pisum sativum* L. var. *saccharatum*). Postharvest Biol. Technol. 21: 213 – 223.

Prussia, S. E., R. L. Shewfelt, M. S. Chinnan and R.B. Beverly. 1990. Moisture loss effects on southern pea color. Transactions of the ASAE 35: 1633 – 1636.

Rakotonirainy, A. M., Q. Wang, and G. W. Padua. 2001. Evaluation of zein films as modified atmosphere packaging for fresh broccoli. J. Food Sci. 66:1108-1111.

Sanz, C, A., Perez, R. Olias and J. Olias. 1999. Quality of strawberries packaged with perforated polypropylene. J. Food Sci. 64: 748 - 752.

Serrano, M., M. Martinez-Madrid, M. Pretil, F. Riquelme, and F. Romojaro. 1997. Modified atmosphere packaging minimizes increases in putrescine and abscisic acid levels caused by chilling injury in pepper fruit. J. Agr. Food Chem. 45:1668-1672.

Shewfelt, R. 1994. Quality characteristics of fruits and vegetables. In: Singh, P. and F. Oliveira (eds.). Minimal processing of foods and process optimization. An interface, pp. 437 - 457. CRC Press Inc.

Shewfelt, R. L. 1986. Postharvest treatment for extending the shelf-life of fruits and vegetables. Food Technol 40:70-79.

Shewfelt, R. L. 2003. Color. In: Bartz, J. and J. Brecht (eds.). Postharvst physiology and pathology of vegetables. Second edition. Revised and expanded. Marcel Dekker, Inc. New York, NY.

Sims, Ch. and R. Golaszeweski. 2003. Vegetable flavor and changes during postharvest storage. In: Bartz, J and J. Brecht (eds.). Postharvst physiology and pathology of vegetables. Second edition. Revised and expanded. Marcel Dekker, Inc. New York, NY.

Singh, D., J. Wang and K. Salunkhe. 1972. Controlled atmosphere storage of lettuce. 2. Effects on biochemical composition of the leaves. J. Food Sci. 52: 52 - 55.

Singh, U., P. Rao, N. Subrahmanyam, and K. Saxena. 1993. Cooking characteristics, chemical composition and protein quality of newly developed genotypes of pigeon pea [*Cajanus cajan* (L.) Millsp.]. J. Sci. Food Agri. 61:395-400.

Singh, U., R. Jambunathan and A. Narayanan. 1980. Biochemical changes in developing seeds of pigeonpea (*Cajanus cajan*). Phytochemistry. 19: 1291 – 1295.

Sinha, S.K. 1977. Food legumes: distribution, adaptability and biology of yield. In: FAO plant production and protection paper 3. pp. 1-102. FAO, Rome.

Skog, L. J. 1996. Prevention of chilling injury of nectarines by controlled atmospheres storage and high temperature conditioning. Thesis M.Sc The University of Guelph, Canada. 198 pp.

Smith, A., K. Waldron, N. Maness and P. Perkins-Veazie. 2003. Vegetable texture: measurement and structural implications. In: Bartz, J and J. Brecht (eds.). Postharvst physiology and pathology of vegetables. Second edition. Revised and expanded. Marcel Dekker, Inc. New York, NY.

Smock, R. M. 1961. Methods of scald control on the apple. Cornell Univ. Agr. Expt. Sta., Itaca, NY Bul. 970.

Sommer, N. 1992. Principles of diseases suppression by handling practices. In: In: A.A. Kader (eds.). Postharvest technology of horticulture crops, pp 109 – 116. University of California Division of Agriculture and Natural Resources, Oakland, CA.

Talasila, P., K. Chau and J. Brecht. 1995. Design of rigid modified atmosphere packages for fresh fruits and vegetables. J. Food Sci. 60: 758 – 769.

Tasneem, A. 2004. Postharvest treatments to reduce chilling injury symptoms in stored mangoes. Thesis M. Sc. McGill University. Canada. 105pp.

Tewfik, S. and L. E. Scott. 1954. Respiration of vegetables affected by post-harvest treatment. J. Agr. Chem. 2: 415-417.

Trail, M. A., A. I. Wahem and J. Bizri. 1992. Snap bean quality changed minimally when stored in low density polyolefin film package. J. Food Sci. 57 (4): 977 – 979.

Valenzuela, H. and J. Smith. 2002. Pigeonpea. http://www.ctahr.hawaii.edu

Viña, S and A. Chaves. 2003. Textura changes in fresh cut celery during refrigerated storage. J. Sci. Food Agri. 83: 1308 - 1314.

Wager, H. G. 1974. The effect of subjecting peas to air enriched with carbon dioxide. I. The path of gaseous diffusion, the content of CO_2 and the buffering of the tissue. J. exp. Bot. 25: 330 - 337.

Wang, C. Y. 1982. Physiological and biochemical responses of plants to chilling stress. HortScience 17: 173 - 185.

Watada, A. 1986. Effects of ethylene on the quality of fruits and vegetables. Food Technol. 40:82-85.

Weichmann, J. 1986. The eefect of controlled atmosphere storage on the sensory and nutritional quality of fruits and vegetables. Hort. Rev. 8: 101 – 127.

Wills, R., T. Lee, D. Graham, W. McGlasson, and G. Hall. 1982. Postharvest. An introduction to the Physiology and Handling of Fruit and Vegetable, pp 166. The AVI Publishing Company Inc. Westport, Conn.

Yadav, S. 1983. Amino acid composition of developing Pigeon Pea (*Cajanus cajan*) seed. J. Agr. Food Chem. 2:1360-1362.

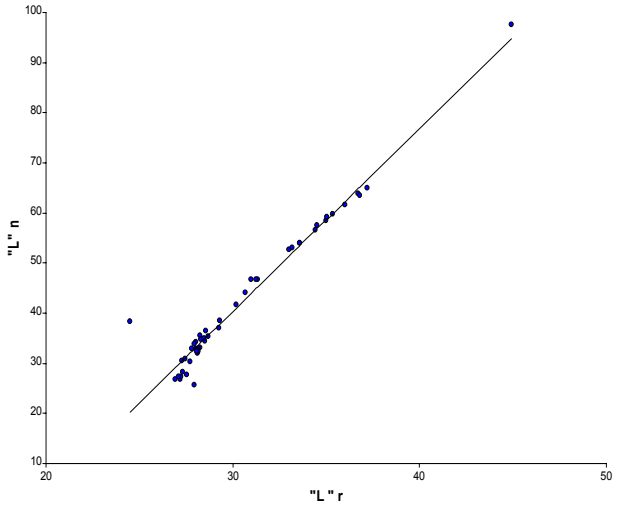
Yashoda, H., T. Prabha and R. Tharanathan. 2006. Mango ripening: changes in cell wall constituents in relation to textural softening. J. Sci. Food Agri. 86: 713 - 721.

Zagory, D. 1998. An update on modified atmosphere packaging of fresh products. Packaging International 117.

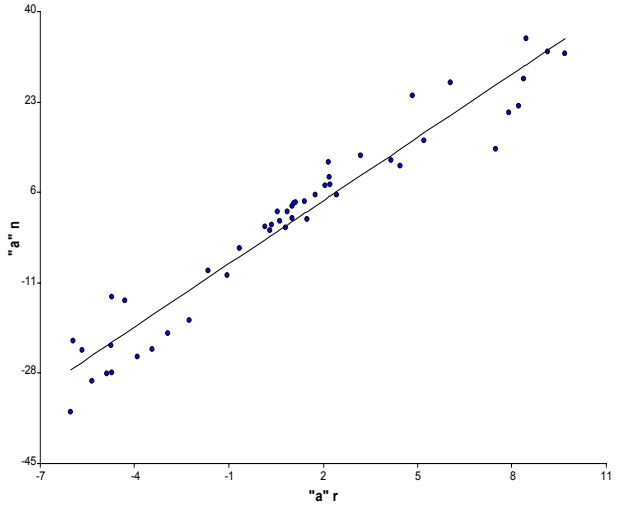
Zagory, D. 2000. What modified atmosphere packaging can and can't do for you. Washington State University.16th Annual Postharvest Conference and Trade Show.

Appendix 1

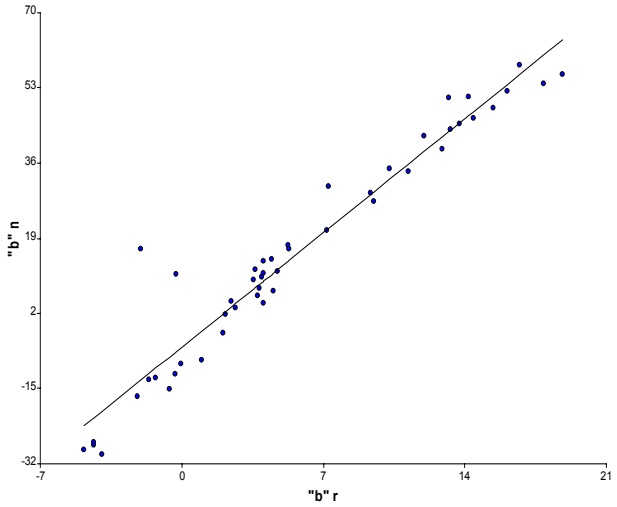
Correlation of color of pigeon pea pod between the normal aperture (n) of the colorimeter and the reduced aperture (r) to 0.8mm (black carton)



	"L"n	"L"r
"L"n	1.00	0.00
"L"r	0.98	1.00



	"a"n	"a"r
"a"n	1.00	0.00
"a"r	0.97	1.00



	"b"n	"b"r
"b"n	1.00	0.00
"b"r	0.97	1.00

CRYOVAC
Sealed Air Corporation

PD-961EZ Bag

CRYOVAC Sealed Air Corporation PD-961EZ Bag

Highlights

Multilayered polyolefin material.

Abuse resistant.

Hermetic factory seals.

Superior seal strength.

Product-specific 0^2 and CO^2 transmission rates.

Excellent clarity.

Superior gloss.

Easy-open feature.

Tough, permeable bag protects freshness of processed lettuce during distribution.

The PD-961EZ Bag is a new bag formulation developed by Cryovac scientists specifically for the packaging of fresh, processed lettuce. It is the result of detailed scientific study of the unique film properties required for extending the freshness of this highly perishable product.

By utilizing advanced multilayer coextrusion technology combined with food science technology, Cryovac was able to design the PD-961EZ Bag for the specific demands of processed lettuce and other produce products such as cabbage, carrots and onions. The bag's gas transmission rates are precisely matched to the product's natural respiration rate. Its controlled permeability allows the package to achieve the proper balance of oxygen and carbon dioxide for maintaining highest lettuce quality.

The PD-961EZ Bag, under proper refrigeration (36° - 38° F), will assure maximum shelf life with optimum flavor, and without pink rib. Although it is only half as thick as many standard poly bags, this 1.25-mil bag is every bit as strong. Its multilayer toughness allows the package to withstand handling abuses in distribution, with fewer leakers. The bag also offers superior heat-sealing parameters.

This new packaging for foodservice processed lettuce provides excellent clarity, high gloss and sheen to enhance the full color and appeal of the product. The bag also has a built-in "easy open" feature which allows quick access to contents without the need for a cutting instrument.

CRYOVAC Sealed Air Corporation PD-961 EZ Bag

PROPERTIES

Gauge (mils)	1.25
Minimum Use Temperature	0°F.
Maximum Storage Temperature (2 years maximum)	90°F.
Density @ 73° F. (g/cc)	0.92
Clarity (%)	75
Haze (%)	6.5
Gloss (%)	84
Oxygen Transmission Rate cc/m2/24 hrs. (73° F., 1 atm)	7,000 (450 cc/100 sq. in)
Carbon Dioxide Transmission Rate, cc/m2/24 hrs. (73° F., 1 atm)	21,000 (1355 cc/100 sq. in)
Water Vapor Transmission Rate gms/100 sq. in./24 hrs. (73° F., 100% RH)	0.90-1.10
Ball Burst Impact Strength (cm/kg)	26
Coefficient of Friction, Film to film, Static	0.25
Tensile Strength (psi)	12,000
Elongation @ Break (%)	105
Modulus of Elasticity (psi)	33,000
Tear Propagation (gms)	30

To find out more about Cryovac's total systems approach to packaging, phone your Cryovac specialist at the nearest regional office.

16201 Commerce Way
Cerritos, CA 90703
(562) 926-0418

Room 250
12600 West Colfax, Suite A-270
Denver, CO 80215
(303) 233-6558

1055 Johnson Drive
Buffalo Grove, IL 60089
(708) 520-4727

157 North Commerce Way
Bethlehem, PA 18017-8933
(610) 694-0606

100 Rogers Bridge Rd., Build. B
Duncan, SC 29334
(864) 433-3800

CRYOVAC
Sealed Air Corporation
Cryovac, Duncan, SC 29334

CRYOVAC
Sealed Air Corporation

PD-941 Bag

Improved multi-ply bag provides greater abuse protection for vacuum-packaged produce.

The PD-941 Bag from Cryovac is a new type of multilayered material specifically developed to extend the freshness life of refrigerated (not iced) produced items such as broccoli and cauliflower florets. These vegetables have a high respiration rate which, without suitable packaging, shortens their shelf life.

The PD-941 Bag is designed with high oxygen and carbon dioxide transmission rates which allow controlled respiration of produce items. There is no odor buildup in the bag, and shelf life is appreciably extended.

This durable, multilayered bag provides greater sanitation during shipping and handling, a benefit that is especially important to health-care foodservice operations which utilize central preparation and packaging. The bag virtually prevents recontamination of the product during distribution, which can occur with mesh netting or perforated bags. It also helps eliminate sanitation problems in the refrigeration areas caused by unclean water and food particles.

The PD-941 Bag also enhances overall merchandisability of fresh produce in the refrigerated display. Because of its merchandising and sanitation benefits, the PD-941 Bag is an ideal alternative to (or replacement for) mesh netting.

It has excellent cold-flex properties and superior seal strength to assure high-integrity packaging. No vacuumizing is required after closure with a hermetic seal. It complies with federal requirements for food packaging and is available in 75 gauge.

Bag Highlights

Type of material.
Multilayered polyolefin.

Toughness.
Durable and noncorrosive.

Seal Strength.
Superior.

Cold-flex Properties.
Excellent.

Transmission Rates.
High O_2 and CO_2.

CRYOVAC Sealed Air Corporation PD-941 Bag

CRYOVAC Sealed Air Corporation PD-941 Bag

PROPERTIES

	ASTM Test Method		Typical Values
Gauge			75
Minimum Use Temp.			-60ºF.
Maximum Storage Temp. (two years maximum)			90ºF.
Density @ 23ºC. (g/cc)	D-1505		.937
Clarity (%)	D-1003		76
Gloss (%)	D-2457		80
Ball Burst Impact Strength (cm/kg)	D-3420		11
Coefficient of Friction Bag-to-Bag, Kinetic	D-1894		.30
Water Vapor Transmission (gm/100 sq. in., 24 hrs.)	F-372		5.0
Oxygen Transmission (cc/sq.M./24 hrs.) (cc/100 sq. in./24 hrs.)	D-1434		16,500 1,065
		LD*	**TD****
Tensile Strength (psi)	D-882	7,000	7,000
Elongation @ Break %	D-882	175	175
Modulus of Elasticity (psi @73ºF.)	D-882	20,000	20,000
Tear Propagation (Grams)	D-1938	16	16

*Longitudinal Direction

**Transverse Direction

The PD-941 Bag complies with the requirements of the Federal Food, Drug and cosmetics Act, as amended, for the packaging of all foods, with the exception of high alcoholic, at room temperature or below.

This information represents our best judgment based on the work done, but the Company assumes no liability whatsoever in connection with the use of information or findings contained herein.

To find out more about Cryovac's total systems approach to packaging, phone your Cryovac specialist at the nearest regional office.

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Cerritos, CA 90703
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Room 250
12600 West Colfax, Suite A-270
Denver, CO 80215
(303) 233-6558

1055 Johnson Drive
Buffalo Drive, IL 60089
(708) 520-4727

157 North Commerce Way
Bethlehem, PA 18017-8933
(610) 694-0606

100 Rogers Bridge Rd., Build. B
Duncan, SC 29334
(864) 433-3800

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Cryovac, Duncan, SC 29334

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