Quality and microbiological changes in minimally processed tropical pumpkin packed in low-density polyethylene bags[1,2]

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ABSTRACT

Minimally processed tropical pumpkin (*Cucurbita moschata*) has considerable potential to create new value-added market opportunities for Puerto Rico. The aim of this work was to evaluate the quality and microbiological changes of minimally processed tropical pumpkin packed in low-density polyethylene (LDPE) bags and stored for 20 days. Pumpkin pieces approximately 2 cm^3 in size were obtained from two cultivars ('Taína Dorada' and 'Soler'). Pieces were immersed in an antimicrobial solution containing citric acid (0.2%) and sodium benzoate (0.1%) for 3 min, centrifuged in a salad spinner, packed in LDPE bags with either vacuum or non-vacuum packaging, and stored at 4° C ± 2 for a period of 20 days. There were minimum effects of storage time on the chemical and physical characteristics of the pumpkin pieces. The percentage of O_2 decreased continuously in non-vacuum packaging while the percentage of CO_2 increased within the first 72 hours of storage. A sensorial panel judged pumpkin pieces stored for a period of 20 days to be of acceptable quality. A minimally processed product based on pumpkin treated with an antimicrobial solution, packed in LDPE bags (either vacuum or non-vacuum packaging) and stored for 20 days at 4° C ± 2 presented safe microbiological levels and acceptable quality for the consumer.

Key words: *Cucurbita moschata,* storage time, vacuum packaging

RESUMEN

Calidad y cambios microbiológicos en calabaza mínimamente procesada y empacada en polietileno de baja densidad

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La calabaza (*Cucurbita moschata*) mínimamente procesada tiene un potencial considerable para crear nuevas oportunidades en el mercado de valor agregado en Puerto Rico. El objetivo de este trabajo fue evaluar la calidad y microbiología de la calabaza tropical mínimamente procesada empacada en bolsas de polietileno de baja densidad (LDPE, por sus siglas en inglés) y almacenada durante 20 días. Pedazos de 2 cm^3 de dos cultivares de calabaza ('Taína Dorada' y 'Soler') fueron tratados por inmersión con una solución antimicrobiana que contenía ácido cítrico (0.2%) y benzoato de sodio (0.1%) durante 3 min, centrifugados en una hilandera de ensaladas, empacados en bolsas de LDPE, sellados al vacío y sin vacío, y almacenados a 4° C ± 2 durante un período de 20 días. El tiempo de almacenamiento tuvo un efecto mínimo sobre las características químicas y físicas de los pedazos de calabaza. El contenido de O_2 en los empaques sellados sin vacío disminuyó, mientras que el contenido de CO_2 tuvo un incremento en las primeras 72 horas de almacenamiento. Los jurados del panel sensorial determinaron que la calabaza almacenada por 20 días mantiene una calidad aceptable. El producto mínimamente procesado tratado con una solución antimicrobial, empacado en bolsas de LDPE (selladas al vacío o sin vacío) y almacenado por 20 días a 4° C ± 2 presentó niveles microbiológicos seguros y una calidad aceptable por el consumidor.

Palabras clave: *Cucurbita moschata*, tiempo de almacenamiento, empaque al vacío

INTRODUCTION

Traditionally, fresh vegetables have been prepared by washing, cutting and peeling immediately or hours before serving. Many modern consumers are unwilling or unable to dedicate the time needed to prepare healthy and nutritious food despite being aware of the importance of consuming fruits and vegetables for their nutritional value. The demand for healthy, fresh and easy-to-prepare products has led to the development of a wide variety of minimally processed fruits and vegetables (Allende et al., 2006). According to the Global Agricultural Trade System (GATS) (USDA, 2014), in 2013, $2.33 billion was generated in worldwide exports of processed vegetables, and that amount increased to $2.58 billion in 2014, an increase of 10.35%.

Pumpkin species *Cucurbita pepo*, *C. maxima* and *C. moschata* are native to subtropical and tropical America, where they are used in a number of food dishes. Fruits of squash and pumpkin are rich in carotenoids, folate and vitamins C and E, and are widely available throughout the year (Azevedo-Meleiro and Rodríguez-Amaya, 2007). In 2011 the top five pumpkin producing countries were: China with 6,905,000 tons, India with 4,695,542 tons, Russia with 1,175,890 tons, Iran with 951,253 tons and the U.S. with 814,330 tons (FAO, 2011). For fiscal year 2011, Puerto Rico produced a total value of $2.4 million of tropical pumpkin (*C. moschata*). In Puerto Rico, pumpkin is traditionally used in preparing beans, with pumpkin pieces added to the sauce. But if pumpkin were sold already cut into pieces, its use would likely increase.

Minimum processing involves washing, removing inedible parts (skin, seeds, etc.), sanitizing, rinsing, drying and packaging. The goal is to reduce consumer labor and at the same time offer a ready-to-use product with a long shelf life, and unaltered nutritional value and sensory quality. The disadvantage of minimal processing is that produce is more susceptible to deterioration when fruit or vegetable tissue is cut into pieces. The biochemical changes that occur accelerate the decomposition process (Sgroppo and Sosa, 2009) and make these products more susceptible to microbial contamination due to the exposure of internal tissues (Sasaki, 2005). For this reason, conservation techniques such as low temperature storage and atmosphere modification techniques are used to extend shelf life and to slow down the deterioration of the sensory quality of minimally processed foods (Habibunnisa et al., 2001). Low temperatures and modified atmosphere reduce the proliferation of spoilage microorganisms and the kinetics of deterioration in a product (Rossaint et al., 2014). Another technique is the use of packaging materials that retard moisture loss and help maintain the quality of minimally processed fruits and vegetables (Schlimme, 1995).

Modified atmosphere packaging is based on modifying the composition of the atmosphere inside the package by reducing the amount of oxygen and replacing it with carbon dioxide and/or nitrogen (Ramos et al., 2013). Oxygen promotes deterioration reactions such as lipid oxidation and pigment darkening (Sandhya, 2009). Passive modified atmosphere packaging involves sealing packages under normal atmospheric conditions, without adding any gas (Tripathi et al., 2011).

Modified atmospheres with higher concentrations of CO_2 and low concentrations of O_2 reduce respiratory rates and minimize deterioration and physiological changes in plant tissues (Kader, 1986; Kader et al., 1989). The increase in plant tissue respiratory rate is due to high levels of ethylene (Brecht, 1995) induced by high temperatures or a wound in the tissue of the fruit or vegetable (Sasaki, 2005). When fruits or vegetables are cut into pieces for packaging, wounded tissue sets off a series of events. The packaged pieces will be exposed to greater amounts of ethylene, which in turn will increase respiration rate and lead to softening of fruit tissue and accelerated senescence (Ohlsson and Bengtsson, 2002). High concentrations of CO_2 and low concentrations of O_2 inhibit ethylene production in fruits and vegetables, delaying product ripening (Sasaki, 2005; Sandhya, 2009).

Plastic or polymer sheets are commonly used for controlling the atmosphere of a product and should have a selective permeability for gases (Oliveira et al., 2011). These sheets retard the rate of moisture loss, and some are used to minimize potential anaerobic conditions developed within the atmosphere packaging (Schlimme, 1995). One material commonly used is polyethylene (Gibe and Kim, 2013), especially low-density polyethylene (LDPE) (Ornelas-Paz et al., 2012). Other available materials are expanded polystyrene, polyvinyl chloride (PVC), polypropylene (PP) and polyethylene terephthalate (PET) (Oliveira et al., 2011). Polyethylene sheets have different degrees of permeability to water vapor and gases such as CO_2, O_2 and ethylene (Batista et al., 2007). LDPE is inexpensive and has high water vapor permeability (Oliveira et al., 2011). Use of appropriate packaging material and storage temperature can reduce the rate of respiration and inhibit spoilage microorganisms, extending the life of the product (Gibe and Kim, 2013).

Lack of proper temperature control during transportation, storage and marketing of fresh products may lead to deterioration due to increased metabolism of the product and microbial growth (Sandhya, 2009). Metabolic reactions in fruits and vegetables are reduced two to three times for each 10° C reduction in temperature (Brecht, 1995). Cooling minimally processed vegetables to 0 to 3° C can extend shelf life 5 to 18 days because of the reduction in respiratory rate (Watada et al., 1990). Maturation and ethylene production of fruit or vegetables increase when stored at high temperatures (Sandhya, 2009). Sasaki and colleagues (Sasaki, 2005; Sasaki et al., 2006) recommended that pumpkin (*C. moschata*) storage temperature should be between 1 and 5° C. Habibunnisa and colleagues (2001) reported that minimally processed pumpkin (*C. maxima*) pieces may be stored for a period of 25 days at 5° C ± 2 in a package under modified atmosphere conditions while suffering a minimum weight loss (0.06%).

Tropical pumpkin pieces preserved in a minimally processed form could promote increased consumption of this important and nutritious crop in Puerto Rico and other places. The objective of this research was to evaluate the effects of sealing methods (vacuum and non-vacuum) in bags of LDPE on the quality and microbiology of two cultivars of minimally processed tropical pumpkin stored for 20 days.

MATERIALS AND METHODS

The pumpkins used in this study were grown at the Lajas ('Taína Dorada') and Isabela ('Soler') Agricultural Experiment Stations of the University of Puerto Rico. After harvest, fruits were taken to the food-processing laboratory of the Food Science Technology Program of the University of Puerto Rico, Mayagüez Campus (UPRM).

Experimental design

Separate experiments for each pumpkin cultivar were conducted as a randomized complete block design (RCB) with seven blocks (runs) and five storage/packaging treatments: (1) raw pumpkin (control, day 0), (2) vacuum sealed and evaluated at day 15, (3) vacuum sealed and evaluated at day 20, (4) non-vacuum sealed and evaluated at day 15, and (5) non-vacuum sealed and evaluated at day 20.

Minimal processing

The first run (block) was carried out in August 2012, using a mature fruit of 'Taína Dorada'. The procedure was repeated in six additional runs from August to December 2012. Whole fruits were washed with water and soap, disinfected with a solution of sodium hypochlorite at 200 mg/L for three minutes and then rinsed again with clean water. Pumpkins were sliced, and the placenta and seeds were removed. Slices were peeled and cut into cubes of about 2 cm^3. Cubes were immersed in an antimicrobial solution (0.2% citric acid and 0.1% sodium benzoate) for approximately three minutes to prolong shelf life. They were then centrifuged in a salad spinner and dried with a disposable paper towel to remove excess water. A sample of 150 g (about seven to nine pieces) was placed in each of five LDPE bags. One bag of raw pieces was left unsealed and was used as the control (day 0). Two bags were vacuum-sealed and two bags were sealed without using vacuum. Vacuum and non-vacuum bags were stored for either 15 or 20 days, at 4° C. The same procedure was used with 'Soler' from January to May 2013.

Data collection

Texture was measured by using a Texture Analyzer TA-XT2 (Stable Micro Systems Ltd., Godalming, Surrey, England)[6]. A 2-mm stainless steel probe measured the force required to penetrate pumpkin pieces. Five cubes from each sample bag (control, sealed under vacuum and without vacuum for 15 or 20 days) were taken for measurements of force in Newtons (N) of force. Color was measured using an EZ ColorFlex colorimeter (Hunter Associates Laboratory, Inc., Reston, Virginia) calibrated with black and white porcelain tiles. Color was measured on five cubes per sample as Hunter values L*, a* and b*, using illuminant D65; L* measures brightness or luminosity on a vertical axis where values range from 0 for black to 100 for white. The coordinates

[6]Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

a* and b* locate the color of a sample on a rectangular-coordinate grid perpendicular to the L* axis at the L* position of the sample. The coordinate a* measures the variation between red and green (+ a = red, -a = green), and b* is a measure of the variation between yellow and blue (+ b = yellow, -b = blue). The parameters a* and b* were used to calculate the chroma and hue angle of each pumpkin sample using the following formulas (McGuire, 1992):

$$\text{Chroma} = \sqrt{a^2 + b^2}$$

$$\text{Hue angle} = \tan^{-1}(a/b)$$

Chroma indicates the degree of departure from gray (at L* axis) toward pure chromatic color. It is an index related to color saturation or intensity. Hue angle categorizes the type of color such as red, yellow, orange, etc. For pumpkin, hue should be in the range of 0 (orange) to 90 (yellow), with a value of 45 corresponding to an orange-yellow color. The pH was determined by a Docu-pH Meter (Sartorius Corporation, Bohemia, New York) with a digital potentiometer, by direct immersion of the electrode previously calibrated with buffer solutions of pH 4.00, 7.00 and 10.00. The pH and total soluble solids were obtained from the supernatant of pieces of pumpkins that were crushed in a coffee bean grinder Model Cuisinart DCG-20N (Cuisinart Inc., East Windsor, New Jersey) and then centrifuged (Damon/ IEC Model HN-SII Thermo IEC, Needham Heights, Massachusetts). Total soluble solids were determined as degree Brix (°Brix) using a hand refractometer. Titratable acidity was measured with a 0.10 N NaOH solution and phenolphthalein as an indicator according to the AOAC 942.15 method (Horwitz and AOAC International, 2003). Results were expressed as a percentage of citric acid.

The percentages of O_2 and CO_2 gases were measured inside the non-vacuum sealed bags after 24, 48 and 72 hours, using the MiniFood-Pack Servomex gas analyzer (5200) (Servomex Company Inc., Brighton, East Sussex, United Kingdom). Bags were pierced with a needle, which sucked out the air inside the packaging for nine seconds.

The microbiological analysis was performed in duplicate for aerobic coliforms/ *Escherichia coli*, lactic acid bacteria, *Staphylococcus aureus*, molds and yeasts. These procedures were carried out according to protocols established by the Bacteriological Analytical Manual (BAM) (Food and Drug Administration Services, 2001). A 25 g sample was taken from each bag and homogenized with 225 mL of 0.1% peptone water in the Stomacher 400 Laboratory Blender (Seward Laboratory Systems Inc., Davie, Florida, USA) for two minutes. Serial dilution samples (1 mL), from 10^{-1} to 10^{-6}, were plated on MRS agar (De Man et al., 1960) to determine lactic acid bacteria (incubated at 35° C ± 1 for 24 h ± 2) and on 3M™ Petrifilm™ plates to determine aerobic bacteria (incubated at 35° C ± 1 for 48 h ± 3), coliforms/*Escherichia coli* (incubated at 35° C ± 1 for 24 h ± 2), *Staphylococcus aureus* (incubated at 35° C ± 1 for 24 h ± 2), and molds and yeasts (incubated at 25° C ± 1 for 3 to 5 d). Observations were recorded and results were expressed as colony forming units (CFU) per gram, then converted to log units.

To determine the composition of unprocessed pumpkin samples, a proximal analysis was carried out on a separate fruit of 'Taína Dorada' and 'Soler.' Methods described by AOAC (Association of Official Analytical Chemists) (Horwitz and AOAC International, 2003) were used to determine the following analyses: AOAC 966.02 for determining moisture, AOAC 923.03 for ash content, and AOAC 991.20 for protein content (using the Kjeldahl method with a conversion factor = 6.25). Crude fat and crude fiber Am5-04 Ba 6-05 were determined according to the official methods described by AOCS (2005) (American Oil Chemists' Society). Total carbohydrate was what remained after subtracting total fat, protein, moisture and ash values.

A sensory evaluation for general taste was performed to determine the acceptability of tropical pumpkin 'Taína Dorada' and 'Soler' that was minimally processed as previously described, and either immediately tested (control treatment) or packed under vacuum and non-vacuum and stored for 20 days. Pumpkin pieces were steamed in a medium size pot with 120 ml of water and 1 g of salt for 10 minutes. The evaluation was conducted by a panel of 30 students and employees of the Science and Food Technology of UPRM who were frequent consumers of pumpkin. A 7-point hedonic scale (1 = extremely dislike; 2 = dislike very much; 3 = dislike; 4 = neither like nor dislike; 5 = like; 6 = like very much; 7 = extremely like) was used following suggestions by Meilgaard et al. (2007).

Statistical analysis

All data except that from the proximal analyses was analyzed by analysis of variance using the statistical package InfoStat (version 2012e, Di Rienzo et al., 2012). Orthogonal contrasts (α = 0.05) were used to compare means of (1) control versus the average of vacuum and non-vacuum at 15 and 20 days of storage, (2) vacuum versus non-vacuum packaging, and (3) 15 versus 20 days of storage. A Tukey's test (α = 0.05) was used to compare means of percentage of O_2 and CO_2. In the proximal analyses, the three determinations made for protein, fat, ash, fiber and total carbohydrates (proximal analyses) were averaged and the standard deviation was determined.

RESULTS AND DISCUSSION

Physical and Chemical Analyses

At the beginning of the storage period (day 0), firmness of pumpkin pieces was 8.06 N for 'Taína Dorada', 10.40 N for 'Soler' (Table 1). For 'Taína Dorada', there were no significant changes throughout the 20-day storage period, but average firmness was significantly higher in the non-vacuum packed treatments (7.74 N) compared with vacuum-packed treatments (7.51 N). Neither the vacuum nor the non-vacuum packed 'Soler' pumpkins showed significant changes during the 20-day storage period. Firmness indicates if there are changes in the structure and cohesion of cells and if biochemical alterations have occurred that impact the texture of the product (Alvés et al., 2010a). Alvés and colleagues (2010a) reported an average firmness of 4.86 N in *C. moschata*. In a study by Habibunnisa and colleagues (2001), firmness of pumpkin pieces packaged under various modified atmosphere treatments and stored at 5° C ± 2 for 25 days in LDPE bags decreased 16.9%. Factors such as temperature and composition of the atmosphere inside the packaging affect the firmness of the plant tissue. High concentrations of ethylene, which is induced by high temperature or the presence of a wound in the tissue of the fruit or vegetable, leads to softening of plant tissue (Ohlsson and Bengtsson, 2002; Sasaki, 2005). Since low O_2 and high CO_2 concentrations reduce ethylene production, packaging that maintains these relative concentrations aid in the maintenance of a stable firmness during 20 days of storage.

Type of packaging and storage period had no effect on chroma of pumpkin pieces in either of the two cultivars, and luminosity (L*) and hue angle were only minimally affected by these treatments (Table 1). In 'Taína Dorada', average luminosity of vacuum-packed pieces was significantly lower (L* = 64.78) than in non-vacuum packed pumpkin (L* = 65.78). In 'Soler', the mean for hue was higher for raw pumpkin at day 0 (67.42) than for treated (vacuum or non-vacuum at 15 or 20 days) pumpkin pieces (66.07). In a previous study, raw pieces of 'Taína Dorada' [designated "PRShortline1" in Wessel-Beaver et al. (2006)] had an intense orange color with hue averaging 65.6 and chroma averaging 74.2. In the same study, 'Soler' tended to be more yellow (average hue = 68.0) with a slightly more saturated color (chroma = 75.0). Color of fruits or vegetables that have undergone minimum processing tends to be affected due to processes such as peeling and chopping. Discoloration occurs when the damaged tissue is scarred after such operations (Angós et al., 2008). The use of a sharp stainless steel knife for chopping followed by immediate cooling of cut pieces are factors that aid in maintaining color stability (Allende et al., 2006; Parzanese,

TABLE 1.—*Mean of physical-chemical analysis values of tropical pumpkin cultivars Taína Dorada and Soler minimally processed, vacuum and non-vacuum packed and stored for 15 and 20 days at 4° C ± 2.*

Cultivar	Treatment	Firmness (N)	Luminosity (L*)	Hue angle	Chroma	pH	°Brix	Acidity (%)
'Taína Dorada'	Control, day 0 (raw pumpkin)	8.06	65.73	64.84	79.15	6.70	7.24	0.04
	Vacuum packed for 15 days	8.06	65.29	64.90	81.98	6.99	7.14	0.04
	Non-vacuum packed for 15 days	8.30	65.79	64.32	80.20	6.99	7.22	0.04
	Vacuum packed for 20 days	6.96	64.28	64.68	80.56	6.96	7.04	0.04
	Non-vacuum packed for 20 days	7.17	65.77	64.84	78.28	7.05	7.15	0.04
	F test	ns	ns	ns	ns	ns	ns	ns
	CV (%)	9.64	1.90	1.36	3.85	2.06	4.08	25.70
	SD	0.74	1.24	0.88	3.08	0.14	0.30	0.01
	Orthogonal Contrasts							
	Control vs. other treatments	ns	ns	ns	ns	*	ns	ns
	Vacuum vs. non-vacuum	*	*	ns	ns	ns	ns	ns
	15 vs. 20 days	ns	ns	ns	ns	ns	ns	ns
	Packaging type x number of days stored (interaction)	ns	ns	ns	ns	ns	ns	ns
	Packaging type x number of days stored (interaction)	ns	ns	ns	ns	ns	ns	ns

* = significant and ns = nonsignificant, respectively, at the p=0.05 probability level

TABLE 1.—(Continued) *Mean of physical-chemical analysis values of tropical pumpkin cultivars Taína Dorada and Soler minimally processed, vacuum and non-vacuum packed and stored for 15 and 20 days at 4° C ± 2.*

Cultivar	Treatment	Firmness (N)	Luminosity (L*)	Hue angle	Chroma	pH	°Brix	Acidity (%)
'Soler'	Control, day 0 (raw pumpkin)	10.40	64.60	67.42	78.61	6.82	9.37	0.03
	Vacuum packed for 15 days	10.61	64.48	66.15	78.29	7.02	9.32	0.02
	Non-vacuum packed for 15 days	10.54	64.89	66.45	77.66	7.09	9.49	0.02
	Vacuum packed for 20 days	10.14	63.88	65.83	79.01	7.04	9.25	0.02
	Non-vacuum packed for 20 days	10.68	63.71	65.83	77.97	7.14	9.51	0.02
	F test	ns	ns	ns	ns	ns	ns	ns
	CV (%)	6.07	1.69	1.06	1.90	1.96	3.26	34.32
	SD	0.63	1.09	0.70	1.49	0.14	0.30	0.01
	Orthogonal Contrasts							
	Control vs. other treatments	ns	ns	*	ns	*	ns	*
	Vacuum vs. non-vacuum	ns	ns	ns	ns	ns	ns	ns
	15 vs. 20 days	ns	*	ns	ns	ns	ns	ns
	Packaging type x number of days stored (interaction)	ns	ns	ns	ns	ns	ns	ns

* = significant and ns = nonsignificant, respectively, at the p=0.05 probability level

2014). These protocols minimize physical damage and the deterioration process.

Raw pieces of both 'Taína Dorada' and 'Soler' had significantly lower pH values than pieces packaged and stored for either 15 or 20 days (Table 1). Type of packaging and length of storage period (15 versus 20 days) had no effect on pH. The increase in pH during storage may be related to the uptake of organic acids by the respiration process (Alvés et al., 2010b). Certain molds and yeasts have the ability to use organic acids, reducing acidity, increasing pH, and creating a favorable environment for the proliferation of pathogenic bacteria (Beuchat, 2002). In general, fruits and vegetables are rich in carbohydrates, low in protein, and have a neutral pH and high water activity, thus making them vulnerable to microbial growth (Ramos et al., 2013). It has been shown that the pH value of pumpkin ranges from 6.11 to 6.77 (Alvés et al., 2010b; Jacobo-Valenzuela et al., 2011). Sgroppo and Sosa (2009) reported pH values of 7.01 to 7.07 in stored pieces of *C. moschata*.

Total soluble solids (°Brix) were unaffected by type of packaging or length of storage period (Table 1). The °Brix ranged from 7.04 to 7.24 for 'Taína Dorada' and from 9.25 to 9.51 for 'Soler.' Type of packaging had no effect on acidity. However, raw pieces of 'Soler' had a significantly higher percentage of acidity compared with stored pieces. A review of the literature shows variable estimates of soluble solids in pumpkin. This is likely due, in part, to the testing of various species (three different species are commonly referred to as "pumpkin" or "squash"). Within the genus *Cucurbita* cultivar differences are also known to occur among genotypes (L. Wessel-Beaver, personal communication). Jacobo-Valenzuela and colleagues (2011) reported a °Brix of 6.42 in pumpkin (*C. moschata* species). Wessel-Beaver (2013) reported a °Brix of 10.75 and 5.35 for 'Taína Dorada' and 'Soler,' respectively, values that appear to be in complete contrast to the current study where 'Soler' was observed to have high amounts of soluble solids. In general, the experience of Wessel-Beaver has been that 'Taína Dorada' generally has higher concentrations of soluble solids compared with 'Soler'. Why our current study had different results is not clear.

Habibunnisa and colleagues (2001) reported that at 25 days of storage the average °Brix of pumpkins in non-vacuum packaging in LDPE was 7.91 while vacuum packaging was 8.52 °Brix. Silva and colleagues (2009) reported concentrations of soluble solids between 9.83 and 10.38 °Brix in minimally processed pumpkin packed in bags of polyvinyl chloride (PVC) and stored at 5 and 10° C for 12 days, respectively. The content of soluble solids in pumpkin is an important quality parameter because they are characterized by high sugar content (Gibe and Kim, 2013). Sugars represent a large part of the soluble solids (85 to 90%) found in most fruits, the rest consisting of vitamins, phenolic compounds, organic acids and pectin (Alvés et al., 2010b).

The percentage of O_2 and CO_2 in the atmosphere within the non-vacuum packaging was measured for 72 hours after processing. In 'Taína Dorada,' the amount of O_2 present in LDPE bags decreased between 24 and 72 hours (Table 2). A similar trend was observed for 'Soler,' although the decrease in O_2 was not significant. The opposite occurred for CO_2 content; the percentage of CO_2 increased significantly between 24 and 72 hours. In general, the coefficients of variation (CV) were much higher for these variables compared with those of other variables measured in this experiment. The increased levels of CO_2 and reduced O_2 is associated with the stress caused by the minimum processing as the tissues are damaged due to mechanical manipulations. This stress promotes metabolic disorders such as ethylene production, accumulation of secondary metabolites and cell disruption (Alvés et al., 2010a). Glycolysis, the tricarboxylic acid cycle and the electron transport system are the metabolic pathways of aerobic respiration, which involves breaking down organic reserves such as carbohydrates, lipids and organic acids to simple molecules (Fonseca et al., 2002). This process results in the consumption of O_2 in a series of enzymatic reactions during the process. In a study by Habibunnisa and colleagues (2001), LDPE bags packed with pieces of minimally processed pumpkins and stored at 5° C had a mean percentage of O_2 of 2% at 60 hours while CO_2 was at 15%. The benefits of having a high content of CO_2 in a modified atmosphere are associated with inhibition of psychotropic spoilage microorganisms (Soliva-Fortuny et al., 2004).

TABLE 2.—*Percentage of O_2 and CO_2 in non-vacuum sealed bags of minimally processed pieces of tropical pumpkin Taína Dorada and Soler 24 to 72 hours after storage at 4° C.*

Cultivar	Storage time (hours)	O_2 (%)	CO_2 (%)
Taína Dorada	24	12.44 a	3.61 c
Taína Dorada	48	6.49 ab	6.09 bc
Taína Dorada	72	3.54 b	10.06 ab
Soler	24	6.46 ab	4.73 c
Soler	48	2.11 b	9.26 ab
Soler	72	0.80 b	13.39 a
LSD		6.02	4.18
CV (%)		69.8	32.8

Within the same column, means with a common letter are not significantly different at p=0.05 according to Tukey's test.

LSD = Tukey's least significant difference at p=0.05.

TABLE 3.—*Counts of aerobic bacteria and molds and yeast in tropical pumpkin 'Taína Dorada' and 'Soler', minimally processed, vacuum and non-vacuum packaged, stored for 15 and 20 days at 4° C ± 2.*

Cultivar	Treatment	Aerobic bacteria (log CFU/g)[1]	Molds and yeast (log CFU/g)
'Taína Dorada'	Control, day 0 (raw pumpkin)	3.15	2.3
	Vacuum packed for 15 days	3.71	2.4
	Non-vacuum packed for 15 days	4.37	2.3
	Vacuum packed for 20 days	4.09	2.6
	Non-vacuum packed for 20 days	4.44	2.7
	F test	NS[2]	NS
	CV (%)	21.45	7.78
	SD	0.85	0.17
	Orthogonal Contrasts		
	Control vs. other treatments	*	NS
	Vacuum vs. non-vacuum	NS	NS
	15 vs. 20 days	NS	NS
	Packaging type x number of days stored (interaction)	NS	NS
'Soler'	Control, day 0 (raw pumpkin)	2.90	2.3
	Vacuum packed for 15 days	3.17	2.6
	Non-vacuum packed for 15 days	3.07	2.4
	Vacuum packed for 20 days	2.76	2.7
	Non-vacuum packed for 20 days	2.96	2.9
	F test	NS	NS
	CV (%)	15.39	11.42
	SD	0.46	0.26
	Orthogonal Contrasts		
	Control vs. other treatments	NS	NS
	Vacuum vs. non-vacuum	NS	NS
	15 vs. 20 days	NS	NS
	Packaging type x number of days stored (interaction)	NS	NS

[1]CFU = colony forming units
[2]NS = non significant at p=0.05

Microbiological analysis

No *Escherichia coli*, coliforms, *Staphylococcus aureus* nor *Lactobacillus* spp. were detected in vacuum or non-vacuum packaged pumpkin pieces sampled after 15 and 20 days of storage (data not shown). Aerobic bacteria, and mold and yeast counts were also generally unaffected by the type of packaging with the exception of a lower aerobic bacteria count in raw pieces of 'Taína Dorada' compared with stored pieces.

Aerobic bacteria counts ranged from 2.76 to 4.44 log CFU/g while mold and yeast counts ranged from 2.19 to 2.41 log CFU/g.

While total counts of bacteria in vegetables are used as parameters of the microbial load, these counts do not indicate whether the population has a beneficial or harmful effect (Alvés et al., 2010b). The counts give an idea of the quality of the product. Roura and colleagues (2004), obtained populations of mesophilic aerobic microorganisms of 8.50 log CFU/g (3.50 x 10^8 CFU/g) at day 15 in minimally processed pumpkin pieces, packed in plastic polyethylene containers and then stored at 10 to 12° C. Sasaki and colleagues (2006) conducted a study in which diced pumpkins chilled to 5° C exhibited an aerobic bacteria count of 0.60 (4.00 x 10 CFU/g), 5.50 (3.40 x 10^5 CFU/g) and 6.90 log CFU/g (7.50 x 10^6 CFU/g) on days 0, 6 and 12, respectively. Habibunnisa and colleagues (2001) applied a 0.2% solution of citric acid and 0.1% potassium metabisulfite to diced pumpkins and obtained a count of aerobic bacteria of 5.50 log CFU/g (32.40 x 10^4 CFU/g) at day 25 when stored at 5° C. Roura and colleagues (2004) obtained values on molds and yeasts of 6.80 log CFU/g and 6.30 x 10^6 CFU/g at day 15 in pieces of minimally processed pumpkins, wrapped in polyethylene and then stored at 10 to 12° C in plastic containers. Food spoilage by yeast is the result of fermentation activity, while mold spoilage is due to the structural degradation of polysaccharides being reduced to simple sugars for use as an energy source (Beuchat, 2002).

Proximal analysis

In general, pumpkin has high moisture content, is low in fat and is an excellent source of vitamins, minerals and dietary fiber (Alvés et al., 2010b). The chemical composition of pumpkins 'Taína Dorada' and 'Soler' was not affected by storage period or the type of packaging (Table 4). The values obtained in chemical composition are similar to those reported by Alvés and colleagues (2010b) and Jacobo-Valenzuela and colleagues (2011) for *C. moschata*.

Sensory evaluation

The acceptability of 'Taína Dorada' and 'Soler' in two types of packaging were evaluated using a 7-point hedonic scale. Evaluations were done on day 0 and 20 of storage (Table 4). 'Taína Dorada' scored 5.73 on day 0, and 5.23 and 5.67 in vacuum and non-vacuum packaging, respectively, at day 20. 'Soler' scored 5.53 on day 0, and 5.20 and 5.80 in vacuum and non-vacuum packaging, respectively, on day 20. These averages indicate that acceptability was between "like" and "like very much." Number of days of storage and type of packaging did not influence the taste of minimally processed tropical pumpkins. The appear-

TABLE 4.—*Chemical composition and acceptability of pieces of minimally processed, tropical pumpkin 'Taína Dorada' and 'Soler' at day 0 (raw) and stored for 20 days at 4° C in vacuum and non-vacuum low-density polyethylene packaging.*

		Proximal analyses[1]					
Cultivar	Treatment	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Total carbohydrates (%)	Acceptance[2]
'Taína Dorada'	Control, day 0	1.21±0.25	0.10±0.05	0.52±0.12	0.72±0.10	4.44	5.73 a
	Vacuum, day 20	1.41±0.13	0.08±0.01	0.61±0.03	0.67±0.02	4.23	5.23 a
	Non-vacuum, day 20	1.42±0.19	0.09±0.01	0.66±0.01	0.71±0.06	4.11	5.67 a
'Soler'	Control, day 0	0.91±0.07	0.14±0.05	0.79±0.05	0.97±0.28	4.19	5.53 a
	Vacuum, day 20	0.90±0.03	0.10±0.02	0.79±0.02	0.88±0.08	4.32	5.20 a
	Non-vacuum, day 20	0.91±0.05	0.09±0.01	0.74±0.02	0.89±0.11	4.36	5.80 a
Tukey (0.05)[3]							0.53

[1]Proximal analysis is reported as the mean of three determinations ± standard deviation.
[2]Acceptance was measured on a 7-point scale where 1=extremely dislike to 7=extremely like
[3]Tukey (0.05) = Tukey's Least Significant Difference at p=0.05. In the same column, means followed by the same letter are not different at p=0.05.

ance of cut fresh vegetables is the first attribute perceived by consumers and strongly affects their purchase decision (Alvés et al., 2010b). Habibunnisa and colleagues (2001) reported that pieces of pumpkins that were treated and packed in LDPE bags stored at 5° C ± 2 remained in good condition for 25 days, retaining the look of freshness, color and flavor.

CONCLUSION

Minimally processed pumpkin pieces treated with an antimicrobial solution containing 0.2% citric acid and 0.1% sodium benzoate, packed in low-density polyethylene bags (vacuum and non-vacuum) and stored at 4° C ± 2 exhibited very few physical or chemical changes over a 20-day storage period, maintained safe levels of microbes and acceptable quality as judged by consumers.

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