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Research Article

Shelf Life of a Mixture of Pumpkin Puree (*Cucurbita moschata*) During Storage at 4°C

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Abstract: Pumpkin is used commonly as an ingredient for many food products because of its nutritional and health benefits. Current studies have been reported about pumpkin puree, but there is no information related to the mixture of flours and other ingredients. The development of a new formulation introduces into the market a different way of commercialization and consumption of pumpkin, favoring its production. The aim of this investigation was to determine the shelf life of the mixture by analyzing the physical-chemical and microbiological parameters of the product using a factorial experimental design of two factors, batches and time, with 3 and 13 levels respectively. The three levels of batches had 26 trays each one and processed with pumpkin puree, wheat flour, sugar, pasteurized egg, cinnamon, salt and butter. Potassium sorbate was added as a preservative agent, gum as a stabilizer and ascorbic acid as an acidulant agent. The mixture was packaged in polypropylene-polyethylene trays and stored at 4°C during 13 weeks. Two samples were taken weekly of each batch for physicochemical and microbial population analysis. The maximum charge of microorganisms reached was 4.07 Log CFU/g for aerobic mesophilic bacteria, 2.92 Log CFU/g for yeasts after day 28 and 1.48 Log CFU/g for molds after day 49. Coliforms were not found. It was observed a decrease in pH, water activity and redox potential. The results demonstrated that the shelf life of the product could be guaranteed up to day 35 when stored at 4°C.

Keywords: Food development, microbiological quality, water activity

INTRODUCTION

The pumpkin *Cucurbita moschata* is the predominant cucurbit in tropical areas of the Caribbean and Latin America (Martínez, 2012). This vegetable is an excellent source of ascorbic acid and carotenoids, which have antioxidant functions as vitamin C and vitamin A precursors, respectively (Pandey *et al*., 2003; Provesi *et al*., 2012). It also contains other vitamins such as niacin, riboflavin, vitamin E, vitamin K and minerals like potassium, phosphorus and calcium (USDA, 2016). Its fat content is low as well as its energy intake. Many authors have reported the health benefits of pumpkin, as it has anti-inflammatory, antibacterial, antiparasitic, antitumoral and analgesic properties (Fu *et al*., 2006; Jacobo-Valenzuela *et al*., 2011; Abd EI-Aziz and Abd El-Kalek, 2011). Also, it has the capability of reducing risky diseases such as diabetes, cancer, hypercholesterolemia, hypertension, atherosclerosis, arthritis, cataracts, cardiovascular and intestinal diseases. Its mesocarp tissue is rich in fiber and provides a basis for the development of functional foods (De Escalada Pla *et al*., 2009). This vegetable can be consumed in various forms, either as a whole vegetable or as an ingredient of stews, sauces, desserts, jellies, jams, purees and other products, however, the primary form of commercialization is as a fresh vegetable. Nonetheless, pumpkin puree can be found on the market as canned food, ready to be eaten.

Currently, many pumpkin-based products are being innovated because of their healthy and nutritious inputs. Besides, it has been identified as a superior and highly preferred vegetable because of its meaty and non-sandy texture, providing smoothness to the food. With the development of a new formulation based on pumpkin puree and sother ingredients such as wheat flour, sugar and egg, among others, fresh food with several facets of preparation is obtained, as it may be consumed in its fried form, such as are pitas, or baked like cakes. In this manner, another kind of commercialization of the pumpkin is introduced in the market, favoring its production.

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There are many investigations related to the study of pumpkin puree (Dragovic-Uzelac *et al*., 2005; Dutta *et al*., 2006; Gliemmo *et al*., 2009; Atef *et al*., 2012; Nawirska-Olszańska *et al*., 2016). However, there is no information on the physicochemical and microbiological characteristics of mixtures based on pumpkin puree with the ingredients mentioned above or their performance under refrigerated storage indicating the time in which the food is fit for consumption. The own characteristics of pumpkin such as its high content of water and nutrients make it a highly perishable food, so it is necessary to implement appropriate measures to develop a stable product during storage. The aim of this study was to determine the useful life of the mixture by studying the physicochemical parameters such as pH, water activity and oxidation-reduction potential and microbiological characteristics such as the count of aerobic mesophilic bacteria, coliforms, molds and yeasts of the product.

MATERIALS AND METHODS

Formulation and processing of the mixture: For the formulation of the product, pumpkin *Cucurbita moschata* variety Soler from a farm in Cabo Rojo, Puerto Rico, was used. The other ingredients were wheat flour, sugar, pasteurized egg, cinnamon, salt and butter obtained from a local supermarket in the city of Mayagüez, P.R. Potassium sorbate was used as a preservative, Tic Gums Ticagel (carrageenan) as a stabilizer and ascorbic acid as an acidulant agent to decrease the pH less than 4.6, which is the minimum to avoid the growth of *Clostridium botulinum* and its production of toxins (Jay *et al*., 2005).

The pumpkins were washed and disinfected with chlorinated water (50 ppm sodium hypochlorite-NaClO) at pH 6.0 for 2 min (FDA, 1998). They were cut into 15 cm long by 25 cm wide fragments to achieve homogeneous cooking in each of them. They were boiled for 15 min, then cooled and dried with absorbent paper to remove excess water. They were passed through the Robot Coupe C-80 pulp extractor to obtain the mash, which was taken to a Barco M-20 mixer along with the ingredients for 10 min until a homogeneous mixture was obtained. Finally, potassium sorbate, ascorbic acid and a stabilizer were added. The pH of the mixture was measured to ensure a value less than 4.6 until equilibrium was reached. Three batches were processed under the same conditions, each with 26 trays. Mixtures of 200 g were packed in polypropylene trays, which were sealed with a film made of polyester with polyethylene using the Koch Ultra Source equipment from ILPRA. The batches were stored under refrigeration temperature at 4°C in a cold room for three months. Two samples from each batch were randomly taken weekly for physicochemical analysis (pH, water activity, oxidation-reduction potential) and microbiological analysis (aerobic mesophilic bacteria, coliform, mold and yeast).

Physicochemical analysis: The pH was determined with the Sartorius Docu-pH Meter, previously calibrated with buffer solutions of pH 4.0, 7.0 and 10.0 from Fisher Scientific. The water activity was determined using the Aqua Lab 4TE equipment from Decagon Devices, previously calibrated with a solution with water activity (a_w) of 0.984. The oxidation-reduction potential was identified with the Sartorius Docu-pH Meter in mV mode. All pH, a_w and potential redox readings were done with three replicates.

Microbiological analysis: Microbiological analysis was carried out using the procedures established by the Bacteriological Analytical Manual (FDA, 2011). 25 g of sample was taken and homogenized with 225 mL of 0.1% peptone water in Seward's Laboratory Blender Stomacher 400 for 2 min. Serial dilutions were made up of 10^{-5}. Inoculations were made in duplicate for aerobic mesophilic bacteria and coliforms and in triplicate for molds and yeasts. It was reported as CFU/g (Colony Forming Units/gram) of a sample. In the determination of aerobic mesophilic bacteria, the Difco™ Plate Count Agar culture medium was used. The dishes were incubated at 35°C±1°C for 48 h±2 h. For coliforms, the Violet Red Bile Agar medium of Difco™ was used. The dishes were incubated at 35°C±1°C for 24 h±2 h. For the mold and yeast counts the Oxoid Potato Dextrose Agar culture medium was used. The dishes were incubated at 25°C±1°C for five days.

Proximate analysis: Proximate analysis was performed using the AOAC official methods (AOAC, 1990). The moisture determination was done by the gravimetric method AOAC 966.02. Ashes were obtained by ignition at 550°C to constant weight, according to AOAC method 923.03. The ether extracts, according to the method of Soxhlet, AOAC 920.39, crude protein by the Kjeldahl method AOAC 991.20 and the total fiber by AOAC method 993.21.

Statistical analysis: Analysis of Variance (ANOVA) and simple linear regression was used to determine significant differences between samples, batches and days, with a significance level of 5% using specialized software in experimental design.

RESULTS AND DISCUSSION

Physicochemical analysis:

***pH*:** For day 0 the average pH of the mixture was 4.57 for batches 1 and 2 and 4.56 for batch 3, these values being maintained on day 7 of sampling. During storage time, a decrease in values is observed (Fig. 1), which tends to stabilize during the last days, with pH fluctuating at the last day between 4.11 and 4.14.

There were no significant differences between samples from the same batch, but there were significant differences between batches and days (p<0.05).

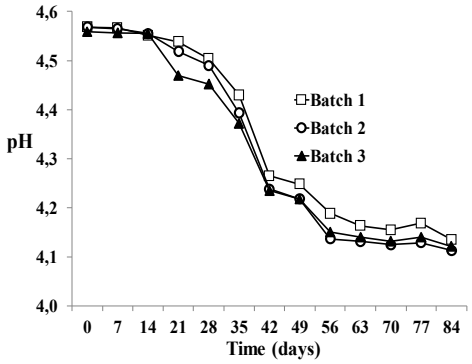

Fig. 1: pH of the mixture during storage at 4°C

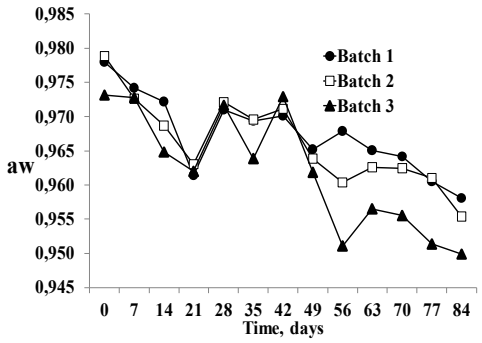

Fig. 2: Water activity (a_w) of the mixture during storage at 4°C

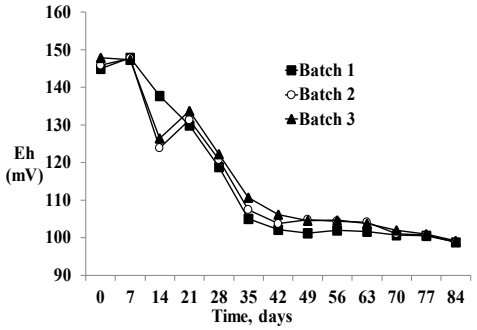

Fig. 3: Oxidation-reduction potential (Eh) of the mixture during storage at 4°C

The tendency to decrease the pH during the storage is a consequence of the accumulation of products that is originated when the microorganisms metabolize carbohydrates producing lactic acid. Also, the native biota of the pumpkin includes Lactic Acid Bacteria (LAB), which can be proliferated by acidic conditions of the product causing a decrease in the pH (Gutiérrez-López *et al*., 2008; De Escalada Pla *et al*., 2009). The same trend in values was found by Gliemmo *et al*. (2010) in pumpkin purees with the pH adjusted to 5.0 which decreased to 4.32 after some days of storage as a consequence of the growth of lactic acid bacteria.

The differences in pH values between batches may be due to the pH of the pumpkin, which ranges from 4.80 to 5.20 (Jay *et al*., 2005). Preliminary studies showed a pH between 5.0 and 5.2. Although the pumpkins used in the processing of the mixtures were from the same crop, there may be differences in the pH value and the soluble solids content. Besides, heat processes affect the pH of the pumpkin, which after scalding had a pH between 6.0 and 6.2. Similar results were found by Gliemmo *et al*. (2009) reporting a pH of 6.1 in pumpkin puree. De Escalada Pla *et al*. (2009) reported a pH of 5.77 after scalding the pumpkin for 8 minutes. Sluka (2016) reported a pH of 7.01 in pumpkin puree. Considering the fact that the influencing factors are days and batches along with their interaction, a mathematical regression for these variables was created with an analysis of variance reflecting that the batch factor and the interaction day and batch are not significant ($p>0.05$) in comparison with the days. Therefore, differences in pH values between batches do not affect the product.

Water activity: The mixture had a water activity (a_w) at day 0 of 0.9780, 0.9789 and 0.9732 in batches 1, 2 and 3, respectively. During the storage time the a_w decreases (Fig. 2). Statistical analysis showed significant differences between samples, batches, days and their interactions ($p<0.05$), but no significant differences were observed when a regression analysis and its respective ANOVA were performed ($p>0.05$).

During storage time, the product maintained values related to high moisture foods, which have an a_w range of 0.90 to 0.999 favoring the growth of bacteria causing deterioration (Jay *et al*., 2005; Ray and Bhunia, 2010). Statistically, the variation of a_w is minimal and does not affect the product. On the other hand, it is important to emphasize that from day 42 phase separation occurred, with water being observed on the surface of the mixture, indicating a syneresis effect; this also influences the decline in a_w and the deterioration of product quality.

Oxidation-reduction potential: The Oxidation-Reduction Potential (ORP) of the mixture at day 0 was on average 146.2 mV. As the storage time elapses, the potential redox decreases (Fig. 3). From day 42 the ORP values tend to stabilize and were close to 100 mV. Statistical analysis showed that there were no significant differences in samples from the same batch ($p = 0.55$), but rather between batches and days ($p<0.05$). When performing a general linear regression analysis of the days and batches with their interaction, there were no significant differences in the batches ($p = 0.872$).

Oxidation-Reduction Potential (ORP) is a determining factor in the presence of aerobic or anaerobic microorganisms. The decrease in ORP is due to oxygen consumption by the microbiota and the formation of reducing compounds in the environment (Reichart *et al*., 2007). On the other hand, the decrease

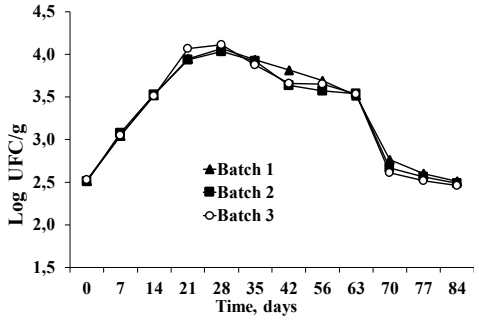

Fig. 4: Aerobic mesophilic bacteria count of the mixture during storage at 4°C

in redox potential in the mixture could be due to the presence of ascorbic acid in the product, which reduces the oxygen present in the air chamber of the package, as well as the oxidation of the potassium sorbate and the carotenoids. Although ORP values decrease, they remain positive, favoring the growth of aerobic or anaerobic microorganisms. It was noted that the oxidation-reduction potential is maintained towards a constant value that is in agreement with the depletion of the number of microorganisms in the product.

Microbiological analysis:

Aerobic mesophilic bacteria count: The average initial bacterial load of the mixture in all batches was 2.50 Log CFU/g (Fig. 4). The increase in the number of microorganisms was observed up to day 28, reaching 4.06 Log CFU/g in batch 1, 4.02 Log CFU/g in batch 2 and 4.10 Log CFU/g in batch 3. By day 84 the average bacterial load was 2.49 Log CFU/g. The analysis of variance showed significant differences between samples, days and batches ($p<0.05$). Regression analysis could not be performed due to the fluctuating behavior of the values.

The bacterial load present on the product in each of the batches reflects suitable hygienic quality. The number of bacteria increases with storage time, so it is expected that there is a significant difference in the days. Regarding batches and samples, the difference may lie in the microbial load that the different ingredients and their arrangement in the mixture can bring to the product. The Log or exponential phase agrees with a slight decrease in the pH values and as the bacteria entered the stationary phase, the pH decline was more significant. The pH conditions of the medium do not favor the growth of most bacteria, except for those that tolerate acidic conditions, such as lactic acid bacteria, which are endogenous microbiota of the pumpkin (Gutiérrez-López *et al*., 2008).

According to Gliemmo *et al*. (2010), the addition of potassium sorbate to pumpkin puree inhibited the aerobic bacterial, fungal and yeast population in approximately 4 Log cycles after 12 days of storage at 25°C compared to puree without potassium sorbate. This was not observed in lactic acid bacteria, which was more resistant to the action of preservatives, especially at pH 4.5 (Jay *et al*., 2005). Gutiérrez-López *et al*. (2008) in their study with a minimally processed pumpkin wrapped in a film of cassava starch containing potassium sorbate and with acidified pH, reported that the mesophilic aerobic bacteria decreased 2 Log cycles, while molds, yeasts and lactic acid bacteria increased significantly after storage. This confirms the resistance of the lactic acid bacteria to the preservative.

Although counts were higher on day 29, the number reached does not represent conditions of poor sanitary quality in the product, in addition, there were no deterioration conditions at that date. According to Gram *et al*. (2002), the deterioration of food by microorganisms is related to growths with counts between 10^7 and 10^9 CFU/g. From day 42 there was visible physical deterioration manifested by water on the surface of the mixture. No foul odors were present in the mixture throughout the storage time (84 days).

Coliform count: The Coliform count at day 0 and during all other storage days in all batches was lower than 10 CFU/g. The values found in the product indicate that no coliforms were detected during the storage time, which is an index of the adequate hygienic quality of the product.

Mold count: The mold count for days 0 and 7 in all batches was lower than 1 Log CFU/g, as shown in Table 1. From day 14, values increased slightly and by day 49 the maximum values were reached: 1.48, 1.45 and 1.39 Log CFU/g in batches 1, 2 and 3, respectively. For the last day of storage, the count in all batches was 0 Log CFU/g. There were significant differences between samples and days ($p<0.05$), but not in the batches ($p = 0.077$).

Although molds are microorganisms associated with deterioration producing bad odors, bad tastes and discoloration, their presence in food is of concern due to the production of toxins by some species (Jay *et al*., 2005; Ray and Bhunia, 2010). The physicochemical characteristics of the product make it susceptible to

Table 1: Mold countof the mixture during storage at 4°C

	Log UFC/g		
Days	Batch 1	Batch 2	Batch 3
0	0.50	0.00	0.50
7	0.00	0.50	0.00
14	1.15	0.65	1.00
21	1.15	1.30	1.15
28	1.39	1.48	1.45
35	1.39	1.39	1.24
42	1.39	1.15	0.50
49	1.48	1.45	1.39
56	1.24	1.15	1.24
63	0.00	0.50	0.00
70	0.00	0.00	0.00
77	0.50	0.00	0.00
84	0.00	0.00	0.00

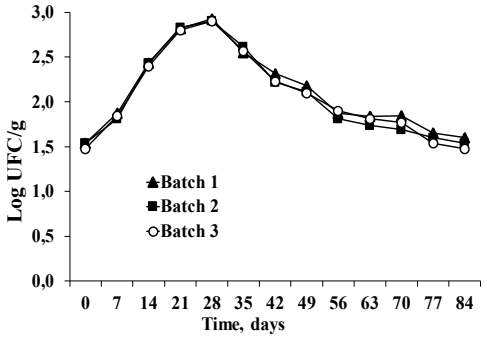

Fig. 5: Yeast count of the mixture during storage at 4°C

deterioration by molds; however, the potassium sorbate has the purpose of limiting this growth. Even though the highest counts were found at day 49, they were less than 1.5 Log CFU/g. Similar results were found by De Escalada Pla *et al*. (2009) where the growth of molds and yeasts in *C. moschata* pumpkin cylinders were lower than 10 CFU/g after day 7 of storage and the maximum found was 100 CFU/g (2 Log CFU/g) after day 45 of storage. The low number of mold in the product may be due to an antagonism caused by lactic acid bacteria, which may inhibit the growth of molds (Dalié *et al*., 2010). During the storage period, no mold was observed in the product that could be visible and cause deterioration.

Yeast count: The mean count at day 0 was 1.52 Log CFU/g (Fig. 5), increasing the values through the days being the most representative counts those between days 14 and 49, where they were higher than 2 Log CFU/g. Significant differences were observed for days and batches ($p<0.05$) but not among the samples ($p = 0.162$).

The initial yeast load in the product may come from wheat flour as these products may contain a high yeast load. A trend was observed to the increase in the number of yeasts up to day 28; however, these did not reach 3 Log CFU/g. The same tendency to increase the number of yeasts was also observed by Gliemmo *et al*. (2010) in pumpkin puree preserved with potassium sorbate, where the yeast and fungus population reached 10^6 to 10^7 CFU/g after three weeks of storage. The pH and a_w conditions of the product were favorable for the development of yeasts, however, the fact that the counts did not reach values as high as 5 Log CFU/g confirms that potassium sorbate exerted a good effect on the yeasts. Guynot *et al*. (2005) studied the impact of three preservatives on bakery products and the results showed that potassium sorbate was the most effective in inhibiting the growth of molds and yeasts at pH 4.5 and a_w of 0.90.

Proximate analysis: Table 2 shows the proximate composition of the dry base mix, with the exception of moisture. The 58.57% of the mixture was water, therefore, it is considered a product of high humidity (>50%). The fat contained in the mixture corresponding to 6.47%, was given by margarine and egg. The protein content was 2.68% especially provided by the egg. The ashes refer to the inorganic residues that remain after the ignition of organic matter (Nielsen, 2003) and are important because they represent the total content of minerals presented in the food. The mixture had 0.76% ash. The carbohydrate content of the mixture was 29.88%, contributed especially by wheat flour and sugar and 1.64% of total fiber, provided mostly by wheat flour.

Table 2: Proximate composition of the mixture

Components	Percentage (%)
Moisture	58.57±0.49
Protein	6.48±0.20
Fat	15.62±1.35
Ash	1.83±0.06
Carbohydrates*	17.49±1.81
Fiber	3.95±0.21

*Calculated by difference

CONCLUSION

The physicochemical properties of the mixture varied significantly through storage days, generally with a tendency to decrease. Microbiologically the product did not reach counts greater than 5 Log CFU/g, which are related to deterioration. The maximum load of microorganisms was 4.07 Log CFU/g for total aerobes and 2.92 Log CFU/g for yeasts at day 28 of storage and 1.48 Log CFU/g for molds at day 49. No coliforms were detected in any sample. It is likely that lactic acid bacteria exist in the mixture because they form part of the endogenous microbiota of the pumpkin. The mixture showed no visible growth of molds throughout the storage time (84 days). The product presented visible physical deterioration at day 42 manifested by the syneresis effect, with water being observed on the surface of the mixture. No odor was detected in any sample analyzed during the entire storage time. The shelf life of the product can be assured at 4°C up to day 35.

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