EFFECT OF SWEET SOLUTES ON THE QUALITY OF A PUMPKIN PUREE (*CUCURBITA MOSCHATA* DUCHESNE EX POIRET) PRESERVED BY THE HURDLE TECHNOLOGY

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ABSTRACT

The effect of glucose and xylitol on the quality of a pumpkin puree preserved by the hurdle technology was studied. It was demonstrated that the addition of glucose or xylitol to depress water activity (A_w) to 0.98 was necessary to assure 3 weeks of microbial stability at 25C of a pH 4.00 pumpkin puree, containing potassium sorbate and submitted to vapor heat treatment. A decrease in a *and* b *color parameters was observed during storage for both humectants. However, the use of a low oxygen permeability packaging such as polyvinyl chloride-polyvinylidene chloride copolymer instead of polyethylene protected the color. From a rheological point of view, xylitol diminished yield stress and consistency coefficient independently of storage time. The results obtained emphasize the advantage of the use of glucose as well as the importance of an appropriate choice of solutes to depress A_w in order to improve the quality of pumpkin puree.*

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PRACTICAL APPLICATIONS

Fresh pumpkins are very sensitive to microbial spoilage and to physiological and biochemical changes; thus, a preservation process is necessary to extend shelf life. In this study, a development of a minimally processed pumpkin puree was proposed and the color stability and rheological behavior were evaluated to improve the quality. The results obtained demonstrated that the addition of glucose (10.00% w/w) to a pH 4.00 pumpkin puree containing potassium sorbate (0.0120% w/w), steam cooked and packed in polyvinyl chloride-polyvinylidene chloride copolymer bags, assures the microbiological and color stability and prevents changes in the rheological behavior during a storage of 3 weeks at 25C.

INTRODUCTION

Pumpkin is a seasonal crop that has been used traditionally for human food and animal feed. There is a wide number of species of the family Cucurbitaceae, most of them with actual or potential economic value. In particular, the production of the species *Cucurbita moschata* Duchesne ex Poiret has been significantly cultivated in the last decades in Argentina. At the same time, its consumption also increased. Furthermore, pumpkin is considered to be a good source of nutrients such as β-carotene, potassium ion, riboflavin and vitamins A, C and E. Moreover, it is rich in fiber and low in calories (González *et al.* 2001; de Escalada Pla *et al.* 2007).

Fresh pumpkins are very sensitive to microbial spoilage due to their pH being close to neutrality and to physiological and biochemical changes even at refrigerated conditions; thus, a preservation process is necessary to extend shelf life. It can be processed by traditional methods such as freezing, drying or canning (Teotia 1992).

The development of new processed pumpkin products can be interesting for the consumers. The addition of solutes by means of solid–liquid infusion may make the vegetable tissue softer and improve its taste, making it more attractive from an organoleptic point of view. In view of these factors, the effects of osmotic dehydration with different solutes have been analyzed (Castilho Garcia *et al.* 2007; Mayor *et al.* 2007).

The use of minimal preservation processes based on the "hurdle" concept sounds interesting to be applied and, to our knowledge, its application has not been previously reported for pumpkin puree. However, many shelf-stable fruit purees have been obtained by an intelligent combination of preservation factors such as the depression of pH, the addition of lipophilic weak acids and the reduction of water activity (A_w) through solute addition (Lopez-Malo *et al.*

1994; Leistner 2000; Soliva-Fortuny *et al.* 2004). The latter stress factor can influence other quality parameters such as color and rheological behavior. The retention of the sensory characteristics mentioned is a must when trying to optimize puree quality and when information about factors affecting the quality of minimally processed vegetable purees is scarce.

β-carotene is mainly responsible for the orange color of pumpkin (Dutta *et al.* 2006). This pigment is unstable due to its highly unsaturated structure and, as a result of its oxidation, the color is lost (Ahmed *et al.* 2002; Meléndez-Martinez *et al.* 2004; Dutta *et al.* 2006). Kinetics of pigment and color degradation of fruits and vegetables during thermal processing and storage have been analyzed by numerous researchers (Ahmed *et al.* 2000, 2002; Meléndez-Martinez *et al.* 2004) and provide useful information so as to improve product quality. In relation to pumpkin puree, no information is available on color degradation during storage.

The knowledge of the rheological behavior of puree is essential for the process design and quality control and it also determines consumer acceptability. Rheological properties of purees depend on temperature, physical state of the dispersion, solid concentration, A_w and the solute added (Sharma *et al.* 1996; Ahmed *et al.* 2007; Nindo *et al.* 2007).

The objective of this research is to study the effect of sweet solutes, such as glucose and xylitol, and the use of two types of packaging materials on the quality of a pumpkin puree preserved by the hurdle technology.

MATERIALS AND METHODS

Pumpkin Purees Processing and Sampling

Fresh pumpkins (*C. moschata* Duchesne ex Poiret) were purchased from the local market (Buenos Aires, Argentina). They were washed thoroughly with soap and drinking water; the last rinsing was done with chlorinated water (200 ppm NaOCl) according to good hygienic practices. From the mesocarp of each pumpkin, 2 cm-thick slices were cut perpendicular to the longitudinal axis. Cylinders of 3 cm of diameter (12.5 g) were cut from each slice. They were exposed to steam for 12 min, cooled by immersion in the chlorinated water for 3 min and dried with tissue paper to remove the excess water. After that, the cylinders were put into flasks (500-mL beaker) with the different additives according to the composition showed in Table 1 and they were homogenized (Sorvall Omnimixer, OMNI Corporation International, Waterbury, CT). The pH was adjusted to 4.00 by citric acid addition. Then, each puree (50 g) was packed in polyethylene (PE) bags (B01018WA, Nasco Whirl-Pak, Modesto, CA; 3.00-mL thick; 3247.3 cm^3/m^2.d.atm [22.8C] oxygen per-

TABLE 1.
PUMPKIN PUREES COMPOSITION

Compostion (% w/w)	System I	II	III	IV
Potassium sorbate	–	0.120	0.120	0.120
Xylitol	–	–	11.00	–
Glucose	–	–	–	10.00
Aw	0.994	0.994	0.980	0.980

All systems had their pH adjusted to 4.00.

meability). In order to evaluate the effect of a packaging material with lower oxygen permeability on color stability, purees were also packed in polyvinyl chloride-polyvinylidene chloride copolymer (PCPC) bags (BB4L, Cryovac Inc., Sealed Air Corporation, Quilmes, Argentina; 59 µm thick; 30.4 cm^3/m^2.d.atm [23C] oxygen permeability). The bags were sealed and stored at 25 ± 1C in a forced convection constant temperature chamber.

Three bags from each formulation proposed were sampled at selected times and used to undertake both microbiological and physicochemical analyses.

Microbiological Analysis

Puree samples (5 g) were taken aseptically from each PE bag and placed in a sterile bag containing 45 mL of 0.1% (w/v) peptone water and homogenized for 1 min in a stomacher. Then, serial dilutions were prepared in 0.1% (w/v) peptone water to determine the level of indigenous flora. Aerobic mesophilic bacteria were investigated in plate count agar and lactic acid bacteria in Man, Rogosa and Sharpe agar. Plates were incubated at 30C for 48 h. Coliforms were investigated in Violet Red Bile Agar after incubation at 30C for 24 h. Yeasts and molds were determined by surface plate on Sabouraud agar after an incubation of 5 days at 25C. All media used were purchased from Biokar (Biokar Diagnostics, Beauvais, France).

Physicochemical Analysis

The pH of each sample was determined through a glass electrode connected to a pH meter (Cole-Parmer, Chicago, IL) into an aliquot of puree.

The A_w was measured at 25C with a Decagon CX-1 hygrometer (Decagon, Pullman, WA). The experimental error in A_w determination is 0.005 A_w units (Roa and Tapia de Daza 1991).

The soluble solids content was determined for the puree free of additives (system I) with a handheld sugar refractometer (Westover, Fisher Scientific, Pittsburgh, PA) and results were expressed as °Brix.

The potassium sorbate (KS) content of each puree formulation was measured according to the AOAC (1990) oxidation method that calls for a steam distillation followed by oxidation to malonaldehyde and measurement at 532 nm of the pigment formed between malonaldehyde and thiobarbituric acid. The precision of the technique, evaluated through the coefficient of variance, was 3.4% as established previously by Campos *et al.* (1991).

The color measurement of each puree was made using a colorimeter (Minolta Co. Ltd., Osaka, Japan) with a D65 illuminant and 10° observer. The instrument was calibrated with black and white reference tiles. After that, each system pouch was placed onto a white tile and the three-color coordinates, namely *L*, *a* and *b*, were read. *L* value quantifies the "lightness" (100 for white and 0 for black), the parameter *a* represents changes from "greenness to redness" (–80 for green and 100 for red) and *b* the change from "blueness to yellowness" (–80 for blue and 70 for yellow). Color measurements were taken in triplicate in three different places of the product packaged in the pouch and average values were calculated.

The color stability of pumpkin puree was also evaluated through the total color difference (ΔE) calculated by the following equation:

$$\Delta \mathrm{E} = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (1)$$

where L_0, a_0 and b_0 represent the reading at time zero, and *L*, *a* and *b* represent the instantaneous individual readings during storage.

The rheological measurements were carried out using a rotational viscosimeter (Rotovisco RV 12, Haake, Buchler Instruments, Inc., Paramus, NJ) equipped with an M-500 measuring head and an MV1P profiled coaxial cylinder sensor system. The sample compartment was held at a constant temperature of 30C using a water bath circulator. The speed of the rotating cylinder was set to increase from 0 to 64 rpm in 1.5 min and the shear stress and shear rate were obtained from the torque and speed readings, respectively (Schramm 1981). Puree samples stored in PE bags were sheared with increasing and, then, decreasing speeds. To characterize puree behavior after eliminating hysteresis, the procedure was repeated until the ascending curve was coincident with the descending one.

Data Processing

Analysis of variance and the least significant difference test were performed to determine significant differences among log microorganism populations, pH values, residual sorbate content and color parameters.

Purees flow curves after tixotropy elimination were fitted to the Herschel and Bulkley model (Holdsworth 1993):

$$\tau = \tau_0 + K\dot{\gamma}^{n} \quad (2)$$

The yield stress (τ_0), the fluid consistency coefficient (K) and the flow index (n) were calculated by nonlinear regression analysis. The average values of the parameters were reported and the overlapping of their standard error was taken as the criterion for the estimation of significant differences among parameters.

In all cases, statistical significance was evaluated at a 5% level ($\alpha = 0.05$) and the analyses were performed using the Statgraphics computer program (Statgraphics Plus for Windows, version 5.0, 2001, Manugistics, Inc., Rockville, MD).

RESULTS AND DISCUSSION

The total soluble solids content of the pumpkin puree free of additives (system I) was 10.5 ± 0.5°Brix and its pH was 6.10. It must be pointed out that, in preliminary experiences, where the pH was not adjusted or was adjusted to a value of 5.00, a pH decrease to 4.32 ± 0.04 was observed after some days of storage, as a consequence of the development of lactic acid bacteria.

The A_w and pH of puree formulated, as described in Table 1, remained almost constant during storage.

Microbiological Analysis

Microbial development in different formulations of pumpkin puree is shown in Fig. 1, panels A, B and C. As can be seen in this figure, in all formulations, the initial microbial count was approximately 10^2–$10^{3.5}$ cfu/g, demonstrating that purees have an adequate hygienic quality. Moreover, coliforms were not detected in any sample during storage.

Microbial populations reached a level of 10^8–10^9 cfu/g in the puree free of additives (system I) after 12 days of storage. These high levels of microbial population were in accordance with visual appreciation of spoilage. The addition of 0.120% (w/w) of KS decreased mesophilic aerobes and yeasts and molds population by approximately 4 log cycles after 12 days of storage (system II versus I). On the contrary, the preservative exerted no effect on lactic acid bacteria population. The effect of KS on indigenous flora reported was as expected since many aerobic bacteria and most yeast and molds are inhibited by this preservative. However, lactic acid bacteria are less sensitive to its action (Stopforth *et al.* 2005; Bozkurt and Erkmen 2007).

After 3 weeks of storage, the lactic acid bacteria population decreased approximately 2 log cycles (system II versus system I). However, the aerobic

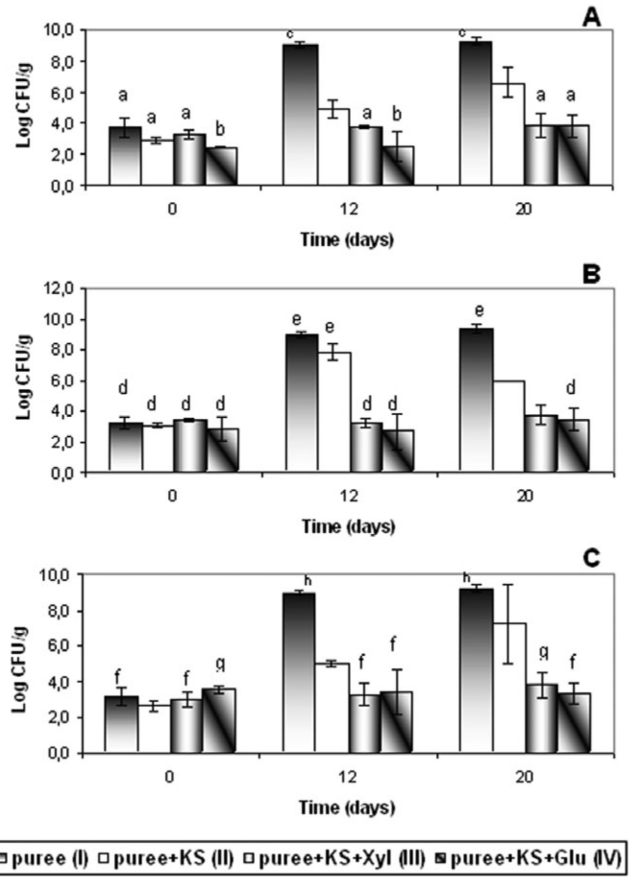

FIG. 1. EVOLUTION OF THE INDIGENOUS FLORA OF PUMPKIN PUREES
Panel A: aerobic mesophiles; panel B: lactic acid bacteria; panel C: yeasts and molds population. Error bars represent standard deviation of log cfu/g mean. cfu, colony-forming units; KS, potassium sorbate; Glu, glucose; Xyl, xylitol. Columns with the same letter are not significantly different ($\alpha = 0.05$)

mesophilic and yeast and mold population reached levels of approximately 10^6–10^7 cfu/g, revealing that an additional hurdle was needed to preserve the puree.

The depression of A_w to 0.98 through xylitol or glucose addition (systems III and IV, respectively) kept microbial population counts below 5.10^4 cfu/g during storage, rendering a microbiologically acceptable puree. Combinations of hurdles herein applied to pumpkin puree had been proved previously as suitable for preservation of many fruits (Leistner 2000).

The results obtained demonstrated that the addition of enough glucose or xylitol to depress A_w to 0.98 for a steam-cooked pumpkin puree of pH 4.00 and containing 0.120% (w/w) of KS assured microbiological stability for 3 weeks at 25C.

Physicochemical Analysis

KS Stability. KS retention, after 3 weeks of storage, was 82% for system II, 84% for system III and 88% for system IV. These retention levels were not significantly different, suggesting that the addition of glucose or xylitol did not influence the preservative degradation. The KS content decrease observed during storage is due to the tendency of this preservative to suffer an oxidative degradation (Gerschenson and Campos 1995). This trend has been previously reported for other foods such as mango and papaya preserved by combined methods (Tapia de Daza *et al.* 1995). It must be remarked that, if significant degradation takes place, residual preservative levels can allow the growth of resistant yeasts.

Color Stability. In pumpkin purees, storage time induced the lost of redness and yellowness as shown by a decrease in *a* and *b* values, respectively (Fig. 2, panels A and B). In all cases, the decrease in *a* values was higher than the one observed for *b* values. This trend suggested that the redness degradation was bigger than the yellowness loss.

A linear correlation between *a* and *b* values and carotenoid concentration was previously reported (Ahmed *et al.* 2002; Dutta *et al.* 2006) and, as a consequence, the color measurement instead of the carotenoid determination can be a useful tool to control not only the sensory but also the nutritional quality in these food products.

The loss of redness and yellowness observed during storage was related to the high oxygen permeability of the PE bags. It is well known that β-carotene is unstable as a result of its highly unsaturated structure; the presence of oxygen promotes its degradation and, as a consequence, the color is lost (Meléndez-Martinez *et al.* 2004; Schieber and Carle 2005). Moreover, the addition of KS promoted a higher decrease of *a* and *b* values during storage (Fig. 2, panels A and B). Probably, KS, which has the structure of a short-chain fatty acid, acted as a prooxidant in studied systems. The same effect has been previously hypothesized for carbonylic compounds produced by the oxidative degradation of that preservative (Gliemmo *et al.* 2009).

In general, depression of A_w through xylitol or glucose addition to purees containing KS did not modify *a* and *b* parameters during storage, as can be observed in Fig. 2, panels A and B.

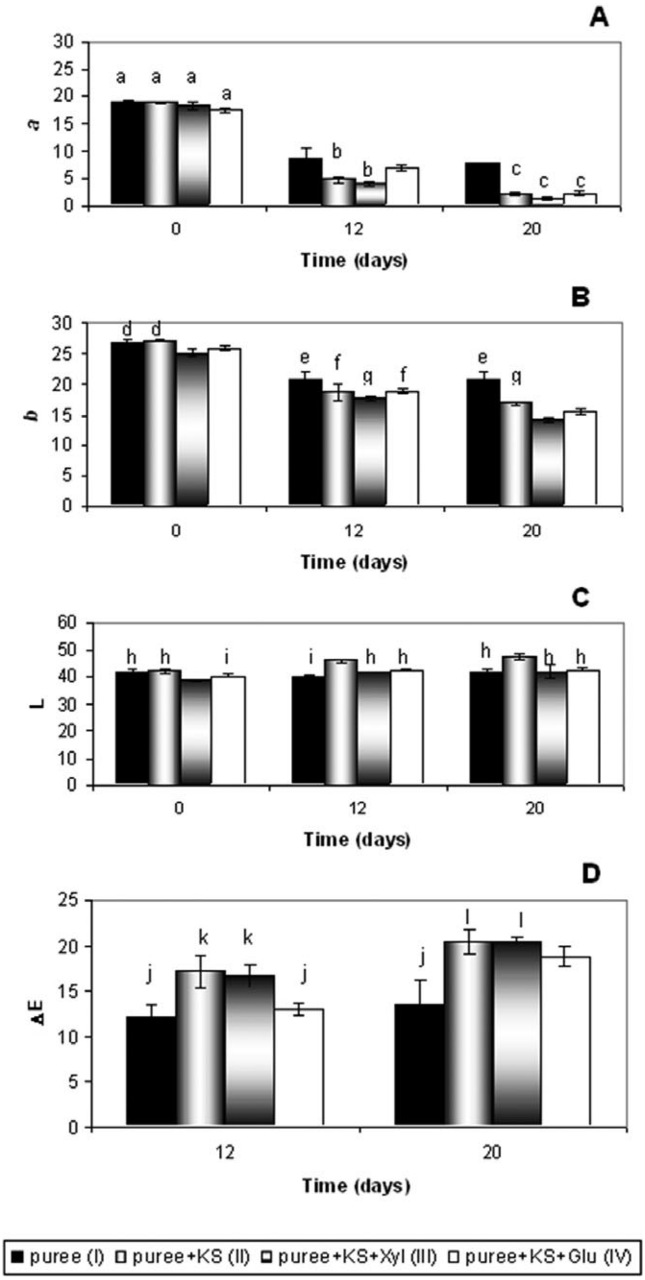

FIG. 2. EVOLUTION OF COLOR PARAMETERS OF PUMPKIN PUREES STORED IN POLYETHYLENE BAGS

Panel A: *a* parameter, panel B: *b* parameter, panel C: *L* parameter, panel D: ΔE parameter. error bars represent standard deviation of mean. KS, potassium sorbate; Glu, glucose; Xyl, xylitol. Columns with the same letter are not significantly different ($\alpha = 0.05$)

The presence of KS promoted a slight increase of lightness as a consequence of the decrease in redness and yellowness previously mentioned (Fig. 2, panel C).

The use of PCPC bags instead of PE ones prevented color loss, as can be seen in Fig. 3. Parameters *a* and *b* remained almost constant during storage; although a slight loss of color was observed after 20 days of storage. However, the magnitude of color loss was minimal in relation to the one observed for PE bags. With this packaging material, in general, no changes in lightness was observed for purees during storage (Fig. 3, panel C). The color stability of pumpkin purees stored in PCPC bags was related to the low oxygen permeability of this packaging material. Moreover, the prooxidant action of KS was not observed in this packaging material.

The ΔE increased as *L, b* and *a* values decreased (Figs. 2 and 3, panel D). The values of puree packed in PCPC were lower than in PE.

It must be taken into account that observations performed by six members of the laboratory staff concluded that a value of ΔE of 7.00 was the maximum value for which visual color differences among puree samples were not detected (data not shown). During storage, all purees packed in PCPC reached ΔE values lower than 7.00 (Fig. 3, panel D). Thus, these results allowed us to inform visual color stability for purees packed in PCPC.

The results obtained demonstrate that, independently of the solute used to depress A_w, the use of PCPC bags prevented carotenoid oxidation and, as a consequence, protected color of pumpkin puree.

Rheological Behavior. All pumpkin purees exhibited a hysteresis loop demonstrating a time-dependent flow behavior and an initial overshoot during start-up. These trends are shown in Fig. 4 for the puree containing KS and glucose (system IV), as an example.

The presence of an initial overshoot was reported for many viscoelastic materials (Navarro *et al.* 1999; Marques *et al.* 2006) and is due to the fact that, when a shear rate is suddenly imposed on a viscoelastic fluid, shear stress produces a transient deformation which is displayed as an initial overshoot before reaching the steady state (Steffe 1996).

Once the thixotropic structure was broken, all purees presented a yield stress and a non-Newtonian behavior which has been previously reported for other food purees (Sharma *et al.* 1996; Ahmed *et al.* 2000, 2004; Dutta *et al.* 2006; Maceiras *et al.* 2007). Experimental data fitted adequately to the Herschel and Bulkley model since the coefficient of determination was always higher than 0.99 with a standard error between 1.30 and 2.00. Parameters of that model obtained are shown in Table 2.

It should be noted that in the puree free of additives (system I), microbial spoilage took place during storage promoting phase separation and syneresis.

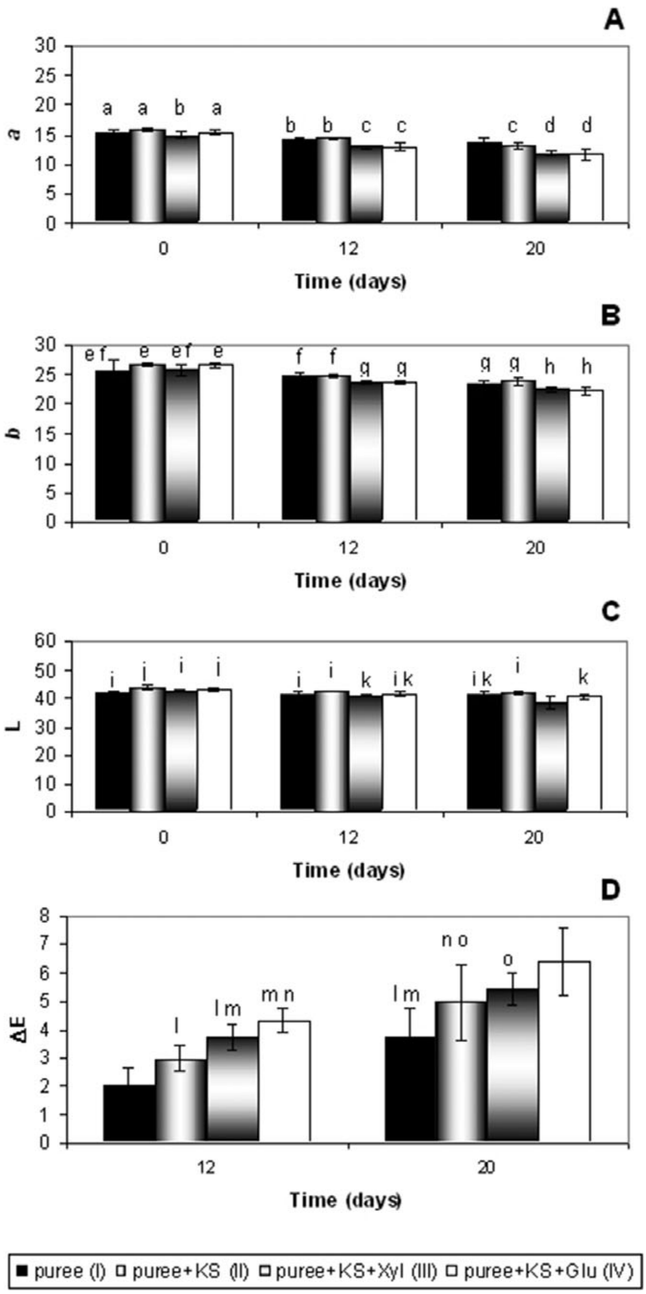

FIG. 3. EVOLUTION OF COLOR PARAMETERS OF PUMPKIN PUREES STORED IN PCPC BAGS

Panel A: *a* parameter, panel B: *b* parameter, panel C: *L* parameter, panel D: Δ*E* parameter. error bars represent standard deviation of mean. KS: potassium sorbate, Glu: glucose, Xyl: xylitol. Columns with the same letter are not significantly different ($\alpha = 0.05$)

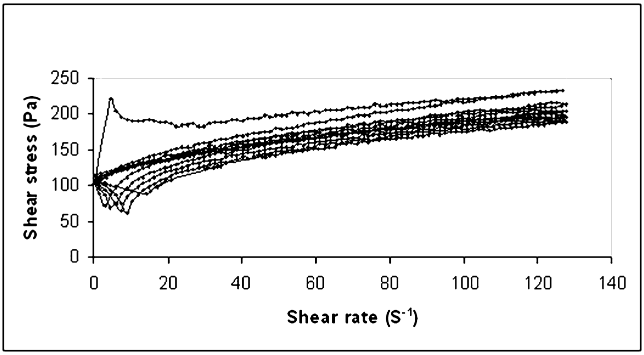

FIG. 4. RHEOGRAM OF PUMPKIN PUREE CONTAINING POTASSIUM SORBATE AND GLUCOSE AFTER 20 DAYS OF STORAGE AT 25C

TABLE 2.
HERSCHEL–BULKLEY MODEL COEFFICIENTS FOR PUMPKIN PUREES THROUGHOUT STORAGE

Pumpkin puree	Storage time (days)	Yield stress* τ_o(Pa) (m ± sd)	Consistency* index, K (Pa. S^n) (m ± sd)	Flow behavior* index, *n* (dimensionless) (m ± sd)
I	0	105.4 ± 3.5a	7.9 ± 0.23b	0.52 ± 0.01c
II	0	98.0 ± 4.2a	7.2 ± 0.46b	0.52 ± 0.01c
II	12	110.2 ± 2.0a	8.1 ± 1.2b	0.50 ± 0.01c
II	20	112.8 ± 2.2a	7.9 ± 0.4b	0.51 ± 0.02c
III	0	87.2 ± 4.9	6.11 ± 0.42	0.54 ± 0.01c
III	12	93.4 ± 2.9	6.61 ± 0.22	0.54 ± 0.01c
III	20	99 ± 1.4a	6.81 ± 0.20	0.53 ± 0.01c
IV	0	97.6 ± 1.8a	7.07 ± 0.63b	0.53 ± 0.02c
IV	12	109.6 ± 1.6a	7.44 ± 0.74b	0.53 ± 0.02c
IV	20	104.9 ± 0.9a	7.14 ± 0.04b	0.53 ± 0.01c

* Parameters with the same letter are not significantly different ($P > 0.05$).
m + sd, mean + standard deviation.

Therefore, for this system, the rheological behavior was evaluated only at time zero.

In all cases, the flow behavior index (*n*) was within the range 0.50–0.54, indicating that the purees behaved as pseudoplastic fluids. No effect of system composition or storage time was observed in *n* value. This trend was also reported for other food products like *dulce de leche* (Rovedo *et al.* 1991), fruit puree (Vitali and Rao 1982) and tomato concentrates (Rao and Cooley 1983).

The addition of KS to the puree free of additives or addition of glucose to the puree containing KS produced no changes in the yield stress and in the consistency coefficient (system II versus I; system IV versus II, respectively). On the contrary, the addition of xylitol diminished the yield stress and consistency coefficient and this effect was observed for all storage times (system III versus II). It can be observed that this system showed an increase of both parameters with storage time.

The effect of solutes on the flow behavior of purees is diverse and different trends were reported in the literature depending on the vegetable in which the puree was based as well as on the solute added. Many authors analyzed the effect of solutes on the rheology and gel formation and proposed that content and proportion of pectin present in the puree determines the behavior (Mizrahi 1979; Van Buren 1991). In pumpkin puree, the pectins found are mainly high methoxyl, water soluble and highly ramified, as was determined by de Escalada Pla *et al.* (2007). It must be remarked that the conditions for gelation were not present in our system because the concentration of solutes was low. However, the solutes added could interact with water and/or with pectins and, therefore, the rheological behavior of the system could be modified in different ways depending on solute characteristics (Tsoga *et al.* 2004).

In the case of xylitol, the diminished the yield stress and consistency coefficient obtained for the puree could be explained taking into account two facts: (1) the addition of xylitol would decrease water immobilization in its surroundings as was stated by Carlevaro *et al.* (1998), that it is a hydrophilic compound that adopts a linear conformation in water (Galema *et al.* 1994; Tsoga *et al.* 2004); and (2) the addition of xylitol would promote dilution of the high molecular weight compounds naturally present in the pumpkin puree that govern the rheology of the system. This effect was observed by Sopade and Filibus (1995) for the addition of sucrose to akamu, a semisolid maize food.

In the case of glucose, its addition produced no changes in the yield stress and in consistency coefficient and, probably, this trend might be the result of the balance of the following effects: (1) the addition of glucose, a compound with a pyranosic structure, would increase water immobilization in its surroundings (Galema *et al.* 1994; Tsoga *et al.* 2004); (2) the addition of glucose would promote a dilution of high molecular weight compounds naturally present in the pumpkin puree which govern the rheology of the system, as previously mentioned for the case of xylitol; and (3) the addition of glucose produces a thickening action in fruit purees and in aqueous solutions containing high methoxyl pectins (Oakenfull 1991; Maceiras *et al.* 2007). Effects (1) and (3) would promote an increase in the yield stress and consistency coefficient and effect (2) would promote a decrease in the yield stress and consistency coefficient. The net balance among precited effects might have determined the null influence of glucose addition on the rheological behavior.

In general, the yield stress and consistency coefficient increased slightly during storage. Probably, polysaccharide chain interactions increased during storage and, as a consequence, the yield stress increased.

CONCLUSION

The addition of glucose or xylitol to depress A_w to 0.98 was necessary to assure microbial stability for 3 weeks at 25C for a pH 4.00 pumpkin puree, containing KS and submitted to a 12-min vapor heat treatment.

Neither glucose nor xylitol presence influenced preservative degradation nor loss of redness and yellowness during storage. The color loss was related to the high oxygen permeability of PE bags which promoted carotene oxidation. The use of a packaging material with low oxygen permeability such as PCPC protected the color of pumpkin; this trend was not modified by the solute added to depress A_w.

From the rheological point of view, the addition of glucose to the puree containing KS produced no changes in the yield stress and in the consistency coefficient. On the contrary, the addition of xylitol diminished the yield stress and consistency coefficient independently of storage time.

The results obtained emphasize the advantage of the use of glucose as well as the importance of an appropriate choice of solutes added to depress A_w in order to improve the quality of pumpkin puree.

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