Plasticized Starch-Based Coatings To Improve Strawberry (*Fragaria* × *Ananassa*) Quality and Stability

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Starch-based coatings were applied to extend storage life of strawberries (*Fragaria* × *ananassa*) stored at 0 °C and 84.8% relative humidity. The effects of amylose content of the starch, the type of plasticizer (glycerol and sorbitol), and the inclusion of antimicrobial agents on coating formulation were analyzed. Microstructure characterization of coatings was related to water vapor permeability (WVP) observations. Coatings made with starches with the higher amylose content decreased WVP and weight losses and retained fruit firmness for longer periods than coatings formulated with medium amylose content starches. Coatings with sorbitol showed lower WVPs than glycerol ones. Both sorbitol and glycerol reduced weight losses and maintained texture and surface color of fruits, with 20 g/L sorbitol being the most effective plasticizer option. Modifications of physiological parameters in strawberries such as anthocyanin content, reducing and nonreducing sugars, titratable acidity, and pH were slowed for coated fruits. The formulations with potassium sorbate reduced microbial counts, extending strawberry storage life from 14 days (for control fruits) to 28 days in coated strawberries. The addition of citric acid enhanced antimicrobial action of potassium sorbate.

Keywords: *Starch coating; plasticizer; strawberry; water vapor permeability; refrigerated storage*

INTRODUCTION

Consumers are choosing minimally processed foods that are prepared for convenient consumption and are distributed in a freshlike state (King and Bolin, 1989). Controlling tissue physiology and microbial growth is critical for minimally processed fruits and vegetables. Both physiological and microbial actions can start biochemical changes that lead to quality loss. Barriers to microorganism growth, in addition to cold temperatures, must be used to produce and market minimally processed fruits and vegetables. The strategy to extend postharvest life of fruit products should be based on reducing three factors: maturation and senescence, dehydration, and onset and rate of microbial growth. The use of semipermeable coatings on fruits has been shown to affect fruit physiology to retard ripening and postharvest metabolism, thus extending fruit storage life (Lowings and Cutts, 1982; Trout et al., 1952; Olorunda and Aworth, 1984; Rolle and Chism, 1987).

Hydrophilic films and coatings, such as those of proteins and polysaccharides, provide under certain conditions of relative humidity (RH) and temperature a good barrier to oxygen and carbon dioxide transmission but a poor barrier to water vapor (Guilbert, 1986; Kester and Fennema, 1986; Gontard et al., 1993). The oxygen and carbon dioxide barrier leads to a reduction in respiration rate by limiting exposure to ambient oxygen and increasing internal carbon dioxide, thus retarding ripening. The poor water vapor barrier allows movement of water vapor across the film, thus preventing water condensation that can be a potential source of microbial spoilage in soft vegetables (Park et al., 1994).

Highly perishable fruits such as berries and tropical fruits are appropriate products to protect with coatings because they are expensive and exhibit a short storage life. Strawberries, as a typical soft fruit, have a high physiological postharvest activity. As a consequence, they have short ripening and senescence periods that make marketing of this high-quality fruit a challenge.

Strawberry fruit (*Fragaria* × *ananassa*) is usually attacked by fungi, mainly *Botrytis cinerea* and *Rhizopus* sp., shortening its storage life (Maas, 1981). To maintain quality and delay strawberry decay, rapid cooling after harvest and storage at low temperatures, typically 0 °C with high humidity, are highly recommended.

El Gaouth et al. (1991a) applied a chitosan-based coating to strawberries. The coating modified the internal fruit atmosphere and decreased water evaporation, resulting in delayed strawberry ripening. Additionally, chitosan has antifungal properties, but it is not considered a generally recognized as safe (GRAS) substance in the United States. Several researchers studied the application of coatings to vegetables such as tomatoes, cucumbers, and red peppers (El Gaouth et al., 1991b, 1992) and to fruits such as bananas (Banks, 1984), apples (Drake et al., 1987), and mangoes (Dhalla and Hanson, 1988).

Edible coatings can be made from food materials regarded as GRAS such as proteins, cellulose derivatives, starch, and other polysaccharides. Although starch is the most commonly used agricultural raw

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material for biodegradable films, few papers have dealt with starch-based coatings (Krochta and De Mulder-Johnston, 1997). Amylose is responsible for the film-forming capacity of starches. In addition, another important component of edible films is the plasticizer, required to overcome film brittleness and improve flexibility and extensibility of the films. Plasticizers reduce intermolecular forces and increase the mobility of polymer chains. They must be compatible with the film-forming polymer; hydrophilic compounds such as polyols (glycerol, sorbitol, polyethylene glycols) and lactic acid are commonly used in hydrophilic film formulations (Gontard et al., 1993). The effect of plasticizer on water vapor and gas permeabilities is controversial, depending on matrix, plasticizer type, and environmental conditions (Herald et al., 1996; Parris et al., 1995; Mc Hugh et al., 1994).

The objectives of the present work were (1) to develop a starch-based coating to improve strawberry storage life and to analyze water vapor permeability and microstructure of the coatings and films; (2) to analyze the effects of starch-based coating formulations (amylose content and glycerol or sorbitol as plasticizers) on strawberry quality attributes (texture, weight loss) and on postharvest parameters (pH, titratable acidity, total and reducing sugars and anthocyanin contents); and (3) to determine the effects of antimicrobial agents included in the coating formulation (potassium sorbate and citric acid) on microbial growth.

MATERIALS AND METHODS

The starches used for coating formulations were selected to analyze the effect of amylose content (due to its film-forming capacity). Easily available starches such as corn and potato with medium amylose content and specific starches such as genetically modified and enriched ones with high amylose content were used. Two groups were selected:

(1) Starches with medium amylose content (MAS) included (1.1) commercial corn starch (Molinos Río de la Plata, Buenos Aires, Argentina) with 250 g of amylose/kg of starch and (1.2) commercial potato starch (Fecofar, Buenos Aires, Argentina) with 230 g of amylose/kg of starch; both were available at a relatively low cost.

(2) Starches with high amylose content (HAS) included (2.1) high amylose corn starch, genetically modified, amylomaize VII (Amaizo, Hammond, IN), with 650 g of amylose/kg of starch and (2.2) high amylose corn starch product (HAP). HAP, an amylose enriched product, was obtained as described in a previous paper (García et al., 1995) by corn starch fractionation with $MgSO_4$. HAP contains 500 g of amylose/kg of starch.

In all cases, aqueous solutions of 20 g/L starch were cold-gelatinized with 10 g/L NaOH to obtain the coatings (Young, 1984; García et al., 1995). These suspensions were then neutralized with 7M H_3PO_4. The plasticizers, glycerol or sorbitol (0, 10, and 20 g/L of formulations, corresponding to 0, 50, and 100 g of plasticizer/100 g of starch), were added after neutralization.

Sample Preparation. Strawberries (*Fragaria* × *ananassa* cv. Selva), at commercial ripening stage (75% red color), grown in greenhouses of a local farm, were harvested and immediately treated. Fruits of uniform size, free of physical damage and fungal infection, were used. Strawberries were dipped in chlorinated water (0.25 g of Cl_2/L), dried with air during ~30 min, dipped for 10 s in the formulated suspensions at ambient temperature, and dried again with air (1.2 m/s, 20 °C, and 84.8% RH) during ~2 h. Uncoated fruits (control) were treated similarly, replacing immersion in starch suspensions by immersion in distilled water. Samples were stored in a cold chamber at 0 °C and 84.8% RH.

In each experiment, 120 fruits were used for the control sample and 120 fruits were used for each tested formulation containing 0, 10, and 20 g of glycerol or sorbitol as plasticizer per liter. Thus, each experimental lot contained 720 fruits. The whole experiment with the corresponding determinations was repeated with two different lots of strawberries. Fruits with coating formulations and the control were tested at the same time.

Microscopic Observations. Coated strawberries (whole sample and cross sections) were monitored under a stereomicroscope Leitz (Leitz, Wetzlar, Germany) to analyze coating integrity. To visualize the coatings, samples were stained with iodine. The stock solution contained 2 g/L I_2 and 20 g/L KI (Lyne, 1976).

Unstained coatings were analyzed by scanning electron microscopy (SEM); to give an insight into system microstructure, samples were prepared on an inert support. HAS and MAS suspensions with 10 and 20 g/L of glycerol or sorbitol as plasticizer were cast on aluminum foil 5 cm in diameter and dried at 60 °C in an oven until constant weight. Samples were then stored in covered storage dishes containing silica gel at 25 °C. SEM was performed with a JEOL JSMP 100 (Japan) electron microscope. Pieces of coating material were mounted on aluminum stubs using double-sided tape and then coated with a layer of gold (40−50 nm); they were examined using an accelerating voltage of 5 kV.

Determination of the Coating Water Vapor Permeability (WVP). WVPs of the different coating formulations were determined using sliced carrots as a biological model system; they were used to get a simple geometrical shape with well-defined dimensions to allow surface area calculations. Slices were 0.5 cm thick and 3.7 cm in diameter and were dipped in the coating formulations and dried with an air stream (1.2 m/s, 20 °C, and 84.8% RH) during ~2 h. Control samples were dipped in distilled water to receive a similar treatment.

Samples were equilibrated for 24 h in a desiccator cabinet maintained at 98.2% RH with a $K_2Cr_2O_7$ (Anedra, Buenos Aires, Argentina) saturated solution at 25 °C. Water activity (a_w) of carrots was measured in a thermoconstanter Humidat TH2/RTD-33/BS, model AG CH-8050 (Novasina, Zurich, Switzerland). Samples were then placed on the bottom of test cups, weighed in an analytical balance, and placed in another cabinet equilibrated at 33.3% RH with $MgCl_2 \cdot 6H_2O$ (Anedra) at 25 °C. At regular intervals samples were weighed to calculate weight loss with time.

Water vapor flux (Fl) was calculated as

$$\mathrm{Fl} = (\Delta w/\Delta t)(1/A) \quad (1)$$

where $(\Delta w/\Delta t)$ is the slope of the weight loss versus time curve in g/s and A is the exposed area ($1.656\ 10^{-3}$ m^2).

The resistance to water vapor transmission through the air surrounding the sample was calculated by considering weight loss versus time data for uncoated samples.

In these parallel surfaces composite systems, water vapor transfer takes place in series through the coating and the surrounding air as follows (Figure 1):

$$\mathrm{Fl} = P(p_i - p_x) = (\mathrm{WVP}/e)(p_i - p_x) = k_{air}(p_x - p_a) \quad (2)$$

In eq 2, Fl is the water vapor flux [g/(m^2 s)], P is the water vapor permeance of the coating [g/(m^2 s Pa)], WVP is the water vapor permeability expressed in g/(s m Pa), e is the coating thickness in m, p_i is the partial water vapor pressure at the vegetable-coating interface (Pa), p_x is the partial water vapor pressure at the coating−air interface (Pa), p_a is the partial water vapor pressure in the environment with 33.3% RH at 25 °C expressed in Pa, and k_{air} is the water vapor mass transfer coefficient in air [g /(m^2 s Pa)].

From eq 2 the following equation is obtained:

$$(p_i - p_a) = \mathrm{Fl}[(1/P) + (1/k_{air})] \quad (3)$$

Thus, coating permeability can be calculated if the value of

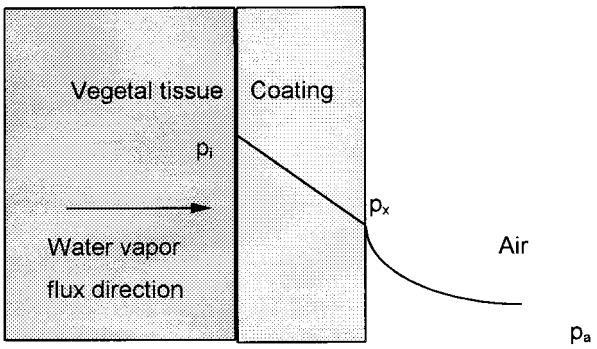

Figure 1. Scheme of water vapor mass transfer through coated vegetable tissue.

k_{air} is known. To determine k_{air}, a control assay was performed with uncoated sliced carrots; water vapor flux through air was measured and $k_{air} = 13 \times 10^{-7}$ g/(m^2 s Pa) was obtained. Considering that a_w of carrots was 0.988, the corresponding p_i value was 3129.18 Pa (total water vapor pressure $P_{tot} =$ 3167.19 Pa, at 25 °C).

In a previous work coating thickness was determined, with values between 40 and 50 μm obtained (García et al., 1996). WVP was calculated according to eq 2:

$$\text{WVP} = Pe \tag{4}$$

Weight Loss. A lot of 10 fruits was used to measure weight loss. The same fruits were weighed at the beginning of the experiment and at 5, 12, 18, 26, and 29 days of storage. The results were expressed as percentage loss of initial weight.

Firmness. The compression force of strawberry flesh was measured with an Instron testing machine (model 11 41, Instron Corp., Canton, MA) using a compression cell of 5 kg and an individual plate 1 cm in diameter, with a 10 cm/min crosshead speed.

Firmness was measured at 0, 5, 12, 18, 22, and 26 days of storage using five fruits each time. Strawberries of uniform size, from which the calyx and its opposite extreme were removed to obtain even surfaces, were used to determine the break force (peak height) expressed in newtons (N). The force was applied perpendicularly to both even surfaces.

Anthocyanin Content. Five strawberries at 1, 7, 14, 22, and 28 days of storage at 0 °C were maintained at −60 °C and were processed in an OmniMixer tissue homogenizer. Anthocyanin content was determined on 300 mg aliquots; pigment was extracted with 10 mL of 10 g/L HCl in methanol at 0 °C. Samples were centrifuged at 577*g* at 0 °C, and the absorbance of the supernatants was measured at 515 nm in a DU 650 Beckman (Beckman, Fullerton, CA) spectrophotometer. Anthocyanin content was calculated as pelargonidin-3-monoglucoside (molar absorptivity = 36000) (Woodward, 1972). Anthocyanin content was expressed in nanomolar anthocyanin per gram of fruit (nM/g).

Surface Color. A lot of 10 fruits was used to perform colorimetric analysis. Color measurements were carried out on the same 10 fruits with a Hunterlab colorimeter, equipped with an optical sensor, model D-25-A3 (Hunter Associates Laboratory, Inc., Fairfax, VA), calibrated with an appropriate device to reduce sampling area. Hunter scale was used: lightness (*L*) and chromaticity parameters *a* and *b* were recorded at 1, 8, 15, and 22 days of storage. The ratio of chromaticity parameters (*a*/*b*) was calculated.

Determination of Reducing and Total Sugar Content and Titratable Acidity. An OmniMixer 17106 (Sorval, DuPont Instruments, Waterbury, CT) was used to obtain homogenates for both titratable acidity and sugar content determinations, using five fruits for each test. Processing time in the OmniMixer was ∼2 min. Samples were analyzed at 1, 7, 14, 22, and 28 days of storage.

Homogenate pH was measured in a pHmeter. Acidity was determined using a 10 g aliquot of the homogenate brought to 100 mL solution with distilled water and titrated with 0.1 M NaOH to an end point of pH 8.1 (AOAC, 1980). Titratable acidity was expressed as milliequivalents of citric acid per 100 g of fruit. Two samples were analyzed for each storage time.

Sugar content of strawberries was determined using a 10 g aliquot of the homogenate and 25 mL of ethanol. The mixture was homogenized again, and then its volume was reduced by heating in a water bath at 90 °C for 10 min. It was centrifuged at 2310*g* for 10 min, and the supernatant was brought to 50 mL in a matrass. The extract was diluted 10 times, and then a 50 μL aliquot was used to determine reducing sugars and another 50 μL aliquot for total sugars. Samples were treated as described for standards. Nonreducing sugars were calculated by the difference between total and reducing sugars. Sugars were expressed as grams of glucose per 100 g of fruit.

The content of reducing sugars was determined spectrophotometrically using a modification of the Somogyi−Nelson method (Southgate, 1976). Calibration curves of glucose (Sigma, St. Louis, MO) were determined with a 0.18 g/L standard solution. Glucose absorptivity was calculated from the calibration curve, being 393.767 ± 5.772 L/(g cm), and the linear correlation coefficient was $r^2 = 0.99914$.

For total sugar determination, a preliminary hydrolysis of the samples was performed with 0.1 M HCl for 10 min; once hydrolyzed, samples were processed as described for reducing sugars. In both cases sample absorbance was measured at 520 nm.

Soluble, Insoluble, and Total solids. To determine soluble and insoluble solids, 2 g aliquots of the homogenate, prepared as described previously, were centrifuged at 2310*g*. Soluble solids of the supernatant were measured with an Abbe (London) 60 refractometer following the AOAC (1980) procedure at 20 °C, and insoluble solids were determined from the pellet. Total solids were determined on another 2 g aliquot of the homogenate. Aliquots for total solids and centrifuged pellets were dried in weighed pans at 60 °C to constant weight during ∼4 days. Soluble solids and solid content were expressed in °Brix and grams per 100 g of fruit, respectively. Assays were performed by triplicate.

Microbiological Assays. Strawberries were washed with chlorinated water; part of them were used as control, and the others were coated with different formulations as previously described. The following eight coating formulations were tested: (a) 20 g/L of potato starch or HAP without plasticizer, (b) 20 g/L of potato starch or HAP with 20 g/L sorbitol, (c) 20 g/L of potato starch or HAP with 20 g/L sorbitol and 0.2 g/L potassium sorbate (Merck) as an antimicrobial agent and (d) the same composition as the formulation c plus citric acid (Parafarm, Buenos Aires) to reach pH 4 in the formulation.

Control and coated strawberry samples were analyzed at 1, 7, 14, 22, and 28 days of storage at 0 °C. Samples of 18 g were homogenized in a Stomacher Seward (London) model 400 with 180 mL of 10 g/L peptone water. Several dilutions were prepared from the homogenate using 10 g/L peptone water. Dilutions were performed in duplicate. Microbial counts were carried out in two ways:

(a) Aerobic mesophilic microorganisms were determined by plating 1 mL of the corresponding dilution in PCA (plate count agar, Merck, Darmstadt, Germany); plates were incubated at 30 °C for 2 days. Aerobic psycrotrophic microorganisms were counted by plating 1 mL of the corresponding dilution in PCA; plates were incubated at 4 °C for 7 days. For yeasts and mold counts 0.1 mL of the corresponding dilution was plated in yeast extract glucose chloramphenicol (YGC; Merck); plates were incubated at 30 °C for 5 days.

(b) Petrifilm plates 6400, 6407, and 6410/6416 (3M, St. Paul, MN) were used to count aerobic mesophilic, psychrotrophic, molds and yeasts, and coliforms, respectively. In all cases, 1 mL of the corresponding dilution was seeded and incubated under the previously described conditions.

Viable counts were expressed as log colony-forming units (CFU) per gram of fruit.

Statistical Analysis. Experiments were designed according to a complete randomized block design. Systat-software

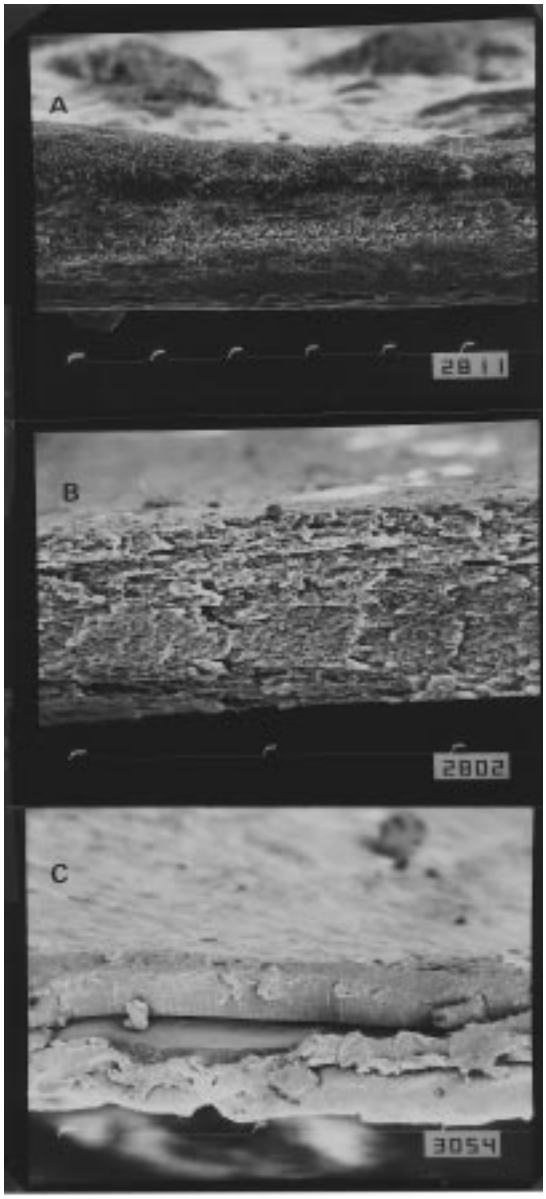

Figure 2. Scanning electron micrograph of (A) high amylose rich product-based coating without plasticizer, (B) corn starch-based coating without plasticizer, and (C) corn starch-based coating with 20 g/L glycerol. Magnification: 100 μm between marks.

(Systat, Inc., Evanston, IL, 1990) version 5.0 was used for all statistical analysis. Analysis of variance (ANOVA), Fisher LSD mean comparison test, and regression analysis were applied. The significance levels used were 0.05 and 0.01.

RESULTS AND DISCUSSION

Microscopic Observations. SEM of plasticized coatings showed smooth surfaces without pores or cracks compared to formulations without plasticizer. HAS coatings were more compact than MAS ones. The higher the amylose content, the more compact the coating (Miles et al., 1985a,b; Noel et al., 1992). Cross sections of coatings without plasticizer showed a multilaminar structure, HAS coatings being thicker than MAS ones. Coatings with plasticizer showed a more compact matrix compared to unplasticized coatings (Figure 2).

Strawberries coated with formulations containing plasticizer were homogeneous and covered the whole surface of the fruit including the achenes. The need of plasticizer addition for coating integrity was evidenced by microscopic observation of iodine-stained coatings (García et al., 1998). Coatings without plasticizer were brittle, and some cracks were observed on them. Donhowe and Fennema (1993) also stressed that amylose molecules without plasticizer produce brittle films.

Table 1. Effect of Plasticizer and Starch Type on Water Vapor Permeability of Coated Strawberries

starch base	plasticizer	water vapor permeability $\times 10^{10}$ [g/(s m Pa)]
potato	WP[a]	3.66 ± 2.32
	2G[b]	2.41 ± 1.29
corn	WP	3.68 ± 2.24
	2G	2.57 ± 1.04
	2S[c]	1.75 ± 0.14
amylomaize VII	WP	2.36 ± 0.90
	2G	1.87 ± 0.82
HAP	WP	2.62 ± 1.39
	1G	2.31 ± 0.54
	2G	2.14 ± 0.75
	1S	1.31 ± 0.07
	2S	1.21 ± 0.15

[a] Coating formulation without plasticizer. [b] Coating formulation with 10 g/L (1G) or 20 g/L (2G) glycerol. [c] Coating formulation with 10 g/L (1S) or 20 g/L (2S) sorbitol.

WVP of the Coatings. Coatings without plasticizer led to higher WVP values than those with plasticizer (Table 1); this was attributed to the presence of pores and cracks. HAS films with a more compact structure as seen by SEM had the lowest values for WVP (Table 1). In the present work, increasing plasticizer concentration, either glycerol or sorbitol, decreased WVP of starch-based coatings; sorbitol gave the lowest WVP values (Table 1). These results agreed with those of McHugh and Krochta (1994a), who found a similar trend for protein films obtained from whey. A previous work showed that the maximum amount of glycerol suitable for these formulations was 20 g/L; higher concentrations led to plasticizer migration to the surface, giving an undesirable sticky aspect (García et al., 1998). Amounts of sorbitol >20 g/L led to inconveniently long drying times. Glycerol is a small molecule and is easily dispersed in film suspensions; it may migrate to the surface when the film is cast. Migration rate depends on the type of functional groups, their polarity and film matrix structure, and plasticizer content (Park et al., 1994).

Generally, plasticizer addition is related to the decrease of intermolecular attractions and the increase of polymer chain mobility. Also, small molecules such as glycerol may fill vacancies within the polymer matrix (Porter, 1980). McHugh and Krochta (1994a,b) found that the addition of glycerol increased protein film permeability. Herald et al. (1996) and Gennadios and Weller (1990) reported that the incorporation of selected additives into corn zein films did not produce significant differences in WVP. Parris et al. (1995) noted that sorbitol reduced WVP of protein films. In polysaccharide films such as those of methylcellulose, Porter (1980) reported that permeability is only slightly modified by glycerol addition. McHugh and Krochta (1994a), using plasticizer concentrations similar to those used in the present work, found that alginate and pectin films with sorbitol exhibited better barrier properties than either lactate or glycerol films.

Differences in permeability of plasticized films depend on several factors such as physical state and molecular

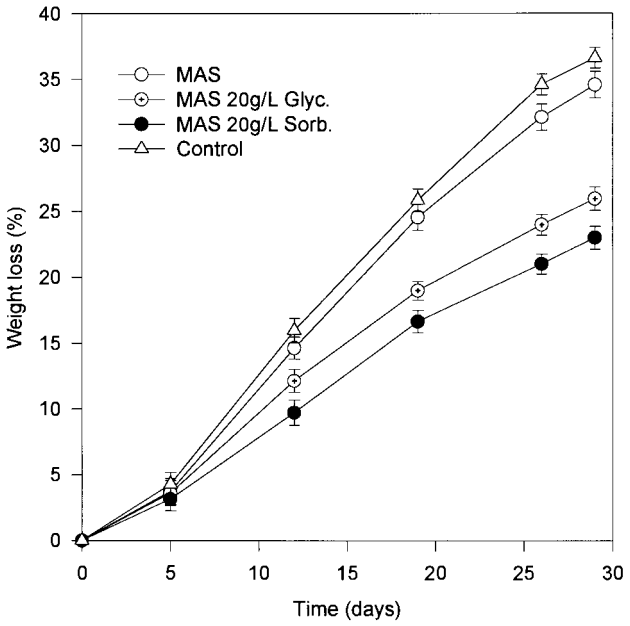

Figure 3. Effect of plasticizer on weight loss during storage at 0 °C of control and starch-based coated strawberries. Compositions of coating formulations: (△) control, uncoated fruits; (○) 20 g/L corn starch (MAS) without plasticizer; (⊗) 20 g/L corn starch (MAS) with 20 g/L glycerol; (●) 20 g/L corn starch (MAS) with 20 g/L sorbitol. Bars indicate standard errors. $LSD_{0.05} = 0.342$.

weight of the plasticizer, chemical interaction between the plasticizer and the permeant, and alterations in film structure. In the present work the higher WVP of glycerol films compared to sorbitol ones can be attributed to glycerol liquid state, its small size, low molecular weight, and high compatibility with amylose matrix, which favored plasticizer migration, facilitating amylose mobility and giving a looser structure.

Weight Loss. Weight loss of strawberries increased as a function of storage time for both control and coated fruits; all coatings provided a beneficial effect on weight loss (Figures 3 and 4). Starch source had a significant effect reducing weight loss; MAS and HAS coatings and control samples differed significantly ($P < 0.05$). HAS formulations showed the lowest weight losses (Figure 4). Because amylose is responsible for network formation (Young, 1984), coating becomes more compact with increasing amylose content.

Weight losses of fruits coated with formulations without plasticizer were similar to those of control fruits; these results were attributed to the presence of pores and cracks on these coatings. Regardless of the plasticizer used, lower weight losses were observed for fruits treated with plasticized coatings (Figure 3).

Plasticizer concentration showed significant differences ($P < 0.05$) at the end of the assay, 20 g/L being the most effective concentration for both sorbitol and glycerol. Sorbitol coatings led to significantly lower fruit weight losses than glycerol for both MAS- and HAS-based coatings (Figure 4).

Firmness. Texture loss and changes in appearance are the most noticeable changes occurring in fruits and vegetables during prolonged storage and are related to metabolic changes and water content. The rate and extension of firmness loss during ripening of soft fruits, such as strawberries, is one of the main factors to determine fruit quality and postharvest shelf life. According to Manning (1993), fruit softening is attributed

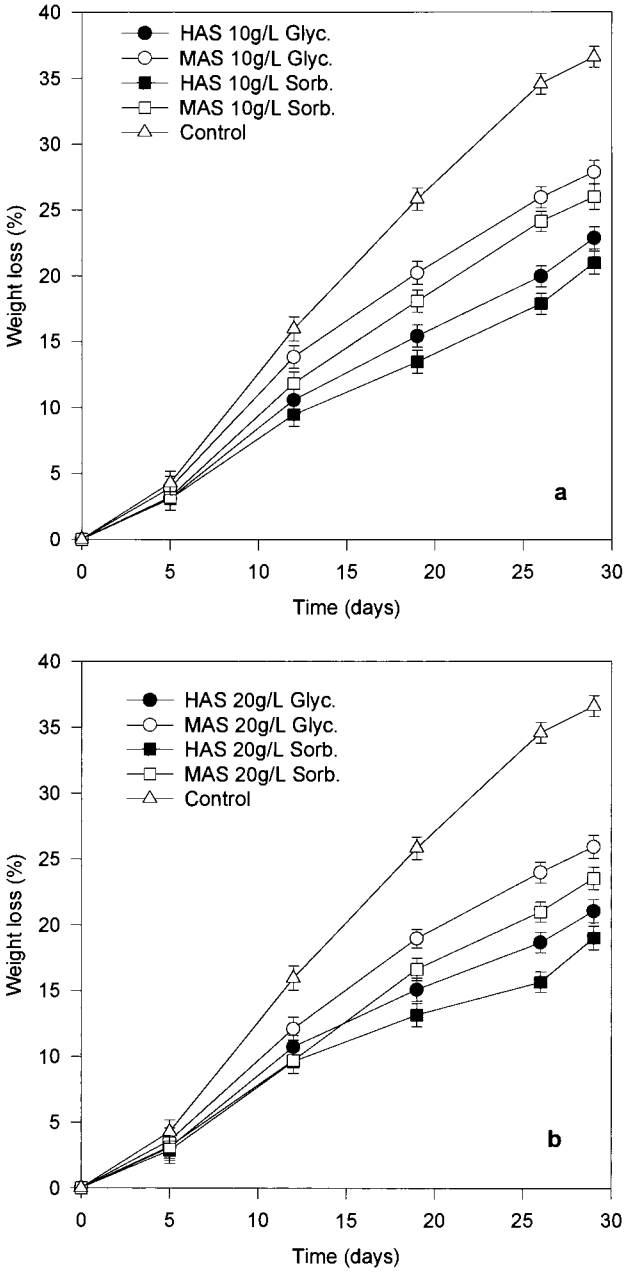

Figure 4. Effect of plasticizer and starch type on weight loss during storage at 0 °C of control and starch-based coated strawberries. Compositions of coating formulations: (a) 20 g/L high amylose rich product (HAS) and corn starch (MAS), both with 10 g/L plasticizer; (b) 20 g/L high amylose rich product (HAS) and corn starch (MAS), both with 20 g/L plasticizer. Bars indicate standard errors. $LSD_{0.05} = 0.342$.

to the degradation of cell wall components, mainly pectins due to the action of specific enzymes activity such as polygalacturonase (PG).

For both control and coated fruits breaking force decreased as a function of storage time (Figure 5). Values of firmness at initial time show no differences among the control and coated samples; thus, symbols in Figure 5 are overlapped.

All coatings showed a beneficial effect on firmness retention. Breaking forces for HAS-coated fruits were significantly higher ($P < 0.05$) than those of MAS-coated fruits regardless of the plasticizer added.

The type and concentration of plasticizer showed significant effects ($P < 0.05$) on firmness. Plasticizer

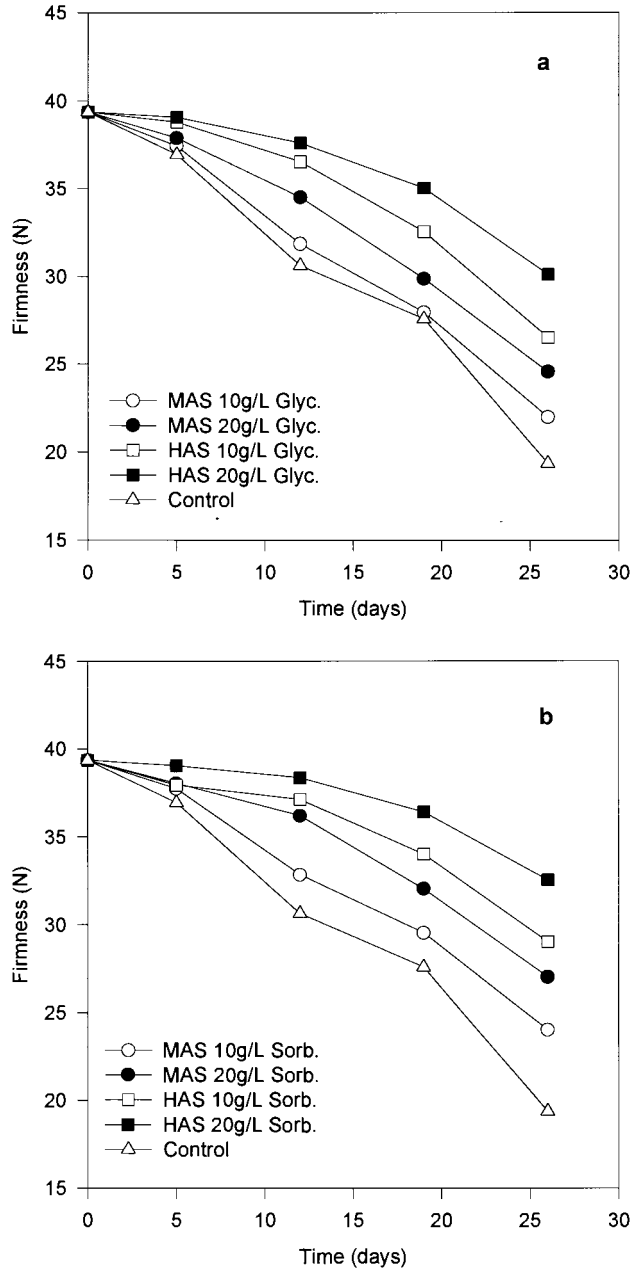

Figure 5. Effect of plasticizer and starch type on firmness during storage at 0 °C of control and starch-based coated strawberries: (a) formulations plasticized with glycerol; (b) formulations plasticized with sorbitol. Standard error: 3.735 N. $LSD_{0.05} = 0.780$.

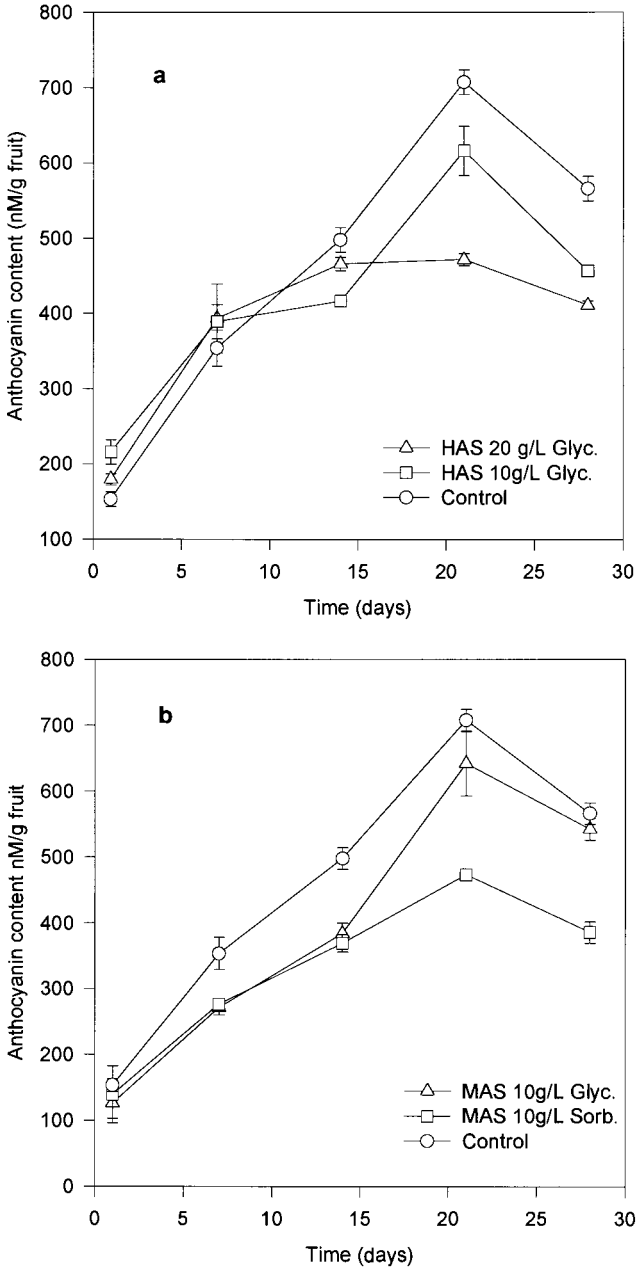

Figure 6. Effect of concentration and type of plasticizer on anthocyanin content of starch-based coated strawberries stored at 0 °C: (a) control and fruits coated with HAS (amylomaize VII) plus 10 or 20 g/L glycerol formulation; (b) control and fruits coated with MAS (potato starch) plus 10 g/L glycerol or sorbitol formulation. Bars indicate standard errors. $LSD_{0.05} = 14.339$.

addition maintained better fruit firmness; best results were obtained with 20 g/L concentration for both glycerol (Figure 5a) and sorbitol (Figure 5b). The formulation containing HAS and 20 g/L sorbitol led to the maximum retention of fruit firmness.

Anthocyanin Content. Color changes of fruits occurring during postharvest are attributed to the synthesis of carotenoids and anthocyanin and the degradation of chlorophyll. Strawberry redness is due mainly to perlargonidin 3-glucoside, the content of which increases during postharvest senescence (Manning, 1993). All of the formulations showed a similar behavior: anthocyanin content increased with time, reaching a maximum at 22 days of storage time and then decreasing (Figure 6); control fruits showed the highest anthocyanin contents. Coatings with 20 g/L of plasticizer showed significantly ($P < 0.05$) lower anthocyanin values than coatings with 10 g/L of plasticizer (Figure 6a). At the maximum, anthocyanin content of fruits coated with sorbitol formulations was significantly lower than those with glycerol (Figure 6b). The effect of starch type was not significant on anthocyanin content.

A linear relationship between anthocyanin content and the *a*/*b* ratio of Hunterlab color parameters was found ($r^2 = 0.9499$). When anthocyanin content increased, *a* values significantly increased while *b* decreased, which led to an *a*/*b* increase as a function of storage time.

The *a*/*b* ratio remained almost constant during the first 8 days of the storage period and then increased with storage time (Figure 7). Control fruits with the highest *a*/*b* values showed highly significant differences

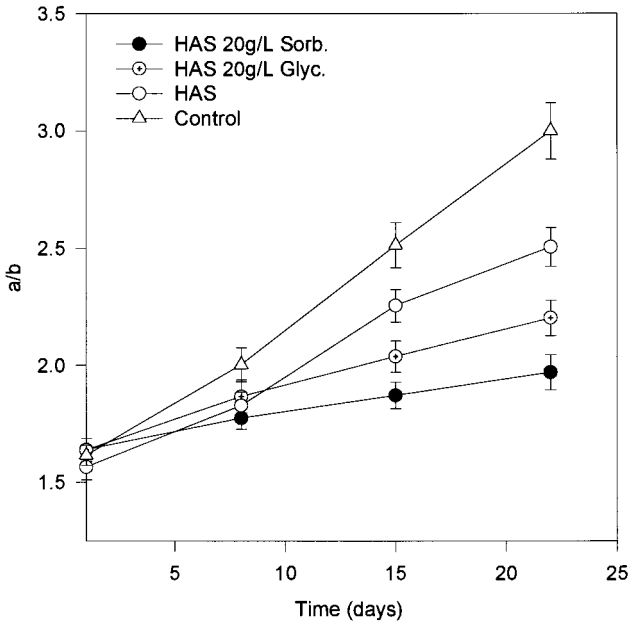

Figure 7. Effect of plasticizer concentration on the ratio of chromaticity parameters *a*/*b* of starch-based coated strawberries stored at 0 °C: (△) control, uncoated fruits; (○) 20 g/L high amylose product (HAS); (⊙) 20 g/L high amylose product (HAS) with 20 g/L glycerol; (●) 20 g/L high amylose product (HAS) with 20 g/L sorbitol. Bars indicate standard errors. $LSD_{0.05} = 0.014$.

($P < 0.01$) with regard to the coated fruits. On the basis of color development, coated fruit showed a ripening delay, which demonstrates the effectiveness of coating treatment.

Changes of Titratable Acidity and pH of Fruits. Citric acid is the most abundant acid in strawberries, followed by malic acid. As a soft fruit, strawberry contains ascorbic acid, biologically important because of its antioxidant activity. Total acidity increases during fruit development until the big-green stage, andthen it decreases toward a minimum at the over-ripened stage, due to a decrease mainly in malic acid (Green, 1971).

As seen in Table 2, as fruit pH increased, accordingly, titratable acidity decreased as a function of storage time. Coated fruits showed higher values of titratable acidity compared to control ones. Acidity decrease demonstrates maturation development. The coating addition delayed the maturation process for >7 days. These results agreed with those found by El Gaouth et al. (1991a) working on strawberries coated with a chitosan-based formulations.

ANOVA showed that the presence and type of plasticizer and storage time were significant effects ($P < 0.05$) on titratable acidity of strawberries. Nonsignificant differences were found between coatings with different amylose contents. Best results were obtained with sorbitol coatings; however, nonsignificant differences were found between sorbitol concentrations ($P > 0.05$).

Soluble and Insoluble Solids. Generally, solid content of soft fruits is related to size and weight of seeds. Although high in number, strawberry achenes do not contribute markedly to the solid content (Green, 1971). During storage, strawberries decreased both soluble and insoluble solid contents. Only storage time showed a significant effect ($P < 0.05$); no significant variations on soluble or insoluble solids content of strawberries were observed due to the coating treatment (Table 2).

Table 2. Effect of Plasticizer on pH, Titratable Acidity, and Soluble and Insoluble Solids on Control and HAP-Based Coated Strawberries

		storage time at 0 °C				
parameter	sample	1 day	7 days	14 days	22 days	28 days
pH	C[a]	3.74	3.97	4.04	4.08	4.19
(0.023)[e]	WP[b]	3.81	3.94	4.12	4.04	4.20
	1G[c]	3.80	3.93	4.02	4.05	4.14
	2G	3.80	3.92	3.98	4.05	4.12
	1S[d]	3.76	3.85	4.00	4.00	4.12
	2S	3.77	3.88	3.97	4.01	4.10
titratable acidity	C	10.89	10.07	9.13	8.13	7.30
(mequiv/100 g)	WP	10.77	10.45	9.73	9.16	8.61
(0.169)[e]	1G	10.85	10.58	9.82	9.46	8.90
	2G	10.87	10.48	9.66	9.64	9.06
	1S	10.79	10.65	10.34	9.84	9.19
	2S	10.77	10.67	10.32	9.82	9.20
soluble solids	C	6.00	6.00	5.50	4.50	3.50
(°Brix)	WP	6.00	6.00	5.50	5.00	4.50
(0.119)[e]	1G	6.00	5.75	5.25	5.00	4.75
	2G	5.75	5.50	5.50	5.00	4.50
	1S	6.00	6.00	5.50	5.25	4.75
	2S	5.75	5.50	5.50	5.25	4.50
insoluble solids	C	8.62	8.46	7.66	6.78	6.66
(g/100 g)	WP	8.64	8.34	7.65	6.82	6.84
(0.207)[e]	1G	8.71	8.36	7.57	6.91	6.95
	2G	8.51	8.41	7.70	7.02	7.11
	1S	8.61	8.31	7.81	7.28	7.16
	2S	8.70	8.33	7.89	7.39	7.28

[a] Control (uncoated fruits). [b] Coating formulation without plasticizer. [c] Coating formulation with 10 g/L (1G) or 20 g/L (2G) glycerol. [d] Coating formulation with 10 g/L (1S) or 20 g/L (2S) sorbitol. [e] Fisher's least significant difference $LSD_{0.05}$.

Sugar Content. Sucrose shows low concentrations during the first 10 days after anthesis and increases rapidly, reaching a maximum when the fruit is ripe and decreases in over-ripened fruits. Generally, in ripe strawberries, glucose and fructose concentrations are 2.3 and 2.2 g/100 g of fresh fruit, respectively, which corresponds to 83% total sugar content (Manning, 1993).

Reducing sugar content increased with storage time. Sugar contents of fruits coated with HAS formulations were significantly lower ($P < 0.05$) than those of fruits coated with MAS formulations (Table 3). Whereas plasticizer presence showed a significant effect, the type of plasticizer was not significantly different.

Nonreducing sugars decreased with storage time (Table 3). These results were confirmed with those of refractive index measurements, both measurements being based on sucrose quantification. The type of plastizicer showed significant effects only on nonreducing sugars.

Microbial Determinations. At pH 4 (fruit pH), mainly yeasts and molds were observed; sugar fermenting bacteria were also detected. Both methods (Petri dish counts and Petrifilm plates) used to determine total counts, mesophilic and psychrotrophic bacteria, and yeasts and molds showed similar results (Table 4).

Washing with chlorinated water (0.25 g/L Cl_2) was effective in reducing microbial counts in one log cycle and in eliminating coliforms. No coliforms were detected during storage of fruits at 0 °C.

Only sorbitol was used as plasticizer for microbial assays because, as described previously, sorbitol coatings were more effective compared to glycerol ones. ANOVA showed a significant effect ($P < 0.05$) for

Table 3. Effect of Plasticizer and Starch Type on Sugar Content of Control and Starch-Based Coated Strawberries

		high amylose content starches (HAP)		medium amylose content starches (corn)	
time (days)	sample	reducing sugars[e]	nonreducing sugars[e]	reducing sugars[e]	nonreducing sugars[e]
1	C[a]	1.50	3.79	1.50	3.79
	WP[b]	1.61	3.62	1.73	3.43
	1G[c]	1.43	3.64	1.61	3.37
	2G	1.74	3.31	1.75	3.48
	1S[d]	1.55	3.45	1.42	3.51
	2S	1.75	3.41	1.54	3.46
7	C	3.63	1.95	3.63	1.95
	WP	3.41	1.97	3.34	1.98
	1G	3.43	1.99	3.03	1.99
	2G	3.31	1.96	3.46	1.98
	1S	3.19	2.30	2.93	2.00
	2S	3.05	2.45	3.10	1.99
14	C	4.73	0.67	4.73	0.67
	WP	4.37	1.14	4.56	1.08
	1G	4.17	1.37	4.15	1.79
	2G	3.86	1.78	4.08	1.75
	1S	3.63	1.87	3.82	1.83
	2S	3.77	1.89	3.92	1.80
22	C	5.39	0.40	5.39	0.40
	WP	4.83	0.64	4.86	0.80
	1G	4.67	0.77	4.55	0.91
	2G	4.37	1.26	4.25	1.57
	1S	4.17	1.57	4.29	1.66
	2S	4.11	1.64	4.16	1.61
28	C	5.97	0.21	5.97	0.21
	WP	5.78	0.37	5.47	0.38
	1G	5.21	0.33	5.33	0.43
	2G	5.09	0.37	5.25	0.30
	1S	4.95	0.43	5.14	0.40
	2S	4.7	0.76	4.72	0.83

[a] Control (uncoated fruits). [b] Coating formulation without plasticizer. [c] Coating formulation with 10 g/L (1G) or 20 g/L (2G) glycerol. [d] Coating formulation with 10 g/L (1S) or 20 g/L (2S) sorbitol. [e] Sugar content expressed in grams of glucose per 100 g of fruit. $LSD_{0.05}$ = 0.105 for reducing sugars and 0.022 for nonreducing sugars.

Table 4. Comparison of Microbial Count Methods Applied to HAP-Based Coated Strawberries at Initial Time

	microbial counts (log CFU/g)	
culture medium	HAP without plasticizer	HAP with sorbitol and potassium sorbate
PCA[a]	4.10 ± 0.139	4.00 ± 0.089
Petrifilm[b] 6400	4.15 ± 0.096	4.11 ± 0.072
YGC[a]	5.00 ± 0.211	4.13 ± 0.173
Petrifilm[b] 6407	4.93 ± 0.115	3.85 ± 0.226

[a] Plate counts: PCA and YGC culture media. [b] Petrifilm: 6400 and 6407 for aerobic and yeasts and molds counts, respectively.

plasticizer presence in decreasing mesophilic and psychrotrophic yeast and mold counts. Amylose content did not have a significant effect ($P < 0.05$) on microbial counts. Only the results corresponding to HAS-based coatings are shown because HAS and MAS coatings did not differ significantly ($P < 0.05$) in microbial counts. Coatings with potassium sorbate decreased significantly ($P < 0.05$) yeast and mold counts compared to both control and fruits coated with 20 g/L sorbitol formulation (Figure 8). However, for total counts (mesophilics and psychrotrophics) the differences were not significant ($P > 0.05$).

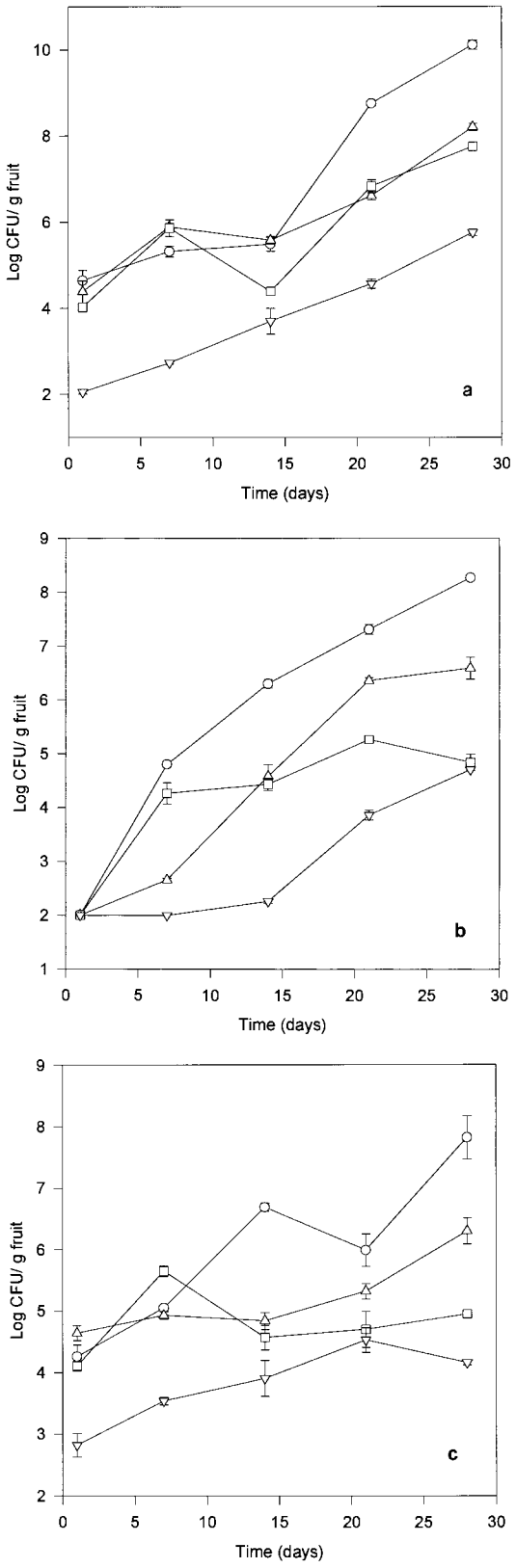

Figure 8. Effect of coating formulation on microbial growth (log CFU/g of fruit) on strawberries stored at 0 °C: (a) mesophilic microorganisms, $LSD_{0.05}$ = 0.367; (b) psychrotrophic microorganisms, $LSD_{0.05}$ = 0.333; (c) yeasts and molds, $LSD_{0.05}$ = 0.317. Compositions of coating formulations: (○) control, uncoated fruits; (△) high amylose product (HAP) and 20 g/L sorbitol; (□) high amylose product (HAP), 20 g/L sorbitol, and 0.2 g/L potassium sorbate; (▽) high amylose rich product (HAP), 20 g/L sorbitol, 0.2 g/L potassium sorbate, and citric acid. Bars indicate standard error.

Sorbic acid action on dehydrogenase enzymes makes yeasts and molds unable to metabolize carbon double bonds in the alpha position with the carboxyl group (Eklund, 1983). Besides, sorbic acid is not toxic for human beings, because it is metabolized similarly as a fatty acid. Sorbic acid is an ideal preservative for acid foods; increasing the content of the nondissociated form, antimicrobial activity is favored. In our case, to increase potassium sorbate effectiveness, citric acid was added to one formulation showing the lowest microbial counts (Figure 8).

Sorbate is considered one of the least harmful preservatives in use; the World Health Organization (WHO) has set its acceptable daily intake (ADI) at 25 mg/kg of body weight (Sofos and Busta, 1983). To prolong storage life of fruits and vegetables, generally the product is dipped in 50−100 g/L potassium sorbate solutions (Restaino et al., 1981). In our coatings, 0.2 g/L potassium sorbate plus critic acid provided a locally high and effective concentration of preservative that allowed a reduction of its total amount in the food. Thus, edible films and coatings act as surface retention agents, particularly when additives are included in the formulation and limit preservative diffusion in the food core (Guilbert, 1986, 1988; Vodjani and Torres, 1989).

To compare coating effectiveness, shelf life of strawberries was limited to the time necessary to reach 10^6 CFU/g of fruit. According to Howard and Dewi (1995), when microbial counts exceed this limit, toxic substances may be produced. Brackett (1994) noted that damaged or defective fruits can contain as many as 10^7 CFU/g of fruits. At 0 °C, shelf life of uncoated fruits was 14 days. Coatings with sorbitol extended fruit shelf life to 21 days. At 28 days (maximum storage time assayed) coatings with sorbitol and potassium sorbate and those with sorbitol, potassium sorbate, and citric acid showed microbial counts $<10^6$ CFU/g of fruit, the lowest counts being obtained with the last one.

CONCLUSIONS

The starch-based coatings developed in the present work proved to extend storage life of strawberries, decreasing water losses and improving fruit quality. Color changes were delayed, and weight and firmness losses were lower in coated fruits than in control ones. Physiological modifications such as anthocyanin content, reducing and nonreducing sugars contents, titratable acidity, and pH of fruits were slowed even though reaching commercially acceptable values.

Microstructure observations by SEM helped to explain WVP differences. Both sorbitol and glycerol avoided cracks, decreased WVP and weight losses, and maintained firmness and surface color of fruits, with a 20 g/L concentration having the best results. However, formulations containing sorbitol were more effective than glycerol ones, regardless of starch amylose content.

Coatings with higher amylose content starches (HAS), particularly our own developed product (HAP), decreased WVP and weight losses and retained fruit firmness for longer periods than coatings with medium amylose starch coatings (MAS), although MAS coating extended considerably storage life and maintained quality parameters satisfactorily. MAS coatings have an additional benefit: they are easily available in every country.

Coating with sorbitol helped to decrease microbial counts. The addition of potassium sorbate made coatings much more effective because storage life was extended to 28 days compared to 14 days of uncoated fruits. Moreover, the addition of citric acid to enhance potassium sorbate antimicrobial action yielded a better coating formulation with a total strawberry storage life of >28 days.

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