Development and Evaluation of a Novel pH Indicator Biodegradable Film Based on Cassava Starch

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ABSTRACT: A pH indicator film based on cassava starch plasticized with sucrose and inverted sugar and incorporated with grape and spinach extracts as pH indicator sources (anthocyanin and chlorophyll) has been developed, and its packaging properties have been assessed. A second-order central composite design (2^2) with three central points and four star points was used to evaluate the mechanical properties (tensile strength, tensile strength at break, and elongation at break percentage), moisture barrier, and microstructure of the films, and its potential as a pH indicator packaging. The films were prepared by the casting technique and conditioned under controlled conditions (75% relative humidity and 23°C), at least 4 days before the analyses. The materials were exposed to different pH solutions (0, 2, 7, 10, and 14) and their color parameters (L^*, a^*, b^*, and *haze*) were measured by transmittance. Grape and spinach extracts have affected the material characterization. Film properties (mechanical properties and moisture barrier) were strongly influenced by extract concentration presenting lower results than for the control. Films containing a higher concentration of grape extract presented a greater color change at different pH's suggesting that anthocyanins are more effective as pH indicators than chlorophyll or the mixture of both extracts. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 120: 1069–1079, 2011

Key words: biodegradable films; mechanical properties; pH indicator; barrier; cassava starch

INTRODUCTION

Packages are used to extend product shelf life with mechanical protection and avoiding biological and chemical contamination.[1,2] New packaging technologies have been developed, and, in recent years, active and intelligent packaging are attracting a lot of attention in the food industry. Packaging may be termed "active" or "intelligent" when it performs some roles. Specifically, active packaging encompasses those that change the condition of the packed food to extend shelf-life or to improve safety and sensory properties while maintaining its quality (e.g., oxygen scavengers, ethylene scavengers, systems releasing antimicrobial compounds or antioxidants). Intelligent or "smart" packaging encompasses those able to monitor or to give information about the quality of the packaged food (e.g., packages containing indicators informing time–temperature conditions, package integrity, and food quality).[3–5] The safety and quality of food products can be related to pH because spoilage is usually accompanied by a pH change. Thus, a packaging system that changes color as the pH alters could allow consumer evaluation of a product before purchasing or opening the package. There are many types of "smart" packages, among which the pH indicators, which report the correlation between the packed product and its pH along the storage period, have great importance, especially for food, pharmaceutical, and cosmetics industries.[3]

Most known active packages are produced with plastics derived from fossil fuels, generating environmental problems.[6] New bio-based materials have been exploited to develop edible and biodegradable films in a great effort to extend shelf life and improve quality of food while reducing packaging waste.[7] Starch, as an example, has received considerable attention as a biodegradable thermoplastic polymer.[4,5,8–11] Flexible films obtained from starch were successfully developed.[12–18] Moreover, antimicrobial activity of edible films has been investigated, as an alternative for active packaging.[19–25]

Because it would be difficult for consumers to detect pH variation in a product, the use of pH indicators presents an extra security for manufacturers and consumers, indicating product spoilage; therefore, the pH-indicator packaging could give information for consumer whether a product is safe without the need to open the packaging.[5] There are few studies related to pH indicators, especially involving natural and edible components. However, there are

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patents reporting pH indicators based on food compounds such as carotenoids[26] and anthocyanins,[27] incorporated in a conventional nonbiodegradable package cooking procedure and for laboratory indicators, respectively. Also, there are no existing patent reports based on different materials and applications than those proposed in this study, such as chlorophylls, which undergo color modification when exposed to different pH environments,[28–30] and can be tested for "smart" packages application.

In this study, a novel biodegradable film with grape and spinach extracts, as sources of pH indicators (anthocyanin and chlorophyll), incorporated into a cassava starch matrix was developed. The mechanical properties, water vapor barrier, and microstructure of the films were evaluated as well as the color change at different pH's.

MATERIALS AND METHODS

Materials

Cassava starch donated by Cargill Agrícola S.A., Brazil), commercial sucrose and inverted sugar (donated by Açúcar Guarani S.A., Brazil), Merlot grape (*Vitis vinifera*) variety (donated by Brasiluvas Agrícola Ltda., Brazil), and spinach (*Spinacea oleracea*) were used as raw materials in this study.

Extracts preparation

The aqueous extracts were prepared from grape skins and seeds as sources of anthocyanin, simulating industrial waste from wineries, and spinach as a source of chlorophyll. The materials were submitted to a blanching process in fluent steam, for 15 min, and then crushed in water, at high speed, for 4 min. The aqueous solution was then filtered and stored in a vertical plasma freezer (Fanem, model 349 FV, São Paulo, Brazil) at −30°C, until use. The total solids content of the extracts was determined gravimetrically, in open oven at 105°C, according to the method described in IAL.[31]

Film preparation

The biodegradable film base suspension (1000 mL) was prepared with 50.0 g of cassava starch, 7 g of sucrose, and 14 g of liquid inverted sugar dissolved in distilled water. The aqueous extracts of spinach (total solids of 1.05 g/100 g of aqueous solution) and of grape (total solids of 8.16 g/100 g of aqueous solution) were added, with concentrations in the film-forming solutions varying from 0.00 to 0.49 g of spinach extract/100 g of filmogenic solution and from 0.00 to 3.79 g of grape extract/100 g of filmogenic solution, according to a second-order central composite design (2^2) with three central points and four star points (Table I).

Film-forming solutions were heated in a domestic microwave oven (Panasonic, Brazil), manually mixed from time to time, up to (70 ± 1)°C for starch gelatinization and then were kept at ambient temperature for at least 3 hr to allow bubbles to dissipate. After that, the suspension was cast onto Petri dishes (150 cm^2), dried under renewable circulated air in a temperature-controlled chamber (FANEM, model 330) at (45 ± 5)°C during 18 to 24 hr, followed by storage at controlled conditions (23°C and 75% of relative humidity) for at least 4 days before testing.

Thickness and tensile properties

Film thickness was measured with a flat parallel surface micrometer (Mituyoyo SulAmericana Ltda., model 103-137, Brazil; precision 0.002 mm), and an average value of five measurements, at random positions, for each filmstrip was used to calculate the tensile properties. A texture analyzer (TA.XT2i; Stable Microsystems, UK) equipped with a self-tightening roller grip (A/TGT) probe was used to measure the tensile strength (TS; MPa), tensile strength at break (TSB; MPa), and percent elongation at break (*E*; %), of specimens. For each formulation, five specimens were tested, with the following parameters: initial grip separation distance of 150 mm, cross-head speed of 0.3 $mm \cdot min^{-1}$, and rate of grip separation of 48 mm min^{-1}, according to D882-09 ASTM standard.[32] TS (nominal) was calculated by dividing the maximum load by the original minimum cross-sectional area of the specimen (related to minimum thickness). TSB (nominal) was calculated in the same way as the TS except that the load at break was used in place of the maximum load. Percent *E* (nominal) was calculated dividing the extension at the moment of rupture of the specimen by its initial gauge length and multiplying by 100.

Water vapor transmission

The water vapor transmission was determined using a desiccant method, at 23°C and at 75% of relative humidity, according to ASTM E96/E 96M-05.[33] This property was reported as water vapor permeability (WVP); that is, the rate of water vapor transmission through unit area of flat material of unit thickness induced by unit vapor pressure difference between two specific surfaces, under specified temperature and humidity conditions (in this case 23°C and 75%). A cell containing silica gel was closed with a specimen of biodegradable film firmly fixed on top, and placed in a desiccator with saturated sodium chloride solution at ambient temperature (23°C and 75% of relative humidity). Control samples were prepared without silica gel to account for the

TABLE I
Quantities of Spinach and Grape Extracts Incorporated in Films Based on Cassava Starch According to a Second-Order Central Composite Design (2^2) with Three Central Points and Four Star Points

	Coded values		Real values[a]	
Film	Spinach extract	Grape extract	Spinach extract	Grape extract
1	−1	−1	0.11	0.82
2	−1	1	0.11	2.77
3	1	−1	0.36	0.82
4	1	1	0.36	2.77
5	−1.41	0	0.00	1.96
6	1.41	0	0.49	1.96
7	0	−1.41	0.25	0.00
8	0	1.41	0.25	3.79
9[b]	0	0	0.25	1.96
10[b]	0	0	0.25	1.96
11[b]	0	0	0.25	1.96

[a] Gram of extract/100 g of filmogenic solution.
[b] Central point.

variation of mass undergone by the film under the same conditions. The samples were weighed every 2 hr for 2 days until constant gained weight was reached. The WVP of biodegradable films, expressed as [g mm m^{-2} d^{-1} kPa^{-1}], was calculated according to eq. (1):

$$\mathrm{WVP} = \frac{w}{\theta} \times \frac{24 \times t}{A \times \Delta p} \tag{1}$$

where w is the weight gain (from the straight line) (g); θ is the time during which w occurred (hr); t is the specimen thickness (mm); A is the test area (cell top area) (m^2); and Δp is the vapor pressure difference (kPa). The tests were carried out in triplicate.

Microstructure

Film microstructure was analyzed using a scanning electron microscope (Philips XL-30, with an integrated EDAX system, SEMTech Solutions, Inc., North Billerica, MA) at a low-intensity beam (<15 kV) to avoid film degradation. Specimens were sputtered with a 16 μg gold layer, and the images were obtained at 10 kV, spotsize 4.1, and ×100 and ×1000 magnification.

Film evaluation as a pH indicator

Films were exposed to solutions at pH's 0, 2, 7, 10, and 14, and the color changes were measured using a color measurement spectrophotometer (Color Quest XE, Hunterlab, Hunter Associates Laboratory, Inc., Reston, VA). The color parameters of films not exposed to pH solutions were also measured and considered as a reference. Film specimens were placed on a white standard plate (L^*) 100, and the CIE *Lab* coordinates were measured, using D65 illuminant and standard observer (10°). Five measurements were taken in each specimen, and three specimens of each film were measured.[34] Transmission haze was measured according to ASTM D 1003-07[35] and expressed as:

$$\mathrm{haze} = \frac{T_d}{T_t} \times 100 \tag{2}$$

where *haze* is, in transmission, the scattering of light by a specimen responsible for the reduction in contrast of objects viewed through it (%); T_d is the light diffusely scattered; and T_t is the total light transmitted.

The total color-difference (ΔE^*_{ab}) between two colors of the films exposed to pH solutions (test specimen) and the color of the film that was not exposed (reference) was calculated by eq. (3), according to ASTM D2244-09b[36]:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{3}$$

where (ΔL^*) is the brightness difference between test specimen and reference; (Δa^*) is the greenness–redness difference between test specimen and reference; and (Δb^*) is the blueness–yellowness difference between test specimen and reference.

For identifying the direction of the color difference between two colors, the hue angle (h_{ab}) (degrees counter-clockwise from the positive a^* axis) and metric chroma (C^*_{ab}) were calculated as well as the differences in chroma (ΔC^*_{ab}) according to ASTM D2244-09b[36]:

$$h_{ab} = 180 - \left(\frac{180}{\pi}\right) \arctan\left(\frac{a^*}{b^*}\right) - 90\,\mathrm{sign}(b^*) \tag{4}$$

$$C^*_{ab} = \sqrt{(a^*)^2 + (b^*)^2} \tag{5}$$

$$\Delta C^*_{ab} = C^*_{ab,S} - C^*_{ab,R} \tag{6}$$

where *sign* is a function that returns the sign of the argument and *arctan* is the inverse tangent function returning angles in units of radians.

For identifying the relative contributions of lightness differences, chroma differences, and hue differences between two colors, the metric hue difference (ΔH^*_{ab}) was calculated according to ASTM D2244-09b[36]:

$$\begin{aligned} &\Delta H^*_{ab} = s\sqrt{2\left(C^*_{ab,S} \times C^*_{ab,R} - a^*_S \times a^*_R - b^*_S \times b^*_R\right)} \\ &\text{If } a^*_R \times b^*_S > a^*_S \times b^*_R, \text{then } s = 1 \\ &\text{If } a^*_R \times b^*_S \le a^*_S \times b^*_R, \text{then } s = -1 \end{aligned} \tag{7}$$

where the subscript S refers to the sample and the subscript R refers to the reference (film that was not exposed to pH solutions).

TABLE II
Tensile Strength (TS), Tensile Strength at Break (TSB), Percent Elongation at Break (*E*), Thickness (*t*), and Water Vapor Permeability (WVP) of Cassava Starch Films Added with Spinach and Grape Extracts According to Second-Order Central Composite Design (2^2) with Three Central Points and Four Star Points

Film	Spinach extract[a]	Grape extract[a]	TS (MPa)[b]	TSB (MPa)[b]	*E* (%)[b]	*t* (μm)	WVP ($\times 10^{-11}$) (g sec^{-1} m^{-1} Pa^{-1})[b]
1	0.11	0.82	$2.93 \pm 0.10^{1,2,3,4}$	$2.88 \pm 0.11^{1,2,3}$	114 ± 15^{3}	116 ± 36^{2}	2.56 ± 0.16^{2}
2	0.11	2.77	$1.88 \pm 0.09^{1,2}$	1.84 ± 0.07^{1}	$99 \pm 17^{2,3}$	$109 \pm 19^{1,2}$	$3.38 \pm 0.44^{3,4}$
3	0.36	0.82	4.19 ± 0.63^{5}	3.82 ± 0.53^{3}	$80 \pm 13^{1,2}$	$105 \pm 19^{1,2}$	$2.90 \pm 0.25^{2,3}$
4	0.36	2.77	$2.19 \pm 0.11^{1,2,3}$	1.81 ± 0.20^{1}	67 ± 13^{1}	$101 \pm 11^{1,2}$	3.64 ± 0.10^{5}
5	0.00	1.96	$2.88 \pm 0.31^{1,2,3,4}$	$2.59 \pm 0.23^{1,2,3}$	65 ± 11^{1}	$106 \pm 34^{1,2}$	$3.25 \pm 0.56^{3,4}$
6	0.49	1.96	$2.08 \pm 0.23^{1,2,3}$	1.86 ± 0.43^{1}	$89 \pm 18^{1,2,3}$	$100 \pm 14^{1,2}$	9.99 ± 0.76^{6}
7	0.25	0.00	$3.69 \pm 0.44^{4,5}$	$3.64 \pm 0.39^{2,3}$	217 ± 19^{4}	$93 \pm 19^{1,2}$	1.89 ± 0.14^{1}
8	0.25	3.79	1.79 ± 0.16^{1}	1.62 ± 0.19^{1}	$76 \pm 10^{1,2}$	$92 \pm 14^{1,2}$	4.92 ± 0.23^{7}
9[c]	0.25	1.96	$2.12 \pm 0.26^{1,2,3}$	$1.99 \pm 0.30^{1,2}$	$84 \pm 6^{1,2,3}$	$99 \pm 14^{1,2}$	5.53 ± 0.67^{7}
10[c]	0.25	1.96	$3.21 \pm 0.28^{c,4,5}$	$2.62 \pm 0.72^{1,2,3}$	$73 \pm 18^{1,2}$	$96 \pm 13^{1,2}$	$3.28 \pm 0.22^{3,4}$
11[c]	0.25	1.96	$3.12 \pm 0.32^{2,3,4,5}$	$2.75 \pm 0.60^{1,2,3}$	$73 \pm 12^{1,2}$	$104 \pm 22^{1,2}$	$2.87 \pm 0.23^{2,3}$
C[d]	0	0	8.49 ± 1.72^{7}	7.75 ± 2.39^{4}	$96 \pm 18^{1,2,3}$	84 ± 18^{1}	1.23 ± 0.16^{1}
Tukey's HSD 5%	1.25	1.71	32	29	0.66		

[a] Gram of extract/100 g of solution.
[b] Means in the same column with the same superscript numbers are not significantly different ($P > 0.05$).
[c] Central point.
[d] Control film produced without extracts.

Statistical analysis

Analysis of variance (ANOVA) ($P < 0.05$) and experimental design analysis were performed using a Statgraphics Centurion program v.15.2.06 (StatPoint Technologies Inc., Warrenton, VA). The Pareto analysis was also performed to observe the significant effect (within 95% confidence interval) of the components on the color change of biodegradable films.

RESULTS AND DISCUSSION

The films produced from cassava starch were homogeneous, transparent, colored with varying color intensity depending on quantity of extracts, and easy to manipulate, with little presence of bubbles. Specimens with bubbles were not tested.

Thickness and tensile properties

Average thickness of the films varied from 92 ± 14 to 116 ± 36 μm and was not influenced by extract addition ($P > 0.05$), as can be observed in Table II. TS of films varied from 1.79 ± 0.16 to 4.19 ± 0.63 MPa (Table II), and the highest value was obtained for films elaborated with 0.36 g of spinach extract/100 g of solution and 0.82 g of grape extract/100 g of solution (Film 3). Compared with the control film (8.49 ± 1.72 MPa), it can be observed that the extracts have significantly ($P < 0.05$) lowered the TS. The same tendency occurred for TSB, whose values varied from 1.62 ± 0.19 to 3.82 ± 0.53 MPa (Table II), inferior to that presented by the control film, 7.75 ± 2.39 MPa. Because TSB values of the films were lower than the TS values, these materials can sustain plastic deformation without rupture.

Tensile properties may vary with specimen thickness, method of preparation, speed of testing, type of grips used, and manner of measuring extension. Consequently, it is difficult to compare with literature data. Data presented in this work are comparable with those reported in literature for biodegradable films.

The analysis of the experimental design indicated that only grape extract, present in different quantities, influenced the TS of the films ($P < 0.05$). As the amount of grape extract increased, TS of the films was lowered (Fig. 1). However, the TSB was significantly influenced by grape extract and by the interaction of both extracts. As can be observed, in Figure 2, this mechanical property of the films decreased by addition of both extracts. Equation (4) presents the fitted model ($r^2 = 0.77$), where the values of variables are specified in original units:

$$\mathrm{TSB} = (2.91 - 0.43 \times G + 0.09 \times G^2 - 2.10 \times G \times S) \pm 0.41 \quad (8)$$

where TSB is the tensile strength at break (MPa); *G* is the quantity of grape extract (g/100 g of filmogenic solution); and *S* is the quantity of spinach extract (g/100 g of filmogenic solution).

The presence of extracts in the films matrix changed the percent *E*, and it can be observed that this property decreased with increasing concentration of grape extract (Fig. 2). A possible explanation is that the sugars naturally present in grape extracts, such as glucose and fructose, have also acted as

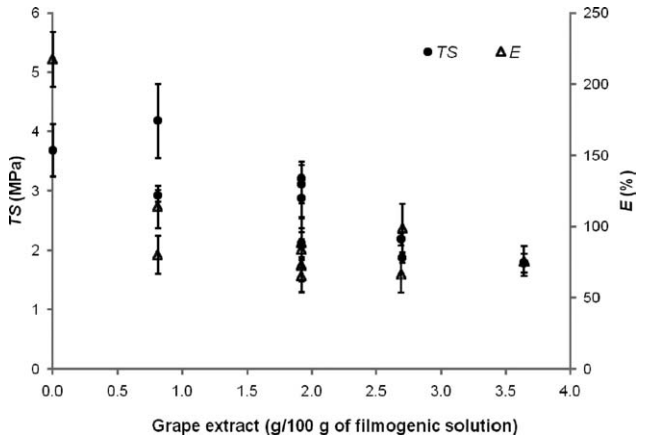

Figure 1 Tensile strength (TS) and percent elongation at break (*E*) of the films according to grape extract concentration.

plasticizers. Because the film base already had added plasticizers (sucrose and inverted sugar), the concentration in the final material may have been too high, resulting in excessive interactions between the film network and the plasticizers, lowering film flexibility.[37] When comparing the films with the control, the additives changed *E*, but some formulations presented higher and others lower values. Such variation may be attributed to a few natural compounds present in the extracts used in this study, such as glucose, sucrose, maltose, and cellulose, which can greatly affect the starch film network and mechanical performance.[38] Also the humidifying ability of such components may have affected the mechanical resistance of the biodegradable materials. The films based on cassava starch, which are already highly hydrophilic materials,[39] could have their hydrophilicity increased by the natural components, absorbing even more water.

For percent *E* of the films, the analysis of the experimental design indicated that grape extract and the interaction between extracts influenced this property significantly, and the fitted model is ($r^2 = 0.84$):

$$E = (140 - 69 \times G - 131 \times G \times S + 18 \times G^2) \pm 19 \quad (9)$$

where the values of variables are specified in original units.

It can be observed in Figure 2 that the *E* of films decreased as the grape extract increased, up to 2 g/100 g of filmogenic solution, and then the value remained constant. The values obtained in this work are comparable with those reported in literature. Pelissari et al.[21] formulated a cassava starch–chitosan film incorporated with oregano essential oil by extrusion process, and the measured TS varied from 1.43 to 2.45 MPa, whereas the percent *E* varied from 21.95% to 48.40%. The authors concluded that the presence of oregano essential oil reduced the films TS and *E* because of plasticizing activity of the oil. Flores et al.[23] formulated tapioca starch–glycerol-based edible films added with xanthan gum and potassium sorbate by extrusion technology. The presence of potassium sorbate as an antimicrobial agent and xanthan gum as a thickening agent and stabilizer influenced significantly decreasing TS of the films from 3.0 to 1.0 MPa and increasing *E* from 19% to 101%. Betül Kayserilioğ et al.[40] measured mechanical properties of glycerol-plasticized wheat gluten films dried at different temperatures (20, 50, and 80°C) and relative humidities (35% and 70%). The TS of the films varied from 3.3 to 8.2 MPa measured according to the ISO standard. Kechichian et al.[25] formulated biodegradable films based on cassava starch with cinnamon and clove powders, as antimicrobial additives, and found that the TS varied from 1.2 to 2.2 MPa, whereas the percent *E* varied from 58% to 140%. The authors verified that the presence of natural additives decreased the TS, and the *E* did not present a clear tendency.

Water vapor permeability

The ANOVA indicated that WVP was affected ($P < 0.05$) by the spinach or grape extract concentration, at the studied values (Table II). Compared with the control film, the extracts concentration increased ($P < 0.05$) WVP particularly for Film 6, which had the highest concentration of spinach extract and also a high concentration of grape extract. From Figure 3, it is clear that the presence of spinach extract affected WVP of the films more strongly than grape extract.

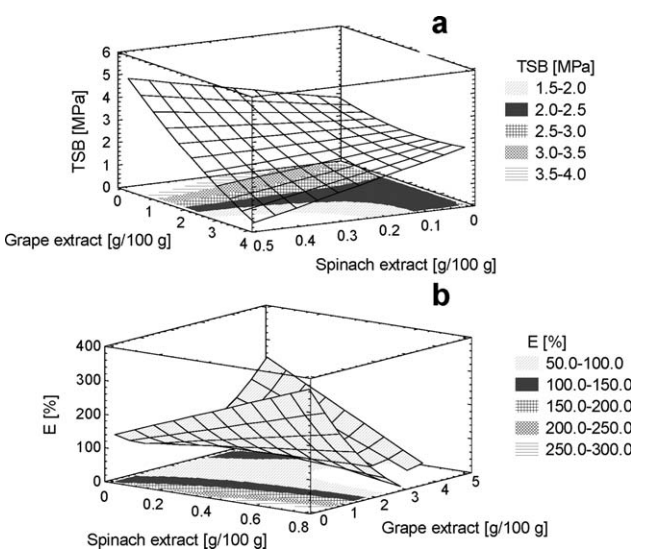

Figure 2 Response surface plots with contours below of tensile strength at break (TSB) and percent elongation at break (*E*) of the films according to grape and spinach extract concentrations.

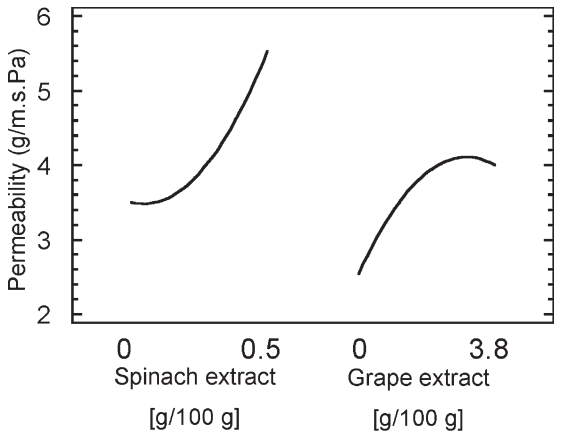

Figure 3 Variation in permeability of the films according to grape and spinach extract concentrations.

Again, the natural compounds present in the natural extracts may be the reason for this increase. Components such as glucose and fructose could have acted as plasticizers, creating regions with higher mobility, allowing a grater interaction with water.[37] The high WVP is characteristic of biodegradable films, mainly for films based on starch, because of a large number of free hydroxyl groups that cause great interaction with water. Pelissari et al.[21] found the WVP of cassava starch film to be 1.39×10^{-10} g sec^{-1} m^{-1} Pa^{-1}, measured at 25°C and RH of 64%, which is 10 times higher than the values found in this study. Tapioca starch–glycerol based edible films added with xanthan gum and potassium sorbate obtained by extrusion technology presented a WVP ranging from 3.72 to 6.40×10^{-10} g sec^{-1} m^{-1} Pa^{-1}, measured at 25°C and RH of 70%.[23]

Microstructure

Figure 4 shows the representative microstructure of the films, indicating their homogeneous surface. Also, it can be observed that there are no differences between the images of the control film, formulated without extracts, and those with the extracts (Film 8).

The films generally showed smooth surfaces; however, some formulations show fissures on the specimen surface, clearly visible with a ×1000 magnification. No clear correlation was established between formulation composition and fissures. They probably occurred during sample preparation for microscopy or because of variations of air velocity during drying of the films.

Film evaluation as pH indicator

The biodegradable films exposed to different pH's reacted with variations of the color parameters "*L**," "*a**," "*b**" as well as *haze* (Fig. 5), indicating correlation between color and pH variation. ANOVA indicated that both extracts (spinach and grape) and pH influenced significantly the *haze* of the films, defined as the cloudy or turbid appearance of an otherwise transparent specimen caused by light scattered from within the specimen or from its surfaces. Materials with *haze* values higher than 30% are considered diffusing. As can be observed from Figure 6, *haze* values increased as the extract content increased; thus, the films became less transparent. This effect is most noticeable with the added quantity of spinach extract.

The experimental design analysis, considering the pure error, indicated that the color parameters a^* and b^* were affected ($P < 0.05$) by grape and spinach extracts concentration and by pH. As can be observed from Figure 7, as the grape extract concentration increased (G), the biodegradable film became redder, and, as the spinach extract (S) increased, film color was greener. This was expected because of the characteristic color presented by the anthocyanin (red) and chlorophyll (green) present in the respective extracts. Equation (6) expresses the fitted model for parameter a^* ($r^2 = 0.82$):

$$a^* = (5.99 - 8.38 \times S - 0.02 \times \text{pH} + 0.88 \times G + 1.95 \times S \times G - 0.21 \times G^2) \pm 0.39 \quad (10)$$

$0 \leq S \leq 0.49$ g/100 g of filmogenic solution

$0 \leq G \leq 3.79$ g/100 g of filmogenic solution

$0 \leq \text{pH} \leq 14$

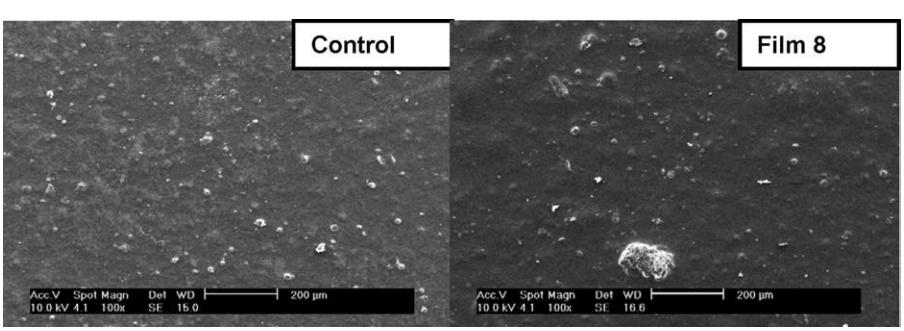

Figure 4 Surface images of biodegrable film 8 in comparison to the control film control (C) obtained by scanning electron microscopy (SEM) with magnification 100×.

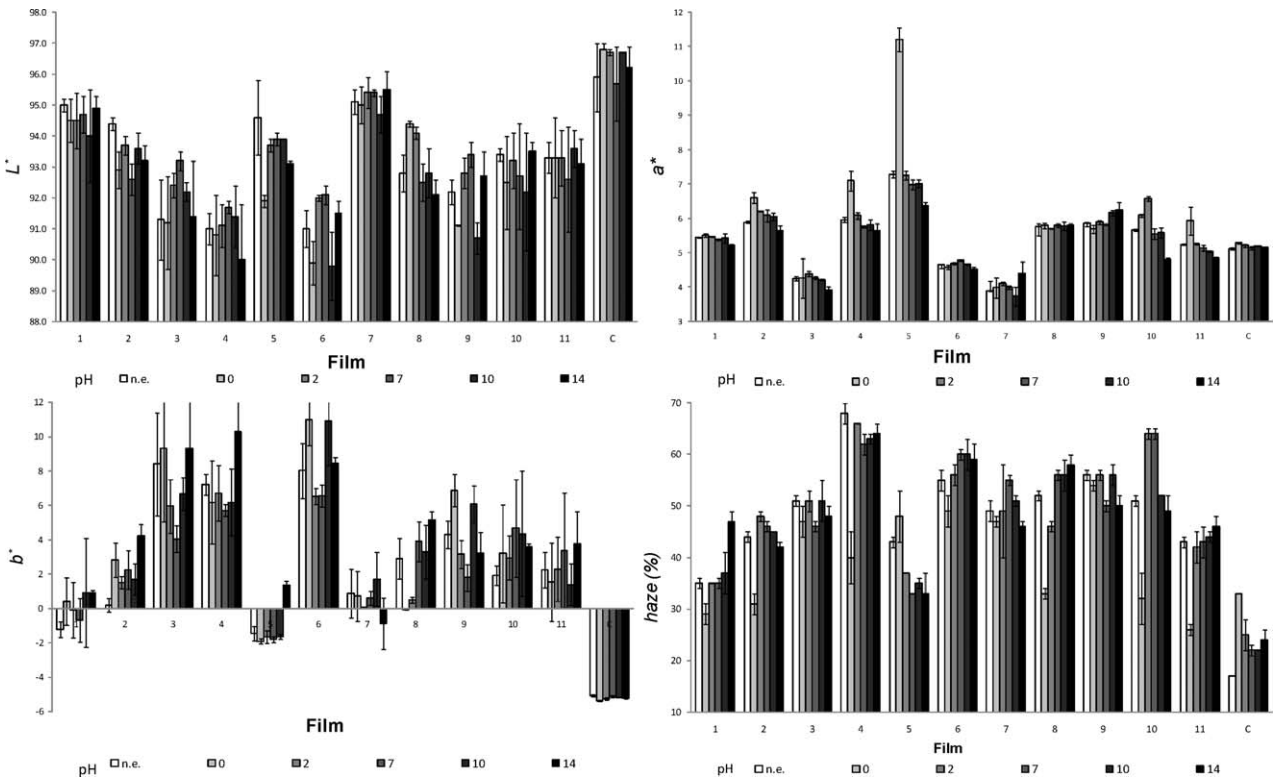

Figure 5 Color parameters (L^*, a^*, and b^*) and *haze* of cassava starch films added with spinach and grape extracts according to a second-order central composite design (2^2) with three central points and four star points, exposed to different pH solutions (0, 2, 7, 10, and 14) or not exposed (n.e.).

The b^* color parameter of the films was strongly affected by spinach extract concentration, becoming more yellow as the spinach extract increased. However, this parameter showed a different tendency with respect to the amount of grape extract, presenting a maximum value at grape extract ranging from 1 to 2 g/100 g of filmogenic solution. The fitted model is expressed by eq. (7) ($r^2 = 0.73$):

$$b^* = (-4.71 + 24.04 \times S - 0.18 \times \text{pH} + 2.84 \times G + 0.08 \times \text{pH} \times G - 0.56 \times G^2) \pm 1.75 \quad (11)$$

$$0 \leq S \leq 0.49 \text{ g/100 g of filmogenic}$$

$$0 \leq G \leq 3.79 \text{ g/100 g of filmogenic solution}$$

$$0 \leq \text{pH} \leq 14$$

The total color-difference (ΔE^*_{ab}) between two colors was significantly influenced by pH, only for Film 5 (with 1.96 g of grape extract of filmogenic solution). The magnitude ΔE^*_{ab} gives no indication of the character of the difference because it does not indicate the relative quantity and direction of *hue*, *chroma*, and *lightness* differences. According to ASTM,[36] differences in *hue* angle (h_{ab}) between the test specimen and reference can be correlated with differences in their visually perceived *hue*, except for dark colors.

Differences in *chroma* (ΔC^*_{ab}) can similarly be correlated with differences in visually perceived *chroma*, and according to ANOVA, pH variation influenced

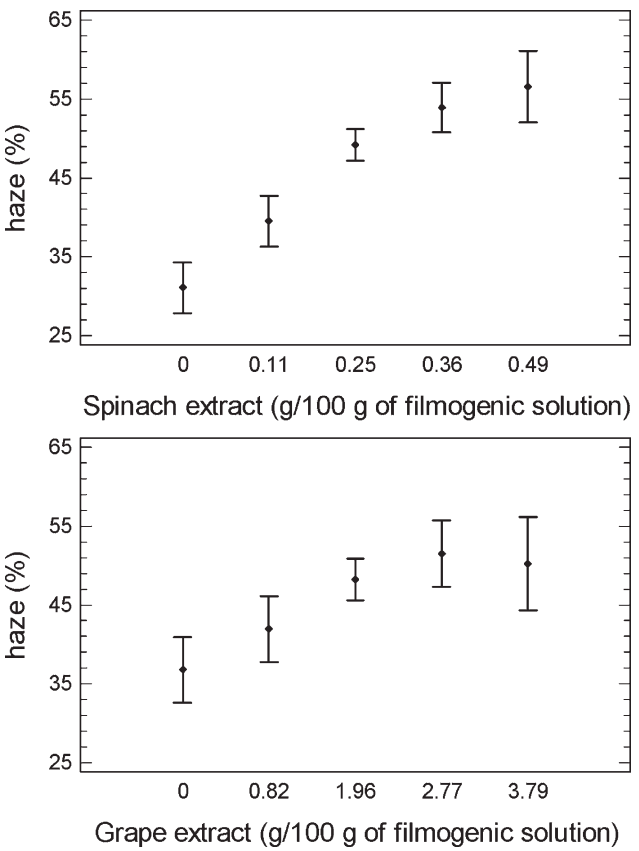

Figure 6 Variation in *haze* of the films according to grape and spinach extracts concentrations.

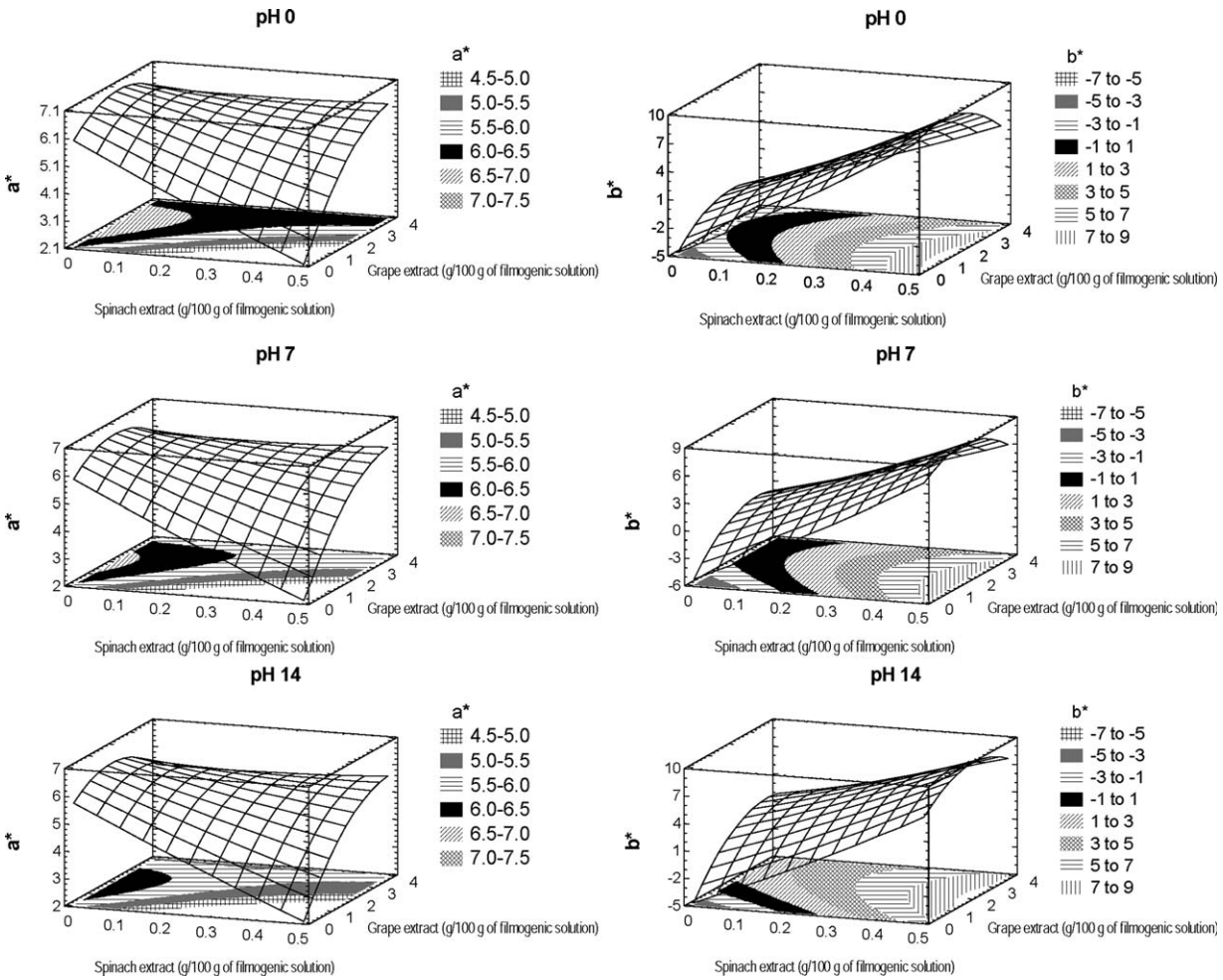

Figure 7 Response surface plots with contours below of color parameters a^* and b^* of the films, according to grape and spinach extracts concentration, exposed to different pH solutions.

the differences in *chroma* of the Films 5 and 8 (Fig. 8). Figure 9 represents the terms for describing differences in *chroma* and lightness of the Films 5 and 8. As the pH increased, the color of Film 8 became deeper (higher in saturation), which can be better observed in the color palette shown in detail in Figure 9. Different results were presented by Film 5; at pH 0 (acid) the chroma was deeper than when the film was exposed to pH 14 (alkali). Film 5 that had only grape extract presented a significant change ($P < 0.05$) in the a^* parameter for acid pH's and a significant change ($P < 0.05$) in the b^* parameter for basic pH's. This was the film that along with Film 8 (that had the highest concentration of grape extract) presented the most significant color change; thus, it can be concluded that the anthocyanin present in the grape extract was more effective as a pH indicator than chlorophyll or a mixture of both indicators.

Films incorporated with grape and spinach extracts when exposed to an alkali pH can present a yellow color (positive b^*) because of the interaction of the colors of the anthocyanin present in grape extract, which can assume a blue color and the green color of the chlorophyll. Terci and Rossi[41] cited that grape extract becomes yellow when exposed to pH 14.

Because the pH solutions were spread onto the film surface after forming, small variations in haze and luminosity values are expected. However, the effect of the extracts on haze and luminosity of the films without exposure to pH solutions can provide important information.

Even though the colorimeter reported a color change when the films were exposed to different pH's, color variation was visible to the naked eye only at the extreme pH's. Terci and Rossi[41] have reported a significant color change for alcoholic extracts of black mulberry (*Morus nigra*), java plum (*Syzygium cuminii*), grape (*V. vinifera*), and jaboticaba (*Myrciaria cauliflora*) when exposed to pH's within the range 1–14. Grape extracts presented color variation that was easily detected by the naked eye. These results, therefore, indicate that further studies should be conducted with different sources of

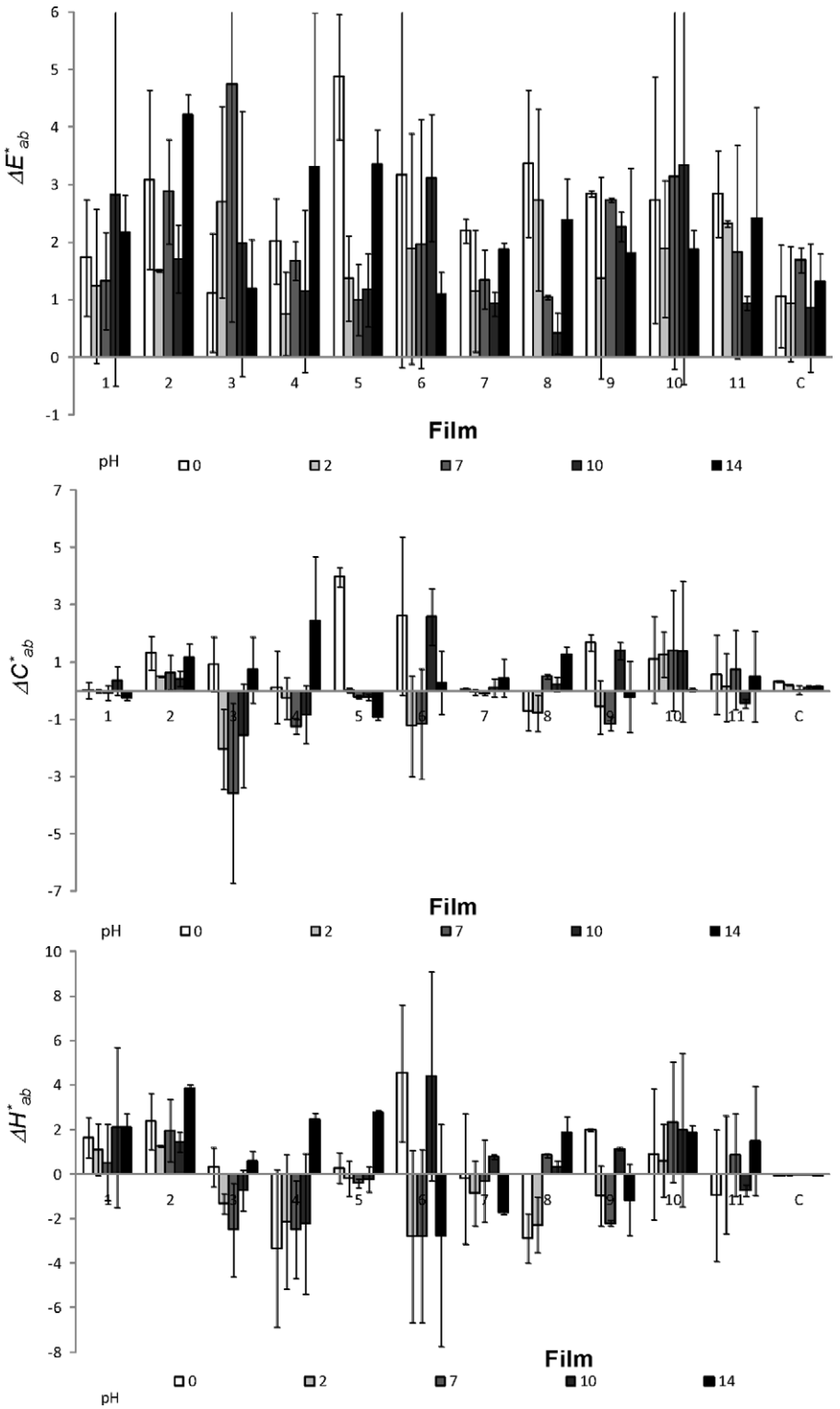

Figure 8 The total color difference (ΔE^*_{ab}), differences in chroma (ΔC^*_{ab}), and the metric hue difference (ΔH^*_{ab}) of cassava starch films added with spinach and grape extracts according to a second-order central composite design (2^2) with three central points and four star points, exposed to different pH solutions (0, 2, 7, 10, and 14) in relation to the film that was not exposed.

anthocyanin, different extraction procedures, or different concentrations, if a commercial application is to be pursued.

CONCLUSIONS

The grape and spinach extracts investigated as pH indicators have affected the mechanical and

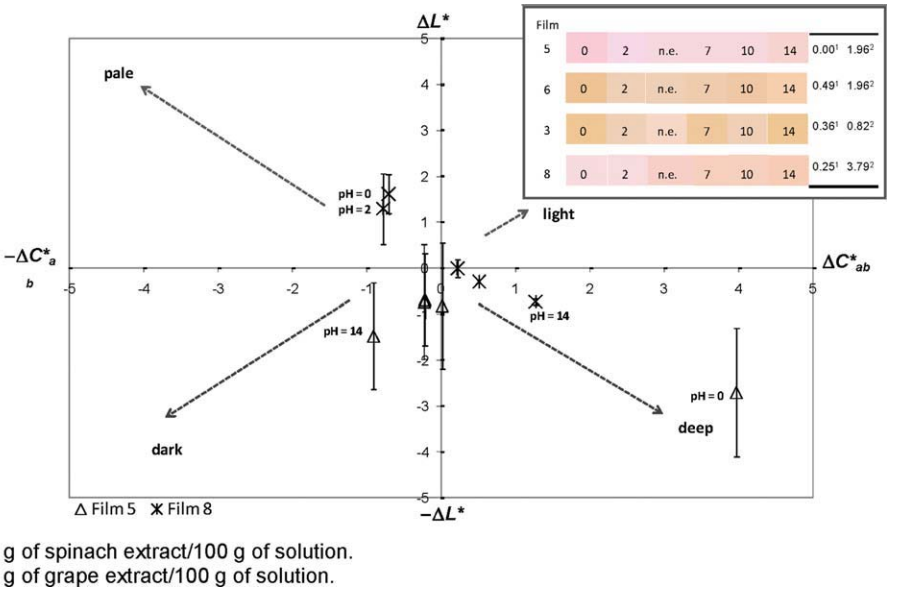

Figure 9 Differences in *chroma* and lightness of films 5 and 8 exposed to different pH solutions. Differences in visually perceived *chroma* of the films 5, 6, 3, and 8 are shown in detail, according to the pH. [1]Gram of spinach extract/100 g of solution. [2]Gram of grape extract/100 g of solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

moisture barrier properties of cassava starch films; thus, their use should be evaluated according to the type of product to be packed by the starch film material. The results indicated that anthocyanin would be a more effective pH indicator than chlorophyll. Although the colorimeter has detected and correlated color variations at different pH's, color changes were visible to the naked eye only at pH's 0.0 and 14.0, indicating that new extract sources or extraction procedures should be researched.

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