Investigation of Natural Additives as pH Indicators for Cassava Starch Biobased Materials

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Abstract. *pH can be used as indicative of food product quality and safety consumption. pH indicators can allow the consumer to evaluate a product without opening the package. With the aim of developing packages with pH indicators, and also biodegradable, this work proposes to study the use of fruit and vegetable pomace as a source of pH indicators (anthocyanin and chlorophyll) on color change of cassava starch biobased films plasticized with sucrose and inverted sugar.*

Cassava starch-plasticized films containing grape and spinach pomace were storage at 75 %RH and 23 °C, at least 4 days prior the analyses. The materials were exposed to different pH solutions (4, 7, 10) and their color parameters (L, a, b and haze) were analyzed by transmittance with a color Quest XE, Hunter Lab equipment. The biofilms were also characterized through their mechanical properties (tensile strength and elongation at break percentage), water vapor permeability, glass transition temperature and total solids content. To evaluate the pH indicator activity and the material characterization, a design experiment (2^2) with 3 central points, totalizing 11 experiments, was applied.

The results were evaluated through ANOVA, considering the pure error. The Pareto analysis was also performed to observe the significant effect (with 95% confidence) of the antioxidant components on the color change of biofilms during storage.

Grape and spinach pomace have affected the material characterization. The results indicated that grape pomace can be used to produce pH indicator films.

Keywords. Active packaging, cassava starch biofilm, pH indicator natural additives.

Introduction

Packages are used to extend product shelf life (Vermeiren, Devlieghere, Beest, Kruijf & Debevere, 1999) with mechanical protection and avoiding biological and chemical contamination (Hotchkiss, 1997). However, in attempt to please ever more demanding consumers, the active or smart packages have appeared (Rooney, 1995) intending to sense internal or external environmental changes and to respond by changing its own properties or attributes (Brody, 2001), aggregating extra benefits to the conventional packages.

Among active packages, the pH indicators, which report the correlation between the packed product and its pH (Hong & Park, 2000), have a great industrial importance, especially for the food and pharmaceutical sectors.

Most known active packages are produced with plastic materials, generating environmental problems (Arvanitoyannis & Biliaderis, 1998). As alternative appear the biodegradable packages, obtained form renewable sources (Lourdin, Coignard, Bizot & Colonna, 1997), such as the starch biobased materials (Guilbert, Gontard & Gossis, 1996 and Krochta & Mulder-Johnston, 1997).

Flexible films obtained from cassava starch were successfully developed (Henrique & Cereda, 1999; Parra, Tadini, Ponce & Lugão, 2004 and Veiga-Santos, 2004), and could be investigated as matrix for pH indicators aggregation. Besides low cost of cassava starch, Brazil is also the second world producer of cassava (FAO, 2004), justifying its investigation by Brazilian researchers.

Studies related to pH indicators are few, especially involving natural and edible components. However there are patents reporting pH indicators based on food compounds such as carotenoids (Bamore, Luthra, Mueller, Pressley & Beckwith, 2003) and anthocyanins (Rossi, 2002), they were proposed for a conventional non biodegradable package cooking procedure and for laboratory indicators, respectively. The existing patent reports are based on different materials and applications than those proposed in this study, also a large amount of natural and edible pigments were not investigated yet, such as chlorophylls, which undergo color modification when exposed to different pH environments (Lee, 1983; Francis, 1985; Belitz & Grosch, 1987), and are also potential pH indicators to be tested for active packages application.

The objective of this project was to evaluate the use of fruit and vegetable pomace as a source of pH indicators (anthocyanin and chlorophyll) on the color change of a biobased cassava starch, sucrose and inverted sugar film. To evaluate the films color parameters (L, a, b and haze) were analyzed as well as mechanical properties (tensile strength and elongation at break percentage), water vapor permeability, glass transition temperature and total solids content.

Material and Methods

Materials

Cassava starch (donated by Cargill Agrícola SA, Brazil), commercial sucrose and inverted sugar (donated by Açúcar Guarani SA, Brazil), *Merlot* grape (donated by Brasiluvas Agrícola Ltda, Brazil) and *Spinacea Oleracea L* (spinach) pomaces.

Film Preparation

Spinach and grape extracts were obtained by extraction with water after vapor blanching (15 min) and then added to cassava starch (5%), sucrose (0.7%) and inverted sugar (1.4%) (Veiga-Santos, 2004). Spinach and grape extract concentration in the film forming solutions varied from 0.00-1.05% and 0.00-8.16%, respectively. Film forming solutions were heated to 71 °C and after casting (45°C), were stored (23°C, 75% RH) for at least 4 days prior to testing.

Films were investigated by an experimental design, second order model (2^2) with 3 central points, according to Table 1.

Table 1. Coded and real values of spinach and grape extracts added to cassava starch biofilms according to a (2^2) second order experimental design with 3 central points.

Assays	Coded values		Real values (%)	
	Spinach Extract	*Grape Extract*	*Spinach Extract* [a]	*Grape Extract*[b]
1	-1	-1	0.22	1.69
2	-1	1	0.22	5.77
3	1	-1	0.74	1.69
4	1	1	0.74	5.77
5	-1.41	0	0.00	4.08
6	1.41	0	1.05	4.08
7	0	-1.41	0.53	0.00
8	0	1.41	0.53	8.16
9	0	0	0.53	4.08
10	0	0	0.53	4.08
11	0	0	0.53	4.08

^concentration (total solids) of the spinach extract
^concentration (total solids) of the grape extract

Films Characterization

Films were characterized through their mechanical properties (ASTM D882-00, 2001) as tensile strength resistance and elongation at break percentage with a TA.XT2i equipped with an A/TGT probe; thickness, through 5 measurements with a digital micrometer, total solids content by constant heating (105°C) until constant weight (Pouplin, Redl & Gontard, 1999), water vapor permeability using a NaCl solution (75%RH) as exterior humidity and silica as inner 0% RH (ASTM E96-80, 1989), glass transition temperature using a DSC Module METTLER TOLEDO instrument, refrigerated with liquid nitrogen circulation (Sobral, Menegalli, Hubinger & Roques, 2001), scanning from -60° to 250°C, with 5°C/min and 10°C/min heating rates.

Film Evaluation as a pH Indicator

Films were exposed to solutions at pHs 0.0, 2.0. 7.0, 10.0 and 14.0, and their color parameters were evaluated by a COLOR QUEST XE, Hunterlab equipment, lecture Ttran D65, 10°, in duplicate (Veiga-Santos, Suzuki, Cereda & Scamparini, 2005).

Results and discussion

Mechanical Properties

Tensile strength of films varied from 1.8 to 4.2 MPa (Table 2), and the ANOVA indicated that, considering the pure error, the concentration of natural extracts added to the cassava starch films did not influence their tensile strength ($p>0.05$). However, when compared to the control films (8.5 MPa), it can be observed that the natural extracts have lowered the tensile strength (up to 78.90%).

The statistical analysis applied to the results has indicated that the increase on the grape pomace extract concentration negatively affected the elongation at break percentage. A possible explanation is that the sugars naturally present in grape pomace, such as glucose and fructose, have acted as plasticizers. As the film base already had added plasticizers (sucrose, fructose and glucose), the concentration in the final material may have been too high, resulting in excessive interactions

between the film network and the plasticizers (Arvanitoyannis, Psomiadou & Nakayama, 1996), lowering film flexibility.

Table 2. Mechanical properties (tensile strength and elongation at break), water vapor permeability rate (WVPR) and water vapor permeability (WVP) of cassava starch biofilms with added spinach and grape extracts according to a (2^2) second order experimental design with 3 central points.

Assays	Tensile Strength (MPa)	Elongation at Break (%)	WVPR ($g/m^2.day$)	WVP ($g.mm/m^2.day.mmHg$)
1	2.9 ± 0.1	114 ± 15	73.24	378.17
2	1.9 ± 0.1	99 ± 17	58.09	369.34
3	4.2 ± 0.6	80 ± 13	82.40	448.42
4	2.2 ± 0.1	67 ± 13	66.48	382.56
5	2.9 ± 0.3	65 ± 11	186.69	1158.52
6	2.1 +0.2	89 ± 18	33.14	219.58
7	3.7 ± 0.4	217 ± 19	96.18	522.88
8	1.8 ± 0.2	76 ± 10	90.87	558.15
9	2.1 +0.3	84 ± 6	69.78	462.99
10	3.2 ± 0.3	73 ± 18	61.66	380.69
11	3.1+0.3	73 ± 12	36.36	185.69
Control	8.5 ± 1.7	96 ± 18	49.51	303.71

Comparing the experimental films with the control, the additives have increased (up to 125%) and lowered (up to 32%) the elongation at break percentage (Table 2). Such variation may be explained due to a few natural compounds present in the extracts used in this study, such as glucose, sucrose, maltose and cellulose, which can greatly affect a starch film network and mechanical performance (Qiu, Ding, Tang & Xu, 1999). Also the humidifying ability of such components may have affected the mechanical resistance of the biodegradable materials. The cassava starch biobased films, which are already highly hydrophilic materials (Avérous, Fringant & Moro, 2001), could have their hydrophilicity increased by the natural components, absorbing even more water.

The ANOVA, considering the pure error, has also indicated that thickness was not affected by the pomace extract concentration ($p>0.05$), varying from 0.09 to 0.10 mm.

Water Vapor Permeability

The ANOVA indicated that water vapor permeability and water vapor permeability rate were not affected ($p>0.05$) by the spinach or grape extract concentration, at the studied values (Table 2). However, when compared to the control film, the extracts concentration increased ($p<0.05$) water vapor permeability (up to 280%) and water vapor permeability rate (up to 410%). 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 (Arvanitoyannis *etai,* 1996).

Glass Transition Temperature

Although the 10 °C/min scanning temperature is usually employed by several authors (Carvalho & Grosso, 2004; Mucha & Pawlak, 2005) to investigate biobased materials glass transition temperature by DSC, a dislocation of the heat loss or gain detection from sample may be observed. A 5 °C/min scanning, also frequently employed (Cuq, Gontard & Guilbert, 1997; Vanin, Sobral, Menegalli,

Carvalho & Habitante, 2005), result in lower resolution, with wider peaks for the heat loss or gain from sample. However, the exact temperature where the glass transition occurs is very important for a material that will be used as packaging.

Table 3 indicates that two glass transition temperatures were observed. Such result indicates phase separation. Since the control film also presented both Tgs, the extract concentration did not influence the material phase separation. The first Tg observed for the assays is negative and its determination was better obtained for the 5 °C/min heating rate and varied from - 39.10°C to - 2.64°C.

For the second Tg (positive) and for the endothermic peak, better correlation coefficients were obtained for the 10 °C/min heating rate. This is probably because of the endothermic peak enlargement at the 5 °C/min heating rate, which has even superimposed itself on the positive Tg. The endothermic peak is probably caused by the gelatinization of the cassava starch grains that were not completely gelatinized during the biobased films preparation step.

ANOVA has indicated, considering the pure error, that the grape pomace extract affected the second (positive) Tg. The glucose and fructose that might be present in the extract is the probable explanation. Such sugars, commonly affect the material Tg due to their plasticizing nature (Vanin *et al,* 2005).

The ANOVA has also indicated that the grape pomace extract has a significant effect ($p<0.05$) on the endothermic peak. Assuming that the endothermic peak is due to the gelatinization temperature of the starch grains, the sugars (glucose and fructose) present on the grape pomace could have affected the endothermic peak. High concentration of sugars may have increased the gelatinization temperature (Veiga-Santos, 2004) of the film forming solution.

Table 3. DSC results obtained for samples 1-11 and the control (C) film through different heating rate scanning.

Assays	5 °C/min						10° C/min					
	T1 [a] ons	T1 [b] I mid	T? [c] 2ons	$T2_{m}$id	T END [e] I	AHENDO	T1 [a] ons	I 1mid	T? [c] 2ons	$T2_{m}$id	T END [e] I	AHENDO
1	X	X	65.93	66.27	126.74	95.68	-11.22	-12.10	30.47	35.34	120.88	245.69
2	-29.15	-25.5	42.75	42.57	115.9	74.93	-32.43	-32.51	30.37	35.75	128.80	194.41
3	X	X	X	X	97.38	275.10	-25.16	-24.09	29.85	32.02	124.99	129.9
4	-39.06	-39.10	38.50	36.02	109.66	256.55	-32.47	-21.97	20.07	30.88	127.52	233.32
5	-13.29	-10.56	53.88	53.93	181.07	403.27	-31.16	-31.40	5.62	21.57	118.43	282.13
6	-30.01	-26.5	14.15	17.44	73.02	99.05	-37.76	-32.25	55.75	54.86	158.39	258.01
7	-0.43	-2.64	45.53	44.99	123.32	68.33	-6.77	-6.32	48.07	49.34	143.27	179.48
8	-29.33	-22.29	11.02	19.89	88.58	374.53	-23.12	-23.11	18.73	25.46	132.00	184.17
9	-10.30	-11.97	17.06	23.07	64.00	2.22	-12.17	-12.72	13.83	24.76	95.07	111.52
10	-22.38	-21.24	35.05	35.29	69.61	106.02	-33.35	-28.19	19.26	22.06	121.90	242.13
11	-7.90	-10.01	13.79	18.49	70.49	4.15	-29.35	-29.6	26.36	39.74	156.07	227.75
Control	-7.36	-4.51	29.45	27.9	155.06	304.2	-9.29	-7.48	28.83	33.95	88.92	221.38

x: results with too much noise, impossible to evaluate the thermic variations; a: 1st Tg onset (°C); b: 1st Tg mid point (°C) ; c: 2nd Tg onset (°C); d: 2nd Tg mid point (°C); e: endothermic peak (°C); f: endothermic peak enthalpy variation ($J.g^{1}$).

Film Evaluation as pH Indicator

To evaluate the biobased materials as pH indicators, the most important parameters are the Hunter Lab scale parameters redness "a" (varying from green to red) and yellowness "b" (varying from yellow to blue).

The biofilms exposed to the different pHs reacted to variations of the color parameters "a" and "b" (Table 4), indicating correlation between color and pH variation.

Table 4. "a" and "b" color parameters presented by samples **(1-11)** and the control films when exposed to different pH solutions (0, 2, 7,10 and 14) or not exposed.

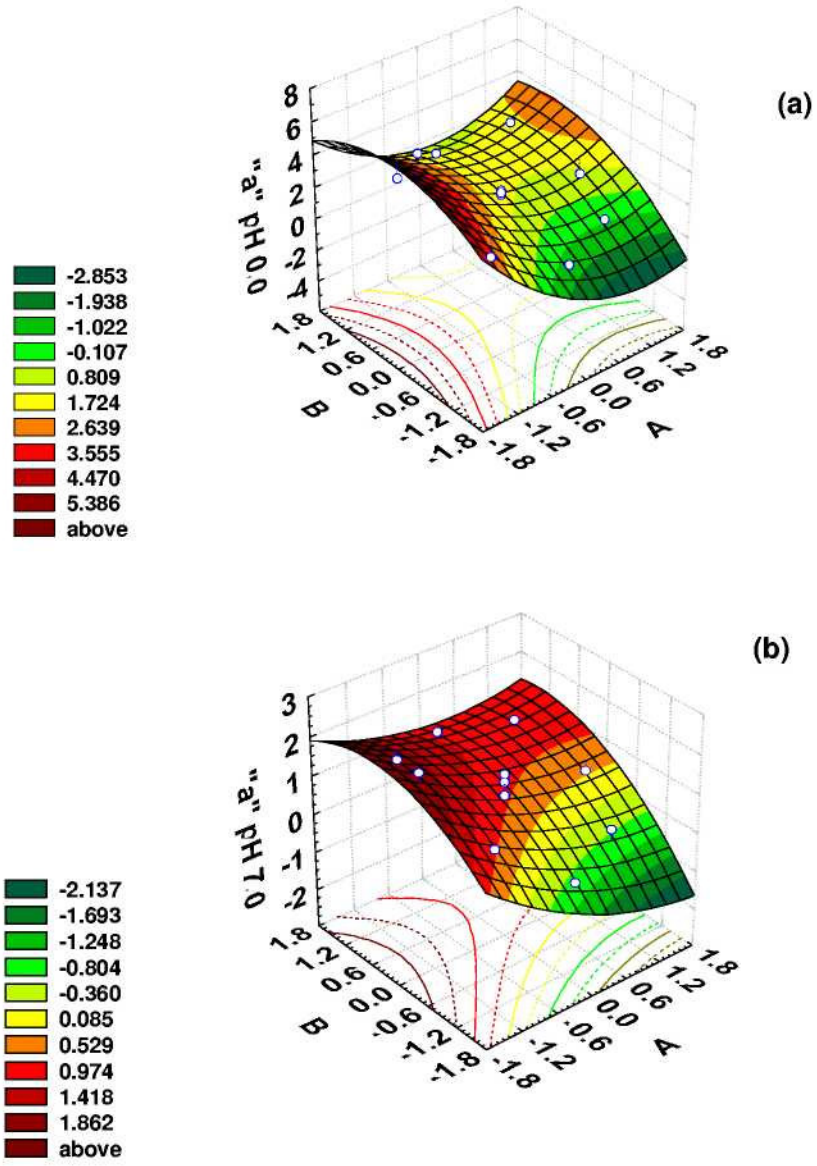
Assays	Not Exposed		pH 0		pH 2		pH 7		pH 1		pH 14	
	a	b	a	b	a	b	a	b	a	b	a	b
1	0.48	3.94	0.56	5 .47	0.52	4.95	0 .44	4.43	0.51	5 .86	0.27	0 .07
2	0.95	5.23	1.75	7 .56	1.32	6.43	1 .23	7.02	1.16	6 .58	0.76	5 .95
3	-0.57	12.30	-0.54	1 .03	-0.47	10.31	-0 .62	8.70	-0.64	1 .94	-0.89	8 .85
4	1.16	11.28	2.32	1 .35	1.29	10.85	0 .93	9.85	1.00	1 .42	0.89	13 .07
5	2.37	3.68	6.51	3 .09	2.37	3.44	2 .09	3.31	2.13	3 .46	1.51	13 .75
6	-0.14	11.95	-0.16	1 .30	-0.15	10.79	-0 .06	10.81	-0.08	1 .20	-0.31	6 .21
7	-1.09	5.95	-1.00	5 .77	-0.91	5.19	-1 .02	5.69	-1.25	6 .67	-0.61	12 .39
8	0.90	7.62	0.86	5 .01	0.77	5.50	0 .94	8.50	0.92	7 .96	0.96	4 .30
9	1.02	8.83	0.90	1 .00	1.03	7.86	0 .94	6.67	1.38	1 .28	1.40	9 .59
10	0.78	6.78	1.23	7 .84	1.72	7.70	0 .69	9.18	0.74	8 .84	-0.09	7 .89
11	0.35	7.06	1.05	6 .41	0.38	7.10	0 .28	8.01	0.14	6 .30	-0.04	8 .31
Control	0.11	0.20	0.25	-0 .04	0.17	0.07	0 .13	0.16	0.15	0 .14	0.14	8 .43

When the biobased films were exposed to 0.0 and 7.0 pH solutions; the extract pomace additives affected ($p<0.05$) the material redness. Such result indicates that both extracts can change colors from green to red when exposed to pH solutions varying from acid (0.0) to neutral (7.0), as can be observed in Figure 1.

Increasing grape pomace extract, increases the red color, and increasing spinach pomace extract, increases the green color. Such fact was expected due to the characteristic color presented by the anthocyanin (red) and chlorophyll (green) present in the respective extracts. In acidic medium, the anthocyanin presents an intensification of the red color, and the chlorophyll, intensification of the green tone.

The ANOVA, considering the pure error, indicates that the biofilm yellowness ("b") was affected by exposition to pHs 2.0; 7.0 and 14.0 ($p<0.05$), but not affected by pHs 0.0 and 10.0. Increasing the spinach pomace extract, increases the yellow color of the biobased films for pHs 2.0; 7.0 and 14.0 ($p<0.05$). The response surface (Figure 2) indicates that the grape pomace also affected the film yellowness. At alkali pH, anthocyanin can assume a bluer color, which associated to the green color of the chlorophyll, could result in a yellow final color.

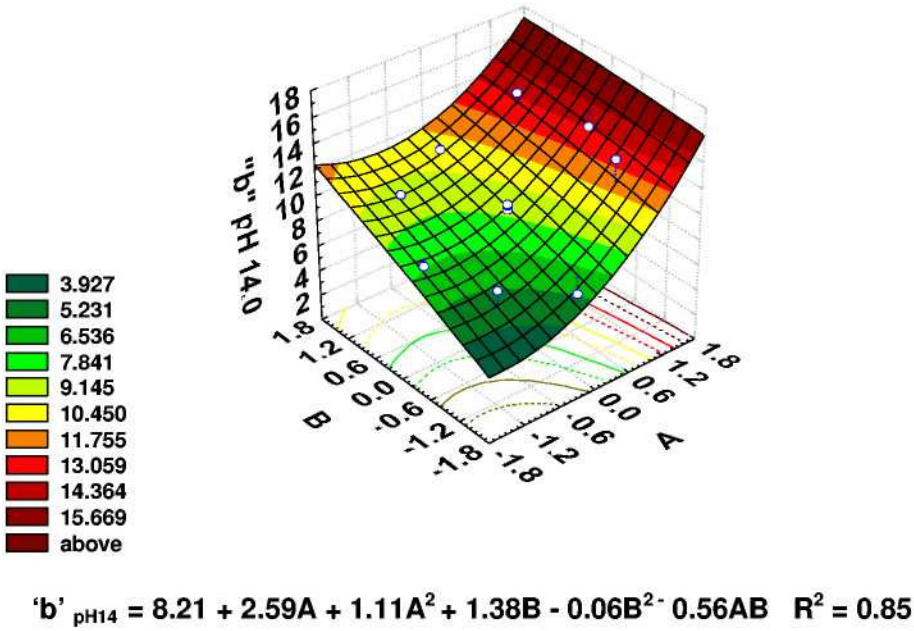
The parameters "L" and haze were also affected by pH modifications, varying from 88 to 95% and 17 to 66%, respectively.



$$\text{'a'}_{pH0} = 1.10 - 1.20A + 0.93A^2 + 0.83B - 0.70B^2 + 0.42AB \quad R^2 = 0.73$$

$$\text{'a'}_{pH7} = 0.64 - 0.55A + 0.19A^2 + 0.64B - 0.34B^2 + 0.19AB \quad R^2 = 0.92$$

Figure 1. Response Surfaces obtained for the effect of spinach (A) and grape (B) pomace extracts on samples redness ("a") when exposed to pH 0.0 (a) and pH 7.0 (b).



$$'b'_{pH14} = 8.21 + 2.59A + 1.11A^2 + 1.38B - 0.06B^{2} - 0.56AB \quad R^2 = 0.85$$

Figure 2. Response Surface obtained for the effect of spinach (A) and grape (B) pomace extracts on samples redness ("b") when exposed to pH 14.0.

Conclusions

The natural extracts investigated as additives have affected the characterization properties of cassava starch films, and so, its utilization should be evaluated according to the type of product to be packed by the starch film material.

The results indicated that chlorophyll and anthocyanin could be used as pH indicators for biodegradable materials. Although the colorimeter has detected and correlated color variations to pH exposition, color changes were only visible with naked eyes at pHs 0.0 and 14.0, indicating that new extracts sources or extraction procedures should be investigated.

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