Investigation of Antioxidant Activity of Cassava Starch Biobased Materials

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Abstract. *The search for antioxidant packages has increased recently in order to increase products shelf life and/or decrease or eliminate antioxidants components from food products. With the aim of developing packages that, besides antioxidant are also biodegradable, this work proposes to study the use fruit and vegetable pomace as a source of antioxidant components (anthocyanin, flavonoids and chlorophyll) in the cassava starch biobased films plasticized with sucrose and inverted sugar.*

Soybean oil was packed in cassava starch-plasticizers materials containing grape and spinach pomace and they were stored at 64% RH and 4CPCto accelerate the oxidation reaction. The antioxidant activity were evaluated through the peroxide content of the soybean oil and analyzed at 0, 7, 14 and 21 days of storage. Unpacked soybean oil and soybean oil packed in LDPE films and cassava starch-plasticizers (with no antioxidant additives) films served as controls. To evaluate the antioxidant activity, an design experiment (2^2) with 3 central points, totalizing 11 experiments, was applied. The extracts addition effect on the biobased material characterization (mechanical properties and water vapor barrier) was also evaluated.

ANOVA was performed and has indicated a significant effect (with 95% confidence) of the antioxidant components on the soybean oil peroxide content during storage. Grape and spinach pomace have affected the material characterization. The results indicated that pomace vegetable and fruits might be added to biodegradable films as source of antioxidant components.

Keywords. Active packaging, cassava starch biofilm, antioxidant natural additives

Introduction

Packaging may be termed active when it performs some role other than providing an inert barrier to external conditions, doing more than just offering protection. They interact with the product, and in some cases, actually respond to changes (Rooney, 1995).

Oxygen is a highly reactive gas, which can cause the deterioration of almost all food products in terms of flavor, color, nutritional value and safety (Rooney, 1995).

Traditional antioxidants applied to delay lipid oxidation by interrupting free radical chain reactions have been incorporated into package materials for many years (Brody, 2001). However, very little has been studied with natural edible compounds and even less are related to biodegradable materials. Most known active packages are produced with plastic materials, generating environmental problems (Arvanitoyannis & Biliaderis, 1998). As an 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 matrices for antioxidant indicators aggregation. Besides low cost of cassava starch, Brazil is also the second world producer of cassava (FAO, 2005), justifying its investigation by Brazilian researchers.

Considering some natural edible components with confirmed antioxidant activity, special attention has been given to flavonoids (Majo, Giammanco, La Guardiã, Trípoli, Giammanco & Finotti, 2005), anthocyanins (Awika, Rooney & Waniska, 2004) and chlorophyll (Lanfer-Marquez, Barros & Sinnecker, 2005), which are commonly found in fruits and vegetables.

The aim of this work was to investigate the antioxidant activity of natural and edible compounds to be used in active antioxidant packages. Spinach and grape pomace extracts were added to cassava starch biobased films and their antioxidant properties were studied.

Materials and Methods

Materials

Cassava starch (donated by Cargill Agrícola SA), commercial sucrose and inverted sugar (donated by Açúcar Guarani SA), *Merlot* grape (donated by Brasiluvas Agrícola Ltda.) 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*	*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℃) 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).

Antioxidant activity evaluation

Soybean oil was packed in cassava starch-plasticizer materials containing grape and spinach pomace extract and stored (64% RH, 40℃) for a total of 3 weeks to accelerate the oxidation reaction. The antioxidant activity was evaluated through the peroxide content of the soybean oil (Azeredo, Faria & Silva, 2004) and analyzed at 0, 7 and 21 days of storage. The antioxidant activity was evaluated through a surface methodology design experiment (2^2) with 3 central points, totalizing 11 experiments. Unpacked soybean oil (control 1) and soybean oil packed in LDPE (control 2) and in cassava starch-plasticizers with no antioxidant additive (control 3) films were used as controls. The results were analyzed through ANOVA, considering the pure error (with 95% confidence).

Results and discussion

Mechanical Properties

The effect of the extracts concentration on the material mechanical properties was evaluated through their tensile strength and elongation at break percentage. Tensile strength varied from 1.8 to 4.2 MPa (Table 2).

The ANOVA, considering the pure error, has indicated that tensile strength is not affected ($p>0.05$) by the additives concentration. Such result indicates that, under the conditions studied, the concentration of natural extracts added to the cassava starch films did not influence tensile strength of the films.

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

However, when compared to the control 3 films (8.5 MPa), it can be observed that the natural extracts have lowered the tensile strength (up to 78.90%).

The elongation at break varied from 67 to 217% (Table 2). The ANOVA has indicated that the grape pomace extract lowered ($p<0.05$) the elongation at break percentage.

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.

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 *etal.,* 1996).

Antioxidant activity evaluation

Table 3 presents the peroxide content increase during the first and third week of storage of samples 1-11 and the unpacked soybean oil (C1), and the soybean oil packed with the LDPE (C2) and the cassava starch (C3) films.

Table 3. Peroxide value (PV) content of the soybean oil packed with samples (1-11), the controls C1 (unpacked soybean oil), C2 and C3 (soybean oil packed with the LDPE and the cassava starch films, respectively).

Assays	PV (mEq .kg-1)[1]	
	at 1-week storage	at 3-weeks storage
1	2.67[a,b]	1.65[b]
2	2.00[a,b]	3.99[b,c]
3	0.67[a]	4.67[b]
4	1.33[a]	2.00[b]
5	1.33[a]	2.67[b]
6	1.33[a]	10.64[c]
7	1.33[a]	4.67[b,c]
8	0.00[a]	2.32[b]
9	0.33[a]	2.66[b,c]
10	0.33[a]	4.31[b]
11	0.33[a]	2.98[b,c]
C1	5.33[b]	1 65.33[a]
C2	2.67[a,b]	10.00[b,c]
C3	2.00[a,b]	5.33[b,c]

[1] Same letters in a column indicate that there is no significant difference between points ($p>0.05$ for the Tukey test).

Observing Table 3 and the ANOVA analysis, a very different oxidation behavior between the unpacked soybean oil control (C1) and the other controls (C2 and C3) after a 3-week storage period can be noticed. Such result indicates that both cassava starch-plasticized film (C3) and the LDPE film (C2) were effective in protecting the soybean oil against oxidation, lowering the peroxide content production. Table 3 also indicates that the cassava starch-plasticized film had a capacity of protecting against oxidation similar to the LDPE film.

Results presented in Table 3 also demonstrated that addition of the pomace extracts has influenced protection against oxidation. The ANOVA, considering the pure error, has indicated that only the spinach pomace extract affected ($p<0.05$) the peroxide content during the 3-week accelerated storage (64% RH. 40°C) period.

Figure 1 represents the effect of the pomace extracts concentration on the soybean oil peroxide value content after 3-week accelerated storage.

Observing Table 3 and Figure 1, it can be concluded that although a little spinach pomace addition may decrease the peroxide value concentration, increasing its concentration above 0.22% total solids increases the peroxide value concentration.

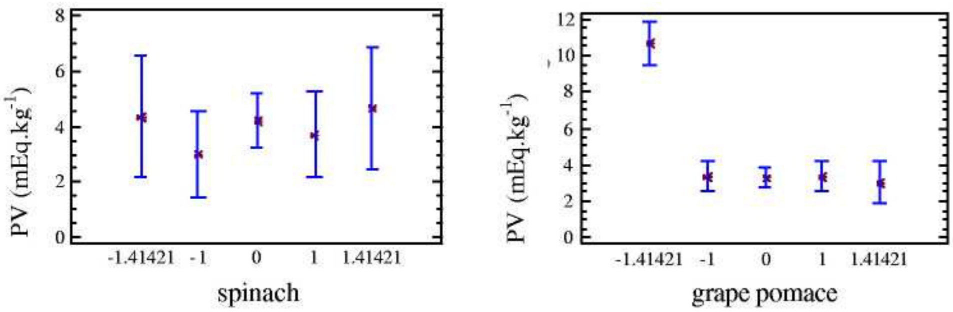

Figure1. Spinach and grape pomace extracts concentration effect ($p<0.05$) on the soybean oil peroxide value content after 3-weeks accelerated storage (60% RH, 40°C).

Also sample 6, containing the maximum level of spinach pomace and no grape pomace extract presented the worst protection against oxidation among the biodegradable biobased films added with the natural antioxidant extract. Sample 6 presented the highest peroxide value (10.64 $mEq.kg^{-1}$) with the exception of the unpacked soybean oil (C1), as can be observed in Table 3. Such result indicates that probably, the chlorophyll present in the spinach pomace acted as an oxidant component. A possible explanation is that although the chlorophyll molecules bonded to oxygen molecules eliminating them from the package environment, the bound oxygen present on the material surface reacted with the free radicals present in the soybean oil, resulting in higher formation of peroxides.

Results also demonstrated that although the grape pomace concentration has not affected the soybean oil peroxide value, there is a clear difference when the grape pomace is present in the biobased polymer. The lowest and highest concentration tested (1.69% and 8.16% grape pomace total solids) had the same effect on the antioxidant activity of the packed product (soybean oil). Figure 1 also shows that the lowest grape extract concentration evaluated was enough to influence the peroxide value of the soybean oil, indicating potential of grape pomace extract use as an antioxidant additive.

Conclusions

The results indicated that grape pomace, commonly discharged by wine industries, may be used as ingredient for producing antioxidant active biodegradable packaging. However, the grape pomace extract has affected a few 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.

Acknowledgments

The authors would like to acknowledge Cargill Agrícola SA, Açúcar Guarani SA and Brasiluvas Agrícola Ltda. for the ingredients donated and the Brazilian Committee for Postgraduate Courses in Higher Education (CAPES) for the scholarships granted.

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