



Contents lists available at ScienceDirect

Active biocomposites of cassava starch: The effect of yerba mate extract and mango pulp as antioxidant additives on the properties and the stability of a packaged product



CrossMark

Letícia Caribé Batista Reis^a, Carolina Oliveira de Souza^{a,*},
 Jania Betânia Alves da Silva^b, Augusto Cezar Martins^a,
 Itaciara Larroza Nunes^c, Janice Izabel Druzian^a

^a Federal University of Bahia, College of Pharmacy, Department of Food Science, Av. Barão de Geremoabo, s/nº, Campus Ondina, Salvador, BA 40171-970, Brazil

^b Federal University of Bahia Recôncavo, Center of Science and Technology, Department of Mechanical Engineering, Rua Rui Barbosa, 710, Cruz das Almas, BA 44.380-000, Brazil

^c Federal University of Bahia, School of Nutrition, Department of Food Science, Rua Araújo Pinho, 32, Canela, Salvador, BA CEP: 40110-150, Brazil

ABSTRACT

There is an increasing interest in the utilization of renewable resources for the production of food packaging. Among the biopolymers, starches from several sources are considered promising materials for this purpose, because they are biodegradable, inexpensive and available worldwide. Antioxidant food packaging films were produced by incorporating mango pulp and yerba mate extract into a cassava starch matrix. The bio-based films were used to pack palm oil (maintained for 90 days of storage) under accelerated oxidation conditions (63% RH/30 °C) in order to simulate a storage experiment. Palm oil packaged in these films exhibited a decreased oxidative process rate, which was attributed to the yerba mate and mango pulp in a concentration-dependent fashion. The evolution of the peroxide value contents indicated that, in general, the films with high concentrations of additives improved palm oil stability. Mechanical, physical and barrier properties of the developed film indicated that the addition of these bioactive compounds modified their properties significantly ($p < 0.05$).

© 2014 The Institution of Chemical Engineers. Published by Elsevier B.V. All rights reserved.

Keywords: Mango pulp; Yerba mate extract; Active films; Palm oil; Characterization; Bio-based films

1. Introduction

Traditional food packages are passive barriers designed to delay the adverse effects of the environment on the food product. Active packaging allows packages to interact with food and the environment and plays a dynamic role in food preservation (Brody et al., 2008, 2010).

In recent years, the development of biodegradable packaging materials made from renewable natural resources (e.g., starch) has received increasing attention. Many new food-packaging concepts have been introduced to satisfy consumer demands (Souza et al., 2013). Plastic materials produced from petrochemicals are widely used due to their versatility, mechanical properties and low cost, but the accumulation of

* Corresponding author. Tel.: +55 71 3283 6907; fax: +55 71 3283 6900.

E-mail addresses: leticiacaribe@yahoo.com.br (L.C.B. Reis), carolinaods@hotmail.com (C.O. de Souza), janiabete@yahoo.com.br (J.B.A. da Silva), gutomartins18@hotmail.com (A.C. Martins), itaciara@yahoo.com (I.L. Nunes), janicedruzian@hotmail.com (J.I. Druzian).

Received 17 July 2013; Received in revised form 30 April 2014; Accepted 7 May 2014

Available online 17 May 2014

<http://dx.doi.org/10.1016/j.fbp.2014.05.004>

0960-3085/© 2014 The Institution of Chemical Engineers. Published by Elsevier B.V. All rights reserved.

large amounts of these conventional synthetic polymers can cause environmental impacts (Brody et al., 2010; Jiménez et al., 2012).

Problems caused by the disposal of these plastics have motivated the development, production and application of biodegradable polymers. Alternatively, there is a growing interest in using biodegradable packaging made from renewable sources, such as films made from starch (Müller et al., 2009).

Oxidation is one of the main mechanisms for the deterioration and reduced shelf life of foods. In addition, oxidation alters the taste (rancidity) and nutritional quality (loss of vitamins and essential fatty acids) of food and generates reactive and toxic compounds that pose a danger to consumers (Lindley, 1998). Antioxidant packaging is an important type of active packaging and represents a very promising food preservation technique for extending food product shelf life (López-de-Dicastillo et al., 2012). The incorporation of antioxidants into biodegradable packaging films has become very studied because oxidation is one of the main causes of food spoilage (Souza et al., 2011; Pereira-de-Abreu et al., 2011).

The incorporation of synthetic antioxidants into packaging materials has recently received increased interest due to toxicological concerns, prompting an increased interest in natural antioxidants (Bonilla et al., 2012). Phenolic acids, terpenes, tocopherols, carotenoids and vitamins are important natural antioxidants that have been proposed for incorporation into packaging to improve the oxidation stability of lipids and to prolong the storage of products (Siripatrawan and Harte, 2010; López-de-Dicastillo et al., 2012; Cavar and Maksimović, 2012). Although many materials with antioxidant activity have been tested, few studies have used natural, edible compounds, and even fewer studies have evaluated how these compounds could be incorporated into bio-based films (Oussalah et al., 2004).

Phytochemical studies of the mango (*Mangifera indica L.*) have shown significant amounts of secondary metabolites that are common components of the human diet. These bioactive constituents, including carotenoids, phenolic compounds and vitamin C, are present in fruits (Ribeiro et al., 2008) and industrialized pulps (Kuskoski et al., 2006) and provide antioxidant protection due to their capacity to scavenge free radicals.

The antioxidants are classified into two types, primary and secondary, depending on the mechanism used to halt the degradation process. The combination of two antioxidants, both primaries or a primary and a secondary, can result in a synergistic effect (Carelli et al., 2005). Studies have showed probable synergistic effect between carotenoids and phenolic compounds (Mertz et al., 2009; Han et al., 2011). However, the understanding of the molecular mechanisms underlying such synergistic effects, as well as the number of the studies, is still limited.

Cassava starch active films containing mango and acerola pulps as additives have already been developed and tested as packaging for palm oil by our research group (Souza et al., 2011). However, acerola pulp exhibited a pro-oxidant effect, likely due to its high concentration of vitamin C. Yerba mate contains high levels of phenolic compounds (Bravo et al., 2007) that can be used as a substitute for acerola pulp, increasing the antioxidant effectiveness of active films. This replacement is important because some of the biological properties of the yerba mate aqueous extract are attributed to the presence of these compounds, including antioxidant properties and protection lipid peroxidation (Murakami et al., 2011).

Yerba mate (*Ilex paraguariensis*, St. Hil.) is a commercially important plant from the subtropical region of South America. It is composed of minerals, vitamins, alkaloids (methylxanthines), phenolic flavonoids (quercetin and rutin) and tannins. The antioxidant properties of yerba mate mainly arise from its phenolic compounds (Bravo et al., 2007).

This study aimed to investigate the antioxidant efficacy and physical, barrier and mechanical property alterations resulting from the incorporation of mango pulp and aqueous yerba mate extract in cassava starch-based edible films using a response surface methodology. The incorporation of these derivatives into packaging produced with cassava starch has great economic importance due to the added value of natural, edible and commercial available raw materials.

2. Materials and methods

2.1. Materials

Cassava starch (Cargill Agrícola SA, Brazil), glycerol (Labsynth, Brazil) and palm oil were obtained from ODELSA SA, Brazil. Mango pulp, yerba mate (*I. paraguariensis*, St. Hil.) and low-density polyethylene (LDPE) films (0.040 mm thickness) were purchased from local markets (Salvador, Bahia, Brazil).

2.2. Characterization of additives

Mango pulp and yerba mate powder were characterized in relation to their chemical composition. Phenolic compounds, flavonoids and carotenoids were measured by UV spectrophotometry according to Roesler et al. (1999), Lee et al. (2003) and Souza et al. (2012a), respectively. Moisture and total solids values were determined by gravimetric measure (AOAC, 2000). The pH values were determined according to the analytical standards (IAL, 1985). The contents of total, soluble and insoluble dietary fiber were analyzed by enzymatic-gravimetric method (AOAC, 2000). Extraction of lipids was performed according to Bligh and Dyer (1959). Fatty acids (FA) profile were separated after hydrolysis and methylation, by gas chromatography (Varian® model 3800), in capillary a column (Elite-WAX 30 m × 0.32 mm × 0.25 mm), and flame ionization detector, the quantification was performed by area normalization (Nascimento et al., 2013). Free sugars of aqueous solution of mango pulp and yerba mate powder were separated by HPLC-IR (PerkinElmer 200 series) using a Polypore Ca pre-column (30 mm × 10 mm × 4.6 mm) followed by a Polypore Ca column (220 mm × 4.6 mm × 10 mm), in an oven at 80 °C. The mobile phase used was chromatographic grade water flowing at 0.1 mL min⁻¹. The quantification was using a standard curve (Assis et al., 2014).

2.3. Preparation of biocomposite films

Preliminary experiments were conducted to evaluate the maximum concentrations of additives that could be incorporated to the films, in order to obtain homogeneous materials, flexible and easy to handle. Therefore, different concentrations of mango pulp (10%, 20% and 30%) and yerba mate (20%, 30% and 40%) were alternately tested. At the end of this stage, the maximum concentrations were fixed in 20% for mango pulp and 30% for the yerba mate. The other concentrations used did not show desirable characteristics in the films obtained.

For films production, film-forming dispersions was prepared by with an aqueous yerba mate extract obtained from

yerba mate powder (0–30%) by method of percolation with 2 L hot water (70 °C) for optimal extraction and preservation of antioxidant compounds (Bravo et al., 2007). The extract was cooled to room temperature and then filtered through Whatman No. 1 filter paper. The yerba mate extract was used to dissolve cassava starch (4%), glycerol (1%) and mango pulp (0–20%). Dispersions were shaken at 70 °C with constant stirring for 10 min and placed under ultrasonic bath for to remove the bubbles and excess air dissolved in the suspension. The film-forming dispersions were poured onto polystyrene Petri dishes and dehydrated at 35 °C in an oven with airflow and circulation (Nova Ética, 400ND, Vargem Grande Paulista, Brazil). Dry films were stored at 23 ± 2 °C and 60 ± 2% relative humidity (RH) in desiccators with a supersaturated solution of magnesium nitrate for 2 days before being characterized (Souza et al., 2011).

2.4. Characterization of biocomposite films

For characterization, cassava starch films without additives (WA) were used as a control.

2.4.1. Moisture content

The moisture contents of films were determined by measuring their weight loss upon drying in an oven at 105 °C until they reached a constant weight (dry sample weight). Samples were analyzed at least in duplicate, and results are expressed as percentage (g/100 g) moisture contents of samples.

2.4.2. Thickness

The thickness of preconditioned film (75% RH, 23 °C) was determined using a Mitutoyo digital micrometer (Tokyo, Japan) (1 µm resolution). Ten measurements were randomly taken at different locations for each specimen, and the mean value is reported.

2.4.3. Water vapor permeability

Water vapor permeability (WVP) tests were conducted using American Society for Testing and Materials, method E96 (ASTM, 2000) with modifications as described in a previous work (Souza et al., 2012b). The films were fixed onto open cells with silica gel inside desiccators containing a saturated NaCl solution (75% RH). For all samples tested, a cell containing just the films (no silica gel) was used as a control to estimate the weight modifications of the material due to its humidity adsorption/desorption arising from its affinity for water. The cells were weighed daily over 5 days to guarantee steady-state permeation.

2.4.4. Mechanical properties

The tensile strength (% TS) and elongation at break (% E) of the films were measured using an Instron Universal Testing Instrument (EMIC – Linha DL 20.000 20KN, PR, Brazil) operated according to ASTM (2001) standard method D882-00. Ten specimens were tested for each sample.

2.5. Biocomposite films used as packaging

Film samples were used to package palm oil for storage evaluations using a response surface methodology. Square-shaped bio-based films (5 cm × 2 cm) were used to simulate the antioxidant capacity and stability of packaged palm oil for 0, 7, 15, 30, 45, 60 and 90 days under storage conditions of 63% RH at 30 ± 2 °C. For the storage of palm oil, films were cut to

10 cm × 4 cm and sealed (Sealer SULPACK Basic SM BL 350, Brazil) at the bottom and sides. Using a volumetric pipette, 10 mL of palm oil was transferred into the packaging. Oxygen bubbles were removed, and the film was sealed on top. Film storage of the palm oil and the analyses were performed in a dark room to avoid the effects of light interference.

2.6. Stability of biocomposite films during storage

The stabilities of the biocomposite films used to package palm oil were evaluated by analyzing the concentration of total polyphenols (TP), total flavonoids (TF) and total carotenoids (TC) during a storage period of 90 days. On day 7, 15, 30, 45, 60 and 90, samples of palm oil packed in 11 formulations containing different concentrations of antioxidant additives were removed from storage for the analyzes. Palm oil stored in the films was removed and used for evaluation of oxidation, while the film samples were used for analysis of the concentrations of antioxidants (TP, TF and TC).

TP concentrations were spectrophotometrically determined at 760 nm (UV/Vis Spectrometer Lambda 20, Perkin-Elmer, Norwalk, USA) using Folin-Ciocalteu reagent (Roesler et al., 1999), and the results are expressed in gallic acid equivalents.

TF concentrations were measured using a spectrophotometer at 510 nm and were calculated using a standard curve prepared with dilutions of an epicatechin standard (Lee et al., 2003).

For TC values, film samples (2 g) were prepared according to Souza et al. (2012a) and were analyzed spectrophotometrically at 440 nm. TC concentrations were determined according to Davies (1976).

2.7. Packaged product oxidative stability during storage

The oxidative stability of the packaged product was determined using the peroxide value (PV) and TC content over a storage period of 90 days. Palm oil samples packaged in low density polyethylene films (LDPE), palm oil without packaging (WP), placed in a Petri dish and palm oil packaged in biocomposite films without antioxidant additives (WA) were used as controls. All controls were subjected to the same storage conditions of the film samples (63% RH at 30 ± 2 °C).

PVs were determined by titration according to the Association of Analytical Communities (AOCS, 1997) methodology. For TC, 0.3 g of the packaged product (palm oil) was dissolved in petroleum ether. TC concentrations were determined spectrophotometrically at 435 nm.

2.8. Experimental design and statistical analysis

A complete factorial experimental design, 2^2 with 3 center points for a total of 11 experiments (Table 2), was applied to enable perception of the influence of different concentrations of the antioxidant additives incorporated into the bio-based films. Mango pulp (0–20%; X_1) and yerba mate extract (0–30%; X_2) were chosen as independent variables. The PV and TC from the packaged product (palm oil) and the TP, TF, TC, mechanical properties and WVPs from the films were used as dependent variables (Y). The data were subjected to variance analysis and Tukey's test for comparison of means at a 5% significance level using Statistica 7.0 software (Minneapolis, USA).

Table 1 – Chemical characterization of additives mango pulp and yerba mate powder.

| Analysis | Mango pulp | Yerba mate powder |
|---------------------------------------|------------|-------------------|
| Total carotenoids ($\mu\text{g/g}$) | 50.17 | 8.04 |
| Total polyphenols (mg/g) | 35.00 | 140.20 |
| Total flavonoids (mg/g) | 3.87 | 50.00 |
| Lipids (%) | 0.29 | 11.66 |
| Moisture (g/100 g) | 86.64 | 5.34 |
| Total solids (g/100 g) | 13.36 | 94.66 |
| pH | 3.27 | 5.80 |
| Total fiber (g/100 g) | 1.51 | 52.76 |
| Free sugar (mg/g) | | |
| Glucose | 39.79 | 12.91 |
| Sucrose | 49.17 | 15.20 |
| Fructose | 92.72 | 9.35 |
| Fatty acids (%) | | |
| C12:0 | 0.76 | ND |
| C14:0 | 5.45 | 1.82 |
| C16:0 | 28.40 | 48.91 |
| C16:1n7 | 12.28 | ND |
| C17:0 | 0.28 | ND |
| C18:0 | 1.91 | 3.65 |
| C18:1n9 | 27.10 | 6.54 |
| C18:2n6 | 6.91 | 5.35 |
| C18:3n3 | 16.66 | 33.73 |
| C22:0 | 0.25 | ND |
| Σ Saturated | 37.05 | 54.38 |
| Σ Unsaturated | 62.95 | 45.62 |

ND, unidentified.

3. Results and discussion

Chemical composition of mango pulp and yerba mate powder are shown in **Table 1**. The results are in agreement with other studies (Medlicott and Thompson, 1985; Salgado et al., 1999; Lima et al., 2006; Ribeiro et al., 2007; Vieira, 2009; Berté et al., 2011; Dartora et al., 2011) showing that both incorporated additives are sources of antioxidants compounds, as well as free sugars. It is worth mentioning that the results presented are relative to yerba mate powder used to prepare the

aqueous extract of this yerba, the extract obtained was used in the preparation of the films.

The effects of incorporating mango pulp and yerba mate extract on the physical properties (thickness, moisture), WVP and mechanical properties (elongation and tensile strength) of the cassava starch-based films are presented in **Table 2**.

The incorporation of additives into cassava bio-based films caused changes in the mechanical and barrier film properties. In general, according to Tukey's test, the formulations with higher additive concentrations exhibited significant differences ($p < 0.05$) in the parameters of thickness, moisture and WVP when compared with controls (**Table 2**). For these parameters, ANOVA indicated that the differences between the formulations were not statistically significant ($p > 0.05$).

Thickness varied from a 1.77% decrease to a 10.62% increase, and the moisture increased 32.68% when compared to control WA (film without additives). The thickness is an important parameter to measure, is the basis for several characteristics of the films, including the mechanical properties. The thickness of the films ranged 0.111–0.125 mm, with significant differences between the formulations, however, there was a low correlation coefficient of this parameter with the elongation ($r^2 = 0.010$) and tensile strength ($r^2 = 0.230$), indicating that this parameter has little influence on the mechanical properties of the films analyzed.

The moisture of the industrialized mango pulp (86.64 g/100 g, **Table 1**) and yerba mate extract (93.14 g/100 g – at 30% yerba mate powder) incorporated into films, may have led to variations of this parameter (11.49–14.86 g/100 g, **Table 2**) observed in films that depended on the levels of each additive incorporated into the matrix.

WVP values ranged from 5.74 to 9.55×10^{-8} g mm/m² h kPa depending on the film formulation, while the control showed a value of 8.6×10^{-8} g mm/m² h kPa ($p < 0.05$) (**Table 2**).

The F1 formulation showed an 11.05% increase in this parameter compared to the film without additives. With the exception of this film, in which there was minimal incorporation of additives (2.90%, w/w, mango pulp and 4.40%,

Table 2 – Real and coded (X_1 and X_2) values of mango pulp and yerba mate extract added to cassava starch filmogenic solutions according to a (2^2) second-order experimental design. Effects of additive incorporation on physical, barrier and mechanical properties.

| F | Mango pulp % (X_1) | Yerba mate extract % (X_2) | Thickness (mm) | Physical, barrier and mechanical properties | | | |
|------------------|------------------------|--------------------------------|------------------------|---|----------------------|--------------------|------------------------|
| | | | | Moisture (g/100 g) | WVP $\times 10^{-8}$ | Elongation (%) | Tensile strength (MPa) |
| WA | – | – | 0.113 ^a | 11.20 ^c | 8.60 ^d | 70.28 ^c | 5.38 ^d |
| F1 | 2.90 (-1.00) | 4.40 (-1.00) | 0.115 ^a | 14.86 ^a | 9.55 ^e | 56.67 ^a | 4.03 ^c |
| F2 | 2.90 (-1.00) | 25.60 (1.00) | 0.117 ^{a,b} | 14.10 ^b | 6.52 ^{a,b} | 55.98 ^a | 2.37 ^a |
| F3 | 17.10 (1.00) | 4.40 (-1.00) | 0.125 ^c | 12.37 ^{b,c} | 6.01 ^b | 69.15 ^c | 2.47 ^a |
| F4 | 17.10 (1.00) | 25.60 (1.00) | 0.120 ^{c,d} | 11.77 ^c | 5.75 ^a | 59.58 ^b | 1.36 ^b |
| F5 | 0.00 (-1.41) | 15.00 (0.00) | 0.114 ^a | 14.80 ^a | 6.86 ^b | 55.15 ^a | 2.67 ^a |
| F6 | 20.00 (1.41) | 15.00 (0.00) | 0.111 ^a | 11.49 ^c | 5.74 ^a | 69.36 ^c | 2.04 ^{a,b} |
| F7 | 10.00 (0.00) | 0.00 (-1.41) | 0.112 ^a | 13.57 ^b | 7.10 ^b | 65.28 ^c | 2.57 ^a |
| F8 | 10.00 (0.00) | 30.00 (1.41) | 0.118 ^{b,d,e} | 12.65 ^{b,c} | 7.70 ^b | 55.50 ^a | 2.00 ^{a,b} |
| F9 ^a | 10.00 (0.00) | 15.00 (0.00) | 0.122 ^{c,e} | 13.12 ^b | 7.84 ^c | 57.38 ^b | 1.37 ^b |
| F10 ^a | 10.00 (0.00) | 15.00 (0.00) | 0.121 ^{c,e} | 13.15 ^b | 7.88 ^c | 58.01 ^b | 1.38 ^b |
| F11 ^a | 10.00 (0.00) | 15.00 (0.00) | 0.124 ^c | 13.14 ^b | 7.85 ^c | 57.94 ^b | 1.37 ^b |

F, formulations; WA, film without additives mango pulp and yerba mate extract; WVP, water vapor permeability (g mm/m² h kPa).

Means with the same letters in the same columns were not statistically different ($p > 0.05$) according to Tukey's test.

^a Central points.

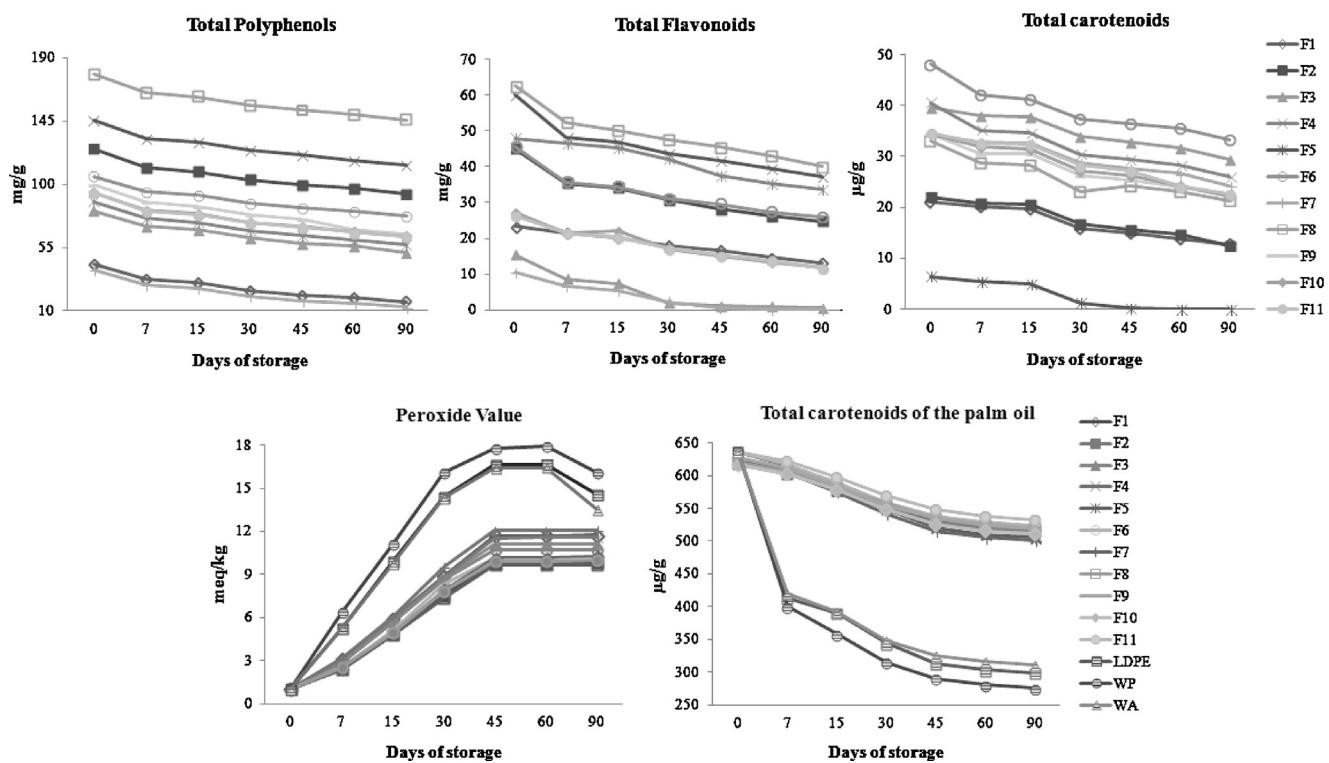


Fig. 1 – Total antioxidant contents in biocomposite film formulations, peroxide value and total carotenoid values in palm oil during storage.

w/w, yerba mate extract), the other formulations exhibited decreased WVP values that ranged between 9.14% and 49.83% compared to the control (Table 2). These results can be explained by the interaction between the mango pulp fibers (Table 1) and starch (Avérous et al., 2001).

The reduction in WVP can be due to the presence of insoluble fibers, which reduce the free space in the polymeric matrix, obstructing the passage of water vapor through the surfaces of films (Müller et al., 2009). Another explanation may be related to the hydrophobic nature of the phenolic compounds, which interrupts the penetration of water molecules. According to Sánchez-González et al. (2011) the same behavior was observed when polyphenols (essential oils) were incorporated into chitosan/hydropropylmethylcellulose composite films. They reported that WVP values of composite films decreased in line with the increase in the concentration of the hydrophobic antioxidant agent.

Mechanical properties were also altered, depending on the levels of mango pulp and yerba mate extracts incorporated (Table 2). In comparison with the control (WA), the elongation reductions ranged from 1.33% to 27.43%. A possible explanation is that the free sugars naturally present in mango pulp and yerba mate (Table 1), as glucose, fructose and sucrose, acted as plasticizers. As the film base already had added plasticizers (glycerol), the concentration in the final material may have been too high, resulting in excessive interactions between the film network and the plasticizers (Arvanitoyannis et al., 1996), reducing film flexibility. The tensile strength was reduced from 33.50% to 259.59% compared to the control. The films produced were not visibly homogeneous; we observed solids from the yerba mate extract on the surface. Therefore, ruptures could occur more easily, justifying the reductions. This study demonstrated that the barrier and mechanical properties of cassava starch biodegradable films could be significantly ($p < 0.05$) altered through the incorporation of mango pulp and yerba mate extracts (Table 2).

Similar results were obtained for bio-based films containing mango and acerola pulp in which the addition of pulps resulted in reductions in the elongation percentage and tensile strength (Souza et al., 2012b). Therefore, replacement of the acerola pulp for yerba mate extract showed similar mechanical properties.

Films of mango puree and other fruits exhibit reduced tensile strength and elongation percentages when compared to films made with fruit starches plasticized with glycerol (Sothornvit and Rodsamran, 2008; Wang et al., 2011). Cassava starch films plasticized with sucrose and inverted sugar and obtained with the casting technique result in poor mechanical properties and high WVP (Veiga-Santos et al., 2005, 2007). However, their low cost and the availability of the glycerin and mango pulp can justify efforts to make starch a feasible raw material for biodegradable film production. Consequently, WVP rates can be reduced.

As expected, initial TP, TF and TC values in the casting-dried biocomposite films are proportional to the mango pulp and yerba mate extract quantities incorporated in the filmogenic solution formulations. The sample supplemented with the lowest concentrations of both additives (F1) resulted in films with low TP (43.41 mg/g), TF (23.24 mg/g) and TC (21.15 µg/g) values (Fig. 1), while the sample incorporated with the highest concentrations of both additives (F4) resulted in higher values of TP (145.33 mg/g), TF (59.92 mg/g) and TC (40.56 µg/g) (Table 2 and Fig. 1).

However, the sample with the highest quantity of yerba mate extract (F8 – 10% mango pulp and 30% yerba mate extract) had the highest TP (178.53 mg/g) and TF (62.54 mg/g) values ($p < 0.05$) (Fig. 1), likely due to the TP (140.20 mg/g) and TF (50.00 mg/g) concentrations of the yerba mate being higher than those of the mango pulp (35.00 and 3.87 mg/g, respectively) according to Table 1.

The same effects were observed when comparing the TC values. Formulation F6 (20% mango pulp and 15% yerba mate

extract) had a high TC concentration of 48.10 µg/g, followed by formulation F4 (17.10% mango pulp and 25.60% yerba mate extract) with a TC concentration of 40.56 µg/g ($p < 0.05$) (Fig. 1), which was due to the fact that the carotenoid concentration of the mango pulp (50.17 µg/g) was higher than that of the yerba mate (8.04 µg/g, Table 1).

Palm oil was packaged in 11 films (Table 3). The TP, TF and TC contents of all biocomposite films decreased during the storage period (90 days). Sample F1 had the lowest concentrations of both additives and presented the least combined decrease in TP, TF and TC contents throughout the storage period (27.04 mg/g, 10.01 mg/g and 8.37 µg/g, respectively) (Table 3). Sample F8 had the highest concentration of yerba mate extract and presented the largest decrease of TP and TF contents (32.50 and 22.56 mg/g, respectively) ($p < 0.05$) after 90 days of storage. Sample F6 had the highest concentration of mango pulp and presented the largest TC content decrease (14.87 µg/g) ($p < 0.05$) after 90 days of storage (Table 3 and Fig. 1). These results indicate that when the additive concentrations increase, the loss of antioxidant compounds during storage also increases, demonstrating that the films provided high levels of protection and resulted in low levels of packaged product oxidation.

When F5 (yerba mate extract only) and F7 (mango pulp only) were compared after 90 days, F7 had smaller decreases in TP (26.69 mg/g) and TF (10.23 mg/g) than F5 (30.49 and 14.29 mg/g, respectively) ($p < 0.05$). F5 had a smaller decrease in TC (6.42 µg/g) than F7 (9.91 µg/g) (Table 3 and Fig. 1).

The PV and TC values of the palm oil packaged in films were analyzed over 90 days (Table 4 and Fig. 1). As expected, all palm oil samples packaged in the different films showed significant decreases ($p < 0.05$) in TC values and concomitant increases in the PV of the oil.

This effect can be considered concentration-dependent because palm oil packaged with F1 (low concentrations of both additives) presented a higher oxidation value (PV = 10.71 meq/kg) compared to oil packaged with F4 (high concentrations of additives) (PV = 8.79 meq/kg; $p < 0.05$) during the same storage period. Palm oil packaged with F8 presented the lowest decrease in PV (8.70 meq/kg; $p < 0.05$) after 90 days (Fig. 1 and Table 4). These results agree with other works indicating that the addition of a high concentration of antioxidants results in higher antioxidant efficacy (Souza et al., 2011; Barbosa-Pereira et al., 2012).

The values of PV in the formulations increased under all conditions during storage, but did not exhibit any significant ($p > 0.05$) between different formulations up to 60 days of storage. However, during the later storage period (90 days), the statistical analysis of the data showed the variations in these values to be significant ($p < 0.05$).

The oil packaged with F5 (yerba mate extract only) presented a lower PV (10.56 meq/kg) than oil packaged with F7 (mango pulp only) (11.09 meq/kg). The statistical test showed that these results were not statistically significant, but analyzing the Pareto chart of standardized effect estimates (absolute values) is possible demonstrate the greater efficacy of the yerba mate extract compared with mango pulp to reduce the oxidative process in the oil (Fig. 2).

The main purpose of using antioxidants in lipid packaging is to delay a significant accumulation of free radicals and thus improve oxidative stability. The results of this study suggest that the protection of packaged products against oxidation can

Table 3 – Periodic reductions of antioxidant contents in biocomposite films activated with mango pulp and yerba mate extract during the storage of palm oil, and model equations of dependent variable reductions (TP, TF, TC) after 90 days of storage.

| F | Decrease in total polyphenols (TP, mg/g) | | | | | | Decrease in total flavonoids (TF, mg/g) | | | | | | Decrease in total carotenoids (TC, µg/g) | | | | | |
|------------------|--|----------------------|----------------------|----------------------|------------------------|------------------------|--|----------------------|----------------------|----------------------|----------------------|----------------------|--|-------------------|----------------------|---------------------|------------------------|----------------------|
| | 7 | 15 | 30 | 45 | 60 | 90 | 7 | 15 | 30 | 45 | 60 | 90 | 7 | 15 | 30 | 45 | 60 | 90 |
| F1 | 10.90 ^e | 13.16 ^e | 19.15 ^{e,f} | 22.33 ^e | 23.87 ^d | 27.04 ^{c,d} | 1.79 ^e | 3.05 ^e | 5.16 ^e | 6.62 ^f | 8.69 ^d | 10.01 ^d | 1.04 ^a | 1.45 ^a | 5.26 ^f | 6.23 ^a | 7.30 ^{c,d} | 8.37 ^{d,e} |
| F2 | 13.54 ^b | 16.24 ^{b,c} | 22.00 ^{b,c} | 25.48 ^b | 28.27 ^a | 32.28 ^a | 9.56 ^b | 10.82 ^{b,c} | 14.22 ^{b,c} | 16.75 ^b | 18.69 ^a | 20.22 ^{a,b} | 1.20 ^b | 1.48 ^a | 5.42 ^b | 6.39 ^b | 7.46 ^{c,d} | 9.59 ^{c,d} |
| F3 | 11.08 ^a | 13.78 ^e | 19.15 ^{a,e} | 22.98 ^a | 25.16 ^{b,c,d} | 29.76 ^{a,b,c} | 6.64 ^a | 7.90 ^e | 13.39 ^e | 14.23 ^d | 14.37 ^b | 14.94 ^c | 1.42 ^c | 1.76 ^b | 5.64 ^{c,d} | 6.61 ^{c,e} | 7.72 ^{b,c,d} | 10.15 ^{c,d} |
| F4 | 12.56 ^d | 15.26 ^d | 21.25 ^d | 24.50 ^d | 28.10 ^a | 31.78 ^a | 11.69 ^d | 12.95 ^d | 16.19 ^d | 18.25 ^c | 20.38 ^a | 22.69 ^a | 5.46 ^d | 5.94 ^c | 10.16 ^{b,c} | 11.13 ^e | 12.34 ^a | 14.52 ^{a,b} |
| F5 | 11.98 ^c | 14.68 ^b | 20.50 ^b | 23.92 ^c | 27.64 ^{b,c,d} | 30.49 ^{a,b} | 1.44 ^c | 2.70 ^b | 5.73 ^b | 10.52 ^b | 12.70 ^{b,c} | 14.29 ^c | 1.01 ^a | 1.40 ^a | 5.23 ^{e,f} | 6.20 ^f | 6.42 ^d | 6.42 ^e |
| F6 | 11.35 ^{b,d} | 13.56 ^c | 19.55 ^{c,d} | 22.71 ^{b,d} | 24.95 ^{c,d} | 28.39 ^{b,d} | 9.92 ^{b,d} | 11.18 ^c | 14.52 ^c | 16.03 ^{c,e} | 18.32 ^a | 19.75 ^b | 6.00 ^d | 6.48 ^c | 10.70 ^d | 11.67 ^c | 12.59 ^a | 14.87 ^a |
| F7 | 10.67 ^a | 12.93 ^a | 18.92 ^f | 22.10 ^a | 23.64 ^d | 26.69 ^d | 3.84 ^a | 5.10 ^a | 8.18 ^a | 9.95 ^a | 10.17 ^{c,d} | 10.23 ^d | 1.32 ^c | 1.64 ^b | 5.54 ^{a,e} | 6.51 ^d | 7.59 ^{b,c,d} | 9.91 ^{c,d} |
| F8 | 13.60 ^d | 16.30 ^{c,d} | 22.29 ^{b,c} | 25.55 ^d | 28.83 ^a | 32.50 ^a | 10.07 ^d | 12.33 ^{c,d} | 15.01 ^{c,d} | 17.11 ^e | 19.51 ^a | 22.56 ^{a,b} | 4.30 ^e | 4.78 ^e | 9.97 ^{b,c} | 9.00 ^b | 10.01 ^{a,b,c} | 11.87 ^{b,c} |
| F9 ^a | 12.50 ^{e,f} | 15.40 ^c | 21.19 ^{e,f} | 24.61 ^{e,f} | 27.78 ^{a,b,c} | 31.27 ^{a,b} | 4.73 ^{e,f} | 5.97 ^c | 9.25 ^c | 11.19 ^{f,g} | 13.18 ^{b,c} | 14.43 ^c | 3.95 ^{e,f} | 3.97 ^f | 8.17 ^f | 9.14 ^f | 10.48 ^{a,b} | 12.05 ^{a,c} |
| F10 ^a | 12.54 ^{e,f} | 15.24 ^c | 21.23 ^{c,f} | 24.45 ^{c,f} | 27.77 ^{a,b,c} | 31.14 ^{a,b} | 5.63 ^{c,f} | 5.02 ^c | 10.21 ^c | 12.07 ^g | 12.71 ^{b,c} | 14.40 ^c | 2.57 ^f | 3.05 ^f | 7.27 ^f | 8.24 ^f | 10.14 ^{a,b} | 12.30 ^{a,c} |
| F11 ^a | 12.43 ^{e,f} | 15.13 ^c | 21.12 ^{e,f} | 24.36 ^{e,f} | 27.91 ^{a,b,c} | 30.91 ^{a,b} | 4.79 ^{e,f} | 6.00 ^c | 9.04 ^c | 10.94 ^f | 12.45 ^{b,c} | 14.48 ^c | 1.86 ^f | 2.13 ^f | 6.35 ^f | 7.32 ^f | 10.41 ^{a,b} | 11.90 ^{a,c} |
| ME | $Y = 31.10 - 0.09X_1 - 0.65X_1^2 + 1.93X_2 - 0.58X_2^2 - 0.85X_1X_2$ ($R^2 = 0.98$) | | | | | | $Y = 14.43 + 1.89X_1 + 1.35X_1^2 + 4.42X_2 + 1.04X_2^2 - 0.61X_1X_2$ ($R^2 = 0.98$) | | | | | | $Y = 12.08 + 2.32X_1 + 0.74X_1^2 + 1.04X_2 - 0.62X_2^2 + 0.78X_1X_2$ ($R^2 = 0.96$) | | | | | |

F, formulations; ME, model equations at 90 days; X_1 , concentration of the independent variable – mango pulp; X_2 , concentration of the independent variable – yerba mate extract; R^2 , coefficient of determination. Means with the same letters in the same columns were not statistically different ($p > 0.05$) according to Tukey's test.

^a Central points.

Table 4 – Increases in peroxide values and decreases in total carotenoids of the palm oil packaged in different films after 90 days of storage, and model equations for the dependent variable increases in the peroxide value.

| Formulations | Increases in peroxide value (PV, meq/kg) | | | | | | Decreases in total carotenoids (TC, µg/g) | | | | | |
|------------------|--|--------------------|--------------------|--------------------|--------------------|------------------------|---|----------------------|----------------------|-----------------------|---------------------|---------------------|
| | 7 | 15 | 30 | 45 | 60 | 90 | 7 | 15 | 30 | 45 | 60 | 90 |
| LDPE | 4.27 ^{a,b} | 8.94 ^a | 13.38 ^a | 15.67 ^a | 15.67 ^a | 13.53 ^{a,b} | 223.83 ^a | 246.66 ^a | 293.67 ^a | 324.06 ^a | 334.06 ^b | 339.06 ^b |
| WP | 5.40 ^a | 10.09 ^a | 15.08 ^a | 17.00 ^a | 17.01 ^a | 15.09 ^a | 237.50 ^b | 279.77 ^b | 322.13 ^b | 347.08 ^b | 357.08 ^a | 362.08 ^a |
| WA | 4.25 ^{a,b} | 8.77 ^a | 13.35 ^a | 15.43 ^a | 15.44 ^a | 12.48 ^{a,b,c} | 214.87 ^a | 243.45 ^a | 287.93 ^c | 309.73 ^c | 319.73 ^c | 324.73 ^c |
| F1 | 2.09 ^b | 4.87 ^b | 7.89 ^b | 10.70 ^b | 10.70 ^b | 10.71 ^{b,c} | 20.89 ^d | 48.73 ^d | 78.85 ^d | 107.00 ^e | 117.10 ^e | 122.10 ^e |
| F2 | 1.42 ^b | 3.87 ^b | 6.49 ^b | 8.75 ^b | 8.75 ^b | 8.79 ^d | 14.23 ^{f,g} | 38.70 ^c | 64.86 ^{e,h} | 87.50 ^f | 97.32 ⁱ | 102.32 ⁱ |
| F3 | 1.90 ^b | 4.77 ^b | 7.68 ^b | 10.09 ^b | 10.09 ^b | 10.10 ^{c,d} | 18.95 ^e | 47.72 ^{d,e} | 76.74 ^{d,g} | 100.86 ^d | 110.87 ^f | 115.87 ^f |
| F4 | 1.44 ^b | 3.90 ^b | 6.73 ^b | 8.75 ^b | 8.75 ^b | 8.79 ^d | 14.40 ^g | 38.92 ^c | 67.30 ^h | 87.50 ^f | 97.51 ⁱ | 102.51 ⁱ |
| F5 | 1.90 ^b | 4.82 ^b | 7.76 ^b | 10.55 ^b | 10.55 ^b | 10.56 ^{b,c,d} | 18.95 ^e | 48.17 ^e | 77.59 ^{c,d} | 105.47 ^{e,d} | 115.47 ^e | 120.47 ^e |
| F6 | 1.82 ^b | 4.67 ^b | 7.59 ^b | 9.69 ^b | 9.69 ^b | 9.72 ^{c,d} | 18.21 ^e | 46.66 ^f | 75.94 ^{f,g} | 96.93 ^{g,d} | 106.93 ^g | 111.93 ^g |
| F7 | 2.17 ^b | 5.10 ^b | 8.49 ^b | 11.04 ^b | 11.04 ^b | 11.09 ^{b,c,d} | 21.63 ^{c,d} | 50.96 ^d | 84.91 ⁱ | 110.40 ^h | 120.44 ^d | 125.44 ^d |
| F8 | 1.40 ^b | 3.79 ^b | 6.35 ^b | 8.68 ^b | 8.67 ^b | 8.70 ^d | 14.00 ^f | 37.89 ^c | 63.42 ^e | 86.72 ^f | 96.72 ⁱ | 101.72 ⁱ |
| F9 ^a | 1.51 ^b | 4.14 ^b | 7.32 ^b | 9.19 ^b | 9.16 ^b | 9.23 ^d | 15.08 ^{f,g} | 41.38 ^e | 73.16 ^g | 91.85 ^g | 101.86 ^h | 106.86 ^h |
| F10 ^a | 1.45 ^b | 3.93 ^b | 6.98 ^b | 9.17 ^b | 9.17 ^b | 9.29 ^d | 14.46 ^{f,g} | 39.25 ^c | 69.79 ^{g,j} | 91.64 ^g | 101.65 ^h | 106.65 ^h |
| F11 ^a | 1.55 ^b | 3.92 ^b | 6.81 ^b | 8.90 ^b | 8.90 ^b | 9.00 ^d | 15.49 ^f | 39.21 ^c | 68.05 ^{j,h} | 88.94 ^{f,g} | 98.95 ^h | 103.95 ^h |
| ME | $Y = 9.17 - 0.22X_1 + 0.38X_1^2 - 0.82X_2 + 0.25X_2^2 + 0.15X_1X_2$ $(R^2 = 0.98)$ | | | | | | - | | | | | |

LDPE, palm oil packaged in low-density polyethylene film; WP, palm oil without packaging; WA, palm oil packaged in film without additives; ME, model equations at 90 days; X_1 , concentration of the independent variable – mango pulp; X_2 , concentration of the independent variable – yerba mate extract; R^2 , coefficient of determination.

Means with the same letters in the same columns were not statistically different ($p > 0.05$) according to Tukey's test.

^a Central points.

be attributed to the concentration-dependent radical scavenging activity of antioxidant compounds present in film-forming dispersions (Tables 3 and 4). This outcome occurred because antioxidants act by inhibiting or interrupting the mechanism of lipid auto-oxidation of free radicals. The protective effect

against lipid oxidation is likely due to a physical and synergistic process mostly from the total polyphenols and total flavonoids in yerba mate. The antioxidant capacity of phenolic compounds is based on their configuration or molecular structure (Han et al., 2011).

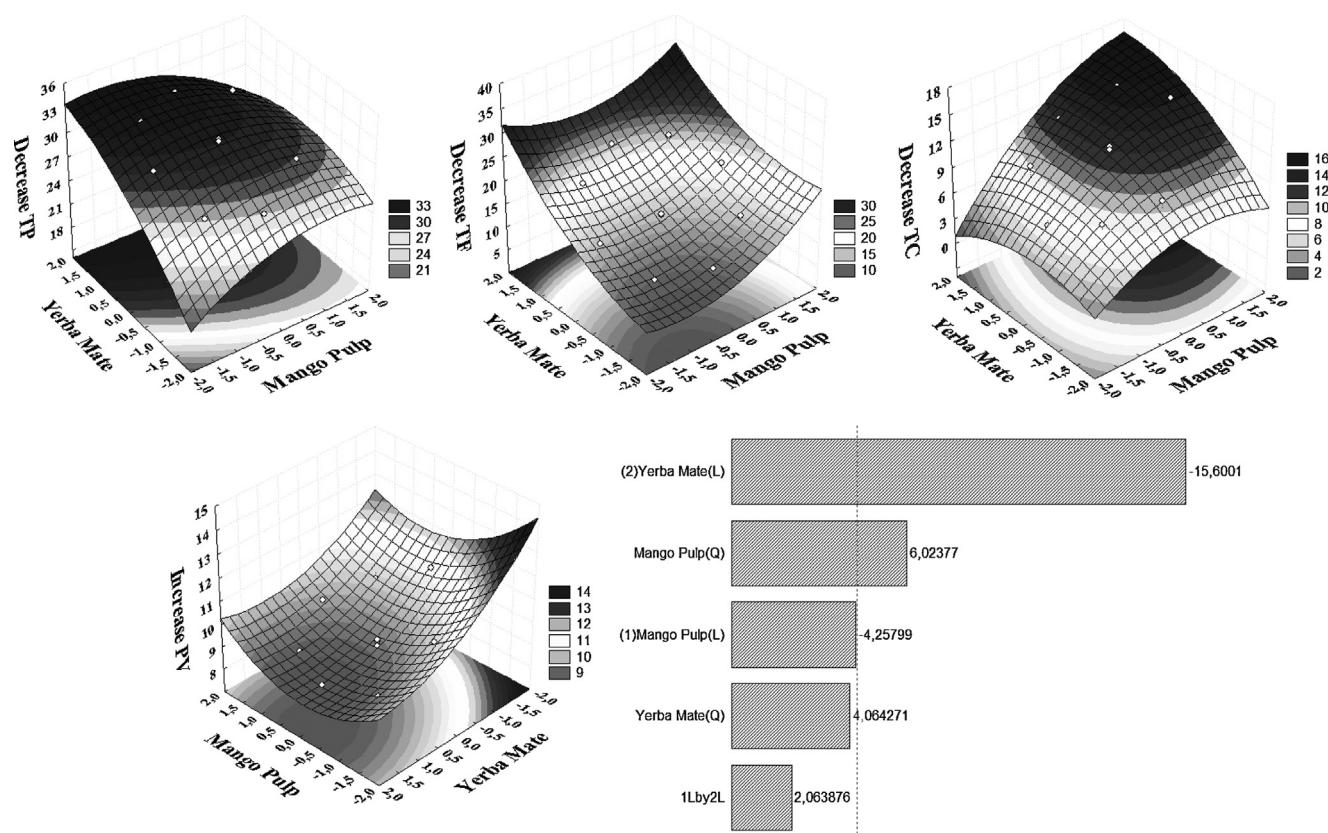


Fig. 2 – Response surface plots and Pareto graphic showing the effects of incorporated additives on the decreases in total polyphenols (TP, mg/g), total flavonoids (TF, mg/g) and total carotenoids (TC, µg/g) in films and the increases in peroxide values (PV, meq/kg) of palm oil after 90 days of storage.

Phenolics act as free radical acceptors, thereby terminating oxidation at the initial stage and preventing further formation of new radicals in the oxidation process (O'Sullivan et al., 2005). Likewise, the antioxidant activity of flavonoids is generally governed by their chemical structures. Flavonoids may act as primary antioxidants by donating a hydrogen atom to act as free-radical acceptors or chain breakers and may also act as metal chelators. The relationship between the structure and antioxidant activity of flavonoids has been well documented (Heim et al., 2002; Silva et al., 2002).

For films made of cassava starch containing mango and acerola pulps, the smallest increases in the PV were associated with the highest concentrations of both additives. However, the antioxidant action is dependent on high concentrations of mango pulp (Souza et al., 2011). In this study, the smallest increases in the PV were shown in the formulations with higher concentrations of yerba mate extract, thus demonstrating the superior antioxidant action of this additive compared with acerola pulp.

The values of TC in the packaged product were not significantly different ($p > 0.05$) between different formulations. However, film formulation F8 showed the lowest decrease in TC (101.72 µg/g) and is therefore most effective for protecting palm oil (Table 4 and Fig. 1). The results confirm the efficacy of bio-based films with mango pulp and yerba mate, likely because antioxidant compounds reduce oxygen levels inside the package, thus reducing carotenoid degradation in the packaged product. Similar results were also reported by Souza et al. (2011) for films with mango and acerola pulps.

The results also showed that cassava starch films without antioxidant additives had larger protective effects ($p < 0.05$) compared to LDPE and can be more effective barriers against oxidation (Table 4). These results are in agreement with some studies that have shown that oxygen can permeate the film and react preferentially with compounds present in the film formulation, allowing the packaged product to be preserved for a longer period of time. It is also worth noting that the palm oil samples packaged with LDPE (low density polyethylene), exposed to air (without packaging, WP) and packaged in the formulation without additives (WA) after 90 days passed through stage 3 oxidation, while palm oil packaged in any formulation containing different levels of additives did not reach this stage. All samples of palm oil packaged with formulations containing antioxidants exhibited delayed induction periods compared with those of the controls (Fig. 1). The PV tends to increase during early stages of oxidation, when the formation rate of hydroperoxides is higher than the rate of decomposition. After the maximum value is reached, the value then decreases as a result of the lower substrate availability and the instability of peroxide molecules, which leads to a formation rate lower than that of decomposition. Pereira et al. (2011) reported that oils and fats are easily broken down during storage; this type of decomposition is demonstrated by the increase and subsequent decrease in the PV, as observed in the control samples in this study.

Experimental results for different film formulations used to package palm oil during storage were significantly different ($p < 0.05$) with regard to TP, TF and TC after 90 days of storage. This outcome in the construction of a second-order polynomial equation, which represents the model equation used to evaluate the decreases in TP, TF and TC (Table 3) in films as a function of the concentrations of mango pulp (% X_1) and yerba mate extract (% X_2) and the interaction between them (X_1 and X_2). The coefficient of determination (R^2) was

calculated to check the adequacy of the models, generally, a number closer to one is preferred. Analysis of variance showed a high coefficient of determination value (R^2) of 0.98 for TP, 0.98 for TF and 0.96 for TC thus ensuring a satisfactory fit of the second order polynomial regression model with the experimental data. In addition, the value indicates that the models for TP, TF, TC and PV did not explain only 0.02%, 0.02% and 0.04% of the total variations, respectively.

The response surface graphs (Fig. 2) display the decreases in TP, TF and TC as functions of the concentrations of mango pulp and yerba mate, which were incorporated as additives in the bio-based films. By analyzing these figures, the combined effects of mango and yerba mate provide data that can be used to visualize the maximal values of these parameters (TP, TF and TC) in the films. In this case, there is an apparent linear relationship between the amount of yerba mate extract added and the protective effect. It is possible to observe that the largest decreases in the values of TF and TP are correlated with the films containing the highest concentrations of yerba mate extract; however, the reductions in TC are influenced by the highest concentrations of both additives. Thus demonstrating that higher concentrations of the additives result in greater antioxidant loss and a more efficient protection of the packaged product.

Due to the incorporation of different proportions of mango pulp (% X_1) and yerba mate (% X_2) in the bio-based films, the increase in the PV (meq/kg) of palm oil after 90 days of storage is expressed using a second-order polynomial equation ($R^2 = 0.98$) (Table 4). The increase in this parameter depends on the concentrations of mango pulp and yerba mate. Fig. 2 represents the response surface graph, showing the increases in palm oil PV packaged with different bio-based film formulations. The graph indicates that the point of minimum PV increase corresponds to the maximum concentration of yerba mate.

The Pareto chart showed the magnitude of the negative effect of yerba mate concentration on increasing PV, thus reinforcing the idea that this factor has a favorable influence on that variable. For the day 90, the Pareto chart demonstrated that the quadratic function of the mango pulp variable showed a slight positive effect on the increase in PV, indicating low antioxidant action of that additive. The influence of the addition of mango pulp was relatively lower than the observed for the yerba, the formulation F7 (only mango pulp) showed the largest increases in PV between the 11 formulations (Table 2 and Fig. 2). Pareto chart also showed that the interaction between yerba mate extract and mango pulp does not influences PV (Fig. 2).

To increasing in concentrations of PV of palm oil packed in cassava starch active films containing mango and acerola pulps, the Pareto chart showed greater negative effect of the independent variable mango pulp compared with pulp variable acerola, demonstrating greater antioxidant effect of mango pulp. The interaction of the variables also showed significant results, however, the negative effect was lower (Souza, 2010).

The PV showed a higher correlation with decreased antioxidant concentrations in the bio-based films (TP 76.22%, TF 60.44% and TC 34.98%) (Fig. 3): the greater the reductions in antioxidants, the lower the PV concentration. Thus, the packaging rather than the packaged product is oxidized (active compound loss). For cassava starch active films containing mango and acerola pulps, with regard to the correlation between vitamin C and PV, the decrease in the vitamin C

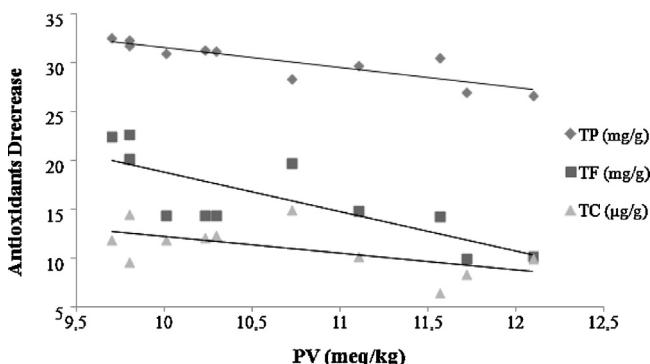


Fig. 3 – Correlation between the reduction of antioxidant contents of the biocomposite films and the peroxide values in palm oil.

values was associated with an increase in the PV; i.e., the results showed a possible pro-oxidant effect of vitamin C.

The results of this study suggest that the protection of packaged products against oxidation can be attributed to the concentration-dependent radical-scavenging activities of antioxidant compounds present in film-forming dispersions. Considering the results for PV in the packaged product, the protective effect against lipid oxidation is likely a consequence of a physical process due to the yerba mate content.

4. Conclusions

The incorporation of mango pulp and yerba mate extract, as additives in film-forming dispersions, are effective in preserving a packaged product against oxidation. The results revealed higher antioxidant efficacy by yerba mate extracts, likely due to phenolic compounds and flavonoids. Film formulations with higher concentrations of this additive exhibited lower rates of oxidation in the packaged palm oil.

The results suggest that a device incorporating natural antioxidants into starch-based films with active compounds may be beneficial in maintaining food quality through antioxidant action. The application of these products represents an innovative concept that is of interest to the food industry.

Acknowledgement

The authors would like to express their sincere thanks to the National Council for Scientific and Technological Development (CNPq) for the financial incentive and postgraduate fellowship (CNPq 505831/2008-2).

References

- American Oil Chemists Society (AOCS), 1997. *Official Methods of Analysis*, Cd 8b-90.
- American Society for Testing and Materials, 2000. *Standard Test Method for Water Vapor Transmission of Materials*, ASTM E96-00.
- American Society for Testing and Materials, 2001. *Standard Test Method for Tensile Properties of Thin Plastic Sheeting*, ASTM D882-00.
- Arvanitoyannis, I., Psomiadou, E., Nakayama, A., 1996. *Edible films made from sodium caseinate, starches, sugars or glycerol*. Carbohydr. Polym. 31, 179–192.
- Assis, D.J., Brandão, L.V., de Sousa Costa, L.A., Figueiredo, T.V.B., Sousa, L.S., Padilha, F.F., Druzian, J.I., 2014. *A study of the effects of aeration and agitation on the properties and production of xanthan gum from crude glycerin derived from biodiesel using the response surface methodology*. Appl. Biochem. Biotechnol. 172, 2769–2785.
- Association of Official Analytical Chemists, 2000. *Official Methods of Analysis of AOAC International*, 17a ed. AOAC International, Gaithersburg.
- Avérous, L., Fringant, C., Moro, L., 2001. *Plasticized starch-cellulose interactions in polysaccharide composites*. Polymer 42, 6565–6572.
- Barbosa-Pereira, L., Cruz, J.M., Sedón, R., Quirós, A.R.B., Ares, A., Castro-López, C., Abad, M.J., Maroto, J., Paseiro-Losada, P.J., 2012. *Development of antioxidant active films containing tocopherols to extend the shelf life of fish*. Food Control 31, 236–243.
- Berté, K.A., Beux, M.R., Spada, P.K., Salvador, M., Hoffmann-Ribani, R., 2011. *Chemical composition and antioxidant activity of yerba-mate (*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae) extract as obtained by spray drying*. J. Agric. Food Chem. 59, 5523–5527.
- Bligh, E.G., Dyer, W.J., 1959. *A rapid method of total lipid extraction and purification*. Can. J. Biochem. Physiol. 37, 911–917.
- Bonilla, J., Atarés, L., Vargas, M., Chiralt, A., 2012. *Edible films and coatings to prevent the detrimental effect of oxygen on food quality: possibilities and limitations*. J. Food Eng. 110, 208–213.
- Bravo, L., Goya, L., Lecumberri, E., 2007. *LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis*, St. Hil.) and its antioxidant activity compared to commonly consumed beverages*. Food Res. Int. 40, 393–405.
- Brody, A., Strupinsky, E.R., Kline, L.R., 2010. *Active Packaging for Food Applications*. CRC Press, Florida.
- Brody, A.L., Bugusu, B., Han, J.H., Sand, C.K., McHugh, T.H., 2008. *Innovative food packaging solutions*. J. Food Sci. 73, 107–116.
- Carelli, A.A., Franco, I.C., Crapiste, G.H., 2005. *Effectiveness of added natural antioxidants in sunflower oil*. Grasas y aceites 56, 303–310.
- Cavar, S., Maksimović, M., 2012. *Antioxidant activity of essential oil and aqueous extract of Pelargonium graveolens L'Her*. Food Control 23, 263–267.
- Dartora, N., De Souza, L.M., Santana-Filho, A.P., Iacomini, M., Valduga, A.T., Gorin, P.A., Sasaki, G.L., 2011. *UPLC-PDA-MS evaluation of bioactive compounds from leaves of *Ilex paraguariensis* with different growth conditions, treatments and ageing*. Food Chem. 129, 1453–1461.
- Davies, B.H., 1976. *Carotenoids*. In: Goodwin, T.W. (Ed.), *Chemistry and Biochemistry of Plant Pigments*. Academic Press, London, pp. 38–65.
- Han, R.M., Li, D.D., Chen, C.H., Liang, R., Tian, Y.X., Zhang, J.P., Skibsted, L.H., 2011. *Phenol acidity and ease of oxidation in isoflavonoid/β-carotene antioxidant synergism*. J. Agric. Food Chem. 59, 10367–10372.
- Heim, E.K., Tagliaferro, A.R., Bobilya, D.J., 2002. *Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships*. J. Nutr. Biochem. 13, 572–584.
- Instituto Adolfo Lutz, 1985. *Normas Analíticas do Instituto Adolfo Lutz. v. 1: Métodos químicos e físicos para análise de alimentos*, 3. ed. IMESP, São Paulo, pp. 27.
- Jiménez, A., Fabra, M.J., Talens, P., Chiralt, A., 2012. *Edible and biodegradable starch films: a review*. Food Bioprocess Technol. 5, 2058–2076.
- Kuskoski, E.M., Asuero, A.G., Morales, M.T., Fett, R., 2006. *Wild fruits and pulps of frozen fruits: antioxidant activity, polyphenols and anthocyanins*. Ciênc. Rural 36, 1283–1287.
- Lee, K.W., Kim, Y.J., Lee, W.J., Lee, C.Y., 2003. *Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine*. J. Agric. Food Chem. 51, 7292–7295.
- Lima, D.M., Colugnati, F.A.B., Padovani, R.M., Rodriguez-Amaya, D.B., Salay, E., Galeazzi, M.A.M., 2006. *Tabela brasileira de composição de alimentos – TACO: versão 2*. In: 2. ed. Unicamp, Campinas, 113 pp.
- Lindley, M.G., 1998. *The impact of food processing on antioxidants in vegetable oil, fruits and vegetables*. Trends Food Sci. Technol. 9, 336–340.

- López-de-Dicastillo, C., Gómez-Estaca, J., Catalá, R., Gayara, R., Hernández-Muñoz, P., 2012. *Active antioxidant packaging films: development and effect on lipid stability of brined sardines*. Food Chem. 131, 1376–1384.
- Medlicott, A.P., Thompson, A.K., 1985. *Analysis of sugars and organic acids in ripening mango fruits (*Mangifera indica L. var Keitt*) by high performance liquid chromatography*. J. Sci. Food Agric. 36, 561–566.
- Mertz, C., Gancel, A.L., Gunata, Z., Alter, P., Dhuique-Mayer, C., Vaillant, F., Brat, P., 2009. *Phenolic compounds, carotenoids and antioxidant activity of three tropical fruits*. J. Food Compos. Anal. 22, 381–387.
- Müller, C.M.O., Laurindo, J.B., Yamashita, F., 2009. *Effect of cellulose fibers addition on the mechanical properties and water vapor barrier of starch-based films*. Food Hydrocoll. 23, 1328–1333.
- Murakami, A., Amboni, R., Prudêncio, E., Amante, E., Zanotta, L., Maraschin, M., Petrus, J., Teofilo, R., 2011. *Concentration of phenolic compounds in aqueous mate (*Ilex paraguariensis A. St. Hil*) extract through nanofiltration*. Food Sci. Technol. 44, 2211–2216.
- Nascimento, I.A., Marques, S.S.I., Cabanelas, I.T.D., Pereira, S.A., Druzian, J.I., Souza, C.O., Nascimento, M.A., 2013. *Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria*. Bioenergy Res. 6, 1–13.
- O'Sullivan, A., Mayr, A., Shaw, N.B., Murphy, S.C., Kerry, J.P., 2005. *Use of natural antioxidants to stabilize fish oil systems*. J. Aquat. Food Prod. Technol. 14, 75–94.
- Oussalah, M., Caillet, S., Salmieri, S., Saucier, L., Lacroix, M., 2004. *Antimicrobial and antioxidant effects of milk protein-based film containing essential oil for the preservation of whole beef muscle*. J. Agric. Food Chem. 52, 5598–5605.
- Pereira, D.A., Losada, P., Maroto, J., Cruz, J.M., 2011. *Natural antioxidant active packaging film and its effect on lipid damage in frozen blue shark (*Prionace glauca*)*. Innov. Food Sci. Emerg. Technol. 12, 50–55.
- Pereira-de-Abreu, D.A., Losada, P.P., Maroto, J., Cruz, J.M., 2011. *Lipid damage during frozen storage of Atlantic halibut (*Hippoglossus hippoglossus*) in active packaging film containing antioxidants*. Food Chem. 126, 315–320.
- Ribeiro, S.M.R., Barbosa, L.C.A., Queiroz, J.H., Knödler, M., Schieber, A., 2008. *Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica L.*) varieties*. Food Chem. 110, 620–626.
- Ribeiro, S.M.R., Queiroz, J.H., de Queiroz, M.E.L.R., Campos, F.M., Sant'Ana, H.M.P., 2007. *Antioxidant in mango (*Mangifera indica L.*) pulp*. Plant Foods Hum. Nutr. 62, 13–17.
- Roesler, R., Malta, L.G., Carrasco, L.C., Holanda, R.B., Sousa, C.A.S., Pastore, G.M., 1999. *Antioxidant activity of cerrado fruits*. Ciência e Tecnologia de Alimentos 27, 53–60.
- Salgado, S.M., Guerra, N.B., Mello Filho, A.B., 1999. *Polpa de fruta congelada: efeito do processamento sobre o conteúdo de fibra alimentar*. Revista de Nutrição 12, 303–308.
- Sánchez-González, L., Chiralt, A., González-Martínez, C., 2011. *Effect of essential oils on properties of film forming emulsions and films based on hydroxypropyl methylcellulose and chitosan*. J. Food Eng. 105, 246–253.
- Silva, M.S., Santos, M.R., Caroço, G., Rocha, R., Justino, G., Mira, L., 2002. *Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity*. Free Radic. Res. 16, 1219–1227.
- Siripatrawan, U., Harte, B.R., 2010. *Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract*. Food Hydrocoll. 24, 770–775.
- Sothornvit, R., Rodsamran, P., 2008. *Effect of a mango film on quality of whole and minimally processed mangoes*. Postharvest Biol. Technol. 47, 407.
- Souza, C.O., (Dissertação (Mestrado em Ciência de Alimentos)) 2010. *Preparação, caracterização e avaliação da eficácia de biofilmes a base de fécula de mandioca (*Manihot esculenta Crantz*) e incorporados com polpas de manga (*Mangifera indica L.*) e de acerola (*Malpighia emarginata*) como aditivos antioxidantes*. Faculdade de Farmácia, Universidade Federal da Bahia, Salvador, BA, 143 p.
- Souza, C.O., Silva, L.T., Silva, J.R., López, J.A., Veiga-Santos, P., Druzian, J.I., 2011. *Mango and acerola pulps as antioxidant additives in cassava starch bio-based film*. J. Agric. Food Chem. 59, 2248–2254.
- Souza, C.O., Menezes, J.D.S., Neto, D.C.R., Assis, J.G.A., Silva, S.R., Druzian, J.I., 2012a. *Carotenoides totais e vitamina A de cucurbitáceas do Banco Ativo de Germoplasma da Embrapa Semiárido*. Ciênc. Rural 42, 926–933.
- Souza, C.O., Silva, L.T., Druzian, J.I., 2012b. *Estudo comparativo da caracterização de filmes biodegradáveis de amido de mandioca contendo polpas de manga e de acerola*. Quim. Nova 2, 262–267.
- Souza, C.O., Veiga-Santos, P., Druzian, J.I., 2013. *Natural ingredients as additive for active antioxidant food packaging*. In: Lima, P.P.G., Vianello, F. (Eds.), *Food Quality, Safety and Technology*. Springer, New York, pp. 179–304.
- Veiga-Santos, P., Oliveira, L.M., Cereda, M.P., Alves, A.J., Scamparini, A.R.P., 2005. *Mechanical properties, hydrophilicity and water activity of starch-gum films: effect of additives and deacetylated xanthan gum*. Food Hydrocoll. 19, 341–349.
- Veiga-Santos, P., Oliveira, L.M., Cereda, M.P., Scamparini, A.R.P., 2007. *Sucrose and inverted sugar as plasticizer. Effect on cassava starch-gelatin film mechanical properties, hydrophilicity and water activity*. Food Chem. 113, 255–262.
- Vieira, M.A., (Tese (Doutorado em Ciência de Alimentos)) 2009. *Análise de hidrocarbonetos policíclicos aromáticos (HPAs) nas etapas do processamento da erva-mate (*Ilex paraguariensis*) e caracterização química dos resíduos da Trituração para o desenvolvimento de produto*. Universidade Federal de Santa Catarina, Florianópolis, SC, 246 pp.
- Wang, X., Sun, X., Liu, H., Li, M., Ma, Z., 2011. *Barrier and mechanical properties of carrot puree films*. Food Bioprod. Process. 89, 149–156.