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Mango and Acerola Pulps as Antioxidant Additives in Cassava Starch Bio-based Film

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ABSTRACT: The objective of this study was to investigate the feasibility of incorporating mango and acerola pulps into a biodegradable matrix as a source of polyphenols, carotenoids, and other antioxidant compounds. We also sought to evaluate the efficacy of mango and acerola pulps as antioxidants in film-forming dispersions using a response surface methodology design experiment. The bio-based films were used to pack palm oil (maintained for 45 days of storage) under accelerated oxidation conditions (63% relative humidity and 30 °C) to simulate a storage experiment. The total carotenoid, total polyphenol, and vitamin C contents of films were evaluated, while the total carotenoid, peroxide index, conjugated diene, and hexanal content of the packaged product (palm oil) were also monitored. The same analysis also evaluated palm oil packed in films without antioxidant additives (C1), palm oil packed in low-density polyethylene films (C2), and palm oil with no package (C3) as a control. Although the film-forming procedure affected the antioxidant compounds, the results indicated that antioxidants were effective additives for protecting the packaged product. A lower peroxide index (36.12%), which was significantly different from that of the control ($p < 0.05$), was detected in products packed in film formulations containing high concentration of additives. However, it was found that the high content of vitamin C in acerola pulp acted as a prooxidant agent, which suggests that the use of rich vitamin C pulps should be avoided as additives for films.

KEYWORDS: Antioxidant, active package, bio-based film, mango, acerola

1. INTRODUCTION

Packages are used to extend the storage of different products, protecting products mechanically and also providing protection from chemical and biological contamination.[1,2] In recent years, many new food packaging concepts have been introduced to satisfy consumer demands. Innovative materials, such as active packages, have been developed. These products are not only useful in their ability to prolong the storage of the product but also in acting to maintain the quality of the products while protecting the packaged product.[1,3] Major active packaging techniques are concerned with antioxidant compounds, which act as O_2 scanvengers and prevent or delay the oxidation process,[3] and are used in a variety of food and pharmaceutical applications.

Lipid oxidation is one of the main reactions causing the deterioration of food products and can result in off-flavors, a reduced nutritional quality of foods, and the production of certain toxic oxidative compounds.[4,5] Synthetic or natural antioxidants and chelating agents are the most effective inhibitors of lipid oxidation and act as free-radical scavengers or discontinue the propagation of oxidation chain reactions.[5,6]

Synthetic antioxidants that have already been incorporated into packaging materials[7] have recently received a great deal of interest for toxicological concerns, and thus, interest in natural antioxidants has steadily increased.[4,8] Phefolic acids, terpenes, tocopherols, carotenoids, and vitamins are important natural antioxidants, and it has been proposed that these antioxidants could be incorporated in packages to improve the oxidation stability of lipids and to prolong the storage of the products.[9,10] The chemical structures of these compounds allow them to receive electrons from reactive species, scavenge free radicals, or interrupt the initial and propagation steps of the oxidation process.[11,12]

Although many materials with antioxidant activity have been tested, few studies have used natural and edible compounds and even fewer have evaluated how these compounds could be incorporated into bio-based films.[13]

Phytochemical studies with mango (*Mangifera indica* L.) and acerola (*Malpighia emarginata* DC.) showed significant amounts of secondary metabolites that are a common component of the human diet. These bioactive constituents, including carotenoids, phenolic compounds, and vitamin C (VC), are present in both fruits[14,15] and industrialized pulps.[16] These components provide antioxidant protection because of their capacity to scavenge free radicals.

Considering the properties of these natural and edible compounds, this work aimed to investigate the efficacy and viability of mango and acerola pulps as antioxidant additives to be incorporated into bio-based cassava starch used to pack palm oil, analyzing its storage stability using some parameters [e.g., peroxide index (PI), conjugated diene (CD), and total carotenoid (TC) content]. The incorporation of these derivatives on films produced with cassava starch has a great economic importance

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because of the added value of these raw materials. Aside from these materials, other fruit industrial waste could potentially be used as additives.

2. MATERIALS AND METHODS

2.1. Materials. Cassava starch (Cargill Agrícola SA, Brazil), commercial sucrose, and inverted sugar were supplied from Açúcar Guarani SA, Brazil, while palm oil was obtained from Odelsa SA, Brazil. Mango and acerola pulps and low-density polyethylene films (0.040 mm thickness) were purchased from local markets (Salvador, Bahia, Brazil).

2.2. Bio-based Film Preparation. Bio-based films were prepared by dispersing cassava starch (4%), sucrose (1.4%), inverted sugar (0.7%), and mango and acerola fruit pulps (0−20%) in distilled water, according to a (2^2) second-order experiment design. Dispersions were shaken at 70 °C with constant stirring for 10 min. To prepare the films, each film-forming dispersion was poured onto polystyrene Petri dishes and then dehydrated at 35 °C in an oven with air flow 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.

2.3. Bio-based Film Used To Pack a Product. Film samples were used to pack palm oil for storage evaluations using a surface response methodology.[17] Square-shaped films (5 × 2 cm) of 0.123 and 0.141 mm in thickness were used to simulate the antioxidant capacity and the stability of packaged palm oil during extended storage at 0, 7, 15, 30, and 45 days under storage conditions of 63% relative humidity (RH) at 30 ± 2 °C. For the storage of palm oil, 10 × 4 cm of films were cut and sealed (Sealer SULPACK Basic SM BL 350, Brazil) at the bottom and side. With the help of a volumetric pipet, 10 mL of palm oil was transferred into the packaging. Subsequently, the bubbles of oxygen were removed, and the film was sealed on top. The film storage with palm oil and analyses were carried out in a dark room to avoid the effects of light interference.

2.4. Packaged Product Oxidative Stability. The oxidative stability from a packaged product was carried out using a PI, as well as the CD and TC content at 0, 7, 15, 30, and 45 days, while the hexanal content (HC) was evaluated at 0 and 45 days. Palm oil samples packed with cassava starch films (without pulps; C1), with low-density polyethylene (LDPE; C2), and without any package (C3) were used as controls.

The PI was determined by titration according to Association of Official Analytical Chemists (AOAC) methodology.[18]

For the quantification of HC, it was used as an adapted methodology[19] and a headspace combined with gas chromatography/mass spectrometry (GC/MS). Briefly, palm oil (200 mg) inside a headspace vial was heated at 140 °C for 30 min. The separation was performed by GC/MS (Clarus 500, Perkin-Elmer, Shelton, CT) coupled to a Turbomatrix Perkin-Elmer headspace and a Wax-FFAP column (50 m × 0.20 mm × 0.2 μm) with 1.0 mL/min helium flow. The following conditions were used during this process: the injector was maintained at 180 °C; the oven temperature was increased from 35 to 160 °C during three ramp-up periods (35 °C for 1 min, 80 °C at 3 °C/min, and 160 °C at 7 °C/min, remaining for 22.57 min). The electron impact ionization energy was 70 eV, and the mass spectra was collected over a mass range of m/z 50−600. Hexanal was identified by comparing the standard area retention time and mass spectra using a National Institute of Standards and Technology (NIST) library. The volatile constituent was quantified using a hexanal external standard (Sigma-Aldrich, St. Louis, MO), diluted in soy oil at five different concentrations (0.40−32.00 μg/mL) of headspace vial, and analyzed as above. A calibration curve of the peak area of standards was determined and expressed in micrograms per milliliter.

CD content was quantified using American Oil Chemists' Society (AOCS) methodology.[20] In this method, 0.2 mg of palm oil was dissolved in isooctane, the absorbance was read at 233 nm (UV/vis spectrometer, Lambda 20, Perkin-Elmer, Norwalk, CT), and the results were expressed as a percentage.

For the TC content, 0.3 g of the packaged product (palm oil) was dissolved in petroleum ether. The TC was determined spectrophotometrically at 435 nm[21] (UV/vis spectrometer, Lambda 20, Perkin-Elmer, Norwalk, CT) and calculated according to eq 1[23]

$$\text{TC}\ (\mu\text{g g}^{-1}) = \frac{\text{Abs} \times \text{Vol} \times 10^4}{(\text{A1\% 1 cm})\text{Wg}} \qquad (1)$$

where TC is the total carotenoid, Abs is absorbance at 435 nm, Vol is dilution volume (mL), A1% 1 cm is the absorptivity coefficient value (2592), and Wg is the sample weight (g).

2.5. Bio-based Film Stability. The bio-based film stability was evaluated by analyzing the TC, total polyphenol (TP), and VC contents during a storage period of 45 days.

For determined TC values in bio-based films, films (2 g) were analyzed according to Silva and Mercadante[22] and the determination was performed spectrophotometrically at 440 nm (UV/vis spectrometer, Lambda 20, Perkin-Elmer, Norwalk, CT). The TC concentration was determined according to eq 1 at 440 nm.[23] The TP content of the film samples (100 mg) was extracted at 0, 7, 15, 30, and 45 days of storage with water after centrifugation (4400 rpm at 5 °C for 3 min; Eppendorf, 5702R, Hamburg, Germany). The TP was spectrophotometrically determined in the supernatant at 760 nm (UV/vis spectrometer, Lambda 20, Perkin-Elmer, Norwalk, CT), using Folin−Ciocalteu reagent,[24] and the results were expressed as gallic acid equivalents.

VC content was determined by titration at days 0 and 45, according to Institute Adolfo Lutz standard procedures.[25]

2.6. Statistical Analysis. A central composite experimental design was used to evaluate the influence of concentration differences of the natural antioxidants incorporated in bio-based films. Mango (%, w/w; X_1) and acerola (%, w/w; X_2) pulps were chosen as independent variables. The real and coded values of these pulps are shown in Table 1.

The packaged product (palm oil) PI, CD, HC, and TC contents, as well as the VC and TP contents of films, were used as dependent variables (Y).

A 2^2-factorial design with four axial points (α = 1.41) and three replications at the center points (for a total of 11 experiments) was employed (Table 1). The second-degree polynomials were calculated to estimate the response of the dependent variable (Stat, Inc., Minneapolis, MN) using eq 2:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}{X_1}^2 + b_{22}{X_2}^2 + b_{12}X_1X_2 \qquad (2)$$

where Y is the predicted response, X_1 and X_2 are the independent variables, b_0 is the offset term, b_1 and b_2 are the linear effects, b_{11} and b_{22} are the squared effects, and b_{12} is the interaction term.

3. RESULTS AND DISCUSSION

3.1. Stability of Additives Incorporated into Bio-based Films during the Storage of the Packaged Product. Mango and acerola pulps were incorporated into cassava starch films as additives to provide a source of active compounds. These pulps contain high concentrations of antioxidant compounds, such as TC, TP, and VC.

At day 0, the TC, TP, and VC values from different film formulations ranged according to the pulp concentrations incorporated into the bio-based films, as expected. When both pulps were used in film-forming dispersion, an increase in these values was found by increasing the concentration of both

Table 1. Coded and Real Values of Mango and Acerola Pulps Added to Cassava Starch Film Formulations According to a (2^2) Second-Order Experiment Design with Three Central Points

film formulations	coded values		real values (%)	
	mango pulp (X_1)	acerola pulp (X_2)	mango pulp	acerola pulp
F1	−1.00	−1.00	2.90	2.90
F2	−1.00	1.00	2.90	17.10
F3	1.00	−1.00	17.10	2.90
F4	1.00	1.00	17.10	17.10
F5	−1.41	0.00	0.00	10.00
F6	1.41	0.00	20.00	10.00
F7	0.00	−1.41	10.00	0.00
F8	0.00	1.41	10.00	20.00
F9[a]	0.00	0.00	10.00	10.00
F10[a]	0.00	0.00	10.00	10.00
F11[a]	0.00	0.00	10.00	10.00

[a] Central points.

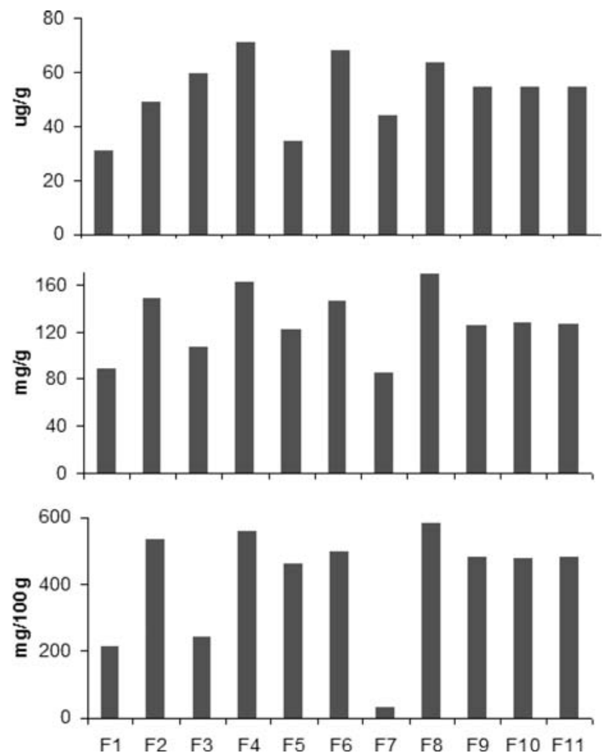

Figure 1. Total antioxidant content in film formulations at day 0 of storage: (top) TC, (middle) TP, and (bottom) VC concentrations.

additives (Figure 1). Thus, a low additive concentration (2.9% of both mango and acerola pulps) resulted in low values of TC and TP (31.15 μg/g and 89.17 mg/g, respectively) in F1 ($p < 0.05$). Conversely, in F4 (17% of both additives, TC = 70.56 μg/g and TP = 162.30 mg/g) and in F8 (containing 20% acerola pulp and 10% mango pulp, TC = 63.13 μg/g and TP = 168.80 mg/g) at higher concentrations, these compounds were detected; therefore, a significant increase in these values was observed ($p < 0.05$).

However, film preparations containing only one additive presented different values of the antioxidant compounds. For example, F7 (only mango pulp) showed a higher concentration of CT (44.22 μg/g) and lower concentration of VC (6.54 mg/100 g), while F5 (only acerola pulp) showed significantly higher values of TP (121.24 μg/g) and VC (94.34 mg/100 g) and low values of CT (34.42 μg/g) (Figure 1).

It was noted that the antioxidant compound concentrations of formulations loaded with high additive concentrations decreased (Table 2). Thus, at 45 days, samples from F1 (low mango and acerola pulp contents) presented a minor decrease on TC and TP (24.53 and 17.80%, respectively), while in F4, the higher additive concentrations caused a significant decrease ($p < 0.05$) in TC (43.60%) and TP (36.12%). These results indicate that the films provided high values of protection and resulted in low values of packaged product oxidation.

With regard to VC values, the opposite situation was observed. F1 showed a higher decrease in VC values (85.00%) than F4 (69.50%). However, there was no significant difference in the decrease in VC values in the different formulations of films between 0 and 45 days ($p > 0.05$).

Table 2 shows that, after 45 days of storage, film samples exhibited decreases ranging from 24.53 to 43.60% for TC, while decreases in TP and VC ranged from 17.80 to 36.12% and from 69.50 to 85.00%, respectively.

However, the antioxidant capacity of additives was maintained throughout the storage period. A similar behavior was observed in films containing palm oil fruit pulp and its oil as added antioxidants, which were used to pack soybean oil. In this case, a decrease in TC ranging from 79.90 to 99.60% was observed during 90 days of storage.[17]

A comparison of F7 and F5 films showed that F7 had a larger decrease in TC (26.38%) in comparison to F5 (24.85%), whereas the opposite occurred with respect to reductions in PT and VC (Table 2). In all cases, the results were statistically significant ($p < 0.05$).

The incorporation of oil palm and palm pulp-like antioxidant additives in packaging of cassava starch was described. The addition of these additives decreased according to the degradation of the packaged product during the storage period.[17] According to Wessling et al.,[26] α-tocopherol incorporated in polyethylene materials showed a greater resistance during 4 weeks of storage than those film controls containing butylated hydroxytoluene (BHT), which degraded in just in 1 week.

Experimental results for the different formulations of films used to pack palm oil during storage showed a significant ($p < 0.05$) difference with regard to TC and TP after 45 days of storage. This

Table 2. Content Decrease of Antioxidants in Film Formulations Prepared with Fruit Pulps[a]

	decrease TC (%)				decrease TP (%)				decrease VC (%)
film formulations	7	15	30	45	7	15	30	45	45
F1	4.55 a	9.86 a	18.06 f	24.53 a	5.70 e	11.08 e	13.61 e,f	17.80 f	85.00 c
F2	5.20 c	11.17 b	20.50 b	27.65 b	10.75 b	20.34 b,c	24.62 b,c	30.18 b	76.21 a
F3	6.65 b,d	13.93 c	25.38 c,d	34.56 c,e	6.00 a	11.64 e	14.29 a,e	18.58 d	72.03 c
F4	8.95 d	17.33 c,d	32.21 b,c	43.60 e	13.40 d	25.00 d	30.03 d	36.12 c	69.50 a,b
F5	4.60 e	10.04 e	18.31 e,f	24.85 f	9.23 c	17.61 b	21.40 b	26.59 b	80.12 b
F6	8.20 d	16.74 d	30.39 d	40.83 c	12.03 b,d	22.61 c	27.26 c,d	33.08 c,e	69.90 b
F7	4.90 a	10.70 e	19.45 a,e	26.38 d	4.41 a	8.63 a	10.64 f	14.57 a	78.21 d
F8	7.65 b	15.78 b,c	28.66 b,c	38.65 b	12.10 d	22.74 c,d	27.37 b,c	33.18 e	70.00 a
F9[b]	6.55 e,f	13.65 f	25.91 f	33.97 f	7.04 e,f	13.58 c	16.63 e,f	21.23 f,g	73.23 b
F10[b]	6.80 f	14.16 f	25.75 f	35.11 f	7.73 c,f	14.86 c	18.15 c,f	22.95 g	73.20 b
F11[b]	6.00 f	13.14 f	25.04 f	34.41 d,f	7.34 e,f	14.14 c	17.29 e,f	21.98 f	73.40 b

[a] TC, total carotenoid; TP, total polyphenol; VC, vitamin C. Means with the same letters in the same columns presented no statistical difference ($p > 0.05$) according to Tukey's test. [b] Central points.

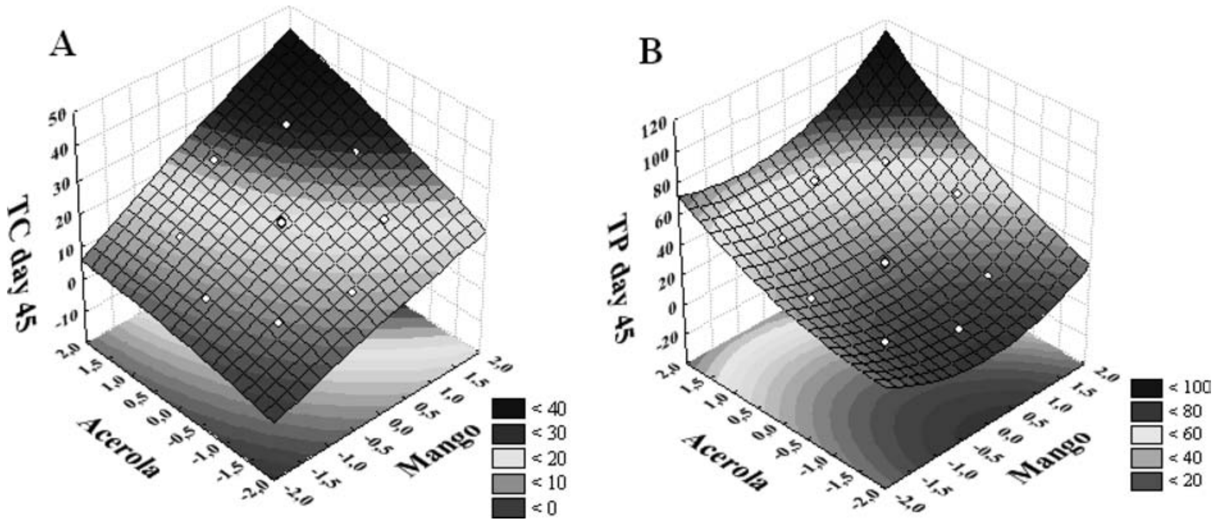

Figure 2. Response surface plots showing the effect of incorporated additives to understand the decrease factor on (A) TC (μg/g) and (B) TP (mg/g) decrease film preparation after 45 days of palm oil storage.

resulted in a second-order polynomial equation, which represents the model equation used to evaluate the decrease of TC (eq 3) and TP (eq 4) in films as a function of the concentrations of mango pulp (%, X_1) and acerola pulp (%, X_2) and the interaction between them (X_1 and X_2). According to eqs 3 and 4, the decrease in the TC concentration in films depends upon the interaction between both independent variables (X_1 and X_2), while the decrease in the TP concentration only depended upon these variables independently.

$$\text{TC (day 45)} = 3.42 + 0.92X_1 + 0.53X_2 + 0.02X_1X_2 \quad (R^2 = 0.87) \tag{3}$$

$$\text{TP (day 45)} = 27.99 + 5.03X_1 + 5.44X_1^2 + 16.13X_2 + 2.49X_2^2 + 2.50X_1X_2 \quad (R^2 = 0.86) \tag{4}$$

The response surface graphs (panels A and B of Figure 2) show reductions in the values of TC and TP in different concentrations of the pulp of mango and acerola, which were incorporated as additives in the films. When these numbers are analyzed, it is observed that the maximum combination of mango and acerola pulps provides data from the largest reductions in TC and TP. These reductions are related to greater protection of the packaged product, demonstrating that there is a linear relationship between the amount of pulps added and the protective effect.

3.2. Monitoring Packaged Palm Oil during Storage. As expected, all palm oil samples packed with different films showed a significant decrease in the initial content of TC and a concomitant increase in the PI. It was noted that palm oil packed in bio-based films containing mango and acerola pulps showed a low PI ($p < 0.05$) compared to the product packed in control films (Table 3). The results indicate the efficacy of pulps as antioxidant additives that act to protect the packaged product. This effect can be considered concentration-dependent; palm oil packed with F1 (low pulp concentrations) presented a high oxidation value (PI = 64.27%) compared to that packed with F4 (high concentrations of pulp), with a lower PI value (31.62%; $p < 0.05$), during the same storage period.

Table 3. Analysis of Palm Oil Samples Packed in Different Film Formulations after 7, 15, 30, and 45 Days of Storage[a]

	packed product (palm oil)									
									increase	
	decrease TC				increase PI				HC	DC
FF[b]	7	15	30	45	7	15	30	45	45	45
C1	4.35 a,b	12.27 a	19.96 a	29.50 a	32.72 a,b	46.18 a	65.37 a	91.93 a	297.96 a	25.54 a
C2	6.98 c	13.28 a	24.81 b	40.92 b	39.05 b	77.48 b	265.23 b	363.41 b	1355.78 b	47.15 b
C3	12.17 d	18.10 b	42.48 c	73.63 c	123.39 c	163.47 c	438.54 c	640.87 c	2145.58 c	108.31 c
F1	4.37 a,e,f,g	10.93 c,d	19.63 e,h,i	27.14 e,h,i	19.90 a,e	32.37 a,d	46.80 a	64.27 a,e	37.33 d	11.39 d
F2	3.12 a,e,f,g	8.79 c	16.66 e,h,i	23.33 d,i	18.50 d,e	30.05 a,d	43.38 a	59.51 d,e	24.50 d	10.17 d
F3	3.47 b,e,g	9.43 d,e	15.89 e,f	22.90 d,i	16.38 d	25.69 d	36.37 a	49.32 d	24.00 d	7.02 e
F4	2.86 g	8.33 e	15.42 f,g,i	20.97 f	10.00 d	16.05 d	23.01 a	31.62 d	16.45 d	4.14 e
F5	4.15 a,e,f,g	10.87 a,f	19.22 a,c	26.88 a,d	19.70 a,d,e	31.97 a,d	46.16 a	63.69 d,e	26,14 d	8.89 e,f
F6	2.96 e,g	8.46 e	15.62 f,g	21.03 f	11.50 d,e	18.47 a,d	26.47 a	35.32 d	19.87 d	5.23 e
F7	3.58 a,e,f,g	10.06 c	17.58 d,h,i	25.49 d,e,i	19.00 a,d,e	30.90 a,d	44.64 a	61.28 d,e	24.34 d	8.32 e,f
F8	2.97 g	8.30 e	15.08 g	21.24 f	12.80 d,e	20.70 a,d	29.75 a	40.78 d	19.90 d	5.35 e
F9[c]	3.12 e,g	8.91 e	15.37 g	22.97 f	15.80 d,e	25.41 a	36.57 a	49.96 d	23.03 d	6.62 e
F10[c]	3.04 a,e,f,g	8.29 c	15.77 f,g,j	21.79 f,g	15.70 d	25.42 a	36.71 a	50.10 d	23.18 d	7.27 e
F11[c]	3.84 a,e,f	9.21 c	15.49 e,h,j	19.36 f,g	15.60 d,e	25.19 a	36.21 a	49.42 d	23.84 d	7.50 e

[a] Data expressed as a percentage indicate PI, TC, HC, and CD. Means with the same letters in the same columns presented no statistical difference ($p > 0.05$) according to Tukey's test. [b] FF = film formulations. [c] Central points.

There was a significant difference (in terms of oxidation; $p < 0.05$) between film-forming dispersions containing only one additive. The oil packed with F7 (only mango pulp) showed the lower PI content (61.28%) compared to oil packed with F5 (acerola pulp only), which showed a PI value of 63.69% (Table 3).

The values of TC in the packaged product showed no significant difference ($p > 0.05$). However, the F4 samples showed a small decrease (20.97%) compared to the product packed with F1 (27.14%). Thus, the formulation film F4 presented the lowest TC decrease and is therefore more effective in protecting palm oil (Table 3).

Because of the incorporation of different proportions of mango pulp (%, X_1) and acerola pulp (%, X_2) in the films, the increase in the PI content (mequiv/kg) of palm oil after 45 days of storage is expressed using a second-order polynomial equation (eq 5). The increase in this parameter depends upon the concentration of the mango and acerola pulps and the interaction from both factors. Figure 3 represents the response surface graph, showing the increase of palm oil PI packed with different film formulations. The graph indicates that the point of maximum increase of PI corresponds to the minimum concentration of both pulps, whereas the point with a minimum increase in PI occurs at the maximum concentration of both pulps.

$$\text{PI (day 45)} = 11.07 - 2.35X_1 - 1.34X_2 - 0.63X_1X_2 \quad (R^2 = 0.87) \tag{5}$$

Moreover, the values of HE and CD analyzed in the product packed with different films showed no significant difference ($p > 0.05$) during storage. However, after 45 days, the packaged product showed a smaller increase in the values of these compounds compared to the controls (Table 3). Mango and acerola pulps, as sources of many quenching agents, VC and phenolic compounds, inhibited off-flavor production. Thus, the protective effect of these compounds increased the time lag of CD and HE formation during storage for controlling the oxidation of palm oil under environmental conditions. This inhibition increased proportionally with the concentration of the antioxidant, as evidenced by the lower CD and HE in formulations F4 and F8.

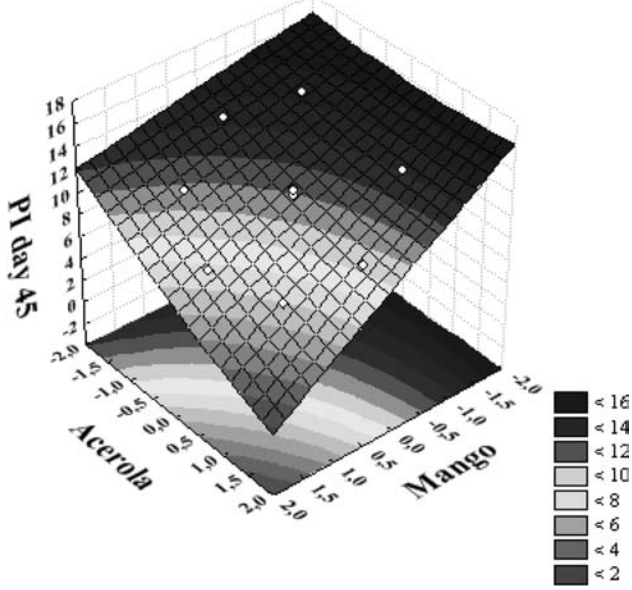

Figure 3. Response surface plot for understanding the increase of PI values (mequiv/kg) after 45 days of palm oil storage.

The results also showed that cassava starch films without antioxidant additives showed a high antioxidant effect ($p < 0.05$) compared to LDPE. Therefore, these results show that cassava starch films can be an effective barrier against oxidation compared to the LDPE film evaluated in this study.

These results are in agreement with some studies that have shown that oxygen can permeate through the film, reacting preferentially with some compounds present in the film formulation. This allows the packaged product to be preserved for a longer period of time.[17] Therefore, mango and acerola pulps, as additives in film-forming dispersions, are effective in preserving a packaged product against oxidation.

3.3. Correlations between Bio-based Films and Packaged Parameter Products during Storage. The results of this study suggest that the protection of packaged products against oxidation can be attributed to the concentration-dependent radical-scavenging activity of antioxidant compounds present in film-forming dispersions.

Considering the results for PI values from the packaged product, the protective effect against lipid oxidation is likely a consequence of a physical process because of the TC content. This is especially true in mango pulp, because the palm oil packed in films containing this additive showed a lower rate of oxidation compared to the controls.

The TC content in the film showed a higher correlation with the evaluated parameters in the packaged product (PI, HE, and CD; 64.09−98.39%) than the TP (31.24−56.99%). This confirms the efficacy of TC incorporated into films in comparison to TP. Because mango pulp possesses a higher value of TC than acerola pulp, the product packed in films containing mango pulp showed less oxidation. Thus, the film formulations constitute a more effective oxygen barrier because the decrease in the TC and TP concentrations in films is correlated with lower PI values in the packaged product. Thus, the packaging rather than the packaged product is oxidized (active compound loss).

With regard to the correlation between VC and PI, the decrease in the VC values was associated with an increase in the PI values; i.e., the results showed a possible prooxidant effect of VC. This effect has been reported in an iron/ascorbic acid system, which induces lipid peroxidation by OH radicals, as well as in a linoleic β-carotene/linoleic acid system and a liposome method.[27] Compounds with antioxidant activity may exhibit prooxidant behavior under certain conditions. The mechanisms of antioxidants being effective via rebalancing the impaired prooxidant/antioxidant ratio are not completely clear.[28] Films can be considered as an *in vitro* system in which the fruit pulp used can contain, besides VC, some ions (e.g., Fe^{3+} and other transition metals), which, in the presence of VC, can generate reactive oxygen species, because of the high VC content that can provoke this imbalance. This process will be controlled *in vivo* by the presence of antioxidant proteins that chelate such metals.[29]

In conclusion, the application of natural products to develop bio-based film with antioxidant activity represents an innovative concept that is of interest to the food industry. This concept could be implemented in response to the continuous changes in current consumer demands and market trends, but there have been few studies on this subject to date. The results presented here show the feasibility of developing antioxidant films by incorporating fruit pulp, a source of bioactive compounds, into a cassava starch packaging for lipid foods. The results provide a positive impact of low PI in packaged palm oil when using film additives. However, it was found that high VC content of the acerola pulp can act as a prooxidant agent, which suggests that this additive could be withdrawn or incorporated into lower concentrations. Further precise investigations are required to verify this question, as well as the effects of incorporating antioxidant additives into other matrixes.

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ABBREVIATIONS USED

PI, peroxide index; HC, hexanal content; CD, conjugated diene; TC, total carotenoid; TP, total polyphenol; VC, vitamin C

REFERENCES

(1) Vermeiren, L.; Devlieghere, F.; Van Beest, M.; Kruijf, N.; Debevere, J. Development in the active packaging of food. *Trends Food Sci. Technol.* **1999**, *10*, 77–86.

(2) Hotchkiss, J. Food packaging interactions influencing quality and safety. *Food Addit. Contam.* **1997**, *14*, 601–60.

(3) Rooney, M. L. Overview of active food packaging. In *Active Food Packaging*; Rooney, M. L., Ed.; Blackie Academic and Professional: London, U.K., 1995; pp 1−37.

(4) Shahidi, F. Antioxidants in food and food antioxidants. *Food Nahrung* **2000**, *44*, 158–163.

(5) Lindley, M. G. The impact of food processing on antioxidants in vegetable oils, fruits and vegetables. *Trends Food Sci. Technol.* **1998**, *9*, 336–340.

(6) Atoui, A. K.; Mansouri, A.; Boskou, G.; Kefalas, P. Tea and herbal infusions: Their antioxidant activity and phenolic profile. *Food Chem.* **2005**, *89*, 27–36.

(7) Brody, A. L. What's active in active packaging. *Food Technol.* **2001**, *55*, 104–106.

(8) Zheng, W.; Wang, S. Y. Antioxidant activity and phenolic compounds in selected herbs. *J. Sci. Food Agric.* **2001**, *49*, 5165–5170.

(9) Broinizi, P. R. B.; Andrade-Wartha, E. R. S.; Silva, A. M. O.; Novoa, A. J. V.; Torres, R. P.; Azeredo, H. M. C.; Alves, R. E.; Mancini-Filho, J. Evaluation of the antioxidant activity of phenolic compounds naturally contained in by-products of the cashew apple (*Anacardium occidentale* L.). *Cienc. Tecnol. Aliment.* **2007**, *27*, 902–908.

(10) Ribeiro, M. A.; Bernardo-Gil, M. G.; Esquível, M. M. *Melissa officinalis* L.: Study of antioxidant activity in supercritical residues. *J. Supercrit. Fluids* **2001**, *21*, 51–60.

(11) Goodwin, T. W. Biosynthesis of carotenoids: An overview. *Methods Enzymol.* **1993**, *214*, 330–340.

(12) Shahidi, F. Natural antioxidants: An overview. In *Natural Antioxidants Chemistry, Health Effects, and Applications*; Shahidi, F., Ed.; American Oil Chemists' Society (AOCS): Champaign, IL, 1996; pp 1−11.

(13) Oussalah, M.; Caillet, S.; Salmieri, S.; Saucier, L.; Lacroix, M. Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. *J. Agric. Food Chem.* **2004**, *52*, 5598–5605.

(14) Lopes, A. S. Surinam cherru and west Indian cherry: study of processing, stability and formulation of a mixed nectar. Doctoral Thesis in Food Science, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil, 2005; p 193.

(15) Kuskoski, E. M.; Asuero, A. G.; Morales, M. T.; Fett, R. Wild fruits and pulps of frozen fruits: antioxidant activity, polyphenols and anthocyanins. *Cienc. Rural* **2006**, *36*, 1283–1287.

(16) Oliveira, M. E. B.; Bastos, S. R.; Feitosa, T.; Branco, M. A. A. C.; Silva, M. G. G. Physico chemical parameters evaluation of acerola, yellow mombin and cashew apple frozen pulps. *Cienc. Tecnol. Aliment.* **1999**, *19*, 326–332.

(17) Grisi, C. V. B.; Veiga-Santos, P.; Silva, L. T.; Cabral-Albuquerque, E. C.; Druzian, J. I. Evaluation of the viability of incorporating natural antioxidants in bio-based packagings. In *Food Chemistry Research Developments*; Papadopoulos, K. N., Ed.; Nova Science Publishers: Lancaster, U.K., 2008; Vol. 1, pp 1−11.

(18) Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis Cd 8b-90*; AOAC: Gaithersburg, MD, 2000.

(19) Amstalden, L. C.; Leite, F.; Menezes, H. C. Identification and quantification of coffee volatile components through high resolution gas chromatoghaph/mass spectrometer using a headspace automatic sampler. *Cienc. Tecnol. Aliment.* **2001**, *21*, 123–128.

(20) American Oil Chemists' Society (AOCS). *Official Method of Analysis Ti 1a-64. Spectrophotometric Determination of Conjugated Dienoic Acid*; AOCS: Champaign, IL, 1993.

(21) Passoto, J. A.; Penteado, M. V. C.; Mancini-Filho, J. Activity of β-carotene and vitamin A: A comparative study with synthetic antioxidant. *Food Sci. Technol.* **1998**, *18*, 624–632.

(22) Silva, S. R.; Mercadante, A. Z. Carotenoid composition of fresh yellow passion fruit (*Passiflora edulis*). *Cienc. Tecnol. Aliment.* **2002**, *22*, 254–258.

(23) Davies, B. H. Carotenoids. In *Chemistry and Biochemistry of Plant Pigments*, 2nd ed.; Goodwin, T. W., Ed.; Academic Press: London, U.K., 1976; pp 38−65.

(24) Roesler, R.; Malta, L. G.; Carrasco, L. C.; Holanda, R. B.; Sousa, C. A. S.; Pastore, G. M. Antioxidant activity of cerrado fruits. *Cienc. Tecnol. Aliment.* **1999**, *27*, 53–60.

(25) Instituto Adolfo Lutz. *Métodos Químicos e Físicos para Análise de Alimentos*; Normas Analíticas do Instituto Adolfo Lutz: São Paulo, Brazil, 2005; Vol. 1, p 1018.

(26) Wessling, C.; Nielsen, T.; Giancin, J. R. Antioxidant ability of BHT and α-tocopherol impregnated LDPE film in packaging of oatmeal. *J. Sci. Food Agric.* **2000**, *81*, 194–201.

(27) Hassimoto, N. M.; Genovese, M. I.; Lajolo, F. M. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *J. Agric. Food Chem.* **2005**, *53*, 2928–2935.

(28) Hsu, P. C.; Guo, Y. L. Antioxidant nutrients and lead toxicity. *Toxicology* **2002**, *180*, 33–44.

(29) Osiecki, M.; Ghanavi, P.; Atkinson, K.; Nielsen, L. K.; Doran, M. R. The ascorbic acid paradox. *Biochem. Biophys. Res. Commun.* **2010**, *400*, 466–470.