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Influence of Blueberry and Jaboticaba Agroindustrial Residue Particle Size on Color Change of Corn Starch Based Films Submitted to Different pH Values Solutions

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Abstract: Corn starch, glycerol and agroindustrial residues were used to produce films by casting. By-products from juice processing, blueberry and jaboticaba in powder with different particle sizes were added in the filmogenic matrix to evaluate its potential as a colorimetric indicator. Blueberry and jaboticaba peels are commonly discarded although contain high amount of important compounds as anthocyanins. These compounds have the ability to color change after immersion in different pH values, demonstrating its potential for the intelligent packaging development. Analyses were performed in a colorimeter after films immersion in different buffer solutions. Visual color changes were perceived; reddish and bluish color in acidic and basic pH values, respectively. Independently of the particle size, the ΔE* values were greater than 5, showing visually perceptible change to the human eye. The results indicate the potential of use of these residues as a pH indicator for the development of renewable and biodegradable sensor of food deterioration.

Keywords: Blueberry; jaboticaba; anthocyanin; casting; colorimetric analysis

1 Introduction

In 2014, around 0.15 billion tons of synthetic polymers were produced in the worldwide. Moreover, in the last years, the global use of plastic is growing at a rate of 12% per year [1]. Accumulation rate of plastic waste in the environment is 25 million tons per year and, consequently, its incorrect disposal generates a serious environmental threat [2-3]. Degradation leads to polymers with lower molecular weight and can cause cracks on the surface of the plastic material [4]. Even when a plastic item degrades under the influence of weathering, it first breaks down into smaller pieces of plastic fragments, but the polymer itself may not necessarily fully degrade in a meaningful timeframe. Consequently, substantial quantities of end-of-life plastics are accumulating in landfills and as they remain in the natural environment, resulting in both waste-management issues and environmental damage [5-6].

Thus, the environmental concern associated with the large disposal of plastic materials, as well as the reduction of the availability of petroleum derived sources, have promoted an increase in researches to find alternative polymers in substitution of the non-biodegradable plastic from fossil sources. Among the biopolymers obtained from renewable sources that have film-formation capacity, the starch can be considered as one of the most promising alternatives because it is abundant, cheap, biodegradable, edible, tasteless and odorless; moreover, starch cultures are available worldwide [7].

It is well known that the global market of the minimally processed foods and/or ready-to-eat foods is growing. The consumers are looking for healthy food in the globalized world; these foods are generally more perishable and require a higher control related to shelf-life. In this context, the concept of intelligent packaging appears as an innovative development technology. By definition, an intelligent packaging is

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based on the quality and/or safety evaluation of the packaged food being able to communicate this information to the external environment [8-9]. This kind of packaging commonly uses different sensor types that are capable of providing visual information to consumers, such as change, appearance or even disappearance of color.

During fruit processing, considerable amounts of husk, bagasse and seeds are produced and most of the times are discarded. Moreover, nutrients and bioactive compounds of the blueberry and jaboticaba fruits are mainly concentrated in the husk and bagasse, not being added to the final product [10]. Within these bioactive compounds, it is possible to highlight the anthocyanins, which have the ability to change its coloration when submitted to different pH values. In acidic condition (pH 1.0) occurs a predominance of the intact structural forms of intense coloration (cation flavílium); at higher pH values (pH 4.5) there is the formation of colorless structures (chalcone); in basic condition (pH > 7) occurs the quinoidal bases formation that have blue color [11-12]. Furthermore, it should be emphasized that studies aiming the use of food industry waste are of great importance, both from the environmental, economic and social viewpoint.

Compared with others agroindustrial residues generate during juice processing (for example, orange, peach, pineapple, among others), the main advantage regarding the reuse of blueberry and jaboticaba residues corresponds to the high content of bioactive compounds present in husks. Among these bioactive compounds are the anthocyanins that have the ability to change its coloration when immersed in solutions with different pH values. In addition, these bioactive compounds have a high antioxidant potential [13-14] and some authors [10-15] have already related this property to prevention of some diseases. However, the main limitation is the small production scale of this type of waste in comparison with others agroindustrial wastes. Thus, it is important to emphasize that studies aiming the reuse of the residues produced in food industry are of great importance.

Moreover, the main novelty of the work is related to the use of jaboticaba and blueberry residue as anthocyanins source. Other authors used pure commercial anthocyanin or in the extract form obtained from different sources [14,16-19]. In order to make feasible the film production with agroindustrial residue, the main idea of this study was to create an alternative way to use it so that could increase the competitiveness in relation to currently pH indicators marketed.

Thus, the objective of the present work was to evaluate the granulometry influence of the jaboticaba and blueberry residue powders, obtained from juice processing, on the color change of corn starch films after immersion in buffer solutions with different pH values. In order to evaluate the potential of use of these films as intelligent packaging, the colorimetric results were correlated with the total anthocyanin content present in the different residue fractions.

2 Material and Methods

The jaboticaba fruits of the variety *Myrciaria cauliflora* (Mart.) O. Berg were directly obtained in the region of Concórdia, SC, Brazil [20]. The blueberries (*Vaccinium corymbosum* L.) fruits were purchased from Italbraz Ltda (Vacaria, RS, Brazil).

The native corn starch (pharmaceutical grade; 28 % of amylose content; 13.5 % of water content; gelatinization temperature ranging between 62°C and 72°C) was purchased from Delaware Company (RS, Brazil). The glycerol used as plasticizer was PA degree (Nuclear, SP, Brazil). All other reagents were of analytical grade.

2.1 The Agroindustrial Residues Production

After sanitization, the jaboticaba fruits were heated (temperature ranging between 60°C and 80°C during 40 min) and the extraction was performed by pressing. The jaboticaba residue powder was produced from the bagasse generated during the production of juice, according the flowchart displayed in Fig. 1. The bagasse (approximately 40% of the processed fruit) was freeze-dried, milled and sieved

(100 *mesh*; with openings of 0.150 mm/μm) in order to obtain different powder fractions (a fraction retained in a sieve of 100 *mesh* and a fraction that passed through the sieve).

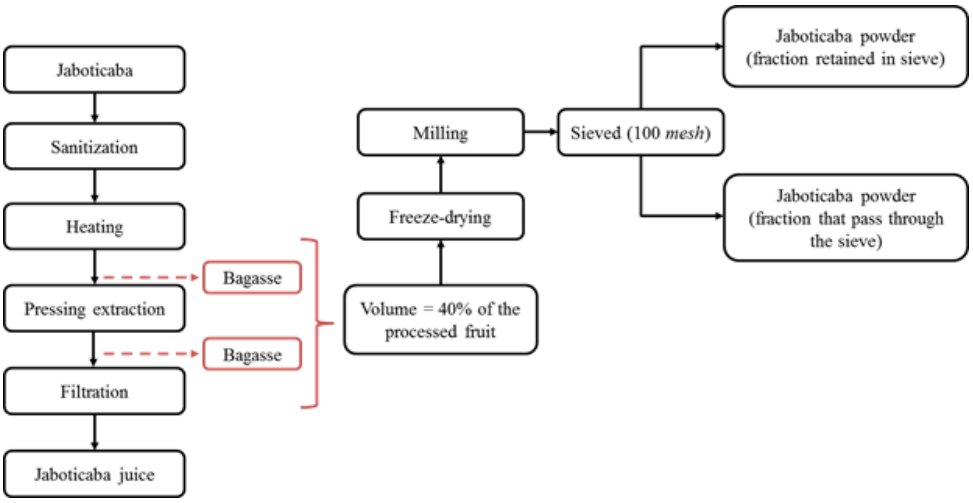

Figure 1: Simplified flowchart of jaboticaba bagasse powder production

To produce the blueberry residue (BR) powder, firstly the samples were defrosted (25°C for 15 hours) and processed in a fruit extractor (Philips Wallita juicer). After juice production, the residue (bagasse fraction) was separated by filtration in a non-woven fabric, freeze-dried for three days (the samples were previously frozen at -40°C), milled and sieved, generating two bagasse fractions: one fraction thinner (passed through the sieve of 100 *mesh*) and other thicker (retained in the sieve of 100 *mesh*). The agroindustrial blueberry fractions of residue were produced according to the simplified flowchart depicted in Fig. 2.

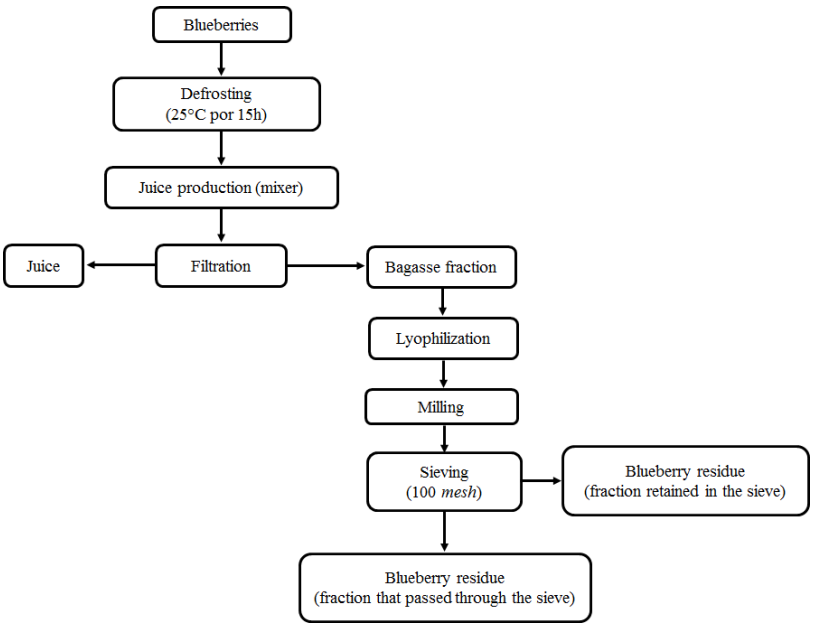

Figure 2: Simplified flowchart of blueberry bagasse powder production

2.2 The Agroindustrial Residues Characterization

Particle size analysis was carried out by laser diffraction (Cilas, model 1180, France) using water as mobile phase for particles dispersion. The total anthocyanin content was quantified by exhaustive extraction using approximately 1 g of sample in 20 mL of methanol and acidified water with 1% citric acid (ratio 50:50) in each extraction until the color disappeared [21]. The quantification of the total anthocyanin content in the different fractions of jaboticaba and blueberry residues was carried out by the pH differential method using spectrophotometric analysis (Pró-Análise, Model UV 1600, Brazil), according to the methodology of Giusti & Wrolstad [22]. Mean values were the average of three replicates.

2.3 Films Preparation

Film forming solutions were prepared by mixing 4 g of corn starch, glycerol (30 wt% based on corn starch mass) and the residues in powder (0.5 g of different fractions of jaboticaba and blueberry) in 100 mL of distilled water. The solutions were submitted to heating in a water bath at 90°C for 35 minutes under mechanical stirring. Then, solutions were casted into Petri dishes (0.34 g cm^{-2}) and dried in a food dehydrator (DeLeo, A5AFD/0915, Brazil) with forced air convection for 24 h at 35°C. The obtained films were easily removed from Petri dishes and conditioned at 25°C and 55% relative humidity for at least 48 hours before testing.

The films were designated as: JAB-containing the jaboticaba powder fraction retained in the sieve (100 *mesh*); JAB100-containing the jaboticaba powder fraction that passed through the sieve (100 *mesh*); BLUB-containing the blueberry powder fraction retained in the sieve (100 *mesh*) and BLUB100-containing the blueberry powder fraction that passed through the sieve (100 *mesh*).

2.4 Colorimetric Test to Evaluate the Use as Intelligent Packaging

The film samples were submerged (pieces of 3 × 2 cm) in commercial buffer solutions (Dinâmica, Brazil) with different pH values to evaluate their potential as colorimetric indicator. Each strip was submerged in a buffer solution with a different pH value (ranging between 2 and 12). Although the samples exhibited an instantaneous color change (visually perceived), they remained submerged in the buffer solutions for up to 20 minutes in order to ensure no further color change. The colorimetric measurements were performed in duplicate for each sample.

2.5 Statistical Analysis

The Tukey test analysis was performed on STATISTICA 8.0 software (Statsoft Inc., Tulsa, USA) at a 5 % level of significance ($p < 0.05$).

3 Results and Discussion

3.1 Residue Characterization

The particle size, measured by laser diffraction, of jaboticaba and blueberry fractions were presented in Tab. 1. The average diameter of the blueberry and jaboticaba fraction that passed through the sieve (100 *mesh*) was 87.2 μm and 104.9 μm, respectively and the average diameter of the blueberry and jaboticaba fraction retained in the sieve was 310.7 μm and 386.3 μm, respectively.

The results of the total anthocyanin content measured by the differential pH method indicated that the residues fractions that passed through the sieve contain more anthocyanin, regardless residue source; values of 3.7 ± 0.2 and 17.5 ± 0.5 mg of anthocyanin per g of dry bagasse were quantified for jaboticaba (JAB100) and blueberry (BLUB100), respectively. Whereas the residual fractions retained in the sieve presented lower total content of anthocyanins: 2.8 ± 0.1 and 14.2 ± 1.6 mg of anthocyanin per g of dry bagasse for jaboticaba (JAB) and blueberry (BLUB), respectively. This result probably occurs because there are more compounds that can interfere in the anthocyanin extraction in the fraction retained in the

sieve. In addition, the particles retained in the sieve are larger (with lower surface area), limiting the contact between particle residue and the extracting solvent.

Similar total anthocyanin content values for different fractions of jaboticaba in powder, obtained as a co-product of juice extraction, were displayed by Gurak et al. [20]: 1.7, 5.1 and 5.7 mg per g of dry whole fruit, jaboticaba peel and jaboticaba pomace, respectively.

The total anthocyanin content values for blueberry bagasse found in the present work were similar that obtained by other investigators. Paes et al. [23] analyzed the anthocyanin content in blueberry residue after freeze-drying and found approximately 16.5 mg per g of dry bagasse. Reque et al. [13] and Khanal et al. [24] found values ranging from 10 to 17 mg of anthocyanins per g of dry bagasse.

Table 1: Particle size analysis and total anthocyanin content of jaboticaba and blueberry residue powder

	Particle size analysis					**Total anthocyanin content**
Residue	**D(0.1) (μm)**	**D(0.5) (μm)**	**D(0.9) (μm)**	**Average Diameter (μm)**	**SPAN**	(mg of anthocyanins per g of dry bagasse**)**
JAB	85.5	393.2	631.3	386.3	1.4	2.8 ± 0.1
JAB100	8.4	66.3	258.6	104.9	3.8	3.7 ± 0.2
BLUB	36.8	304.5	566.8	310.7	1.7	14.2 ± 1.6
BLUB100	11.1	60.2	192.3	87.2	3.0	17.5 ± 0.5

**JAB-fraction of jaboticaba residue powder retained in the sieve of 100 *mesh*; JAB100-fraction of jaboticaba residue powder that passed through the sieve of 100 *mesh*; BLUB-fraction of blueberry residue powder retained in the sieve of 100 *mesh*; BLUB100-fraction of blueberry residue powder that passed through the sieve of 100 *mesh*.

*D(0.1): particle diameter corresponding to 10 % of the cumulative distribution (μm); D(0.5): particle diameter corresponding to 50 % of the cumulative distribution (μm); D(0.9): particle diameter corresponding to 90 % cumulative distribution (μm); average diameter: average diameter by volume (μm); SPAN = measurement of particle size dispersion.

3.2 Film Characterization and Colorimetric Test Results

At macroscopic scale, the film samples containing the fractions retained in the sieve presented a more heterogeneous structure in comparison with the films produced using the fractions that passed in the sieve, regardless the residue type. All films were flexible and easy to handle. Film thickness values (mean ± standard deviation) were 0.24 ± 0.02 mm, 0.18 ± 0.02 mm, 0.22 ± 0.02 mm and 0.18 ± 0.03 mm, for JAB, JAB100, BLUB and BLUB100, respectively.

In Fig. 3, it was possible to verify that JAB100 and JAB presented $\Delta E^* = 8.3$ while BLUB100 and BLUB presented $\Delta E^* = 4.7$. The total color differences (ΔE^* values) between the samples previously to immersion in buffer solutions (with different pH values) can be confirmed by the photographs displayed in Fig. 3. The ΔE^* values demonstrate that there were visually perceptible differences to the human eye between the samples containing different fractions of the agroindustrial residues, since the ΔE^* values were greater than 3.0 [25-26].

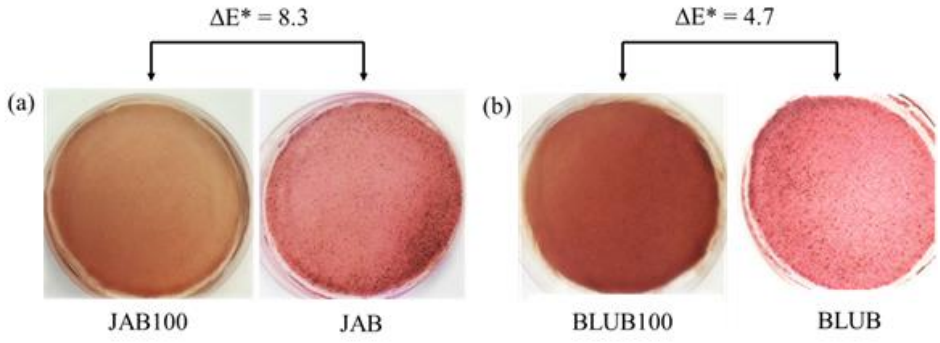

Figure 3: Visual aspect and total color difference values between the corn starch based films: (a) JAB100 and JAB (ΔE* = 8.3) and (b) BLUB100 and BLUB (ΔE* = 4.7)

The colorimetric results (Fig. 3) can be corroborated by the total anthocyanin content in the different fractions of the jaboticaba and blueberry residues. The fractions that passed through the sieve (JAB100 and BLUB100) presented a more intense reddish coloration due to the higher anthocyanin content in comparison with the fractions retained in the sieve (JAB and BLUB).

In Fig. 4 are displayed the visual aspect and colorimetric analysis results using the CIELab* color scale [27] found in the color change tests. These analyses were performed in order to verify the influence of the jaboticaba and blueberry residue fractions on the film color after immersion in buffer solutions with different pH values. It is important to emphasize that after 20 min of immersion in buffer solutions, all samples maintained their structural integrity. Furthermore, regardless of the residue source added, the films presented visual color changes when subjected to different pH values, showing reddish color when immersed in acid pH values and bluish color at basic pH values.

The total color difference (ΔE*) was calculate using as standard the initial sample, that is the color sample measured previously the films immersion in buffer solutions and the results are presented in Fig. 4. In Fig. 4(a) are displayed the films containing the jaboticaba residue fraction that passed through the sieve (JAB100) and in Fig. 4(b), the films containing the jaboticaba residue fraction retained in the sieve (JAB). In Fig. 4(c) are displayed the films containing the blueberry residue fraction that passed through the sieve (BLUB100) and in Fig. 4(d), the films containing the blueberry residue fraction retained in the sieve (BLUB).

Corn starch based films containing blueberry residue without and with previous fruit bleaching, produced by Luchese et al. [21], presented similar color alteration after being submitted to different pH values. Luchese et al. [28] also displayed similar results of color modification in native and modified *pinhão* starch incorporated with jaboticaba residue after colorimetric tests. Similar color variation results were also found by Zhai et al. [18] and Choi et al. [17] using roselle and sweet potato anthocyanins extracts in starch/PVA (polyvinyl acetate) based films and potato starch/agar based films, respectively.

The films containing the jaboticaba and blueberry powder with larger particle size, i.e., those powder fractions that were retained in the sieve of 100 mesh, demonstrated a more pronounced color change. However, it was not possible to directly correlate this result with the total anthocyanins content present in the agroindustrial residues, since the jaboticaba powder of higher grain size originally presented lower anthocyanin content in the matrix (2.8 ± 0.1 mg of anthocyanins per g of dry bagasse). Likewise, the blueberry powder fraction retained in the sieve of 100 mesh also presented lower total anthocyanins content (14.2 ± 1.6 mg of anthocyanins per g of dry bagasse) in comparison with the blueberry powder fraction that passed through the sieve. This result indicates that the initial anthocyanins content may not be the only factor that determines the color change, since the interaction between the filmogenic starch matrix and the agroindustrial residues could also influence this color modification.

The films produced with blueberry residue powder had higher values of total color difference (ΔE*) when compared to that manufactured using jaboticaba residue powder, probably due to the higher total anthocyanins content in the blueberry residue (almost 4 times).

Another aspect that should be considered is the particle size of the fraction that passed through the sieve of 100 *mesh*, since it has a smaller size and, therefore, greater surface area exposed during the manufacturing and drying film process. Thereby, the anthocyanins may have been more easily degraded during these steps. For this reason, less intense color difference after the immersion test in buffer solutions have been detected.

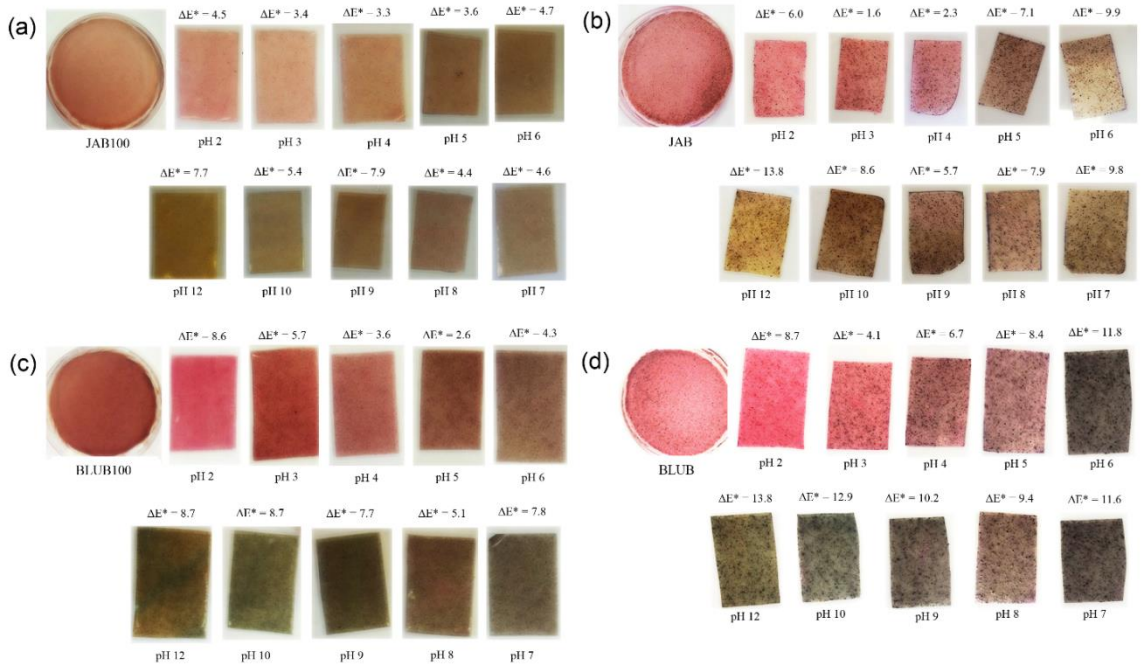

Figure 4: Visual aspect and total color difference after immersion in different pH values between the corn starch based films: (a) JAB100 (jaboticaba residue fraction that passed through the sieve of 100 mesh), (b) JAB (jaboticaba residue fraction retained in the sieve of 100 mesh), (c) BLUB100 (blueberry residue fraction that passed through the sieve of 100 mesh), and (d) BLUB (blueberry residue fraction retained in the sieve of 100 mesh)

4 Conclusion

Colorimetric changes in corn starch films containing jaboticaba and blueberry agroindustrial residues were visually perceptible to the human eye after immersion in buffer solutions with different pH values. In general, greater particle sizes had minor anthocyanin content. All the residues tested were efficient to act as a colorimetric indicator (samples presented ΔE* values higher than 5). Nevertheless the films with greater residue particle size were more efficient as a colorimetric indicator (higher values of ΔE*) although of its minor anthocyanin content. Therefore, anthocyanin compounds from the residue of jaboticaba and blueberry juice production exhibited a high potential to be incorporated during starch-based films fabrication, aiming the development of intelligent packaging. However, although the samples evaluated in the present work provided qualitative information about color changes to the external environment, more tests should be performed to correlate these results with the shelf life of food products.

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