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Microcapsules of 'jabuticaba' byproduct: Storage stability and application in gelatin

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ABSTRACT
This study aimed to produce microcapsules using 'jabuticaba' byproduct through lyophilization, and evaluate the stability of the powder, intended for use in gelatin. Three formulations were prepared, including the pure lyophilized extract (E), capsule with maltodextrin (CapM), and capsule with maltodextrin and xanthan gum (CapMG). The storage stability was evaluated at 4 and 25 ºC, and in the presence and absence of light, regarding color parameters, phenolic compounds and monomeric anthocyanins. The microencapsulated powders were applied in gelatin and analyzed for sensorial acceptance. Following an investigation of the storage stability of the three powders at 4 °C for 60 days, the sample that presented the best result was then analyzed at 25 ºC in the presence/absence of light. Microcapsules, independent of the encapsulant agent, exhibited lower values of degradation (2.82% phenolic compounds and 14.5% monomeric anthocyanins) than extract E when exposed to light and at 25 °C. Regarding color stability, CapMG showed smaller variations in the total color difference (ΔE) than the other samples. The lowest ΔE value corresponded to the gelatin sample with CapM, stored in the absence of light. It is possible to conclude that microencapsulation is an alternative for the preservation of 'jabuticaba' byproduct extracts, facilitating their use, retaining some compounds and protecting them against adverse conditions.

Palavras-chave:
Myrtaceae
compostos bioativos
liofilização
maltodextrina
goma xantana

Estabilidade de subproduto de jabuticaba armazenada em microcápsulas e com aplicação de gelatina

RESUMO
Objetivou-se neste estudo produzir microcápsulas usando o subproduto do processamento de jabuticaba (casca) através da liofilização e avaliar a estabilidade do pó, visando sua aplicação em gelatina. Obteve-se diferentes amostras: extrato puro liofilizado (E), cápsula com maltodextrina (CapM) e cápsula com maltodextrina e goma xantana (CapMG). A estabilidade foi avaliada ao longo do armazenamento a 4 e 25 ºC, com ausência e presença de luz, para parâmetros de cor, compostos fenólicos (CF) e antocianinas monoméricas (AM). Os pós microencapsulados foram aplicados em gelatina e analisou-se a aceitação sensorial em duas etapas para a estabilidade, no primeiro os três pós foram armazenados a 4 °C por 60 dias e no segundo, a amostra que apresentou o melhor resultado foi analisada em relação à presença/ausência de luz. As microcápsulas, independentemente do agente encapsulante, apresentaram menores valores de degradação (CF 2,82% e AM 14,5%) em relação ao extrato E quando expostos à luz e a 25 °C. Em relação à estabilidade da cor, o CapMG apresentou menores variações de ΔE. A menor diferença de cor (ΔE) foi encontrada na amostra de gelatina com CapM na ausência de luz. É possível concluir que a microencapsulação é uma alternativa para preservar extratos de subproduto de jabuticaba, facilitando seu uso, mantendo seus compostos e protegendo contra condições adversas.

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Introduction

Fruit and fruit byproduct consumption has increased over the years. Important research has been done, showing the benefits of berries against cardiovascular disease, obesity, urinary tract infection and renal diseases (Leite-Legatti et al., 2012; Silva et al., 2017).

'Jabuticaba' (Myrtaceae family) is included in the Brazilian berry group. It has beneficial effects on health and a high potential as a functional food. Studies have revealed the importance of antioxidant activity, mainly attributed to flavonoids and anthocyanins, which are mostly present in the byproduct (skin and seeds) (Reynertson et al., 2006; Leite et al., 2011).

Considering that phenolic compounds and anthocyanins are unstable in the presence of light, oxygen and high temperatures (Fang & Bhandari, 2011; Rutz et al., 2013), it is valuable to evaluate alternative methods for increasing the stability of such bioactive compounds against adverse conditions during processing and storage. Microencapsulation (ME) of the bioactive can provide its controlled release, thereby extending the products shelf life (Favaro-Trindade et al., 2008; Mahdavi et al., 2016). Nonetheless, this process presents technological challenges, especially for the ME of sensitive bioactive compounds in the food industry (Paulo & Santos, 2017).

Spray drying and lyophilization are the most common encapsulation techniques (Wilkowska et al., 2016). Both processes generate a powder that can be readily packaged, stored, commercialized and handled. Besides, the low water activity of the powdered product prevents the growth of microorganisms and the occurrence of biochemical reactions (Silva et al., 2014).

A mixture of encapsulating agents may result in increased ME efficiency and capsule stability (Mahdavi et al., 2016). Maltodextrins are widely used in ME because of their high solubility in water, low viscosity and because they are colorless solutions (Ahmed et al., 2010; Robert et al., 2010; Silva et al., 2010). Thus, ME of the aqueous extract of 'jabuticaba' byproduct emerges as an alternative to facilitate the use of this fraction of the fruit, for its functional properties.

The objectives of this study were to obtain microcapsules from 'jabuticaba' byproduct aqueous extract by lyophilization, evaluate the stability of the microcapsules under different temperature and light exposure conditions when applied in gelatin, and assess the sensorial acceptance and color stability of the product.

Material and Methods

The residue of the processing of 'jabuticaba' pulp (composed by skin) was purchased from one batch of Sítio do Bello native fruits, Bairro Paraibuna, SP, Brazil (23° 27` 53.94"), harvested in December 2015. Maltodextrin (DE 10) was supplied by Cargill (SP, Brazil) and xanthan gum was procured from a local commerce in Maringá, PR, Brazil. All reagents used in the study were of analytical grade.

In order to obtain the aqueous extract, 'jabuticaba' byproducts were thawed and weighed in the ratio of 1:2 (w/v) skin:water, defined by preliminary tests. The solution was placed in a water bath until the temperature reached 80 °C, with constant stirring for 45 min, according to preliminary results obtained in an experimental design. The solution was filtered, and the total solid content of the final solution was measured.

The appropriate amount of the encapsulating agents was added at a 1:1 (w/w) agent:extract ratio, as described by Ferrari et al. (2012). Different solutions were prepared to contain only maltodextrin (100%) and the other containing maltodextrin (99.5%) and xanthan gum (0.5%). The mixtures were magnetically stirred for 20 min and frozen for 48 h at -10 °C, before lyophilization.

Samples were lyophilized (L108, Lyova) for approximately 30 h. The pure lyophilized extract (E), used as the control sample, was obtained under the same conditions as the samples. The final products were in the form of a dry powder and were stored in thermo-weldable plastic packages, for future analysis. The experiments were performed in triplicate for the three different samples: pure lyophilized extract (E), capsule with maltodextrin (CapM), and capsule with maltodextrin and xanthan gum (CapMG).

The microcapsules were stored in a temperature-controlled chamber under different light and temperature conditions, following Rutz et al. (2013) with modifications. Some were stored in the dark and others were exposed to the presence of two 20 W fluorescent lamps, as an artificial light source, both at 25 °C. According to preliminary tests for temperature stability, the samples were monitored at 4 and 25 °C for 36 days, for color parameters, phenolic compounds and monomeric anthocyanins.

The powder color was measured using a Minolta® CR400 portable colorimeter, with integration sphere and view angle of 3º, that is, d/3 illumination and illuminant D65. The CIELAB three-dimensional color space system was used. In this color model, L* indicates the luminosity on a scale from 0 (black) to 100 (white); a* represents a scale of shade ranging from red (positive a*) to green (negative a*) and b* represents a scale from yellow (positive b*) to blue (negative b*). The color difference (ΔE) was calculated according to Eq. 1 below, following the method detailed by da Silva et al. (2017):

$$\Delta E = \sqrt{\left(L \cdot day_x - L \cdot day_0\right)^2 + \left(a \cdot day_x - a \cdot day_0\right)^2 + \left(b \cdot day_x - b \cdot day_0\right)^2} \quad (1)$$

where:

L, a*, and b* - color parameters;

day_0 - initial; and,

day_x - 9, 18, 27 and 36 days of storage, respectively.

The total phenolic content was determined by the Folin-Ciocalteu spectrophotometric method (Singleton & Rossi, 1965). Measurements were performed in triplicate, using a calibration curve of gallic acid as the standard. The absorbance readings were acquired using a spectrophotometer at 725 nm, and the results were expressed as milligrams of gallic acid equivalents per gram of dry sample (mg GAE g^{-1}).

Total monomeric anthocyanins were determined using the differential pH method proposed by the Association of Analytical Chemists' (AOAC, 2005). Two buffer systems were

used, including 0.025 mol L^{-1} potassium chloride (KCl) and 0.4 mol L^{-1} sodium acetate buffer (CH_3COONa), and the pH values were corrected to 1.0 and 4.5 with hydrochloric acid (HCl). In test tubes containing 2.0 mL of each buffer solution, 0.5 mL of sample was added. After 20 min, the spectrophotometric absorbances were read at wavelengths of 520 and 700 nm. The results were expressed as milligrams of cyanidin-3-glucoside per gram of dry sample (mg cyanidin-3-glucoside g^{-1}), using the cyanidin molar mass of 449.2 g mol^{-1} and a molar absorptivity of 26900 L mol^{-1} (Giusti & Wrolstad, 2001).

The percentage of total loss (loss %) of anthocyanins and phenolic compounds in the storage period was calculated by dividing the amount at the last storage day (d_{36}), by the initial quantity (d_0), as mentioned in Souza et al. (2014).

The microcapsules were applied in a colorless powdered gelatin (Dr. Oerker®). Samples of pure gelatin were pre-solubilized in water according to the manufacturer's recommendations. Next, the powder samples were added directly into the gelatin solutions and shaken manually until complete dissolution. To determine the amount of microcapsule to be used, preliminary tests were performed with proportions between 0.5 and 2.9%. Based on the results, 1.8% of the capsule was used in 98.2% dissolved gelatin. Afterward, samples were poured into Petri dishes and stored at 4 °C until analysed. This stage was divided into two tests. First, the gelatin samples combined with E, CapM and CapMG were refrigerated (4 °C) for 60 days, and the color parameters were evaluated at 1, 15, 30, 45 and 60 days. Then, the gelatin sample that presented the best results was stored in the presence of light (two 20 W fluorescent lamps as an artificial light source) and the dark, respectively, at 20 ± 1 °C. During 60 h of storage, the color parameters were observed, as a measure of stability. The assay and readings were performed in duplicate. The results were expressed as a function of the ΔE value (see Eq. 1).

Sensorial acceptance was evaluated after the gelatin production (d_1), using the nine-point hedonic scale test (1 = dislike extremely; 5 = indifferent, and 9 = like extremely) to assess the overall appearance. The trial involved a single session of 100 non-trained tasters. Each taster received (in individual cabins) the gelatin with microcapsules (GCapM and GCapMG, respectively) simultaneously. The samples were encoded with three digits and given to the tasters in a random order, along with a glass of water at room temperature to rinse the mouth between samples.

All tests were performed in replicate and the analysis in triplicate. Statistical difference was obtained through analysis of variance (ANOVA) and Tukey's test at 0.05 significance level, by using Sisvar 5.0.

Results and Discussion

Among the fractions of 'jabuticaba', it is known that the byproduct (skins) concentrates most of the bioactive compounds. Among those, anthocyanins are responsible for the red-purple appearance (Lima et al., 2011).

For color and bioactive compounds, samples were appraised during 36 d, under two different storage conditions: temperature (4 and 25 °C) and presence/absence of light at 25 °C (Table 1). After light exposure, the extract (E) presented anthocyanin degradation of 24.0 to 34.0%. In comparison, the microcapsules exhibited significantly less degradation (values between 5.45 and 14.85%) ($p < 0.05$).

It is probable that the use of encapsulated agents (CapM and CapMG) delayed the deterioration of the anthocyanins by protecting the bioactives from the adverse environmental conditions. Most anthocyanin degradation occurred at 25 versus 4 °C. At 25 °C, extract E showed a reduction of 33.85%, CapM 10.62% and CapMG 5.66%, whereas at 4 °C, the corresponding values were relatively lower, varying from 0 to 2.33%. Similarly, da Silva et al. (2017) observed the same trend when a liquid extract of 'jabuticaba' byproduct was exposed

Table 1. Total phenolic compounds (TPC) and total monomeric anthocyanins (TMA) during 36 days of storage in the presence/absence of light, and at 4 and 25 °C (in the dark)

Storage time (d)	E	CapM	CapMG	E	CapM	CapMG
	TMA (mg cyanidin-3-glucoside g^{-1})					
	Light			No light		
1	3.09 a ± 0.11	2.57 b ± 0.21	2.76[b] ± 0.15	3.22 a ± 0.15	2.73 b ± 0.01	2.65 b ± 0.12
18	2.66 a ± 0.07	2.42 a ± 0.10	2.56 a ± 0.16	2.88 a ± 0.20	2.35 a ± 0.05	2.66 a ± 0.02
36	2.36 a ± 0.18	2.43 a ± 0.03	2.35 a ± 0.04	2.72 a ± 0.10	2.44 a ± 0.12	2.50 a ± 0.06
(loss %)	24.00	5.45	14.85	15.53	10.62	5.67
	25 °C			4 °C		
1	3.22 a ± 0.15	2.73 b ± 0.01	2.65 b ± 0.12	3.43 a ± 0.12	2.69 b ± 0.12	2.75 b ± 0.10
18	2.88 a ± 0.20	2.35 a ± 0.05	2.36 a ± 0.02	3.34 b ± 0.10	2.69 a ± 0.05	2.34 a ± 0.04
36	2.13 a ± 0.10	2.44 a ± 0.12	2.50 a ± 0.06	3.35 b ± 0.10	2.64 a ± 0.11	2.75 a ± 0.08
(loss %)	33.85	10.62	5.66	2.33	1.86	ND
	TPC (mg GAE g^{-1} product)					
	Light			No light		
1	125.40 a ± 1.08	55.51 b ± 0.08	54.22 b ± 1.87	115.4 a ± 1.08	52.44 b ± 1.24	54.69 b ± 1.50
18	103.10 a ± 1.70	46.00 b ± 2.71	45.36 b ± 1.58	120.5 a ± 1.89	47.60 b ± 1.51	39.29 b ± 0.94
36	114.70 a ± 3.76	53.90 b ± 0.49	46.76 b ± 0.91	95.11 a ± 1.20	50.21 b ± 0.29	48.27 b ± 0.83
(loss %)	8.53	2.90	13.76	17.58	4.25	11.73
	25 °C			4 °C		
1	115.4 a ± 1.08	52.44 b ± 1.24	54.69 b ± 1.50	89.79 a ± 1.74	40.22 b ± 2.23	45.09 b ± 1.02
18	120.5 a ± 1.89	47.60 b ± 1.52	39.29 b ± 0.94	104.7 a ± 2.07	47.60 b ± 1.13	43.16 b ± 1.13
36	95.11 a ± 1.20	50.21 b ± 0.29	48.27 b ± 0.83	137.2 a ± 4.34	51.33 b ± 0.67	48.81 b ± 0.79
(loss %)	17.58	4.25	11.74	ND	ND	ND

Mean values ± standard deviation (n = 3 replicates); Different letters in the same line indicate statistical difference between samples ($p < 0.05$). Time difference was evaluated by % of the loss. ND – Not detected; GAE – Gallic acid equivalents

to 4-10 and 23-25 °C, respectively, whereby the monomeric anthocyanin content was lower at 25 °C at 48 and 72 h.

The storage conditions affected the content of phenolic compounds in the samples (Table 1). The major difference was between the pure extract (E) and the microcapsules (CapM and CapMG). Alves et al. (2014) evaluated the stability of 'jabuticaba' byproduct flour at room temperature and noticed a decrease in anthocyanin content. Santos et al. (2013) investigated the use of supercritical CO_2 to encapsulate 'jabuticaba' byproduct extract, and the stability of the encapsulated anthocyanins was monitored for 14 days. Light and temperature affected the free extract more than the systems encapsulated by a conventional method and, also, when the extract was isolated by rapid supercritical extraction.

In the current study, the extract (E) presented a higher degradation of phenolic compounds (around 8.5%) in the presence than absence of light and at 25 °C (17.6%) compared to refrigeration. The lowest degradation values corresponded to the capsule with maltodextrin (CapM) in the presence/absence of light (2.9 to 4.2%), while the CapMG samples showed degradations ranging from 11.7 to 13.7%. Rutz et al. (2013) mentioned that xanthan-coated microparticles exhibited less antioxidant activity when stored at 25 than 4 °C for 84 days.

Powders stored in the dark at 4 °C did not suffer phenolic compound degradation during 1–36 days. In contrast to their findings, Rutz et al. (2013) stated that low temperatures provide greater stability to phenolic compounds. The discrepancy was considered to be due to factors that were not analyzed, such as the relative humidity of the environment. Herein, some phenolic values increased during storage, which may have occurred due to the degradation of other compounds present in the sample (Silva et al., 2017).

The color change during storage for 36 days was studied in the presence and absence of light at 25 °C (Figure 1A) and at different temperatures in the dark (Figure 1B). The ΔE parameter was used to describe the color variation relative to the control (d_0).

According to Obón et al. (2009), color changes can be measured as the modulus of by the distance vector between the initial color values and the actual color coordinates in the three-dimensional CIELAB color space (ΔE). Also, differences in perceivable color can be categorized as small (ΔE < 1.5), distinct (1.5 < ΔE < 3) and highly distinct (ΔE > 3). Although, Obón et al. (2009) considered color variation is only evident for ΔE > 5.

At 36 day of storage (Figure 1A), the lowest color changes observed were for CapMG microcapsules under both storage conditions, with and without light. Extract (E) presented a high color variation, irrespective of the evaluated storage parameters. Silva et al. (2013) emphasized that low ΔE values are largely desirable for the overall color variation, as it indicates that the powdered pigment retains the color of the original extract after reconstitution.

The powders were stored in the dark and at two different temperatures (4 and 25 °C). At 36 days (Figure 1B), the extract (E) at 25 °C presented the highest color variation, and CapMG microcapsule at 4 and 25 °C had the least. Thus, CapMG was considered the best formulation because it offered the smallest color variation under the three different storage conditions.

In order to observe sample had the lowest color variation (ΔE) during long-term storage (60 days), colorless gelatin

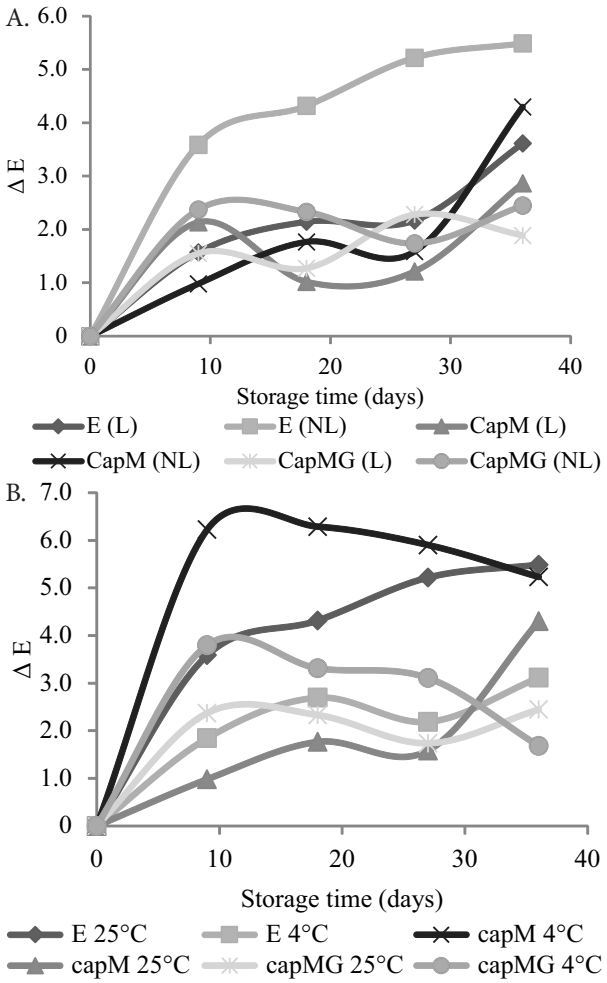

Figure 1. (A) Powder color variation (ΔE) during 36 days of storage at 25 °C in the presence (L)/absence (NL) of light. (B) Powder color variation (ΔE) during 36 days of storage in the dark at 4 and 25 °C

was used as the matrix for the application of the powders (E, CapM and CapMG). Notably (Figure 2A), the extract (E) demonstrated the greatest color variation, whereas, contrastingly, the incorporation of the capsules with gelatin had the lowest change. Little difference was observed between CapM and CapMG, indicating that the type of encapsulating agent had no marked influence on color. CapM was chosen to be incorporated in gelatin, as it is not only the cheapest formulation, using just one encapsulating agent, it maintained ΔE < 5, which is a desirable factor for food applications (Obón et al., 2009). Figure 2B presents the color stability behavior of CapM in the presence/absence of light for 60 h.

Figure 2B indicates that the gelatin CapM (GCapM) sample had a greater color variation during 60 h when stored in the presence than absence of light. It is important to emphasize that although storage at 25 ºC resulted in the greatest color difference, it remained below ΔE of 5. Therefore, when applied in a food matrix, it is recommended that the product is appropriately packaged to eliminate light exposure. This variation of color may be correlated to the levels of bioactive compounds present, in which degradation or alteration of pigments may indicate changes in phenolic compounds (Zhang et al., 2008).

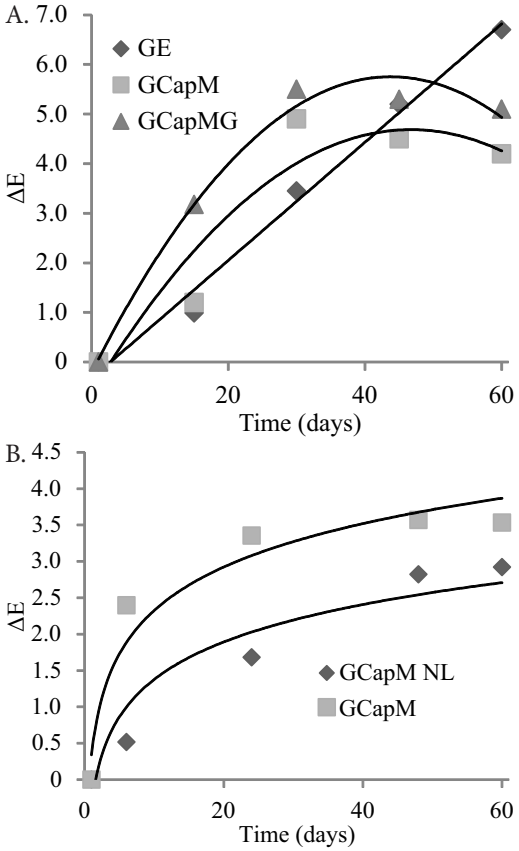

Figure 2. (A) Gelatin color variation (ΔE) for 60 days at 4 °C. (B) Color difference (ΔE) of gelatins with CapM in presence of light (GCapM L) and in dark (with no light) (GCapM NL), during 60 h at 20 °C

Regarding the sensorial evaluation, the overall acceptance presented no significant differences ($p < 0.05$), obtaining scores of 5.48 for GCapM and 5.25 for GCapMG. Theses scores represented a satisfactory result, considering flavoring agents were not incorporated in the formulation, which was intentionally done to evaluate if the microcapsules influenced this parameter.

Conclusions

1. Overall, the presence or absence of light negatively impacted the extract (E), regarding both its color and bioactive compounds, whereas the microcapsules exhibited less degradation, regardless of the type of encapsulant used. Storage at 4 °C for 36 days, had a positive impact on all stability parameters evaluated.

2. The color stability of the microcapsule with maltodextrin (CapM) improved when incorporated into a gelatin matrix, and it was particularly stable when stored under darkness.

3. The microencapsulation by lyophilization can be indicated as a method for preserving bioactive compounds of the aqueous extract of 'jabuticaba', enabling the use of the byproduct as an antioxidant source.

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