Blackberry pomace microspheres: An approach on anthocyanin degradation

Microesferas de bagaço de amora-preta: Uma abordagem sobre a degradação de antocianina

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ABSTRACT
Blackberry pomace is a rich source of antioxidant compounds, including anthocyanins, but these compounds degrade easily in the presence of high temperatures. Therefore, the present study aimed to evaluate the effect of temperature on anthocyanin degradation in spray-dried blackberry pomace extract. Maltodextrin was used as a drying aid agent in a spray drying process to produce microspheres. The experiment was set up at Maringá-PR, Brazil (23º 25' 31" S, 51º 56' 19" W, 596 m altitude). The thermal stability of anthocyanins was evaluated in the presence and absence of copigments at different temperatures ranging from 70 °C to 100 °C using degradation kinetics. The role of maltodextrin in protecting anthocyanins during the spray drying process was studied at high temperatures. The highest anthocyanin stability was found at 70 °C. We also studied the effect of copigment phytic acid on the stability of anthocyanin and found that the copigment plays an important role in anthocyanin protection at high temperatures. The spray drying process with maltodextrin is a feasible technique for the preservation of food products and can improve anthocyanin's thermal stability. The reuse of industrial wastes, such as blackberry pomace along with preservation techniques, can be a good strategy to reduce their negative impact on the environment.

Index terms: Rubus fruticosus; antioxidant analyses; spray dryer; kinetics.

INTRODUCTION
Fruit and vegetable residues have been recognized as a rich source of bioactive compounds. Thus, the use of agro-industrial residues such as blackberry pomace can be feasible for the development of functional foods. Several studies have highlighted that fruit processing produces about 20% to 60% of byproducts such as peels, seeds, stems, and pulp that can be used to extract bioactive compounds (Machado et al., 2018).

The research on the blackberry (Rubus fruticosus) composition has revealed that the typical red color of the fruit is because of anthocyanins, mainly, including cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside. Besides anthocyanins, the fruit also contains flavonoids,
Some studies have shown that anthocyanins are less stable at a high temperature. Heat treatment can negatively affect the stability of anthocyanins. Therefore, the study of an alternative technique that can increase anthocyanin stability would be significant. Thermal degradation of anthocyanins results in their color loss and appearance of brown compounds, possibly because of chalcone formation followed by loss of glycosyl moieties and formation of α-diketone compounds (Maciel et al., 2018; Pedro; Granato; Rosso, 2016; Reyes; Cisneros-Zevallos, 2007). The flavylum nucleus of anthocyanins can provide a number of colors because of its interaction with copigments existing in an aqueous medium. Van der Waals forces and hydrophobic interactions result in a strong association between the anthocyanin molecules and copigment (Maciel et al., 2018; Pedro; Granato; Rosso, 2016; Grajeda-Iglesias et al., 2016).

The use of maltodextrin as a drying aid agent can be used to increase the stability of bioactive compounds when applied to food products (Rezende; Nogueira; Narain, 2018; Ye; Georges; Selomulya, 2018). A spray drying technique can improve the shelf-life of food and food ingredients. The technique is commonly used in the food industry because of its simplicity, flexibility, and economical feasibility, along with its application in drying bioactive compounds. The technique transforms a liquid solution, suspension, or emulsion into a dried particle (Lisboa; Duarte; Cavalcanti-Mata, 2018; Ramos et al., 2019; Salminen et al., 2019).

The objective of this study was to produce and characterize microspheres from blackberry pomace extract, and evaluate the effect of storage (or processing) temperature on anthocyanin degradation.

**MATERIAL AND METHODS**

**Materials**

Blackberry (*Rubus fruticosus*) pomace was purchased from the producer of Paraibuna-SP, Brazil (23° 25' 31" S, 51° 56' 19" W, 596 m altitude) from a single batch and was frozen (−18 °C) until use. Maltodextrin (M) DE10 was provided by Cargil® (Campinas-SP). Other reagents were of analytical grade and purchased from Sigma Aldrich®.

**Samples preparation and encapsulation of bioactive compounds**

The experiment was set up at Maringá-PR, Brazil (23° 25’ 31” S, 51° 56’ 19” W, 596 m altitude).

Initially, the blackberry pomace (BP) was defrosted. Considering the results of a preliminary experiment, the BP was diluted with water up to the concentration of 500 mg mL⁻¹. Bioactive compounds were extracted using an ultrasonic cleaner bath (Ultracleaner 1650 Unique, 40 kHz frequency, 120 Watts RMS power) for 45 min at the temperature-controlled manually at around 60 ± 3 °C. The variation was controlled by considering the increase in temperature caused by the ultrasound treatment (Santos et al., 2017a). The solution was filtered subsequently to obtain the blackberry pomace extract (BE).

The blackberry microspheres (BM) were prepared using maltodextrin (DE 10) as a drying aid agent. Maltodextrin was directly mixed with the extract in the ratio of 1:1 (w/w), by using mechanical agitation. The samples of BE and BM were dried in a spray dryer using the following conditions: Inlet drying air temperature 150 °C and outlet 110 °C, atomization pressure 0.08 to 0.14 bar, average drying airflow 3.5 m³ h⁻¹, average feed rate 0.3 L h⁻¹ in the LM-MSD 1.0 Mini Spray-dryer equipment (Santos et al., 2017a). The sample BP was frozen for 48 h at −10 °C and subsequently submitted to freeze-drying for two days to ensure complete drying (freeze L108, Liobras).

The dried samples of BP, BE, and BM were stored in plastic containers and kept frozen (-18 °C) for further analysis.

**Effect of temperature on copigmentation**

The effect of temperature on anthocyanin stability was evaluated for BE and BM samples in the presence and absence of copigments. Anthocyanin stability in the presence of copigment was evaluated using citrate buffer (pH 3.0) and copigment phytic acid (4.0 x 10⁻⁴ mol L⁻¹), whereas anthocyanin stability in the absence of copigment (control) was evaluated using only citrate buffer (pH 3.0) (Maciel et al., 2018).

The solutions of BE and BM with and without phytic acid were kept into a thermostatic bath (sensor PT-100 Nova Orgânica) at 70 °C, 80 °C, 90 °C, and 100 °C. The absorbance was recorded using a spectrophotometer at 513 nm at intervals of 30 min. Using the data, kinetic parameters such as rate constant (k) and half life time (t₁/₂) were mathematically calculated using Equations 1 and 2, respectively (Pedro; Granato; Rosso 2016).

\[
\ln \left(\frac{A_t}{A_0}\right) = -k \times t
\]  

(1)
Total phenolic compounds (TPC), total monomeric anthocyanins (TMA), and total flavonoids (TF)

Total phenolic compounds (TPC) analysis was carried out by using a reaction between Folin-Ciocalteu, sodium carbonate (Na₂CO₃), and sample. The reaction mixture was incubated for 30 min at 25 °C, and the absorbance was measured using a spectrophotometer at 725 nm (Pierpoint, 2004). Results were expressed as µg of gallic acid equivalent (GAE) per mg of dry matter.

Total monomeric anthocyanins (TMA) were determined using the differential pH method using reagents potassium chloride (KCl) and sodium acetate (C₂H₃NaO₂). The reaction mixture was incubated for 20 min at 25 °C, and the absorbance was measured using a spectrophotometer at 520 and 700 nm (Lee, Durst, and Wrolstad 2005). The results were expressed in µg of cyanidin-3-glucoside per mg of dry matter.

Total flavonoids (TF) analysis was performed using aluminum chloride (AlCl₃), sodium nitrite (NaNO₂), and sodium hydroxide (NaOH). The absorbance of the reaction mixture was measured immediately using a spectrophotometer at 510 nm (Alothman; Bhat; Karim, 2009). Results were expressed in µg of quercetin equivalent (QE) per mg of dry matter.

Antioxidant activity analysis

Reduction in the stable DPPH radicals concentration was measured by a spectrophotometric assay by using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) reagent. The reaction mixture was incubated for 1 h at 25 °C and absorbance was measured using a spectrophotometer at 515 nm (Thaipong et al., 2006).

Antioxidant activity was measured using the ABTS method with the help of the colorimetric assay. Reagents such as ABTS (2, 2’-AZINO-BIS (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt and potassium persulfate (K₂S₂O₈) were used for the reaction, and absorbance was measured using a spectrophotometer at 734 nm after 6 min of incubation at 25 °C (Nenadis et al., 2004).

Antioxidant activity was measured using the FRAP method by mixing the sample directly with distilled water and FRAP (Ferric Reducing Antioxidant Potential) reagent. The reaction mixture was maintained at 37 °C in a water bath for 30 min. The absorbance was measured using a spectrophotometer at 595 nm (Pulido; Bravo; Saura-Calixto 2000).

Results of all antioxidant analyses were expressed in µM of Trolox equivalent (TE) per mg of dry matter.

Colorimetric analysis

Colorimetric analysis of the samples was performed by using the portable Minolta® CR400 colorimeter. The system used was CIELAB (L*, a*, b*, C + and H°).

Morphological characterization by scanning electron microscopy (SEM)

The particle morphology of microspheres was characterized using a scanning electron microscope (JEOL model JSM-6060 LV). Metal support with a double-sided tape of carbon was used to fix the samples, which was covered with gold. The samples were visualized with the magnification of 250 to 10000 times using the excitation voltage of 12.5 kV.

Statistical analysis

All readings were subjected to statistical analysis using the analysis of variance and Tukey’s test with the p-value of <0.05. Statistical analyses were performed using the statistical program Sisvar 5.6. Calibration curves for the antioxidant analyses were plotted in the Graph Pad Prism 5 software.

RESULTS AND DISCUSSION

Antioxidant and color analyses

Table 1 shows the analyses of TPC, TMA, antioxidant, and color of BP, BE, and BM samples.

The values of TPC, TMA, TF, DPPH, ABTS, and FRAP were higher for sample BE compared to sample BP, corroborating with other similar studies (Santos et al., 2017a; Rodrigues et al., 2018). This could be due to the high temperatures used in the spray dryer, which can improve the antioxidant activity of samples by the formation of phenolic compounds resulting from the Maillard reaction, which generally exhibit high antioxidant properties (Murador et al., 2018; Pitalua et al., 2010).

A comparison between TMA values of samples BM and BE (Table 1) showed that maltodextrin protected anthocyanins during the drying process. Both samples showed similar values since the BM sample represented only 50% of the BE. BM sample, as expected, showed around 50% of the values for TPC, TF, DPPH, ABTS, and FRAP analyses compared to BE. Also, the spray drying technique had a great impact on encapsulation characteristics, including stability (Santos et al., 2019; Santos et al., 2017b).
Moreover, the present study reported higher TMA and TPC values of microspheres than those reported by other studies on microcapsules of blackberry pomace. One study reported 1.18 µg cyanidin-3-glucoside mg⁻¹ for TMA and 23.13 µg GAE mg⁻¹ for TPC (Santos et al., 2017b). Another study has reported 1.66 µg cyanidin-3-glucoside mg⁻¹ for TMA (Santos et al., 2019).

A comparison between the colorimetric values of BM and BE samples showed an increase in the value of L*. Probably because of the use of maltodextrin, the colorimetric intensity of the BM sample was lower than that of BE. A similar trend was observed for chromaticity (C). The C-value is the relative strength of any given color; the higher the C, greater is the saturation of colors that are perceptible to humans (Cuadros et al., 2020).

The value of a* (red intensity) for the BM sample was higher than that of the BE sample, indicating that maltodextrin encapsulation protected the coloring compounds in the sample during the drying process. The parameter H° is an angle specifying the hue of a color and is considered as the qualitative attribute of the color, which traditionally defines as reddish, greenish, etc. Graphically, 0° is attributed to red, 90° to yellow, 180° to green, and 270° to blue (Cuadros et al., 2020). Thus, the H° angle of sample BM (Table 1) was lower than that of sample BP and BE, indicating its proximity to red.

Table 1: Analyses of TPC, TMA, antioxidant, and color of BP, BE, and BM samples.

|        | BP   | BE   | BM   |
|--------|------|------|------|
| TPC¹   | 5.43 ± 0.73 | 54.08 ± 6.43 | 27.67 ± 2.91 |
| TMA²   | 0.46 ± 0.07  | 2.52 ± 0.12  | 2.50 ± 0.05  |
| TF³    | 18.02 ± 2.34 | 178.47 ± 27.42 | 85.48 ± 5.11 |
| DPPH⁴  | 2.27 ± 0.36  | 22.44 ± 0.72  | 11.41 ± 1.98 |
| ABTS⁵  | 36.70 ± 2.16 | 600.50 ± 8.13 | 246.90 ± 5.13 |
| FRAP⁶  | 150.69 ± 3.45 | 1773.16 ± 48.07 | 759.42 ± 30.60 |
| L*     | 26.29 ± 1.84 | 16.46 ± 1.75  | 41.77 ± 4.06 |
| a*     | 24.70 ± 0.98 | 26.91 ± 1.74  | 43.10 ± 0.87 |
| b*     | 12.25 ± 0.56 | 11.21 ± 0.36  | 15.01 ± 0.01 |
| C      | 27.58 ± 1.11 | 28.54 ± 0.72  | 45.65 ± 0.83 |
| H°     | 26.55 ± 0.29 | 23.14 ± 0.46  | 19.24 ± 0.30 |

Means followed by the same letters on line did not differ among themselves by Tukey's test (p<0.05). BP: blackberry pomace; BE: blackberry pomace extract (100% extract); BM: blackberry pomace microsphere (50% maltodextrin + 50% extract); Iµg GAE. mg⁻¹ of dry matter; ²µg cyanidin-3-glucoside.mg⁻¹ of dry matter; ³µg EQ.mg⁻¹ of dry matter; ⁴, ⁵ and ⁶µM TE.mg⁻¹ of dry matter. TPC: total phenolic compounds; TMA: total monomeric anthocyanins; TF: total flavonoids.

Means followed by the same letters on line did not differ among themselves by Tukey's test (p<0.05). BP: blackberry pomace; BE: blackberry pomace extract (100% extract); BM: blackberry pomace microsphere (50% maltodextrin + 50% extract); Iµg GAE. mg⁻¹ of dry matter; ²µg cyanidin-3-glucoside.mg⁻¹ of dry matter; ³µg EQ.mg⁻¹ of dry matter; ⁴, ⁵ and ⁶µM TE.mg⁻¹ of dry matter. TPC: total phenolic compounds; TMA: total monomeric anthocyanins; TF: total flavonoids.

Morphological characterization by scanning electron microscopy (SEM)

Figure 1 shows the morphologies of blackberry pomace samples characterized by a scanning electron microscopy.

BP samples dried by lyophilization (Figures 1A and 1B) showed morphology similar to broken glass. They were in the form of amorphous particles of different sizes with porosity due to the structural rigidity, characteristic of this drying technique (Ezhilarasi et al., 2013; Kuck; Noreña, 2016). On the other hand, BE samples (Figures 1C and 1D) showed amorphous nature and irregular shape compared to the BM samples (Figures 1E and 1F). Microspheres showed circular morphology with different sizes and porosity due to the structural rigidity, characteristic of this drying technique.

Effect of temperature in the presence and absence of copigment

Table 2 and Figure 2 show the effect of temperature and kinetic parameters involved in the stability of samples BE and BM in the absence and presence of
copigments. It was observed that the degradation of anthocyanins in samples BE and BM increased with an increase in the heating temperature and time. This linear relationship between the logarithm of the concentration of anthocyanins and time indicates the first-order kinetics for anthocyanin degradation (Sinela et al., 2017).

Figure 1: Samples (A) BP: blackberry pomace with an increase of 500x and (B) 1000x; (C) BE: blackberry pomace extract with an increase of 250x and (D) 500x; (E) BM: blackberry pomace microsphere with an increase of 2000x and (F) 10000x.
Table 2: Kinetic parameters of the effect of temperature on the stability of BE and BM in the absence and presence of copigments.

| Temperature (°C) | t₁/₂ (h) | k (min⁻¹) | t₁/₂ (h) | k (min⁻¹) |
|-----------------|----------|-----------|----------|-----------|
|                 | Blackberry extract (BE) | Blackberry microsphere (BM) | Blackberry extract (BE) | Blackberry microsphere (BM) |
| Control         |          |           |          |           |
| 70              | 12.27    | 9.42 x 10⁻⁴ (0.8809) | 16.57    | 6.97 x 10⁻⁴ (0.8235) |
| 80              | 7.72     | 1.50 x 10⁻³ (0.9388) | 8.20     | 1.41 x 10⁻³ (0.9156) |
| 90              | 3.86     | 2.99 x 10⁻³ (0.9831) | 4.39     | 2.63 x 10⁻³ (0.9643) |
| 100             | 1.85     | 6.23 x 10⁻³ (0.9864) | 1.90     | 6.07 x 10⁻³ (0.9820) |
| Phytic acid     |          |           |          |           |
| 70              | 12.42    | 9.30 x 10⁻⁴ (0.8458) | 16.94    | 6.82 x 10⁻⁴ (0.8025) |
| 80              | 7.91     | 1.46 x 10⁻³ (0.9340) | 8.65     | 1.34 x 10⁻³ (0.9000) |
| 90              | 4.19     | 2.76 x 10⁻³ (0.9880) | 4.50     | 2.57 x 10⁻³ (0.9717) |
| 100             | 2.21     | 5.23 x 10⁻³ (0.9805) | 2.33     | 4.95 x 10⁻³ (0.9832) |

*t₁/₂*: half-time life; *k*: rate constant of anthocyanins degradation; Coefficient of determination (*R²*) shown in parentheses.

Figure 2: Degradation kinetics of anthocyanins; (a) Control BE: blackberry extract; (b) Phytic acid BE: blackberry extract; (c) Control BM: blackberry microsphere; (d) Phytic acid BM: blackberry microsphere.

The highest half-life time (Table 2) was found at 70 °C, and *t₁/₂* for BE control and BE copigment were 12.27 and 12.42 h, respectively. On the other hand, *t₁/₂* for BM control and BM copigment were 16.57 and 16.94 h, respectively, indicating the highest levels of anthocyanins’ thermal stability at this temperature.
The use of phytic acid as a copigment provided a reduction in the rate of anthocyanin degradation, which was less evident at 70 °C. According to Table 2, $t_{1/2}$ for copigment BE sample increased by 1.22% at 70 °C, 2.46% at 80 °C, 8.55% at 90 °C, and 19.46% at 100 °C compared to the control BE sample. Moreover, $t_{1/2}$ for the copigment BM sample increased by 2.23% at 70 °C, 5.49% at 80 °C, 2.51% at 90 °C, and 22.63% at 100 °C compared to the control BM sample. Thus, the effect of copigment was more evident at high temperatures for both BE and BM.

This behavior can be explained using intermolecular interactions that are present between the copigment phytic acid and the flavylium cation of anthocyanins. Some studies emphasize that copigments are rich in pi (π) systems and are capable of associating with flavylium ions that provide protection to the anthocyanin molecule (Pedro; Granato; Rosso, 2016; Grajeda-Iglesias et al., 2016).

A copigmentation study on black rice anthocyanins reported $t_{1/2}$ as 14.06 h for control and 15.72 h for phytic acid copigment in the presence of light. It showed an increase of 11.79% in half-time life (Pedro; Granato; Rosso, 2016).

Some studies have reported that anthocyanin thermal degradation is due to loss of flavylium cation and hydrolysis of a double bond in the C-ring of the anthocyanin molecule. Application of maltodextrin is a suitable alternative in this degradation process since maltodextrin is a good film-forming, water-based factor and used as a coating molecule for polar solid matrices. This drying aid agent is capable of trapping hydrophilic compounds like anthocyanins by protecting their flavylium cations (Mehran; Masoum; Memarzadeh, 2020; Mahdavi et al., 2016; Burin et al., 2011).

Use of maltodextrin as a drying aid agent provided an increase of 36.39% in half-time life compared to BE (12.42 h) and BM (16.94 h), at 70 °C in the presence of copigment. The increase was 9.36% at 80 °C, 7.40% at 90 °C, and 5.43% at 100 °C. The first-order reaction rate constants (k) ranged between 6.82 x 10^{-4} at 70 °C and 4.95 x 10^{-3} at 100 °C. A study on anthocyanins degradation from blackberry juice reported $t_{1/2}$ values of 8.8, 4.7, and 2.9 h, and k values of 1.32 x 10^{-3}, 2.47 x 10^{-3}, and 3.94 x 10^{-3} at temperatures of 70 °C, 80 °C, and 90 °C, respectively (Wang; Xu, 2007). Our study emphasizes the relevance of the use of maltodextrin to increase the half-life time of blackberry compounds. The compounds could be useful as supplement food ingredients in several types of products. It is important to mention that microsphere could be highly applicable for products that could not tolerate high processing temperatures, and could help to increase the half-life time of anthocyanins and product shelf life.

**CONCLUSIONS**

By comparing the microsphere with extract, we found that the microsphere maintained anthocyanin content and increased red color ($a^*$) by around 60%, indicating that maltodextrin protects anthocyanin degradation under high temperatures. Moreover, the use of maltodextrin as a drying aid agent provided an increase of 36.39% in half-life time in the microsphere at 70 °C in the presence of phytic acid as a copigment. To summarize, blackberry pomace is feasible to use as a functional food because of the bioactive compounds present in it. The high stability of these compounds is appropriate for technological food applications, and the use of blackberry pomace is essential for agribusiness in order to reduce their negative impacts on the environment.

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