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Original article

# Effect of differently processed açai (*Euterpe oleracea* Mart.) on the retention of phenolics and anthocyanins in chewy candies

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**Summary** This study investigated the effects of processing and storage on the physicochemical properties and retention of antioxidant compounds of no-added sucrose chewy candies (NASC) incorporated with differently processed açai (frozen pulp, spray-dried and freeze-dried powders). NASC containing freeze-dried açai had the highest softness and recoveries of total phenolic (TP) and total anthocyanin (TA) immediately after production. Colour parameters and antioxidant capacity by ABTS and ORAC assays had no significant differences after 6 months of NASC storage, except for ORAC in NASC containing spray-dried açai, whereas $DPPH^{\bullet}$ in all samples significantly increased. Water activity and hardness also increased after storage, whereas TP and TA contents decreased, despite presenting good retentions (approx. 72–78% TP and 84–99% TA). This study suggests that açai has a great potential to be used as a natural pigment and antioxidant source in candy manufacturing, meeting consumption trends towards healthier products.

**Keywords** Açai, anthocyanins, antioxidant activity, chewy candy, colour, phenolics, processing effects, storage.

## Introduction

The food industry has been challenged to find innovative strategies to meet the growing demand of consumers for nutritionally balanced, healthy, natural and additive-free products, while maintaining their indulgent features. According to Munawar & Jamil (2014), there is a strong preference for natural sources to provide colour in processed foods rather than synthetic ones, which are less preferred by consumers because of a variety of allergic reactions associated with their consumption, besides attention-deficit hyperactivity disorder (ADHD) in children. Considering that most confectionery products are still coloured artificially in many countries, açai could be used as a natural alternative to red-violet colouring additives because of its anthocyanin profile. Red pitaya fruit and grape skin were already used to provide natural colour in confections, showing high application potentiality in the confectionery industry (Cappa *et al.*, 2015; Hani *et al.*, 2015). Paz *et al.* (2015) and Yamaguchi *et al.* (2015) reported that açai is a promising functional food ingredient and besides being a great source of pigments, it provides additional health benefits as enhanced nutritional and antioxidant profiles.

Açai (*Euterpe oleracea* Mart.) is a Brazilian tropical fruit rich in phenolic compounds with many biological activities, mostly antioxidant (Yamaguchi *et al.*, 2015). The regular consumption of polyphenol-rich foods has been positively associated with beneficial health effects like prevention of numerous chronic and degenerative diseases because of their potential antioxidant capable of combating or preventing oxidative stress and consequent cellular damages caused by the excessive production of free radicals in the human organism (Chan *et al.*, 2016; Lai *et al.*, 2016). The total antioxidant capacity of a food may be evaluated through a large number of *in vitro* spectrophotometric methods using several free radical sources and involving different reaction mechanisms based on free radical scavenging capacity or reducing ability. The use of different chemical assays to measure the antioxidant capacity is recommended aiming to ensure more reliable responses (Nora *et al.*, 2014; Paz *et al.*, 2015; Chan *et al.*, 2016). ABTS, $DPPH^{\bullet}$ and ORAC are some of the most widely used methods to investigate the antioxidant capacity of food extracts (Haminiuk *et al.*, 2012).

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Currently, there is limited scientific information on the inclusion of açai in processed products submitted to high temperatures, particularly chewy candies, and there is no data regarding the impact of açai on the physicochemical properties, especially colour stability, and retention of bioactive compounds of confections from the conditions of processing and storage. Considering that bioactive compounds can be sensitive to manufacturing operations, Mongia (2014) emphasises the importance to monitor their retention levels from the raw material to the finished product. The aim of this study was to investigate the effects of processing and storage on the physicochemical properties and retention of antioxidant compounds of no-added sucrose chewy candies containing differently processed açai.

## Materials and methods

### Chemicals and standards

The 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid) (ABTS), potassium persulphate, Folin-Ciocalteu phenol reagent, sodium carbonate, gallic acid, 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) and fluorescein sodium salt were obtained from Sigma-Aldrich. All other chemical reagents were of analytical or HPLC grade.

### Materials

No-added sucrose açai chewy candies (NASC) were formulated with maltitol syrup (Polyglobe® 1351; purity ≥50% (db); Ingredion, Mogi Guaçu, Brazil), isomalt powder (*C**IsoMaltidex 16500; purity ≥98%; Cargill, Krefeld, Germany), erythritol powder (Zerose™ Erythritol STD GRAN; purity ≥99.5%; Cargill, Blair, NE, USA) and açai (*Euterpe oleracea* Mart.). The types of açai added in the chewy candies were as follows: frozen açai pulp (FZ) purchased from De Marchi (Jundiaí, Brazil), freeze-dried açai powder (FD) donated by Liotécnica (Embu das Artes, Brazil) and spray-dried açai powder (SD) produced in a pilot spray-dryer (Gea Niro Atomizer, CB3104D, Soborg, Denmark) from a mixture of frozen açai pulp (FZ) (De Marchi) and maltodextrin 20DE (MOR-REX® 1920; Ingredion) according to the method described by Silva *et al.* (2016).

### Physicochemical characterisation of açai

Açai samples were characterised by moisture content according to an adaptation of the AOAC Official Method 934.06 and 920.151 (AOAC, 2012), determined in a vacuum oven (RVT360, Heraeus, Hanau, Germany) at 70 °C for 24 h, in triplicate; water activity, determined after sample equilibrium at 25 °C (AquaLab 4TEV, Decagon Devices Inc., Pullman, WA, USA), in triplicate; total lipid content, determined by acid hydrolysis method (Zenebon *et al.*, 2008), in triplicate; chromatic properties, quantified by instrumental measurement (CR 410, Konica Minolta, Inc., Osaka, Japan) in the CIELAB system ($L^*a^*b^*$ colour space), performed in ten replicates, whereas saturation ($C^*$, chroma) and hue angle ($h$) were determined according to eqns (1) and (2), respectively (Minolta, 1998).

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$h = \arctan(b^*/a*) \quad (2)$$

### Production of chewy candies

The NASC formulation and manufacturing processes were based on Silva *et al.* (2016), with slight modifications. Briefly, the dissolution of isomalt (30%, w/w, db) and erythritol (30%, w/w, db) in water (ratio of 1:3, water/polyol powders) together with maltitol syrup (40%, w/w, db) occurred in a cooker with direct-flame heating under atmospheric condition and took approx. 15 min until the mass reaches 130 °C (for a batch of 1.7 kg). The mass was transferred to an atmospheric batch cooker (Ecoline Staredition RE212, Lauda, Lauda-Königshofen, Germany) with heating by thermal oil (Xiameter® PMX-200, Dow Corning, Midland, MI, USA) and adapted stirring system (TE039/1, Tecnal, Piracicaba, Brazil) to be cooked up to 132 °C. The mass took approx. 6 min to reach this temperature. The slow heating rate (0.3–0.4 °C $min^{-1}$) during cooking allowed an accurate control of the final temperature and, consequently, the standardisation of the residual moisture content of the mass to 6%, minimising variations among NASC. Then, the mass was cooled down to 45–50 °C, pulled mechanically at 40 rpm for 20 min, formed, cut, individually wrapped (BOPP/BOPP metallised with water vapour permeability at 38 °C/90% RH of 0.78 g water $m^{-2}$ $day^{-1}$) and stored for 6 months at room temperature (25 ± 2 °C) and relative humidity of 60%. Açai (5% of açai solid content, w/w) was added at different steps: FZ in the dissolution to evaporate excessive water from the pulp, resulting in greater heat exposure (21 min until the mass reaches 130 °C); SD and FD in the cooling step to avoid their heat exposure. Isomalt (90 μm) (5%, w/w) was added in the cooling step as recommended by Sentko & Willibald-Ettle (2012), who reported the use of less than 10% (w/w) of

seeding crystals as advantageous in reducing stickiness and increasing shape stability of sugar-free chewy candies. The quantities of both ingredients were calculated based on the chewy candy mass after the cooking step.

### Physicochemical characterisation of chewy candies

No-added sucrose chewy candies samples were characterised at 0 and 6 months of storage by water activity ($a_w$) and chromatic attributes as described for the characterisation of açai; moisture content, determined by the Karl Fischer volumetric titration method (Titrando 901; Metrohm Pensalab, São Paulo, Brazil) (Bruttel & Schlink, 2003), in triplicate; instrumental texture for hardness parameter (TA.XT2i Texture Analyser; Stable Micro Systems Ltd., Godalming, UK) using a P/4 probe (4 mm dia. cylinder), pretest and post-test speed of 2 mm $s^{-1}$, test speed of 1 mm $s^{-1}$, 0.05 N trigger force and penetration distance of 4 mm (Silva *et al*., 2016), in ten replicates.

### Extraction methods of phenolic and anthocyanin compounds

Açai and NASC extracts were prepared by using the solid–liquid extraction technique at 25 °C, performed in triplicate for each sample. For the extraction of phenolic compounds, preliminary trials were performed according to the following methods: Kähkönen *et al*. (1999) using acetone/water (70:30, v/v), Canuto *et al*. (2010) using methanol/water (80:20, v/v) and Rufino *et al*. (2010) using methanol/water (50:50, v/v) and acetone/water (70:30, v/v), in addition to adaptations (1, substitution of 1 h in orbital shaker for 1 min in Ultra Turrax®; 2, equal to 1 plus defatting pretreatment). Adaptation 1 of Rufino *et al*. (2010) was used to obtain extracts for the determinations of TP and antioxidant capacity. For the extraction of anthocyanins, the method described by Teixeira *et al*. (2008) was followed to obtain extracts for the determination of TA, in which a solution containing ethanol/water (70:30, v/v) and HCl (pH 2.0) was used.

### Determination of TP and TA

The TP content of açai and NASC extracts was determined by the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965), in triplicate, measured spectrophotometrically (Cary 50, Varian Australia Pty Ltd., Mulgrave, Vic., Australia) at 750 nm. Results were expressed as gallic acid equivalents (GAE) in mg per 100 g. The TA content was determined from açai and NASC extracts by the pH differential method (Teixeira *et al*., 2008), in triplicate, measured spectrophotometrically (Cary 50, Varian Australia Pty Ltd.) at 535 nm. Results were expressed in mg per 100 g, representing the mean values obtained in both pH 1.0 and 4.5. After the storage of NASC, the analyses were repeated. The retentions (or recoveries) of TP after the açai drying processes were calculated comparing the TP analytically found in the dried powders with an estimated one, which considered the TP and the açai solid content of the frozen pulp that originated the dried açai powders. Similarly, the retentions of TP obtained immediately after NASC production were calculated comparing the TP analytically found in NASC with an estimated one, which considered the TP and the açai solid content of the fruit ingredient used in NASC production. The storage retentions were calculated comparing the TP content found at 0 and 6 months. The same calculations were taken into account for obtaining the retentions of TA.

### Determination of *in vitro* total antioxidant capacities

Antioxidant capacities ($DPPH^{\cdot}$, ABTS and ORAC) were determined for açai and NASC. The assays were repeated after the storage of NASC.

The $DPPH^{\cdot}$ (free radical scavenging capacity) assay was performed according to Brand-Williams *et al*. (1995), with modifications. It was based on $DPPH^{\cdot}$ scavenging (61 μM in methanol), which expresses a percentage of inhibition by antioxidants, resulting in a decreased absorbance at 515 nm, measured spectrophotometrically (Cary 50, Varian Australia Pty Ltd.) after incubation in dark conditions for 1 h. Results were obtained in triplicate and expressed as μmol of Trolox equivalents (TE) per g.

The ABTS (free radical scavenging capacity) assay, also known as Trolox equivalent antioxidant capacity (TEAC), was performed as described by Re *et al*. (1999). It was based on the ability of antioxidants to scavenge the ABTS radical cation ($ABTS^{\bullet+}$) produced by reacting 7 mM of ABTS solution with 2.45 mM of potassium persulphate. After 16 h in dark conditions (25 °C), $ABTS^{\bullet+}$ absorbance was adjusted to $0.70 \pm 0.02$ using ethanol. The absorbance was measured spectrophotometrically (Cary 50, Varian Australia Pty Ltd.) at 750 nm after 10 min of incubation in dark conditions. Results were obtained in triplicate and expressed as μmol TE per g.

The oxygen radical absorbance capacity (ORAC) assay towards peroxyl radical (AAPH) was measured through hydrophilic $ORAC_{FL}$ assay with fluorescein (Dávalos *et al*., 2004). Fluorescence emission was monitored every 2 min up to 100 min at 37 °C using a 96-well microplate fluorometer (Fluostar Omega, BMG LABTECH GmbH). Excitation and emission wavelengths were 485 and 520 nm, respectively. Results were expressed as μmol TE per g, measured in five replicates for each extract.

### Statistical analyses

To establish the statistical differences among means, the data were treated by one-way analysis of variance through the Tukey HSD test. Additionally, two-way analysis of variance was performed aiming to evaluate the impact of selected factors and their interactions. Pearson's correlation coefficient between selected parameters was also calculated. The statistical software Statistica® 12 (StatSoft Inc., 2012) was used considering a 95% confidence level for all analyses.

## Results and discussion

### Physicochemical characterisation of açai and chewy candies

The physicochemical properties of the açai samples are shown in Table 1. The SD and FD were originated from different FZ types (FZ-1 and FZ-2, respectively). The FD, SD and FZ-2 presented vivid colour with a reddish-purple hue, while FZ-1 appeared dull with a blue hue. The $a^*$ values were well correlated with anthocyanins ($r = 0.84$, $P < 0.05$, $n = 10$), indicating that the higher the TA, the more intense the red colour is, resulting in the following rankings for both $a^*$ and TA values (Tables 1 and 2, respectively): FD>SD>FZ-2>FZ-1.

Table 3 presents the characterisation of NASC at 0 and 6 months of storage, besides the effects of the type of açai ingredient added in NASC, the period of storage and these factors' interaction on the responses. Moisture content showed no significant difference ($P < 0.05$) after storage. In general, NASC $a_w$ ranged from 0.47 to 0.51, showing similar results to those reported by Ergun *et al.* (2010) (0.45–0.60). The increased $a_w$ values after NASC storage probably happened because of a crystallisation process (Ergun *et al.*, 2010), in which a decrease in the concentration of dissolved solids from the liquid phase occurs (Silva *et al.*, 2016). The NASC presented intermediate results of hardness (90.50–101.45 N for NASC-FD and 127.08–156.70 N for NASC-FZ and NASC-SD) in comparison with sugared chewy candies containing 5% (50.14 N) and 10% (192.11 N) of spray-dried strawberry (Fadini *et al.*, 2003). The NASC-FD had the highest softness compared to the other NASC probably due to the highest lipid content of FD (54.6 g per 100 g, db) compared to SD and FZ-1 (32.6–34.8 g per 100 g, db) (Table 1). Regarding the chromatic characterisation, açai type and storage period showed significant effect on the evaluated parameters as shown by two-way ANOVA (Table 3), except the açai type on lightness, and no interaction between factors was observed at $P < 0.05$. As indicated by the comparison of means test, colour parameters did not differ statistically at $P < 0.05$ after NASC storage, showing good colour stability. The only differences found among samples were coordinate $b^*$ and colour saturation ($C^*$), with NASC-FZ going in the yellowish direction (+$b^*$) and presenting a more intense vivid colour. High $L^*$ values indicate all samples were bright.

### Selection of the extraction method of phenolic compounds

The TP content found in the freeze-dried açai using the extraction method of Rufino *et al.* (2010) and adaptations 1 and 2 (3247.53–3343.14 mg GAE per 100 g) presented no significant difference ($P < 0.05$)

**Table 1** Physicochemical characterisation of açai

| Analysis | FZ-1 | FZ-2 | SD | FD |
|---|---|---|---|---|
| Moisture content (g per 100 g) | 89.02 ± 0.01[a] | 82.60 ± 1.61[b] | 2.13 ± 0.06[c] | 3.25 ± 0.01[c] |
| Açai solid content (g per 100 g) | 10.98 ± 0.01[d] | 17.40 ± 1.61[c] | 47.49*± 0.03[b] | 96.75 ± 0.01[a] |
| Water activity | 1.002 ± 0.001[a] | 1.000 ± 0.001[a] | 0.274 ± 0.014[c] | 0.448 ± 0.004[b] |
| Total lipid content (g per 100 g, db) | 34.8 ± 0.6 | 51.8 ± 2.0 | 32.6† ± 0.8 | 54.6 ± 0.8 |
| Colour parameters | | | | |
| $L^*$ (lightness) | 25.71 ± 0.32[a,b] | 27.89 ± 1.54[a] | 23.40 ± 0.82[a,b] | 19.43 ± 0.41[b] |
| $a^*$ (green to red) | 1.23 ± 0.07[b] | 8.61 ± 0.44[a] | 9.98 ± 0.36[a] | 10.63 ± 0.30[a] |
| $b^*$ (blue to yellow) | −1.23 ± 0.09[b] | 1.70 ± 0.12[a] | 2.18 ± 0.23[a] | −0.27 ± 0.07[b] |
| $C^*$ (chroma) | 1.74 ± 0.04[b] | 8.77 ± 0.91[a] | 10.22 ± 0.77[a] | 10.63 ± 0.53[a] |
| $h$ (hue angle) | −44.78 ± 8.07[b] | 11.11 ± 0.67[a] | 12.27 ± 1.50[a] | −1.48 ± 0.84[a] |

FZ-1, frozen açai pulp used to produce SD; FZ-2, frozen açai pulp used to produce FD; SD, spray-dried açai powder; FD, freeze-dried açai powder. Values followed by different letters in the same row are significantly different ($P < 0.05$) according to Tukey's test.

*The açai solid content of SD had maltodextrin content (50.38 g per 100 g) ignored.

†The total lipid content of SD is from the açai fraction.

**Table 2** Total phenolic, total anthocyanin and antioxidant activities of açai based on fresh matter

| Analysis | FZ-1 | FZ-2 | SD | FD |
|---|---|---|---|---|
| Total phenolic (mg GAE per 100 g) | 537.79 ± 6.14$^{c}$ | 480.34 ± 27.07$^{c}$ | 1936.81 ± 31.13$^{b}$ | 2669.88 ± 46.46$^{a}$ |
| Total anthocyanin (mg per 100 g) | 62.58 ± 0.31$^{d}$ | 135.15 ± 1.62$^{c}$ | 274.21 ± 19.58$^{b}$ | 713.14 ± 7.12$^{a}$ |
| DPPH$^{\cdot}$ (μmol TE per g) | 8.27 ± 0.40$^{c}$ | 6.85 ± 0.16$^{c}$ | 32.01 ± 2.73$^{b}$ | 54.99 ± 0.80$^{a}$ |
| ABTS (μmol TE per g) | 34.39 ± 2.94$^{c}$ | 14.00 ± 0.26$^{d}$ | 189.86 ± 10.39$^{a}$ | 162.74 ± 5.31$^{b}$ |
| ORAC (μmol TE per g) | 173.54 ± 9.19$^{c}$ | 128.85 ± 9.79$^{c}$ | 604.64 ± 23.14$^{b}$ | 1254.98 ± 87.51$^{a}$ |

FZ-1, frozen açai pulp used to produce SD; FZ-2, frozen açai pulp used to produce FD; SD, spray-dried açai powder; FD, freeze-dried açai powder. Values followed by different letters in the same row are significantly different ($P < 0.05$) according to Tukey's test.

**Table 3** Characterisation of no-added sucrose açai chewy candies containing differently processed açai during storage

| Sample | Moisture content (g per 100 g) | Water activity | Hardness (N) | Colour parameters | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | *L** (lightness) | *a** (green to red) | *b** (blue to yellow) | *C** (chroma) | *h* (hue angle) |
| NASC-FZ | | | | | | | | |
| t0 | 6.11 ± 0.41$^{a}$ | 0.49 ± 0.00$^{c}$ | 130.39 ± 5.33$^{b}$ | 71.87 ± 1.10$^{a}$ | −2.60 ± 0.13$^{a,b}$ | 10.54 ± 0.26$^{a,b}$ | 10.86 ± 0.31$^{a}$ | −76.15 ± 1.37$^{a,b,c}$ |
| t6 | 6.03 ± 0.19$^{a}$ | 0.50 ± 0.00$^{a,b}$ | 156.59 ± 4.79$^{a}$ | 69.62 ± 0.58$^{a}$ | −1.88 ± 0.20$^{a}$ | 11.07 ± 0.25$^{a}$ | 11.23 ± 0.29$^{a}$ | −80.33 ± 1.72$^{c}$ |
| NASC-SD | | | | | | | | |
| t0 | 5.98 ± 0.14$^{a}$ | 0.47 ± 0.00$^{d}$ | 127.08 ± 5.67$^{b}$ | 71.85 ± 0.72$^{a}$ | −3.45 ± 0.22$^{b}$ | 8.66 ± 0.18$^{c,d}$ | 9.33 ± 0.12$^{b,c}$ | −68.26 ± 2.36$^{a}$ |
| t6 | 5.90 ± 0.17$^{a}$ | 0.50 ± 0.01$^{b,c}$ | 156.70 ± 5.85$^{a}$ | 69.36 ± 0.89$^{a}$ | −2.45 ± 0.13$^{a,b}$ | 9.48 ± 0.21$^{b,c}$ | 9.79 ± 0.24$^{b}$ | −75.49 ± 1.48$^{a,b,c}$ |
| NASC-FD | | | | | | | | |
| t0 | 6.00 ± 0.13$^{a}$ | 0.49 ± 0.00$^{c}$ | 90.50 ± 4.26$^{d}$ | 70.03 ± 0.77$^{a}$ | −2.73 ± 0.32$^{a,b}$ | 7.73 ± 0.23$^{d}$ | 8.20 ± 0.15$^{d}$ | −70.50 ± 3.72$^{a,b}$ |
| t6 | 6.12 ± 0.17$^{a}$ | 0.51 ± 0.00$^{a}$ | 101.45 ± 7.33$^{c}$ | 67.84 ± 0.55$^{a}$ | −1.76 ± 0.20$^{a}$ | 8.39 ± 0.13$^{c,d}$ | 8.58 ± 0.12$^{c,d}$ | −78.14 ± 2.10$^{b,c}$ |
| Two-way ANOVA - F | | | | | | | | |
| Factor I (açai type) | 0.6* | 49.0 | 461.1 | 3.2* | 7.6 | 85.1 | 143.4 | 8.0 |
| Factor II (storage period) | 0.0* | 59.7 | 235.3 | 12.8 | 27.4 | 14.9 | 9.9 | 23.5 |
| Factor I x Factor II | 0.4* | 62.2 | 15.7 | 0.02* | 0.3* | 0.2* | 0.1* | 0.7* |

NASC-FZ, chewy candies produced with frozen açai pulp (FZ-1); NASC-SD, chewy candies produced with spray-dried açai powder; NASC-FD, chewy candies produced with freeze-dried açai powder; t0, 0-month storage; t6, 6-month storage at 25 °C/60% RH. Values followed by different letters in the same column are significantly different ($P < 0.05$) according to Tukey's test.
*Not significant at the 0.05 level of confidence.

among them and showed the highest results compared with the use of Kähkönen *et al.* (1999) (3073.57 mg GAE per 100 g) and Canuto *et al.* (2010) (2446.81 mg GAE per 100 g) methods. The removal of nonpolar compounds (lipids) from açai (adaptation 2) did not affect the TP results, thus indicating that the defatting process was unnecessary. As adaptation 1 provided a faster analysis compared to the original method, it was selected to obtain extracts for further TP and antioxidant capacity assays.

The batch of the freeze-dried açai used in the selection of the extraction method was different from the one which was added in NASC, thus explaining the variation in the TP results found in the selection stage (3343.14 mg GAE per 100 g) and in the characterisation of the FD used in NASC production (2269.88 mg GAE per 100 g, Table 2).

### TP, TA and antioxidant capacity in açai

The TP, TA and antioxidant capacity in açai samples are shown in Table 2. The FZ-2 and FZ-1 presented higher TP (480.34–537.79 mg GAE per 100 g) than the one found by Hassimotto *et al.* (2005) (328 mg GAE per 100 g). Deducting the carrier agent content from SD and converting the result into dry basis

(4078 mg GAE per 100 g), a great difference in TP was observed in relation to the value shown by Tonon *et al.* (2009) (13542 mg GAE per 100 g, db). Converting the result of TP from FD into dry basis (2760 mg GAE per 100 g), this study presented lower TP compared to Kang *et al.* (2012) (3120 mg GAE per 100 g, db) and Rufino *et al.* (2010) (3268 mg GAE per 100 g, db). However, FD presented higher TP in comparison with Paz *et al.* (2015) (1808 mg GAE per 100 g, db). Regarding anthocyanins, Pacheco-Palencia *et al.* (2009) found higher TA (205.6 mg per 100 g) for açai pulp than FZ-1 and FZ-2 (62.58–135.15 mg per 100 g), whereas Rufino *et al.* (2010) reported a TA value (111 mg per 100 g) close to FZ-2 (135.15 mg per 100 g). Tonon *et al.* (2010) reported a notably greater TA (3402.30 mg per 100 g açai juice, db) for spray-dried açai than the one shown in the present study (577 mg per 100 g, result considering the açai juice dried matter).

The dehydration techniques led to the concentration of TP and TA levels in the resulting powders and also to an increase in the antioxidant activities compared to the frozen pulps, which considerably varied among the samples according to the assay ($DPPH^{\cdot}$: 6.85–54.99 μmol TE per g; ABTS: 14.00–189.86 μmol TE per g; ORAC: 128.85–1254.98 μmol TE per g). The ORAC values were higher than those obtained from other chemical methods, as it combines time and degree inhibitions, besides resulting in a free radical completed action (Haminiuk *et al.*, 2012). For freeze-dried açai, Kang *et al.* (2012) found 985.9 μmol TE per g (db) by the ORAC method (hydrophilic fraction), Cohen *et al.* (2009) found 232.76 μmol TE per g by the ABTS method and Paz *et al.* (2015) found 15.74 μmol TE per g (db) by the DPPH method. In this study, the antioxidant capacities of FD were 1297 μmol TE per g (converted into dry basis), 162.74 μmol TE per g and 56.84 μmol TE per g (converted into dry basis) by the ORAC, ABTS and DPPH methods, respectively. For spray-dried açai, Tonon *et al.* (2009) verified an antioxidant capacity by the ORAC method of 2390.46 μmol TE per g of juice dried matter from a sample produced with maltodextrin 20DE, whereas in this study, SD reached 1273 μmol TE per g (result considering açai juice, db).

Discrepancies among results obtained in this study compared to the literature were expected and may be assigned to different intrinsic features of the fruits/physiological factors, cultivated area and agronomic interferences from which they were originated (Paz *et al.*, 2015), besides differences in the processing/storage conditions and methodologies used.

Antioxidant capacity by the three assays and TP were highly correlated positively ($0.96 \leq r \leq 0.99$, $P < 0.05$). A strong linear correlation was also observed among the antioxidant capacity assays ($0.88 \leq r \leq 0.99$, $P < 0.05$), showing the açai extracts were capable of scavenging free radicals in different reaction mechanisms. The linear relationship among all these assays is in agreement with other studies (Harakotr *et al.*, 2014; Chan *et al.*, 2016). Rufino *et al.* (2010) attributed stronger antioxidant capacity in fruits to a higher TP level, which indicates the phenolic compounds are greatly responsible for the antioxidant effects of the extracts.

### TP, TA and antioxidant capacity in chewy candies

The results of two-way ANOVA (Table 4) indicated that the type of açai added in NASC and the storage period significantly influenced TP and TA content of the samples at $P < 0.05$. The interaction term between açai type and storage period was statistically significant only for TA parameter. TP and TA levels of the chewy candies decreased significantly ($P < 0.05$) after their storage, except for NASC-FZ, where TA content remained constant, with 99.21% of retention of anthocyanins (Fig. 1b). It probably occurred because more labile anthocyanin compounds were lost (51.47%, Fig. 1b) during cooking step. During NASC storage, certain groups of nonanthocyanin polyphenolic cofactors naturally occurring in açai may have conferred additional stability to the remaining anthocyanins by the intermolecular copigmentation reactions among these compounds (Pacheco-Palencia & Talcott, 2010). Although NASC-FD presented the lowest TP content compared to the other samples, it showed the greatest results for TA and had the highest recovery of bioactive compounds (TP and TA) (Fig. 1a, b), thus indicating the best performance in using freeze-dried açai to retain those compounds in the chewy candy immediately after its manufacturing. Cappa *et al.* (2015) reported that changes in phenolic compounds during processing had no impact on the antioxidant capacity of fruit candy enriched with red grape skin. In the present study, the antioxidant activities of NASC remained stable after their storage (Table 4), except for $DPPH^{\cdot}$ in all samples and ORAC in NASC-SD, which revealed significant increased results. The storage period was the most pronounced factor which impacted $DPPH^{\cdot}$, whereas the type of açai added in NASC was the factor which most influenced ABTS and ORAC.

### Effects of processing and storage on the retentions of TP and TA

The effects of processing of the FZ subjected to spray-drying and freeze-drying techniques and also the manufacturing and storage of NASC on the retention of bioactive compounds (TP and TA) are presented in Fig. 1a, b.

A significant and positive correlation ($r = 0.97$, $P < 0.05$, $n = 10$) was observed between TP and TA

**Table 4** Total phenolic, total anthocyanin and antioxidant activities of no-added sucrose açai chewy candies during storage

| Sample | Total phenolic (mg GAE per 100 g) | Total anthocyanin (mg per 100 g) | DPPH· (μmol TE per g) | ABTS (μmol TE per g) | ORAC (μmol TE per g) |
|---|---|---|---|---|---|
| NASC-FZ | | | | | |
| t0 | 252.65 ± 15.72$^{a}$ | 13.28 ± 0.23$^{e}$ | 3.83 ± 0.02$^{d,e}$ | 8.69 ± 0.69$^{b}$ | 65.79 ± 2.20$^{a,b}$ |
| t6 | 191.19 ± 7.92$^{b}$ | 13.17 ± 0.20$^{e}$ | 4.97 ± 0.57$^{b,c}$ | 9.20 ± 0.24$^{a,b}$ | 66.27 ± 0.52$^{a,b}$ |
| NASC-SD | | | | | |
| t0 | 250.89 ± 5.62$^{a}$ | 19.41 ± 0.19$^{c}$ | 2.98 ± 0.37$^{e}$ | 10.11 ± 0.82$^{a}$ | 63.30 ± 0.93$^{b}$ |
| t6 | 195.81 ± 6.28$^{b}$ | 16.32 ± 0.28$^{d}$ | 6.85 ± 0.33$^{a}$ | 9.25 ± 0.56$^{a,b}$ | 66.65 ± 0.91$^{a}$ |
| NASC-FD | | | | | |
| t0 | 204.25 ± 8.40$^{b}$ | 27.75 ± 0.37$^{a}$ | 4.10 ± 0.16$^{c,d}$ | 8.63 ± 0.16$^{b}$ | 44.07 ± 0.97$^{c}$ |
| t6 | 146.29 ± 3.46$^{c}$ | 23.73 ± 0.01$^{b}$ | 5.44 ± 0.37$^{b}$ | 8.51 ± 0.21$^{b}$ | 43.07 ± 1.00$^{c}$ |
| Two-way ANOVA - F | | | | | |
| Factor I (açai type) | 58.1 | 4163.2 | 3.5* | 7.2 | 660.5 |
| Factor II (storage period) | 197.1 | 451.3 | 165.0 | 0.4* | 2.8* |
| Factor I x Factor II | 0.2* | 108.8 | 28.3 | 2.7* | 5.0 |

NASC-FZ, chewy candies produced with frozen açai pulp (FZ-1); NASC-SD, chewy candies produced with spray-dried açai powder; NASC-FD, chewy candies produced with freeze-dried açai powder; t0, 0-month storage; t6, 6-month storage at 25 °C/60% RH. Values followed by different letters in the same column are significantly different ($P < 0.05$) according to Tukey's test.
*Not significant at the 0.05 level of confidence.

contents. According to Fig. 1a, the açai drying processes showed good percentages of recovery of TP (83.27–99.96%), in which the freeze-drying technique did not cause any significant change in TP compared to the original TP level found in FZ, while a loss of TP of 16.73% was observed after the açai spray-drying. Greater performance regarding the retention of TP after the açai freeze-drying was expected as this technique is well known to provide high-quality dried products because of the nutrients preservation. Regarding the açai spray-drying, the temperature used in the process (inlet/outlet air temperatures of 170/80 °C, respectively) may have contributed to the partial degradation of the phenolic compounds. Nora *et al.* (2014) reported that drying and freeze-drying treatments may decrease or even increase the content of bioactive compounds. The decrease in this compounds level may be due to several factors such as heat, oxygen and light exposure, physicochemical parameters influence (i.e. pH, moisture) and degradative enzymatic reactions, whereas their increase may be related to the loss of cellular integrity by the effects of heating (in drying process) or freezing followed by ice sublimation (in freeze-drying process) and a consequent contribution to the better extraction of the bioactive compounds (Wu *et al.*, 2010).

Considering the production of NASC, samples presented recoveries of TP ranging from 107.24 to 153.88% (Fig. 1a). Probably, such results were a consequence of the hydrolysis of conjugated polyphenols in açai during the production of NASC. Haminiuk *et al.* (2012) reported that processing affects the content of phenolic compounds and may result in the loss or enrichment of some polyphenols. Harakotr *et al.* (2014) observed thermal treatment does not always imply in bioactive compound destruction and suggested cooking may lead to the forming of novel compounds and the improvement of antioxidant properties. They also related the increase in the level of these compounds to their better extractability or release from the food matrix after exposure to heat. The lower recovery of TP presented by NASC-FZ compared with the other chewy candies was expected and could be explained by simultaneous occurrences: partial heating degradation of phenolic compounds once they were subjected to more severe treatment (addition of açai in the cooking step) and release of compounds from the bound fraction followed by their interaction with macromolecules of the matrix.

Concerning the storage of NASC, recoveries of TP varied between 71.62 and 78.05% (Fig. 1a). As polyphenols are the major cofactors in açai and considering anthocyanin intermolecular copigmentation reactions (Pacheco-Palencia & Talcott, 2010), a decrease in TP was expected after the storage period, although this positively influenced the anthocyanin stability (recoveries of TA from 84.08 to 99.21%, Fig. 1b).

According to Fig. 1b, recoveries of TA were above 94.89% in açai drying processes and were within the range of 48.53–78.42% and 84.08–99.21% in the production and storage of NASC, respectively.

Tonon *et al.* (2008) found an anthocyanin retention of 84.06% after the açai spray-drying process at inlet/outlet air temperatures of 170/99 °C and using maltodextrin 10DE (30 g per 100 g) as a carrier agent. In this study, the great retention of TA (101.30%,

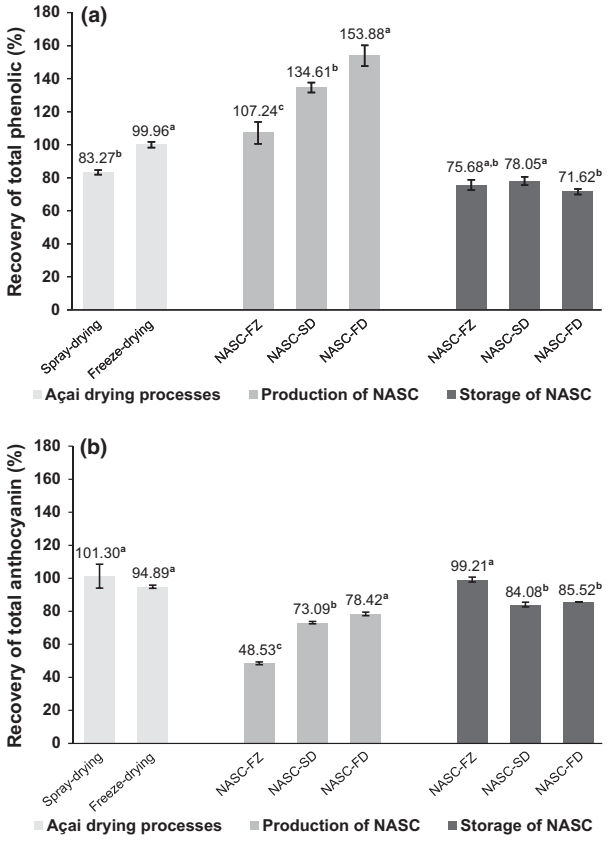


**Figure 1** Recovery of total phenolic (a) and total anthocyanin (b) after drying processes of açai, production and storage of chewy candies [NASC, no-added sucrose açai chewy candies; FZ, frozen açai pulp; SD, spray-dried açai powder; FD, freeze-dried açai powder; bars represent the standard deviations ($n = 3$); different letters within the same column group indicate significant differences ($P < 0.05$)].

Fig. 1b) may be attributed to the effect of the maltodextrin concentration (50.38 g per 100 g) and its dextrose equivalent (20DE). Ersus & Yurdagel (2007) confirmed that, in black carrot spray-drying at 160 °C, maltodextrin 20DE conferred a higher pigment (anthocyanins) retention compared to the use of maltodextrins 10 and 30DE. Tonon *et al.* (2010) reported that spray-drying of açai juice with carrier agents of high molecular weight may provide good anthocyanin retention and increase the glass transition temperature of the resulting powder, avoiding structural changes, which could lead to nutritional and quality losses. Anthocyanins may also be protected against degradation during spray-drying because of the agglomeration of powders caused by their higher moisture content when lower inlet air temperatures are used, reducing exposure to oxygen (Tonon *et al.*, 2008). Powders with higher water activity may imply in higher molecular mobility inside the food, which allows easier oxygen diffusion and, consequently, increases the anthocyanin degradation rate due to the occurrence of oxidation reactions (Tonon *et al.*, 2010). The low $a_w$ value of SD (< 0.3, Table 1) was very positive for its stability. A great retention of TA (94.89%) in açai after employing the freeze-drying technique was also observed and probably happened because of the low temperature and vacuum inherent to the process, protecting the compounds from oxidation. In general, spray-drying and freeze-drying were efficient techniques to preserve açai.

The production of NASC considerably influenced the retentions of TA (Fig. 1b). The NASC-FZ presented the lowest percentage of recovery of TA, showing a negative effect on the stability of anthocyanins (51.47% of degradation) mainly because of the longer cooking time at high temperature (27 min until the mass reaches 132 °C). Cappa *et al.* (2015) found a lower recovery of anthocyanins due to fruit candy manufacturing, in which degradation by about 80% occurred mainly during the concentration step (95–100 °C per 40 min). Patras *et al.* (2010) reported that the degradation of anthocyanins follows a logarithmic course according to an arithmetic increase in temperature. The NASC-FD and NASC-SD had a recovery of TA of 73.09–78.42%, being less affected by heat exposure as the açai powders were added in the cooling step when the mass was at approximately 80 °C, being quickly reduced to 45–50 °C. Therefore, the results showed the addition of açai powders in the cooling step resulted in better preservation of anthocyanins. The losses of TA in the NASC may be attributed not only to thermal degradation, but also to oxygen incorporation into the mass during the pulling step and to the vulnerability of anthocyanin to light during manufacturing. Anthocyanins were more susceptible to degradation by the production of NASC than by the period of storage.

Focusing mainly on the stability of colour and retention of bioactive compounds after the conditions of processing applied to both fruit ingredients and finished products, this study showed the selection of the type of processed fruit as an ingredient in confectionery application is an essential step to be considered by manufacturers. Mongia (2014) also reported other important criteria for fruit ingredient selection, such as its easy incorporation, preservation of flavour and nutritional benefits of the fruit close to its natural form, delivery of the desired sensory experience, variation of fruit content, compatibility of the water activity between the processed fruit and the product it will be incorporated into aiming to minimise interactions, fruit stability in terms of specific requirements of the storage system and cost. Despite freeze-dried açai having shown better performance in the retention of bioactive compounds from its inclusion in the production of chewy candy, in general, all the processed açai

tested presented satisfactory physicochemical properties, TP/TA retentions and antioxidant activities.

## Conclusion

This study reported that all the processed açai (frozen pulp, spray-dried and freeze-dried powders) incorporated into chewy candies showed a positive performance on the physicochemical behaviour and retention of bioactive compounds by the effects of processing and storage of the products. No changes in the manufacturing process of chewy candy were needed by the use of açai ingredient. Chewy candies presented a stable colour after 6 months of storage, proper texture, moisture content and $a_w$, great retention/stability of total phenolic and anthocyanin content, besides antioxidant capacity according to the methodologies used. Among the chewy candies, the one containing freeze-dried açai showed higher softness and retentions of phenolic and anthocyanin compounds immediately after its production compared to those added by spray-dried and frozen açai pulp. Anthocyanins in chewy candies were more susceptible to degradation during the processing than during the storage and also more susceptible to degradation in the cooking step than in the cooling step. Açai pulp and powders can be exposed under high temperature in industrial application providing a satisfactory colour to the product and retaining considerable bioactive compound levels. They possess great potential to be used as an ingredient source of natural pigments and antioxidants resulting in a product meeting global consumption trends towards healthier and natural purposes.

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## References

AOAC. (2012). *Official Methods of Analysis of AOAC International*, 19th edn. Gaithersburg, USA: Association of Official Analytical Chemists.

Brand-Williams, W., Cuvelier, M.E. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, **28**, 25–30.

Bruttel, P. & Schlink, R. (2003). *Monograph: Water Determination by Karl Fischer Titration*. Switzerland: Metrohm Ltd. (Chapter 11).

Canuto, G.A.B., Xavier, A.A.O., Neves, L.C. & Benassi, M.T. (2010). Caracterização físico-química de polpas de frutos da Amazônia e sua correlação com a atividade anti-radical livre. *Revista Brasileira de Fruticultura*, **32**, 1196–1205.

Cappa, C., Lavelli, V. & Mariotti, M. (2015). Fruit candies enriched with grape skin powders: physicochemical properties. *LWT - Food Science and Technology*, **62**, 569–575.

Chan, C.-L., Gan, R.-Y. & Corke, H. (2016). The phenolic composition and antioxidant capacity of soluble and bound extracts in selected dietary spices and medicinal herbs. *International Journal of Food Science and Technology*, **51**, 565–573.

Cohen, K.O., Mattietto, R.A., Chisté, R.C. & Oliveira, M.S.P. (2009). Caracterização físico-química e funcional da polpa extraída de frutos da cultivar de açaizeiro BRS Pará. *Boletim de Pesquisa e Desenvolvimento*, **70**, 1–22.

Dávalos, A., Gómez-Cordovés, C. & Bartolomé, B. (2004). Extending applicability of the oxygen radical absorbance capacity (ORAC-Fluorescein) Assay. *Journal of Agricultural and Food Chemistry*, **52**, 48–54.

Ergun, R., Lietha, R. & Hartel, R.W. (2010). Moisture and shelf life in sugar confections. *Critical Reviews in Food Science and Nutrition*, **50**, 162–192.

Ersus, S. & Yurdagel, U. (2007). Microencapsulation of anthocyanin pigments of black carrot (*Daucuscarota* L.) by spray drier. *Journal of Food Engineering*, **80**, 805–812.

Fadini, A.L., Facchini, F., Queiroz, M.B., Anjos, V.D.A. & Yotsuyanagi, K. (2003). Influência de diferentes ingredientes na textura de balas moles produzidas com e sem goma gelana. *Bol. Centro Pesqui. Process*, **21**, 131–140.

Haminiuk, C.W.I., Maciel, G.M., Plata-Oviedo, M.S.V. & Peralta, R.M. (2012). Phenolic compounds in fruits – an overview. *International Journal of Food Science and Technology*, **47**, 2023–2044.

Hani, N.M., Romli, S.R. & Ahmad, M. (2015). Influences of red pitaya fruit puree and gelling agents on the physicomechanical properties and quality changes of gummy confections. *International Journal of Food Science and Technology*, **50**, 331–339.

Harakotr, B., Suriharn, B., Tangwongchai, R., Scott, M.P. & Lertrat, K. (2014). Anthocyanin, phenolics and antioxidant activity changes in purple waxy corn as affected by traditional cooking. *Food Chemistry*, **164**, 510–517.

Hassimotto, N.M.A., Genovese, M.I. & Lajolo, F.M. (2005). Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *Journal of Agricultural and Food Chemistry*, **53**, 2928–2935.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J. *et al*. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*, **47**, 3954–3962.

Kang, J., Thakali, K.M., Xie, C. *et al*. (2012). Bioactivities of açaí (*Euterpe precatoria* Mart.) fruit pulp, superior antioxidant and anti-inflammatory properties to *Euterpe oleracea* Mart. *Food Chemistry*, **133**, 671–677.

Lai, Q., Wang, H., Guo, X. *et al*. (2016). Comparison of phytochemical profiles, antioxidant and cellular antioxidant activities of seven cultivars of *Aloe*. *International Journal of Food Science and Technology*, **51**, 1489–1494.

Minolta, K. (1998). *Precise Color Communication: Color Control from Perception to Instrumentation*. Japan: Konica Minolta, Inc. (Chapters 1, 4).

Mongia, G. (2014). Fruit ingredients in confectionery applications. *The Manufacturing Confectioner*, **94**, 73–82.

Munawar, N. & Jamil, H.M.T.H. (2014). The Islamic perspective approach on plant pigments as natural food colourants. *Procedia - Social and Behavioral Sciences*, **121**, 193–203.

Nora, C.D., Müller, C.D.R., Bona, G.S. *et al*. (2014). Effect of processing on the stability of bioactive compounds from red guava (*Psidium cattleyanum* Sabine) and guabiju (*Myrcianthes pungens*). *Journal of Food Composition and Analysis*, **34**, 18–25.

Pacheco-Palencia, L.A. & Talcott, S.T. (2010). Chemical stability of açai fruit (*Euterpe oleracea* Mart.) anthocyanins as influenced by naturally occurring and externally added polyphenolic cofactors in model systems. *Food Chemistry*, **118**, 17–25.

Pacheco-Palencia, L.A., Duncan, C.E. & Talcott, S.T. (2009). Phytochemical composition and thermal stability of two commercial açai species, *Euterpe oleracea* and *Euterpe precatória*. *Food Chemistry*, **115**, 1199–1205.

Patras, A., Brunton, N.P., O'Donnell, C. & Tiwari, B.K. (2010). Effect of thermal processing on anthocyanin stability in foods;

mechanisms and kinetics of degradation. *Trends in Food Science & Technology*, **21**, 3–11.

Paz, M., Gúllon, P., Barroso, M.F. *et al*. (2015). Brazilian fruit pulps as functional foods and additives: evaluation of bioactive compounds. *Food Chemistry*, **172**, 462–468.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, **26**, 1231–1237.

Rufino, M.S.M., Alves, R.E., Brito, E.S., Pérez-Jiménez, J., Saura-Calixto, F. & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, **121**, 996–1002.

Sentko, A. & Willibald-Ettle, I. (2012). Isomalt. In: *Sweeteners and Sugar Alternatives in Food Technology*. (edited by K. O'Donnell & M.W. Kearsley) Pp. 243–273. Oxford: Wiley-Blackwell.

Silva, L.B., Queiroz, M.B., Fadini, A.L., Fonseca, R.C.C., Germer, S.P.M. & Efraim, P. (2016). Chewy candy as a model system to study the influence of polyols and fruit pulp (açai) on texture and sensorial properties. *LWT - Food Science and Technology*, **65**, 268–274.

Singleton, V.L. & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**, 144–158.

StatSoft Inc. (2012). *Statistica v.12 for Windows*. Tulsa, USA: Statsoft (software).

Teixeira, L.N., Stringheta, P.C. & Oliveira, F.A. (2008). Comparação de métodos para quantificação de antocianinas. *Revista Ceres*, **55**, 297–304.

Tonon, R.V., Brabet, C. & Hubinger, M.D. (2008). Influence of process conditions on the physicochemical properties of açai (*Euterpe oleraceae* Mart.) powder produced by spray drying. *Journal of Food Engineering*, **88**, 411–418.

Tonon, R.V., Brabet, C., Pallet, D., Brat, P. & Hubinger, M.D. (2009). Physicochemical and morphological characterisation of açai (*Euterpe oleraceae* Mart.) powder produced with different carrier agents. *International Journal of Food Science and Technology*, **44**, 1950–1958.

Tonon, R.V., Brabet, C. & Hubinger, M.D. (2010). Anthocyanin stability and antioxidant activity of spray-dried açai (*Euterpe oleracea* Mart.) juice produced with different carrier agents. *Food Research International*, **43**, 907–914.

Wu, R., Frei, B., Kennedy, J.A. & Zhao, Y. (2010). Effects of refrigerated storage and processing technologies on the bioactive compounds and antioxidant capacities of 'Marion' and 'Evergreen' blackberries. *LWT - Food Science and Technology*, **43**, 1253–1264.

Yamaguchi, K.K.L., Pereira, L.F.R., Lamarão, C.V., Lima, E.S. & Veiga-Junior, V.F. (2015). Amazon acai: chemistry and biological activities: a review. *Food Chemistry*, **179**, 137–151.

Zenebon, O., Pascuet, N.S. & Tiglea, P. (2008). *Métodos Físico-Químicos Para Análise de Alimentos*. Pp. 119–121. São Paulo: Instituto Adolfo Lutz.