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# Effect of freezing and atomization on bioactive compounds in cagaita (*Eugenya dysenterica* DC) fruit

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**Abstract**

The aim of this study was to evaluate the effect of freezing and atomization on bioactive compounds in cagaita fruit. The levels of total phenolics, total flavonoids, condensed tannins, vitamin C, β-carotene, the antioxidant potential assessed by DPPH and ABTS, sugar profile, and mineral profile were all evaluated. High levels of total polyphenols (881.95 mg/100 g), total flavonoids (42.93 mg/100 g) and condensed tannins (67.00 mg/100 g) were detected in atomized cagaita pulp. A higher content of vitamin C was found in fresh cagaita pulp (29.75 mg/100 g), compared to frozen pulp, or atomized pulp, which had levels of 24.64 mg/100 g and 20.38 mg/100 g, respectively. Atomized pulp had the highest antioxidant activity as assessed using the ABTS method (517.04 μmol Trolox/g), compared with frozen pulp (357.73 μmol Trolox/g) and fresh cagaita pulp (276.07 μmol Trolox/g). The drying method demonstrated the best performance with respect to fruit preservation.

**Keywords:** *Eugenia dysenterica* DC; spray drying; conservation methods; bioactive compounds.

**Practical Application:** Conservation methods for new fruit, in particular fruit from the savannah, are important so that high value nutritional foods can be provided to the food industry. The development of new food products with high added value has become a challenge for the food industry, since the nutritional quality of the same, will depend on the raw material used, contributing in the preservation of savannah fruits.

## 1 Introduction

Cagaita (*Eugenia dysenterica* DC) is an economically important fruit belonging to the Cerrado biome. It can be used as a raw material in the production of jams, juices, jellies, amongst other foods (Martinotto et al., 2008). The high perishability and seasonality of cagaita fruit has encouraged the development of technological processes to improve its use and increase its post-harvest shelf life. Among these processes, the production of frozen pulp, which is an important agro-industrial activity, stands out in particular since it adds economic value to the fruit, minimizing losses that may occur during marketing of the fresh product, and allowing for the extension of shelf-life while maintaining the fruit's nutritional quality. The production of frozen pulp results in a reduction in the rate of degradative reactions (Evangelista & Vieites, 2006) thereby prolonging shelf-life. Spray-drying, also known as atomization, is another method of fruit preservation, that involves three phases, namely: the gas phase (drying air), the liquid phase (droplets), and the solid phase (particles). The atomization process for food, initially in a liquid or paste form, involves the use of continuous flow of hot air to promote the rapid evaporation of water. This dynamic process allows for the drying of heat-sensitive products, reducing losses in fruit quality (Mezhericher et al., 2010).

A knowledge of the nutritional value of native fruit is vital, especially because of the need to make available to the population a greater variety of nutrients and bioactive compounds. In addition, increased productivity and better conservation of fruit not only increases their availability in the market-place, but also contributes to the conservation of the Cerrado biome, by encouraging its exploitation in a sustainable manner (Silva et al., 2013).

Given the above, and in order to add value to fruit from the Brazilian Cerrado by extending post-harvest shelf-life, this study aimed to evaluate the effect of freezing and atomization of cagaita pulp on the levels of bioactive compounds, compared to those in fresh pulp.

## 2 Materials and methods

### *2.1 Fruit acquisition*

Mature cagaita fruit (*Eugenia dysenterica* DC) were harvested in the morning from plants grown in the experimental area of the School of Agronomy, Federal University of Goiás, Brazil. After collection, the fruit was transported in low-density polyethylene bags (LDPE) to the Laboratory of Vegetables at the same institution, where fruit were selected for use in the study.

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The fruit were washed in water and sanitized with a sodium hypochlorite solution (200 mg $L^{-1}$) for 20 minutes. Following this, the fruit were divided into two groups; the first group was composed of fresh fruit that was submitted to physical and chemical analyses. The second group was processed for freezing and atomization before the same analyses.

*Preparation of frozen and atomized pulp*

Frozen pulp

Pulp was obtained by mechanical agitation of the fruit using a fruit stripper (Bonina, 025DFA8 model, Itabuna city – BA), and, immediately afterwards the fruit was packed in 500 g capacity low-density polyethylene bags (LDPEs), and rapidly frozen by placing into an ultra-freezer at -40 °C in order to preserve the physical and chemical characteristics of the fruit.

Atomized pulp

For the atomization process, 41 kg of cagaita pulp (crop 2013/2014), previously collected from a native area with a typical cerrado formation, located in the city of Abadia-GO, latitude −16°45'26" and longitude −49°26'15". The frozen pulp was thawed in a refrigerator (7-8 °C) for seven days. After thawing, the pulp was sieved twice, (M18, 40 cm, mash 3) to reduce pulp viscosity in order to facilitate spraying. The resulting yield of 31.21 kg of pulp was transported to the Laboratory of Pharmaceutical Chemistry Education, University of Brasilia-DF/Brazil, for atomization.

Prior to initiating the atomization process, the pulp was transferred to a beaker and a carrier agent (10% gum arabic) was added, after which the mixture was placed on a magnetic stirrer for 24 h to maintain a homogeneous suspension. Atomization was performed using a Spray-dryer atomizer ($LM_{MSD}$ 1.0, LABMAQ do Brasil Ltda, Nº de série: $LM_{MSD}$ 102010), under the following conditions: flow of 8.5 mL/min, inlet and outlet temperature of 175 °C ± 10 °C and 110 °C ± 10 °C, respectively. The atomized product was packed in LDPE bags and in polyester bags with polyethylene, ESP007z - Stand up - Silver with zipper, metallized structure with the following dimensions: 230 mm (height) × 185 mm (width) × 90 mm (Sanf) for subsequent physical and chemical analyses.

*Physical and chemical analyses*

Physical and chemical analyses were performed in triplicate using fresh cagaita (control) and frozen and atomized pulps. The following analyses were performed.

Water activity

The water activity was determined using an Aqualab apparatus (Dew Point model, Binh Thanh Dist., Ho Chi Minh City, Vietnam) at 25 °C.

Moisture content

The moisture content was determined using a drying oven at 105 °C until a constant weight was achieved, as described by the Association of Official Analytical Chemists (2010).

Vitamin C and β-carotene content

The vitamin C and β-carotene contents were determined using high performance liquid chromatography (HPLC) equipped with an RP-18 Lichrospher 100 chromatography column (dimensions; 250 × 4 mm, 5 μm). The eluate was analyzed using a diode array detector (Shimadzu SOD-M10 AVP, Kyoto, Japan) with a wavelength set for each vitamin (i.e. provitamin A (β-carotene) and vitamin C) to be analyzed, with each one being identified based on their known retention time, as described by Ball (2006).

Determination of total polyphenols

About 5 g of either fresh cagaita or frozen pulp, or 1 g of atomized pulp, were added to 40 mL of 50% methanol and incubated for 1 hour in the absence of light. Total polyphenols were then measured using the Folin-Ciocalteu method as described by Roesler et al. (2007). The data were expressed as mg of gallic acid equivalents (GAE) per 100 g of sample.

Total flavonoids

About 5 g of fresh cagaita or frozen pulp, or 1 g of atomized pulp, were used to assess total flavonoids according to the method described by Lees & Francis (1982). The data were expressed as mg per 100 g of sample.

Condensed tannins

One g of each sample was added to 10 mL of 70% acetone, and the extract stirred for 3 min, after which it was allowed to rest for 60 min followed by stirring for another 3 min. The condensed tannin content was determined using the vanillin method according to Broadhurst & Jones (1978). The data were expressed as mg of equivalent catechin per 100 g of sample.

DPPH assay

About 5 g of fresh cagaita or frozen pulp, or 1 g of atomized pulp, were added to 40 mL of 50% methanol, 40 mL of 70% acetone, and the volume was filled to a final volume of 100 mL with distilled water. The DPPH assay followed the protocol of Brand-Williams et al. (1995), with modifications according to Sanchez-Moreno et al. (1998), and the results were expressed as $EC_{50}$ g sample/g DPPH.

ABTS assay

One g of each sample was added to 40 mL of 50% methanol and homogenized, after which the homogenate was incubated at room temperature for 60 minutes in the dark. The solution was then centrifuged at 15,000 rpm for 15 minutes and the supernatant filtered into a 100 mL volumetric flask.

Forty mL of 70% acetone was then added to the extract and following homogenization the extract was incubated for 60 minutes at room temperature in the dark. The solution was centrifuged again at 15,000 rpm for 15 minutes and the supernatant was filtered back into the same volumetric flask and the volume was filled to 100 mL with distilled water.

The extract (approximately 30 µL) was added to test tubes containing 3 mL of the ABTS $^{+}$ radical and homogenized. The absorbance was read at at 734 nm after 6 minutes. Ethanol was used to calibrate the spectrophotometer (Shimadzu Cooperation 05441 – serial A116353, Kyoto, Japan).

The calibration curve used to express the results was $y = 0.010x + 0.009$ with $R^2 = 0.998$, and the results were expressed as µmol Trolox/g of sample.

Mineral profile

The mineral composition of the different fruit samples, specifically the phosphorus, calcium, copper, iron, manganese, potassium, magnesium, zinc, sodium, and sulfur contents were determined using methodologies described by Malavolta et al. (1997).

Sugar content: identification and quantification of sugars by HPLC-RID

The sugar content of the different fruit samples was determined by HPLC according to the method described by Macrae (1998), using an HPLC Shimadzu apparatus (Shimadzu Cooperation Analytical & Measuring Instruments Division Kyoto, Japan), composed of a refractive index detector (Model RID-10A), a pump (Model LC-20AD), an autosampler (SIL-20A Model HT), an oven (Model CTO-20A), and the LC Solution software (Shimadzu). Sugars were separated on a Shimadzu column (CLC $NH_2$ (M), 15 cm × 6.0 mm), with amine groups chemically bonded to silica, and a pre-column (Shimadzu CLC-ODS). The sample analysis method used a mobile phase containing 75% acetonitrile at a flow rate of 1.3 mL/min, an injection volume of 30 µL, a temperature of 40 °C, and a run time of 20 min. Sugar identification was performed by means of the refraction index provided by the RID detector compared with standards (sugar content). The results were expressed as % sugar on a wet basis. Fructose, glucose, and sucrose were used as standards.

### *2.2 Statistical analysis*

The experiment was conducted in a completely randomized design (CRD) in a 3 × 4 factorial design, namely three products (fresh cagaita, frozen pulp, and atomized pulp) and four replicates. For comparison of means, an analysis of variance (ANOVA) and Tukey test were performed, using the R software. The level of significance for differences among the means was set at 5% ($p < 0.05$).

## 3 Results and discussion

The data pertaining to the levels of bioactive compounds, sugars (reducing and non-reducing), moisture content, and water activity are shown in Table 1. When compared to fresh and frozen pulp, atomized pulp had significant levels of total polyphenols (881.95 mg/100 g), total flavonoids (42.93 mg/100 g), condensed tannins (67.00 mg/100 g), ABTS antioxidant activity (517.04 µmol Trolox/g) and DPPH scavenging activity ($EC_{50}$ 8.94 g/g DPPH). These data may have been obtained as a result of the spray dryer method used, since during this process water is eliminated and hence compounds become more concentrated. Bennett et al. (2011) have reported that atomization is one of the most efficient drying techniques, which can produce end products with a high content of bio-active compounds. With respect to the atomized pulp here, its polyphenol content was lower than that found by Daza et al. (2016) who studied the effect of drying using a spray dryer on the physical properties of cagaita fruit extracts, and found values ranging from to 1630-4710 mg/100 g in cagaita powders stored at 25 °C for 120 days. The difference between this study and ours could be related to the species analyzed, the soil type, and the climate conditions, which can directly interfere with the content of polyphenols. Factors such as season, climate, soil composition, maturity stage, preparation, processing, and storage conditions can also directly influence the concentrations of total flavonoids (Kim et al., 2003). In fact, the flavonoid content was reduced (31%), whereas total phenolics and condensed tannins increased (28%) upon freezing. This reduction in flavonoid content was also reflected in the antioxidant activity (ABTS - 23% reduction) upon freezing the

**Table 1**. Levels of bioactive compounds, sugar content, moisture content, and water activity of fresh cagaita, frozen cagaita pulp, and atomized cagaita pulp.

| Analyses | Fresh cagaita | Frozen pulp | Atomized pulp |
|---|---|---|---|
| Total polyphenols (mg/100 g) | 134.88 ± 4.53c | 171.76 ± 2.27b | 881.95 ± 8.92a |
| Total flavonoids (mg/100 g) | 7.07 ± 0.22b | 4.86 ± 0.24b | 42.93 ± 3.40a |
| Condensed tannins (mg/100 g) | 16.62 ± 1.86b | 21.30 ± 4.31b | 67.00 ± 4.28a |
| Vitamin C (mg/100 g) | 29.75 ± 1.33a | 24.64 ± 2.41b | 20.38 ± 1.84b |
| β-carotene (µg/100 g) | 88.67 ± 4.63a | 91.63 ± 5.39a | 86.68 ± 4.57a |
| ABTS (µmol Trolox/g) | 357.73 ± 8.03b | 276.07 ± 27.05c | 517.04 ± 31.47a |
| $EC_{50}$ (g/g DPPH) | 5.16 ± 0.01a | 6.13 ± 0.02a | 8.94 ± 0.89b |
| Fructose (%) | 47.18 ± 2.45b | 54.76 ± 0.78a | 12.84 ± 0.41c |
| Glucose (%) | 42.68 ± 1.57b | 45.09 ± 0.85a | 5.55 ± 0.11c |
| Sucrose (%) | 2.02 ± 0.10 | ND | ND |
| Moisture (%) | 89.84 ± 0.13a | 89.65 ± 0.11a | 6.30 ± 0.26b |
| Water activity | 0.989 ± 0.002a | 0.996 ± 0.002a | 0.236 ± 0.013b |

Means followed by the same letter on the same row do not differ by the Tukey test at 5% probability. % = g/100 g; ND = not detected.

cagaita pulp. Moreover, this decrease in flavonoids is similar to the 35% reduction in total phenolic compounds (as measured by the Folin-Ciocalteau method) in a spray dried ethanol extract of cagaita pulp observed by Daza et al. (2016).

The difference between fresh pulp and frozen pulp with respect to total polyphenols, tannins, fructose and glucose, may be associated with the synthesis of these compounds, since the frozen pulp was not pasteurized to inactivate enzymes, which could lead to differences between the fruit treatments studied here.

With respect to the vitamin C content, we found that the preservation methods used here caused a notable decrease in vitamin C levels, presumably because vitamin C is highly unstable in the presence of heat and light (Table 1). The differences compared to the control could be related to sample processing, both for freezing and for atomization, facilitating the degradation of vitamin C present in the samples. According to Zhang & Hamauzu (2004), the temperature used during the atomization process can be detrimental to the ascorbic acid present, since it is extremely thermo-labile. The vitamin C content of fresh cagaita was however similar to that reported for Goiás (27.46 mg/100 g) (Silva et al., 2008 apud Cardoso et al., 2011). The β-carotene values for cagaita pulp were within the range reported for whole cagaita pulp (110 µg/100 g) (Gomes et al., 2011 apud Ribeiro et al., 2013). Melo & Araújo (2011) have reported that the loss of vitamin C following freezing of fruit is mainly due to exposure to oxygen and light during fruit manipulation. The amounts of vitamin C found both in the frozen or atomized pulp were lower than those found by Oliveira et al. (2011), and Cardoso et al. (2011) in fresh cagaita, who detected values of 40.11 mg/100 g and 34.11 mg/100g, respectively. These differences may be a result of different geographical origins, cultivation area (climate, soil types) and the maturity stage of the fruit, all of which could influence their nutritional value (Chitarra & Chitarra, 2005). Facundo et al. (2015), when studying the effect of storage at low temperature on the color and carotenoid composition of two cultivars of banana, did not observe significant differences, showed values inferior to those found in this experiment.

With respect to the β-carotene content, both preservation methods studied (freezing and atomization) showed no significant losses compared to fresh fruit, which may be related to the stability of β-carotene, and so therefore both are viable preservation methods for maintaining β-carotene levels.

Cardoso et al. (2011) found higher β-carotene values in fresh cagaita (390 mg/100 g) which originated from the northern state of Minas Gerais, Brazil. Charoensiri et al. (2009) and Zanatta & Mercadante (2007) found lower β-carotene values for the guava variety Panseetong (13.8 µg/100 g) and camu-camu (72.8 µg/100 g), both of which belong to the same family of cagaita, namely the Myrtaceae.

The reduction in antioxidant potential assessed by the ABTS method in the frozen pulp may have been due to fruit opening, as a result of physical manipulation, exposing the antioxidants to light, oxygen, and the action of enzymes. The increase in antioxidant potential, assessed by the ABTS method, compared to control is likely to be related to the accumulation of polyphenols, flavonoids, and tannins that were noted previously (Table 1). The antioxidant capacity, assessed using the DPPH method, was low in all of the samples. According to Kim et al. (2003), antioxidant capacity may be influenced by factors such as maturity, species, cultivation practices, geographical origin, growth stage, crop conditions, and storage process, as well as preservation method, and type of carrier agent, which explains the difference in the antioxidant potential of fresh cagaita and its derivatives compared to other fruit and their products. The antioxidant potential (assessed using the ABTS method) of atomized cagaita pulp was lower than that of atomized jabuticaba pulp (gum arabic/maltodextrin) belonging to the same family of cagaita, with temperatures of 140, 160, and 180 °C, which varied with temperature, from 6520 µmol Trolox/g to 9800 µmol Trolox/g, according to Silva et al. (2013). When comparing the results obtained using the atomization method, the levels of vitamin C and phenolic compounds, amongst others were higher than with the other methods. For example Escobedo-Avellaneda et al. (2015), analyzing the effect CUT and HHP on the functional components of oranges showed that some processing conditions caused changes. The AOA and total vitamin C indices of most samples treated with HHP increased and were not efficient for flavonoids and phenolics.

With respect to the sugar content, sucrose was only found in fresh fruit, which suggests that during freezing and thawing of the pulp prior to atomization, the enzyme invertase converts sucrose into fructose and glucose (Leininger & Kilpatrick, 1938). The sugar content of cagaita pulp is comparable to that found in fruit by other researchers (Ribeiro et al., 2013) who analyzed overview about macro end micro components and technological of cagaita (*Eugenia dysenterica* DC). The moisture and water activity of the atomized pulp were within the range reported for a spray dried ethanol extract of cagaita pulp (Daza et al., 2016).

Regarding the mineral profile (Table 2) of fresh cagaita, frozen cagaita pulp, and atomized cagaita pulp, it was noted that the preservation methods were optimal for specific minerals; for example, the atomization process was optimal for Fe, Zn, and Na. The increases observed may be related to the concentration that occurs following the significant removal of water.

For several minerals there were no significant differences between fresh cagaita, frozen cagaita pulp, and atomized cagaita pulp. In this regard, the levels of phosphorus (0.10%, 0.12% and 0.05%); calcium (0.36%, 0.45% and 0.55%); and magnesium (0.10; 0.15% and 0.05%), respectively, were of particular note. Boron

**Table 2**. Mineral profile of fresh caigata pulp, frozen caigata pulp, and atomized cagaita pulp.

| Mineral | Fresh pulp | Frozen pulp | Atomized pulp |
|---|---|---|---|
| K (%) | 1.22 ± 0.10b | 1.64 ± 0.04a | 0.85 ± 0.08c |
| S (%) | 0.03 ± 0.01c | 0.07 ± 0.01b | 0.20 ± 0.01a |
| Na (ppm) | 353.45 ± 6.65b | 347.18 ± 2.22b | 511.33 ± 1.47a |
| Cu (ppm) | 17.75 ± 0.35a | 16.50 ± 0.00a | 8.26 ± 0.35b |
| Mn (ppm) | 3.85 ± 0.35b | 15.15 ± 0.35a | 6.20 ± 1.70b |
| Zn (ppm) | 14.10 ± 0.14a | 15.05 ± 0.49a | 16.60 ± 0.93b |
| Fe (ppm) | 61.55 ± 6.72b | 44.30 ± 3.75b | 112.00 ± 34.08a |

% = g/100 g; 1 = 0.0001% ppm. The mean of samples followed by the same letter on the same row do not differ by the Tukey test at 5% probability.

was not detected in any of the cagaita samples analyzed in this study. Lower levels of minerals were observed by Leterme et al. (2006), who studied the mineral content of various tropical fruit from Colombia that belonged to the same family and genus of cagaita, namely *Eugenia malaccensis* L (jambo-vermelho), *Eugenia stipitata* Mc Vaugh (Araça-boi), *Eugenia uniflora* L (pitanga), who reported calcium levels of 0.015%, 0.025%, and 0.048%, respectively; phosphorus levels of 0.006%, 0.007%, and 0.028%, respectively; potassium levels of 0.164%, 0.078%, and 0.165% (notably higher than fresh cagaita), respectively; magnesium levels of 0.025%, 0.009%, and 0.038%, respectively; and sulfur levels of 0.009%, 0.014%, and 0.015%, respectively. These differences may be due to different climate conditions, types of soil, degree of fruit maturation, amongst other factors.

## 4 Conclusion

Among the different drying methods, spray-drying gave the best performance, preserving the total phenolic, total flavonoid and tannin contents, while increasing the antioxidant potential (ABTS). The food preservation methods studied in this experiment were not however effective for the preservation of vitamin C.

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