

Effect of Drying Method in the Maintenance of Bioactive Compounds and Antioxidant Activity of Feijoa Pulp (*Acca sellowiana*)

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Abstract:

The aim of this work was to evaluate the bioactive compounds contents and the antioxidant activity preserved in the obtained powders of dehydrated feijoa pulp after two drying methods applied: Spray drying and Freeze-drying methods. Ascorbic acid, total phenolic and total flavonoids content, antioxidant activity (by DPPH, ABTS and FRAP methods) were determined and the obtained data were evaluated using ANOVA and Tukey test ($p < 0.05$). Spray drying method produced powders with higher amounts of total phenolic and total flavonoid contents, while Freeze-drying method presented higher amounts of ascorbic acid and higher antioxidant activity. Both applied methods were effective in maintaining the antioxidant properties of Feijoa fruits.

Keywords: antioxidant; freeze-drying; phenolics; spray-drying; vitamin C

1. Introduction

Feijoa (*Acca sellowiana*) is a plant from Myrtaceae family, native from Southern Brazil, Paraguay, Argentina and Uruguay [1]. Feijoa fruits have oval shape, green color even when ripe, and vary in their aspect between smooth and rough skin [2]. Feijoa presents several bioactive properties, due to the high amounts of Vitamin C, phenolics, flavonoids, terpenes, tannins, among others [3]. These substances are recognized as natural antioxidants, acting as Reactive Oxygen Species eliminators in the human organisms, preventing the oxidative damage on cellular tissues [4, 5].

Although Feijoa fruit has presented high amounts of bioactive compounds, the fruits are climacteric and present high levels of ethylene production. These characteristics result in the fast ripening, making it difficult to preserve the quality of the fruits for extended times after

harvest. The storage time of Feijoa fruit is about four weeks under refrigeration. After this period, the pulp starts to brown, have alterations in flavor and lose its nutritional quality [6, 7, 8].

In view of the fast ripening of Feijoa fruits, Spray drying, and Freeze-drying methods bring alternatives for the conservation of taste, aroma and nutritional value of Feijoa pulp, presenting a solution for the food industry [9, 10].

Spray drying technique is widely used as a tool to maximize the storage time, conversing the pulp into powders of high quality and low water activity [11]. This methodology allows the microencapsulation of sensitive substances, adding a thin cover of solid particles, liquids and dispersions that act as a protector film, protecting the pulp from oxidation, chemical, and microbiological reactions [12].

Freeze drying method is based on the process of sublimation, in which the

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thermosensitive and volatile compounds are frozen and exposed to low temperatures under vacuum. It removes the moisture contained in the pulp, reducing degradation reactions and preserving the biological properties of the pulp [13].

This work aimed to determine the total phenolic, total flavonoids and ascorbic acid contents, and antioxidant activity of Feijoa pulp extract dehydrated by Spray and Freeze drying methods, as well as to identify and quantify their phenolic composition using High-Performance Liquid Chromatography.

2. Results and Discussion

2.1 Bioactive Compounds

The results obtained for total phenolic, total flavonoids, and ascorbic acid content of methanolic extracts of dehydrated Feijoa pulp are presented in Table 1.

Table 1. Total phenolic compounds (TPC), total flavonoids (TFC), and ascorbic acid (Vit C) amounts of methanolic extracts from Feijoa pulp dehydrated by Spray and Freeze drying methods.

Drying Method	TPC (mg GAE g ⁻¹)	TFC (mg QE g ⁻¹)	Vit C (mg 100 g ⁻¹)
Spray drying	67.39 ^a ±4.70	78.78 ^a ±6.56	21.94 ^b ±0.07
Freeze drying	49.45 ^b ±0.01	7.80 ^b ± 0.82	38.09 ^a ±3.46

Values expressed as mean ± SD (n = 3). Means followed by equal letters in the same column did not differ statistically from each other (p < 0.05) by Tukey test; GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent

Spray drying method showed to be more efficient than Freeze drying method on the preservation of total phenolic and total flavonoid contents (p < 0.05). On the other hand, Freeze-drying method showed to be a better method to preserve the levels of ascorbic acid (p < 0.05). Although the inlet temperature of the Spray dryer equipment is high, the process installs almost instantaneously and, therefore; the losses of bioactive compounds due to drying are associated with the outlet temperature (85 °C), which is lower and does not influence on the degradation of phenolic compounds [22].

However, ascorbic acid is more thermosensitive to this temperature than phenolic compounds, fact that explain the higher loss of this compound when the Spray drying method was applied. Regarding to the Freeze-drying method, the losses in this process are associated with the grinding of the material after the drying process [23, 24]. The lower amounts of total phenolic compounds and total flavonoids content in Freeze dried powder may also be attributed to the low temperatures and the use of vacuum in the process. Another possible explanation for these results is the long drying time required by the Freeze-drying technique.

Nonetheless, Freeze-drying is considered a gentle technique, since lower temperatures are used in this drying process, as several studies have reported lower losses of vitamin C in Freeze dried products compared to Spray dried ones [9, 26, 27].

Rezende, Nogueira and Narain [25] determined the concentration of total phenolic and ascorbic acid contents in extracts obtained from acerola (*Malpighia emarginata* DC) dried pulp and residue using Spray and Freeze drying methods. The authors observed the same trend in the retentions of these compounds: higher amounts of total phenolic compounds in the treatments with Spray drying and higher levels of ascorbic acid in the treatments with Freeze drying.

Saikia, Mahnot and Mahanta [28] used Spray drying and Freeze-drying methods to preserve the phenolic compounds extracted from *Averrhoa carambola* pomace. Their results showed higher amounts of phenolic compounds when the Spray drying method was applied.

No reports have been found regarding to the comparison between spray and freeze drying methods applied to Feijoa pulp.

Pasquariello et al. [2] obtained total phenolic compounds amounts between 0.10 and 2.51 mg GAE g⁻¹ and total flavonoids content values between 0.14 and 0.33 mg CE g⁻¹ for twelve different cultivars of fresh Feijoa pulp from several countries. Tuncel and Yulmaz [29] presented total phenolic compounds values between 7.6 and 16.2 mg GAE g⁻¹ for Feijoa pulp hydroalcoholic extract. One of the decisive factors on the total phenolic compounds and total

flavonoids amounts is the maturation state of the fruits. According to Cecilia *et al.* [30], the higher the maturation state of Feijoa, the lower the content of total phenolic compounds.

Krishnaiah *et al.* [31] developed an optimization process of Spray drying conditions of *Morinda citrifolia* L. fruit extract, obtaining total phenolic compounds values (18.00 to 54.00 mg TAE g⁻¹) and total flavonoids content (21.00 to 45.00 mg CE g⁻¹), expressed in mg of tannic acid equivalent and catechin equivalent, respectively.

Barbosa *et al.* [27] presented ascorbic acid values of 6.2 and 6.5 mg 100 mL⁻¹ in orange powders obtained by Freeze and Spray drying methods, respectively. Marques, Silveira and Freire [9] obtained ascorbic acid value of 19.72, 30.00, 33.29 mg 100 g⁻¹ for Freeze dried mango, pineapple and guava pulp, respectively.

The development of microparticles with higher concentrations of phenolic compounds from the use of the Feijoa pulp has a wide range of applications in food preparation and food industry. These products can be considered highly nutritious, since they are excellent sources of vitamin C.

2.2 Antioxidant Activity

The antioxidant activity of the samples according to the drying methods employed are shown in Table 2.

Table 2. Antioxidant activity (DPPH, ABTS and FRAP) of dehydrated Feijoa pulp methanolic extracts by Spray and Freeze drying methods.

Drying Method	DPPH (μmol de Trolox g ⁻¹)	ABTS (μmol Trolox g ⁻¹)	FRAP (μmol Fe ²⁺ g ⁻¹)
Spray drying	106.00 ^a ±1.51	315.36 ^a ±4.72	756.90 ^a ±10.75
Freeze drying	77.44 ^b ±0.22	17.49 ^b ±0.02	578.43 ^b ±33.58

Values expressed as mean ± SD (n = 3). Means followed by equal letters in the same column did not differ statistically from each other by Tukey test (p < 0.05).

Comparing the applied methods, the sample dried by Spray drying method presented higher antioxidant activity, while the Freeze-drying method produced powders with higher ascorbic acid content. Thus, the higher antioxidant

potential may be related to the presence of phenolic compounds, conferring greater protection against oxidation, showing a positive correlation, since an increase in the concentration of these compounds increases the antioxidant activity when compared both drying methods.

The obtained results suggest the importance of testing different methods for the safe and conclusive determination of the antioxidant activity, since each method has its own specificity and acts at a particular site of action. In general, it was observed that the Freeze dried powder showed lower antioxidant activity compared to the Spray dried powder, indicating that the method was efficient and satisfactory.

Souza and Nunes [34] obtained DPPH values of 14.6 μg Trolox mL⁻¹ for Feijoa leaves aqueous extract. Tuncel and Yulmaz [29] showed ABTS values between 63.4 and 125.5 mmol Trolox g⁻¹ for fresh Feijoa fruit. Pasquariello *et al.* [2] presented antioxidant values between 1.41 and 2.82 (μmol TE g⁻¹) for different cultivars of Feijoa pulp from several countries.

Several studies have been shown that Feijoa is rich in phenolic compounds with high antioxidant activity. Isobe *et al.* [35] evaluated the antioxidant activity of Feijoa and other tropical fruits ethanolic extracts and verified that Feijoa showed the highest content of phenolic compounds and antioxidant activity among the studied fruits. Moreover, in addition to the high values for Feijoa pulp, other parts of the plant, such as skin and leaves, also showed great bioactive properties [36].

Koley *et al.* [39] evaluated the antioxidant activity in Freeze dried fruit genotypes of Indian jujube (*Zizyphus mauritiana* Lamk.) and found values between 15.18 and 29.69; 12.74 and 29.45; and 7.41 and 13.93 μmol Trolox g⁻¹ applying DPPH, ABTS and FRAP methods, respectively.

Ramírez, Giraldo and Orrego [40] explain that the use of high temperatures in Spray drying method presents a risk for the bioactive compounds. However, this technique does not cause a drastic degradation of polyphenols, contributing to the high antioxidant activity of the obtained powders.

Although Freeze-drying is considered a low

nutrient degradation drying method due to low temperature, some losses of bioactive compounds and volatile substances occur mainly during the sublimation stage of ice [25]. In the sublimation phase, compounds with vapor pressure higher than water molecules are excluded and evaporated from the frozen materials when the sample matrix exceeded its glass transition temperature [41,42], leading to lower antioxidant activity when compared to Spray drying method.

2.3 HPLC-DAD-UV-Vis profile

Chromatography analysis allows to separate, identify and quantify phenolic compounds present in the sample [43]. Thus, High-Performance Liquid Chromatography of Feijoa pulp extracts was carried out using nine standards (gallic acid, caffeic acid, syringic acid, trans-cinnamic acid, coumaric acid, catechin, epicatechin, rutin and quercetin) for phenolic acids and flavonoids identification in the samples.

The Feijoa pulp extracts, after undergoing Spray drying and Freeze drying processes, were separated and identified by RP-HPLC profile analysis. Three flavonoids: rutin ($52.45 \mu\text{g g}^{-1}$), quercetin ($28.82 \mu\text{g g}^{-1}$), and catechin ($81.72 \mu\text{g g}^{-1}$) as well as a phenolic acid: trans-cinnamic acid ($32.98 \mu\text{g g}^{-1}$) were obtained from the methanolic extract of Spray dried Feijoa pulp.

The methanolic extracts of Freeze dried Feijoa pulp did not show phenolic compounds under the studied conditions.

Figure 2 shows the chromatogram of the identified compounds in Feijoa pulp extracts dehydrated by Spray drying method.

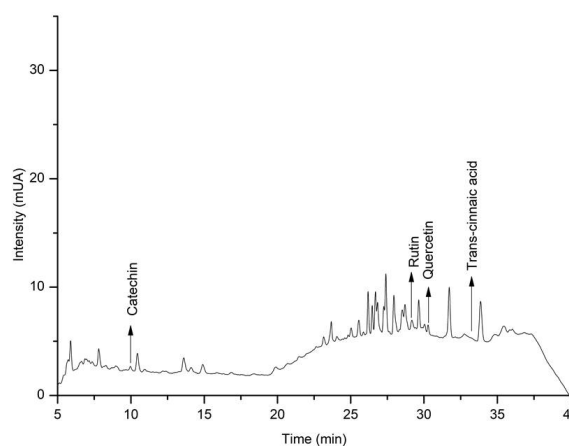


Figure 2. Chromatogram of compounds identified in Spray dried Feijoa pulp methanolic extracts.

The calibration curves obtained using the standards gave rise to the regression equations applied to calculate the concentrations of the compounds identified in the extract samples. Table 3 shows the retention time, regression equation, and coefficient value of the three flavonoids and phenolic acid identified in the samples.

Tabela 3. Wavelength (λ), retention time (R.T.), regression equation and determination coefficient (R^2) for each identified phenolic compound in Feijoa pulp dried by Spray drying method.

Phenolic Compounds	λ (nm)	T.R. (min)	Equation regression	R^2
Trans-cinnamic acid	275	32.04	$y = 6.211x - 13.051$	0.9625
Catechin	276	10.00	$y = 0.1794x - 0.1560$	0.9311
Rutin	256-355	24.73	$y = 0.355x - 0.7723$	0.9551
Quercetin	254-371	30.20	$y = 0.9346x - 1.0338$	0.9616

It is important to point out that may there are other types of phenolic compounds present in Spray dried Feijoa pulp, in addition to the standards used.

Monforte *et al.* [44] determined the phytochemical composition of Feijoa varieties and identified catechin (385.3 to 613.1 mg kg^{-1}), quercetin (3.7 to 12.3 mg kg^{-1}), rutin (4.7 to 15.7 mg kg^{-1}), and other compounds, such as eriodictiol, pyrocatechol, eriocitrin, ellagic acid,

gallic acid and syringic acid.

Other Myrtaceae family fruits were studied by Golçalves, Lajolo and Genovese [45] as to the presence of flavonoids in their composition. Quercetin and kaempferol were identified in Cambuci ($21.6 \text{ mg } 100 \text{ g}^{-1}$ and $0.4 \text{ mg } 100 \text{ g}^{-1}$), Araçá-boi ($14.4 \text{ mg } 100 \text{ g}^{-1}$ e $2.5 \text{ mg } 100 \text{ g}^{-1}$), Camu-camu ($42.0 \text{ mg } 100 \text{ g}^{-1}$ and $2.1 \text{ mg } 100 \text{ g}^{-1}$), and Araçá ($40.0 \text{ mg } 100 \text{ g}^{-1}$ and $0.7 \text{ mg } 100 \text{ g}^{-1}$), respectively.

Corroborating the findings of this study, other authors have identified quercetin in Jaboticaba ($0.04 \mu\text{g g}^{-1}$), Pitomba ($0.04 \mu\text{g g}^{-1}$), Camu-Camu (0.24 mg g^{-1}), Jambo ($0.01 \mu\text{g g}^{-1}$), Jamelão ($0.01 \mu\text{g g}^{-1}$), Pitanga ($20.06 \text{ mg } 100 \text{ g}^{-1}$), and Cherry ($0.04 \mu\text{g g}^{-1}$). In addition, rutin was identified in Jaboticaba (0.21 mg g^{-1}), Pitomba (0.18 mg g^{-1}), Camu-Camu ($0.13 \mu\text{g g}^{-1}$), Cereja (0.48 mg g^{-1}) and Jamelão (0.13 mg g^{-1}), being all these fruits also belonging to the Myrtaceae family [46, 47].

Considering the thermal stability of the phenolic compounds, the loss of some polyphenols in the Spray dried powder produced at temperatures of $150 \text{ }^\circ\text{C}$ was probably due to the release and oxygen decomposition of the phenolic molecules through the cracked walls of the microspheres obtained at those temperatures.

3. Material and Methods

3.1 Materials

Acca sellowina fruits were obtained in the city of Agua Doce, state of Santa Catarina, Brazil, in April 2017. The pulp was manually extracted, triturated in a food processor and passed through a sieve to remove the seeds. Maltodextrin 20 DE (Dextrose Equivalent) was employed as encapsulating agent (DAXIA, São Paulo). For total phenolic compounds, total flavonoids content, DPPH, ABTS and FRAP analysis, the used solvents were all analytical grade, and standards were all obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

3.2 Preparations of emulsions

Maltodextrin solution was prepared by dissolving 10% (w/V) in distilled water at $80 \text{ }^\circ\text{C}$ under stirring until complete dissolution. After cooling to room temperature, the solution was added to Feijoa pulp (1:1) under stirring to obtain a content of 11.4% of total soluble solids.

3.3 Microencapsulation by Spray drying method

For the microencapsulation of Feijoa pulp, a bench Spray dryer (Model B-290 Buchi, Flawil,

Switzerland) was employed. The process was conducted in co-current flow using spray nozzle double fluid of 0.7 mm diameter with a maximum air-drying flow of $35 \text{ m}^3 \text{ h}^{-1}$, compressed air flow of 473 L h^{-1} and atomization relative pressure of 7 bar. The inlet air temperature was set at $150 \text{ }^\circ\text{C}$.

3.4 Freeze-drying method

Part of the Feijoa pulp was frozen in ultra-freezer (Model IULT 205D Indrel Scientific – Londrina, Brazil) at $-40 \text{ }^\circ\text{C}$ for following Freeze-drying, which was performed using a bench Freeze-dryer (Model L101 Liotop, São Carlos, Brazil) for 72 h.

3.5 Rupture of microcapsules obtained by Spray drying

For the microcapsules rupture, 0.5 g of the powder was added to 15 mL of 80% methanol (V/V) acidified with 0.1% HCl. The mixture was stirred using a vortex apparatus (Model AP56, Phoenix, São Paulo, Brazil) for 2 minutes, placed into an ultrasound equipment (Model Cuba 2.5-25, Spencer, São Paulo, Brazil) for 15 minutes and then centrifuged at 6000 rpm for 5 min at room temperature. The release of the phenolic compounds from the microcapsules followed the method proposed by Robert *et al.* [14], with modifications. The supernatant was used for later analysis with the microencapsulated Feijoa pulp obtained by Spray dryer.

3.6 Methanolic extraction of Freeze-dried Feijoa pulp

The extraction of Freeze dried pulp was performed adding 3 g of dried Feijoa pulp into 30 mL of 80% methanol (V/V) and placed in heating bath for 30 minutes at $60 \text{ }^\circ\text{C}$ [15]. The extract was passed through a qualitative filter paper and used for later analysis.

3.7 Total phenolic compounds content

The Total Phenolic Compounds (TPC) was determined according to Castro *et al.* [16], using Folin-Ciocalteu method. The assay consisted of the addition of 2.5 mL of 10% Folin-Ciocalteu

reagent into 0.5 mL of diluted samples and 2 mL of 4% sodium carbonate. The tubes were kept in the dark for 2 hours until the reading at 740 nm. The results were expressed as gallic acid equivalent (mg GAE g⁻¹).

3.8 Total flavonoids content

For the Total Flavonoid Content (TFC) evaluation, the methodology described by Felhi *et al.* [17] was applied. The tests were carried out adding 0.5 mL of the samples into 4.3 mL of 80% ethanol (V/V), 0.1 mL of 0.5 mol L⁻¹ aluminum nitrate and 0.1 mL of 1 mol L⁻¹ potassium acetate. The mixture was protected from light for 40 minutes and the absorbance was then read at 415 nm and the results were expressed as Quercetin equivalent (mg QE g⁻¹).

3.9 Ascorbic acid (Vit C) content

The ascorbic acid content of Feijoa pulp samples was determined by titration with 1% iodine solution, using 1% starch solution as indicator. Approximately 5 g of each sample was added to 25 mL of distilled water and 10 drops of the starch indicator solution. This mixture was then titrated with the iodine solution until the final point was reached [18]. The results were expressed as mg of ascorbic acid per 100 g of sample.

3.10 Antioxidant activity

DPPH radical scavenging

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) was determined according to Alothman, Bhat and Karim [19], through the addition of 0.5 mL of the samples into a test tube containing 3.0 mL of absolute ethanol and 0.3 mL of 0.5 mM DPPH radical. The absorbance was verified at 517 nm after 30 minutes reaction. The reduction of the radical DPPH to hydrazine in the presence of an antioxidant compound was verified through UV-VIS spectroscopy due to the change in the color of the mixture from violet to yellow. The results were expressed as Trolox equivalent (μmol TEAC g⁻¹).

ABTS radical scavenging

2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging was also expressed using a Trolox standard curve according to Floegel *et al.* [20]. ABTS radical formation occurs during the reaction of the 7 μmol ABTS aqueous solution with 140 mM potassium persulfate diluted in absolute ethanol until de absorbance of 0.700 at 734 nm. The analysis was performed adding 0.03 mL of the samples into 3 mL of ABTS solution kept in the dark for 6 minutes and the absorbance was read at 734 nm using a UV-VIS spectroscopy

Ferric reducing antioxidant power (FRAP)

FRAP analysis was performed according to Rufino *et al.* [21], through iron reduction. FRAP reagent was prepared at the time of the assay by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of TPTZ solution (10 mM TPTZ into 40 mM HCl) and 2.5 mL of 20 mM FeCl₃ in aqueous solution. For the FRAP determination, 0.1 mL of samples were added into 3 mL of FRAP reagent and placed in a water bath at 37 °C for 30 minutes. The absorbance was read at 595 nm using a UV-VIS spectroscopy and the results were expressed as μmol Fe²⁺ g⁻¹.

3.11 HPLC-DAD-UV-Vis profile

The analysis of the phenolic compounds profile of the Spray dried and Freeze dried Feijoa pulp extract were performed using a C-18 (250 mm x 4.6 mm, 5 μm) column and a liquid chromatograph (Varian Inc. Walnut Creek, CA US) with a flow rate of 1.0 mL min⁻¹ coupled to a photodiode arrangement detector (HPLC / PDA), composed of quaternary pumps, a self-injection system with diode arrangement detector (DAD). The samples were filtered through 0.45 μm membranes (Millipore, Massachusetts, USA) and the injected volume was 10 μL. The mobile phase consisted of a mixture of 95% acetic acid solution (eluent A) and 5% acetonitrile (eluent B) in isocratic mode. Nine standards (gallic acid, caffeic acid, syringic acid, trans-cinnamic acid, coumaric acid, catechin, epicatechin, rutin and quercetin) were used to identify and quantify the phenolic acids and flavonoids in the samples.

3.12 Statistics

All the trials were performed in triplicate and the data were evaluated employing analysis of variance (ANOVA). The means were compared by the Tukey test, considering the level of significance of 95% ($p < 0.05$), using the STATISTICA software version 12.0 (StatSoft, USA) and the ASSISTAT software version 7.6.

4. Conclusions

The Spray drying method showed to be more efficient in the retention of phenolic compounds, while ascorbic acid was better extracted employing the Freeze-drying method. The Feijoa pulp dehydrated by Spray drying presented higher antioxidant activity by the three tested methods. The atomized dry powder presented a profile of phenolic compounds and it was possible to identify and quantify trans-cinnamic acid, catechin, rutin and quercetin. The Spray drying of the feijoa pulp proved to be a viable option to maintain the bioactive compounds of Feijoa pulp, presenting greater antioxidant potential for its use as functional food and nutraceuticals.

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