

**Non-fermented and fermented Jabuticaba (*Myrciaria cauliflora* Mart.) pomaces as valuable sources of functional ingredients**

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**Running title:** Jabuticaba fruit pomace as valuable sources of functional ingredients

## **Abstract**

Jaboticaba (*Myrciaria cauliflora*. Mart) is a highly perishable fruit native to Brazil, which is consumed both fresh and industrially processed in the form of juices, jams, wines and distilled liqueurs. These processing generate large amount of waste by-products, which represent approximately 50% of the fruit weight. The by-products are of interest for obtaining valuable bioactive compounds that could be used as nutraceuticals or functional ingredients. In this study, fermented and non-fermented jaboticaba pomaces were studied regarding their hydrophilic and lipophilic compounds, and antioxidant properties, including: soluble sugars, by HPLC-RI; organic acids, by UHPLC-DAD; phenolics and anthocyanins, by LC-DAD-MS/ESI; tocopherols, by HPLC-FL; fatty acids, by GC/FID. The analytical data demonstrated that jaboticaba pomaces are rich source of bioactive compounds such as tocopherols, polyunsaturated fatty acids and phenolic compounds (namely hydrolyzable tannins and anthocyanins) with antioxidant potential. Therefore, jaboticaba pomaces may have good potential as functional ingredients in the fabrication of human foods and animal feed.

**Keywords:** Jaboticaba pomace, by-products, phytochemical, functional ingredients, antioxidant capacity

## 1. Introduction

Jaboticaba (*Myrciaria cauliflora*. Mart) is a non-climacteric, highly perishable fruit native to Brazil, and commonly named as Brazilian Grape Tree, Jabotica, Guaperu, Guapuru, Hivapuru, Sabará and Ybapuru. The fruit is a black spherical berry with a thin and fragile peel, and with a white pomace that is slightly acidic and sweet, with an astringent taste. The composition and nutritional value of jaboticaba fruits and products are characterized by their high content of carbohydrates (mainly glucose and fructose), dietary fiber, minerals like iron, calcium and phosphorus, vitamins, and also other bioactive compounds such as ascorbic acid, carotenoids, glycosides and phenolic compounds (Ascheri, Ascheri & Carvalho, 2006a; Ascheri, Andrade, Carvalho & Ascheri, 2006b; Reynertson, Basile, & Kennelly, 2008; Alezandro, Dubé, Desjardins, Lajolo, & Genovese, 2013a; Alezandro, Granato & Genovese, 2013b; Wu, Long, & Kennelly, 2013a; Wu et al. 2013b).

The jaboticaba fruits are consumed both fresh and industrially processed in the form of juices, jams, wines and distilled liqueurs. In the manufacture of jams and fermented products, jaboticaba peels and seeds represent approximately 50% of the fruit, being considered as a waste (Asquieri, Silva & Candido, 2009) and no subsequently used for other productive purposes. However, in recent years there has been great interest to obtain and study natural antioxidants in waste by-products of agribusiness, in order to use them as functional food ingredients or as nutraceuticals, acting in the prevention of degenerative and cardiovascular diseases, and various types of cancers (Guo, Yang, Wei, Li, Xu & Jiang, 2003; Soong & Barlow, 2004; Ajila, Bha & Rao, 2007; Morales, Barros, Ramírez-Moreno, Santos-Buelga & Ferreira, 2014; Morales, Barros, Ramírez-Moreno, Santos-Buelga & Ferreira, 2015). Another important aspect in the potential utilization of waste by-products of agribusiness is the emphasis that has been given to

the identification and purification of new compounds with antioxidant activity, which can act alone or synergistically with other components in preventing the oxidative deterioration of foods. Traditionally, an astringent decoction of the sun-dried jabuticaba's skins has been used in the treatment of asthma and diarrhoea, and gargled for chronic inflammation of the tonsils (Morton, 1987). Moreover, some recent research studies have demonstrated that jabuticaba displays high *in vitro* antioxidant capacity to scavenge free radicals, increases HDL-cholesterol and the antioxidant potential of blood plasma, improves insulin resistance in animal studies, and controls oxidative stress in pathological conditions, which might contribute to prevention of various diseases including neurodegenerative disorders (Leite, Malta, Riccio, Eberlin, Pastore, & Marostica, 2011; Leite-Legatti et al. 2012; Lenquiste, Batista, Marineli, Dragano, & Maróstica, 2012; Wu et al. 2013a; Alezandro et al. 2013b). Additionally, fermented jabuticaba beverages are traditionally used in folk medicine to treat asthma, throat inflammation and gastrointestinal disturbances (Boscolo & Senna-Valle, 2008; Giraldi & Hanazaki, 2010); showing also vasorelaxant capacity, associated with the antioxidant potential of the phenolic compounds present (de Sá et al. 2015).

Nowadays, there is limited information about jabuticaba fruit by-products. There is only some studies about jabuticaba's peel fraction (Gurak, Bona, Tessaro & Marczak, 2014). However, studies about the pomace fraction, which is a waste from the industrial processing of jabuticaba in the form of juices, jams, wines and distilled liqueurs, is basically non-existent. Even though, the pomace fraction contains most of the fruit nutrients and bioactive compounds (Alezandro et al. 2013a; Lima, Corrêa, Alves, Abreu & Dantas-Barros, 2008).

Research studies are required to evaluate the potential use of jabuticaba by-products, such as the pomace, as value-added ingredients for the development of novel functional

foods, such as ready-to-eat (RTE) snacks, breakfast cereals or other food products. With this aim, by-products of fermented and non-fermented jabuticaba fruit, in the form of pomaces, were characterized regarding their phytochemical composition and antioxidant capacity in order to evaluate their potential as functional ingredients that could be used in the fabrication of human foods and animal feed.

## **2. Material and methods**

### *2.1. Standards and reagents*

The fatty acids methyl ester (FAME) standard mixture (standard 47885-U), sugars (glucose, fructose and sucrose) and organic acid standards (L (+)-ascorbic, oxalic, malic, citric and quinic acid) were purchased from Sigma (St. Louis, MO, USA), as also formic and acetic acid. Tocol in n-hexane (50 mg/mL) and tocopherols ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -isoforms) were purchased from Matreya (Plesant Gap, PA, USA). Phenolic compound standards (ellagic acid, gallic acid and quercetin-3-*O*-glucoside) were purchased from Extrasynthese (Genay, France). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Methanol, of analytical grade purity, was supplied by Pronalab (Lisbon, Portugal). HPLC grade acetonitrile (99.9%), n-hexane (97%) and ethyl acetate (99.8%) were purchased from Fisher Scientific (Lisbon, Portugal).

### *2.2. Plant materials*

The Jabuticaba pomace, a by-product of jabuticaba's liquor and juice industries, was provided by the Food Laboratory of the Federal University of Goiás (Goiás, Brazil). The non-fermented and fermented pulp was obtained from the jabuticaba fruit, according to the indicated flow chart in **Figure 1**. The pomace of non-fermented and fermented jabuticaba, was uniformly distributed on trays and dehydrated, in a forced-air

drying oven (Imperial IV Microprocessor Oven., Lab-Line Instruments, Inc., Melrose Park, Ill) at 50 °C, to 7.0% moisture. The dried pomaces were then ground on a Wiley mill (Thomas Scientific, New Jersey, NY, USA) equipped with a 0.5 mm screen and the resulted flour was packed in polyethylene bags and stored under refrigeration (4-5 °C) until use.

### *2.3. Hydrophilic phytochemicals*

*2.3.1. Soluble sugars.* Soluble sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI; Knauer, Smartline system 1000, Berlin, Germany), as previously described by the authors ([Heleno, Barros, Sousa, Martins, Santos-Buelga & Ferreira, 2011](#)). Their identification was carried out by chromatographic comparisons with commercial standards, while their quantification was performed using the internal standard (melezitose) method. The results were expressed in g per 100 g of dry weight.

*2.3.2. Organic acids.* Organic acids namely oxalic, quinic, malic, ascorbic and citric acid, were determined following a procedure previously described by [Pereira, Barros, Carvalho & Ferreira \(2013\)](#). Their analysis was performed by ultra-fast liquid chromatography coupled to photodiode array detection (UFLC-PDA; Shimadzu Coperation, Kyoto, Japan), using 215 nm and 245 nm (for ascorbic acid), as preferred wavelengths. Their quantification was performed by comparing the area of the peaks, recorded at the corresponding wavelength, with the area of the calibration curves obtained from commercial standards of each compound. The organic acids determined were: oxalic acid ( $y=9\times 10^6 x + 377946$ ;  $R^2=0.994$ ); quinic acid ( $y=612327x + 16563$ ;  $R^2=1$ ); malic acid ( $y=863548x + 55591$ ;  $R^2=0.999$ ); ascorbic acid ( $y=1\times 10^8x + 751815$ ;

$R^2=0.999$ ) and citric acid ( $y=1\times 10^6x + 16276$ ;  $R^2=1$ ). The results were expressed in mg per 100 g of dry weight.

2.3.3. *Phenolic compounds non-anthocyanins*. Phenolic compounds were extracted, from jaboticaba pomaces, twice in methanol:water (80:20, v/v) at 25 °C and 150 rpm for 1 h and then filtered through Whatman paper. The combined extracts were evaporated at 35 °C under reduced pressure (Büchi R-210, Flawil, Switzerland) and lyophilized (FreeZone 4.5, Labconco, Kansas, USA). Afterwards, 10 mg of each lyophilized extract was re-dissolved in 2 mL of 20% aqueous methanol and filtered through a 0.22- $\mu$ m disposable LC filter disk for subsequent HPLC analysis.

Chromatographic analyses were carried out on a Spherisorb S3 ODS-2 C<sub>18</sub> column (3  $\mu$ m, 4.6  $\times$  150 mm, Waters, Milford, MA, EUA) thermostatted at 35 °C. The mobile phase consisted of two solvents: (A) 0.1% formic acid in water and (B) acetonitrile, using the following gradient: 15% B for 5 min, 15-20% B over 5 min, 20-25% B over 10 min, 25-35% B over 10 min, 35-50% B for 10 min. Then, the column was re-equilibrated with a flow rate of 0.5 mL/min and the samples were injected at a volume of 100  $\mu$ L. Spectral data for all peaks were recorded at 280, 330 and 370 nm, as preferred wavelengths. The HPLC-DAD-MS/ESI analyses were carried out using a Hewlett-Packard 1100 series chromatograph (Hewlett-Packard 1100, Agilent Technologies, Santa Clara, CA, US) equipped with a diode-array detector (PDA) and mass detector (API 3200 Qtrap, Applied Biosystems, Darmstadt, Germany) connected to the HPLC system via the PDA cell outlet. The HPLC consisted of a quaternary pump, an autosampler, a degasser and a photodiode-array detector. The HPLC system was controlled by HP Chem Station (rev. A.05.04). The mass detector was a triple quadrupole ion trap spectrometer equipped with an ESI source and controlled by the

Analyst 5.1 software. Air (zero grade) served as the nebulizer gas (30 psi) and turbo gas for solvent drying (400 °C, 40 psi). Nitrogen served as the curtain (20 psi) and a medium collision gas was used. The quadrupoles were set at unit resolution. The ion spray voltage was set at -4500V in the negative mode. The mass spectra (MS) detector was programmed for recording in two consecutive modes: Enhanced MS (EMS, employed to show full scan spectra) and enhanced product ion (EPI, fragmentation pattern of the parent ion(s) in the previous scan) analysis. Settings used for EMS and EPI were: declustering potential -45 V and -50 V; entrance potential -6 V; collision energy -10V and -25V, respectively. Spectras were recorded in negative ion mode between  $m/z$  100 and 1700. The phenolic compounds were identified by comparing their UV-vis and MS retention times with those obtained with standard compounds, when available. Otherwise, compounds were tentatively identified comparing the obtained information with available data reported in the literature. For quantitative analysis, a calibration curve for each available phenolic standards: ellagic acid ( $y=38.466x+35.44$ ;  $R^2=0.999$ ); gallic acid ( $y=365.2x+38.923$ ;  $R^2=1$ ); quercetin-3-*O*-glucoside ( $y=336.36x+358.06$ ;  $R^2=0.998$ ), was constructed based on the UV signal. For the identified phenolic compounds, for which a commercial standard was not available, the quantification was performed through the calibration curve of another compound from the same phenolic group. The quantification of peaks 3 and 11 were obtained using the calibration curves of gallic acid and quercetin-3-*O*-glucoside, respectively. For quantification of the remaining compounds, an ellagic acid calibration curve was used. The results were expressed as mg per g of extract.

*2.3.4. Anthocyanins.* Each sample was extracted twice in methanol containing 0.5% trifluoroacetic acid (TFA) at 25 °C and 150 rpm for 1 h, and filtered through Whatman

paper. The combined extracts were evaporated at 35 °C under reduced pressure, in order to remove the methanol, and re-dissolved them in water. Each sample was further purified by depositing the dissolved extract onto a C-18 SepPak® Vac 3 cc cartridge (Phenomenex), previously activated, and anthocyanins were further eluted with 5 mL of methanol containing 0.1% TFA. The extract was concentrated under vacuum, re-dissolved in 2 mL of 20% aqueous methanol and filtered through a 0.22- $\mu$ m disposable LC filter disk for HPLC analysis.

Anthocyanins were determined using the HPLC equipment described above for the phenolic compounds non-anthocyanins and as previously described by [Bastos et al. \(2015\)](#). Separation was achieved on an AQUA® (Phenomenex) reverse phase C<sub>18</sub> column (5  $\mu$ m, 150  $\times$  4.6 mm) thermostatted at 35 °C using a gradient elution. The solvents used were: (A) 0.1% TFA in water, and (B) 100% acetonitrile, with the following elution gradient: 10% B for 3 min, from 10–15% B for 12 min, isocratic 15% B for 5 min, from 15–18% B for 5 min, from 18–30% B for 20 min and from 30–35% B for 5 min, at a flow rate of 0.5 mL/min. Double detection was carried out as mentioned above and the spectral data for all peaks was recorded at 520 nm, as the preferred wavelength. The MS conditions were: nebulizer gas, 40 psi; turbo gas, 600 °C and 50 psi; curtain nitrogen, 100 psi; collision gas, high. The quadrupols were set at unit resolution. The ion spray voltage was set at 5000V in the positive mode. The EMS and EPI settings were: declustering potential -41 V; entrance potential -7.5 V; collision energy -10V. Spectras were recorded in positive ion mode between  $m/z$  100 and 1000.

The anthocyanins were tentatively identified by comparing their UV-vis and MS retention times with those obtained from available standards and data from our compound library and the literature. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal: cyanidin-3-

*O*-glucoside ( $y=630276x-153.83$ ;  $R^2=0.999$ ) and delphinidine-3-*O*-glucoside ( $y=557274x+126.24$ ;  $R^2=0.999$ ). The results were expressed in  $\mu\text{g}$  per g of extract.

#### 2.4. *Lipophilic phytochemicals*

2.4.1. *Tocopherols*. Tocopherols were determined following a procedure previously described by [Morales, Ramírez-Moreno, Sanchez-Mata, Carvalho & Ferreira \(2012\)](#), using HPLC coupled to a fluorescence detector (FP-2020; Jasco, Easton, USA) programmed for excitation at 290 nm and emission at 330 nm. Their identification was performed by chromatographic comparisons with commercial standards, while the quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g of sample on dry weight basis.

2.4.2. *Total fat and fatty acids*. Fatty acids were determined, after a trans-esterification process, by gas-liquid chromatography with flame ionization detection (GC-FID; DANI model GC 1000 instrument, Contone, Switzerland) as previously described by [Morales, et al. \(2012\)](#). Fatty acids identification were made by comparing the relative retention times of FAME peaks from samples with commercial standards at a concentration of 10 mg/mL. The results were recorded and processed using Clarity Software (DataApex, Prague, The Czech Republic) and expressed as relative percentage of each fatty acid.

#### 2.5. *Antioxidant activity*

2.5.1. *Methanolic extracts preparation*. One gram of dried pomace powder was extracted twice with methanol at 25 °C for 1 h and filtered through Whatman filter paper. The combined methanolic extracts were evaporated at 35 °C under reduced

pressure (Büchi R-210 R-210; Flawil, Switzerland) and re-dissolved in methanol at a concentration of 10 mg/mL.

2.5.2. *Antioxidant assays.* The *in vitro* antioxidant activity assays were performed following the previously described methodology by [Morales et al. \(2015\)](#). The sample concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC<sub>50</sub>), were calculated from the graphs of antioxidant activity percentages (DPPH,  $\beta$ -carotene/linoleate and TBARS assays) or absorbance at 690 nm (reducing power assay) against sample concentrations. The commercial standard trolox was used as positive control.

DPPH radical-scavenging activity was performed using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA), according to the procedure described by [Morales et al. \(2015\)](#). The reaction mixture, in each one of the 96-wells, consisted of different concentrations (ranging from 0.156 to 10 mg/mL methanolic extract) of the extracts (30  $\mu$ L) and aqueous methanolic solution (80:20 v/v, 270  $\mu$ L) containing DPPH radicals ( $6 \times 10^{-5}$  mol/L). The mixture was left to stand for 60 min in the dark, before analysis. The reduction of the DPPH radical was determined by measuring the absorbance at 515 nm.

In the evaluation of reducing power assay, different concentrations of the methanolic extracts (0.5 ml) were mixed with sodium phosphate buffer (200 mmol/l, pH 6.6, 0.5 ml) and potassium ferricyanide (1% w/v, 0.5 ml). The mixtures were incubated at 50 °C for 20 min, and trichloroacetic acid (10% w/v, 0.5 ml) was added. Then, 0.8 ml of the mixtures, deionised water (0.8 ml) and ferric chloride (0.1% w/v, 0.16 ml) were poured in the 48-wells and the absorbance was measured at 690 nm in the Microplate Reader.

For Inhibition of  $\beta$ -carotene bleaching assay, a solution of  $\beta$ -carotene was prepared by dissolving  $\beta$ -carotene (2 mg) in chloroform (10 mL). Two millilitres of the solution

were pipetted into a round-bottom flask. After the chloroform was removed at 40 °C under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg) and distilled water (100 mL) were added to the flask under vigorous shaking. Aliquots (4.8 mL) of the emulsion were transferred into test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50 °C in a water bath for 120 minutes. Absorbance at 470 nm was measured at zero time (as soon as the emulsion was added to each tube) and after 120 minutes.

### *2.6. Statistical analysis*

All assays were carry out in triplicate; the results are expressed as mean values and standard deviation (SD). Each analyzed parameter was compared by means of a Student's *t*-test to determine the significant difference between samples, with  $\alpha = 0.05$ . This statistical treatment was carried out using SPSS v. 22.0 program.

## **3. Results and discussion**

### **3.1. Chemical compounds in jabuticaba by-products**

#### *3.1.1. Hydrophilic compounds*

The soluble sugars and organic acids in the jabuticaba by-products, in the form of non-fermented and fermented pomaces, are shown in **Table 1**. Results showed that soluble sugars were identified only in the non-fermented pomace, since alcoholic fermentation transforms all soluble sugars in alcoholic derivatives (Beliz, Grosh & Schieberle, 2009). In the non-fermented jabuticaba pomace, fructose and glucose were the major soluble sugars with values around 3 g/100 dw, while sucrose was present in minor amount (0.2 g/100 g). However, Leite et al. (2011) reported higher sucrose concentration (18.09 g/100 g) in depulping residue of jabuticaba fruit (peel by-product). Since, jabuticaba

pomace contained the seeds and pulp, beside the skin (peel), of the jabuticaba fruit, this difference in composition would account for such observed difference. Regarding organic acids, oxalic, quinic, malic, shikimic and citric acids were quantified in both the non-fermented and fermented pomaces. Citric acid was the major organic acid with values of 14.5 and 13 mg/100 g dw in the non-fermented and fermented pomaces, respectively. Similarly, citric acid was also the predominant organic acid in fermented beverages of jabuticaba berry (de Sá et al. 2014).

Fourteen individual phenolic compounds (ten gallic or ellagic acid/HHDP derivatives, one flavonol and three anthocyanins) were detected and tentatively identified in the methanolic extract of the non-fermented and fermented jabuticaba pomaces (**Table 2**). The phenolic profiles of the two jabuticaba pomaces were quantitatively and qualitatively very similar. An example of the phenolic profile of the jabuticaba methanolic extract, recorded at 280 and 370 nm, is shown in **Figure 2**. The largest group of compounds found in the methanolic extracts of the non-fermented and fermented jabuticaba pomaces were ellagic acid derivatives. Peak 10 was positively identified as ellagic acid according to its retention time, mass and UV characteristics by comparison with a commercial standard. Peaks 8 and 9 were designated as ellagic acid hexoside ( $[M-H]^-$  at 463  $m/z$ ) and ellagic acid pentoside ( $[M-H]^-$  at 433  $m/z$ ), respectively, based on their UV spectra very similar to ellagic acid, pseudomolecular ions and the production in both cases of a fragment ion  $[M-H]^-$  at  $m/z$  301 from the respective loss of the hexosyl and pentosyl moieties. The presence of free ellagic acid (Reynertson et al. 2006; Abe et al. 2011; Wu, Dastmalchi, Long & Kennelly, 2012; Alezandro et al. 2013; Inada et al. 2105) and ellagic acid pentoside (Wu et al. 2012; Wu, Long & Kennelly, 2013a) in jabuticaba fruits, has been consistently reported. Peaks 1, 3, 5, 6 and 7 were identified as hydrolysable tannins. Moreover, peaks 5 and 6

presented a pseudomolecular ion [M-H]<sup>-</sup> ion at *m/z* 633, releasing MS<sup>2</sup> fragment ions at *m/z* 481 (loss of a galloyl moiety, 152 mu), *m/z* 463 (loss of gallic acid, 170 mu) and *m/z* 301 ([M-H-162]<sup>-</sup>, further loss of a glucosyl moiety), were designated as HHDP-galloyl-glucose isomers. Peak 7 presented a pseudomolecular ion [M-H]<sup>-</sup> ion at *m/z* 785, with the successive loss of two galloyl moieties (-152 mu; *m/z* at 633 and 481), which further loss of neutral gallic acid (-170 mu; *m/z* at 463) and a hexosyl moiety (-162 mu; *m/z* at 301), respectively, was designated as HHDP-digalloyl-glucose. These results agreed with those of [Wu, Dastmalchi, Long and Kennelly \(2012\)](#) who reported the presence of similar compounds in samples of jabuticaba fruits. The mass spectrum of peak 1, with a pseudomolecular ion [M-H]<sup>-</sup> ion at *m/z* 783 and MS<sup>2</sup> fragment ions at *m/z* 481 ([M-H-302]<sup>-</sup>, loss of an HHDP unit) and *m/z* 301 ([M-H-302-180]<sup>-</sup>, further loss a hexose), allowed assigning it as a bis-HHDP-glucoside, which might correspond to the ellagitannins previously identified in jabuticaba fruits as casuariin, pedunculagin ([Wu et al. 2012](#)) and (iso)oenothien C ([Wu et al. 2013a, b](#)). The authors reported that all those compounds presented the same molecular weight. Peaks 2 and 4 showed the same mass spectra characteristics, with a pseudomolecular ion [M-H]<sup>-</sup> at *m/z* 469 and MS<sup>2</sup> product ions at *m/z* 425 ([M-H-44]<sup>-</sup>; loss a carboxyl moiety) and *m/z* 301 (ellagic acid), matching a valoneic acid dilactone, reported in jabuticaba fruits by [Wu et al. \(2012 and 2013a\)](#). Peak 3 presented a pseudomolecular ion [M-H]<sup>-</sup> at *m/z* 483, with MS<sup>2</sup> fragment ions at *m/z* 313, 271, and 169, which are typical in the fragmentation of gallotannins ([Sanz et al. 2010](#)), allowing the identification of the compound as digalloyl-glucose.

Peak 11 represented the only flavonol found in both jabuticaba's pomace samples, which was regarded as quercetin-*O*-rhamnoside, presenting a pseudomolecular ion [M-H]<sup>-</sup> at *m/z* 447 and a fragment at *m/z* 301 ([M-H-146]<sup>-</sup>, loss of a rhamnosyl moiety). The presence of quercetin-3-*O*-rhamnosido (quercitrin) has been described previously in

jaboticaba by [Reynertson et al. \(2006\)](#) and [Wu et al. \(2012\)](#); thus, it was tentatively identified as such compound herein.

Peaks 12-14 corresponded to anthocyanins. Compounds 12 and 13 were identified as delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside, respectively, by comparison of their chromatographic characteristics and absorption, and mass spectra, with our library data. These compounds were previously reported in hydromethanolic extracts of jaboticaba fruits ([Wu et al. 2012](#); [Wu et al. 2013b](#)) and fruit peels ([Leite et al. 2011](#); [Inada et al. 2015](#)). However, [Inada et al. \(2015\)](#) failed to detect delphinidin-3-*O*-glucoside in the jaboticaba fruit's pomace. Finally, peak 14 ( $[M-H]^-$  at  $m/z$  623) released  $MS^2$  fragments at  $m/z$  461 ( $[M-H-162]^-$ , loss of a hexosyl moiety) and 287 ( $[M-H-132-42]^-$ , further loss of a pentosyl and acetyl moieties). This fragmentation pattern suggested different location for the hexosyl and acetylpentoside residues. Therefore, the compound was tentatively identified as cyanidin-*O*-hexosyl-*O*-acetylpentoside. To our knowledge, this anthocyanin has not been reported in any previous jaboticaba study.

The fermented jaboticaba pomace presented slightly higher amount of phenolic compounds than the non-fermented sample. The main phenolic compound present in the fermented pomace was HHDP-galloyl-glucose (peak 6), while in the non-fermented pomace was digalloyl-glucose (peak 3). Peak 4 was not detected in the non-fermented pomace, suggesting that it was produced during the fermentation process from other ellagic acid derivatives.

### *3.1.2. Lipophilic bioactive compounds*

**Table 3** shows fat and fatty acids profile of the analysed the non-fermented and fermented jaboticaba pomaces. The total fat content in the fermented pomace was slightly higher than the non-fermented sample. Moreover, the total fat content values in

the non-fermented and fermented jabuticaba pomaces were somehow comparable to the ranges on fat content reported for the whole fruit (0.1 to 1.8 g/100 g; [Leite-Legatti et al. 2012](#); [Inada et al. 2015](#)), pomace fraction (0.2 to 0.63 g/100 g; [Gurak et al. 2014](#); [Inada et al. 2015](#)) and peel by-product (0.6 to 1.72 g/100 g; [Leite-Legatti et al. 2012](#); [Gurak et al. 2014](#); [Inada et al. 2015](#)). However, to the authors best of knowledge, this is this is the first time that the fatty acids and tocopherols profiles are reported for jabuticaba, and particularly on its non-fermented and fermented pomaces. Saturated fatty acids (SFA) were the predominant lipid fraction in both non-fermented and fermented pomaces, followed by polyunsaturated fatty acids (PUFA) fraction. The monounsaturated fatty acids (MUFA) represented the minor fatty acid fraction. Similar percentages of each SFA, MUFA and PUFA were determined in the two types of pomace (**Table 3**). Palmitic acid (C16:0) was the major SFA, with 34% and 31% in the non-fermented and fermented pomaces, respectively; oleic acid (C18:1) was the major MUFA in the non-fermented pomace, while palmitoleic acid (C16:1) was the predominant MUFA in the fermented pomace. As for PUFA, the major fatty acid was linoleic acid (C18:2) with 33% in both types of pomace.

The total tocopherols content (**Table 3**) was similar in the non-fermented and fermented pomaces. The isoforms  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocopherol were identified in both by-products, being  $\alpha$ -tocopherol the majority isoform in the non-fermented and fermented pomaces.

The obtained results indicated that the fermentation process did not extensively affect the content and distribution of lipophilic bioactive compounds. It also demonstrated, that both jabuticaba by-products are good sources of those compounds.

### **3.2. Antioxidant properties of non-fermented and fermented jabuticaba pomaces**

The antioxidant capacity of the non-fermented and fermented jabuticaba pomace extracts was evaluated by DPPH radical scavenging ability, reducing power of  $\text{Fe}^{3+}$  into  $\text{Fe}^{2+}$ , and inhibition of lipid peroxidation using the  $\beta$ -carotene–linoleate model system (**Table 4**). The fermented pomace extracts presented significantly ( $p < 0.001$ ) higher antioxidant activity, represented by lower  $\text{EC}_{50}$  values of DPPH radical scavenging capacity (130  $\mu\text{g/mL}$ ) and reducing power (131  $\mu\text{g/mL}$ ), than the non-fermented pomace extracts (262  $\mu\text{g/mL}$  and 503  $\mu\text{g/mL}$ , respectively). The highest antioxidant effect, exerted by both types of pomace extracts, was observed in the  $\beta$ -carotene bleaching inhibition assay. This result could be related to the high concentrations of phenolic compounds and lipophilic compounds, namely tocopherols, present in those pomace extracts.

Comparing the results obtained in the DPPH assay, [Leite-Legatti et al. 2012](#) reported higher antioxidant activity (lower  $\text{EC}_{50}$  values) in peel by-products (45.38  $\mu\text{g/mL}$ ), compared with the jabuticaba pomace (262  $\mu\text{g/mL}$ ) analysed herein. Moreover, [Inada et al. \(2015\)](#) reported relevant antioxidant values on different jabuticaba by-products measured by different methods (Folin-Ciocalteu, FRAP, TEAC and ORAC) on depulped residues (9 mg GAE/100g; 167.1 mmol  $\text{Fe}^{2+}$ /100g; 92.6 mmol Trolox/100 g and 71.6 Trolox/100 g, respectively), peel (12.1 mg GAE/100 g; 192 mmol  $\text{Fe}^{2+}$ /100 g; 97.6 mmol Trolox/100 g and 82.7 Trolox/100 g, respectively) and seeds (10.7 mg GAE/100 g; 186.6 mmol  $\text{Fe}^{2+}$ /100 g; 97.9 mmol Trolox/100 g and 65.4 Trolox/100 g, respectively), and compared them to the whole fruit (6 mg GAE/100 g; 7.2 mmol  $\text{Fe}^{2+}$ /100 g; 43.5 mmol Trolox/100 g and 36.3 Trolox/100 g, respectively), highlighting the antioxidant potential of those by-products.

#### 4. Conclusions

The results of the present study revealed that non-fermented and fermented jaboticaba pomaces represent a rich source of bioactive compounds, such as tocopherols, PUFA and phenolic compounds, with high antioxidant potential. Therefore, the jaboticaba pomaces may be a suitable option, to be added as functional ingredients, in the fabrication of human foods and animal feed with the subsequent reduction of waste from the industrial processing of jaboticaba fruits into juice and fermented beverages.

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**Table 1.** Soluble sugars and organic acids in non-fermented and fermented jabuticaba (*Myrciaria cauliflora* Mart.) pomaces (Mean  $\pm$  SD, n=3)

	<b>Non-fermented pomace</b>	<b>Fermented pomace</b>	Student's <i>t</i> -test <i>p</i> -value
<b>Soluble sugars (g/100 g dw)</b>			
Fructose	3.8 $\pm$ 0.1	nd	-
Glucose	3.1 $\pm$ 0.2	nd	-
Sucrose	0.2 $\pm$ 0.01	nd	-
Total soluble sugars	7.1 $\pm$ 0.3	nd	-
<b>Organic acids (mg/100 g dw)</b>			
Oxalic acid	0.33 $\pm$ 0.02	0.21 $\pm$ 0.02	0.001
Quinic acid	0.60 $\pm$ 0.02	0.41 $\pm$ 0.03	0.002
Malic acid	0.11 $\pm$ 0.01	0.010 $\pm$ 0.001	<0.001
Shikimic acid	0.41 $\pm$ 0.01	0.170 $\pm$ 0.001	<0.001
Citric acid	14.5 $\pm$ 0.3	13.0 $\pm$ 0.1	0.001

nd (non detected)

**Table 2.** Retention time (Rt), waveleghts of maximum absorption in visible region ( $\lambda_{max}$ ), mass spectral data, tentative identification and quantification of phenolic compounds (mg/g methonolic extract) and anthocyanins ( $\mu\text{g/g}$  methanolic extract) in non-fermented and fermented jabuticaba (*Myrciaria cauliflora* Mart.) pomaces.

Peak	Rt (min)	$\lambda_{max}$ (nm)	[M-H] <sup>-</sup> (m/z)	MS <sup>2</sup> (m/z)	Tentative identification	Non-fermented pomace	Fermented pomace	Student's t-test p-value
<b>Phenolic compounds (non-anthocyanins)</b>								
1	5.22	260	783	765(10),481(10),301(29)	bis-HHDP-glucose	0.35 ± 0.03	1.3 ± 0.1	<0.001
2	5.4	-	469	425(100),407(10),301(13)	Valoneic acid dilactone isomer	nd	1.3 ± 0.1	-
3	5.6	-	483	331(7),313(12),271(22),169(16)	Digalloyl glucose	4.3 ± 0.1	1.3 ± 0.2	<0.001
4	6	260,sh294	469	425(100),407(23),301(23)	Valoneic acid dilactone isomer	0.41 ± 0.04	1.1 ± 0.1	<0.001
5	6.6	280	633	481(3),463(3),301(37)	HHDP-galloyl-glucose isomer	0.70 ± 0.02	1.1 ± 0.1	<0.001
6	6.9	232,sh280	633	481(3),463(18),301(23)	HHDP-galloyl-glucose isomer	0.86 ± 0.01	1.7 ± 0.1	<0.001
7	8	264	785	633(9),483(14),301(17)	HHDP-digalloyl-glucose	0.7 ± 0.1	1.1 ± 0.2	0.005
8	13.9	254,sh368	463	301(100)	Ellagic acid hexoside	0.83 ± 0.01	0.6 ± 0.1	0.001
9	18.3	254,sh362	433	301(100)	Ellagic acid pentoside	0.25 ± 0.01	0.4 ± 0.1	0.010
10	20.5	254,sh370	301	284(8),229(4),185(4)	Ellagic acid	1.2 ± 0.1	0.83 ± 0.03	0.002
11	24	354	447	301(100)	Quercetin-3- <i>O</i> -rahmnoside (quercitrin)	0.17 ± 0.01	0.17 ± 0.01	0.293
<b>Non-anthocyanin phenolic compounds</b>						9.7 ± 0.1	10.9 ± 0.3	0.001
<b>Phenolic compounds (anthocyanins)</b>								
12	15.3	524	465	303(100)	Delphinidin-3- <i>O</i> -glucoside	0.13 ± 0.01	0.16 ± 0.01	0.002
13	18.1	518	449	287(100)	Cyanidin-3- <i>O</i> -glucoside	2.05 ± 0.01	1.88 ± 0.04	0.003

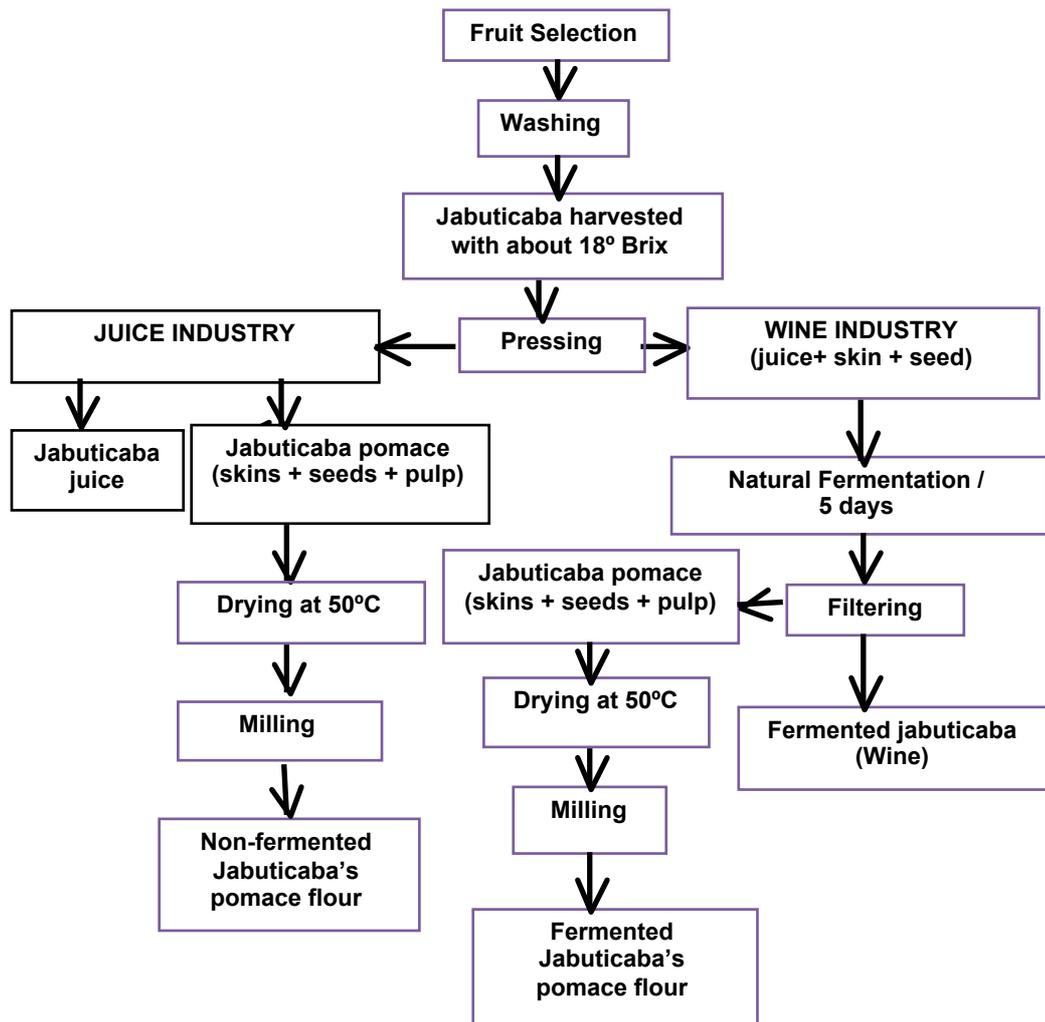
**Table 3.** Tocopherols and fatty acids in non-fermented and fermented jabotic (*Myrciaria cauliflora* Mart.) pomaces (Mean  $\pm$  SD, n=3).

	Non-fermented pomace	Fermented pomace	Student's <i>t</i> -test <i>p</i> -value
<b>Fatty acids (relative percentage)</b>			
C6:0	0.5 $\pm$ 0.1	0.050 $\pm$ 0.001	<0.001
C8:0	0.10 $\pm$ 0.01	0.10 $\pm$ 0.01	0.519
C10:0	0.10 $\pm$ 0.01	0.6 $\pm$ 0.1	<0.001
C11:0	4.95 $\pm$ 0.04	3.0 $\pm$ 0.3	<0.001
C12:0	0.40 $\pm$ 0.01	0.95 $\pm$ 0.04	<0.001
C13:0	0.15 $\pm$ 0.04	0.10 $\pm$ 0.04	0.482
C14:0	1.2 $\pm$ 0.2	1.85 $\pm$ 0.04	0.005
C14:1	nd	0.50 $\pm$ 0.01	-
C15:0	1.05 $\pm$ 0.04	0.8 $\pm$ 0.1	0.018
C15:1	nd	0.45 $\pm$ 0.04	-
C16:0	34 $\pm$ 2	31 $\pm$ 1	0.055
C16:1	2.4 $\pm$ 0.4	4.90 $\pm$ 0.01	<0.001
C17:0	0.70 $\pm$ 0.01	0.40 $\pm$ 0.01	<0.001
C18:0	3.9 $\pm$ 0.1	4.20 $\pm$ 0.01	0.016
C18:1n9	5.3 $\pm$ 0.2	6.2 $\pm$ 0.2	0.012
C18:2n6	33 $\pm$ 1	32.9 $\pm$ 0.4	0.924
C18:3n3	9.9 $\pm$ 0.4	9.70 $\pm$ 0.01	0.595
C20:0	0.95 $\pm$ 0.04	0.75 $\pm$ 0.01	0.008
C20:2	nd	0.20 $\pm$ 0.01	-
C20:3n3+C21:0	0.15 $\pm$ 0.01	0.10 $\pm$ 0.01	0.158
C22:0	0.75 $\pm$ 0.04	0.50 $\pm$ 0.01	0.001
C23:0	0.20 $\pm$ 0.01	0.10 $\pm$ 0.01	<0.001
C24:0	0.6 $\pm$ 0.1	0.40 $\pm$ 0.01	0.026
<b>Total fat (g/100 g dw)</b>	1.1 $\pm$ 0.1	1.5 $\pm$ 0.2	0.085
SFA	49 $\pm$ 2	45 $\pm$ 1	0.024
MUFA	8 $\pm$ 1	12.0 $\pm$ 0.3	0.001
PUFA	43 $\pm$ 1	43 $\pm$ 1	0.953
<b>Tocopherols (mg/100 g dw)</b>			
$\alpha$ -tocopherol	2.64 $\pm$ 0.01	2.81 $\pm$ 0.04	0.003
$\beta$ -tocopherol	0.23 $\pm$ 0.02	0.26 $\pm$ 0.00	0.141
$\gamma$ -tocopherol	0.38 $\pm$ 0.02	0.23 $\pm$ 0.02	0.010
$\delta$ -tocopherol	0.22 $\pm$ 0.01	0.13 $\pm$ 0.01	<0.001
Total tocopherols	3.2 $\pm$ 0.1	3.3 $\pm$ 0.1	0.163

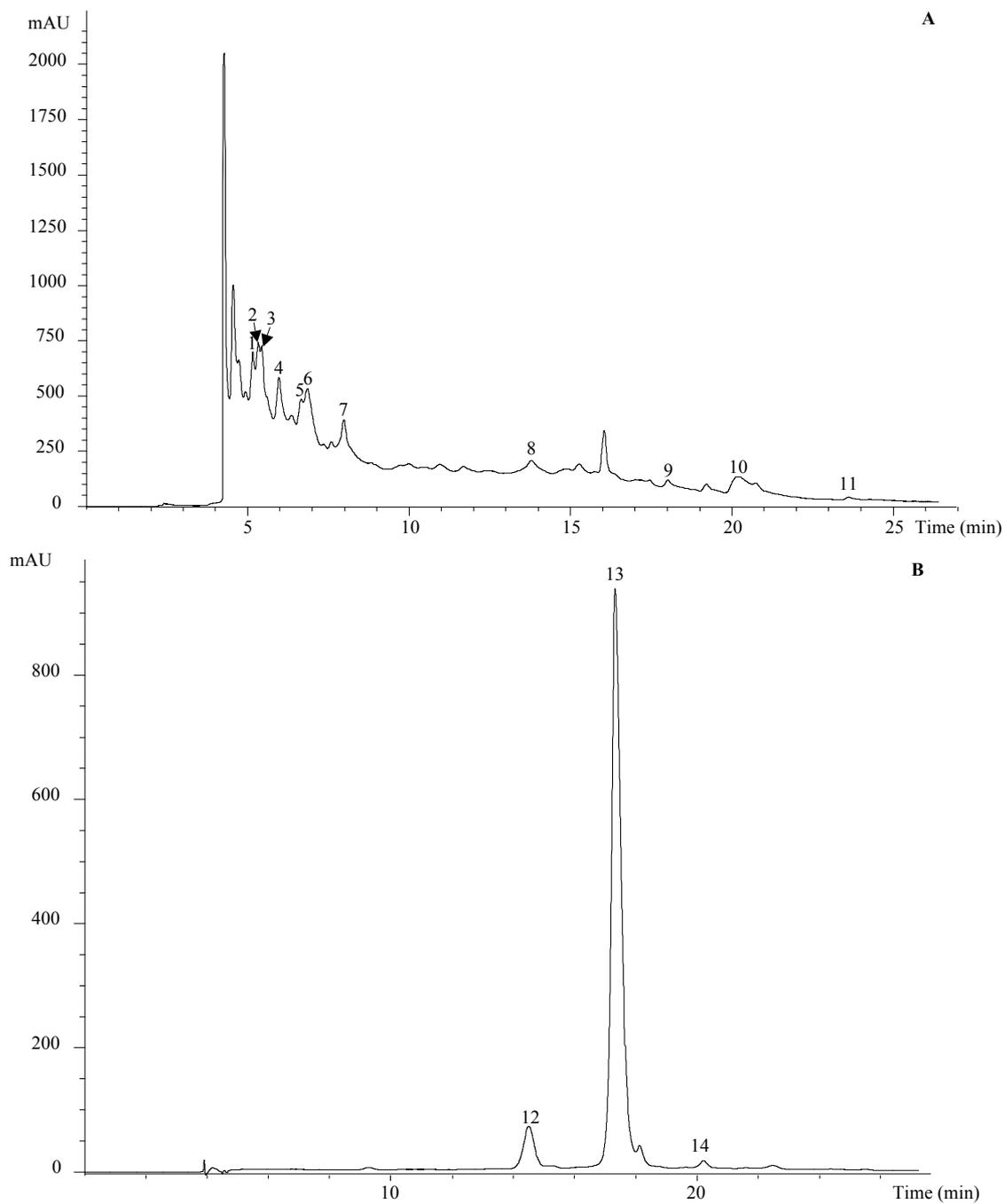
nd (not detected); SFA (saturated fatty acids); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids); dw (dry weight).

**Table 4.** Antioxidant activity in non-fermented and fermented jabuticaba (*Myrciaria cauliflora* Mart.) pomaces (Mean  $\pm$  SD. n=3).

<b>EC<sub>50</sub> values (<math>\mu\text{g/mL}</math> methanolic extract)</b>	<b>Non-fermented pomace</b>	<b>Fermented pomace</b>	<b>Student's <i>t</i>-test <i>p</i>-value</b>
DPPH scavenging activity	262 $\pm$ 8	130 $\pm$ 2	<0.001
Reducing power	503 $\pm$ 1	131 $\pm$ 1	<0.001
$\beta$ -carotene bleaching inhibition	32 $\pm$ 1	33 $\pm$ 1	0.253



**Figure 1.**– Flow diagram for obtaining non-fermented and fermented Jabuticaba's pomace flour, as a waste of the juice and wine industries.



**Figure 2.** HPLC phenolic profile of the methanolic extract obtained from fermented jaboticaba (*Myrciaria cauliflora* Mart.) pomace, recorded at 280 nm (A) and 520 nm (B).