An Acad Bras Cienc (2024) 96(1): e20230174 DOI 10.1590/0001-3765202420230174
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CHEMICAL SCIENCES

Sequential extraction of anthocyanins and pectin from jabuticaba (*Plinia cauliflora*) peel: Peel pretreatment effect and ultrasound-assisted extraction

TAINAH M. BUENO, FABIANA QUEIROZ, JHENIFER CRISTINA C. DOS SANTOS, MARIA LUIZA B. FURTADO, MARIA CECÍLIA E.V. SCHIASSI, SORAIA V. BORGES & JAYNE A. FIGUEIREDO

Abstract: The jabuticaba bark is rich in anthocyanins and fibers, and its use may be of industrial interest. In the food sector, its used as an ingredient in the production of fermented products, liqueurs or enriched flours. It also has pharmaceutical and cosmetic applications. The objective was to evaluate the effect of pretreatment and *fresh* use of jabuticaba peels in the extraction of total phenolic compound (TPC) and total anthocyanin (TA) contents with and without ultrasound assistance and in the sequential extraction of pectin from the residue. In the TPC and TA extraction, a 3x2 factorial design was used. For conventional anthocyanin extraction (CAE), occurred in an incubator under agitation. For ultrasound-assisted anthocyanin extraction (UAE) was utilized an ultrasonic homogenizer with probe (20 kHz, 160 W). The extracts were quantified (TPC, TA, antioxidant activity and color). The residues were characterized and used for sequential pectin extraction, which was quantified and characterized. The results were subjected to analysis of variance. Fresh jabuticaba peel is a residue that can be used to sequentially extract phenolic compounds, particularly anthocyanins and pectin. The use of ultrasound (UAE) was less efficient than CAE for extracting TPC and TA or performing sequential extraction on all pretreatment peels.

Key words: Waste, pigments, sustainable, waste processing, dietary fibre.

INTRODUCTION

In Brazil, different native fruits, including jabuticaba, have been the target of research to fully investigate their nutritional properties. Jabuticaba is highly appreciated for natural consumption and can be used for the industrial production of jellies, wines, sweets, nectars, liqueurs and frozen pulps (Benvenutti et al. 2021, Ferreira et al. 2020).

During processing, the peel and seeds of a fruit, which may represent up to 40% of the fruit *fresh*, are usually discarded (Martins et al. 2011). Researchers are seeking to develop innovative and functional (bioactive) products from agro-industrial byproducts or coproducts that have nutritional and functional properties as a new alternative to reduce waste. The jabuticaba peel is a rich source of anthocyanins and other natural pigments with antioxidant properties, which have several industrial applications, as shown in pharmaceutical (Silva et al. 2014, Wu et al. 2013) and cosmetics products (Lima et al. 2011).

Bioactive or phytochemical compounds are substances derived from the secondary metabolism of plants and benefit human health.

Among these compounds, anthocyanins deserve special attention because they are natural pigments that can be used in food processing and are potentially beneficial to health in terms of disease prevention (Meregalli et al. 2020).

Extraction, in which a solvent acts on the plant cell structure, thus solubilizing the compound of interest, is one of the most used processes to obtain bioactive compounds. In anthocyanin extraction, an effective process should enhance the recovery of these pigments with minimal degradation and result in an extract with high antioxidant capacity using clean technologies and low-cost raw materials materials (Santos et al. 2010). Ultrasound-assisted solvent extraction has emerged as a promising technique from an economic perspective because it is a relatively inexpensive and simple procedure that can result in greater efficiency, reduced time and lower energy and solvent consumption (Trojanowska et al. 2019).

The jabuticaba peel has attracted increased interest in the food industry because it is rich in TA and fibre (pectin), which can be extracted and used in the preparation of various products. TA can be used as a dye and pectin can be used to increase viscosity and acts as a stabilizing colloid in foods and beverages. These compounds have applications in sweets and jellies, fruit preparations for yogurts, beverages and concentrated fruit juices. The extraction of pectin is performed in an acidic aqueous medium, followed by the purification of the extracted liquid and the isolation of pectin by precipitation in the presence of alcohol (Canteri et al. 2012). The highest pectin extraction yields are found when the temperature and extraction time are increased and the pH is reduced, which is probably due to the increase in hydrolysis and mass transfer of pectin from the cell to the solvent and to the increase in solubility of pectin in the extracted solvent (Hosseini et al. 2016, Samavati 2013, Samavati & Manoochehrizade 2013).

Although the extraction of these two compounds, anthocyanin and pectin, occurs individually due to their particularities in the extraction processes, possible forms of sequential extraction have been studied for a better use of the raw material (Koubala et al. 2008, Mugwagwa & Chimphango 2019).

The objective of this study was to evaluate the effect of *fresh* use and pretreatments (drying and freezing) on jabuticaba peels for the sequential extraction of anthocyanins and other compounds in conventional extraction, ultrasound-assisted extraction and the sequential extraction of pectin from waste.

MATERIALS AND METHODS

Materials

Sabará jabuticabas were purchased at the Fair of Rural Producers of Lavras (Lavras, MG, Brazil) at the time of harvest (from August to October 2021).

All solutions were prepared from analytical reagent grade chemicals (> 95 per cent of purity). Ethanol, hydrochloric acid, potassium chloride, sodium acetate, Folin–Ciocalteu Phenol reagent, ABTS radical, DPPH radical, citric acid, and other chemical products (analytical grade) were provided by Sigma–Aldrich (São Paulo, Brazil). The experiment was conducted in the laboratories of the Department of Food Science at the Universidade Federal de Lavras (UFLA).

Processing of jabuticaba and physicochemical analyses of the fresh peel

The fruits were checked for defects and pests, washed with running water and sanitized in a sodium hypochlorite solution (100 mg/L) for 10 minutes. Then, they were washed in distilled water and drained for 10 minutes. After this

step, the fruits were manually pulped and then separated into pulp, seeds and peel. The peels were the material used in this study. Fig. 1 shows the flowchart of the experiment.

In the manually pulped peels, the moisture content (method No. 967.08), ash (method No. 94205), proteins (method No. 988.05), lipids (method No. 2003.06) and dietary fibre (method No. 991.43) were analysed according to the methodology described by the Association of Official Analytical Chemicals (AOAC 2016). The carbohydrate levels were calculated using the following formula: 100 - (moisture + lipid + protein + ash + fibre). The results were expressed as the percentage (%) of carbohydrates in the whole matter.

The total and soluble pectin contents were also determined (McCready & McComb 1952), and the insoluble pectin content was obtained by the difference between the total and soluble pectin contents. The results were expressed in mg of galacturonic acid per 100 g of whole matter (g/100 g).

The determination of water activity was performed using a 3TE Aqualab water activity meter (Decagon Devices, São José dos Campos, SP, Brazil) at 25 ± 0.3 °C, and the hydrogen potential was determined using a Q-400 A digital potentiometer (Quimis, Diadem, SP, Brazil) calibrated with buffer solutions of pH 4 and 7.

Colorimetric analysis

The color was determined using a CM5 colorimeter (Konica Minolta Spectrophotometer, São Paulo, SP, Brazil) operating in the International Commission on Illumination (CIE) (luminance-chroma-hue) LCH system to measure the parameters L*, C*, h° and a*, according to Gennadios et al. (1996). These color parameters were evaluated using fresh, dry and frozen peels and the extracts and extraction residues.

The L*, C* and h° coordinates were converted to the red (R), green (G) and blue (B) coordinates using the ColorMine converter, which is available free of charge at colormine.org/delta-e-calculator, to reconstruct the color (Muhammad Zair et al. 2020).

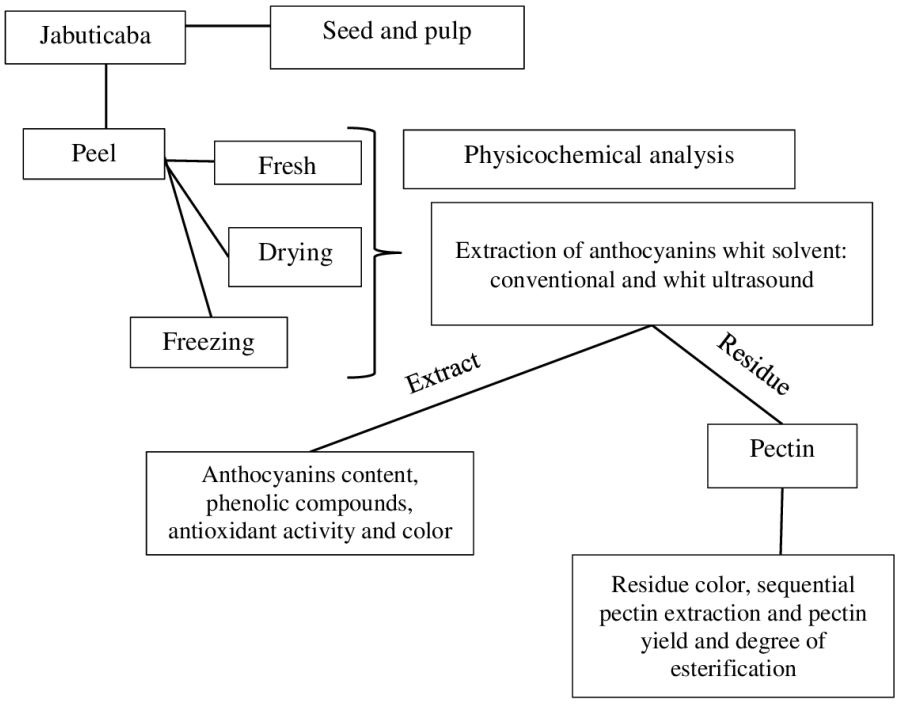

Figure 1. Flowchart of the anthocyanin and phenolic compound extraction methods (conventional and ultrasound-assisted) and sequential pectin extraction method.

Effect of pretreatment of the raw material and use of ultrasound on the sequential extraction of anthocyanins and pectin

Experimental design

The experiments were performed according to a full factorial experimental design 3x2, completely randomized and conducted in triplicate. The first factor was the type of pretreatment, with three levels (dry, frozen and fresh peels), and the second factor was the anthocyanins extraction methods, with two levels (conventional and ultrasound-assisted extraction). The response variables were TA content, TPC content, antioxidant activity and extract and residue colors, yield and degree of esterification of the pectin sequentially extracted from the residue.

The results were subjected to analysis of variance (ANOVA), and the means were compared using Tukey's test with a 5% level of significance using *Statistica* 10.0 (StatSoft, Tulsa, Oklahoma, EUA, 2010).

Preparation of jabuticaba peels

To evaluate the effect of the pretreatment of the jabuticaba peel, after pulping, the peels were prepared in three ways: fresh peel, dried peel and frozen peel.

The fresh peels were ground in a multiprocessor (Master Duo RI7638, Walita) and immediately extracted and analysed.

Peels were also stored in a plastic bag and placed in a Brastemp GE Frost Free Refrigerator (for slow freezing at -18 °C) until extraction. Before extraction, these peels were thawed in a refrigerator (8 °C) until they lost their characteristic firm consistency from freezing. The exudate was discarded, and the peels were ground in a multiprocessor (Master Duo RI7638, Walita) and extracted.

The dehydrated peels were placed on stainless steel trays and subjected to dehydration in an oven with forced air circulation at 38 ± 1 °C to avoid the degradation of thermolabile compounds until a constant mass was obtained (± 72 hours). After drying, the samples were ground in a blender, sieved through 60- and 80-mesh sieves and extracted. The mean particle size was 0.215 mm.

For standardization, the results of the analyses were expressed on a dry basis, and the moisture content of the fresh, thawed and dried samples was determined.

Extraction and quantification of phenolic compounds and anthocyanins and determination of antioxidant activity

Extraction of phenolic compounds and anthocyanins

For conventional anthocyanin extraction (CAE - without ultrasound), the methodology by Meregalli et al. (2020) was followed with some modifications: 5 grams of the sample was weighed and placed in an Erlenmeyer flask with 50 mL of ethanol acidified with 1% hydrochloric acid and covered with aluminium foil, so that the samples are protected from light. The extraction occurred in an incubator (Marconi MA830/A) for 2 hours at a temperature of 25 ± 1 °C under agitation at 180 rpm. After the time elapsed, the samples were centrifuged at 3500 rpm for 10 minutes under refrigeration. The extract was collected and analysed.

For ultrasound-assisted anthocyanin extraction (UAE), 5 grams of sample was weighed and placed in an Erlenmeyer flask, 50 mL of ethanol acidified with 1% hydrochloric acid was added and then the flask was covered with aluminium foil, so that the samples are protected from light. The flasks were immersed in an ultrasonic homogenizer with a continuous pulse probe (Branson DigitalSonifier, Model S-450D, Branson Ultrassonics Corporation, Dun

Bury, USA) at a frequency of 20 kHz, amplitude of 40%, and power of 160 W. The temperature of the solution was controlled with an ice bath. After 12 minutes of extraction (time defined in preliminary tests, at the same frequency and power, with 3, 6, 12 and 30 minutes and peel/solvent ratio 1:10), the samples were centrifuged at 3500 rpm for 10 minutes, and the extract was collected and analysed.

Anthocyanins, total phenolic compounds, antioxidant activity and color analysis were quantified from the extracts. The waste from the CAE and UAE was left in a fume hood for evaporation of the residual solvent for 24 hours, and color analysis was performed. The waste was then used for sequential extraction and quantification of pectin and degree of esterification.

Quantification of anthocyanins

Total anthocyanin (TA) content was determined by the differential pH method proposed by by Giusti & Wrolstad (2001). This method is based on the structural transformation of anthocyanin as a function of pH (TECNAL pH metre, Tec 3MP) in two buffer solutions: potassium chloride at pH 1.0 (0.025 M) and sodium acetate at pH 4.5 (0.4 M).

According to the method, the difference in absorbance of the pH 1.0 and 4.5 solutions is directly proportional to the TA concentration. The absorbance values of the samples were measured at 510 nm and 700 nm wavelengths using a spectrophotometer (UV1600 Pro-analysis). It was necessary to perform a 1:10 dilution with distilled water for the absorbance value to be within the desired range. The concentration of total anthocyanins (TA) was expressed as mg of total anthocyanins per 100 g of dry peel, as shown in Equation 1.

$$AT = \frac{\left[\left(A_{520} - A_{700}\right)pH_{1,0} - \left(A_{520} - A_{700}\right)pH_{4,5}\right] * M * DF * 1000}{\varepsilon * L} * 100 \quad (1)$$

where M is the molar mass of cyanidin-3-glycoside (449.2 g/mol), DF is the dilution factor of the extracted sample (1:10; g/mL), ε is the molar extinction coefficient of cyanidin-3-glucoside (26900 L/(mol.cm)), 1000 represents the conversion from g to mg, and L is the optical path of the cuvette (1 cm).

Determination of total phenolic content (TPC)

The total phenolic content was determined according to the method adapted from Folin–Ciocalteu (Waterhouse 2002). It was necessary to perform a 1:10 dilution of the extract obtained from the jaboticaba peel with distilled water to fit the absorbance value in the desired range. Absorbance was measured at a wavelength of 750 nm using a spectrophotometer (UV1600 Pro-analysis). The results were expressed in mg of gallic acid equivalent (GAE)/g of jabuticaba peel on a dry basis (mg GAE/g db).

Antioxidant activities

The determination of antioxidant activity by the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)·+ method was adapted from Mareček et al. (2017) and the spectrophotometer readings (UV1600 Pro-analysis) were performed at a wavelength of 734 nm. The results were expressed in micromoles of Trolox per gram of sample on a dry basis (μmol Trolox/g db).

The free radical scavenging capacity of 2,2-diphenyl-1-picryl hydrazyl (DPPH) was estimated by the methodology modified by Brand-Williams et al. (1995). The readings were taken at 515 nm using a spectrophotometer (UV1600 Pro-analysis), and the results were expressed in half maximal effective concentration (EC_{50}) (g of peel db/g of DPPH), i.e., the sample mass needed to decrease the concentration of · DPPH in 50% in the extract.

Sequential extraction of pectin from the residue and determination of its degree of esterification

Sequential extraction and quantification of pectin

After evaporating the residual solvent from the jabuticaba peel residue from the extraction of phenolic compounds and anthocyanins in a fume hood for 24 hours, pectin was extracted according to the methodology of Ranganna (1997). The solvent used for pectin extraction was an aqueous solution of citric acid. The samples (4 g) were dissolved in 200 mL of distilled water. The extractions were performed at constant temperature (97 °C) and time (95 minutes) under acid conditions (3.5% of the sample mass of the peel residue). After acid extraction, the samples were cooled at 4 °C for 2 hours and filtered in polyester fabric, and the supernatant was discarded. Ethanol (95%) was added to the filtrate containing pectin at a 1:2 ratio (one part pectin solution and two parts ethanol). After one hour, the pectin was separated into a precipitate and separated by filtration. The pectin obtained was washed twice with 95% and 70% ethanol and dried in a drying oven at 55 °C until constant weight. The pectin yield was obtained from the initial mass of jabuticaba peel (sample mass from the first extraction) on a dry basis (Equation 2).

$$Yield = \frac{Weight\ of\ extracted\ pectin}{Weight\ of\ initial\ sample} * 100 \quad (2)$$

The mass of pectin extracted = mass of pectin obtained after extraction and dried (in grams), and the initial sample mass = mass of jabuticaba peel in the initial extraction of TPC and TA on a dry basis.

Determination of the degree of esterification (DE) of pectin

The degree of esterification of the extracted pectin was determined by the titration method proposed by Jafari et al. (2017) with minor modifications. A pectin sample (100 mg) was suspended in 2 mL of ethanol and 20 mL of distilled water. This suspension was stirred at 40 °C and then titrated with 0.1 N NaOH solution (first titration, V_1) until pH 8.5 based on a pH metre. Then, 10 mL of 0.1 N NaOH solution was added, and the system was stirred for 30 minutes at 40 °C. Then, 10 mL of 0.1 N HCl solution was added and stirred for 30 minutes at 40 °C again.

The resulting solution was titrated with 0.1 N NaOH solution to pH 8.5 (second titration, V_2). The degree of pectin esterification was determined as described in Equation 3 (Hosseini et al. 2016):

$$DE(\%) = \frac{V_2(mL)}{V_1(mL) + V_2(mL)} * 100 \quad (3)$$

RESULTS AND DISCUSSION

Physicochemical composition of fresh peel

The moisture content on the wet basis of the jabuticaba peel was 68.93 ± 1.03%. The proximate composition of the jabuticaba peel and pectin, water activity and pH values of the jabuticaba peel used is shown in Table I.

Values close to the measured moisture, lipid and carbohydrate compositions in the peel were found by Miranda et al. (2020). Leite-Legatti et al. (2012) and Ferreira et al. (2020) found lower values of ash in the peel. Higher moisture values were found in the literature (Cavalcanti et al. 2011, Ferreira et al. 2020), which can be explained by the difference in the precipitation regime that precedes the harvest. Ferreira et al. (2020) found similar values of protein in the peel. The difference found between authors for centesimal composition is justified because the content of the plants varies according to genetics, maturation stages, climatic conditions,

Table I. Proximate composition of the jabuticaba peel on a dry basis and pectin, water activity and pH values of the jabuticaba peel.

Chemical composition (g/100 g of bark on a db)	
Lipids	5.03 ± 2.59
Protein	6.56 ± 0.14
Crude fibre	6.77 ± 0.33
Ashes	3.30 ± 0.11
Carbohydrates	78.33 ± 2.28
Pectin, water activity and pH values of the jabuticaba peel.	
Total pectin (g/100 g of bark)	6.42 ± 0.59
Insoluble pectin (g/100 g of bark)	5.08 ± 0.52
Soluble pectin (g/100 g of bark)	1.34 ± 0.12
Water activity	0.988 ± 0.001
pH	2.88 ± 0.01

Mean ± standart deviation; n= 3.

planting location and soil type (Bugianese et al. 2004).

The crude fibre shown in Table I (6.77 g/100 g of dry sample) can be compared with the pectin content present in the peel, since the peel has fibres that are formed by insoluble parts (cellulose, hemicellulose and lignin) and soluble parts (pectin, gum and mucilage). Note that 94.8% of the crude fibre found in the peel corresponds to pectin (20.9% soluble pectin and 79.1% insoluble pectin).

Similar water activity values and higher pH values were found in the literature (Ferreira et al. 2020, Miranda et al. 2020). The jabuticaba peel is highly perishable with high water activity. The water activity of a food is related to the availability of water susceptible to various chemical and enzymatic reactions or to the use of microorganisms present. The higher the water activity is, the higher the multiplication rate of microorganisms, so this parameter is related to food conservation. In addition to Aw, pH is another key parameter limiting the types of microorganisms that are capable of developing in food (Belitz et al. 2009).

Extraction and quantification of phenolic compounds and anthocyanins and their antioxidant activity and sequential extraction of pectin from the residue and determination of its degree of esterification

Pretreatment (freezing, drying or using *fresh*), the use of ultrasound-assisted extraction and their interactions significantly affected all response variables, except for the TA content, in which the interaction between the type of pretreatment and the use of ultrasound showed no significant effect (Table VII in the Appendix). A p value less than 0.05 was considered to evaluate the significance of the independent variables and their interaction (Tables VI to XI in the Appendix).

Table II shows the results obtained for TPC content, TA content and antioxidant activity of the extracts from *fresh*, dried and frozen peels using CAE and UAE and the yield of the sequential extraction of pectin from the residue from the extraction of bioactive compounds (TPC and TA) and the degree of esterification. The results are expressed in g of the peel on a dry basis, with moisture contents of 68.93%, 63.62% and 0.98% for the *fresh*, frozen and dried peel samples, respectively.

It is known that temperature is one of the factors that most influences the degradation of bioactive compounds. Preventing the thermal degradation of phenolic compounds is a very important aspect and therefore has been studied with great intensity (Cavalcanti et al. 2011, Huarancca-Huarcaya et al. 2022).

Regarding the pretreated jabuticaba peels subjected to ultrasound, their TPC contents did not differ from each other. For the *fresh* and frozen jabuticaba peels, the use of ultrasound was ineffective, while for the dried peel, there

Table II. Total phenolic compound (TPC) content, total anthocyanin (TA) content and antioxidant activity (DPPH and ABTS) for jabuticaba peels in the first extraction and pectin yield levels (%) and degree of esterification (%) for jabuticaba peel in the second extraction.

Pretreatment	Fresh	Freezing	Drying
Type of extraction	**TPC (mg GAE/g dry sample) - CV (%) = 3.46**		
CAE	51.41 ± 1.37Aa	33.94 ± 0.04Ab	17.85 ± 0.40Bc
UAE	25.57 ± 1.28Ba	17.81 ± 0.15Bb	17.36 ± 1.31Bb
	TA (mg/100 g dry sample) - CV (%) = 10.55		
CAE	427.65 ± 10Aab	401.81 ± 2Ab	484.65 ± 80Aa
UAE	186.93 ± 17Ba	163.07 ± 0.2Ba	231.08 ± 28Ba
	EC_{50} (g of sample/g of DPPH) - CV (%) = 5.55		
CAE	161.18 ± 2.8Aa	418.20 ± 1.3Ab	448.67 ± 16Ab
UAE	215.94 ± 3.3Ba	395.63 ± 1.8Ab	499.00 ± 15.4Bc
	ABTS (Trolox µmol/g dry sample) - CV (%) = 0.85		
CAE	1920.67 ± 34Bb	3336.24 ± 4Aa	1383.58 ± 19Ac
UAE	2971.29 ± 24Ab	3106.10 ± 8Ba	1305.59 ± 12Bc
	% pectin in the bark (db) - CV (%) = 6.80		
CAE	5.86 ± 0.16Aa	4.64 ± 0.25Ab	2.16 ± 0.17Ac
UAE	4.92 ± 0.16Ba	4.51 ± 0.17Aa	1.13 ± 0.06Bb
	Degree of esterification (DE) - CV (%) = 7.49		
CAE	65.20± 0.98Ba	45.13 ± 1.98Bb	60.07 ± 3.84Aa
UAE	89.70 ± 3.82Aa	54.53 ± 3.59Ab	62.37 ± 3.02Ab

Mean; n=3. The mean values with common lowercase letters in the same row and common uppercase letters in the same column indicate that there is no significant difference between the samples ($p \leq 0.05$) by the Tukey test for the analyzed variable. CAE= conventional extraction; UAE = ultrasound-assisted extraction; CV = coefficient of variation.

was no significant difference between CAE and UAE. The highest efficiency of TPC extraction was obtained using CAE on the *fresh* jaboticaba peel.

Higher TPC values in dried jabuticaba skins were found by other authors, as shown in Fig. 2. Paludo (2022) also compared conventional and ultrasound-assisted extractions of lyophilized peels and found a significant difference, with better results obtained using conventional extraction.

For the TA content, there was no significant effect on the interaction between pretreatment type and extraction type. The UAEs were unfavourable to TA yield, which can be explained by the degradation of anthocyanins through excessive exposure to ultrasonic waves, which was verified in preliminary tests at the same frequency and power for 3, 6, 12 and 30 minutes and peel ratio:solvent of 1:10. When comparing the types of pretreatments, it was noted that with the use of ultrasound, there were no significant differences between the TA values. Conversely, when using conventional extraction, the highest TA value was obtained for the dried peel, which was significantly equal to that of the *fresh* peel.

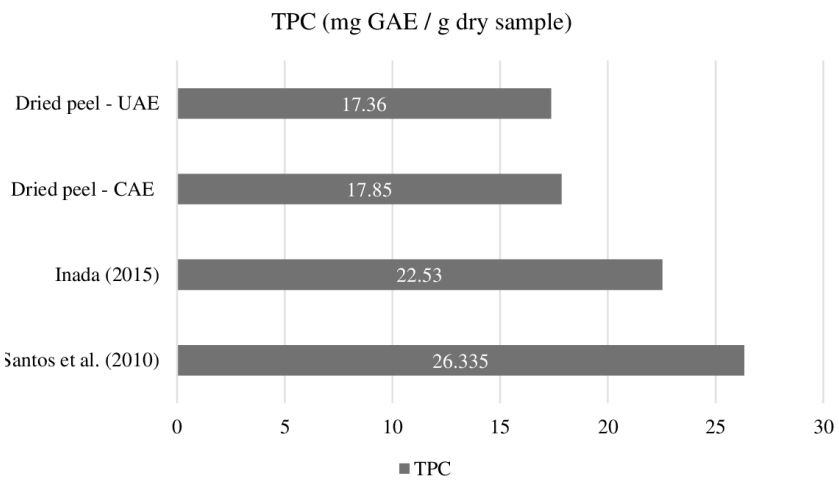

Figure 2. Comparison between different TPCs obtained in the extraction of dried jabuticaba peels.

The range of TA values using conventional extraction was higher than the levels reported for frozen açaí pulp (135.15 mg/100 g of dried pulp sample) for the whole blueberry fruit (118 mg/100 g of dried fruit sample), for whole black raspberries (179 mg/100 g of dried fruit sample) and for black currant (207 mg/100 g of dried peel sample), with jabuticaba peel being a source of anthocyanins (Huarancca-Huarcaya et al. 2022).

The differences in particle size between the dried, frozen and *fresh* jabuticaba peels could also have influenced the extraction of phenolic compounds and anthocyanins since the peels after drying were ground and passed through sieves, while the frozen and *fresh* peels were ground in a multiprocessor only, yielding larger and nonuniform particle sizes.

The antioxidant activity was determined by the DPPH and ABTS methods. For the DPPH method, the result was expressed in EC_{50}, which is defined as the sample mass required to reduce the initial concentration of DPPH by 50%. The lower the EC_{50} value is, the greater the antioxidant activity of the product, i.e., the lower the sample mass required to inhibit the activity of free radicals by 50%. There was no significant difference in the EC_{50} values using CAE for the pretreated (drying and freezing) peels, and these values were higher than those obtained for the *fresh* peels, which was the extract with the highest antioxidant activity value by this methodology. The EC_{50} concentrations of the UAE extracts for all pretreatments differed from each other. For *fresh* and dry peels, CAE was more effective. For the frozen peel, there was no difference between CAE and UAE.

Difficulties were encountered during the analysis of DPPH, since both the jabuticaba peel extract and DPPH radical are both purplish/reddish in color. It was observed that the high antioxidant activity present in the jabuticaba peel hindered the dilution of the extract and the stabilization time for reading in the spectrophotometer.

For ABTS, the treatment with the highest antioxidant activity was the frozen peel obtained by CAE. For the two types of extraction (CAE and UAE), the antioxidant activities of the peel followed the same decreasing order: frozen peel, *fresh* peel and dried peel. A higher antioxidant activity (ABTS) was obtained only in the *fresh* peels using UAE.

Therefore, UAE was effective only for the antioxidant activity value of the extract by the ABTS method for the *fresh* sample and did not affect the extraction of TPC from dry samples or the EC_{50} value of the extracts from the frozen samples. In the other extractions, the use of

ultrasound was unfavourable in the extraction of compounds.

The extracts obtained from the *fresh* samples had higher TPC values and higher antioxidant activity by the EC_{50} method. The TA values obtained did not differ significantly from those of the dried and frozen peels. Thus, for the industrial extraction of anthocyanins from jabuticaba peels, if extraction is performed immediately after the processing of jabuticaba, pretreatment is not necessary. However, if immediate extraction is not possible, drying is an alternative for storage and handling of the peel until extraction, due to its high perishability.

Regarding the sequential extraction of pectin from the residue and determination of its degree of esterification, presented in Table II, it can be seen that the pretreatment (freezing, drying and using fresh), type of extraction (CAE and UAE) and their interaction showed statistically significant differences for the response variables. A p-value less than 0.05 was considered to evaluate the significance of the independent variables and their interactions (Tables XII to XIX in the Appendix).

The treatment with the highest pectin extraction yield was the *fresh* peel with CAE. There was no significant difference in the results using UAE between *fresh* and frozen peels, while for CAE, all treatments differed from each other. For *fresh* and dried peels, the use of UAE in the first extraction resulted in lower pectin yields. In general, the drying pretreatment negatively influenced the pectin extraction yield, and the use of ultrasound was not effective.

Although the pectin content found in jabuticaba peel (1 to 5%) is low when compared to the pectin content extracted from passion fruit peel (14%), apple bagasse (24%) and banana peel (10%) (Kulkarni & Vijayanand 2010, Khamsucharit et al. 2018), it should be taken into account that the anthocyanin extraction residue is commonly discarded and therefore is an option for pectin extraction. Benvenutti et al. (2021) reported similar and higher yields for pectin extraction of dried jabuticaba peel, obtaining 7.5% and 9.3% for extraction with hot water by stirring with and without citric acid, respectively; 12.1%, 17.8% and 19.4% for extraction with subcritical water without and with citric acid and in the extraction with subcritical water modified by deep eutectic solvent, respectively.

The proposed sequential extraction of pectin was effective in recovering 91% of the pectin quantified in the initial raw material, showing its potential application in the fractionation of other residues that are rich in bioactive compounds and pectin. In addition, the pectins extracted from the *fresh* peel residue showed 63% higher extraction yield for CAE and 77% higher extraction yield for UAE when compared to pectins extracted from dry peel residues.

The degree of esterification is considered a parameter that characterizes pectin polymers, as it is specific to each plant, in addition to being influenced by the change in pectin during the ripening of fruits and vegetables by the action of the enzyme pectinesterase (Koubala et al. 2008). Generically, pectins are subdivided into two classes, one with a high degree of methoxylation (> 50%), ATM, and the other with a low degree of methoxylation (<50%), BTM. Commercially, pectins with a high degree of methoxylation have levels in the range of 55% to 75%, whereas in those with a low degree of methoxylation, the levels vary in the range of 15% to 45%. ATMs have gelling power and are widely used in the gelation of jams and are of greatest interest to the industry. BTMs are used in the manufacture of diet products (Brandão & Andrade 1999, Chan & Choo 2013). The degree of esterification of pectin from the dry jabuticaba peel was not affected by the type of extraction (CAE and UAE), indicating that more studies

with higher powers or longer exposure time to ultrasound are perhaps necessary for the difference between treatments to be significant, once, ultrasound is capable of modifying the structure of the cell wall of the plant, facilitating the extraction of the soluble fiber under study (Oliveira et al. 2016).

Fresh and frozen peels obtained by UAE resulted in higher DE. Similar values were found by Oliveira et al. (2016), in passion fruit peel, when evaluating the influence of ultrasound on pectin extraction. It could be said that exposure to ultrasound facilitated pectin extraction with higher DE. The lowest DE was found for the frozen peel obtained using CAE, which differed significantly from the other treatments obtained by CAE.

In the present study, it was noted that the most promising treatment was *fresh* peel obtained by CAE. For antioxidant activity via DPPH, the best treatment was *fresh* peel (lowest EC_{50} value). For TA, the ultrasound treatments showed lower values, and there was no difference between the pretreatments, with no significant effect of the interaction. In general, the use of freezing resulted in median values, which are related to the cellular damage caused by the formation of ice crystals and loss of turgor of the jabuticaba peel and exudate upon thawing. In the treatments with drying, the lowest results were obtained for the studied variables (except TA and DE), a fact that can be explained by the thermal degradation of the compounds during the drying process, even though this treatment has a smaller particle size in the extraction. Thus, it is noted that if the jabuticaba peel is used immediately after pulping, it is not necessary to use pretreatments, which, due to energy expenditures, are expensive; thus, the *fresh* peel can be used for the extraction of the bioactive compounds studied and subsequently the extraction of pectin from the residue.

If the objective of extraction is anthocyanin extraction, the yield obtained in the drying process at a temperature of 38 °C is similar to the treatment of extract obtained from *fresh* peel, but it prevents the sequential extraction of pectin, which has lower yields than those obtained using *fresh* or frozen samples.

Contrary to expectations, the treatments subjected to ultrasound showed yields less than or equal to the treatments subjected only to agitation.

Colorimetric analysis

Through colorimetric analysis, it is possible to correlate the coordinate values with the concentration of anthocyanins in the extract and in the extraction residue. Table III shows the results of the colorimetric analysis for pretreated and *fresh* jabuticaba peels. In the color analysis of the raw material (Table III), the parameters L*, C* and a* showed higher values for the dried peel, followed by the *fresh* peel and finally the frozen peel. For the parameter h°, the frozen peel showed the highest value and differed significantly from the others.

It should be noted that the color of the dried peel is lighter and more intense (L* and C* larger), while the color of the frozen peel is darker and less intense (L* and C* smaller). This indicates that the pretreatment influences the color of the jabuticaba peel, since compounds can be degraded or modified during pretreatment. The parameter a* shows that the dried peel has a more reddish color, which can be explained by the fact that during drying, biochemical reactions occur and there is a concentration of compounds that were previously solubilized in water.

Table IV show the color results for the extracts and residues from the first extraction. The results of the analysis of variance of the extract color parameters (L*, C* and h°) indicated that only the type of extraction and the interaction for the L*

Table III. Color parameterrs L*, C*, h°, a* and color reconstitution for pretreated and fresh jaboticaba peels.

Treatment	L*	C*	h°	a*	Reconstituted Color
Fresh	23.74b	10.09b	7.31b	10.00b	
Freezing	17.26c	7.88c	15.42a	7.79c	
Drying	35.79a	19.04a	7.83b	18.36a	

Mean; n=3. Mean values with common letters in the same column indicate that there is no significant difference between samples ($p \leq 0.05$) by Tukey's test. L*= luminosity. C*= chroma. hº= tone angle. a*= color variation from green to red.

parameter was not significant (Tables XII to XV in the Appendix).

Regarding the L* parameter, low values were found (Table IV), which indicates dark samples, and there are no significant differences between the use of ultrasound. Extracts obtained from dry and *fresh* peels using CAE showed lower values and were therefore darker than the others.

The color intensity parameter (C*) indicates the intensity of the main compost (Minolta 2007), and the extract obtained from the *fresh* peel with agitation has the highest value, showing a more intense color. In both CAE and UAE, the *fresh* peel had the highest C* value. For *fresh* and frozen peels, UAE resulted in less intense extracts; for the dry peel, there was no significant effect of the type of extraction. Regarding the hue angle (h°), the dry peel extracts did not show significant differences in the extraction type (CAE or UAE). Conversely, for *fresh* and frozen peels, CAE resulted in greater angles. Extracts obtained from frozen peels showed lower values of shade angles in both types of extraction.

Higher values for the L* parameter and lower values for the C* and h° parameters of jabuticaba peel extracts in extractions using ethanol solvent (100%) and variations in ultrasound intensities (1.1; 3.7; 7.3 and 13.0 W/cm^2) were found by Tarone (2021). Values close to those of L* for the dry peel extract were found by Resende et al. (2020).

When evaluating the three parameters, L*, C* and h°, individually, it is not possible to visualize the differences between the samples, but it is possible to observe a relationship between these parameters and reconstitute the color in a more didactic and visible manner. Thus, it should be noted that the extracts obtained by UAE for *fresh* and frozen peels showed less intense colors and less hue than those obtained by CAE, as previously mentioned.

In the color analysis of the residue, the type of extraction and the interaction of the parameters L* and h° did not show significant effects (Tables XVI to XIX in the Appendix).

From the color analysis data of the residues (Table IV), it is observed that for L*, the treatments with the dry peel showed higher values, indicating lighter colors, followed by frozen and *fresh* peels. Regarding the use of ultrasound, no significant difference was found for each pretreatment.

Regarding the C* values of the residues, a significant difference was reported between the *fresh* and frozen peel samples regarding the type of extraction (CAE or UAE), and UAE resulted in less intense residues. For the dry peel, there was no significant difference between the type of extraction in the C* values, and the same behaviour was observed for the extracts (Table IV). The residue obtained from the CAE from the *fresh* peel had a higher C* value, showing a more intense color. Regarding the pitch angle (h°), there was no significant difference between the use of ultrasound. The largest angles were found for the dry peel residues.

Through the relationship between the three parameters (L*, C*, h°) to obtain a visible color

Table IV. Color results obtained for the parameters L*, C* and h° and color reconstitution for jabuticaba bark extracts and residues in the first extraction.

Pretreatment	*Fresh*	Freezing	Drying
Extract			
	L* - CV (%) = 18.90		
CAE	38.65ab	44.48a	25.10b
UAE	47.79a	55.14a	26.49b
	C* - CV (%) = 2.68		
CAE	91.59Aa	77.03Ab	60.75Ac
UAE	83.45Ba	50.32Bc	62.88Ab
	h° - CV (%) = 4.18		
CAE	41.92Aa	31.02Ab	42.46Aa
UAE	27.03Bb	12.26Bc	43.18Aa
	Reconstituted Colour		
CAE			
UAE			
Residue			
	L* - CV (%) = 6.14		
CAE	19.27c	25.16b	33.40a
UAE	17.78c	22.61b	32.66a
	C* - CV (%) = 12.55		
CAE	22.33Aa	6.78Ac	13.42Ab
UAE	14.82Ba	2.20Bb	13.52Aa
	h° - CV (%) = 17.59		
CAE	14.80ab	11.78b	19.84a
UAE	10.20b	13.78ab	17.36a
	Reconstituted Colour		
CAE			
UAE			

Mean; n=3. The mean values with common lowercase letters in the same row and common uppercase letters in the same column indicate that there is no significant difference between the samples ($p \leq 0.05$) by the Tukey test for the analyzed variable. CAE= conventional extraction; UAE = ultrasound-assisted extraction; CV = coefficient of variation. L*= luminosity. C*= chroma. hº= tone angle.

for the residue from the first extraction, it should be noted that the *fresh* peel treatments showed a more intense and darker color. Conversely, the treatments with drying showed less intense colors, indicating possible degradation of the compounds initially present in the jabuticaba peel.

Comparing the results of extracts and residues (Table IV), for the saturation (C*), the results were quite consistent with the reconstituted color, that is, the extracts presented higher values than the residues, since the extract is the liquid filtered from the extraction and the residue retains on the filter.

Table V presents the results for the parameter a* found in the extract and in the residue.

The red color indicates the presence of pigments from this spectrum, in the case of jabuticaba peel, the anthocyanins. In this study, high values were obtained in the extract and proportionally lower in the residue because, during the extraction, the pigments present in the peel were extracted and found in the extract. This occurred for the *fresh*, dried and frozen treatments. The value of a* followed the ascending order of freezing, drying and *fresh* for CAE, both in the extract and in the residue. In the UAE, the *fresh* and dried peels did not differ from each other, followed by the frozen peel, and this behaviour occurred for the extract and residue.

Table V. Color results obtained for a* of the extracts and residues.

Pretreatment	Fresh	Freezing	Drying
	Extract - CV (%) = 2.68		
CAE	68.15Aa	34.00Bc	44.77Ab
UAE	44.26Ba	39.16Ab	45.81Aa
	Residue - CV (%) = 12.16		
CAE	21.58Aa	6.60Ac	12.62Ab
UAE	14.58Ba	2.18Bb	12.88Aa

Mean; n=3. The mean values with common lowercase letters in the same row and common uppercase letters in the same column indicate that there is no significant difference between the samples ($p \leq 0.05$) by the Tukey test for the analyzed variable. CAE= conventional extraction; UAE = ultrasound-assisted extraction; CV = coefficient of variation. a*= color variation from green to red.

CONCLUSIONS

The sequential extraction of anthocyanins and pectin from *fresh* jabuticaba peel is a viable option, since similar efficiencies for TA and an efficiency greater than 63% was obtained for pectin compared to extraction of the dried peel. When comparing the *fresh* peel extracts obtained by different types of extraction, it was verified that ultrasound-assisted extraction was ineffective for extraction of TPC, TA, antioxidant activity by DPPH and pectin yield, and the addition of this step in the process under the conditions used was not justified. Regarding the degree of pectin esterification, only the treatment of *fresh* peel with UAE showed an DE above 75%. The most suggested pretreatments for anthocyanin extraction are those that use CAE. Regarding the sequential extraction of pectin, the use of UAE in the first step decreased the yield of the sequential pectin extraction from the *fresh* and dry peels and had no significant effect on the extraction of the frozen peel. The CAE of the *fresh* peel yielded the highest pectin extraction; therefore, for the sequential extraction of TA and pectin, the best treatment was CAE of the *fresh* peel.

Acknowledgments

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, Brazil) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes, Brasília, Brazil) by granting the scholarship and financial support in the acquisition of equipment, and the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG, Belo Horizonte, Brazil) by financial support for this research.

REFERENCES

AOAC - Association of Official Analytical Chemists. 2016. Official Methods of analysis of AOAC International. 18th ed., Washington: AOAC.

BELITZ H-D, GROSCH W & SCHIEBERLE P. 2009. Food Chemistry. 4th. ed., Berlin: Springer Verlag, 1114 p.

BENVENUTTI L, ZIELINSKI AAF & FERREIRA SRS. 2021. Jaboticaba (*Myrtaceae cauliflora*) fruit and its by-products: Alternative sources for new foods and functional components. Trends Food Sci Tech 112: 118-136.

BRANDÃO EM & ANDRADE CT. 1999. Effects of structural features on the gelling process of high methoxyl pectins. Polimeros 9(3): 38-44.

BRAND-WILLIAMS W, CUVELIER ME & BERSET C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT – Food Sci Technol 28(1): 25-30.

BUGIANESE R, SALUCCI M, LEONARDI C, FERRACANE R, CATASTA G, AZZINI E & G MAIANI. 2004. Effect of domestic cooking on human bioavailability of naringenin, chlorogenic acid, lycopene and carotene in cherry tomatoes. Eur Jour Nutr 43(6): 360-366.

CANTERI MHG, MORENO L, WOSIACKI G & SCHEER AP. 2012. Pectin: from raw material to the final product. Polimeros 22(2): 149-157.

CAVALCANTI RN, SANTOS DT & MEIRELES MAA. 2011. Non-thermal stabilization mechanisms of anthocyanins in model and food systems – An overview. Food Res Int 44(2): 499-509.

CHAN S-Y & CHOO W-S. 2013. Effect of extraction conditions on the yield and chemical properties of pectin from cocoa husks. Food Chem 141(4): 3752-3758.

FERREIRA SPL, JARDIM FBB, DA FONSECA CR & COSTA LL. 2020. Whole-grain pan bread whit the addition of jabuticaba peel flour. Cien Rural 50(8): e20190623.

GENNADIOS A, WELLER CL, HANNA MA & FRONING GW. 1996. Mechanical and barrier properties of egg albumen films. J Food Sci 61(3): 585-589.

GIUSTI MM & WROLSTAD RE. 2001. Anthocyanins. Characterization and Measurement with UV-Visible Spectroscopy. Curr Prot Food Anal Chem Unit F1.2, 1-13.

HOSSEINI SS, KHODAIYAN F & YARMAND MS. 2016. Aqueous extraction of pectin from sour orange peel and its preliminary physicochemical properties. Int J Biol Macromol 82: 920-926.

HUARANCCA-HUARCAYA R, PAREDES-QUIROZ LR, ESTRADA NMP & BARRAGÁN-CONDORI M. 2022. Kinetic of termal degradation of alaybilí (*Vaccinium floribundum Kunth*) and macha-macha (*Gaultheria glomerata* (Cav.) Sleumer) anthocyanins. Braz J Food Technol 25: e2021106.

INADA KOP ET AL. 2015. Screening of the chemical composition and occurring antioxidants in jabuticaba (*Myrciaria jaboticaba*) and jussara (*Euterpe edulis*) fruits and their fractions. J Funct Food 17: 422-433.

JAFARI F, KHODAIYAN F, KIANI H & HOSSEINI SS. 2017. Pectin from carrot pomace: Optimization of extraction and physicochemical properties. Carbohyd Polym 157: 1315-1322.

KHAMSUCHARIT P, LAOHAPHATANALERT K, GAVINLERTVATANA P, SRIROTH K & SANGSEETHONG K. 2018. Characterization of pectin extracted from banana peels of different varieties. Food Sci Biotechnol 27: 623-629.

KOUBALA BB, KANSCI G, MBOME LI, CRÉPEAU M-J, THIBAULT J-F & RALET M-C. 2008. Effect of extraction conditions on some physicochemical characteristics of pectins from "Améliorée" and "Mango" mango peels. Food Hydrocol 22(7): 345-1351.

KULKARNI SG & VIJAYANAND P. 2010. Effect of extraction conditions on the quality characteristics of pectin from passion fruit peel (*Passiflora edulis f. flavicarpa* L.). LWT – Food Sci Technol 43(7): 1026-1031.

LEITE-LEGATTI AV ET AL. 2012. Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities. Food Res Int 49(1): 596-603.

LIMA AJB, CORRÊA AD, SACZK AA, MARTINS MP & CASTILHO RO. 2011. Anthocyanins, pigment stability and antioxidant activity in jabuticaba [*Myrciaria cauliflora* (Mart.) O. Berg]. Rev Bras Frutic 33(3): 877-887.

MAREČEK V, MIKYSKA A, HAMPEL D, CEJKA P, NEUWIRTHOVÁ J, MALACHOVÁ A & CERKAL R. 2017. ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. J Cereal Sci 73: 40-45.

MARTINS S, MUSSATO SI, MARTÍNEZ-AVILA G, MONTAÑEZ-SAENZ J, AGUILAR CN & TEIXEIRA JA. 2011. Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. Biotechnol Adv 29(3): 365-373.

MCCREADY RM & MCCOMB EA. 1952. Extractions and determination of total pectin materials in fruits. Anal Chem 24(12): 1986-1988.

MEREGALLI MM, PUTON BMS, CAMERA FDM, AMARAL AU, ZENI J, CANSIAN RL, MIGNONI ML & BACKES GT. 2020. Conventional and ultrasound-assisted methods for extraction of bioactive compounds from red araçá peel (*Psidium cattleianum Sabine*). Arab J Chem 13: 5800-5809.

MINOLTA. 2007. Precise color communication: color control from perception to Instrumentation. Japan: Minolta Co. Ltd. Available from: https://www.konicaminolta.com/instruments/knowledge/color/pdf/color_communication.pdf.

MIRANDA BM, DI-MEDEIROS MCB, BATISTA KA, CARBONERO ER, FERNANDES KF & SILVA FA. 2020. A galactose-rich heteropolysaccharide extracted from "jaboticaba" (*Plinia cauliflora*) peels. Carbohyd Polym 249: 116821.

MUGWAGWA LR & CHIMPHANGO AFA. 2019. Box-Behnken design based multi-objective optimisation of sequential extraction of pectin and anthocyanins from mango peels. Carbohyd Polym 219: 29-38.

MUHAMMAD ZAHIR SAD, YAHAYA OKM & OMAR AF. 2020. Correlating the natural color of tropical fruit juice whit its pH. Color Res Appl 46(2): 467-476.

OLIVEIRA CF, GIORDANI D, LUTCKEMIER R, GURAK PD, CLADERA-OLIVEIRA F, MARCZAK LDF. 2016. Extraction of pectin from passion fruit peel assisted by ultrasound. LWT – Food Sci Technol 71: 110-115.

PALUDO MC ET AL. 2022. Phenolic composition of peels from different Jaboticaba species determined by HPLC-DAD-ESI/MS^n and antiproliferative activity in tumor cell lines. Current Plant Biology 29: 100233.

RANGANNA S. 1997. Manual of analysis of fruit and vegetable products. New Delhi: Tata McGraw Hill Publishing Company Ltd., 634 p.

RESENDE LM, OLIVEIRA SO & FRANCA AS. 2020. Characterization of jaboticaba (*Plinia cauliflora*) peel flours and prediction of compounds by FTIR analysis. LWT – Food Sci Technol 133: 110135.

SAMAVATI V. 2013. Polysaccharide extraction from Abelmoschus esculentus: Optimization by response surface methodology. Carbohyd Polym 95(1): 588-597.

SAMAVATI V & MANOOCHEHRIZADE A. 2016. Polysaccharide extraction from Malva sylvestris and its anti-oxidant activity. Int J Biol Macromol 60: 427-436.

SANTOS DT, VEGGI PC & MEIRELES MA. 2010. Extraction of antioxidant compounds from Jabuticaba (*Myrciaria cauliflora*) skins: Yield, composition and economical evaluation. J Food Eng 101(1): 23-31.

SILVA MC ET AL. 2014. Use of the jabuticaba (*Myrciaria cauliflora*) depulping residue to produce a natural pigment powder with functional properties. LWT - Food Sci Technol 55(1): 203-239.

STATSOFT. 2010. Tulsa, Oklahoma, EUA, Data Analysis Software System 10.0. Available from: https://www.spotfire.com/products/data-science.

TARONE AG, SILVA EK, BARROS HDFQ & CAZARIN C. 2021. High-intensity ultrasound-assisted recovery of anthocyanins from jabuticaba by-products using green solvents: Effects of ultrasound intensity and solvent composition on the extraction of phenolic compounds. Food Res Int 140: 110048.

TROJANOWSKA A, TSIBRANSKA I, DZHONOVA D, WRÓBLEWSKA M, HAPONSKA M, JOVANCIC P, MARTURANO V & TYLKOWSKI B. 2019. Ultrasound-assisted extraction of biologically active compounds and their successive concentration by using membrane processes. Chem Eng Res Des 147: 378-389.

WATERHOUSE AL. 2002. Polyphenolics: Determination of total phenolics. In: WROLSTAD RE (Eds), Current Protocols in Food Analytical Chemistry, New York: John Wiley & Sons, Units 11.1.1-11.1.8.

WU SB, LONG C & KENNELLY EJ. 2013. Phytochemistry and health benefits of jaboticaba, an emerging fruit crop from Brazil. Food Res Int 54(1): 148-159.

APPENDIX

ANOVA tables for 3 x 2 factorial design responses

Table VI. ANOVA for total phenolic contente response.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	1327,22	2	663,61	742,06	0,000
X2	901,11	1	901,11	1007,64	0,000
X1 x X2	490,09	2	245,05	274,01	0,000
Residue	10,73	12	0,89		
Total	2729,16	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not.

Table VII. ANOVA for total anthocyanins response.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	18147,04	2	9073,52	7,11	0,009
X2	265744,07	1	265744,07	208,38	0,000
X1 x X2	206,37	2	103,18	0,08	0,923
Residue	15303,16	12	1275,26		
Total	299400,64	17			

X1 = petreatment (fesh, freezing and drying); X2 = use of ultrasound or not.

Table VIII. ANOVA for antioxidant activity via DPPH response.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	249451,39	2	124725,70	329,29	0,000
X2	4974,47	1	4974,47	13,13	0,004
X1 x X2	3563,60	2	1781,80	4,70	0,031
Residue	4545,31	12	378,78		
Total	262534,78	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not.

Table IX. ANOVA for antioxidant activity via ABTS response.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	10700097,73	2	5350048,86	13597,57	0,000
X2	283278,65	1	283278,65	719,98	0,000
X1 x X2	1527944,02	2	763972,01	1941,70	0,000
Residue	47721,48	12	393,46		
Total	12516041,87	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not.

Table X. ANOVA for pectin yield response.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	44,16	2	22,08	326,62	0,000
X2	2,78	1	2,78	41,15	0,000
X1 x X2	1,02	2	0,51	7,52	0,008
Residue	0,81	12	0,07		
Total	48,77	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not.

Table XI. ANOVA for esterification degree response.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	2308,64	2	1154,32	52,18	0,000
X2	656,43	1	656,43	29,67	0,000
X1 x X2	386,87	2	193,44	8,74	0,005
Residue	265,45	12	22,12		
Total	3617,39	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not.

Table XII. ANOVA for L* response of the extract.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	1847,76	2	9223,88	17,99	0,000
X2	224,65	1	224,65	4,38	0,058
X1 x X2	74,12	2	37,06	0,72	0,506
Residue	616,12	12	51,34		
Total	2762,65	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not. L*= luminosity.

Table XIII. ANOVA for C* response of the extract.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	2465,40	2	1232,70	339,82	0,000
X2	535,19	1	535,19	147,54	0,000
X1 x X2	641,53	2	320,77	88,43	0,000
Residue	43,53	12	3,63		
Total	3685,65	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not. C*= chroma.

Table XIV. ANOVA for hº response of the extract.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	1366,42	2	683,21	358,83	0,000
X2	541,97	1	541,97	284,65	0,000
X1 x X2	319,10	2	159,55	83,80	0,000
Residue	22,85	12	1,90		
Total	2250,34	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not. hº= tone angle.

Table XV. ANOVA for a* response of the extract

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	1160,07	2	580,03	379,97	0,000
X2	156,29	1	156,29	102,38	0,000
X1 x X2	740,94	2	370,47	242,69	0,000
Residue	18,32	12	1,53		
Total	2075,62	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not. a*= color variation from green to red.

Table XVI. ANOVA for L* response of the residue.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	345,33	2	322,66	135,24	0,000
X2	11,44	1	11,44	4,80	0,049
X1 x X2	2,50	2	1,25	0,52	0,605
Residue	28,63	12	2,38		
Total	687,90	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not. L*= luminosity.

Table XVII. ANOVA for C* response of the residue.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	609,72	2	304,86	130,60	0,000
X2	71,92	1	71,92	30,811	0,000
X1 x X2	44,20	2	22,10	9,47	0,003
Residue	28,01	12	2,33		
Total	753,85	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not. C*= chroma.

Table XVIII. ANOVA for hº response of the residue.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	142,40	2	71,20	10,76	0,002
X2	12,99	1	12,99	1,96	0,187
X1 x X2	34,05	2	17,03	2,57	0,118
Residue	79,43	12	6,62		
Total	268,88	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not. hº= tone angle.

Table XIX. ANOVA for a* response of the residue.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F	p-value
X1	571,30	2	285,65	140,12	0,000
X2	62,31	1	62,31	30,57	0,000
X1 x X2	40,76	2	20,38	10,00	0,003
Residue	24,46	12	2,04		
Total	698,83	17			

X1 = petreatment (*fesh*, freezing and drying); X2 = use of ultrasound or not. a*= color variation from green to red.

How to cite

BUENO TM, QUEIROZ F, SANTOS JCC, FURTADO MLB, SCHIASSI MCEV, BORGES SV & FIGUEIREDO JA. 2024. Sequential extraction of anthocyanins and pectin from jabuticaba (*Plinia cauliflora*) peel: Peel pretreatment effect and ultrasound-assisted extraction. An Acad Bras Cienc 96: e20230174. DOI 10.1590/0001-3765202420230174.

Manuscript received on February 24, 2023;
accepted for publication on May 27, 2023

TAINAH M. BUENO
https://orcid.org/0000-0002-4255-5659

FABIANA QUEIROZ
https://orcid.org/0000-0002-8958-5476

JHENIFER CRISTINA C. DOS SANTOS
https://orcid.org/0000-0002-9060-3141

MARIA LUIZA B. FURTADO
https://orcid.org/0000-0001-6204-9801

MARIA CECÍLIA E.V. SCHIASSI
https://orcid.org/0000-0001-8704-5815

SORAIA V. BORGES
https://orcid.org/0000-0002-1612-1363

JAYNE A. FIGUEIREDO
https://orcid.org/0000-0002-9403-5655

Universidade Federal de Lavras, Departamento de Ciência dos Alimentos, Campus Universitário, Caixa Postal 3037, 37200-900 Lavras, MG, Brazil

Correspondence to: **Tainah Morais Bueno**
E-mail: tainahmbueno1997@gmail.com

Author contributions

Tainah Morais Bueno is the main author of the project and participated fully in the entire project with the guidance of Fabiana Queiroz. Jhenifer Cristina Carvalho dos Santos and Maria Luiza Bianchetti Furtado collaborated in carrying out analyses, data collection and data interpretation. Maria Cecília collaborated with the writing of the article and critical review. Soraia Vilela Borges and Jayne de Abreu Figueiredo collaborated in carrying out the analyzes with ultrasound assistance.