Research Article

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# Sequential extraction of flavonoids and pectin from yellow passion fruit rind using pressurized solvent or ultrasound

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## Abstract

**BACKGROUND: Passion fruit rind (PFR) represents 90% of the total fruit weight and is wasted during juice processing. Passion fruit rind is known to contain flavonoids and pectin. An alternative use for this fruit juice industrial residue is to obtain these compounds. This study aimed to verify the influence of pressurized solvent extraction (PSE) or ultrasound assisted extraction (UAE) of flavonoid and pectin in a sequential process.**

**RESULTS: The PSE using ethanol at 60:40 (v/v) yielded a total polyphenol content of 4.67 g GAE $kg^{-1}$ PFR, orientin-7-*O*-glucoside (1.57 g $kg^{-1}$ PFR) and luteolin-6-*C*-glucoside (2.44 g $kg^{-1}$ PFR). Pectin yield was 165 g $kg^{-1}$ PFR, either in PSE or UAE. Pectin characterization indicates that the pectic structure has basically homogalacturonans and galacturonate followed by a galacturonic acid ester unit, with methylation degree of 70%.**

**CONCLUSION: With this study it can be concluded that mixtures of alcohols with water favor the extraction of bioactive compounds of passion fruit peel. Both PSE and UAE were effective in sequentially extracting flavonoids and pectin. The preferred solvent is ethanol due to its lower toxicity.**


**Keywords:** luteolin-6-*C*-glucoside; orientin-7-*O*-glucoside; NMR; *Passiflora*; PSE, waste; UAE

## INTRODUCTION

The genus *Passiflora* comprises between 450 and 600 passion fruit species.[1,2] Passion fruit rind (mesocarp and pericarp) corresponds to nearly 90% of the fruit weight, which is discarded as waste during juice processing.[3] The use of this residue represents an opportunity to create income sources by producing new products, simultaneously minimizing environmental impacts.[4] Products from tropical fruit residues contain bioactive compounds (vitamins, minerals, phenolic compounds, and dietary fibers), which are beneficial to health.[5] They may also be used as food additives (antioxidants, antimicrobials, dyes, flavorings, and thickeners).[6]

Several *C*-glucoside flavonoids have been found in the leaves, rind and in the juice of *Passiflora* species.[2,7,8] These compounds boast great diversity in biologic activity such as antioxidant roles, vasodilation effects, antiallergic, antitumoral, anti-inflammatory, antihepatotoxic, antiulcerogenic, antiplatelet, antimicrobial, and antiviral activities.[9,10] Another chemical structure found in passion fruit rind is pectin. The primary pectic structure is a galacturonic acid homopolymer bound at $\alpha$-(1,4) with a variable level of methyl esterified carboxyl groups. The pectic structure has a gel-forming property, offering opportunities for its application. Under certain conditions, pectins are used as gelling and stabilizing agents in the pharmaceutical, cosmetic and food industries, particularly in the production of sweets, jellies, and other products,[11–14] besides replacing fat in ice cream and salad dressings.[15] The pectin extracted from passion fruit rind meets the industrial technological requirements for pectic products.[16] Passion fruit pectin also has therapeutic properties, for example, helps in reduction of total cholesterol, reducing fractions of low density lipoproteins; assists in reducing blood glucose; slows gastric emptying, which leads to lower digestion and absorption, increasing the feeling of satiety, consequently assists in weight loss.[17]

Alcoholic solvents are often used in the extraction of phenolic compounds, since they provide a high yield of total extract. In particular, mixtures of alcohols and water have proved to be more efficient in extracting phenolic constituents than a system containing only one solvent.[18] This mixture of solvent with water provides an increase in the polarity of the solvent, whereby higher phenolic compound contents can be extracted.[19–21]

Several extraction processes have been developed in recent years, in particular, pressurized solvent extraction (PSE) and ultrasound assisted extraction (UAE).[22] The PSE is an automated

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extraction technique that uses high temperature and pressure to quickly obtain extracts. The high temperature improves solubilization, increasing diffusion rates and, consequently, weakens the solute–matrix interactions. High pressure enables working with the solvent above its boiling point, which speeds up the extraction process.[23] In PSE less solvent is used during the process, simultaneous extractions can be carried out, solvent combinations can be programmed, and the extracted compounds are quickly recovered.[24,25]

UAE uses acoustic energy and a solvent to extract the target compounds of several plant structures.[26] The extraction mechanism involves diffusion, which forms cavitation bubbles resulting in better cell rupture due to the formation of micro jets close to the plant cell wall. This enables better solvent penetration into the cellular matrix, thus increasing the mass transfer rate.[27] Some factors that impact the UAE process are the sound wave intensity, exposition time to the sound frequency, process temperature and product granulometry.[28] UAE is used to extract a broad range of food compounds such as pigments, aromas, antioxidants, and other organic and mineral compounds from different matrices (animal origin tissue, foods, and plant material).[27]

For pectin extraction, the most commonly employed methods include direct boiling and microwave heating. New pectin extraction methods are being employed aiming to obtain better yield, pectic structure quality, reduce environmental pollution by using less solvents and increase energy efficiency. Some techniques employed are the ultrasound-assisted extraction, supercritical fluid extraction, microwave-assisted extraction, and accelerated solvent extraction.[15,29]

The traditional extraction processes use large volumes of solvents and long periods of time to extract only one group of target substance. The sequential extraction is important in the recovery of the substances coming from agro-industrial waste, since this type of matrix has a variety of industrially important functional substances.[30] Thus the sequential extraction system is advantageous, since it can extract more than one target molecule consecutively. There are no reports in the literature on sequential extraction of flavonoids and pectin from passion fruit rind using the UAE and PSE techniques. Consequently, the present study aimed to evaluate a sequential extraction process of flavonoid and pectin from *Passiflora edulis* rind.

## MATERIALS AND METHODS

### Chemicals

Folin–Ciocalteu reagent, sodium carbonate, citric acid, and ethanol were purchased from Vetec (Duque de Caxias, RJ, Brazil). Water was obtained using a Milli-Q water purifier system from Millipore (Billerica, MA, USA). Gallic acid, quercetin and diatomaceous earth were purchased from Sigma–Aldrich (St. Louis, MO, USA), methanol and acetonitrile from Tedia (Rio de Janeiro, RJ, Brazil), and phosphoric and formic acids from Fluka (Buchs, Switzerland).

### Plant material

The *Passiflora edulis ssp. fruits* were purchased at Central de Abastecimento S/A (CEASA) in the city of Fortaleza, CE, Brazil. The fruits were hygienized and depulped. The rinds were chopped and dried in an air circulation oven at 60 °C for 48 h, and then ground in a grinding mill. The material used for extraction was sieved using a vibratory machine (Fristsch Analysette 3, Idar-Oberstein, Germany), with 1 Hz frequency. The material with a particle size $\leq 50\ \mu$m was used for extractions.

### Conventional extraction

Phenolics were extracted based on an adapted procedure[31] described as follows. Samples were weighed (2 g) in 50-mL centrifuge tubes and extracted sequentially with 40 mL of 50% (v/v) methanol in water solution at room temperature for 1 h. Tubes were centrifuged at $2540 \times g$ for 15 min and the supernatant was recovered. Then, 40 mL of 70% (v/v) acetone in water was added to the residue, extracted for 60 min at room temperature, and centrifuged for a second time ($2540 \times g$ for 15 min). Ethanol and acetone extracts were combined, made up to 100 mL with distilled water and used for Folin–Ciocalteu analysis.[32] For pectin quantification, a standard procedure was employed.[33] The passion fruit peel (2 g) was weighed and mixed with 120 mL of a 1% citric acid solution, heated for 6 h at 70 °C. The sample was centrifuged at $2540 \times g$ for 15 min. The supernatant was cooled to 4 °C for 24 h and filtered with a silk screen. The pectin was precipitated with 95% ethanol (1:2, extract:alcohol, v/v). After 1 h the pectin precipitate was separated by filtration, lyophilized and weighed.

### Sequential extraction

In the present study, sequential extractions for the recovery of different compounds were performed (Fig. 1), using a pressurized or an ultrasound process. For each process, detailed below, passion fruit rind underwent three cycles of extraction in order to extract flavonoids. The influence of the type of solvent and its concentration were tested. Sequentially, after the extraction of flavonoids, the residue underwent through more three cycles (4$^{th}$, 5$^{th}$ and 6$^{th}$ cycles) of extractions, detailed below, for pectin recovery.

### Pressurized solvent extraction

The PSE was carried out in an automated extractor Dionex ASE 350 system (Dionex, Sunnyvale, CA, USA). Initially, 2 g of the sample were weighed, mixed with 4 g of diatomaceous earth, and placed in a 66 mL stainless steel extraction cell. The process conditions were: pressure 10 342 to 11 721 kPa; temperature at 80 °C; 5 min rinsing; 10 min extraction time at each cycle; and 200 s purge. The solvents used were methanol/water or ethanol/water solutions at 80:20, 70:30, 60:40, and 50:50 (v/v). A solvent volume of 40 mL was used in the three extraction cycles. After three extraction cycles the extracts were combined, concentrated under reduced pressure and lyophilized. The lyophilized extracts were stored at −18 °C. Next, three more pectin extraction cycles were carried out for each sample (see Pectin extraction). All extraction experiments were performed in triplicate.

### Ultrasound-assisted extraction

The UAE process conditions were based on the same parameters used in the PSE. Initially, 2 g of sample were weighed and 40 mL of an extracting solution were added for each extraction cycle. The extraction was performed on an ultrasound bath model USC-1400 (Indaiatuba, SP, Brazil) whose standard ultrasound frequency was 40 kHz, power of 135 W and a water volume of 0.0012 m$^3$, resulting in a power density of 112 500 W m$^{-3}$. The extraction time was 10 min per cycle. After that, the samples were centrifuged for 15 min at $2254 \times g$ and the supernatant was collected. The extractions were repeated three times resulting in a 120 mL sample. Next, three more pectin extraction cycles were carried out for each sample (see Pectin extraction). All trials were carried out in triplicate and the samples obtained were evaporated and lyophilized. The lyophilized extracts were stored at −18 °C.



  

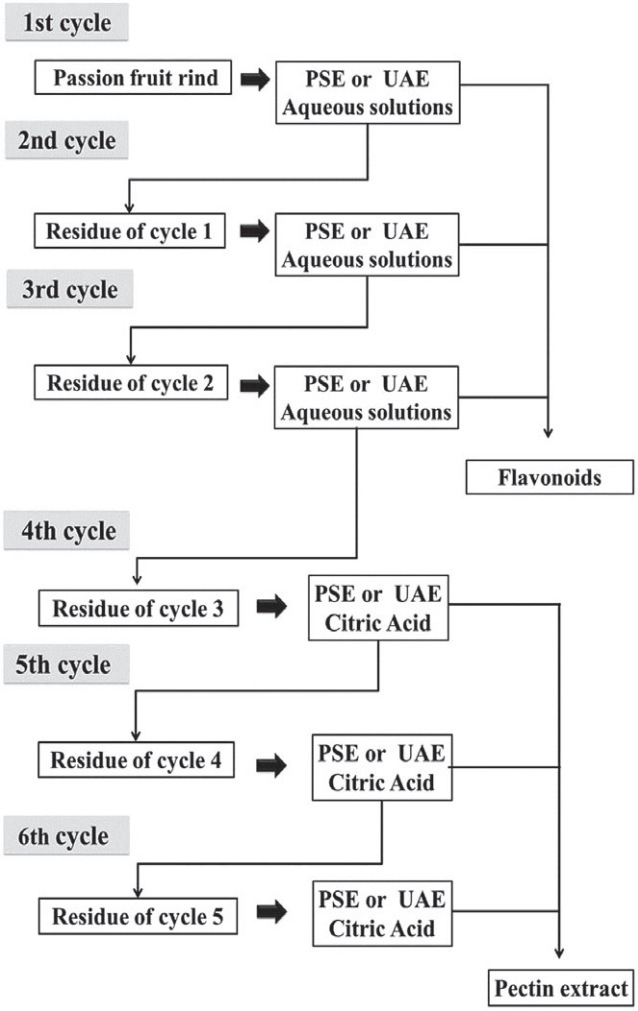


**Figure 1.** Flowchart of the sequential extraction of flavonoids and pectin from passion fruit rind.

### Pectin extraction

Pectin was sequentially obtained after the phenolic compounds extraction, i.e. the residual mass of the flavonoid extraction was used to extract pectin (Fig. 1). The solvent used to extract pectin was a 1% citric acid aqueous (w/v) solution. The extractions were performed following the same process conditions described above for PSE and UAE. After acid extraction, the sample was cooled to 4 °C for 24 h and filtered with a silk screen. The pectin was precipitated with 99.8% ethanol at 1:2 (extract: alcohol, v/v). After 1 h the pectin precipitated was separated by filtration, lyophilized and weighed.[33]

### Total polyphenols analysis

The methodology described by Singleton and Rossi was adapted to determine total polyphenol content.[32] The extracts were previously diluted with a 10% ethanol solution in water and aliquots ranging from 0.1 to 0.5 mL were used. The aliquots were added to test tubes and the volume was completed up to 0.5 mL with the 10% ethanol solution. The Folin–Ciocalteu reagent (0.5 mL) was added and, after 3 min, 0.5 mL 20% sodium carbonate. Next, the volume was completed to 5 mL with water and the tubes were shaken. After 90 min the absorbance was read in a UV spectrophotometer (Cary 300; Varian, Palo Alto, CA, USA) at 725 nm. The standard solution used was gallic acid at 0.001 to 0.012 mg $mL^{-1}$ in 10% ethanol. The results were expressed in gallic acid equivalents as g GAE $kg^{-1}$ PFR.

### Flavonoid profile

Liquid chromatography with diode array detection and electrospray ionization mass spectrometry (LC-DAD-ESI-MS) was used as the overall procedure to investigate phenolic compounds. A methodology adapted from Lin and Harnly was used.[34] The LC-DAD-ESI-MS device consisted of a Varian 250 HPLC (Varian, USA) coupled to a diode array detector (DAD) and a 500-IT mass spectrometer (Varian, USA). A Symetry C18 column (Waters Inc., Lake Forest, CA, USA) (5 $\mu$m, 250 × 4.6 mm) was used at a 0.4 mL $min^{-1}$ flow rate. The column oven temperature was set to 30 °C. The mobile phase employed was a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient varied linearly from 10% to 26% B (v/v) at 40 min, to 65% B at 70 min, and, finally, 100% B at 71 min and remained at 100% B until 75 min. The DAD was set to 190–650 nm. The mass spectra were acquired simultaneously using positive and negative electrospray ionization and at 80 V fragmentation tension for a 100–1000 amu mass range. Drying gas pressure of 241 kPa, spray gas pressure of 275 kPa, drying gas temperature of 370 °C, capillary tensions of 3500 V for both PI and NI, and spray shield tension of 600 V were used. The LC system was coupled to the MS detector with a 50% split. The compounds were tentatively identified based on their MS and UV–visible spectra, compared with literature data for *Passiflora* phenolics.

### Orientin-7-*O*-glucoside and luteolin-6-C-glucoside quantification

Orientin-7-O-glucoside and luteolin-6-C-glucoside were quantified by HPLC 920/940-LC (Varian Australia PTY Ltd, Melbourne, Australia) equipped with a quaternary pump and diode array detector (DAD). A Microsorb 100 (Varian, CA) C18 analytical column (5 $\mu$m, 250 mm × 4.6 mm), protected by a pre-column with the same composition (4 mm × 4.6 mm) was used. Mobile phase A consisted of HPLC-grade methanol while mobile phase B consisted of a 20:80 (v/v) 0.1% phosphoric acid ($H_3PO_4$) aqueous solution in methanol, flow rate of 1.0 mL $min^{-1}$, and oven temperature of 30 °C. Injection volume was 20 $\mu$L and the elution was carried out in gradient mode as follows, 0–5 min, 20% A; 5–18 min, 27% A; 18–24 min, 47% A; 24–25 min, 61% A; 25–29 min 100% A; 29–35 min, 20% A. Approximately 30 mg of sample extract was weighed and diluted in a 60:40 (v/v) MeOH/$H_2O$ solution in a 5 mL volumetric flask and filtered in a teflon membrane filter (0.45 $\mu$m). The wavelength used was 350 nm and the substances were quantified using a quercetin standard curve and the results expressed in g quercetin $kg^{-1}$ PFR.

### Nuclear magnetic resonance of pectin

In order to characterize the pectic polymer using nuclear magnetic resonance (NMR), the pectin obtained after flavonoid extraction using PSE with ethanol/water (60:40, v/v) was dialyzed (D9652, Sigma–Aldrich) to remove the citric acid. The dialyzed pectin extract was lyophilized and stored at room temperature until analysis.

The NMR spectra were obtained in a 600 MHz Agilent DD2 device (Agilent, Santa Clara, CA, USA) (for $^1H$ nucleus) equipped with a 5 mm inner diameter inverse detection One Probe (H-F/15 N-31P)



  

**Table 1.** Analysis of the influence of the sequential extraction process on the total polyphenol content and on pectin yield (polyphenols extraction: 1st to 3rd cycles; pectin extraction: 4th to 6th cycles)

| | | 1st to 3rd cycles | | |
|---|---|---|---|---|
| Solvent | Technique | Solvent/water ratio (v/v) | Total polyphenols (g GAE $kg^{-1}$) | 4th to 6th cycles Pectin yield (g $kg^{-1}$) |
| Methanol 50% and acetone 70% | Conventional | | $2.05 \pm 0.04^{j}$ | $280 \pm 18^{a}$ |
| Methanol | PSE | 80:20 | $3.66 \pm 0.06^{ed*}$ | $188 \pm 16^{b}$ |
| | | 70:30 | $4.06 \pm 0.02^{cd}$ | $149 \pm 17^{b}$ |
| | | 60:40 | $5.77 \pm 0.10^{a}$ | $160 \pm 13^{b}$ |
| | | 50:50 | $3.99 \pm 0.02^{cd}$ | $141 \pm 24^{b}$ |
| | UAE | 80:20 | $2.86 \pm 0.07^{gh}$ | $137 \pm 35^{b}$ |
| | | 70:30 | $4.21 \pm 0.01^{cd}$ | $160 \pm 9^{b}$ |
| | | 60:40 | $2.80 \pm 0.02^{gh}$ | $142 \pm 1^{b}$ |
| | | 50:50 | $3.01 \pm 0.01^{g}$ | $137 \pm 19^{b}$ |
| Ethanol | PSE | 80:20 | $2.57 \pm 0.01^{hi}$ | $138 \pm 35^{b}$ |
| | | 70:30 | $3.66 \pm 0.09^{ed}$ | $187 \pm 7^{b}$ |
| | | 60:40 | $4.67 \pm 0.04^{b}$ | $165 \pm 9^{b}$ |
| | | 50:50 | $2.34 \pm 0.07^{ij}$ | $132 \pm 2^{b}$ |
| | UAE | 80:20 | $2.05 \pm 0.01^{j}$ | $144 \pm 14^{b}$ |
| | | 70:30 | $2.77 \pm 0.08^{gh}$ | $159 \pm 25^{b}$ |
| | | 60:40 | $3.18 \pm 0.02.0^{fg}$ | $160 \pm 23^{b}$ |
| | | 50:50 | $3.48 \pm 0.03^{ef}$ | $136 \pm 19^{b}$ |

Means with the same letter do not significantly differ regarding the solvent system and techniques used according to Tukey's test ($P < 0.05$).
GAE, gallic acid equivalent.

and field gradient in the *z* axis. The samples were prepared by dissolving 5 mg of pectin in 500 $\mu$L deutered water with 1% sodium 3-(trimethylsilyl)propionate-2,2,3,3-d4 (TSP) for spectral calibration. The one-dimensional $^{1}H$ spectra were obtained at 80 °C with 20 s wait time between each acquisition, receptor gain set to 20, acquisition of 128 transients in a 22 ppm spectral window, and 66 k points. In order to aid in signal assignment, two-dimensional heteronuclear experiments were carried out with HSQC $^{1}H-^{13}C$ and HMBC $^{1}H-^{13}C$. For HSQC, 56 transients were acquired with 1 s wait time between each acquisition, 200 ppm and 16 ppm windows in the $^{13}C$ (F1) and $^{1}H$ (F2) dimensions, and 200 and 1974 points for F1 and F2, respectively. For HMBC, 64 transients were acquired with 1 s wait time between each acquisition, 240 ppm and 21.9 ppm windows in the $^{13}C$ (F1) and $^{1}H$ (F2) dimensions, and 200 and 1974 points for F1 and F2, respectively.

### Statistical analysis

The data obtained were subjected to one-way analysis of variance using the software SigmaPlot 12.0 (Systat Software Inc., Chicago, IL, USA). Significant differences among the sample means were determined using Student's *t*-test at 95% confidence level.

## RESULTS AND DISCUSSION

The total polyphenol content for each extraction system is shown in Table 1. The PSE with 60:40 (v/v) methanol/water had 5.77 g GAE $kg^{-1}$ PFR and a significant total polyphenol content was also found for an ethanol/water system (60:40, v/v, PSE), with a polyphenol content of 4.67 g GAE $kg^{-1}$ PFR. For the UAE, the best condition was the 70:30 (v/v) methanol/water system, with a polyphenol content of 4.21 g GAE $kg^{-1}$ PFR. The results indicate that the 60:40 (v/v) ratio, either with methanol or ethanol and PSE, led to a considerable increase in total polyphenol recovery compared to the other solvent systems and UAE. The mixture of alcoholic solvents and water changes the solvent polarity in such way that improves significantly the extraction of phenolic compounds.[33] A low total polyphenol content (1.50 g $kg^{-1}$) passion fruit rind was reported using a methanol/acetone system[35] and for *Passiflora tripartita* rind an amount of 0.56 g GAE $kg^{-1}$ was reported.[7] A comparison between PSE and conventional extraction to obtain phenolics from passion fruit bagasse showed that the PSE technique is more efficient resulting in a higher total phenolic content (5.50 g $kg^{-1}$ passion fruit bagasse).[33] In general, all the solvent systems as well as extraction technique produced a higher polyphenol content when compared to conventional extraction.

The phenolic compounds in the PFR extract were tentatively identified by comparing the data obtained in the LC-DAD-ESI-MS analysis (Table 2) and the data found in the literature. The compounds found in expressive amounts were identified as orientin-7-*O*-glucoside and luteolin-6-*C*-glucoside. The *C*-glucoside flavonoids identified in the present study were also found in the leaves and pericarp of *Passiflora edulis* var. *flavicarpa*, *Passiflora alata*, *Passiflora edulis* var. *edulis*, and *Passiflora tripartita* var. *molíssima*.[2,7,8] The results of LC–MS revealed that the different extraction conditions employed, as well as the type of solvent and their concentrations, did not impact the profile of the compounds obtained (data not shown). It was not observed degradation and any metabolic changes. On the contrary, the use of higher temperatures provided a better solubilization of the compounds, increasing their diffusion rates and therefore weakening the solute–matrix interactions. The high pressure allows working with the solvent above its boiling point, accelerating the overall extraction process.

The results of orientin-7-*O*-glucoside and luteolin-6-*C*-glucoside concentration are shown in Table 3. The treatments with the highest orientin-7-*O*-glucoside were the PSE using a 60:40 (v/v)



  

**Table 2.** Compounds putatively assigned found in the extract obtained using PSE with a 60:40 ethanol/water solution

| Retention time (min) | λ max (nm) | $[M-H]^-$ | $[M+H]^+$ | Compounds[a] |
|---|---|---|---|---|
| 10.26 | 270, 350 | 609 | 611 | Luteolin-(7-*O*-glucopyranosyl)-8-*C*-8-glucopyranoside (orientin-7-*O*-glucoside) |
| 12.64 | – | – | 611 | Luteolin-6,8-di-*C*-*β*-D-glucopyranoside (leucenin II) |
| 13.60 | 270, 350 | 593 | 595 | Apigenin-6,8-di-*C*-*β*-D-glucopyranoside (vicenin II) |
| 14.23 | 270, 350 | 579 | 581 | Luteolin-(6-*C*-pentosyl)-8-*C*-*β*-D-glucopyranoside isomer |
| 17.28 | 270, 350 | 563 | 565 | (6-*C*-*α*-L-arabinopyranosyl)-8-*C*-*β*-D-glucopyranoside (isoschaftoside) |
| 17.80 | 270, 350 | 447 | 449 | Luteolin-6-*C*-glucoside (isoorientin) |
| 19.33 | 265, 350 | – | 449 | Luteolin 8-*C*-glucoside (orientin) |
| 19.65 | 270 | 577 | 579 | Apigenin-7-rutinoside |
| 24.65 | 270, 350 | 461 | 463 | 4′-Methoxyluteolin-8-*C*-*β*-D-glucopyranoside |
| 28.84 | 270, 350 | 431 | 433 | Apigenin-8-*C*-*β*-D-glucopyranoside (vitexin) |

[a] All compounds were previously reported in the literature for *Passiflora* species.[7,8]

methanol/water system (1.71 g quercetin $kg^{-1}$ PFR); PSE using a 70:30 and 60:40 (v/v) ethanol/water systems (1.55 and 1.57 g quercetin $kg^{-1}$ PFR, respectively); and UAE using a 70:30 (v/v) methanol/water system (1.66 g quercetin $kg^{-1}$ PFR). The present result suggests that alcoholic solutions at 70:30 and 60:40 (v/v) ratios led to a significant recovery of orientin-7-*O*-glucoside due to solvent polarity, which effectively increased the flavonoid solubility. The luteolin-6-*C*-glucoside concentration in the PSE treatments using a 70:30 (v/v) methanol/water system was 2.57 g quercetin $kg^{-1}$ PFR and for the ratios of 70:30 and 60:40 (v/v) ethanol/water were 2.72 and 2.44 g quercetin $kg^{-1}$ PFR, respectively. For UAE, the extraction using a 50:50 (v/v) ethanol/water system yielded 2.56 g quercetin $kg^{-1}$ PFR.

The pectin yield ranged between 132 and 188 g $kg^{-1}$ PFR (Table 1). Such values suggest that pectin recovery was not influenced either by the previous flavonoid extraction or by the extraction techniques. However, it was significantly lower than the pectin yield obtained by a conventional extraction. Probably, due to the longer and exhaustive extraction procedure employed. However, these results stand out when compared to the research by Yapo on the effects of the type of extracting acid on the production and characterization of passion fruit rind pectin, in which citric acid yielded 51 g $kg^{-1}$ passion fruit rind,[36] 3.7 times less compared to the best yield obtained using the residual mass from the PSE treatment carried out with 80:20 (v/v) methanol solution. The use of nitric acid led to a higher pectin yield (139 g $kg^{-1}$ sample) while sulfuric acid yielded (102 g $kg^{-1}$ sample). Seixas *et al.* analyzed the influence of the solvent and their concentrations in pectin recovery from passion fruit rind.[13] The best extracting agent reported was tartaric acid with yields between 153 and 302 g $kg^{-1}$, followed by acetic acid at 94 to 129 g $kg^{-1}$ and nitric acid at 95 to 131 g $kg^{-1}$. However, despite tartaric acid promoting a high yield, the pectin extracted with this agent presented undesirable qualities such as low molar mass and lower uronic acid content. An optimized passion fruit rind pectin extraction (HCl, pH 2, 1:30 rind:solvent (m/v), 98.7 °C, 60 min extraction time, and two consecutive extractions)[16] gave results close to the pectin yields reported here.

Pectin composition varies according to the source as well as the conditions used on the extraction, isolation and purification.[37,38] For instance, pectins with low degree of methoxylation (DM) form gels in the presence of calcium ions while those with a high DM tend to form gels with the addition of sugars. Therefore, NMR comes as a valuable technique since it quickly provides information on the chemical composition, as well as the DM, that can be obtained with no need for the exhausting commonly employed methodologies to prepare the samples for analysis.[37,38]

The resonance data for galacturonic acid and the other residues are summarized in Table 4. The NMR spectrum of HSQC $^1H-^{13}C$ depicted the attributions of galacturonic acid signals, which are the main unit in the pectin obtained. This suggests that passion fruit pectin is formed mainly by homogalacturonans.[39] In the region of anomeric hydrogens, hydrogen 1′ is observed at $\delta$ 5. 15 with carbon at $\delta$ 102.4 (GalA′-H1) inherent to the two consecutive $\alpha$-1,4-GalA galacturonate units (GG).[14,37,40] Hydrogen at $\delta$ 4.96 with carbon at $\delta$ 103.1 (GalA-H1) refers to the system composed of one galacturonate unit followed by a galacturonic acid ester unit (GE).[37,40] The signal at $\delta$ 5.06 with carbon at $\delta$ 73.7 (GalA-H5) belongs to the proton H-5 adjacent to the ester group. This is confirmed with the HMBC $^1H-^{13}C$ spectrum, where the binding of H-5″ with the carboxyl at $\delta$ 173.6 is observed. The signal at $\delta$ 4.66 with carbon at $\delta$ 74.5 (GalA-H5″) belongs to the hydrogen H-5″ adjacent to the free carboxyl. This observation is also confirmed with the HMBC $^1H-^{13}C$ spectrum, where the binding of the hydrogen of the GalA-H5″ residue with the free carboxyl at $\delta$ 177.4 is observed. Moreover, the signal at $\delta$ 4.46 with carbon at $\delta$ 82.1 (GalA-H4) refers to the proton H-4 of galacturonic acid, the signal at $\delta$ 3.98 with carbon at $\delta$ 71.8 (GalA-H3) refers to the proton H-3; the signal at $\delta$ 3.74 with carbon at $\delta$ 71.4 (GalA-H2) refers to the proton H-2, and the signal at $\delta$ 3.81 with carbon at $\delta$ 56.2 (GalA-$OCH_3$) refers to the methoxyl of the ester group. In addition, small resonances are observed at $\delta$ 2.09 with carbon at $\delta$ 23.2 inherent to the acetyl group and at $\delta$ 1.25 with carbon at $\delta$ 19.7 referring to the rhamnose of the of the rhamnogalacturonans. These results match the previously published data.[38–40]

A method to determine the DM of pectins using the NMR $^1H$ spectrum was proposed by Rosenbohm *et al.*[38] The DM results in the portion of the ester groups related to the total amount of carboxyls present. To that end, the region from 4.86 to 5.22 inherent to the anomeric hydrogens (H1 and H1′) and to the hydrogen H5 adjacent to the $COOCH_3$ group is integrated and the signal inherent to the hydrogen H5′ adjacent to the $COO^-$ group is subtracted from it. This calculation allows inferring that passion fruit pectin has a DM of 70.2%. Compared to the data found in the literature, this degree of esterification of the pectic structure obtained was close to (78.59% with citric acid)[11] and (64.56% with acetic acid and 64.15% for nitric acid).[13]



  

**Table 3.** Influence of extraction type on flavonoid concentration

| Solvent | Technique | Solvent/water ratio (v/v) | Orientin-7-*O*-glucoside (g quercetin $kg^{-1}$ PFR) | Luteolin-6-*C*-glucoside (g quercetin $kg^{-1}$ PFR) |
|---|---|---|---|---|
| Methanol | PSE | 80:20 | $1.09 \pm 0.03^{cde*}$ | $2.37 \pm 0.03^{bcd*}$ |
| | | 70:30 | $1.29 \pm 0.09^{bc}$ | $2.57 \pm 0.08^{ab}$ |
| | | 60:40 | $1.71 \pm 0.24^{a}$ | $2.23 \pm 0.15^{cdef}$ |
| | | 50:50 | $1.10 \pm 0.07^{cde}$ | $1.60 \pm 0.08^{g}$ |
| | UAE | 80:20 | $1.01 \pm 0.02^{de}$ | $1.29 \pm 0.06^{h}$ |
| | | 70:30 | $1.66 \pm 0.15^{a}$ | $2.24 \pm 0.09^{cdef}$ |
| | | 60:40 | $1.17 \pm 0.01^{cd}$ | $2.34 \pm 0.02^{cde}$ |
| | | 50:50 | $1.14 \pm 0.04^{cde}$ | $2.09 \pm 0.04^{ef}$ |
| Ethanol | PSE | 80:20 | $0.87 \pm 0.03^{f}$ | $1.98 \pm 0.03^{f}$ |
| | | 70:30 | $1.55 \pm 0.07^{b}$ | $2.72 \pm 0.1^{a}$ |
| | | 60:40 | $1.57 \pm 0.01^{a}$ | $2.44 \pm 0.01^{bc}$ |
| | | 50:50 | $0.73 \pm 0.04^{f}$ | $0.88 \pm 0.05^{i}$ |
| | UAE | 80:20 | $0.68 \pm 0.01^{f}$ | $1.60 \pm 0.02^{g}$ |
| | | 70:30 | $1.10 \pm 0.01^{cde}$ | $2.15 \pm 0.02^{def}$ |
| | | 60:40 | $1.18 \pm 0.01^{cd}$ | $2.38 \pm 0.02^{bcd}$ |
| | | 50:50 | $1.28 \pm 0.06^{c}$ | $2.56 \pm 0.05^{ab}$ |

*Means with the same letter do not significantly differ regarding the solvent system and techniques used according to Tukey's test ($P > 0.05$).
PFR, passion fruit rind.

**Table 4.** $^{13}C$ NMR and $^{1}H$ NMR data for pectin from passion fruit rind

| Compound | Galacturonic acid and other residues $^{13}C/^{1}H$ (ppm) |
|---|---|
| [a]1 | 103.1/4.96 |
| [b]1′ | 102.5/5.15 |
| 2 | 71.2/3.74 |
| 3 | 71.8/3.98 |
| 4 | 82.1/4.46 |
| 5 | 73.7/5.03 |
| 5″ | 74.5/4.66 |
| *O*-Me | 56.2/4.81 |
| Acetyl | 23.2/2.09 |
| Rhamnose | 19.7/1.25 |

[a] One galacturonate unit followed by a galacturonate acid ester (GE).
[b] Two consecutive galacturonate units (GG).

## CONCLUSION

The sequential extraction method developed in this study showed it to be viable and advantageous; due to the use of the same plant material, it was possible to extract different target molecules, without loss of yield or chemical properties. It was also possible to decrease organic solvent consumption, increasing the percentage of water on extraction solvent. The alcohol/water mixtures favor the extraction of bioactive compounds from passion fruit rind. The 60:40 (v/v) ethanol/water system was the solvent of choice for flavonoid extraction, since it performed as well as methanol, but has lower toxicity. Both extraction techniques, PSE and UAE, were affordable for sequential extraction of flavonoids and pectin.

This result confirms that pectin extraction can be carried out after the extraction of bioactive compounds with no quality loss of the pectic structure obtained. Using passion fruit rind to produce commercial pectin could not only solve the issues with eliminating this residue, but also provide the industry with a new source of pectin. The NMR characterization of the pectic polymer shows it is composed basically of homogalacturonans GG (consecutive galacturonates) and GE (galacturonate followed by a unit of the galacturonic acid ester). Furthermore, the high degree of methylation indicates passion fruit pectin could be applied to gel forming products.

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