

An eco-friendly extraction method to obtain pectin from passion fruit rinds (*Passiflora edulis* sp.) using subcritical water and pressurized natural deep eutectic solvents

Débora Tamires Vitor Pereira^{a,c}, Pablo Méndez-Albiñana^{b,d}, Jose A. Mendiola^c, Mar Villamiel^b, Alejandro Cifuentes^c, Julian Martínez^a, Elena Ibáñez^{c,*}

^a State University of Campinas (Unicamp), School of Food Engineering, Department of Engineering and Food Technology, Laboratory of High Pressure in Food Engineering, Campinas, SP 13083 – 862, Brazil

^b Grupo de Química y Funcionalidad de Carbohidratos y Derivados, Instituto de Investigación en Ciencias de la Alimentación (CIAL, CSIC-UAM), Nicolás Cabrera 9, Campus de Cantoblanco, 28049 Madrid, Spain

^c Foodomics Laboratory, Instituto de Investigación en Ciencias de la Alimentación (CIAL, CSIC-UAM), Nicolás Cabrera 9, Campus de Cantoblanco, 28049 Madrid, Spain

^d Department of Physiology, School of Medicine, Universidad Autónoma de Madrid, 28029 Madrid, Spain

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ABSTRACT

This work evaluated the efficiency of Subcritical Water Extraction (SWE) and Pressurized Natural Deep Eutectic Solvents (P-NaDESs) under different temperatures (100, 120, 140 and 160 °C) in obtaining pectin from Passion Fruit Rinds (PFR) and its residual biomass (PFR - UAPLE), and compare the results with those of Conventional Extraction (CE). The highest pectin yields, 19.1 and 27.6 %, were achieved using P-NaDES (Citric Acid:Glucose: Water) at 120 °C for PFR and its PFR-UAPLE, respectively. Regarding the Degree of Esterification (DE), pectin obtained with SWE and CE had DE below 50 %, while with P-NaDES (Citric Acid: Glucose:Water), DE was above 50 %. Higher Molecular Weights (MW) (98 and 81 kDa) were obtained with SWE and P-NaDES from PFR compared to PFR-UAPLE and CE. Galacturonic acid was the most abundant (74 to 78 %) monosaccharide obtained by SWE. In terms of morphology, water extraction provided pectin with more uniform textures, whereas extraction with acidified mixtures led to more heterogeneous surfaces. Overall, comparing SWE and P-NaDES, the obtained pectins differed in terms of monomeric composition, MW and DE. These results indicate that pectins obtained by both methods can have different applications depending on their structural characteristics.

1. Introduction

Brazil is the world's largest producer and consumer of passion fruit (FAO, 2018), and according to the Brazilian Institute of Geography and Statistics – IBGE, production in 2021 reached 683,993 tons (IBGE, 2023). Passion fruit rind is rich in pectin, one of the most important polysaccharides used mainly as source of nutritional fiber and prebiotic. Pectin is a heteropolysaccharide and its main component is D-galacturonic acid (GalA), which forms polymers linked in chains through α-1,4 covalent glycosidic bonds forming its main domain which is Homogalacturonan (HG). Pectin can also contain other monosaccharides like L-Rhamnose (Rha), D-Arabinose (Ara) and D-Galactose (Gal) (Chan et al., 2017; Nussinovitch, 1997).

Pectins have different degrees of methyl esterified carboxyl groups

that can be classified according to their Degree of Methyl Esterification (DM). Pectins can be classified as highly esterified pectin (HM ≥ 50 %) or low esterified pectin (LM < 50 %) (Chatjigakis et al., 1998; Manrique & Lajolo, 2002). The content of GalA, DM and Molecular Weight (MW) are directly related to the gelling capacity and technological properties of pectin, thus affecting its commercial applications.

Pectin is widely used in the food industry for gelling, emulsion stabilization and as a thickening agent, and also for cosmetic, pharmaceutical and biomedical applications (Abboud et al., 2019, 2020; Adetunji et al., 2017). Thus, passion fruit rind has great potential to be used as a valuable raw material for the production of new commercial sources of pectin.

Currently, pectin is industrially obtained by a conventional method, which is based on heating over long extraction times using toxic mineral

* Corresponding author.

E-mail address: elena.ibanez@csic.es (E. Ibáñez).

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acids at low pH that may cause corrosion in the system (Adetunji et al., 2017; Nussinovitch, 1997; Rolin & Vries, 1990). Yields between 6 and 16 % were obtained using nitric and hydrochloric acid in conventional pectin extraction from passion fruit rind (de Oliveira et al., 2015; Huo et al., 2023; Kulkarni & Vijayanand, 2010; Oliveira et al., 2016; Vasco-Correa & Zapata Zapata, 2017; Yapo, 2009). In order to find an alternative to the drawbacks of the conventional method, works have investigated enzymatic ultrasound-assisted or microwave-assisted extraction, pulsed electric field and reflux by condensation to obtain pectin from passion fruit rinds (Canteri et al., 2010; de Oliveira et al., 2015; de Souza et al., 2018; Liew et al., 2016; Oliveira et al., 2016; Pinheiro et al., 2008; Seixas et al., 2014). In these works, pectin yields varied between 6 and 20 %. Although these technologies present advantages in terms of environmental issues, the cost of enzymes and scale-up can be an obstacle to their industrial application (Adetunji et al., 2017). The study of new extraction technologies is interesting to overcome this problem.

High pressure extraction has gained ground in pectin recovery because they can use GRAS (Generally Recognized as Safe) solvents, besides requiring less energy consumption since these methods are faster and scalable (Banerjee et al., 2017; Galanakis et al., 2013; Ramos et al., 2002). Subcritical Water Extraction (SWE) operates at temperatures below the critical point (374 °C) and above the boiling point (100 °C) of water, and at pressures in the range of 2–20 MPa, thus ensuring that the solvent remains in the liquid state even at high temperatures (Adetunji et al., 2017; Herrero et al., 2013; Klinchongkon et al., 2018). Under these extraction conditions, water has advantages such as lower viscosity and surface tension, higher vapor pressures and diffusivity, which lead to improved mass transfer rates (Naqash et al., 2017).

According to Adetunji et al. (2017), promising results for pectin extraction were obtained using Subcritical Water Extraction (SWE) from several plant matrices, including beet pulp, unripe papaya, cacao pod husk, citrus peel, yuzu and apple pomace (Chen et al., 2015; Muñoz-Almagro et al., 2019; Pedraza-Guevara et al., 2021; Ueno et al., 2008; Wang et al., 2014). To our knowledge, only one work (de Souza et al., 2018) has been reported in the literature dealing with SWE of pectin from passion fruit rind. In this work, the authors performed three SWE cycles in static mode, leading to greater solvent consumption. In static systems, extraction efficiency will be limited when the phase equilibrium between the compounds in the plant matrix and those solubilized in the solvent is achieved, thus requiring several process batches.

The efficiency of SWE can be improved by adding co-solvents such as Natural Deep Eutectic Solvent (NaDES). NaDESs are binary or ternary mixtures of hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA), forming intermolecular hydrogen bonds. They are composed of two or more natural compounds, such as acids, sugars and amino acids, forming a eutectic mixture (Zainal-Abidin et al., 2017). In comparison with the conventional solvents used in pectin extraction, NaDESs stand out for being non-toxic, low cost and highly biodegradable (Benvenuti et al., 2019; Paiva et al., 2014). Promising results have been obtained in the extraction conventional of pectin with NaDES from *Averrhoa bilimbi*, pomelo, *Myrciaria cauliflora*, dragon fruit peels and apple pomace (Benvenuti et al., 2020; Chen et al., 2021; Liew et al., 2018; Shafie et al., 2019; Tien et al., 2022). Huo et al. (2023) simultaneously extracted pectin and phenolics from passion rinds by a conventional method using NADES (L-proline: citric acid) as solvent, obtaining a pectin yield of 22 %. This yield is attractive, leading us to consider that NADES at high pressure could intensify the extraction mechanism.

Given this background, we hypothesized that Pressurized Natural Deep Eutectic Solvents (P-NaDES) could intensify extraction and produce high pectin yields from passion fruit rinds compared to SWE and conventional extraction. The confirmation of this hypothesis may encourage researchers and investors to study and apply this technology in pilot and industrial scale. To the best of our knowledge, the extraction of pectin from passion fruit rinds using subcritical dynamic extraction with water and Pressurized NaDES (P-NaDES) has not been reported to

date. High molecular weight phenolic compounds can complex with proteins, forming aggregates that precipitate (Cheynier, 2012; Halbwirth et al., 2019; Spencer et al., 1988) during pectin extraction. One strategy to avoid this problem would be a prior removal of the phenolic compounds from the passion fruit rinds. Furthermore, an extract with bioactive properties and high added value would be obtained in this previous step. Therefore, the aim of this work was to evaluate the efficiency of subcritical extraction of pectin from passion fruit rinds and their residual biomass using water and P-NaDESs under different temperatures, in order to achieve a technically viable process. A comparison of process performance and characterization of pectin obtained by subcritical extraction and conventional method was also performed. The extracted pectins were characterized in terms of their molecular weight, monomeric composition, degree of methyl esterification, changes in functional groups and morphology.

2. Material and methods

2.1. Chemicals

Standard monosaccharides (galactose, rhamnose, fructose, glucose, galacturonic acid (GalA), mannose, xylose and arabinose), citric acid monohydrate, hexamethyldisilazane, β -phenylglucoside, Pullulan Standard (805, 200, 10, 1.3 and 0.34 kDa), citric acid, hydroxylamine chloride sodium hydroxide, sodium citrate, ammonium acetate, hydroxylamine chloride, β -phenylglucoside, and trifluoroacetic acid (TFA) were acquired from Sigma (St. Louis, MO, USA). Ethanol (96 %) was provided by VWR Chemicals (Barcelona, Spain). Hydrochloric acid and pyridine were supplied by J.T.Baker Fisher Scientific (Strasbourg, France). Sulfuric acid, nitric acid, and phenol were purchased from Panreac (Darmstadt, Denmark). Citric Acid, D-Glucose Anhydrous, and L-Lactic Acid were acquired by VWR Chemicals (Llinars del Vallès, Spain). L-Lactic Acid and Choline Chloride were provided by TCI Chemicals (Zwijndrecht, Belgium).

2.2. Materials

Passion fruit rinds (*Passiflora edulis* sp.) were kindly provided by Minasfruit Agroindustria (Visconde do Rio Branco, SP, Brazil). Fresh Passion Fruit Rinds (PFR) and their residual biomass were obtained after Ultrasound-Assisted Pressurized Liquid Extraction (PFR - UAPLE) in a previous study (Pereira et al., 2022). The PFR was ground in a cutter mill (Marconi, MA340, Piracicaba, SP, Brazil). After, PFR and PFR - UAPLE were dried in a stove (Fanem, 320-SE, São Paulo, Brazil) at 60 °C until a dry matter having a water content of 11 % was obtained. The dried PFR and PFR - UAPLE were ground using a mill (IKA®, M20, Staufen, Germany) and sieved using a digital sieve shaker (CISA, BA200N, Barcelona, Spain) to have a particle size <1 mm, between 0.5 and 0.9 mm.

2.3. Physico-chemical characterization of PFR and PFR - UAPLE

Previous to pectin extraction, a study of the chemical composition of PFR and PFR - UAPLE was carried out. The following analyses were performed, according to AOAC (1997) methods: moisture (method 931.04); lipids (method 963.15); proteins (method 970.22); ash (method 972.15); and carbohydrates determined by difference.

2.4. Pectin extraction

Conventional, subcritical water and pressurized NaDES pectin extractions were carried out using PFR and PFR - UAPLE as raw materials. The optimal condition applied in the UAPLE procedure for obtaining phenolic compounds in a first step was reported by Pereira et al. (2020): 246 g of PFR, ultrasonic intensity of 360 W/cm², constant pressure and temperature (10 MPa, 60 °C), solvent mass flow rate of 10 g/min, and solvent to feed (S/F) ratio of 14.61 kg solvent/kg of dried rinds. With

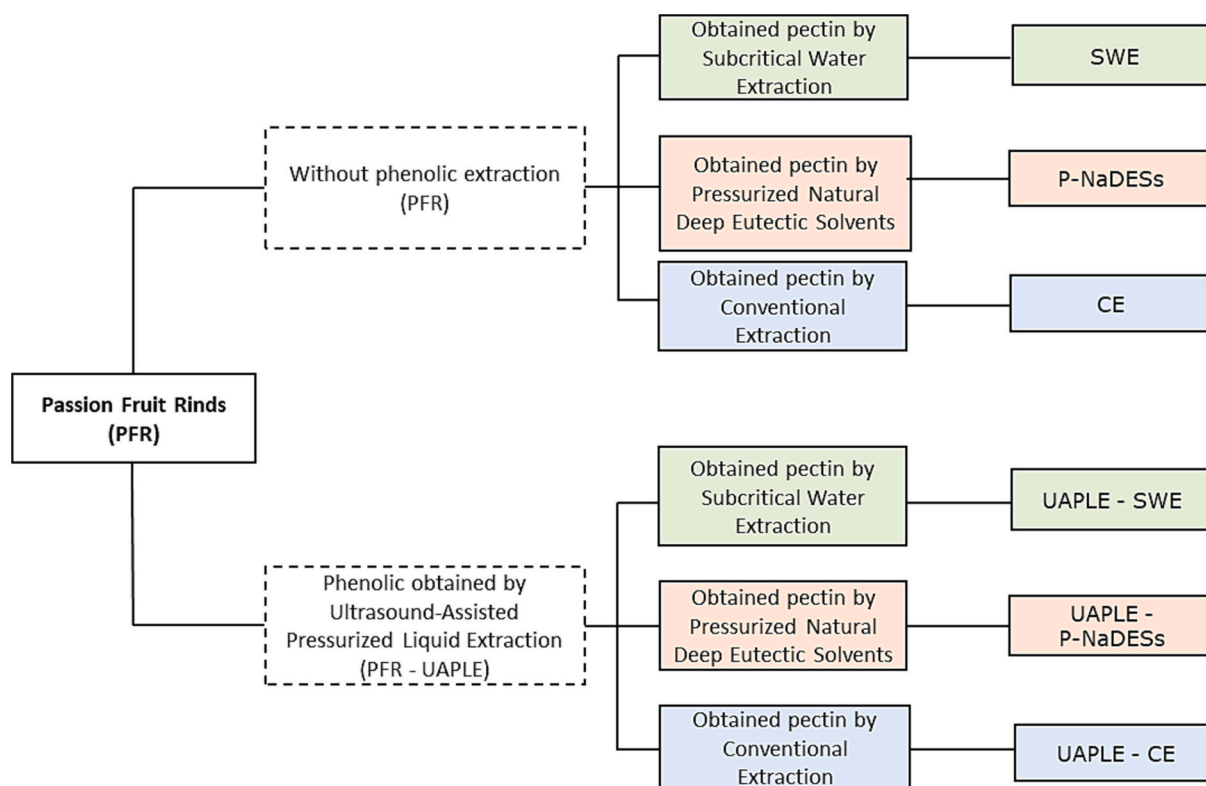


Fig. 1. General scheme of pectin recovery by Subcritical Water Extraction (SWE), aqueous solutions Pressurized Natural Deep Eutectic Solvents (P-NaDESS) and Conventional Extraction (CE) from Passion Fruit Rinds (PFR) and its residual biomass after phenolic extraction (PFR – UAPLE).

this process, the residual biomass of the passion fruit rinds was obtained and stored at -18°C until sample preparation for subsequent pectin extraction.

The general scheme of conventional and subcritical extractions of pectin from PFR and PFR-UAPLE is shown in Fig. 1.

2.4.1. Conventional extraction (CE)

Pectins were extracted from PFR and PFR – UAPLE following the method optimized by Vriesmann et al. (2012) with some modifications (Muñoz-Almagro et al., 2019). PFR and PFR – UAPLE powder (1:30 w/v solid-to-liquid ratio) were suspended in 4 % (w/v) citric acid solution adjusting the pH to 3.0. Afterwards, the mixture was kept under continuous stirring at 95°C for 95 min. Next, to separate the residual solid biomass from the supernatant, the mixture was centrifuged (SORVALL LYNX 6000 Centrifuge, Thermo Scientific, Massachusetts, USA) at 3700g and 4°C for 15 min. To precipitate pectin, ethanol (96 %) in a proportion (1:2 v/v) was mixed with the supernatant and kept at 4°C for 16 h. Then, centrifugation at 3700g for 30 min at 4°C was performed to recover the pectin residues. After centrifugation, pectin residues were washed and centrifuged at 1700g three times with ethanol (96 %) 20 min at 4°C . Pectin samples were partially dried in a vacuum oven at 45°C (Heraeus VTR 5022 vacuum oven Heraeus Holding GmbH Heraeusstraße, Hanau, Germany) to remove residual ethanol. Afterward, they were frozen at -16°C and lyophilized. Extractions were performed in duplicate.

2.4.2. Subcritical water extraction (SWE)

SWE of pectin was performed in a custom-made PLE (Pressurized Liquid Extraction) unit described by Fagundes et al. (2021), equipped with Jasco pump (Jasco Inc., Hachioji, Japan), a Finnigan oven (Thermo-Finnigan, Santa Clara, USA). Approximately 3.0 g of PFR and PFR – UAPLE were used in each extraction forming a fixed bed inside a 25 mL stainless steel jacketed column. The extraction conditions were constant pressure of 10 MPa, solvent to feed (S/F) ratio of 30 kg solvent/

Table 1

Code, composition, molar ratio, water dilution of the four aqueous solutions of NaDESs evaluated in pressurized pectin extraction.

Code	Components	Molar ratio	Water dilution	References
Ca:Glu:Wa	Citric acid:glucose:water	1:1:3	1:9 (w/w)	(Benvenuti et al., 2020)
Cc:La	Choline chloride:lactic acid	1:2	–	(Chen & Lahaye, 2021)
Cc:Glu:Wa	Choline chloride:glucose:water	5:2:5	1:3 (v/v)	(Tien et al., 2022)
Cc:Ca	Choline chloride: citric acid	1:1	3.74 % (w/v)	(Shafie et al., 2019)

kg dried PFR and PFR – UAPLE, solvent mass flow rate of 3 g/min and water purified with a Milli-Q system from Millipore was used as solvent. Different temperatures (100, 120, 140 and 160°C) were evaluated on the PFR and PFR – UAPLE to verify their influence on the pectin extraction yield. The extractions were carried out for 30 min. After the extractions, all extracts were precipitated, centrifuged and dried as described in Section 2.4.1. Extractions were performed in duplicate.

2.4.3. Pressurized natural deep eutectic solvents (P-NaDES)

A preliminary study with the screening of four different mixtures of P-NaDESs (Ca:Glu:Wa, Cc:La, Cc:Glu:Wa, Cc:Ca) was carried out with PFR that obtained a higher pectin yield using subcritical water (Section 2.4.2). It was considered that PFR-UAPLE would provide the same pectin yield trend, justifying the use of only PFR in the preliminary study. The choices of NaDESs were based on published works (see Table 1), in which eles were successfully applied in the extraction of pectin from different plant materials (jaboticaba and apple pomace, dragon fruit peels and *Averrhoa bilimbi*). Após a triagem inicial dos solventes, the P-NaDES that obtained the highest pectin yield was used in the PFR at temperatures of 100, 140 and 160°C and in the PFR – UAPLE at 120 and

140 °C.

The extractions with P-NaDESs were performed in the same equipment described in Section 2.4.2, maintaining the same pressure (10 MPa), S/F ratio (30 kg/kg dried PFR or PFR - UAPLE), solvent mass flow rate (3 g/min) and amount of material (3 g de PFR or PFR - UAPLE). The extraction time was 30 min. After the processes, all extracts were precipitated, centrifuged and dried as described in Section 2.4.1. Extractions were performed in duplicate.

2.5. Pectin yield (Y_{pec})

Pectin yields obtained by the conventional method, SWE and aqueous solutions of P-NaDESs from PFR and PFR - UAPLE were calculated according to Eq. (1):

$$Y_{pec}(\%) = \frac{\text{Mass of dry pectin (g)}}{\text{Dry mass of PFR or PFR - UAPLE used for extraction (g)}} \times 100 \quad (1)$$

2.6. Structural analysis of pectin

2.6.1. Molecular weight (MW) estimation

Pectin MW distribution was estimated following the method described by Muñoz-Almagro et al. (2018). Separation was achieved by HPSEC with two TSK-Gel columns G5000 PWXL (7.8 mm × 300 mm, 10 μm) and G2500 PWXL (7.8 mm × 300 mm, 6 μm) connected in series with a TSK-Gel guard column (6.0 mm × 400 mm) (Tosoh Bioscience, Stuttgart, Germany). The HPSEC-ELSD analysis was carried out on a LC chromatograph Agilent Technologies 1220 Infinity and a detector ELSD 1260 Infinity (Agilent Technologies, Boeblingen, Germany). Samples (50 μL) were eluted with 0.1 M NH₄Ac as mobile phase at a flow rate of 0.4 mL/min for 50 min at 40 °C. A mixture of pullulans was used as standard. Data obtained was processed with Agilent Open Lab CDS Data System software.

2.6.2. Determination of galacturonic acid and neutral sugars

Prior to Gas Chromatography with Flame ionization detector (GC-FID), pectin was derivatized after hydrolysis following the method reported by Muñoz-Almagro et al. (2018). Analyzes were performed on a gas chromatograph (GC7890A), equipped with a flame ionization detector (FID) and a 7693 autosampler (Agilent Technologies Ing., Palo Alto, CA) Sample injection was done in split mode 1:5 and eluted using a VF-5HT column (30 m × 0.25 mm × 0.10 μm, J&W- Agilent, Folson, California, USA). The flow of nitrogen was 1 mL/min. Detector and injector temperatures were kept at 382 and 280 °C, respectively; the temperature gradient started at 120 °C, and raised at 240 °C at 3 °C/min and up to 300 °C at 3 °C/min Data processing and integration were carried out in Agilent software. ChemStation (Washington, DE, USA). The quantitative analysis was performed using the internal standard method calculating carbohydrate response factors standards (xylose, arabinose, rhamnose, galactose, mannose, glucose, GalA, sucrose and fructose), previously injected at known concentrations (0.125–2 mg/mL), with respect to the internal standard β-phenyl-glucoside.

2.6.3. Degree of methyl esterification (DM)

For DM determination, all the pectins obtained from PFR and PFR - UAPLE were mixed with bromide potassium and analyzed by FT-IR in a Bruker IFS66v (Bruker, US) in a wide frequency range from 450 to 4000 cm⁻¹ and with 4 cm⁻¹ of resolution according to the method described by Singthong et al. (2004). The DM of pectin was determined as the average of the ratio of the peak area at 1740 cm⁻¹ (COO-R) over the sum of the peak areas of 1740 cm⁻¹ (COO-R) and 1630 cm⁻¹ (COO-) (Chatjigakis et al., 1998).

2.6.4. Morphological analysis of the extracted pectin

Morphological observations of pectin of PFR and PFR - UAPLE

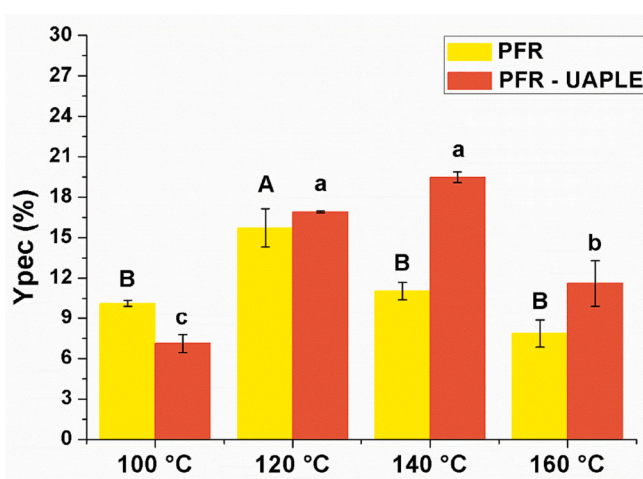


Fig. 2. Pectin yields (Y_{pec}) obtained at different temperatures by Subcritical Water Extraction (SWE) from Passion Fruit Rinds (PFR) and its residual biomass (PFR - UAPLE). Equal capital or lowercase letters indicate that there is no significant difference ($p < 0.05$) as estimated by Tukey's test between pectin yields. Results are expressed as mean $\pm$ standard deviation.

obtained by conventional, subcritical water extraction and pressurized NaDES were performed using a S-3000 N Scanning Electron Microscopy (SEM, HITACHI, Tokyo, Japan) at 15 kV accelerating voltage, a magnification of 5000 $\times$ and a distance of 10 mm.

2.7. Statistical analysis

The results were statistically evaluated by the one-way analysis of variance (ANOVA), and significant differences at the 5 % level were analyzed by Tukey's test. The results are shown as mean values $\pm$ standard deviation (SD). All extractions were performed in duplicate, and analyses were performed in triplicate.

3. Results and discussion

3.1. Chemical characteristics of passion fruit rinds and their residual biomass

The passion fruit rinds presented the following composition: moisture: 81.1 ± 0.2 g/100 g wet basis (w.b.); lipids: 0.76 ± 0.06 g/100 g dry basis (d.b.); proteins: 8.0 ± 0.1 g/100 g (d.b.); ash: 5.69 ± 0.01 g/100 g (d.b.); carbohydrates: 90.7 ± 0.1 g/100 g (d.b.). The residual biomass of passion fruit rinds obtained after UAPLE of phenolic compounds obtained the composition: moisture: 11.4 ± 0.6 g/100 g (w.b.); lipids 0.56 ± 0.05 g/100 g dry basis (d.b.); proteins: 6.0 ± 0.2 g/100 g (d.b.); ash: 3.85 ± 0.05 g/100 g (d.b.); carbohydrates: $89.6 \pm 0.5/100$ g (d.b.).

3.2. Pectin extraction yields

3.2.1. Subcritical water extraction

Fig. 2 shows the influence of temperature (100, 120, 140 and 160 °C) on pectin yield applying SWE from PFR and PFR-UAPLE. The highest yield for PFR (15.7 %) was obtained at 120 °C. For PFR - UAPLE, high yields (16.9 % and 19.5 %) were obtained at 120 and 140 °C, respectively, with no statistical difference ($p < 0.05$) between these temperatures. It can be observed that the pectin yield trends were different for PFR and PFR-UAPLE. In PFR there was an increase in pectin yield up to 120 °C and then a decline as the temperature increased. On the other hand, for PFR-UAPLE the pectin yield increased up to 140 °C, followed by a decrease.

The plant cell wall was more accessible to solvent penetration in PFR-UAPLE, due to the previous treatment to remove phenolic

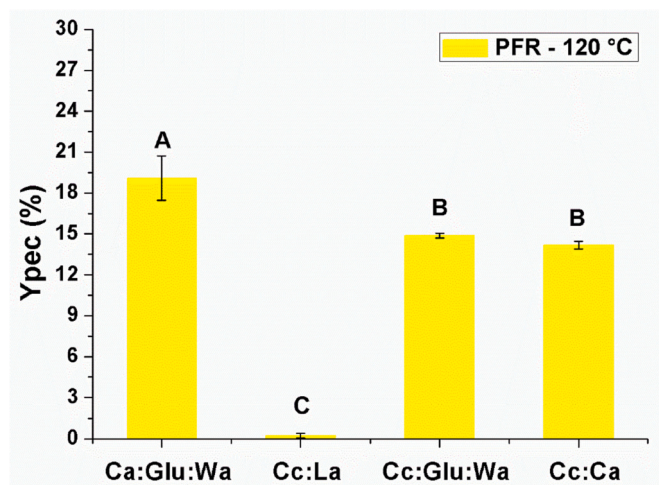


Fig. 3. Pectin yields (Y_{pec}) obtained from passion fruit rinds (PFR) using different aqueous solutions of Pressurized Natural Deep Eutectic solvents (P-NaDESs). See Table 1 for NaDES composition. Equal capital letters indicate that there is no significant difference ($p < 0.05$) as estimated by Tukey's test between pectin yields. Results are expressed as mean $\pm$ standard deviation.

compounds. This promoted greater diffusion of the solvent, impacting the hydrolysis and desorption of pectin at temperature up to 140 °C. As the cell wall remained intact in PFR, the diffusivity of the solvent was limited to a temperature of 120 °C.

High extraction temperatures reduce water's dielectric constant and viscosity, promoting also the rupture of the cellular matrix (Benvenuti et al., 2022). Therefore, solvent penetration and pectin diffusion is enhanced. However, it is necessary to consider that extremely high temperatures can promote the hydrolysis of polysaccharides into monosaccharides and smaller particles, and the degradation of pectin, reducing its yield (Wang & Lü, 2014). This may explain the low pectin yield obtained at 140 °C for PFR and 160 °C for both PFR and PFR-UAPLE. Furthermore, the extracts obtained by SWE under this condition showed a dark brown color, also observed by Liew, Ngoh, et al. (2018) and Liew, Teoh, et al. (2018) in the extraction of pomelo pectin at temperatures above 120 °C, indicating possible thermal degradation of polymers or Maillard reaction products. Several authors (Benvenuti et al., 2022; Chen et al., 2015; Dias et al., 2020; Ma et al., 2020; Wang et al., 2014) also reported that the pectin yield obtained with SWE from different plant matrices (berry, citrus peel, sunflower heads, sugar beet, berg fruits) decreased when temperatures below and above 120 °C were used.

3.2.2. Preliminary extraction with different types of pressurized natural deep eutectic solvents

Based on the results obtained in Section 3.2.1, 120 °C was chosen to evaluate pectin extraction from PFR using different aqueous solutions of P-NaDESs named in Table 1, which yields are shown in Fig. 3. It was considered that PFR-UAPLE would present the same pectin yield trend, justifying the use of only PFR in the preliminary study. Among the evaluated solutions, Ca:Glu:Wa provided the highest yield (19.1 %). According to Yapo (2009), concentration and acid type influence pectin yield. Furthermore, different DES can interact with polysaccharides in different ways, affecting extraction efficiency.

The pectin yield obtained with Ca:Glu:Wa was slightly higher than using subcritical water as solvent (see Section 3.2.1) at the same temperature, probably due to the acidic medium and the change of water polarity achieved with P-NaDES, favoring pectin extraction. The low yield obtained with Cc:La may be due to its higher viscosity compared to the other used NaDES. As reported by Chen et al. (2021), viscous solvents can limit mass transfer, reducing the extraction yield.

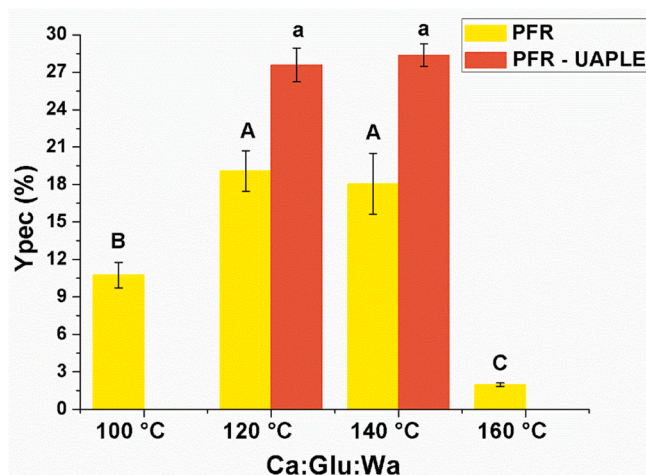


Fig. 4. Pectin yields (Y_{pec}) obtained from Passion Fruit Rinds (PFR) and its residual biomass (PFR - UAPLE) by pressurized aqueous solution of natural deep eutectic solvent (Citric Acid:Glucose:Water). Equal capital or lowercase letters indicate that there is no significant difference ($p < 0.05$) as estimated by Tukey's test between pectin yields. Results are expressed as mean $\pm$ standard deviation.

3.2.3. Extraction with selected pressurized deep eutectic liquid solvent

Based on the results reported in Section 3.2.2, aqueous Ca:Glu:Wa solution was chosen as solvent for the temperature optimization using P-NaDES from PFR and PFR-UAPLE. Fig. 4 shows the pectin yields obtained at 100, 120, 140 and 160 °C for PFR and at 120 and 140 °C for PFR-UAPLE. It can be seen that for both PFR (19.1 and 18.1 %) and PFR - UAPLE (27.6 % and 28.4 %) no significant difference between yields from the same material was found. This result confirms that 120 °C is the lowest temperature at which the highest pectin yield can be achieved.

Extractions using PFR - UAPLE and Ca:Glu:Wa provided increases of 45 and 57 % in pectin yields at temperatures of 120 and 140 °C, respectively, compared with extraction from PFR under the same temperature and solvent conditions. A possible explanation could be that pressurized extraction with Ca:Glu:Wa enabled accessing more polysaccharides in the cell walls already damaged by the previous extraction of phenolics by UAPLE. This led to a considerable improvement in extraction yields.

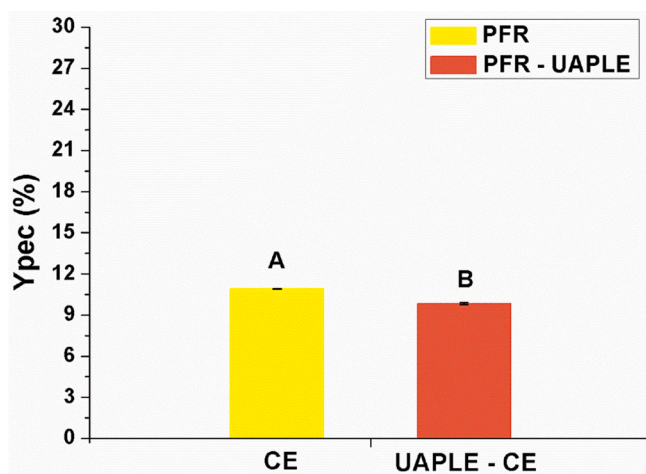


Fig. 5. Pectin yields (Y_{pec}) obtained from Passion Fruit Rinds (PFR) and its residual biomass (PFR-UAPLE) by Conventional Extraction (CE) using 4 % citric acid solution (w/v) as solvent. Equal capital letters indicate that there is no significant difference ($p < 0.05$) as estimated by Tukey's test between pectin yields. Results are expressed as mean $\pm$ standard deviation.

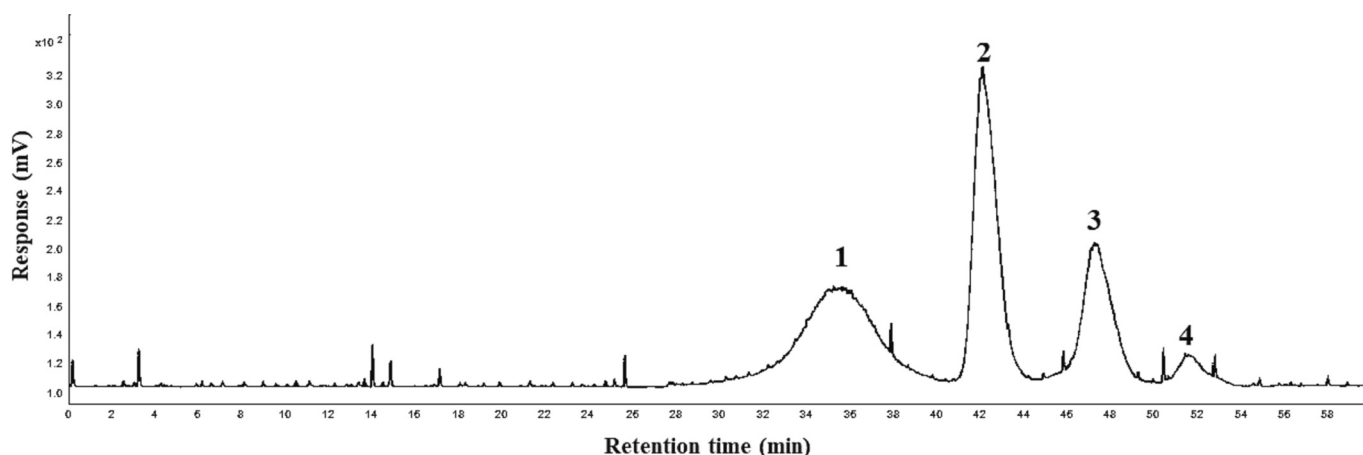


Fig. 6. Chromatographic HPSEC-ELSD profile for pectin extracted from PFR with pressurized aqueous solution of natural deep eutectic solvent (Citric Acid:Glucose: Water) at 120 °C.

Huo et al. (2023) investigated the simultaneous conventional extraction of pectin and phenolics from passion fruit rinds using DESs as solvent. After screening different DESs in pectin extraction, the authors obtained the highest yield (22.63 %) with proline-citric acid (Pro:Ca) at 95 °C. This yield was lower than those obtained in the present work using Ca:Glu:Wa with PFR-UAPLE (27.6 % and 28.4 %) and higher than the yields obtained with PFR (19.1 and 18.1 %). Some factors must be considered to justify these results. The prior removal of phenolic compounds from the passion fruit rinds with UAPLE may have favored greater accessibility to the pectin present in the cell structure and pressurized extraction at temperatures above 95 °C may have contributed to intensify this process. Furthermore, the Ca:Glu:Wa composition may have more affinity with passion fruit rinds than Pro:Ca. Therefore, the highest pectin yield obtained by the sequential process UAPLE + P-NaDESs with Ca:Glu:Wa at 120 °C, compared to the other methods used, stands out as a promising process for pectin extraction.

3.2.4. Conventional extraction

Pectin yields obtained by conventional extraction from PFR and PFR-UAPLE are shown in Fig. 5. The pectin yield obtained from PFR (10.9 %) was significantly higher than the obtained with PFR-UAPLE (9.8 %). However, in both CE extractions, pectin yields were lower than those of non-conventional extraction methods (SWE at 120 °C and P-NaDESs at 120 and 140 °C) using water and Ca:Glu:Wa as solvents. This result proves the efficiency of high pressures in disrupting the cellular matrix and increasing mass transfer.

In addition, the Ca:Glu:Wa eutectic mixture seems more efficient than any of its individual components, such as the citric acid used in CE and CE-UAPLE. This behavior was also observed by Elgharrawy et al. (2019) in pectin extraction assisted by different Natural Deep Eutectic Solvents (NaDESs) from pomelo peel using sonoreactor. These authors reported that NaDES provided higher pectin yields than salt or HBD used individually.

Works that also used conventional extraction with citric acid to obtain pectin from passion fruit rinds were found in literature. Yapo (2009) obtained pectin yields of 2.8 % and 5.1 % in the conventional extraction by heating at 75 °C for 60 min, using a Solid-Liquid (S/L) ratio of 1:25 (m/v), and citric acid as solvent at pH's of 1.8 and 2.5, respectively. Liew et al. (2014) achieved a yield of 14.60 % by heat extraction with stirring and citric acid at pH 2.0, at 70 °C in 75 min, and S/L of 1:25 (m/v). The differences in the yields obtained in the different works can be explained by the extraction conditions employed (pH, S/L, time, temperature) or edaphoclimatic factors, cultivar, maturation stage and plant genetics that directly influence the nutritional composition of the plants (Corrêa et al., 2016; Freitas et al., 2020).

3.2.5. Comparison of extraction with SWE, P-NaDES and CE

The results show that pectin yields using P-NaDES are higher than with SWE and CE. This is probably due to a possible synergistic effect between passion fruit rinds, P-NaDES and high pressure. The biocompatibility and high solubility of NaDES in plant materials (Huo et al., 2023) combined with high pressures may have favored the penetration of the solvent into the cellular matrix of PFR and PFR-UAPLE. The penetration of P-NaDES was even more evident when using PFR-UAPLE, since its structure had been previously damaged by ultrasound. This resulted in a more pronounced interaction between solvent and sample, solubilizing the pectin and promoting greater extraction yield. Ultrasound forms bubbles that grow and collapse, in a phenomenon known as cavitation. When a rupture occurs in the structure of the samples, it favors the penetration of the solvent, solubilization of the sample and intensification of mass transfer, resulting in greater extraction yield (Esclapez et al., 2011; Mason, 1996).

Conventional extraction obtained the lowest pectin yields. The pressure and temperature of the process may have played a fundamental role in this result. Extraction at pressure greater than atmospheric and temperatures above 95 °C, used in SWE and P-NaDES, may have provided more efficient pectin hydrolysis and recovery. According to Huo et al. (2023), high temperatures are important to break the pectin bonds with the cell wall and increase its solubility. It is known that extractions under subcritical and pressurized conditions favor the increase in solvent transport by decreasing its viscosity and surface tension (Mustafa & Turner, 2011) and, consequently, greater desorption of the target compound.

3.3. Pectin structural characterization

3.3.1. Molecular weight (MW)

The Molecular Weight (MW) of the pectins varied with extraction temperature, solvent, and type of raw material. Most of the pectins extracted with P-NaDESs were more heterogeneous, presenting four peaks, being one of larger MW and three of lower MW, as exemplified in Fig. 6. In Table 2, it can be noted that the MWs decreased as temperature increased in the pectins obtained with SWE and P-NaDESs, indicating a possible degradation of the pectic chain at high temperatures.

Results obtained for CE show a pectin MW of 38 kDa, obtained from PFR-UAPLE, which was significantly lower to that obtained from PFR. This is probably related to the previous extraction of phenolic compounds by UAPLE, which may have resulted in the depolymerization of the pectic chains due to the effect of ultrasound under high pressure. Pectins that achieved the highest MW values (SWE at 100 and 120 °C) and (Ca:Glu:Wa at 120 °C) are likely to produce stronger gels, since pectin gelation is directly related to its molecular weight, among other

Table 2
Molecular weight (kDa) of pectin obtained from PFR and PFR- UAPLE by conventional extraction (CE), subcritical extraction with water (SWE) and P-NaDESs.

Extraction type	SWE		P-NaDESs				CE		P-NaDESs									
	PFR	PFR	PFR-UAPLE		PFR	PFR-UAPLE	PFR	PFR-UAPLE	PFR	PFR								
Material	Water				Ca:Glu:Wa		Acidified Water		Cc: Glu: La Wa									
Solvent	Water				Ca:Glu:Wa		Acidified Water		Cc: Glu: La Wa									
Temperature (°C)	100	120	140	160	100	120	140	160	120	120								
Molecular weight (kDa)	Peak 1	98 ^A ± 7	74.4 ^{BC} ± 0.02	40 ^{DEFGH} ± 4	12 ^I ± 6	58 ^{CDE} ± 4	38 ^{DEFGH} ± 7	36 ^{FGH} ± 11	22 ^{HI} ± 2	81 ^{AB} ± 3	50 ^{DEF} ± 2	22 ^{HI} ± 2	34 ^{FGHI} ± 10	29 ^{GHI} ± 8	76 ^{ABC} ± 2	38 ^{DEFGH} ± 10	68 ^{BCD} ± 10	49 ^{DEFG} ± 4
	Peak 2	0.17 ^B ± 0.01	0.16 ^B ± 0.02	0.31 ^B ± 5.9	0.027 ^B ± 0.001	0.026 ^B ± 0.005	0.03 ^B ± 0.005	0.0268 ^B ± 0.0002	0.023 ^B ± 0.004	2.203 ^A ± 0.001	2.1 ^A ± 0.3	2.133 ^A ± 0.005	2.14 ^A ± 0.01	2.17 ^A ± 0.02	1.7 ^A ± 0.1	0.027 ^B ± 0.001	2.0 ^A ± 0.5	2.14 ^A ± 0.06
	Peak 3	0.03 ^B ± 1.27	0.03 ^B ± 0.01	–	–	–	–	–	–	0.19 ^A ± 0.06	0.20 ^A ± 0.01	0.18 ^A ± 0.03	0.1835 ^A ± 0.0004	0.18 ^A ± 0.01	–	–	0.16 ^A ± 0.03	0.17 ^A ± 0.05
	Peak 4	–	–	–	–	–	–	–	–	0.02 ^A ± 0.01	0.03 ^A ± 0.01	0.028 ^A ± 0.004	0.033 ^A ± 0.003	–	0.025 ^A ± 0.001	–	0.026 ^A ± 0.001	0.032 ^A ± 0.003

^a The recovered amount of pectin was insufficient for this analysis.

factors.

Seixas et al. (2014) extracted pectins with average MWs between 230 and 497 kDa from passion fruit rinds under microwave heating using nitric acid, acetic acid and tartaric acid. Yapo (2009) obtained MWs between 104 and 287 kDa in pectins extracted from yellow passion fruit rinds, varying the acidification of the solvent (nitric acid, sulfuric acid and citric acid), concentration (0.01 or 0.03 M) and pH (1.8 or 2.5) at 75 °C for 60 min with two sequential extractions. The mentioned studies showed higher values of MWs compared to the results obtained in this work. This fact may be related to the extractions performed at high pressures, which may have favored the depolymerization of pectic chains.

3.3.2. Neutral sugars and galacturonic acid

The chromatographic profile of the monosaccharides of pectin extracted with P-NaDES (Ca:Glu:Wa) from PFR-UAPLE obtained by GC-FID is shown in Fig. 7. Galacturonic Acid (GalA), glucose, xylose, rhamnose, galactose, mannose, fructose, and arabinose were the main monomers found in pectins obtained with SWE, P-NaDESs and CE. The quantitative analysis of these carbohydrates is presented in Table 3. The solvent type, temperature, pressure and the extraction method affected the monosaccharide composition of the recovered pectin fractions. The most abundant monosaccharide in almost all extractions was GalA, mainly in SWE (74 to 78 %) with PFR (160 °C) and PFR – UAPLE (100 °C), respectively.

Huo et al. (2023) obtained 59.41 % and 50.24 % of GalA in pectin extracted from passion fruit rind using DES (proline-citric acid) and HCl, respectively. These values were lower than most GalA levels obtained under the extraction conditions and methods used in the present work. Additionally, the authors identified galactose (Gal) as the most abundant neutral monosaccharide, 15.93 % for DES and 17.78 % for HCl, followed by arabinose (Ara) and rhamnose (Rha).

Kulkarni and Vijayanand (2010) investigated the effect of conventional pectin extraction conditions from passion fruit rind using HCl as solvent and found GalA content of 88.2 g/100 g using the spectrophotometric method estimated by the modified uronic acid carbazole reaction (Bitter & Muir, 1962). According to Blumenkrantz & Asboe-Hansen, the carbazole reaction described by Dische (1947) and modified by other authors (Bitter & Muir, 1962; Galambos, 1967) has the disadvantage of not allowing differentiation between uranic acids and hexoses.

Seixas et al. (2014) used tartaric, acetic and nitric acids to extract pectin from passion fruit rinds under microwave heating and obtained 58.50 %, 62.5 % and 82.3 % of uronic acids, respectively. These authors used a method for determining uranic acids with *m*-hydroxydiphenyl, through an unexplained mechanism (Blumenkrantz & Asboe-Hansen, 1973).

The GalA content obtained by Kulkarni and Vijayanand (2010) and Seixas et al. (2014) was higher than in other works on pectin extraction from passion fruit rinds. The replacement of chromatographic analysis by spectrophotometric methods not commonly used for the determination of monosaccharides may have contributed to this result. The GalA content may also have been affected by the removal of phenolics glycosylated with monosaccharides. The flavonoids, including phenolic compounds, are generally linked to one or more sugar units, i.e., a glycosylated. Glucose is the most common sugar, but galacturonic and glucuronic acids, galactose, rhamnose, xylose or arabinose can also be present (Bravo, 1998; Giada, 2013).

In general, in extractions applying P-NaDESs (except using Ca:Glu:Wa at 100 °C and Cc:Glu:Wa, Cc:Ca at 120 °C), higher glucose contents were obtained compared to other solvents, reducing the relative amount of GalA. This suggests that the NaDESs used in this work had a negative impact on GalA recovery and was able to hydrolyze the main pectin chain, homogalacturonan (HG), formed by repeats of [α-1,4-D-galacturonic acid]_n units. The ionic dissociation of water in subcritical extraction promotes the hydrolysis of monosaccharides (Klinchongkon

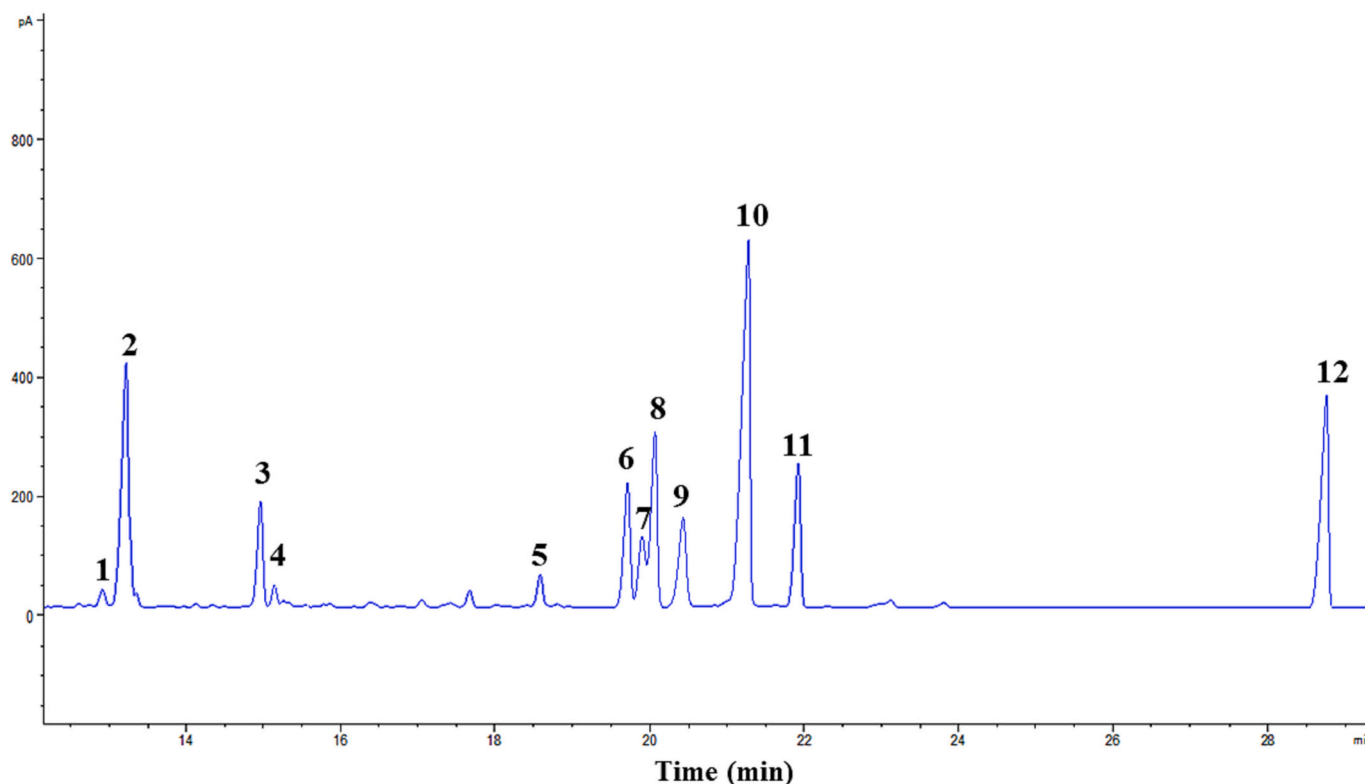


Fig. 7. Chromatographic profile obtained by GC-FID of TMS oximes of monosaccharides after hydrolysis with 2 M TFA of extracted pectin from PFR – UAPLE by pressurized Natural Deep Eutectic Solvents (P-NaDESs) extraction with Ca:Glu:Wa at 120 °C. 1: Xylose; 2: Arabinose; 3 and 4: Rhamnose; 5: Fructose; 6: Galactose; 7: Mannose; 8: Glucose; 9: Galactose + Mannose + Glucose; 10 and 11: Galacturonic acid; 12: Internal standard.

et al., 2017), and this effect may have been enhanced by the eutectic mixture.

Klinchongkon et al. (2017) used subcritical water (static mode, S/L ratio 1:16, 100–245 °C) for the extraction of oligosaccharides from passion fruit rinds and found glucose as the major monosaccharide in all obtained hydrolysates, followed by galactose and mannose. Glucose, xylose and mannose are monosaccharides found in the cellulose and hemicellulose fractions linked to the pectin side chains (Muñoz-Almagro et al., 2018; Scheller & Ulvskov, 2010; Wang et al., 2016).

3.3.3. Degree of methyl esterification (DM)

FTIR analysis was performed to investigate possible changes in functional groups and molecular structures of the obtained pectins, as shown in Fig. 8, and also to evaluate their degree of methyl esterification (DM) obtained with different solvents (acidified water, pure water and NaDESs) and extraction methods (SWE, P-NaDESs, CE), as presented in Table 3.

The O—H stretching vibration of the hydroxyl groups located in the absorption band at 3400 cm^{-1} was more pronounced in the pectins obtained by SWE (160 °C) and P-NaDESs (Ca:Glu:Wa, 120 and 140 °C) than those extracted at other temperatures and conventional extractions (Fig. 8A). The breaking of O—H bonds using subcritical water at high temperatures (Muñoz-Almagro et al., 2019) and the greater dissociation power of the eutectic Ca:Glu:Wa mixture at high pressures may have influenced this result. According to Chen and Lahaye (2021) and Benvenuti et al. (2022), NaDES with acid promote the breaking of chemical bonds and severe extraction temperatures favor the depolymerization and de-esterification of pectic substances.

The bands close to 2930 cm^{-1} are characterized by the C—H stretching of the CH_2 groups (Chatjigakis et al., 1998). Bands below 1500 cm^{-1} are known as “fingerprint region” and are specific for each polysaccharide. However, these bands correspond to complex interaction groups, which makes impracticable to assume a vibration group of

atoms in this region (Chatjigakis et al., 1998; Manrique & Lajolo, 2002).

Bands between 1800 and 1500 cm^{-1} refer to the absorption of carboxylic acid and ester groups present in pectin molecules. From the analysis of this spectral region it is possible to distinguish two bands; one close to 1740 cm^{-1} , which corresponds to the vibration of the C=O stretching of the methyl esterified carboxylic groups of the pectin molecules, and another one around 1630 cm^{-1} , related to the asymmetric vibrations of stretching of the carboxylate group. The area of these two bands is proportional to the DM.

The type of solvent used in the different extraction methods affected the DM. The pectin fractions recovered with P-NaDES (Ca:Glu:Wa) showed DM above 50 % and, therefore, are classified as high esterification pectin (HM). Nevertheless, in the other solvents (water, acidified water and other NaDESs) low esterified pectins (LM) were obtained. A possible hypothesis for this finding is the conversion of non-methyl-esterified carboxyl groups into methyl-esterified carboxyl groups in the extraction using Ca:Glu:Wa as solvent, since SWE under the same process conditions produced LM. Other authors (Liew, Teoh, et al., 2018; Wang et al., 2014) have also described this possible conversion of carboxylic groups during pectin extraction in the presence of HCl from pomelo and citrus peels.

Another explanation for the recovery of HM with P-NaDES (Ca:Glu:Wa) is its lower percentage of citric acid compared to the conventional extraction method. Pinheiro et al. (2008) investigated the relation between citric acid concentration and DM from passion fruit rinds. These authors found that lower concentrations of citric acid, within the applied range (0.086 to 2.914 % w/v), contributed to the higher percentage of DM.

Shafie et al. (2019) also obtained HM (54 %) from *Averrhoa bilimbi* using Cc:Ca as NaDES. Chen and Lahaye (2021) used Cc:La as a pre-treatment of apple pomace before mineral acid extraction, and obtained HM, ranging from 56.9 to 78.3 %. Tien et al. (2022) applied the Cc:Glu:Wa solvent system to dragon fruit peels using Microwave

Table 3

Molecular parameters and sugar composition of pectin extracted from PFR and PFR- UAPLE using conventional extraction (CE), subcritical extraction with water (SWE) and pressurized natural deep eutectic solvents P-NaDESS.

Extraction type	SWE								P-NaDESS								CE		P-NaDESS		
Material	PFR				PFR-UAPLE				PFR				PFR-UAPLE				PFR	PFR-UAPLE	Preliminary Study		
Solvent	Water								Ca:Glu:Wa								Acidified Water		PFR		
																			Cc:La	Cc:Glu:Wa	Cc:Ca
Temperature (°C)	100	120	140	160	100	120	140	160	100	120	140	160	120	140	95		120	120	120		
DM (%)	15.5	15.1	17.4	13.9	27.8	29.2	27.5	28.6	66.3	73.3	61.7	^a	78.3	78.5	18.9	37.5	^a	14.7	42.8		
Monosaccharides composition (%) over total carbohydrates																					
Xyl	6.03 ^C ± 0.02	6.0 ^{CDE} ± 0.4	3.46 ^{FGH} ± 0.04	3.2 ^{GH} ± 0.1	4.2 ^{EFG} ± 0.2	5.59 ^{CD} ± 0.02	4.7 ^{DE} ± 0.1	0.983 ^{JK} ± 0.001	4.9 ^{CDE} ± 0.8	1.7 ^{IJ} ± 0.1	1.45 ^{JK} ± 0.02	^a	2.02 ^{IJ} ± 0.01	4.58 ^{DEF} ± 0.07	10.3 ^B ± 0.5	13.8 ^A ± 0.2	^a	0.32 ^K ± 0.05	2.8 ^{HI} ± 0.3		
Arab	0.16 ^{EF} ± 0.01	0.35 ^{DE} ± 0.01	0.45 ^D ± 0.02	1.09 ^C ± 0.05	0.25 ^{DEF} ± 0.01	0.16 ^{EF} ± 0.00	0.3 ^{DEF} ± 0.0	5.02 ^B ± 0.07	0.20 ^{DEF} ± 0.02	0.07 ^F ± 0.01	0.23 ^{DEF} ± 0.01	^a	0.11 ^{EF} ± 0.01	0.15 ^{EF} ± 0.01	0.24 ^{DEF} ± 0.06	0.22 ^{DEF} ± 0.02	^a	6.4 ^A ± 0.3	0.07 ^F ± 0.01		
Rham	6.54 ^D ± 0.07	6.4 ^D ± 0.5	4.53 ^{DE} ± 0.09	2.08 ^E ± 0.07	4.2 ^{DE} ± 0.1	4.55 ^{DE} ± 0.01	3.75 ^{DE} ± 0.06	2.03 ^E ± 0.05	7 ^{CD} ± 1	21 ^A ± 1	10.3 ^C ± 0.8	^a	17.5 ^{AB} ± 0.6	14.4 ^B ± 0.3	5.4 ^{DE} ± 0.4	4.3 ^{DE} ± 0.3	^a	4.7 ^{DE} ± 0.2	15 ^B ± 3		
Fru	0.73 ^{ABCDEF} ± 0.03	0.8 ^{ABCDE} ± 0.2	0.97 ^{AB} ± 0.09	1.05 ^A ± 0.07	0.85 ^{ABCD} ± 0.06	0.95 ^{ABC} ± 0.08	0.85 ^{ABCD} ± 0.04	0.84 ^{ABCD} ± 0.01	0.6 ^{BCDEFG} ± 0.4	0.51 ^{CDEFG} ± 0.01	0.46 ^{DEFG} ± 0.03	^a	0.49 ^{DEFG} ± 0.02	0.31 ^{EFG} ± 0.01	0.79 ^{ABCD} ± 0.02	0.3 ^{FG} ± 0.1	^a	0.99 ^{AB} ± 0.03	0.20 ^G ± 0.01		
Gal	6.8 ^{AB} ± 0.2	6.4 ^{BCD} ± 0.5	6.2 ^{BCDE} ± 0.1	5.3 ^{ACDEF} ± 0.1	4.5 ^{FGH} ± 0.1	5.79 ^{BCDEF} ± 0.02	6.4 ^{BCD} ± 0.1	5.45 ^{BCDEF} ± 0.04	8 ^A ± 1	3.11 ^{HI} ± 0.06	3.28 ^{GHI} ± 0.08	^a	3.27 ^{GHI} ± 0.09	2.41 ^I ± 0.04	6.5 ^{ABC} ± 0.4	5.0 ^{DEF} ± 0.3	^a	6.4 ^{ABCD} ± 0.2	4.7 ^{EFG} ± 0.3		
Man	2.39 ^{FGH} ± 0.02	3.2 ^{CDE} ± 0.2	3.95 ^{BC} ± 0.07	2.6 ^{EFG} ± 0.1	2.1 ^{GHI} ± 0.1	3.20 ^{CDE} ± 0.04	5.7 ^A ± 0.3	4.24 ^B ± 0.04	3.1 ^{DEF} ± 0.5	0.00 ^J ± 0.00	0.00 ^J ± 0.00	^a	0.00 ^J ± 0.00	0.00 ^J ± 0.00	1.4 ^I ± 0.2	1.7 ^{HI} ± 0.2	^a	3.7 ^{BCD} ± 0.2	0.00 ^J ± 0.00		
Glu	14.02 ^{EF} ± 0.08	14 ^{EF} ± 1	11.4 ^{FGH} ± 0.2	10.7 ^{FGHI} ± 0.3	6.3 ^{IJ} ± 0.3	8.52 ^{HIJ} ± 0.09	9.3 ^{GHI} ± 0.2	9.96 ^{FGHI} ± 0.04	13 ^{EFG} ± 2	42.7 ^A ± 0.7	30.1 ^C ± 0.8	^a	36.7 ^B ± 0.9	31.4 ^C ± 0.5	20 ^D ± 2	4.7 ^J ± 0.4	^a	16.47 ^{DE} ± 0.06	41 ^{AB} ± 3		
GalA	63.3 ^{CD} ± 0.4	63 ^{CD} ± 3	69.0 ^{BC} ± 0.3	74.0 ^{AB} ± 0.9	78 ^A ± 1	71.24 ^{AB} ± 0.15	69.0 ^{BC} ± 0.8	71.5 ^{AB} ± 0.2	68 ^{CDE} ± 7	31.1 ^J ± 0.2	54.16 ^{FG} ± 0.07	^a	39.9 ^{HI} ± 0.4	46.7 ^{GH} ± 0.09	55 ^{EF} ± 3	70.0 ^{ABC} ± 0.9	^a	61.0 ^{DEF} ± 0.9	36.2 ^{IJ} ± 0.8		

^a The recovered amount of pectin was insufficient for this analysis. Equal capital letters in the same line indicate that there is no significant difference ($p < 0.05$) as estimated by Tukey's test between the values. PFR: fresh passion fruit rinds; PFR - UAPLE: residual biomass obtained after Ultrasound-Assisted Pressurized Liquid Extraction. SWE: subcritical water extraction; P-NaDESS: pressurized natural deep eutectic solvents; CE: conventional extraction. Ca:Glu:Wa; citric acid:glucose:water; Cc:La:Choline chloride:lactic acid; Cc:Glu:Wa: choline chloride:glucose:water; Cc:Ca: choline chloride:glucose:water. DM: degree of methyl esterification. Xyl: xylose; Ara: arabinose; Rha: rhamnose; Fru: fructose; Gal: Galactose; Man: mannose; Glu: glucose; GalA: galacturonic acid. Results are expressed as mean ± standard deviation.

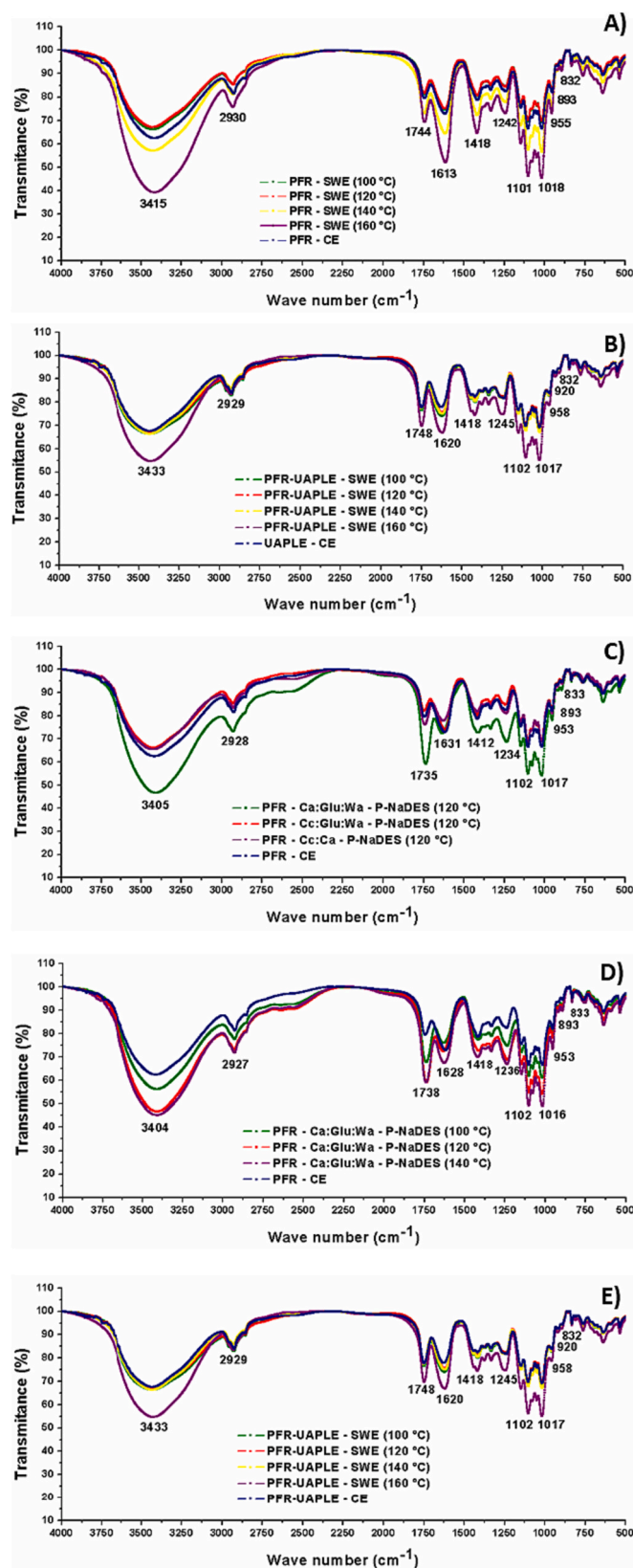


Fig. 8. FT-IR spectra of pectin extracted from A) PFR by subcritical water extraction; B) PFR with previous UAPLE by subcritical water extraction; C) PFR with different NaDESs and pressurized extraction conditions; D) PFR with NaDES (Ca:Glu:Wa) and pressurized extraction conditions; E) PFR with previous UAPLE, NaDES (Ca:Glu:Wa) and pressurized extraction conditions subcritical.

Ultrasound Assisted Extraction (MUAE) and found that the pectin obtained was highly methoxylated (50.77 %). Benvenuti et al. (2022) obtained HM, 67.2 % and 77.9 %, respectively, from jaboticaba by-product using P-NaDES (Ca:Glu:Wa) and heat-stirred extraction.

3.3.4. Morphological analysis of the passion fruit rinds extracted pectins

Scanning Electron Microscopy (SEM) was performed to investigate the effects of extraction conditions (temperature and solvent) on pectins obtained by SWE, P-NaDESs and CE. As shown in Fig. 9, there are differences between the samples that underwent SWE A) and B) and P-NaDESs C), D), E) and F), and CE with acidified water G) and H). Only the pectins obtained by SWE presented smoother, more homogeneous and uniform textures. However, pectins obtained with acidified water showed more heterogeneous, deformed and scaly surfaces. This effect was even more pronounced using NaDESs (see Fig. 9 C, D, E and F), in which fragmentation and disruption on the pectins' surfaces can be observed. Zhou et al. (2023) also observed that the surface of dietary fiber extracted with DES (1:2 chloride: lactic acid) was rough, irregular and with cracks on the particle surface.

Muñoz-Almagro et al. (2019) found that pectins obtained from cacao pod husks with citric acid had surfaces with some long cracks, probably due to acid hydrolysis. Liu et al. (2023) reported that the residue from *Dendrobium devonianum* Paxt stem showed a damaged cell wall after mechanochemical extraction of polysaccharides using different DES. This was not observed in the residue extracted with water, as it still presented a structure similar to that of the cell wall. These authors further inferred that the damage caused to the cell wall using DES could have led to the dissolution of cellulose, increasing the efficiency in the extraction of polysaccharides. According to Gu et al. (2023) the wolfberry residue obtained after conventional extraction with water presented a relatively intact and slightly peeled surface. On the other hand, the extraction with DES (1:2 choline chloride-propylene glycol) exhibited a rough and wrinkled surface probably because of the erosion and permeation of DES into the cell wall.

Comparing the pectin samples obtained with PFR and PFR-UAPLE that underwent the same extraction method, temperature and solvent (Fig. 9 A and B, C and D, and G and H), no modification was noted.

3.4. Sequential process: biorefinery of passion fruit rinds

Based on the results obtained in this work, two processes to obtain pectin with SWE and P-NaDES from passion fruit rinds or its residual biomass are proposed, as shown in Fig. 10. The scheme suggests a sequence of steps to obtain two types of pectins (LM and HM) with different applications. LM pectin can be used in low-calorie or dietary products and fat substitutes. HM pectin can be used as gelling agent, stabilizer, emulsifier and thickener in food processing (Marić et al., 2018).

In the proposed process, first, fresh passion fruit rinds (PFR) or its residual biomass (PFR - UAPLE) are dried at 60 °C to a moisture content of 15 %, ground and sieved to achieve particle size between 0.5 and 0.9 mm. The obtained powder is then subjected to SWE at 120 °C if the objective is to obtain LM pectin or P-NaDESs (Ca:Glu:Wa) at 120 °C to obtain HM. Pressure (10 MPa) and S/F (30 kg solvent/kg PFR or PFR - UAPLE) conditions should be constant for both extractions. Next, the extracts are precipitated, centrifuged, dried and ground. Both final products can be applied in the food, cosmetic and pharmaceutical industries. Furthermore, the residue obtained after SWE and P-NaDESs can be reused for bioremediation, bioconversion processes and for the production of biodegradable and edible films.

The possibility of obtaining two different types of pectin (LM and HM) is advantageous, as it eliminates the need to de-esterify HM into LM pectin. Through the same process, but only replacing the aqueous solutions of the natural deep eutectic solvent (Ca:Glu:Wa) by pure water, it is possible to eliminate a deesterification stage and its involved costs regarding solvents. In addition, pectin can be produced with fast and

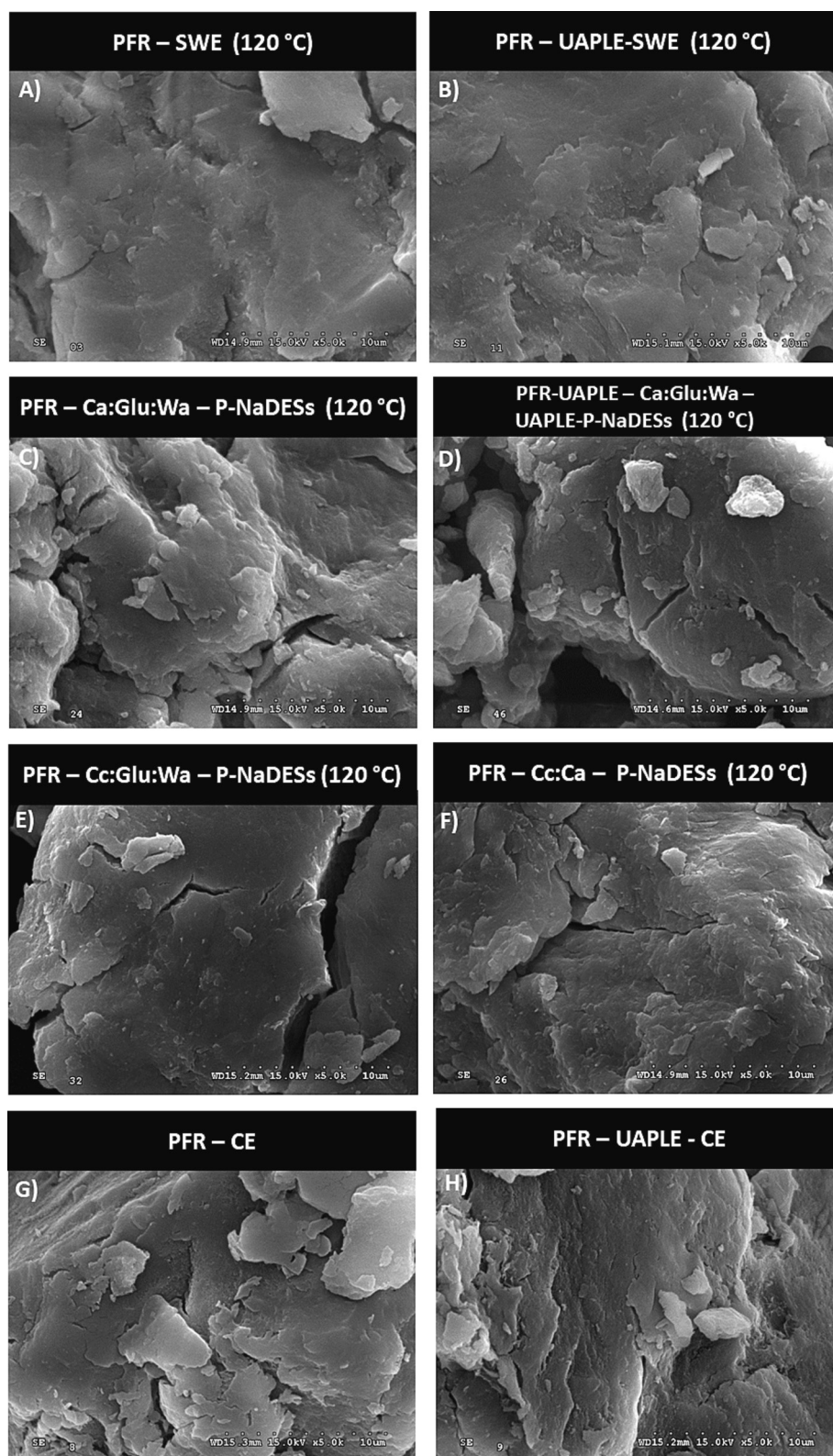


Fig. 9. Scanning electron micrographs ($\times 5000$ magnification) of pectin from passion fruit rinds subjected to: subcritical water extraction (SWE) at 120 °C with (A) PFR and (B) PFR -UAPLE; pressurized natural deep eutectic solvents (P-NaDESS) at 120 °C with Ca:Glu:Wa (C) PFR and (D) PFR – UAPLE, (E) Cc:Glu:Wa and PFR; (F) Cc:La and conventional extraction (CE) with (G) PFR and (H) PFR -UAPLE.

sustainable extraction techniques, using GRAS solvents and without generating toxic waste for the environment.

4. Conclusion

This work presents the yields and structural characterization of pectins obtained from passion fruit rinds using sustainable extraction

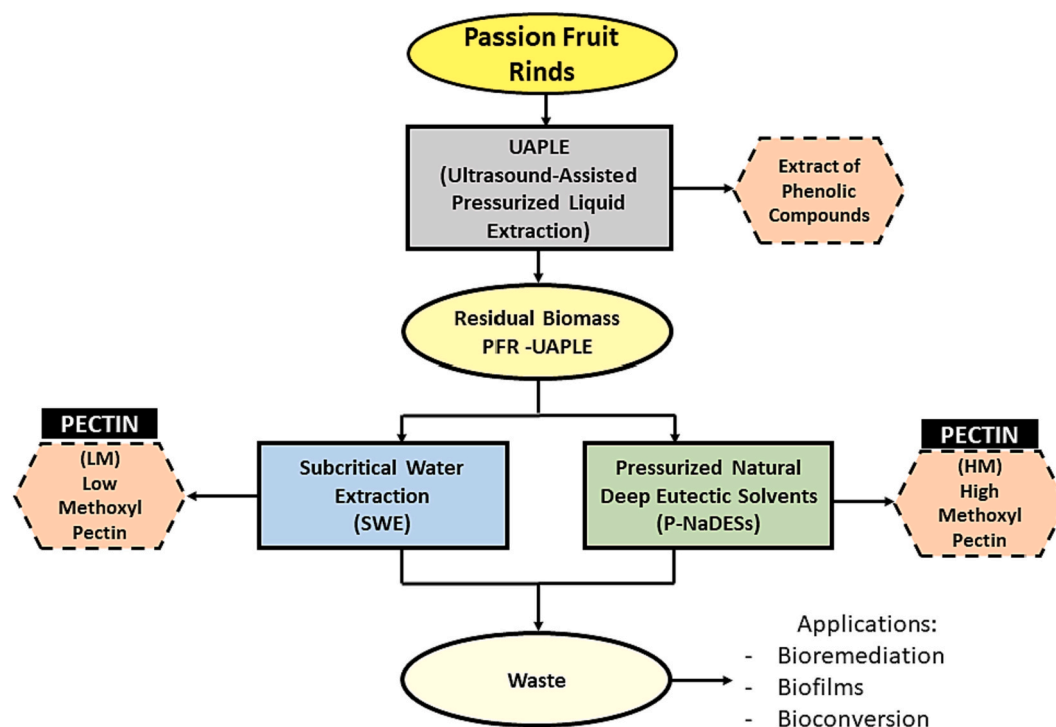


Fig. 10. Proposal of a scheme to obtain high and low DE pectin using SWE and P-NaDESs from passion fruit rinds or its residual biomass.

technologies and green solvents, such as SWE and P-NaDESs. The comparison of these methods with CE by heating using acidified water is also reported. Extraction using P-NaDES (Ca:Glu:Wa) at 120 °C was more effective than SWE and CE, leading to a higher pectin recovery (19.1 and 27.6 %) from passion fruit rinds and its residual biomass, respectively. However, P-NaDES (Ca:Glu:Wa) led to the extraction of more non-pectic monosaccharides, such as glucose (13–42 %).

Regarding the monomeric composition, galacturonic acid was the main monosaccharide found in pectin in most extractions, mainly using subcritical water (74–78 %). The molecular weight was also evaluated and the highest values (98 and 81 kDa) were achieved with SWE and P-NaDES (Ca:Glu:Wa), respectively, at 120 °C from passion fruit rinds. The residual biomass had the lowest molecular weights, probably due to the depolymerization of the polysaccharide chain in the previous extraction of phenolic compounds with ultrasound.

The degree of esterification was affected by the extraction solvent, with pectins with a high degree of esterification (61.7 to 78.5 %) being obtained with P-NaDES (Ca:Glu:Wa) and pectins with a low degree of esterification (13.9 to 37.5 %) with pure water and acidified. Regarding the pectins' morphology, SWE provided more uniform surfaces, unlike the deformed surface obtained with acidified water and with fissures using NaDESs.

The results obtained in work encourage proposing two different promising processes, SWE at 120 °C to obtain LM pectin and P-NaDESs (Ca:Glu:Wa) at 100 °C to obtain HM pectin from passion fruit rinds or its residual biomass. Thus, pectins with different structural characteristics can give rise to several food applications depending on the temperature and solvent applied. More investigation must be carried out to evaluate the technological properties of the pectins obtained in the two suggested processes.

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CRediT authorship contribution statement

Débora Tamires Vitor Pereira: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Pablo Méndez-Albiñana:** Validation, Investigation, Writing – review & editing. **Jose A. Mendiola:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Project administration. **Mar Villamiel:** Validation, Resources, Writing – review & editing, Supervision, Funding acquisition. **Alejandro Cifuentes:** Resources, Writing – review & editing, Supervision, Funding acquisition. **Julian Martínez:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition. **Elena Ibáñez:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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