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Author statement

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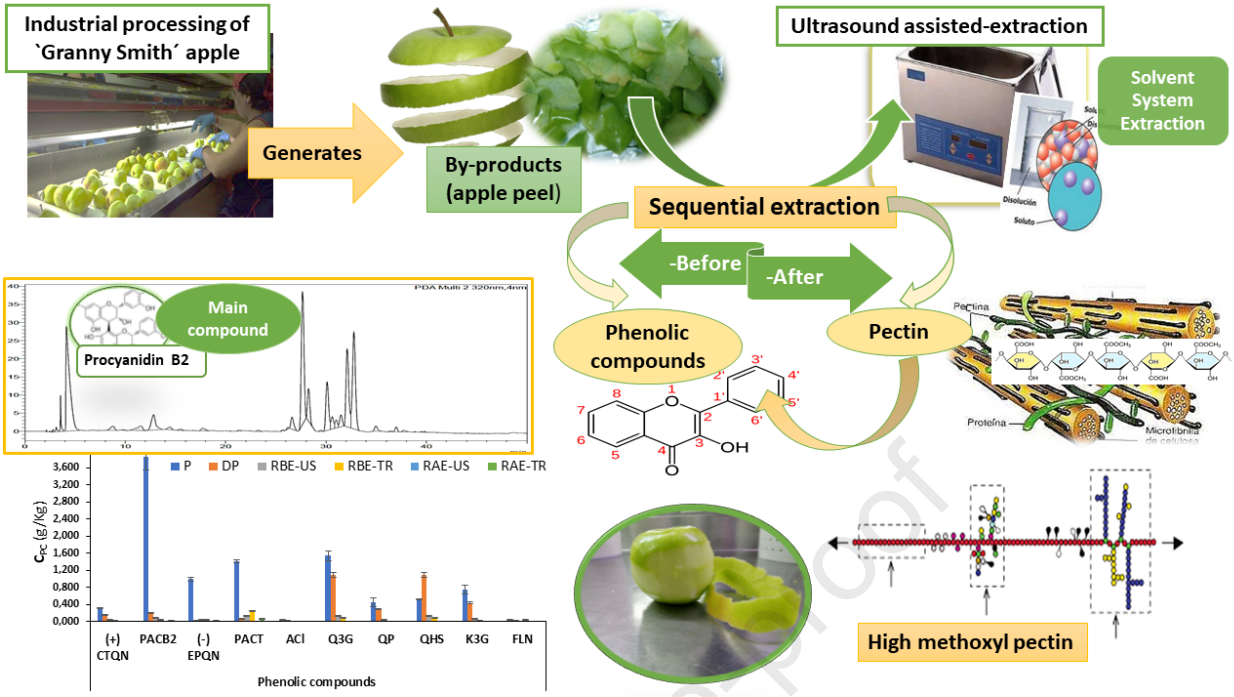

SEQUENTIAL ULTRASOUND-ASSISTED EXTRACTION OF PECTIN AND PHENOLIC COMPOUNDS FOR THE VALORISATION OF `GRANNY SMITH´ APPLE PEEL

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Abstract

This study aims to evaluate the impact of the sequential extraction system for phenolic compounds and pectin from waste `Granny Smith´ apple peel using ultrasound. The effects of solvent, formic acid concentration (C_{FA}), and the number of extraction steps on the content of individual and total phenolic compounds (TPC), total flavonoids, and antioxidant capacity, were determined. Then, pectin was obtained from apple peel before (BE) and after (AE) phenolic compounds extraction, using conventional (TR) or ultrasound-assisted procedures (US). The two-steps 80% Acetone (0% C_{FA}) extraction system had the highest TPC (3.47 g GAE/Kg). Procyanidin B2 (0.03-0.77 g/Kg) was the major phenolic compound extracted from `Granny Smith´ apple peel. AE pectin extraction yield (6.38% for US and 4.92% for TR) was higher than BE. Pectin obtained had 57-60 % DE, 9.3-10.3 % Methoxyl content, and 436-460 equivalent weight. Wasted apple peel is a great low-cost source of phenolic compounds and pectin. Furthermore, it is possible to achieve the highest yields of both compounds through appropriate extraction sequences (AE: phenolic compound extraction followed by pectin extraction) and alternative technologies like ultrasound-assisted extraction.

Keywords:

Fruit waste by-products; circular economy; bioactive compounds; cavitation; procyanidins; antioxidants.

Abbreviations

(-)EPQN: Epicatechin; (+)CTQN: Catechin, Ac: acetone 80%; ACL: Chlorogenic acid, AE: Pectin extraction process after obtaining phenolic compounds; AERUS: Phenolic compounds obtained from RAE-US; BE: Pectin extraction process before obtaining phenolic compounds; BERTR: Phenolic compounds obtained from RBE-TR; BERUS: Phenolic compounds obtained from RBE-US; C_{FA}: formic acid concentration; C_{PC}: Phenolic

compound concentrations; DE: Degree of esterification; DP: Dried Peel; DPPH: antioxidant capacity by DPPH; DRT: Dried Residual Tissue, ES: number of extraction steps; EtOH: Ethanol 80%; FLN: Phloretin, FRAP: ferric reducing antioxidant power; GAE: gallic acid equivalents; HMP: high-methoxyl pectins; K3G: Kaempferol-3-glucuronide, LMP: low-methoxyl pectin; MeOH: methanol 80%; P: Fresh peel; PACB2: Procyanidin B2, PACT: Procyanidin tetramer; Q3G: Quercetin-3-glucuronide; QE: quercetin equivalent; QHS: Quercetin hexoside; QP: Quercetin pentoside; RAE-TR: residual tissue of AE pectin TR-extraction; RAE-US: Phenolic compounds obtained from RAE-TR, RAE-US: residual tissue of AE pectin US-extraction; RBE-TR: residual tissue of BE pectin TR-extraction; RBE-US: residual tissue of BE pectin US-extraction; S: type of solvent; TF: total flavonoids; TPC: total phenolic content; TPC_{HPLC}: Total phenolic compounds by HPLC; TR: conventional pectin extraction; US: ultrasound-assisted pectin extraction; W: water.

1. Introduction

The agro-industrial business generates large amounts of wasted by-products in its production processes, representing a critical problem for the environment and public health (Kumari et al., 2018; Ravindran et al., 2018). In developing countries, 40% of these by-products come from the industrial processing steps (Garcia-Amezquita et al., 2018; Ravindran et al., 2018). Their composition includes essential nutrients such as vitamins, fibre, amino acids, and bioactive and functional compounds such as lignocellulose, terpenes, alkaloids, and phenolic compounds (Campos et al., 2020; Girotto et al., 2015; Santagata et al., 2021). Therefore, several studies have determined these by-products´ bioactive and techno-functional properties and the application of different technologies for extracting their bioactive molecules (Cano-Lamadrid & Artés-Hernández, 2021; Kumari et al., 2018; Maina et al., 2017).

The industrial processing of apples generates a substantial amount of non-avoidable residues such as peel, seeds, and core, which account for 16-36%.(Piagentini & Pirovani, 2017; Garcia-Amezquita et al., 2018). Apple wasted by-products contain many valuable compounds, including pectin and phenolic compounds (Henríquez et al., 2014; Kalinowska et al., 2014; Massini et al., 2016).

Pectin is part of the primary cell walls present in the middle lamellae of plants (Luo et al., 2017). The structural and functional properties of pectin depend on the methoxylation degree, galacturonic acid content, sugar composition, and molecular weight. These properties vary according to the source and the extraction methodology (Güzel & Akpınar, 2019). In the industrial pectin extraction process, the apple peel is dried to avoid enzymatic degradation and simplify storage and handling. High methoxyl pectin is extracted using an acidified solid-liquid extraction system that breaks the polygalacturonic acid chains, solubilising the protopectin (Maran et al., 2017). This process may also degrade other

secondary metabolites, such as phenolic compounds, due to the long extraction times and high temperatures required, which increases the rate of oxidation and condensation of phenolic compounds (Mieszczakowska-Frąc et al., 2016). It also generates aqueous residues that can be harmful to the environment and may lead to a loss of nutritional quality of the product (Bhatia et al., 2016). Therefore, other more efficient and environmentally friendly techniques have been applied for pectin extraction, such as ultrasound-assisted extraction (Maran et al., 2017), supercritical fluid extraction (Azwanida, 2015), and microwave-assisted extraction (Sarah et al., 2018).

The apple peel can have 2-5 times more phenolic compounds than flesh, depending on the cultivar, environmental conditions, and type of production (Piagentini & Pirovani, 2017). These compounds have biological (anti-inflammatory, anticancer, antimicrobial, and antioxidant activities) and technological activities and can be applied as replacements for synthetic antioxidant compounds at the industrial level (Kalinowska et al., 2014; Rodríguez-Arzuaga et al., 2021).

Solid-liquid extraction is widely used to obtain phenolic compounds from plant tissues, and it is mainly affected by solvent, acidity, temperature, time, particle size, agitation conditions, and solid-liquid ratio (Mourtzinos & Goula, 2019). There is no single solvent that ensures total phenolic compound extraction. The solvatochromic and macroscopic properties of the solvents will determine the solvent-solute and solvent-solvent interactions (Villamil-Galindo et al., 2020). The capacity of the extraction solvent to donate hydrogen bonds and the ability to accept hydrogen bonds vary the solvation of the different phenolic compounds and their derivatives (Mourtzinos & Goula, 2019). Ultrasound-assisted extraction has proven to be a technology with high industrial projection for obtaining bioactive compounds, improving extraction yields and reducing extraction costs (Zhang et al., 2018).

Some authors have studied the profile and the extraction of phenolic compounds from `Granny Smith´ apples (Henríquez et al., 2014; Piagentini & Pirovani, 2017). Others have studied the extraction of pectin from apple peel (Bhatia, Sharma, & Alam, 2016; Constenla et al., 2002; Güzel & Akpınar, 2019). However, there are no current studies about the sequential extraction of pectin and phenolic compounds from wasted `Granny Smith´ apple peel. The ultrasound-assisted extraction appears as a prominent option for the full use of these fruit wasted by-products recovering more phenolic compounds and pectin. Therefore, the main objective of this work was to revalorise apple peel waste by evaluating the impact of different solvent systems on the total phenolic content and the antioxidant activity of apple peel extracts. Moreover, the effect of the ultrasound-assisted extraction step of pectin, and the sequence of the extractions steps for obtaining phenolic compounds and pectin from apple peel, were studied.

2. Material and methods

2.1. Plant material

`Granny Smith´ apples were obtained in a local market (Santa Fe, Argentina) and stored at 1.5°C and 95% RH. The fruits were selected, washed, and disinfected with sodium hypochlorite (100 ppm, 2 min). The peel was removed (1 mm thickness) with a sharp stainless-steel knife, and the moisture content was determined in triplicate (80.4 ± 0.52%) using a thermogravimetric analyser (RADWAG PMR 50, Poland) at 80°C, 60 min. Part of the apple peel (P) was packed in polyethylene bags, frozen at -20°C for studying the extraction of phenolic compounds and the extraction of pectin AE (After the Extraction of phenolic compounds, Fig. 1). Another part of the apple peel was dried in a laboratory oven (50 °C, 24 h, up to 9% of moisture), then it was milled and sieved to a particle size of < 1

mm (DP: dried peel, Fig. 1). These samples were stored in 40 µm polypropylene bags (100 g) for obtaining pectin BE (Before the Extraction of phenolic compounds, Fig. 1).

2.2. Phenolic compounds extraction.

2.2.1. Experimental design

The effect of the type of solvent [water (100%) and ethanol, methanol, and acetone (80%)]; the formic acid concentration (C_{FA}) [0 and 0.5%]; and extraction steps [1 (1/10 w/v) and 2 (1/5 w/v)] were determined through a factorial design, on the total phenolic content (TPC), total flavonoid content (TF), phenolic compound profile, and the antioxidant capacity of `Granny Smith´ apple peel extracts, for selecting the best extraction system.

2.2.2. Phenolic Compound Extraction

The phenolic compound extraction was carried out according to Villamil-Galindo et al. (2020) to study the one-step and two-step extraction process. For the one-step extraction, the mixture of ground apple peel (P, Fig 1) with the solvent (1:10 w/v) was sonicated (Ultrasonic Cleaner, Testlab, Buenos Aires, Argentina) at 160 W and 40 kHz for 15 min and centrifuged at 12000 g for 20 min at 20°C (Neofuge 18R Heal Force centrifuge, Shanghai, China). The supernatant was collected and reserved until analysis.

Two-step extraction consisted of sonicating for 15 min 20°C, a mixture of the ground sample (P) plus the solvent (1:5 w/v) (first step), and then centrifuged at 12000 g for 20 min 20°C. The supernatant was collected in a volumetric flask, and the residue was re-extracted with fresh solvent solution (1:5 w/v) (second step). Then, the mixture was sonicated, centrifuged, and the supernatant was separated. Both supernatants were pooled and analysed.

The extraction system with the highest phenolic compound yield was used for analysing the polyphenols extraction impact on pectin extraction and vice-versa.

155

156 **2.3. Pectin Extraction**

157 2.3.1. Experimental design

158 The sequence of phenolic compound extraction steps and the method for pectin extraction
159 could affect pectin yield. Regarding the phenolic compounds extraction step, the pectin was
160 obtained before (BE) and after (AE) the phenolic compound extraction from apple peels. The
161 pectin BE was extracted from dried apple peel (DP), and the pectin AE was obtained from
162 the dried residual tissue obtained after phenolic extraction (DRT). Moreover, two extraction
163 methodologies were evaluated for pectin extraction, both BE and AE, with ultrasound-
164 assisted extraction (US) and without ultrasound (conventional process, TR) (Fig. 1).
165 After each extraction assay, pectin was characterized by determining pectin extraction yield,
166 degree of pectin esterification (DE), methoxyl content, and pectin equivalent weight.

167

168 2.3.2. Pectin extraction methodologies

169 The pectin was extracted from dried apple peel, DP (pectin BE) and from the dried residual
170 tissue (DRT) obtained after phenolics extraction (pectin AE), using ultrasound-assisted
171 extraction (US), following the methodology of Maran et al. (2017) with some modification
172 (Fig. 1). Five grams of sample (DP or DRT) were placed into an Erlenmeyer flask with a
173 citric acid solution (pH 2.00) to complete an extraction ratio of 1:18. The mixture was
174 sonicated in an ultrasound bath (Testlab) with 40 kHz and 160 W at 50°C for 1 h. After
175 centrifugation at 12000 g for 20 min 4°C (Neofuge 18R Heal Force), the supernatant was
176 filtered, added to the same volume of absolute ethanol, and allowed to precipitate the pectin
177 for 30 min 4°C. The pectin pellet was centrifuged (20 min, 12000g, 4°C), collected, and
178 washed three times with ethanol. The pectin pellet was left to repose for 12 h in ethanol 70%

and dried in a laboratory oven (50°C, 24h). Pectin BE-US, and pectin AE-US were obtained (Fig. 1).

The conventional pectin extraction method (TR) was also performed similarly, but replacing the ultrasound step with a thermostatic bath (80°C, 2 h) to determine the effect of ultrasound-assisted extraction. Pectin BE-TR and pectin AE-TR were obtained (Fig. 1).

The yields of extractions were calculated as followed (equation 1):

$$Yield\ (\%) = pectin\ weight(g)\ /\ Sample\ weight\ \ (g)\ \times 100 \quad (1)$$

2.4. Analytical determinations

2.4.1. Total phenolic content (TPC)

TPC was measured on the extracts obtained from apple peel during the phenolic compound extraction assays, before (P) and after the apple peel drying (DP), and pectin extraction processes (BE_{RUS}, BE_{RTR}, $AE_{RUS,}$ and AE_{RTR}) (Fig. 1). The TPC determination followed the Folin-Ciocalteu method according to Piagentini and Pirovani (2017). Three replicates were performed by sample, and TPC was expressed as gallic acid equivalents (g GAE/Kg).

2.4.2. Total flavonoid content (TF)

TF was determined in triplicate according to Villamil-Galindo et al. (2020), using aluminium chloride solution as the specific reagent for flavonoid determination. Results were expressed as quercetin equivalents (g QE/Kg).

2.4.3. Phenolic compound profile

The phenolic compound profile was determined in an LC-20AT high-performance liquid chromatography with a photodiode array detector (Shimadzu Co., Kyoto, Japan) with a Gemini 5 µ C18 110 Å 250 × 4.6 mm hybrid reverse phase column attached to a guard

column (Phenomenex Inc, CA, USA). The identification and quantification methodologies of phenolic compounds were similar to those applied by Villamil-Galindo et al. (2021). Identification of phenolic compounds was performed by comparing retention times and UV–Vis absorption spectra of standard phenolic compounds. The identified compounds were quantified using the external standard method with the corresponding calibration curves of analytical standards (Sigma-Aldrich Inc.; St. Louis, MO, USA), and the phenolic concentrations were reported as g/Kg.

2.4.4. Antioxidant Capacity

The free radical scavenging capacity (DPPH), evaluated by the DPPH assay, was determined in triplicated according to Villamil-Galindo et al. (2021). The results were expressed as mmol Trolox/Kg. Furthermore, the total antioxidant capacity of the apple peel extracts, using the ferric reducing antioxidant power (FRAP) assay, was performed according to Rodríguez-Arzuaga and Piagentini (2018). FRAP results were expressed as mmol Fe^{2+}/Kg.

2.4.5. Degree of esterification and methoxyl content of pectin

The degree of esterification (DE) and the methoxyl content (MC) of pectin were determined by a volumetric method, according to Gazala et al. (2017) and Doesburg (1966). Pectin (200 mg) was diluted in water (20 mL) and stirred (2 h, 40°C) until completely dissolved. The pectin solution was titrated with 0.1 M NaOH until a pH of 8.1 (V1: volume expended). Then, 10 mL of 0.1 M NaOH was added, and the homogenized solution was left to stand for 120 min at room temperature. Finally, 10 mL of 0.1 M HCl was added, homogenized, and titrated with 0.1 M NaOH until pH 8.1 (V2). The DE and MC were calculated using equations 2 and 3, respectively:

$$DE\ (\%) = \frac{V2}{(V1 + V2)} \times 100 \quad (2)$$

$$MC(\%) = \frac{V2\ \times normality \times 3.1}{weight\ of\ sample\ (g)} \quad (3)$$

2.4.6. Pectin equivalent weight

The equivalent weight of pectin (EW), calculated according to Doesburg (1966), was the number of grams of pure polygalacturonic acid that corresponds with an equivalent of free carboxyl groups. EW was calculated with equation 4, where DE (%) was the pectin degree of esterification (equation 2):

$$EW = \frac{17600 + 14 * DE}{100 - DE} \quad (4)$$

2.5. Statistical analysis

Data were subjected to analysis of variance (ANOVA to determine the effect of extraction variables on the analytical responses. First, we investigated the assumptions underlying the ANOVA test. The Kolmogorov-Smirnov test determined (with 95% confidence) that responses had normal distributions. Homoscedasticity was assessed through Levene's test ($p>0.05$), verifying the homogeneity of variance for all response variables. Statistical differences among treatment means were determined by Tukey´s multiple range test (at a 5% significance level). Also, a correlation analysis between the studied variables was performed using the Pearson correlation coefficients. The statistical analyses were performed with STATGRAPHICS Centurion XV (StatPoint Technologies Inc., Warrenton. VA, USA).

3. Results and discussion

3.1. Phenolic compound extraction and its antioxidant capacity

The solvent (S), formic acid concentration (C_{FA}) and the number of extraction steps (ES) significantly affected the total phenolic content and the antioxidant capacity ($p \leq 0.001$) of the apple peel extracts (Tables 1 and S1). The interaction of S.C_{FA} was highly significant ($p \leq 0.001$) for all responses, and the S. C_{FA}.ES interaction significantly affected TPC, TF, and DPPH. The effect of the extraction variables on the phenolic compounds and antioxidant capacity cannot be analysed separately, as most of the interactions were highly significant (Table S1).

A C_{FA} of 0.5% in the extraction solution improved the TPC in the extracts obtained with polar protic solvent solutions (100% water - W, 80% methanol – MeOH, and 80% ethanol - EtOH). In the case of TF, the increased acid concentration reduced the flavonoid content in all extraction systems. On the other hand, the TPC and the antioxidant capacity (FRAP) were higher in the extracts obtained two- than one-step extractions. In the same way, the systems with polar protic solvent showed more yields of TPC and more antioxidant capacity in two-step extraction (Table 1).

For one-step extracts (C_{FA} = 0%), acetone 80% (Ac) showed the major TPC ($p<0.05$) with 1.94 g AGE/Kg (Table 1). Although having a low capacity to donate hydrogen bonds, the dipole moment of acetone allows it to solvate phenolic compounds as it has a high acceptance of hydrogen bonds, explaining their solvatochromic properties. In acetone-water solutions, water facilitates diffusion and solute-solvent interactions (Villamil-Galindo et al., 2020). The TPC increased ($p<0.05$) around 15%, when C_{FA} = 0.5% in one-step extraction with MeOH and Ac solutions (Table 1). The pH reduction in the extraction solutions allows hydrolysing of the plant matrix, stabilising the charges of certain phenolic compounds and their solvation (Takeuchi et al., 2008).

Besides, the use of acetone 80%, in two steps without formic acid, provides the highest ($p<0.05$) TPC (3.47 g AGE/Kg) among the extracts (Table 1). This yield was 44% higher

than the obtained with acetone 80% in one-step and 9.5% higher than the extract with C_{FA}=0.5% in two-step extraction. Regarding the mass transfer phenomenon occurring in the different extractions performed on the apple peel, the solvent-solute ratio generates the driving force for this phenomenon to occur (Takeuchi et al., 2008). These phenolic compound contents were higher than those reported by Drogoudi and Pantelidis (2011) for `Granny Smith´ peel (3.03 g AGE/Kg) and Guyot et al. (2002) (3.15 g AGE/Kg). They were even higher than those reported for peel from other cultivars such as `Golden delicious´ (3.04 g AGE/Kg) and `Red Delicious´ peel obtained with acetone 80% with a solid-solvent ratio of 1:10 (2.48 g AGE/Kg) (Piagentini & Pirovani, 2017). Phenolic compounds present a broad structural diversity, contributing to their different properties, such as polarity, demonstrating no single solvent can guarantee a complete extraction of phenolic compounds (Azwanida, 2015). Compared with previous studies performed using the same extraction systems on the strawberry by-products, the extraction with methanol 80% with C_{FA}=0.5% in two steps showed the highest TPC yield with 15 g/Kg (Villamil-Galindo et al., 2020). These results confirm the variation of phenolic compounds and their concentrations among the different fruit waste by-products. Besides, the diverse molecular structures of these metabolites confer them different polarities, hence generating a preferential solvation phenomenon. Consequently, the phenolic compounds of strawberry by-products were better extracted with MeOH, while the extraction yields of phenolic compounds of the `Granny Smith´ apple peel were better with Ac (Table 1).

Therefore, the use of binary solutions of organic solvents and water extends the range of solvation due to the change in polarity, viscosity dielectric constant, acidity, surface tension, and solvatochromic properties, and consequently, allowing a higher recovery of phenolic compounds (Takeuchi et al., 2008). The acetone-water solution was used with excellent results, obtaining higher yields than pure solvent or mixtures of other organic solvents

(Stavrou et al., 2018). The dipole moment of acetone gave it an excellent ability to accept hydrogen bonds from the hydroxyl groups of phenolic compounds (Villamil-Galindo et al., 2020).

The flavonoids have been reported as the main phenolic compounds in many apple cultivars (Kalinowska et al., 2014). The extraction in one-step and C_{FA}=0 % produced the highest TF content (0.28-0.27 g QE/Kg) with EtOH and Ac, without differences between them ($p>0.05$) (Table 1). TF extracted with EtOH and Ac was 2.5 times higher than W in the same conditions. These results show the possibility of using green solvents, such as ethanol, for flavonoids recovery from agro-industrial waste by-products. Regarding W, EtOH and Ac, the use of C_{AF} =0.5% did not significantly improve the TF extraction yield, probably, because the structure of the heterocyclic linking the A and B rings of the flavonoids could have been altered by the use of acid, making it difficult to recover and quantify (Kalinowska et al., 2014).

The two-step extracts with C_{FA}=0% showed the highest TF content among all studied conditions, being MeOH and Ac, the best solvents (0.43-0.45 g QE/Kg) ($p>0.05$) to recovery flavonoids from `Granny Smith´ apple peel. Savatović et al. (2008) reported similar results for the `Granny Smith´ pomace methanolic extract (0.51 g/Kg).

Regarding antioxidant capacity, Table 1 shows that in the extracts obtained with one-step and C_{FA}=0%, Ac had the highest anti-radical activity (DPPH*) (17.60 mmol Trolox/Kg), being up to 17 times higher than W and MeOH, both similar ($p>0.05$) and with the lowest DPPH. In the two-steps extractions, the addition of formic acid 0.5% did not improve significantly the antioxidant capacity (DPPH* and FRAP) of the extracts obtained with green solvents like water and ethanol 80% (Table 1). This result was similar to the reported by Lončarić et al. (2020) for `Granny Smith´ apple (18 mmol Trolox/Kg) and higher than `Golden Delicious´ peel (15 mmol Trolox/Kg). Concerning the FRAP antioxidant capacity,

the extract obtained with Ac in one step without formic acid had 7.70 mmol Fe^{+2}/Kg. The use of two-step extraction improves FRAP significantly by 30% (11 mmol Fe^{+2}Kg). The use of a C_{FA}= 0.5% increased FRAP significantly ($p<0.05$), having the two-steps extract obtained with acetone 80% the highest FRAP value (13.4 mmol Fe^{+2}/Kg), 2.4 times higher than the EtOH extract in one-step. FRAP values of Ac extracts show the importance of `Granny Smith´ apple peel extracts as an excellent source of antioxidant compounds. These values were higher than those reported for other agro-industrial by-products such as pistachio hull (6.6 mmol Fe^{+2}/Kg) obtained with acetone 100% (Rezaie et al., 2015).

3.2. `Granny Smith´ apple peel phenolic compound profile

Ten phenolic compounds were identified and quantified (Tables 2, S3, and S4). Flavan-3-ols, flavonols, phenolic acids, and dihydrochalcones were the principal phenolic compound families determined in `Granny Smith´ apple peel. Flavan-3-ols (procyanidins) were the main phenolic compounds (59.6%), followed by flavonols (38.3%), phenolic acids (0.85%), and dihydrochalcones (0.67% of the identified phenolic compounds). Massini et al. (2016) reported similar results for `Bramley´ apple peel, with the procyanidins (64%) as the principal family of phenolic compounds, followed by flavonols (26%), using ethanol 80% as the extraction solvent.

Among the flavan-3-ols, the (+) catechin [(+) CTQN] was identified in concentrations of 0.005-0.082 g/Kg, being the MeOH in two-steps the extraction system with higher yields, and the formic acid concentration did not affect ($p>0.05$) the extraction yield (Table S3). Besides, the (-) Epicatechin [(-) EPQN] was extracted in 0.006-0.199 g/Kg. MeOH with C_{FA} = 0.5% in two-steps, and Ac with C_{FA}= 0% in two-steps (Table 2) showed the major yields of (-) EPQN (0.196 and 0.199 g/Kg, respectively). The (+) catechin and (-) epicatechin concentrations obtained herein were higher than those reported for `Idared´apple flesh (0.05

g/Kg (+) CTQN, and 0.137 g/Kg (-) EPQN) (Mieszczakowska-Frąc et al., 2016). The (+) CTQN correlated significantly with the FRAP antioxidant capacity by assay in one-step extraction, C_{FA}=0-0.5% (R^2 0.72 and 0.89, respectively), and also in the two-steps extraction C_{FA} =0% (R^2 0.73). On the other hand, the (-) EPQN showed a significant correlation with the FRAP antioxidant capacity in all the extraction systems (Tables S5 to S8), and for two-step C_{FA}= 0.5% extraction correlated with both antioxidant capacities (R^2 0.86 for DPPH* and 0.88 for FRAP). These results suggested the significant contribution of the condensed tannins present in the `Granny Smith´ apple peel, such as (+) Catechin and (-) Epicatechin in the antioxidant capacity, due to their radical scavenging capacity, redox properties, and the capacity to chelate transition metals. Likewise, He and Liu (2008) reported the high antioxidant activity of the catechin obtained from `Red Delicious´ apple peel extracted with acetone 80%. Two procyanidins were also identified, the procyanidin tetramer (PACT) and procyanidin B2 (PACB2). The latter is the principal phenolic compound extracted from the `Granny Smith´ apple peel, representing 23-38% of the total phenolic compounds (Table 2). The extracts obtained with Ac and C_{FA}=0%, in two-steps, showed the highest PACB2 concentration (0.77 g/Kg) followed by the MeOH extract (C_{FA} = 0%, two-steps) with 0.73 g/Kg. The B procyanidins were dimers of epicatechins, characterised by their medium-low polarity. They had a single inter-flavan bond between carbon-4 of the B-ring and either carbon-8 or carbon-6 of the C-ring (Massini et al., 2016). This fact facilitated solubilisation with medium polarity solvents such as methanol and acetone. Moreover, the cavitation generated by the ultrasound allowed the rupture of the chloroplasts of the plant cell where the proanthocyanidins were stored, facilitating the extraction process (Dzah et al., 2020). In this study, the PACB2 content of the EtOH, MeOH, and Ac extracts obtained in two steps was higher than the reported by Almeida et al. (2017) for `Granny Smith´ (0.28 g/Kg) and `Golden Delicious´ (0.38 g/Kg) apple peel methanolic extract, without ultrasound-assisted

extraction. Moreover, the PACT showed a significant correlation with FRAP antioxidant capacity in all the extraction systems (Tables S5 to S8). Besides, in the extraction with two-steps C_{FA} = 0.5%, the PACB2 and PACT significantly correlated with DPPH and FRAP antioxidant capacities ($R^2 > 0.8$).

The flavonols, the second main family of phenolic compounds identified in `Granny Smith´ apple peel, were the Quercetin-3-o-glucuronide (Q3G), Quercetin pentoside (QP), Quercetin hexoside (QHS) and Kaempferol-3-glucuronide (K3G). EtOH, one of the protic polar solvents used, showed the best flavonols recovery, representing up to 40% of total phenolics, with the one-step extraction system of EtOH, C_{FA} =0.5%. Moreover, a C_{FA}=0.5% improved the yields by up to 18% in the case of QP (0.048 g/Kg) (Table S4). Apples were one of the most significant flavonoid sources in the human diet (Almeida et al., 2017). The extracts made in two-step with C_{FA} = 0.5% showed a highly significant correlation with DPPH and FRAP activities (R^2 >0.9) (Tables S5 to S8). Their structures and the hydroxyl groups disposition in this kind of extraction conferred great potential as a natural antioxidant source at a low cost (He & Liu, 2008). These results brought valuable information about the flavonoid extraction with eco-friendly solvents such as water or ethanol 80%, allowing the valorisation of these by-products with minimal cost from `Granny Smith´ apple peel.

Regarding dihydrochalcones, phloretin (FLN) was identified and quantified in apple peel extracts. FLN came from several metabolic products, such as phlorizin, trilobactin, phloretin 20-O-xyloglucoside, sieboldin, 3-hydroxyphlorizin, and 3-hydroxyphloretin. It was a compound of interest, especially in the apple peel, where its synthesis was higher, and it could be present in concentrations of 0.02-0.42 g/Kg (Mariadoss et al., 2019). Similar concentrations were obtained in EtOH extracts (two-steps, C_{FA} = 0%) (0.019 g/Kg), being higher than the reported by Kschonsek et al. (2018) for `Granny Smith´ apple flesh (0.007 g/Kg). On the other hand, the chlorogenic acid (ACl), a phenolic acid, was identified in

`Granny Smith´ apple peel (0.006-0.33 g/Kg) (Table S3) in lower concentrations than the reported for the flesh (1.34 g/Kg) (Almeida et al., 2017). The total phenolic content determined by HPLC (TPC_{HPLC}) for two-step extraction with Ac and MeOH showed the highest concentration (1.99 and 1.90 g/Kg, respectively) for C_{FA}=0 (Table 2), in agreement with TPC (Table 1).

Apple industrial processing has been generating most fruit waste, and the use of `Granny Smith´ apple peel as a phenolic compound source became an important issue (Scarano et al., 2022). The average daily intake of quercetin and phloretin in developed countries was approximately 34 mg/100 g fresh food portion and 0.7 mg/100 g diet, respectively, lower than the intake of other compounds such as hesperidin (100 mg/diet) (Koch et al., 2015). Besides, these amounts were lower for most of the population in developing countries. For this reason, it was of interest to have a low-cost source of different phenolic compounds that brought the consumer a bioactive compound source for improving the antioxidant and healthy characteristics of food.

3.3. Impact of the extraction processes on pectin and phenolic compounds of `Granny Smith´ apple peel

The principal industrial use of apple peels was for pectin production. As part of the conventional process for obtaining pectin, the peel must be dried to avoid enzymatic alterations (Constenla et al., 2002). However, this process not only affected the pectin quality but also affected the polyphenol content. Fig. 2 shows the TPC_{HPLC} retention of the `Granny Smith´ apple peel during the conventional (TR) and ultrasound-assisted (US) pectin extraction processes (BE: before phenolic compound extraction, and AE: after phenolic compound extraction). The fresh apple peel (P), with 80.1% moisture and TPC_{HPLC} of 9.9 g/Kg dw, was dried at 50°C for 24h to obtain DP. The drying process reduced the TPC_{HPLC}

by 66% (3.4 g/Kg dw) (Fig. 2). The phenolic compound extraction from residual tissue of BE pectin US-extraction ($R_{BE\text{-}US}$) allowed the recovery of the 7.12% of TPC_{HPLC} initially present on de fresh apple peel and the 6.15% of TPC_{HPLC} from the residual tissue of BE pectin TR-extraction ($R_{BE\text{-}TR}$), losing a large number of valuables phenolic compounds. Otherwise, in the AE sequential extraction process, the larger quantity of phenolic compounds was obtained from P (9.9 g/Kg dw), and the remanent TPC_{HPLC} on $R_{AE\text{-}US}$ was 0.04 g/Kg dw (0.36%); and on the $R_{AE\text{-}TR}$ was 0.15 g/Kg dw (1.49%). These results showed that the best TPC_{HPLC} recovery yields corresponded to the AE process (Fig. 2).

Fig. 3 shows the phenolic compound content changes during the sequential extraction of phenolic compounds and pectin. During the drying process (DP, Fig. 3), procyanidins were the most affected phenolic compound. The drying process produced losses of up to 94% of PACB2 and 96% of PACT. Similarly, Heras-Ramírez et al. (2012) reported that drying apple pomace at 60°C significantly reduced the content of phenolic compounds (mainly chlorogenic acid and (-) epicatechin). In contrast, the flavonols (Q3G, QP, QHS, K3G) were the least affected among phenolic compounds, even in the DP extracts. QHS concentration increased up to 50% compared to the P extract, becoming the main compound of the DP extract. Probably, the drying process stabilised these glycosides, allowing a better recovery in the extraction. Schieber et al. (2003) reported that drying apple pomace in a three-step drum dryer increased the recovery of flavonols by about 6% but negatively affected the procyanidins by up to 22%. This phenomenon could occur by external factors, like high temperature, although some authors reported that most phenolic compounds were stable up to 150-200°C (Huaman-Castilla et al., 2019). Nevertheless, the polyphenol thermal degradation followed a first-order kinetic, and some compounds, such as kaempferol, could be unstable at temperatures below 50°C (Setyaningsih et al., 2016). Henríquez et al. (2014) studied the thermal degradation of phenolic compounds from `Granny Smith´ apple peel

452 during drum-drying and reported a loss of 27% of phenolic compounds at 110°C for 250 s.
453 Moreover, internal factors can participate in the phenolic degradation through anaerobic
454 pathways like the benzoyl-CoA pathway, the resorcinol pathway, and the phloroglucinol
455 pathway, promoted by low drying temperatures and prolonged drying time (Schink et al.,
456 2000). Otherwise, the conventional BE-TR pectin extraction process allowed a higher
457 recovery of PACT and FLN (0.246 and 0.05 g/kg, respectively) from the residual RBE-TR
458 tissue than the one recovered in DP (0.058 and 0.02 g/Kg) (Fig. 3). In plant tissue, the
459 metabolic route of shikimic acid produced the deamination of phenylalanine by the enzyme
460 Phenylalanine ammonium lyase (PAL, EC 4.3.1.5), generating cinnamic and coumaric acids.
461 Later, chalcones and hydrochalcones, such as phloretin FLN present in `Granny Smith´ apple
462 peel, were obtained by malonyl CoA and the chalcone synthase (EC 2. 3.1.74). From
463 chalcones and hydrochalcones, condensed tannins (such as epicatechin homo-oligomers like
464 procyanidins, PACT), could be synthesised by hydroxylases and isomerases (Rue et al.,
465 2018). These phenolic compounds could be covalently linked to cell wall structural
466 components in the food matrix. The pectin extraction process with citric acid (pH 2) could
467 extract these insoluble-bound polyphenols, increasing the concentration of these phenolic
468 compounds in RBE-TR compared with DP (Azwanida, 2015; Mariadoss et al., 2019).
469 Regarding the pectin extraction in the BE process, the use of ultrasound-assisted extraction
470 (US) showed a higher yield of pectin (5.35%), 22% higher than the extraction yield obtained
471 without the ultrasound process (TR, 4.17%, $p<0.05$) (Table 3). However, these results are
472 similar to those reported for pectin extraction from `Granny Smith' peel (4.2%) with nitric
473 acid pH 2.5 and with citric acid at 80°C (5.25%) (Constenla et al., 2002; Kumar et al., 2020).
474 The ultrasonic frequencies generated micro-jets that moved with the acoustic flow and then
475 cycles of contraction-expansion in the citric acid solution. This cavitation produced a
476 swelling of the plant material that absorbed more of the extraction solution, facilitating the

hydrolysis of the cell walls, thus improving extraction yields (Maran et al., 2017; Minjares-Fuentes et al., 2014).

The pectin extraction after the extraction of the phenolic compounds (AE) increased ($p<0.05$) the pectin yields, when compared with BE process, for both studied pectin extraction methodologies, US (6.38%) and TR (4.92%). Due to the previous two-step ultrasound-assisted extraction of phenolic compounds with 80% acetone, the above mention swelling occurred and subsequently facilitated the hydrolysis of the glycosidic bonds of the middle lamella of the cell wall by the citric acid solution (pH 2) and the ultrasound cavitation (Bhatia et al., 2016; Dranca & Oroian, 2018). These values were higher than the pectin yields obtained with other technologies like microwave-assisted extraction. Yeoh et al. (2008) reported a 5.2% pectin yield from orange peel using microwave-assisted extraction. The characteristics of the extracted pectin were related to the different extraction conditions and raw materials used (Bhatia et al., 2016). Commonly, pectin was classified according to its degree of esterification (DE). If DE > 50%, more than 50% of the carboxyl groups of polygalacturonic acid were methylated, and the pectins were called high-methoxyl pectins (HMP). If DE < 50%, pectins were called low-methoxyl pectins (LMP) (Bhatia et al., 2016; Güzel & Akpınar, 2019). The degree of pectin esterification (DE) obtained herein by the different processes varied between 52 and 58% (high-methoxyl pectin) (Table 3). These degrees of esterification were higher than those reported by Gazala et al. (2017) for pectin from concentrated apple juice (49% DE). The use of different sequential extraction processes did not significantly affect the molecular characteristics of the pectin obtained (Table 3). The methoxyl content of the pectin obtained in this study (9.30-10.7%, Table 3) was higher than the reported for pectin from other sources, such as cocoa hulls (using 5% citric acid for pectin extraction) (Sarah et al., 2018). Nevertheless, the equivalent weight values determined for pectin extracted herein were lower (436-462) than the reported for commercial pectin

(1666.67) (Kumar & Chauhan, 2010). Probably, the lower pH values in the citric acid solution degraded pectin. Minjares-Fuentes et al. (2014) reported that the use of acidic solutions (pH<2.5) could lead to partial degradation of the homogalacturonan chains of pectin. These results suggested that the obtained pectin could form gels through hydrogen bonds and hydrophobic interactions at pH < 3.5 and sugar content greater than 55%. The low equivalent weight of pectin allowed to generate these interactions in less time, but its stability could be lower than that of commercial pectin. Further research is needed to determine the appropriate conditions for implementing sustainable alternative technologies for obtaining phenolic compounds and pectin with better functional properties through the extraction process AE-US.

4. Conclusions

The highest total phenolic compound content was extracted from fresh apple peel with acetone (80%) in two-step extraction. Flavan-3-ols were the majority class of phenolic compounds determined in the apple peel extracts representing 59% of total phenolic compounds. The procyanidin B2 was the main phenolic compound extracted and significantly correlated with the antioxidant capacity of DPPH (R^2 0.86) and FRAP (R^2 0.88). The procyanidins were the compounds more affected by the drying process, with reductions of up to 96%, thus reducing the content of total phenolic compounds in the BE pectin extraction process (before the phenolic compound extraction).

The ultrasound-assisted extraction improves the pectin yields significantly (22%). The pectin obtained after the phenolic compound extraction (AE pectin extraction process, both ultrasound-assisted) increased pectin yield from 5.35 to 6.38 % and TPC yield from 1.37 to 11.92 g AGE/Kg dw for the BE and AE pectin extraction processes, respectively.

526 The valorisation of the wasted apple peels could be possible through the sequential
527 extraction of phenolic compounds and pectin, using alternative technologies such as
528 ultrasound. The sequential extraction of these compounds could help the conversion of
529 agribusiness from a linear economy to a circular economy by reducing and employing the
530 fruit waste by-products currently going to landfills.
531
532
533 **Author statement**
534 Esteban Villamil-Galindo: Conceptualization, Data curation, Validation, Investigation,
535 Writing - original draft, Formal analysis.
536 Andrea Marcela Piagentini: Conceptualization, Methodology, Writing - Reviewing and
537 Editing, Supervision, Project administration, Funding acquisition.
538
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540 The authors confirm that they have no conflicts of interest with respect to the work described
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548
549 **Appendix A. Supplementary data**
550 The following is the Supplementary data to this article:

551

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Figure Captions

Fig. 1. Flow-sheet for phenolic compounds and pectin extraction processes from `Granny Smith´ apple peel

BE: Pectin extraction process before obtaining phenolic compounds; **AE:** Pectin extraction process after obtaining phenolic compounds; **P:** Fresh peel; **DP:** Dried Peel; **DRT**: Dried Residual Tissue; **TR:** conventional pectin extraction; **US:** ultrasound-assisted pectin extraction; **$R_{BE\text{-}US}$:** residual tissue of BE pectin US-extraction; **$R_{BE\text{-}TR}$:** residual tissue of BE pectin TR-extraction; **$R_{AE\text{-}US}$:** residual tissue of AE pectin US-extraction; and **$R_{AE\text{-}TR}$:** residual tissue of AE pectin TR-extraction; **BE_{RUS}:** Phenolic compounds obtained from $R_{BE\text{-}US}$; **BE_{RTR}:** Phenolic compounds obtained from $R_{BE\text{-}TR}$; **AE_{RUS}:** Phenolic compounds obtained from $R_{AE\text{-}US}$; **$R_{AE\text{-}US}$:** Phenolic compounds obtained from $R_{AE\text{-}TR}$.

Fig. 2: Total Phenolic Content (TPC) during de different steps of pectin extraction processes from `Granny Smith´ apple peel.

P: Fresh peel; DP: Dried Peel; $R_{BE\text{-}US}$: residual tissue of BE pectin US-extraction; $R_{BE\text{-}TR}$: residual tissue of BE pectin TR-extraction; $R_{AE\text{-}US}$: residual tissue of AE pectin US-extraction; and $R_{AE\text{-}TR}$: residual tissue of AE pectin TR-extraction; BE: Pectin extraction process before obtaining phenolic compounds; AE: Pectin extraction process after obtaining phenolic compounds; TR: conventional pectin extraction; US: ultrasound-assisted pectin extraction.

Fig. 3: Phenolic compound concentrations (C_{PC}) for the different steps of pectin extraction from `Granny Smith´ apple peel.

(+)CTQN: Catechin, **PACB2**: Procyanidin B2, **(-)EPQN**: Epicathechin, **PACT**: Procyanidin tetramer, **ACL:** Chlorogenic acid, **Q3G**: Quercetin-3-*O*-glucuronide, **QP:** Quercetin penstoside, **QHS:** Quercetin Hexoside, **K3G**: Kaempferol-3-*O*-glucuronide, **FLN:** Phloretin P: Fresh peel; DP: Dried Peel; $R_{BE\text{-}US}$: residual tissue of BE pectin US-extraction; $R_{BE\text{-}TR}$: residual tissue of BE pectin TR-extraction; $R_{AE\text{-}US}$: residual tissue of AE pectin US-extraction; and $R_{AE\text{-}TR}$: residual tissue of AE

764 pectin TR-extraction; BE: Pectin extraction process before obtaining phenolic compounds; AE:
765 Pectin extraction process after obtaining phenolic compounds; TR: conventional pectin extraction;
766 US: ultrasound-assisted pectin extraction.

Table 1. Total phenolic compounds (TPC), Total flavonoid content (TF), and Antioxidant capacity by DPPH* and FRAP of different extracts of `Granny Smith´ apple peel

S	ES	TPC (g GAE/Kg)		TF (g quercetin/Kg)		DPPH (mmol Trolox/Kg)		FRAP (mmol Fe^{2+}/Kg)	
		C_{FA} (%)		C_{FA} (%)		C_{FA} (%)		C_{FA} (%)	
		0	0.5	0	0.5	0	0.5	0	0.5
W	1	0.78 ± 0.01 eA	0.78 ± 0.01 fA	0.11 ± 0.05 cA	0.12 ± 0.006 dA	1.20 ± 0.40 eB	2.30 ± 0.10 eA	4.40 ±0.09 dA	6.30 ±0.04 dB
	2	1.25 ± 0.08 dA	1.60 ± 0.004 dB	0.23 ± 0.01 bA	0.22 ± 0.01 cA	4.90 ± 0.50 dA	4.910 ± 0.10 dA	5.60 ±0.20 cA	6.10 ±0.50 dA
EtOH	1	1.39 ± 0.01 dA	1.40 ± 0.02 eA	0.28 ± 0.01 bA	0.20 ± 0.001 cB	5.30 ± 0.10 dA	2.90 ± 0.60 eB	5.30 ±1,00 cdA	5.60 ±0.50 dA
	2	2.09 ± 0.01 bcB	2.27 ± 0.01 cA	0.33 ± 0.01 bA	0.38 ± 0.01 aA	7.20 ± 0.80 cA	6.80 ± 0.30 cA	7.60 ±0.50 bA	8.10 ±0.07 cA
MeOH	1	1.39 ± 0.07 dB	1.64 ± 0.06 dA	0.22 ± 0.001 bB	0.28 ± 0.002 bA	1.00 ± 1.10 eA	1.00 ±0.01 fA	5.50 ±0.08 cdB	8.50 ±0.30 cA
	2	2.19 ± 0.10 bB	3.00 ± 0.02 bA	0.43 ± 0.001 aA	0.37 ± 0.002 aB	6.30 ±0.20 dB	13.10 ± 1.00 bA	7.30 ±0.02 bB	10.80 ±0.10 bA
Ac	1	1.94 ± 0.07 cB	2.23 ± 0.01 cA	0.27 ± 0.002 bA	0.28 ± 0.01 bA	17.60 ±0.40 aB	12.08 ±0.90 bA	7.70 ±0.40 bB	11.20 ±0.50 bA
	2	3.47 ± 0.06 aA	3.14 ± 0.10 aB	0.45 ± 0.06 aB	0.21 ± 0.01 cA	16.10 ±1.00 bA	14.79 ± 2.00 aB	11.0 ±0.30 aB	13.40 ±0.04 aA

S: solvent, C_{FA}: formic acid concentration, ES: extraction steps. Mean (n=3). W: water 100%, EtOH: ethanol 80%, MeOH: methanol 80% Ac: acetone 80%. Different capital letters and lowercase letters indicate significant differences ($p < 0.05$) by Tukey's test, between formic acid concentration, and among extraction systems, respectively.

Table 2. Content of the principal phenolic compounds from different `Granny Smith´ apple peel extracts

S	ES	PACB2 (g/Kg)		PACT (g/Kg)		(-)EPQN (g/Kg)		Q3G(g/Kg)		TPC_{HPLC}(g/Kg)	
		C_{FA} (%)		C_{FA} (%)		C_{FA} (%)		C_{FA} (%)		C_{FA} (%)	
		0	0.5	0	0.5	0	0.5	0	0.5	0	0.5
W	1	0.034±0.031 abA	0.057 ±0.040 cA	0.010± 0.009 dA	0.079±0.070 cdA	0.005 ±0.008 eA	0.014 ±0.006 eA	0.010± 0.002 cA	0.048 ±0.002 dB	0.092±0.060 cA	0.268±0.040 deA
	2	0.010 ±0.001 bA	0.029 ±0.009 cA	0.002±0.000 dA	0.004±0.000 dB	0.006±0.000 eA	0.016±0.004 deA	0.001 ± 0.001 cA	0.018 ±0.008 dA	0.05±0.001 cA	0.106 ±0.040 eA
EtOH	1	0.192 ±0.002 abA	0.197±0.015 bcA	0.07 ±0.014cdA	0.070±0.030 cdA	0.076±0.005 dA	0.090±0.002 cdA	0.143 ± 0.020 bA	0.154±0.006 cA	0.680 ±0.007 bcA	0.753 ±0.060 cdA
	2	0.424 ±0.180 abA	0.304 ±0.023 abcA	0.172 ±0.025 bA	0.150±0.030abcdA	0.136 ±0.008 bA	0.127 ±0.007 abcA	0.193 ± 0.020 bA	0.184 ±0.028 cA	1.221±0.260 abA	0.965±0.130 bcA
MeOH	1	0.298 ±0.042 abA	0.374 ±0.080 abA	0.120±0.050 bcA	0.140 ±0.040bcdA	0.100 ±0.010 cdA	0.122 ±0.060 bcA	0.159 ± 0.026 bA	0.212 ±0.020 bcA	0.960±0.040 bA	1.160 ±0.090 bcA
	2	0.734 ±0.382 abA	0.596 ±0.080 aA	0.270±0.009 aA	0.307 ±0.050 aA	0.177 ±0.008 aA	0.196 ±0.040 aA	0.332 ± 0.040 aA	0.276 ±0.030abA	1.903 ± 0.430 aA	1.756 ±0.145 aA
Ac	1	0.299 ±0.060 abA	0.397±0.136 abA	0.160 ±0.010 bcA	0.190 ±0.002 abcA	0.119 ±0.003 bcA	0.148 ±0.020 abcA	0.188 ±0.013 bA	0.215 ±0.033 bcA	1.035 ±0.100 bA	1.288 ±0.220 abA
	2	0.772 ±0.319 aA	0.528 ±0.010 aA	0.280 ±0.006 aA	0.251 ±0.044 abA	0.199 ±0.010 aA	0.199 ±0.036 abA	0.310±0.020 aA	0.340 ±0.024 aA	1.988 ± 0.242 aA	1.737 ±0.181 aA

S: solvent, C_{FA}: formic acid concentration, ES: extraction steps, PACB2: Procyanidin B2, (-)EPQN: Epicatechin, PACT: Procyanidin tetramer, Q3G: Quercetin-3-glucuronide, TPC_{HPLC}: Total phenolic compounds by HPLC. Mean (n=3). Different capital letters and lowercase letters indicate significant differences ($p< 0.05$) by Tukey's test, between formic acid concentration, and among extraction systems, respectively.

Table 3: Characterization of apple peel pectin obtained by ultrasound-assisted (US) and conventional (TR) extraction processes

Parameters	***Before TPC extraction (BE)***		***After TPC extraction (AE)***	
	US	**TR**	**US**	**TR**
Yield (%)	5,35 ± 0,2 abA	4,17 ± 0,3 cB	6,38 ± 0,2 aA	4,92 ± 0,3 bcB
DE (%)	57,64 ± 4aA	57,84 ± 2aA	60,01 ± 2aA	58,15 ± 1aA
Methoxyl content (%)	9,30 ± 0 aA	10,23 ± 0,9 aA	10,70 ± 0,2 aA	10,39 ± 0,2 aA
Equivalent weight	436,23 ± 39,1 aA	437,22 ± 22,4 aA	461,69 ± 23,4 aA	440,1 ± 35,6 aA

DE: Degree of esterification, TPC: total phenolic compounds. Different capital letters and lowercase letters indicate significant differences ($p < 0.05$) by Tukey's test, among extraction systems, and between US and TR extraction methods, respectively.

(80% moisture)

(P)

BE

AE

Drying 50° 24h
(DP)

Pectin extraction
(BE)

Ultrasound assisted pectin extraction
(US)

PECTIN (BE-US)

Residual tissue
(R_{BE-US})

PHENOLIC COMPOUNDS EXTRACTION (BE_{RUS})

Conventional pectin extraction
(TR)

PECTIN (BE-TR)

Residual tissue
(R_{BE-TR})

PHENOLIC COMPOUNDS EXTRACTION (BE_{RTR})

PHENOLIC COMPOUNDS EXTRACTION

Dried Residual Tissue (50°C-24h)
(DRT)

Pectin extraction
(AE)

Ultrasound assisted pectin extraction
(US)

PECTIN (AE-US)

Residual tissue
(R_{AE-US})

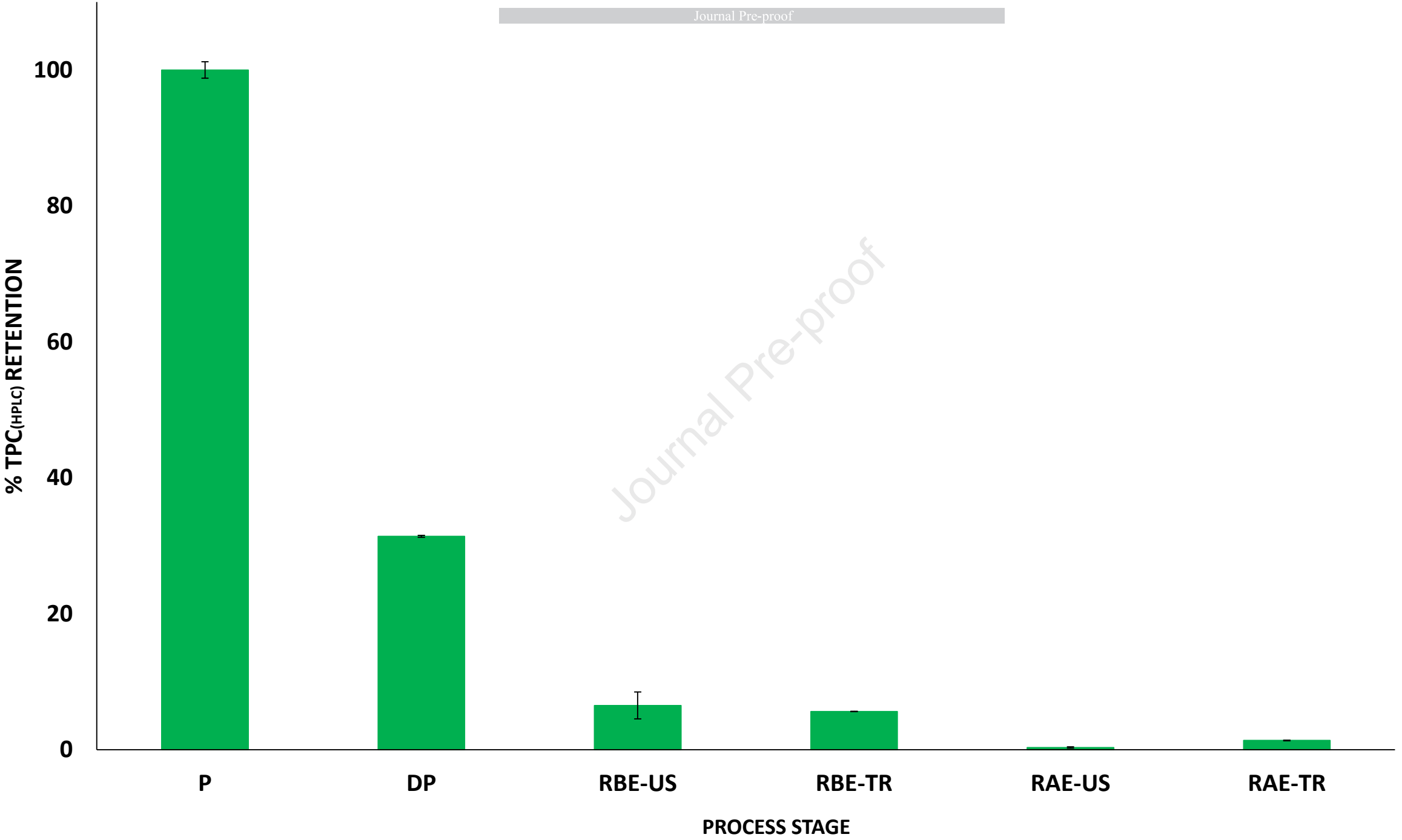
Phenolic compounds extraction
(AE_{RUS})

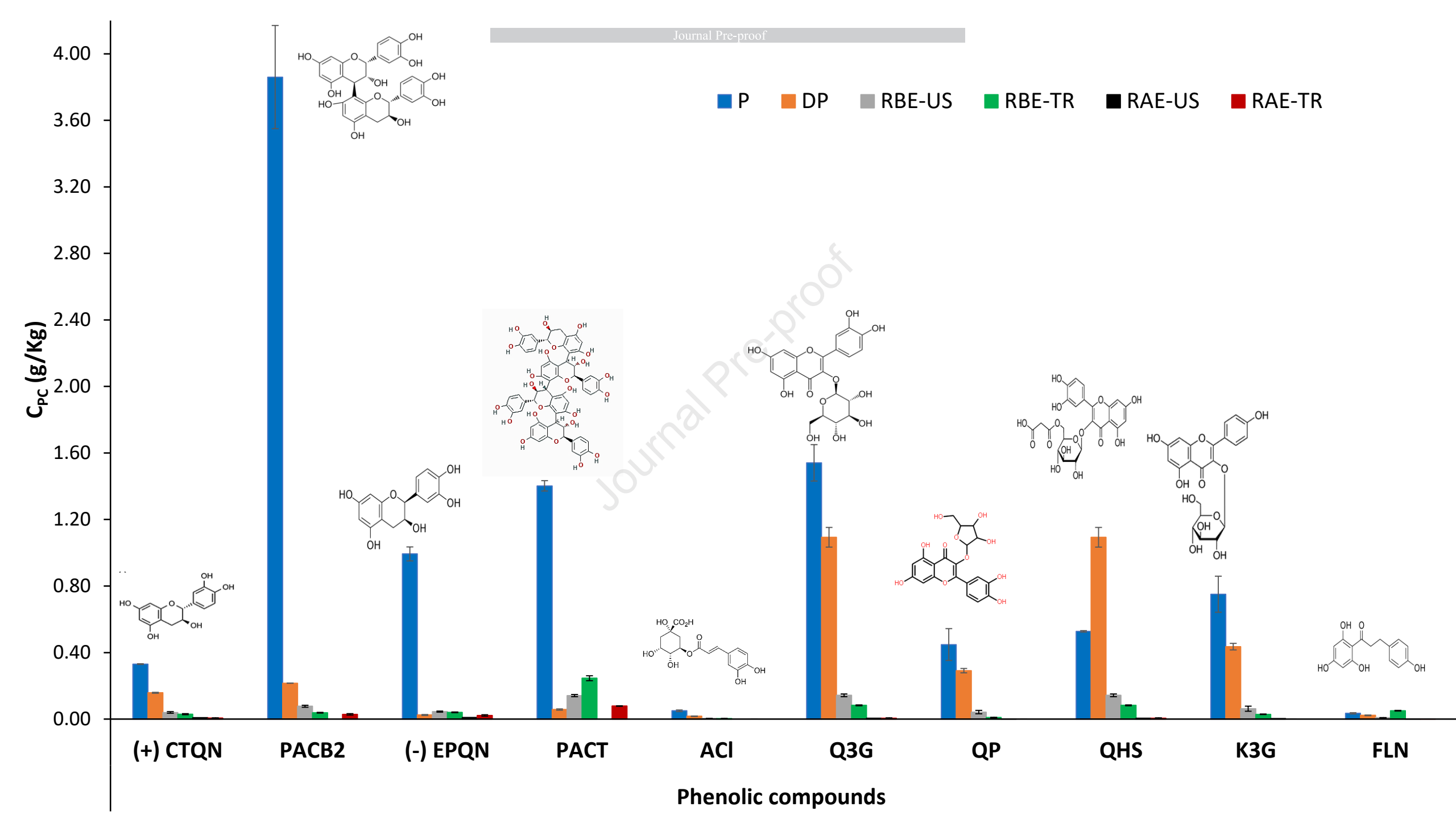
Conventional pectin extraction
(TR)

PECTIN (AE-TR)

Residual tissue
(R_{AE-TR})

Phenolic compounds extraction
(AE_{RTR})





Highlights

- Apple peel waste is a great low-cost source of phenolic compounds and pectin
- Ultrasound-assisted extraction enhances the phenolic compounds and pectin recovery
- Extraction with Acetone 80% in two-step produces the highest phenolic compound yield
- Procyanidin B2 is the main phenolic compound extracted from Granny Smith apple peel
- Phenolic compounds extraction followed by pectin extraction provided the best yield

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.