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Phenolic Compounds Extraction of *Arbutus unedo* L.: Process Intensification by Microwave Pretreatment

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Abstract: *Arbutus unedo* L., commonly known as the strawberry-tree fruit, is an endemic species of the Mediterranean flora. Microwave extraction technology has been considered as a fast and "green" method for the production of extracts rich in bioactive compounds, although the energy consumption is high. To overcome this bottleneck, microwave was used as a pretreatment procedure in short time periods. This technique promotes the burst of intracellular vacuoles leading to an increase in the lixiviation of phenolic compounds. Different approaches were tested, namely a solvent-free irradiation (SFI), a solvent-assisted irradiation (SAI) and a pressurized solvent-assisted irradiation (PSAI). After irradiation, a solid–liquid extraction procedure was performed using a mixture of water and ethanol. A kinetic evaluation of the total phenolic content (TPC) was performed using the Folin–Ciocalteu method. For the total anthocyanin content, a UV-spectrophotometric method was used. HPLC-UV and LC-MS were used for TPC and identification of present compounds. Microwave irradiation led to an increase in TPC of extracts after SAI (52%) and PSAI (66%) along with a reduction in time of extraction from 30 min to less than 2 min. The anthocyanin content also increased by 66% for the SAI and PSAI extractions.

Keywords: *Arbutus unedo*; microwave pretreatment; process intensification; phenolic compounds

1. Introduction

The Mediterranean berry *Arbutus unedo*, known as strawberry-tree fruit [1,2], is produced in different zones of Portugal. *A. unedo* fruits are spherical berries with a rough surface that vary in color from yellow to red when fully ripe. Due to their perishable character, the fresh fruits are difficult to commercialize and are mostly used to prepare jams and marmalades or a characteristic distillate beverage [3–6]. Several authors have investigated the phytochemical composition of the strawberry-tree fruits in terms of phenolic composition, thus depicting its content in flavonoids as anthocyanins, non-flavonoids, such as gallic and quinic acid and their derivatives, and also other bioactive components such as carotenoids and triacylglycerols [2,7–10]. The strawberry-tree fruit not adequate for fresh consumption, or the residues obtained from the distillation process used in the preparation of the traditional beverage, can be used as a source of bioactive molecules [11,12], namely phenolic compounds.

Phenolic compounds are secondary metabolites produced by plants for their protection against environmental stress such as herbivores, UV radiation and/or severe weather conditions [13,14].

Their presence in fruits, vegetables or related products [15,16] is important in the human diet as they protect against free radicals that are known to be responsible for heart and neurodegenerative diseases, such as Alzheimer or Parkinson's diseases, and also certain types cancer. These natural bioactive compounds can be seen as a complement to traditional therapeutics acting in the prevention of diseases.

Different extraction procedures have been developed in order to recover these compounds from natural matrices [13,17–22]. From conventional solid–liquid extraction like maceration, Soxhlet extraction or reflux extraction to Clevenger extraction, all of these methods present disadvantages. There is a need to introduce processes that are more environmentally acceptable, more efficient and less time and resource consuming [15,21,23]. Amongst recent extraction procedures, the use of supercritical fluid technology (SFE), pressurized liquid extraction (PLE) [24,25], ultrasound-assisted extraction (UAE) [26] and microwave-assisted extraction (MAE) [13,27] have gained attention of researchers, as they improve extraction efficiency and effectiveness and, at the same time, reduce time of extraction and solvent consumption, thus being in accordance with the principles of green chemistry [28].

MAE is a well-known and implemented methodology, mainly due to its low cost, availability, eco-friendliness and reduced time of operation, as well as low solvent consumption [29]. The use of microwave energy for process intensification was first reported by Ganzler et al. [30]; since then, many other authors have used it for the recovery of phenolic compounds from a wide variety of natural sources such as tea, grape marc, blackberries and medicinal plants [13,17–20]. Briefly, microwaves are nonionizing electromagnetic waves with frequency between 300 MHz and 300 GHz that are normally used to heat materials [27]. Heating occurs in a selective way throughout all the sample, leading to a structural damage of the matrix, at the same time that heat and mass transfer occur in the same direction, thus enhancing extraction [31]. Microwave accelerates energy transfer and reduces thermal gradient and so that thermolabile compounds can be preserved [32].

In this work, microwave irradiation was used to promote the extraction of phenolic compounds form *A. unedo*. Despite some authors having already discussed the use of this technique to intensify their recovery [33,34], our work intended to evaluate the effect of microwave as a pretreatment to increase extraction process from strawberry-tree fruits. Different approaches, namely solvent-free irradiation (SFI), solvent-assisted irradiation (SAI) and pressurized solvent-assisted irradiation (PSAI) were tested as pretreatments before performing a conventional solid–liquid extraction, using a water:ethanol mixture as extracting solvent. The phenolic content improvement associated with the implementation of these pretreatments was assessed in terms of yield and time of extraction, as well as phenolic content evaluated using different methodologies.

2. Materials and Methods

2.1. Plant Material

A. unedo fruits were collected in October 2016 from Serra da Gardunha, Portugal (40°07′15.17″ N, 7°27′58.66″ W) at approximately 625 m altitude.

The strawberry-tree fruits were frozen immediately at −20 °C and kept at this temperature until the experiments were performed. Samples were defrosted at 4 °C overnight before experiments.

Moisture Content Determination

Total water content of fruits was determined by drying the fruits in an oven (GALLENKAMP, model OHF050.XX1.5, Loughborough, UK) at 105 °C, until constant weight was reached. The total water content was found to be 75% (*w*/*w*).

2.2. Chemicals

Solvents used for extractions were absolute ethanol (99.9% Carlo Erba Reagents, Val de Reuil, France) and bidistilled water (Milli-Q Integral, Burlington, MA, USA). Chemicals used in the determination of total phenolic content were sodium carbonate (Sigma-Aldrich, St. Quentin

Fallavier, France), Folin–Ciocalteu reagent (Panreac, Barcelona, Spain) and gallic acid (Fluka, Steinheim, Germany). To determine total anthocyanin content, potassium chloride (Riedel-de Haën, Seelze France), sodium acetate trihydrate (≥99.0%, Sigma-Aldrich, France) and hydrochloric acid (≥37%, puriss. p.a., Riedel-de Haën, France) were used to prepare the buffer solutions in bidistilled water. Chromatographic analyses were performed using acetonitrile (99.9%, Sigma-Aldrich, Steinheim Germany), ultrapure water purified with a Milli-Q water purification system (Merck Millipore, Burlington, MA, USA) and formic acid (99–100%, VWR-CHEM, Llinars del Vallés, Spain).

2.3. Extraction Procedures

2.3.1. Conventional Solid–Liquid Extractions

Solid–liquid (S-L) extractions were performed as previously described [9]. Briefly, a water:ethanol 50:50 (*v*/*v*) solution was used as a solvent to produce a phenolic-rich extract from strawberry-tree fruits. The solid–liquid ratio used was 1:20, and a comparison was done between the extractions performed with and without microwave irradiation. As the raw material used was fresh and the amount of water for the fruits was found to be 75% (*w*/*w*), the solid:liquid ratio was reduced to 1:5, which corresponds to 1:20 if using dried fruits. Extractions were done for 30 min at room temperature for all the extraction procedures studied. Samples were collected at different time points, namely at 1, 2, 3, 4, 5, 7.5, 10, 15, 20 and 30 min. Extracts were then centrifuged for 10 min at 6000 rpm, filtered through a 0.22 µm pore filter and stored at −20 °C before analysis.

2.3.2. Microwave Pretreatment

The microwave pretreatment was done using a commercial monomode oven working at a frequency of 2.45 GHz, CEM Discover One microwave (CEM Corp., Matthews, NC, USA). Three pretreatments were used, before a conventional solid–liquid extraction was performed: a solvent-free irradiation (SFI), where only the fruit was irradiated until the temperature of 100 °C, without the presence of any added solvent; solvent-assisted irradiation (SAI), where solvent water:ethanol 50:50 (*v*/*v*) was added to the fruit before irradiation until a temperature of 80 °C was reached; and finally a pressurized solvent-assisted irradiation (PSAI), where the fruit and solvent were placed inside a closed vessel and irradiated until a pressure of 3 bar was achieved.

For the SAI and PSAI pretreatments, 5 g of strawberry-tree fruit was mixed with 25 mL of the solvent. In all pretreatments, after being homogenized, the mixture or the fruit was irradiated with maximum power (300 W). According to Sólyom et al. [35] the power level of the radiation does not have a significant effect on phenolic extraction yield. Temperature during SFI and SAI was continuously recorded with an optic fiber thermometer (FoTEMP 4, OPTPcon GmbH, Dresden, Germany), calibrated in an ice-water bath. For the PSAI, a glass pressure vessel (QianCap, QLabtech, East Lyme, CT, USA) was used to maintain the solvent in a liquid phase under pressure. When irradiation was over, the vessel used for each pretreatment was cooled rapidly down to room temperature under tap water. A conventional solid–liquid extraction in a water bath at 20 °C was than performed.

The total amount of irradiated energy absorbed by the sample was determined as described by Sólyom et al. [35], where heat losses to the environment were dismissed, due to their low contribution to the final value.

2.4. Extract Characterization

2.4.1. Total Mass Yield

Samples, 500 µL from each extract, were evaporated until dryness using a vacuum centrifuge (Centrivap concentrator, Labconco, Kansas City, MO, USA) with a MD 4C NT vacuum pump (Vacuubrand, Wertheim, Germany). Results were expressed in mg/g d.w. Assays were done in triplicate.

2.4.2. Total Phenolic Content (TPC)

Total phenolic content was measured by Folin–Ciocalteu method [36]. Briefly, 40 µL of diluted sample was mixed with 3000 µL of distillate water Milli-Q Integral, Burlington, MA, USA) and 200 µL of Folin–Ciocalteu reagent (Panreac, Barcelona, Spain). After 5 min shaking, 600 µL of 20% sodium carbonate (Sigma-Aldrich, St. Quentin Fallavier, France), was added. Samples were left 30 min at 40 °C. Then, absorbance was measured at 765 nm (Shimadzu UV/vis Spectrophotometer, Kyoto, Japan). Results were expressed in gallic acid equivalents (mg GAE/g d.w.). Assays were done in triplicate.

2.4.3. Anthocyanin Content

Anthocyanin content was determined by the pH differential method [37]. Samples were diluted in two buffers (potassium chloride (Riedel-de Haën, Seelze, France) 0.025 M, pH 1 and sodium acetate (≥99.0%, Sigma-Aldrich, Seelze, France) 0.4 M, pH 4.5. Absorbance was calculated as the increment in absorbance at 520 min the increment at 700 nm between the two buffers. An extinction coefficient of 26 900 µL/mol/cm was used. Results were expressed as cyanidin-glucoside equivalents (mgCGE/g d.w.). Assays were done in triplicate.

2.5. Analysis of Phytochemical Composition of Extracts by HPLC-DAD

All extracts obtained were analyzed [9] using a high-performance liquid chromatography (HPLC) system (Thermo Finnigan Surveyor model, San Jose, CA, USA) equipped with an autosampler, pump and photodiode-array detector (PDA) (Thermo Finnigan, San Jose, CA, USA). Chromatographic separation of compounds was carried out on a Lichrocart RP-18 column (250 × 4 mm, 5 µm, Merck, Darmstadt, Germany) and a Manu-cart RP-18 pre-column (Merck, Darmstadt, Germany) at 35 °C. Photodiode-array detector performed scans between 200 and 798 nm at a speed of 1 Hz with a bandwidth of 5 nm. Three individual channels, 280, 360 and 520 nm, were used for monitoring phenolic content, flavonoids and anthocyanin content at a speed of 10 Hz with a bandwidth of 11 nm. The injection volume was 20 µL. The autosampler temperature was set at 12 °C. The eluents used were: (A) formic acid solution (0.5%, *v*/*v*) (99–100%, VWR-CHEM, Llinars del Vallés, Spain) in Milli-Q water; and (B) 5 mL formic acid + 900 mL acetonitrile (99.9%, Sigma-Aldrich, Steinheim Germany) HPLC gradient grade + 95 mL Milli-Q H_2O (0.5:90:9.5), at a flow rate of 0.3 mL min^{-1}. The gradient conditions applied consisted of 100% A at time 0 min and 94.4% A at 15 min, 83.3% A at 20 min, 77.8% A at 55 min, 66.7% A at 80 min, 44.4% A at 120 min. After, column was washed for 20 min with 100% eluent B and re-equilibrated for 10 min with 94.4% of eluent A. Chromquest software version 4.0 (Thermo Finnigan Surveyor, San Jose, CA, USA) was used to control the system for data acquisition and processing.

2.6. Analysis of Phytochemical Composition of Extracts by HPLC-MS/MS

Identification of the most important compounds present in the strawberry-tree extracts was done by analyzing a representative extract by HPLC-MS/MS, as described by the authors in a previous work [9]. The HPLC–MS/MS equipment consisted of a Waters Alliance HPLC system (Waters, 2695 separation module, Dublin, Ireland) comprising a quaternary pump, an on-line solvent degasser, autosampler and column oven. A reversed-phase column LiChrospher 100 RP-18 (250 × 4.0 mm, 5 µm) (Merck, Darmstadt, Germany) was used for the separation of compounds at 35 °C using an injection volume of 20 µL. The mobile phase consisted of A (0.5% formic acid) and B (acetonitrile). Flow rate was 0.3 mL min^{-1}, and the gradient conditions applied consisted of 100% A at time 0 min until 99% A and 1% B at 15 min, 85% A at 20 min, 80% A at 55 min, 70% A at 80 min, 50% A at 120 min, 10% A at 140 min. The column was washed for 15 min with 99% B and was re-equilibrated with 100% A for 10 min. Tandem mass spectrometry (MS/MS) detection was performed on a Micromass Quattro Micro triple quadrupole (Waters, Wexford, Ireland) using an electrospray ionization (ESI−/ESI+) source operating at 120 °C and desolvation temperature of 350 °C. Capillary voltage applied was of 3.0 kV, with a cone voltage of 30.0 V. Extractor voltage was 3.0 V, and RF Lens voltage was 1.0 V. Cone gas

flow was 50 L/h and desolvation gas flow was 750 L/h MassLynx software (4.1, Waters, Milford, MA, USA, 2005) was used to control the system for data acquisition and processing.

2.7. Data Treatment

All the experimental data obtained were expressed as means ± SD. Statistical analyses were performed by using analyses of variance (ANOVA). Differences among extracts were detected by analysis of variance with Tukey honest significant difference (HSD) multiple comparison test ($\alpha = 0.05$). Statistical significance was considered between mean values when $p < 0.05$.

3. Results

3.1. Extract Mass Yield and TPC

Optimization of the extraction procedure for phenolic compounds from strawberry-tree fruits, using conventional solid–liquid extraction, had already been performed by the authors in a previous work [9]. Results from mass yield and TPC obtained when different solvents and their mixtures, as well as different solid:liquid ratios were used were compared. In order to enhance the yield of phenolic compounds, an extracting solution consisting in a water:ethanol 50:50 (*v*/*v*) mixture and a solid–liquid ratio of 1:20 were the conditions used in the present work to prepare extracts of strawberry-tree fruit.

The different conditions tested for the microwave irradiation had already been studied by other authors [18,38] using the same equipment, although with different raw materials. Nevertheless, this information was crucial in order to choose the irradiation conditions for this study. Each pretreatment consisted of different irradiation times, namely, for SFI it was around 25 s, 40 s for SAI and around 100 s for PSAI. These times of irradiation were found after observing that the fruit temperature had risen to 100 °C for the SFI, to 80 °C when considering the SAI (the lower temperature is due to the fact that there is a mixture of ethanol and water) and finally to reach the pressure of 3 bar for the PSAI. The different absorbed energies for SFI, SAI and PSAI were found to be 0.04, 0.32 and 0.53 kJ/kg, respectively, and, as previously mentioned, were calculated according to Sólyom et al. [35].

In Figure 1, the results obtained for the total mass yield and TPC for the extracts obtained when no pretreatment or different pretreatment procedures were used, before conventional solid–liquid extractions were performed, are compared.

Results show that microwave irradiation per se (SFI) without any solvent present has no effect in the extract mass yield or TPC, when comparing to the application of only a conventional extraction ($p > 0.05$). This is probably due to the fact that no solvent beside the fruit´s water content is present, which may not be enough to promote lixiviation of compounds. However, the use of microwave irradiation in fruits immersed in solvent, either in SAI or PSAI, leads to increases in total extract yield of 13% and 19% respectively, when comparing to the conventional extraction without irradiation. Although total yield increase is not always directly related to an increase in TPC, as other compounds could be lixiviated to the solvent, in this case an increase in TPC per gram of extract was also observed. When results from TPC obtained for conventional extraction (23.65 mg GAE/g dry extract) are compared with the ones obtained after irradiation with microwaves, once again significant differences are only noticed for SAI and PSAI. In fact, SAI increases TPC content by 52%, to 36.64 mg GAE/g dry extract, compared with conventional extraction without irradiation, and when considering PSAI, this value represents an increase of 66% in TPC (39.22 mg GAE/g dry extract). Results presented show that a microwave pretreatment before solid–liquid extraction increased the amount of TPC in the final extract when the matrix was immersed in the extraction solvent.

This effect was predictable, as microwave energy acts directly towards polar solvents. Even though microwave is better absorbed by water due to its permanent dipole, ethanol also presents a high heating efficiency in relation to its dissipation factor [27]. Although water is present in the matrix (~75%), it seems that it is necessary to have a larger volume of liquid for a more efficient and rapid

lixiviation of the phenolic compounds directly to the solvent, thus increasing TPC in the final extract as well as the extract mass yield.

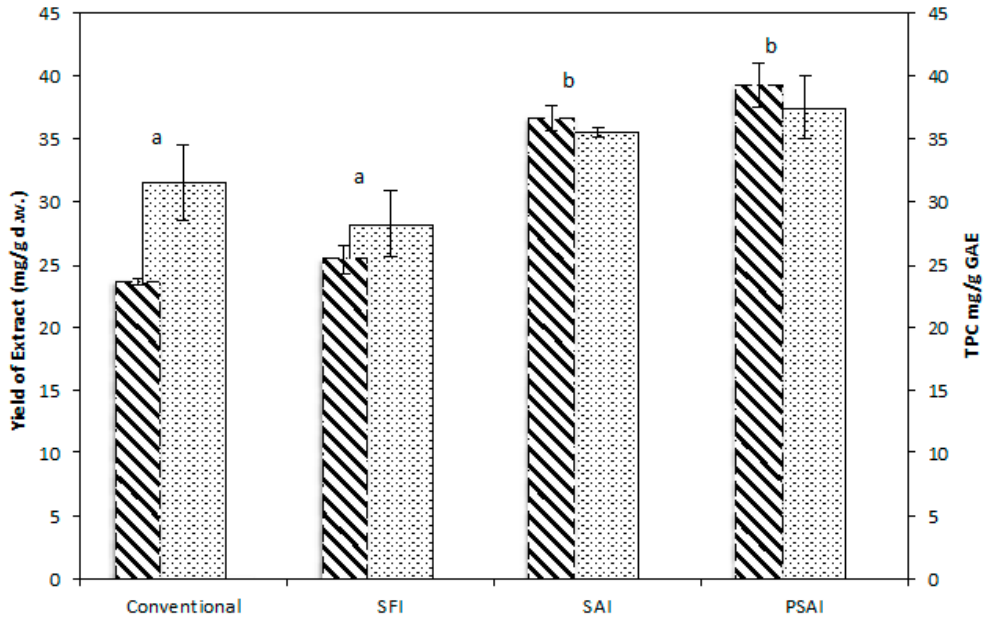

Figure 1. Comparison between total yield (mg/g d.w.) (lined bars) and total phenolic content (TPC) expressed as mg/g gallic acid equivalents (GAE) (dashed bars) obtained from a conventional extract and extracts obtained using the different pretreatments (solvent-free irradiation (SFI), solvent-assisted irradiation (SAI) and pressurized solvent-assisted irradiation (PSAI)). Lowercase letters (a,b) represent significant differences ($p < 0.05$) between yield of extract and TPC.

Kinetic Extraction of TPC

As time reduction in the extraction process is also a parameter to be considered in relation to the green chemistry principles, a kinetic study was performed while monitoring the TPC during the first 5 min and at 7.5, 10, 15, 20 and 30 min of extraction. Results obtained are presented in Figure 2.

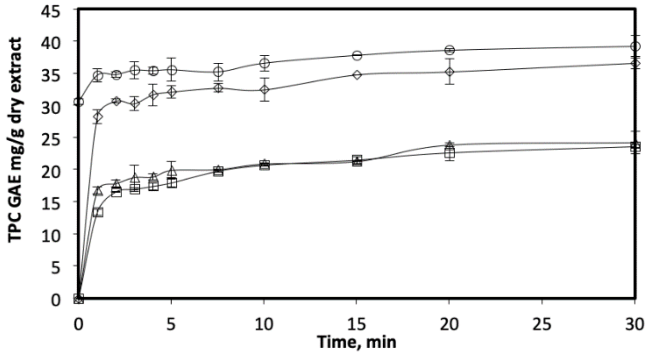

Figure 2. Total phenolic content (TPC) monitorization at different time points using different extraction processes: conventional (□), SFI (Δ), SAI (◇) and PSAI (○).

The profiles are similar in trend and show that most of TPC is extracted in the first 5 min, followed by a slow increase of the content. For the conventional process, 75% of the TPC at timepoint 30 min was obtained after 5 min of extraction. For SFI, SAI and PSAI, results were 82%, 91% and 92%, respectively. As mentioned before, few or no differences were noticed when SFI pretreatment was used against no irradiation, and we concluded that SFI does not represent a feasible alternative to the extraction process intensification.

The use of SAI allows to obtain, in only 2 min, 1.3 times the amount of TPC extracted after 30 min of conventional extraction. For PSAI, this value increases to 1.5 times. The intensification of the extraction process of phenolic compounds after the microwave pretreatments can be attested by the HPLC chromatograms presented in Figure 3, where, despite that there is not a difference in composition between extracts, it is clear that the amount of compounds present is higher when considering extractions after SAI and PSAI pretreatment. The analytical conditions used are not optimal for this type of matrix, as different compounds overlap, but the method was used as a screening method. This method is implemented at our laboratory to analyze other matrices. As there are anthocyanins present in the extracts, the broad peaks that were identified in the chromatographic profile correspond to these compounds. The total peak areas of the chromatograms were measured at 280 nm (characteristic of phenolic compounds) and 520 nm (characteristic of anthocyanins) and are presented in Table 1. There was an increase in the peak area at 280 nm characteristic of phenolic compounds. These results confirm the ones obtained by Folin–Ciocalteu method, although it is known that this colorimetric method is not accurate due to interfering compounds such as vitamin C, sugars, some amino acids and peptides that can be present in these complex natural matrices [39]. Compounds detected in Figure 3 are identified in Table 2. The identification has been done by comparison with chromatograms previously reported [9], where mass spectra for each compound are also available.

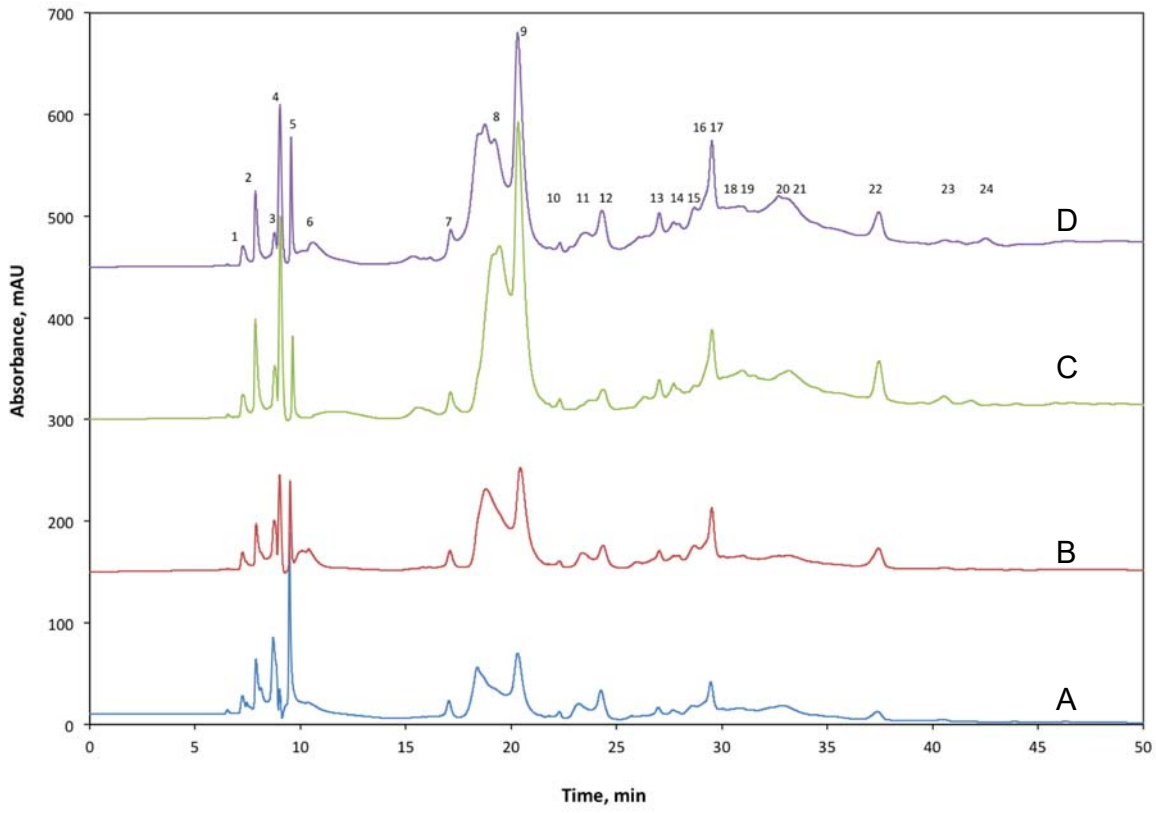

Figure 3. Chromatograms obtained by HPLC-DAD at 280 nm after analysis of extracts prepared with solid–liquid (S-L) procedure, without microwave irradiation (**A**) and after SFI (**B**), SAI (**C**) and PSAI (**D**) pretreatments followed by S-L procedure. For identification purposes see Table 2.

Table 1. Total area of peaks in the chromatograms corresponding to the extracts obtained using the different pretreatment procedures, at 280 nm for phenolic compounds and 520 nm for anthocyanins.

	Extracts			
mAU × min	**Conventional**	**SFI**	**MAI**	**PMAI**
280 nm	265.3	398.8	1027.6	1743.8
520 nm	-	0.081	0.617	0.909

Table 2. Putative identification of compounds presented in the chromatograms and their corresponding retention time (Rt) and maximum absorption wavelength (λ_{max}).

Peak No.	Rt (min)	λ_{max} (nm)	Putative Identification
1	7.3	226	Glucaric acid
2	8.5	227	Quinic acid
3	9.2	227	n.i.
4	9.6	240	Malic acid
5	9.9	229	Ascorbic acid
6	11.12	238	Quinic acid derivative
7	17.5	227	Galloyl-hexoside
8	18.5	268	Gallic acid glucoside
9	19.4	274	5-*O*-Galloylquinic acid
10	22.1	227	Galloyl-hexoside
11	23.5	274	Galloyl shikimic acid
12	24.3	280	Protocatechuic acid
13	26.7	276	3,5-Di-*O*-galloylquinic acid
14	26.9	278	Procyanidin B2
15	28.3	278	Catechin
16	29.6	276	Strictinin elagitannin
17		276	Digalloyl shikimic acid
18	30.5	525	Delphinidin-3-*O*-glucoside
19	30.7	277	Coumaric acid derivative
20	32.5	513	Cyanidin-3-*O*-glucoside
21	33.9	278	n.i.
22	38.8	277	Gallotannin
23	40.8	271	Ellagic acid
24	46.6	360	Ellagic acid xyloside

Although the differences between PSAI and SAI for total yield and TPC are not significant, when considering kinetic rate of extraction overall, there are differences after the first minutes of extraction. The use of PSAI does favor TPC in the extract when compared to SAI. However, the total gain in TPC decreases with time from 1.23- to 1.07-fold. If we consider the energetic usage, this difference may not be so significant to prefer PSAI to SAI, as PSAI takes twice the time of irradiation. Moreover, PSAI requires a different and specific vessel that sustains pressure alongside temperature, which can also be a limiting factor when scaling up to industrial production. Other authors [34] who have tested the use of microwave pretreatments against conventional extraction did not find differences between the two methods, in fact their work attests that the maceration process is the best alternative to bioactive compound extraction without any microwave pretreatment. These results may be due to the fact that these authors used lyophilized fruit in their extractions. As depicted by [31], the extraction efficiency is sensitive to many variables, namely biomass characteristics and solvent. Therefore the pre-existence of water in the irradiated matrix is crucial to take advantage of the full power of microwaves, however it is not enough to promote the best results, as seen before. This could also be an advantage to industrial processes as it eliminates the drying step of the matrix, which is time- and money-consuming.

In a previous work, intensification of phenolic extraction from strawberry-tree fruits has already been focused upon [9]. The authors achieved yields of TPC similar to the ones obtained in this work when they used high-pressure carbon-dioxide-assisted extraction (HPCDAE) to boost the amount of

phenolic compounds with an identical solvent and solid:liquid ratio. As the yields are similar and both SAI and PSAI present lower time of processing compared to HPCDAE, microwave was revealed as a more convenient alternative green process of extraction for intensification of phenolic compounds from strawberry-tree fruits. Moreover, HPCDAE was unable to enhance anthocyanin extraction in opposition to microwave pretreatments, as depicted below in Section 3.2.

3.2. Anthocyanin Content

Several authors have used microwave pretreatment to enhance anthocyanin extraction. The work of Romero-Díez et al. [38], performed in the same microwave equipment using wine lees as raw material, proved that microwave pretreatment led to a double increase in anthocyanin yield, when compared to a conventional S-L extraction. Other authors [17] have described the use of MAE in a similar process to the SAI pretreatment presented in this work, to enhance anthocyanin extraction from black currant marc. The work developed showed that a 20% increase in anthocyanin content was observed after MAE.

As shown in Figure 4, the use of SFI before conventional extraction did not show any significant difference between the anthocyanin content in the extracts obtained. An increase of 66% was noticed when SAI and PSAI were used as pretreatment. As there were no significant differences between SAI and PSAI, once again SAI should be considered over PSAI due to time, energy savings and equipment requirements.

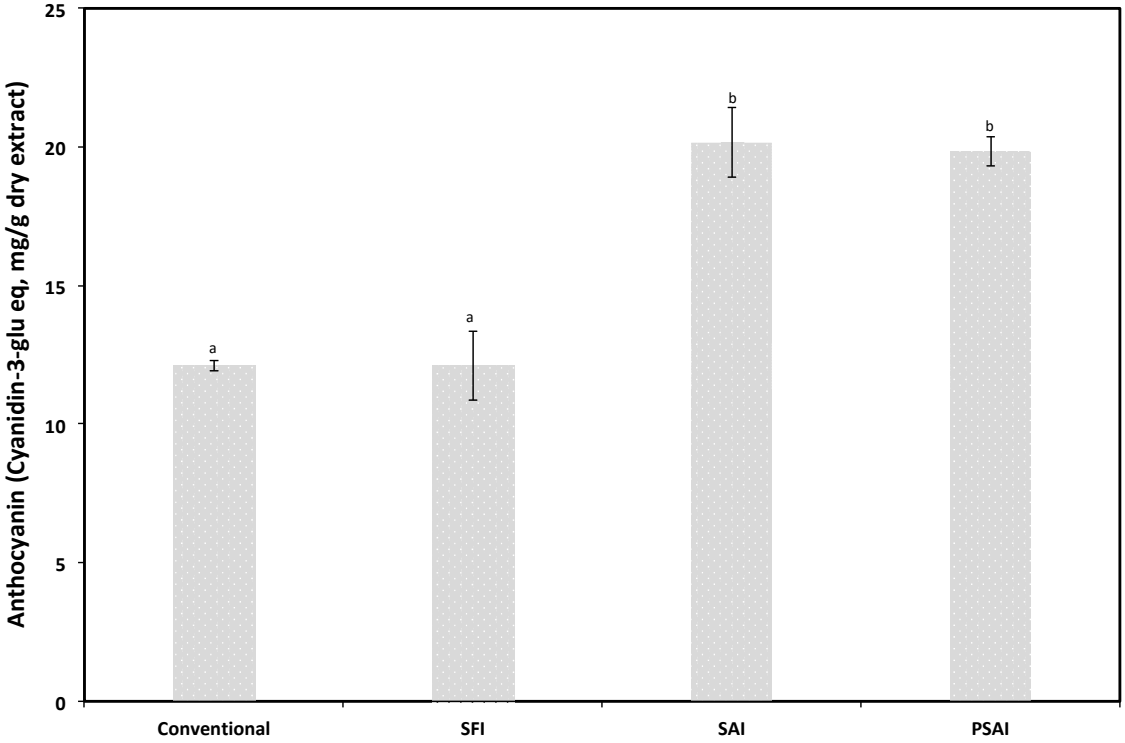

Figure 4. Anthocyanin content expressed in equivalents of cyanidin-3-glucoside for each extract obtained using different pretreatments. Lower case letters (a,b) represent significant differences between the anthocyanin content present in each extract submitted to different pretreatment conditions ($p < 0.05$).

Table 3 presents the putative identification of the different anthocyanins found in the extracts obtained. In our previous work [9], only cyanidin glucoside, cyanidin arabinoside and delphinidin glucoside were identified. The extraction conditions used in the present work enable the extraction of a higher content of anthocyanins, and it was possible to identify more anthocyanins. The results

depicted in Table 3 correspond to the anthocyanins identified in extracts of SAI and PSAI, extracts where the effect of the microwave clearly led to a differentiation of the compounds present in the outcoming extract.

Table 3. Putative identification of anthocyanins present in the chromatograms obtained after analysis by HPLC-MS/MS of the extracts of strawberry-tree fruit obtained after microwave irradiation and their corresponding retention time (Rt), maximum absorption wavelength (λ_{max}), precursor ion ($[M + H]^+$) and product ions obtained by MS/MS.

Retention Time (min)	λ_{max} (nm)	$[M + H]^+$ m/z	MS/MS	Putative Identification	Reference
25.9	525	433	301	Peonidin arabinoside	[40]
26.5	525	433	271	Pelargonidin glucoside	[40]
26.9	525	463	301	Peonidin glucoside	[40]
29.9	555	403	271	Pelargonidin arabinoside	[40]
30.5	525	465	303	Delphinidin glucoside	[1]
32.5	513	449	287	Cyanidin glucoside	[1]
35.4	513	419	287	Cyanidin arabinoside	[1]
33.6	525	435	303	Delphinidin arabinoside	[1]

4. Conclusions

This work demonstrated that phenolic extraction from strawberry-tree fruit may be intensified through the use of a microwave irradiation as pretreatment, for a short time, before a conventional solid–liquid extraction. The extraction conditions used in the SAI pretreatment process enabled the extraction of 1.55 times more phenolic compounds in less than one minute. This value showed a tendency to increase to 1.66 if using PSAI, although the differences among both processes were not statistically significant ($p > 0.05$). The energy consumption was increased in PSAI (0.53 kJ/kg) compared to SAI (0.32 kJ/kg). In addition to higher energy consumption, PSAI requires special equipment to sustain both temperature and pressure, since a flammable solvent is used in the extraction process. Therefore, the questionable TPC gain for PSAI over SAI does not justifies its use. The anthocyanin content is also favored by the use of both SAI and PSAI, with both processes leading to the same increase in the anthocyanin content (66%). Thus, once more, SAI should be considered over PSAI. Moreover, this increase in content enabled the identification of more anthocyanins, compared with previous work. The use of a microwave irradiation procedure prior to fruit processing when preparing jams may eventually cause a higher bioaccessibility of phenolic compounds from strawberry-tree fruit, making these compounds more available for absorption after ingestion in a diet. Further work must be done in order to evaluate this effect.

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