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# Development of a green ultrasound-assisted procedure for the extraction of phenolic compounds from avocado peel with deep eutectic solvents

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ABSTRACT

Avocado (*Persea americana* Mill.) is a tropical fruit grown in different areas of the world. Avocado pulp is of interest to food companies while seed and peel are wasted. In 2021, about 25 thousand tons of avocado peel were produced as waste. In accordance with circular economy and green chemistry principles, avocado peel could be considered a source of bioactive molecules, specifically phenolic compounds, to be recovered using green methods. Deep eutectic solvents (DESs) are green solvents whose extractive efficiency for phenolic compounds from food and waste products of the agri-food chain has already been demonstrated. In this work, an ultrasound-assisted solid-liquid extraction, using DESs, was developed to recover phenolic compounds from avocado (Hass variety) peel. For this purpose, twelve DESs were tested as extraction solvents considering the provided total phenolic content. Then, in order to obtain the largest amount of phenolic compounds, the most relevant factors affecting solid-liquid extraction were evaluated. Matrix-to-solvent ratio of 1:30 (w/v), 25°C and 15 min as temperature and time of extraction, respectively, were found as optimal conditions to guarantee an extracted amount of phenolic compounds of 8.29 ± 0.07 g GAE/100g of dry avocado peel. Through high-performance liquid chromatography coupled with photodiode array and mass spectrometry detection, some flavonoids and phenolic acids were identified in the avocado peel extract.

## 1. Introduction

Avocado (*Persea americana* Mill.) belonging to the *Lauraceae* family and the genus *Persea*, is a tropical fruit that grows in different areas of the world [1]. The fruit is a drupe consisting of the epicarp (peel) for 11-15%, the mesocarp (pulp) for 65-73% and the endocarp (seed) which accounts for 16-20%, of which size, shape, color and phytochemical content depend on the genotype [2]

The pulp is the only edible component of the fruit arousing the interest of industries that use it for the production of oils and sauces. In fact, avocado pulp is rich in proteins, fats, carbohydrates, fiber, minerals and vitamins such as: C, E, and K. The processing of avocado fruit produces a large amount of waste represented by seeds and peel. The discarded avocado peel corresponds to 17 % of the weight of the total fruit [3].

In Europe in 2021, about 25 thousand tons of avocado peel were produced as waste from the production of about 151 thousand tons of avocados [4]. Recent studies present avocado peel as a source of carbohydrates, lipids, proteins, minerals and bioactive molecules such as phenolic compounds [5]. Phenolic compounds are a class of strongly heterogeneous molecules, structurally characterized by the presence of a phenolic group with one or more hydroxyl groups. Many studies have confirmed the antioxidant properties of these bioactive molecules and their consequent beneficial activities for human health [6]. Even if the avocado peel is a waste product of the processing industry of the fruit, it can be considered a valuable resource [7]. In terms of circular economy and environmental sustainability, it is possible to recover these bioactive molecules from food waste products with the aim of using them in the production of food supplements and nutraceuticals [8]. The total phenolic content (TPC), determined in avocado peel extracts, is strongly

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variable depending on the avocado variety, type of procedure and extraction solvent. Avocado peel TPC has resulted to be in the range of 0.4-9 g GAE/100 g of sample [1,9,10]. Phenolic profile of avocado peel includes different molecules belonging to the classes of flavonoids (catechin, epicatechin, kaempferol, phloridzin, quercetin) and phenolic acids (caffeic acid, syringic acid, chlorogenic acid) [11,12].

Some works in literature tested the possibility to recover phenolic compounds from avocado peel through maceration [11], solid-liquid extraction (SLE) [9,13], ultrasound-assisted extraction [14] or microwave-assisted extraction (MAE) [11]. The most often used solvents for this purpose are methanol, ethanol, acetone and their water mixtures [9,11–13]. With the aim to respect green chemistry principles, research is focused on replacing classical organic solvents with greener and more environmentally friendly ones such as deep eutectic solvents (DESs) [15]. DESs are solvents derived by the union of two or more molecules that can act as hydrogen bond acceptor (HBA) or hydrogen bond donor (HBD). Usually, HBA is a quaternary ammonium salt, such as choline chloride (ChCl), while HBD could belong to several chemical classes such as sugars, organic acids, amines or polyalcohols. Considering specific molar ratios, these molecules form a eutectic mixture characterized by a lower transition temperature than that of the individual molecules [16]. When both HBA and HBD are natural, the related mixture is called natural deep eutectic solvent (NADES) [16]. The application of DESs as extraction solvents is an effective strategy for recovering phenolic compounds from food wastes, also in comparison with organic solvents. Kalogioury et al. [17] improved an extraction procedure for phenolic compounds from lemon peel using as extraction solvent a DES composed of ChCl and glycerol. Fanali et al. [18] optimized and validated a SLE procedure for phenolic compounds from hazelnut skin using ChCl-lactic acid DES as the extraction solvent. The same DES resulted to be efficient also for phenolic compounds extraction from barley malt rootlets [8]. Recently, Rodriguez-Martinez et al. [19] developed an extraction procedure for phenolic compounds from avocado peel using DESs. Specifically, they tested five DESs proving that, using a ChCl and acetic acid or lactic acid-based DESs as extraction solvent, a TPC value of 92.03 $\pm$ 2.11 mg GAE/g was obtained, higher than that obtained using ethanol as extraction solvent. However, the authors did not perform an optimization of the extraction procedure [19].

The aim of this work was to optimize a green extraction procedure for the recovery of phenolic compounds from avocado peel. Firstly, the extraction procedure optimization involved the selection of the best extraction solvent, among different tested DESs and common organic solvents. Subsequently, matrix-to-solvent ratio, temperature and time of extraction were optimized to guarantee the highest recovery of PCs. Finally, PC profile in the avocado extract was defined through high-performance liquid chromatography (HPLC) analysis, coupled with photodiode array (PDA) and mass spectrometry (MS) detection.

The greenness of the extraction procedure was evaluated through a Green Analytical Procedure Index (GAPI) complex analysis.

For the best of our knowledge the present work rapresents the first example of analytical method optimization based on the use of a DES for phenolic compounds recovery from avocado peel.

## 2. Materials and methods

### 2.1. Chemicals

ChCl, glycerol and urea were supplied by Sigma-Aldrich (Milan, Italy), while betaine anhydrous, ethylene glycol, lactic acid and malic acid were supplied by Carlo Erba (Milan, Italy). The standards used for the phenolic profile determination (gallic acid, protocatechuic acid, procyanidin B2 (PB2), epicatechin, epicatechin gallate, kaempferol, myricitrin, quercetin and afzelin) were purchased by Sigma-Aldrich (Milan, Italy). Ethanol (EtOH) and solvents used for HPLC-MS, methanol (MeOH) (99.9%), water (HPLC-MS grade), acetonitrile (99.9%), and formic acid (95-97%) were provided by Sigma-Aldrich (Milan, Italy). All reagents are of analytical grade and have been used without further purification steps.

### 2.2. Samples

The avocado peel was obtained from a commercial avocado of the Hass variety. Prior to the experiments, an aliquot was pre-cooled at -80°C and then freeze-dried (LIO5PD6T, 5pascal, Milan, Italy). The lyophilized sample was blended in a mixer (Johnson Blend, 200 W, Rome, Italy) to obtain a homogeneous powder prior to the extraction.

### 2.3. DESs preparation

HBA and HBD were weighed according to established molar ratios and placed in a falcon. Then, mixtures were heated at 80 °C until homogeneous and colorless liquids were obtained. Finally, an amount of water (30% v/v) was added, and mixtures were vortexed and cooled at room temperature. In Table 1 are reported HBAs, HBDs and molar ratios of the tested DESs for the phenolic compound extraction from avocado peel.

### 2.4. Reference extraction method

The extraction procedure was developed starting from a previously extraction method from the literature, with some modifications [13].

An ultrasound-assisted extraction was carried out on 5 g of avocado peel lyophilized powder using 5 mL of extraction solvent in a centrifuge tube. The mixture was placed in an ultrasonic bath (Elmasonic S30H, Elma Schmidbauer GmbH, Singen, Germany) at 55°C, with a frequency of 37 kHz and a thermal power of 200 W, for 30 min. The tube is centrifuged at 5000 rpm for 5 min and the extract was recovered.

In order to select the best extraction solvent, the same procedure was carried out with selected DESs, water and mixture of water with EtOH and MeOH (50:50 v/v).

### 2.5. Extraction procedure optimization

The extraction optimization was realized through one factor at time experiments. At each step, the best value of each parameter studied was selected, keeping the values of the others fixed and varying only the values of the parameter considered. Best parameters were selected considering the TPC of the extracts through Folin-Ciocalteu assay.

First, the best extraction solvent was selected among twelve DESs (Table 1). In order to evaluate the extraction efficiency of each DES, the reference extraction procedure was carried out maintaining the same extraction parameters and varying the extraction solvent.

Then, with the aim of reducing the amount of starting sample, the extraction procedure was carried out making smaller the matrix quantity and maintaining the same matrix-to-solvent ratio of the reference method. Specifically, 0.1 g, 0.3 g and 0.5 g of avocado peel were weighed and 1, 3 mL and 5 mL of selected DES were added, respectively.

**Table 1**
HBA and HBD of tested DESs.

| HBA | HBD | Molar ratio |
|---|---|---|
| ChCl | Urea | 1:2 |
| ChCl | Lactic acid | 1:2 |
| ChCl | Glucose | 2:1 |
| ChCl | Glycerol | 1:2 |
| ChCl | Etylen glycol | 1:2 |
| ChCl | Malic acid | 1:2 |
| Betaine | Urea | 1:2 |
| Betaine | Lactic acid | 1:2 |
| Betaine | Glucose | 1:2 |
| Betaine | Glycerol | 1:2 |
| Betaine | Etylen glycol | 2:1 |
| Betaine | Malic acid | 1:2 |

Regarding the matrix-to-solvent ratio optimization, 1:5, 1:10, 1:20, 1:30, 1:40 and 1:50 (w/v) were considered.

Once selected the extraction solvent, the amount of starting matrix to weight and the matrix-to-solvent ratio, time and then temperature of extraction were studied to select those that guaranteed the highest phenolic compounds recovery. Nine different extraction times between 1 min and 90 min (1, 5, 10, 15, 30, 45, 60, 75, 90 min) were considered. Finally, the optimal extraction temperature was selected among the following tested: 25, 40, 60, 80°C.

### 2.6. Determination of Total Phenolic Content

The total phenolic content (TPC) was determined by the oxidation of phenolic compounds using Folin–Ciocalteu's reagent [20]. Briefly, 20 μL of the sample were mixed with 100 μL of Folin–Ciocalteu's reagent and 1580 μL of EtOH:$H_2O$ (50% v/v) mixture and kept in the dark for 10 min. Then, 300 μL of an aqueous solution of $Na_2CO_3$ 0.2 g $mL^{-1}$ were added and put back in the dark for 2 h under continuous stirring. Finally, the mixture was centrifuged for 2 min at 10.621 g and 200 μL of the sample was put in a Greiner microplate. The absorbance was measured with the Infinite M200 PRO Tecan microplate spectrophotometer (Tecan Trading AG, Switzerland) at 765 nm. The concentration of the samples was calculated by interpolating the result in a calibration curve made using gallic acid as an analytical standard in a range from 0 to 2000 μg $mL^{-1}$. Results were expressed as grams of gallic acid equivalent (g GAE) per 100 $g^{-1}$ of freeze-dried peel.

### 2.7. Extraction procedure using DES

The optimized extraction procedure, applied for phenolic compounds extraction from avocado peel, involved to weigh 0.1 g of lyophilized sample in a centrifuge tube added with 3 mL of a DES composed of ChCl and lactic acid, in a molar ratio of 1:2. The mixture was placed in an ultrasonic bath (Elmasonic S30H, Elma Schmidbauer GmbH, Singen, Germany) at ambient temperature, with a frequency of 37 kHz and a thermal power of 200 W, for 15 min. The tube is centrifuged at 5000 rpm for 5 min and the extract was recovered and analyzed.

### 2.8. HPLC/ESI-MS qualitative analysis

The extract characterization was performed using a Shimadzu Prominence LC-20A chromatograph (Shimadzu, Milan, Italy) equipped with two LC-20 AD XR pumps-a SIL-10ADvp pump and the CTO-20 AC-column furnace and a DGU-20 A3 degasser coupled with PDA SPD-detectorM10Avp PDA and mass spectrometer (LCMS-2010, Shimadzu, Tokyo, Japan) equipped with electrospray interface (ESI). MS data was acquired using Shimadzu LC solution version 3.7 (Shimadzu, version 3.7). Analyte separation was carried out using an Ascentis® Express F5 HPLC Column (150 × 2.1 mm I.D., 2.7 μm) (Merck KGaA, Darmstadt, Germany). The elution of the analytes was carried out at the constant flow of 1 mL $min^{-1}$ at the temperature of 40°C. The mobile phase (A) was an acidified aqueous solution with 0.1% (v/v) HCOOH and (B) acetonitrile 0.1% (v/v) HCOOH. The gradient used for the separation of the phenolic compounds present in the extract was: 0-40 min 0-30% B, 40-41 min 100% B. The injected volume was 2 μl. The data was acquired with PDA in the range between 200-400 nm and the chromatograms were extracted at 280 and 360 nm. MS chromatograms were recorded according to the ionization in negative mode, using the following parameters: gas flow for nebulization (N2): 1.5 ml min 1; event time: 1 s; range of m/z acquired: m/z 100-800; scanning speed: 1000 amu/s; detector voltage: 1.5 kV; interface temperature: 250 C; CDL temperature: 300 C; heated block temperature: 300 C; interface voltage: 3.50 kV; Q-array voltage: 0.0 V; Q-array RF: 150.0 V.

### 2.9. GAPI index analysis

The GAPI analysis was conducted through ComplexGAPI software. The GAPI metric defines the greenness of each step of the analytical process, from collection of samples until the final analysis. The main advantage of GAPI method is its capacity to take into account the greenes of the entire analytical method avoiding the use of several tools [21]. The five pentagrams described the greenes of each step of the analytical procedure represent sample collection, preservation, transport and storage, the sample preparation, including, the reagents and solvent used, the instrumentation and the qualitative or quantitative purpose of the analytical method. Concerning the first steps of the analytical procedure, the highest greenness is achieved by an in-line sampling, whenever it is unpractable some storaging apporoaches must be done to preserve the target analytes, affecting the method greenness [22]. Despite the implementation of the analytical methods over the years, sample preparation resulted to be necessary to guarantee the analysis of a large amount of analytes, concentrating anaytes and reducing matrix effect. Conseguentially, develop miniaturized method, based on the use of a low quantity of organic solvents, or replace organic solvents with green once let to decrease the environmental impact of the method. Moreover the analytes derivatization should be avoid [23]. The last step of the analytical procedure is the quali-quantitative analytes determination. To respect the green analytical chemistry requiriments multi-analytes method and rapid and simple analysis are preferred [21].

Pentagram takes a color on the base of its greenness: a sustainable step of the analytical method is represented with green color while steps with a medium and high environmental impact have yellow and red color, respectively.

### 2.10. Statistical analysis

Analyses were performed in triplicate. Means and standard deviations were calculated, and statistical differences were analyzed by One-way analysis of variance (ANOVA) followed by Tukey's test using GraphPad Prism version 4 for Windows (GraphPad software).

## 3. Results

### 3.1. Selection of the DES for phenolic compounds extraction from avocado peel

In order to develop a sustainable extraction method to recover phenolic compounds from avocado peel using environmentally friendly solvents, different DESs were tested as possible extraction solvent and also compared with traditional solvents reported in the literature. The extraction procedure was performed as previously described in section 2.4. Extracts were analyzed through Folin-Ciocalteu assay. The total concentration of phenolic compounds in the extracts, obtained using the selected different DESs, was expressed as g GAE per 100 g of lyophilized

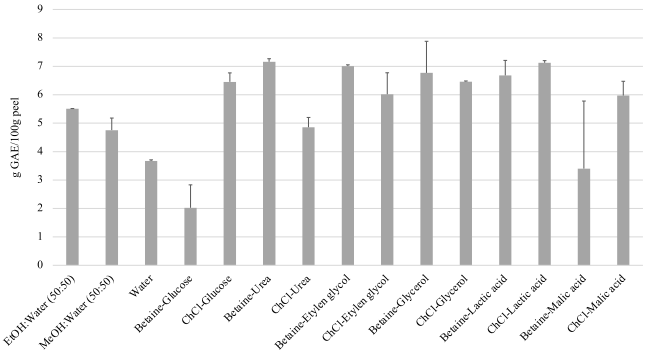


**Fig. 1.** TPC obtained using tested DESs and conventional solvents.

sample. As possible to see in Fig. 1, among the traditional solvents used, the $H_2O$:EtOH mixture was found to be the most efficient, ensuring 5.51 g GAE 100 $g^{-1}$ of lyophilized sample. Results were in accordance with data present in the literature [9,10,19]. All the tested DESs guaranteed an extraction efficiency better or equal than $H_2O$:EtOH, except for betaine-glucose and betaine-malic acid DESs. As reported in the literature, betaine-glucose and betaine-malic-based DESs are characterized by high viscosity that could cause a reduction in mass transfer with a decrease in extraction efficiency [15,24]. Betaine-urea DES, betaine-ethylene glycol DES and ChCl-lactic acid DES resulted to be the three extraction solvents that guaranteed the best phenolic compounds extraction, providing 7.2, 7.0 and 7.1 g GAE per 100 $g^{-1}$ dry matter. Among these, ChCl-lactic acid-based DES is considered a NADES, with very low toxicity and high extraction efficiency for phenolic compounds [1,3,18], for these reasons, it has been selected as extraction solvent for the following optimization. In addition the price for the production of 1 Kg of this DES is about 130 €, lower than the price of the same quantity of EtOH (green organic solvent).

### 3.2. Selection of matrix-to-solvent ratio

Several parameters could affect the extraction efficiency of a SLE procedure, including the matrix-to-solvent ratio. Since each plant matrix has unique properties, both in terms of structure and composition, when it is dispersed in a solvent it tends to have a characteristic behavior, so this makes it impossible to establish which quantity of matrix-volume of solvent ratio should be adopted to achieve extraction optimum [25]. Before the quantity of matrix-volume of solvent ratio optimization, some experiments were performed with the aim of reducing the quantity of sample used for each analysis, making the extraction easier and faster. Specifically, following the reference method, three experiments were carried out with some modifications: maintaining the same matrix-to-solvent ratio and using ChCl-lactic acid DES as extraction solvent, three extractions starting from 0.1 g, 0.3 g and 0.5 g of lyophilized sample were carried out. Each procedure was conducted in an ultrasonic bath at 55°C for 30 min.

No statistical differences were revealed in TPC obtained (results not shown). For this reason, 0.1 g of lyophilized avocado peel was used as matrix amount for the subsequent matrix-to-solvent ratio optimization. Six matrix-to-solvent ratios being 1:5, 1:10, 1:20, 1:30, 1:40, and 1:50 (w/v) were tested, considering the previously described extraction procedure.

As is possible to see in Fig. 2, the extraction carried out with 1:5 and 1:10 ratios did not guarantee good extraction repeatability, probably because the volume of solvent was too low to wet the solid matrix evenly.

Increasing the ratio from 1:20 to 1:50 no statistically significant difference in terms of TPC was found. For the final procedure, 1:30 ratio was selected because it ensured operatively an easier recovery of the solvent from the matrix that was deposited on the bottom of the tube after centrifugation, compared to the 1:20.

Thus, the optimized matrix-to-solvent ratio and amount of starting material conditions involved weighing 0.1 g of lyophilized sample and performing the extraction with 3 mL of ChCl-lactic acid DES.

### 3.3. Optimization of the extraction time and temperature

Time and temperature of the extraction procedure could strongly affect the extraction yield. According to the literature, increasing the extraction time and therefore increasing the contact time between matrix and solvent, increases the extractive yield. At the same time increasing the temperature facilitates the extraction process of the analytes. However, long time of extraction if combined with high temperatures could cause hydrolysis of phenolic compounds in the extract with consequently TPC reduction [26]. Firstly, the time of the extraction procedure was optimized testing the extraction at 1, 5, 10, 15, 30, 45-, 60-, 75- and 90-min. Extractions were performed as follows: 0.1 g of lyophilized peel was weighed and extracted with 3 mL of ChCl-lacitic acid DES. The mixture was placed in an ultrasonic bath (Elmasonic S30H, Elma Schmidbauer GmbH, Singen, Germany) at 55°C, with a frequency of 37 kHz and a thermal power of 200 W, for the selected time to test. The tube is centrifuged at 5000 rpm for 5 min and the extract was recovered.

As is possible to see in Fig. 3, from 1 min to 15 min of extraction time a statistically significance increase in the TPC was revealed. Testing the extraction considering a time of 30 min or higher of 30 min did not guarantee changes in extraction yield. Fifteen min were selected as time for the extraction procedure.

Performing the previously described extraction procedure, considering 15 min as time of extraction, temperature of extraction was optimized. Specifically, 25°C, 40°C, 60°C and 80°C, were tested as possible extraction temperatures but as is possible to see in Fig. 4, no statistically significant difference in TPC was obtained.

The final optimized extraction procedure resulted to be as follow: weigh 0.1 g of lyophilized sample in a centrifuge tube added with 3 mL of a DES composed by ChCl and lactic acid, in a molar ratio of 1:2. Put the mixture in an ultrasonic bath (Elmasonic S30H, Elma Schmidbauer GmbH, Singen, Germany) at ambient temperature, with a frequency of 37 kHz and a thermal power of 200 W, for 15 min. Centrifuge the tube at 5000 rpm for 5 min and recover the extract for the subsequent analysis.

### 3.4. HPLC/ESI-MS qualitative analysis

In order to identify phenolic compounds in avocado peel, the extracts obtained with the optimized extraction method were analyzed through the HPLC/ESI-MS method previously described. The identification was done considering: $t_R$ and UV spectra data, MS spectra, use of standard compounds and data available in the literature. In total 7 phenolic compounds were revealed: 5-O-caffeoyl-quinic acid, procyanidin B2,

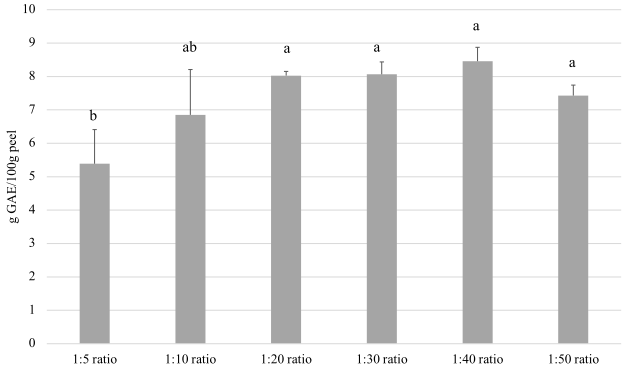


**Fig. 2.** TPC obtained considering different quantities of matrix-to-solvent ratio.

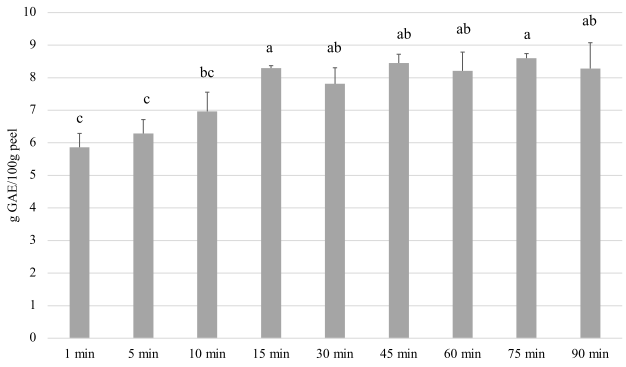


**Fig. 3.** TPC obtained testing the extraction at different time.

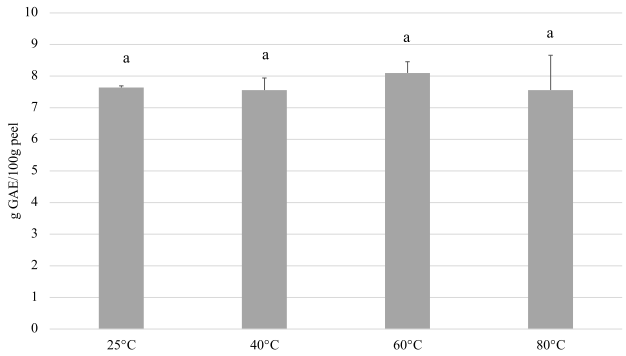


**Fig. 4.** TPC obtained testing the extraction under different temperature.

(-)-epicatechin, procyanidin B trimers, kaempferol O-glucosyl rhamnoside, myricetin-3-o-alpha-l-rhamnopyranoside and Kaempferol.

In Fig. 5 the chromatogram extracted at λ=280 nm is reported. Numbers in the chromatogram are referred to the Table 2 in which analyte number, analyte name and m/z $[M-H]^-$ ion are listed. 5-O-caffeoyl-quinic acid was identified considering its m/z $[M-H]^-$ and comparing the retention time with which of an available standard molecule. This chlorogenic acid was previously detected in avocado peel by Rosero, J.C. et al. [11] and Figueroa, J.G. et al. [12]. In accordance with data present in the literature, the presence of procyanidin B2 and procyanidin B trimers, belonging to the class of proanthocyanidins, was confirmed [11,27]. Procyanidin B2 identification was performed considering its m/z $[M-H]^-$ and comparing the retention time with which of an available standard molecule, while procyanidin B trimers was tentatively identified considering its m/z $[M-H]^-$ since the standard molecule was not available. Two molecules belonging to the class of flavonoid-3-o-glycosides were identified: kaempferol O-glucosyl rhamnoside and myricetin 3-rhamnoside. Kaempferol O-glucosyl rhamnoside was previously detected in avocado peel [27] while, to our knowledge, myricetin 3-rhamnoside had never been identified before in the avocado peel; in this work, its presence has been confirmed considering its molecular ion and its retention time based on co-chromatography of available standard molecule. (-)-Epicatechin and kaempferol, previously identified in avocado peel, were revealed considering their m/z $[M-H]^-$ and comparing the retention time with which of an available standard molecules [11,13].

### 3.5. GAPI index analysis

The optimized analytical method involved to extract phenolic compounds starting from 0.1 g of lyophilized avocado peel with 3 mL of ChCl-lactic acid DES, in an ultrasonic bath (Elmasonic S30H, Elma Schmidbauer GmbH, Singen, Germany) for 15 min at ambient temperature.

**Table 2**
number in order of elution, compound name, retention time and m/z ion of phenolic compounds identified in the avocado peel extract.

| N | Analyte | $t_R$ | m/z $[M-H]^-$ |
|---|---|---|---|
| **1** | 5-O-caffeoyl-quinic acid | 19.7 | 353 |
| **2** | Procyanidin B2 | 20.2 | 577 |
| **3** | (-)-epicatechin | 22.1 | 289 |
| **4** | Procyanidin B trimers | 22.8 | 865 |
| **5** | Kaempferol O-glucosyl rhamnoside | 27.5 | 593 |
| **6** | Myricetin 3-rhamnoside | 27.9 | 463 |
| **7** | Kaempferol | 44.5 | 285 |

The extraction procedure meets the green analytical chemistry main principles since it involves the use of a NADES composed by ChCl and lactic acid and in a very low quantity, as extraction solvent. Moreover, the extraction is performed at room temperature and with a low time of extraction (15 min). According to our knowledge, the methods from the literature require long extraction times to extract phenolic compounds from avocado peel [11,25,26]. The only work in which a DES is used for the extraction of phenolic compounds from avocado peel needed 120 min of extraction, starting from 6 g of sample in presence of 100 mL of DES [1].

To evaluate the greenness of the optimized method a GAPI analysis was performed. GAPI pictogram (Fig. 6) of the new optimized method reveals ten zones filled with green color with two yellow zones and four red ones for the proposed methods. Zones related to sample preservation, amount, health and safety hazard of reagents and solvents are green since solvents characterized by a low toxicity and in a low

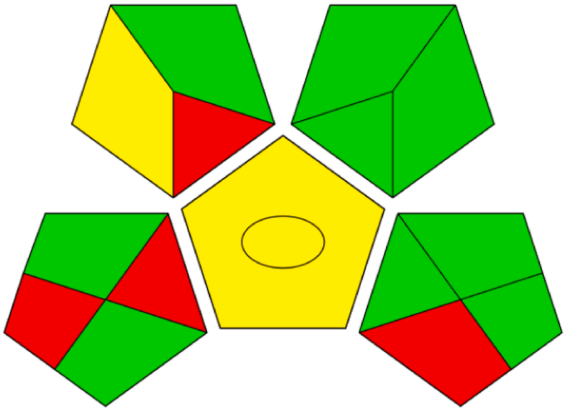

**Fig. 6.** GAPI pictogram.

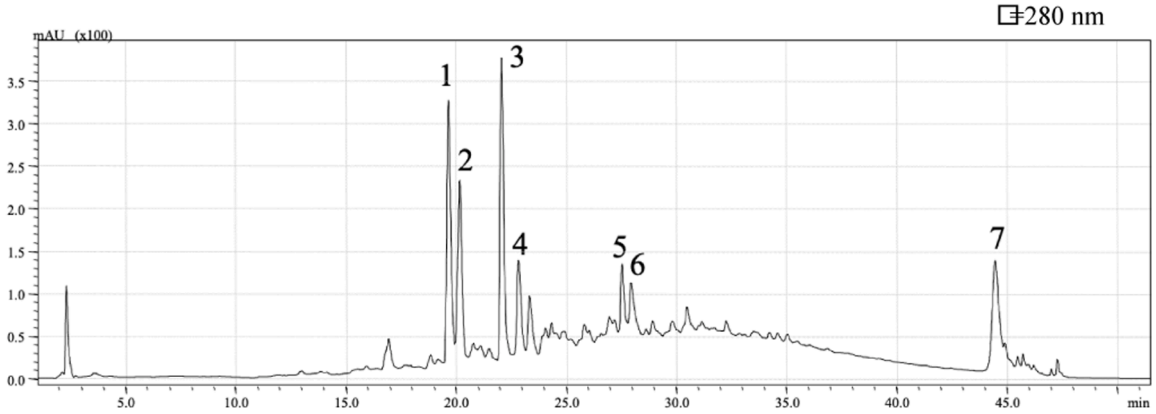


**Fig. 5.** Phenolic compounds chromatogram (λ=280 nm) of avocado peel extraction.

quantity were used. Since only the qualitative analysis of the extracts was performed through HPLC-MS instrument, also zones related to the instrument are mostly green. In general, GAPI pictogram confirms that the whole analytical method has a good greenness.

## 4. Conclusion

An eco-friendly method in line with the principles of green chemistry has been developed and optimized. The capacity of DES in the extraction of phenolic compounds has been confirmed and DES consisting of ChCl-lactic acid has shown a higher extractive efficiency than that achieved by the organic solvents traditionally used. The optimized extraction method let to extract 8.29 ± 0.07 g GAE per 100 $g^{-1}$ freeze-dried peel, by using a reduced amount of solvent and time. Moreover, the optimized procedure resulted to be energy-effective avoiding the use of high temperature during the extraction. The phenolic compounds isolated belong to the class of chlorogenic acids, proanthocyanidins and flavonoid-3-O-glycosides. This green method can be considered as an alternative to conventional techniques in which organic solvents, characterized by a high impact on the environment and operator health, are used for the recovery of reusable phenolic compounds in nutraceutical and cosmeceutical field.

## 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.

## Aknowledgements

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## References

[1] B. Rodríguez-Martínez, P. Ferreira-Santos, I.M. Alfonso, S. Martínez, Z. Genisheva, B. Gullón, Deep Eutectic Solvents as a Green Tool for the Extraction of Bioactive Phenolic Compounds from Avocado Peels, Molecules 27 (2022) 6646, https://doi.org/10.3390/molecules27196646.
[2] M. Calderón-Oliver, H.B. Escalona-Buendía, O.N. Medina-Campos, J. Pedraza-Chaverri, R. Pedroza-Islas, E. Ponce-Alquicira, Optimization of the antioxidant and antimicrobial response of the combined effect of nisin and avocado byproducts, LWT - Food Sci. Technol. 65 (2016) 46–52, https://doi.org/10.1016/j.lwt.2015.07.048.
[3] R.G. Araujo, R.M. Rodríguez-Jasso, H.A. Ruíz, M. Govea-Salas, M. Pintado, C. N. Aguilar, Recovery of bioactive components from avocado peels using microwave-assisted extraction, Food Bioprod. Process. 127 (2021) 152–161, https://doi.org/10.1016/j.fbp.2021.02.015.
[4] FAOSTAT, Food and Agriculture Organization of the United Nations. https://www.fao.org/faostat/en/#data/QCL/visualize, 2023, (accessed February 6, 2023).
[5] R.G. Araújo, R.M. Rodriguez-Jasso, H.A. Ruiz, M.M.E. Pintado, C.N. Aguilar, Avocado by-products: Nutritional and functional properties, Trends Food Sci. Technol. 80 (2018) 51–60, https://doi.org/10.1016/j.tifs.2018.07.027.
[6] M.R. Segura-Campos, Bioactive compounds: health benefits and potential applications, Woodhead Publishing, Duxford, United Kingdom, 2019.
[7] S.M. Ferreira, L. Santos, From by-product to functional ingredient: Incorporation of avocado peel extract as an antioxidant and antibacterial agent, IFSET 80 (2022), 103116, https://doi.org/10.1016/j.ifset.2022.103116.
[8] S. Della Posta, V. Gallo, L. Dugo, L. De Gara, C. Fanali, Development and Box–Behnken design optimization of a green extraction method natural deep eutectic solvent-based for phenolic compounds from barley malt rootlets, Electrophoresis 43 (2022) 1832–1840, https://doi.org/10.1002/elps.202200059.
[9] W. Wang, T.R. Bostic, L. Gu, Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars, Food Chem. 122 (2010) 1193–1198, https://doi.org/10.1016/j.foodchem.2010.03.114.
[10] J.-G. Rodríguez-Carpena, D. Morcuende, M.-J. Andrade, P. Kylli, M. Estévez, Avocado (Persea americana Mill.) Phenolics, In Vitro Antioxidant and Antimicrobial Activities, and Inhibition of Lipid and Protein Oxidation in Porcine Patties, J. Agric. Food Chem. 59 (2011) 5625–5635, https://doi.org/10.1021/jf1048832.
[11] J.C. Rosero, S. Cruz, C. Osorio, N. Hurtado, Analysis of Phenolic Composition of Byproducts (Seeds and Peels) of Avocado (Persea americana Mill.) Cultivated in Colombia, Molecules 24 (2019) 3209, https://doi.org/10.3390/molecules24173209.
[12] J.G. Figueroa, I. Borrás-Linares, J. Lozano-Sánchez, A. Segura-Carretero, Comprehensive identification of bioactive compounds of avocado peel by liquid chromatography coupled to ultra-high-definition accurate-mass Q-TOF, Food Chem. 245 (2018) 707–716, https://doi.org/10.1016/j.foodchem.2017.12.011.
[13] D.R. Morais, E.M. Rotta, S.C. Sargi, E.M. Schmidt, E.G. Bonafe, M.N. Eberlin, A.C. H.F. Sawaya, J.V. Visentainer, Antioxidant activity, phenolics and UPLC–ESI(–)–MS of extracts from different tropical fruits parts and processed peels, Food Res. Int. 77 (2015) 392–399, https://doi.org/10.1016/j.foodres.2015.08.036.
[14] I. Trujillo-Mayol, C.L. Cespedes-Acuña, F. Silva, J. Alarcon, Improvement of the polyphenol extraction from avocado peel by assisted ultrasound and microwaves, J. Food Process Eng. 42 (2019), https://doi.org/10.1111/jfpe.13197.
[15] S.Della Posta, V. Gallo, A. Gentili, C. Fanali, Strategies for the recovery of bioactive molecules from deep eutectic solvents extracts, TrAC Trends Anal. Chem. 157 (2022), 116798, https://doi.org/10.1016/j.trac.2022.116798.
[16] H. Vanda, Y. Dai, E.G. Wilson, R. Verpoorte, Y.H. Choi, Green solvents from ionic liquids and deep eutectic solvents to natural deep eutectic solvents, Comptes Rendus Chim. 21 (2018) 628–638, https://doi.org/10.1016/j.crci.2018.04.002.
[17] N.P. Kalogiouri, E. Palaiologou, E.N. Papadakis, D.P. Makris, C.G. Biliaderis, I. Mourtzinos, Insights on the impact of deep eutectic solvents on the composition of the extracts from lemon (Citrus limon L.) peels analyzed by a novel RP-LC–QTOF-MS/MS method, Eur. Food Res. Technol. 248 (2022) 2913–2927, https://doi.org/10.1007/s00217-022-04100-0.
[18] C. Fanali, V. Gallo, S. Della Posta, L. Dugo, L. Mazzeo, M. Cocchi, V. Piemonte, L. De Gara, Choline Chloride–Lactic Acid-Based NADES As an Extraction Medium in a Response Surface Methodology-Optimized Method for the Extraction of Phenolic Compounds from Hazelnut Skin, Molecules 26 (2021) 2652, https://doi.org/10.3390/molecules26092652.
[19] B. Rodríguez-Martínez, P. Ferreira-Santos, B. Gullón, J.A. Teixeira, C.M. Botelho, R. Yáñez, Exploiting the Potential of Bioactive Molecules Extracted by Ultrasounds from Avocado Peels—Food and Nutraceutical Applications, Antioxidants 10 (2021) 1475, https://doi.org/10.3390/antiox10091475.
[20] J.D. Everette, Q.M. Bryant, A.M. Green, Y.A. Abbey, G.W. Wangila, R.B. Walker, Thorough Study of Reactivity of Various Compound Classes toward the Folin−Ciocalteu Reagent, J. Agric. Food Chem. 58 (2010) 8139–8144, https://doi.org/10.1021/jf1005935.
[21] J. Płotka-Wasylka, A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index, Talanta 181 (2018) 204–209, https://doi.org/10.1016/j.talanta.2018.01.013.
[22] M. Tobiszewski, A. Mechlińska, B. Zygmunt, J. Namieśnik, Green analytical chemistry—theory and practice, Trends Anal. Chem. 28 (2009) 943–951, https://doi.org/10.1039/B926439F.
[23] A. Gałuszka, Z.M. Migaszewski, P. Konieczka, J. Namieśnik, Analytical Eco-Scale for assessing the greenness of analytical procedures, Trends Anal. Chem. 37 (2012) 61–72, https://doi.org/10.1016/j.trac.2012.03.013.
[24] F. Mohd Fuad, M. Mohd Nadzir, A. Harun Kamaruddin, Hydrophilic natural deep eutectic solvent : A review on physicochemical properties and extractability of bioactive compounds, J. Mol. Liq. 339 (2021), 116923, https://doi.org/10.1016/j.molliq.2021.116923.
[25] M. Pinelo, M. Rubilar, M. Jerez, J. Sineiro, M.J. Núñez, Effect of Solvent, Temperature, and Solvent-to-Solid Ratio on the Total Phenolic Content and Antiradical Activity of Extracts from Different Components of Grape Pomace, J. Agric. Food Chem. 53 (2005) 2111–2117, https://doi.org/10.1021/jf0488110.
[26] S. Nipornram, W. Tochampa, P. Rattanatraiwong, R. Singanusong, Optimization of low power ultrasound-assisted extraction of phenolic compounds from mandarin (Citrus reticulata Blanco cv. Sainampueng) peel, Food Chem. 241 (2018) 338–345, https://doi.org/10.1016/j.foodchem.2017.08.114.
[27] A. Rojas-García, E. Fuentes, M. de la L. Cádiz-Gurrea, L. Rodriguez, M. del C. Villegas-Aguilar, I. Palomo, D. Arráez-Román, A. Segura-Carretero, Biological Evaluation of Avocado Residues as a Potential Source of Bioactive Compounds, Antioxidants 11 (2022) 1049, https://doi.org/10.3390/antiox11061049.