Combined Effects of Deep Eutectic Solvents and Pulsed Electric Field Improve Polyphenol-Rich Extracts from Apricot Kernel Biomass

Ioannis Makrygiannis, Vassilis Athanasiadis, Eleni Bozinou, Theodoros Chatzimitakos, Dimitris P. Makris and Stavros I. Lalas*

Department of Food Science & Nutrition, University of Thessaly, Terma N. Temponera Str., GR-43100 Karditsa, Greece
* Correspondence: slalas@uth.gr; Tel.: +30-24410-64783

Abstract: Apricots are one of the most important fruits in the Mediterranean region for both their nutritional and economic value. They are widely cultivated and consumed fresh or dried or are used in the food industry for the production of jams, juices, etc. In any case, the seeds they contain constitute waste. The kernels are very rich in bioactive compounds such as polyphenols, a fact that makes them very appealing in cosmetology. However, the extraction of the bioactive compounds of apricot kernels is poorly examined. In this study, the preparation of polyphenol-rich extracts from apricot kernel biomass is discussed. To this end, a common extraction procedure with water as a solvent was employed. To enhance the extraction yield, the use of a deep eutectic solvent (DES) was examined. In addition, the use of pulsed electric field (PEF) either as a standalone extraction method or as a complementary step was also examined. According to the results, it was evident that when PEF was applied before the extraction procedure, an increase of 88% in the total polyphenol content (TPC) was recorded. Likewise, the use of a glycerol:choline chloride (2:1, w/w) DES increased the TPC by ~70%. When the two approaches were combined, a 173% increase was recorded. According to the above, it can be concluded that apricot kernel biomass is a very good source of polyphenols, especially using the proposed extraction procedure.

Keywords: apricot; kernel; biomass; polyphenols; antioxidants; deep eutectic solvents; pulsed electric field

1. Introduction

Apricots (Prunus armeniaca L.) belong to the Rosaceae family [1]. They derived from China and were transferred to the Mediterranean region [2]. Apricots have been cultivated for many years and are considered important fruits for human nutrition [3]. They are consumed either fresh or dried, canned, or used for the preparation of juices. Around 40 million tons of apricots are produced each year, with Turkey and Iran being the top producers in the world [4]. Apricots are rich in carotenoids, polyphenols, and vitamins that bestow a high nutritional value to the fruit [5]. The seed of the fruit makes up around 7% of the fruit and is considered a waste material. Apricot kernels (contained within the pit/stone) account for about 18 to 38% of the seed [6] and have commercial value since they are used for oil production and also have applications in the cosmetics industry. Moreover, apricot kernels are used for thermal energy storage, food product production, and antimicrobial film preparation [7].

The preparation of antimicrobial films lies among the efforts made to prevent deaths due to microbial infections, which are known to be a major cause of many deaths worldwide [8,9]. It has also been demonstrated in the literature that polyphenols are beneficial to human health due to their anti-cancer and heart disease-preventing properties [10–13]. Fruits and vegetables are a rich source of polyphenolic compounds. For instance, the total
polyphenol content (TPC) in grapes, apples, and pears can range from 200 to 300 mg gallic acid equivalents (GAE) per 100 g [10,11]. Likewise, apricots are a good source of polyphenol compounds [12], but it is noteworthy that apricot kernels exhibit significantly higher antioxidant activity and have an increased TPC compared to the flesh of the fruit [14]. In a previous study, it was reported that the kernel of the Raktsey Karpo Chenmo variety has a TPC that ranges from 92.2 to 162.1 mg GAE per 100 g [3]. Variables such as soil, cultural practices, weather, and geographical region can have an impact on the TPC of apricot kernels, and the genotype is also a major factor that affects the TPC [3,15].

Due to their health effects, much effort is being placed into the extraction of polyphenols. Water and ethanol are the most commonly employed solvents for their extraction on an industrial scale [16], although the extraction efficiency in such cases may be low. Thus, alternative options are being examined and proposed.

Deep eutectic solvents (DESs) have gained popularity in the scientific community as a way to maximize extraction effectiveness while simultaneously lowering the cost of extract preparation. Low transition temperature mixtures, also known as DESs, are innovatively designed liquids made of inexpensive, recyclable, and non-toxic components such as natural compounds (e.g., sugars, organic acids, salts, etc.) [17–20]. Some of these compounds act as hydrogen bond donors and others as hydrogen bond acceptors. They constitute a promising novel approach for the green extraction of polyphenols [18,21,22].

Another important parameter that should be taken into account for the extraction of polyphenols is temperature. Increasing the temperature during the extraction process may be advantageous by lowering the extraction time and increasing the yield. However, it has been demonstrated that as the temperature increases, the quality of the extract deteriorates due to the degradation of the extracted compounds [23]. Consequently, there is a need for innovative extraction methods that achieve similar or even better results without increasing the temperature of the extract and that also have the potential to be used on an industrial scale. To this end, pulsed electric field (PEF) is a new technique that has been used both as a pretreatment step to increase the TPC in fruit and vegetable extracts [24–27] and as a standalone extraction technique for the isolation of polyphenols [28,29]. In comparison to conventional extraction techniques, PEF extraction achieves a better yield and minimizes the use of solvents and other resources, while its application duration is significantly short [30]. The electroporation that takes place during PEF is a unique process that makes the cell membranes of the plant tissue more permeable, facilitating the diffusion of the compounds into the solvent. Due to the above, a growing number of industries, including the food and pharmaceutical ones, are using PEF to prepare extracts, with the aforementioned advantages [31].

In light of the above, our aim was to valorize the biomass of apricot kernels, after their defatting, in order to produce polyphenol-rich extracts. To this end, a common extraction procedure with water as a solvent was examined. Moreover, the use of PEF was examined, both as a standalone extraction technique and as a complementary step to the aforementioned extraction. In an effort to further increase the extraction yield, the use of a DES was also examined, and its combination with PEF was determined as well.

2. Materials and Methods
2.1. Chemicals

The Folin–Ciocalteu reagent, sodium carbonate anhydrous (99%), gallic acid hydrate (≥99.0%), and solvents (ethanol, n-hexane, and methanol) were obtained from Panreac Co. (Barcelona, Spain). Petroleum ether (40–60 °C) was obtained from Chem Lab (Zedelgem Belgium). Rutin (quercetin 3-O-rutinoside) (≥95.0%), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (≥99.0%), L-ascorbic acid (≥99.0%), hydrochloric acid, and 2,2-diphenylpicrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Darmstadt, Germany). Sodium acetate trihydrate (≥99.0%) was obtained from Penta (Praha, Czechia). Aluminum chloride (≥99.0%) and iron chloride hexahydrate (≥99.0%) were purchased from Merck (Darm-
stadt, Germany). All reagents and solvents, unless otherwise specified, were of at least analytical grade.

2.2. Synthesis of DES

For the preparation of the DES solvent, glycerol and choline chloride were used as a hydrogen bond donor and a hydrogen bond acceptor, respectively. Glycerol was mixed with choline chloride at a molar ratio of 2:1 (w/w). The mixture was placed in a sealed glass vial and heated at 80–90 °C for 90 min under stirring until a transparent liquid was formed. Finally, the DES was diluted with water at a concentration of 80% (w/w) and used for further extraction [32].

2.3. Material Defatting and Oil Content

The apricot seeds were collected from an industrial processing facility (ELBAK S.A., Falani, Larissa, Greece) and were of the variety *Prunus armeniaca* ‘Bebeco’. The apricot seeds were frozen at −40 °C upon collection. Next, the moisture was removed by freeze-drying using a Biobase BK-FD10P freeze-dryer (Jinan, China) for 24 h. After manual crushing by hand, the moisture content of the apricot kernels was calculated to be 7.1 ± 0.4%. Then, the dried apricot kernels were ground to a fine powder using a blender.

To obtain the apricot kernel oil, 80 g of apricot kernel powder was added to a glass bottle along with 400 mL of petroleum ether. The mixture was stirred at 500 rpm for 2 h. Then, the mixture was centrifuged at 4500 rpm for 10 min using a NEYA 16R centrifuge (Remi Elektrotechnik Ltd., Palghar, India). The supernatant was retracted, and a second defatting step was carried out on the solid residue. After the second defatting procedure, the supernatants were pooled. Finally, the solvent was evaporated using a rotary evaporator (Laborota 4000 efficient, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany), and the apricot kernel oil was obtained. The oil content of the apricot kernels was calculated to be 34.6 ± 2.2% of dry weight.

The solid residue after the centrifugation step was placed in an oven at 50 °C for 1 h to remove solvent residues. Finally, the defatted apricot kernel biomass (AKB) was sieved (Analysette 3, Fritsch GmbH, Oberstein, Germany), and the particles with an average diameter of 92 μm were used for further study.

2.4. Pulsed Electric Field (PEF) Treatment

Full details about the PEF system used are given in our previous study [33]. PEF was applied to the samples for 15 min, with an electric field strength of 1.0 kV/cm, a pulse duration of 10 μs, and a period of 1000 μs.

2.5. Polyphenol Extraction of Defatted AKB

The extraction of total polyphenols from the AKB was carried out using either water or the DES. Ethanol was also employed as a means of comparison. In all cases, 1 g of dry sample was mixed with 10 mL of solvent, and the mixture was stirred for 15 min at room temperature. This mixture was then used in the extraction procedures. For the main extraction step, the mixture was stirred at 500 rpm for 3 h at 60 °C. In all cases, after extraction was completed, the extracts were centrifuged for 5 min at 10,000 rpm and the samples were directly analyzed. A flow diagram showing the detailed methodology can be seen in Figure 1.
PEF was also employed either as a pretreatment step (before the main extraction procedure) or as a complementary step (after the main extraction procedure). In the first case, the AKB–solvent mixture was placed in the PEF cell, with applied PEF for 15 min, and then extracted, as described above. In the latter case, after the main extraction step, the mixture was placed in the PEF cell, with PEF applied for 15 min. When PEF was used as a standalone extraction procedure, the mixture was placed in the PEF cell, with PEF applied for 15 min. For comparison with the PEF procedure, control samples were prepared. For the preparation of the control samples, the solvent was mixed with the AKB, stirred for 15 min at room temperature, and then placed for 15 min in the PEF cell without PEF application.

2.6. Determinations
2.6.1. Total Polyphenol Content (TPC)

The TPC was determined using the Folin–Ciocalteu assay [33]. In brief, 0.1 mL of sample was mixed with 0.1 mL of Folin–Ciocalteu reagent. After 2 min, 0.8 mL of Na₂CO₃ solution (5% w/v) was added, and the mixture was heated at 40 °C for 20 min. Then, the absorbance was recorded at 740 nm using a spectrophotometer (Shimadzu UV-1700 PharmaSpec Spectrophotometer, Kyoto, Japan). Determination of the total polyphenol concentration ($C_{TP}$) was performed by preparing a calibration curve with gallic acid. The results are expressed as mg of gallic acid equivalents (GAE) per L. The extraction of TPC is expressed as mg GAE per g dry weight (dw), using the following equation:

$$TPC \ (\text{mg GAE/g dw}) = \frac{C_{TP} \times V}{w}$$

where $V$ is the volume of the extraction medium (in L) and $w$ is the dry weight of the sample (in g).

2.6.2. Total Flavonoid Content (TFC)

A method that has been previously described was used for determining the TFC [34]. In an Eppendorf tube, 100 µL of the sample was added, along with 860 µL of a 35% (v/v) ethanol in water mixture and 40 µL of a solution containing 5% (w/v) aluminum chloride and 0.5 M sodium acetate. The mixture was incubated for 30 min at room temperature in the absence of light. Then, the absorbance was measured at 415 nm. The total flavonoid...
concentration ($C_{TF}$) was calculated with a calibration curve prepared using rutin. The extraction of TFC is expressed as mg rutin equivalents (RtE) per g dw, according to the following equation:

$$TFC \text{ (mg RtE/g dw)} = \frac{C_{TF} \times V}{w}$$

(2)

where $V$ is the volume of the extraction medium (in L) and $w$ is the dry weight of the sample (in g).

2.6.3. Ferric-Reducing Antioxidant Power (FRAP) Assay

For the FRAP study, a previously described technique was used [32]. In an Eppendorf tube, 50 µL of the sample was mixed with 50 µL FeCl$_3$ solution (4 mM in 0.05 M HCl), and the mixture was incubated for 30 min at 37 °C. Then, 900 µL of TPTZ solution (1 mM in 0.05 M HCl) was added, and the absorbance was recorded at 620 nm after 5 min. The ferric-reducing power ($P_R$) was calculated with a calibration curve prepared with ascorbic acid ($C_{AA}$, 50–500 µmol/L in 0.05 M HCl). The $P_R$ is expressed as µmol ascorbic acid equivalents (AAE) per g of dw, using the following equation:

$$P_R \text{ (µmol AAE/g dw)} = \frac{C_{AA} \times V}{w}$$

(3)

where $V$ is the volume of the extraction medium (in L) and $w$ is the dry weight of the sample (in g).

2.6.4. Antiradical Activity (DPPH$^\bullet$ Assay)

A previously mentioned method was applied for the DPPH$^\bullet$ assay [32]. In an Eppendorf tube, 25 µL of the extract was added along with 975 µL of DPPH$^\bullet$ solution (100 µM in methanol). The absorbance at 515 nm was recorded immediately after mixing ($A_{515(i)}$) and after 30 min ($A_{515(f)}$). The capacity to scavenge the DPPH$^\bullet$ radical is expressed as:

$$\text{Inhibition (\%)} = \left( \frac{A_{515(i)} - A_{515(f)}}{A_{515(i)}} \right) \times 100$$

(4)

Antiradical activity ($A_{AR}$) was determined as µmol ascorbic acid equivalents (AAE) per g of dw, using an ascorbic acid calibration curve, with the following equation:

$$A_{AR} \text{ (µmol AAE/g dw)} = \frac{C_{AA} \times V}{w}$$

(5)

where $V$ is the volume of the extraction medium (in L) and $w$ is the dry weight of the sample (in g).

2.7. Statistical Analysis

All the sample preparation procedures and extracts described above were repeated three times. Moreover, all determinations were carried out in triplicate. Thus, a total of nine measurements were used per condition for statistical analysis. The results are expressed as the mean values of the nine measurements ± standard deviation. The Shapiro–Wilk test was used to examine the normal distribution of the results. The Mann–Whitney U test and the Kruskal–Wallis test were used to assess statistically significant differences between the samples. All statistical analyses were carried out using SPSS (version 26) (SPSS Inc., Chicago, IL, USA) software.

3. Results and Discussion

3.1. Polyphenol Extraction

The use of a proper solvent is a major issue in polyphenol extraction since it has a big impact on the extraction yield of polyphenols [35]. It is reported that polyphenols are
more readily dissolved in organic solvents, such as ethanol and acetone. However, it is also reported that in some cases, water is a more appropriate solvent [36]. Furthermore, regardless of the extraction yield, the solvent must also be safe for human consumption (in case the extract will be used in such a way), or it must be removed until no traces of the solvent can be detected. Such a procedure would be time-consuming and cost-inefficient. Therefore, selecting safer solvents is a better option. In our case, the first step was to examine the effect of using water for polyphenol extraction from AKB. The results are presented in Figure 2.

As can be seen, when water was employed, ~4 mg GAE/g dw was detected in AKB. The results are similar to a previous report [37] in which the authors examined 32 cultivars of apricots and the AKB was examined for their TPC by using a methanol:water (3:1) mixture as a solvent while the extraction time was 24 h. In 31 cultivars, the TPC of the AKB was found to be in the range of 0.6–4.2 mg GAE/g dw, with an average value of 2.67 mg GAE/g dw. In one case, the TPC was found to be 12.77 mg GAE/g dw, which was considered an outlier. Similar results were also reported in the study of Göttingerová et al. [38]. Therefore, the TPC recorded in our case is in close agreement with this study. Next, we evaluated the performance of ethanol, which is commonly employed for polyphenol extraction. As can be seen, when ethanol was used, the total polyphenol content was nearly halved compared to that obtained with the aqueous extract. Therefore, it can be inferred that the polyphenols contained in the AKB are more polar; thus, they are better dissolved in water.

The next step was to evaluate whether the use of PEF would increase the extraction yield. For this reason, PEF was first examined as a standalone extraction technique. According to the results, a 55% increase in the TPC was recorded compared to the case where water and the common extraction procedure were employed (statistically significant for \( p < 0.05 \)). This is a major increase considering that the increased TPC can be achieved in 15 min compared to the 3-hour extraction procedure. A similar increase of about 44% was recorded when PEF was used for the extraction of polyphenols from Vitis vinifera, Sideritis scardica, and Crocus sativus [39]. In the study of Frontuto et al. [40], the authors examined...
the use of PEF in order to increase the TPC of potato peel extracts. Under the optimum conditions (solvent, temperature, and time of extraction), the use of PEF achieved a 10% increase in the TPC. In our case, when PEF was combined with the extraction procedure, a further increase in the TPC was recorded. More specifically, when PEF was employed after the extraction step, a 78% increase in the TPC of the extract was recorded compared to the extract prepared with the common extraction procedure (statistically significant for \( p < 0.05 \)). When PEF was employed prior to the extraction step, an 88% increase in the TPC of the extract was recorded compared to the extract prepared with the common extraction procedure (statistically significant for \( p < 0.05 \)). These percentages are relatively higher compared to other studies [39,40].

To avoid the aforementioned problems with the presence of solvents in food products, it was chosen to examine the use of a DES for the extraction of polyphenols from AKB. More specifically, a choline-based DES was selected for two main reasons: (I) choline is a low-cost solvent, compatible with food and cosmetics, and (II) reports are stating that choline-based DESs exhibit an enhanced performance for polyphenol extraction compared to common organic solvents [41–43]. Furthermore, the extraction process is significantly impacted by the amount of water added to the DES [22]. As can be seen in Figure 2, the TPC in the DES-based extract was nearly 70% increased compared to the aqueous extract (statistically significant for \( p < 0.05 \)). Moreover, it was found to be comparable with the aqueous extracts prepared with PEF. This suggests that the examined DES is more suitable to extract the polyphenols contained in the AKB. This may be due to its high polarity (higher than water) and due to the fact that the DES can form more extended networks of hydrogen bonds, thus better solubilizing and stabilizing polyphenols [44]. Since the polarity of the DES is higher than that of water, it can be speculated that compounds of lower polarity may not be well dissolved, thus making the solvent more “selective” towards polyphenols. Saini et al. [21] reported that the use of DESs considerably increases the efficacy of extraction and recycling of both the bioactive components and the solvent. In our previous study, it was showcased that the use of a DES can yield extracts with a 540% higher TPC compared to using water [32]. Likewise, DES-based extracts of \textit{Cistus creticus} had an increased TPC by up to 75% compared to aqueous extracts [44].

Similar to the above case, when PEF was used as a standalone procedure, a \(~30\%\) increase in the TPC was recorded. When PEF preceded the extraction procedure, the TPC increased by 61% compared to the simple extraction procedure, while a 173% increase in the TPC was recorded compared to the simple extraction with water (\( p < 0.05 \)). The above content is significantly higher compared to those reported in previous reports [3,37]. For instance, when PEF was employed as a pretreatment step before the extraction of polyphenols from \textit{Cistus creticus} with a DES, it was found that PEF increased the TPC by up to 16% [44]. This suggests not only that DESs are superior for the extraction of polyphenols from AKB, but also that the use of PEF can significantly enhance the extraction yield. On the industrial scale, the PEF continuous extraction method produced encouraging outcomes in terms of the extraction rate of phytoconstituents [30].

3.2. Determination of Flavonoids

The flavonoids content of the extracts was also examined. The results are summarized in Figure 3. As can be seen, the extracts’ total flavonoid content (TFC) follows the same trend as the TPC. More specifically, when water was used, a content of 3.78 mg RtE/g dw was determined. This value is higher compared to previous reports [37,45]. In the case of Dulf et al. [45], the apricot kernel extract was found to contain \(\sim 2.5\) mg of quercetin equivalents, while Rampackova et al. [37] reported a maximum content of 1.53 mg of Trolox equivalents. In both cases, the reduced concentration of flavonoids can be attributed to inadequate extraction. In the first case, extraction was carried out for 30 min at 40°C, and in the second case, extraction was carried out for 24 h without stirring or heating [37,45]. As regards the use of the DES, a 69% increase (\( p < 0.05 \)) in the TFC was recorded, suggesting that the DES is more appropriate for flavonoids extraction. Li et al. [46] reported that the
The antioxidant activity of the extracts was evaluated using the FRAP and DPPH• assays. The results can be seen in Figures 4 and 5, respectively. In both cases, the antioxidant activity followed the same pattern as the polyphenol content. More specifically, in the FRAP assay, the water extract exhibited a $P_R$ of 10.8 μmol AAE/g dw, while the extract prepared with ethanol exhibited 20% less activity ($p < 0.05$). When PEF was employed, the highest $P_R$ value was recorded in the case of PEF prior to the main extraction step. The $P_R$ value was 52% higher compared with the respective control sample ($p < 0.05$). When the DES was employed, a $P_R$ value similar to that obtained with water was recorded. However, when PEF and the DES were combined (PEF prior to the extraction step), the $P_R$ value of the extract increased by 120% compared to the respective control ($p < 0.05$). In a previous study, it was found that the use of PEF increased the FRAP activity of white grape pomace extracts by 36% [47]. In another study, the use of a DES improved the FRAP activity of apricot pulp waste extracts by 590% [32]. In both cases, the FRAP values increased the same way the TPC of the extracts increased. This was somewhat anticipated since the antioxidant activity is attributed, mainly, to the polyphenols contained within an extract. Our results are also in line with the previous findings. More specifically, the extract with the highest TPC content also exhibited the highest FRAP activity.
Overall, it can be inferred that the use of the DES resulted in extracts with increased antioxidant activities. Similarly, the use of PEF increased the extracts’ antioxidant activity, while when the two methods (DES + PEF) were combined, enhanced antioxidant activity was recorded.

**Figure 4.** Ferric-reducing power (PR) assay of the extracts, expressed as μmol AAE/g dw with different solvents and extraction procedures; standard deviations are presented with error bars, and means with different superscript letters (a–i) are significantly (p < 0.05) different. PEF: pulsed electric field; EX: extraction for 3 h at 60 °C.

**Figure 5.** Antiradical activity (AR) or DPPH• assay of the extracts, expressed as μmol AAE/g dw with different solvents and extraction procedures; standard deviations are presented with error bars, and means with different superscript letters (a–i) are significantly (p < 0.05) different. PEF: pulsed electric field; EX: extraction for 3 h at 60 °C.

As regards the antiradical activity (AR) of the samples, the extract prepared with water exhibited a value of 6.84 μmol AAE/g dw, while the extract prepared with ethanol exhibited 43% less activity. When PEF was employed, the highest AR was recorded in the case of PEF prior to the main extraction step. The AR was 84% higher compared with the respective control sample (p < 0.05). When the DES was employed, an AR increase of 20%
was recorded (compared to that with water as the solvent). However, when PEF and DES were combined (PEF prior to the extraction step), the \( A_{AR} \) of the extract increased by 75\% compared to the respective control \( (p < 0.05) \).

Overall, it can be inferred that the use of the DES resulted in extracts with increased antioxidant activities. Similarly, the use of PEF increased the extracts' antioxidant activity, while when the two methods (DES + PEF) were combined, enhanced antioxidant activity was recorded.

4. Conclusions

In this study, the extraction of polyphenols from AKB was described. A common extraction procedure with water was employed as a reference. In order to produce polyphenol-rich extracts, the use of a DES and PEF was examined. According to the results, the use of the DES increased the TPC of the extracts by 70\%. Similarly, the use of PEF increased the TPC of the extracts by up to 88\% when PEF was used prior to the extraction step. It is noteworthy that when PEF and the DES were used in combination, the TPC increased by up to 173\%. Moreover, increases in the TFC, ferric-reducing power, and antiradical activity were also recorded. Based on the above, it can be concluded that the AKB resulting after the oil extraction of kernels (used for industrial purposes) is a valuable source of polyphenols. Moreover, the use of PEF and a DES contributes significantly to the enhancement of the extraction yield. Therefore, this combination can be used as an alternative route for the production of polyphenol-rich extracts.

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