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Original article

Pressurised hot water extraction of phenolic compounds with a focus on eriocitrin and hesperidin from lemon peel

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Summary In this study, the extraction of bioactive compounds from lemon peel, a by-product of the food industry, was investigated using pressurised hot water extraction (PHWE) at different extraction temperatures (40–200 °C) and times (5–30 min) under 10.34 MPa pressure. The selectivity of the PHWE process on eriocitrin and hesperidin extraction was also tested. The highest total phenolic content (TPC) (59.57 mg gallic acid equivalents g^{-1}), total flavonoid content (TFC) (8.22 mg catechin equivalents g^{-1}) and antioxidant capacity by DPPH (42.59 mg Trolox equivalents (TE) g^{-1}) were obtained at 160 °C for 30 min. The maximum eriocitrin (30.41 mg g^{-1}) and hesperidin (25.90 mg g^{-1}) contents were achieved at 160 °C for 5 min with a 5-hydroxymethyl furfural content of 0.07 mg g^{-1}. PHWE provided better results for individual compounds and antioxidant capacities than conventional extraction. The results indicated the potentiality of PHWE in the selective extraction of eriocitrin and hesperidin from lemon peel by controlling the extraction temperature and time.

Keywords Bioactive compounds, eriocitrin, hesperidin, lemon peel, pressurised hot water extraction.

Introduction

Lemon (*Citrus limon* L.), belonging to the family *Rutaceae*, is one of the most valuable citrus fruits in the world (González-Molina et al., 2010; Mahato et al., 2018). Lemon is widely consumed as fresh fruit in household applications such as salad dressings. However, more amount of lemon is processed into some products such as lemonade, juice, jams, jellies, molasses and marmalades. Both domestic consumption and industrial processing of lemon generate a substantial amount of by-products such as peels, pulp and seeds. Lemon peel is well-known as a good source of polyphenols including phenolic acids and flavonoids (Xi et al., 2017). Flavanones are the most abundant flavonoids in lemon peel. The flavanones exhibit a wide range of promising bioactivity, that is antioxidant, anti-inflammatory, anticancer, antimicrobial and anticholesterolemic activity (Barreca et al., 2017). Two main flavanones in lemon peel are eriocitrin (Eriodictyol-7-*O*-rutinoside) and hesperidin (Hesperetin-7-*O*-rutinoside) (Patrón-Vázquez et al., 2019; Peiró et al., 2019). The principal sources of flavanones are citrus species (Chanet et al., 2012; Li et al., 2006). Therefore, the selective extraction of these compounds from lemon peel is an important issue due to their potential health benefits.

Selective extraction is an application that focuses on the extraction of specific compounds (Lefebvre et al., 2021). Conventional extraction techniques (*e.g.* Soxhlet extraction and maceration) with organic solvents (methanol, ethanol and acetone) or water have commonly been used to recover polyphenols from plant materials. In these applications, selective extraction can be achieved using organic solvents having different polarities, and water or solvent mixtures. However, some of the organic solvents are toxic, poorly biodegradable, flammable and costly. Even though organic solvents such as ethanol, ethyl acetate and ethyl lactate are considered green solvents, the greenest option is the use of water in the extraction process. In general, water dissolves polar compounds at room temperature and atmospheric pressure due to its nature. Therefore, water is considered a poor solvent for the extraction of less polar compounds (Teo et al., 2010). However, this drawback of water can be overcome by applying the appropriate pressure and temperature.

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Pressurised hot water extraction (PHWE) is an emerging technology based on the use of water as an extraction solvent at elevated temperatures and pressures (Alaşalvar & Çam, 2019). In this technology, water is heated to high temperatures and kept under sufficient pressure to maintain its liquid state. Under these conditions, the viscosity, surface tension and dielectric constant of water decrease significantly (Kronholm et al., 2007). The decrease in viscosity and surface tension of water allows it better penetrate the plant matrix and increases the mass transfer rate (Munir et al., 2018; Teo et al., 2010). As the dielectric constant of water also decreases, the polarity of water approaches near to that of organic solvents such as ethanol and methanol (Herrero et al., 2006; Kheirkhah et al., 2019; Teo et al., 2010). This improves the extractability of less polar or nonpolar compounds using water. Moreover, PHWE could be a promising method for the selective extraction of specific compounds by changing the temperature-dependent polarity of water. However, there are some concerns regarding the PHWE of carbohydrate-rich materials due to the formation of Maillard reaction products such as 5-hydroxymethyl furfural (5-HMF) (Özkaynak Kanmaz, 2018; Tomšik et al., 2017).

Based on these considerations, the purpose of this study was to recover bioactive compounds with a focus on eriocitrin and hesperidin from lemon peel using PHWE. The effects of extraction temperature (40–200 °C) and extraction time (5–30 min) were investigated by determining total phenolics, total flavonoids, eriocitrin, hesperidin and 5-HMF contents of pressurised hot water extracts along with antioxidant capacity. Besides, the efficiency of PHWE was compared with that of conventional solvent extraction.

Materials and methods

Plant material

Fresh lemon fruits were purchased from a local market in Kayseri, Turkey. Lemon peel (flavedo and albedo) was manually removed from the fruit. The peels were spread on trays and dried without direct sunlight contact at room temperature for 7 days. The dried peels were ground to fine particles (500–3550 μm) using a Waring blender and stored at 4 °C before extraction.

Chemicals

Catechin and eriocitrin were supplied from Extrasynthese (Lyon, France). Other chemicals were purchased from Sigma Aldrich Co. (St. Louis, MI, USA) or Merck (Germany) unless otherwise stated. All chemicals and solvents were of analytical grade. Deionised water (resistivity 18.2 MΩ.cm) was prepared using Millipore Simplicity 185 water purification system (Darmstadt, Germany).

Pressurised hot water extraction and experimental design

PHWE of phenolic compounds from lemon peel was performed using an accelerated solvent extractor (ASE 350, Dionex, Sunnyvale, CA, USA). The dried lemon peel (10 g) was loaded into a 100-mL stainless steel extraction cell containing a 30 mm cellulose filter (Dionex, Sunnyvale, CA, USA). Then, extractions were done with the following fixed parameters: Pressure (10.34 MPa, default setting), rinse volume (30%), purge (90 s with N_2 gas) and extraction solvent (deionised water). In experiments, independent variables were extraction temperature (40, 70, 100, 130, 160 and 200 °C) and static extraction time (5, 10, 20 and 30 min). Dependent variables or responses were selected as total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity by DPPH (AC_{DPPH}), ABTS (AC_{ABTS}), eriocitrin, hesperidin and 5-HMF contents. The effects of independent variables on dependent variables were evaluated by using a one variable at a time approach. Firstly, the effect of extraction temperature on PWHE of phenolic compounds from lemon peel was tested for an extraction time of 10 min by changing the level of extraction temperature. Secondly, the effect of static extraction time was evaluated at the temperature selected in the first part by changing the level of static extraction time.

Conventional solvent extraction

Conventional extraction was done to evaluate the effectiveness of PHWE. Briefly, 1 g of lemon peel was mixed with 100 mL of distilled water or 72% ethanol and placed in a water bath (ST30; Nüve, Ankara, Turkey) at 80 °C for 6 h (Li et al., 2006). The resulting extracts were filtered and taken to the analyses.

Determination of total phenolic content, total flavonoid content and antioxidant capacity

TPC was estimated by using the Folin–Ciocalteu's phenol reagent, based on procedures described by Çam & Hişil (2010). Gallic acid was used as a standard to express the TPC as mg gallic acid equivalents per gram (mg GAE g^{-1}). TFC was measured according to the aluminium chloride colourimetric assay (Zhishen et al., 1999). The TFC of the extracts was expressed as mg catechin equivalents per gram (mg CE g^{-1}).

AC_{ABTS} of extracts was estimated following the procedure of Re et al. (1999) with some modifications (Çam et al., 2009). AC_{DPPH} analysis was performed

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according to Brand-Williams et al. (1995). The results were expressed as mg Trolox equivalent per gram (mg TE g^{-1}) using a six-level linear calibration curve of Trolox.

HPLC analysis of eriocitrin and hesperidin

Identification and quantification of eriocitrin and hesperidin were done by an HPLC system (Shimadzu, Kyoto, Japan) equipped with an SPD-M20A photodiode array detector. Chromatographic separations were achieved using a Brisa LC2 C18 column (150 mm × 4.6 mm, 5 μm; Teknokroma, Barcelona, Spain). The injection volume was 20 μL. The extracts were filtered through a membrane filter with a 0.45 μm pore size before analyses. The mobile phase was a mixture of 0.1% glacial acetic acid in distilled water (solvent A) and 0.1% glacial acetic acid in acetonitrile (solvent B) (Peiró et al., 2019). Gradient elution was programmed as follows: from 0 to 5 min, 10% B; from 5 to 85 min, 10–80% B; from 85 to 90 min, 80–10% B. The column temperature was maintained at 30 °C and the flow rate was 0.6 mL min^{-1}. Eriocitrin and hesperidin were detected at 283 nm. Peak purities were checked by software (LC Solution 1.25) using UV–vis spectra (200–400 nm). Quantifications of eriocitrin and hesperidin were based on external standards.

HPLC analysis of 5-HMF

The extracts were prepared and analysed as described by Garcia-Villanova et al. (1993). 5-HMF content was determined by an HPLC system (Shimadzu) coupled with an SPD-M20A photodiode array detector and a SilUR KC18 (150 mm × 4.6 mm, 5 μm, USeM Ar-Ge, Kayseri, Turkey). The mobile phase consisted of distilled water (95%) plus acetonitrile (5%). The flow rate, column temperature and injection volume were 1 mL min^{-1}, 32 °C and 40 μL, respectively. The samples were filtered through a membrane filter with a 0.45 μm pore size before injection. 5-HMF was monitored at 284 nm. The 5-HMF contents of the extracts were calculated using an external standard method.

Statistical analyses

Analyses were done minimally in duplicate. The results were expressed as mean ± standard deviation. The significance of differences among extracts was determined by one-way analysis of variance (ANOVA). Comparisons were performed by Tukey's *post hoc* test using a level of 95% confidence ($\alpha = 0.05$) *via* the SPSS 24 statistical package for Windows (SPSS Inc., Chicago, IL, USA). Also, differences between conventional extraction solvents were determined using the t-test.

Results and discussion

Effect of temperature

Table 1 shows TPC, TFC, AC_{DPPH}, AC_{ABTS}, eriocitrin, hesperidin and 5-HMF values obtained from the extractions at various temperatures. The representative chromatogram of eriocitrin and hesperidin is displayed in Figure S1. TPC, TFC, AC_{DPPH} and AC_{ABTS} values of extracts ranged from 10.12 to 54.78 mg GAE g^{-1}, 1.36 to 6.23 mg CE g^{-1}, 3.26 to 47.35 mg TE g^{-1} and 15.04 to 75.05 mg TE g^{-1}, respectively. TPC, TFC, AC_{DPPH} and AC_{ABTS} values gradually increased with extraction temperature increasing from 40 to 200 °C. The highest results for these responses were obtained at 200 °C. These significant increases at 200 °C can be attributed to the decrease in polarity, viscosity and surface tension of pressurised hot water. The changes in these characteristics of water improve diffusion and hydrolytic degradation (Teo et al., 2010). Phenolic compounds are found in soluble free, soluble esters or conjugated and insoluble-bound forms in plant matrix. Insoluble-bound forms of phenolic compounds are linked with the cell wall structural components (Zhang et al., 2020). Pressurised hot water, depending on the final properties of water, might hydrolyse and depolymerise cell wall structural components of plant materials (Mayanga-Torres et al., 2017; Prado et al., 2014). This can allow the extraction and release of bound phenolics as well as increase the presence of the total bioactive substances in the extracts.

On the other hand, Çam & Hişil (2010) found that punicalagin can be hydrolysed to ellagic acid during PHWE of pomegranate peel. In another study, the conversion of conjugated isoflavones to their non-conjugated aglycones (Nkurunziza et al., 2019) was also reported. In this study, new compounds derived from the degradation or conversion of phenolic compounds in the lemon peel can have a higher antioxidant capacity than their intact forms. The antioxidant capacity of phenolic compounds is not only related to their concentration but also their structural properties. In addition to the formation or conversion of phenolic compounds, new compounds with antioxidant properties that are derived from Maillard, caramelisation and thermoxidation reactions during PHWE can contribute to the overall antioxidant capacity of the extracts (Plaza et al., 2010). In parallel to the above statement, the 5-HMF formation was observed in the extracts of this study obtained at 160 and 200 °C. The higher the 5-HMF content, the extracts displayed better antioxidant capacity results. On the contrary, no effect of Maillard products such as 5-HMF on antioxidant capacity was reported in some studies (Herrero et al., 2012; Tomšik et al., 2017). As for variations in the 5-HMF content depending on the temperature, no

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Table 1 Effect of temperature on extraction responses at the fixed time of 10 min

Temperature (°C)	TPC (mg GAE g^{-1})	TFC (mg CE g^{-1})	AC_{DPPH} (mg TE g^{-1})	AC_{ABTS} (mg TE g^{-1})	Eriocitrin (mg g^{-1})	Hesperidin (mg g^{-1})	5-HMF (mg g^{-1})
40	10.12 ± 0.10^{a}	1.36 ± 0.23^{a}	3.26 ± 0.67^{a}	15.04 ± 0.96^{a}	4.46 ± 0.29^{a}	0.97 ± 0.03^{a}	n.d.
70	11.04 ± 0.12^{b}	1.35 ± 0.05^{a}	3.42 ± 0.61^{a}	18.47 ± 0.90^{a}	8.14 ± 0.57^{b}	1.57 ± 0.13^{a}	n.d.
100	12.86 ± 0.39^{c}	1.93 ± 0.02^{ab}	4.50 ± 1.43^{a}	32.36 ± 0.93^{b}	8.83 ± 0.24^{b}	2.07 ± 0.01^{a}	n.d.
130	14.26 ± 0.23^{d}	2.18 ± 0.10^{b}	5.35 ± 0.30^{a}	44.22 ± 1.18^{c}	21.34 ± 2.19^{d}	4.97 ± 0.69^{b}	n.d.
160	53.51 ± 0.52^{e}	5.41 ± 0.12^{c}	26.38 ± 3.00^{b}	71.02 ± 0.59^{d}	18.29 ± 1.84^{c}	18.29 ± 1.13^{c}	0.41 ± 0.02^{a}
200	54.78 ± 0.22^{f}	6.23 ± 0.74^{d}	47.35 ± 8.21^{c}	75.05 ± 1.31^{e}	7.17 ± 0.44^{ab}	5.93 ± 0.35^{b}	1.03 ± 0.01^{b}

Different lowercase letters in the same column indicate statistically significant differences ($P < 0.05$). n.d., not detected.

5-HMF formation was detected up to 130 °C, whereas its amount was 0.41 and 1.03 mg g^{-1} for extracts obtained at 160 and 200 °C, respectively. Özkaynak Kanmaz (2018) investigated 5-HMF formation from lemon peel using PHWE. In that study, the 5-HMF was detected in the extracts with the application of temperatures higher than 140 °C depending on the time.

Eriocitrin and hesperidin contents in the extracts displayed a distinctive behaviour compared to TPC, TFC and antioxidant capacity (Table 1). The highest results for eriocitrin (2.34 mg g^{-1}) and hesperidin (18.29 mg g^{-1}) were obtained at 130 and 160 °C, respectively. Increases beyond these temperatures significantly ($P < 0.05$) decreased the amount of eriocitrin and hesperidin in the extracts. The temperature-dependent polarity of water and differences in their thermal stability could be the reason for the effective extraction of these two compounds at different temperatures. Similar results for extraction temperatures of eriocitrin and hesperidin were reported in the studies of Çam et al. (2019) and Cheigh et al. (2012), respectively. In the study of Çam et al. (2019), 130 °C was reported to be an effective temperature for eriocitrin extraction from peppermint using PHWE. According to Cheigh et al. (2012), 160 °C is the most suitable temperature for the PHWE of hesperidin from *Citrus unshiu* peel. On the contrary, the effectiveness of different temperatures for hesperidin extraction has been reported in some previous studies. For example, 150 °C was found as the optimum temperature for hesperidin extraction by Lachos-Perez et al. (2018), who evaluated the subcritical water extraction of flavanones from defatted orange peel. In that study, however, temperatures beyond 150 °C were not tested. In the study by Ko et al. (2014) in which hesperidin was extracted from orange peel, 170 °C was the most effective temperature. The authors evaluated the effect of temperature on hesperidin in increments of 20 °C in the range of 110–200 °C, and the effect of 160 °C remained unknown.

Overall, total phenolics, total flavonoids and antioxidant compounds were more soluble in pressurised hot water than eriocitrin and hesperidin at relatively higher temperatures. TPC and TFC as well as the antioxidant capacity of the extracts were not directly related to eriocitrin and hesperidin contents. Also, the use of high temperatures led to the formation of 5-HMF. Consequently, considering the high extraction efficiency on total bioactive compounds, relatively high selectivity on eriocitrin and hesperidin, and low 5-HMF content of 160 °C; it was selected to be used further experiments.

Effect of time

The effects of extraction time on TPC, TFC, AC_{DPPH}, AC_{ABTS}, eriocitrin, hesperidin and 5-HMF results are presented in Table 2. In this step, it was desired to find the suitable static extraction time among selected static times; 5, 10, 20 and 30 min. As in the case of extraction temperature, the prolonged extraction time had a positive effect on the TPC, TFC and antioxidant capacities of pressurised hot water extracts. TPC, TFC and AC_{ABTS} increased continuously from 5 to 20 min, although no statistically significant difference ($P > 0.05$) was observed between 20 and 30 min. However, an increase from 20 to 30 min was significant for AC_{DPPH}. The highest TPC (59.57 mg GAE g^{-1}), TFC (8.22 mg CE g^{-1}) and AC_{DPPH} (42.59 mg TE g^{-1}) were determined for 30 min, whereas the highest AC_{ABTS} (87.28 mg TE g^{-1}) were observed at 20 min. The results indicated that there might be increases in the values of TPC, TFC and antioxidant capacities if the static extraction time is extended beyond 30 min. However, this will bring about another drawback which is the loss in the contents of eriocitrin and hesperidin as the time is extended. The highest results for eriocitrin (30.41 mg g^{-1}) and hesperidin (25.90 mg g^{-1}) were determined at 5 min extraction time. This trend was consistent with the findings of Song et al. (2018) who evaluated the total phenolic and luteolin contents of pressurised water extract of carrot leaves. The authors reported that the highest TPC was observed at 210 °C for 113.5 min, whereas the highest luteolin content was measured at 120 °C

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Table 2 Effect of time on extraction responses at the fixed temperature of 160 °C

Time (min)	TPC (mg GAE g^{-1})	TFC (mg CE g^{-1})	AC_{DPPH} (mg TE g^{-1})	AC_{ABTS} (mg TE g^{-1})	Eriocitrin (mg g^{-1})	Hesperidin (mg g^{-1})	5-HMF (mg g^{-1})
5	35.65 ± 0.43[a]	5.30 ± 0.26[a]	18.23 ± 0.76[a]	60.80 ± 5.80[a]	30.41 ± 0.95[c]	25.90 ± 1.73[d]	0.07 ± 0.00[a]
10	52.01 ± 1.18[b]	7.17 ± 0.24[b]	29.14 ± 2.54[b]	71.16 ± 7.45[b]	17.92 ± 0.94[b]	18.37 ± 1.01[c]	0.30 ± 0.02[b]
20	58.18 ± 1.46[c]	8.20 ± 0.45[c]	38.13 ± 3.02[c]	87.28 ± 9.31[c]	14.98 ± 1.14[b]	12.98 ± 0.44[b]	0.62 ± 0.00[c]
30	59.57 ± 1.79[c]	8.22 ± 0.34[c]	42.59 ± 3.01[d]	84.71 ± 7.55[c]	4.36 ± 0.66[a]	4.82 ± 0.37[a]	1.06 ± 0.01[d]

Different lowercase letters in the same column indicate statistically significant differences ($P < 0.05$).

Table 3 Conventional extractions of lemon peel with water and aqueous ethanol

Solvent	TPC (mg GAE g^{-1})	TFC (mg CE g^{-1})	AC_{DPPH} (mg TE g^{-1})	AC_{ABTS} (mg TE g^{-1})	Eriocitrin (mg g^{-1})	Hesperidin (mg g^{-1})	5-HMF (mg g^{-1})
72% ethanol	52.01 ± 1.18[b]	1.87 ± 0.07[b]	6.20 ± 0.51[b]	16.83 ± 1.30[b]	10.46 ± 0.74[b]	2.80 ± 0.21[b]	n.d.
Water	35.65 ± 0.43[a]	1.14 ± 0.05[a]	4.12 ± 0.76[a]	11.41 ± 1.18[a]	7.58 ± 0.42[a]	0.48 ± 0.05[a]	n.d.

Different lowercase letters in the same column indicate statistically significant differences ($P < 0.05$). n.d., not detected.

for 10 min. In this study, the extension of the extraction time resulted in significant decreases in eriocitrin and hesperidin contents (Table 2). This can be attributed to prolonged extraction time which might have resulted in the destruction or hydrolysis of eriocitrin and hesperidin to their respective aglycones called eriodictyol and hesperetin. As the amount of eriocitrin and hesperidin declined, the antioxidant activity-related terms began to increase which indicated that hydrolysis was the most probable situation. The changes in the amounts of hesperidin observed in this study are consistent with the findings of Grohmann et al. (2000), who reported that 140–160 °C and 0.05–0.5% H_2SO_4 (no info about time) in the reaction medium were suitable conditions for obtaining hesperetin-7-glucoside from hesperidin that was normally quite resistant to hydrolysis at ambient conditions. In previous studies, the effective extraction of hesperidin from various citrus peels was achieved at 160 °C for 10 min (Cheigh et al., 2012), 170 °C for 10 min (Ko et al., 2014) and 153 °C for 15 min (Šafranko et al., 2021). Also, Šafranko et al. (2021) performed an optimisation process to minimise 5-HMF content and maximise hesperidin content. Then, the optimal conditions were 130 °C for 14 min. In this study, the 5-HMF content of the extracts, ranging from 0.07 to 1.06 mg g^{-1}, increased with the extension of time (Table 2). A similar observation in the PHWE of artichoke leaf, lemon peel and flaxseed meal was reported by Özkaynak Kanmaz (2018). The effects of temperature and time on eriocitrin have been evaluated in limited studies. Eriocitrin (Eriodictyol-7-*O*-rutinoside), a disaccharide (rutinose) form of eriodictyol, may produce 2 different forms depending on hydrolysis conditions called eriodictyol-7-*O*-β-glucoside and eriodictyol. Yamamoto & Muto (2015) studied the conversion conditions of eriocitrin to eriodictyol-7-*O*-β-glucoside and eriodictyol. The authors reported that eriocitrin was converted to eriodictyol-7-*O*-β-glucoside and eriodictyol with conversion yields of 25.4 and 70.2%, respectively, when eriocitrin was treated with 1 N HCl at boiling temperature for 30 min. Very limited conversion yields (4.8% for eriodictyol-7-O-β-glucoside and 1.2% for eriodictyol) were reported at 0.01 N HCl at boiling temperature for 30 min (Yamamoto & Muto, 2015).

Comparison between pressurised hot water extraction and conventional solvent extractions

The efficiency of PHWE and conventional extractions was compared in terms of TPC, TFC, AC_{DPPH}, AC_{ABTS}, eriocitrin, hesperidin and 5-HMF contents. The results of conventional solvent extractions are presented in Table 3. Among organic solvents, methanol is one of the best solvents for the extraction of phenolics from citrus peel. However, the use of ethanol is recommended due to the higher toxicity of methanol compared to ethanol (M'hiri et al., 2014). Generally, aqueous ethanol is preferred in the extraction of phenolic compounds, specifically for the extracts that are to be used in subsequent food enrichment applications. In this study, the application of 72% ethanol as an extraction solvent showed higher results for all responses compared to water extraction. Nevertheless, the results showed that 72% ethanol resulted in lower extraction efficiency than the PHWE results in Table 2, except for TPC. Eriocitrin (10.46 mg g^{-1})

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and hesperidin (2.80 mg g^{-1}) contents of the extracts obtained at 80 °C for 6 h were remarkably lower than those of PHWE at 160 °C for 5 min (Table 2). A shorter extraction time is beneficial for industrial applications from an economic point of view. Also, 5-HMF was not detected in 72% ethanol and water extracts of lemon peel.

Conclusion

In this study, the PWHE technique was investigated to evaluate the effects of extraction conditions on phenolic antioxidant compounds and individual compounds from lemon peels. Increasing temperature and time during PHWE increased TPC, TFC and antioxidant capacities of pressurised water extracts. The results indicated that eriocitrin and hesperidin were successfully extracted using PHWE together with high efficiency and a shorter time. Future studies are required to elucidate the effects of PHWE on other individual phenolics in lemon peel. As the glycosidic and aglycone forms of phenolics have diverse biological activities, the optimum extraction conditions should be studied and determined based on the target form of phenolics are to be in the final extracts.

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Author contributions

Hamza Alaşalvar: Conceptualization (equal); investigation (equal); methodology (equal); writing – original draft (equal). **Murat Kaya:** Formal analysis (equal); investigation (equal); methodology (equal); validation (equal); writing – review and editing (equal). **Serap Berktaş:** Formal analysis (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Bülent Başyiğit:** Investigation (equal); validation (equal); writing – original draft (equal). **Mustafa Cam:** Conceptualization (equal); funding acquisition (lead); investigation (equal); methodology (equal); resources (lead); supervision (lead); writing – review and editing (equal).

Conflict of interest

There is no conflict of interest.

Ethical approval

Ethics approval was not required for this research.

Data availability statement

Research data are not shared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Representative HPLC chromatogram of eriocitrin (Peak 1) and hesperidin (Peak 2) at 280 nm.

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