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This is the peer reviewed version of the following article:
Papoutsis, K. et al. 2016. Enhancement of the total phenolic compounds and antioxidant activity of aqueous Citrus limon L. pomace extracts using microwave pre-treatment on the dry powder. *Journal of Food Processing and Preservation*

which has been published in final form at doi: 10.1111/jfpp.13152

Enhancement of the total phenolic compounds and antioxidant activity of aqueous *Citrus limon* L. pomace extract using microwave pre-treatment on the dry powder

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Abstract

The effect of microwave pre-treatment on the levels of total phenolic compounds, flavonoids, proanthocyanidins and individual major compounds as well as the total antioxidant activity of the dried lemon pomace was investigated. The results showed that microwave pre-treatment significantly affected all the examined parameters. The total phenolic content, total flavonoids, proanthocyanidins, as well as the total antioxidant activity significantly increased as the microwave radiation time and power increased (e.g., 2.5 folds for phenolics, 1.4 folds for flavonoids and 5.5 folds for proanthocyanidins), however irradiation more than 480 W for 5 min resulted in the decrease of these parameters. These findings indicate that microwave irradiation time and power may enhance higher levels of the phenolic compounds as well as the antioxidant capacity of the dried lemon pomace powder. However, higher and longer irradiation may lead to a degradation of phenolic compounds and lower the antioxidant capacity of the dried lemon pomace.

Practical Applications

Lemon pomace could be a good source of bioactive compounds and antioxidants. Microwave radiation could be applied for the enhancement of the total phenolic compounds and antioxidants of the lemon pomace dried powder. The findings of this study can be applied for enhancing the bioactive compounds and the antioxidant activity of the dried lemon pomace for further extraction, isolation and utilisation.

Keywords: microwave pre-treatment; flavonoids; proanthocyanidins; antioxidants; lemon pomace

Introduction

Lemon *(Citrus limon L.)* is the third most important *Citrus* species after orange and mandarin, with a high commercial value, generating a large amount of waste. Lemon peel representing the main component of waste, accounts for 50 to 65% of the whole fruit weight (González-Molina *et al.* 2010). Lemon peel contains bioactive compounds, such as vitamin C (ascorbic acid), flavonoids (flavanones, flavonols, flavones) and phenolic acids (ferulic, p-coumaric and sinapic acids) (Bocco *et al.* 1998; González-Molina *et al.* 2010), which have been linked to antimicrobial (Dhanavade *et al.* 2011), anticancer (Rawson *et al.* 2014) and antioxidant activities (Proteggente *et al.* 2002; Wilmsen *et al.* 2005).

Several studies have reported that *Citrus* peels contain more bioactive compounds compared to the pulp (Abeysinghe *et al.* 2007; Goulas and Manganaris 2012). Gorinstein *et al.* (2001) reported that the contents of the total phenolics in peels of lemons, oranges, and grapefruit were higher than in those of the peeled fruits. Guimarães *et al.* (2010) also showed that orange, lemon, lime, and grapefruit peels contain higher total phenolic and flavonoid content compared to those measured in the juice.

Phenolic compounds are secondary metabolites derived from the amino acid phenylalanine through the shikimate pathway and phenylpropanoid metabolism (Robards and Antolovich 1997) which can be classified into water-soluble compounds (phenolic acids, phenylpropanoids, flavonoids and quinones) and water-insoluble compounds (condensed tannins, lignins and cell-wall bound hydroxycinammic acids) (Tomás-Barberán and Espín 2001).

The phenolic compounds contained in lemon peel include phenolic acids (hydroxyl cinnamic acids) (Ma *et al.* 2008) and flavonoids (flavanones, flavones and flavonols) (Bilbao *et al.* 2007). Phenolic acids are often linked to various plant components through ester, ether, or acetal bonds (Xu *et al.* 2007). González-Molina *et al.* (2010) showed that lemon flavonoids can be present in the aglycones or glycoside forms. The liberation of phenolic compounds of *Citrus* peels may result in extracts with increased antioxidant activity and could be achieved by different treatments (Jeong *et al.* 2004; Xu *et al.* 2007; Kim *et al.* 2008; Hayat *et al.* 2010).

Microwaves are electromagnetic radiation with a frequency of 0.3 to 300 GHz (Kaufmann *et al.* 2001). Because of their electromagnetic nature, microwaves have electric and magnetic fields which are perpendicular to each other (Kaufmann and Christen 2002). The electric field results in heating via dipolar rotation and ionic conduction which happens simultaneously (Kaufmann and Christen 2002). This heat may result in the liberation of some

phenolic compounds, such as phenolic acids from the matrix (Hayat *et al.* 2010). Bioactive compounds from lemon peel have been studied for their utilization by the food and pharmaceutical industries (González-Molina *et al.* 2010; Dhanavade *et al.* 2011; Dahmoune *et al.* 2013). Therefore, it is necessary to enhance the levels of the bioactive compounds as well as the antioxidant capacity of the lemon pomace for further extraction and isolation.

The aim of this study was to examine the effect of microwave pre-treatment on the levels of phenolic compounds, flavonoids, and proanthocyanidins as well as the antioxidant activity of the dried lemon pomace.

Materials and Methods

Material

Lemon (*Citrus limon* L.) waste including peel (flavedo and albedo) and seeds was obtained from a commercial juicing factory at Kulnura, NSW Australia in September 2015. After collection, the seeds were removed and the remaining peel and pomace flesh were stored immediately at -18 °C, until used. The frozen lemon waste was dipped into liquid nitrogen and freeze dried (FD3 freeze dyer, Thomas Australia Pty. Ltd., Seven Hills, NSW, Australia). The dried waste was ground using a commercial blender (John Morris Scientific, Chatswood, NSW, Australia) and sieved using a steel mesh sieve (1.4 mm EFL 2000; Endecotts Ltd., London, England). The ground lemon waste was kept in a sealed and labeled container at -18 °C for further analysis.

Microwave pre-treatment

A household microwave oven (1200 W, Frequency 2450 MHz, Sharp Carousel, Abeno-ku, Osaka, Japan) was used for the irradiation of *Citrus* pomace dried powder. Lemon (*Citrus limon* L.) pomace powder had water content of 14.29% ± 0.33%. Powder of lemon pomace (1.5 g) was put into a 100 mL glass beaker and heated in a microwave oven at 120, 240, 360, 480, and 600 W, for 2 and 5 min. Radiation times of 2 and 5 min were selected according to preliminary experiments. 2 min was selected as the minimum time while 5 min as the maximum for retaining the phenolic compounds in the treated samples. After microwave treatment the treated powder was allowed to cool at ambient temperature. Non-heated (untreated) powder was used as a control.

Extraction procedure

Ultrasound-assisted extraction was applied with water as a solvent at a sample-to-solvent ratio of 1:100 g/mL. The extraction process was conducted using an ultrasonic bath (Soniclean, 220 V, 50 Hz and 250 W, Soniclean Pty Ltd., Thebarton, Australia) with pre-set conditions at temperature of 30 °C, time of 20 min and power of 60 W. Agitation was conducted for 10 sec once every 5 min using a Vortex (Bacto Laboratories Pty Ltd., NSW, Australia). After completion of the extraction, the extracts were immediately cooled on ice and then centrifuged at 3500 x g for 10 min at 14 °C. Then the supernatants were collected and stored in the dark at -18 °C until used for quantitative analysis and antioxidant determination.

Total phenolic compounds (TPC)

The TPC was measured as described by Vuong *et a*l. (2013). Briefly, 5 mL of 10% (v/v) Folin-Ciocalteu reagent were mixed with 1 mL of sample and 4 mL of 7.5% (w/v) Na_2CO_3 and incubated in the dark at room temperature for 1 h before the absorbance was measurement (λ= 760 nm) by UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). The results were expressed as mg gallic acid equivalents per g of sample dry weight (mg GAE/g dw).

Total flavonoid content (TF)

The total flavonoid content was measured as described by Zhishen *et al.* (1999). Briefly, 2 mL of H_2O and 0.15 mL of 5% (w/v) $NaNO_2$ were added to 0.5 mL of sample and left at room temperature for 6 min. Then 0.15 mL of 10% (w/v) $AlCl_3$ was added and left at room temperature for 6 min. Subsequently, 2 mL of 4% (w/v) NaOH and 0.7 mL of H_2O were added and the final volume of the mixture left at room temperature for a further 15 min before the absorbance was measured (λ = 510 nm) using a UV spectrophotometer. The results were expressed as mg catechin equivalents per g of sample dry weight (mg CE/g dw).

Proanthocyanidins

The Proanthocyanidins were determined according to a method described by Li *et al.* (2006). Vanillin (3 mL, of 4% w/v) was added to 0.5 mL of sample. Subsequently, 1.5 mL of concentrated HCl was added to the mixture and left for 15 min at room temperature before

the absorbance was measured at (λ = 500 nm) using a UV spectrophotometer. The results were expressed as mg catechin equivalents per g of sample dry weight (mg CE/g dw).

Methods for the determination of antioxidant properties

DPPH assay: DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used for the determination of the antioxidant activity as described by Thaipong *et al.* (2006), with some modifications. A stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and then stored at −20 °C until required. The working solution was then prepared fresh by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 (λ = 515 nm). Subsequently, 2.85 mL of working solution was added to 0.15 mL of sample and left under darkness at room temperature for 30 min before measuring the absorbance (λ = 515 nm) using a UV spectrophotometer. The results were expressed as mg trolox equivalents per g of dry weight (mg TE/g dw).

FRAP assay: FRAP (Ferric reducing antioxidant power) was measured as described by Thaipong *et al.* (2006) with some modifications. A working FRAP solution was prepared by mixing 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM $FeCl_3$ in the ratio of 10:1:1. Working FRAP solution (2.85 mL) was added to 0.15 mL of sample and incubated at room temperature in the dark for 30 min before its absorbance was read (λ = 593 nm) using a UV spectrophotometer. The results were expressed as mg trolox equivalents per g of dry weight (mg TE/g dw).

CUPRAC assay: CUPRAC (cupric reducing antioxidant capacity) was determined as described by Apak *et al.* (2004) with some modifications. $CuCl_2$ (1 mL) was mixed with 1 mL of neocuproine and 1 mL of NH_4Ac. Subsequently 1.1 mL of sample was added to this mixture. The mixture was incubated at room temperature for 1.5 h before measuring the absorbance (λ = 450 nm) using a UV spectrophotometer. The results were expressed as mg trolox equivalents per g of sample dry weight (mg TE/g dw).

Analysis of major individual bioactive compounds by HPLC

Major individual bioactive compounds were determined using high-performance liquid chromatography (HPLC) (Shimadzu Australia, Rydalmere, NSW, Australia). C_{18} reversed-phase column (Gemini 110A 5 µm, 150 × 4.6 mm Phenomenex Australia Pty., Ltd., Lane Cove, NSW, Australia) was used. The working solution (20 µL) was injected onto the HPLC

system under the following conditions: flow rate of solvents = 0.5 mL/min, UV detector = 280 nm. Gradient elution (two solvent systems) was utilized: system A: water: acetonitrile: formic acid, 95:4:1 (v:v:v) and system B: acetonitrile. Gradient elution schedule: 100% A from 0 to 5 min; a linear gradient from 100% A to 100% B from 5 to 45 min, then remaining at 100% B to 50 min; then back to 100% A at 60 min with a post-run re-equilibration time of 10 min with 100% A, prior to the next injection. A representative HPLC chromatogram for lemon pomace extract solution is shown in Fig. 3. As the current study only determined the effect of microwave irradiation on the major individual bioactive compounds, the name of each compound has not yet identified. Five peaks were identified and their quantities were quantified based on gallic acid standard ($R^2 = 0.98$) and reported in mg GAE/g.

Statistical analysis

The one-way ANOVA and the Least Significance Difference (LSD) were conducted using the SPSS statistical software version 23. Data were reported as means ± standard deviations. Differences between the mean levels of the components in the different experiments were taken to be statistically significant at $p < 0.05$. The Pearson correlation test was employed to determine the correlation coefficients among bioactive compounds and different antioxidant assays at $p < 0.01$.

Results and discussion

Effect of microwave pre-treatment on the total phenolic compounds

The effect of microwave pre-treatment on the total phenolic compounds of lemon pomace are presented in Fig. 1(a). The microwave pre-treatment had a significant effect on the total phenolic content ($p < 0.05$, coefficient of variation (CV) = 12.16%). At the irradiation time of 2 min, the total phenolic content increased more than double from 10.73 to 22.05 mg GAE/g dw when the irradiation power increased from 120 to 480 W, however the total phenolic content significantly decreased when the irradiation power continued to increase to 600 W. At the irradiation time of 5 min, the total phenolic content increased 60% when increasing irradiation power from 120 to 360 W. However, the total phenolic content significantly decreased from 22.05 to 11.71 mg GA/g dw when the irradiation power exceeded 360 W. The increase of phenolic content can be explained by the liberation and cleavage of phenolic acids and flavonoids during the microwave treatment due to the electromagnetic radiations (Hayat *et al.* 2010). These results are in agreement with Xu *et al.* (2007) who reported that

heat treatment of *Citrus* peel powder resulted in an increased phenolic compound content. However, irradiation at higher power might result in lower level of phenolic content due to degradation by heat. In the current study irradiation at 2 min with power greater than 480 W or irradiation at 5 min with power greater than 360 W resulted in lower level of phenolic content. Hayat *et al.* (2010) also found that the levels of phenolic acids in mandarin pomace decreased when increasing microwave power from 125 to 500 W. However, it should be noted that the effect of irradiation power depends on species because phenolic compounds and their stability to heat varied depending on species (Jeong *et al.* 2004).

It is interesting to note that at the irradiation power of 360 W or lower, lemon pomace sample treated with longer irradiation time (5 min) had higher levels of total phenolic content in comparison with shorter irradiation time (2 min). In contrast, at the irradiation power of greater than 360 W, the lemon pomace sample treated with longer irradiation time (5 min) had lower levels of total phenolic content in comparison with shorter irradiation time (2 min). The variation can be explained by the impact of temperature on phenolic compounds. At low power, more phenolic compounds could be liberated when longer time applied. However, at high power level, longer irradiation time could increase the loss of phenolic compounds due to heat degradation. Overall, these findings show that pre-treatment could increase the levels of phenolic content of lemon pomace and the highest content of total phenolic compounds was obtained when lemon powder was treated at 360 W for 5 min (57% higher than the phenolic content of untreated sample).

Effect of microwave pre-treatment on the total flavonoids

The microwave pre-treatment had a significant effect on the total flavonoid content ($p < 0.05$, CV = 3.06%) (Fig. 1(b)). At the irradiation time of 2 min, the total flavonoid content increased with increasing power from 120 to 480 W. However, the total flavonoid content decreased when the irradiation power exceeded 480 W. At the irradiation time of 5 min, the total flavonoid content increased when increasing power from 120 to 360 W. However, the total flavonoid content decreased when the irradiation power exceeded 360 W.

At irradiation power of 360 W or lower, the sample treated with longer irradiation time had higher level of total flavonoid content. These findings indicated that flavonoids were also affected by the heat generated by the electromagnetic radiations, which resulted in the liberation and cleavage of flavonoids during the microwave treatment (Hayat *et al.* 2010). However, at irradiation power of 480 W or higher, the sample treated with longer irradiation time (5 min) had significant lower levels of flavonoids in comparison with those treated with

shorter irradiation time (2 min). This can be explained by the degradation of some flavonoid compounds due to their exposure at high temperature for longer time. These results are in agreement with Hayat *et al.* (2010) who reported that the flavonoid content of mandarin pomace increased as the microwave power increased, while at higher irradiation and longer treatment time the flavonoids were reduced. Xu *et al.* (2007) also reported that long exposure of *Citrus* pomace powder at high temperature resulted in the reduction of narirutin, naringin, hesperidin, and neohesperidin which are flavanone glycosides presenting in *Citrus*. In general, the highest flavonoid content was achieved at 360 W for 5 min and 480 W for 2 min (6.28 and 6.21 mg CE/g dw, respectively) which was 29 and 28%, respectively higher than the untreated sample.

Effect of microwave pre-treatment on the proanthocyanidins

Proanthocyanidins (also known as condensed tannins) are secondary metabolites derived from the flavonoid pathway and have been linked to antioxidant, antibacterial, antiviral, and anticancer activities (Hellström *et al.* 2009; Khanal *et al.* 2010). Since proanthocyanidin levels of *Citrus* are very low, it is important to increase the concentration of these compounds by using different methods (Hellström *et al.* 2009). This study examined the impact of various irradiation powers and times on the levels of proanthocyanidins.

The microwave pre-treatment had a significant effect on the proanthocyanidin content of the lemon pomace powder ($p < 0.05$, CV = 2.17%) (Fig. 1(c)). The levels of proanthocyanidins increased with increasing power. However, irradiation more than 360 W for 5 min resulted in the decrease of proanthocyanidin content. The highest proanthocyanidin content was achieved at 360 W for 5 min (7.40 mg CE/g dw) which was 82% higher compared to the untreated sample. At higher irradiations (480 and 600 W) and longer treatment time the proanthocyanidin content decreased, indicating that prolong treatment time at high irradiation may lead to a degradation of proanthocyanidins. These results are in agreement with Khanal *et al.* (2010) who reported that heating decreased proanthocyanidins content in blueberry and grape pomace.

Effect of microwave pre-treatment on the antioxidant activity

The microwave pre-treatment had a significant effect on the antioxidant activity of the lemon pomace extracts as measured by DPPH, FRAP and CUPRAC ($p < 0.05$, CV = 12.69%, 10.54% and 6.24%, respectively) (Fig. 2(a), 2(b), 2(c)). The antioxidant activity of the extracts increased with increasing microwave power, however irradiation more than 480 W

for 5 min resulted in the decrease of the antioxidant activity of the three assays. The highest antioxidant activity (DPPH, FRAP and CUPRAC) was achieved at 360 W for 5 min (0.23, 19.40 and 50.70 mg TE/g dw, respectively) which was 76%, 69% and 58% higher compared to the antioxidant activity of the untreated sample. These results are in agreement with Hayat *et al.* (2010) who showed that the antioxidant activity of mandarin peel extracts increased as the microwave power applied in the powder increased due to the liberation of phenolic acids. Jeong et al. (2004) also reported that the antioxidant activity of *Citrus unshiu* peels increased as the heating temperature applied on the powder increased. Indeed when microwave irradiation passes through the medium, its energy may be absorbed and converted into thermal energy (Zhang *et al.* 2011). In this study as the microwave power and time increased, the colour of the powder turned to brown, indicating the possible production of Maillard reaction products which have been reported to have antioxidant properties (Vhangani and Van Wyk 2013). The reduction in the antioxidant activity could be attributed to the degradation of some phenolic compounds, due to the high temperature created by the applied microwave energy, since high correlation between phenolic compounds and antioxidant activity of *Citrus* has been observed (Lagha-Benamrouche and Madani 2013; Garcia-Castello *et al.* 2015). For instance, Hayat *et al.* (2010) reported that increasing microwave treatment on the mandarin powder from 250 W, 10 min to 500 W, 5 min resulted in the degradation of hesperidin, vanillic acid, p-coumaric acid and ferulic acid. Overall, appropriate microwave treatment on the powder could enhance the antioxidant activity of lemon pomace extracts.

Effect of microwave pre-treatment on individual compounds

The current study only determined the effect of microwave pre-treatment on five individual bioactive compounds of lemon pomace, therefore the name of each bioactive compound has not yet identified. The results showed that microwave pre-treatment had a significant effect on the individual bioactive compounds ($p < 0.05$) (Table 1). In general, at both irradiation times of 2 min and 5 min, the content of each bioactive compound significantly increased when increasing irradiation power from 120 to 480 W, however, the levels of individual compounds decreased when irradiation power exceeded 480 W. These findings further illustrated that these individual bioactive compounds were significantly affected by the heat generated by the electromagnetic radiations.

It is interesting to note that at each irradiation power, the levels of the individual compounds varied depending on the irradiation time. For example, at the powers of 120, 240 or 480 W, the content of compounds 1 and 2 was not significantly different when increasing

irradiation time from 2 to 5 min. However, at the power of 360 W the content of compounds 1 and 2 was doubled when irradiation time increased from 2 to 5 min. The content of these compounds significantly decreased when the irradiation time increased from 2 to 5 min at the power of 600 W. The variation could be explained by the liberation as well as the degradation of these bioactive compounds due to the heat generated by the electromagnetic irradiation. Hayat *et al.* (2010) reported that microwave treatment of *Citrus* mandarin powder, resulted in the increase of the free fraction of phenolic acids and the decrease of bound fractions, whereas the content of the flavonoids catechin and hesperidin increased with power but, at longer irradiation time, it declined. Similarly, Xu *et al.* (2007) reported that heat treatment of *Citrus* powder resulted in the increase of the p-coumaric acid, ferulic acid and vanillic acid content, while chlorogenic acid content, as well as narirutin, naringin, hesperidin and neohesperidin decreased with increasing heating time and temperature. These findings further confirm that microwave irradiation power and time may lead to the liberation of the bound phenolic compounds of *Citrus* peels resulting in higher antioxidant capacity, but also might cause their degradation. Therefore, it is necessary to determine the appropriate microwave pre-treatment conditions to obtain high content of some individual phenolic compounds in lemon pomace.

Correlation between TPC, TF, proanthocyanidins and antioxidant capacity

The contribution of TPC, TF, and proanthocyanidins to the total antioxidant activity can be evaluated by their correlation between each component and the antioxidant assays (Candrawinata *et al.* 2015). The correlation (*r* values) between TPC, TF, proanthocyanidins and DPPH, FRAP and CUPRAC indicated high contribution of TPC, flavonoids and proanthocyanidins to the antioxidant properties of the lemon pomace extracts ($p < 0.01$) (Table 2). These results are in agreement with previous studies which indicated that TPC had a close correlation with the antioxidant character and were the major contributor to the antioxidant properties of *Citrus* extracts, because of their potential electron donor capacity, due to the usual presence of multiple hydroxyl groups (Lagha-Benamrouche and Madani 2013; Garcia-Castello *et al.* 2015). One the other hand Goulas and Manganaris (2012) and Anagnostopoulou *et al.* (2006) have mentioned weak correlation between total phenolic compounds and antioxidants in different *Citrus* species. These differences should be attributed to the different solvents and extraction methods used in each experiment, since solvent type and extraction method have been shown to play an important role for the recovery of the phenolic compounds (Khoddami *et al.* 2013).

Conclusion

Microwave pre-treatment was found to significantly affect the levels of the total phenolic compounds, total flavonoids, proanthocyanidins, individual bioactive compounds as well as the antioxidant activity of the dried lemon pomace. Microwave pre-treatment of the dried lemon pomace at irradiation time of 5 min and power of 360 W could increase the levels of TPC, flavonoids and proanthocyanidins 2.5, 1.4 and 5.5 folds respectively, compared to the control (untreated powder). Microwave power and time were also found to significantly increase the levels of major bioactive compounds of the dried lemon pomace. Similarly, microwave pre-treatment was found to significantly increase the antioxidant capacity of the aqueous extracts as there was a close correlation between bioactive compounds and antioxidant capacity. However, higher and longer irradiation times were found to decrease the levels of TPC, flavonoids, proanthocyanidins, major individual compounds as well as the antioxidant capacity. This study found that microwave pre-treatment could significantly increase the levels of bioactive compounds and antioxidant capacity of the dried lemon pomace and future studies are recommended to comprehensively study on the impact of a wide range of microwave treatments on the different types of lemon pomace bioactive compounds.

Acknowledgements

This research was supported by the University of Newcastle and Australian Research Council (ARC) Training Centre for Food and Beverage Supply Chain Optimisation (IC140100032). NSW Department of Primary Industries is a partner organisation in the Training Centre.

Conflict of interest

The authors declare no conflict of interest.

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Figures

Fig. 1. Effect of different microwave pre-treatment conditions on the total phenolic compounds (TPC) (a), total flavonoids (TF) (b) and proanthocyanidins (c) of aqueous lemon pomace extracts. The values are the mean of three replications. Columns not sharing the same superscript letter are significantly different at $p < 0.05$.

Fig. 2. Effect of different microwave pre-treatment conditions on the antioxidant activity (DPPH (a), FRAP (b) and CUPRAC (c)) of aqueous lemon pomace extracts. The values are the mean of three replications. Columns not sharing the same superscript letter are significantly different at $p < 0.05$.

Fig. 3. High-Performance Liquid Chromatogram of lemon (*Citrus limon* L.) pomace extract. Detection was measured at 280 nm and all peaks were designated from 1 to 5 as the retention time increased to 70 min.

Tables

Table 1. Effect of different microwave pre-treatment conditions on individual bioactive compounds of aqueous lemon pomace extracts.

Treatment	mg GAE/g				
	1	2	3	4	5
Control	0.64±0.12^{d}	0.05±0.01^{e}	0.61±0.11^{f}	8.93±0.16^{efgh}	2.38±0.52^{def}
120W2min	0.63±0.17^{d}	0.05±0.01^{e}	0.98±0.03^{def}	7.99±0.84^{fgh}	2.46±0.63^{cde}
120W5min	0.67±0.08^{d}	0.10±0.01^{e}	0.69±0.12^{ef}	6.92±1.08^{hij}	1.61±0.31^{ef}
240W2min	0.94±0.16^{cd}	0.33±0.05^{de}	1.46±0.26^{cd}	7.65±0.59^{ghij}	1.49±0.12^{e}
240W5min	1.53±0.28^{c}	0.91±0.19^{d}	1.82±0.37^{bc}	5.91±1.49^{j}	2.89±0.39^{bcd}
360W2min	2.91±0.46^{b}	1.67±0.36^{c}	2.04±0.45^{b}	9.59±1.24^{defg}	3.29±0.30^{abc}
360W5min	4.15±0.53^{a}	2.65±0.39^{b}	2.32±0.40^{b}	10.14±0.70^{abcd}	3.67±0.52^{ab}
480W2min	4.44±0.05^{a}	3.31±0.19^{a}	2.97±0.12^{a}	11.18±0.19^{abc}	4.12±0.33^{a}
480W5min	4.32±0.47^{a}	3.32±0.32^{a}	1.46±0.07^{cd}	9.86±0.69^{bcdef}	3.09±0.24^{bcd}
600W2min	3.93±0.43^{a}	3.76±0.38^{a}	1.24±0.0.04^{de}	9.15±0.41^{defg}	3.26±0.11^{abcd}
600W5min	2.51±0.44^{b}	1.61±0.29^{c}	0.83±0.08^{ef}	5.94±0.91^{ij}	2.72±0.45^{cd}

The 5 bioactive compounds detected by high-performance liquid chromatography using UV detector at 280 nm as shown in Fig. 1. The values are the mean ± standard deviation for at least triplicate experiments and those in the same column not sharing the same superscript letter are significantly different from each other at $p < 0.05$.

Table 2. Correlation between bioactive compounds and antioxidant properties of lemon peels ($p < 0.01$).

	TPC		TF		Proanthocyanidins	
	r	*p* value	*r*	*p* value	*r*	*p* value
DPPH	0.91	***	0.82	***	0.81	***
FRAP	0.89	***	0.86	***	0.92	***
CUPRAC	0.90	***	0.87	***	0.87	***