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Impact of different solvents on the recovery of bioactive compounds and antioxidant properties from lemon (*Citrus limon* L.) pomace waste

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Abstract The effects of different solvents on the recovery of (i) extractable solids (ES), (ii) total phenolic compounds (TPC), (iii) total flavonoid content (TFC), (iv) vitamin C, and (v) antioxidant activity from lemon pomace waste were investigated. The results revealed that solvents significantly affected the recovery of ES, TPC, TFC, and antioxidant properties. Absolute methanol and 50% acetone resulted in the highest extraction yields of TPC, whereas absolute methanol resulted in the highest extraction of TFC, and water had the highest recovery of vitamin C. 50% ethanol, and 50% acetone had higher extraction yields for TPC, and TFC, as well as higher antioxidant activity compared with their absolute solvents and water. TPC and TFC were shown to be the major components contributing to the antioxidant activity of lemon pomace.

Keywords: lemon peel, total flavonoid, ascorbic acid, extractable solid, antioxidant

Introduction

Citrus fruits from the family *Rutaceae* include oranges, lemons, limes, grapefruits, and tangerines and are well known for their nutritional value as they are good sources of dietary fiber, vitamin C, vitamin B group, carotenoids, flavonoids, and limonoids (1). Several recent studies have demonstrated anti-inflammatory activity (2) and have linked citrus extracts with the prevention of colon cancer (3).

Worldwide citrus production has exceeded 88×10^6 tons (2), and approximately 34% of this production has been used by the juice industry, resulting in high amounts of waste (4). Citrus pomace includes peel composed from flavedo, albedo, and seed. These have been found to be good sources of phenolic acids, flavonoids, vitamin C (ascorbic acid), molasses, essences, seed oil, and pectins (4,5).

Lemon (*Citrus limon* L.) is considered as the third most important citrus species after orange and mandarin, with a strong commercial value, generating a large amount of waste. Lemon peel represents the main component of waste, i.e., between 50% and 65% of the whole fruit weight (6). Lemon peel contains bioactive compounds such as vitamin C, flavonoids (flavanones, flavonols, and flavones), and phenolic acids (ferulic, p-coumaric, and sinapic acids) (6,7), which have been linked to antimicrobial (8) and antioxidant activities (9).

Several studies have examined the recovery of bioactive compounds from lemon peel for valorization by food and pharmaceutical industries (6,8). Solvent type has been shown to play an important role for the optimum recovery of these compounds (10). Several solvents have been used for the recovery of bioactive compounds from citrus, with methanol known as a solvent commonly used for the recovery of phenolic compounds from citrus (11). To the best of our knowledge, there is no study investigating the effect of different solvents on the recovery of phenolic compounds, flavonoids, vitamin C, and extractable solids (ES) from lemon pomace waste. Therefore, the aim of this study was to investigate the effects of different solvents including water, methanol, ethanol, and acetone and the combination between these organic solvents with water at a ratio of 50:50 (v/v) on the recovery of total phenolic compounds, total flavonoids, vitamin C, and antioxidant activity of lemon pomace.

Materials and Methods

Lemon waste including peel and seeds was obtained from a commercial juicing factory in Kulnura, NSW, Australia. After collection, the seeds were removed and the remaining peel and pomace flesh were stored immediately at −18°C. The frozen lemon waste was

dipped in liquid nitrogen and freeze-dried (FD3 freeze dryer; Thomas Australia Pty. Ltd., Seven Hills, Australia). The dried waste was ground using a commercial blender (Waring 2-speed blender, John Morris Scientific, Chatswood, 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.

Extraction process Seven extraction solvents were used for comparison, including water, absolute methanol, ethanol, acetone, 50% methanol, 50% ethanol, and 50% acetone. An ultrasonic bath (Soniclean, 220 V, 50 Hz, and 250 W; Soniclean Pty Ltd., Thebarton, Australia) was used for the extraction. Briefly, 1 g of dried lemon pomace was mixed with 100 mL of solvent and exposed to 60 W ultrasonic power for 20 min at a temperature of 30°C. Agitation was conducted for 10 s once every 5 min using a vortex. After completion of the extraction process, the extracts were centrifuged at 3,500× *g* for 10 min at 14°C. Then, the supernatants were collected using pipet and diluted 10 folds (for the determination of total phenolic content (TPC), vitamin C, 2,2-diphenyl-1-picrylhydrazyl (DPPH), cupric reducing antioxidant capacity (CUPRAC), and 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assays), whereas sample without dilution was used for the determination of total flavonoid content (TFC) and ES. Subsequently, they were stored in the dark at −18°C until used for quantitative analysis and antioxidant determination.

Extractable solids ES of lemon pomace were estimated according to the method reported by Vuong (12) with a minor modification. 2 mL of the supernatant was kept in an oven (set at 110°C) until the solvent being completely evaporated. ES were expressed as percentage, and the equation, ES (%)=W x 100/2 (W: Weight of 2 mL after drying in g), was used for the calculation.

Total phenolic content TPC was measured as described by Vuong (13). 5 mL of 10% (v/v) Folin-Ciocalteu reagent was mixed with 1 mL of diluted sample and 4 mL of 7.5% (w/v) Na_2CO_3 and incubated in the dark at room temperature for 1 h. The absorbance was measured at 760 nm using a UV spectrophotometer (Cary 50 Bio; Varian Australia Pty. Ltd., Victoria, Australia). The results were expressed as mg of gallic acid equivalents per g of sample dry weight (mg GAE/g dw).

Total flavonoid content TFC was measured as described by Zhishen (14). 2 mL of H_2O, 0.15 mL of 5% (w/v) $NaNO_2$, and 0.5 mL of sample were mixed and left for 6 min at room temperature and then 0.15 mL of 10% (w/v) $AlCl_3$ was added and left for 6 min. Subsequently, 2 mL of 4% (w/v) NaOH and 0.7 mL of H_2O were added and kept at room temperature for 15 min before the absorbance was measured at 510 nm. The results were expressed as mg of catechin equivalents per g of sample dry weight (mg CE/g dw).

Total vitamin C The total vitamin C was measured according to the method described by Vuong (15) with a minor modification. A solution was prepared by mixing 500 mL of 0.6 M sulfuric acid with 5.3218 g of sodium phosphate and 2.471 g of ammonium molybdate. 3 mL of the solution was mixed with 0.3 mL of diluted sample and incubated at 95°C for 90 min in a water bath. After incubation, they were left at room temperature for 30 min and the absorbance was measured at 695 nm. The results were expressed as mg ascorbic acid equivalents per g of sample dry weight (mg AAE/g dw).

Assays for measurement of antioxidant activity

***DPPH assay*:** DPPH was used for the measurement of antioxidant activity, as reported by Thaipong (16), with minor modifications. A stock solution was prepared and stored at −20°C until used. A working solution was prepared by mixing 10 mL of the stock solution with 45 mL methanol to obtain an absorbance of 1.1±0.02 at 515 nm. 2.85 mL of the working solution was mixed with 0.15 mL of diluted sample and left in the dark for 3 h before measuring the absorbance at 515 nm. The results were expressed as mg of trolox equivalents per g of sample dry weight (mg TE/g dw).

***CUPRAC assay*:** CUPRAC was performed as described by Apak (17) with some modifications. 1 mL of $CuCl_2$, 1 mL of neocuproine, 1 mL of NH_4Ac, and 1.1 mL of diluted sample were mixed. The mixture was left at room temperature for 1.5 h before the absorbance was measured at 450 nm. The results were expressed as mg of trolox equivalents per g of sample dry weight (mg TE/g dw).

***ABTS assay*:** ABTS assay was used for the determination of antioxidant activity, as described by Thaipong (16), with some modifications. A stock solution was prepared and stored at −20°C until used. A working solution was prepared by diluting 1 mL of the stock solution with 60 mL of methanol to obtain an absorbance value of 1.1±0.02 at 734 nm. 2.85 mL of the working solution was mixed with 0.15 mL of diluted sample and left in the dark at room temperature for 2 h before the absorbance was measured at 734 nm. The results were expressed as mg trolox equivalents per g of sample dry weight (mg TE/g dw).

Statistical analysis A one-way analysis of variance was conducted using SPSS (version 23, SPSS Inc., Chicago, IL, USA). Least significant difference was applied for the comparison of means at $p<0.05$. Data were reported as means±standard deviations. 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 solvents on extractable solids ES comprise all soluble compounds such as sugars, proteins, pectins, vitamins, minerals, and phytochemicals, which are extracted during the extraction process.

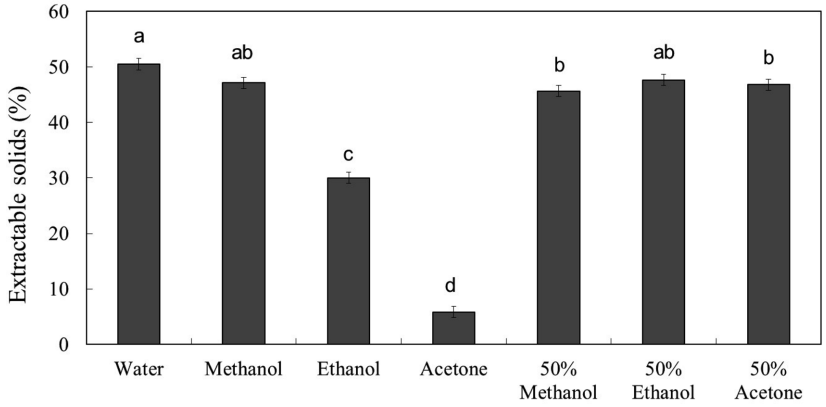

Fig. 1. Effect of solvents on the recovery of extractable solids (ES) from lemon peels. The values are the mean average of three replications for each solvent±standard deviation. Columns not sharing the same superscript letter are significantly different at $p<0.05$.

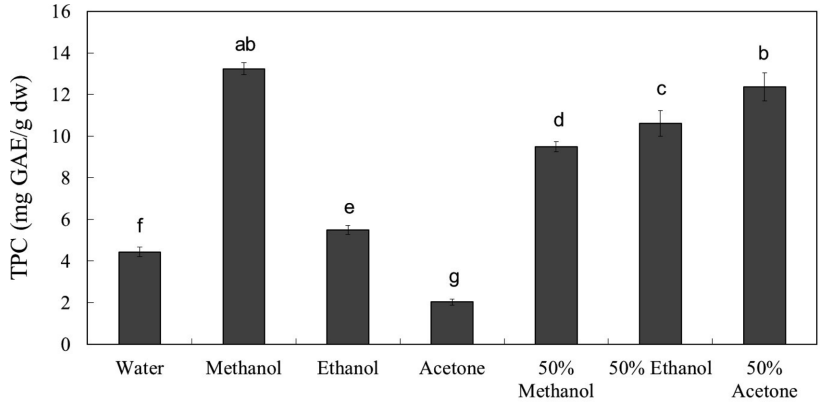

Fig. 2. Effect of solvents on the recovery of total phenolic compounds (TPC) from lemon peels. The values are the mean of three replications for each solvent±standard deviation. Columns not sharing the same superscript letter are significantly different at $p<0.05$.

The solvents had a significant effect on the ES content ($p<0.05$) (Fig. 1). Water, absolute methanol, and 50% ethanol had the highest levels of ES, whereas absolute acetone had the lowest content of ES. Variation in the ES content can be explained via different types of bioactive compounds present in lemon peels, such as carotenoids, phenolic compounds, ascorbic acid, fibers, and pectins and their different solubilities in various types of solvents. For instance, lipophilic compounds, such as carotenoids, can be easily extracted via organic solvents (18), whereas hydrophilic compounds, such as ascorbic acid, pectins, and sugars, can be extracted via water or aqueous alcohols (19,20). The results are supported by the results of a previous study in almond hulls, which reported that ES were significantly affected by the extraction solvents (21).

Effect of solvents on total phenolic content Phenolic compound extraction yields can be influenced by the choice of extraction solvents, ranging from polar to non-polar solvents (10). The type of solvent had a significant effect on the extraction yields of total phenolic compounds from lemon peel ($p<0.05$). Results can be seen in Fig. 2 and are in accord with the findings in a previous study, which reported that extraction solvents significantly affected the extraction yields of TPC from citrus materials (11). Absolute methanol and 50% acetone had the highest recovery yields with 13.24 and 12.37 mg GAE/g dw, respectively, whereas water had less phenolic compound yields compared with 50% acetone but higher compared with absolute acetone. These results are in agreement with Nayak *et al*. (22), who found that 51% acetone had the highest recovery yield of TPC (12.20 mg GAE/g dw) from orange peels. Park *et al*. (23) also reported that methanol gave higher extraction yield of TPC from orange peel in comparison with other solvents. The differences in the extraction efficiency of TPC can be attributed to the variation in polarity of the tested solvents, which selectively extracted phenolic compounds with different polarities. The highest extraction yield obtained by methanol and 50% acetone can be due to the reduced polyphenol oxidase (PPO) activity in these extracts, because polar solvents result in reduced PPO activity, which is an enzyme responsible for the oxidation of phenolic compounds (11). In summary, among the different solvents examined in this study, absolute methanol and 50% acetone were found to be the most efficient solvents for the recovery of TPC from lemon pomace.

Effect of solvents on total flavonoid content TFC was significantly

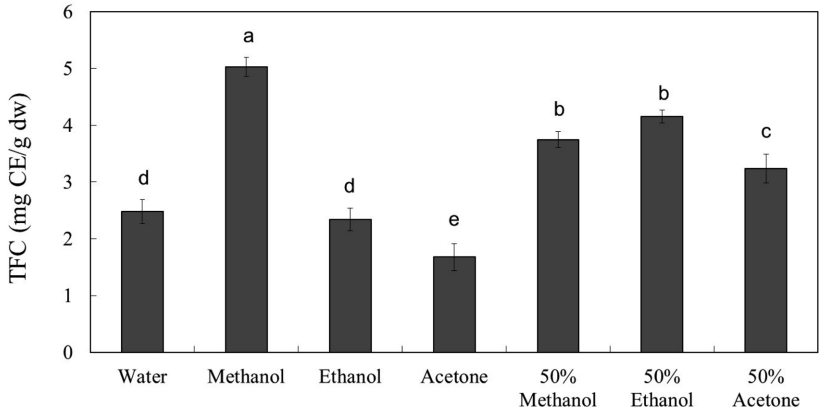

Fig. 3. Effect of solvents on the recovery of total flavonoid content (TFC) from lemon peels. The values are the mean of three replications for each solvent±standard deviation. Columns not sharing the same superscript letter are significantly different at $p<0.05$.

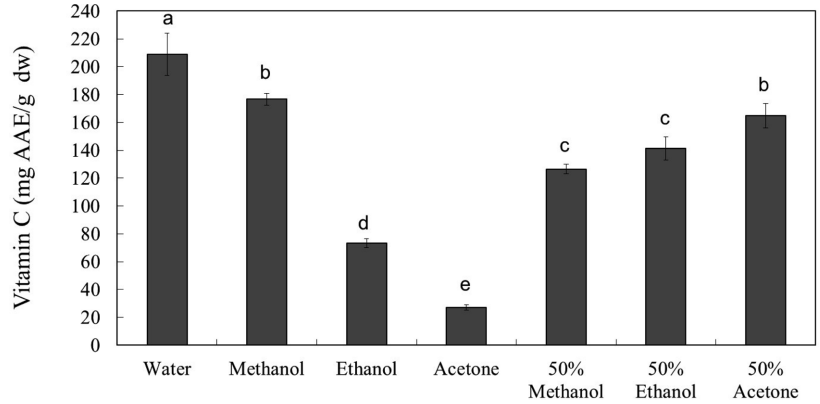

Fig. 4. Effect of solvents on the recovery of vitamin C from lemon peels. The values are the mean of three replications for each solvent±standard deviation. Columns not sharing the same superscript letter are significantly different at $p<0.05$.

affected by the extraction solvents (Fig. 3). Absolute methanol extract had the highest extraction yield of TFC (5.03 mg CE/g dw), followed by 50% ethanol and 50% methanol (4.15 and 3.75 mg CE/g dw, respectively). These findings are in agreement with Ma *et al*. (24), who reported that methanol was the most effective extraction solvent for hesperidin, which is a flavonoid compound (flavanone), and are different to the previous results reported by Lou *et al*. (25), who mentioned that hot water was an efficient solvent for the extraction of TFC from calamondin (*Citrus mitis* Blanco) compared with absolute methanol, ethanol, or their combination with water. The differences in the extraction efficiency of TFC can be related to the different polarity of solvents and the different polarity, class (flavanones, flavones, and flavonols), and form (glycoside or aglycone) of flavonoids in lemon peels (6). It has been mentioned that flavonoid glycosides and more polar aglycones can be extracted with alcohols or alcohol-water mixtures, whereas low polarity solvents are suitable for the extraction of less polar flavonoids, e.g., isoflavones, flavanones, methylated flavones, and flavonols (26).

Effect of solvents on total vitamin C Solvents had a significant effect on the extraction yields of vitamin C ($p<0.05$) (Fig. 4). As we expected, water extract had the highest content of vitamin C (209 mg AAE/g dw), followed by absolute methanol and 50% acetone (177 and 165 mg AAE/g dw, respectively), because vitamin C is a cyclic polar molecule and its solubility increases as the solvent polarity increases (27). Higher extraction yields obtained by 50% acetone and 50% ethanol compared with their absolute solvents can be attributed to the presence of water, which may increase the polarity of the solvents. These results are in accord with the results mentioned by Shalmashi and Eliassi (27), who found that the solubility of vitamin C is in decreasing order as follows: water, methanol, ethanol, acetone, acetonitrile, and ethyl acetate.

Effect of solvents on antioxidant activity The effect of solvents on the antioxidant activity of lemon pomace was determined using three antioxidant assays. The results showed that the tested solvents had a significant effect on the antioxidant properties of the extracts (Fig. 5).

For the DPPH assay, the extracts obtained using 50% acetone proved to have the highest antioxidant properties (0.15 mg TE/g dw), followed by methanol, whereas water and absolute acetone extracts had the lowest antioxidant properties (0.03 and 0.02 mg TE/g dw,

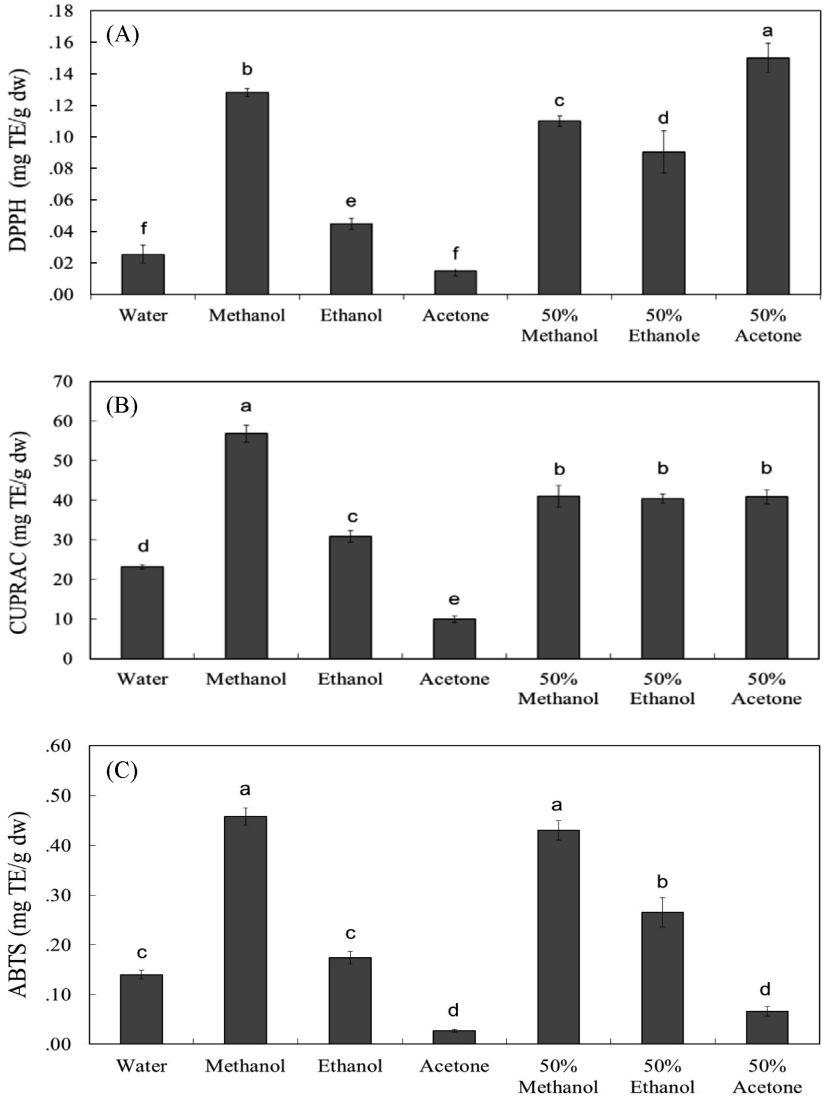

Fig. 5. Effect of solvents on the recovery of antioxidant properties from lemon peels using various antioxidant assays such as DPPH (A), CUPRAC (B), and ABTS (C). The values are the mean of three replications for each solvent±standard deviation. Columns not sharing the same superscript letter are significantly different at $p<0.05$.

Table 1. Correlation between bioactive compounds and antioxidant properties of lemon peels ($p<0.01$)

	Total phenolic compounds		Total flavonoids		Vitamin C	
	r	*p* value	*r*	*p* value	*r*	*p* value
DPPH	0.95	***	0.75	***	0.45	*
CUPRAC	0.94	***	0.91	***	0.55	*
ABTS	0.59	**	0.80	***		

respectively) (Fig. 5A) ($p<0.05$). The high antioxidant activity of 50% acetone and methanol extracts given by the DPPH assay may be due to the high level of TPC and TFC extracted with these solvents. In addition, the low antioxidant activity of water extracts can be explained by the ability of the DPPH assay to mainly measure the antioxidants that are soluble in organic solvents (28). As DPPH is an electron transfer assay (29), the high value obtained by methanol can be attributed to the very fast electron transfer from the phenoxide anion to the radical because of partial ionization.

For the CUPRAC assay, the extracts obtained using absolute methanol proved to have the highest antioxidant properties (57 mg TE/g dw), whereas absolute acetone had the lowest antioxidant properties (10 mg TE/g dw) (Fig. 5B) ($p<0.05$). As methanol is an alcohol that enhances ionization (29,30), the high antioxidant activity of methanol extracts could be explained by the high level of TPC and TFC, which are compounds with antioxidant activity, and partial ionization of the phenols, resulting in a very fast electron transfer. These findings are in agreement with Çelik *et al.* (30), who mentioned

that CUPRAC values are higher in absolute methanol compared with other solvents.

For the ABTS assay, the extracts obtained using absolute methanol and 50% methanol had the highest antioxidant properties (0.46 and 0.43 mg TE/g dw, respectively), followed by 50% ethanol (0.27 mg TE/g dw) (Fig. 5C) (p<0.05), whereas 50% acetone and absolute acetone extracts had the lowest antioxidant properties. These findings are in agreement with Van den Berg *et al*. (31), who reported different ABTS values among the different solvents. These differences can be explained by the limited solubility of some antioxidants at these solvents. The high ABTS value obtained by methanol can be attributed to the very fast electron transfer from the phenoxide anion to the radical due to partial ionization (29). 50% acetone extracts showed a large variation in their antioxidant activities among the different assays. These variations should be attributed to the different reaction mechanisms of the antioxidants extracted by 50% acetone with the different antioxidant assays (30). This is in accord with Çelik *et al*. (30), who reported that the antioxidant activity of catechin solved in dichloromethane/ethanol, measured by ABTS was the lowest among the different solvents, whereas its antioxidant activity in the same solvent measured by CUPRAC was quite similar with those obtained by the other solvents.

Correlation between bioactive compounds and antioxidant properties

The antioxidant properties of lemon pomace can be contributed by bioactive compounds such as TPC, TFC, and vitamins C and E. In this study, TPC and TFC had a strong correlation with the antioxidant properties of the extracts prepared from lemon pomace (Table 1). The r values between TPC and DPPH, CUPRAC, and ABTS were 0.95, 0.94, and 0.59 (p<0.01), respectively, revealing that TPC was a major contributor to the antioxidant properties of lemon pomace extracts. Similarly, the r values between TFC and DPPH, CUPRAC, and ABTS were 0.75, 0.91, and 0.80 (p<0.01), respectively, indicating that TFC was also a major antioxidant contributor. The r values between vitamin C and antioxidant properties measured by DPPH and CUPRAC were 0.45 and 0.55 (p<0.01), respectively, whereas no correlation observed between vitamin C and the ABTS assay, indicating that vitamin C contributed to the antioxidant properties of lemon pomace extracts but not significantly. These findings are supported by a previous study which showed that TPC had close correlation with antioxidant properties and were the major contributor to the antioxidant properties of citrus extracts because of being potential electron donors due to their hydroxyl groups (32). However, these findings are different to those reported by Ghasemi *et al*. (33), who found no correlation between phenolic compounds or flavonoids and the antioxidant activity of the citrus peel. These findings were also in accord with a previous study, which found that antioxidant power of plant extracts is largely contributed by phenolic compounds rather than ascorbic acid (34). However, another study found that vitamin C contributed to the antioxidant capacity of citrus fruits more than phenolic compounds (35). The differences can be explained by the potency of each phenolic compound contained in the extracts and their levels in the extracts, which could be linked with the correlation with the antioxidant properties (30).

To summarize, the type of solvent significantly affected the recovery of ES, TPC, TFC, vitamin C, and antioxidant properties from lemon pomace. Water, methanol, and 50% ethanol resulted in the highest extractable solids. Methanol and 50% acetone resulted in the highest extraction yields of TPC, methanol provided the highest extraction yield of TFC, and water had the highest recovery of vitamin C. Methanol, 50% methanol, 50% ethanol, and 50% acetone were found to provide the most potent antioxidant properties. TPC and TFC were strongly correlated with antioxidant properties, whereas vitamin C had a relatively low correlation with antioxidant properties, revealing that lemon pomace waste is a great source of TPC and TFC, which are the major source of antioxidants.

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