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

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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 $88\times10^6$ 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 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 $-18^\circ$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) Na₂CO₃ 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 H₂O, 0.15 mL of 5% (w/v) NaNO₂, 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) AlCl₃ was added and left for 6 min. Subsequently, 2 mL of 4% (w/v) NaOH and 0.7 mL of H₂O 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 AAЕ/g dw).

**Assays for measurement of antioxidant activity**

**DPPH assay**: DPPH assay 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 of 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 CuCl₂, 1 mL of neocuproine, 1 mL of NH₄Ac, 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 of 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.