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Extraction of phenolics from citrus peels I. Solvent extraction method

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Abstract

The total phenolic contents of five citrus peels (Yen Ben lemon, Meyer lemon, grapefruit, mandarin and orange) extracted either by ethanol or by simple aqueous extraction were evaluated using the Folin-Ciocalteu assay and compared. The main parameters that affected the yield of phenolics included the condition of the peels, temperature of the extraction, solvent concentration and species of citrus. Generally, grapefruit peel had the highest total phenolic contents, followed by mandarin, Yen Ben lemon, orange and Meyer lemon peel. High extraction (about 74%) was obtained using ethanol as solvent and the percentage extraction could further be increased using a higher temperature of 80 °C. In addition, the total antioxidant activity of the phenolic contents extracted from different citrus peels were investigated using the FRAP assay. The phenolics in grapefruit peels had the highest total antioxidant activity, followed by Yen Ben lemon, mandarin, orange and Meyer lemon.

Keywords: Total phenolic contents; Citrus peels; Antioxidant activity; Solvent extraction; Folin-Ciocalteu assay; FRAP assay

1. Introduction

Phenolic compounds are widely distributed in plants. They offer important sensory and nutritional qualities that responsible for the colours, flavours and tastes of many plants [1]. Recently, phenolics, especially flavonoids [2], have gained much attention, due to their antioxidant activities and free radical scavenging abilities, which potentially have beneficial implications in human health [3,4].

Citrus is an important crop with production estimated at 80 million tonnes per year [5,6]. The main uses of citrus in food industries include fresh juice or citrus-based drinks [5]. Since the juice yield of citrus (mainly orange and grapefruit) is less half of the fruit weight, very large amounts of byproduct wastes, such as peels are formed every year [7]. Citrus byproduct wastes have been traditionally valorised as molasses for animal feed [6], fiber (pectin) production [8,9] and fuel production [10]. Recently, a number of studies have proposed that some fruit or vegetable byproducts could be a source of natural antioxidants in order to valorise these wastes [6,7,10,11]. Citrus processing byproducts potentially represent a rich source of natural flavonoids, owing to the large amount of peel produced, and that citrus peels contain a high concentration of phenolic compounds [7,12]. Moreover, while flavonoids are abundant elsewhere in the plant kingdom, there are several compounds (e.g., flavanones, flavanone glycosides and polymethoxylated flavones) unique to citrus, which are relatively rare in other plants [13,7].

Many valuable natural materials have traditionally been extracted with organic solvents. However, some of the organic solvents are believed to be toxic, and the extraction conditions are often harsh. A simple method using ethanol (a food-grade solvent) instead of methanol was applied for the extraction of phenolic compounds from various citrus peels. The effects of the following parameters: the conditions of the peel samples, effect of repeated extraction, different types of organic solvents, the concentration of the solvent and temperature of extraction were examined.

The objectives of this research were (1) to quantify the extracted phenolic contents in five types of citrus peels, (2) to compare the amounts obtained using solvent extraction and (3) to evaluate the antioxidant activities of selected extracts.

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2. Materials and methods

2.1. Plant materials

Lemons (*Citrus limon* cv. Meyer), Lemon (*C. limon* cv. Yenben), grapefruit (*C.* x *paradisi*, unknown cultivar), mandarin (*C. reticulata* cv. Ellendale) and sweet orange (*C. sinensis* cv. Navel) were obtained from a local fruit shop in Auckland, New Zealand. All the fruits were of eating quality, and without blemishes, or damage. Fruits were transported to the University of Auckland, and on arrival immediately peeled. The peels were stored at −18 °C before any further treatments.

2.2. Chemicals

Folin-Ciocalteu phenol reagent, TPTZ (2,4,6-tripridyl-s-triazine) was purchased from Sigma Chemical Co., St. Louis, MO, USA. Sodium carbonate was purchased from Riedel-de Haën AG, Seelze-Hannover, Germany. Gallic acid was from Eastman Organic Chemicals Kingsport, Tennessee, USA. Milli-Q water was used throughout and had a conductivity <18.2 MΩ cm^2 (obtained through a Milli-Q Millipore filter system, Millipore Co., Bedford, MA, USA). Liquid nitrogen was obtained from BOC Gases Otahuhu, Auckland, New Zealand. Ferrous sulphate ($FeSO_4 \cdot 7H_2O$) was obtained from BDH Laboratory Supplies (Poole, England). Anhydrous sodium acetate was from AJAX Chemicals (Auburn, NSW, Australia). Ferric chloride ($FeCl_3 \cdot 6H_2O$) was from Panreac Quimica SA Montcada (Barcelona, Spain). Acetic acid was from May & Baker Ltd. (Dagenham, England). All chemicals and reagents used in the study were of analytical grade.

2.3. Sample preparation

Fruits were immediately peeled after purchased. The tissue removed was the pericarp region (peel), which includes the *epicarp* and *mesocarp*. The peels were stored at −18 °C before any further treatments. Frozen citrus peels were dipped in liquid nitrogen and ground into a fine powder using a pre-chilled mortar and pestle. To achieve a standard size of particles, the ground material was sieved through a 1 mm metal sieve. Large particles remaining on the sieve were further ground. The process was repeated until all the material passed through the sieve.

2.4. Extraction of phenolics

Two systems: ethanol extraction and aqueous extraction were studied and compared. Frozen citrus peel powder (2 g) was placed in a 50 ml centrifuge tube, and 16 ml of solvent or aqueous phase was added. The preparation was left to stand at different temperature (various from 20 to 80 °C) for 3 h. The mixtures were then centrifuged using a Mistral 1000 centrifuge (MSE Labsupply Pierce, Loughborough, Leicestershire, UK) at 500 × *g* for 10 min at room temperature. After centrifugation, the supernatants were filtered through Whatman No. 42 filter paper (Whatman Inc., Clifton, NJ, USA). Following filtration, a 10 ml aliquot of the filtrate was concentrated by evaporation of the solvent, using a rotary evaporator (BÜCHI Rotavapor R-114, BÜCHI, Lausanne, Switzerland) under partial vacuum at 40 °C until less than 1 ml of filtrate remained. The extract was then re-dissolved in 10 ml of Milli-Q water and stored at 4 °C prior to purification step. All the extracts were prepared in triplicate.

2.5. Purification of the crude extract

Sugars and organic acids can contribute to the absorbance measurement in the Folin-Ciocalteu assay [14,15]. Purification of the crude extracts is necessary. Sugars and organic acids were removed from the crude extract using the method of [16] with some modifications. As shown in Fig. 1, firstly, the C18 Sep-Pak cartridge (Maxi-Clean™ Cartridge 300 mg, Alltech Associates Inc., Deerfield, IL, USA) was preconditioned by sequentially passing 10 ml absolute methanol and 10 ml of 0.01N aqueous HCl through the cartridge. Secondly, a 10 ml portion of crude extract (prepared as described in Section 2.2) was loaded onto the cartridge. Then, the cartridge was washed by adding 6 ml of 0.01N HCl. Following the wash, the cartridge was dried by flushing with a gentle stream of nitrogen gas. Finally, 5 ml methanol with 0.1% (v/v) HCl was added to elute the phenolic compounds, and the fraction was collected.

2.6. Determination of total phenolic content

Total pehnolic contents in citrus extract were evaluated using the Folin-Ciocalteu assay, which was adapted from [17] with some modifications as described by [18]. Briefly, 250 μl of citrus extract (in triplicate), a gallic acid calibration standard, or Milli-Q water (as blank) was placed in a separate 25 ml volumetric flask, followed by the addition of 15 ml

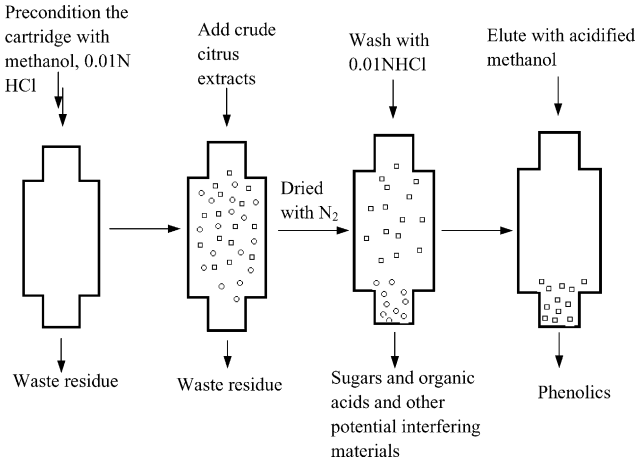

Fig. 1. Removal of sugars and organic acids from crude citrus extract using C18 cartridges (squares: phenolic compounds; circles: sugar and organic acids) (redrawn from Ref. [14] with some modifications).