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Liberation and separation of phenolic compounds from citrus mandarin peels by microwave heating and its effect on antioxidant activity

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ABSTRACT

The effect of microwave treatment on the phenolic compounds and antioxidant capacity of citrus mandarin peels was evaluated. After microwave treatment, methanol extracts of citrus peels were prepared and the contents of phenolic acids (free and bound) and flavanol, flavanone and flavonol compounds (FCs) were determined by HPLC. Antioxidant capacity of peel extracts was measured using DPPH radical scavenging assay, hydroxyl radical scavenging assay and reducing power. After microwave treatment, the free fraction of phenolic acids increased, whereas the bound fractions decreased and antioxidant activity was increased. The content of total FCs increased with power but at longer irradiation time it was declined which meant that some FCs might be degraded. The results indicated that appropriate microwave treatment could be an efficient process to liberate and activate the bound phenolic compounds and to increase the antioxidant capacity of citrus mandarin peels.

1. Introduction

Polyphenolic compounds comprising flavonoids and phenolic acids are widely distributed in foods of plant origin and count to the most abundant antioxidants in our diet [1]. As antioxidants, polyphenols play an important role in the prevention of human pathologies. Moreover, they have many industrial applications, for example, can be used as natural colorants and preservatives for foods, and applied in the production of paints, paper, and cosmetics [2].

Citrus is an important crop mainly used in food industries for fresh juice production and peel is the main by-product during its processing. Citrus peel which represents roughly half of the fruit mass, is a rich source of bioactive compounds [3,4]. Many antioxidative phenolic compounds in plants, however, are usually presented in a covalently bound form [5]. Therefore, reliable and practical methods for liberation of natural antioxidants from plant materials are of considerable interest. Microwave energy can potentiate the bioavailability of free pharmacologically active natural compounds by preventing the binding of polyphenols to the plant matrix [6]. Microwave processing has distinct advantages in the treatment of materials which contain a mixture of absorbers and transparent components. Microwave energy is absorbed by the substances with a high dielectric loss factor whilst passing through the low loss (transparent) material, resulting in selective heating. In this case significant energy savings are possible since the dielectric material can be heated without heating the entire matrix. The internal temperature distribution of a material subjected to conventional-heating depends on its thermal conductivity, whereas microwave-heating results in the heating of all the individual elements of a material instantaneously. Consequently, heating time using microwave can be significantly reduced as compared to conventional-heating methods [7].

Several methods such as heat treatment, far-infrared radiation (FIR), fermentation, and protease treatment have been studied to liberate and activate low-molecular weight natural antioxidants [5,8,9]. However, our comprehensive literature review revealed that there was no report of detailed study on the application of microwaves to liberate phenolic compounds in plant materials and especially in citrus peels.

This research evaluates the effect of microwave treatment on phenolic compounds and antioxidant activity of citrus mandarin peels. Kinnow (*Citrus reticulata* Blanco cv Kinnow) fruit peels were chosen for this study, since it is one of the main citrus mandarin cultivars in Pakistan, besides being common in the whole South Asian region. The aim of this work was to propose a suitable method for the liberation, and separation of phenolic compounds from citrus mandarin peels and evaluation of antioxidant activity before and after microwave treatment.

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2. Materials and methods

2.1. Materials

Kinnow fruits were purchased from a local market in Jhang, Pakistan. Fruits were washed and peeled off with hands. Peels were sun-dried for about 15 days and transported to the Sate Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, China, with in shortest possible time (3 days). The dried peels were ground into fine powder in a mill (DFY-500 samples mill, Da De Zhong Yao Ji Xie Company, Wenling, China) and the material that passed through a 60-mesh sieve was stored in airtight plastic bags at room temperature.

2.2. Chemicals

Standards of gallic acid, *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, ferulic acid, catechin, naringin, naringenin, hesperidin, rutin, kaempferol and isorhamnetin were purchased from Sigma (St. Louis, MO, USA). All other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.3. Microwave treatment

A multimode cavity house-hold microwave oven (700 W, 2450 MHz, Foshan Midea Microwave Oven Manufacture Co., Fo Shan, Guang Dong China) was used for heating citrus peels powder. Microwave output power was measured according to the method of [10]. Citrus peels powder (4 g, moisture content 10.7%) was put into a 500 mL glass beaker and placed in the central position of microwave oven in order to minimize the effect of field pattern variation in the oven. Samples were heated at 250 W for 5, 10 and 15 min and at 125 and 500 W for 5 min. Non-heated powder was used as a control.

2.4. Extraction of phenolic compounds from citrus peels

The extraction was performed according to a modified method of [11]. Peels powder (4 g) was extracted with 80 mL of 80% methanol in an ultrasonic device (200 W, 59 kHz, Shanghai Kudos Sonication Machine Company Ltd., China) for 60 min at room temperature. The mixture was then filtered through Whatman filter paper No. 4 and the filtrate was evaporated under reduced pressure at 30 °C until its volume was about 40 mL. The final volume of the extract was made to 50 mL with the extraction solvent and then it was taken out for the analysis of FCs, free phenolic acids and evaluation of antioxidant capacity.

2.5. Isolation of phenolic acids

Phenolic acids were isolated from the extracts according to the method as described by [11], with modifications. A 20 mL aliquot of the extract was used for sequential hydrolysis with base followed by an acid for the determination of ester-bound and glycoside-bound phenolic acid content, respectively. Twenty milliliters of 4 M NaOH were added to the extract and the mixture was stirred for 1 h at ambient temperature. The reaction mixture was adjusted to pH 2 using 6 M HCl and extracted three times with 25, 20 and 20 mL of diethyl ether-ethyl acetate (DE/EA, 1:1) at room temperature. The DE/EA extracts were combined and evaporated to dryness under vacuum at 30 °C. The dry residue was dissolved in 5 mL of methanol and analyzed for ester-bound phenolic acid content. Afterward, 10 mL of 6 M HCl were added to the aqueous phase and the mixture was hydrolyzed at 85 °C for 30 min. Then it was cooled to room temperature and the glycoside-bound phenolic acids were extracted with DE/EA as described above. After evaporation under vacuum, the residue was re-dissolved in 5 mL methanol and analyzed for glycoside-bound phenolic acids. Each sample was done in duplicate.

2.6. HPLC analysis

The analysis of phenolic acids and FCs was carried out according to the method of [12]. The chromatographic system consisted of a Waters 1525 binary pump and a Waters 2996 photodiode array detector (Waters, Milford, MA, USA). C18 column (SunFire™ 5 μm, 4.6 mm × 150 mm, Waters, USA) was used for separation. Solvent A consisted of 0.1% formic acid, and solvent B consisted of 100% methanol and the flow rate was 1 mL/min. The solvent gradient in volume ratios was as follows: 0–13 min, 15–33% B; 13–21 min, 33–39% B; 21–23 min, 39–45% B; 23–25 min, 45% B; 25–27 min, 45–15% B; 27–33 min, 15% B. Phenolic acids were quantified from peak area at 270 nm.

The contents of FCs were determined with an HPLC system module 1100 (Agilent) using a Diamonsil C18 column (4.6 mm × 250 mm). The mobile phase consisted of 0.05% phosphoric acid and 100% methanol. The elution gradient was carried out as follows: 0 min, 80% B; 5 min, 60% B; 20 min, 30% B; 23 min, 20% B; 26 min, 20% B; 27 min, 80% B; 30 min, 80% B. The flow rate was maintained at 0.8 mL/min and the eluent was monitored at 280 nm. All the identified phenolic compounds were quantified by external standard method using calibration curves, and their concentrations were expressed as microgram per gram of dry weight (μg/g DW).

2.7. DPPH radical scavenging activity

The effect of extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated according to the procedure described by [13], with some modifications. An aliquot of 1.5 mL of sample solution (1 mg/mL) was mixed with 1.5 mL of methanolic solution of DPPH (0.2 mM). The reaction mixture was incubated for 30 min in the darkness at room temperature. The absorbance of the resulting solution was measured at 517 nm with spectrophotometer. For the control, the assay was conducted in the same manner but ethanol was used instead of sample solution. DPPH scavenging capacity of the tested samples was measured as a decrease in the absorbance and was calculated by using the following equation:

$$\text{Scavenging activity}\,(\%) = \frac{Ac - As}{Ac} \times 100$$

where *Ac* and *As* are the absorbances at 517 nm of the control and sample, respectively.

2.8. Hydroxyl radical (OH) scavenging activity

Hydroxyl radical scavenging activity of extracts was determined according to the modified method of [14]. The reaction mixture containing 0.1 ml of extract (1 mg/mL) dissolved in phosphate buffer (50 mM, pH 7.4), 0.1 mL of 60 mM 2-deoxy-D-ribose in phosphate buffer, 0.2 mL of a premixed 100 mM $FeCl_3$ and 1.04 mM EDTA (1:1, v/v) solution, 0.1 mL of 10 mM H_2O_2 and 0.1 mL of 2 mM ascorbic acid, was incubated at 37 °C for 1 h. After incubation, 1 mL of 20% trichloroacetic acid (TCA) and 1 mL of 0.8% thiobarbituric acid (TBA) were added. The reaction mixture was heated in a boiling water bath for 20 min. The absorbance of the pink color developed was measured at 532 nm using a spectrophotometer. Distilled water in place of extracts was used as blank and the sample solution without adding deoxyribose as sample blank. The percentage of hydroxyl radical scavenging activity was calculated as follows:

$$\text{Scavenging activity}\,(\%) = \frac{Ab - (As - Asb)}{Ab} \times 100$$