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Antioxidant activity of a hydrothermal extract from watermelons

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ABSTRACT

In this study, the antioxidant activity of hydrothermal extracts of different parts of watermelons (flesh, white rind, and green rind) was evaluated. Each part of the watermelons was extracted at temperatures ranging from 100 °C to 300 °C for 10, 30, or 60 min. The highest total phenol content was observed for the green rind extracted at 300 °C for 30 min with 7626.52 μg gallic acid equivalents (GAE)/g, whereas the phenol content of the untreated green rind extract was 715.15 μg GAE/g. The antioxidant activity of the watermelon parts increased with increase in treatment temperature and time. In GC–MS analysis, catechol, 4-methylcatechol, pyrogallol, and 1,2,4-benzenetriol were detected after hydrothermal extraction at 300 °C for 30 min. These results indicate that hydrothermal extraction is an efficient process for increasing the antioxidant activity of watermelon extracts.

1. Introduction

The watermelon (*Citrullus lanatus*) is a commonly consumed fruit in many countries. Watermelons contain various nutrient compounds such as ascorbic acid, β-carotene, citrulline, and lycopene, and they show significant antioxidant activity (Akashi, Nishimura, Ishida, & Yokota, 2004; Ko et al., 2005; Tlili et al., 2011) as well as a protective effect against carbon tetrachloride-induced toxicity (Altas, Kızıl, Kızıl, Ketani, & Haris, 2011). For effective extraction of bioactive compounds from watermelons, various techniques such as thermosonication (Rawson et al., 2011), carbon dioxide and heat treatment (Liu, Hu, Zhao, & Song, 2012), as well as treatment with supercritical fluids (Katherine, Edgar, Jerry, Luke, & Julie, 2008) have been applied.

The use of water under high temperature and pressure below supercritical conditions during the extraction procedure is called hydrothermal extraction. Hydrothermal extraction techniques use water as the extractant at temperatures between 100 °C and 374 °C and under high pressure. In this condition, water is maintained in the liquid state during the extraction process; however, the hydrogen bonds of water become weak and break. Thus, water becomes less polar in the hydrothermal state, shows good solubility for various organic compounds, and can hydrolyze ester and ether bonds. Hydrothermal extraction has been successfully used for the extraction of different antioxidant and functional compounds from natural matrices (Dharmaraj & Malleshi, 2011; Herrero, Cifuentes, & Ibanez, 2006; Wiboonsirikul & Adachi, 2008). For instance, it has been used for the extraction of nutraceutical compounds with antioxidant activities from canola meal (Roudsari, Chang, Pegg, & Tyle, 2009), bitter melon (Budrat & Shotipruk, 2009), winery by-products (García-Marino, Rivas-Gonzalo, Ibáñez, & García-Moreno, 2006), and rice hull (Park & Lee, 2009).

In this study, hydrothermal extraction of 3 parts of the watermelon, that is, the flesh, green rind, and white rind, was performed at several temperatures for 3 time periods, and the antioxidant activity of and several bioactive compounds from the extracts were identified.

2. Materials and methods

2.1. Materials and reagents

Watermelons were purchased from a local market at Changwon, Korea. Ferric chloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) tablets, trichloroacetic acid (TCA), buthylated hydroxytoluene (BHT), and 5,5′-dithiobis (2-nitrobenzoic acid) were from Sigma–Aldrich (St. Louis, MO, USA). L-Ascorbic acid, hydrogen peroxide, lycopene, β-carotene, sodium chloride, sodium hydroxide, potassium chloride,

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potassium phosphate, and vanillic acid were also purchased from Sigma–Aldrich. Methanol, tetrahydrofuran, and hexane (HPLC grade) were purchased from J.T. Baker (J.T. Center Valley, PA, USA). Catechol, 4-methylcatechol, pyrogallol, 1,2,4-benzenetriol, *p*-hydroxybenzoic acid, and ferulic acid were products of Kishida Chemical (Osaka, Japan). *n*-Docosane and Folin-Ciocalteu reagent were purchased from Wako Pure Chemical (Osaka, Japan). Stainless steel tube (12.7 mm OD × 1.2 mm Wall × 2 Meters) and caps were purchased from Swagelok Co. (Solon, OH, USA). The water used in this study was prepared with a super purity water system (Purite Ltd., Oxon, UK) with a resistance of>17.5 MΩ cm. All the other organic solvents and chemicals used in this study were of analytical grade.

2.2. Preparation of hydrothermal extracts from watermelons

Watermelons were divided into 3 different parts: flesh, white rind, and green rind. The core of the flesh, that is, approximately 3 cm from the perimeter of the white rind, was homogenized. The white rind was mixed with an equal volume of water, and the green rind was carefully peeled off and mixed with 10 times its volume of water.

The reaction vessels for the hydrothermal extraction were prepared from stainless steel tubes with a length of 15 cm (inner volume, 12.5 mL). After the homogenized sample (10 mL) was placed in the vessel, the stainless steel cap was tightly closed and the vessel was placed in a muffle furnace (Daeil Engineering, Seoul, Korea). Each part of the watermelons was extracted at 100 °C, 150 °C, 200 °C, 250 °C, and 300 °C for 10, 30, or 60 min. After the desired conditions were achieved, the vessel was immediately removed from the furnace and cooled to room temperature for 30 min. The hydrothermal extracts were filtered through Whatman No. 3 filter paper (Advantec, Tokyo, Japan), and the filtrate was stored in a deep freezer (Operon Co., Seoul, Korea) at −70 °C for further experiments.

2.3. Total phenolic content

Total phenolic content (TPC) in the each extract were determined according to the method of Gutfinger (1981). Each extract (1.0 mL) was mixed with 1.0 mL of Na_2CO_3 solution (2 g/100 mL H_2O), and then the mixture was allowed to stand at room temperature for 3 min. After the addition of 0.2 mL Folin-Ciocalteu reagent (2-fold diluted with H_2O), the reaction was kept for 30 min in the dark room, followed by centrifugation at 13,400 × *g* for 5 min. The absorbance of supernatant was measured at 750 nm by using a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan), and TPC were expressed as garlic acid equivalents.

2.4. DPPH radical scavenging activity

To determine the DPPH radical scavenging activity (RSA) of the hydrothermal extracts, the extract (0.1 mL) was mixed with 0.9 mL of 0.041 mmol DPPH/L ethanol for 30 min and then the optical density (OD) of the sample was measured at 517 nm using a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). RSA was expressed as a percentage inhibition and it was calculated by the following formula:

$$\%\ \text{DPPH RSA} = \left[1 - \left(A_{\text{sample}} \middle/ A_{\text{control}}\right)\right] \times 100$$

where A_{control} is the absorbance of the control reaction (the mixture of 0.1 mL of distilled water and 0.9 mL of the DPPH solution was used as the control), and A_{sample} is the absorbance of the reaction under the presence of extracts. Lower absorbance of the reaction mixture indicates higher free RSA (Lee et al., 2003).

2.5. ABTS RSA

The determination of the ABTS RSA was performed as described by Muller (1985). Extracts (0.1 mL), potassium phosphate buffer (0.1 mL, 0.1 mol/L, pH 5.0), and hydrogen peroxide (20 μL, 10 mmol/L) were mixed and pre-incubated at 37 °C for 5 min. After pre-incubation, ABTS (30 μL, 1.25 mmol/L, in 0.05 mol/L phosphate-citrate buffer, pH 5.0) and peroxidase (30 μL, 1 unit/mL) were added to the mixture and then it was incubated at 37 °C for 10 min. The OD value of mixture was measured by a multiplate reader (Sunrise RC/TS/TS Color-TC/TW/BC/6Filter, Tecan Austria GmbH, Grφdig, Austria) at 405 nm against a blank. L-ascorbic acid were used as positive controls and all tests were carried out in triplicate, and the ABTS RSA was calculated by the following formula:

$$\%\ \text{ABTS RSA} = \left[1 - \left(A_{\text{sample}} \middle/ A_{\text{control}}\right)\right] \times 100$$

2.6. Reducing power

The reducing power of each extract was determined according to the method described procedure by Oyaizu (1986). One mL of the extract (concentrations: 0.05, 0.1, 0.5, and 1 mg/mL), 1 mL of sodium phosphate buffer (0.2 mol/L, pH 6.6), and 1 mL of 1% potassium ferricyanide solution were mixed and incubated at 50 °C for 20 min. One mL of 10% TCA was added to the mixture, followed by centrifugation at 14,240 × *g* for 5 min. One mL of supernatant was mixed with 1 mL of distilled water and 0.1 mL of 0.1% ferric chloride, and then its absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

2.7. Lycopene and β-carotene analysis

Lycopene and β-carotene extraction was conducted as described by the Fish group (Fish, Perkins-Veazie, & Collins, 2002). One gram of each watermelon sample was correctly weighed into a 50-mL Falcon tube. The tube was then wrapped with aluminum foil to prevent the entry of external light. The sample was then supplemented with a mixture consisting of 5 mL of a solution of 0.05 g BHT/100 mL ethanol, 5 mL of acetone, and 10 mL of hexane; subsequently, it was agitated for 10 min by vortexing. Then, 3 mL of distilled water was added into each Falcon tube, and the samples were vortexed for another 3 min. The resulting solution was allowed to stand for 10 min. The polar and non-polar layers were separated, and the suspension (upper hexane layer) was collected. The hexane phase was filtered using a 0.20-μm filter (Dismic-25, Toyo Roshi Kaisha Ltd., Japan) for HPLC analysis.

The levels of lycopene and β-carotene were measured by HPLC (Sadler, Davis, & Dezman, 1990). The HPLC system consisted of Shimadzu LC-6AD pumps (Shimadzu Co. Ltd., Kyoto, Japan) with a 2-pump gradient system, a Shimadzu SPD-10AVP UV–VIS detector, and a Shimadzu SIL-10ADVP auto sample injector. The column was a Shim-pack VP ODS column (5 μm, 150 × 4.6 mm, Shimadzu Co. Ltd.) equipped with a Shim-pack CLC guard column (10 × 4 mm, Shimadzu Co. Ltd.). The mobile phase was methanol:tetrahydrofuran:water (67:27:6), and the flow rate was 1 mL/min. The sample injection volume was 10 μL. Detection was accomplished using a UV–Vis detector, and chromatograms were recorded at 475 nm. Peaks were identified by comparing the retention times with authentic standards. Standards were prepared using purified