Food Chemistry 123 (2010) 878–885

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Pressurised water extraction of polyphenols from pomegranate peels

Mustafa Çam [a,*], Yaşar Hışıl [b]

[a] Erciyes University, Department of Food Engineering, 38039 Kayseri, Turkey
[b] Ege University, Department of Food Engineering, 35100 Izmir, Turkey

ARTICLE INFO

Article history:
Received 27 October 2009
Received in revised form 6 April 2010
Accepted 1 May 2010

Keywords:
Pomegranate peel
Pressurised water extraction
Total phenolic content
Punicalagin
Ellagic acid

ABSTRACT

Pomegranate peels are one of the most valuable by-products of the food industry in terms of polyphenols which are conventionally extracted from plant materials by organic solvents, especially with methanol. Pressurised water extraction was investigated for the extraction of polyphenols from pomegranate peels. The most important factors affecting the extraction results were found to be particle size, temperature, and static time. The results indicated that pressurised water extraction was as effective as conventional methanol extraction for the recovery of polyphenols from pomegranate peels. Total phenolic contents of pomegranate peels obtained by pressurised water extraction at optimised conditions and conventional solid–liquid methanol extraction were determined as 264.3 and 258.2 mg/g tannic acid equivalents, respectively. Hydrolyzable tannins were the predominant polyphenols of pomegranate peels corresponding to 262.7 mg/g tannic acid equivalents. Punicalagin content of pomegranate peels by pressurised water extraction was found to be 116.6 mg/g on dry matter basis.

1. Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest edible fruits and has been used extensively in the folk medicine of many cultures (Li et al., 2006a). Popularity of pomegranate has increased tremendously especially in the last decade because of anti-microbial, anti-viral, anti-cancer, potent anti-oxidant, and anti-mutagenic effects of the fruit (Negi, Jayaprakasha, & Jena, 2003). Pomegranate fruit is composed of three parts: the seeds, the juice, and the peels (Lansky & Newman, 2007). Pomegranate juice has been proposed as chemopreventive, chemotherapeutic, antiatherosclerotic, and anti-inflammatory agent. Accordingly, its consumption has grown tremendously (Çam, Hışıl, & Durmaz, 2009). The peels of some fruits have higher anti-oxidant activity than the pulps (Guo et al., 2003). Pomegranate is a good example for this type of fruits. Pomegranate peels constitute approximately 40% of the whole fruit and are rich in ellagic acid derivatives such as the ellagitannins, punicalagin, and punicalin. In addition, some ellagic acid derivatives (ellagic acid hexoside, -pentoside, etc.) are also present, although in lesser amounts (Cerda, Ceron, Tomas-Barberan, & Espin, 2003b; Seeram et al., 2005). The most abundant of these polyphenols is punicalagin which is extracted from pomegranate juice during juice processing and which is responsible for more than 50% of the pomegranate juice's potent anti-oxidant activity (Adams et al., 2006).

Synthetic anti-oxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytolune (BHT) have restricted use in foods as these synthetic anti-oxidants are suspected to be carcinogenic. Therefore, the importance of the search for and exploitation of natural anti-oxidants, especially of plant origin, has greatly increased in recent years (Singh, Murthy, & Jayaprakasha, 2002). Plant based anti-oxidants are extracted from raw materials or waste products of food industry by organic solvents such as methanol, diethyl ether, and acetone. Methanol is an effective extractant for a broad range of polyphenols, therefore it is a frequently used solvent for both a laboratory scale and an industrial extraction process. Methanol is cheap and easily accessible and the manufacturing of herbal medicine usually uses methanol as a solvent to extract natural ingredients, a fact that will also concern us about the residual level of methanol in these products (Wang, Wang, & Choong, 2004).

Polyphenols of pomegranate peels have been extracted mostly by methanol and/or combinations of methanol and other organic solvents by classical extraction techniques. Water is not an effective solvent for the extraction of polyphenols in pomegranate peels compared to methanol at normal atmospheric pressure conditions. Pressurised water extraction (PWE) is a technique that uses water as extractant at elevated pressure. PWE was used for extraction of thermally labile components, including terpene trilactones from *Ginkgo biloba* leaves (Lang & Wai, 2003) and naphtodianthrones from *Hypericum perforatum* (Manila & Wai, 2003).

The aim of this study was to investigate the suitability of PWE for extraction of polyphenols in pomegranate peels and subsequent use of the extracts as natural anti-oxidant. Effective

* Corresponding author. Tel.: +90 352 437 4901x32757.
E-mail addresses: mcam@erciyes.edu.tr, mustafa.cam@nofima.no (M. Çam).

0308-8146/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.05.011

extraction parameters upon PWE were determined and ineffective ones were eliminated by a univariate statistical approach.

2. Materials and methods

2.1. Materials

Seven kilograms of nine different pomegranate cultivars widely grown in Turkey were harvested in October 2006 from the orchard of Aegean Agricultural Research Institute when all greenness had disappeared from the fruit rind surface and red or yellow colour appeared. Injured and sunburnt fruits were discarded. After peeling the fruit, pomegranate peels were sun-dried and then the dried peels were ground by a hammer mill (Brook-Crompton, England). Pomegranate peels grinded by hammer mill were passed through four different standard sieves (Prüfsieb Jel 200, Germany). Four different particle size fractions were collected (45–65, 65–212, 212–560, and 560–1400 μm). The fractions of each cultivar were kept in a dry glass bottle in a freezer at −25 °C.

2.2. Chemicals

Methanol, ethanol, ethyl acetate, acetone, Na_2CO_3, $NaNO_2$, $AlCl_3$, HCl, KIO_3, NaOH, and sea sand were purchased from Mallinckrodt Baker (New Jersey, USA). β-Carotene, linoleic acid, butylated hydoxyanisole (BHA), butylated hydroxytoluene (BHT), Folin–Ciocalteu's phenol reagent, catechin hydrate, gallic acid, ellagic acid, vanillin, and tannic acid were obtained from Sigma–Aldrich Chemical Company (St. Louis, USA). Punicalagin standard was purified from pomegranate peels according to the method of Cerda, Llorach, Ceron, Espin, and Tomas-Barberan (2003a). Deionized water (18.2 MΩ cm) was prepared using an Aquamax-Ultra 370 water purification system (Young Lin Corporation, Anyang, Korea). Syringe filters made of nitrocellulose (Millipore, Bedford, USA) or polytetrafluoroethylene (Sartorious, Goettingen, Germany) with pore size of 0.45 μm were used to filter aqueous or organic extracts, respectively.

2.3. Extraction

PWE of pomegranate peels was carried out by a pressurised liquid extraction system (ASE 300, Dionex Corporation, Sunnyvale, CA, USA). Deionized water was used throughout all experiments as extraction solvent. Deionized water was degassed for 1 h by a continuous nitrogen gas stream. All extractions were performed at the following basic conditions: 10 g of dried pomegranate peels (particle size of 65–212 μm) were mixed with 40 grams of sea sand and placed in a 100 ml volume of stainless steel extraction cell. A circular cellulose filter (size 30 mm, Dionex) was placed at the bottom of the extraction cell in order to prevent suspended particles from entering the collection bottles. Extraction process was carried out hereafter automatically by an ASE 300 system. Once the oven temperature reached the set point (40 °C), the extraction cell was moved into the oven and the pump filled the cell with water. The static valve was closed and the pump kept on pumping until the pressure of the extraction cell reached 102.1 atm. The cell was preheated at 40 °C for 5 min, followed by a 5 min static extraction step. After 5 min static extraction, the extracts were pumped into 250 ml of collection vials. Fresh solvent (60% of the cell volume) was pumped again into the cell and nitrogen gas purged for 100 s. The extracts collected in vials were transferred into a 250 ml of volumetric flasks and the total volume was adjusted to 250 ml with water. One extraction cycle was applied for the determination of the effective extraction factors. Particle size, temperature, static time, and flush volume were investigated as independent factors. Effects of independent factors on system responses were determined by changing the level of each factor and keeping the other factors constant. After determination of the optimum levels of independent factors, the number of optimum extraction cycles was determined at optimum extraction conditions.

Pomegranate peels were also extracted with different solvents, including methanol, ethanol, ethyl acetate, acetone, and water using a magnetic stirrer in order to compare the results with the results obtained by PWE. 10 grams of pomegranate peels were extracted with 100 ml of methanol at 40 °C for 1 h. The extract was filtered through a Whatman No. 1 filter paper. The residue was re-extracted with the same volume of the same solvent. The extracts were pooled and the volume adjusted to 250 ml by the same solvent. The same procedure was applied for the other solvents. Extracts were filtered by a 0.45 μm filter prior to analysis.

2.4. Analysis of the extracts

2.4.1. Total phenolic content

Total phenolic content (TPC) of the extracts was determined according to the method of Li et al. (2006a). Briefly, 0.5 ml of a 100-fold diluted extract was mixed with 2.5 ml of 10-fold diluted Folin–Ciocalteu's phenol reagent and incubated for 1 min, before 2 ml of 7.5% Na_2CO_3 was added. The mixture was allowed to stand for 30 min. The absorbance versus prepared blank was read at 760 nm. Six different concentrations of tannic acid solutions (25–150 mg/l) were used for calibrations. The final results were expressed as mg tannic acid equivalent (TAE) per g of dry matter.

2.4.2. Radical scavenging activity

Radical scavenging activity (RSA) of the extracts was determined by the method of Singh et al. (2002). Briefly, 0.1 ml of 100-fold diluted extracts was taken in different test tubes. Five millilitres of a 0.1 mM methanolic solution of DPPH was added to the test tubes and vortexed for 10 s. The tubes were kept in a water bath at 27 °C for 20 min. The control was prepared as above using 0.1 ml of water instead of the extract. Absorbance of the resulting violet coloured mixture was read at 517 nm after baseline correction with methanol. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\text{Radical scavenging activity } \% = [\text{Abs}_{\text{control}} - (\text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}})] \times 100$$

2.4.3. Total flavonoid content

Total flavonoid content of the extracts was determined by the method of Zhishen, Mengcheng, & Jianming, 1999. Briefly, 1 ml of 25-fold diluted extracts was added into a 10 ml volumetric flask containing 4 ml of water. At zero time, 0.3 ml of 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 ml of 10% $AlCl_3$ was added into the flask. At 6 min, 2 ml of 1 M NaOH was added to the mixture. Immediately, the reaction flask was diluted to volume with the addition of 2.4 ml of distilled water and thoroughly mixed. Absorbance of the pink coloured mixture was read at 510 nm versus the prepared water blank. Six different concentrations of catechin solutions (20–100 mg/l) were used for calibrations. The final results were expressed as mg catechin equivalent (CE) per g of dry matter.

2.4.4. Condensed tannins

Condensed tannins were determined by the vanillin assay with slight modifications (Sun, Ricardo-da-Silva, & Spranger, 1998). Extracts obtained with PWE contains water and water in the reaction