Food Chemistry 168 (2015) 21–26

Contents lists available at ScienceDirect

Food Chemistry

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

Extraction characteristics of subcritical water depending on the number of hydroxyl group in flavonols

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ARTICLE INFO

Article history:
Received 2 April 2014
Received in revised form 18 June 2014
Accepted 7 July 2014
Available online 14 July 2014

Keywords:
Subcritical water extraction
Myricetin
Quercetin
Kaempferol
Hydroxyl group

ABSTRACT

This study compared the efficiencies of using subcritical water, hot water, and organic solvents to extract flavonols from black tea, celery, and ginseng leaf. The effect of key operating conditions was determined by varying the temperature (110–200 °C), extraction time (5–15 min), and pressure (about 10 MPa) and the extracts were analysed quantitatively using HPLC. The yields of myricetin, quercetin, and kaempferol from plants were maximal at extraction temperatures of 170 °C, 170 °C and 200 °C, respectively, and they depend on the number of hydroxyl groups included in the chemical structure of the flavonols, with more of those with fewer hydroxyl (—OH) groups attached being extracted at higher temperatures. The results also showed that the yields of flavonols by subcritical water extraction were 2.0- to 22.7- and 1.8- to 23.6-fold higher than those obtained using the ethanol and methanol as traditional extraction methods, respectively.

1. Introduction

Fruits, vegetables, and plants contain numerous physiologically active substances, such as vitamin C (ascorbic acid), vitamin E (tocopherol), and polyphenols (Pérez-Jiménez et al., 2008), whose main role is to inhibit oxidization and the effects thereof, including the prevention of cell aging and cell loss (Bazzano et al., 2002; Brighenti et al., 2005; Trichopoulou, Costacou, Bamia, & Trichopoulos, 2003). Approximately 4000 kinds of flavonoids have been identified, with the most well-known among them including flavonols, flavonones, flavanols, anthocyanidins, isoflavones, dihydroflavonols, and chalcones (Herrmann, 2006). Flavonoids are vegetable components with various structural characteristics, and their antioxidant activities are attributable to the hydroxyl (—OH) group and double bond of carbon C2 and C3 of the carbon group in the benzene ring, and the hydroxyl group fused into the A and B rings and the carbonyl group of carbon 4 (Middleton & Kandaswami, 1994).

Many methods are used to extract physiologically active substances from plants (Dai & Mumper, 2010; Katalinic, Milos, Kulisic, & Jukic, 2006; Kerchev & Ivanov, 2008; Sultana, Anwar, & Ashraf, 2009). Various organic solvents such as alcohols, alcohol-water mixtures, ethyl acetate, and chloroform dichloromethane are traditionally used for extraction, but their adverse effects on both the environment and the food ingredients have led to a recent focus on using subcritical water (SW) as a solvent. Subcritical water extraction (SWE) is considered a "green" technology, and is based on using water as an extraction agent in various forms, such as hot water, pressurised (hot) water, pressurised low-polarity water, and superheated water (Smith, 2002). SWE is performed in the liquid state at various pressure from 100 °C (the boiling point of water) to 374 °C (the critical point of water), which are the critical temperature and pressure, respectively (Ayala & Luque de Castro, 2001; Ju & Howard, 2005; Ramos, Kristenson, & Brinkman, 2002). That is, the dielectric constant of SW becomes similar to that of organic solvents; for example, $\varepsilon = 30$ for water at about 220 °C and $\varepsilon = 33$ for methanol at room temperature. This makes it possible to use water to selectively extract polar, mid-polar, and nonpolar substances simply by changing its temperature and pressure (Anekpankul, Goto, Sasaki, Pavasant, & Shotipruk, 2007; Ko, Cheigh, & Chung, 2014; Teo, Tan, Yong, Hew, & Ong, 2010).

SWE has been used to extract polyphenolic or antioxidant compounds from rosemary (Ibañez et al., 2003), laurel (Fernández-Pérez, Jiménez-Carmona, & Luque de Castro, 2000), *Terminalia chebula* Retz. fruits (Rangsriwong, Rangkadilok, Satayavivad, Goto, & Shotipruk, 2009), canola meal

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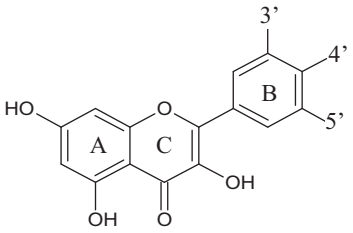
http://dx.doi.org/10.1016/j.foodchem.2014.07.047
0308-8146/

Table 1
Chemical structure of flavonols used in this study.

Flavonol skeleton



Compounds	Position		
	3′	4′	5′
Myricetin	OH	OH	OH
Quercetin	OH	OH	H
Kaempferol	H	OH	H

(Hassas-Roudsari, Chang, Pegg, & Tyler, 2009), olive leaves (Ghoreishi & Shahrestani, 2009), bitter melon (*Momordica charantia*) (Budrat & Shotipruk, 2009), grape seeds (García-Marino, Rivas-Gonzalo, Ibáñez, & García-Moreno, 2006), onion skin (Ko, Cheigh, Cho, & Chung, 2011), and *Citrus unshiu* peel (Cheigh, Chung, & Chung, 2012).

The goals of this study were to identify any change in extraction behaviour according to the number of hydroxyl groups in flavonols using SWE, determine the optimum extraction conditions such as extraction temperature and time, and compare the efficiencies of SWE and traditional extraction methods. Accordingly, this study selected myricetin, quercetin, and kaempferol which have different numbers of hydroxyl groups – in order to identify how the extraction efficiency varies with the chemical structure of a flavonol (Table 1), and also, as materials for the experiments, black tea leaves (*Thea sinensis* L.), celery (*Apium graveolens*), and ginseng leaves (*Panax ginseng* C.A. Meyer), which are common plants that contain flavonols (Han, Shen, & Lou, 2007; Hollman & Arts, 2000; Miean & Mohamed, 2001).

2. Materials and methods

2.1. Sample preparation

Black tea obtained from a local market was ground using a high-speed mixer (Blender Model 7012S, Waring, Torrington, CT, USA), freeze-dried celery powder was purchased from Sanmaeul (Changnyeong, Gyeongsangnam-do, Korea), and ginseng leaf was obtained from a ginseng farm (Yeoungdong, Chungchungbuk-do, Korea) as a by-product of the manufacture of ginseng goods. The ginseng leaf was freeze-dried and then ground using a high-speed mixer. All dried samples were stored at 4 °C in a sealed state before being used in extraction processes.

2.2. Subcritical water extraction

The accelerated solvent extractor (Model 350, Dionex, Sunnyvale, CA, USA) was used for SWE by the following procedure (Cheigh et al., 2012). The dried sample (1 g) was mixed with diatomaceous earth (3 g, Dionex) and placed in a stainless-steel sample cell (22 ml, Dionex). The pressure was maintained at 10.1 MPa while various extraction temperatures were used: 110 °C, 130 °C, 150 °C, 170 °C, 190 °C, and 200 °C. The accelerated solvent extractor comprised a heating block in an oven, a cell containing the specimen to extract, and a surrounding thermostat. In the experimental setup the solvent in the vial passed through a pump valve and then pressure-relief valve before entering the oven where the extraction process was performed. The extract was finally collected in a collection vial through a static valve. The obtained extract was freeze-dried for 24 h before being subjected to quantitative analysis.

2.3. Traditional extraction methods

Traditional extractions were performed using three kinds of solvent: hot water, methanol (extra pure grade, Duksan Co., Ansan, Gyeonggi-do, Korea), and ethanol (extra pure grade, Duksan Co.). The sample-to-solvent ratio was 4.17% (wt/vol) and the volume of extraction was 48 ml of solvent. Hot-water extraction was performed at 90 °C for 2 h. The temperature was controlled using a water bath (C-WB1, Changshin Scientific, Seoul, Korea), and vortexing was performed every 20 min. Methanol and ethanol were extracted at 60 °C and 70 °C for 2 h, respectively. After filtering, the hot-water extract was freeze-dried, and the organic solvent extracts were evaporated using an evaporator (Cheigh et al., 2012). These extracts were stored at 4 °C in a sealed state before being used in the HPLC analysis.

2.4. HPLC analysis methods

Myricetin (hexahydroxyflavone, MW 318, purity grade: ⩾96%), quercetin (pentahydroxyflavone, MW 302, purity grade: ⩾98%), and kaempferol (tetrahydroxyflavone, MW 286, purity grade: ⩾96%) were purchased from Sigma–Aldrich (Yongin, Gyunggi-do, Korea). HPLC grade solvents for the HPLC analysis such as methanol, acetonitrile, and water were obtained from J.T. Baker (Phillipsburg, NJ, USA). Each freeze-dried sample (0.1 g) extracted by SWE and hot-water extraction was dissolved in 5 ml of 80% methanol and vortexed. Each concentrated extract obtained using methanol or ethanol was added to 30 ml of 80% methanol, vortexed, and then filtered through a 0.45-μm PVDF 13-mm filter (Whatman, England). HPLC was performed using an Agilent system (1200 series, Agilent Technologies, Santa Clara, CA, USA) with a Zorbax Eclipse XDB-C_{18} column (4.6 × 150 mm, 5 μm pore size; Agilent Technologies) and a UV detector (Variable Wavelength Detector, Agilent Technologies). HPLC analysis method was applied by modifying the method of Schmidt et al. (2010). The mobile phase consisted of 1% acetic acid (solvent A) and 100% acetonitrile (solvent B). The gradient consisted of solvent B at the following concentrations: 10% during 0–2 min, 15% during 2–18 min, 50% during 18–20 min, 10% during 20–25 min, and post-time 5 min before next injection at a flow rate of 1 ml/min. The volume of sample injected was 20 μl. The mobile phase was pumped at room temperature, and the UV spectrum was recorded at 370 nm.

2.5. Data analysis

The calibration curves of standard flavonoids were used for calculating the extraction yield (expressed in units of mg/kg dried sample). Spiking experiments were conducted for qualitatively identifying all peak chromatograms of extracts. The optimum SWE conditions (i.e., extraction temperature and extraction time) from each plant were chosen based on the highest flavonoid contents.

3. Results and discussion

3.1. HPLC chromatograms

Each standard curve for measuring the myricetin, quercetin, and kaempferol contents was obtained using the following five concentrations: 0.03125, 0.0625, 0.125, 0.25, and 0.5 mg/ml. The