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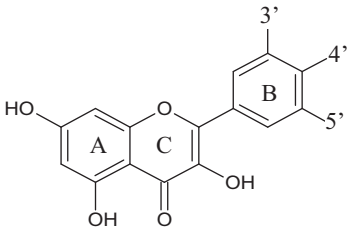
http://dx.doi.org/10.1016/j.foodchem.2014.07.047
0308-8146/

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

standard curve obtained was $y = 44.142x - 325.13$ ($R^2 = 0.9994$) for myricetin, $y = 31.243x - 121.24$ ($R^2 = 0.9997$) for quercetin, and $y = 27.174x - 77.589$ ($R^2 = 0.9997$) for kaempferol. The flavonol contents of all the extracts were measured using HPLC, and a spiking test was used to ensure that an exact qualitative analysis was performed. Fig. 1A shows a chromatogram of a standard substance comprising myricetin, quercetin and kaempferol mixed at a concentration of 0.5 mg/ml, and Fig. 1B–D show chromatograms of SW extracts obtained at 170 °C for 15 min from black tea (Fig. 1B), celery (Fig. 1C), and ginseng leaf (Fig. 1D), respectively. The chromatogram of the standard substance allowed us to confirm that the peak times of myricetin, quercetin, and kaempferol were 11.7, 14.1, and 16.3 min, respectively.

3.2. Optimum SWE conditions for extracting flavonols from plants

We analysed the myricetin, quercetin, and kaempferol contents of black tea extracts for SWE times of 5, 10, and 15 min at temperatures of 110 °C, 130 °C, 150 °C, 160 °C, 170 °C, 190 °C, and 200 °C. The contents obtained in these conditions are listed in Fig. 2. The myricetin (Fig. 2A, 568 ± 3 mg/kg of dry weight) and quercetin (Fig. 2B, 1179 ± 54 mg/kg of dry weight) contents of black tea

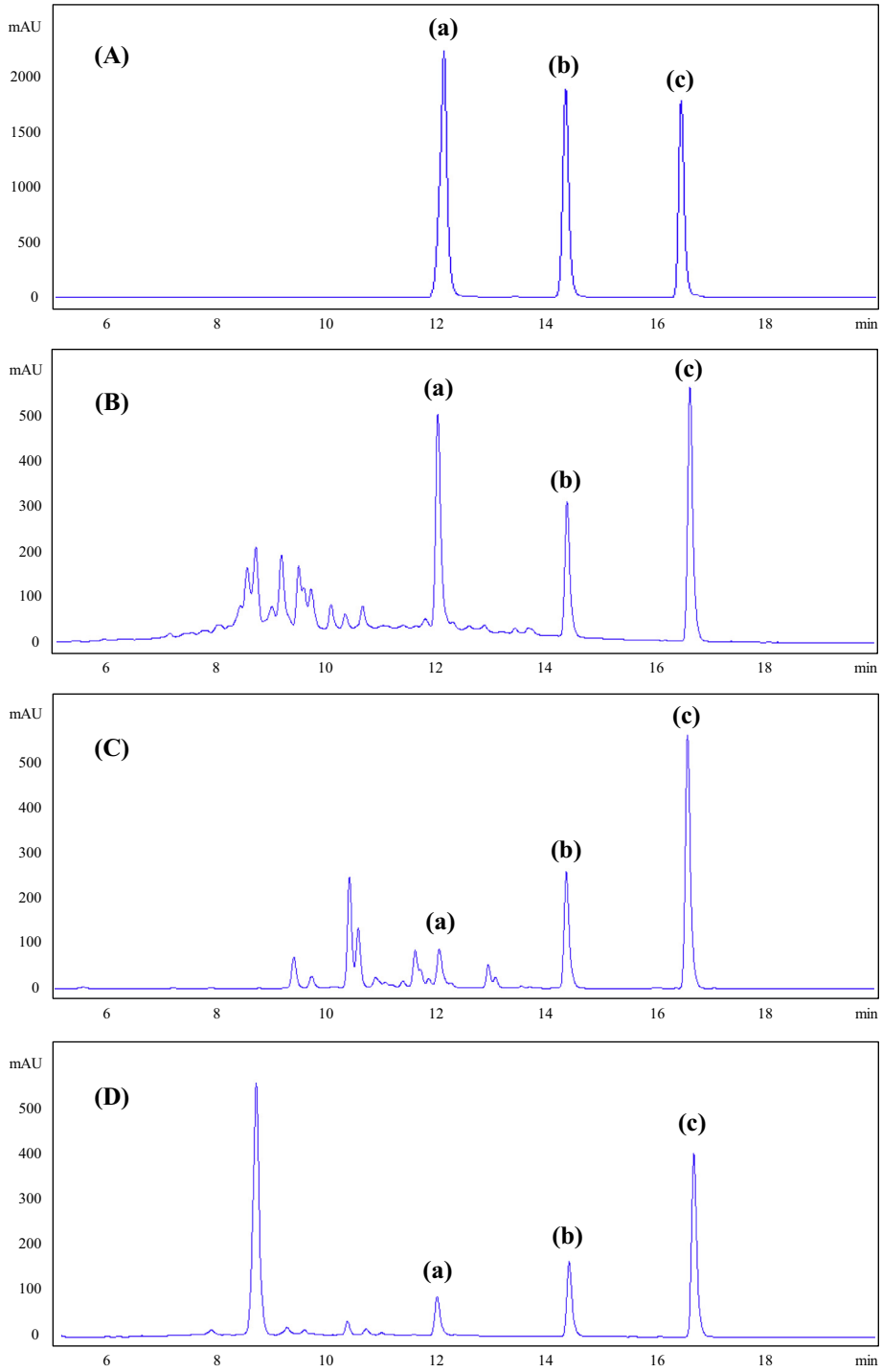


Fig. 1. HPLC chromatogram of standard substances (A) that are mixed at a concentration of 0.5 mg/ml and SW extracts at 170 °C and 15 min from black tea (B), celery (C), and ginseng leaf (D), respectively. Peaks: (a) myricetin; (b) quercetin; (c) kaempferol.

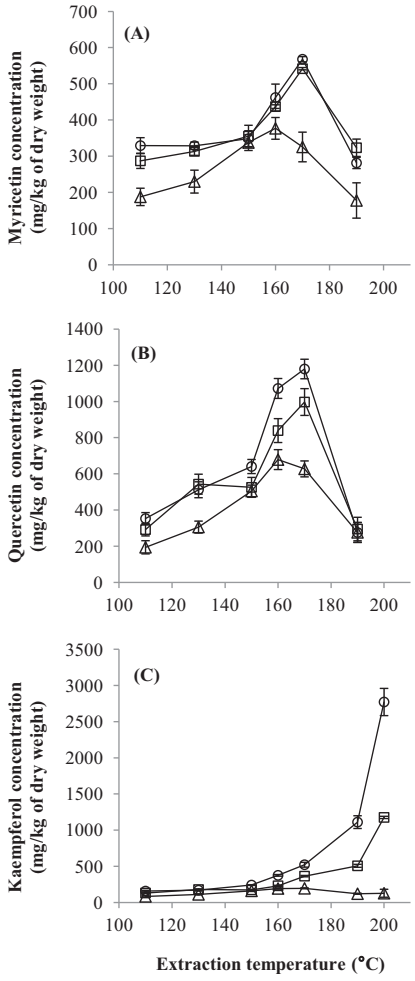


Fig. 2. Change of flavonol myricetin (A), quercetin (B), and kaempferol (C) contents according to temperature and time change of black tea extracts by SWE. △, extraction time of 5 min; □, 10 min; ○, 15 min.

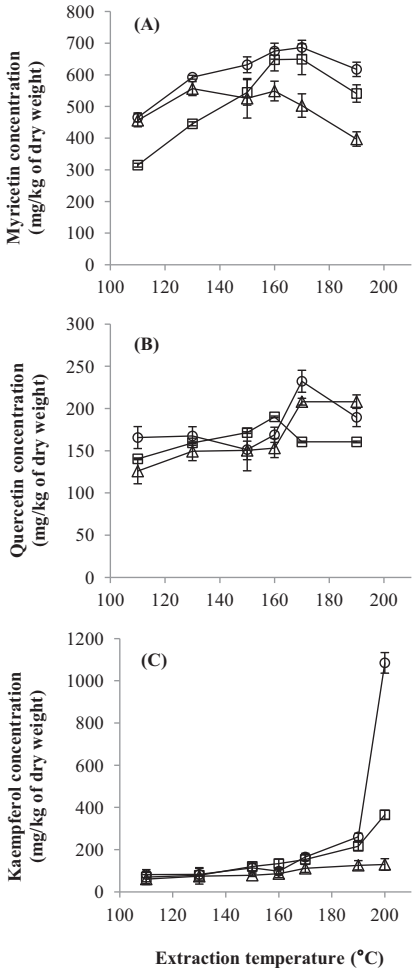


Fig. 3. Change of flavonol myricetin (A), quercetin (B), and kaempferol (C) contents according to temperature and time change of celery extracts by SWE. △, extraction time of 5 min; □, 10 min; ○, 15 min.

extracts were maximal for extraction at 170 °C for 15 min, and the kaempferol (Fig. 2C, 2770 ± 72 mg/kg of dry weight) content was maximal at 200 °C for 15 min. The SWE of celery was performed using the same methods as for the myricetin, quercetin, and the kaempferol contents of black tea extracts. The contents obtained in these conditions are listed in Fig. 3. The myricetin (Fig. 3A, 686 ± 30 mg/kg of dry weight) and quercetin (Fig. 3B, 232 ± 13 mg/kg of dry weight) contents of celery extracts were maximal for extraction at 170 °C for 15 min, and the kaempferol (Fig. 3C, 1085 ± 49 mg/kg of dry weight) content was maximal at 200 °C for 15 min, both as for black tea extracts. The SWE of ginseng leaf was performed using the same methods. The contents obtained in these conditions are listed in Fig. 4. The myricetin (Fig. 4A, 467 ± 32 mg/kg of dry weight) and quercetin (Fig. 4B, 285 ± 13 mg/kg of dry weight) contents of ginseng leaf extracts were maximal at 170 °C for 10 min, and the kaempferol (Fig. 4C, 2042 ± 25 mg/kg of dry weight) content was maximal for 200 °C for 15 min. These graphs show the changes in the flavonol content of each substance produced by altering the extraction temperature and time of subcritical water extraction.

3.3. Change of flavonol concentrations according to different numbers of hydroxyl groups from SW extracts

The flavonol content generally increased as the extraction temperature increased to 170 °C for myricetin and quercetin and to 200 °C for kaempferol. Water at a higher temperature has a lower dielectric constant, which reduces the energy required for divisions in solute–matrix interactions and also the polarity due to weakened hydrogen bonds (Ho, Cacace, & Mazza, 2007). Moreover, water in its subcritical state exhibits hydrolytic properties as its ionization constant increases, producing an increase in the flavonol extraction efficiency as temperature increases. Accordingly, the myricetin and quercetin contents increased proportionally with temperature rises, from 110 to 170 °C, and dropped after 170 °C; meanwhile, kaempferol content increased proportionally with temperature from 110 to 200 °C. These results (Figs. 2–4) confirm that the myricetin and quercetin contents peaked at 170 °C, and moreover, the kaempferol yield was the maximum at extraction temperature of 200 °C. The increase in extract yield with water temperature is due to the associated reduction in the water polarity improving the dissolution of nonpolar compounds. Also, the lower myricetin and quercetin contents at 200 °C relative to 170 °C are probably due to their loss and/or degradation (Kubátová, Lagadec, Miller, & Hawthorne, 2001).

The extraction characteristics of subcritical water according to the number of hydroxyl groups could be explained as follows: as the temperature of water is increased, the polarity of water decreases because the continuous network of hydrogen bonds is broken (Kalinichev & Bass, 1997). Therefore, the dielectric constant, which represents the polarity, can be described by the hydrogen bond and acceptor strength in subcritical water. The

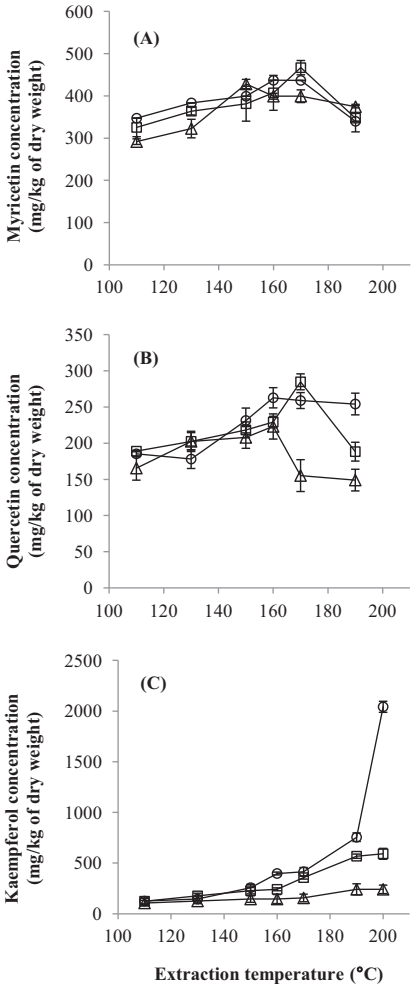


Fig. 4. Change of flavonol myricetin (A), quercetin (B), and kaempferol (C) contents according to temperature and time change of ginseng leaf extracts by SWE. △, extraction time of 5 min; □, 10 min; ○, 15 min.

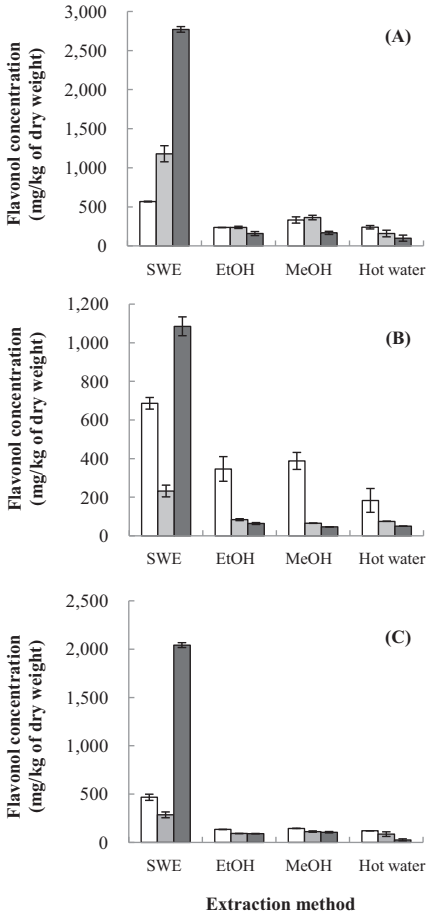


Fig. 5. Comparison of flavonol contents between SW extracts (170 °C and 15 min) and traditional extracts from black tea (A), celery (B), and ginseng leaf (C). □, myricetin; ■, quercetin; ■, kaempferol.

hydrogen-bonding strength can vary depending on the oxygen charges remains and a function of the *R* group (Marten et al., 1996). Ko et al. (2014) also reported that hydrogen bonds affect the interaction between the polarity of water solvent and the structure of the flavonoid solute in the SWE. In this study, flavonols having a greater number of hydroxyl groups (B ring of flavonols shown in Table 1) were optimally extracted at relatively lower temperature that represents higher dielectric constant since hydrogen bonds can be easily formed, so more interaction between hydroxyl groups and subcritical water is possible. These mechanisms explain why the optimum temperature for the SWE was lower for myricetin and quercetin (160–170 °C) with 6 and 5 hydroxyl groups than for kaempferol (200 °C) with 4 hydroxyl groups. These results were similar to a previous study which showed that the tri-ol type flavanols were more unstable than the di-ol types at high temperature (Suzuki et al., 2003). Carr, Branch, Mammucari, and Foster (2010) also suggested that the addition of multiple hydroxyl groups onto simple organic aromatic hydrocarbon chains would improve the solubility of organic compounds in subcritical water extraction.

Consequently, subcritical water, depending on temperature, can be effective to selectively extract a variety of polarity organic compounds from many different matrices. A temperature-dependent associating energy of dielectric constant and associative interactions of hydrogen bonds greatly improve solubility of compounds (Fornari, Stateva, Señorans, Reglero, & Ibañez, 2008).

3.4. Comparison of flavonol concentrations with traditional extraction methods

We compared the flavonol extraction efficiency of SWE with those of traditional extraction methods using ethanol, methanol, or hot water. The sample-to-solvent ratio was kept at 1:24 in order to maintain identical conditions with SWE in this research; therefore, 2 g of each sample was added to 48 ml of each solvent. Fig. 5 shows that the extracts obtained from black tea (Fig. 5A), celery (Fig. 5B), and ginseng leaf (Fig. 5C) using SWE had higher flavonol contents than those obtained using the traditional extraction methods. The myricetin yields obtained from the materials using SW were 2.0- to 3.7-, 1.8- to 3.4-, and 2.4- to 4.0-fold higher than those obtained using the ethanol, methanol, and hot water, respectively. The quercetin yields by SW were 3.1- to 6.1-, 2.6- to 3.6-, and 3.0- to 7.4-fold higher than those by ethanol, methanol, and hot water, respectively. Also, the kaempferol yields by SW were 17.2- to 22.7-, 16.6- to 23.6-, and 21.6- to 81.7-fold higher than those by ethanol, methanol, and hot water, respectively. An increased water temperature will decrease both the surface tension and viscosity, which will increase the speed of diffusion and mass transfer during extraction. This characteristic was apparent in the kaempferol analysis (Ko et al., 2014; Nakahara, Matubayasi, Wakai, & Tsujino, 2001).

4. Conclusion

This study aimed to find out how the differences of chemical structures of flavonols affect their extraction by using black tea,

celery and ginseng leaf, which contain various flavonols. To this end, myricetin, quercetin, and kaempferol, which are all the same except for the number of their —OH groups, were tested to evaluate at what temperature and time they show the best extraction results and why. Our analyses have revealed that the extraction yield was maximal at a higher temperature (i.e., 200 °C) for kaempferol than for myricetin and quercetin (i.e., 170 °C), which is due to the former having with a smaller number of hydroxyl groups, so the extraction of flavonols is strongly affected by extraction temperature of solvent and the hydroxyl group characteristics. Our results also showed that it is more efficient to use subcritical water than ethanol, methanol, and hot water to extract flavonols. Besides, SWE is not only effective and ecofriendly, but is also a highly selective and rapid method for extracting flavonols from plants. Therefore, SW could be an excellent alternative to organic solvent as a medium for extracting flavonols.

Acknowledgements

This research was supported by a Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology (No. 2013R1A1A2A10060325).

References

Anekpankul, T., Goto, M., Sasaki, M., Pavasant, P., & Shotipruk, A. (2007). Extraction of anti-cancer damnacanthal from roots of *Morinda citrifolia* by subcritical water. *Separation and Purification Technology, 55*, 343–349.

Ayala, R. S., & Luque de Castro, M. D. (2001). Continuous subcritical water extraction as a useful tool for isolation of edible essential oils. *Food Chemistry, 75*, 109–113.

Bazzano, L. A., He, J., Ogden, L. G., Loria, C. M., Vupputuri, S., Myers, L., et al. (2002). Fruit and vegetable intake and the risk of cardiovascular disease in US adults: The first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. *The American Journal of Clinical Nutrition, 76*, 93–99.

Brighenti, F., Valtueña, S., Pellegrini, N., Ardigò, D., Del Rio, D., Salvatore, S., et al. (2005). Total antioxidant capacity of the diet is inversely and independently related to plasma concentrations of highsensitive C-reactive protein in adult Italian subjects. *British Journal of Nutrition, 93*, 619–625.

Budrat, P., & Shotipruk, A. (2009). Enhanced recovery of phenolic compounds from bitter melon (*Momordica charantia*) by subcritical water extraction. *Separation and Purification Technology, 66*, 125–129.

Carr, A. G., Branch, A., Mammucari, R., & Foster, N. (2010). The solubility and solubility modeling of budesonide in pure and modified subcritical water solutions. *The Journal of Supercritical Fluids, 55*, 37–42.

Cheigh, C. I., Chung, E. Y., & Chung, M. S. (2012). Enhanced extraction of flavanones hesperidin and naririutin from *Citrus unshiu* peel using subcritical water. *Journal of Food Engineering, 110*, 472–477.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules, 15*, 7313–7352.

Fernández-Pérez, V., Jiménez-Carmona, M. M., & Luque de Castro, M. D. (2000). An approach to the static-dynamic subcritical water extraction of laurel essential oil: comparison with conventional techniques. *Analyst, 125*, 481–485.

Fornari, T., Stateva, R. P., Señorans, F. J., Reglero, G., & Ibañez, E. (2008). Applying UNIFAC-based models to predict the solubility of solids in subcritical water. *Journal of Supercritical Fluids, 46*, 245–251.

García-Marino, M., Rivas-Gonzalo, J. C., Ibáñez, E., & García-Moreno, C. (2006). Recovery of catechins and proanthocyanidins from winery by-products using subcritical water extraction. *Analytica Chimica Acta, 563*, 44–50.

Ghoreishi, S. M., & Shahrestani, R. G. (2009). Subcritical water extraction of mannitol from olive leaves. *Journal of Food Engineering, 93*, 474–481.

Han, X., Shen, T., & Lou, H. (2007). Dietary polyphenols and their biological significance: A review. *International Journal of Molecular Sciences, 8*, 950–988.

Hassas-Roudsari, M., Chang, P. R., Pegg, R. B., & Tyler, R. T. (2009). Antioxidant capacity of bioactives extracted from canola meal by subcritical water, ethanolic and hot water extraction. *Food Chemistry, 114*, 717–726.

Herrmann, K. (2006). Flavonols and flavones in food plants: A review. *International Journal of Food Science and Technology, 11*(5), 433–448.

Hollman, P. C. H., & Arts, I. C. W. (2000). Flavonols, flavones and flavanols – Nature, occurrence and dietary burden: A review. *Journal of the Science of Food and Agriculture, 80*, 1081–1093.

Ho, C. H. L., Cacace, J. E., & Mazza, G. (2007). Extraction of lignans, proteins and carbohydrates from flaxseed meal with pressurized low polarity water. *LWT – Food Science and Technology, 40*, 1637–1647.

Ibañez, E., Kubátová, A., Señoráns, F. J., Cavero, S., Reglero, G., & Hawthorne, S. B. (2003). Subcritical water extraction of antioxidant compounds from rosemary plants. *Journal of Agricultural and Food Chemistry, 51*, 375–382.

Ju, J. Y., & Howard, L. R. (2005). Subcritical water and sulfured water extraction of anthocyanins and other phenolics from dried red grape skin. *Journal of Food Science, 70*(4), 270–276.

Kalinichev, A. G., & Bass, J. D. (1997). Hydrogen bonding in supercritical water. 2. Computer simulations. *Journal of Physical Chemistry A, 101*, 9720–9727.

Katalinic, V., Milos, M., Kulisic, T., & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry, 94*, 550–557.

Kerchev, P., & Ivanov, S. (2008). Influence of extraction techniques and solvents on the antioxidant capacity of plant material. *Biotechnology & Biotechnological Equipment, 22*, 556–559.

Ko, M. J., Cheigh, C. I., Cho, S. W., & Chung, M. S. (2011). Subcritical water extraction of flavonol quercetin from onion skin. *Journal of Food Engineering, 102*, 327–333.

Ko, M. J., Cheigh, C. I., & Chung, M. S. (2014). Relationship analysis between flavonoids structure and subcritical water extraction (SWE). *Food Chemistry, 143*, 147–155.

Kubátová, A., Lagadec, A. J. M., Miller, D. J., & Hawthorne, S. B. (2001). Selective extraction of oxygenates from savory and peppermint using subcritical water. *Flavour and Fragrance Journal, 16*, 64–73.

Marten, B., Kim, K., Cortis, C., Friesner, R., Murphy, R., Ringnalda, M., et al. (1996). New model for calculation of solvation free energies: Correction of self-consistent reaction field continuum dielectric theory for short-range hydrogen-bonding effects. *Journal of Physical Chemistry A, 100*, 11775–11788.

Middleton, E., Jr., & Kandaswami, C. (1994). The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In J. B. Harborne (Ed.). *The flavonoids: Advances in research since 1986* (1st ed.), pp. 619–652). London: Chapman & Hall.

Mean, K. H., & Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry, 49*, 3106–3112.

Nakahara, M., Matubayasi, N., Wakai, C., & Tsujino, Y. (2001). Structure and dynamics of water: from ambient to supercritical. *Journal of Molecular Liquids, 90*, 75–83.

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz-Rubio, M. E., Serrano, J., Goñi, I., et al. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International, 41*, 274–285.

Ramos, L., Kristenson, E. M., & Brinkman, U. A. T. (2002). Current use of pressurised liquid extraction and subcritical water extraction in environmental analysis. *Journal of Chromatography A, 975*, 3–29.

Rangsriwong, P., Rangkadilok, N., Satayavivad, J., Goto, M., & Shotipruk, A. (2009). Subcritical water extraction of polyphenolic compounds from *Terminalia chebula* Retz. Fruits. *Separation and Purification Technology, 66*, 51–56.

Schmidt, S., Zietz, M., Schreiner, M., Rohn, S., Kroh, L. W., & Krumbein, A. (2010). Genotypic and climatic influences on the concentration and composition of flavonoids in kale (*Brassica oleracea* var. *sabellica*). *Food Chemistry, 119*, 1293–1299.

Smith, R. M. (2002). Extractions with superheated water. *Journal of Chromatography A, 975*, 31–46.

Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules, 14*, 2167–2180.

Suzuki, M., Sano, M., Yoshida, R., Degawa, M., Miyase, T., & Maeda-Yamamoto, M. (2003). Epimerization of tea catechins and O-methylated derivatives of (−)-epigallocatechin-3-O-gallate: Relationship between epimerization and chemical structure. *Journal of Agricultural and Food Chemistry, 51*, 510–514.

Teo, C. C., Tan, S. N., Yong, J. W., Hew, C. S., & Ong, E. S. (2010). Pressurized hot water extraction (PHWE). *Journal of Chromatography A, 1217*, 2484–2494.

Trichopoulou, A., Costacou, T., Bamia, C., & Trichopoulos, D. (2003). Adherence to a mediterranean diet and survival in a greek population. *The New England Journal of Medicine, 348*(26), 2599–2608.