Journal of Applied Pharmaceutical Science Vol. 6 (12), pp. 202-205, December, 2016
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2016.601230
ISSN 2231-3354

The potential use of citrus juice waste as sources of natural phenolic antioxidants

Ji Hye Kim, Min Young Kim*

Toxicology Laboratory, Major in Biomaterials, College of Applied Life Science, SARI, Jeju National University, Jeju, Republic of Korea.

ARTICLE INFO

Article history:
Received on: 29/08/2016
Revised on: 19/09/2016
Accepted on: 12/10/2016
Available online: 28/12/2016

Key words:
Antioxidant capacity, Citrus juice processing waste, Total phenolic and flavonoid contents.

ABSTRACT

The methanolic extract of citrus juice processing waste was evaluated for its potential applications in medicine or as nutraceutical materials. Determination of total phenolic and flavonoid contents was done by microplate reader, whereas UPLC-mass detection was used for the analysis of individual flavanone (naringin, hesperidin, hesperetin, neohesperedin and narirutin) and flavonol (rutin). In addition, free radicals (DPPH, O_2^-, H_2O_2 and NO) scavenging and ferrous ion chelating assays were used to determine the antioxidant capacity. The contents of total phenolic and flavonoid were more prevalent in citrus juice processing waste than the level found in the pulp of citrus fruit. The results of UPLC analyses indicate that citrus juice processing waste was rich in mainly hesperidin and neohesperedin, and it had different flavonoid composition in comparison with the pulp of citrus fruit. The citrus juice processing waste was also found to possess an evident antioxidant capacity. The results showed that citrus wastes could be economic and readily accessible source of natural antioxidants and as a possible food and pharmaceutical supplement.

INTRODUCTION

Citrus is the main fruit crop in the world, with a total production of 122 million tons in 2008 (Terol *et al*., 2010). In Korea, Jeju island is well known for its important production of citrus fruits (0.6 million tons in 2008/2009) (Department of Citrus Policy, Jeju Special Self-Governing Providence, Korea). Citrus species are rich sources of vitamin C, folate, dietary fibre, and minerals as well as many antioxidant phytophenolics, including flavonoids, amino acids, triterpenes, phenolic acids and carotenoids that can potentially protect health (Gypta *et al*., 2015). Recently, there is much biomedical interest in citrus fruits because their consumption appears to be associated with lower risk of colorectal, esophageal, gastric and stomach cancers and stroke (Roussos, 2011). Citrus fruits are utilized primary for juice recovery, where about half of the processed citrus including peels, segment membrane and seeds ends up as wastes. These solid residues are referred to as citrus wastes with estimated worldwide production of 15 million tons per year (Marı´n *et al.*, 2007). Only a very small part of the wastes has been properly converted into useful or high-value products and most are disposed in landfills, constituting severe economic and environmental problems (Montgomery, 2004). In Jeju island, more than 50,000 tons of citrus wastes including citrus peels generated by juice processing facilities and canning industries, and premature fruit drops in orchards annually have been incinerated in disposal yards and dumped into the ocean (Yang *et al*., 2009). The by-products industry has a potential for growth since products have also been produced from citrus fruit residues. The peel of citrus fruits, the primary by-product, is a rich source of flavones as well as many polymethylated flavones, they could be which are very rare in other plants (Nogata *et al*., 2006). These beneficial actions suggest new value-added uses for these compounds as nutraceuticals and specialty ingredients, and studies of the occurrence of the polymethoxylated flavones in by-products generated from citrus juice processing or premature fruit drops caused by poor condition from climate change, some diseases and other stresses are currently of considerable interest to both pharmaceutical and food industries. Therefore, this study was performed to test the potentiality of citrus juice processing waste as a functional nutraceutical and

** Corresponding Author*
Min Young Kim, Toxicology Laboratory, Major in Biomaterials, College of Applied Life Science, SARI, Jeju National University, Jeju, Republic of Korea Email: jeffmkim@jejunu.ac.kr; Tel: 82-64-754-3349.

pharmaceutical source on the basis of their biomolecules with antioxidant property. Here we report a comparative analysis of the polyphenolic compounds and *in vitro* antioxidant properties of the citrus pulp extract and citrus peel waste after juice extraction.

MATERIALS AND METHODS

Plant material and extraction

The peel waste of citrus fruit (Citrus unshiu Marc.) after juice extraction was obtained from a local food processing company (Jeju Provincial Development Co., Jeju, Korea). Samples were extracted twice according to the method described by Im *et al*. (2014).

Analysis of total phenolic and flavonoid contents

Contents of total phenol and flavonoid were determined by the method described previously (Im *et al*., 2014).

UPLC-MS analysis of flavonoids

UPLC analysis of five major flavonoids (naringin, hesperidin, hesperetin, neohesperedin, narirutin and rutin) in citrus pulp and citrus waste extracts were performed on an Acquity Waters Ultra Performance Liquid Chromatographic system equipped with an Acquity photodiode array (PDA) detector coupled with a triple quadrupole tandem mass spectrometer (Micromass® Quattro microTM API, Waters, Milford, MA, US) and electrospray ionization (ESI). Flavonoid standards were separated on a UPLC BEH C18 column (50 mm x 2.1 mm, 1.7 µm particle size). The column temperature was kept at 33 °C. Optimum separation was achieved with a binary gradient of 0.1% formic acid (vol/vol) in water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.2 mL/min under the following program: 0–0.63 min, 2% B; 0.63–3.50 min, 2–60% B; 3.50–4.00 min, 60% B; 4.00–4.50 min, 60–2% B; 4.50–5.00 min, 2% B (equilibration of column).

The injected sample volume was 2 µL and the UV spectra by PDA were recorded between 210 and 410 nm. MS detection was performed directly after PDA measurements. The ESI source was optimized with positive ionization mode as follows: scan spectra from m/z 100 to 400, capillary voltage 3.30 kV, cone voltage 20 V, source temperature 120 °C and desolvation temperature 360 °C. Nitrogen was used as the desolvation and cone gas with a flow rate of 850 and 50 L/h, respectively. For quantitative determination of the five flavonoids, the MS detection was operated in negative ESI mode with multiple reaction monitoring (MRM). Argon was used as the collision gas at a pressure of 3.82×10^{-3} mbar. Instrument control, data acquisition, and quantification were performed by Mass Lynx 4.1 software (Waters).

Free radical scavenging and ferrous ion chelating assays

Radical scavenging (1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide, hydrogen peroxide and nitric oxide) and chelating ability was determined by the method described previously (Im *et al*., 2014; Kim and Kim, 2011).

Statistical analysis

Comparisons of all results between citrus pulp and citrus waste samples were made by using a nonparametric test (Mann-Whitney U test) with $p < 0.05$ (SPSS, ver. 12.0; SPSS Inc., Chicago, IL, US). For each measurement, three replicate samples were tested. A dose response curve was plotted to determine the IC_{50} values which are defined as the concentration sufficient to obtain 50 % of a maximum scavenging capacity.

RESULTS AND DISCUSSION

Phenolic compounds act as free radical terminator, and mostly include flavonoids, phenolic acids, stilbenes, coumarins and tannins (Winkel-Shirley, 2002). In many cases, flavonoids may increase antioxidant activity as part of a general stress response (Winkel-Shirley, 2002). The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler *et al*., 2003). In the present study, citrus juice processing waste extract (41.6 and 13.2 g/100 g) had significantly higher total phenolic and flavonoid contents than the pulp extract of citrus fruit (8.3 and 5.7 g/100 g), respectively ($p < 0.05$) (Figure 1).

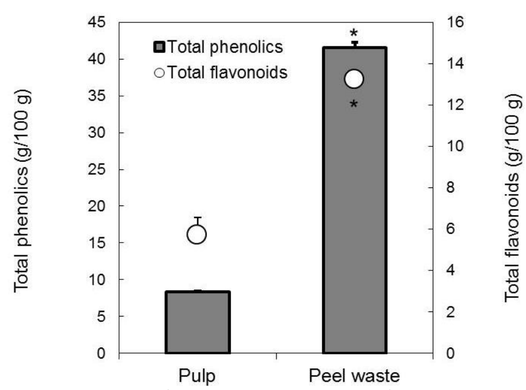

Fig. 1: Total phenolic and flavonoid compositions of the methanolic extracts obtained from citrus pulp and citrus juice processing waste. Each value is expressed as mean ± standard deviation (n = 3). $^{*}p < 0.05$, compared to pulp.

Flavonoids are a widely distributed group of phenolic compounds, which have a wide range of biological effects, such as inhibition of key enzymes in mitochondrial respiration, protection against coronary heart disease and anti-inflammatory, antitumor, and antimicrobial activities (Wang *et al*., 2008). Four types of flavonoids occur in citrus: flavanones, flavones, flavonols and anthocyanidins (Benavente-García *et al*., 1997). Citrus fruit contains high levels of the flavanones, as well as flavonol, which are very rare in other plants (Gattuso *et al*., 2007). Some of these flavanones and flavonol are of commercial interest because they are used in the pharmaceutical and food industries. In this study, obtained results showed that naringin, hesperidin, hersperetin, neohesperedin, neohesperedine and narirutin were the main flavanones in citrus juice processing waste extract (Table 1).

Table 1: Flavonoid content (g/100 g dry weight) of citrus pulp and citrus juice processing waste.

Group	Flavanone					Flavonol glycoside
	Naringin	**Hesperidin**	**Hesperetin**	**Neohesperedin**	**Narirutin**	**Rutin**
Pulp	nd[†]	0.179 ± 0.0004	nd	nd	0.092 ± 0.0006	0.058 ± 0.0009
Peel waste	0.072 ± 0.0005	0.104 ± 0.0005*	nd	0.105 ± 0.0006	0.055 ± 0.0005*	0.0008 ± 0.00006*

[†] Not detected; * Values are significantly different from corresponding pulp ($p < 0.05$).

Table 2: IC_{50} value in free radical scavenging and ferrous ion chelating properties of citrus pulp and citrus juice processing waste.

Group	IC_{50} value (mg/mL)				
	DPPH	**Superoxide**	**Hydrogen peroxide**	**Nitric oxide**	**Chelating effect on ferrous ions**
Pulp	2.2 ± 0.03	1.6 ± 0.09	2.6 ± 0.24	2.3 ± 0.13	0.9 ± 0.27
Peel waste	1.1 ± 0.08*	1.6 ± 0.03	2.0 ± 0.18*	2.8 ± 0.10	1.0 ± 0.02

IC_{50} was obtained by interpolation from linear regression analysis. Each values is expressed as mean ± standard deviation ($n = 3$).

In comparison with the pulp of citrus fruit, citrus waste contained significantly lower concentration of hesperidin, narirutin and rutin ($p < 0.05$), but naringin and neohesperedin were present only; the flavanone glycoside pattern consisted predominantly of hesperidin (0.104 g/100 g) and neohesperedin (0.105 g/100 g) (Table 1).

The interest in natural antioxidants has grown in the recent years with the awareness of several fatal diseases. Antioxidants act in the various ways, binding metal ions, scavenging radicals and decomposing peroxides. Often more than one mechanism is involved. In the present study, citrus juice processing waste extract and the pulp extract of citrus fruit were evaluated for their antioxidant capacity by free radical scavenging (DPPH, superoxide, hydrogen peroxide and nitric oxide) and ferrous ion chelating assays, as shown in Table 2. The citrus juice processing waste extract showed slightly higher or similar hydrogen peroxide and superoxide scavenging activities compared with those of the pulp extract of citrus fruit (Table 2).

Other antioxidant assays need to be undertaken to verify this point and to understand the full potential of citrus juice processing waste extract as an antioxidant agent. Therefore, we examined the DPPH and ferrous ion chelating activities of citrus juice processing waste extract (Table 2). In the DPPH radical scavenging assay, IC_{50} value of the citrus juice processing waste extract (1.1 mg/mL) was significantly lower than that of the pulp extract of citrus fruit (2.2 mg/mL) ($p < 0.05$) (Table 2). The DPPH assay is often used to evaluate the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damages caused by oxidative stress. DPPH radical involves a hydrogen atom transfer process (Kaviarasan *et al*., 2007). In this assay, the antioxidant activity on DPPH radicals of the citrus juice processing waste extract may be attributed to a direct role in trapping free radicals by donating hydrogen atom.

The methanolic extract of the citrus juice processing waste was also tested for ferrous ion chelating activity (Table 2). It exhibited 1.0 mg/mL IC_{50} value, they were similar to that of the pulp extract of citrus fruit, indicating that the reductive capacity of antioxidants may affect ion, especially Fe^{2+} and Cu^{2+} (Senevirathne *et al*., 2010). Ferrous is a vital substance for normal physiology, but an excess of it may result in cellular damage. If iron undergoes the Fenton reaction, these reduced metals may form highly reactive hydroxyl radicals and thereby contributing to oxidative stress (Senevirathne *et al*., 2010).

The results from the present work, citrus juice processing waste possessed the higher total phenolic content and exhibited strong antioxidant activity and it, therefore, may be developed as natural antioxidant for food industry and other fields. The bioactive components and antioxidative actions of citrus juice processing waste extract warrant further studies in both *in vitro* and *in vivo* models.

ACKNOWLEDGEMENT

Financial support and sponsorship: This work was supported by Basic Science Research Program (2016R1A6A1A03012862 and 2014R1A1A2056292) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, Republic of Korea.

Conflict of Interests: There are no conflicts of interest.

REFERENCES

Benavente-García O, Castillo J, Marin FR, Ortuño A, Del Río JA. Uses and properties of citrus flavonoids. Journal of Agricultural and Food Chemistry. 1997; 45: 4505-4515.

Gattuso G, Barreca D, Gargiulli C, Leuzzi U, Caristi C. Flavonoid composition of citrus juices. Molecules. 2007; 12: 1641-1673.

Gupta P, Ray J, Aggarwal BK, Goyal P. Food processing residue analysis and its functional components as related to human health: Recent developments. Austin Journal of Nutrion and Food Sciences. 2015; 3(3): 1068.

Im SJ, Kim JH, Kim MY. Evaluation of bioactive components and antoxidant and anticancer properties of citrus wastes generated during bioethanol production. Natural Product Communication. 2014; 9(4): 483-486.

Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha CV, Priyadarsini KI. In vitro studies on antiradical and antioxidant activities of fenugreek (Trigonella foenum graecum) seeds. Food Chemistry. 2007; 103: 31-37.

Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. Journal of Pharmacy and Pharmacology. 2003; 55: 131-142.

Kim JH, Kim MY. Effect of citrus waste substrate on the production of bioactive component, and antioxidant and antitumor activity of Grifola frondosa. Life Science Journal. 2011; 8: 564-571.

Marı´n FR, Soler-Rivas C, Benavente-Garcı´a O, Castillo J, Pe´rez-Alvarez JA. By-products from different citrus processes as a source of customized functional fibres. Food Chemistry. 2007; 100: 736-741.

Montgomery R. Development of biobased products. Bioresource Technology. 2004; 91: 1-29.

Nogata Y, Sakamoto K, Shiratsuchi H, Ishii T, Yano M, Ohta H. Flavonoid composition of fruit tissues of citrus species. Bioscience Biotechnology and Biochemistry. 2006; 70: 178-192.

Roussos PA. Phytochemicals and antioxidant capacity of orange (Citrus sinensis (l.) Osbeck cv. Salustiana) juice produced under organic and integrated farming system in Greece. Scientia Horticulturae. 2011; 129: 253-258.

Senevirathne M, Kim SH, Kim YD, Oh CK, Oh MC, Ahn CB, Je JY, Lee WW, Jeon YJ. Effect of far-infrared radiation drying of citrus press-cakes on free radical scavenging and antioxidant activities. Journal of Food Engineering. 2010; 97: 168-176.

Terol J, Soler G, Talon M, Cercos M. The aconitate hydratase family from Citrus. BMC Plant Biology. 2010; 10: 222.

Wang YC, Chuang YC, Hsu HW. The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. Food Chemistry. 2008; 106: 277-284.

Winkel-Shirley B. Biosynthesis of flavonoids and effects of stress. Current Opinion in Plant Biology. 2002; 5: 218-223.

Yang EJ, Kim SS, Oh JS, Baik JS, Lee NH, Hyun CG. Essential oil of citrus fruit waste attenuates LPS-induced nitric oxide production and inhibits the growth of skin pathogens. International Journal of Agricultural and Biology. 2009; 11: 791-94.

How to cite this article:

Kim JH, Kim MY. The potential use of citrus juice waste as sources of natural phenolic antioxidants. J App Pharm Sci, 2016; 6 (12): 202-205.