Food Chemistry 167 (2015) 358–362

Contents lists available at ScienceDirect

Food Chemistry

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

Short communication

Antioxidant and cytoprotective activities of extracts prepared from fruit and vegetable wastes and by-products

Faisal Kabir, Wei Wei Tow, Yasunori Hamauzu, Shigeru Katayama, Sachi Tanaka, Soichiro Nakamura *

Graduate School of Agriculture, Shinshu University, 8304 Minamiminowamura, Kamiina, Nagano 399-4598, Japan

ARTICLE INFO

Article history:
Received 6 March 2014
Received in revised form 19 May 2014
Accepted 25 June 2014
Available online 10 July 2014

Keywords:
Wastes
By-products
Polyphenols
Antioxidant activity
Free radical scavenging activity

ABSTRACT

In this study, fruit and vegetable wastes and by-products were tested for polyphenol content and their antioxidant activity. The highest content of polyphenols as assessed by the Folin–Ciocalteu assay was the hot-water extract of grape seed, followed by the ethanol extract of buckwheat hull. The highest antioxidant activity measured by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assays was also detected in the hot-water extract of grape seed, followed by the ethanol extract of immature prune. Most of samples showed protective effects against oxidative stress induced by 2,2′-azobis-(2-amidinopropane) dihydrochloride (AAPH) peroxyl radical generator in African monkey kidney (MA 104) cells. Samples containing high amounts of phenolics (more than 30 mg ChAE/g) generally showed high antioxidant activity and a protective effect against AAPH-induced oxidative stress. This study demonstrates that fruit and vegetable wastes and by-products are good sources of high amounts of phenolics with antioxidant properties.

1. Introduction

Polyphenols are common constituents of the human diet, with fruits and vegetables being the major dietary source of these bioactive compounds. The possible health benefits of polyphenol consumption have been suggested to derive from their antioxidant properties (Leopoldini, Russo, & Toscano, 2011). Dietary antioxidants are indeed believed to play a very important role in the human body defense system, protecting, as in plants, against oxidative damage induced by reactive oxygen species, which are known to be involved in the pathogenesis of ageing and many degenerative diseases (Andre, Larondelle, & Evers, 2010). Current evidence strongly supports a contribution of polyphenols to the prevention of several chronic degenerative diseases such as cancer, atherosclerosis and cardiovascular diseases and central nervous system disorders, as well as ageing (Giovannini & Masella, 2012).

Fruit and vegetable production and processing generates substantial quantities of waste/by-products. It has previously been reported that wastes and by-products of fruits may be an abundant source of antioxidant polyphenols (Peschel et al., 2006; Vasco, Ruales, and Kamal-Eldin 2008; Wijngaard, Rößle, & Brunton, 2009). At the present time, fruit and vegetable wastes and by-products are often discarded at the expense of the manufacturer. Use of the wastes as a source of polyphenols may be of considerable economic benefit to food processors. In addition, the antioxidant and cytoprotective activities of polyphenols in fruit and vegetable wastes and by-products are of utmost importance to substantiate their potential health benefits in human nutrition.

The aim of the present study was to measure the relative content of phenolics in extracts prepared from fruit and vegetable wastes and by-products and to evaluate their antioxidant capacity and cytoprotective activity under the same conditions. The contents of total phenolics in extracts prepared from (I) under-utilised fruits: Chinese quince (*Pseudocydonia sinensis*), quince (*Cydonia oblonga*), and hardy kiwi (*Actinidia arguta*); (II) fruit wastes: immature apple (*Malus pumila*), immature peach (*Prunus persica*), immature prune (*Prunus domestica*), immature pear (*Pyrus pyrifolia*), grape skin (*Vitis labruscana*: *V. labrusca* × *Vitis vinifera*), and grape seed (*V. labruscana*: *V. labrusca* × *V. vinifera*); (III) vegetable wastes: broccoli leaf (*Brassica oleracea* Italica group), broccoli stem (*B. oleracea* Italica group), asparagus stem (*Asparagus officinalis*), cabbage outer leaf (*B. oleracea* Capitata group), Chinese cabbage outer leaf (*Brassica rapa* Pekinensis group), lettuce outer leaf (*Lactuca sativa* Capitata group), and cornhusks (*Zea mays*); (IV) by-products: persimmon peel (*Diospyros kaki*), apple pomace (*M. pumila*), wine pomace (*Vitis spp.*), grape bunchstem (*V. vinifera*), Chinese quince pomace (*P. sinensis*), quince pomace (*C. oblonga*), and perilla pomace (*Perilla frutescens*); (V) hull: cowpea hull (*Vigna unguiculata*), black azuki bean hull (*Vigna angularis*), and buckwheat hull (*Fagopyrum esculentum*) were analysed by Folin–Ciocalteu assay. Antioxidant activities of all samples were measured by

* Corresponding author. Tel./fax: +81 265 77 1609.
E-mail address: snakamu@shinshu-u.ac.jp (S. Nakamura).

http://dx.doi.org/10.1016/j.foodchem.2014.06.099
0308-8146/

2,2-diphenyl-1-picryl-hydrazyl (DPPH) assays. Furthermore, cytoprotective activities of extracts were evaluated by their inhibitory effects against oxidative stress induced by 2,2′-azobis-(2-amidinopropane) dihydrochloride (AAPH) peroxyl radical generator in MA 104 cells.

2. Materials and methods

2.1. Chemicals

Folin–Ciocalteu phenol reagent, chlorogenic acid hemihydrate (3-caffeoylquinic acid hemihydrate), and AAPH were purchased from Wako Pure Chemical Industries (Osaka, Japan). DPPH and 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Plant materials

The samples investigated were (I) under-utilised fruits: Chinese quince (*P. sinensis*), quince (*C. oblonga*), and hardy kiwi (*A. arguta*); (II) fruit wastes: immature apple (*M. pumila*), immature peach (*P. persica*), immature prune (*P. domestica*), immature pear (*P. pyrifolia*), grape skin (*V. labruscana*: *V. labrusca* × *V. vinifera*), and grape seed (*V. labruscana*: *V. labrusca* × *V. vinifera*); (III) vegetable wastes: broccoli leaf (*B. oleracea* Italica group), broccoli stem (*B. oleracea* Italica group), asparagus stem (*A. officinalis*), cabbage outer leaf (*B. oleracea* Capitata group), Chinese cabbage outer leaf (*B. rapa* Pekinensis group), lettuce outer leaf (*L. sativa* Capitata group), and cornhusks (*Z. mays*); (IV) by-products: persimmon peel (*D. kaki*), apple pomace (*M. pumila*), wine pomace (*Vitis spp.*), grape bunchstem (*Vitis vinifera*), Chinese quince pomace (*P. sinensis*), quince pomace (*C. oblonga*), and perilla pomace (*P. frutescens*); (V) hull: cowpea hull (*V. unguiculata*), black azuki bean hull (*V. angularis*), and buckwheat hull (*F. esculentum*). Fruit and vegetable waste and by-products were obtained from different agro-industries of Nagano prefecture, Japan.

2.3. Hot-water extraction

Hot-water extraction was performed as follows: each plant material (100 g) was put into 4 times its volume of boiling water and boiled for 1 h in a flask equipped with a reflux condenser. The suspended solution was filtered using two layers of cheesecloth and a filter paper (ADVANTEC, No. 2, Tokyo, Japan), concentrated using a rotary evaporator, then lyophilised (Hot-water extracts). Samples were re-dissolved using distilled water. In the case of the cowpea hull, the extraction was conducted in 8 times its volumes of water (800 mL) because of very high water absorbability.

2.4. Ethanol extraction

Ethanol extraction was performed as follows: each plant material (100 g) was immersed in 4 times its volumes of ethanol (for wet materials) or 80%(v/v) ethanol (for dried materials) and after 1 week at room temperature, the ethanol solution was filtered using a filter paper (ADVANTEC, No. 2). The filtrate was concentrated using a rotary evaporator (below 40 °C), then lyophilised (Ethanol extracts). Samples were re-dissolved using distilled water. In the case of persimmon peel and apple pomace, the extraction was conducted in a hot-80% (v/v) ethanol (boiled for 1 h) in a flask equipped with a reflux condenser. The other procedures were the same as described previously.

2.5. Determination of total phenolics content

Total phenolic concentrations were measured using the Folin–Ciocalteu assay (Singleton & Rossi, 1965). Briefly, 10 μl of sample or a standard solution of chlorogenic acid hemihydrate was added into a 96-well microplate, followed by 40 μl of distilled water and 50 μl of Folin–Ciocalteu reagent diluted with distilled water (1:7 v/v). After 5 min, 50 μl of 9% of sodium carbonate aqueous solution was added and the contents were mixed thoroughly. The mixtures were allowed to stand for 90 min at room temperature in the dark. The absorbance was measured at 590 nm using a Model 680 Microplate Reader (Bio-Rad Laboratories, Hercules, CA, USA). Results (average of three replicates ± SD) were expressed as milligrammes of chlorogenic acid hemihydrate equivalent per gram (mg ChAE/g) of extracted sample.

2.6. Free radical scavenging activity (DPPH assay)

The free radical scavenging activity using DPPH reagent was measured using the Brand-Williams, Cuvelier, and Berset (1995) method with some modification. Briefly, 10 μl aliquots of sample solution with a different concentration or phosphate buffer saline (PBS) were added into a 96-well micro plate, followed by 40 μl of 0.5 mM Tris–HCl (pH 7.2). The reaction was initiated by the addition of 100 μl/well of DPPH solution in ethanol or pure ethanol for the colour control. The mixtures were allowed to stand for 30 min at room temperature in the dark. The absorbance was measured at 520 nm using a microplate reader. The results (average of three replicates ± SD) were expressed as the EC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%.

2.7. Cell culture

MA 104 cells, an African monkey kidney cells, purchased from the ATCC (American Type Culture Collection), were maintained at 37 °C in an incubator with a humidified atmosphere of 5% CO_2. The MA 104 cells were cultured in GIT medium (Wako Pure Chemical Industries) containing streptomycin (100 μg/ml) and penicillin (100 units/ml).

2.8. Cell viability

Cell viability was estimated using the MTT assay, which is based on the cleavage of a tetrazolium salt by mitochondrial dehydrogenases in viable cells (Hansen, Nielsen, & Berg, 1989). MA104 cells were seeded in a 96-well plate at a density of 8.0×10^3 cells/well. At 2 or 3 days after plating, cells were treated with 100 μg/ml of sample extract. After incubation for 3 h, the samples were removed and cells were treated with 10 μl of sample, 10 μl of 0.8 mM AAPH solution, and 80 μl of PBS for 3 h. After removal of the AAPH solution, the cells were incubated for an additional 3 days and then treated with 70 μl of MTT solution containing 0.5 mg/ml of MTT in Eagle's minimum essential medium (Gibco, Grand Island, NY, USA) for 50 min at 37 °C. The samples were then extracted with 90 μl of 0.04 N HCl–isopropanol and the absorbance was measured at 570 nm. The relative cell viability was determined by the amount of MTT converted into the insoluble formazan salt. The optical density of the formazan formed in the untreated control cells was taken as 100% viability. Data are mean percentages of six replicates of viable cells relative to the respective controls.

2.9. Statistical analysis

Statistical significance between the results was analysed using Student's *t* test. *P* values <0.05 were considered statistically significant.