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ScienceDirect
Food Chemistry 109 (2008) 340–347
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Assessment of polyphenolic content and *in vitro* antiradical characteristics of apple pomace

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Received 31 July 2007; received in revised form 12 December 2007; accepted 17 December 2007

Abstract

Apple pomaces, a by-product in the apple juice processing, were subjected to evaluation as potential sources of antioxidant phytochemicals on the basis of their total content of phenolics (from 4.22 to 8.67 mg/g), total flavonoids (from 0.45 to 1.19 mg/g) and total flavan-3-ols (from 2.27 to 9.51 mg/g), and *in vitro* antiradical activities. Some individual phenolic compounds including caffeic and chlorogenic acids, (+)-catechin and (−)-epicatechin, rutin, quercetin glycosides and phloridzin were identified and quantified by HPLC. The antiradical activity of apple pomaces was tested by measuring their ability to scavenge DPPH and hydroxyl radicals by ESR spectroscopy. The highest DPPH ($EC_{50}^{DPPH} = 6.33$ mg/ml) and hydroxyl ($EC_{50}^{OH} = 26.11$ mg/ml) radical scavenging activities were obtained in the case of Reinders pomace. The regression analysis produced moderate to high correlation coefficients between the antiradical activities ($1/EC_{50}^{DPPH}$ and $1/EC_{50}^{OH}$), and total phenolics, total flavonoids, total flavan-3-ols, and some individual phenolic compounds.

Keywords: Apple pomace; Phenolic compounds; HPLC; Antiradical activity; DPPH radical; Hydroxyl radical; ESR

1. Introduction

The diet plays an important role in the morbidity and mortality associated with the chronic diseases such as cardiovascular disease, cancer, hypertension and obesity. Several investigations have estimated that one-third of all cancer cases and one-half of cardiovascular diseases and hypertension can be attributed to diet (Lee & Smith, 2000; Willet, 1994; Wolfe, Wu, & Liu, 2003). The possible beneficial health effects of diets including fruits, vegetables and their products have been attributed to their phytochemicals (Block, Patterson, & Subar, 1992; Lampe, 1999; Liu, 2003). Fruits and vegetables contain many different dietary phytonutrients which contribute to the prevention of degenerative diseases caused by oxidative stress (Kaur & Kapoor, 2001). Polyphenols are one of the phytochemical groups whose "protective" properties include antioxidant, antimicrobial, anticancer and cardiovascular-protective activities (Bendini et al., 2006; Chu, Chang, & Hsu, 2000; Hertog et al., 1995; Liu, 2002; Süzgeç, Meriçli, Houghton, & Çubukçu, 2005).

Apples are well-known and widespread fruit of the genus Malus (about 25 species) belonging to the family Rosaceae. In numerous diets, apples are a very significant part and represent an important source of bioavailable polyphenolic compounds such as flavonols (with quercetin glycosides as the main representative), monomeric and oligomeric flavanols, dihydrochalcones (e.g., phloridzin), anthocyanidins, *p*-hydroxycinnamic and *p*-hydroxybenzoic acids (Escarpa & Gonzalez, 1998). The contents of phenolic compounds vary greatly among different varieties of apples, and between the peel and the flesh; apple peels contain a higher concentration of phenolic compounds (Escarpa & Gonzalez, 1998; Vrhosek, Rigo, Tonan, & Mattivi, 2004).

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0308-8146/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2007.12.046

The antioxidant compounds from waste product of food industry could be used for increasing the stability of foods by preventing lipid peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen free radicals (Makris, Boskou, & Andrikopoulos, 2007). Apple pomace is a by-product in the apple juice processing, which is a rich source of polyphenols, minerals and dietary fibre (Boyer & Liu, 2004; Figuerola, Hurtado, Estevez, Chiffelle, & Asenjo, 2005; Schieber, Keller, & Carle, 2001; Sudha, Baskaran, & Leelavathi, 2007). Processing apples into juice has been found to affect to phenolic content. Conventional apple juice production (straight pressing of apple pulp or pressing after pulp enzyming) resulted in a juice poor in phenolics and with only 3–10% of the antioxidant activity of the fruit they were produced from (van der Sluis, Dekker, Skrede, & Jongen, 2002).

In view of the fact that most of the phenolic compounds remained in the apple pomace, our interest is focused on the apple pomaces (obtained from apple varieties – Pinova, Reinders, Jonagold, Iduna, Braeburn and sample obtained from factory Nectar) as a potential source of bioactive phenolics, which can be used for various purposes in the food, pharmaceutical and cosmetic industry. The objective of this study was to examine phenolic composition using the spectrophotometrical determination of total phenolics (TP), total flavonoids (TFd) and total flavan-3-ols (TFl), and individual phenolic compounds by HPLC. In effort to establish the antioxidative activity of apple pomace extracts against stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reactive hydroxyl radicals, a very sensitive analytical method, electron spin resonance (ESR) spectroscopy, was evaluated. The total phenolic, flavanoid and flavan-3-ol contents, and also a content of individual phenolic compounds of investigated apple pomaces, were correlated to their antioxidant activities.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), Folin–Ciocalteu reagent, vanillin, caffeic acid, chlorogenic acid, (+)-catechin and (−)-epicatechin, quercetin, rutin and phloridzin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). These chemicals were of analytical reagent grade. Other used chemicals and solvents were of the highest analytical grade and obtained from "Zorka" Šabac (Serbia).

2.2. Pomace preparation

Apple varieties (Pinova, Reinders, Jonagold, Iduna, Braeburn) harvested in Serbia in the 2005 season, were collected from the Department for Fruit Growing and Viticulture, Faculty of Agriculture, University of Novi Sad.

Apples (1 kg) of each variety were cleaned by washing, stalks were removed, and the fruits were cut in four pieces and apple pulp was prepared by quick slicing in a domestic food processor (Bosch, Compact Kitchen Machine 4420, Gerlingen-Stuttgart, Germany). Straight pressed apple juice was prepared by immediate pressing of apple pulp. Apple pulps were pressed in a manual cider juice press to separate apple juice. The samples of the obtained apple pomace were taken. One sample of apple pomace was procured from a fruit juice industry (D.O.O. "Nectar", Bačka Palanka, Serbia). Moisture of each sample of apple pomace was determined using drying oven method, by drying a representative 10 g sample in a forced air oven (Sterimatic ST-11, Instrumentaria, Zagreb, Croatia) at 60 °C until the constant mass.

The yields of apple pomace such as moisture contents are shown in Table 1.

2.3. Extraction procedure

Samples of apple pomace (20 g) were extracted at room temperature using an ultrasonic bath, Heidolph DIAX 900 (Heidolph Instruments GmbH, Kelheim, Germany). The extraction was performed three times with different amounts of 80% methanol: 160 ml in 60 min, 80 ml in 60 min, 80 ml in 30 min at room temperature. The total extraction time was 150 min. The obtained three extracts were combined and evaporated to dryness under reduced pressure.

The yields, average of triplicate analysis, of extracts were: Pinova, $m = 2.87 \pm 0.14$ g; Reinders, $m = 2.68 \pm 0.13$ g; Jonagold, $m = 2.99 \pm 0.14$ g; Iduna, $m = 2.88 \pm 0.14$ g; Braeburn, $m = 2.52 \pm 0.13$ g; Nectar, $m = 1.65 \pm 0.08$ g.

2.4. Spectrophotometrical determination

2.4.1. Total phenolics

Total phenolics in apple pomace extracts were determined using the Folin–Ciocalteu reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999). The reaction mixture was prepared by mixing 0.1 ml of methanolic solution (concentration 50 mg/ml) of extract, 7.9 ml of distilled water, 0.5 ml of the Folin–Ciocalteu's reagent and 1.5 ml of 20% sodium carbonate. After 2 h, the absorbance at 750 nm (spectrophotometer Camspec M105, Cambridge, UK) was obtained against blank that had been prepared in a similar manner, by replacing the extract with distilled water. The total phenolic content, expressed as mg chloro-

Table 1
The yields and moisture contents of apple pomaces

Apple varieties	Yield (g)	Moisture content (%)
Pinova	288.8	77.2
Reinders	216.1	79.8
Jonagold	342.6	80.1
Iduna	306.5	76.5
Braeburn	295.7	78.8
Nectar[a]	–	74.9

[a] Industrial apple pomace obtained from factory Nectar.