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Chemical composition and antioxidant capacity of different anatomical parts of pear (*Pyrus communis* L.)

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ABSTRACT

Sugar, organic acid, triterpenoid and phenolic composition as well as antioxidant capacity of different anatomical parts of pear were studied. Fruits and leaves of 'Radana' pear (*Pyrus communis* L.) were used. The results showed great quantitative differences in the composition of the pear pulp, peel, leaves and seeds. Fructose was the major sugar in pulp, seeds and peel (227.46, 45.36 and 67.49 g/kg dry mass [DM], respectively), while sorbitol was predominant in leaves (40.66 g/kg DM). Malic acid was the major organic acid, followed by citric and shikimic acids. The highest concentration of triterpenoids (3460.5 µg/g DM) was determined in pear peel, and ursolic acid was predominant. Leaves were characterized by the highest amount of phenolics (5326.7 mg/100 g DM) and the highest DPPH and FRAP values (2027.9 and 3539.6 µmol TE/100 g DM, respectively). Pear leaves and seeds may be selected as potential sources of phytochemicals.

1. Introduction

Pear (*Pyrus* spp.) is one of the most important stone fruits, widely consumed fresh when fully mature, and in processed forms such as in purées, jams, dried, and so on. There is a great diversity of pear cultivars, due to it being commercially grown in more than 50 countries around the world (Nikićević, 2005). In 100 g of the edible portion, European pears (*Pyrus communis*) provide about 54 calories of food energy. Pear consists of 85% water, 14% carbohydrate, 2% fiber, 0.3% protein and 0.1% fat. Of vitamin contents, there are about 3 mg of ascorbic acid, and trace amounts of other vitamins (Standard Tables of Food Composition., 2003). Being high in fiber, low in calorie, and rich in vitamins and minerals, pear is a nutrient-dense fruit. Contents of free sugars and organic acids play an important role in the nutritive value and quality of fruit, due to the formation of sour taste and regulation of chemical reactions in the body (Chen, Wang, Wu, Wang, & Hu, 2007).

Pears are a good source of phytochemicals, especially phenolics, which are important bioactive compounds known for their health benefits. Those health benefits of polyphenol consumption derive from their antioxidant and anti-inflammatory properties (Borges, Mullen, & Crozier, 2010). Other pro-health properties that are attributed to pears, are related to the content of triterpenoids, due to their antioxidative, anti-inflammatory and anticancer properties (Lesselier, Destandau, Grigoras, Fougère, & Elfakir, 2012).

Phytochemicals are not equally distributed within the fruit (Escarpa & Gonzales, 2000). The peel is a rich source of anthocyanins, flavan-3-ols, including polymeric procyanidins, and flavonols. Also triterpenoids are located in cuticle waxes (Szakiel, Paczkowski, & Huttunen, 2012). Chlorogenic acid is mainly present in the core, whereas lower levels are found in the pulp and peel. In pear leaves, arbutin and hydroxycinnamic acids are the dominating compounds (Andreotti, Costa, & Treutter, 2006).

Therefore, the present work evaluated pears for selected chemical parameters (composition of sugars and organic acids), bioactive compounds (phenolic compounds and triterpenoids), and antioxidant activities, in pear pulp, peel, seeds and leaves. Additionally, the correlations between the content of phenolic compounds, triterpenoids and antioxidant activities were investigated. An additional goal of this study was to find the most valuable source of bioactive substances. Taking into consideration the considerable economic importance of the disposal of by-products of the fruit industry, it is important to identify the potential application of new sources of phytochemicals. In this study, pear anatomical parts, including seeds and leaves, have been compared from this perspective for the first time.

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2. Material and methods

2.1. Reagents and standards

Acetonitrile, formic acid, methanol, DPPH (1,1-diphenyl-2-picrylhydrazyl radical), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tri(2-pyridyl)-*s*-triazine), acetic acid, phloroglucinol, arbutin, caffeic acid, betulinic, oleanolic and ursolic acid, fructose, glucose, sorbitol and sucrose, malic, oxalic, citric, shikimic, succinic and fumaric acid were purchased from Sigma-Aldrich (Steinheim, Germany). Quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, cyanidin 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside, apigenin 7-*O*-glucoside, quinic, *p*-coumaric and ferulic acid, (+)-catechin and procyanidin B_2 were purchased from Extrasynthese (Lyon, France).

2.2. Plant material

Fruits and leaves of 'Radana' pear (*P. communis* L.) were used in this study. These were obtained from the Research Station for Cultivar Testing in Zybiszów near Wrocław, Poland (51°3′51.11″ N, 16°54′43.56″ E). Fruits were collected at the optimum ripening stage recommended for consumption (based on the internal ethylene concentration and starch index). Leaves were picked up on the same day in early August, 2014. In the course of the measurements, 10 replications (for each 30 randomly chosen leaves and about 3 kg of fruit) from three trees, i.e., 30 replications, were established.

After harvest, the fruits were peeled (skin thickness approx. 0.5–0.7 mm), and cut (cube size 0.5 cm), and the seeds were separated. The leaves were cut (pieces of approx. 0.5–1 cm). Flesh, peel, seeds and leaves were frozen in liquid nitrogen and crushed by a laboratory mill to a homogeneous powder in liquid nitrogen (IKA 11A; Staufen, Germany) and freeze-dried for 24 h (Christ Alpha 1–4 LSC, Germany). Powders were kept in a refrigerator (−80 °C) until extract preparation.

2.3. Analysis of sugars by the HPLC-ELSD method

The extract for analysis of sugars was prepared as described by Oszmiański, Kolniak-Ostek, Lachowicz, Gorzelany, & Matłok, 2015. Chromatographic analysis was carried out with a Merck-Hitachi L-7455 liquid chromatograph with an evaporative light scattering detector (ELSD) (Polymer Laboratories PL-ELS 1000) and L-7100 quaternary pump, equipped with the D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan), L-7200 autosampler and a Prevail Carbohydrate ES HPLC Column-W (250 mm × 4.6 mm, 5 µm) (Alltech, US). Calibration curves ($R^2 = 0.9999$) were created for glucose, fructose, sorbitol and sucrose. All data were obtained in triplicate. The results were expressed as grams per kilogram of dry matter (DM).

2.4. Analysis of organic acids by the HPLC method

For the extraction of samples, the protocol described by Nawirska-Olszańska, Biesiada, Sokół-Łętowska, and Kucharska (2014) was followed. Analysis of organic acids was carried out with a Dionex (USA) liquid chromatographer equipped with the Ultimate 3000 LED detector, LPG-3400A pump, EWPS-3000SI autosampler, TCC-3000SD column thermostat, an Aminex HPH-87 H (300 mm × 7.8 mm) column with IG Cation H (30 mm × 4.6 mm) Bio-Red precolumn and the Chromeleon v.6.8 computer software. Separation was conducted at 65 °C. The elution solvent was 0.001 N sulfuric acid. Samples (10 µl) were eluted isocratically, at the flow rate of 0.6 ml min^{-1}. The compounds were monitored at 210 nm. All data were obtained in triplicate. The results were expressed as grams per kilogram of dry matter (DM).

2.5. Analysis of triterpenoids by the UPLC–PDA–MS method

Sample extraction was performed as described by Farneti et al. (2015). Identification and quantification of ursolic, oleanolic, and betulinic acids was performed using an ACQUITY Ultra Performance LC system with binary solvent manager (Waters Corp., Milford, MA, USA), a UPLC BEH C18 column (1.7 µm, 2.1 mm × 150 mm, Waters Corp., Milford, MA, USA), and a Q-Tof Micro mass spectrometer (Waters, Manchester, UK) equipped with an ESI source, operating in negative mode. The elution solvent was methanol-acetonitrile (15:85, v/v), at a flow rate of 0.1 ml min^{-1}. The m/z for betulinic acid was 455.3452, for oleanolic acid 455.3496, and for ursolic acid 455.3365, and the retention times were 6.80, 7.50 and 7.85 min, respectively. The compounds were monitored at 210 nm. All data were obtained in triplicate. The results were expressed as milligrams per gram of dry matter (DM).

2.6. Identification and quantification of polyphenols by the UPLC–PDA–MS method

The extract of polyphenol analysis was prepared as described by Kolniak-Ostek and Oszmiański (2015). Analysis of polyphenols was carried out using an ACQUITY Ultra Performance LC system (UPLC) equipped with binary solvent manager (Waters Corp., Milford, MA, USA), a UPLC BEH C18 column (1.7 µm, 2.1 mm × 50 mm, Waters Corp., Milford, MA, USA) and a Q-Tof Micro mass spectrometer (Waters, Manchester, UK) with an ESI source operating in negative and positive modes. The mobile phase consisted of aqueous 0.1% formic acid (A) and 100% acetonitrile (B). Samples (10 µl) were eluted according to the linear gradient described previously by Oszmiański, Kolniak-Ostek and Biernat (2015). The conditions of the mass spectrometer were: a source block temperature of 130 °C, desolvation temperature of 350 °C, capillary voltage of 2.5 kV, cone voltage of 30 V, and a desolvation gas (nitrogen) flow rate of 300 l/h.

The compounds were monitored at 280 nm (flavan-3-ols and hydroquinones), 320 nm (phenolic acids), 340 nm (flavones), 360 nm (flavonol glycosides) and 520 nm (anthocyanins). Calibration curves were determined experimentally for caffeic, *p*-coumaric, quinic and ferulic acids, procyanidin B_2, (+)-catechin, kaempferol 3-*O*-glucoside, quercetin 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside, apigenin 7-*O*-glucoside, cyanidin 3-*O*-glucoside and arbutin. Caffeic acid derivatives were expressed as caffeic acid, *p*-coumaric acid derivatives were expressed as *p*-coumaric acid, ferulic acid derivatives were expressed as ferulic acid, monomeric catechins were expressed as (+)-catechin, polymeric procyanidins were expressed as procyanidin B_2, kaempferol derivatives were expressed as kaempferol 3-*O*-glucoside, quercetin derivatives were expressed as quercetin 3-*O*-glucoside, isorhamnetin derivatives were expressed as isorhamnetin 3-*O*-glucoside, derivatives of apigenin were expressed as apigenin 7-*O*-glucoside, and cyanidin derivatives were expressed as cyanidin 3-*O*-glucoside. All experiments were done in triplicate. The results were expressed as milligrams per 100 g of dry matter (DM).

2.7. Analysis of proanthocyanidins by the phloroglucinolysis method

Phloroglucinolysis of powder samples was performed according to the protocol described by Oszmiański, Wojdyło, and Kolniak (2011). Phloroglucinolysis products were separated on a Cadenza CD C18 (75 mm × 4.6 mm, 3 µm) column (Imtakt, Japan). Analysis was carried out using a Waters (Milford, MA) system equipped with Waters 474 diode array and scanning fluorescence detectors