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Content of bioactive compounds and antioxidant capacity in skin tissues of pear

Joanna Kolniak-Ostek *

Department of Fruit, Vegetable and Cereal Technology, Wroclaw University of Environmental and Life Sciences, 37 Chelmonskiego Street, 51-630 Wroclaw, Poland

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ABSTRACT

The amounts of bioactive compounds (phenolic compounds, triterpenoids and carotenoids) and antioxidant activities of peels from 10 different pear cultivars were studied. Polyphenols, triterpenoids and carotenoids were identified and quantified by liquid chromatography photodiode array quadrupole time-of-flight mass spectrometry (LC-PDA-QTOF/MS), while antioxidant capacities were evaluated *in vitro* using scavenging assay of 1,1-diphenyl-2-picrylhydrazyl radical and ferric reducing power. A total of 44 polyphenolic compounds were identified: 18 hydroxycinnamic acids, 13 flavan-3-ols, 10 flavonols, 1 flavone, 1 hydroquinone and 1 anthocyanin. Three triterpenoids (betulinic, oleanolic and ursolic acid), 4 carotenoids and 7 chlorophylls were identified. It was found that the cultivars had different phenolic, triterpenoid and chromoplast pigment concentrations. In addition, a positive correlation between antioxidant capacity and content of bioactive compounds was found.

1. Introduction

In recent years increasing attention has been paid to the role of oxidative stress and inflammation in several chronic diseases. Positive correlations between oxidative stress and formation of transmissible chronic diseases (HIV-1 and HPV type A and B), autoimmune and neurodegenerative diseases (Parkinson's disease and multiple sclerosis), metabolic diseases (atherosclerosis, obesity, hypertension and heart diseases), cancer (breast, head and neck cancer), and cachexia have been reported (Dichi, Breganó, Colado Simão, & Cecchini, 2014). Intake of food rich in antioxidants has been suggested to protect against chronic diseases and oxidative damage (Dandona, Aljada, & Bandyopadhyay, 2004). Fruits and vegetables are a good source of bioactive compounds, which strongly influence their nutritional value. Among the bioactive compounds, phenolics, which possess strong antioxidant activity, display a broad spectrum of health-promoting benefits (Galvis Sánchez, Gil-Izquierdo, & Gil, 2003). Phenolics are present in all parts of plants including roots, stems, leaves, and fruits (peel, flesh, seeds) and are associated with resistance to pathogens in the environment (Li et al., 2014). In comparison to the flesh, many fruits, such as pears, contain more phenolics in the peel (Li et al., 2014; Kolniak-Ostek & Oszmiański, 2015). The addition of stable polyphenolics to products such as desserts, yogurts, beverages and snacks makes it possible to not only improve the colour but also to enrich them in the bioactive compounds. In addition, the use of solid residues that remain after the fruit processing can significantly reduce the amount of underutilised wastes.

Other health benefits attributed to the pear fruit are connected with the content of triterpenoids and carotenoids. Wang et al. (2015) in their studies on *Pyrus* peel and pulp extracts re-

* *E-mail address:* joanna.kolniak-ostek@up.wroc.pl.
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ported them as a good source of triterpenes. Triterpenoids, which are mainly located in cuticular waxes, due to their antioxidative properties, exhibit anti-HIV activity, showed significant cytotoxicity in lymphocytic leukaemia cells and possess anti-inflammatory and antiarthritic properties (Claude, Morin, Lafosse, & Andre, 2004).

Apart from anthocyanins, responsible for the red colour of peels, chromoplast pigments – carotenoids and chlorophylls – contribute to fruit colouration. The structure of most carotenoids includes a system of conjugated double bonds (Britton, 1995). This structure influences their antioxidant and provitamin A activity, inhibition of carcinogenesis, the defence response against free radicals and reactive oxygen species (ROS), and the reduction of the risk of developing degenerative diseases (Britton, Liaaen-Jensen, & Pfander, 2004). Also chlorophylls exhibit antioxidant and antimutagenic properties and can prevent degenerative diseases and cancer (Delgado-Pelayo, Gallardo-Guerrero, & Hornero-Méndez, 2014).

Due to the presence of bioactive compounds, pears possess anti-inflammatory, antitussive, diuretic and antihyperglycemic activities (Li et al., 2014), which have been used in folk medicine for over two thousand years.

Although pear peels could be a promising source of bioactive nutraceuticals with potential health benefits, a large amount of solid residues, generated during fruit processing, remains underutilised. Therefore, in the present work bioactive compounds (phenolics, triterpenoids, carotenoids and chlorophylls) and antioxidant capacity (DPPH and FRAP) were evaluated in pear skins from 10 different cultivars. Although the chemical composition and antioxidant capacity have already been reported in pears, detailed characterisation of polyphenolics, triterpenoids and chromoplast pigments of pear peels and their antioxidant activity has not yet been performed. An additional goal of this study was to find the most promising pear cultivars with respect to bioactive compounds content and health-promoting properties, in order to develop a selection procedure suitable for a pear breeding programme for new cultivars for use in the Polish food industry. No cultivars of pears have been analysed from this perspective until now.

2. Materials and methods

2.1. Reagent and standards

Acetonitrile, formic acid, methanol, acetic acid, phloroglucinol, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), arbutin, caffeic acid, betulinic, oleanolic and ursolic acid, all-*trans*-β-carotene, chlorophyll a, all-*trans*-lutein and pheophorbide a 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, 1-caffeoylquinic acid, (+)-catechin and procyanidin B_2 and A_2 were purchased from Extrasynthese (Lyon, France).

2.2. Plant material

Ten pear (*Pyrus communis* L.) cultivars ('Bonkreta Williamsa', 'Carola', 'Dicolor', 'Faworytka', 'Hortensia', 'Konferencja', 'Lukasówka', 'Nojabrska', 'Radana' and 'Uta') were used. Fruits were collected in 2014 from the Research Institute of Horticulture in Skierniewice, Poland (51° 55′ 24″ N, 020° 5′ 58″ E). Fruits were harvested at the optimum ripening stage recommended for consumption (based on internal ethylene concentration and starch index). In the course of the measurements, 10 randomly chosen fruits from three trees, i.e. 30 replications per cultivar, were used. For each cultivar approximately 3 kg of fruits were collected.

After harvest, the fruits were peeled. The peels were directly frozen in liquid nitrogen and freeze-dried (24 h; Christ Alpha 1–4 LSC; Martin Christ GmbH, Osterode am Harz, Germany). The freeze-dried samples were crushed using a closed laboratory mill (IKA 11A; Staufen, Germany). Powders were kept in a refrigerator (–80 °C) until extract preparation.

2.3. UPLC–PDA–MS system

All analyses were performed using an ACQUITY Ultra Performance LC system (UPLC) with binary solvent manager (Waters Corp., Milford, MA, USA) and a Micromass Q-Tof Micro mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionisation (ESI) source. The analyses were carried out using MS scanning from *m/z* 100 to 2500. Leucine enkephalin at a concentration of 500 pg/μL and a flow rate of 2 μL/min was used as the reference compound. The lock mass correction was ±1.000 for the mass window. The mass spectrometer was operated in negative- and positive-ion mode, set to the base peak intensity (BPI) chromatograms, and scaled to 12,400 counts per second (cps) (100%). The optimised MS conditions were: source temperature of 100 °C, desolvation temperature of 300 °C, desolvation gas (nitrogen) flow rate of 300 L/h, capillary voltage of 2500 V and cone voltage of 30 V, and collision gas (argon) flow rate of 0.1 L/h. The data were subsequently entered into the MassLynx 4.0 ChromaLynx Application Manager software (Waters).

2.4. Identification and quantification of polyphenols

For the extraction and determination of polyphenols, a protocol described before by Kolniak-Ostek and Oszmiański (2015) was followed. The column used was a UPLC BEH C18 column (1.7 μm, 2.1 mm × 100 mm, Waters Corp.). The mobile phase consisted of aqueous 0.1% formic acid (A) and 100% acetonitrile (B).

The compounds were monitored at 280 nm (flavan-3-ols and hydroquinones) (Fig. 1), 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 1-caffeoylquinic acids, procyanidin B_2 and A_2, (+)-catechin, kaempferol 3-*O*-glucoside, quercetin 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside, apigenin 7-*O*-glucoside, cyanidin 3-*O*-glucoside and arbutin. *p*-Coumaroylquinic acids were expressed as *p*-coumaric acid, quercetin derivatives were expressed as quercetin 3-*O*-glucoside, kaempferol derivatives were expressed as kaempferol 3-*O*-glucoside, isorhamnetin derivatives were expressed as isorhamnetin 3-*O*-glucoside, derivatives of apigenin were expressed as apigenin 7-*O*-glucoside, A-type procyanidin derivatives were expressed as procyanidin A_2, and B-type procyanidin derivatives were