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Research Paper

Phenolic antioxidant profiles in the whole fruit, flesh and peel of apple cultivars grown in Lithuania

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ABSTRACT

In this study total phenolic content (TPC), total ferric reducing antioxidant power, individual phenolics and their contributed antioxidant activity were determined by Folin-Ciocalteu, FRAP and HPLC-DAD coupled to FRAP post-column assays in the whole fruit, flesh and peel of six Lithuanian grown apple cultivars. The greatest TPC and total antioxidant activity were determined in 'Aldas' and 'Auksis' cultivars. Significant amounts of flavonols were determined in peels, while flavan-3-ols in apple parts varied. (−)-Epicatechin, procyanidins B2, C1 and chlorogenic acid were predominant reducing compounds in whole apple fruits. Hyperoside, avicularin and quercitrin were significant reducing compounds in peels. The cultivars with highly expressed flavan-3-ol fraction could be used in preparation of highly antioxidant active natural products.

1. Introduction

Apples are one of the most consumed fruits all over the word (Feliciano et al., 2010). They contain biologically active compounds of various classes: pectins, dietary fibres, vitamins, oligosaccharides, triterpenic acids and phenolic compounds (Figuerola et al., 2005; Hyson, 2011; Vasco et al., 2009). Due to its high accessibility apples are one of the largest contributors of phenolic intake in European and USA diet (Wolfe and Liu, 2003). Apple intakes have been associated with reduced risk of cardiovascular diseases, certain cancers, diabetes. Additionally apples have been shown to affect weight and can reduce cholesterol levels (Kalinowska et al., 2014; Koch et al., 2009). The main health effects have been ascribed to a complex of phenolic compounds (flavonols, dihydrochalcones, flavan-3-ols, anthocyanins, hydrocinnamic acids) (Duda-Chodak et al., 2010; Kalinowska et al., 2014; Koch et al., 2009; Serra et al., 2012). These compounds possess antioxidant activity, therefore apple extracts can be incorporated in various convergent products of health, nutrition and cosmetics providing nutraceuticals, cosmeceuticals and nutricosmetics (Barel et al., 2014). The main factor determining the amount of bioactive compounds is the apple cultivar. Scientific research has proven significant differences and intraspecific variations of phenolic complex in different apple cultivars (Ceymann et al., 2012; Minnocci et al., 2010; Serra et al., 2012). Their quantitative traits vary considerably and depend on environmental parameters, cultivation practices, harvestingand storage conditions(Feliciano et al., 2010; Kalinowska et al., 2014; Koch et al., 2009; Marks et al., 2007; McGhie et al., 2005).

A number of studies have determined total antioxidant activities of the phenolic rich extracts of various apple fruits (Serra et al., 2010; Vieira et al., 2009; Wojdyło et al., 2008). Nevertheless, information regarding the impact of individual compounds on antioxidant activity, as well as, their feasibility becoming activity markers, is still lacking. It is important to identify individual phenolic compounds possessing significant antioxidant activity for the proper choice of apple materials for functional products. HPLC-DAD-FRAP modern post-column system comprehensively characterized a full picture of phenolic and antioxidant profiles of whole fruits, flesh and peels of 'Aldas', 'Auksis', 'Connel Red', 'Ligol', 'Lodel', 'Rajka' cultivars during this multiannual study.

2. Materials and methods

2.1. Reagents and standards

All the reagents and standards were of analytical grade. HPLC-grade acetonitrile, acetic acid, iron(III) chloride hexahydrate ($FeCl_3 \times 6H_2O$), 2,4,6-tripyridyl-s-triazine (TPTZ), sodium

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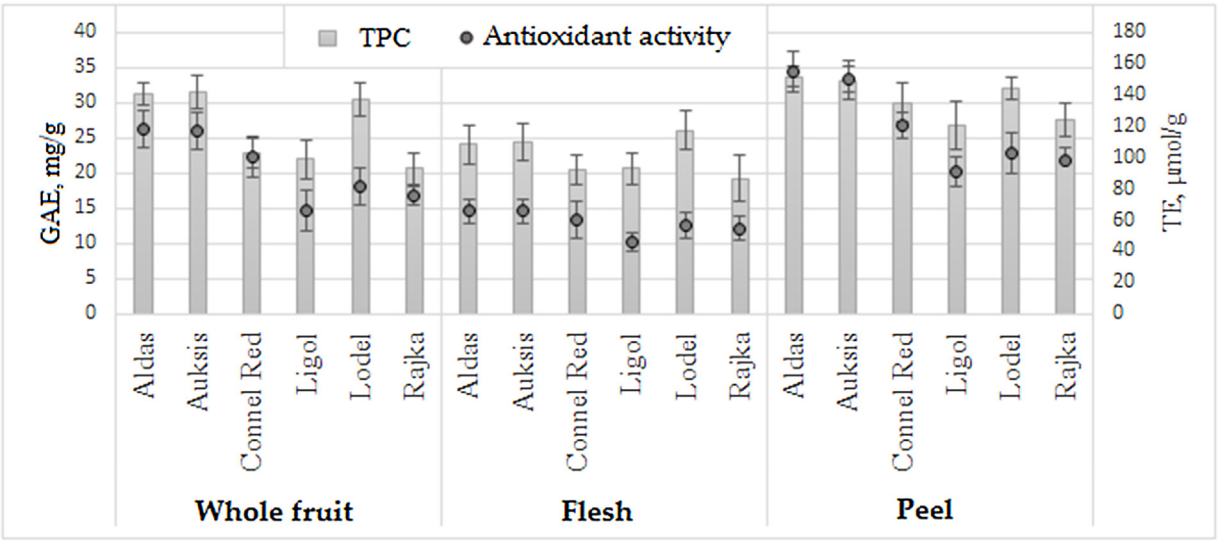

Fig. 1. Total phenolic content (GAE, mg g^{-1} dw) and total antioxidant activity (TE, µmol/g of dw) in the whole fruit, flesh and peel of six apple cultivars. (Results as mean ± S.E.).

acetate trihydrate ($C_2H_3NaO_2 \times 3H_2O$), Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and gallic acid monohydrate were obtained from Sigma-Aldrich GmbH (Buchs, Switzerland); ethanol from Stumbras AB (Kaunas, Lithuania). Hyperoside, rutin, quercitrin, phloridzin, procyanidin B1, procyanidin B2, procyanidin C1, and chlorogenic acid were obtained from Extrasynthese (Genay, France); (+)-catechin, (−)-epicatechin and reynoutrin were obtained from Fluka (Buchs, Switzerland); avicularin and isoquercitrin were obtained from Chromadex (Santa Ana, USA). Ultrapure water (18.2 m cm^{-1}) was prepared by Millipore (USA) water purification system.

2.2. Plant material and growing conditions

Six apple cultivars were included in the study: 'Aldas', 'Auksis', 'Connel Red', 'Ligol', 'Lodel', 'Rajka'. The apples were harvested in 2011–2013 years. The apples were grown at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai, Lithuania.The altitude of the experimental area is 57 m above sea level. The climatic conditions were described by Kviklys et al., 2014. The rainfall and the temperature of the years 2012 and 2013 were about the long term average values. Pest and disease management was carried out according to integrated plant protection practices. The experimental orchard was not irrigated. Tree fertilization was performed according to soil and leaf analysis. Nitrogen was applied before flowering at the rate of 80 kg ha^{-1}, and potassium was applied after harvest at the rate of 90 kg ha^{-1}. Soil conditions of the experimental orchard were the following: clay loam, pH: 7.3, humus: 2.8%, P_2O_5: 255 mg kg^{-1}, and K_2O: 230 mg kg^{-1}. Apples were harvested at the optimal harvest time individually established for every cultivar (Kvikliene et al., 2006).

2.3. Preparation of samples, spectrophotometric assays and HPLC-DAD-FRAP post-column assay

The apple powder sample of each cultivar was composed of20 ripe apples. The extracts were prepared according to the methods described by Liaudanskas et al. (2015). Lyophilized apple powder (exact weight, 2.5 g) was added to 30 mL of ethanol (70%, v/v), and extracted in a Sonorex Digital 10 P ultrasonic bath (Bandelin Electronic GmbH & Co. KG, Berlin, Germany) for 20 min at 40 °C.

TPC was evaluated using a modified spectrophotometric method described by Singleton and Rossi (1965). In this study, 0.50 mL of the extract was mixed with2.5 mL of Folin-Ciocalteau reagent (10-fold diluted). Then 2 mL of sodium carbonate (7.5%) was added. The absorbance was read after 30 min at 765 nm using an UV–vis spectrophotometer DU-70 (Beckman, USA). The TPC was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} dw).

The FRAP spectrophotometric method used the working FRAP reagent that was mixed with 10 µL aliquots of each extract. After 30 min, the absorbance was read at 593 nm using a UV–vis spectrophotometer. The results were expressed as µmol Trolox equivalent per gram of dry weight (µmol TE g^{-1} dw). The working FRAP reagent comprised TPTZ (0.01 M dissolved in 0.04 M HCl), FeCl3 × 6H2O (0.02 M in water) and acetate buffer (0.3 M, pH 3.6) in the ratio of 1:1:10 (Benzie and Strain, 1996).

A Waters 2695 chromatograph equipped with a Waters 2998 photodiode array detector (Milford, USA) was used for the HPLC analysis. Chromatographic separations were carried out by using YMC-Pack ODS-A (5 µm, C18, 250 × 4.6 mm i.d.) column equipped with YMC-Triart (5 µm, C18, 10 × 3.0 mm i.d.) pre-column (YMC Europe GmbH, Dinslaken, Germany). The flow rate was 1 mL min^{-1}. The mobile phase consisted of 2% (v/v) acetic acid in water (solvent A) and 100% (v/v) acetonitrile (solvent B). The following conditions of elution were applied: 0–30 min, 3%–15% B; 30–45 min, 15%-25% B; 45–50 min, 25%–50% B; and 50–55 min, 50%–95% B (Liaudanskas et al., 2015).

On-line post-column working FRAP solution was added by chromatograph Beckman programmable solvent module 126 (Fullerton, CA). The working FRAP reagent for post-column analysis comprised TPTZ, FeCl3 × 6H2O and acetate buffer in the ratio of 1:1:25 (Raudonis et al., 2012). The flow rate was set at 0.5 mL min^{-1}. The post-column reactor was made of 0.4 mL (PEEK, 0.25 mm i.d., 8 m) coil. The reactor temperature was set at 25 °C. The product chromatograms after the FRAP post-column reaction were registered at 593 nm, using Waters 2487 dual λ absorbance (UV–vis) detector (Milford, USA). The antioxidant activity of sample compounds was assessed by standard antioxidant Trolox. The antioxidant activity of phenolic compounds was expressed as µmol Trolox equivalent (TE) for 1 g of dry weight (dw). TE was calculated using the following formula:

$$TE = c \times V\,m^{-1}(\mu mol\,g^{-1});$$