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Chemical composition of apple fruit, juice and pomace and the correlation between phenolic content, enzymatic activity and browning

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ABSTRACT

Primary and secondary metabolites were evaluated in apple fruit, juice and pomace of scab resistant and leading European apple cultivars. The primary goal was to study the chemical composition of apple fruit fractions in correlation with their enzymatic browning. Additional goal was to assess the suitability of apple pomace for the extraction of phenolic compounds. Furthermore, the cultivars were grouped according to their suitability for fresh-cut fruit slices by measuring their enzymatic activity, total phenolic content (TPC) and the rate of browning. Highest TPC was determined in 'Granny Smith' pomace suggesting its optimal suitability for phenolic extraction among the analyzed apple cultivars. 'Majda' fruit (investigated for the first time) can be offered to the market as fresh-cut slices as almost no changes in pulp color have been detected due to oxidation. The correlation test showed that oxidation of apple pulp is highly dependent on TPC and weakly correlated to the activity of polyphenol oxidase. Contrary, no significant correlation has been determined between the rate of oxidation and the activity of peroxidase.

1. Introduction

In recent years consumers have become more aware of diverse health benefits of non-processed or low processed fruit (Rico, Martin-Diana, Barat, & Barry-Ryan, 2007). In addition to a fast growing market for locally produced apple fruit and juice, an increasing interest for fresh-cut apple slices or cubes has been recorded as consumers are in search for fresh and nutritious snacks. However, apple slices often develop unattractive brownish color due to oxidative processes in the fruit. Genetically modified apple cultivars that do not change color due to oxidation (Prakash, 2014) are not suitable for European markets due to the ban of genetically modified organisms in most European countries. Therefore, this trait must be ascertained in existing and resistant apple cultivars, which have been introduced into modern apple orchards. These cultivars are often organically cultivated and contain high levels of nutrients and phenolic compounds (Mikulic-Petkovsek, Slatnar, Stampar, & Veberic, 2010; Mikulic-Petkovsek, Stampar, & Veberic, 2007). The later are involved in natural defensive reactions of plants against herbivores and plant pathogens (Korkina, 2007). The content of phenolic compound is highly dependent on the apple cultivar and various cultivation practices (Mikulic-Petkovsek et al., 2010; Slatnar et al, 2010; Veberic et al., 2005; Zupan, Mikulic-Petkovsek, Cunja, Stampar, & Veberic, 2013; van der Sluis, Dekker, de Jager, & Jongen, 2001). On the other hand, phenolics are also partially responsible for deterioration of fresh-cut apple fruit.

The main setback in fresh-cut industry is rapid product deterioration due to enzymatic browning. This does not only affect flavor and nutrient content, but also reduces visual quality of the product (Quevedo, Jaramillo, Diaz, Pedreschi, & Aguilera, 2009). Browning of fresh-cut fruit can be ascribed to oxidative reactions of enzymes and phenolic compounds, while non-enzymatic browning usually occurs in heat-processed products. Polyphenolics are oxidized mainly by polyphenol oxidase and to a lesser extent by peroxidase. Goupy et al. (1995) correlated the rate of browning with substrate content and enzyme activity. Contrary, Robards, Prenzler, Tucker, Swatsitang, and Glover (1999) summed up opposing results on this subject and reported high cultivar dependency of phenolic profile and enzymatic activity.

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Phenolic compounds have been well studied in apple and apple juice, yet on the other hand leftover pulp or pomace of apple juice production has not been sufficiently tested for potential extraction of phenolic compounds (Candrawinata, Golding, Roach, & Stathopoulos, 2013). van der Sluis, Dekker, Skrede, and Jongen (2002) suggested a second-stage extraction of polyphenols from apple pomace and subsequent addition of phenolics to juice in order to make "enhanced apple juice" with high marketable value. Moreover, phenolic extracts from apple pomace are not only interesting as an addition to the juice but could commercially be used as nutritional supplements or added to other products and functional foods (Lu & Foo, 2000). Phenolic extraction of apple pomace could therefore be considered as added commercial value of the juice-making process.

In the present research traditional, native, resistant and susceptible apple cultivars have been evaluated for their suitability for extraction of phenolics from pomace and their use as fresh-cut fruit. Phenolic profiles and the content of organic acids and sugars have been compared among cultivars in fresh apple fruit, juice and pomace. Additionally, enzymatic activity of polyphenol oxidase and peroxidase have been investigated in apple pulp, peel and juice to determine the link between total phenolic content, enzymatic activity and the rate of browning in fresh-cut apples. To our knowledge, the fruit of 'Majda' cultivar has not been chemically characterized until now.

2. Materials and methods

2.1. Plant material and sample preparation

The research was carried out on eight apple cultivars: three widespread European cultivars - 'Jonagold' (JG), 'Golden Delicious' (GD) and 'Granny Smith' (GS); two traditional cultivars - 'Boskoop' (BO) and 'Kronprinz Rudolf' (KR), two scab resistant cultivars - 'Topaz' (TO) and 'Florina' (FL) and a local Slovenian cultivar - 'Majda' (MA). All apple cultivars were harvested at technological maturity and were grown according to guidelines for integrated production. Apples of all examined cultivars were classified by size and 20 medium-sized apples were used for measurements and further analysis. The measurements were carried out at room temperature (22 ± 2 °C) and 50–60% relative humidity.

Ten apples per cultivar were washed and cut in half. One half of each apple was used for juice extraction and the other half was peeled with a ceramic fruit peeler. Pulp and peel were weighted separately to calculate the peel to pulp ratio of each studied cultivar.

The apple juice was prepared using automatic juice extractor AE 3150 (Clatronic, Kempen, Germany). The juice was immediately filtrated into vials and frozen at −20 °C until further analyzes of phenolic compounds, organic acids and sugar content. The pomace was retrieved from the juicer and analyzed according to the same procedure as apple pulp.

The rest of the apples (10 per cultivar) were used for measurements of enzymatic activity and browning. Each apple was cut in half and one half was used for measuring the enzymatic activity of peel and pulp. Juice was extracted from the other half and immediately analyzed for enzymatic activity.

2.2. Extraction and determination of sugars and organic acids

Sugars and organic acids were extracted from apple pulp according to the method described by Mikulic-Petkovsek et al. (2007). For the extraction of sugars and organic acid, 25 g of fresh apple pulp (in five repetitions per cultivar) was homogenized with an Ultra-Turrax T-25 (Ika-Labortechnik, Stauden, Germany) in 25 mL of double distilled water. The extracts were left for 30 min at room temperature with continuous stirring. After the extraction, samples were centrifuged and filtered through 0.20 μm cellulose mixed ester filters (Macherey-Nagel; Düren, Germany), into vials. Sugars and organic acids from the pomace were extracted in the same way as in fresh apple pulp. Samples were further analyzed for the content of individual sugars and acids using the Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA). Chromatographic conditions were as described by Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, and Veberic (2012). The content of individual sugars and organic acids were calculated from calibration curves of corresponding standards. Sugar/acid (S/A) ratio was calculated from the obtained results.

2.3. Extraction and determination of individual and total phenolics

Phenolic compounds were extracted from apple pulp, peel, juice and pomace according to the method described by Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, and Veberic (2015) with slight modifications. For determination of individual phenolic compounds and total phenolic content (TPC), 10 g of pulp, 10 g of pomace and 5 g of peel was extracted with 10 mL of methanol containing 3% of formic acid. The extraction lasted one hour and was facilitated with ultrasonic waves. Subsequently, samples were centrifuged and filtered through 0.20 μm Chromafil AO-20/25 polyamide filters (Macherey-Nagel, Düren, Germany) into vials. Identification of individual phenolic compounds was carried out using Thermo Scientific Dionex UltiMate 3000 Series UHPLC+ (Thermo Scientific, San Jose, Calif., U.S.A.) under conditions as described by Wang, Zheng, and Galletta (2002). Concentration of individual phenolic compounds was calculated from corresponding calibration curves. Individual phenolic compounds were grouped into corresponding phenolic classes (flavanols, flavonols, dihydrochalcones and hydroxycinnamic acids) and their levels were calculated from the sum of all identified compounds. Phenolics were also identified in fresh apple juice, which was filtrated directly into vials and frozen until further analysis. TPC measurements were carried out according to the method described by Singleton, Orthofer, and Lamuela-Raventos (1999) and modified by Mikulic-Petkovsek et al. (2007). The content of individual phenolic group (i.e. flavanols, dihydrochalcones, hydroxycinnamic acids and flavanols) in the entire apple fruit (x) was calculated using the following formula

$$x = a\ (\mathrm{mg/kg}) \times ps\ (\%) + b\ (\mathrm{mg/kg}) \times pp\ (\%)$$

where a is the content of individual phenolic group in apple peel, b is the content of individual phenolic group in apple pulp, ps is the percentage of peel and pp is the percentage of pulp in the entire apple fruit.

2.4. Enzymatic activity and browning

Activity of the polyphenol oxidase (PPO) and peroxidase (POX) enzymes was measured spectrophotometrically. The activity of PPO was assessed as described in Worthington manual (Worthington Enzyme Manual, 1972) and the activity of POX was measured according to the method described by Halbwirth et al. (2002). For measurements of enzymatic browning, approx. 1 cm thick longitudinal slices (from stem to calyx) of apple fruit were cut with a ceramic knife. The color of the pulp was immediately recorded with a portable colorimeter (CR-10 Chroma; Minolta, Japan) and the slices were left to oxidize on plastic plates for one hour. Subsequently, the color was measured again. a^* (redness), b^* (yellowness) and L^* (lightness) parameters were obtained from the first and second measurement and ΔE parameter was calculated according