Scientia Horticulturae 214 (2017) 214–220

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Importance of metabolite distribution in apple fruit

Anka Cebulj*, Vlasta Cunja, Maja Mikulic-Petkovsek, Robert Veberic

University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Chair for Fruit, Wine and Vegetable Growing, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 24 August 2016
Received in revised form 7 November 2016
Accepted 29 November 2016
Available online 4 December 2016

Keywords:
Malus domestica
Sugars
Organic acids
Phenolics
Dihydrochalcones

ABSTRACT

Apples (*Malus domestica* Borkh.) are one of the most studied fruits, however when we look closely we have inadequate information on how the metabolites are distributed inside the fruit. We performed a study of twelve different sampling positions in apple, six on each side of the fruit (sunlit and shaded). We sampled two cultivars: 'Jonagold' and 'Granny Smith'. Samples along the equatorial plane of the fruit varied more than those along the height of the fruit. What grabbed our attention was the flesh sample in the vascular region with higher content of phenolic compounds, especially dihydrochalcones in comparison to other flesh sampling positions. Also the distribution of sugars and organic acids varied between positions. The results can help with more accurate sampling of apple fruits and are the basis for better understanding of metabolite distribution in apple fruit.

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1. Introduction

Apples (*Malus domestica* Borkh.) are one of the most studied fruits, likely due to their second place in world fruit production (FAOSTAT, 2016). Due to their excellent storability, they are available all year around and are highly appreciated by consumers. The longevity of the fruit in storage is influenced by many factors and storage itself has influence on apple fruits. Therefore it is not surprising to find numerous studies regarding primary and secondary metabolite synthesis (Treutter, 2001; Li et al., 2012; Zhang et al., 2010), content (Li et al., 2013; Łata et al., 2009; Renard et al., 2007; Tsao et al., 2003; Veberic et al., 2005) and degradation (Bizjak et al., 2013; Golding et al., 2001; Zhu et al., 2013). An abundance of methods was developed to analyze those metabolites (Filip et al., 2016; Ignat et al., 2011; Sturm et al., 2003), therefore there are various ways to prepare samples for extraction. A now well-known fact that the skin of apple fruits contains a higher proportion of phenolic compounds compared to apple flesh led the researches to separately sample peel and flesh.

Secondary metabolites in the peel have been thoroughly studied: the influence of the position of fruits in the tree crown and hail nett influence (Jakopic et al., 2009; Solomakhin and Blanke, 2010), metabolic response to apple scab infection (Slatnar et al., 2010), the color (Greer, 2005), the content of phenolics in peels of apples with physiological disorders such as bitter pit (Zupan et al., 2013) and sunburn (Felicetti and Schrader, 2009; Zupan et al., 2014) and its health beneficial composition (Łata and Tomala, 2007) and many more. On the other hand, information about sugar and organic acid content in the peel is scarce (Filip et al., 2016; Li et al., 2013).

The apple flesh is also meticulously investigated. From its sugar and organic acid content (Zhang et al., 2010) to phenolics (Treutter, 2001; Tsao et al., 2003), the enzymes involved in their pathways (Kasai and Arakawa, 2010; Li et al., 2012) and physiological disorders (Zupan et al., 2013, 2016). Most of the studies are made from apple juice or chopped fruit flesh, notwithstanding the position of flesh in the fruit. There are some studies that take sun exposure into consideration and the sun exposed and shaded flesh was sampled separately (Opara et al., 1997), but what about different positions within the fruit flesh? There is a recent study about L-ascorbic acid distribution with a new sampling technique (Tang and Lee, 2016), but other than that, the studies on primary and secondary metabolites distribution in apple fruit have been scarce.

The aim of this study was to establish if there are any differences between fruit flesh samples taken from different positions inside the fruit and on the basis of these results to determine the best way of sampling the fruits. To confirm this hypothesis we acquired two dissimilar apple cultivars 'Jonagold' and 'Granny Smith', sampled five different positions of the flesh on the sunlit side and five on the shaded side, the peel was also sampled on both sides and all samples were extracted and analyzed to determine the content of sugars, organic acid and phenolic content.

* Corresponding author.
E-mail address: anka.cebulj@bf.uni-lj.si (A. Cebulj).

http://dx.doi.org/10.1016/j.scienta.2016.11.048
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2. Materials and methods

2.1. Plant material

Apples of two cultivars, 'Jonagold' and 'Granny Smith', were harvested for the purpose of this study. The apples from cv. 'Jonagold' were harvested on 15th of September in the University experimental orchard in Ljubljana (latitude 46,05° N, longitude 11,47° E, altitude 289 m) and the 'Granny Smith' apples were harvested on 18th of September in Horticultural Center of Biotechnical faculty in Orehovlje (latitude 45,9° N, longitude 13,6° E, altitude 48 m). For each cultivar, 60 apples were randomly collected from an area of one hectare; six fruits were used per repetition to ensure enough material for the analysis.

Flesh plugs (about 1 cm long) were collected at three positions along the height of the apple fruit from three different slices A, B and C (Fig. 1a) and, at three positions in the equatorial plane of the fruit (slice B) (Fig. 1b) on each side of the fruit (sunlit and shaded). The sampling positions are marked with F1–F5. Position F2 marks the same sampling point along the height of the fruit and in the equatorial plane (thus only five positions instead of six). The peel of the equatorial plane was also sampled on both sides. Immediately after sampling the plugs were immersed in liquid nitrogen and stored at −80 °C until extraction.

2.2. Chemicals

The water for sugar, organic acid extraction and for mobile phases was double distilled and purified with a Mili-Q Millipore Direct 8 system (Merck Millipore, Billerica, USA). Methanol for the extraction of the phenolics was acquired from Sigma-Aldrich (Steinheim, Germany). Formic acid and HPLC–MS grade acetonitrile from Fluka Chemie (Buch, Switzerland) were used for the mobile phase. The following standards were used for quantification of sugars, organic acids and phenolic compounds: Fluka Chemie (Buch, Switzerland): glucose, fructose, sucrose, sorbitol, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-rhamnoside, (−)-epicatechin, procyanidin B1 and B2, caffeic acid, *p*-coumaric acid and phloridzin dehydrate; Apin Chemicals (Abingdon, UK): quercetin 3-*O*-xyloside and quercetin 3-*O*-arabinofuranoside; Sigma-Aldrich citric acid, quercetin 3-*O*-rutinoside (rutin) and chlorogenic acid; Roth (Karlsruhe, Germany): (+)-catechin; Merck (Darmstadt, Germany): malic acid.

2.3. Analysis of individual sugars and organic acids

The frozen apple pulp plugs and peel were taken out of the freezer. Two grams of pulp were homogenized in 10 mL of double distilled water using Ultra-Turrax T-25 (Ika-Labortechnik, Stauden, Germany) and 1.5 g of chopped peel was extracted with 7.5 mL double distilled water. The further extraction and analysis was made as described by Zupan et al. (2016). The results are presented in g kg^{-1} fresh weight (FW).

2.4. Extraction and determination of individual phenolic compounds

Frozen apple pulp plugs and peel were chopped into small pieces. Two grams of pulp was extracted with 2 mL and 0.5 g of peel was extracted with 2.5 mL of methanol containing 3% (v/v) formic acid. The further extraction and analysis was made as described by Zupan et al. (2016). The results are presented in mg kg^{-1} FW.

2.5. Statistics

The statistical analyses were made with the Statgraphics Centurion (Manugistics Inc.; Maryland, USA). A one-way analysis of variance (ANOVA) and Duncan test were used to determine the differences between positions of pulp samples of apple fruit, for both cultivars separately. Shaded and sunlit sides were compared using LSD test. Ten repetitions were made for each position (on both sides of the fruit).

3. Results and discussion

3.1. Sugars and organic acids

Four sugars (sucrose, glucose, fructose and sorbitol) were quantified in six sampling positions on either side of the apple (sunlit and shaded) (Fig. 1). Peel is characterized with P1 and five different positions of flesh are marked with F1–F5. In figures and graphs (Figs. 2 and 3) for sugars, F2 is shown in both, for better visualization of the results. P1 contained higher content of sucrose, glucose and sorbitol in comparison to all positions of flesh, irrespective of the cultivar. Peel has quite different individual sugar ratio compared to sampling positions of flesh. Even though the metabolism of apple peels is manly supported by sugars imported from leaves, a different ratio could be the consequence of photosynthesis in the peels (Chen and Cheng, 2007), and thus the higher content of sorbitol and sucrose as the main transport form, could be explained. While F1 and F2 mostly didn't differ from each other, they had either higher (fructose) or lower (glucose) individual sugar content than P1, F3 showed notable differences in comparison to all 4 positions in flesh. The content of sucrose in F3 was about 23% lower than in F1 (in both cultivars), whereas glucose was higher for 38% and 20% in comparison to F1 in 'Jonagold' and 'Granny Smith', respectively. Fructose content, main sugar in apple fruit at harvest (Li et al., 2012), was the highest in F1 and F2 (if comparing only the equatorial sampling positions). These two samples represent the cortex of the apple. There are probably multiple causes of these variations, but one possible explanation for higher fructose and sucrose content and consequently total sugar content in comparison to P1 and F3 is, that the cells in cortex are larger than in the vascular and epidermis regions (Bain and Robertson, 1951) and may thus contain larger amount of stored sugars. Regardless of the explanation behind the individual sugar content, these results show that it is important how we take the samples of flesh (and peel) for total and even more so for individual sugar content. Between five flesh positions, F3 had the lowest total sugar content (110 and 94 g kg^{-1} FW in 'Jonagold' and 'Granny Smith', respectively), and F1 and F5 had the highest total sugar contents (123 and 122 g kg^{-1} FW in 'Jonagold' and 113 and 109 g kg^{-1} FW in 'Granny Smith').

There were slightly more differences in sunlit positions than in shaded ones. The shaded side of apple flesh of cv. 'Jonagold' did not differ in fructose and sorbitol content, whereas in sunlit they did (Fig. 2). Cultivar 'Granny Smith' had higher glucose content in sunlit F1 and F2 in comparison to P1, while in the shaded side only the F3 stood out. While the positions along the equatorial plane showed diversity, positions along the height of the fruit did not show statistical differences (Figs. 2 and 3). Hence the radial distance from the core is the one which influences the content of sugars and not the distance from stem to calyx.

Four organic acids were determined in both cultivars, however fumaric and shikimic acid were not included in our paper due to their small contents (less than 1% share of total organic acids). Malic and citric acid contents in sunlit and shaded side in different sampling positions are shown in Fig. 4 for 'Jonagold' and Fig. 5

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