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Influence of Post-Harvest Ripening on the Levels of Selected Compounds in Various Cherry Cultivars

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Abstract

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The stability of the main sugars and the organic acids neochlorogenic acid, *p*-coumaroylquinic acid and rutin was assessed in the Vanda, Napoleonova, Kordia, New Moon, Sweetheart, and Regina sweet cherry (*Prunus avium* L.) cultivars during post-harvest storage. Neochlorogenic acid was the predominant phenolic acid in these sweet cherry cultivars followed by *p*-coumaroylquinic acid. Rutin concentrations ranged from 0.5 ± 0.38 to 12.35 ± 2.84 mg/kg of homogenate and its concentration was higher in the Sweetheart, Kordia, and Regina cultivars in the postharvest time.

Keywords: neochlorogenic acid; organic acids; *p*-coumaroylquinic acid; rutin; storage; sugars; sweet cherries

While sweet cherries have a very attractive overall appearance, they are a highly perishable commodity with a short shelf life. Achieving the longer post-harvest shelf lives of cultivars requires the balanced content of sugars and organic acids in order to delay all negative processes that affect external quality features such as the wilting of stems and darkening of the surface of the fruits. Soluble solids content (SSC) in cherries ranges from 11 to 25% and titratable acidity (TA) ranges from 0.4 to 1.5%; differences may be detected in the fruits of the same cultivar if they are harvested under different climatic conditions (Remón *et al*. 2000; Bernalte *et al*. 2003; Serradilla *et al*. 2011).

A stable SSC will influence the SSC/TA ratio, but that does not reflect post-harvest loss of quality. Esti *et al.* (2002) showed that total sugar levels, i.e., glucose, sorbitol and fructose contents, are related to the sensory perception of sweetness. Total acidity and malic acid content correlated well with the sensory perception of sourness. The maturation index (TSS/TA) (TSS total soluble solids) exhibits a clear tendency for greater values in response to increasing maturity, which confirms that the various ripening stages are appropriately defined (Serrano *et al*. 2009; Garcia-Montiel *et al*. 2010).

Many studies have analysed the polyphenol content and the distribution of these compounds in cherries, and the results have shown that the majority of polyphenols are found in the skin (Tomás-Barberán & Espín 2001), although they may also occur in the flesh. Caffeoyltartaric acid and *p*-coumaroylquinic acid were identified as the two dominant polyphenols in cherries (Robards *et al*. 1999), but according to Mozetič *et al.* (2002) and Ballistrery *et al.* (2013) the dominant phenolic acids in cherries are neochlorogenic acid and *p*-coumaroylquinic acid and their levels depend on the variety and planting

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site. In the Pico Negro sweet cherry cultivar, the content of *p*-coumaroylquinic acid reached 1.3 g/kg in fresh material (González-Goméz *et al.* 2010). Studies of ripening are of special interest because they allow the identification of the optimum point of maturity for harvesting and enable delivery of fruit to consumers in its best condition in terms of nutritional and functional properties. Bioactive compounds and sources of energy for respiration in sweet cherries at the time of harvest differ considerably among cultivars and in the post-harvest. The aim of this article was therefore to determine the changes in the quality and stability of bioactive compounds during the storage of the selected sweet cherry cultivars. For this purpose, two distinct states of edible ripeness were established.

MATERIAL AND METHODS

Plant materials. The samples of sweet cherry (*Prunus avium* L.) used in this study were obtained from 10- to 12- year old sweet cherry trees in orchards in Stošíkovice (South Moravia). Cultivars were harvested sequentially, Vanda and Kordia on June 28, New Moon and Napoleonova on July 5, and Regina and Sweetheart on July 11, respectively. The fruits were harvested by hand and transported within several hours to the technological laboratory of the Institute of Postharvest Technology at Mendel University Lednice where they were sorted again in order to remove mechanically damaged fruits and fruits without stems.

One portion of the material was frozen (harvest time designed as 'I'), while the remainder of the fruits were stored for eight days at the temperature of 20°C and relative humidity of 90–94% (post-harvest time designed as 'II'). After eight days of storage this material was frozen. Homogenate was prepared by removing the stones from the thawed cherries and then mashing them to form a homogenous mixture.

Analysis of sugars and organic acids with HPLC. Sugars were analysed on a Watrex liquid chromatograph with a DeltaChrom SDS pump, Watrex Delta Chrom™ UVD 200 detector and Knauer K-2301 refractive index (RI) detector using Watrex 250 × 8 mm polymer IEX H form (Watrex Prague, Czech Republic). The mobile phase was degassed demineralised water with a flow rate of 0.7 ml/minute. For each analysis, a homogenised sample prepared from 25 defrosted fruits was used and filtered before injection with a 5 µl sample loop. The concentrations of sucrose, glucose, fructose and sorbitol were expressed as mg/kg of the original sample.

Organic acids were analysed on the same column, but the mobile phase was 0.05 N methanesulfonic acid at a flow rate of 0.7 ml/min with the UV detector set at 210 nm. Calibrations were carried out for each acid: tartaric, malic, citric and succinic acids. The acids were purchased from Sigma Aldrich (Czech Republic). Acid concentrations were expressed as mg/kg of the original sample.

Analysis of phenolic compounds with HPLC. For the analysis of phenolic compounds, the homogenate was defrosted at 4°C and mixed with methanol 1 : 1 (v/v). The mixture was centrifuged in a cooled centrifuge and the supernatant was stored at –20°C. The samples were analysed using an HPLC 1050 apparatus (Hewlett Packard, USA) with a diode array detector (DAD Agilent G1315B; Agilent Technologies, Czech Republic) and Phenomenex Luna C18(2) column (3 µm, 2 × 150 mm) (Phenomenex, USA). The volume of injected sample was 5 µl.

Mobile phase A: acetonitrile/*o*-phosphoric acid/water (5 : 0.1 : 94.9), mobile phase B: acetonitrile/*o*-phosphoric acid/water (80 : 0.1 : 19.9). For separation, a gradient from 2% to 25% of mobile phase B was used for 35 min; the flow rate was 0.25 ml/min, sample injection volume was 5 µl. Column temperature was 25°C. The concentration of neochlorogenic acid was calculated using a calibration curve for chlorogenic acid; the concentration of *p*-coumaroylquinic acid was calculated using a calibration curve for coumaric acid; the concentration of rutin was calculated from a calibration curve for rutin. Identification of compounds was verified on APCI-LC/MS (LCQ Accela Fleet; Thermo Fisher Scientific, USA) with the same column as in HPLC. Mobile phases were acidified using formic acid.

Rutin, chlorogenic acid and *p*-coumaric acid were purchased from Sigma-Aldrich (Czech Republic), methanol and acetonitrile were from Merck (Czech Republic), *o*-phosphoric acid was from Fluka and formic acid was acquired from Sigma-Aldrich (Czech Republic). All results were expressed as mg/kg of homogenate.

Statistical analysis. All data were subjected to the one-way ANOVA method ($P < 0.05$). Statistical tests were performed using JMP software. Tukey's test was used to determine the level of significance ($P < 0.05$). All measurements were performed in triplicate and the results are expressed as mean ± standard error. For PCA analysis, the Canoco program (Ter Braak & Šmilauer 2002) was used.

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RESULTS AND DISCUSSION

Contents of sugars and organic acids. The contents of glucose, fructose and sorbitol as the main sugars found in the sweet cherries are given in Figure 1A. Sucrose content in all cultivars was negligible; therefore, it is not possible to perceive any values even when the concentration units were changed from grams to milligrams for kilogram of homogenate. Cornwell *et al*. (1982) described trace amounts of sucrose and 2.6–3.9 g of sorbitol/100 g of cherry homogenate. It is widely accepted that the most important parameters determining sweet cherry acceptability by consumers are changes in sugars and acids during post-harvest storage. At the harvest time, the ratio of the major sugars fructose/glucose/sorbitol in all cultivars is 1 : 1 : 0.75 and this ratio is maintained in the post-harvest period. Later ripening cultivars such as Sweetheart have higher contents of fructose and glucose (4003 ± 209 mg/kg and 3705 ± 189 mg/kg of homogenate, respectively). Differences are also evident among cultivars and maturity stages in relation to the content of sugars (glucose, fructose, and sorbitol) and organic acids (malic, tartaric, citric, succinic, and fumaric acids) at harvest time, with values of 7593–10573 and 8258–10277 mg/kg of homogenate, respectively, for New Moon and Sweetheart, the cultivars with the highest acidity levels.

During storage, the most significant differences were observed in malic acid content; on average, a reduction of 4.4% was observed (Figure 1B). In agreement with this observation, in other cherry cultivars a general decrease in acidity content during post-harvest storage was reported (Esti *et al*. 2002; Bernalte *et al*. 2003; Alique *et al*. 2005). Malic acid is the predominant organic acid in all cherry cultivars, and its content ranges from 3561 to 6009 mg/kg of homogenate. The content of both citric (from 276 mg/kg to 425 mg/kg of homogenate) and tartaric acids (from 530 mg/kg to 773 mg/kg of homogenate) is relatively stable during post-harvest storage; larger differences among cultivars are observed for succinic and fumaric acids. In comparison to the recent publication of Nawirska-Olszańska *et al*. (2017), the contents of fumaric acid were higher in the varieties examined here. The fresh weight of fruits increases slightly during storage, which is attributed to transpiration. The weight increase was higher in cultivars that ripened earlier but did not exceed 2%.

Phenolic composition of sweet cherries. Three phenolic compounds were analysed and quantified in the sweet cherries (Figure 2). These compounds included phenolic acids, which were the main type of phenolic compounds found at higher concentrations in the cvs Vanda, Kordia, and Regina as *p*-coumaroylquinic acid and its content significantly increased during post-harvest storage. The levels of this acid were positively correlated with the antioxidant potency of cherry extracts on human low-density lipoproteins (LDL) (Gonçalves *et al*. 2004a). The second most abundant acid was neochlorogenic acid, which fluctuated between 16 and 147 mg/kg of homogenate; its concentration increased in the last ripening stages. In fact, the antioxidant capacity of chlorogenic acid itself is higher than those of vitamin C and vitamin E. Phenolics like chlorogenic acid and neochlorogenic acid carry out antioxidative functions by chelating metal ions, inhibiting lipid oxidation and radical forming enzymes and eliminating free radicals (Thurow 2012). Our results contradict those of other authors, who measured higher levels of neochlorogenic acid than *p*-coumaroylquinic acid (Serrano *et al*. 2009;

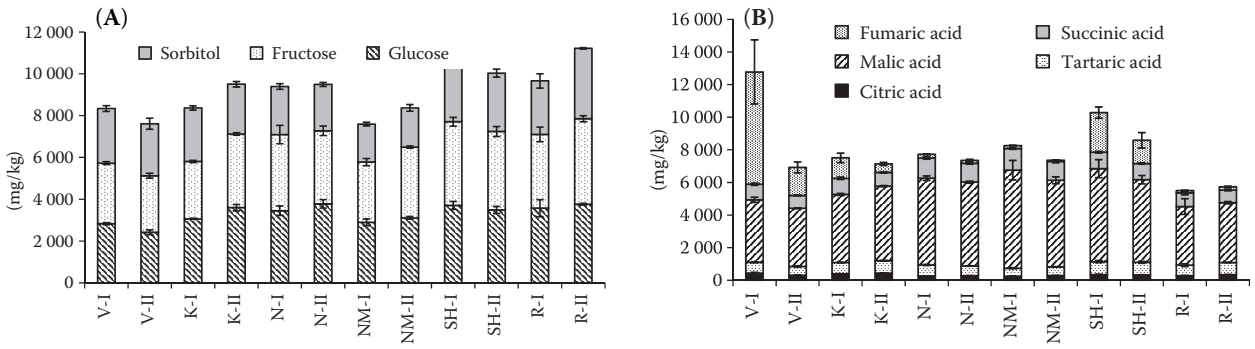

Figure 1. Mean (**A**) sugar and (**B**) organic acid content (mg/kg homogenate) of sweet cherry cultivars

V – cv. Vanda; K – cv. Kordia; N – cv. Napoleonova; NM – cv. New Moon; SH – cv. Sweetheart; R – cv. Regina; I – harvest time; II – postharvest time

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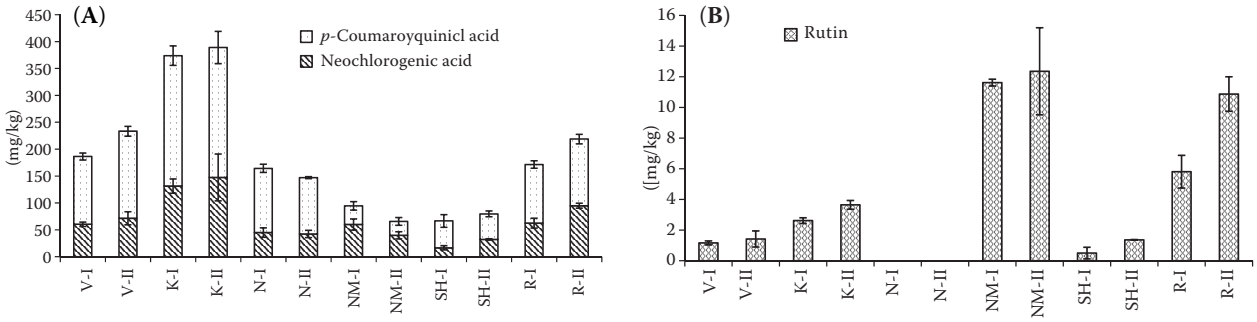

Figure 2. *p*-Coumaroylquinic acid and neochlorogenic acid (**A**) and (**B**) rutin content (mg/kg homogenate) of sweet cherry cultivars

V – cv. Vanda; K – cv. Kordia; N – cv. Napoleonova; NM – cv. New Moon; SH – cv. Sweetheart; R – cv. Regina; I – harvest time; II – postharvest time

Usenik *et al*. 2010). As reported by Mozetic *et al.* (2002), the contents of *p*-coumaroylquinic acid and neochlorogenic acid vary among different cultivars. Concentrations of neochlorogenic acid and *p*-coumaroylquinic acid ranged from 19.5 mg/100 g FW to 53.0 mg/100 g FW and from 7.5 mg/100 g FW to 50.6 mg/100 g FW, respectively. The relative amounts of these two phenolic acids varied widely between the cherry cultivars. As a fruit flavonoid, rutin was also detected in 13 cultivars, with content ranging from 0.7–8.7 mg/100 g FW; its levels were highly correlated (R^2 = 0.97) with the content of cyanidin-3-rutinoside, a major anthocyanin in sweet cherries (Sugawara & Igarashi 2008). The flavonol content of the different cultivars as measured by the levels of rutin was significantly different (P < 0.001) among the cherry cultivars, but not among harvest years. The Saco cultivar was richest in this compound, containing 14 mg/100 g of FW, while cvs Burlat and Summit had a lower value of 3 mg/100 g of FW that represented 5 and 3% of the total phenolic contents, respectively (Gonçalves *et al*. 2004b).

Rutin was found in all cultivars with the exception of the cv. Napoleonova where the concentration was under the detection limit of the method; it showed a consistent tendency to increase with post-harvest ripening. Rutin concentrations (Figure 2B) were in the range from 0.5 ± 0.38 mg/kg to 12.35 ± 2.84 mg/kg of homogenate and the highest concentration was found in the late ripening New Moon and Regina cultivars. Statistically significant differences between the rutin concentrations at harvest and post-harvest

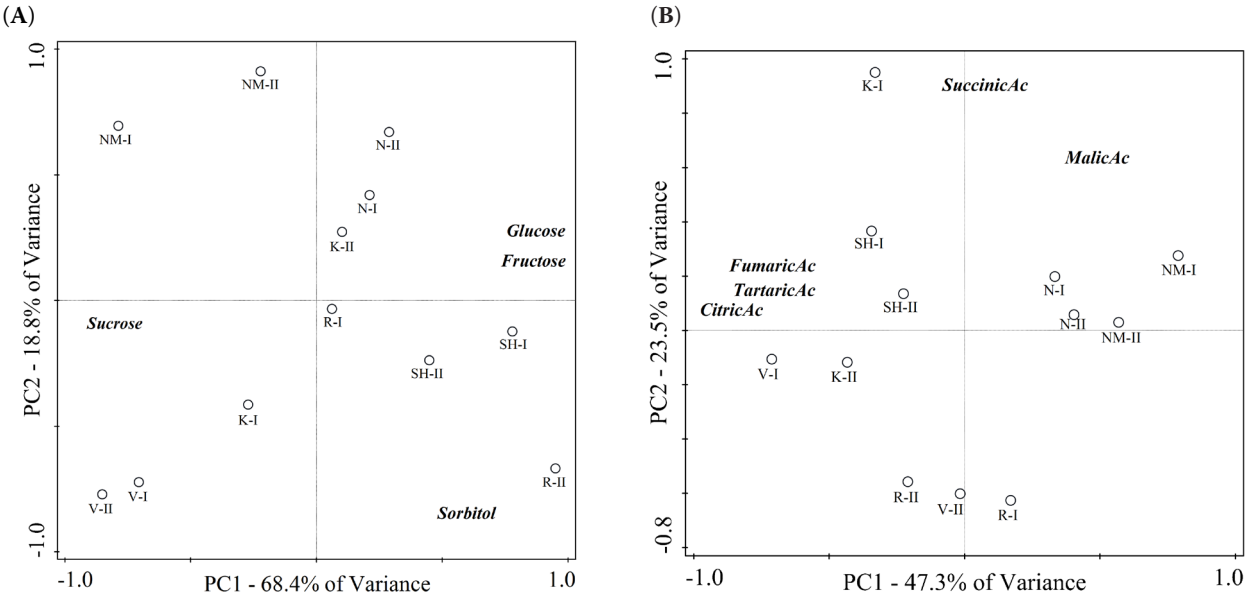

Figure 3. Loading plot and score plot after principal component analysis of sugars (**A**) and organic acids (**B**) defined by the two first principal components (PC1 and PC2)

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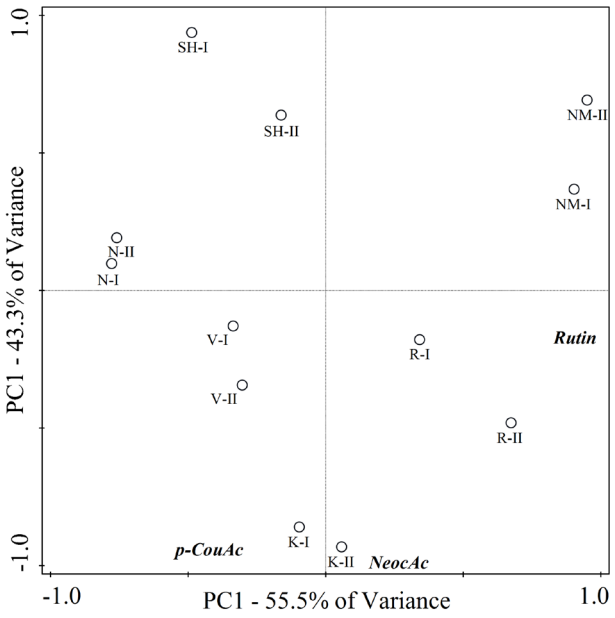

Figure 4. Loading plot and score plot after principal component analysis of phenolic compounds defined by PC1 and PC2

time were found in the Sweetheart, Kordia, and Regina cultivars.

Principal component analysis (PCA). A principal component analysis (PCA) was performed for all studied variables in order to evaluate the ripening of cultivars. In Figure 3A, the different variables are represented on the plane of the first two principal components (PC1 and PC2). PC1 is defined on the positive axis by glucose, fructose and sorbitol. The variation in PC1 on the negative axis is explained by cultivars with lower sugar concentrations at harvest, such as cvs Vanda, Kordia, and New Moon. The cultivars can be clearly distinguished on the basis of their organic acid concentrations (Figure 3B). Only two cultivars are distinguished (New Moon and Napoleonova) at two ripening stages. All three phenolic compounds are clearly detected (Figure 4), and their levels are elevated for the Sweetheart and Napoleonova cultivars. We originally hypothesised that it would be possible to distinguish, based on the PCA analysis, not only all varieties, but also their harvest and post-harvest quality. Unfortunately, the PCA analysis was not successful in this regard.

CONCLUSIONS

The differences in the concentrations of three sugars, six organic acids and three phenols were evaluated at two stages of ripeness in six cultivars of sweet cherries. Post-harvest differences in the concentrations of sugars and organic acids were only slight when compared to the level of rutin. Malic acid was the main organic acid; levels of organic acids were observed to be higher for the late cultivars with the exception of fumaric acid whose levels were highest at harvest time in the cv. Vanda. *p*-Coumaroylquinic acid and neochlorogenic acid were identified as the main phenolic compounds in all cultivars.

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