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Phenolics and antioxidant activity of freeze-dried sour cherry puree with addition of disaccharides

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ABSTRACT

This study was carried out to determine the influence of disaccharides on phenolics and antioxidant activity of freeze-dried sour cherry puree. The freeze-dried sour cherry purees were prepared without and with addition of maltose, sucrose or trehalose in amounts of 5, 10 or 20%. Addition of maltose and trehalose had a positive effect on preservation of anthocyanins, especially on cyanidin-3-glucosylrutinosid. Positive effect of sugars was the most pronounced for cyanidin-3-glucosylrutinosid and the least for cyanidin-3-glucoside. The highest content of individual anthocyanins except cyanidin-3-glucoside was measured in sample with addition of 20% of maltose. Results of antioxidant activity (AA) measured with ABTS and FRAP followed the same trends with a positive correlation of $r = 0.824$. Antioxidant activity measured with ABTS also showed the positive correlation with total phenolics, flavonoids and with sum of individual anthocyanins ($r = 0.745$, 0.709 and 0.814, respectively). The AA measured with all three methods (DPPH, ABTS, and FRAP) showed that sample with addition of 20% of maltose had the highest AA, 65.46, 28.45, 62.24 mg GAE/100 g, respectively.

1. Introduction

Sour cherries (*Prunus cerasus* L.) are popular as fruit crop and in fruit industry. They are rich source of anthocyanins, pigments that are responsible for red color of fruit (Mazza & Miniati, 1993). Anthocyanins are also responsible for strong antioxidant and anti-inflammatory properties of sour cherries (Šarić et al., 2009). The major anthocyanin pigments in sweet and sour cherries are cyanidin-3-glucoside, cyanidin-3-glucosylrutinoside, cyanidin-3-rutinoside, cyanidin-3-sophoroside (Chandra, Nair, & Iezzoni, 1992; Chandra, Rana, & Li, 2001; Chaovanalikit & Wrolstad, 2004; Gao & Mazza, 1995). The distribution of individual anthocyanins in skins, flesh and pits has not been explored in detail, however all cherry cultivars have some anthocyanin pigmentation in the skin but the amounts could vary tremendously. The content and stability of anthocyanins in sweet and sour cherries is strongly affected by pre-harvest factors and temperature, light intensity, fruit crops maturity etc. (Jakobek, Šeruga, Voća, Šindrak, & Dobričević, 2009; Usenik, Kastelec, & Štampar, 2005; Wang, 2006). Post-harvest factors such as transport and storage can also influence phytochemical composition of food crops. The anthocyanin content in cherries is generally stable during storage although cold storage may lead to an increase in concentration of anthocyanins (Gonçalves et al., 2007; Serrano et al., 2009). Since the sour cherry season is short, they are usually processed as frozen, canned, dried, or used for juice production. The primary use of processed sour cherries is in snacks, baked products or they can be rehydrated for pies. Obviously that processing which can include operations such as disintegration of fruit tissue, increasing or lowering temperature, high pressure, addition of natural and/or chemical additives has an impact on compounds contained in sour cherries especially on anthocyanins (Chaovanalikit & Wrolstad, 2004; Kirakosyan, Seymour, Llanes, Kaufman, & Bolling, 2009; Wrolstad et al., 2003). To prevent loosing such valuable compounds milder processing methods should be considered. Freeze-drying is considered as one of the best method of water removal with final products of highest quality compared to other methods of food drying such as conventional, convective air, vacuum oven, microconvection, solar drying, etc. (Marques, Silveira, & Freire, 2006). Biological and sensory qualities of the freeze-dried material are mainly retained in the product. Furthermore, natural food ingredients, such as sugars may influence anthocyanin stability. The problem with current research on anthocyanin stability upon sugar addition is that they are showing contradictory results, and there is no scientific consensus regarding this matter. It is well known that sugar

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degradation products may promote the degradation of anthocyanins, but addition of sugars or invert sugar syrups showing from no influence to positive or negative effects on anthocyanin stability (Calvi & Francis, 1978; Debicki-Pospisil, Lovrić, Trinajstić, & Sabljić, 1983; Rababah, Ereifej, & Howard, 2005; Tsai, Hsieh, & Huang, 2004). For example, study conducted by Kirakosyan et al. (2009) showed that sour cherries products processed with sugar (15% of total fresh weight) had lower concentrations for both anthocyanins and phenolic than dry cherry without sugar addition which is result of smaller amount of fruit in final product. Wrolstad, Skrede, Lea, and Enersen (1990) reviled protective effects of sucrose with a higher half-life value for pelargonidin glycosides in strawberry juice as compared to strawberry concentrate. It is clear that impact of sugars on anthocyanins depends on many factors among which the most important are: processing type and conditions, the type of fruit or vegetable, the type and concentration of added sugar. Selected sugars for this study were the sucrose, as the sugar that is commonly used in fruit product formulation, and maltose and trehalose. Maltose and trehalose are less sweet than the sucrose and previous studies have shown a positive effect of these sugars on the preservation of compounds such as polyphenols. In addition, it has been proven that sugars are behaving differently at low temperature and that behavior is related to the structure of the disaccharides (Wang & Haymet, 1998; Magazù, Migliardo, & Ramirez-Cuesta, 2005; Magazù, Migliardo, & Telling, 2008; Cesaro, Magazù, Migliardo, Sussich, & Vadalà, 2004; Magazù, Migliardo, & Ramirez-Cuesta, 2007; Uchida, Nagayama, Shibayama, & Gohara, 2007; Magazù, Migliardo, Gonzalez, Mondelli, Parker, & Vertessy 2012). Chosen disaccharides are isomers and were used for preparation of the freeze-dried sour cherry puree to investigate their influence on phenolic compounds and antioxidant potential. In addition, influence of their amount, 5, 10 or 20% on the mentioned parameters was investigated.

2. Materials and methods

2.1. Freeze-dried puree preparation

Sour cherries (*Prunus Cerasus cv. Maraska*) were both from the local grower in season 2015 and stored at −20 °C prior preparation of puree. Before puree preparation, sour cherries were thawed at room temperature for 6 h and pits were removed. Fruits were disintegrated in blender (Braun Multiquick Professional 600 Watt Turbo) for 5 min. After disintegration, 5, 10 or 20% (w/w) of maltose, sucrose or trehalose were added and homogenized. Freeze-drying was conducted according to the procedure described by Loncaric, Dugalic, Mihaljevic, Jakobek, and Pilizota (2014) with slight modification. Before freeze-drying sour cherry puree mixture was frozen at −18 °C for 24 h and then freeze-dried in a laboratory freeze-dryer (Christ Freeze Dryer, Gamma 2–20, Germany). Conditions for drying process were as follows: freezing temperature was adjusted at −55 °C; the temperature of sublimation from −35 °C to 0 °C; and the vacuum level 0.220 mbar. The temperature of the isothermal desorption varied from 0 °C to 22 °C under the vacuum of 0.060 mbar. Freeze-drying lasted about 48 h until the total solids content was 95–97%. Prepared samples were left for stabilization for 30 days at room temperature, and after that period of time analyses were conducted.

2.2. Extraction of phenolics and anthocyanins

Samples of the freeze-dried puree were homogenized, weighed (1 g), and extracted (10 mL) in pure methanol solution acidified with 1% hydrochloric acid. After 1 h at the room temperature extracts were filtered through plated filter paper. Extracts were used for total phenolic content and antioxidant activity determination.

Extraction of anthocyanins from freeze-dried puree (100 mg) for HPLC determination were carried out with 2 mL of acidified methanol (1% hydrochloridric acid) by placing samples in an ultrasonic bath (Bandelin Sonorex Digitech, Berlin, Germany) at room temperature for 15 min, then centrifuging for 15 min (Microspin, Grant-bio, England) (6596.2 × *g*) and filtrated through 0.45 μm PTFE syringe filters. Extraction has been done in triplicate.

2.3. HPLC determination of anthocyanin compounds

Anthocyanins were identified and quantified by Varian LC system (USA) equipped with a ProStar 230 solvent delivery module, and ProStar 330 PDA Detector. Anthocyanin separation was done in an OmniSpher C18 column (250 × 4.6 mm inner diameter, 5 μm, Varian, USA) protected with guard column (ChromSep 1 cm × 3 mm, Varian, USA) using 0.5% water solution of phosphoric acid as a solvent A and 100% HPLC grade methanol as a solvent B (elution conditions: 0–38 min from 3 to 65% B; 38–45 min, 65% B; flow rate 1 mL min^{-1}, injection volumes were 20 μL) (Jakobek, Seruga, Novak, & Medvidovic-Kosanovic, 2007). Prior to injection into the HPLC system, extracts were diluted with high quality water at a ratio of 1:9. For each sample, three replicated HPLC analyses were performed, and results were given in mg per kg of sour cherry (mg/kg). UV–Vis spectra were recorded in wavelength range from 190 to 600 nm (detection wavelength was 520 nm). Quantification has been performed by external standard calibration. Standards, cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside, cyanidin-3-glucoside and cyanidin-3-rutinoside were purchased from Sigma-Aldrich (Germany).

2.4. Determination of total phenolics

The total phenolic content in the samples were determined by Folin-Ciocalteu method; briefly 0.2 mL of extract and 1.8 mL of deionizer water were mixed with 10 mL (1:10) of Folin-Ciocalteu reagent and 8 mL of 7.5% solution of sodium carbonate (Singleton & Rossi, 1965). Prepared mixtures were left in the dark for 120 min to develop the color, and the absorbance was read at 765 nm by spectrophotometer (Cary 60, UV–Vis, Agilent Technologies, Malaysia). For each sample, the measurements were performed in triplicates and values were interpolated on a gallic acid calibration curve and expressed as g of gallic acid equivalents per kg of sour cherry (g GAE/kg).

2.5. Determination of flavonoids

The total flavonoid content was determined according to Makris, Boskou, and Andrikopoulos (2007). Briefly, the 0.5 mL of extract was mixed with 4 mL distilled water, than 0.3 mL 5% $NaNO_2$ was added and allowed to react for 5 min. Following this, 0.3 mL 10% $AlCl_3$ was added and the mixture was allowed to react for a further 5 min. At the end, 2 mL 1 M Na_2CO_3 and 2.4 mL distilled water were added to the reaction mixture and the absorbance at 510 nm was read against a blank. For each sample, the measurements were performed in triplicates and values were interpolated on calibration curve using catechin as a standard, and expressed as g catechin equivalents per kg of sour cherry (g CE/kg).

2.6. Polymeric color

Percentage of polymeric color was determined using the method described by Giusti and Wrolstad (2001). For analysis, 0.2 mL of sodium bisulfite was added to 2.8 mL of diluted sample and 0.2 mL of water was added to 2.8 mL of diluted sample. After