TOTAL ANTHOCYANINS IN FRESH FRUIT AND COMPOTE OF "OBLACINSKA" SOUR CHERRY (*PRUNUS CERASUS L.*)

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ABSTRACT

Anthocyanins are secondary metabolites of plants in the flavonoid family. Red and blue fruits are the main sources of anthocyanins, because cyanidin-3-glucoside is one of the most common and gives the red colour of the fruit. They are soluble in water and are 100 % absorbed by the human body. Anthocyanins are powerful antioxidants that protect our body from free radicals. They have a unique ability to resist ultraviolet light and reduce the risk of serious illness. Nutritionists recommend the use of anthocyanins in amounts of 10 to 15 mg per day. Sour cherry (*Prunus cerasus* L.) contains high levels of anthocyanins that have strong antioxidant and anti-inflammatory properties. In this paper, for the determination of the content of total anthocyanins, fresh fruits from the sour cherry "Oblacinska" variety and compotes of the same variety sweetened with sucrose and stevia are used. Anthocyanins are examined according to the following method: AOAC Official Method 2005.02, Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines, pH Differential Method. The content of anthocyanins is in the following order: 32.00 mg cyanidin-3-glucoside equivalents / 100 g in fresh sour cherry fruits, 16.10 mg cyanidin-3-glucoside equivalents / 100 g in sour cherry compote with sucrose and 16.12 mg cyanidin-3-glucoside equivalents / 100 g in sour cherry compote with stevia.

Key words: anthocyanins, sour cherry, compote, spectrophotometry.

INTRODUCTION

Anthocyanins (Greek anthos = flower and kyáneos = blue) are polyphenolic pigments that belong to the flavonoid group and are characterized by a red-orange and blue-violet colour present in fruits. To date, more than 700 structurally different anthocyanin derivatives have been identified in nature, of which 27 are aglycones, known as anthocyanidins (Wallace & Giusti, 2015).

Anthocyanins belong to the group of pigments that are soluble in water. Their colour changes from red to blue with increasing concentration of OH^- ions, and from blue to red with

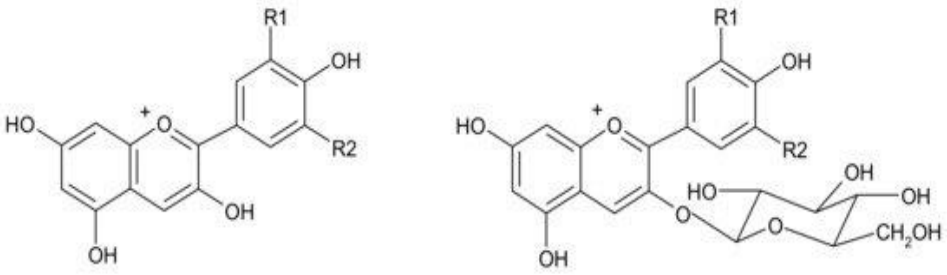
increasing concentration of H^+ ions. The colour of anthocyanins depends on pH values (Mazza, 2007).

Foods rich in anthocyanins are an important part of a healthy diet. But researchers are still investigating their role in the treatment and prevention of certain diseases. Overall, evidence suggests that sour cherry anthocyanins possess strong antioxidant and anti-inflammatory activities. They inhibit tumor development and growth, and thus may provide a range of health benefits (Wallace & Giusti, 2015, Alrgei et al., 2016).

Sour cherries are a popular fruit mainly because of their excellent organoleptic characteristics. They are an important source of nutrients, bioactive food and antioxidants, so for these reasons, sour cherry should be included as an essential part of human nutrition (Pantelić et al., 2014, Sokół-Łetowska et al., 2020).

The total anthocyanin content of sour cherries ranges between 27.80 and 80.40 mg/100 g of fresh fruit (Blando et al., 2004). However, the total anthocyanin content and anthocyanin fractions differ according to the sour cherry variety and range between 2.70 to 28.00 mg/100 g (Wanget et al., 1997; Kim et al., 2005; Šimunic et al., 2005). The content of total anthocyanins in sour cherry fruits and products has always been the subject of research by several authors. Total anthocyanins according to the authors range in the following limits from 20.25 to 99.45 mg/100 g (Vereset et al., 2008), from 11.30 to 93.50 mg/100 g (Papp et al., 2010), from 27.80 to 210.00 mg/100 g (Filimonet et al., 2011), from 16.14 to 34.06 mg/100 g (Bijelić et al., 2014), from 21.00 to 48.60 mg/100 g (Pantelić et al., 2014), from 22.00 to 41.00 mg/100 g (Yılmazet al., 2015). Several clones of the cultivar "Oblacinska", studied by Alrgei et al. (2016), presented significant levels of total anthocyanin content above 100.00 mg cyanidin 3-O-glucoside per 100 g of fresh fruit. Sokół-Łetowska et al. (2020) determined the content of total anthocyanins in sour cherry fruits ranging from 17.97 to 131.28 mg/100 g. Numerous studies have shown that the total content of anthocyanins is influenced by many factors, such as: harvest season, variety, harvest phase, as well as climatic conditions (Sass-Kiss et al., 2005).

The main anthocyanins found in sour cherry (*Prunus cerasus* L.) are: cyanidin (cy)-3-glucoside, cy-3-glucosylrutinoside, cy-3-sophoroside and cy-3-rutinoside. In addition to these anthocyanins, in lower concentrations can be found: cy-3-xylosylrutinoside, peonidin-3-glucoside, peonidin-3-rutinoside and cy-3-gentiobioside (Figure 1.) (Wallace & Giusti, 2015).



(a) (b)

Anthocyanidin	R1	R2
Cyanidin	–OH	–H
Delphinydin	–OH	–OH
Pelargonidin	–H	–H
Malvinidin	$–OCH_3$	$–OCH_3$
Peonidin	$–OCH_3$	–H
Petunidin	–OH	$–OCH_3$

Figure 1. Chemical structure of the main anthocyanins (a) and anthocyanidin-3-O-glucosides present in the fruits (b)

The aim of this paper is to determine the total anthocyanins in fresh sour cherries and compotes of the "Oblacinska" variety using spectrophotometric method with cyanidin-3-glucoside as the reference standard.

MATERIALS AND METHODS

Materials

For the determination of the total content of anthocyanins, fresh fruits and compote of the sour cherry variety "Oblacinska" (Figure 2) are used as raw materials.

Figure 2. "Oblacinska" sour cherry variety

The plantations from which fresh cherry samples are taken are located in the village of Lakavica, Municipality of Stip on an area of 35 hectares and are under the management of the company Euro Lozar.

The production process of sour cherry compote from the "Oblacinska" variety, with sweeteners (sucrose and steviol glycoside) took place at the fruit and vegetable processing plant Fructana D.O.O. in the village of Argulitsa - Shtip.

Methods

The analyses for the total content of anthocyanins in the fresh fruits of the sour cherry "Oblacinska" variety and in the compote are made at the Institute of Public Health in Skopje.

The total anthocyanin content is determined according to AOAC Official Method 2005.02, Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines, pH Differential Method.

A. Principle

Monomeric anthocyanin pigments reversibly change colour with a change in pH, the coloured oxonium form exists at pH = 1.0, and the colourless hemiketal form predominates at pH = 4.5. The difference in the absorbance of the pigments at 520 nm is proportional to the pigment concentration. Results are expressed as mg (cyanidin-3-glucoside equivalents) / 100 g. Degraded anthocyanins in the polymeric form are resistant to colour change regardless of pH and are not included in the measurements because they absorb at pH = 4.5 as well as pH = 1.0.

B. Apparatus

(a) pH meter - Standardized with pH = 4.0 and pH = 7.0 standard buffer solutions.

(b) Visible spectrophotometer (model UV – 1800, Shimadzu). Performance of the spectrophotometer at 520 nm should be verified with reference standards for wavelength accuracy, photometric accuracy, photometric linearity, and stray light.
(c) Glass or disposable cuvets for spectrophotometer - 1 cm path length.
(d) Volumetric flasks of 50 mL.
C. Reagents
(a) Buffer with pH = 1.0 (potassium chloride, 0.025 M): 1.86 g KCl is weighted into a beaker and distilled water to ca 980 mL is added. The pH is measured, and then adjusted pH to 1.0 (± 0.05) with HCl (ca 6.3 mL). It is transferred to a 1 L volumetric flask, and diluted to volume with distilled water.
(b) Buffer with pH = 4.5 (sodium acetate, 0.4 M). 54.43 g $CH_3CO_2Na \cdot 3H_2O$ is weighted in a beaker, and distilled water to ca 960 mL is added. The pH is measured, and then adjusted pH to 4.5 (± 0.05) with HCl (ca 20 mL). It is transferred to a 1 L volumetric flask, and diluted to volume with distilled water.
D. *Preparation of Test Solution*
All dilutions are performed in 50 mL volumetric flasks (B(d)). For addition of the test portion volumetric pipets are used. The maximum test portion added should be ≤ 10 mL (the volume ratio of test portion and buffer = 1 : 4) so as not to exceed the buffer capacity of the reagents.
The appropriate dilution factor is determined by diluting the test portion with pH = 1.0 buffer (C(a)), until absorbance at 520 nm is within the linear range of the spectrophotometer. For most spectrophotometers, the absorbance should be between 0.2 and 1.4 AU (Absorption Unit). Using this dilution factor, 2 dilutions of the test sample are prepared, one with pH = 1.0 buffer and the other with pH = 4.5 buffer.
E. *Determination*
The absorbance of test portion diluted with pH = 1.0 buffer (C(a)), and pH = 4.5 buffer (C(b)), at both 520 and 700 nm is determined. The diluted test portions are read versus a blank cell filled with distilled water. The absorbance is measured within 20 - 50 min of preparation.

RESULTS AND DISCUSSION

"Oblacinska" is the leading variety of sour cherry in Macedonia, and originates from the village of Oblačina - Republic of Serbia. This sour cherry variety is a collection of various clones. It ripens at the end of the second decade of June. The fruit of this sour cherry variety is dark red in colour and round in shape. Depending on the intensity of fertilization and the weather conditions, the mass of the fruit varies from 2.80 to 4.90 g (Puškar, 2002), and the yield from 68.00 to 91.00 % (Vuković, 2014) (Figure 2).

The high quality of the fruit makes this sour cherry variety suitable for various industrial processing, such as compote. Compote is a non-alcoholic drink with pieces of fruit in sugar syrup. The syrup is a non-alcoholic clear juice obtained by boiling the fruit in a large volume of water. Other than sour cherries, compote is also made from other fruit, such as cherries, strawberries, apricots, peaches, *etc*. (Vračar, 2001, Jašić, 2007).

The fresh fruits of sour cherry "Oblacinska" variety and the obtained processing, i.e. the compotes sweetened with sucrose and steviol glycoside, are analysed using the pH-differential method in order to examine the total content of anthocyanins.

The anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents (CGE), is calculated as follows:

$$\text{Anthocyanin pigment (CGE mg/L)} = \frac{A \; x \; MW \; x \; DF \; x \; 10^3}{\varepsilon \; x \; 1}$$

where A = (A_{520nm} – A_{700nm}) pH = 1.0; (A_{520nm} – A_{700nm}) pH = 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor established in D; *l* = pathlength in cm; ε = 26 900 molar absorption coefficient, in L x mol^{-1}x cm^{-1}, for cyanidin-3-glucoside; and 10^3 = factor for conversion from g to mg (Lee et al., 2005).

The content of total anthocyanins are expressed as mg CGE / 100 g. The obtained results are given in Table 1, from which it can be concluded that the fruits of the fresh sour cherry from the "Oblacinska" variety are characterized by a higher value of 32.00 mg CGE / 100 g, and the two compotes with lower values of 16.12 and 16.10 mg CGE / 100 g.

Table 1. Mean values and standard deviations of total anthocyanin content

	Oblacinska (fresh fruits)	*Oblacinska* (compote with sucrose)	*Oblacinska* (compote with steviol glycoside)
Total anthocyanins (mg CGE / 100 g)	32.00 ± 0.1	16.10 ± 0.0	16.12 ± 0.1

Sour cherry fruit is mainly used for processing. The applied sweeteners had no influence on the content of total anthocyanins. During thermal processing of sour cherries, two parameters should be taken into account, namely temperature and time, in order to obtain a product with nutritious components. For that reason, short-term heating at a higher temperature is recommended in order to maintain higher concentrations of anthocyanins and to achieve better quality parameters for the products, i.e. sour cherry compotes.

Foods rich in anthocyanins have traditionally been recommended in Europe and Asia for the treatment of atherosclerosis, chronic venous insufficiency and other health effects (Wallace & Giusti, 2015).

CONCLUSIONS

Sour cherries represent an important source of nutrients and bioactive food.

The sour cherry from the "Oblacinska" variety is characterized by a relatively high content of anthocyanins, which is a positive feature for the production of compote.

For the production of cherry compote, two types of sweeteners are used: sucrose and steviol glycoside. From the applied sweeteners, it has been established that they have no influence on the anthocyanin content.

Spectrophotometric method with cyanidin-3-glucoside as the reference standard is used for the determination of total anthocyanins in fresh sour cherries and compotes of the “Oblacinska” variety.

The results obtained within this research for the content of total anthocyanins revealed that the fruits of the fresh cherry of the variety "Oblacinska" are characterized by a higher value (32.00 mg CGE / 100 g), compared to the two compotes (16.12 and 16.10 mg CGE / 100 g, respectively).

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