INFLUENCE OF PROCESSING TECHNOLOGY ON BIOACTIVE COMPONENTS OF SOUR CHERRY

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Cherry (*Prunus cerasus var. Oblačinska*) is one of the most common varieties in Bosnia and Herzegovina and the region. Owing to the excellent sensory and physico-chemical characteristics and the very high concentration of biologically active ingredients, it is a very good potential for obtaining value added products or products with high content of bioactive, nutritionally and biologically valuable ingredients. The processing techniques of fresh fruit have a large influence on the content of bioactive components.

The aim of this study was to analyse the effects of processing technologies of sour cherries on the content of bioactive compounds (total phenols, total flavonoids and anthocyanins) and the antioxidant capacity. Products that were analysed were juice, concentrate, extra jam and lyophilisate. The content of bioactive components and antioxidant capacity of these products was compared with fresh cherries. The results showed that the greatest influence on the content of bioactive ingredients has process where vacuum concentration is applied. More sophisticated technology such as lyophilization would contribute to the retention of bioactive components and could increase product competitiveness at the market.

Keywords: sour cherries, processing, bioactive components, antioxidant capacity

INTRODUCTION

Owing to the fact that different types of fruit show a positive health effects, many studies were conducted to determine the properties of certain bioactive compounds and to confirm the correlation between the chemical composition and the favourable health effects[1]. Phytochemicals as secondary plant metabolites are biologically active compounds of plant. In the human body they have a protective effect towards various diseases, particularly cardiovascular diseases and cancer[2]. Phytochemicals include a large number of structurally different compounds: organosulfur compounds (glucosinolates and their breakdown products, allyl and analogues in the onion), polyphenolic compounds (phenolic acids, flavan-3-ols, flavanones, flavanolic glucosides), carotenoids, isoflavones, dietary fiber in fruits, vegetables and grains. Many phytochemicals exhibit antioxidant activity and contribute to protecting cells against oxidative damage[3]. Fruits are considered a natural source of antioxidants that include anthocyanins and polyphenols, which reduce the risk of diseases related to oxidative stress, such as cancer and cardiovascular diseases[4]. The steady increase in the market for functional foods that is rich in antioxidants has led to increased interest in natural sources of antioxidants and their potential utilization as a nutrient and functional food ingredients. Flavonoids are perhaps the most beneficial phytochemicals found in food. It has been shown that flavonoids have antibacterial, sedative, anti-allergic, antimutagenic, antiviral and other effects[5]. Studies have shown that flavonoids have an excellent antioxidative and antiradical activity and thus play an important role in the pharmaceutical and food industry as antioxidants[6]. More than 6400 flavonoids have been identified[7].

The protective role of flavonoids in biological systems is attributed to their multiple actions such as capacity to capture electrons of free radicals, the ability of chelating binding of transition metal ions (Fe^{2+}, Cu^{2+}, Zn^{2+} and Mg^{2+})[8], activation of antioxidant enzymes[9] and inhibition of oxidase[10]. The mechanism of action of flavonoids on the molecular level in biological systems is not completely understood, due to significant

differences in chemical properties and also because of their great structural heterogeneity of flavonoids.

Anthocyanins are water soluble pigments in plants. They structurally belong to flavonoids and are well known for their health effects[11].

Cherries are taxonomically classified in the genus Prunus, which is part of the Rosaceae family. Cherry is an industrial fruit species and it is a significant raw material for the production of high quality syrup, tart and jam. The fruit of cherry is juicy and due to the presence of anthocyanins is red in color. Cherry (Prunus cerasus var. Oblačinska) is one of the most common varieties in Bosnia and Herzegovina and the region. Owing to the excellent sensory and physico-chemical characteristics and very high concentration of biologically active ingredients, it is very good potential for obtaining value added products, or products with the high content of bioactive, nutritionally and biologically valuable ingredients. In order to avoid a loss of nutritional value of fruits, it is best to consume fresh fruit. However, it is often not possible, due to the need of transportation, storage, extending durability and the like. Therefore, the fruit should be processed. Since processing of fresh fruit have a large influence on the content of bioactive components, it is necessary to conduct studies to determine the effects of processing on the content of phytochemicals.

The aim of this study was to analyse the effects of different ways of processing of sour cherries, such as freeze drying, vacuum concentration and concentration on the content of bioactive compounds, particularly phenols, total flavonoids and anthocyanins. Also, the influence of processing on the antioxidant capacity of cherries was compared. The products that were analysed included lyophilisate (obtained by lyophilization process), dried sour cherry (obtained by vacuum drying) and concentrate (obtained by evaporation concentrating). The content of bioactive components and antioxidant capacity of these products was compared with fresh cherries and sour cherry jam.

MATERIALS AND METHODS

Cherry

Autochthonous sort of cherry “Oblačinska” was used. Fresh cherry, cherry juice, concentrate juice cherry, extra jam, jam and freeze-dried cherries were analysed and compared. Cherry juice was prepared by pressing and squeezing fresh cherries. The concentrate (juice concentrate) cherry was obtained on a laboratory BUCHI Rotavapour R-210 by evaporation of cherry juice at 50°C and pressure 75 mbar. Cherry extra jam was prepared by using sucrose and pectin as a gelling agent. For extra jam 60 g fruit/100 g jam was used. Pectin preparation was added in an amount of about 0,3-0,5% and the amount of sucrose and cooking time were set so that the final dry matter content of the product was at least 65% (Brix). The freeze-dried (lyophilisate) cherry was obtained by freeze drying in the lyophilizer Leybold-Heraeus GT2. Lyophilization was carried out at -40°C. The initial pressure was 2,5 mbar and after completion of lyophilization process it was 1,0 mbar. Duration of lyophilizing process was 47 hours.

Samples

For further analysis extract of fresh sour cherries, extra jam and dried cherry were prepared in triplicate. 100 g edible part of the fruit or jam was mixed in a blender to obtain a homogeneous sample. 10 g of the homogenized sample was extracted in an ultrasonic bath with 30 ml of methanol/HCl (95:5, v/v). After 30 min the solution was filtered and the residue on the filter paper was extracted again in the same way. The extracts were combined and diluted to 50 ml with a solution of methanol/HCl. Cherry juice and juice concentrate were analysed directly or diluted if necessary. Experiments were repeated at least three times. All results are given as mean ± standard deviation (SD).

Total phenolic content (TPC)

Total phenol content in the extracts was determined spectrophotometrically after reaction with Folin-Ciocalteu phenol reagent[12]. Samples were dissolved in methanol to a final concentration of 0,25 mg/ml. 50 ml of extracts, 450 ml deionized water and 2,5 ml of Folin-Ciocalteu reagent were mixed and incubated for 5 min. 2 ml of 7% sodium bicarbonate solution was added, filled with water up to 100 ml and incubated for 1,5 hours at 30°C. Absorbance of resulting blue colored liquids was measured at 765 nm. Quantitative analysis is performed based on the standard calibration curve of gallic acid in methanol. The concentrations of gallic acid in the solution from which the curve was prepared were 50, 100, 150, 250 and 500 mg/L (y=9,0135x-0,1935, R^2=0,9869). The result is expressed as mg of gallic acid equivalent per gram of dry weight of sample (mg GAE/100 g edible part of fruit).

Total flavonoid content (TFC)

Total flavonoids content (TFC) was determined by 24 h precipitation reaction with formaldehyde[13]. The remaining phenolic compounds, evaluated as non-flavonoid content (TNFC), were determined according to the previously mentioned procedure for TPC determination. TFC was calculated as subtraction of TPC and TNFC. In a 50 ml flask 5 ml of sample, 5 ml 1:4 HCl and 2,5 ml of formaldehyde solution was added, incubated for 24 hours at room temperature and filtered.

Determination of total anthocyanins

The content of anthocyanins was evaluated by the pH differential method spectrophotometrically[14]. Anthocyanins showed a maximum absorbance at 520 nm by pH 1.0 when coloured oxonium form was dominant, whereas colourless hemiacetal form was dominant by pH 4,5. The difference in absorbance was proportional to the anthocyanins content. Two solutions of fruit extract were prepared, one with potassium chloride buffer (0,2 M KCl) pH 1,0 and the other with sodium acetate buffer (1,0 M Na-Ac) pH 4,5 diluting each by the determined dilution factor of 1:20 (v/v). After 15 min incubation at room temperature the absorbance of each solution was measured at 514 nm and at 700 nm. The absorbance of the diluted samples is calculated as follows:

$$A = (A_{514\ nm} - A_{700\ nm})_{pH1.0} - (A_{514\ nm} - A_{700\ nm})_{pH\ 4.5}$$

$$\text{Antocyane (mg)} = \frac{A \times MW}{\varepsilon} = \frac{A \times 449{,}2}{29600} = \frac{A \times MW}{\varepsilon} = \frac{A \times 449{,}2}{29600}$$

$$\text{Total antocyane (mg/100 g fruit)} = \frac{\text{Antocijane (mg)} \times F \times V\ (\text{ml}) \times 100\ \text{g}}{\text{sample mass (g)}}$$

where is:

F=dilution factor (1:20)
V=volumen for solution for extraction (ml)

Determination of antioxidant capacity using DPPH (2,2-diphenyl-1-picrylhydrazyl) method

In the DPPH method[15], reaction solution was prepared by mixing 50 µl of diluted extract with 0,5 ml of 0,5 mM solution of DPPH and filled by methanol to 2 ml. The mixture was incubated for 30 min in the dark at room temperature. The absorbance was measured against the prepared blank (50 ml diluted fruit juice, 2950 ml methanol) at 517 nm. For DPPH blank solution preparation 1 ml of 0,5 mM DPPH solution was dissolved with 4 ml of methanol. Percent inhibition of DPPH radical is calculated according to the equation:

$$\text{Radical scavenging effect (\%)} = \left[1 - \frac{\text{absorbance of sample}}{\text{absorbance of blank}}\right] \times 100$$

Results are expressed as the IC 50 value (mg/ml) or the concentration of sample that causes 50% neutralization of DPPH radicals.

HPLC method

HPLC analysis of phenolic components in extracts was performed on an Agilent Series 1100

HPLC-system equipped with an Agilent 1200 Series DAD detector. The liquid samples filtered with 0,45 mm filter was introduced into the HPLC system using the Agilent 1100 Series auto sampler. System management and processing chromatograms was performed using Agilent LC Chem-Station software for data analysis. For HPLC analysis Zorbax SB C18 column was used, mobile phase ethanol/water 20/80 % v/v, flow rate 0,4 ml/min, detection: 254 nm (520 nm), injected volume 20 ml, room temperature[16]. As reference standards solution cyanidin-3-rutinoside chloride and cyanidin-3-glycoside chloride were used.

RESULTS AND DISCUSSION

Quantitative measurements of total phenols are based on the standard calibration curve of different concentrations of gallic acid. The results of determining TPS in the analyzed samples of fresh sour cherries and processed cherries are shown in the Table 1.

Table 1. Content of total phenols, flavonoids and anthocyanins in fresh cherry, juice, concentrate, extra jam and lyophilised cherry

Sample	Total phenols (mg GAE/100 g)	Total flavonoids (mg/100 g)	Total anthocyanins (mg/100 g)
Cherry	179,5 ± 9,7	37,5 ± 2,1	82,2 ± 7,3
Juice	199,7 ± 5,4	35,7 ± 3,8	22,4 ± 2,9
Concentrate	44,9 ± 7,6	7,5 ± 3,3	17,7 ± 5,7
Extra jam	116,7 ± 3,1	33,8 ± 5,4	30,1 ± 4,8
Lyophilisate	130,3 ± 8,6	38,7 ± 6,3	47,5 ± 7,8

The results show that fresh cherry has a relatively high content of phenol (179,5 mg GAE/100 g edible part of the fruit cherries). The result is consistent with the literature data[17, 18]. The total phenol content in the products obtained from processing of sour cherries shows that cherry juice has maintained a high phenolic content (199,7 mg GAE/100 g), while the amount of TPC is somewhat preserved in lyophilized cherries (130,29 mg GAE/100 g). The production process of extra jam reduces TPC and this value is 116,67 mg GAE/100 g. During the process of concentration of cherry juice there is a significant loss of TPC (44,9 mg GAE/100 g). It can be concluded that the vacuum processing of cherries greatly influences the value of TPC. Total flavonoids are calculated from the difference between total phenol and non-flavonoid content. It can be seen from Table 1 that the highest content of flavonoids have fresh cherries, followed by freeze-dried cherry, cherry juice and extra jam (33,75 mg GAE/100 g). The results show that lyophilisation process preserves the content of flavonoids. The flavonoid content of cherry juice is much higher compared to the concentrated juice, which means that the process of vacuum concentrating causes a loss of flavonoids. Process of jam production where high temperature is applied do not greatly influence the content of flavonoids.

The content of anthocyanins has long been used as to assess adulteration by the addition of juices from different fruit sources[19]. The profile of anthocyanins is characteristic for different types of fruits and all these variations affect the way of determination. Table 1 shows the results of total anthocyanins per 100 g of sample. Anthocyanins are quite reactive and unstable compounds. The highest content of anthocyanins is measured in fresh cherries (82,2 mg/100 g edible part). The results are similar with the results obtained by other authors[20]. Lyophilisation of cherries most preserves the anthocyanins content (47,50 mg/100 g). Because the anthocyanins loss may occur in the presence of oxygen and at elevated

temperatures, cherry processing causes the reduction of the content of anthocyanins. The lowest content of anthocyanins is registered in the concentrated juice and amounts to 17,70 mg/100 g sample.

Antioxidant capacity depends on the composition of the extract and methods that are used for their determination. DPPH is a stable radical and is often used to assess the antioxidant capacity of different natural products. The results in Figure 1 are expressed as the IC 50 value (mg/ml) or the concentration of sample that causes 50% neutralization of DPPH radicals.

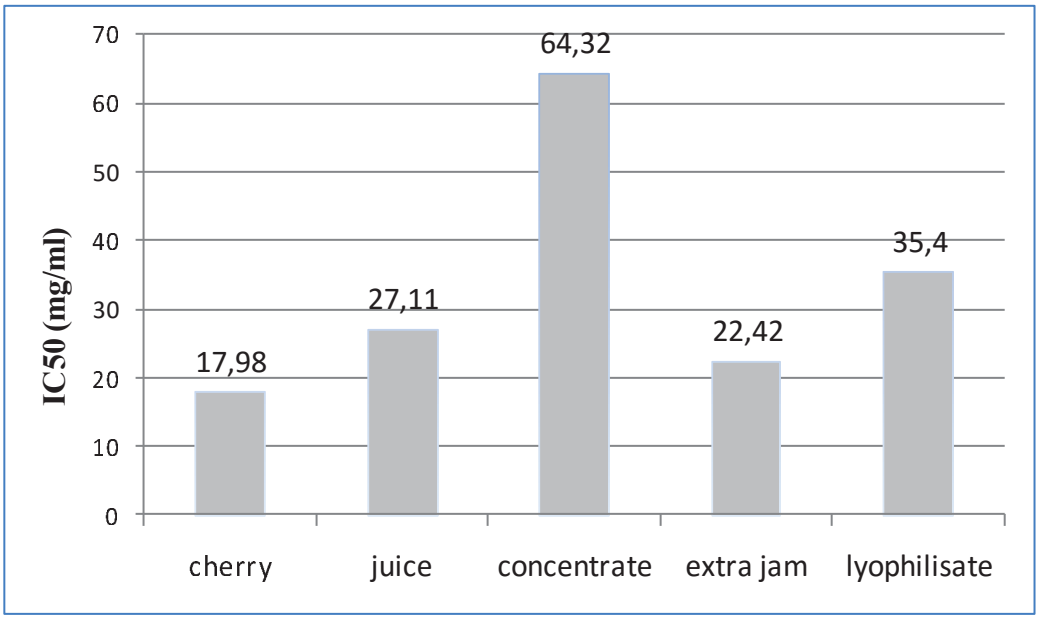

Figure 1. Antioxidant capacity of cherries and processed cherries determined by DPPH method

The results in Figure 1 show that all samples have similar values of antioxidant capacity or mass of the sample needed to achieve 50% inhibition of DPPH radicals. The cherry concentrate shows the weakest antioxidant capacity (64,32 mg/ml). It should be considered that fresh cherry has a significant content of vitamin C which is one of the most powerful antioxidants. During the technological process, especially during the preparation of concentrate by evaporation, part of vitamin C is lost, which lowers antioxidant capacity of the concentrate.

Reversed phase RP-HPLC is the most used method for detection and identification of anthocyanins. Anthocyanins could be separated according to their polarity and detected on the chromatogram at different retention times. Most of methods for the analysis of anthocyanins are qualitative or semi-quantitative and provide information about identification of anthocyanins in their source. The big challenge is the quantification of anthocyanins by HPLC due to the absence of most commercial standards. The composition of anthocyanins in all samples was analyzed by HPLC (Figure 2). Cyanidin-3-rutinosid and cyanidin-3-glycoside are used as reference standards. Retention time (RT) of cyanidin-3-glycoside is 7,55 min and retention time of cyanidin-3-rutinosid is 7,86 min.

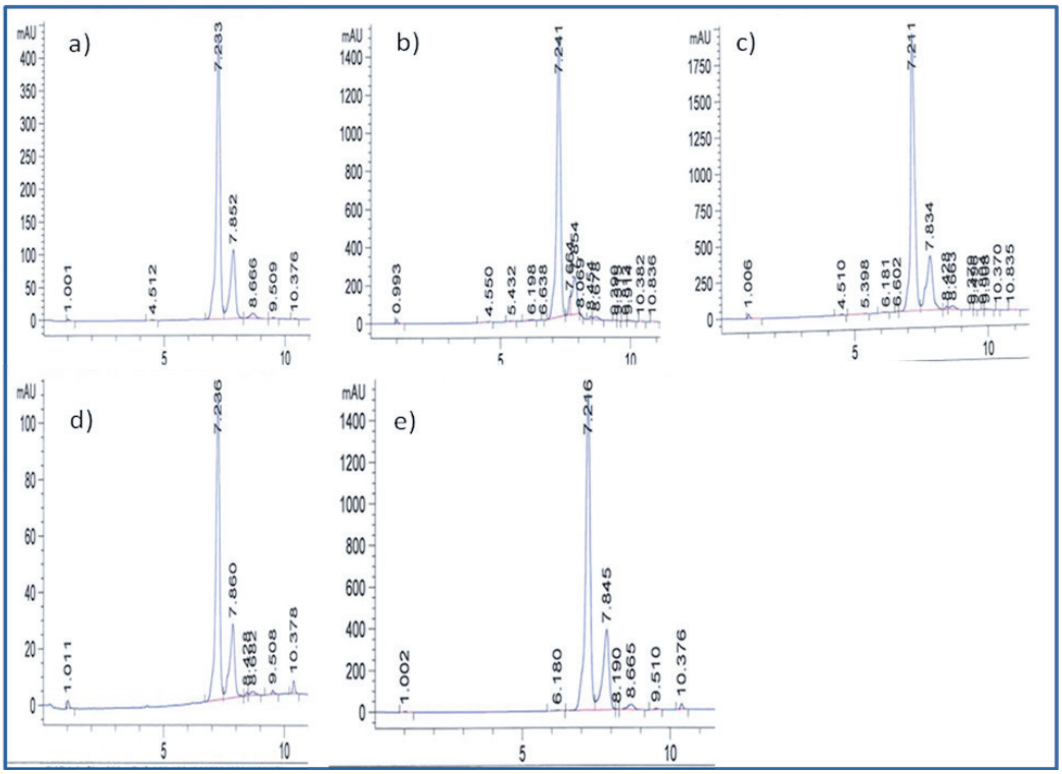

Figure 2. HPLC diagrams of anthocyanins profile of fresh cherry (a), juice (b), concentrate (c), extra jam (e) and lyophilised cherry (d)

On HPLC diagrams of all samples (Figure 2) there is a peak detected at RT 7,86, which corresponds to cyanidin-3-rutinosid. None of the sample diagrams show peak at RT 7,55, which corresponds to cyanidin-3-glycoside. However, the intensity of this peak is different, where juice, concentrate and lyophilisate show the strongest intensity. Since the peak intensity corresponds to the amount of anthocyanins in the sample, it can be concluded that the technological process of production of jam causes the loss of anthocyanins. In all diagrams there is the high intensity peak at RT 7,24 min detected, which cannot be identified. Also, several other peaks of lesser intensity are registered that, due to the lack of a reference standard, are not possible to identify.

CONCLUSION

Since the trend of manufacturing of value added products in the world is increasing, there is need to affirm application of sophisticated technology such as freeze-drying and vacuum concentration in order to increase the competitiveness of food products at the local level in Bosnia and Herzegovina and in such way to increase the export potential of these products. The aim of this study was to demonstrate the effect of different ways of cherries processing technologies on the content of bioactive compounds, especially of total phenols, flavonoids and anthocyanins. Also, the impact of processing on the antioxidant capacity of cherries and processed cherries was compared. The analysed products were obtained by lyophilization (lyophilisate) and vacuum concentration (concentrate) and compared to the content of bioactive components and antioxidant capacity of these products with the product obtained by conventional processes (extra jam).

The results showed that the processes of cherries affect the content of bioactive ingredients. This study showed that the greatest influence on the

content of bioactive ingredients has process where vacuum concentration is applied. Conventional technologies used for production have a negative impact on the bioactive components. The use of more sophisticated technologies such as lyophilization would contribute to the retention of bioactive components and in that way increase product competitiveness and at the same time meet constant market growth for functional foods that is rich in antioxidants.

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