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Influence of storage time on the content of vitamin C and phenol compounds in sour cherry compote

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Abstract
Fruits, as well as fruit products, play a very important role in human nutrition. However, a large number of different types of fruit are not available to consumers throughout the year, mostly due to the lack of storage capacity and expensive transport in cold stores. For this reason, significant quantities of different types of fruit are processed in the industrial plants of the food industry. However, during fruit processing there are various changes in the nutritive composition of fruit, and most often due to the influence of high temperatures during technological operations of fruit processing into various food products, such as fruit compotes, juices, etc.
This paper investigates the influence of sour cherry compote storage time on the content of some nutritional components important in human nutrition, such as vitamin C content, phenolic compounds content, and some physico-chemical parameters that affect the quality and durability of food products (dry matter, acidity, pH).

Keywords: vitamin C, phenol compounds, sour cherry compote

Introduction
Sour cherry (*Prunus cerasus L.)* is one of the oldest fruits used by mankind in their daily diet. Sour cherry is an industrial fruit, suitable for processing into juices, jams, fruit purees, compotes and syrups. In terms of industrial processing, sour cherries are an important raw material, and a significant number of varieties of sour cherries are suitable for fresh consumption (Cvrk R, *et al*.,(2019) [1]; Trees of noble sour cherry varieties live 25 to 30 years, while individual trees up to 50 years. On average, sour cherries contain about 83 to 85% water, between 15 and 17% dry matter. They contain the highest total sugar, about 10%, of which invert sugar accounts for 9%. Acid on average contains 1.9%, while minerals contain about 0.6%. The content of vitamin C has a value of 21 to 60 mg/%, which mostly depends on the characteristics of each variety. The most current varieties of sour cherries are: Maraska, Oblačinska, Hajmanov Rubin, Satenmorele, (Jašić M, 2011) [2].

Materials and methods
Samples of sour cherry compote (*Oblačinska* variety), produced and stored in industrial conditions, were used as materials for this research.
The products are packed in glass packaging with a volume of 720 ml, with a metal Twist-off lid that has an evenly distributed plastic coating on the inside that ensures hermetic sealing. After closing the jars, the sour cherry compote was pasteurized at a temperature of 80°C for 30 minutes. The samples were collected for a period of 18 months and stored in an industrial warehouse.

Determination of vitamin C content in sour cherry compote
L-ascorbic acid (vitamin C) in this experiment was determined by titration with 2.6 dichlorophenol-indolphenol (Hernández Y, al., (2006)[3] In the presence of the enzyme ascorbic acid oxidase, oxidation of ascorbic acid to dehydroascorbic acid occurs, so the extraction of ascorbic acid from the tissue is performed using metaphosphoric acid, which inactivates the oxidase present in the cells. During the analysis of vitamin C, a solution of 2.6 dichlorophenol-indolfenol 0.25 mg/mL was used (50 mg of DIF is dissolved in 150 mL of hot water, cooled, filtered through filter paper and made up to 200 mL with water). Standard solution of ascorbic acid 0.1 mg/mL (in a 100 ml volumetric flask, weigh 5-10 mg of ascorbic acid, dissolve and make up to the mark with 2% oxalic acid solution. Then pipette 10 mL of standard solution into a 100 mL Erlenmeyer flask and titrate with 2.6 dichlorophenol-indolphenol to a pink color which must be stable for 10-20 seconds and calculate the titer of the 2.6 dichlorophenol-indolphenol solution according to expression, where A is the amount of ascorbic acid in 10 mL of standard solution (mg), Z is the amount of 2.6 dichlorophenol-indolphenol used to titrate 10 mL of standard ascorbic acid solution:

$$E = A / Z$$

Determination procedure: Take 10 ml of the sample previously homogenized with 2% oxalic acid and titrate with 2.6 dichlorophenol-indolphenol until a pink color appears which must be maintained for 15-20 seconds. The total amount of ascorbic acid is calculated by the following formula:

$$c = \frac{V \times E \times 10}{G}$$

Where:
c – mass of ascorbic acid (mg/100 g of the sample), V – volume of 2.6 dichlorphenol-indolphenol (mg/mL), E – titer of 2.6 dichlorphenol-indolphenol (mg/mL), G – weighed amount of the sample.

Determination of dry matter content refractometrically
The dry matter content in the tested samples of sour cherry compote was determined refractometrically, using a table refractometer according to ABBE (Japan), and the results were expressed as degrees Brix. For each sample analyzed, the refractometer was calibrated, using distilled water, and set to zero, after which a homogenized compote sample was prepared, which was applied to the refractometer prism and read the dry matter value.

Determination of pH value of the sour cherry compote
Determination of the pH value of homogenized compote samples was performed by direct measurement using a tabletop, laboratory pH meter. During the analysis of sour cherry compote homogenized samples, a laboratory, table pH meter "FiveEeasy-FE20" (Mettler Toledo - AG; 8603 Schwerzenobach, Swit Zealand) was used.

Determination of antioxidant capacity by DPPH method
This method was developed to determine the antioxidant oxidability of compounds in food using a stable 2.2 diphen-1-picryl hydrazyl radial. Due to the unpaired electron, the DPPH radical achieves an absorption max in the visible part of the spectrum (517 μm), a change from purple to yellow results from pairing of the unpaired electron DPPH radical with hydrogen antioxidants, creating a reduced form of DPPH-H, the color change is in stoichiometric relation to the number of paired electrons (Ferretti G., et. al, 2010 [4] Miletić N, *et al*, [2012) [5].
Used as reagents: 0.5 mM solutions of *DPPH (2.2-d*iphenyl-1-picrylhydrazyl radical), 100% methanol and formic acid.

Reagent preparation - 0.5 mM DPPH solution
0.02 g - 2.2 diphenyl-1-picryl hydrazyl radical (DPPH) is weighed into a plastic weighing pan and quantitatively transferred and dissolved in 100% methanol and made up to 100% with methanol in a 100 ml volumetric flask.

Preparation of extracts
From 2 g of sample prepare 10 ml of extract. The extraction is carried out in an ultrasonic bath preheated to 50 ° C.
The extraction solvent is 1% formic acid in 80% methanol, the resulting extract is centrifuged for 10 min at 5500 rpm, decanted and made up to 10 ml with extraction solvent. The resulting extract was stored until analysis at -18°C under an inert gas atmosphere.

Analysis procedure
Pipette 2 ml of extract, 2 ml of methanol and 1 ml of 0, 5 mM DPPH solution into a test tube. The tubes with the contents were left in the dark at room temperature for 20 min, after which the absorbance at 517 μm was measured with methanol as a blank test.
Two parallels were made for each individual sample.
The measured absorptions did not exceed 1.0, so it was not necessary to dilute the sample extracts.
Preferred absorbance values are from 0.1 to 0.9.

Calibration direction construction
To prepare the calibration direction, prepare 100 ml of a 0.02 M solution of Trolox (6-hydroxy-2, 5, 6, 7, 8-tetramethylchroman-2-carboxylic acid) from which the diluted concentrations were prepared: 0.25; 50; 100; 200; 300 μM by pipetting in the 50 ml volumetric flasks 0, 6; 25; 125; 250; 500 i 750 μL of Trolox aliquot solution and fill up to the mark with 100% methanol.
Pipette 200 μL of Trolox solution, 3.8 M methanol and 1 ml of 0.5 mM DPPH solution into the tube, stir the contents and leave in the dark for 20 minutes. The absorbance was then measured at 517 μm with methanol as a blank.
From the measured values of the absorbance of the Trolox solution, the calibration direction was obtained using Microsoft Office Excel with the values of the concentration of Trolox (μm) on the abscissa and the absorbance values applied on the ordinate.

Based on the results obtained, the equation of the direction is as follows:
y = - 0, 00123814*X+1, 2031
r^2= 0, 9965

From the accompanying direction equation, the DPPH (X) of the extract is calculated based on the measurements determined by the DPPH method, where:

Y is absorption of the sample at 517μm
X is Troloxa equivalent (μmol)/l
1, 2031 = section of the line on the y axis
The antioxidant activity of the Trolox sample / 100g sample is recalculated taking into account weigh the mass of the sample and dilution.

Determination of total phenols by the Follin-Ciocalteu method

The Follin-Ciocalteu method is a spectrometric method based on the oxidation of phenolic compounds by reagents, i.e. Follin-Ciocalteu solution. Due to oxidation, the solution changes color to intense blue. The blue color of the oxide is stable. The color intensity is proportional to the concentration of phenolic compounds and is measured spectro photometrically at a wavelength of 750 μm.
The extract (2 ml) was evaporated on a rotary evaporator to remove the solvent. The residue was dissolved in methanol (10 ml). The methanol solution (0.1 ml) was then pipetted into a test tube where water (7.9 ml), Follin-Ciocalteu reagent (0.5 ml) and 20% Na2CO3 solution (1.5 ml) were then added. This content of the tube thus obtained was mixed on a Vortex mixer and after 30 min the absorbance of the solution thus obtained was measured at λ = 750 μm. A blank test was performed with methanol. The calculation of the content of total phenols calculated on chlorogenic acid was performed on the basis of the absorbance and the equation obtained by the mass of chlorogenic acid, and the content of total phenols calculated on chlorogenic acid was calculated. A UV spectrophotometer - UV mini 1240 (Shimzdu) with a UV interval of 320-560 was used to measure the absorbance.

Determination of total acidity

The method is based on titration with sodium hydroxide solution with phenolphthalein as an indicator or potentiometrically. It is used to determine the total acidity in fruits and vegetables and their products. The results are expressed in grams of free or total acidity per 100 g or 100 cm^3 of the sample. For analysis, 25 g of the sample was weighed with accuracy of 0, 01 g and quantitatively transferred to an Erlenmeyer flask with a ground neck using 150 cm^3 of distilled water. Mix the contents until homogeneous. Connect the flask with the reflux condenser and heat on a water bath for 30 minutes. Cool the contents and transfer to a 250 cm^3 volumetric flask, fill up to the mark with freshly boiled and cooled distilled water, then filter through plain filter paper. Pipette 50 cm^3 of the test sample into a 100 cm^3 Erlenmeyer flask, add a few drops of indicator and titrate with NaOH until a light pink color appears which must be stable for at least 30 seconds. If the sample has an intense dark color make a dilution. The dilution made for this analysis was 1:5 (5 cm^3 of filtrate: 25 cm^3 of distilled water). The total acidity of the samples was determined according to the following formula:
In the above formula, V1 represents the consumption of NaOH solution (cm^3); c -concentration of NaOH (mol/l); V_0 - amount of dilution taken (cm^3).

Results and discussion

The results of the research were obtained by analyzing samples of sour cherry compote, stored for 18 months in an industrial warehouse. Analyzes were performed by random sampling with random samples of compote from the warehouse. The results are expressed as the mean values of three consecutively repeated measurements for each analyzed parameter. The mean values of the obtained results for the examined parameters are shown in Table 1.

Table 1: Chemical composition of sour cherry compote

Sample	Vitamin C (mg/100 g of sample)	% of dry material refectometric	% acid	pH	% DPPH	mg polyphenols/100 g of samples
S1	4,64	19,43	1,55	3,43	77,43	23,64
S2	6,720	19,67	1,70	3,59	75,80	26,49
S3	9,150	19,39	1,60	3,643	81,94	20,91
S4	8,89	19,51	1,60	3,677	79,89	24,32
S5	9,43	18,40	1,60	3,706	88,32	16,83

Results of vitamin C content

Observing the results from Table 1, it can be concluded that the vitamin C content is highest for sample S5 (9.43 mg/100 g of product), then in sample S3 (9.150 mg/100 g of product), sample S4 (8.89 mg/100 g of product) while sample 1 contains the least amount of vitamin C (4.64 mg/100 g of product), then sample S1 (4.64 mg/100 g of product). The lowest content of vitamin C was obtained in samples 1 (4.64 mg/100 g of product) and sample 2 (6,720 mg/100 g of product). According to the obtained results, it can be concluded that the samples of sour cherry compote S1 and S2, which have been stored for the longest period of time, have the lowest content of vitamin C. Comparing the content of vitamin C, in samples of cherry compote with Ferretti *et al*., (2010)[4] which found 7 (mg /100 g) vitamin C for sweet cherries, and 10 mg/100g for vitamin C for sour cherries). Therefore, it can be concluded that the content of vitamin C is significantly preserved during the production and storage of compote (Table 1).

Results of dry matter (refractometric)

Dry matter results (Table 1) show that there is no significant difference in terms of results for dry matter values (Brix). The obtained results are in accordance with the prescribed legislation for compotes, since the Ordinance on fruit products requires a dry matter of at least 18 - 22 Brix.

Results of the acidity content of the sour cherry compote]]

The acidity of sour cherry compote in this study was 1.55% - 1.70%, which are characteristic values for sour cherry compote, since sour cherries as a fruit species contain a significant amount of fruit acids, which gives sour cherry products a specific refreshing taste. According to the obtained values of compote acidity, the measured pH values of sour cherry compote were in accordance (Table 1).

Determination results of antioxidant capacity of DPPH for sour cherry compote

The results of testing the antioxidant capacity of sour cherry compote show the highest percentage of DPPH quenching in sample S5 (88.32%), then in sample S3 (81.94) while the lowest percentage of quenching was obtained in the sample in compote S2 (75.80%), Table1. (Antolovich M, *et al*., 2012) [6] (Bondet V, *et al*., 1997) [7]

Determination results of polyphenolic compounds in sour cherry compote

By comparing the results of polyphenolic compounds in the tested samples of sour cherry compote, it can be seen that sample S2 (26.49 mg of polyphenol/100 g product), then sample S4 (24.32 mg of polyphenol/100 g product), while the lowest content of polyphenolic components obtained from sample S5 (16.83 mg polyphenols/100 g product), Table 1.

Also, Ferretti *et al*., 2010 [4] considered the phenoles content in different varieties of fresh cherries, and found different contents of polyphenol content in fresh fruits of different varieties of cherries (from 60 ± 13 to 312 ± 8 (mg GAE/100g fresh cherries.

Comparing the polyphenol content of sourcherry compote with the polyphenol content of fresh sour cherry, it can be concluded that the polyphenols content is well preserved, if we take into account the processing of cherries during the production of compote, and the storage period (Antolovich *et al*., 2012) [6] (Moharram H. And Youssef M. 2014)) [8]

Conclusion

Based on the conducted research, it can be concluded that the tested samples of sour cherry compote showed a very high level of quality during storage. Taking into account the length of storage, and process parameters during processing, as well as the manipulation of raw materials during the compote production. It is extremely important to maintain relatively high levels of vitamin C during the storage of compote, and a relatively high content of polyphenols in the tested samples, as well as the DPPH value.

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