



## Comparative analysis of the content of polyphenol compounds and vitamin C in rosehip jam and plum jam

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### Abstract

Due to the fact that most fruits cannot be consumed fresh all year round, the processing of fruits into different types of food has a very long tradition. The fruit is most often processed into fruit juices, compotes, jams and other products. The chemical composition of fruits is important both from the point of view of nutrition and from the point of view of technology. Which technological procedure will be applied, which reactions can be expected during processing, what kind of product and its quality will be obtained, is most often related to the chemical composition of the raw material. This paper analyzes plum jam and rosehip jam, and primarily considers the content of vitamin C, the content of polyphenolic compounds in rosehip jam and plum jam, and parameters important for the correctness of the product (pH, dry matter, acidity, etc.).

**Keywords:** polyphenol compounds, vitamin C, rosehip jam and plum jam

### Introduction

Plum (*lat. Prunus domestica*) from the genus *Prunus*, is a type of fruit that grows on the tree. As a raw material, it is very important in the daily diet, but also as a raw material for the food industry. Today, plum production is based on scientific principles and modern agricultural techniques (Niketić-Aleksić G, (1994) <sup>[1]</sup>.

Large plantations are being planted. New varieties with increasing economic and biological potential are being created and introduced into production. A large number of food products are produced from plum fruits, but the most famous are certainly prunes, plum jams, juices and other products. Plum fruits are used as a first-class fruit and as a raw material for industrial processing. The fruits contain up to 75% water and up to 25% dry matter. The taste of the fruit depends on the ratio of sugar and organic acids. Glucose predominates in the plum fruit, while fructose is less, and malic and citric acids are the most abundant. The percentage of acid and sugar is different in fruits of the same variety, depending on climatic and soil conditions. Plums have a significant amount of vitamin C, and are a significant source of vitamins B1, B2, B6, beta carotene, vitamin E, potassium and dietary fiber (Jašić M, (2007) <sup>[2]</sup>.

Vitamin C is a very strong reducing agent, it can prevent lipid peroxidation caused by hydroxyl radicals and some other pathophysiological factors of oxidative stress, such as activated neutrophils, cigarette smoke or xanthine oxidase system. Vitamin C can act as an antioxidant and as a coantioxidant. It has been proven that vitamin C participates in the process of vitamin E recovery, i.e. in its translation from oxidized to reduced form, (Stomatovska V, *et.al.* (2013) <sup>[3]</sup> Levaj B. (2003) <sup>[4]</sup>.

Plum is sensitive to wind during the growing season, especially before fruit ripening, because it causes premature decline. Compared to other fruit species, plums tolerate lower temperatures up to -30°C relatively better. The choice of soil for plums should be made taking into account the specific requirements of the substrate and variety. The variety is the most important factor in the economic cultivation of plums. Some of the most famous varieties are: *Požegača* (Bistrica, Mađarica), *Stanly*, *Italijanka* (Italian Prune), *Čačanka Ljepotica* etc. (Jašić M. 2007) <sup>[2]</sup>.

### Rosehip as raw material

Rosehip, cynorrhodon, a fruit characteristic of plants of the botanical genus *Rosa*. The names wild rosehip, wild rose, etc. are often used for the plant, but also for its fruits. Today, however, most of the industrial needs for these fruits are met by cultivation, and such varieties have slightly larger fruit, are easier to pick, are more resistant to disease, but their quality and chemical composition are far worse than natural fruits (Niketić – Aleksić G, 1989) <sup>[5]</sup>.

The rosehip fruit is usually oval in shape, orange-red in color, but can vary from yellow to dark purple and black. Sweet and sour taste, harvested in September, October and November. For preservation purposes, they are dried at temperatures up to 50 °C to retain their natural color. Rosehip as a raw material is mostly used for the production of marmalades and teas.

### Materials and methods

Samples of plum jam and rosehip jam, produced and stored in industrial conditions, were used as materials for this research. The products are packed in glass packaging with volume of 720 ml, with Twist-off lid that has an evenly distributed plastic coating on the inside that ensures hermetic sealing. The lids used are breathable, with the possibility of creating a vacuum in the jar with the product. The samples were collected for a period of 18 months and stored in an industrial warehouse.

### Determination of vitamin C content in the products

L-ascorbic acid (vitamin C) in this experiment was determined by titration with 2,6-dichlorophenol-indolphenol (Hernández Y, *et al.*, (2006)<sup>[6]</sup>). 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-indolphenol 0.25 mg/mL was used (50 mg of DIF is dissolved in 150 mL of hot water, cooled, filtered through filter paper and filled 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 fill 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 the expression (1), 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-dichlorophenol-indolphenol (mg/mL), E – titer of 2,6-dichlorophenol-indolphenol (mg/mL), G – weighed amount of the sample.

### Determination of dry matter content refractometrically

The dry matter content in the tested samples of the products 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 sample was prepared, which was applied to the refractometer prism and read the dry matter value.

### Determination of pH value of the products

Determination of the pH value of homogenized product samples was performed by direct measurement using a tabletop, laboratory pH meter. During the analysis of homogenized samples of the products, a laboratory, table pH meter "FiveEeasy-FE20" (Mettler Toledo - AG; 8603 Schwerzenbach, Switzerland) 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-diphenyl-1-picrylhydrazyl radical. Due to the unpaired electron, the DPPH radical achieves an absorption max in the visible part of the spectrum (517 nm), 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 (Prior *et al.*, 2005; Braca *et al.*, 2001)

Used as reagents: 0.5 mM solutions of DPPH (2,2-diphenyl-1-picrylhydrazyl radical), 100% methanol and formic acid (Miletić N, *et al.* [2012]<sup>[7]</sup>).

### Reagent preparation - 0.5 mM DPPH solution

0.02 g - 2,2-diphenyl-1-picrylhydrazyl 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  $\mu\text{m}$  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 route, 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  $\mu\text{M}$  by pipetting in the 50 ml volumetric flasks 0,6; 25; 125 ;250 ; 500 and 750  $\mu\text{L}$  of Trolox aliquot solution and fill up to the mark with 100% methanol.

Pipette 200  $\mu\text{L}$  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  $\mu\text{m}$  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 ( $\mu\text{m}$ ) 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 517  $\mu\text{m}$

X is Trolox equivalent ( $\mu\text{mol}/\text{l}$ ).

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 (Antolovich M, *et al.*,2012)<sup>[8]</sup> ; (Bondet V, *et al.*, 1997)<sup>[9]</sup>.

### 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 spectrophotometrically at a wavelength of 750  $\mu\text{m}$ .

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%  $\text{Na}_2\text{CO}_3$  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  $\lambda = 750 \mu\text{m}$ . 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  $\text{cm}^3$  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  $\text{cm}^3$  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  $\text{cm}^3$  volumetric flask, fill up to the mark with freshly boiled and cooled distilled water, then filter through plain filter paper. Pipette 50  $\text{cm}^3$  of the test sample into a 100  $\text{cm}^3$  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  $\text{cm}^3$  of filtrate: 25  $\text{cm}^3$  of distilled water). The total acidity of the samples was determined according to the following formula:

$$\text{Total acidity (\%)} = \frac{1000 \times V1 \times c}{V0}$$

In the above formula, V1 represents the consumption of NaOH solution ( $\text{cm}^3$ ); c -concentration of NaOH ( $\text{mol}/\text{l}$ );  $V_0$  - amount of dilution taken ( $\text{cm}^3$ ).

## Results and discussion

The results of the research were obtained by analyzing the samples of plum jam, stored for 18 months in an industrial warehouse, and the samples of rosehip jam stored in the same conditions. Both jam analyses were obtained in the same plant, with the same technological conditions. Analyses were performed by random sampling from the warehouse. The results are expressed as the mean values of three consecutively repeated measurements for each analyzed parameter. The mean value of the obtained results for the examined parameters are shown in Table 1. and Table 2. Also, the comparison of the research parameters is given for the prune jam and the rosehip jam.

**Table 1:** Results of the examined parameters for plum jam

Sample	Vitamin C (mg/100 g of sample)	% of dry material refractometric	% acid	pH	% DPPH	mg polyphenols/100 g of samples
S1	5,60	59,48	2,16	3,84	82,40	111,70
S2	12,83	59,62	2,19	3,79	90,85	102,26
S3	8,14	57,61	2,12	3,78	74,97	78,44
S4	6,93	57,43	2,37	3,78	90,91	122,12
S5	10,44	61,24	2,16	3,64	81,15	89,70

### Results of Polyphenols content in the jams

According to the obtained results of polyphenols content in **plum jam samples** (Table 1), the high polyphenol content was found in the sample S4 (122,12 mg polyphenols/100 g jam), then in sample S1 (111.70 mg polyphenols /100 g jam), and sample S2 (102.26 mg polyphenols / 100 g jam. Samples S3 (78.44 mg polyphenols /100 g) and S5 (89.70 mg polyphenols / 100 g) showed lower values of phenolic compounds content.

Results and content of phenolic compounds in rosehip jam samples (Table 2), show significantly lower values compared to plum jam (17.24 mg polyphenols /100 g jam), while the highest content was observed in sample S1 (36.96 mg polyphenols / 100 g jam). Considering the results for polyphenolic compounds in rosehip jam, was found lower content found phenol content than expected in this study was expected.

The relatively low content of phenol in bar jam was probably influenced by the degree of maturity of the bar, the time of picking, the length and the method of storage in refrigerators. In general, fruit ripeness significantly affects the content of polyphenolic components and air temperature during storage (Miletić N., at al (2012) [7].

**Table 2:** Results of the examined parameters for rosehip jam

Sample	Vitamin C (mg/100 g of sample)	% of dry material refractometric	% acid	pH	% DPPH	mg polyphenols/100 g of samples
S1	6,91	67,55	0,76	4,195	52,25	36,96
S2	9,28	65,88	0,76	4,263	71,82	28,86
S3	9,15	68,39	0,76	4,352	79,04	17,24
S4	9,02	66,74	0,90	4,276	71,73	22,92
S5	9,26	68,75	0,76	4,306	74,89	24,00

### Results of vitamin C content in the jams

Comparing the results of vitamin C for the analyzed plum jams and rosehip jams, one can see the significant potential of vitamin C, if we take into account the storage time of raw materials, technological processing of raw materials and storage time of finished products.

According to the obtained results, the content of vitamin C in plum jam is slightly lower in sample S1 (5.60 mg/100 g of the sample) and sample S4 (6.93 mg/100 g of the sample) while other samples had significantly higher amounts of vitamin C, (8.14 - 12.83 6 mg/100 g of the sample) which is probably achieved by the appropriate plum assortment and test parameters. The vitamin C content for rosehip jam (Table 2) has a relatively high and stable vitamin C content, with a slightly lower vitamin C content having a S1 sample. Other samples have a relatively high content of vitamin C (9.01 - 9.28 mg/100 g of the sample).

### Results of dry matter content (Refractometric)

Dry matter results (Table 1 and Table 2) show that the dry matter values are lower at plum jams and are 57,43-61,24 Brix, while the rosehip jam samples have significantly higher dry matter values 65,88 Brix to 68,75 Brix (Table 1 and Table 2).

### Results of acidity of jams

When considering the acidity of jams, a significant difference in acidity content was observed between the analyzed samples of the jam, given the fact that rosehip as a raw material has a naturally high acidity compared to plums. In any case, these types of jams are acceptable to consumers in Bosnia and Herzegovina, primarily due to the specific tastes and traditions of production and consumption (Vračar O. Lj. 2001) [8].

### Results of pH of jams

The pH values of plum jam ranged from 3.64 to 3.84, while the pH of the bar jam had a value of 4.195 - 4.306. Slightly higher acidity is characteristic of the bar melody, since the acidity affects the sensory properties, gelling properties and other parameters of the jam.

### Results of antioxidant capacity of DPPH for plum jams

According to the obtained results for antioxidant capacity for plum jam (Table 1), it can be seen that the percentage of quenching DPPH has a potential of 74.97% to 90.85%, while the content of phenolic compounds in the sample of plum jam had a value of 78, 44 to 122.12 (Table 1), while samples of rosehip jam have a content of polyphenolic compounds of 17.24 - 36 mg of polyphenols/100 g of the jam.

### Conclusion

Based on the conducted research, it can be concluded that the tested samples of rosehip jam and plum jam showed significant antioxidant potential, as well as the content of polyphenic compounds in the tested jams. The obtained result is significant, especially due to the fact that these are products that were obtained in industrial conditions, and stored in a warehouse for a long time. It should be emphasized that plum jam preserved a higher amount of vitamin C during processing and storage, as well as the content of polyphenolic compounds.

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