

UDK 577.164.2:582.711.714
535.234:577.164.2

DETERMINATION OF VITAMIN C IN SOME BOSNIAN *CRATAEGUS* L. SPECIES BY SPETROPHOTOMETRIC METHOD

Određivanje vitamina C kod nekih bosanskohercegovačkih vrsta roda *Crataegus* L. spektrofotometrijskom metodom

Azra Tahirović¹, Amira Čopra – Janićijević², Nedžad Bašić¹, Danijela Vidić²,
Lejla Klepo², Delila Delić²

Abstract

Vitamin C or ascorbic acid content has been determined by spectrophotometric method in fruits of some Bosnian hawthorns (*Crataegus* L). species. Spectrophotometric method used in this study is based on the kinetic reaction between Vitamin C and methylene blue. Measurements were carried out at absorption maximum, $\lambda_{\max} = 665$ nm. Titrimetric method was used as the reference method for comparison of the obtained results. We found that the lowest content of vitamin C was 106.12 mg/100 g of a dry sample in fruits of the *C. monogyna*, and the highest level of Vitamin C was found in the *C. microphylla* (231.96 mg/100 g of a dry sample) fruits. Recoveries of the results obtained by the spectrophotometric method were 101.4% - 108.0% with relative standard deviation (RSD) values ranging between 0.40% - 3.37%.

Obtained results showed that fruits of studied *Crataegus* L. species are a good source of vitamin C with its potential to be used in food industry as the natural antioxidant.

Keywords: ascorbic acid, *Crataegus*, hawthorns, methylene blue, spectrophotometry,

INTRODUCTION – Uvod

Crataegus L. is a polymorphic genus from Rosaceae family, widespread in temperate regions of the Northern Hemisphere. Mostly, its species are shrubs or small trees growing to 5-15 m tall with pome red fruit and thorny branches. Only a few researchers studied populations of *Crataegus* from Bosnia and Herzegovina (BECK, 1927; MALÝ, 1919, 1940; FUKAREK, 1974; JANJIĆ, 1998, 2002). According to recent investigations, four autochthonic species of *Crataegus* and their few hybrid complexes can be found in Bosnia and Herzegovina (BAŠIĆ, 2004). One of the most abundant species, with highly wide ecological amplitude, is *Crataegus monogyna* Jacq. Contrary, *C. microphylla* Koch subsp. *malyana* (CHRISTENSEN AND JANJIĆ, 2006) and *C. rhipidophylla* Gand. are the species which are recently included on the list of Flora of Bosnia and Herzegovina. According to JANJIĆ AND CHRISTENSEN (2006) *C.*

microphylla subsp. *malyana* is endemic to Bosnia and Herzegovina and it represents isolated enclaves at the end of its west areal. The fourth autochthonic species *C. laevigata* (Poiret) DC, which grows on meadows in the north, was not a subject of our investigations. All previously mentioned species are joined to ser. *Crataegus*, which comes inside sect. *Crataegus* by CHRISTENSEN (1992). Since interspecies breedings are frequent in hawthorns, in this study we also analysed and species *C. × subsphaericea* Gand., originated from *C. rhipidophylla* × *C. monogyna* (JANJIĆ, 2002)

Crataegus monogyna is traditional medicinal plant with many health benefits. The hawthorn fruits of this specie are considered to be generally safe and well tolerated in the treatment of cardiovascular diseases and angina pectoris of coronary heart (PRYOR ET AL., 1991; RIGELSKY AND SWEET, 2002). The extract is clinically effective in reducing blood pressure and total plasma cholesterol (HANACK AND BRUCKEL, 1983). Phenolic profiles and antioxidative effects of cell suspensions, fresh fruits and medicinal dried parts from common hawthorn has been investigated (BAHORUN ET AL., 1994; ZHANG ET AL., 2001). Preparations from hawthorn fruits are used for teas, dry and liquid extracts, tinctures and juices. The main biological active substances detected in the medicinal vegetal raw materials of common hawthorn are flavonoids and their glycosides, oligomeric procyanidins, catechines and phenolic acids (CUI ET AL., 2006; BAHORUN ET AL., 2003). The monograph on “hawthorn fruits”, which refers to *C. monogyna* and *C. laevigata*, is included in the European Pharmacopoeia. Many diseases such as cardiovascular diseases, diabetes mellitus, and cancers are related to the oxidative stresses, where many antioxidants (e.g. vitamins C and E, carotenes, phenolic acids) may terminate the attack of reactive oxygen species and reduce the risk of correlative diseases (PRYOR ET AL., 1991; LAMPE, 1999; GUO AND YANG, 2001). Recently, there has been a considerable interest in finding natural antioxidants to replace synthetic ones.

Vitamin C, also known as ascorbic acid (AA), is valuable food component because of its antioxidant and therapeutic properties. Biochemical functions are largely based upon the oxidation-reduction characteristics of the vitamin. It helps the body in forming connective tissues, teeth, and bones. Also it plays a major role as an antioxidant that forms part of the body defence system against reactive oxygen species and free radicals thereby preventing tissue damage (ENGLAND AND SEIFTER 1986.).

A wide variety of analytical techniques is available for the determination of ascorbic acid, including titrimetric analysis (AOAC, 2005), spectroscopy (ARAYA ET AL., 1998), chromatography (EIRENMILLER, 2008, BUSHWAY ET AL., 1998) and electroanalysis (OGUNLESI ET AL., 2010).

Presented spectrophotometric method is based on the measurement of decreasing of the absorption intensity of coloured methylene blue (MB^+) due to the reaction between vitamin C and MB, where MB is reduced to colourless leucomethylene blue (LMB^+) (MOWRY AND ORGEN, 1999). Measurements were carried out at absorption maximum, $\lambda_{max} = 665 \text{ nm}$.

Up to date, no analytical study of active compounds has been performed on the Bosnian *Crataegus* species.

The aims of this work were:

- to investigate content of vitamin C in fruits of selected *Crataegus* species collected from different localities on mountain Trebević in Sarajevo region;
- to investigate applicability of the spectrophotometric method based on the reaction between AA and MB in the determination of AA in the biological samples such are investigated samples of fruits.

The findings from this work will be helpful in understanding of possible medicinal use or food industry applications of these fruits.

MATERIALS AND METHODS – *Materijali i metode*

Plant material – *Biljni materijal*

Hawthorn fruit material was collected from natural populations on several localities near Sarajevo during September 2011 (Table 1). Identification and taxonomic determination of the analysed species was carried out with comparative-morphological analysis.

The fresh fruits were sorted out and dried in the drying room with ventilation at ambient temperature for 15 days. All voucher specimens are deposited in the, Faculty of Forestry, Department of Ecology, Botanic Laboratory Herbarium.

Table 1. Investigated *Crataegus* species and sampling localities

Tabela 1. Ispitivane vrste glogova i mjesta uzorkovanja

Number	Hawthorn species	Localities
1.	<i>C. monogyna</i>	Zlatište 1a
2.	<i>C. monogyna</i>	Zlatište 1b
3.	<i>C. monogyna</i>	Dobre vode
4.	<i>C. × subsphaericea</i>	Dobre vode
5.	<i>C. microphylla</i>	Dobre vode
6.	<i>C. rhpidophylla</i>	Brus

Reagents – *Reagensi*

L(+)-ascorbic acid; citric acid monohydrate; starch and methylene blue were purchased from Merck, acetic acid (glacial) and potassium iodide from Semikem, iodine resublimated from Zorka. All chemicals were of analytical grade.

Preparation of solution – *Priprema rastvora*

Iodine solution (0.005 mol L⁻¹). Potassium iodide (2.0 g) and iodine (1.3 g) were dissolved in 100 mL distilled water. This solution is diluted ten times. The concentration of prepared iodine solution was more accurately determined by titration with a standard solution of AA.

Starch indicator solution (0.5%). Soluble starch (0.25 g) was added to 50 mL of near boiling distilled water. It was stirred to dissolve and cooled before use.

Stock solution of ascorbic acid (0.1 molL^{-1}) was prepared by dissolving appropriate amount of ascorbic acid with distilled water.

Methylene blue solution (0.4 mmolL^{-1}) was prepared by dissolving appropriate amount of methylene blue in distilled water.

Preparation of samples – Priprema uzoraka

Six samples of selected *Crataegus* L. species were used for the determination of ascorbic acid. Fruits samples (5 g) were coarsely powdered and glacial acetic acid (2 mL) was added, then filtered, and the volume of the sample is made up to 100 mL with distilled water. The samples were analyzed with two methods, spectrophotometric and titrimetric in very short time after sample preparation.

Spectrophotometric determination AA – Spektrofotometrijsko određivanje AA

The spectrophotometric study was carried out by Perkin Elmer UV/VIS Spectrometer (Lambda 25) to determine the amounts of AA in the samples. Fifty microliters of a sample solution was mixed with 125 μL of MB ($c=0.4 \text{ mmolL}^{-1}$) solution and diluted up to 10 mL with distilled water. Decrease of absorption was measured at $\lambda_{\text{max}} = 665 \text{ nm}$. A linear relationship was obtained between the decreasing absorbance intensity and the concentration of AA in the concentration range of the analyte between 0.001 molL^{-1} and 0.05 molL^{-1} . All analysis were carried out in triplicates.

Titration conditions – Titracijski uslovi

The procedure for titration of AA was as follows: 10 mL of sample solutions, 50 mL of distilled water and 1 mL of 0.5% starch solution was titrated with iodine solution. All samples were analyzed in triplicate. Results are expressed in mg of ascorbic acid per 100 g of dry sample.

Influence of the MB, AA and HCl concentrations on the absorption intensity- Uticaj koncentracija MB, AA i HCl na intenzitet apsorpcije

Three sets of mixtures were prepared from stock solutions. In each set one specie was varied while concentrations of other two were kept fixed. Each component were mixed together and portion was transferred to the cuvette (1 cm) to record a change in absorbance intensity of methylene blue. The change in absorbance intensity of methylene blue was monitored by spectrophotometer ($\lambda_{\text{max}} = 665 \text{ nm}$) at room temperature.

Preparation of calibration curve –Priprema kalibracione krive

A stock solution of AA (0.1 molL^{-1}) was used for preparation of working solutions in concentration range: 0.001, 0.005, 0.01, 0.025 and 0.05 molL^{-1} .

Reaction mechanism – *Reakcioni mehanizam*

Methylene blue (MB^+) is a water soluble dye molecule. Under acidic conditions it can be easily reduced to colourless hydrogenated molecule leucomethylene blue (LMB^+) by ascorbic acid as it shown in Figure 1. The stoichiometry of the reaction was 1:1.

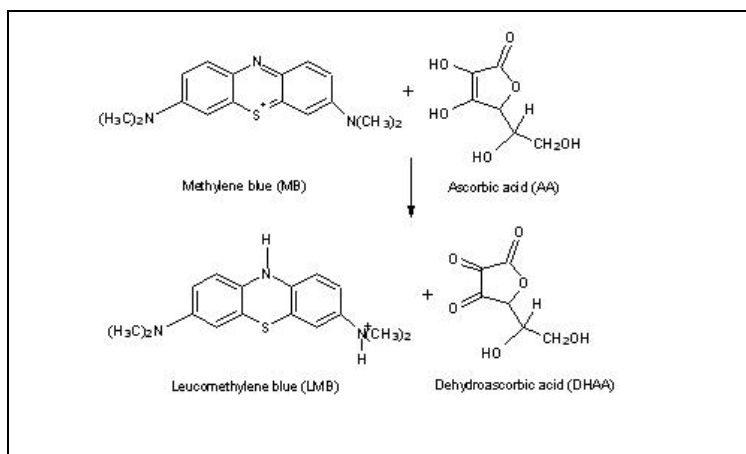


Figure 1. Reaction mechanism of MB with AA

Slika 1. Reakcioni mehanizam MB sa AA

RESULTS AND DISCUSSION – *Rezultati i diskusija*

Fruits and vegetables are the best sources of vitamin C in plants. The *Rosaceae* family of plants includes many well-know edible fruits reach in vitamin C. Relative abundance of vitamin C in some members of this family, given as mg of ascorbic acid per 100 g of fruits is: rose hips (2000), strawberry (60), raspberry (30), apricot (10), plum (10), peach and apple (7). Any fruits that have more than 30 mg of vitamin C per 100 g can be considered as an excellent source of vitamin C. Recommendations for vitamin C intake have been set by various national and international agencies. The UK Food Standards Agency and the World Health Organisation (WHO) recommend approximately 40-45 mg per day, while USA Dietary Reference Intake advocates higher amounts, about 90 mg per day for an adult male and 75 mg for an adult female (WHO/FAO, 2004).

An accurate and specific determination of the nutrient content of fruits is extremely important to understand relationships of nutrient intake and human health. For these reasons great caution should be exercised in the employment of the methods that have been developed for the analysis of specific plant tissue types (DAVEY ET AL. 2000).

Absorption maximum was investigated at different concentrations of methylene blue. Four concentrations of methylene blue were investigated. From the obtained results we can see that absorption maximum for all investigated

concentrations of methylene blue was at 665 nm (Figure 2). This value of λ_{\max} was selected as an optimal and used in further studies.

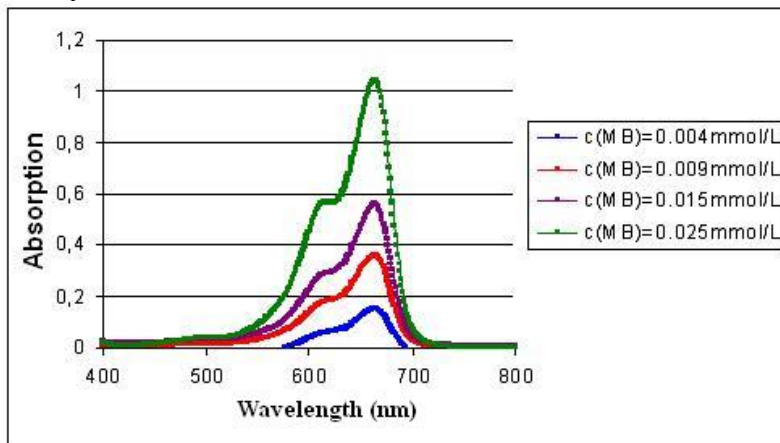


Figure 2. Absorption spectra for different concentrations of methylene blue

Slika 2. Apsorpcioni spektar različitih koncentracija metilenskog plavog

Influence of the MB, AA and HCl concentration on the absorption intensity ***Uticaj koncentracije MB, AA i HCl na intenzitet apsorpcije***

Reduction of methylene blue with ascorbic acid was studied with variable concentrations of MB, AA and HCl acid keeping one parameter varied and other constant. The colour-fading reaction was followed by measuring the absorbance of MB^+ complex ion over time at $\lambda_{\max}=665$ nm, and each curve can be obtained in only 1-10 min. It was observed how the rate law depends on MB^+ concentration, on ascorbic acid concentration, and on HCl concentration.

Stock solutions of MB (0.4 mmolL^{-1}), AA (0.5 molL^{-1}) and HCl acid (2.0 molL^{-1}) were prepared. Fifteen sample solutions were prepared from these stock solutions according to the defined conditions.

In the first set MB concentration was varied between 0.035 and 0.004 mmolL^{-1} in presence of HCl acid, $c=0.3 \text{ molL}^{-1}$ and AA, $c=0.025 \text{ molL}^{-1}$.

In second set AA concentration was varied between 0 to 0.036 molL^{-1} in presence of HCl acid, $c=0.2 \text{ molL}^{-1}$ and MB, $c=0.015 \text{ mmolL}^{-1}$.

In third set concentration of HCl acid was varied between 0 to 0.5 molL^{-1} in presence of AA, $c=0.045 \text{ molL}^{-1}$ and MB, $c=0.015 \text{ mmolL}^{-1}$.

In all three cases were followed absorbance decays under those conditions.

The change in absorbance of methylene blue at 665 nm plotted as a function of time is shown in Figure 3.

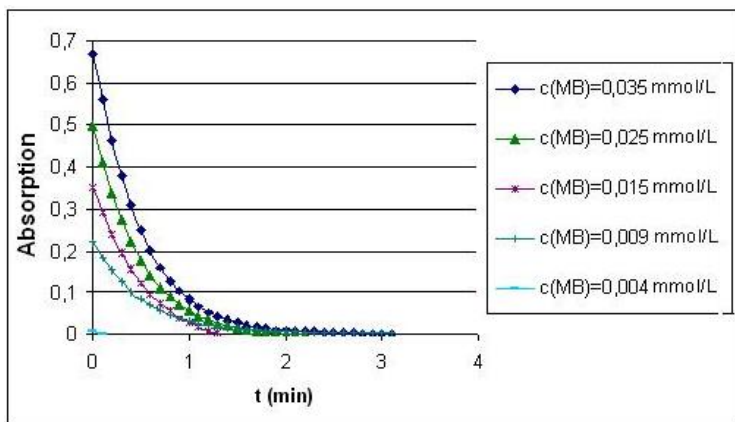


Figure 3. Absorbance decrease of methylene blue in time for investigated solutions (first set): $c(\text{HCl}) = 0.3 \text{ molL}^{-1}$; $c(\text{AA}) = 0.025 \text{ molL}^{-1}$; $c(\text{MB}) = 0.035 - 0.004 \text{ mmolL}^{-1}$.

Slika 3. Smanjenje apsorbance metilenskog plavog u vremenu u ispitivanim rastvorima (prvi set): $c(\text{HCl}) = 0.3 \text{ molL}^{-1}$; $c(\text{AA}) = 0.025 \text{ molL}^{-1}$; $c(\text{MB}) = 0.035 - 0.004 \text{ mmolL}^{-1}$.

From the obtained results, we noticed that under applied conditions, disintegration of methylene blue is finished after 1.3 minutes for the lowest concentration of MB, and after 3.3 minutes for the highest concentration of MB (Figure 3).

To determine the effect of ascorbic acid on the reaction rate, the concentrations of AA varied according to conditions described in second set. Figure 4. presents change in absorbance of methylene blue applied at fixed concentration of 0.015 mmolL^{-1} in the presence of different concentrations of ascorbic acid $c(\text{AA}) = 0 - 0.036 \text{ molL}^{-1}$ in acidic reaction medium $c(\text{HCl}) = 0.2 \text{ molL}^{-1}$.

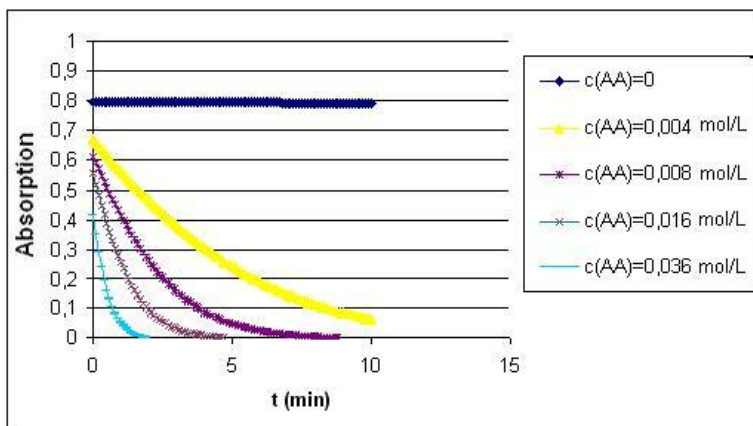


Figure 4 Absorbance decrease of methylene blue in time for investigated solutions (second set): $c(\text{MB}) = 0.015 \text{ mmolL}^{-1}$, $c(\text{HCl}) = 0.2 \text{ molL}^{-1}$; $c(\text{AA}) = 0 - 0.036 \text{ molL}^{-1}$.

Slika 4. Smanjenje apsorbance metilenskog plavog u vremenu u ispitivanim rastvorima (drugi set): $c(\text{MB}) = 0.015 \text{ mmolL}^{-1}$; $c(\text{HCl}) = 0.2 \text{ molL}^{-1}$; $c(\text{AA}) = 0 - 0.036 \text{ molL}^{-1}$.

We noticed that increasing of AA concentration in reaction medium causes faster decrease of absorbance. Reaction is slower at lower concentrations of the analyte (10 minutes), while with highest concentration of AA ($c = 0.036 \text{ molL}^{-1}$) absorbance reaches zero after 2.5 minutes. These data indicate that the reaction rate is highly dependent on the concentration of ascorbic acid present in reaction solution.

Decay of methylene blue under different acidity in solutions containing methylene blue in concentration of 0.015 mmolL^{-1} and ascorbic acid in concentration of $c = 0.045 \text{ molL}^{-1}$ was investigated. Concentrations of hydrochloric acid were varied from 0 to 0.5 molL^{-1} (Figure 5).

In Figure 5. it can be seen that MB^+ decay rates are much faster, if reaction is acid-catalyzed. Reaction time is about 10 minutes in absence of HCl acid. In the case that HCl acid is present, reaction time is increasing up to 0.5 minutes with increasing concentration of the acid up to 0.5 molL^{-1} . In our case it was crucial that MB reduction by AA goes slower, so for that reason, hydrochloric acid was omitted from the reaction solution.

Solutions of ascorbic acid in concentrations of 0.001 molL^{-1} up to 0.050 molL^{-1} were used for preparation of a calibration curve. Figure 6. presents absorbance decrease intensity of investigated solutions versus concentrations of AA standards. Linear correlation was obtained between absorbance and investigated AA concentrations with following parameters: $a = 0.2664$; $b = -1,139$, and correlation factor of $r^2 = 0.9983$.

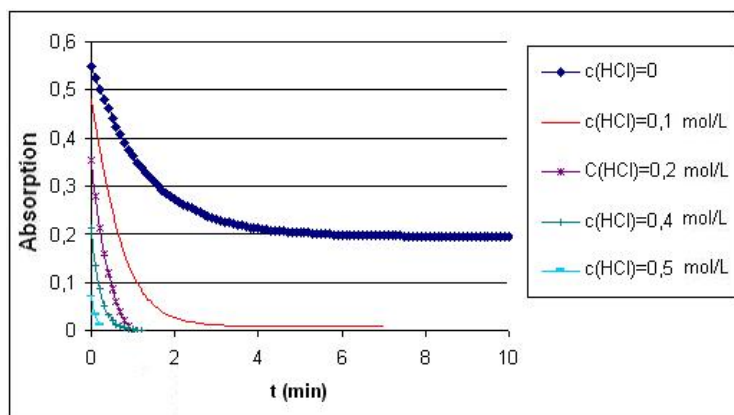


Figure 5 Absorbance decrease of methylene blue in time in investigated solutions (third set): $c(\text{MB}) = 0.015 \text{ mmolL}^{-1}$, $c(\text{HCl}) = 0 - 0.5 \text{ molL}^{-1}$; $c(\text{AA}) = 0.045 \text{ molL}^{-1}$.

Slika 5. Smanjenje apsorbance metilensko plavog u vremenu u ispitivanim rastvorima (treći set): $c(\text{MB}) = 0.015 \text{ mmolL}^{-1}$, $c(\text{HCl}) = 0 - 0.5 \text{ molL}^{-1}$; $c(\text{AA}) = 0.045 \text{ molL}^{-1}$.

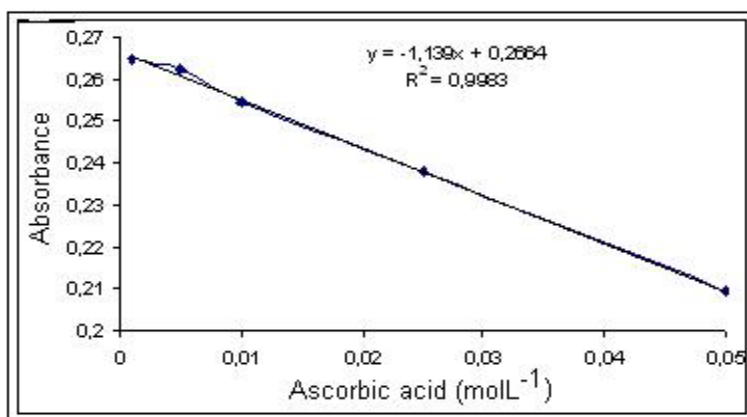


Figure 6. The standard curve of ascorbic acid
Slika 6. Standardna kriva askorbinske kiseline

Samples analysis – Analiza uzoraka

One of the main antioxidant substance mostly found in fruits and plants is ascorbic acid (ŠAVIKIN ET AL., 2009). Ascorbic acid is ubiquitous antioxidant present in animal and plant cells. It plays a key role in the detoxification of activated oxygen acting as an antioxidant either by reducing superoxide, hydrogen peroxide and hydroxyl radicals or by quenching singlet oxygen (FOYER ET AL., 1991).

Fruits of selected *Crataegus* species were investigated regarding their content of ascorbic acid. Contents of ascorbic acid were determined in the fruits by spectrophotometric and titrimetric methods. The second method was used as a reference method for comparison of the obtained results (Table 2). The results were expressed as mg ascorbic acid per 100 g of dry sample (DW).

The content of ascorbic acid ranged between 102.25 and 142.16 mg /100 g of dry sample for *C. monogyna* species collected from different localities (Table 1). Content of ascorbic acid in the fruits of *C. rhipidophylla* was 142.79 mg /100 g of sample which was very close to the content of ascorbic acid found in *C. monogyna* and their hybrid *C. × subsphaerice* fruits collected at the same site. Also we noticed that content of ascorbic acid in the hybrid is similar to content of AA in *C. rhipidophylla*. Only *C. microphylla* had much higher content of ascorbic acid (228.97 mg/100 g of dry sample) compared to all other investigated species.

Generally, the ascorbic acid content in fruits of *C. monogyna* are much higher than those found by some researchers e.g. EGEA ET AL.(2010). It is also demonstrated that among investigated species some of them, such as *C. microphylla*, have higher AA content than *C. monogyna*. To the best of our knowledge, there are no literature data concerning content of AA in fruits of *C. rhipidophylla* and *C. × subsphaerice* so that comparison of our results with the results other authors was not possible.

These finding on determination of ascorbic acid were consistent with the results obtained by titrimetric referent method. Since excellent results are obtained, they indicate that spectrophotometric method based on methylene blue can be used as an alternative to titrimetric method. This method is fast, simple and its advantage is that it does not require standardisation of the reagent. Recovery of the results obtained by spectrophotometric method was 101.4% - 108.0% with RSD values ranging from 0.40% to 3.37 %.

Table 2. Content of AA (mg/100 g) in fruits of six samples of *Crataegus* L. species determined by spectrophotometric and titrimetric methods. Results are expressed as mean \pm SD (n=3).

Tabela 2. Sadržaj AA (mg/100g) u voću šest uzoraka glogova određene sa spektrofotometrijskom i titrimetrijskom metodom.. Rezultati su izraženi kao srednja vrijednost \pm SD (n=3).

	Samples and sites	Spectrophotometric method (mg/100g DW)	Titrimetric method (mg/100g DW)
1.	<i>C. monogyna</i> (Zlatište 1b)	106.12 \pm 2.87	102.25 \pm 1.48
2.	<i>C. monogyna</i> (Zlatište 1a)	114.38 \pm 2.44	104.95 \pm 1.50
3.	<i>C. \times subsphaerice</i> (Dobre vode)	138.04 \pm 0.57	135.79 \pm 1.75
4.	<i>C. monogyna</i> (Dobre vode)	144.39 \pm 4.87	142.16 \pm 1.26
5.	<i>C. rhipidophylla</i> (Brus)	144.81 \pm 1.38	142.79 \pm 0.79
6.	<i>C. microphylla</i> (Dobre vode)	231.96 \pm 3.41	228.97 \pm 1.87

CONCLUSIONS - Zaključci

- The proposed method provides a simple and sensitive spectrophotometric procedure by using MB for the determination of AA.
- Results showed good agreement between spectrophotometric and titrimetric methods.
- This investigation shows the potential value of *Crataegus* L. species as a good source of natural antioxidant vitamin C.
- According to our best knowledge, this is the first time that content of ascorbic acid was investigated in some Bosnian *Crataegus* species.
- Also we gave first data for content of ascorbic acid for fruits of *Crataegus rhipidophylla*, *C. \times subsphaerice* and *C. microphylla* of Bosnian species.
- The lowest content of vitamin C was found in *C. monogyna*, while the highest content of vitamin C was found in *C. microphylla*.
- All investigated hawthorn species had higher content of vitamin C than the medicinal species *C. monogyna*. These results suggest that investigated hawthorn species are good source of vitamin C with the potential of application in pharmaceutical and food industry as the natural antioxidant.

- Some further investigations can be focused on antioxidant activities in correlation to chemical composition of the fruits such as: phenolic acid and procyanidins in order to estimate some possible medicinal use of the investigated species.

REFERENCES – *Literatura*

- AOAC International, official methods of analysis, 18th ed., AOAC International, Arlington, VA, 2005.
- ARAYA, S. P., MAHAJN, M., JAIN, P. (1998): Photometric methods for the determination of vitamin C. *Analytical Sciences*, 14: 889-894.
- BAHORUN, T., TROTIN, F., POMMERY, J., VASSER, J., PINKAS, M. (1994): Antioxidant activities of *Crataegus monogyna* Extracts. *Planta Medica*, 60: 323-328.
- BAHORUN, T., AUMJAND, E., RAMPHUL, H., RYEHA, M., LUXIMON-RAMMA, A., TROTIN, F. (2003): Phenolic constituents and antioxidant capacities of *Crataegus monogyna* (Hawthorn) callus extracts. *Nahrung*, 47 (3): 191-8.
- BAŠIĆ, N. (2004): Morfološko-taksonomska istraživanja glogova (*Crataegus* L.) na području Bosne i Hercegovine. Magistarski rad. Sarajevo.
- BECK, G. (1927): Flora Bosne, Hercegovine i oblasti Novoga Pazara. III . Choripetalae (Kaj): 169-172. Beograd-Sarajevo.
- BUSHWAY, R. J., KING, J. M., PERKINS, B. M., KRISHNAN, M. (1988) High-performance liquid chromatographic determination of ascorbic acid in fruits, vegetables and juices. *Journal of Liquid Chromatography* 11: 3415 - 3423.
- CHRISTENSEN, K. I. (1992): Revision of *Crataegus* Sect. *Crataegus* and Notosect. *Crataegineae* (Rosaceae-Maloideae) in the old world. *Systematic Botany Monographs*, 35: 1-199.
- CHRISTENSEN, K. I., JANJIĆ, N. (2006): Taxonomic notes on European taxa of *Crataegus* (Rosaceae). *Nordic Journal of Botany*, 24: 143-147.
- CUI, T., NAKAMURA, K., TIAN, S., KAYAHARA, H., TIAN, Y. L. (2006): Polyphenolic content and physiological activities of Chinese hawthorn extracts. *Bioscience Biotechnology and Biochemistry*, 70 (12): 2948-56.
- DAVEY, M. W., VAN MONTAGU, M., INZÉ, D., SANMARTIN, M., KANELIS, A., SMIRNOFF, N., BENZIE, I. J. J., STRAIN, J. J., FAVELL, D., FLETCHER, J. (2000): Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture*, 80, 825-860.
- EGEA, I., SANCHEZ-BEL, P., RAMOJARO, F., PRETEL, M. T. (2010): Six edible wild fruits as potential antioxidant additives or nutritional supplements. *Plant Foods for Human Nutrition*, 65:121-129.
- EITENMILLER, R. R., YE L., LANDEN, W. O. (2008): Vitamin analysis for the health and food sciences. CRC Press, Taylor & Francis Group, UK, 231-274.

- ENGLAND, S., SEIFTER, S. (1986): The biochemical functions of ascorbic acid. Annual Review of Nutrition, 6: 365-406.
- WHO/FAO (2004): Vitamin and mineral requirements in human nutrition. Second edition, World Health Organisation and Food and Agricultural Organisation of the United Nations, Sung-Fung, China, Chapter 7, 130-139.
- FUKAREK, P. (1974): Neke vrste drveća i grmlja koje su pogrešno navedene u Flori Bosne i Hercegovine i susjednih krajeva. ANU BiH-Radovi LIV, Odjeljenje prirodno-matematičkih nauka, 15: 45-60.
- FOYER, C.H., LELANDIAS, M., EDWARDS, E.A., MULLINEAUX, P. M. (1991): Current topics in plant physiology, 6, ASPP, Rockville, 131.
- GUO, C. J. AND YANG, J. J. (2001): Progress in the study of antioxidant capacity of fruits and vegetables. China Public Health, 17: 87-88.
- HANACK, T., BRUCKEL, M. H. (1983): The treatment of mild stable forms of angina pectoris using crategutt novo. Therapiewoche, 33: 4331-4333.
- JANJIĆ, N. (1998): Neki zanimljivi dendrološki nalazi iz sarajevskog područja. Radovi Šumarskog Fakulteta Univerziteta u Sarajevu, 28(1): 85-103.
- JANJIĆ, N. (2002): Nova kombinacija u lepezolisanog ili krivočašičnog gloga, *Crataegus rhipidophylla* Gand. (Rosaceae). Radovi Šumarskog Fakulteta Univerziteta u Sarajevu, 32(1): 1-7.
- LAMPE, J. W. (1999): Health effects of vegetables and fruits: Assessing mechanisms of action in human experimental studies. American Journal of Clinical Nutrition, 70: 475S-490S.
- MALÝ, K. (1919): Prilozi za floru Bosne i Hercegovine 5 i 6. Glasnik Zemaljskog Muzeja BiH, Sarajevo, 31: 61-92.
- MALÝ, K. (1940): Notizen zur Flora von Bosnien-Herzegovina. Glasnik Zemaljskog Muzeja BiH, Sarajevo, 52: 21-46.
- MOWRY, S., ORGEN, P. J. (1999): Kinetics of methylene blue reduction by ascorbic acid, Journal of Chemical Education, 76(7): 970-974.
- OGULNESI, M., OKIEI, W., AZEEZ, L., OBAKACHI, V., OSUNSANMI, M., NKENCHOR, G. (2010): Vitamin C contents of tropical vegetables and foods determined by voltammetric and titrimetric methods and their relevance to the medicinal uses of the plants. International Journal of Electrochemical Science, 5: 105-115.
- PRYOR, W. A., DAS, B., CHURCH, D. F. (1991): The ozonation of unsaturated fatty acids: aldehydes and hydrogen peroxide as products and possible mediators of ozone toxicity", Chemical Research in Toxicology, 4: 341-348.
- RIGELSKY, J. M., SWEET, B. V. (2002): Hawthorn: pharmacology and therapeutic uses. American Journal of Health and Systematic Pharmacology, 59: 417-422.
- ŠAVIKIN, K., ZDINIĆ, G., JANKOVIĆ, T., TASIĆ, S., MENKOVIĆ, N., STEVIĆ, T., ĐORĐEVIĆ, B. (2009): Phenolic content and radical scavenging capacity of berries and related

jams from certificated area in Serbia. Plant Foods for Human Nutrition, 64: 212-217.

ZHANG, Z., CHANG, Q., ZHU, M., HUANG, Y., HO, W. K., CHEN, Z. (2001): Characterization of antioxidants present in hawthorn fruits. The Journal of Nutritional Biochemistry, 12: 144-152.

SAŽETAK

U ovoj studiji su prikazani rezultati određivanja vitamina C u plodovima odabranih bosanskih vrsta glogova (*Crataegus* L). Određivanje vitamina C je vršeno primjenom spektrofotometrijske metode, zasnovane na kinetičkoj reakciji askorbinske kiseline (AA) sa metilenskim plavim (MB⁺). Mjerenja intenziteta apsorpcije su vršena na apsorpcionom maksimumu, $\lambda_{\max} = 665$ nm. U studiji je korištena i poredbeni titracijska metoda analize sadržaja vitamina C u cilju komparacije i evaluacije dobivenih rezultata. Sadržaj vitamina C u ispitivanim uzorcima plodova različitih vrsta glogova se kretao u rasponu 106,12 – 231,96 mg vitamina C na 100 g suhog uzorka ploda. Dobivene vrijednosti iskorištenja (recovery) za spektrofotometrijsku metodu, su se kretale od 101,4% do 108,0%, dok je vrijednost relativne standardne devijacije (RSD) iznosila 0,40 % - 3,37 %. Iz dobivenih rezultata određivanja sadržaja vitamina C u ispitivanim uzorcima plodova odabranih vrsta glogova može se uočiti da su sadržaji vitamina C u svim ispitivanim uzorcima znatno visoki. Vrste *C. rhipidophylla*, *C. microphylla* i *C. × subsphaerice*, koje nisu uvrštene u oficijelnu Evropsku farmakopeju, imaju čak i veće vrijednosti vitamina C u odnosu na priznatu ljekovitu vrstu, *C. monogyna*. Najveći sadržaj vitamina C je određen u plodu endemične vrste, *C. microphylla* i u odnosu na druge ispitivane vrste je veći za približno dva puta.

Dobiveni rezultati jasno ukazuju da su plodovi ispitivanih vrsta glogova dobar izvor vitamina C kao prirodnog antioksidanta.