FACTA UNIVERSITATIS
Series: **Physics, Chemistry and Technology** Vol. 20, Nº 1, 2022, pp. 79 - 86
https://doi.org/10.2298/FUPCT2201079M

Original scientific paper

ROSA CANINA L. FRUIT AND JAM MADE OF IT – NATURAL FOOD COLORS E160A AND E160D CONTENT AND ANTIOXIDANT CAPACITY

UDC 547.97 : 582.734.4

Vojkan Miljković[1], Milica Nešić[2], Jelena Mrmošanin[2], Ivana Gajić[1], Bojana Miladinović[3], Dušica Stojanović[4]

[1]University of Niš, Faculty of Technology, Leskovac, Serbia
[2]University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Niš, Serbia
[3]University of Niš, Faculty of Medicine, Department of Pharmacy, Niš, Serbia
[4]University of Niš, Faculty of Medicine, Institute of Public Health, Niš, Serbia

Abstract. *Rosehip is one of the richest sources of vitamin C, and the jam made from it tastes delicious. In this work, the content of food colors E160a (lycopene) and E160d (β-carotene) was determined, as well as the antioxidant capacity of these food products. By applying the UV-Vis spectrophotometric method and using the Lambert-Beer law, a system of two linear equations with two unknowns was set up, which was used to determine the concentrations for the colors E160a and E160d. The content of lycopene and β-carotene in dried rosehip fruit and jam was 2.34 and 0.85, and 7.25 and 2.01 mg in 100 g of fruit/jam, respectively. The antioxidant capacity of the samples was determined by the DPPH test. The antioxidant capacity of fruit and rosehip jam was 6.84 and 4.17 μmol of Trolox/100 g of fresh sample. Thermal processing affects the content of lycopene, β-carotene, and antioxidant capacity. The obtained results reveal beneficial effects of the everyday consumption of dried rosehip fruit and jam.*

Key words: *E160a (β-carotene), E160d (lycopene), cynosbati fructus, jam, antioxidative capacity, DPPH assay*

1. INTRODUCTION

Rosehip (*Rosa canina* L.) belongs to the Rosaceae family. It is also known under names such as dog brier, wild briar, witch's briar, hip fruit, and hip tree. There are 70 different species of the genus *Rosa* known in the world, with 47 growing in Europe

Received: December, 23rd, 2022; accepted: December, 27th, 2022
Corresponding author: Vojkan Miljković, Faculty of Technology, Bulevar Oslobođenja 124, 16000 Leskovac, Serbia; e-mail: vojkan@tf.ni.ac.rs

(Davis, 1972; Tutin et al., 1992), and also in Western Asia, North Africa, the Middle East, and North America (Nilsson, 1997). The plant is a perennial shrub 2-3 m in height, with elongated stems and curved or arched branches, and has pink, red, or white flowers (Ercisli, 2005). Fruit ripens in August-September (Turkben et al., 2005). The color of the fruit (Cynosbati fructus) is red to brick-red, to a deep red. The fruit has small hairy achenes inside of it (Iancu et al., 2020). It weighs between 1.25-3.25 g, of which 71% belongs to the pericarp and 29% to seeds (Zahara et al., 2020).

Cynosbati fructus is considered an important source of food and medicinal plant in many different nations. It has found usage in the preparation of: juices, teas, wine (high phenolic content) (Razungles et a., 1989), jam, jelly, mixture with dried salmon eggs (Moerman, 2002), desserts, soups, marmalade, purees, dried rose hip fruits (Turkben et al., 2010). The powdered form is available in Denmark and it is made from dried fruits, and ready-made soups are made in Sweden (Gaik, 2011). This fruit has also been used for food and pharmaceutical purposes, yogurts, and health supplementation. It is in focus in recent years because of confirmed health benefits (Chrubasik et al., 2008; Larsen et al., 2003).

The fruits of wild briar are effective in the treatment of arthritis, inflammation, diabetes, heart ailments, pathogens, and gastric ulcers (Iancu et al., 2020) and have anti-inflammatory and anti-mutagen effects (Kilicgun and Altiner, 2009). Success in the treatment of osteoarthritis has been clinically confirmed (Christensen et al. 2008; Chrubasik et al., 2006, 2008).

Natural orange food color is marked as E160a and consists of α-, β- and γ-carotene mixture. When the term "carotene" is used it means a natural mixture of alpha, beta and gamma carotene. It is most often obtained by chemical extraction from various plants and contains about 85% of β-carotene, about 15% of α-carotene and 0.1% of γ-carotene (Meléndez-Martínez et al., 2022). Differing from plants and microorganisms that can synthesize these compounds, humans, and other animals cannot (Eggersdorfer and Wyss, 2018).

In plants, carotene biosynthesis is important for growth and development; they play a role in photosynthesis, preventing photo-oxidative damage to plants, and serve as precursors of phytohormones. Carotenes in the body are converted into vitamin A. The reference daily intake (RDI) for E160a is 5.0 mg per kg body weight (European Food Safety Authority (EFSA), 2012.).

E160d (lycopene) belongs to the carotenoid group and it is a natural red-orange dye. It is mostly found in tomatoes, pomegranates, watermelons, and red grapefruit. Lycopene has the ability to inactivate free radicals that cause oxidative damage to lipids, proteins, and DNA in living cells. Studies also show that lycopene reduces the risk of lung, stomach, and prostate cancer, breast and colon, ovarian, endometrial, and pancreatic and bladder cancers, as well as cardiovascular disease. The risk of prostate cancer is reduced by almost 10-20% by consuming about 6 mg of lycopene per day. The absorption of lycopene from processed tomato products is incomparably better because, in raw tomatoes, lycopene is bound to other compounds and is more difficult to separate from them. The content of these valuable compounds is lowered after thermal processing, i.e. tomato and tomato juice (Miljković et al., 2021).

Functional foods attracted the interest of scientists because they are able to protect human health from diseases whose origin is oxidative stress in cells (Salminen et al., 2005). Cynosbati fructus is classified as a functional food because of reported results in promoting health as a result of the lutein presence, lycopene, and β-carotene (Fan et al.,

2014). As an antioxidant, lycopene is able to fight carcinogenesis (Khachik et al., 1995). Also, it stops LDL cholesterol oxidation and prevents oxidative damage to DNA (Gerster, 1997). This work aims to determine the content of lycopene, β-carotene, and antioxidant capacity in dried cynosbati fructus and the jam made of it.

2. MATERIALS AND METHODS

For this experimental research fresh cynosbati fructus bought from the local market in Niš, Serbia was used. The fruits were washed and left to dry in the shade. After that, dried fruits were sliced and homogenized in a Brown® blender. The jam was produced from dried cynosbati fructus. Fruits were put in water and heated until boiling started. Continuous stirring was performed. During the preparation process, the addition of water was done. One-half kg of sugar was added per 1 kg of cynosbati fructus and the mixture was cooked at a temperature of 220 °C for 150 minutes. The cooking was stopped when the jam got a thick consistency. After the cooking was done, it was packed into a jar and kept in the dark.

Acetone was obtained from Fisher Scientific (Loughborough, United Kingdom). Hexane, butylated hydroxytoluene, sodium acetate, ethanol, glacial acetic acid, 2,2-diphenyl-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Steinheim, Germany).

2.1. Determination of E160a (β-carotene) and E160d (lycopene) content

Herein conventional solvent extraction methods were applied (Perkins-Veazie et al., 2001; Sadler et al.,1990). Specifically, 10 g of each sample (dried rosehip fruit - sample 1 and jam made of it - sample 2) were mixed with a solution consisting of 250 ml hexane, 125 ml of acetone, 125 ml of ethanol (2:1:1, v/v/v), and 0.05 % (w/v) butylated hydroxytoluene (BHT). The stoppered mixture was placed on an orbital shaker to mix at 180 rpm for 150 minutes (the temperature of mixing was 5 °C). After mixing, 75 ml of cold deionized water was added and the mixture was agitated for another 5 min. In order to allow the separation of polar and non-polar layers, the suspension was left at room temperature for 10 minutes. The extract was re-dissolved in hexane. Then, the hexane extracts were scanned in the visible light wavelength range of 400-750 nm using Jenway 6105 UV/Vis spectrophotometer (Jenway, United Kingdom) in 1 cm path length quartz cuvette blanked with n-hexane and the maximum absorbances were observed at 450, 472, and 503 nm, respectively for the lycopene/β-carotene hexane layer mixture. The molar extinction coefficient of 172 000 L mol^{-1} cm^{-1} at 503 nm was used to estimate E160d (lycopene) concentration, using the Beer-Lambert law (Ravelo-Perez et al., 2008; Zechmeister and Polgar, 1943).

2.2. Determination of antioxidant capacity - DPPH assay

The total antioxidant capacity of samples 1 and 2 was determined by applying DPPH modified method as described by Kaneda et al. (1995). For that purpose, approximately 10 g of samples 1 and 2 were dissolved in 30 ml of ethanol solution (70 %, v/v). The samples were then mixed for 10 minutes at 5 °C and after that centrifuged at 9000 rpm for 10 minutes. Then the supernatant was poured off and the pulp was reextracted with 15

ml of ethanol solution by the same procedure. The supernatants obtained were then combined and the total volume was adjusted with 70% (v/v) ethanol solution to 50 ml. Each sample of extracts (0.2 ml) was added to the DPPH solution (2.8 ml) (mixture of $1.86x10^{-4}$ mol/L DPPH in ethanol and 0.1 M acetate buffer (pH 4.3) in ration 2:1) and mixed vigorously. After 60 minutes of incubation in a dark place, the absorbance was measured at 525 nm. The standard curve was constructed and the results were expressed as μmol per 100 g of fruit/jam weight.

2.3. Statistical analysis

All measurements were conducted in triplicate and data was expressed as mean ± standard deviation. The significance of differences among means was tested using t-test for independent samples.

3. Results and Discussion

Figure 1 shows typical overlap spectra of the hexane extract of the E160a/E160d β-carotene-lycopene mixture in samples 1 and 2 with absorption maxima at 450 nm, 472 nm, and 503 nm. It is known that carotenoids exhibit absorbance maxima at three wavelengths in a three-peak spectrum and that when the number of conjugated double bonds increases, the λ_{max} shifts to longer wavelengths. Therefore, it is not surprising that lycopene (E160d), which possesses 11 conjugated double bonds and is red-colored with absorption maximums (λ_{max} at 443, 471, 503 nm) (Rodriquez-Amaya and Kimura, 2004). The cyclization results in steric hindrance between the methyl group at C-5 of the ring and the hydrogen atom at C-8 of the polyene chain. This process takes the n electrons of the ring double bond out of plane with respect to those of the chain, and is resulting in a hypsochromic effect (displacement of λ_{max} to shorter wavelength), a hypochromic effect (decrease in absorbance), and loss of fine structure (spectrum with less defined peaks).

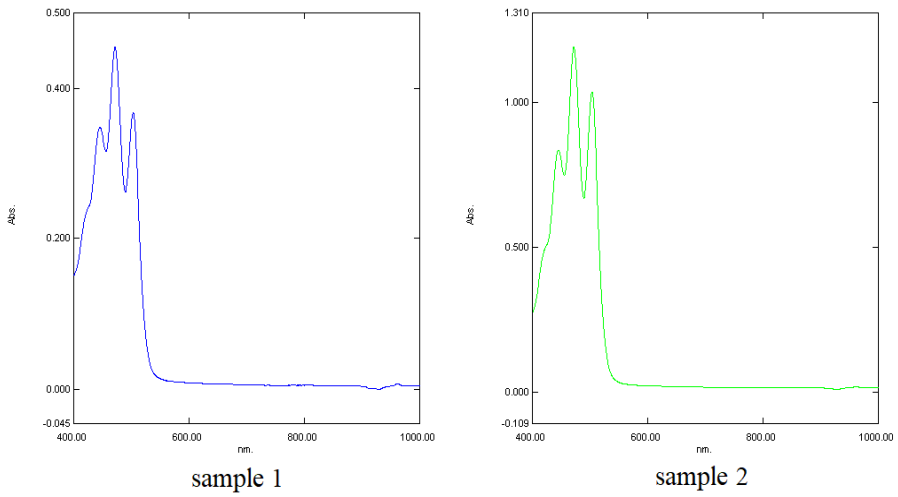

Fig. 1 UV/Vis spectrum of the hexane extract of the E160a/E160d β-carotene-lycopene mixture)

However, the molecule of β-carotene is yellow-orange colored and despite possessing the same number of conjugated double bonds as lycopene, shows absorption peaks at 450 and 472 nm and a shoulder at 425 nm (Rodriguez-Amaya and Kimura, 2004). The explanation for this is that both carotenoids absorb greatly in the overlapping wavelength ranges. The choice of 503 nm to quantify lycopene is acceptable regardless of the fact that this wavelength value is not equal to λ_{max} (Ravelo-Perez et al., 2008).

A final calculation of the lycopene content was done using the following equation:

$$A= \varepsilon \cdot b \cdot c \quad (1)$$

where (ε) is the reported molar extinction coefficient of 17.2 x 104 M/cm (Zechmeister and Polgar, 1943), b is a 1 cm path-length glass cuvette and c is the concentration of lycopene. The results are shown in Table 1 (see Fig 2, left).

Table 1 The content of lycopene and β-carotene content as determined by UV/Vis spectrophotometry

Compound	*Sample*	
	Dried cynosbati fructus	Jam
Lycopene (mg/100 g FW)	2.34 ± 0.06 (RSD = 2.35%)	0.85 ± 0.04 (RSD = 2.4%)
β-Carotene (mg/100 g FW)	7.25 ± 0.09 (RSD = 7.04%)	2.01 ± 0.08 (RSD = 8.6%)

It was calculated that the content of lycopene in dried cynosbati fructus and jam was 2.34 mg/100 g FW and 0.85 mg/100 g FW, respectively. The content of lycopene in fruit is 2.75 and β-carotene 3.6 times higher than in its processed product. The reason for such a difference could be in difference in composition. Namely, dried fruit had seeds in it, and for the jam preparation, the seeds were removed. Also, cooking at high temperatures and the addition of sugar in the preparation of jam could affect the lycopene content (Uylaser, 2000). The visual comparison of the result of lycopene and β-carotene can be seen in figure 2 (left).

Principally, the content of lycopene and β-carotene is affected by different varietal factors such as agronomic factors (Martinez-Valverde et al., 2002), climatic conditions, and geographical location (Kotíková et al., 2011). Right correlation between all factors and imperative for healthy food.

For comparison, in rosehip marmalade prepared with sugar, the lycopene content was 6.6 mg/100 g, and 1.33 mg/100 g in the sugar-free jam (Turkben et al., 2010). In the fresh rosehips, lycopene content was calculated to be in the range of 12.9-35.2 mg/100 g FW, and 2.3-5.2 mg/100 g FW in products made from it.

The chemical composition of *Rosa canina* fruit varies depending on the cultivar, cultivating region, climate differences, and maturity (Ercisli, 2007). Lower lycopene content in products made by cooking is not unexpected. The carotenoids, where lycopene belongs, are sensitive to light exposure, SO_3^{2-}, metal ions, changes in pH, high temperatures, and oxygen exposure during food processing. When sources with lycopene content are exposed to higher temperatures for a recent time, degradation and decolorization happen (Uylaser, 2000). Lower lycopene content in the jam and marmalades is the result of oxidative degradation during processing (Turkben et al., 2010).

Razungles et al. compared lycopene content in *Rosa canina* and other berries. The results of their research showed that lycopene is present in an amount of 11.12 mg/100 g

of deseeded fruit *Rosa canina*, in *Rosa rugosa* Thunb. 4.34 mg/100 g and in *Aronia melanocarpa* (Michx.) Britton 0.6 mg/100 g (Razungles et al., 1989).

The antioxidant capacity of the tested samples was determined using the spectrophotometric method – DPPH assay. The results are expressed as μmol per 100 g of fruit (jam) weight (FW) and the mean value of the three testing samples (C_{sr} ± SD, n = 3). They are shown in Table 2, and a comparison can be seen in Fig. 2, right.

Table 2 Comparison of antioxidant activity values of tested cynosbati fructus (1) and jam (2) samples by using DPPH assay

Sample	DPPH C_{sr} ± SD (μmol/100g FW)	RSD (%)
1	6.84 ± 0.03	0.42
2	4.17 ± 0.09	2.08

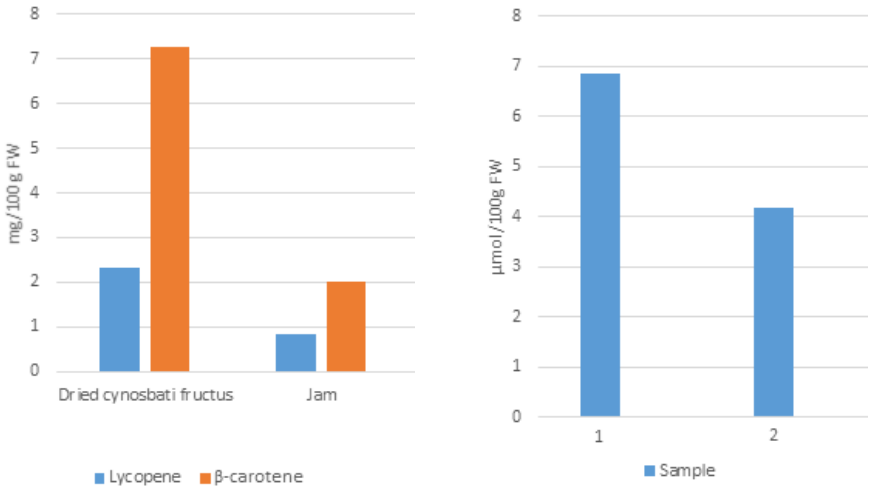

Fig. 2 The comparison char for E160a and E160 content in dried cynosbati fructus and jam made of it (left); Antioxidative capacity of samples (1) and (2) (right)

4. CONCLUSION

Determination of lycopene content in dried cynosbati fructus and jam was performed. *Rosa canina* fruit is a valuable source of lycopene and β-carotene. They are a good source of lycopene and β-carotene with 2.75- and 3.6-times higher amounts in cynosbati fructus than in the jam. The spectrophotometric method is an appropriate method for lycopene determination and provides accurate results. The results of DPPH assay showed that the dried cynosbati fructus possesses higher antioxidant capacity in comparison to the jam made of it. Food processing at high temperatures affects the content of lycopene, β-carotene, and antioxidant capacity. The results obtained in this work are recommending this as a supplement to a regular diet.

Acknowledgement: *The research leading to these results has received funding from the Republic of Serbia - Ministry of Education, Science and Technological Development, Program for Financing Scientific Research Work, no. 451-03-68/2022-14/200133.*

REFERENCES

Christensen, R., Bartels, E.M., Altman, R.D., Astrup, A., Bliddal, H., 2008. Osteoarthr. Cartil., 16**,** 965–72. doi:10.1016/j.joca.2008.03.001

Chrubasik, C., Duke, R.K., Chrubasik, S., 2006. Phytother. Res., 20, 1–3. doi:10.1002/ptr.1729

Chrubasik, C., Roufogalis, B.D., Muller-Ladner, U., Chrubasik, S., 2008. Phytother. Res., 22, 725–733. doi: 10.1002/ptr.2400

Davis, P.H., 1972. Flora of Turkey and East Aegean Islands (vol 4). University Press, Edinburg, pp. 106–128.

Ercisli, S., 2005. Genet. Resour. Crop. Evol., 52, 787–795. doi:10.1007/s10722-003-3467-8

European Food Safety Authority (EFSA), P. I., 2012. Scientific Opinion on the re-evaluation of mixed carotenes (E 160a (i)) and beta-carotene (E 160a (ii)) as a food additive. *EFSA Journal*, p. 1. doi:10.2903/j.efsa.2012.2593

Fan, C., Pacier, C., Martirosyan, D.M., 2014. Funct. Foods Health Dis.**,** 4, 493–509. doi:10.31989/ffhd.v4i12.159

Gaik, M. K., 2011. "Bioactive plant foods," Ph.D thesis, Aarhus University.

Gerster, H. 1997. J. Am. Coll. Nutr. 16, 109–26. doi:10.1080/07315724.1997.10718661

Iancu,P., Soare, R., Dinu, M., Soare, M., Dorina Bonea, D., Popescu, M., 2020. Sci. Papers. Ser. B, Hortic., LXIV, 325–331. Print ISSN 2285-5653, 325-332.

Kaneda, H., Kobayashi, N., Furusho, S., Sahara, S., Koshino, S., 1995. Tech. q. - Master Brew. Assoc., 32, 90–94.

Khachik, F., Beecher, G.R., Smith, J.C., 1995. J. Cell. Biochem. Suppl., 22, 236–46. doi:10.1002/jcb.240590830

Kilicgun, H., Altiner, D., 2009. Pharmacogn. Res., 1, 417–20. doi:10.4103/0974-8490.58034

Kotíková, Z., Lachman, J., Hejtmánková, A., Hejtmánková, K., 2011. LWT - Food Sci. Technol., 44, 1703-1710. doi:10.1016/j.lwt.2011.03.015

Larsen, E., Kharazmi, A., Christensen, L.P., Christensen, S.B.J., 2003. Nat. Prod., 66, 994–995. doi: 10.1021/np0300636

Martinez-Valverde, I., Periago, J.M., Provan, G., Chesson, A., 2002. J. Sci. Food Agric., 82, 323-330. doi:10.1002/jsfa.1035

Meléndez-Martínez, J.A., Mandić, I.A., Bantis, F., Böhm, V., Borge, A.G.I., Brnčić, M., Bysted, A., Eggersdorfer, M., Wyss, A., 2018. Arch. Biochem. Biophys., 652, 18-26. doi:10.1016/j.abb.2018.06.001

Ercisli, S., 2007. Food Chem., 104, 1379–84. doi:10.1016/j.foodchem.2007.01.053

Miljković, V., Nešić. M., Gajić, I., Urošević, M., Mrmošanin, J., Miljković, M., 2021. FU Phys. Chem. Tech., 19, 91–98. doi: 10.2298/FUPCT2102091M

Moerman, D. E., 2002. Native American ethnobotany, Timber Press, Portland, pp. 482–486.

Nilsson O., 1997. "*Rosa* L.," in P.H. Davis (Ed.), Flora of Turkey and the East Aegean Islands. Edinburgh University Press, Edinburgh, pp. 106–128.

Perkins-Veazie, P., Collins, J.K., Pair, S.D., Roberts, W., 2001. J. Sci. Food Agric., 81, 983–987. doi:10.1002/jsfa.880

Ravelo-Perez, L.M., Hernandez-Borges, J., Rodriguez-Delgado, M.A., Borges-Miquel, T., 2008. Chem. Educat., 13, 11–13.

Razungles, A., Ozszmianski, J., Sapis, J. –C., 1989. J. Food Sci., 54, 774–775. doi:10.1111/j.1365-2621.1989.tb04709.x

Rodrigues-Aamaya, D.B., Kimura, M., 2004. HarvestiPlus Handbook for Carotenoid Analisys, HarvestiPlus Technical Monograph Series, 2, Washington, DC USA, pp 63.

Sadler, G., Davis, J., Dezman, D., 1990. J. Food Sci., 55, 1460–1461. doi:10.1111/j.1365-2621.1990.tb03958.x

Salminen, J.K., Karonen, M., Lempa, K., Liimatainen, J., Sinkkonen, J., Lukkarinen, M., Pihlaja, K., 2005., J. Chromatogr. A., 1077**,** 170–80. doi:10.1016/j.chroma.2005.04.073

Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Valters, S.M., Webb D. A., 1992. Flora Europaea, Rosaceae to Umbelliferae (vol 2). University Press, Cambridge, pp. 469.

Turkben, C., Barut, E., Copur, O.U., Durgut, E., Himelrick, D.G., 2005. Int. J. Fruit Sci., 5, 113–121. doi:10.1300/J492v05n02_11

Turkben, C., Uylaser, V., Incedayi, B., 2010. Chem. Asian J., 22, 2309–2318.

Uylaser, V., 2000. Dünya Gıda Dergisi, 5, 79–84

Zahara, K., Bibi, Y., Riaz, I., Sardar, N., Sadaf, H.M., Bibi, F., 2020. J. Herbs Spices Med. Plants, 24, 92–96.

Zechmeister, L., Polgar, A., 1943. J. Am. Chem. Soc., 65, 1522 –1528. doi:10.1021/ja01248a025

PLOD BILJNE VRSTE *ROSA CANINA* L. I DŽEM NAPRAVLJEN OD NJEGA - SADRŽAJ BILJNIH BOJA E160A I E160D I ANTIOKSIDATIVNI KAPACITET

Šipak je jedan od najbogatijih izvora Vitamina C a džem napravljem od njega među najukusnijima. U ovom radu je određen sadržaj prehrambene boje E160a (likopena) i E160d (β-karotena) kao i antioksidativni kapacitet ovih prehrambena proizvoda. Primenom UV-Vis spektrofotometrijske metode i korišćenjem Lambert-Beer-ovog zakona postavljen je sistem dve linearne jednačine sa dve nepoznate koji je poslužio za određivanje koncentracija za boje E160a i E160d. Sadržaj likopena i β-karotena u osušenom plodu šipka i džemu napravljenom od njega je 2,34 i 0,85 odnosno 7,25 I 2,01 mg u 100 g voća/džema, redom. Antioksidativni kapacitet uzoraka određen je primenom DPPH testa. Antioksidativni kapacitet ploda i džema od šipka je 6,84 i 4,17 μmol/100g svvežeg uzorka. Termička obrada utiče na sadržaj likopena, β-karotena i antioksidativni kapacitet. Dobijeni rezultati preporučuju konzumiranje osušenog ploda i džema od šipka u svakodnevnoj ishrani.

Ključne reči: *E160a (β-karoten), E160d (likopen), šipak, džem, antioksidativni kapacitet, DPPH test*