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Determination of Vitamin C Content in *Cornus mas* L. Fruits from Bosnia and Herzegovina

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Abstract

Cornelian cherry (*Cornus mas* L.), known for its strong antioxidant and antiproliferative activity, is rich in vitamin C, polyphenols, flavonoids, anthocyanins, and iridoids. Its fruits exhibit a wide range of biological and pharmacological properties and are used in the food and cosmetic industries. Considering the importance of vitamin C for the immune system and the protection of cells from oxidative stress, the aim of this study was to determine the vitamin C content in fresh fruits collected from five different locations across Bosnia and Herzegovina. After harvesting and cleaning, the fruits were stored at −18 °C until analysis. Total ascorbic acid (AA + DHA), which includes both the reduced and oxidised forms of vitamin C, was determined using the spectrophotometric DNPH method. Vitamin C concentrations were calculated using a calibration curve and expressed as $mg\,g^{-1}$ of fresh mass.

The obtained concentrations ranged from 0.031 to 0.105 $mg\,g^{-1}$, with the highest vitamin C content found in fruits from Sarajevo and the lowest in fruits from Bileća. The observed differences among locations are the result of various factors, including climate conditions, genetic variations, fruit maturity, and the susceptibility of ascorbic acid to degradation during storage and sample preparation. Although the values of certain concentrations were lower in comparison to those reported in the literature, the results indicate that cornelian cherry remains a valuable source of vitamin C, and contribute to a better understanding of the nutritional profile of autochthonous populations of *Cornus mas* in Bosnia and Herzegovina.

Keywords

Cornus mas, vitamin C, DNPH, ascorbic acid, Bosnia and Herzegovina

1 Introduction

Cornus mas L. is a species belonging to the *Cornaceae* family that has been used for centuries both in the human diet and in folk medicine. The species originates from the Caucasus region but is now distributed throughout Central and South-Eastern Europe, including Bosnia and Herzegovina.[1] The fruits are edible and rich in nutrients and bioactive compounds. They are used in the food industry for the production of jams, liqueurs, compotes, juices, and other fruit-based products. Cornelian cherry fruits are also used in the cosmetic industry.[2,3]

Beyond their nutritional value, cornelian cherry fruits exhibit significant pharmacological potential, including pronounced antioxidant, antimicrobial, and antiproliferative effects, as confirmed by numerous studies.[4]

The fruits and leaves of *Cornus mas* L. contain considerable amounts of polyphenols, flavonoids, anthocyanins, and iridoids. These groups of phytochemicals are associated with the strong antioxidant capacity of the plant, including free-radical scavenging and protection of cellular structures.[5]

Cornelian cherry is also recognised as an exceptionally rich source of vitamin C, with some studies reporting concentrations exceeding those found in oranges, kiwifruit, or strawberries.[6,7]

Vitamin C (L-ascorbic acid, AA) is considered one of the most important water-soluble antioxidants and is an essential nutrient that cannot be synthesised endogenously in humans; therefore, dietary intake of fresh fruit represents the primary source of this vitamin. Its antioxidant function is manifested through the neutralisation of free radicals and the protection of DNA, proteins, and lipids from oxidative stress.[8,9]

Dehydroascorbic acid (DHA) is also a biologically active form of vitamin C and can be reversibly reduced to ascorbate *in vivo*. Therefore, the determination of total vitamin C content (AA + DHA) in plant material is important for assessing the nutritional value of the fruit.[10]

The concentration of vitamin C in fruit varies significantly and depends on genotype, agroecological conditions, degree of fruit maturity, and storage conditions. Previous studies have reported substantial differences in ascorbic acid content among various cornelian cherry genotypes.[5]In order to evaluate the nutritional quality and potential of this fruit, it is essential to accurately quantify vitamin C concentration.[11]

Vitamin C can be determined using various analytical techniques, including titrimetry,[12] amperometry,[13] fluorimetry,[14] high-performance liquid chromatography (HPLC),[15] enzymatic assays,[16] and spectrophotometry.[10]

Among these methods, spectrophotometry using 2,4-dinitrophenylhydrazine (DNPH) represents a simple, sensitive, accurate, and widely applied technique for the determination of total vitamin C in food products.

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The aim of this study was to determine the total vitamin C content in fresh *Cornus mas* L. fruits harvested from different areas in Bosnia and Herzegovina using the DNPH spectrophotometric method, and to evaluate variations in concentration according to geographical origin of the samples. This study contributes to a better understanding of the nutritional profile of autochthonous *Cornus mas* populations and their potential applications in the food and pharmaceutical industries.

2 Materials and methods

2.1 Reagents

A metaphosphoric acid solution (Carl Roth, Germany) in acetic acid (Merck, Darmstadt, Germany) was prepared by dissolving 8.15 g of metaphosphoric acid in a mixture of 20 ml of acetic acid (96 %) and 220 ml of distilled water in a 250 ml volumetric flask. The resulting solution was filtered prior to use.

The 2,4-dinitrophenylhydrazine (DNPH, Sigma-Aldrich, Germany) reagent was prepared by dissolving 1 g of DNPH in 12 ml of 4.5 M sulphuric acid (Fisher Scientific, U.K.). A standard ascorbic acid solution with a concentration of 1.0 mg ml^{-1} was prepared by dissolving 10 mg of L-ascorbic acid (Carl Roth, Germany) in 10 ml of the metaphosphoric acid/acetic acid solution. A series of standard solutions with concentrations ranging from 0.005 to 0.100 mg ml^{-1} was prepared by appropriate dilution of the stock solution with the same solvent. All reagents were of high analytical purity, and all solutions were prepared immediately before use.

2.2 Sample collection

Cornelian cherry fruits were collected during July and September 2024 from different localities in Bosnia and Herzegovina for analysis. The coordinates of the sampling locations and sample codes are presented in Table 1. Tuzla, Bijeljina, Bileća, Mostar, and Sarajevo were selected in order to encompass diverse geographical, climatic, and pedological conditions across Bosnia and Herzegovina. These locations represent different regions of the country, including the north-east, south-east, south, central, and north-west regions, enabling the assessment of potential variations in the chemical composition of *Cornus mas* influenced by environmental factors. At each location, several subsamples were collected from different branches of the same tree. Approximately 10 branches from one healthy tree per location were sampled and homogenised to obtain a representative sample for analysis. The total homogenised sample mass was 500 g of fruit. This sampling strategy reduces intra-individual variability and allows for a representative assessment of chemical composition.[17] However, as samples were collected from only one tree *per* location, inter-individual variability within each population was not taken into account, which represents a limitation of this study. Nevertheless, this approach is suitable for preliminary analysis.

Immediately after harvesting and cleaning, the fruits were stored at −18 °C until analysis. Botanical identification of the collected material was performed at the Laboratory for Cormophyte Systematics, Faculty of Science and Mathematics, University of Sarajevo, using standard literature.[18,19]

Herbarium specimens of the investigated species have been deposited in the scientific collection of the National Museum of Bosnia and Herzegovina (SARA) under inventory numbers 54 050–54 056.

Table 1 – Analysed *Cornus mas* samples: sampling areas, geographic coordinates, and sample codes
Tablica 1 – Analizirani uzorci *Cornus mas*: Područje uzorkovanja, geografske koordinate i kodovi uzoraka

Sampling area	Coordinates	Sample code
		Flesh (F)
Tuzla	44°32′17″N 18°40′34″E	TZ-F
Bijeljina	44°45′24″N 19°12′56″E	BI-F
Bileća	42°52′N 18°26′E	BL-F
Mostar	43°20′37″N 17°48′27″E	MO-F
Sarajevo	43°51′23″N 18°24′47″E	SA-F

2.3 Sample preparation

For each analysis, 2 g of fresh cornelian cherry fruit were macerated in a mortar with 10 ml of the metaphosphoric acid/acetic acid solution. The resulting homogenate was centrifuged for 20 min at 4000 rpm. The supernatant was decanted, filtered, transferred into a 10 ml volumetric flask, then supplemented up to the mark with the same solution. Prior to analysis, all sample extracts were diluted tenfold.

2.4 Spectrophotometric determination of vitamin C

After centrifugation, extracts of fresh fruit (2 ml) were transferred into 10 ml vials, followed by the addition of:

- 115 µl of 3 % bromine water (Merck, Darmstadt, Germany) to oxidise ascorbic acid to dehydroascorbic acid;
- 65 µl of 10 % thiourea (Sigma-Aldrich, USA) to eliminate excess bromine;
- 500 µl of 2,4-DNPH reagent to form the osazone.

The resulting solutions were heated at 37 °C for 3 h and then cooled on ice for 30 min. After cooling, 2.5 ml of 85 % H_2SO_4 was added with constant stirring, resulting in the formation of a red-coloured complex. The absorbance of the coloured solution was measured at 521.5 nm using a UV-Vis spectrophotometer (Shimadzu UV-1280). Each sample was analysed in triplicate. A reagent blank was prepared in the same manner, except that 2 ml of the metaphosphoric acid/acetic acid solution was used instead of the sample solution.[10]

Standard solutions of ascorbic acid (0.005–0.100 mg ml^{-1}) were prepared as described in Section 2.1 and treated identically to the fruit samples.

3 Results and discussion

3.1 Reaction and calibration curve

For the quantitative analysis of vitamin C in fresh cornelian cherry fruit, UV-Vis spectrophotometry was employed. Vitamin C in fruit extracts reacts with acidic 2,4-dinitrophenylhydrazine (DNPH). The DNPH method is among the simplest, most accurate, and reliable techniques for determining the vitamin C content in foods such as fruits and vegetables. In this method, vitamin C (ascorbic acid) is oxidised to dehydroascorbic acid by the addition of bromine water in the presence of acetic acid. Subsequently, L-dehydroascorbic acid reacts with DNPH to form an osazone, which, when treated with 85 % sulphuric acid, produces a red-coloured solution (Fig. 1). After cooling, the absorbance was measured at 521.5 nm.

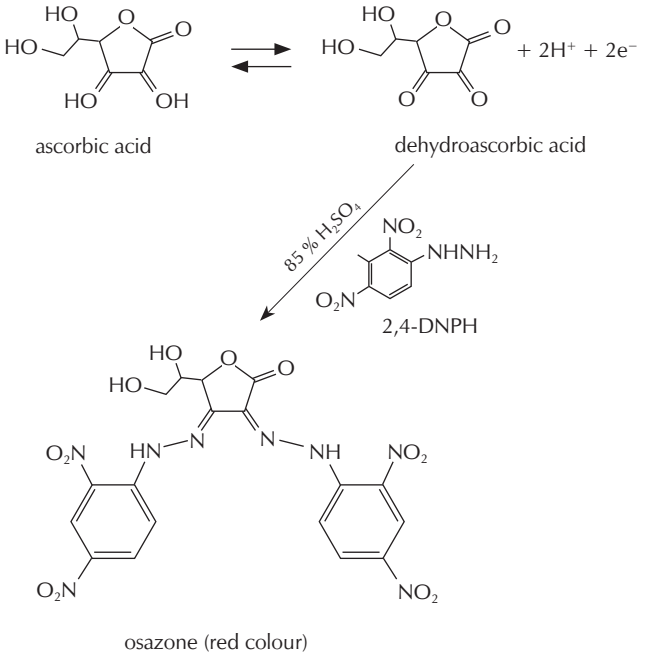

Fig. 1 – Reaction of osazone formation through oxidation of ascorbic acid
Slika 1 – Reakcija nastanka osazona oksidacijom askorbinske kiseline

After determining the maximum absorbance wavelength of the coloured complex (λ_{max} = 521.5 nm), the absorbances of standard vitamin C solutions (0.005–0.100 mg ml^{-1}) were used to construct a calibration curve (Fig. 2). Linear regression had the form $y = 9.9503x–0.0037$ ($R^2 = 0.9761$), confirming good linearity of the method within the tested concentration range. This calibration curve was used to calculate the concentration of ascorbic acid in the analysed samples.

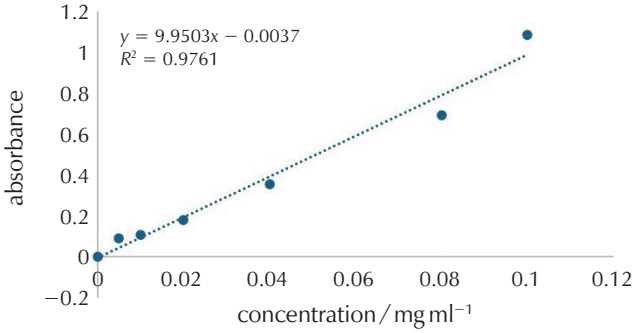

Fig. 2 – Calibration curve for the determination of vitamin C concentration
Slika 2 – Kalibracijska krivulja za određivanje koncentracije vitamina C

3.2 Vitamin C content in samples

The obtained vitamin C concentrations in fruit extracts (c / mg ml^{-1}) and the calculated values in mg vitamin C *per* gram of fresh sample (γ / mg g^{-1}) are presented in Table 2.

Table 2 – Vitamin C content in Cornus mas samples
Tablica 2 – Sadržaj vitamina C u uzorcima Cornus mas

Samples	Absorbance ± SD	Vitamin C c / mg ml^{-1}	Vitamin C γ / mg g^{-1}
BL-F	0.058 ± 0.003	0.006	0.031
BI-F	0.189 ± 0.017	0.019	0.097
TZ-F	0.148 ± 0.001	0.015	0.076
SA-F	0.206 ± 0.003	0.021	0.105
MO-F	0.130 ± 0.001	0.013	0.067

Absorbance values are shown as mean ± SD calculated from three measurements (n = 3).

The results show that the vitamin C content in cornelian cherry fruits varied between 0.031 and 0.105 mg g^{-1} depending on the sampling location. The highest concentration was found in the sample collected in Sarajevo, while the lowest was observed in the fruit collected in Bileća. Based on these results, it can be concluded that the analysed samples contained lower amounts of vitamin C compared with some previously published studies. Thus, *A. Martinović* and *I. Cavoski*[20] reported values ranging from 0.48 to 1.08 mg g^{-1}, depending on different genotypes. In another study, *M. De Biaggi et al.*[5] reported a vitamin C concentration of 0.61 mg g^{-1} in fresh mass, while *S. Tural* and *I. Koca*[21] reported values ranging from 0.16 to 0.88 mg g^{-1}. Differences in vitamin C concentrations between studies may result from various factors, such as variety, genotype, climatic conditions, degree of fruit ripeness at harvest, sample processing techniques, and the analytical methods used to determine vitamin C content.[22]

The climatic characteristics of the location may also influence the accumulation of ascorbic acid. According to the

literature, fruits grown in areas with cooler nights often contain higher concentrations of vitamin C, while those from warmer regions contain lower concentrations.[23] Sarajevo is characterised by a continental climate (Cfb) with cooler nights and more pronounced daily temperature oscillations, whereas Bileća has a Mediterranean climate (Csb) with warmer nights and smaller day-night temperature differences.[24] Climatic parameters are based on long-term climatological normals (1991–2020) obtained from the World Meteorological Organization (WMO).[25] This climatic contrast could explain why the highest concentration of vitamin C was found in samples from Sarajevo compared with those from Bileća.

Another significant factor is the sensitivity of ascorbic acid to temperature, light, and pH, which can lead to its degradation during storage, particularly during freezing, thawing, and sample preparation. These factors can accelerate the oxidation of ascorbic acid to dehydroascorbic acid, which then undergoes irreversible lactone ring opening and hydrolysis to diketogulonic acid, resulting in a loss of vitamin activity. Diketogulonic acid, which naturally occurs in stored or frozen fruits, has a reactive carbonyl (keto) group capable of forming osazone in reaction with DNPH, potentially causing deviations in measured vitamin C values.[26]

M. M. Rahman et al.[10] reported that the initial concentration of ascorbic acid was reduced by 76 % at 5 °C and 64 % at −10 °C over a two-month storage period. Since the samples in this study were stored frozen until analysis, partial degradation of ascorbic acid was expected, which could to some extent explain why the measured values were lower than those reported in the literature. However, this does not suggest that the natural vitamin C content in the fresh fruit is lower.

4 Conclusion

This study provides insight into the total vitamin C content in fresh *Cornus mas* fruits collected from different geographical locations in Bosnia and Herzegovina. The obtained concentrations ranged from 0.031 to 0.105 mg g^{-1}, with significant differences between locations. Although these values are lower than most data available in the literature, our results confirm that cornelian cherry fruits represent a valuable source of vitamin C and a potentially significant source of natural antioxidants.

Ecological conditions, genetic diversity, fruit maturity, and storage conditions, particularly sample freezing, may have a key impact on ascorbic acid content. The discrepancies observed in comparison with previous studies highlight the need for standardisation of sampling and storage procedures, especially since ascorbic acid is highly sensitive to oxidation and degradation during preservation.

The findings of this study enhance our understanding of the nutritional profile of *Cornus mas* in Bosnia and Herzegovina and may serve as a basis for further studies on its bioactive compounds and functional value as a pharmacologically and nutritionally significant species.

The spectrophotometric method used proved to be simple, sensitive, and suitable for determining total vitamin C content in fruit, making it appropriate for routine analyses.

Overall, the results indicate that cornelian cherry possesses a valuable, yet still insufficiently researched, nutritional and biological potential, broadening the scope for further research on autochthonous species in Bosnia and Herzegovina.

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List of abbreviations and symbols
Popis kratica i simbola

AA	– L-ascorbic acid – L-askorbinska kiselina
DHA	– dehydroascorbic acid – dehidroaskorbinska kiselina
DNPH	– 2,4-dinitrophenylhydrazine – 2,4-dinitrofenilhidrazin
H_2SO_4	– sulphuric acid – sumporna kiselina

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SAŽETAK

Određivanje sadržaja vitamina C u plodovima *Cornus mas* L. iz Bosne i Hercegovine

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Drijen (*Cornus mas* L.), s izrazitim antioksidativnim i antiproliferativnim djelovanjem, bogat je vitaminom C, polifenolima, flavonoidima, antocijaninima i iridoidima. Njegovi plodovi imaju širok spektar bioloških i farmakoloških svojstava, te se upotrebljavaju u prehrambenoj i kozmetičkoj industriji. S obzirom na važnu ulogu vitamina C u normalnom funkcioniranju imunološkog sustava i zaštiti stanica od oksidativnog stresa, cilj ove studije bio je odrediti sadržaj vitamina C u svježim plodovima koji su prikupljeni na pet različitih lokaliteta u Bosni i Hercegovini. Plodovi su nakon prikupljanja i čišćenja skladišteni na −18 °C do analize.

Ukupna askorbinska kiselina (AA + DHA), koja obuhvaća reducirani i oksidirani oblik vitamina C određena je spektrofotometrijskom DNPH metodom. Koncentracija vitamina C u uzorcima izračunata je pomoću kalibracijske krivulje te izražena u $mg\,g^{-1}$ svježeg uzorka.

Dobivene koncentracije kretale su se od 0,031 do 0,105 $mg\,g^{-1}$, pri čemu je najviša zabilježena u plodovima iz Sarajeva, a najniža u plodovima iz Bileće. Uočene razlike među lokalitetima posljedica su različitih faktora, kao što su klimatski uvjeti, genetske varijacije, stupanj zrelosti ploda te osjetljivost askorbinske kiseline na razgradnju tijekom skladištenja i pripreme uzorka. Iako su vrijednosti određenih koncentracija bile niže u odnosu na literaturne podatke, dobiveni rezultati potvrđuju da drijen ostaje vrijedan izvor vitamina C te doprinose boljem razumijevanju nutritivnog profila autohtonih populacija *Cornus mas* u Bosni i Hercegovini.

Keywords
Cornus mas, vitamin C, DNPH, askorbinska kiselina, Bosna i Hercegovina

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