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# INFLUENCE OF AGRO-ECOLOGICAL FACTORS ON PHENOTYPIC AND CHEMICAL CHARACTERISTICS OF CHOKEBERRY FRUIT IN THE REGION OF BOSNIA AND HERZEGOVINA

## ***Jasna Hasanbegović***

PhD, Assistant Professor, Dzemal Bijedic University of Mostar, Agromediterranean Faculty,  
Sjeverni logor b.b. 88104, Mostar, Bosnia and Herzegovina, email: [jasna.hasanbegovic@unmo.ba](mailto:jasna.hasanbegovic@unmo.ba)

## ***Aleksandra Šupljeglav Jukić***

PhD, Assistant Professor, Dzemal Bijedic University of Mostar, Agromediterranean Faculty,  
Sjeverni logor b.b. 88104, Mostar, Bosnia and Herzegovina; email: [aleksandra@unmo.ba](mailto:aleksandra@unmo.ba)

## ***Jasmina Aliman***

PhD, Full Professor, Dzemal Bijedic University of Mostar, Agromediterranean Faculty,  
Sjeverni logor b.b. 88104, Mostar, Bosnia and Herzegovina; email: [jasmina.aliman@unmo.ba](mailto:jasmina.aliman@unmo.ba)

## ***Semina Hadziabulić***

PhD, Full Professor, Dzemal Bijedic University of Mostar, Agromediterranean Faculty,  
Sjeverni logor b.b. 88104, Mostar, Bosnia and Herzegovina; email: [semina.hadziabulic@unmo.ba](mailto:semina.hadziabulic@unmo.ba)

## ***Semira Dedić***

PhD, Assistant Professor, University of Bihać, Biotechnical Faculty, Luke Marjanovića bb. 77000 Bihać,  
Bosnia and Herzegovina; email: [semira.dedic@yahoo.com](mailto:semira.dedic@yahoo.com)

## ***Azra Skender***

PhD, Full Professor, University of Bihać, Biotechnical Faculty, Luke Marjanovića bb. 77000 Bihać,  
Bosnia and Herzegovina; email: [skender.azra71@gmail.com](mailto:skender.azra71@gmail.com)

## ***Boris Dorbić***

PhD, Associate Professor, University of Split, Agromediterranean Faculty, Zrinsko-Frankopanska 38,  
21000 Split, Croatia; email: [bdorbic@unist.hr](mailto:bdorbic@unist.hr)

## ***Sezai Ercişli***

PhD, Full Professor, Ataturk University, Agricultural Faculty, Department of Horticulture, 25240 Erzurum,  
Türkiye; email: [sercisli@gmail.com](mailto:sercisli@gmail.com)

## **ABSTRACT**

*Aronia is known for its medicinal properties due to its unique chemical characteristics, which are influenced by various factors, including growing conditions, soil quality and harvest time. Like most fruit species with high water content, aronia has a low caloric value. The specific composition of aronia*

may vary slightly in different ecological conditions due to influencing factors. The research included the most common aronia variety Nero, at three different growing locations: Mostar (Gornji Jasenjani), Bugojno and Kupres. The research results indicate that the average height and width of the Nero variety fruit was the highest at the Kupres location with 10.92 mm and 12.01 mm, and the lowest at the Mostar (Gornji Jasenjani) location with 9.19 mm and 10.02 mm. The chemical composition of the Nero variety fruits varied at different locations. The Nero variety had the highest content of total sugars at the Kupres location (6.35%) and total acids (1.36%), while at the Mostar location (Gornji Jasenjani) it had slightly lower values of total sugars (5.59%) and total acids (1.06%). Regarding the content of dry matter, minerals and pH values, the Nero variety had higher average values at the Mostar location (Gornji Jasenjani) compared to Bugojno. The lowest pH value (3.45) was recorded for the Nero variety at the Kupres location. The results of morphological and chemical characteristics of chokeberry in different agro-ecological conditions indicate the potential for increased production of this attractive fruit species in the mountainous areas of Bosnia and Herzegovina.

**Keywords:** *Aronia melanocarpa*, Nero, fruit morphology, chemical composition, agroecological conditions

## 1. INTRODUCTION

Chokeberry is a plant native to North America, specifically Canada and the USA, where it grows in the wild on poor, acidic soils with annual rainfall of 1000 to 1200 mm. Although well known to the Native Americans, chokeberry was not commercially grown in the USA until the 20<sup>th</sup> century (Smith and Ringenberg, 2003).

The cultivation and consumption of chokeberry fruit originated in North America and then spread globally. The genus consists of three species: *Aronia arbutifolia* [L.] Elliot (red chokeberry), *Aronia melanocarpa* [Michx.] Elliot (Black chokeberry), and *Aronia prunifolia* (Marsh.) Rehd. (Purple chokeberry). Chokeberry fruit is valued for its distinct taste, texture, and color, as well as its high antioxidant, vitamin, mineral, fiber, and folic acid content. In addition to being eaten fresh, chokeberry is used in various food products such as drinks, ice cream, yogurt, jams, and jellies (Poyraz Engin and Mert, 2020; Celik et al., 2022; Boyaci et al., 2023).

Bossert (2012) stated that chokeberry was introduced to Europe by explorers and sailors of the Imperial Russia in the 18th century, who brought it from North America. It was initially planted in Siberia and later spread to eastern and western parts of Europe in the 19th century. Currently, chokeberry is predominantly cultivated in northern Russia in Europe. Additionally, new plantations are being established in countries such as Poland, the Czech Republic, Slovakia, as well as in the northern regions of Germany and France.

According to Strik et al. (2003), red chokeberry populations can be found in various habitats such as swamps, ponds, lowland forests, and at the edge of water ecosystems. In contrast, black chokeberry populations are typically found on sandy slopes, dry rocky slopes, flat terrain, grassy areas, and slightly wetter locations. Additionally, black chokeberry plants are able to thrive in

mountainous regions due to their high resistance to low winter temperatures and late flowering in spring, which prevents their generative organs from freezing due to late spring frosts.

Skender *et al.* (2017) noted that the first orchards in Bosnia and Herzegovina were established in recent years. The cultivation of chokeberry originally began in the former USSR, where chokeberry was officially recognized as a fruit in 1946 and recommended for planting in the Altai district. By 1971, chokeberry was being planted in Russia on 5400 hectares of land, with cultivation also spreading to Moldova, Belarus and Ukraine. By the early 1980s, experimental cultivation of chokeberry began in Bulgaria, former Czechoslovakia, and Scandinavian countries. The Aron, Nero and Viking varieties, which are relatively new, were introduced from Slovakia and Scandinavia.

According to multiple studies, the chemical composition of chokeberry suggests that it may have beneficial effects on the human body. Specifically, polyphenol components have been identified as having various biological activities including antioxidant, antimutagenic, cardioprotective, anticarcinogenic and antihyperglycemic properties (Kokotkiewicz *et al.*, 2010).

Chokeberry is highly valued for its medicinal properties due to its unique chemical composition, which can vary depending on factors such as climate, soil quality and harvest timing. The amount of specific components in chokeberry may differ slightly in various literature sources. Chokeberry is a low-calorie fruit with a low content of reducing sugars such as glucose and fructose (13-18%) and no detectable sucrose. It contains sorbitol and has a low-fat content (0.14%) and moderate protein content (0.7%). The fruit also has a relatively low total organic acid content (1-1.5%) compared to other fruits. Chokeberry is a good source of dietary fiber, with 5.62 g per 100 g of fresh fruit, and a pectin content of 0.3-0.6%. The fruit's aroma is primarily due to the presence of amygdalin, which gives a bitter almond-like taste and slight aroma, as well as pungent tannins (Kulling and Rawel, 2008).

Chokeberry fruit contains important chemical constituents with the following ranges: dry matter (15-20%), total sugars (9-15%), reducing sugars (7-13%), total polyphenols (1,500-2,500 mg gallic acid/100 g), and total anthocyanin content (800-1,000 mg/100g) (Ochmian *et al.*, 2012). Tomić *et al.* (2016) emphasized that chokeberry fruits and products derived from processing are a valuable source of polyphenols. Kulling and Rawel (2008) highlighted the high content of proanthocyanidins and anthocyanins in chokeberry products. According to Čujić *et al.* (2013), the content of anthocyanins in chokeberry fruits or freshly squeezed juice ranges from 300 to 2000 mg/100g. Zheng and Wang (2003) suggested that anthocyanins contribute up to 40% to the antioxidant activity of chokeberry juice. Considering the previously discussed findings and the limited information available on chokeberry cultivation across diverse agroecological conditions in Bosnia and Herzegovina, the aim of the research was to compare the morphological and chemical characteristics of the Nero aronia variety under different agroecological conditions at the Mostar (Gornji Jasenjani) Bugojno, and Kupres locations.

## **2. MATERIALS AND METHODS**

### **2.1 Plant material**

The study involved an analysis of the morphological and chemical properties of chokeberry (*Aronia melanocarpa* L.) variety Nero. Samples were collected from Kupres (Latitude: 44° 04' 59.88" North, Longitude: 17° 13' 0.18" East), Bugojno (Latitude: 44° 03' 25.97" North, Longitude: 17° 27' 2.97" East) and Mostar (Gornji Jasenjani Latitude 43.50134° or 43° 30' 5" North, Longitude 17.78913° or 17° 47' 21" East) in 2022 from cultivated bushes of the Nero variety. Thirty fruits and leaves were collected from each locality at full ripeness and transported to the laboratories of the Faculty of Agriculture in Mostar for morphological analysis and the Faculty of Biotechnology at the University of Bihać for chemical analysis. Morphological parameters of the fruits were examined using a mechanical sliding scale and analytical balance.

The methodology utilized is outlined extensively in the work methods chapter. During the trial period, agronomically important traits for the Nero variety were observed and evaluated at the specified locations. These traits included fruit weight (g), fruit height (mm), fruit width (mm), and average number of berries per bunch. Additionally, chemical analyses were conducted on parameters such as total sugars %, total acids %, dry matter %, mineral matter % and pH value.

### **2.2 Chemical analyses of chokeberry fruits**

Chemical analysis of Nero chokeberry samples involved thawing and homogenizing the fruit samples. The samples were then analyzed for dry matter, mineral matter (ash), total sugars, and total acidity. Dry matter content was determined by drying the samples with the addition of quartz sand, which increases the sample's surface area and facilitates water evaporation. The samples were dried at 105°C until a constant mass was achieved and measured. The ash content was analyzed through annealing at 550°C until a white powder was produced. Total sugars were measured using the Luff-Schoorl method as outlined by Vračar (2001). Total acidity was determined using the potentiometric technique also described by Vračar (2001), with results reported as a percentage equivalent to citric acid. The pH value was determined by immersing a combined electrode into the sample and recording the reading on a pH meter.

### **2.3 Sampling and soil analysis**

The soil sample analysis was conducted in accordance with the Methodology Manual for Monitoring the State of Agricultural Land, Instruction on unique methodology for classification of agricultural land in bonitet categories (2009). After collecting composite soil samples from two depths, the analysis was performed at the Federal Institute for Agro-Pedology in Sarajevo. Soil texture or mechanical composition was determined using the international B-method for soil texture classification, while a three-component diagram according to Ilijanić (1977) was used for soil classification.

Soil reaction (pH) was determined based on the ratio of H<sup>+</sup> and OH<sup>-</sup> ion concentrations and expressed as pH value. Active acidity was measured in distilled water and expressed as pH in H<sub>2</sub>O. The measurement was carried out electrometrically using a pH meter. Exchange acidity was determined in a 1M KCl solution and expressed as pH in KCl or physiological active acidity. The measurement was performed electrometrically with a pH meter, and the results obtained were compared with the pH in KCl scale according to the Schfer-Schachatschabel.

The content of organic matter was determined gravimetrically. The carbonate (CaCO<sub>3</sub>) content was determined volumetrically. Humus content was expressed as a percentage, following the Springer method (dichromate method), in accordance with FZAPL U 5.4.06. Total nitrogen (N) content was expressed as a percentage, determined by the Kjeldahl method in accordance with BAS ISO 11261 (2000). The available phosphorus (P<sub>2</sub>O<sub>5</sub>) and potassium (K<sub>2</sub>O) contents were determined using the Al method.

## **2. 4 Statistical analysis of results obtained**

In the processing and analysis of the data on pomological traits, various statistical methods were utilized. This included analysis of variance to determine the impact of analyzed factors on statistical parameters, the Tukey test to verify the significance of observed differences, and the calculation of the Pearson correlation coefficient to analyze the relationship between two variables. The evaluation of morphological characteristics of chokeberry fruit in different areas was conducted through principal component analysis (PCA) using a computer program R v. 3.2.3, based on a correlation matrix. The methodology was adapted from Hotelling (1936) and implemented in accordance with the guidelines of the R core team (2016).

Principal component analysis (PCA) was conducted using mean values of 12 morphological and chemical traits following fruit analysis. The spatial distribution of the analyzed chokeberry fruit based on the first two components of the experimental factors is presented graphically. The principal components succinctly elucidate the data variability and variable interrelations. The graphical illustrations (Figs. 1, 2, 3, 4) were generated using the computer program R version 3.2.3 R core team (2016).

## 2.5 Meteorological data

The following tables show meteorological data for three localities

Table 1. Average precipitation sums and average monthly temperature by location

Month	Rainfall sums in mm			Mean monthly temperature °C		
	Bugojno	Mostar	Kupres	Bugojno	Mostar	Kupres
January	25.9	9.4	13	-1.1	6.3	-2.9
February	42.5	82	56.1	3.9	8.8	-0.1
March	12.7	41	8.1	3.9	9.8	-0.6
April	79.8	226.6	160.8	9.3	13.7	4.6
Maj	87.8	28.5	49	16.6	20.9	12.4
June	55.8	30.8	12.1	21.8	26.8	17.6
July	63.9	23.6	44.3	22.1	28.8	17.9
August	59.2	16.1	86.4	20.1	27.3	16.5
September	74.1	101.6	106.5	14.9	20.1	11.2
October	10.4	4.5	15.2	12.5	18.1	9.5
November	143.4	247.8	163.8	6.9	12.4	3.8
December	95.5	180.1	165.6	5.6	9.6	3.0
SUM	725.10	992.00	880.90	136.50	202.60	92.90
AVERAGE	62.96	73.81	65.03	11.90	17.55	8.17

Source: Federal Hydrometeorological Institute of Bosnia and Herzegovina

In 2022, the total precipitation levels recorded across three distinct locations within Bosnia and Herzegovina exhibited notable variability, contingent upon the specific site and month. At the Bugojno site, the annual cumulative rainfall ranged from a minimum of 12.7 mm observed in March to a maximum of 143.4 mm registered in November. These data indicate that November was the most precipitation-rich month for this location, whereas March experienced the lowest rainfall. In Mostar, precipitation levels were even more pronounced, with the highest average monthly rainfall reaching 247.8 mm in November. During this month, Mostar recorded its peak precipitation for the year. Conversely, October experienced the least rainfall, totaling merely 4.5 mm, suggestive of a comparatively drier period during that month. Regarding Kupres, the annual precipitation sum varied from 13 mm in January to 165.6 mm in December. December emerged as the wettest month on this high-altitude site, while January was the driest. According to the data presented in Table 1, Mostar recorded the highest annual total precipitation, amounting to 992 mm, significantly surpassing the other locations. In contrast, Bugojno experienced the lowest total annual precipitation, totaling 751 mm. Turning to temperature patterns, Bugojno's lowest average monthly temperature in 2022 was  $-1.1$  °C in January, reflecting cold winter conditions. The highest average temperature was recorded in July, reaching  $22.1$  °C, characteristic of summer months. In Mostar, the warmest

month was July, with an average temperature of 28.8 °C, while January experienced the coldest average temperature at 6.3 °C, typical for winter. At Kupres, the highest average temperature in 2022 was 17.6 °C in June, whereas the coldest month was January, with an average temperature of –2.9 °C, indicative of the harsh winter conditions characteristic of this high-altitude region. These data provide valuable insights into climatic variability and differences across the studied sites, which can be instrumental in informing agricultural planning, water resource management, and other activities dependent on climatic conditions.

Table 2. Average maximum and minimum monthly temperatures by location

Month	Maximum monthly temperature °C			Minimum monthly temperature °C		
	Bugojno	Mostar	Kupres	Bugojno	Mostar	Kupres
January	16.0	20.0	17.0	–18.5	–3.7	–13.7
February	16.4	19.0	15.1	–8.2	0.2	–8.1
March	21.3	24.6	20.3	–10.3	–1.6	–10.8
April	25.3	26.9	22.7	–4	2.4	–4.5
Maj	30.8	33.8	29.5	1.6	8.8	1.4
June	36.0	41.0	36.7	7.7	16.2	8.3
July	38.2	41.5	36.1	7.6	17.3	6.8
August	34.3	39.4	34.2	10.4	17	9
September	28.7	33.8	27.5	2	8.3	–0.3
October	27.4	28.4	26.2	1.2	9.8	1.2
November	25.2	27.4	25.5	–4.5	2.4	–4.8
December	16.3	17.8	13.9	–4.6	0.8	–6.6

Source: Federal Hydrometeorological Institute of Bosnia and Herzegovina

Analyzing the data on maximum monthly temperatures for 2022, we observe notable differences based on location. In the area of Bugojno, the lowest average maximum temperature recorded was 16 °C, which occurred in January, reflecting the colder winter period. Conversely, the highest average maximum temperature was observed in July, reaching 38.2 °C, indicating the peak of the summer heat. This demonstrates a significant temperature variation throughout the year in Bugojno, with summer months experiencing quite high temperatures. Moving to Mostar, the data shows that the hottest month was July, with an average maximum temperature of 41.5 °C, making it the warmest period of the year for this location. The coldest month was December, with an average maximum temperature of 17.8 °C, indicating milder winter conditions compared to Bugojno. This suggests that Mostar experiences more pronounced summer heat and somewhat milder winters relative to Bugojno. In Kupres, the maximum average temperature in June was 36.7 °C, marking the hottest month for this area. The coldest month in terms of maximum temperature was February, with an average of 15.1 °C. These figures highlight that Kupres, like the other

locations, experiences warm summers and relatively cool winters, though its maximum temperatures tend to be slightly lower than those in Mostar. When examining the minimum monthly temperatures for 2022, we notice further differences. In Bugojno, the coldest average minimum temperature was  $-18.5\text{ }^{\circ}\text{C}$  in January, reflecting severe winter conditions, while the warmest minimum was  $10.4\text{ }^{\circ}\text{C}$  in August, indicating milder summer nights. For Mostar, the highest average minimum temperature was  $17.3\text{ }^{\circ}\text{C}$  in July, typical of warm summer nights, whereas the lowest was  $-3.7\text{ }^{\circ}\text{C}$  in January, pointing to milder winter conditions compared to Bugojno. In Kupres, the highest average minimum temperature was  $9\text{ }^{\circ}\text{C}$  in August, and the coldest was  $-13.7\text{ }^{\circ}\text{C}$  in January, showing that the winters are quite cold but not as extreme as in Bugojno. Overall, this data illustrates distinct climatic patterns across these locations, with Bugojno experiencing more severe winter lows, Mostar having hotter summers, and Kupres showing moderate temperatures with notable seasonal variation. These insights are crucial for understanding local climate conditions, planning agriculture, infrastructure and preparing for seasonal weather variations.

### 3. RESULTS AND DISCUSSION

#### 3.1 Results of soil pedological analyses

The results of soil pedological analyses at three localities are presented in Table 3.

Table 3. Chemical analysis of basic soil fertility parameters at three localities or basic soil fertility parameters

Chemical properties	T1- Bugojno, Sandy-peat soil	T2- Mostar, Ilovača	T3-Kupres, Loam
	T1 - Bugojno	T2 - Mostar	T3 - Kupres
pH in H <sub>2</sub> O	7.66	7.59	8.13
pH in KCl	7.05	6.94	7.25
Content of CaCO <sub>3</sub> (%)	1.98	2.08	20.49
Humus content (%)	5.94	5.5	4.39
Total Nitrogen (%)	0.36	0.39	0.28
Available P <sub>2</sub> O <sub>5</sub> mg/100 g	12.61	166.4	20.95
Available K <sub>2</sub> O mg/100g	11.8	45.7	67.70

Source: Authors

The reaction of the soil samples to potassium chloride (KCl) is observed to be neutral in both cases, indicating neither acidity nor alkalinity in response to this reagent. Conversely, when measured in water, the pH values tend to be slightly alkaline, suggesting a weakly basic reaction of the soils under aqueous conditions.

Analyzing the chemical composition, the contents of calcium carbonate ( $\text{CaCO}_3$ ), humus, and total nitrogen in both samples are quite similar. The  $\text{CaCO}_3$  content measures approximately 1.98% in one sample and 2.08% in the other, reflecting a modest presence of carbonates. The humus content is 5.94% in the first sample and slightly lower at 5.5% in the second, indicating a rich organic matter presence that significantly influences soil fertility and structure. Total nitrogen levels are also comparable, recorded at 0.36% and 0.39%, respectively.

The supply of  $\text{CaCO}_3$  in both soils is below 8%, which classifies these soils as having a weak carbonate reserve. Such low carbonate content suggests limited buffering capacity against acidity and influences on nutrient availability. The humus content values point to a soil rich in organic matter, which supports robust microbial activity and nutrient retention. Additionally, the total nitrogen levels exceeding 0.3% confirm the soils' high nitrogen status, conducive to plant growth.

Regarding plant nutrient availability, the proportion of accessible phosphorus in sample T1 is 12.61 milligrams per 100 grams of soil. This indicates a moderate level of phosphorus availability, sufficient for many crops. In contrast, sample T2 demonstrates a significantly higher phosphorus content, with 166.4 mg/100 g, classifying it as very well supplied with available phosphorus. For potassium, accessible  $\text{K}_2\text{O}$  content in T1 is 11.8 mg/100 g, representing an average level of potassium availability. Meanwhile, the T2 sample exhibits a substantially higher  $\text{K}_2\text{O}$  content of 45.7 mg/100 g, which is considered to provide a very high level of potassium nutrition to plants.

The pH measurements in both water and KCl solutions for sample T3 reveal an alkaline soil environment, consistent with its calcium carbonate content of 20.49%. This substantial carbonate presence classifies the soil as medium carbonate, contributing to its alkalinity. The humus content in T3 is 4.39%, indicating a very humus-rich soil, while the nitrogen content of 0.28% further highlights its richness in total nitrogen. Moreover, the levels of accessible phosphorus and potassium—20.95 mg/100 g and 67.70 mg/100 g, respectively—demonstrate that this soil is well supplied with active forms of these essential nutrients, supporting healthy plant growth and development.

In summary, the soils studied exhibit a range of chemical and biological properties that strongly influence their fertility and suitability for agricultural use. Their neutral to alkaline pH, high humus and nitrogen content, and varying levels of available P and K, provide valuable insights for soil management and crop cultivation strategies.

### **3. 2 Results of morphological characteristics of chokeberry fruit**

The results of the morphological characteristics of Nero variety chokeberry fruits at three localities are shown in Table 4.

Table 4. Morphological characteristics of Nero variety chokeberry fruit at three localities

Location	Fruit weight (g)	Fruit height (mm)	Fruit width (mm)	Number of berries
Mostar (Gornji Jasenjani)	0.50±0.11c	9.19±0.61c	10.02±0.61b	9±0.88c
Bugojno	0.75±0.16b	10.02±0.67b	11.87±0.67a	11±0.76b
Kupres	0.80±0.14a	10.92±0.73a	12.01±0.73a	13±0.99a

\*Note: Different letters indicate significant differences between means at  $P \leq 0.05$  by Tukey test.  $\pm$  standard deviation

Source: Authors

The morphological assessment of black chokeberry (*Aronia melanocarpa*) fruits, specifically of the Nero cultivar, yielded insightful data regarding fruit size and weight across different geographic locations. The analysis revealed that the highest average fruit mass was observed at the Kupres site, reaching 0.80 grams per berry. In contrast, the lowest average fruit mass was recorded at the Mostar (Gornji Jasenjani) site, with berries averaging 0.50 grams. In addition to fruit weight, measurements of fruit dimensions showed that the largest average height and width for the Nero variety were also obtained at Kupres, with mean values of 10.92 mm and 12.01 mm, respectively. Conversely, at the Mostar (Gornji Jasenjani) location, the fruits exhibited slightly smaller sizes, with an average height of 9.19 mm and width of 10.017 mm. The number of berries per sample varied between sites, with the Mostar (Gornji Jasenjani) sample containing an average of 8 berries, while the Kupres sample had approximately 13 berries per sample (see Table 4).

These findings align with previous research conducted by Ochmian *et al.* (2012), who examined various black chokeberry Nero cultivars and reported significantly higher average fruit weights, averaging 91.7 grams per fruit, with an average width of 14.1 mm. Furthermore, Jeppsson (2000a, b) classified fruits from the Galicjanka, Nero and Viking varieties as relatively large, with individual fruit weights ranging from approximately 65 grams to nearly 95 grams.

The study of fruit weights extended to the evaluation of 100-fruit samples, which provides a cumulative perspective on fruit size. For instance, Kawecki and Tomaszewski (2006) documented fruit weights ranging from 84 grams to 98 grams in certain years. Conversely, other studies, such as those by Smolarz and Chlebowska (1997) and Strik *et al.* (2003), reported considerably higher average weights for 100 fruits, approximately 280 grams, indicating possible differences in cultivar, cultivation practices or measurement methodologies.

In a more specific investigation by Skender *et al.* (2017), it was noted that under control conditions without particular fertilization regimes, the average weight of individual Nero berries was approximately 1.02 grams. This variation underscores the influence of environmental factors, cultivation techniques and experimental treatments on fruit development metrics.

In summary, the morphological parameters of Nero chokeberry fruits demonstrate notable variability depending on geographic location and cultivation conditions. These findings

contribute to a broader understanding of the cultivar's growth characteristics and can inform optimal cultivation practices to maximize fruit size and yield.

### 3.3 Results of leaf morphological characteristics

The results of the morphological characteristics of the Nero variety chokeberry leaves in three localities are shown in Table 5.

Table 5. Morphological characteristics of Nero variety chokeberry leaves

Location	Leaf length (mm)	Leaf width (mm)	Stem length (mm)
Mostar (Gornji Jasenjani)	81.96±1.55 c	41.94±1.55 c	11.07±0.89 b
Bugojno	85.87±1.68 a	49.38±1.68 a	10.10±0.89 a
Kupres	84.87±1.64 b	48.55±1.64 b	10.33±0.89 a

\*Note: Different letters indicate significant differences between means at  $P \leq 0.05$  by Tukey test  $\pm$  standard deviation

Source: Authors

The comprehensive analysis of the morphological characteristics of the Nero variety's leaves, conducted across two distinct localities, reveals notable variations in key parameters. Specifically, the highest average leaf length and width were recorded at the Bugojno site, measuring 85.87 mm and 49.38 mm, respectively. In contrast, at the Mostar location (Gornji Jasenjani), the leaf length was slightly shorter at 81.96 mm, with a width of 41.94 mm.

Furthermore, the length of leaf petiole exhibited minor differences between sites, ranging from 11.07 mm at the Mostar (Gornji Jasenjani) site to 10.33 mm at Kupres site. Statistical analyses, including analysis of variance (ANOVA), confirmed that these differences in all measured leaf parameters—length, width and petiole length—were statistically significant across the different localities (see Table 5).

These findings are consistent with prior research by Skender *et al.* (2017), who reported an average leaf width of 50.13 mm and leaf length of 85.12 mm within control groups, with petiole lengths averaging 11.13 mm. Similarly, Ochmian *et al.* (2012) in Poland documented leaf dimensions of approximately 53 mm in width and 87 mm in length, indicating comparable morphological traits across different geographic regions.

Additionally, in a recent study by Skender *et al.* (2023), the average fruit weight of the Nero chokeberry was assessed under various soil fertilization regimes. The results indicated mean fruit weights of 0.89 g, 0.80 g, and 0.68 g corresponding to treatments involving plastic films, mulch and control conditions, respectively. This suggests that soil management practices can influence fruit development and overall crop yield in Nero chokeberry cultivation.

### 3. 4 Results of chemical characteristics of fruit

The results of the chemical characteristics of Nero variety chokeberry fruits at three localities are shown in Tables 6 and 7 and 8.

Table 6. Chemical characteristics of Nero variety chokeberry fruits

Locality	Total sugars (%)	Total acids (%)	Dry matter (%)	Mineral matter	pH
Mostar (Gornji Jasenjani)	5.59±0.93 c	1.06±0.02 c	29.05±0.35 a	1.77±0.06 a	4.20±0.03 a
Bugojno	6.18±0.08 b	1.23±0.03 b	16.90±0.21 c	0.67±0.08 c	3.78±0.06 b
Kupres	6.35±0.06 a	1.36±0.03 a	20.65±0.27 b	1.49±0.16 b	3.45±0.04 c

\*Note: Different letters indicate significant differences between means at  $P \leq 0.05$  by Tukey test  $\pm$  standard deviation

Source: Authors

The chemical analysis results of the Nero variety across three different localities reveal notable variations in key parameters. The highest average sugar and acid contents were recorded at the Kupres locality, with values of 6.35% and 1.36%, respectively. Conversely, the Mostar locality (Gornji Jasenjani) exhibited lower levels, with sugars at 5.59% and acids at 1.06%. The average dry matter content at the Mostar locality (Gornji Jasenjani) was 29.05%, markedly surpassing the 16.90% observed at the Bugojno locality for the same variety.

Similarly, mineral substance content was greater at the Mostar locality (Gornji Jasenjani), reaching 1.77%, compared to just 0.67% at the Bugojno locality. In terms of pH, the Nero variety showed its highest average at the Mostar locality (Gornji Jasenjani) with a value of 4.20, while Bugojno recorded a lower pH of 3.78. Statistical analysis of variance confirmed that these differences among localities are significant, underscoring the influence of geographical factors (see Table 6).

Aligning with previous research, Skender *et al.* (2017) reported that fruits from the control variant contained the highest dry matter at 21.78%, total sugars at 13.41% and total acidity at 1.34%. Our findings corroborate this trend: Bugojno demonstrated significantly higher dry matter, whereas Mostar (Gornji Jasenjani) exhibited lower levels.

The dry matter content observed in our study closely matches the 15.7% reported by Ochmian *et al.* (2012), and falls within the range of 18.92% to 20.14% documented by Skupień *et al.* (2008). Additionally, the total sugar content of 6.91% aligns with their findings.

Recent research by Skender *et al.* (2023) examined the effects of different land treatment methods—plastic film, mulch and control—on chemical properties. The dry matter content varied from 24.13% (plastic film) to 26.05% (control). Total sugars ranged from 4.48% (control) to 21.32% (plastic film), with sucrose levels at 1.59% (control), 7.59% (plastic film), and 4.47% (mulch). Ash content was lowest in the mulch group at 0.36%, higher in the control at 0.65% and 0.47% with plastic film. Total acidity remained relatively stable across treatments, measuring between 1.19% and 1.30%.

Table 7. Eigenvalues, proportion of variance and cumulative variance associated with first two principal components (PCA), estimated from correlation matrix with 12 variables at three localities

Variables	PCA1	PCA2
Eigenvalue	10.362	1.638
Percentage of variance	86.3487	13.651
Cumulative percentage of variance	86.349	100.000

Source: Authors

Table 8. Analysis of 12 quantitative characteristics of chokeberry in total variability of experiment (significant sources of variability in bold)

Characteristics	PCA1	PCA2
Fruit weight - FW	<b>9.602</b>	0.309
Fruit length - FL	7.734	<b>12.126</b>
Fruit width - FWW	<b>9.646</b>	0.029
Number of berries - NB	7.910	<b>11.008</b>
Leaf length - LL	<b>8.615</b>	6.550
Leaf width - LW	<b>9.317</b>	2.109
Leaf petiole length - PL	<b>8.731</b>	5.820
Total sugars - TS	<b>9.489</b>	1.022
Total acidity - TA	<b>8.442</b>	7.646
Ashes - A	8.248	<b>8.873</b>
Mineral compound - MC	3.872	<b>36.550</b>
pH	<b>8.393</b>	7.958

Variables with the highest eigenvector values in first two main components are presented:

PCA1 – fruit mass, fruit thickness, leaf length, leaf width, leaf petiole length, total sugars, total acids and pH;

PCA2 – leaf width, number of berries, ash, mineral matter.

Source: Authors

After examining the results of the two primary elements of the PCA evaluation as demonstrated in Table 7, it can be observed that each of the 12 examined characteristics plays a significant role in the overall variability seen in the three specific regions. In the initial two principal components, all 12 traits exhibit a substantial eigenvector value.

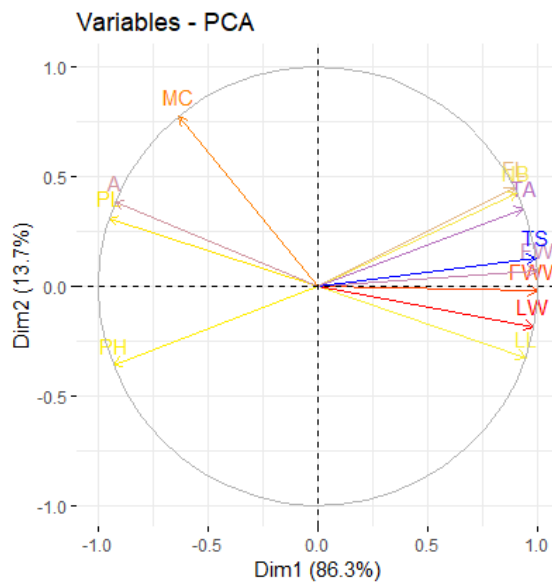
After analyzing the results, it can be inferred that the dominant eigenvectors in the first principal component (PCA) played a significant role, explaining 86.348% of the total variance in the study. This component mainly focused on the morphological features of the fruit and leaf, as well as the chemical properties of the chokeberry fruit. The key properties of the first component included fruit mass (9.602), fruit thickness (9.646), leaf width (9.317) and total

sugars (9.489). Conversely, traits such as leaf length (8.615), leaf petiole length (8.731), total acid (8.442) and pH (8.393) displayed lower values of eigenvectors (Table 8).

In the second main component, which accounts for 13.651% of the total variability in the experiment, a majority of the properties with high eigenvector values are linked to the morphological and chemical characteristics of chokeberry. Specifically, traits such as leaf width, number of berries, ash content and mineral matter (with eigenvector values of 12.126, 11.008, 8.873 and 36.550, respectively) stand out. To visually represent the statistical significance of the separation between individual locations, ellipsoids were included in the first three graphs to indicate where the different localities were analyzed (Table 7).

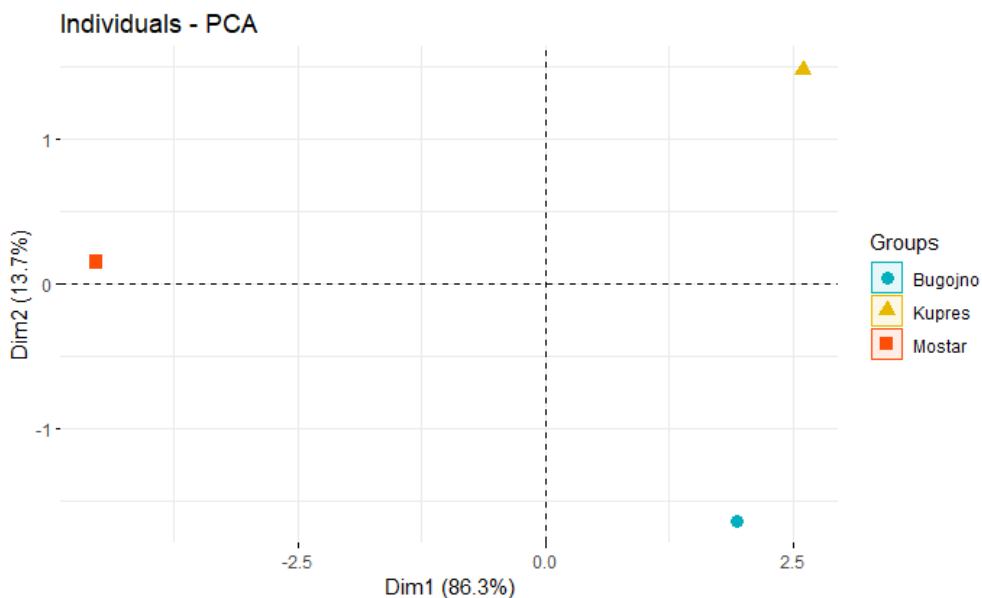
Figures 1 and 2 display the geographical distribution of chokeberry cultivation locations, determined through the utilization of the first two principal components derived from a correlation matrix involving 12 morphological and chemical traits. The analysis indicates a clear distinction among the locations.

Figure 1. Grouping and interrelationships of 12 variables analyzed in three localities, against the first two main components (PC1 and PC2)



Source: Authors

Figure 2. Presentation of locality in relation to the first two main components (PCA1 and PCA2), calculated via correlation matrix for 12 characteristics



Source: Authors

Figure 1 and Figure 3 show that there is a strong positive correlation between the content of total acids, content of total sugars, fruit weight, fruit length and number of berries, also a positive correlation is visible between the content of mineral substances, ash content and the length of leaf petiole. The presence of a negative correlation was recorded between leaf width, leaf length, fruit width and pH.

The vectors representing the original properties are illustrated in the biplot, where the direction signifies the property's value, and the length represents variability level. Along with illustrating the separation of various sites, Fig. 3 further serves as an analytical depiction of the initial characteristics examined.

Figure 3 - Biplot of 3 localities that were the subject of chokeberry research based on morphological and chemical properties of chokeberry by analysis of main components.

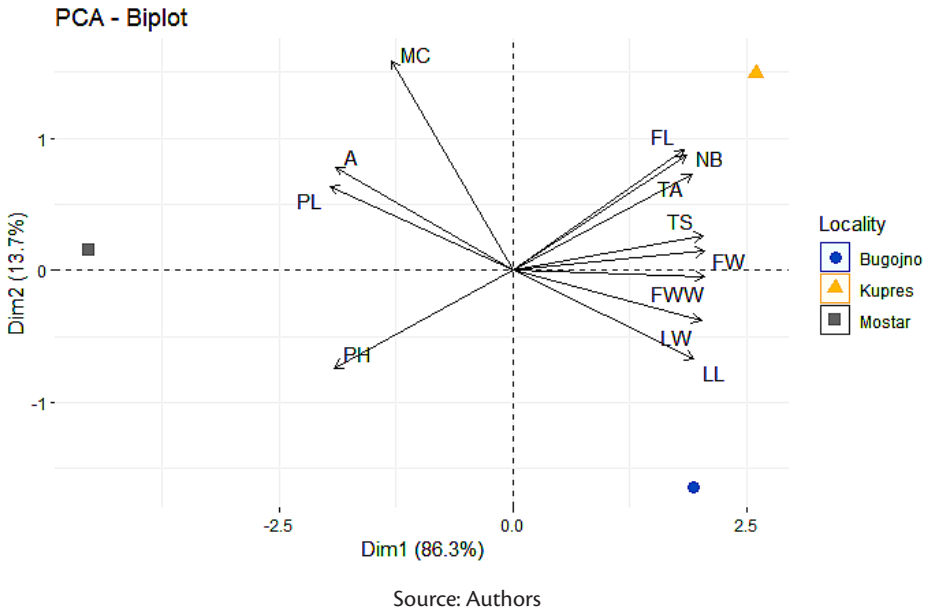
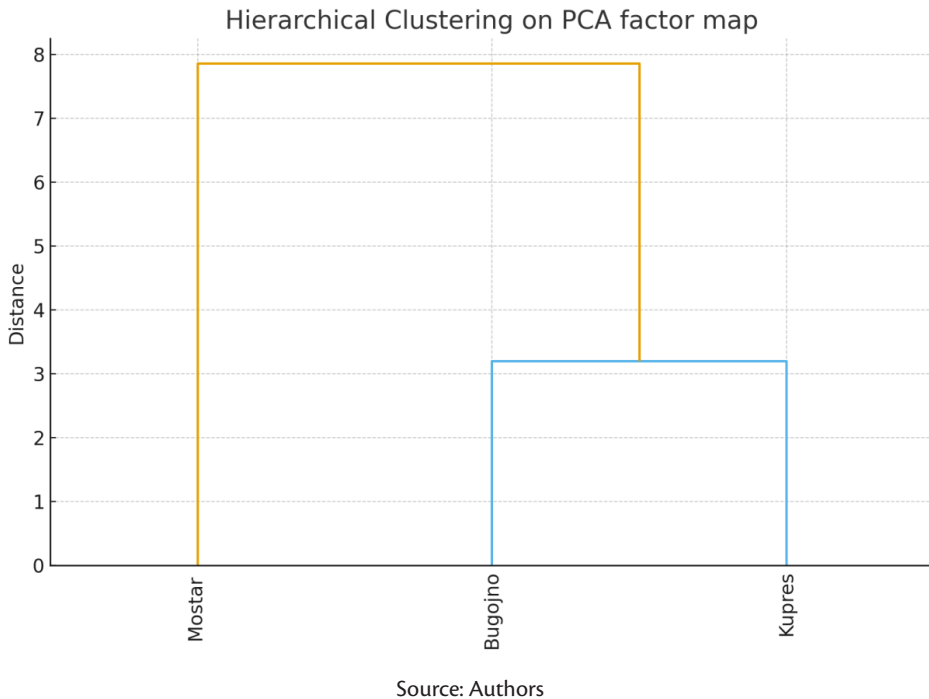


Figure 4. Dendrogram created on the basis of Euclidean matrix - Euclidean distance between average values for 12 quantitative traits in three localities



After the hierarchical cluster analysis was carried out, the existence of locality divergence was evident, which resulted in the separation of all analyzed characteristics into two different clusters (Figure. 4). Only one locality was classified into the first cluster. Two localities were classified into the second largest cluster.

#### 4. CONCLUSION

This study demonstrated that the morphological and chemical characteristics of chokeberry (*Aronia melanocarpa*), Nero cultivar, vary significantly depending on the cultivation site in Bosnia and Herzegovina. At the Kupres locality, fruits were characterized by larger size as well as higher sugar and acid content, while at the Mostar site (Gornji Jasenjani) higher levels of dry matter, minerals and pH were recorded. These findings confirm the strong influence of agroecological conditions on chokeberry quality traits.

The results provide clear evidence that the ecological diversity of Bosnia and Herzegovina offers highly favorable conditions for chokeberry cultivation, with site-specific advantages that can be strategically utilized to optimize fruit production and quality. From a broader perspective, the intensive cultivation of chokeberry in mountainous regions is justified not only due to its considerable commercial value and stable market demand but also because of its potential to improve the efficient use of natural resources and strengthen local agricultural production.

Future research should focus on the long-term monitoring of yield stability, detailed evaluation of bioactive compounds and the development of cultivation practices tailored to specific agroecological zones. Such studies would provide a more comprehensive understanding of chokeberry adaptation and further support its sustainable expansion as a nutritionally and economically valuable fruit crop in Bosnia and Herzegovina.

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#### DECLARATIONS

**Conflicts of Interest** The authors declare that no competing interest exists.

**Ethics Approval** Not applicable.

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# UTJECAJ AGROEKOLOŠKIH ČIMBENIKA NA FENOTIPSKE I KEMIJSKE KARAKTERISTIKE ARONIJE U REGIJI BOSNE I HERCEGOVINE

**Jasna Hasanbegović**

Dr. sc, docentica, Sveučilište "Džemal Bijedić" u Mostaru, Agromediteranski fakultet, Sjeverni logor b. b. 88 104, Mostar, Bosna i Hercegovina; e-mail: jasna.hasanbegovic@unmo.ba

**Aleksandra Šupljeglav Jukić**

Dr. sc, docentica, Sveučilište "Džemal Bijedić" u Mostaru, Agromediteranski fakultet, Sjeverni logor b. b. 88 104, Mostar, Bosna i Hercegovina; e-mail: aleksandra@unmo.ba

**Jasmina Aliman**

Dr. sc, redovita profesorica, Sveučilište "Džemal Bijedić" u Mostaru, Agromediteranski fakultet, Sjeverni logor b. b. 88 104, Mostar, Bosna i Hercegovina; e-mail: jasmina.aliman@unmo.ba

**Semina Hadžiabulić**

Dr. sc, redovita profesorica, Sveučilište "Džemal Bijedić" u Mostaru, Agromediteranski fakultet, Sjeverni logor b. b. 88 104, Mostar, Bosna i Hercegovina; e-mail: semina.hadziabulic@unmo.ba

**Semira Dedić**

Dr. sc, redovita profesorica, Sveučilište u Bihaću, Biotehnički fakultet, Luke Marjanovića bb, 77 000 Bihać, Bosna i Hercegovina; e-mail: semira.dedic@yahoo.com

**Azra Skender**

Dr. sc, redovita profesorica, Sveučilište u Bihaću, Biotehnički fakultet, Luke Marjanovića bb, 77 000 Bihać, Bosna i Hercegovina; e-mail: skender.azra71@gmail.com

**Boris Dorbić**

Dr. sc, izvanredni profesor, Sveučilište u Splitu, Agromediteranski fakultet, Zrinsko-Frankopanska 38, 21 000 Split, Hrvatska; e-mail: bdorbic@unist.hr

**Sezai Ercişli**

Dr. sc, redovni profesor, Sveučilište Atatürk, Poljoprivredni fakultet, Odjel za hortikulturu, 25 240 Erzurum, Turska; e-mail: sercisli@gmail.com

## SAŽETAK

Aronija je poznata po svojim ljekovitim svojstvima zbog jedinstvenih kemijskih karakteristika, na koje utječu različiti čimbenici, uključujući uvjete uzgoja, kvalitetu tla i vrijeme berbe. Kao i većina voćnih vrsta s visokim udjelom vode, aronija ima nisku kalorijsku vrijednost. Specifičan sastav aronije može se neznatno razlikovati u različitim ekološkim uvjetima zbog utjecajnih čimbenika. Istraživanje je obuhvatilo najčešću sortu aronije Nero, na tri različite lokacije uzgoja: Mostar (Gornji Jasenjani), Bugojno i Kupres. Rezultati istraživanja pokazuju da je prosječna visina i širina ploda sorte Nero bila najveća na lokaciji Kupres s 10,92 mm i 12,01 mm, a najniža na lokaciji Mostar (Gornji Jasenjani) s 9,19 mm i 10,02 mm. Kemijski sastav plodova sorte Nero varirao je na različitim lokacijama. Sorta Nero imala je najveći sadržaj ukupnih šećera na Kupresu (6,35%) i ukupnih kiselina (1,36%), dok je na sorti Mostar (Gornji Jasenjani) imala nešto niže vrijednosti ukupnih šećera (5,59%) i ukupnih kiselina (1,06%). Što se tiče sadržaja suhe tvari, minerala i pH vrijednosti, sorta Nero imala je više prosječne vrijednosti na sorti Mostar (Gornji Jasenjani) u usporedbi s Bugojnom. Najniža pH vrijednost (3,45) zabilježena je kod sorte Nero na Kupresu. Rezultati morfoloških i kemijskih karakteristika aronije u različitim agroekološkim uvjetima ukazuju na potencijal za povećanu proizvodnju ove atraktivne voćne vrste u planinskim područjima Bosne i Hercegovine.

**Ključne riječi:** *Aronia melanocarpa*; Nero; morfologija ploda; kemijski sastav; agroekološki uvjeti

