Characteristics of elderberry (*Sambucus nigra* L.) fruit

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Summary

The purpose of this paper is to highlight certain characteristics of the fruits of *Sambucus nigra* L. harvested from five different locations in Iasi County (N-E Romania). The following features were considered: histological (epicarp and mesocarp analysis), morphological (number of fruits, fruits fresh weight), physicochemical (refractometric index and total soluble solid substances - TSS) and biochemical (water and dry matter content, total mineral elements). Referring to the histological characteristics, epidermal cells were found to be rectangular, being prominently elongated in sample L2; the size of the hypodermic cells varied according to the analyzed samples and the mesocarp was thick in L1 and thin in L4 and L5. The number of fruits in corymbiform cymes showed value between 126.4±10.66 and 354.4±30.42. The fruits fresh weight showed values between 0.0967 g and 0.1468 g. The fruits harvested from the location 5 are characterized by high values for the parameters number of fruit/ corymbiform cymes, TSS, organic substance, and low values for the content of water and ashes. The above specified parameters indicate specific variations related to the fruits source location, a high content in water and solid matter, a moderate content in mineral elements, and a strong negative correlation between the water content and the soluble solid matter content.

Key words

Sambucus nigra, epicarp and mesocarp structure, biochemistry

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Introduction

Elderberry (*Sambucus nigra* L.) belongs to family Viburnaceae. It is a shrub species that grows spontaneously in Europe, Asia, North Africa, and USA (Veberic et al., 2009). In Romania, from the plain area up to the low mountain area, the species is frequent in forests (margins and gaps), shrub, and abandoned fields, being mesophilous-mesohydrophilous and eutrophic species (Pârvu, 2006; Sârbu et al., 2013). It is grown in commercial plantations in Romania (Mihaescu, 2007) as well as in some countries in Europe (e. g. Austria, Denmark, Germany, and Hungary) (Vítová el al., 2013).

S. nigra has alimentary, medicinal, ornamenthal, meliferous, and anti-erosional importance (Grădinaru and Istrate, 2004; Pârvu, 2006). The fruits are spheric berries, violet-black, 6-8 mm, ripening in August – September; contain bioactive compounds (carbohydrates, organic acids, vitamins, anthocyanins, etc.) with laxative, analgesic, vitaminizing, antioxidant, and diuretic properties (Pârvu, 2006; Muntean et al., 2007).

Recent studies have highlighted that *S. nigra* fruits extracts have immunomodulatory, antiviral, antibacterial, antioxidant (Osgen et al., 2010), and cardioprotective effects (Ciocoiu et al., 2012). Fruits are also used to make syrups, jam, alcoholic drinks, phytopharmaceuticals, food colorants, etc. (Grădinaru și Istrate, 2004; Pârvu, 2006; Mihaescu, 2007).

Fruit color and fruit pedicle color (in some cases) is due to the presence of anthocyanins, a group of pigments located in vacuoles as glycosides. *S. nigra* fruits are a source of anthocyanins and other polyphenolic compounds with high antioxidant potential (Ősgen et al., 2010; Denev et al., 2013).

Early studies on elderberry fruits highlight the fact that fruit pedicle colour has a specific indicator of fruit quality, being a mediator of communication between these fruits and birds that play a role in seed dispersal. Birds have a preference for red pedicel fruits with higher contrast and indicate higher and more stable rewards than green pedicel fruits (Schaefer and Braun, 2009).

Data on some physico-chemical and biochemical characteristics of *Sambucus nigra* fruits are presented in various studies (Mratinić and Fotirić, 2007; Vulić et al., 2008; Akbulut et al., 2009; Veberic et al., 2009; Denev et al., 2013; Vítová et al., 2013; Diviš et al., 2015).

The knowledge of the morphology, structure, micromorphology of *S. nigra* fruits and the accumulation of bioactive substances in these organs is rather poor.

Anatomical, micromorphological and ultrastructural investigations were performed on fruits from other species of the Viburnaceae family. Studies conducted by Konarska and Domaciuk (2018) on fruits of *Viburnum opulus* and *Viburnum lantana* revealed differences between fruit cuticle thickness and structure, cuticular wax structure, thickness of pericarp layers and cells contained therein, and thickness of the cell walls in these tissues. There were also differences in the content of pigments responsible for the fruit colour as well as the occurrence, location, and content of biologically active compounds (Konarska and Domaciuk, 2018).

The aim of this paper was to analyze the structural and physico-biochemical features of *Sambucus nigra* fruits harvested from different locations in Iasi County (Romania).

The characteristics (morphological, histological, physico-chemical, and biochemical) investigated in the present study are preliminary, representing a starting point for a more detailed study of fruits from different *S. nigra* shrubs from the NE region of Romania.

Material and methods

Plant material

Fruits of *S. nigra* (black elderberry) were harvested from the end of August to beginning of September 2017 at biological maturity from plants growing wild at five different locations situated in Iasi County (the NE part of Romania): location 1 - located in Iași City in area away from pollution sources; location 2 - CA Rosetti Forest – periurban area; location 3 – Pădureni locality; location 4 – Bârnova Forest; location 5- Strunga Forest. Fruits were analyzed immediately after harvesting.

Description of sampling locations

Locations 1 and 2 are positioned in the SE part of Iași County; locations 3 and 4 in S part and location 5 is positioned in SW part of Iași County. The territory of Iași County presents higher areas in the form of massive hills and plateaus with average altitudes between 300-350 m (in the west and south) and lower areas with hilly plain and with medium altitudes of 100-150 m (in the north and northeast) (Report on the State of the Environment in the Iași County (2013).

Table 1. General characteristics of topoclimates in the locations studied

Sampling locations	Topoclimate type	Meteorological station	Average annual temperature	Average annual amplitudes	Frosty days a year	Annual precipitation	Days per year with snow cover
Location 1	Moldavia Plain	Iasi	8-9° C	>24°C	120-140	450-550 mm	< 75
Location 2							
Location 3	Bârlad Plateau	Bârnova Negrești	8-9° C	>24-25°C	110-120	500-650 mm	< 75
Location 4							
Location 5	Suceava Plateau	Cotnari Strunga	8-9° C	>22-24°C	140	500-700 mm	< 75

Under climatic aspects (Bogdan et. al., 1977; Apostol, 1990; Margarint, 2010), the five locations belong to an area that is located in the type of temperate transition climate specific to the east of Europe. The relief of plateaus and plains from this area induce local climate characteristics. Both the varied arrangement of the slopes to the general direction of the movement of the sun, as well as the level differences of approx. 250-300 m altitude lead to the generation of topoclimates. The studied area climate is monitored by five meteorological stations: Iași (located near to the airport of Iași), Bârnova (located in the Bârnova Forest), Cotnari (located in Cotnary, NE to the Strunga Forest), Roman (located north of the Roman City, at the SW of the Strunga Forest) and Negrești (Vaslui County, south of the Bârnova Forest).

There are three topoclimates in this area: topoclimate of Moldavia Plain (locations 1 and 2), topoclimate of Bârlad Plateau (locations 3 and 4) and topoclimate of Suceava Plateau (location 5). The general characterization of these three topoclimates is presented in Table 1.

The pedological cover (Margarint, 2010) associated with the three topoclimates fit to the specific forest soil from the nemoral area, belonging to the class of luvosols for the areas occupied by the Bârnova Forest formations and the area of Padureni (locations 3 and 4), a mixture of luvosols and cernisols (cambic chernozems) in the area of the Strunga Forest (location 5) and cambic chernozems and erosion regosols in the area of locations 1 and 2.

For the year 2017 climate data was downloaded from the website Meteomanz.com (www.meteomanz.com).

The fruits were analysed histologically, morphologically, physiochemically and biochemically.

Histological analyses

The fruit samples were analysed through specific methods of histo-anatomy. For histological analysis of epicarp and mesocarp cross sections were made through fruits using a microtome and a botanical razor. The sections obtained were stained with green iodine and red ruthenium, analyzed with optical microscope Holland (Novex) and photographed with a digital camera Sony Cyber-shot. For each fruits sample three sections were analyzed.

Morphological and physical-chemical characteristics of the fruits

The morphological characteristics analysed were: the average number of fruits in corymbiform cymes, the average of fruit fresh weight, the weight of 100 fruits. The fresh weight was determined by digital balance. Ten inflorescences were taken in the study for each sampling location.

Physico-chemical parameters established by the experimental model were: the refractometric index and the total soluble solids content (TSS) of juice obtained by manual pressing of fruit. These indicators were determined with by Abbe refractometer AR4; the results are expressed in % Brix.

Biochemical parameters

The following parameters were determined: the dry matter content and water – through gravimetrical methods; total mineral elements contents (ash) – through dry calcinations at 550°C (Boldor et al., 1983); the organic substance content (calculated through the difference between the dry matter content and ash). The obtained results are expressed in: g dry matter / 100 g fresh material analysed and respectively, g calcined residue / 100 g dry material analysed. The obtained results for physico-chemical and biochemical parameters represent the average of six consecutive determinations.

Statistical analyses

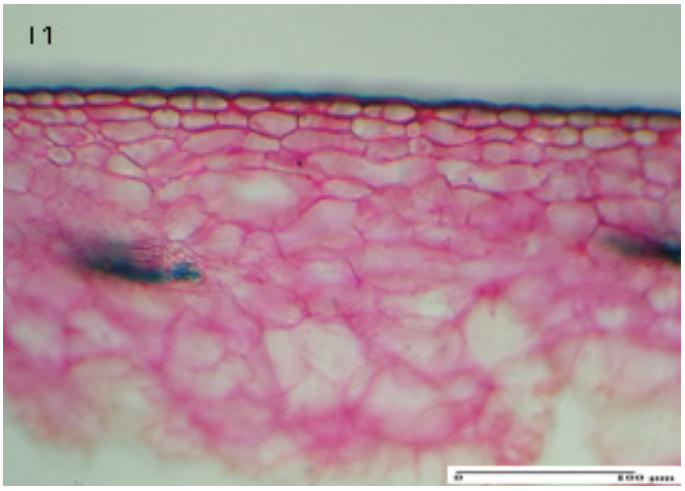
Means and standard error (±SE) and deviations (±SD), coefficient of variation, Person's coefficient of correlation (for some parameters) were calculated using the Microsoft Excel software. The statistical analysis was made for morphological, physico-chemical and biochemical characteristics.

Results and discutions

Histological characterization of fruits

Fruits harvested from the location 1 (L1) - Fig. 1.

The epidermis consists of small, rectangular cells, covered by a relatively thin cuticle. Hypodermis is relatively thin, consisting of tangential elongated cells. The mesocarp is thick consisting of relatively large cells (L1).



Fruits harvested from the location 2 (L2) - Fig. 1.

The epidermis was composed of cells elongated tangentially, covered by a relatively thick cuticle. The hypodermis is thin, formed by tangential elongated cells (L2).

The mesocarp has an average thickness, with smaller cells.

Fruits harvested from the location 3 (L3) - Fig. 1.

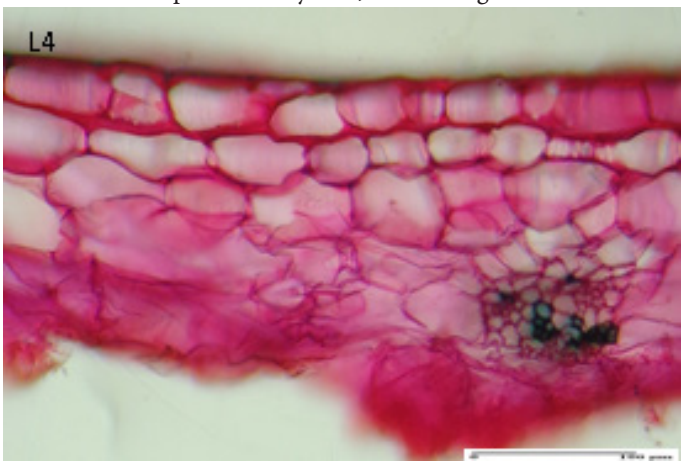
The epidermis consists of relatively large, slightly elongated cells, covered with a thin cuticle. Hypodermis is relatively thin, consisting of slightly elongated tangential cells (L3).

The mesocarp has average size cells.

Fruits harvested from the location 4 (L4) - Fig. 1.

The epidermis consists of slightly elongated cells tangentially, covered with a thin cuticle. Hypodermis is relatively thin, consisting of slightly elongated tangential cells (L4).

The mesocarp is relatively thin, with average size cells.



Fruits harvested from the location 5 (L5) - Fig. 1.

The epidermis consists of relatively small cells, slightly elongated tangentially, and covered with a thin cuticle. Hypodermis is relatively thick, consisting of isodiametric cells (L5).

The mesocarp is thin, with average size cells.

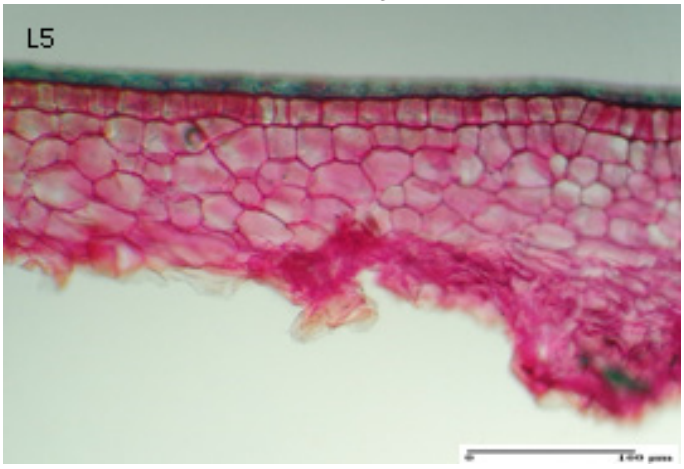


Figure 1. Cross sections through fruits of *Sambucus nigra* collected from different areas: L1. Iași City: L2. C.A. Rosetti Forest; L3. Pădureni locality; L4. Bârnova Forest; L5. Strunga Forest

Based on the information above, it can be observed that there are variations in the ratio of epicarp and the mesocarp of the analyzed samples from the five locations.

This reflects the complementary development of the protective peripheral complex (with a role in ensuring fruit resistance and storage capacity) in relation to the deeper tissue layers of the fruit (with larger cells, with vacuoles having a more water-rich vacuolar juice). On the other hand, these variations may be correlated with possible mechanisms of adaptation to the microhabitat conditions from which the fruits originated. At the same time, these tendencies of histological structure may be correlated with the genetics of taxa taken in the analysis.

However, the observed histological characteristics require further detailed investigations.

Overall, comparative histological analysis of fruits from the five locations highlights the following features: the epidermal cells

Table 2. Morphological and physical-chemical parameters of *Sambucus nigra* fruit collected from different locations

Sampling locations	The average number of fruit / corymbiform cymes	The average fresh mass of fruit (g)	The fresh mass of 100 fruit	Refractometric index	TSS (% Brix)
Location 1	126.4±10.66	0.1019±0.0	10.19	1.3510±0.0	11.8±0.33
Location 2	142.8±17.51	0.1110±0.0	11.10	1.3532±0.0	13.46±0.30
Location 3	173.8±25.85	0.0967±0.0	9.67	1.3530±0.0	13.5±0.29
Location 4	141.9±12.81	0.1468±0.0	14.68	1.3530±0.0	13.63±0.31
Location 5	354.4±30.42	0.1076±0.0	10.76	1.3594±0.0	17.16±0.09
Mean	187.66	0.1128	11.28	1.3539	13.91
CV %	50.21	16.96	17.46	0.22	14.05

C V % - coefficient of variation; mean values ± standard error

Table 3. Biochemical parameters of *Sambucus nigra* fruit collected from different locations

Sampling locations	Water (g %)	Dry matter (g %)	Total mineral elements (g %)	Organic substances (g %)
Location 1	83.93 ±0.38	16.06±0.38	6.36±0.19	9.70±0.47
Location 2	82.12 ±0.33	17.87±0.33	6.16±0.45	11.70±1.04
Location 3	78.55 ±0.05	21.44±0.05	6.73±0.36	14.70±0.45
Location 4	81.10±0.76	18.89±0.76	9.19±1.27	8.76±1.60
Location 5	71.36±0.3	28.48±0.3	5.93±0.33	21.68±0.72
Mean	79.41	20.54	6.87	13.30
CV %	6.17	23.56	19.21	39.09

C V % - coefficient of variation; mean values ± standard error

were in all cases rectangular, being visibly elongated tangentially in sample L2. Large epidermal cells were found in samples L3, L4, and smaller in epidermal cells of samples L1 and L5. The hypodermis cells increased in the order: L1, L2, L3, L5, L4. The mesocarp was thick in L1 and thin in L5 and L4.

Morphological, physical-chemical and biochemical characteristics of fruit

Morphological, physical-chemical and biochemical parameters of fruits harvested from *S. nigra* are presented in the Tables 2 and 3.

The average number of fruits in corymbiform cymes presented the lowest average value in the samples of fruit harvested in location 1 and the highest value in the samples of fruits harvested in location 5. Other authors have reported similar or lower values for this parameter: 100-220 by Ważbińska and Puczel (2002) and 131-280 by Mratinić and Fotirić (2007).

The average fresh mass of fruit was from 0.0967±0.0 g (location 3) to 0.1468±0.0 g (location 4) (Table 2). Mratinić and Fotirić (2007) have reported average fresh mass from 0.13 g to 0.21 g. Akbulut et al. (2009) have determined values from 0.14 g to 0.20 g in fruits from samples grown wild in Turkey.

The fresh mass of 100 fruits was the highest in the samples harvested from location 4 (14.68 g) (Table 2). Ważbińska and Puczel (2002) have reported the mass of 100 fruit being from 13.2 g to 16.2 g in the samples grown wild in Poland (research from 1998 to 2000). Propaczi and Laszlo (1984, quoted by Wasbinska et al., 2007) have reported a higher variation of the fresh mass of 100 fruits (from 9 g to 45 g).

According to Wasbinska et al., (2007), black elderberry grown on soil poor in mineral elements and humus soil produces less and smaller fruit.

There were lower value variations in refractometric index in this study.

The content of total soluble solid substances (TSS) had lower average values in the samples from the locations 1-4 comparing with the samples from the location 5. The soluble solid substances in the cellular juice are represented by simple carbohydrates and other groups of substances (organic acids, tannins, pigments, mineral salts, etc.). The content of total soluble solid substances is considered an indicator for fruit maturation. The results obtained are in compliance with the data presented in other studies (Mratinić and Fotirić, 2007; Akbulut et al., 2009; Garofulic et al., 2012; Vukovic et al., 2016).

The analysis of the results obtained regarding the water content in fresh fruits indicates values between 71.96±0.3% and 83.93±0.38% (Table 3). In general, the fruits of the shrubs with edible fruits have a high content of water (Mladin and Mladin 1992; Marjanovic–Balaban et al., 2012). There was a strongly significant negative correlation (r = -0.960*) between the water content and TSS.

The content of dry substance presented amplitudes opposite to those described in relation with the water content (Table 3). Vulic et al. (2008) has reported a value of 20.22% for dry substance in fruit of elderberry in samples grown in Serbia.

The content of total mineral elements was from 5.93±0.33% (location 5) to 9.19±1.27% (location 4) (Table 3). In the literature, it is mentioned that the mineral elements present in elderberry fruit are potassium, calcium, magnesium, phosphor, sodium, iron, manganese, zinc and copper (Vulic et al., 2008; Diviš et al., 2015). The mineral elements are found in various forms: anions or cations, salts of inorganic and organic acids, and complex organic combinations (Neamțu et al., 1993). The content of ashes can be a criterion for estimation of the biological value of fruit (Vulić et al., 2008).

The content of organic substances had lower average values in the samples from the locations 1-4 compared with the samples in location 5. The black elderberry fruits are considered a good source of organic compounds (Divis et al., 2015). The organic substances in the black elderberry fruits are carbohydrates (simple, soluble, poly-carbohydrates), lipids, amino acids, proteins, organic acids, vitamins, phenolic compounds (flavonoids, phenolic acids, anthocyanins), etc. (Pârvu, 2006; Muntean et al., 2007; Veberic et al., 2009).

The fruits harvested from the location 5 were characterised by high values for the following parameters: number of fruits/ corymbiform cymes, TSS, and organic substance; and low values for the content of water and ashes.

The coefficient of variation had moderate values, from 14.05% to 23.56% for most of the analysed parameters. The parameters number of fruits/ corymbiform cymes crown and the content of organic substance had higher variability (CV% = 50% and 39.9%, respectively), and the water content had low variability (CV% = 6.17%).

Table 4. Characteristics of topoclimates in the studied locations in 2017

Sampling locations	Topoclimate type	Meteorological station	Average annual temperature	Average annual amplitudes	Frosty days a year	Anual precipitations	Days per year with snow cover
Location 1	Campiei Moldovei	Iasi	11.3° C	> 27.6° C	77	510 mm	35
Location 2							
Location 3	Podișului Bârladului	Bârnova	9.8° C	> 26.7° C	89	704.4 mm	31
Location 4		Negrești	10.6° C	> 27.9° C	91	513.8 mm	24
Location 5	Podișului Sucevei	Cotnari	11.3° C	> 27.4° C	72	509.4 mm	32
		Strunga	10.4° C	> 27.9° C	90	580.1 mm	13

Meteorological parameters

In 2017 for the meteorological stations that characterize the topoclimate of locations 1-5 there were recorded average annual values (Table 4) above and below the general values characteristic of each type of topoclimate (presented in Table 1): the average annual temperature and average annual amplitudes were higher; the number of frost days per year and the number of days with snow cover per year were much lower and the annual rainfall ranged from the range of values characteristic of each type of topoclimate.

In research area black elderberry leaves appear in March, the flowers in May-June and the fruits reach maturity at the end of August and beginning of September. The average monthly temperature values for the vegetation season, the March-August interval, aproximately for the 5 locations are presented in Fig. 2. The average (monthly and maximum) temperatures showed the highest values for locations 1 and 2 and the lowest values for locations 3 and 4; the temperature difference between these locations did not exceed 1.5°C. The amount of precipitation during the vegetation season was the highest for locations 3 and 4 and the lowest for location 5. The lowest amount of precipitation was recorded in August (Fig. 3).

It is known that temperature and precipitation are factors that influence plant growth and development. After Mladin and Mladin (1992), black elderberry are characterized by a great adaptation to climate and soil conditions. In the present study we can not determine the influence of climatic conditions on the characteristics of the analyzed fruits.

Previous studies of various black elderberry and american elderberry genotypes (Thomas et al., 2013, 2015) show that black / american elderberry genotypes can be highly responsive to environmental conditions that vary from one season to another and from one site to another. Studies conducted in other species of fruit trees indicate that climatic conditions during the year may influence the characteristics of the fruit (Chiarucci et al., 1993). The chemical composition (water and dry matter, ash, organic compounds, etc.) of the fruits is influenced by the environmental conditions in the plant growing area, genotype characteristics and stage of fruit ripening (Özcan, 2008).

Based on literature data, we consider that specific variations of the parameters analyzed may be due to the interrelationship between several factors: specific microhabitat conditions, genotype, plant age, and fruit maturation stage.

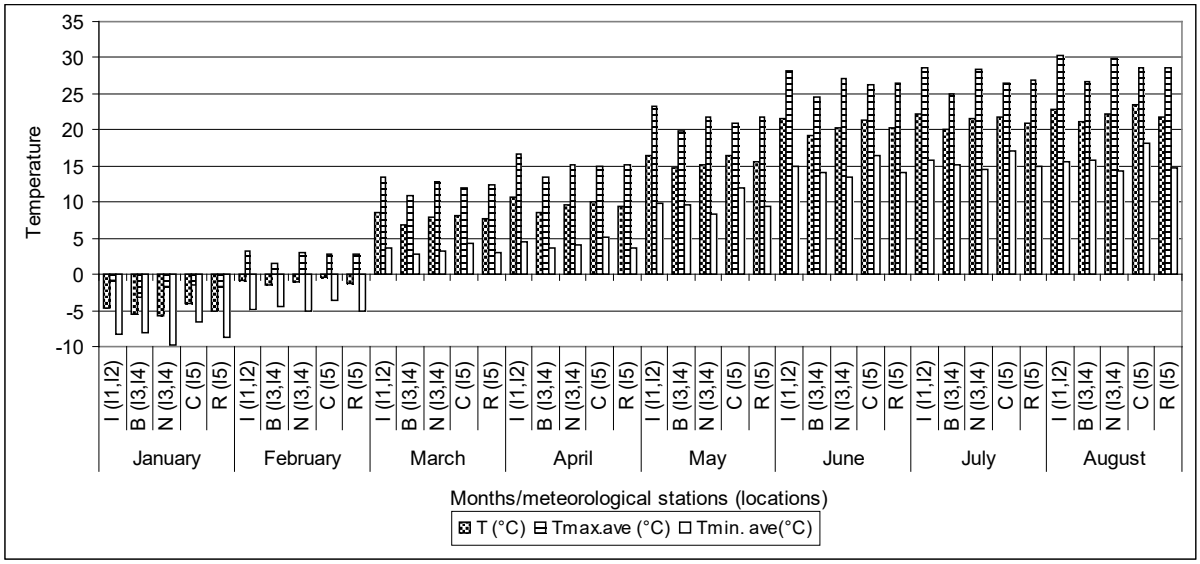


Figure 2. Air temperature for 2017 (I, B, N, C, R- meteorological stations: Iași, Bârnova, Negrești, Cotnary, Roman; l1, l2, l3, l4, l5 – locations)

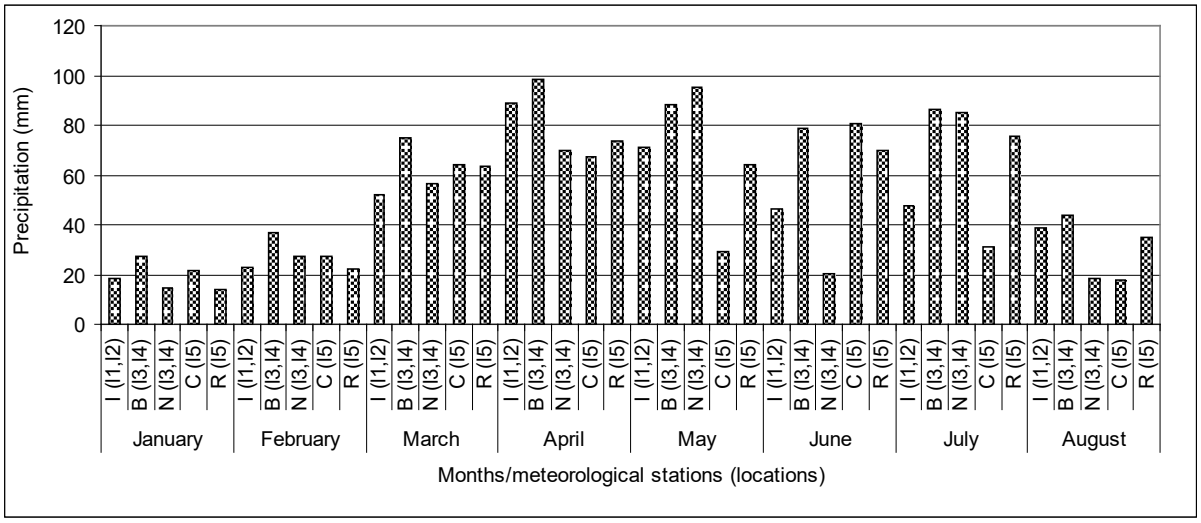


Figure 3. Precipitation for 2017 (I, B, N, C, R- meteorological stations: Iași, Bârnova, Negrești, Cotnary, Roman; l1, l2, l3, l4, l5 – locations)

Conclusions

The results obtained underline the existence of a possible interrelation between the analysed characteristics of the fruits collected from the five different locations.

The results obtained underline, for all the samples of fruits investigated, specific variations of the parameters researched, the values determined presenting comparable amplitudes for each parameter separately.

We believe that additional, more complex investigations over several years and sites with different pedoclimatic conditions are needed to highlight a possible interrelation between the studied characteristics of the fruits and pedoclimatic conditions.

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