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ORIGINAL RESEARCH PAPER

BILBERRY (*VACCINIUM MYRTILLUS*) AND ELDERBERRY FRUIT (*SAMBUCUS NIGRA* L.) FROM ROMANIA PROCESSED AS CONFITURE. EVOLUTION OF COLOR, TOTAL CONTENT OF ANTHOCYANIN AND VITAMIN C

Maria Lidia Iancu[1,*], Valentina Mădălina Moga[2], Mihaela Adriana Tița[1], Ovidiu Tița[1]

[1]*Lucian Blaga University of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environmental Protection, 5-7, Ion Raţiu Street, Sibiu, 550012, Romania*
[2]*Doctoral school of the Lucian Blaga University of Sibiu, 5-7, Ion Raţiu Street, Sibiu, 550012, Romania*

*Corresponding author: *maria.iancu@ulbsibiu.ro*

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Abstract: The subject of the present paper is the influence of sugar preservation and heat treatment on the total content of anthocyanins, vitamin C, and the color of the confiture. In Romania, bilberry confiture is in demand while that made from wild black elderberry is almost non-existent for most people. Thus, a comparative analysis of the two varieties of confiture has been carried out taking into consideration the similarity of the macroscopic characteristics of the raw materials, namely the forest fruits. Thus, it has been established that the vitamin C content decreased by 81 % in the bilberry confiture (BC) and by 52 % in that of wild elderberry, as compared to the vitamin content in the fruit. The total content of anthocyanins decreases by 45 % in the BC sample and by 25 % in the elderberry confiture (EC). The values of the color parameters showed a trend towards dark brownish-red with a distinct color difference (ΔE^* = 2.01) between the two varieties of confiture. Despite the heat treatment and the addition of sucrose in the BC and the EC made from Romanian fruits, wild flora can still be considered to be a valuable source of phytonutrients. The values of the primary quality indicators of the wild elderberry confiture are very similar to those of the variety made from Romanian bilberries.

Keywords: *bilberry, color, elderberry, preserving with sugar, total anthocyanin content, vitamin C*

INTRODUCTION

Confiture is an extremely popular food product which is consumed all over the world. According to the Codex Alimentarius, confiture is defined as the concentrated product brought to an appropriate consistency, made from whole fruits that are mixed with sugar and preserved by anabiosis, osmoanabiosis, where the method of long-term preservation is based on using sugar [1]. Bilberries and respectively, black elderberries can be used highly successfully in the preparation of confiture.

Bilberries (*Vaccinium myrtillus*) are known in Europe and grow in mountain areas, being harvested from July to August. They are used both in the food and pharmaceutical industries [2]. The annual production in Europe is 1,113.26 t including both forest and cultivated blueberries [3]. They are especially valuable because they are rich in phenolic compounds, anthocyanins, proanthocyanidins [4]. Their health benefits are well known, as they contribute to improving vision, reducing glycemia, and improving peripheral blood circulation, with substantial antitumoral activity [5 – 8]. The anthocyanin content is present in the whole bilberry [9]. In Romania, bilberries are harvested every year and are used for the preparation of confiture, alcoholic beverages, juice, dried or processed as powder, and natural anthocyanin dyes.

Wild black elderberries (*Sambucus nigra* L.) have entered the people's diet in recent years. They are small, and black, resembling forest blueberries, and can be successfully used in traditional medicine [10]. Similar to the blueberries, the elderberries are rich in phytonutrients and contribute to the alleviation of the same diseases, such as diabetes, poor venous circulation, and cancer [11]. Black elder is used the most in the world, traditionally in the preparation of drinks, jam, and jelly [12, 13]. However, it is recommended not to consume it raw because it contains sambunigrin (*cyanogenic glycoside*) which is an antinutrient with a toxic potential. But this component can be degraded through thermal treatment and thus unwanted side effects can be eliminated [14].

Therefore, if bilberry confiture is a very popular food product in Romania, it seems that elderberry confiture is not. The fact that the elderberry and the bilberry are very similar in terms of their morphological characteristics, led to the idea of conducting this research.

The main objective of the research is to conduct a comparative analysis of the values of the total content of anthocyanins, and vitamin C, to observe the evolution of the color parameters from the wild elderberries, and Romanian forest bilberries to the confiture made from these fruits The collateral objectives were the production of the confitures, the analysis of the raw material and the finished products, the observation and comparison of the values of other primary and specific physico-chemical indicators, and the sensory analysis.

MATERIALS AND METHODS

Materials

For the confiture preparation and analysis, black elderberries and wild bilberries were used. They were harvested in August 2023 from the mountain area around Sibiu and the meadow of Cibin river in Romania. They were sorted, washed and cryopreserved, and kept frozen at - 18 ºC for 2 months. The sugar used was commercially purchased. The raw materials used for the working variants in the present study are forest bilberry (FB) and forest elderberry (FE).

Obtaining confiture

The two varieties of confiture, that of bilberry (BC) and that of elderberry (EC) were made according to a common recipe. Elderberries and separately bilberries were mixed with sugar in a 1:1 ratio. Acid was excluded from the recipe due to the high acidity of fruit as a raw material. The amount of water used in the recipe was 30 % of the amount of sugar. The technological scheme used was similar in both cases, as was the installation, for the two varieties of sweetness. The concentration was carried out at atmospheric pressure in a concentrator with mixing ability and a maximum adjustable temperature of 106 ºC. The hot diffusion technique was used after the first 20 minutes of boiling. The operation was complete when the value of the soluble dry matter content determined refractometrically showed minimum 65 %. The final product was packed in jars when cold to maximize the diffusion effect of sucrose into the plant cell.

Methods of analysis

The appropriate choice of method of analysis was made in such a way as to highlight the quality indicators that change the most. Analyses were carried out on the raw material, during the stages of production as well as on the finished product. The determinations carried out were the following: the technological characterization of the fruits [15]; the titratable acidity (TA)(g/100 g expressed as citric acid) [16]; the total soluble solid (TSS) (ºBx) (the *Krüss* refractometer connected to a *Brookfield* ultra-thermostat bath with external circulation, Germany) [17]; the moisture (u) (%) and dm (%) (100-u) (the thermobalance A&D MF 50) [18]; the *p*H (pH-meter Orion type 2-STAR-England) [19]; the available moisture (a_w) (LabMaster, Novasina) [20]; the consistency, (cm) (Bostwick consistometer [21], the vitamin C content (iodometric method) [22], the net weight (kg) and the fruit percentage [23].

Total anthocyanin content (TAC)

The differential *p*H method was used, both for the fruits used and for the prepared confiture [24]. Samples were taken from the obtained extracts and read using a UV/VIS spectrophotometer, showing the following values: the absorbance value at *p*H 1 and at *p*H 4.5, at the wavelength λ = 510 nm and λ = 700 nm. The results were expressed in mg·L^{-1} cyanidin-3-glycoside, using formula 1:

$$TAC = \frac{A \cdot MW \cdot DF \cdot 10^3}{\varepsilon \cdot l}, \text{ mg}\cdot\text{L}^{-1} \text{ cyanidin - 3 - glucoside} \quad (1)$$

where: A– the absorbance calculated with the absorbance values read on the spectrophotometer and calculated using formula 2:

$$A = [(A_{510} - A_{700})_{pH=1} - (A_{510} - A_{700})_{pH=4,5}] \quad (2)$$

MW is the molecular weight of the corresponding anthocyanin (449.2 g·mol^{-1} for cyanidin-3-glucoside (cyd-3-glu); DF is the dilution factor (15); 10^3- conversion from g to mg; l - optical path (1 cm); ε is the molar extinction coefficient 3 glucoside (26900 L·mol^{-1}·cm).

Instrumental analysis of color

EasyMatch QC (A60-1012-402) was used. It is a comprehensive color quality control package designed for Windows operating systems. It is a spectrophotometer used for color measurements. Thus, the measurements were made in the CIE lab coordinate system: L^*CIE lightness coordinate (0 = blackness; 100 = whiteness), a^* CIE red (+)/green (-) color attribute, b^* CIE yellow (+)/ blue (-) color attribute. The following measures were calculated: C*– the chroma using equation 3 [25]; h*- the hue angle, using equation 4. To capture this variation in a single index that would be correlated to a brown color, BI is calculated using expressions 5 and 6 [26].

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{3}$$

$$h^* = tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{4}$$

$$\text{X} = \frac{(a^*+1{,}75L^*)a^*}{5{,}645L^*+(a^*-3{,}012b^*)} \tag{5}$$

$$BI = \frac{100(X-0.31)}{0.17} \tag{6}$$

Total color difference

Color changes can be measured as the vector modulus of the distance between the initial color values and the actual color coordinates. This concept is called the total color difference. Total color difference (ΔE*) indicates the difference in color from one sample to another, calculated as ΔE* [27- 28].

$$\Delta E^* = (\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})^{1/2} \tag{7}$$

Perceptible color differences can be analytically classified as ”very distinct” if $\Delta E^* > 3$, ”distinct” if $1.5 < \Delta E^* < 3$, and ”little distinction” if $1.5 < \Delta E^*$ [29].

Sensory analysis

The sensory analysis was carried out to choose the favorite assortment of confiture and to identify which one of the two samples is the elderberry one. Questionnaires were filled in by a total number of 21 panelists, aged between 18 and 38 years, boys and girls, students of the *Faculty of Agricultural Sciences, Food Industry and Environmental Protection* from the "*Lucian Blaga*" University in Sibiu. The training of the panelists lasted 30 days, 2 hours per week. Work was done under sensory analysis laboratory conditions. The characteristics of the elderberry and bilberry confiture as well as those of the fresh fruit were assessed. The characteristics were defined, the limits were fixed, and the differences between the samples were identified. The method with the hedonic scale is preferential and presents a high degree of subjectivity. Each characteristic (color, smell, taste, aroma, appearance) of the finished product was evaluated [30]. The level of assessment of the characteristic will be done according to a 9-point hedonic scale, as follows: 9- like extremely; 8- like very much; 7- like moderately; 6- like slightly; 5- neither like nor dislike; 4- dislike slightly; 3- dislike moderately; 2- dislike very much; 1- dislike extremely. The samples to be analyzed were coded (148 - bilberry confiture and 119 - elderberry confiture) and presented for tasting in small quantities in sealed, transparent containers.

Statistical analysis

The values that were obtained for each determined parameter were statistically analyzed using the factor analysis of variance (type of confiture). The mean value from n = 3, the deviation from the mean, the squared probable error, the mean squared error, the mean squared error of the selection mean, and confidence interval were calculated, the tabular "*t*" test was used for a 0.05 significance level and two degrees of freedom and then the actual value of the quality indicator was calculated.

RESULTS AND DISCUSSION

Physico-chemical characteristics of bilberries and elderberries

Table 1 shows the values of the primary physicochemical indicators of forest bilberry and black elderberry fruits and Figure 1 shows their macroscopic characteristics.

***Table 1**. Physico-chemical characteristics of the bilberry and elderberry fruits*

Characteristic	Fruit	
	FB	FE
Weight 100 berry [g]	40.8 ± 0.209	39.26 ± 0.19
Volume [cm^3]	40 ± 0.87	38 ± 0.63
Average diameter [mm]	7 ± 2.29	4.9 ± 0.11
Shape	spherical	spherical
Available moisture [a_w]	0.912 ± 0.00005	0.912 ±0.00048
Moisture [%]	81.46 ± 2.3	72.6 ± 0.41
Total dry Matter [%]	18.54	27.4
Total solubile solid (TSS) [°Bx]	10.6 ± 0.19	10 ± 0.11
Titratable acidity (TA)^b [g x100^{-1}]	1.38 ± 0.026	1.16 ± 0.12
*p*H	4.26 ± 0.07	5.46 ± 0.05
(TSS/TA)^c	7.68	8.62

Bilberry fruit (FB); Elderberry fruit (FE)
a - (100 – u) calculated from the average humidity value
b - expressed as citric acid calculated using the average values and therefore a variation of it does not appear
The student's "*t*" test was used and each experiment was observed 3 times (n = 3) resulting in the mean ± standard deviation
c - Total soluble solid (TSS); Titratable acidity (TA)

These values are real and influenced by the genotype, the time of the harvest, and the storage conditions. The values of some of these indicators were found in the literature, such as those for the elderberries: the total soluble solid 8.6 - 15.8 %, the titratable acidity 0.6 - 1.7 g/100 g, the total dry matter 18.5 - 22.9 w/w [31]. In the case of the bilberries, the total dry matter content is 14.5 g/100 g [31]. A great similarity between the fruits chosen for this research is observed.

The manufacturing technological schemes were applied for the bilberry confiture, and for the elderberry one. The production yield was 84.46 % for the bilberry confiture and 86 % for the elderberry confiture. The total losses were insignificant because the technological loss of removing the inedible parts is non-existent for these types of fruit.

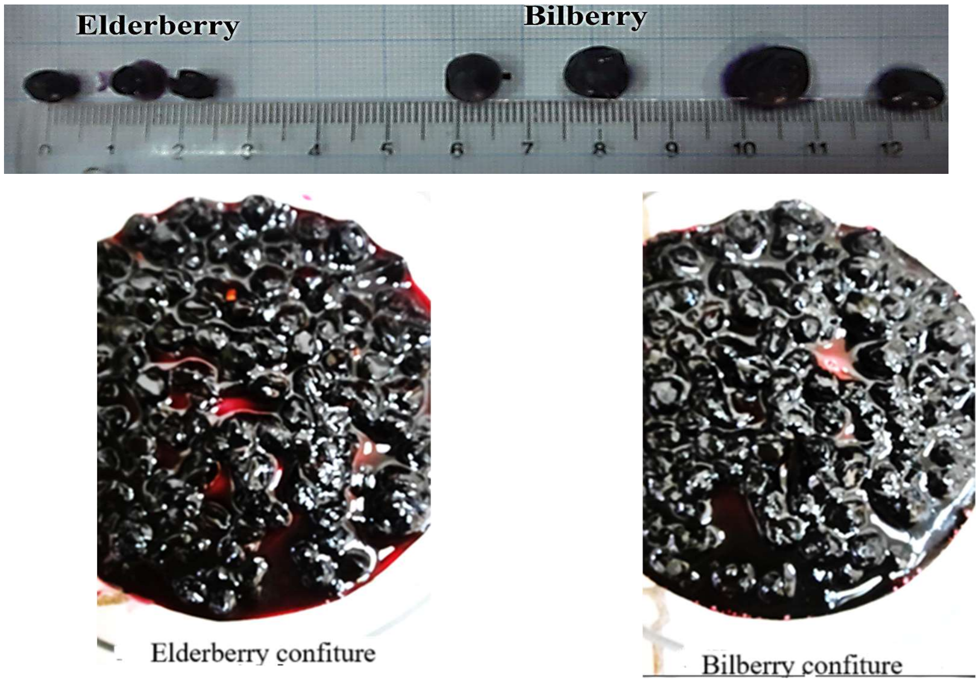

Figure 1. *Macroscopic characteristics of the bilberry, respectively elderberry and the finished product 119 (elderberry confiture-CFE) and 148 (bilberry confiture-CFB)*

Physico-chemical characteristics of the confiture

The primary indicators for the bilberry confiture and the elderberry confiture are separately presented. They are the first to change at the slightest change in the manufacturing recipe or the used raw material. Some of them are shown in Table 2.

Table 2*. Physico-chemical characteristics of the elderberry confiture and the bilberry confiture*

Characteristics	Sample	
	CFB	CFE
Net weight [kg]	0.224 ± 2.74	0.226 ± 2.1
Fruit content [%]	57.12 ± 0.0005	57.84 ± 0.0048
Total solubile solid (TSS) [ºBx]	64.46 ± 0.96	66 ± 0.81
Available moisture [a_w]	0.648 ± 0.015	0.682 ± 0.004
Consistency [cm]	16.4 ± 0.47	12 ± 0.5
Titratable acidity (TA)[a] [g x 100^{-1}]	0.32 ± 0.002	0.33 ±0.001
(TSS/TA)[b]	201.43	200

Bilberry confiture (BC); Elderberry confiture (EC)
a - as citric acid
b - calculated using average values, Total soluble solid (TSS); Titratable acidity (TA)
The student's "*t*" test was used and each experiment was observed 3 times (n = 3) resulting the mean ± standard deviation

The preservative action of the sugar is seen in the value of the water activity (a_w) which decreased by 28.9 % in the processing of bilberries and by 25.2 % in the case of elderberries. The value of this indicator gets lower as the content of the soluble dry matter gets higher. The evolution of the main quality indicators is as follows: increased consistency, decreased water activity, and increased soluble dry matter. This behavior is valid for both experimental variants. It could not be said that these values are influenced by the biological nature of the fruit. As it is observed, they are within the same range of specific values for the confiture.

The total content of anthocyanins, vitamin C

The evolution of the vitamin C content is significant. These raw materials are sources of vitamin C. Maintaining vitamin C under boiling conditions, under the action of oxygen, with a high concentration of sugar is a challenge. These results can be seen in Figure 2. It is a small content of vitamin C compared to the one in fresh fruits, but it must be taken into account that the fruits have been frozen and kept as such for 2 months. Vitamin C is an important quality indicator, sensitive to the action of oxygen and temperature. A decrease in the vitamin C content by 81 % in the forest bilberry confiture and by 52.8 % in the elderberry confiture is observed compared to the vegetable raw material. Parveez [32] argued that there is a decrease in vitamin C content by 32 - 67 % when processing the bilberries by drying After 2 hours of heating at acid *p*H, the content of vitamin C in the elderberry extract is 10.33 mg·L^{-1} [33].

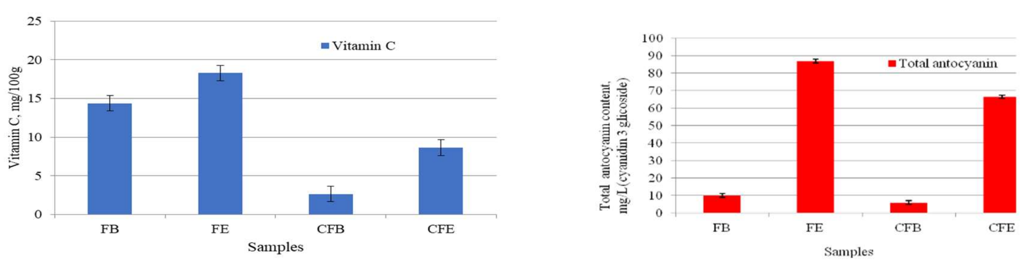

Figure 2. *Evolution of the content of vitamin C and total anthocyanins content from raw material to bilberry and elderberry confiture: Bilberry confiture (CFB); Elderberry confiture (CFE)*

The student's "t" test was used and each experiment was observed 3 times (n = 3) resulting the mean ± standard deviation

The evolution of the total content of anthocyanins, which are important biochemical components and considered natural pigments, is shown in the graph in Figure 2. The content of anthocyanins in the bilberries used in this research is low (10.05 mg·L^{-1} cyanidin-3-glucoside equivalent) due to the decrease during storage but may also be influenced by the genotype and the time when they were harvested. In the literature, a study on drying at high temperatures, showed that the values of this indicator in bilberries range from 6.74 to 17.30 mg·g^{-1} cyanidin-3-glucoside equivalent [32].

The anthocyanin content of the elderberries used in this research is 86.91 mg·L^{-1} cyanidin-3-glucoside equivalent. These values are comparable to those in literature. It has been proven that the values of this indicator in the elderberries range from 8.33 to

89.9.30 mg·g^{-1} cyanidin-3-glucoside equivalent [31] or 3071 mg·g^{-1} /·100 cyanidin-3-glucosides equivalent [12] and in the bilberries from 6.74 to17.41 mg·g^{-1} cyanidin-3 glucosides and are influenced by the genotype, the time of harvest, the total dry matter and the processing temperature [32]. The loss of total anthocyanins is 41% in the forest blueberry confiture and 25 % in the elderberry confiture. The boiling temperature and the high sugar content influence the decrease in the anthocyanin content.

Color parameters for bilberry and elderberry confiture

They were determined using the CIELAB technique uniform space in which two color coordinates, a* and b*, as well as a psychometric brightness index are read. These read and calculated values are shown in Table 3.

***Table 3**. Measured color parameters (L*; a*; b*) and calculated parameter C*; h*; ΔE*, BI for elderberry confiture and bilberry confiture*

Colour parameter	Samples	
	BC	**EC**
L*	12.13 ± 0.15	13.36 ± 0.14
a*	6.41 ± 0.14	4.87 ± 0.346
b*	2.91 ± 0.035	2.65 ± 0.66
[a]C*	7.03	5.51
[b]h*	24.22	28.36
[c]x[a]	0.42	0.31
[d]BI[b]	64.7	23.52
Color difference between BC and EC confiture samples		the significance
[e]ΔE*	2,01	< 3 >1.5, distinctive

Bilberry confiture (BC); Elderberry confiture (EC)
a, b, c, d, e - the value was calculated after reading the L*a*b* values on the colorimeter and calculating their actual value
b - value was calculated after calculating the value of x,
d - is expressed in %
The student's "*t*" test was used and each experiment was observed 3 times (n = 3) resulting the mean ± standard deviation

The color parameters of the elderberry confiture or the forest bilberry one with very high sugar content (from the 1:1 manufacturing recipe), thermally treated (106 ºC) and in an acidic *p*H have the following values which are rendered in Table 3.
The Luminance L* has comparable values for the two samples. Its maximum value is 100 and its minimum value is 0 (darkness). Parameter a* has positive, low values that show a trend towards the red color. The value of a* parameter is lower in the EF sample, so the trend is more obvious. The parameter b* (yellow +/ blue -) has low, positive values close to 0, which means that the color tends towards the yellow component. They are similar in both samples.
The Chroma C* as an attribute of color fullness is positive and has a low value, below 10, which means that the color intensity that is perceived by the human eye is small. The hue angle h* has values between 20º and 30º for both samples, higher in the elderberry sample, which shows the reddish trend of the color. The perceptible color differences can be analytically classified as *very distinct* if ΔE* > 3, *distinct* if 1.5 < ΔE* < 3, and there is a *little distinction* if 1.5 < ΔE*[29]. A distinct difference between

the sample of bilberry confiture and that of elderberry confiture has been noticed following the experiments.

The color difference, ΔE*, was calculated between the two varieties of confiture. It is distinct according to calculations, lower than 1.5 and higher than 3. BI is used for the brown color ensemble. It is the best color indicator that is used for the products containing sugar and is modified depending on the L*, a*, and b* values. For the elderberry sample, the value of 23.52 was obtained which was lower than that from the bilberry sample (64.7). This may also indicate a different management of the technological manufacturing process. In the literature, the L* value of the bilberries is 24 and that of the elderberries is 77.34; a* = 0.3; b*=1.18. In the fresh elderberry fruits, h* equals 20.54 and C* is 21.21 [32, 33].

When we consider these values, we notice they are different from the samples studied here, in the same range of values but influenced by the cooking conditions. The color evolves from blue-red to dark brownish red. The heat opens the plant cell membranes and the *p*H of the contents is acidic. The anthocyanins responsible for the color of the fruits of these plant species easily leak out of the cell where the *p*H is acidic. Thus, the reddish shade of the confiture samples is observed, compared to the bluish shade of the fresh fruits. For example, bilberries can give a greenish color if used in the preparation of muffins where the *p*H is different [34].

Sensory analysis

The graphical results of the sensory analysis using the hedonic scale method are shown in Figure 3.

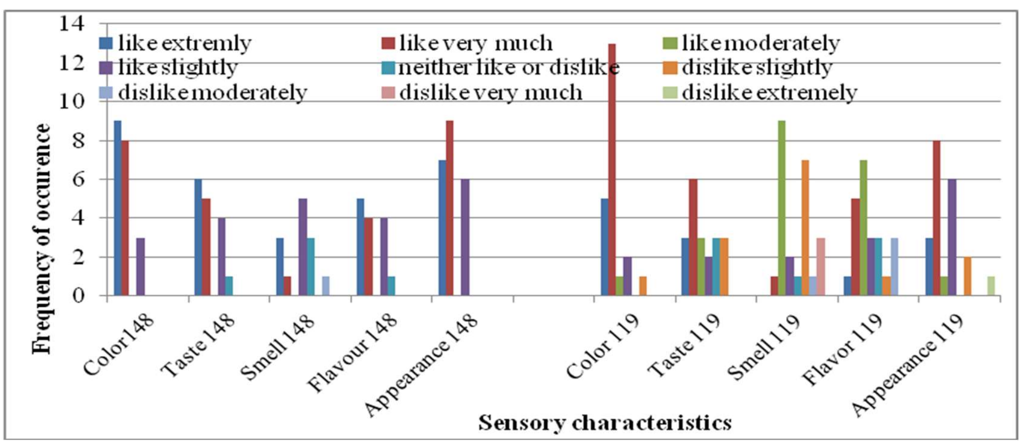

Figure 3. *Graphic representation of the results of the preferential sensory analysis*

For sample 148 the variety of the panelists' options is clearer with more votes for the *"like extremely"* and the *"like very much"* levels. For variant 119 the options span all stages of the hedonic scale for all the assessed characteristics. This shows that this confiture is not very popular. The most valued sensory characteristic was color 27 % and appearance 17 %. Most of the panel members identified the elderberry sample out of the two samples. It seems that the bilberry confiture was preferred by the panelists compared to the elderberry confiture. The members of the panel said that the flavor of sweet candies stood out in the elderberry sample. Of course, it has been shown in the literature that this flavor is given by what remains of the flavor components of the elder fruits as 2 and 3 Methyl -*1*-butanol with a sweet aroma which is an amino acid and is present in the fresh fruit in an amount of 323.2 ng·mL^{-1} and the benzaldehyde with a concentrated candy flavor. This is the shikimic acid and is found in an amount of 271 ng·mL^{-1} [35]. Myrtenal, *D*-limonene, and linalool have been identified in bilberries which give an unmistakable uniqueness to the bilberry confiture [36].

CONCLUSIONS

In this research, obtaining confiture from elderberries proved to be a good choice and it was demonstrated that from a physico-chemical point of view, its values are very similar to those of the confiture made from Romanian forest bilberries. The content of vitamin C decreases during processing with sugar and at high temperatures. However, it is not completely lost, but in a proportion of 52 to 81 %. The total anthocyanin content decreases from fruit to confiture. The color evolves from bluish-red to dark brownish-red. The sensory analysis revealed that the consumers' favorite was the bilberry confiture compared to the elderberry confiture because they thought it was more familiar. Some of the consumers, for example, did not even know that elderberries can be used for such a thing. Although in this type of osmoanabiosis preservation, the heat treatment is intense, the concentration time is prolonged, and the amount of added sugar is for preservation purposes, according to this research, the bilberry confiture and the elderberry confiture can be good sources of vitamin C and anthocyanins as very valuable phytonutrients.

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