



Physico-Chemical and Sensorial Properties of a New Beverages Obtained from Wild Mountain Cranberry (*Vaccinium Vitis-Idaea*)

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Abstract: *This study aimed to characterize a new product obtained from wild mountain cranberry, namely mountain cranberry cider, in terms of physico-chemical properties, antioxidant activity, polyphenols content, mineral elements and sensory acceptability and the results obtained were compared with those of mountain cranberry juice. The characterization of these products gives the opportunity to valorization the precious bioactive compounds from mountain cranberries in all the seasons. HPLC results of the mountain cranberry cider show a higher content of chlorogenic (25.11 mg/100 mL) and gallic (16.04 mg/100 mL) acids than cranberry juice which has lower values (10.21 mg/100 mL chlorogenic and 7.16 mg/100 mL gallic acids). Sensorial analyses results of the both products indicated that the characteristics of the mountain cranberry cider have a higher score for the most of sensory characteristics than the juice, except for the color. Statistical analysis of Unpaired Student-T test was used to determine if there are significant differences between the obtained data for the two products; a significant difference at a level of $p < 0.001$ was emphasis for color parameters, concentration of soluble substances, turbidity and a lower difference ($p < 0.01$) was recorded for radical scavenging effect.*

Keywords: *wild mountain cranberry cider; bioactive compounds; polyphenols; tannins; minerals*

1. Introduction

Berries are defined as functional food [1] they are small, round, brightly colored, sweet or sultry fruits which occupy an important place between forest products, others than wood. People harvest this fruits since ancient times because they represent a significant source of food [2] containing a large variety of different phenolic compounds such as anthocyanins, flavonols, quercetin, hydroxycinnamic acids, hydroxybenzoic acids, a high level of vitamin C (ascorbic acid), vitamin A, essential minerals, tannins, dietary fiber (cellulose, hemicellulose, including soluble fibers such as pectins) and sugars like glucose and fructose [3-5]. Between European countries Romania and Finland have the largest collection areas, for wild berries, followed by Albania, Bulgaria and Iceland [6]. Mountain cranberry (*Vaccinium Vitis-idaea* L., *Ericaceae*) has become one of the most consumed berries in Nordic countries inclusive Romania, being used in different forms in human diet such as jam, sauce, compote, juice, syrup, preserved by drying or freezing [7].

Mountain cranberry, also known as lingonberry, partridgeberry or cowberry is one of the most significant wild berries wide-spread in countries of Northern and Central Europe, also in Russia and Canada, this berries being classified as “super fruits” rich in antioxidants [8]. Arbutin can be found in leaves and stems and it is used by pharmaceutical companies for skin care products [9,4]. The intake of mountain cranberry and its products has been associated with anti-inflammatory, antioxidant and antimicrobial activities, also presenting the highest antiproliferative properties among the berries, helps in curing gastric diseases and lowering the cholesterol levels [10,11]. Recent studies have substantiated that the consumption of wild berries on different forms has a positive and strong effect on human body

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[1]. Most berries cultures throughout the world have traditionally consumed fresh but some in the form of alcoholic beverages. Cranberries are processed by the food industry to extend shelf-life, delivering more options and opportunities for consumers to eat and drink these special fruits. Conventional thermal processing is an effective method for producing safe extended shelf-life and shelf-stable products; however, the harsh temperatures impact nutritive quality, destroying essential nutrients and non-essential nutrients/components [12]. Developing new products from cranberries is intended to diversify the offer to the potential consumers, representing a business opportunity allowing producers to obtain value-added products, such as juice concentrates, wine, puree, cranberry sauce, cranberry paste, jam, dehydrated cranberries [13].

Wild mountain cranberries are perishable seasonal fruits that are found in quite large quantities between August and September consequently their consumption in fresh state is limited, but transforming them into beverages represents a great opportunity for diversify and augment the consumption of such fruits due their biologically active constituents. Therefore, the aim of this study was to process and conserve these nutritive products, namely, wild mountain cranberry into beverage which can be consumed more time. Also, this aim includes to characterize the main chemical composition, the total polyphenol content, total anthocyanin content, the antioxidant activity, as well as the mineral elements, total phenolic acids and flavonols of the two beverages - mountain cranberries juice and mountain cranberry cider.

2. Materials and methods

2.1. Samples

The mountain cranberry samples were picked from Suceava (Romania) area by the end of September and divided into two equal parts, one was preserved by freezing and transform into juice while the other was processed into a mildly alcoholic beverage - "mountain cranberry cider". Wild cranberry cider was obtained by the fermentation of these highly nutritious fruit at a temperature of 8°C.

2.2. Reagents

Folin–Ciocalteu reagent, Na₂CO₃, gallic acid, DPPH, ethanol, HCl, HNO₃, perchloric acid, orthophosphoric acid, monopotassium phosphate, vitamin C were purchased from Sigma-Aldrich (Germany).

2.3. Methods

The physico-chemical parameters analyzed were: pH using a Hach pH-meter (HQ11d), electrical conductivity measured with an Accumet conductivity meter XL30, the concentration of soluble substances (°Brix) and refraction index were determined by a Leica Mark II Plus refractometer, turbidity with 2100AN Turbidimeter (ISO METHOD 7027) total acidity and density were also measured [14]. The Konica Minolta CR-400 Chroma meter (Konica Minolta, Japan) was used for color determination. The color difference (ΔE^*), hue angle or tone (h^0) and chroma or color intensity (C^*) was calculated [15]:

$$\Delta E^* = [(\Delta L^2) + (\Delta a^2) + (\Delta b^2)]^{1/2} \quad (1.1)$$

where:

ΔL^* - the difference of brightness between two samples,

Δa^* - red-green coordinates difference,

Δb^* - yellow-blue coordinates difference

$$h^0 = \tan^{-1}(b^*/a^*) \quad (1.2)$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (1.3)$$



- The total polyphenol content was measured according to Sripakdee et al. (2015), thus 2 mL of sample, 10 mL of 10% Folin–Ciocalteu reagent and 8 mL of 7.5% Na₂CO₃ were added, then mixed for 5 min and stored in the dark at ambient temperature for 60 min before measuring the absorbance at 765 nm. The results were expressed as mg gallic acid equivalents/100 mL sample [16].

- Phenolic Compounds

For phenolic compounds separation was used a HPLC Shimadzu (Tokyo, Japan) equipped with a diode array detector and an Alltech column (250 × 4.6 mm, with particle size of 5 μm). Acidified water with hydrochloric acid at pH 2.00 was used for preparation of 40% methanol solution (solute). Each sample consisted of 1 mL of juice and cranberry cider dissolved in 5 mL of solute and shaken on the magnetic stirrer, then filtered through a membrane of 0.45 μm. The sample volume was 10 μL, temperature was 30°C and the flow rate of 1.0 mL/min, acetic acid 0.1% and acetonitrile representing the mobile phases were the condition for HPLC analysis. The resulted peak area for each analyzed phenolic compound was compared with the standard, allowing for their quantitative determination of phenols at 280 and 320 nm. Phenols contents were expressed as mg/100 mL [17].

- Total anthocyanin content

The total anthocyanin content was performed by spectrophotometric assay following the protocol described by Oroian (2017). 5 mL of each sample was mixed and extracted with 50 mL of ethanol for 40 minutes and expressed as cyanidin-3-glucoside equivalents [18].

- DPPH free radical scavenging assay.

Scavenging activity of mountain cranberry cider and juice was determined according to the method described by Ulewicz-Magulska et al (2018). A volume of 1 mL of sample was mixed with 5 mL DPPH ethanol solution (6·10⁻⁵ M) and after 5 minutes the absorbances were measure at 517 nm. Scavenging activity was calculated with equation:

$$\text{DPPH scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (1.4)$$

where A₀ was the absorbance of the control sample and A₁ was the absorbance of the sample [19, 20].

- Determination of Total Tannins content

The content of total tannins (TT) was determined according to Diaconeasa et al., (2015). The mountain cranberry new beverage and juice were diluted to 1/50 with double distilled water. Thus 4.0 mL of the solution was mixed with 2.0 mL double distilled water and 6.0 mL of 12M HCl. The mixture was divided in two parts. First part was heated in boiled water and cooled on bath ice for 30 min (A₁), whereas the second part was stored at ambient temperature (A₂). Then 1 mL of 95% ethyl alcohol was added to both samples. The absorbance value was measured at 550 nm for each tube, Abs A₁ and Abs A₂. Total tannins concentration (g/L) was calculated as follows [21]:

$$TT = 19.33 \times (AbsA_1 - AbsA_2) \quad (1.5)$$

- Determination of Mineral elements

The determination of minerals from juice and the beverage obtained from mountain cranberry was performed by inductively coupled plasma mass spectrometer (ICP-MS), 7500cs Series Agilent Technologies and following the protocol describe by Fernandez-Turiel et al (2000) [22] which implies the acidification of the sample with HNO₃ (1% v/v HNO₃). Double deionized water 18.2 MΩ cm⁻¹ prepared by a water purification system ThermoFisher- Germany was used for preparation and/or dilution of solutions, if necessary. All reagents were purchased from Sigma Aldrich (Germany).

- Vitamin C determination

Vitamin C concentration of juice and new beverage from mountain cranberry was analyzed by HPLC method according to Pădureț et al. (2016). Therefore 8 mL of juice or mountain cranberry cider with 24 mL acid solution (10% HClO₄ and 1% H₃PO₄) were mixed and transferred into a 100 mL volumetric flask. The solution obtained was mixed for approximatively 2 minutes and added up to the



mobile phase (0.02 mol/L KH_2PO_4 acidified to $\text{pH}=3.5$ with 10% H_3PO_4) until the flask's mark was reached [23].

- Alcohol content

After distilling the cranberry cider sample, the distilled liquid was transferred to measuring cylinder; the alcohol meter and thermometer were inserted and measurements were taken when the readings stabilized at a liquid temperature of 20°C [24].

- Sensorial analysis

For the evaluation, all samples were tasted and examined by five-point ordinal category scales, where 1 represents the lowest standard and 5 represent the highest standard of the given property (color, odor, flavor, taste, acidity). In order to perform the analysis 78 persons have participated, totaling 156 responses, and during the sensory evaluation rinsed their mouth with water in-between the testing of samples. The beverages were given as the coded samples to judges [25,26].

2.4. Statistical analysis

The obtain data were subjected to unpaired Student-t test by Statgraphics Centurion XVI (Trial Version).

3. Results and discussions

The Unpaired Student-T test results of physico-chemical analysis are conducted in Table 1. Concentration of soluble substances expressed in $^\circ\text{Brix}$ is lower in the cider product (0.5) obtained by mountain cranberry (*Vaccinium vitis-idaea*) due to the fact that fermentation process occurred but also to the added water content, while mountain cranberry juice has a high value (10.9).

Table 1. Unpaired Student-T test of mountain cranberry physico-chemical parameters

Physico-chemical parameters	Mountain cranberry-mean (SD)		T- value	P- value	
	Cider	Juice			
Soluble substances ($^\circ\text{Brix}$)	0.5 (0.100)	10.9(0.100)	127.373	$p < 0.001$	
Refractive index	1.3334 (0.001)	1.3486 (0.001)	93.080	$p < 0.001$	
Conductivity (mS/cm)	1.27 (0.007)	2.96 (0.009)	232.997	$p < 0.001$	
Acidity ($\text{cm}^3\text{NaOH}/100\text{cm}^3$)	6.56 (0.53)	34.00 (1.000)	46.971	$p < 0.001$	
pH	3.13 (0.038)	2.86 (0.010)	12.237	$p < 0.001$	
Density (g/cm^3)	1.0021 (0.001)	1.0452 (0.003)	21.589	$p < 0.001$	
Turbidity (NTU)	22.2 (0.200)	454.3 (3.215)	232.391	$p < 0.001$	
Alcoholic content (%volume)	4.8	0	45.938	$p < 0.001$	
Total tannins (g/L)	18.5 (0.897)	30.6 (1.035)	16.6705	$p < 0.001$	
Vitamin C (mg/L)	2.5 (0.496)	40.7 (1.857)	34.431	$p < 0.001$	
DPPH radical scavenging (%)	89 (0.360)	88 (0.353)	6.586	$p < 0.01$	
Total polyphenols (mg/100ml)	195.70 (2.137)	798.32 (2.867)	12.905	$p < 0.001$	
Total anthocyanin content (mg/100mL)	16.4 (0.53)	61.3 (0.45)	112.444	$p < 0.001$	
Color	L	25.21 (0.240)	19.85 (0.038)	38.175	$p < 0.001$
	a*	14.11 (0.880)	1.70 (0.087)	24.317	$p < 0.001$
	b*	10.19 (0.353)	3.67 (0.015)	31.958	$p < 0.001$
	ΔE	15.01 (0.77)		-	-
	h	0.62 (0.013)	1.13 (0.018)	39.163	$p < 0.001$
	C	17.41 (0.920)	4.05 (0.050)	25.134	$p < 0.001$

Refractive index as well as concentration of soluble substances is higher for the juice than for the mountain cranberry cider. Electrical conductivity (EC) in juice and mountain cranberry cider is dependent on ion compounds and is proportional to their contents, so the results of the conductivity analysis shown similar results (1.27 mS/cm) for cranberry cider with other studies [27]. Total titratable acidity of the juice sample is approximately $34 \text{ cm}^3 \text{ NaOH}/100 \text{ cm}^3$ which was relatively high as compared with the acidity of the mountain cranberry cider ($6.56 \text{ cm}^3 \text{ NaOH}/100 \text{ cm}^3$). Concentrations of hydrogen ion of the mountain cranberry cider resulted (3.13) and mountain cranberry juice (2.86) are

similar with other fruit wines or ciders [27] and juice studies [28]. Generally, juice and fruit ciders contain polyphenolic compounds which influence the color parameters and usually are directly proportional to their content. The brightness (L^*), color intensity (C), a^* and b^* parameters of mountain cranberry juice is lower than the new mildly alcoholic beverage (mountain cranberry cider) while the hue angle or tone (h_0) is higher. The color difference – ΔE , between these two mountain cranberry beverages is greater than three (15.01), which mean that the color difference between samples is perceptible by human eyes.

The measured density for the juice was 1.0452 g/cm^3 and for new mountain cranberry beverage 1.0021 g/cm^3 . Turbidity has been determined because it is used as a means of assessing the particle level in a cider or juice (visual clarity) and the results shown extremely different values for juice 454.3 NTU (Nephelometric Turbidity Units) and for mountain cranberry cider 22.2 NTU.

The alcoholic content of the new cranberry-based product was 4.8 % volume. Content of total tannins analyzed are approximatively 18.5 g/L in the case of new beverage and 30.6 g/L for the classical mountain cranberry juice.

DPPH scavenging effect indicated high values of the two products 89% for mountain cranberry cider, respectively 88% for juice, similar values to those obtained by Pop et al. (2008) [29]. Spectrophotometric determination of the total phenolic content as well as Vitamin C content presented very different values for the two beverages, meaning that the juice has a higher concentration compared to the cider product.

The analysis of unpaired Student-t test shows that between these two mountain cranberry beverages is a significant difference at a level of $p < 0.001$, a smaller difference being recorded at DPPH radical scavenging, both beverages exhibiting high antioxidant capacity.

Regarding the polyphenols content, a chromatographic analysis to determine which polyphenols predominate in this two samples was performed. So, from Figure 1 it can be observed that the presence of phenolic acids as hydroxybenzoic, rosmarinic, vanilic and miricetin acids from the mountain cranberry is found also abundant in juice and to a lesser extent in the new beverage. The most abundant phenolic acid in mountain cranberry cider was rosmarinic acid (91.48 mg/100 mL) followed by miricetin (31.36 mg/100 mL). Gallic acid (16.04 mg/100 mL) and chlorogenic acid (25.11 mg/100 mL) are found to be in higher quantities in mountain cranberry cider than mountain cranberry juice. Some polyphenols, such as quercetin, luteolin, kaempferol and cafeic acid have been found to be absent from the two samples. From the identified phenolic acids in juice only two acids did not find in the new beverage, respectively vanilic and protocatecuic acids.

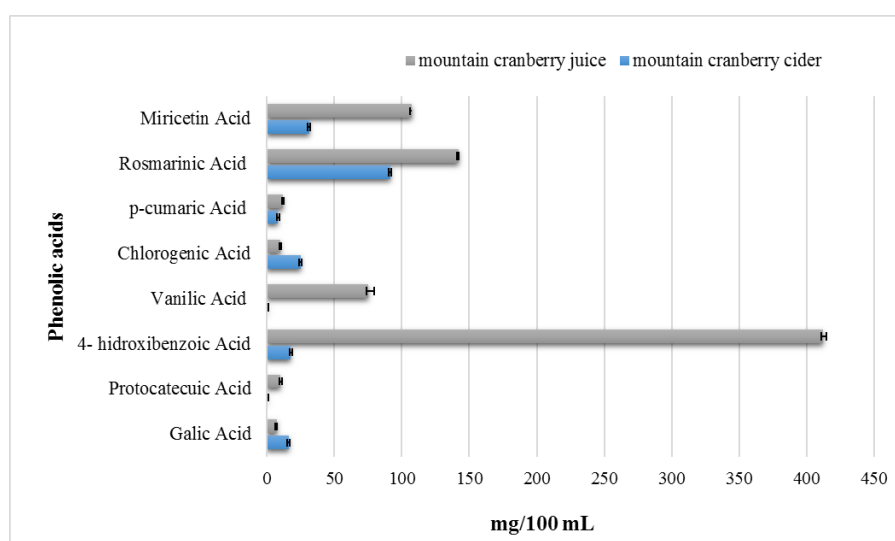


Figure 1. The content of phenolic acids (mg/100mL)

Anthocyanins are valuable compounds that confer the red-purple color to a lot of fruits and are well

known for their antioxidant activity; the total anthocyanin content of mountain cranberry juice (61.3 mg/100mL) expressed as cyanidin-3-glucoside equivalents was higher than the new develop beverage (16.4 mg/100mL), which is slightly less than the amount found in red wine [30].

Sensory analysis is shown in Figure 2 and the results obtained indicated that the characteristics of mountain cranberry cider are constantly for the most of sensory characteristics with a higher score than the juice except the color. Regarding the sensory analysis results of juice, the acidity was rated with the lowest score (1.60 ± 0.82) while the color has been evaluated with maximum (4.75).

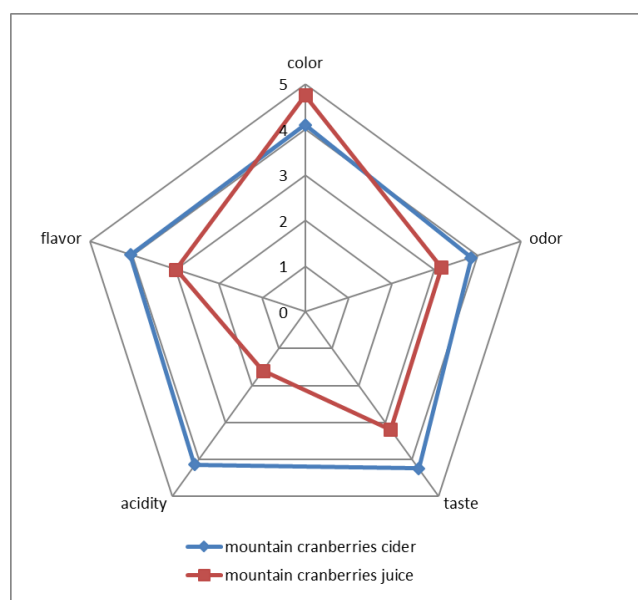


Figure 2. Sensorial analysis of mountain cranberry beverages

The analysis of mineral elements content shows close values for both juice and mountain cranberry cider, with only a few exceptions when the juice has higher values. The abundant mineral element in mountain cranberries juice and cider is sodium (66.19/20.6 mg/L), followed by magnesium (55.00/11.59 mg/L), chromium (19.5/1.31 mg/L), and calcium (18.8/6.25 mg/L). Mercury, lead and cadmium ions are not detected which provides an advantage for this two mountain cranberries beverages consumption. In contrast to the juice which has a relatively low content of aluminum (4.10 mg/L), manganese (7.43 mg/L) and iron (0.21 mg/L), in the mountain cranberry cider those minerals are not detected. The concentration of nickel and copper ions are within acceptable limits for the two products, so in the mountain cranberries juice is 0.27 mg/L nickel, respectively 1.28 mg/L copper and in the mountain cranberry cider is 0.23 mg/L nickel and 0.38 mg/L copper. As a result of mineral content determination, from total minerals present in frozen mountain cranberries, 93.35% are found in juice while in cider there are found in a proportion of 22.77%, but if we take into account the dilution factor ($df=4$) from cider making process we can conclude that minerals are found in percent of 91.8%.

According to the results presented above the wild mountain cranberry processing into a low-alcoholic beverage is an opportunity for both large producer countries and especially for consumers who can enjoy the exceptional properties of this beverage throughout the year, most of the bioactive compounds of mountain cranberry being found in mountain cranberry cider too. Statistical analysis of unpaired Student-T test shown a significant difference between the cranberry juice and the new beverage mountain cranberry-based at a level of $p < 0.001$; which is in agreement with sensorial analysis that emphasis the different sensorial characteristics for both beverages. In contrast to the cranberry juice, the new developed beverage based on mountain cranberry with clearly and effervescent character presents a low level of total acidity, a slightly alcoholic content and a significant polyphenols content. Considering the content of soluble substances of both beverages alongside with the other



physico-chemical properties it can be concluded that the mildly alcoholic beverage is less caloric product compared with the cranberry juice and it can be recommended for consumption in diets but not only.

4. Conclusions

This study presents the opportunity to develop a new low alcoholic beverage based on wild mountain cranberry, which can be consumed throughout the year, even if the fruits are seasonal. The analysis results of mountain cranberry cider were compared with mountain cranberry juice and fruits and most of the bioactive compounds such as phenolic acids, tannins, vitamin C, minerals like sodium, magnesium or calcium are also present in this new beverage; the mountain cranberry cider presenting high antioxidant capacity too. Fermentation in tightly sealed containers along with the exceptional qualities of the mountain cranberry made this mountain cranberry cider to be more appreciated by the trained evaluators than the classical juice. The new mountain cranberry cider offers a solution to market demands, less processed products and free additives, whereas maintaining the compositional attributions that have allowed to promote the health benefits associated with berries.

Acknowledgments: “This work was supported from contract no. 18PFE/16.10.2018 funded by Ministry of Research and Innovation within Program 1 - Development of national research and development system, Subprogram 1.2 -Institutional Performance -RDI excellence funding projects;

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Manuscript received: 21.07.2019