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Article

Formulation of a Wort-Based Beverage with the Addition of Chokeberry (*Aronia melanocarpa*) Juice and Mint Essential Oil

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Featured Application: This research resulted in a potentially functional non-alcoholic beverage applicable to all age groups. With the addition of chokeberry juice, a potent antioxidative beverage with no added sugars, this drink has the potential to become a functional beverage. This preparation could have a promising application in the food and beverage industries, providing health benefits due to its high antioxidant potential.

Abstract: In Croatia, the production of non-alcoholic wort-based beverages is in its initial stages. The main goal of this research was to produce wort that could be then used in the production of a wort-based beverage with the addition of chokeberry (*Aronia melanocarpa*) juice. Sensory characteristics and consumer acceptance was also analyzed. Worts were prepared by the standard mashing process, using Munich and pale ale malt. Chokeberry juice was added to the cooled worts. For the purpose of this research, two versions of wort with different concentrations of chokeberry juice (10%, 20%, 30%) were formulated, and all of the beverages were subjected to sensory analysis. Sensory analysis showed that wort with the addition of 30% chokeberry juice was the most accepted. This concentration of chokeberry juice gave the beverage a pleasant taste, but needed some improvement. To address this, mint essential oil was added to the mixture and carbonation using gaseous CO_2 was conducted. This beverage received better acceptance when carbonated and mixed with mint essential oil. In addition to the sensory analysis, the polyphenol content of the chosen beverage was also analyzed, along with its nutritional value. Polyphenol content was 2621.47 mg/L and antioxidative activity was 2.28 mmol Trolox/L. The energy value was determined to be 57 kcal. However, in order to optimize the production of this wort-based beverage, further research should be conducted.

Keywords: chokeberry; wort; mint essential oil; malt; polyphenols; anthocyanins; antioxidant capacity

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1. Introduction

Beer production has been practiced for more than 5000 years, as evidenced by numerous legends and records on clay tablets of the inhabitants of former Mesopotamia. Additionally, Greek and Roman literature refer to beer, mocking people who drank beer instead of wine. This point of view regarding beer as a less valuable drink than wine changed during the reign of Charlemagne, with beer production even extending to monasteries. It was at this time, at the turn of the seventh century, that beer hopping began [1].

Modern times demand modern approaches and innovations in the food and beverage industries. Recent decades have imposed certain choices regarding the health demands of consumers. Increases in food allergies, food intolerance, and healthy food and lifestyle choices have led to a rise in healthy food production. Functional foods and beverages that aid and preserve the health of consumers is a current trend [2]. Since cereals are such a great source of various beneficial components such as phenols, vitamins B and E, fibers, carbohydrates, and phytoestrogens, it is reasonable to employ them in the production of food and beverages [3–5].

Wort is a semi-product in the production of beer, obtained after the thermal and enzymatic decomposition of basic raw materials, i.e., barley or wheat malt. Standard all-malt wort commonly contains around 12% monosaccharides, 5% sucrose, 47% maltose, 15% maltotriose, and 25% higher saccharides, such as dextrins [6]. Wort does not contain alcohol since no yeasts have been added to it, omitting the fermentation process. However, it does contain many minerals and vitamins such as vitamin C. Interestingly, Captain Cook is known to have used wort to suppress the occurrence of scurvy during his long voyages at sea. After his second voyage (1772–1775), he reported that wort was effective as an antiscorbutic medicine [7]. Thus, wort is a great beverage that can potentially and easily be transformed into a functional drink.

As for chokeberry (*Aronia melanocarpa*), its products are recognized as foods that have a beneficial effect on health and are designated as functional food. Due to its antioxidant properties, such as its rich polyphenols content, chokeberry is used as a medicine. Polyphenolic compounds are commonly found in all plants. They are a significant antioxidant available in food [8]. Many studies have investigated polyphenols due to their therapeutic effects related to oxidative stress [9]. Among polyphenols, anthocyanins and proanthocyanidins are the most biologically active components in *A. melanocarpa* berries [9,10]. Chokeberry is also a source of glucose, fructose, sucrose, sorbitol and pectic, K and Zn, along with certain amounts of Na, Ca, Mg and Fe. Vitamins B1, B2, B6, and C, niacin, pantothenic acid, folic acid, alpha and beta tocopherol and carotenoids are also found in chokeberry fruits, in addition to citric, malic, shikimic, and ascorbic acid [9,11]. The antioxidative activity of black chokeberry have also been confirmed, as well as its other related benefits such as antihypertensive, lipid-lowering, and anti-inflammatory effects [12–14]. Most importantly, no harmful effects from chokeberry juice, fruit and extracts have been reported [9]. The aim of this study was to produce a polyphenol-rich beverage based on the combination of wort and chokeberry that has no added sugars or sweeteners and is suitable for everyone (children, sportsmen and older people). Since wort is a naturally sweet beverage and chokeberry juice is rich in polyphenols and antioxidative activity, it was reasonable to assume such a mixture would result in a drink acceptable to consumers. The combination of wort and chokeberry juice resulted in a product with an increased content of polyphenols, anthocyanins, and antioxidant activity.

2. Materials and Methods

2.1. Production of Wort

For the purpose of this research, light and dark worts were produced. Grounded malt was mashed at 69 °C for 1 h in a 20 L Speidel Braumeister (Speidel Tank- und Behälterbau GmbH, Ofterdingen, Germany). The first batch was produced with 5 kg of Munich malt (Badass barley malt, Nova Gradiška, Croatia) with the addition of 50 g black wheat malt (Wheat chocolate—Weyermann®, Bamberg, Germany) to achieve a darker color. The second batch was produced using pale ale malt (Badass barley malt, Nova Gradiška, Croatia), also 5 kg. The production process was the same for both worts. Water for mashing was prepared by reverse osmosis and hardness was adjusted by adding 1 g $CaSO_4$, 2.5 g $CaCl_2$, and 2 g $MgSO_4$ for darker wort and 6.5 g $CaSO_4$, 14 g $CaCl_2$, and 9 g $MgSO_4$ for pale ale wort. At the end of the mashing process, spent grains were rinsed with 4 L of the same water used for mashing. The rinsing water temperature was 72 °C. After the separation of spent grains, worts were boiled at 100 °C for 1 h. This step was followed by cooling to 23 °C, using a Speidel cooling coil. Worts were then transferred to a container. The obtained volume of worts was 19 L each. Chokeberry juice (Encian d.o.o. Donji Stupnik, Croatia) was then added in each keg in concentrations of 30% to the volume of wort. For example, since keg has the volume of 18 L, the volume of wort was 13.8 L and the added juice was 4.2 L for 30% beverage mixture.

The optimal amount of chokeberry juice was determined previously, testing out the three mixtures with the addition of chokeberry juice in 10, 20, and 30% to 5 L of lighter and darker non-carbonated wort mixtures.

In order to improve the sensory properties of the prepared wort-based drink with the addition of chokeberry juice to soften the taste of the wort itself, 0.25 mL of mint essential oil was added to 18 L of the prepared drinks. The beverages were then left in the cooler at 2 °C. The prepared wort-based drinks were then carbonized using CO_2 at 2.5 bar.

2.2. Sensory Evaluation

Upon production, a sensory evaluation was needed to decide which beverage is more appealing to consumers. Primarily, the addition of chokeberry juice in concentrations of 10, 20 or 30% in darker and lighter wort was subjected to evaluation to decide which drink would be the most acceptable. An evaluation was conducted as described in Section 2.2.1. A table presenting the evaluated properties can be found in the Supplementary Material.

After determining the most suitable addition of chokeberry juice, another sensory evaluation was carried out to determine which mixture would be better accepted among consumers. A drinkability test was conducted to determine which version of the prepared mixture would be accepted better among consumers, as described in Section 2.2.2.

2.2.1. Sensory Evaluation of Basic Characteristics

A sensory evaluation was conducted by 20 untrained consumers (13 females and 7 males). The panel consisted of beer consumers of different age groups (aged 21–59). The test and sensory descriptors were adjusted from the general evaluation sheer for beer [15]. The scoring of each sensory attribute was conducted on a five- or four-point intensity scale, where 1 point means "fault" and higher points mean "excellent", as can be seen from the Supplementary Material, Table S1. Smell, taste, bitterness, astringency, carbonation, and mouthful were determined in the second round of testing, as opposed to the first round of testing where carbonation was omitted since the beverages were not carbonated. Tastings were performed in an appropriate room. Samples were kept at room temperature for 10 min before the test and poured into a clean transparent glass. All samples were served under a number and every sample was tested in triplicate. Evaluators were offered flat mineral water between the samples, together with plane white bread. Tastings were conducted twice; the first tasting was carried out with the beverage being offered to the consumers at room temperature, and for the second tasting, the evaluators were offered a cold (4 °C) beverage.

2.2.2. Drinkability Test

After the sensory evaluation, a drinkability test was performed in order to determine which version of the beverage would be more acceptable. The drinkability test involved the two produced beverages. The same panelists who participated in the sensory evaluation were called up again (20 consumers, aged 21–58). Panelists were initially offered to try all beverages (200 mL in a glass marked with numbers), cooled at 4 °C. The tasting atmosphere provided a relaxed environment, with a person waiting on the participants. After the initial tasting of the beverages, panelists were left to choose on their own between the samples. The test lasted for 2 h and was conducted in the afternoon (5–7 PM) and was performed as described by Habschied et al. [16].

2.3. Determination of Polyphenols Index

The total polyphenolic index (TPI) was determined using Folin–Ciocalteu reagent (FC) according to a procedure described by [17]. The changes in the color of the radical from light blue to dark blue were measured after 30 min at 760 nm using a UV-Vis spectrophotometer (Shimadzu UVmini-1240, Kyoto, Japan). The TP was quantified from gallic acid calibration curve (3–20 mg/L, R^2 = 0.9961). The TPI was calculated and expressed as mg gallic acid equivalent (GAE) per L of beverage.

2.4. Determination of Antioxidant Activity

The antioxidant activity was measured using a DPPH radical according to a modified method described by [18]. The reaction mixture consisted of 0.2 mL of the beverage sample and 3 mL of DPPH radical solution 0.1 mM in methanol. The changes in the color of the radical from deep violet to light violet were measured after 30 min at 515 nm using a UV-ViS spectrophotometer (Shimadzu UVmini-1240, Kyoto, Japan). The antiradical activity (AA) was determined using the following equation ($y = 0.9548x + 0.0294$; $R^2 = 0.9914$) obtained from linear regression after plotting the A515 nm of known solutions of trolox against concentration (0.1–0.9 mM). The results were expressed as mmol of Trolox® equivalents (TE) per one liter of beverage (mmol TE/L).

2.5. Determination of Anthocyanins and Flavonoid Content

Monomeric anthocyanins were determined using the method described by Giusti et al. [19]. Total monomeric anthocyanins were expressed as cyanidin-3-glucoside, and the obtained values were expressed as mg/L.

The total flavonoid content was determined according to Makris et al. [20]. Briefly, 0.5 mL of the beverage sample was mixed with 4 mL of distilled water, then 0.3 mL 5% $NaNO_2$ was added and allowed to react for 5 min. Following this, 0.3 mL 10% $AlCl_3$ was added and the mixture was allowed to react for a further 5 min. At the end, 2 mL 1 M Na_2CO_3 and 2.4 mL distilled water were added to the reaction mixture and the absorbance at 510 nm was read against a blank. For each sample, the measurements were performed in triplicates and values were interpolated on a calibration curve using catechin as a standard and expressed as g catechin equivalents per L of beverage (g CE/L).

2.6. Nutritional Value Determination

All analyses are in-house methods and can be retrieved from the Institute of Public Health of the Osijek-Baranja County upon request; however, a short description the analyses carried out in this study is provided here. Fat content was determined using the Röse–Gottlieb method. The method is commonly used to determine fats in foods that mainly also contain proteins (milk, butter, cheese, meat, whole meal, etc.) [21].

Ash was determined according to the standard method based on burning the sample at a temperature of 550 ± 10 °C and weighing the obtained residue [22].

Crude fiber determination was carried out using the Scharrer–Kürschner method [23].

Sugar content was determined according to Luff [24].

Salt was determined as follows: 10–50 g of the homogenized sample was transferred to a 250 mL volumetric flask and filled to the mark with distilled water. Then, it was shaken and filtered. Twenty-five mL of the filtrate was transferred into a 100 mL flask and subjected to titration with 0.1 M $AgNO_3$ with the addition of 10 drops of potassium chromate until the color changed from yellow to red, using an in-house method.

Brix, dry matter and refraction index was determined using a refractometer (Carl Zeiss Abbe, Oberkochen, Germany), which is part of an in-house method.

Protein content was determined as described by Lim [25].

2.7. Statistical Analysis

Analysis of variance (ANOVA) and Fisher's least significant difference test (LSD) were conducted, with the least statistical significance set to $p < 0.05$. Statistica 13.1. (TIBCO Software Inc., Palo Alto, CA, USA) was the software of choice for this data set.

3. Results and Discussion

To determine which version of the beverage would be preferred among the consumers, a sensory evaluation was conducted. Upon preliminary sensory evaluation, as shown in Table 1, it appeared that the addition of 30% chokeberry juice is more acceptable than other combinations (10 and 20%) for both versions of wort. Smell, taste, bitterness, astringency, and mouthful were evaluated for all mixtures, but the overall result was taken into account and is presented in Table 1.

Table 1. Table presenting evaluating scores/acceptance of formulated beverages.

Formulation	Score
Temperature	20 °C
Munich 30%	26.50 ^{a}
Pale ale 30%	23.05 ^{b}
Munich 20%	22.65 ^{b}
Pale ale 20%	20.95 ^{bc}
Munich 10%	21.55 ^{bc}
Pale ale 10%	20.00 ^{c}

Means within rows with different superscripts ^{a,b,c} are significantly different ($p < 0.05$).

From this table, it is visible that for both worts, lighter and darker, the addition of 30% of chokeberry juice gave a pleasant taste to the wort, and scored the highest. The highest score of 26.50 points was received for Munich malt wort, with 30% of added chokeberry juice. The similar formulation with pale ale malt and 30% chokeberry juice followed with a slightly but statistically significantly lower score of 23.05 points. In general, the darker beverage received better scores than the pale ale malt beverage, as can be seen from Table 1. However, the panelists noted that the addition of mint, cooling, and carbonation would probably add t a more enjoyable and refreshing kick to the beverage. In further evaluations, mint essential oil and carbonation were added. Such beverages, prepared as described in the Section 2, were then offered to the panelists in order to determine the preferable mixture.

The next stage of this research was set up as a second sensory analysis performed in order to evaluate the consumers' preference among the produced wort mixtures. According to the results presented in Table 2 and Figures 1 and 2, sample M (Munich malt with 30% chokeberry juice and mint oil) had an overall better result in all evaluated categories than sample P (pale ale malt with chokeberry juice and mint oil). Since the panelists previously declared that a cooled beverage would provide a more refreshing kick, we served them the same beverages at two temperatures (20 °C and 4 °C).

Table 2. Table presenting evaluating scores/acceptance of formulated beverages drank at different temperatures.

Temperature	20 °C	4 °C
Sample	Score	
Munich 30%	24.60 ^{aB}	26.50 ^{aA}
Pale ale 30%	21.40 ^{bB}	24.50 ^{bA}

Means within rows with different superscripts ^{a,b} are significantly different ($p < 0.05$); Means within columns with different superscripts ^{A,B} are significantly different ($p < 0.05$).

Smell, bitterness, taste, carbonation, and mouthful all received higher scores in sample M, while astringency received a lower score, as opposed to the sample P, where astringency was rated a higher score. Astringency is an important property in this research since the intention was to achieve a moderate bitterness with just a hint of astringency originating from chokeberry. The results indicate that consumers prefer drinks stored at lower temperatures (4 °C), as can be seen in Table 2 and Figures 1 and 2.

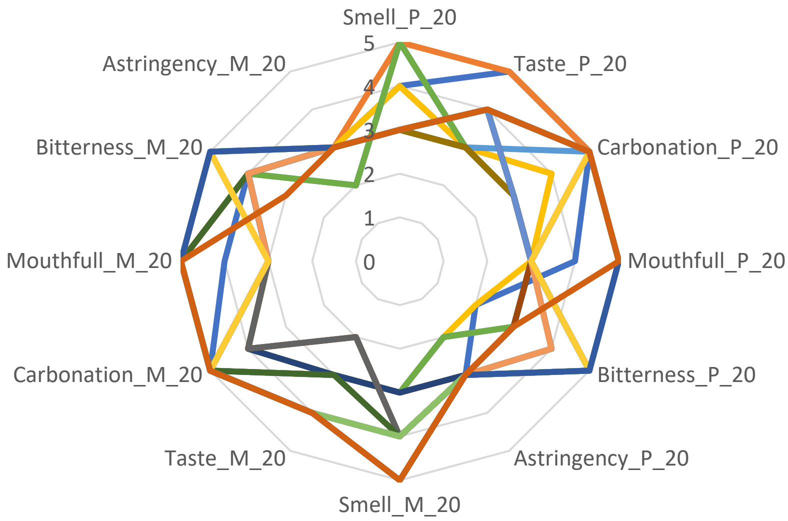

Figure 1. Sensory analysis of samples at 20 °C; Sample produced with pale ale malt is marked as "P" and sample produced with Munich malt is marked with "M".

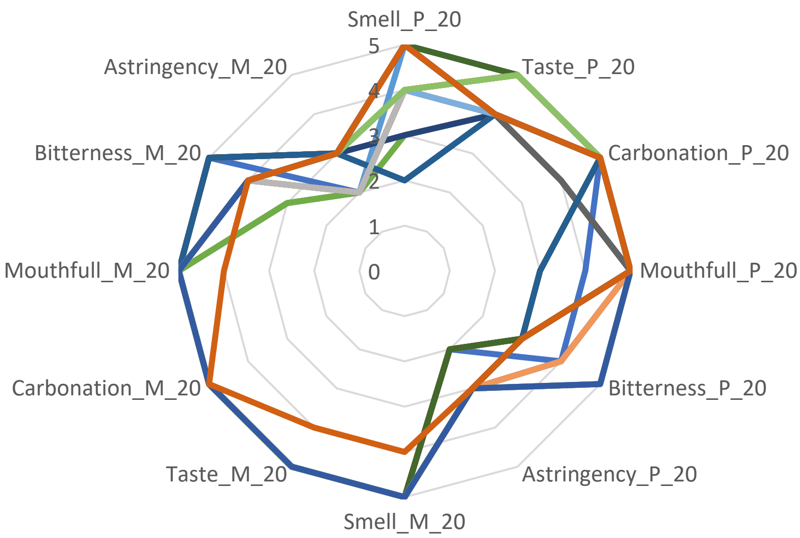

Figure 2. Sensory analysis of samples at 4 °C; Sample produced with pale ale malt is marked as "P" and sample produced with Munich malt is marked with "M".

Consumers prefer a less bitter, more carbonated drink, which is provided with a beverage formulated with Munich malt. During storage at a lower temperature, the feeling of astringency and the feeling of bitterness decreased, which is evident from Figures 1 and 2. Additionally, there were tasters who commented that they liked the sweetness, i.e., that the drink was not too sweet for them, unlike commercially available soft drinks. Lower temperature (4 °C) also contributed to the mouthful score and the feeling of carbonation for both mixtures.

The taste of wort was dominant in this drink, and most of the tasters had never tasted wort. However, this research showed that potential consumers, who had the opportunity to try a similar wort-based drink earlier (the tested drink was commercially available as a wort-based beverage with lemon or orange juice), exhibited a better acceptance of the wort-based drink with the addition of chokeberry juice and mint essential oil. This indicates that the taste of wort, or wort-based drinks, is a habit-forming taste in the sense that consumers can develop a fondness for this drink over time through more frequent consumption of the product. As mentioned before, the sweetness of this drink was acceptable to individuals who follow a healthier way of life, with reduced or no-sugar content in their diet.

This narrowed the choice to two possible mixtures that were acceptable to consumers: lighter and darker versions of wort enriched with chokeberry juice (30%), and mint essential oil and carbonated, which were tested using the drinkability test. This test was set up to determine which drink was more acceptable to consumers. The drinkability test revealed that, as shown in Table 3, the darker mixture (marked as sample 2) prepared with Munich malt was better accepted among users than the lighter color version prepared with pale ale malt (sample 1). It appeared that the darker wort was more acceptable to the eyes of the end consumers when mixed with chokeberry juice and was more pleasant in taste than the lighter color version.

Table 3. Drinkability test results.

Sample	V (L) of Consumed Beer
1	7.8 [a]
2	10.8 [b]

Means within rows with different superscripts [a,b] are significantly different ($p < 0.05$). Sample 1—pale ale malt wort with the addition of 30% chokeberry juice and mint oil; sample 2—Munich malt wort with the addition of 30% chokeberry juice and mint oil.

The drinkability test was performed in order to determine which version of the wort mixture would provide a better, more pleasant beverage. According to the results (Table 3), consumers seemed to prefer sample 2 (darker wort mixture), as they drank 10.8 L of beer, while sample 1 was less desirable, with 7.8 L of consumed beverage.

Research conducted by Habschied et al. [26] reported that dark and black beers contain higher amounts of polyphenols and exhibit higher antioxidative activity. According to this report, a darker wort formulation would presumably contain a higher amount of antioxidative activity and polyphenols.

Since it was statistically confirmed that the more desirable beverage mixture was darker wort with chokeberry, additional analyses were carried out accordingly, as described in the following sections.

The values of antioxidant activity and polyphenols obtained from the analysis of wort-based drinks with the addition of chokeberry juice and mint essential oil are presented in Table 4. Since there are no scientific literature data about wort-based beverages in that regard, the obtained results were compared with the antioxidant activity and polyphenol value of industrially-produced dark and black beer [26]. The hypothesis was that the prepared beverage would have higher antioxidant activity, i.e., a higher proportion of polyphenols, than the dark or black beers. This is especially anticipated due the additions of potent antioxidant chokeberry juice. The results shown in Table 4 confirmed the stated hypothesis, i.e., the antioxidant activity of dark, i.e., black of beer, is lower than determined in this beverage by an average of four times. Maximal values for dark and black beer were around 0.6 mmol Trolox/100 mL, while for this beverage, the antioxidative activity was determined to be 2.28 mmol Trolox/100 mL. As for the content of polyphenols, their content in dark beers averaged to 682.79 mg/L (maximal value was determined for black beer sample and amounted to 855 mg/L), which is also four times lower than the value found in the prepared wort-based beverage with the addition of chokeberry juice and mint essential oil. The proportion of polyphenols in black beers was slightly higher than in dark beers, i.e., the average value of polyphenols in the analyzed samples of dark beer is 809.43 mg/L, which shows that the proportion of polyphenols in the prepared drink is three times higher than in black beer. Namely, the content of polyphenols in the mixture was 2621.47 mg/L. Anthocyanins and flavonoids have also been analyzed. The content of anthocyanins was 87.67 mg/L, while flavonoids reached 1110.00 g/kg. These concentrations are significant and surely add to the biological value of the chosen mixture. Anthocyanins in chokeberry juice can be found in concentrations of 200–480 mg/L [27–29]. The results obtained in this research are within these values, considering this is a 30% chokeberry juice solution. Chokeberry juice can have a wide range of polyphenol content; according to several authors, it can range from 3000 mg GAE/L to 11,000 mg GAE/L [30,31]. Antioxidative activity in commercially available juices was 19.02–106.13 mmol Trolox/L [32]. The obtained results of our research fit with the values of polyphenols and antioxidative activity that can be found in the literature. Namely, since this beverage contains 30% chokeberry juice, the values for polyphenols are within the range reported in the aforementioned literature. However, antioxidative activity falls slightly short in comparison to the 100% chokeberry juice, probably due to the dilution with wort.

Table 4. Determined antioxidative activity, polyphenols, anthocyanins and flavonoids content in the best-chosen beverage, Munich malt beverage with 30% of chokeberry juice.

	Value
Polyphenols	2621.47 mg/L
Anthocyanins	87.67 mg/L
Antioxidative activity	2.28 mmol Trolox/L
Flavonoids	1110.00 mg/L

Since there is no alcohol in this drink, it is rich in antioxidants, and there are no added sugars or sweeteners, it can also be recommended for children.

Similar research, using caramel and darker malts, was conducted by Shopska et al. [33], who developed a wort-based beverage with increased biological value. They developed a beverage using 24.2% Vienna, 51.8% Melanoidin, 20% Caramel pils, and 4% Special X malts. This formulation was rated as optimal in regard of antioxidative activity. Additionally, research by Latifova et al. [34] investigated the production of a lactic acid beverage based on wort and mint (*Menta piperita* L.), which resulted in increased antioxidant activity.

To determine the nutritive value of the composed beverage, an analysis of nutritional properties was also carried out. The results are presented in Table 5. The caloric or energy value of the produced drink was 239 kJ or 57 kcal. Artificial flavors and sweeteners have not been added to this drink; all carbohydrates and sugars originate from malt and chokeberry juice.

Table 5. Determined nutritive value of the best-chosen beverage, Munich malt beverage with 30% of chokeberry juice.

Energy Value (in 100 g of Sample)	**239 kJ/57 kcal**
Dry matter	14.5%
Ashes	0.25%
Raw fibers	0.00%
Fat	0.00%
Proteins	0.28%
Salt	0.09%
Total sugars	6.65%
Carbohydrates	13.97%

A small amount of salt (0.09%) and no fat content provides a good nutritional option for consumers who wish to avoid such ingredients. Additionally, as mentioned before, the sweetness of the beverage was rated as pleasant, especially by individuals who choose to avoid sugars in their diet.

4. Conclusions

Trends in the food and beverage industry are dependent on the increase in healthy lifestyles of the public, especially among younger people. Thus, novel formulations are being developed and subjected to research. No sugar, no alcohol and the efficient addition of the potent antioxidative juice of chokeberry is a desirable formulation, accessible to younger and older people.

Dark wheat malt was added to increase the number of polyphenols in the drink, but also to improve the color of the drink itself. Namely, as reported in the preliminary conducted sensory evaluation, using only light, pale ale malt, for the formulation, the drink was not visually appealing to consumers. After determining the acceptable formulation with 30% chokeberry juice and the addition of mint essential oil and CO_2, the taste significantly improved. The fizziness provided by CO_2 contributes to the feeling of refreshment during consumption. This beverage is rich in polyphenols, mainly anthocyanins, and it can be consumed by children, older people, or even sportsmen, as it is a non-alcoholic beverage.

This research resulted in the formulation of a potentially functional beverage mixture with an energy value of only 239 kcal, which makes it ideal for use in personalized nutrition, nutrition for children, sportsmen, and older people, as was set in the objectives of the study. However, further research is needed to determine the true functionality of this beverage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13042334/s1, Table S1. Evaluation sheet used for sensory evaluation

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