Journal homepage: http://fens.usv.ro/index.php/FENS
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XX, Issue 4 - 2021, pag. 403 - 410

FOOD AND ENVIRONMENT SAFETY

BLUEBERRY, SEA BUCKTHORN FRUITS AND APPLE BEVERAGE: BIOCHEMICAL AND SENSORIAL CHARACTERIZATION

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Received 5th September 2021, accepted 20th December 2021

Abstract: *This work aimed at optimizing the extraction of total phenolic (TP) and total monomeric anthocyanin (TMA) content for the development of a potentially functional wild blueberry smoothie (BP) with apple puree (AP), apple juice (AJ) and sea buckthorn puree (SBJ) in various proportions using conventional extraction (CE) and advanced extraction techniques [ultrasound-assisted (UAE)]. There were also analyzed the rheological behavior and the color attributes (lightness and redness respectively) of smoothie samples. S7 smoothie sample [30 AP/30 AJ/20 SBJ/20 BP (UAE)] provided a beverage with significant higher amount (21.40%) of anthocyanins (TMA) compared to sample S10 [30 AP/30 AJ/20 SBJ/20 BP (CE)] in which the extraction of anthocyanins was by the classical method. Further, different color attributes, particularly brightness (L *), redness (a *) and yellow (b *) decreased considerably for the samples with 15 and 20% addition of blueberry puree. Therefore, ultrasound-assisted extraction had a significant effect on the amount of anthocyanins extracted, compared to the classical extraction method.*

Keywords: *Fruit Smoothies; ultrasound; extraction; anthocyanin content; CieLab Method.*

1. Introduction

Although, it is recognized that a healthy diet can reduce the risk of many common chronic diseases, the prevalence of diet-related diseases is still increasing. Unhealthy eating habits can affect nutrient intake, and these habits include: lack of natural foods or eating too many foods and beverages that are low in fiber or high in fat, salt and / or sugar. Nutritional status is very important to maintain a strong immune system against the coronavirus disease 2019 (COVID-19) pandemic, so there is great interest in foods rich in substances that help strengthen the body's immune system.

Various studies have shown changes in eating habits, consuming food of a poorer quality and a decrease in fruit consumption during the quarantine period [1-3]. Smoothies are blended beverages containing fruits, vegetables and liquids such as water, milk, fruit juices and yogurt. Wild blueberries, sea buckthorn and apples are fruits native to northern Bukovina, and are an important part of the usual diet and have practically identical historical uses and known health benefits.

The use of both berries and apples has its roots in food traditions, which used fresh or sun-dried fruit to make juices, syrup, jams, cakes, dairy products or season food. The presence of micronutrients and phytochemical compounds, such as vitamins, fibers and phenolic compounds (anthocyanin and non-anthocyanin compounds), with nutritional and functional properties, that justify the growing interest in these berries, not only

DOI: https://doi.org/10.4316/fens.2021.041

for food applications, but also in the pharmaceutical industry [4].

Bilberries *(Vaccinium myrtillus L.)*, belong to the most popular fruits, are a dark blue fruit and an interesting source of phenolic compounds, mainly anthocyanins, sugars, vitamins, fibers, minerals, making them valuable to the food, pharmaceutical industries, or they can be used in the development of functional foods [5, 6]. The content of anthocyanins in bilberries varies between 1017 mg per 100 g fresh weight, and 1610 mg/L to 5963 mg/L in juice [7]. Blueberry with tall (cultivated) shrub is a branched, bushy subshrub, which reach maturity after about 6-8 years from planting. There are many varieties of blueberries early, seasonal and late varieties. Wild blueberries have a more pronounced aroma than cultivated fruits; they are shrubs that grow in non-forest habitats and mountain hills, over the Carpathians, especially on shady slopes [8].

The fruits of sea buckthorn (*Hippophaë L., family Eleagnaceae*) are berries of orange to red colour and have the taste sour, bitter and astringent; they have a special aroma, which cannot be compared with the aroma of any other fruit [9]. The presence of total phenolic_compounds and tocopherols in sea buckthorn fruits [10, 11], vitamin C and minerals [12] antioxidant activity and flavonoids [13], which are considered as major preventive agents against several degenerative diseases, with health-promoting effects, have been reported. Sea buckthorn berries are also used in traditional medicine and has become an ingredient in natural products for food, pharmaceutical and cosmetic products.

The apple is a well-known fruit, and a major fruit crop in Eurasia, that belongs to the species *Malus Domestica* from the *Rosaceae family*. Thousands of apple varieties are grown worldwide and have multiple uses in the food industry (dried, juices, purees, cider, ingredients from various preparations), but can also be used fresh or in various forms of preservation [14]. Nowadays, the apple are considered to be a good source of a large number of bioactive compounds, including phenolic compounds, flavanols, and anthocyanins, vitamins (ascorbic acid), minerals [15,16], that ensure nutritional and health-promoting effects as an important part of our daily food. Apple is a widely consumed and epidemiological studies show that eating apples reduces the risk of of colorectal cancer [17], cardiovascular disease [18] asthma and diabetes [19].

Ivanovic et al. [20] studied the ultrasound-assisted extraction on the colour stability and the total content of anthocyanins (TAC) of the blackberry fruit purée, cultivar "Čačanska Bestrna" widely grown in Serbia. The greatest increasing of anthocyanin content was obtained for extract isolated at 40 ^{0}C after 30 min of sonication (1.38 g/100 g DW) [20]. The effects of ultrasonic treatment (for 15, 30 and 60 minutes at 25 ^{0}C, frequency 40 kHz, 130 W) have already been described for freshly squeezed Chokanan mango juice, in which was observed after sonication, an increase in brightness (L^*) and a decrease in redness (+ a^*) and yellowing (+ b^*) was observed [21]. In a recent study, Potter et al. [22] tested a beverage of blueberry-soy with 12% blueberry juice and 2.8% soy protein isolate, and this new formula represented a great source of anthocyanins. Thus, new smoothie products rich in bioactive compounds can be developed to meet the requirements of consumers while facing the modern lifestyle.

The aim of this paper is to obtain new formulas for smoothie sources of natural antioxidants, with special sensory, biochemical and textural properties.

2. Materials and Methods

Plant Material and sample preparation

Fruits of Bilberries *(Vaccinium myrtillus L.)* and sea buckthorn (*H. rhamnoides L.)* were harvested optimally ripe, in Suceava County, Romania. Fruits were cleaned and then stored at 4 ^{0}C until analysis; the juice was extracted by cold pressing. Apples *Malus Domestica* 'Fălticeni' were obtained from a local retailer. The fruits were blended in a homogenizer.

Smoothie composition by weight was whole apple, apple juice, sea buckthorn, and blueberry mixed immediately after obtaining, in appropriate proportions (50/30/10/10, 40/30/15/15 and 30/30/20/20). Finally, seven different products were obtained: four semi products (100% apple puree (AP); 100% apple juice (AJ); 100% sea buckthorn puree (SBJ); 100% blueberry puree (BP) and three smoothie's samples [S5 - 50AP/ 30AJ/ 10SBJ/ 10BP; S6 - 40AP/ 30AJ/ 15SBJ/ 15BP; S7 - 30AP/ 30AJ/ 20 SBJ/ 20SB.

Total monomeric anthocyanin determination

Extraction procedure

5 g smoothie samples were extracted with 10 ml of 80% methanol aqueous solution containing 1 g / 100 g HCl in an ultrasonic bath for 30 minutes 20 kHz, as described before by Dranca and Oroian [24] with a slight modification. A conventional 1-hour extraction was performed at room temperature with a magnetic stirrer. The beverage samples were then centrifuged for 10 min at 10000 rpm.

After extraction, the whole extracts were filtered through a filter paper and placed in a volumetric flask (25 mL) and the volume was completed with the 80 % methanol.

Total monomeric anthocyanin (TMA) content in smoothie samples was determined by UV–VIS spectrophotometer, (Ocean Optics, Largo, FL, USA) according to Leahu et al. [23]. Absorbance was measured at 530 and 657 nm, and TMA content was expressed as cyanidin-3-glucoside (mg/g) using the following Eq. (1):

$$TMA\,(mg/g) = (A\,net/29600) * MW * DF * W/Wt \quad (1)$$

where: *MW*= 449.1, A_{net}= A_{530}− 0.25 A_{657}, *DF*-dilution factor, *V*-total volume (mL), *Wt*-sample weight (g)

The total phenolic content of smoothie methanol extract was measured, by using the Folin-Ciocalteu method [8]. The data was reported as mg gallic acid equivalents (GAE)/100 g fresh-weight (FW).

Brix degree determination

This method evaluates the total soluble sugar (TSS) content, by measuring index of refraction with a digital handheld Brix-Hanna HI96801. The refractive index was recorded and converted to °Bx.

Colour measurement

Colour components of obtained products, were measured using the CIELAB-system. L*, a* and b* (where: L* (100 = white; 0 = black), a* (+ red, – green), b* (+ yellow; – blue), with a Minolta Chroma Meter CR-200 (tristimulus method) [23].

Sensory Evaluation

Taste, flavor, color, sweetness, acidity, and overall acceptability on a 9-point hedonic scale were estimated in the fruit smoothie samples compared to the control fruits puree by twenty panelists (12 females, 8 males) untrained assessors between 18 and 50 years of age. Following the processing

smoothie samples were coded and served at room temperature (aprox 20 ^{0}C) in aliquots of 20 mL together with non-salted table biscuits and still water (Bucovina, Romania) to panelists for evaluation of sensory attributes.

Statistical analysis

The obtained data were analyzed with Minitab 17 statistical software. Data were reported as means ± standard deviation. In order to observe if there is any correlation between the parameters Pearson correlation was used.

3. Results and Discussion

Effect of ultrasound on the total monomeric anthocyanin content

The anthocyanin contents of the apple peel extract, S1 (5.84 mg/ g fresh peels) were measured (Figure 1). The results showed that the total anthocyanin content increased after 30 min of sonication using the 20 kHz system, compared to the conventional extraction control samples. The blueberry puree (37.56 mg cyanidin 3-glucoside/g fresh weight) had a similar content to the S7 (30/30/20/20) smoothie (32.89 mg cyanidin 3-glucoside/ g fresh weight), because it has the highest addition of blueberries.

Total monomeric anthocyanin (TMA) content of S7 30/30/20/20 smoothie sample increased by 14.43 % at 20 kHz (Figure 1), 30 min sonication compared to the conventional S10 extraction sample.

Ultrasound assisted extraction (UAE) of red raspberry puree prepared from crushed berries was recently observed by Golmohamadi et al. [25]. Total monomeric anthocyanin content of red raspberry puree increased by 12.6% at 20 kHz and by 6.7% at 490 kHz after 10 min sonication ($p < 0.05$) [25].

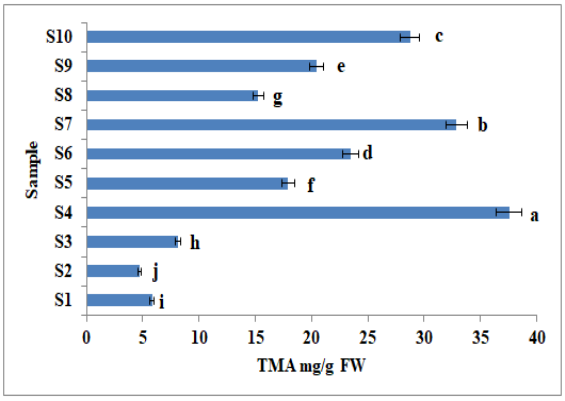

Fig. 1: Anthocyanin content of smoothie samples. Results are the means of three analyses and bars show the standard error

S1 100% apple puree (AP); S2 100% apple juice (AJ); S3 100% sea buckthorn puree (SBJ); S4 100% blueberry puree (BP); S5 - smoothie sample 50/30/10/10 (UAE); S6 smoothie sample 40/30/15/15 (UAE); S7 smoothie sample 30/30/20/20 (UAE); S8 smoothie sample 50/30/10/10 (CE); S9 smoothie sample 40/30/15/15 (CE); S10 smoothie sample 30/30/20/20 (CE). Values are mean ± standard deviation (n = 3). Means that do not share a letter (a-j) are significantly different ($p \leq 0.05$).

Total Phenolic Content

Smoothie samples were analyzed for their total phenolic content using Folin–Ciocalteu assay. The total phenolic compounds (TP) values in the smoothie extracts analyzed were in the range 186.16 – 376.3 mg GAE/100 g FW fresh weight (Figure 2). Among all the samples analyzed, the S7 sample revealed the highest TP concentration at 376.3 mg GAE/100 g FW.

Total soluble sugar (TSS) content

S2 100% apple juice (AJ) showed an initial TSS of 12.8 ^{0}Bx, which tended to decrease in the smothie samples. However, S6 (40 AP/30 AJ/15 SBJ/15 BP) smoothie samples showed the highest TSS (12.6 ° Bx) and were not significantly different from the control samples. The total soluble solids content, or sugar index, is an important indicator of quality for fruit and vegetable smothie drinks [26].

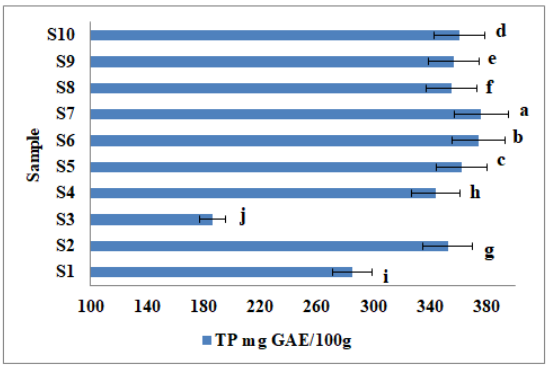

Fig. 2: Total phenolic compounds content of smoothie samples. Results are the means of three analyses and bars show the standard error

S1 100% apple puree (AP); S2 100% apple juice (AJ); S3 100% sea buckthorn puree (SBJ); S4 100% blueberry puree (BP); S5 - smoothie sample 50/30/10/10 (UAE); S6 smoothie sample 40/30/15/15 (UAE), S7 smoothie sample 30/30/20/20 (UAE); S8 smoothie sample 50/30/10/10 (CE); S9 smoothie sample 40/30/15/15 (CE); S10 smoothie sample 30/30/20/20 (CE). Values are mean ± standard deviation (n = 3). Means that do not share a letter (a-j) are significantly different ($p \leq 0.05$).

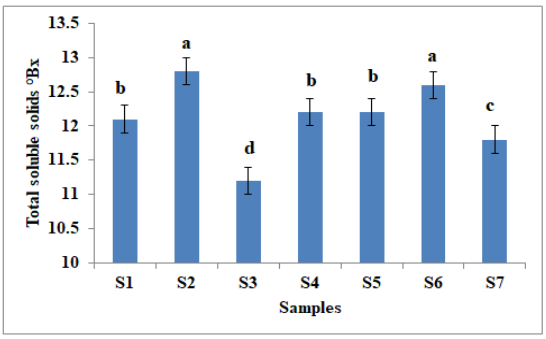

Fig. 3: Content of total soluble sugar (TSS) of smoothie samples

S1 100% apple puree (AP); S2 100% apple juice (AJ); S3 100% sea buckthorn puree (SBJ); S4 100% blueberry puree (BP); S5 - smoothie sample 50/30/10/10; S6 smoothie sample 40/30/15/15; S7 smoothie sample 30/30/20/20. Values are mean ± standard deviation (n = 3). Means that do not share a letter (a-d) are significantly different ($p \leq 0.05$).

Color measurement in the CIE L*a*b* system

In general, the acceptability of a product is influenced by color. Color properties of smoothie samples are illustrated in Figure 4.

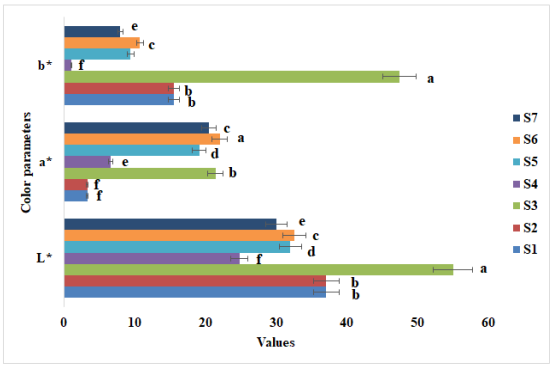

Fig. 4: Color parameters of smoothie samples. Different lowercase letters (a–f) show significant differences between the groups ($p < 0.05$)

L* values, indicating the luminosity level, ranged from 24.80±0.01 to 55.03±0.02. In the case of a* the samples presented values situated in the pozitive region (ranged between 3.29±0.03 and 22.04±0.03), more towards red and in the case of b* they were situated in the positive region (7.98±0.02 - 47.42±0.06), more towards yellow.

Sensory evaluation

Members of the sensory panel evaluated a total of seven smoothie products (four semi products and three smoothie beverages). The sensory scores of the four semi products (100% apple puree (AP); 100% apple juice (AJ); 100% sea buckthorn puree (SBJ); 100% blueberry puree (BP) and three smoothie samples are presented in Figure 5. In general, all samples received high scores of preferences and all smoothie samples were characterized by fruit flavour, smoothness and sweetness.

The S5 smoothies sample, which is the sample with the smallest addition of sea buckthorn, received the highest overall impression score, along with the highest taste and texture scores (Figure 5). Smoothies S7 was defined as dense, astringent which could result from the presence of fiber with high water holding capacity and apparent viscosity.

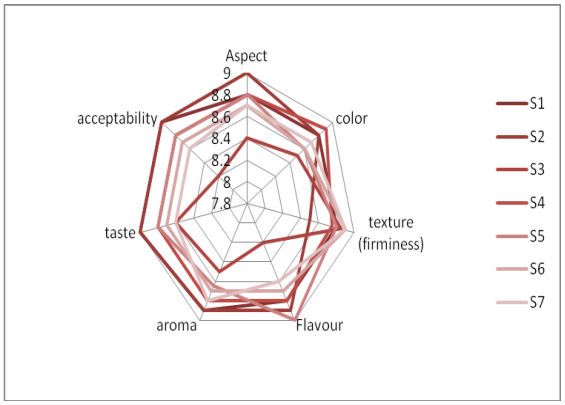

Fig. 5: Sensory evaluation of the smoothie samples

S1 100% apple puree (AP); S2 100% apple juice (AJ); S3 100% sea buckthorn puree (SBJ); S4 100% blueberry puree (BP); S5 - smoothie sample 50/30/10/10; S6 smoothie sample 40/30/15/15; S7 smoothie sample 30/30/20/20.

Pearson correlation and principal component analysis

From Table 1, it can be observed that the Pearson correlation between TMA and TP is 0.517 which indicates that is a moderate positive relationship between these two variables and a strongly negative relationship between TMA and L^* (−0.709) and b^* (-0.617) (p-value < 0.05). A strongly positive relationship can be observed between TP and TSS (0.733) and strongly negative relationship between TP and b^* (-0.898) and L^* (-0.881) (p-value < 0.05). Strongly negative relationship was observed between TSS and color parameters.

Table 2 show the results obtained after performing the analysis of the principal components and it was observed that the first two components have eigenvalues greater than 1.

Table 1

Correlation matrix (Pearson correlation)

	TMA	TP	TSS	L*	a*
TP	0.517				
TSS	-0.012	0.733			
L*	-0.709	-0.881	-0.585		
a*	0.254	-0.032	-0.439	0.22	
b*	-0.617	-0.898	-0.654	0.992	0.283

Table 2

Principal component analysis: TMA, TP, TSS, L^*, a^*, b^*

Eigenvalue	3.7777	1.4716	0.6156	0.103	0.0318	0.0002
Proportion	0.63	0.245	0.103	0.017	0.005	0
Cumulative	0.63	0.875	0.977	0.995	1	1
Eigenvectors						
Variable	PC1	PC2	PC3	PC4	PC5	PC6
TMA	-0.318	-0.571	-0.401	-0.602	0.186	-0.12
TP	-0.483	-0.053	0.385	0.269	0.737	0.032
TSS	-0.372	0.45	0.475	-0.615	-0.234	-0.015
L*	0.504	0.089	0.18	-0.216	0.353	-0.731
a*	0.127	-0.679	0.647	0.049	-0.32	-0.004
b*	0.507	0.003	0.122	-0.37	0.375	0.671

The first component has a proportion of 0.63, which means that it explains 63% of the variability in the data and the second component has 24.5% and can both be included in the evaluation. The other components have lower proportions and may not be important enough to be included into the evaluation. The first component has a positive association with L^* (0.504) and b^* (0.507) and large negative association with TP, while the second component has a large negative association with TMA (−0.571) and a^* (−0.679). There is a statistically significant association between the indicators.

4. Conclusion

The investigation of the smoothie samples revealed a special biochemical and sensory properties. This indicates that apple, blueberry and sea buckthorn are a promising source of health-promoting biochemical compounds with antioxidant activity.

These results illustrate that the use of ultrasound-assisted extraction is suitable for the extraction and quantification of anthocyanin compounds and total polyphenols to improve the quality of fruit-based beverages for consumers.

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