

Stability of Carotene and Phenols of Sea Buckthorn (*Hippophae rhamnoides* L.) Juice with Pomace during Storage

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Abstract

Sea Buckthorn (SB) juice is rich in biologically active compounds. It has considerable health benefits; thus, it can serve as functional food ingredient. The aim of this study was to determine and compare the total content of polyphenols, antioxidant capacity (FRAP), soluble solid content, pH value, β -carotene content and colour parameters (L^* , a^* , b^*) in berry juice of cultivar 'Leikora' Sea Buckthorn (SB) (*Hippophae rhamnoides* L.). The treatment was made as control sample of Sea Buckthorn juice (C), and Sea Buckthorn juice with 0.5 % (P0.5); 1 % (P1); 2 % (P2) dried pomace of Sea Buckthorn. The samples were stored at room temperature for physicochemical analysis at interval of 2 months for a total period of 14 months.

According to our results, the β -carotene (C to P2 was 3.71, 4.82, 5.49 and 6.52 mg 100 mL⁻¹) as well as the antioxidant capacity of the samples increased with the growth of the pomace content. During storage, degradation occurred in the polyphenol content and antioxidant capacity, but the β -carotene content increased. The increase of the β -carotene content was 94.6 % (C) and 32.7 % (P0.5). The smaller reduction in antioxidant compounds was the higher the sample of pomace content is. The FRAP and total polyphenol values measured during storage confirm that the pomace has antioxidant effect. There is a close correlation between the two parameters, including a positive correlation ($r = 0.8614$), which indicates that a significant part of the antioxidant capacity of buckthorn is due to the presence of different polyphenols.

Keywords

Hippophae rhamnoides, pomace, β -carotene, total polyphenol, FRAP

1 Introduction

Biologically active compounds are of great importance in several scientific fields (plant science, food science, modern pharmacology, agrochemistry, etc.), their research is booming nowadays. The biologically active compounds are capable to interact with the living tissue, and have (possibly positive) impact on it. Although several possible sources of these compounds are known (plant, animals, microorganisms, or even not natural sources), plant materials (especially fruits and vegetables) are the most relevant [1, 2].

Sea Buckthorn (SB) (*Hippophae rhamnoides* L.) is a unique medicinal plant and belongs to the family of *Elaeagnaceae* [3]. It is most commonly found in Asia and North America, but also found in Europe, especially along the banks of rivers and the coasts of Finland, Sweden, Poland, and Germany. Sea Buckthorn is native to Asia

and very large Eurasian area at different altitudes. It is currently domesticated in different countries, in particular China, Russia, Germany, Finland, Romania, France, Nepal, Pakistan and India [4].

The berries of SB are rarely consumed fresh, but the fruit juice, pulp, and peel and the seed oil are widely used in several countries. The SB is a good source of bioactive compounds as C-vitamin, carotenoid, flavonoid, polyphenolic content [5–11]. Due to these components, the SB offers many health benefits [12]. Processing of SB produces high amount of pomace, which are utilized rather efficiently or discarded as a waste, so considerable amounts of nutrients are lost [13]. Carotenoids give Sea Buckthorn typical yellow to orange colour. Therefore, the oil from the pulp contains more carotenoids than seed oil. The most active

representative of carotenoids is β -carotene. In addition to β -carotene in Sea Buckthorn, there are also lycopene, zeaxanthin, β -kryptoxanthin [5]. The Sea Buckthorn juice is yellow; the high amount of carotene is responsible for this yellow colour. The presence of some other pigments also contributes to the colour. Granules or clumps are embedded in the juice, which are actually the material containing spherical droplets that are yellow-brown in colour [14].

The nonedible portion of fruits and vegetables after processing (waste), such as peels, pods, seeds, skins, etc., accounts for about 10–60 % of the total weight of the fresh produce. Because of the significant presence of pectin, minerals, vitamins, and bioactive molecules content, this waste offers a huge potential for its conversion into useful products, such as enzymes, ethanol, and biocolours [15]. The management of food processing by-products and wastes with reference to their reuse and recycling creates value [16]. Processing of SB produces high amount of pomace, which are utilized rather inefficiently or discarded as a waste, so considerable amounts of nutrients are lost [17]. The pomace, which is left when berries are squeezed, is also a good source of vitamins and contains compounds that show antioxidant effects like flavonols, and phenolic acids and also is a good source of linoleic and α -linolenic acid which is beneficial for human health [18, 19].

In addition, there is an increasing interest towards functional food products, made of fruit that are rich in bioactive compounds such as Sea Buckthorn [20].

Our goal is to obtain enriched Sea Buckthorn juice by using Sea Buckthorn pomace and investigate a delicious enriched apple juice containing this pomace extracts during the storage period of 14 months. The target of the statistical analysis was to determine whether the storage interval or the different quantities of pomace have an effect on changes of biologically valuable compounds during the test period.

2 Materials and methods

2.1 Sea Buckthorn berries collection and processing

The berries of the Sea Buckthorn (*Hippophae rhamnoides* L.) cultivar 'Leikora' was collected from a commercial orchard near Szolnok (North latitude 47° 57' 28", East longitude. 18° 51' 53") located in middle Hungary in November 2017. The berries were cleaned to remove damaged, diseased, or pest-infected fruits.

2.2 Preparation of samples

During the separation of juice, seeds and shells of the berries we followed the technological process used in industrial practice. First, berries were heated to 80–85 °C

next to continuous mixing to inactivate the enzymes. The berries were squeezed using fruit pulping and squeezer equipment, during which the juice and the pomace were separated. Based on the experiments of Furulyás et al. [21] the pomace was dried at 80 °C until moisture content became lesser than 10 % by atmospheric dryer (LMIM, Esztergom, Hungary). The moisture content was determined by RADVAG.MAC-50 equipment. The separated juice was added to different amounts of dried SB pomace. In this experiment, four types of samples were made with increasing SB pomace content: Control (C) without SB pomace, SB juice + pomace 0.5 % (P0.5), SB juice + pomace 1 % (P1) and SB juice + pomace 2 % (P2). Juice from each treatment was filled into sterilized 125 mL glass jars and sealed. After heat treatment (90 °C and 10 min), all samples were cooled with the cool water bath and stored at room temperature for physicochemical analysis at an interval of 2 months for a total period of 14 months.

2.3 Analytical methods

The Soluble Solid Content (SSC) measurements were carried out by ATAGO DBX-55 digital refractometer according to the Codex Alimentarius 558/93/EEC [22]. The pH measuring was realized by Testo 206 pH/temperature measuring instrument.

The color parameters were determined by CIE Lab Color Measuring System with a Konica Minolta CR 410 manual digital color meter. The results were expressed in the CIE LAB system with L^* (the lightness coordinate), a^* (the red/green coordinate, with $+a^*$ indicating red, and $-a^*$ indicating green) and b^* (the yellow/blue coordinate, with $+b^*$ indicating yellow, and $-b^*$ indicating blue) colorimetric coordinates. Calculation of color difference between two samples using Eq. (1) [23]:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

The evaluation of ΔE^* is shown in Table 1. Extraction pre-treatment was used to the total polyphenolic content and antioxidant activity measurement. During the extraction, 1 g of each sample was dissolved in 30 mL solution consisting of 60 % distilled water, 39 % methanol and 1 % formic acid. The mixture was put in ultrasonic

Table 1 Summary of color difference

ΔE^*	Sensible difference
0–0.5	Not noticed
0.5–1.5	Hardly noticeable
1.5–3.0	Noticeable
3.0–6.0	Clearly visionable

bath for 15 minutes, the homogenate was centrifuged at 5000 rpm for 10 min. The spectrometric measurements were carried out with Hitachi U-2900 equipment (Hitachi High-Technologies Europe GmbH, Krefeld, Germany). All reagents were purchased in analytical grade from Sigma-Aldrich Chemical Co. (3050 Spruce Street, St. Louis, MO 63103, USA).

Total Polyphenolic Content (TPC) was determined according to the Singleton and Rossi [24] method. Samples were prepared with Folin-Ciocalteu's reagent and sodium-sulphate solution. The colour change during the reaction was detected on 765 nm by spectrophotometer, and the results can be expressed as gallic acid equivalent (μg Gallic Acid Equivalent (GAE) mL^{-1}).

Antioxidant capacity (FRAP) was determined by Ferric Reducing Ability of Plasma (FRAP) assay according to the method of Benzie and Strain [25]. The iron ion (Fe^{3+}) reducing ability of the antioxidants in the samples is shown by blue colour change ($\lambda = 593 \text{ nm}$). The antioxidant capacity can be expressed as ascorbic acid equivalent after calibration (μg ascorbic acid (AA) mL^{-1}).

The β -carotene [7235-40-7; Sigma-Aldrich Chemical Co.] was determined by Waters Co. (USA) HPLC instrument according to Ficzek et al. [26]. The sample preparation was carried out by tetrahydrofuran (THF) under subdued light for 12 h at $+4 \text{ }^\circ\text{C}$, and Edmund Bühler SM 30 control shaker (150 rpm). Each extraction was performed in triplicate. During the measurement we used dual absorbance detector (analytical wavelength 450 nm), Symmetry C18, 5 μm , $4.6 \times 150 \text{ mm}$ column, Empower TM2 software. The mixture ACN:MeOH:THF (50:45:5, V/V/V) flowing at 1 mL min^{-1} was used as eluent.

2.4 Statistical analysis

All experiments were conducted in more than triplicates. There was two factors pomace treatment and storage time. Treatment factor had four levels 0 %, 0.5 %, 1 % and 2 % of pomace concentration represented as *C*, *P0.5*, *P1* and *P2* respectively. Time factor had 8 levels 0, 2, 4, 6, 8, 10, 12, and 14 months. Assumptions for Normal distribution of data and homogeneity of variance were checked. Normality was proved by Kolmogorov-Smirnov test. Homogeneity of variance was checked by Levene's test for each factor. Two factor complete randomized design ANOVA was selected for analysis since two factors were involved with one dependent variable. Turkey post hoc test was run for significant variables. Statistical evaluation was done by using IBM SPSS V25 in 95 % confidence interval.

3 Results and discussion

3.1 Change of soluble solid content, pH-value and colour parameters

The brix changes of the control sample juice, *P0.5*, *P1* and *P2* samples are illustrated in Table 2. As expected, the water-soluble solids content of the sample without pomace was the lowest, and the more pomace is in the juice, the higher refraction was detected. The soluble solid content values of samples (*P0.5* to *P2*) on initial day was 9.82, 10.02 and 10.32 $^\circ\text{Brix}$, which were gradually increased to 10.07, 10.23 and 10.47 $^\circ\text{Brix}$ respectively during the 14 months storage. Only the control sample *P0* on initial day was 9.5 $^\circ\text{Brix}$ and 9.23 $^\circ\text{Brix}$ during last month storage.

During the storage maximum increase was observed in sample *P0.5* (2.56 %) followed by *P1* (2.18 %), while minimum increase was recorded in sample *P2* (1.42 %). Both pomace treatment and storage time had significant effect on soluble solids of buckthorn juice. Pomace treatment, $F(3, 64) = 165.777$, $P < 0.001$ and storage time, $F(7, 64) = 5.921$, $P < 0.001$.

The pH change measures at room temperature for control juice sample (*C*), *P0.5*, *P1* and *P2* juice samples are tabulated in Table 2. During the storage maximum increase was observed in sample *P0.5* (6.87 %) followed by *P2* (3.17 %), while minimum increase was stated in sample *P1* (1.31 %) followed by *C* (3.12). Pomace treatment, storage time had significant effect on pH of buckthorn juice. Pomace treatment, $F(3, 64) = 118.930$, $P < 0.001$ and storage time, $F(7, 64) = 66.279$, $P < 0.001$. Our results are in agreement with the finding of Khan et al. [27], who noticed a reduction in pH at 3.10 between 2.06 of 90 days on the storage of mango-Sea Buckthorn blended juice, and Chirila et al. [28] also obtained similar results (pH 2.53) in buckthorn juice.

The results of the determination of the L^* value can be seen in Table 2. The mean L^* values decreased from 9.59 % to 7.61 % during storage. The degree of change in L^* was reduced with the addition of pomace, thus the samples became less dark. The brightness factor (L^*) was affected by the amount of pomace added: the degree of darkening was reduced addition of pomace, and the samples containing pomace became less dark compared to the control. Both pomace treatment and storage time had significant effect on L^* color value of buckthorn juice. Pomace treatment, $F(3, 64) = 9.131$, $P < 0.001$ and storage time, $F(7, 64) = 58.407$, $P < 0.001$.

Colour change in redness of control sample juice, *P0.5*, *P1* and *P2*. During the storage maximum decrease

Table 2 Change of Soluble Solid Content (SSC), pH and color parameters (L^* , a^* , b^*) during 14 months storage (Control (C) without SB pomace, SB juice + pomace 0.5 % (P0.5), SB juice + pomace 1 % (P1) and SB juice + pomace 2 % (P2))

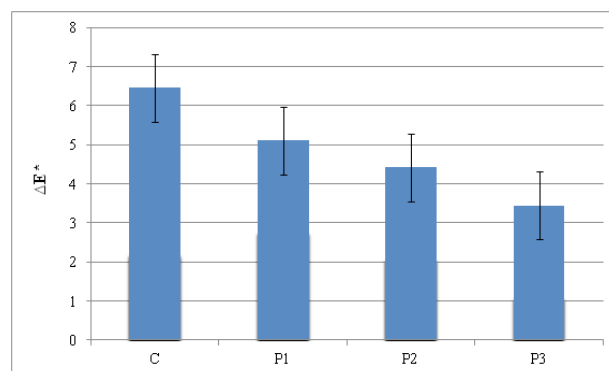
	month	C	P0.5	P1	P2
SSC	0	9.50 ± 0.17 ^A	9.82 ± 0.02 ^{abcAB}	10.02 ± 0.12 ^{abB}	10.32 ± 0.33 ^B
	6	9.18 ± 0.13 ^A	9.88 ± 0.11 ^{abcB}	9.98 ± 0.37 ^{abBC}	10.50 ± 0.17 ^C
	14	9.23 ± 0.15 ^A	10.07 ± 0.06 ^{bcB}	10.23 ± 0.21 ^{bb}	10.47 ± 0.50 ^B
pH	0	2.35 ± 0.01 ^{aA}	2.33 ± 0.02 ^{aA}	2.43 ± 0.01 ^{abB}	2.48 ± 0.01 ^{bcC}
	6	2.42 ± 0.02 ^{bcA}	2.45 ± 0.02 ^{baB}	2.51 ± 0.04 ^{deB}	2.45 ± 0.04 ^{abAB}
	14	2.43 ± 0.02 ^{bcA}	2.47 ± 0.03 ^{ba}	2.55 ± 0.02 ^{eB}	2.55 ± 0.01 ^{dB}
L^*	0	47.93 ± 0.79 ^{eA}	47.92 ± 0.05 ^{ba}	47.64 ± 0.02 ^{eA}	47.86 ± 0.01 ^{dA}
	6	46.10 ± 0.77 ^{bcA}	46.37 ± 0.76 ^{abA}	46.08 ± 0.12 ^{bcA}	46.11 ± 0.02 ^{bcA}
	14	43.34 ± 0.06 ^{aA}	44.75 ± 0.34 ^{aC}	44.01 ± 0.26 ^{aB}	44.94 ± 0.12 ^{abC}
a^*	0	17.19 ± 0.26 ^{bcC}	17.11 ± 0.05 ^{cC}	15.59 ± 0.04 ^{bcB}	14.57 ± 0.02 ^{ba}
	6	16.96 ± 0.72 ^{bcB}	15.73 ± 0.64 ^{abcAB}	15.25 ± 0.91 ^{abAB}	13.95 ± 0.36 ^{abA}
	14	14.91 ± 0.22 ^{aA}	14.41 ± 0.07 ^{aB}	13.96 ± 0.10 ^{abAB}	13.56 ± 0.19 ^{abAB}
b^*	0	41.02 ± 0.25 ^{bc}	45.21 ± 0.04 ^{bd}	39.72 ± 0.03 ^{bcA}	40.43 ± 0.02 ^{abB}
	6	42.29 ± 1.99 ^{bcA}	42.36 ± 1.34 ^{abA}	40.86 ± 2.02 ^{eA}	39.52 ± 1.24 ^{abA}
	14	36.57 ± 0.54 ^{aA}	39.46 ± 0.42 ^{aC}	37.84 ± 0.13 ^{abB}	39.08 ± 0.14 ^{abC}

Value means more than three replicates and their corresponding standard errors. Superscripts with small case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. (Control (C) without SB pomace, SB juice + pomace 0.5 % (P0.5), SB juice + pomace 1 % (P1) and SB juice + pomace 2 % (P2))

was observed in a^* values of sample P0.5 (15.79 %) followed by C (13.24 %), while minimum decrease was recorded in sample P2 (6.92 %) followed by P1 (10.46 %). Both pomace treatment and storage time had significant effect on a^* color value of buckthorn juice. Pomace treatment, $F(3, 64) = 135.654$, $P < 0.001$ and storage time, $F(7, 64) = 24.190$, $P < 0.001$. The b^* values of samples were gradually decreased. During the storage maximum decrease was observed in sample P0.5 (12.73 %) followed by C (10.85 %), while minimum decrease was recorded in sample P2 (3.34 %) followed by P1 (4.72 %). The yellow-blue factor (b^*) decreased almost three times less in the 1 and 2 % pomace-containing juice than in the ones containing 0 and 0.5 % pomace. Both pomace treatment and storage time had significant effect on b^* color value of buckthorn juice. Pomace treatment, $F(3, 63) = 24.494$, $P < 0.001$ and storage time, $F(7, 63) = 28.288$, $P < 0.001$.

The change in the parameters of the sample or its deviation from a given colour pattern can be characterized by the spatial distance between the two colour points, the total colour difference (ΔE^*). During these investigations the parameters measured 14 month of all samples were compared with the initial ones, so the ΔE^* values were determined with respect to the starting colour coordinates. The results obtained are illustrated in Fig. 1.

The colour change of the pomace-containing juice was "clearly visible" at the end of storage, since ΔE^* values were between 3.0 and 6.0. In the control sample, ΔE^* was


Fig. 1 The color difference parameters (ΔE^*) on the storage period

greater than 6.0, thus the difference was large. Increasing the amount of pomace, the colour difference decreased, including a negative correlation ($r = -0.9681$). Within the Microsoft Excel data analysis program, the correlation was determined by Pearson's correlation, which measures the strength of the linear relationship between two variables, and the correlation coefficient (r) can be between -1 and 1 .

3.2 Change of β -carotene content

Carotenoids are an important food constituent, responsible for colour and nutritional value as provitamin A. The Vitamin A plays a central role in both daytime (photopic) and nighttime (scopic) vision, in the reproductive biology, the bone formation and the growth, its proper daily intake is important and the pomace-enriched juice can be greatly contributed, respectively [29].

The results of the determination of the β -carotene can be seen in Table 3. The values were different at baseline, and the more turf the marrow contained, the higher the β -carotene content. This tendency was observed throughout the storage period.

Initially the β -carotene values of the samples (*C* to *P2*) was 1.91, 3.61, 4.37 and 5.87 mg 100 mL⁻¹. The initial data were gradually increased to 3.71, 4.82, 5.49 and 6.52 mg 100 mL⁻¹ respectively after 14 months storage. The increase of the β -carotene content was 94.6 % for *C* sample and 32.7 % for *P0.5* sample. With the increase of the pomace content, the increase of the β -carotene content was slower during storage. In the *P1* sample it was only 29.4 %, while in the *P2* sample it was 15.3 %.

The initial β -carotene content of the control sample (1.91 mg 100 mL⁻¹) almost doubled at the end of storage (3.71 mg 100 mL⁻¹). The highest value was reached by month 10, then a slight decrease was observed (not significant, $p = 0.985$), and the situation is the same with the 0.5 % pomace-containing sample ($p = 0.961$).

Both pomace treatment and storage time had significant effect on β carotene content of buckthorn juice. Pomace treatment, $F(3, 102) = 230.45$, $P < 0.001$ and storage time, $F(7, 102) = 19.024$, $P < 0.001$. Moreover, there was significant interaction between pomace treatment and storage on the β carotene content of the juice, $F(21, 102) = 2.214$, $P = 0.01$.

Sea Buckthorn fruits are rich in carotenoids, the most active representative is β -carotene, whose average content was mentioned by Bajér [30] 1.8–3.9 mg 100 g⁻¹. Yang and Kallio [5] state the total content of carotenoids in Sea Buckthorn berries from 1.0 to 200.0 mg 100 g⁻¹ and β -carotene content from 0.2 to 17.0 mg 100 g⁻¹. Eccleston et al. [18] determined the total content of carotenoids in Sea Buckthorn juice at the amount of 7.3 mg 100 g⁻¹ and β -carotene formed 3.3 mg 100 mL⁻¹. In the study of Raffo et al. [31], in Germany varieties

of Sea Buckthorn 'Ascola', 'Hergo' and 'Leikora' a presence of major carotenoid zeaxanthin (3–15 mg 100 g⁻¹), β -carotene (0.3–5 mg 100 g⁻¹) were found.

In samples *C* and *P0.5*, the β -carotene content in the juice increased significantly, similar to data of Lessin et al. [32], who examined the geometric isomers of carotenoids in processed fruits and vegetables. Processing resulted growth (16–50 %) in the total amount of carotenoids with provitamin A activity compared to fresh samples. The reasons for this increment include increased extraction efficiency, inactivation of carotenoid-degrading enzymes, and loss of soluble solids in the liquid heat treatment medium [32].

3.3 Change of Total Polyphenol Content (TPC)

All polyphenol contents show a similar trend in all samples (Table 4). The more pomace is in the juice, the higher polyphenol content. For all samples, the initial polyphenol content decreased by the end of storage but not in the same extent. Increasing pomace content of the samples resulted in a smaller reduction (20.65 % for *C*, 13.84 % for *P0.5*, and 10.68 % for *P2*).

In terms of time TPC content slightly increased after month 6 and month 8, which lasted until month 10. Thereafter, the downward trend continues by the end of storage, except in the case of 2 % pomace containing juice, which reached 1563.63 $\mu\text{g GAE mL}^{-1}$ value by month 14. Storage time, pomace treatment and their interaction had significant effect on total polyphenol content: pomace treatment $F(3, 190) = 9.65$, $P < 0.001$, storage time $F(7, 190) = 42.47$, $P < 0.001$ and interaction of pomace treatment and storage time $F(21, 190) = 15.20$, $P < 0.001$.

Based on the results the addition of pomace in the buckthorn juice arrested the deterioration of TPC for the time period. This effect was in direct proportion to concentration of pomace added to the juice.

The polyphenol content of the SB is depended on the species of SB. In the research of Rop et al. [11], 6 different

Table 3 Effect of pomace treatment and storage time (months) on β -carotene of buckthorn juice

Treatment	β -carotene (mg 100 g ⁻¹)							
	0	2	4	6	8	10	12	14
<i>C</i>	1.93 ± 0.22 ^{aA}	2.40 ± 0.22 ^{abA}	2.46 ± 0.15 ^{abA}	3.04 ± 0.21 ^{bcA}	3.28 ± 0.09 ^{bcA}	3.89 ± 0.03 ^{cA}	3.21 ± 0.24 ^{bcA}	3.71 ± 0.28 ^{cA}
<i>P0.5</i>	3.61 ± 0.11 ^{abB}	3.75 ± 0.15 ^{abB}	4.29 ± 0.16 ^{abcB}	4.61 ± 0.10 ^{bcB}	4.99 ± 0.12 ^{cbB}	5.16 ± 0.13 ^{cbB}	4.35 ± 0.11 ^{abcAB}	4.82 ± 0.38 ^{acAB}
<i>P1</i>	4.37 ± 0.00 ^{abB}	4.66 ± 0.05 ^{abC}	4.29 ± 0.16 ^{abB}	4.98 ± 0.21 ^{acbB}	5.26 ± 0.26 ^{abcB}	5.69 ± 0.05 ^{cbC}	6.03 ± 0.80 ^{cdBC}	5.49 ± 0.35 ^{acBC}
<i>P2</i>	5.87 ± 0.33 ^{abC}	5.76 ± 0.14 ^{abD}	5.59 ± 0.18 ^{abC}	5.23 ± 0.21 ^{abB}	6.11 ± 0.04 ^{abC}	6.24 ± 0.13 ^{cbD}	5.89 ± 0.16 ^{abC}	6.52 ± 0.47 ^{bdC}

Value means more than three replicates and their corresponding standard errors. Superscripts with small case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns.

(Control (*C*) without SB pomace, SB juice + pomace 0.5 % (*P0.5*), SB juice + pomace 1 % (*P1*) and SB juice + pomace 2 % (*P2*))

Table 4 Effect of pomace treatment and storage time (months) on total polyphenol content of buckthorn juice

Treatment	Total Polyphenol Content (TPC); $\mu\text{g GAE mL}^{-1}$							
	0	2	4	6	8	10	12	14
<i>C</i>	1649.98 $\pm 165.59^{\text{Aa}}$	1605.45 $\pm 40.37^{\text{Aa}}$	1331.93 $\pm 33.59^{\text{Ab}}$	1277.75 $\pm 6.55^{\text{Abc}}$	1095.20 $\pm 16.85^{\text{Ac}}$	1191.12 $\pm 32.75^{\text{Abc}}$	1135.79 \pm 23.57 ^{Ac}	1142.21 $\pm 36.11^{\text{Abc}}$
<i>P0.5</i>	1750.59 $\pm 31.02^{\text{Aa}}$	1607.65 $\pm 40.96^{\text{Aab}}$	1441.76 $\pm 43.21^{\text{Abc}}$	1143.61 $\pm 18.19^{\text{ABde}}$	1083.29 $\pm 38.74^{\text{Ae}}$	1276.63 $\pm 28.70^{\text{Acd}}$	1286.97 \pm 29.35 ^{BCcd}	1207.05 $\pm 21.74^{\text{Ade}}$
<i>P1</i>	1439.55 $\pm 43.81^{\text{Aa}}$	1302.34 $\pm 27.06^{\text{Babc}}$	1049.16 $\pm 34.56^{\text{Bd}}$	1032.53 $\pm 23.80^{\text{Bd}}$	1212.57 $\pm 28.98^{\text{Bcd}}$	1433.27 $\pm 43.33^{\text{Bab}}$	1229.13 \pm 63.85 ^{ABbcd}	1286.18 $\pm 105.07^{\text{Aabc}}$
<i>P2</i>	1400.99 $\pm 37.46^{\text{Ab}}$	1408.53 $\pm 25.50^{\text{Bb}}$	1068.88 $\pm 65.04^{\text{B}}$	1097.22 $\pm 30.69^{\text{Bc}}$	1320.16 $\pm 18.99^{\text{Cbc}}$	1459.26 $\pm 35.74^{\text{Bab}}$	1374.44 \pm 29.05 ^{Cb}	1624.41 $\pm 42.95^{\text{Ba}}$

Value means more than three replicates and their corresponding standard errors. Superscripts with small case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. (Control (*C*) without SB pomace, SB juice + pomace 0.5 % (*P0.5*), SB juice + pomace 1 % (*P1*) and SB juice + pomace 2 % (*P2*))

varieties of SB were compared. The total polyphenol content of Leikora SB, which we also examined, was $9.74 \pm 1.20 \text{ g GAE kg}^{-1}$ of fresh mass and had the 3rd highest polyphenol content of the studied varieties.

Various fruits were studied by Nowak et al. [9], in which the total polyphenol content of SB was determined to be $4784 \pm 35 \mu\text{g GAE mL}^{-1}$, which showed a higher result, as Gojiberry and Cranberry.

3.4 Change of antioxidant capacity (FRAP)

By the FRAP values, in all four cases (*C*, *P0.5*, *P1*, *P2*) a decreasing tendency was observed during storage, however, the higher pomace content of the juice was, the smaller decrease of FRAP values was between the initial and the final state (43.7 %, 43.2 %, 32.2 % and 29.4 %). The more pomace contained the juice, the higher FRAP value. At the end of the 14 months storage period, the samples could be separated into two groups. Samples *C* and *P0.5* had lower antioxidant capacities, 598.5 and $648.2 \mu\text{g AA mL}^{-1}$, while the samples *P1* and *P2* had higher values, 789.2 and $849.4 \mu\text{g AS mL}^{-1}$, respectively.

Both pomace treatment and storage time had significant effect on antioxidant activity of buckthorn juice. Pomace treatment, $F(3, 179) = 74.343$, $P < 0.001$ and storage time, $F(7, 179) = 82.192$, $P < 0.001$. Interaction of pomace treatment and storage time was not significant, $F(21, 179) = 0.833$, $P = 0.677$.

Sea Buckthorn juice have phytochemicals that lead to antioxidant activity. Treating the juice with 1 % and 2 % of pomace significantly increased the antioxidant activity of the buckthorn juice compared to control and treatment by 0.5 %. Increase of antioxidant activity could be resulted phytochemicals mainly phenolic compounds from the pomace.

Early days after juice preparation had significant higher amount of antioxidant activity compared to late days. The antioxidant activity decreased during the storage (Table 5). Decrease of antioxidant in relation to storage time could be due to degradation of phytochemicals that lead to loss of antioxidant activity of the juice.

There is a correlation between TPC and FRAP values, as also shown by Makovics-Zsohár et al. [33], who found strong correlation between TPC and FRAP values.

Table 5 Effect of pomace treatment and storage time (months) on antioxidant activity of buckthorn juice

Treatment	FRAP; $\mu\text{g AA mL}^{-1}$							
	0	2	4	6	8	10	12	14
<i>C</i>	1066.04 $\pm 12.52^{\text{aA}}$	991.05 $\pm 10.52^{\text{bA}}$	929.40 $\pm 29.45^{\text{bA}}$	955.98 $\pm 24.84^{\text{bA}}$	825.25 $\pm 22.49^{\text{cA}}$	811.73 $\pm 24.05^{\text{cA}}$	679.64 $\pm 22.37^{\text{dA}}$	598.50 $\pm 17.76^{\text{dA}}$
<i>P0.5</i>	1141.45 $\pm 30.82^{\text{aA}}$	957.33 $\pm 52.52^{\text{bA}}$	910.89 $\pm 24.53^{\text{bA}}$	976.37 $\pm 50.82^{\text{bA}}$	881.02 $\pm 21.79^{\text{cA}}$	844.29 $\pm 23.51^{\text{cA}}$	709.60 $\pm 18.31^{\text{dA}}$	648.22 $\pm 11.09^{\text{dA}}$
<i>P1</i>	1164.74 $\pm 27.34^{\text{aB}}$	1058.35 $\pm 31.00^{\text{bB}}$	1063.95 $\pm 29.92^{\text{bB}}$	1033.15 $\pm 19.10^{\text{bB}}$	940.12 $\pm 30.76^{\text{cB}}$	917.90 $\pm 24.37^{\text{cB}}$	773.66 $\pm 17.58^{\text{dB}}$	801.56 $\pm 16.52^{\text{dB}}$
<i>P2</i>	1202.37 $\pm 17.09^{\text{aC}}$	1194.75 $\pm 33.91^{\text{bC}}$	1152.20 $\pm 24.72^{\text{bC}}$	1165.93 $\pm 64.72^{\text{bC}}$	1053.88 $\pm 27.36^{\text{cC}}$	1035.72 $\pm 31.57^{\text{cC}}$	890.83 $\pm 22.02^{\text{dC}}$	859.44 $\pm 35.06^{\text{dC}}$

Value means more than three replicates and their corresponding standard errors. Superscripts with small case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. (Control (*C*) without SB pomace, SB juice + pomace 0.5 % (*P0.5*), SB juice + pomace 1 % (*P1*) and SB juice + pomace 2 % (*P2*))

The closeness of the linear relationship between the two parameters was investigated by Pearson's correlation coefficient for each sample separately, the results are shown in Table 6.

The values of the correlation coefficient give a value close to 1, $r = 0.8614$ on average. This means that there is a positive correlation between the total polyphenol content and the antioxidant capacity based on the iron reducing ability, that is, if one parameter increases, the other increases. The relationship would be completely linear if r were 1 (or -1 for negative correlation).

The correlation between FRAP and TPC indicates that a significant part of the antioxidant capacity of Sea Buckthorn is due to different polyphenols in the berry [33]. Overall, FRAP and TPC measurements confirm that the pomace has an antioxidant effect, because in each case higher values were obtained for the juice with higher pomace content.

4 Conclusion

The aim of this research was to set up a technological process to obtain high-value biologically active extracts from Sea Buckthorn pomace, thereby helping to reduce waste from the juice industry. On one hand, these could be retrieved using the proper method, and used again by the food industry. In our study, we have successfully recovered and recycled the antioxidant compounds from pomace of Sea Buckthorn to produce more valuable and Sea Buckthorn juice. These new aspects concerning the use of the pomace as by-products for further exploitation on the production of food additives with high nutritional value, their recovery may decrease quantity of a waste of valuable components and may be economically attractive.

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Table 6 Values of Pearson's correlation coefficient between TPC and FRAP

Sampling	Correlation coefficient (R)
0	0.7851
2	0.6487
4	0.9293
6	0.9135
8	0.9502
10	0.9922
12	0.8635
14	0.8087

These experiments prove that the added pomace has a protective effect for the valuable components of the juice (antioxidant compounds, polyphenol-type compounds, β -carotene), since the amount of these decreased to a lesser extent in the pomace-containing juice ($P1$, $P2$), as in the control sample (C). Similar results were obtained by Pilizota et al. [34] who also enriched cranberry jam with an extract rich in antioxidants (tea or pine nuts) and concluded that these extracts can be used as a protective agent against the thermal degradation of anthocyanins.

Experiments have shown that Sea Buckthorn has a remarkable antioxidant effect, which can neutralize free radicals and helps maintain health [35]. In order to avoid wastage of significant quantities of nutrients by treating the pomace as waste, it would be worth to use it not only for feeding purposes, but also in functional foods for human consumption.

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