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OPTIMIZING OF EXTRACTION OF BIOACTIVE COMPONENTS FROM SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.) POMACE AND DEVELOPE OF ANTIOXIDANT-ENRICHED APPLE JUICE

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ABSTRACT

In our study, we were looking for an alternative to use of sea buckthorn pomace to open new opportunities. The dried pomace was extracted in a different method to achieve the highest antioxidant content. Ethanol and acetone were used as solvents, applied in different concentration. For the selection of the optimum extraction method, four spectrophotometric measurements were performed. With the best extract, three types of apple juice were made from apple juice concentrate by diluting it with different percentages of water and pomace extracts. The spectrophotometric measurements were also performed for juice samples to check the increase in amount of antioxidant components in the apple juice. Acceptability of enriched juices was determined by sensory evaluation and short market research. Based on the results the optimum extraction agent is 40 m/m % acetone for enriching apple juice. The results showed that there is a demand for the consumption of enriched juice with sea buckthorn pomace extracts and the fruit juice enriched with the 1:1 ratio of extract: water mixture is the most optimum according to sensory evaluators. Further examination could reveal whether the extracted antioxidant content of the pomace could be used as bio-preservatives in the food industry.

Keywords: sea buckthorn, pomace, apple juice, antioxidant, by-product, product-development

1. INTRODUCTION

The food waste is a critical subject in every industry, in every household; but in many cases by-products should not be considered as waste [1-3]. Fruit and vegetable wastes are produced in large quantities in food industry and constitute a source of nuisance in landfills because of their high biodegradability [4]. 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 biocolors [5]. The management of food processing by-products and wastes with reference to their reuse and recycling through value addition [6].
The European sea buckthorn (SB, *Hippophae rhamnoides* L.) is a deciduous shrub, which belongs to the *Eleagnaceae* family (*Rosales*). 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 [7-9]. 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 [10]. 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 are bioactive compounds. There is a growing interest in the utilization of antioxidant-rich plant extracts as dietary food supplements [11-12], so our goal is to find a method to extract this valuable antioxidant components and develop a delicious enriched apple juice with contain this pomace extracts.

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2. MATERIALS AND METHODS

The "Ascora" sea buckthorn was collected from agricultural plots of Hungary at 2017. For the measurements the all chemicals were purchased by Sigma-Aldrich Chemie Ltd. All reagents used were of analytical grade.

2.1. Pre-treatment of sea buckthorn

Sea buckthorn was destemmed and heated to 80°C to inactivate their enzymes. The berries were squeezed, resulting in juice and pomace (skin and seed). In the next step, the sea buckthorn pomace (SBP) was dried by atmospheric dryer (LMIM, Esztergom, Hungary) at 80°C until moisture content became lesser than 10 (m/m) %. The moisture content was check by drying until constant weight at 121 °C using a MAC-50 moisture analyser (Radwag Waagen GMBH, Hilden, Germany). After that, the pomace was grinded. All SBP were stored in a freezer at -20 °C until ready for extraction, which was performed at room temperature.

2.2. Optimization of extraction condition

Optimization of conditions for the extraction of antioxidants and phenolics components from dried SBP was determined using water, ethanol and acetone as solvents, applied in different concentration (in case of ethanol and acetone: 20 (m/m) % and 40 (m/m) %). The ratio between pomace and solvent was 1:30 proportions. After half-an-hour of extraction, the samples were placed into a supersonic bath for another 30 minutes **(***Bandelin Sonorex RK52)*, to intensify the process. The tube is centrifuged (SIGMA 204) at 5000 1/min for 10 min to the phases separate and the supernatant is recovered. After the solvents were removed (by heating at 60°C) and replaced with water, the evaporated and back diluted sample was treated with bentonite to give translucent, completely clear liquid.

2.3. Measurements of antioxidant status of pomace extracts

Various spectrophotometric measurements were carried out to select the highest antioxidant content of pomace extracts. All spectrophotometry measurements were performed in triplicate.

- **TPC:** Total Polyphenol Content (TPC) was evaluated using a method by Singleton and Rossi [16]. The absorbance was measured at 765 nm. Results were specified in mg gallic acid equivalent/ 100 g dried pomace (mg GA/100g).
- **FRAP:** The antioxidant capacity of samples was determined by Benzie and Strain [13]. This method is based on reduction of Fe3+, TPTZ (2,4,6-tripyridyl-s-triazine) complex to the ferrous Fe2+ form at low pH. This reduction is followed by the measurement of absorption change at 593 nm. Ferric reducing antioxidant power assay was defined in ascorbic acid equivalent (mg ascorbic acid equivalent/ 100 g dried pomace; mg AA/100g).
- **DPPH:** The samples were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (2,2-Diphenyl-1-picrylhydrazyl) [14]. The results were expressed as milligrams of Trolox equivalents (TE) per 100g dried pomace (mg TE/100g). Higher absorbance of the reaction mixture indicates lower free radical scavenging activity.
- **TEAC:** The free radical-scavenging activity was estimated by ABTS radical cation decolorization assay according to the procedure described by Re et al. [15]. The results were corrected for dilution and expressed as milligrams of Trolox equivalents (TE) per 100 grams of dried pomace (mg TE/100g).

2.4. Processing of enrichment of apple juice

With the best extraction method, three types of apple juice (11,2 Brix°, ATAGO PR-301) were made from apple concentrate (70 Brix°) by diluting it to minimum 11,2 Brix° [according to Hungarian regulation of 152/2009] with different mixture of water and pomace extracts (Tab. 1.).

Table 1. Ingredients and name of juices

Sample name		Apple juice	Water	Pomace extract
Control apple juice	CA	70 Brix°	100%	0%
50% - enriched apple juice	EA1		50%	50%
100% - enriched apple juice	EA2		0%	100%

The spectrophotometry methods were carrying out also for the juices, the results expressed mg per litre juice (mg/L).

2.5. Sensory evaluation of apple juice

Acceptability of enriched juices was determined by sensory evaluation and short market research. The sensory evaluation was carried out with 30 lay judges, the samples were coded with three-digit numbers. The evaluation was based on 100-point system, during the analysis the attributes were as follows: colour (max. 20 point) turbidity (max. 10 point) flavoured (max. 10 point) taste (max. 40 point) sea buckthorn taste (max. 10 point) of juices and overall impression. Evaluations of the samples were carried out separately and independently without any influence.

2.6. Statistical data evaluation

Single-factor ANOVA was used for monitoring the relations of the results of the individual analytical methods on different extraction conditions, separately. Correlation analysis was used to analyse the relation of the results of the antioxidant measurements. The significance level of all tests was specified to be $P=0.05$. The statistical evaluations were performed using Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1. Results of antioxidant measurements

Dried SBP was extracted with different solvents (water, 20 and 40 (m/m) % ethanol and acetone), the samples were analysed to define total polyphenol content, as well as their antioxidant capacity (FRAP, DPPH, TEAC), to find the most effective method of extraction to achieve an extract rich in biological activity components (Tab. 2.).

Table 2. The average results of the spectrophotometry measurements of the pomace extracts (mg/100g pomace)

	Water	Acetone		Ethanol	
		20%	***40%***	***20%***	***40%***
TPC	1026.26±100.23	1587.54±164.61	2265.99±598.66	917.51±86.06	1146.46±338.38
FRAP	324.11±114.65	687.13±182.72	1925.59±49.52	486.47±403.95	1295.21±197.13
DPPH	4464.29±4.46	6422.99±6.42	10796.13±10.79	7047.99±7.05	5630.58±5.63
TEAC	302.36±0.30	687.86±0.68	9708.45±9.71	4931.78±4.93	7276.74±7.28

x±SD(x: mean, SD: standard deviation

Previous studies had also shown, that the fruits of SBT cultivars have strong antioxidant effect, and these results were right also in case of pomace extracts [17-19]. The results showed significant differences between the species (p_{TPC}=2.5*10^{-3}, p_{FRAP}=2.8*10^{-5}, p_{DPPH}=7.4*10^{-14}, p_{TEAC}=6.3*10^{-19}). Based on the results the water as a solvent was not efficiently, these results of antioxidant measurements were the lowest value in most cases. The optimum extraction agent is acetone to the highest antioxidant content of pomace extracts. Between the extraction efficient of two concentration of acetone was significantly difference ($p<0.05$), so the best extraction solvent was the 40 m/m % acetone for extracting the valuable components from the pomace.

Table 3. The average results of the spectrophotometry measurements of the juice samples (mg/L)

	CA	**EA1**	**EA2**
TPC	372.80±11.57	329.68±72.63	462.15±19.79
FRAP	4338.54±477.06	10494.78±1039.99	14315.44±769.65
DPPH	150.67±2.01	196.61±1.70	398.56±1.87
TEAC	40.71±1.16	197.04±0.70	242.71±1.91

x±SD(x: mean, SD: standard deviation

Based on the results of juices (Tab 3.), our desired goal - the enrichment of apple juice - was met, the antioxidant compounds, the antioxidant capacity value of FRAP, DPPH and TEAC method greatly increased by added the pomace extract. The all measurements showed outstanding results compared to the control sample. The antioxidant content of the sample increases in direct proportions with the amount of added pomace extract. The sample EA2 (enriched apple juice, with 100% pomace extract) had the most antioxidant content.

Table 4. Results of correlation analysis between the antioxidant methods.

	TPC	FRAP	DPPH	TEAC
TPC	1			
FRAP	0.79	1		
DPPH	0.84	0.78	1	
TEAC	0.49	0.87	0.74	1

This study assessed antioxidant potential of both extracts by using four different methods, which gave different antioxidant activity values (Tab. 2-3). From our values in Tab. 4, the greatest mutual correlation is shown between the results obtained by FRAP and TEAC methods, with the correlation coefficient being 0.87. In case of the most methods the coefficient was greater than 0.74, expect, the low correlation between TPC and TEAC was found.

3.2. Results of sensory analysis and market research

The enriched apple juices had beneficial sensory properties (Fig.1), the tasters were satisfied with finished products.

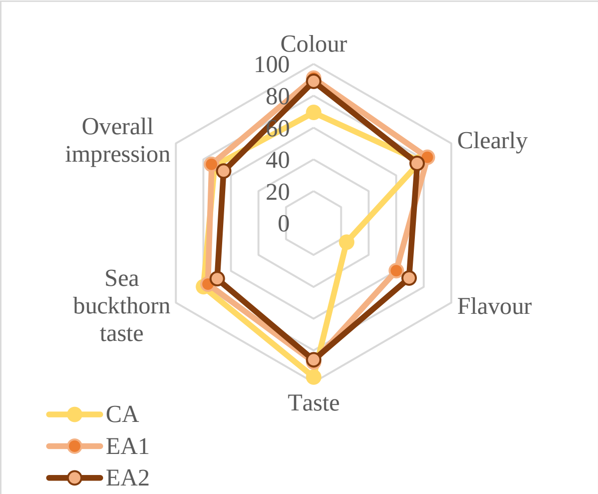

Figure 1. **Results of sensory analysis**

During product development, the flavour and sour flavour from SBP should be improved and decreased because the participants were the least satisfied with these parameters. According to the Fig. 2., apple concentrates dilute with 50% pomace extract (EA1 sample) is the most optimum according to evaluators. In case of this sample, the typical acidic flavour of the sea buckthorn did not yet negatively affect the taster and the ratio of apple and SBP was optimal and harmonic.

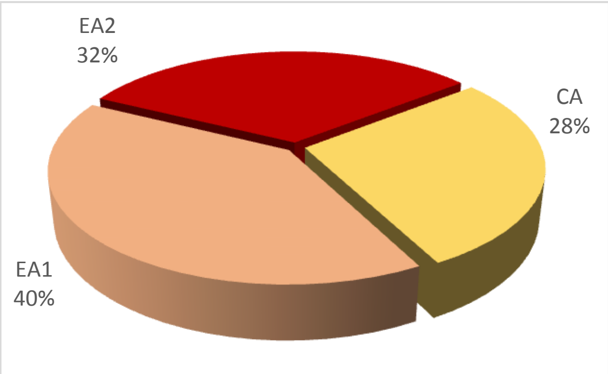

Figure 2. Results of market research, percentage distribution of popularity level

The results of the market research showed that there is a demand for the consumption of enriched juice. Overall, according to these data, consumers are open to new products, especially if they have a health benefit and are made up of natural materials. The 86 % of tasters are happy to buy the finished products. It can be assumed that consumers have strong connect between the healthy diet and sea buckthorn, so the young and health conscious consumer can be targeted with this product.

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4. CONCLUSIONS

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. In the past few years, treating waste coming from the food industry, has become a remarkably important issue, due to environmental and economic reasons. Recycling waste should mean a satisfactory alternative, particularly, if we consider the amount of valuable components remaining in the waste of certain plants. 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 special apple 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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