DEVELOPING OF ANTIOXIDANT-ENRICHED APPLE JUICE WITH ELDERBERRY (*SAMBUCUS NIGRA* L.) POMACE

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Abstract
The aim of this study to develop a healthy enriched apple juice and utilization of by-product of elderberry pomace. The elderberries and their pomace contain high biological activity components, primarily polyphenols, anthocyanins, flavanols which compounds are known to have potential antioxidant properties. In this study the most efficiently extraction method was explored by using different solvents and using the chosen best method, our goal is to develop a delicious enriched apple juice with contain this pomace extracts.

Introduction
The food waste is a very important subject in the food industry, especially since in many cases by-products should not be considered as waste [1-3]. Our aim is that we are looking for an alternative to the use of fruit pomace to open new opportunities for the food industry. The chosen cultivar, Haschberg is from Hungary. Black elderberry (*Sambucus nigra* L.) is from Adoxaceae family, its shrubs are multi stemmed, they have weak, grey coloured branches on which fruit clusters develop with 5-9 mm (d) glossy, dark purple berries [4-5]. These elderberries contain high biological activity components which compounds are known to have potential antioxidant properties [6-8]. The berries can be utilized as coloring food, jam, or, because of its favorable composition, as dietary supplements [9]. Processing of elderberry fruit 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 elderberry is pressed, is also a good source of vitamins and contains compounds that show antioxidant effects like anthocyanins, flavonols, and phenolic acids are bioactive compounds present in the elderberry. There is a growing interest in the utilization of antioxidant-rich plant extracts as dietary food supplements [11-12]. Our goal, when conducting this experiment, was to find the right extraction method for extracting the antioxidant compounds from black elderberry pomace and produced apple juice were improved by using the extract of pomace which contain high biological active compounds.

Experimental
The “Haschberg” elderberry was collected from agricultural plots of Hungary at 2017. Chemicals were purchased by Sigma-Aldrich Chemie Ltd. All reagents used were of analytical grade.

Elderberry was destemmed, and then heated to 80°C, to inactivate enzymes. The material was squeezed, resulting in juice and pomace. Drying the pomace was the next step by atmospheric dryer (LMIM, Esztergom, Hungary) at 80°C until moisture content became lesser than 10%. After this step the pomace was grinded. All samples were sealed in bag and stored in a freezer at -20 °C until ready for extraction, which was performed at room temperature.

The optimum conditions for the extraction of antioxidants, anthocyanins and phenolics components from dried elderberry pomace 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, supersonic bath was used for another 30 minutes, to intensify the process. The tube is centrifuged at 2500g 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 a translucent, completely clear liquid. Various spectrophotometric measurements were performed to select the highest antioxidant content of pomace extracts. All measurements were performed in triplicate.

- The antioxidant capacity of samples was estimated according to the procedure described by Benzie and Strain [13]. Ferric reducing antioxidant power assay (FRAP) was defined in ascorbic acid equivalent (mg ascorbic acid equivalent/ 100 g dried pomace – mg AA/100g).
- 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 millimoles of Trolox equivalents (TE) per 100g dried pomace (mM TE/100g). Higher absorbance of the reaction mixture indicates lower free radical scavenging activity.
- The free radical-scavenging activity was determined by ABTS radical cation decolorization assay described by Re et al. [15]. The results were corrected for dilution and expressed as millimoles of Trolox equivalents (TE) per 100 grams of dried pomace (mM TE/100g).
- Total Polyphenol Content (TPC) was evaluated using a method by Singleton and Rossi [16]. Results were specified in mg gallic acid equivalent/ 100 g dried pomace (mg GA/100g).
- Total Anthocyanin Content (TAC) measurement was based on Lee's pH differential method [17], results are given in mg/ 100 g dried pomace (mg/100g).

Table 1. Ingredients and name of juices
(in case of TPC, TAC and FRAP the results were expressed as mg/100g dried pomace, in case of DPPH and TEAC method, they were given is mM/100g dried pomace)

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%

With the best extraction method, three types of apple juice (12 Brix°) 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 (Table 1.). The spectrophotometry methods were carrying out also for the juices sample, the results expressed per milliliter juice. Acceptability of enriched juices was determined by sensory evaluation and short market research. The sensory evaluation has been carrying out with 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: color (max. 20 point) turbidity (max. 10 point) flavored (max. 10 point) taste (max. 40 point) elderberry taste (max. 10 point) of juices and overall impression. Evaluations of the samples were carried out separately and independently without any influence.

Results and discussion

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

Table 2. The average results of the spectrophotometry measurements of the pomace extracts
(in case of TPC, TAC and FRAP the results were expressed as mg/ml, in case of DPPH and TEAC method, they were given is mM/ml)

Extraction solvents		TAC	TPC	FRAP	TEAC	DPPH
Water		54.21	1166.33	1570.27	2645.50	34100.02
Acetone	**20 %**	411.70	1400.13	1927.75	3424.85	38155.56
	40 %	418.22	1305.67	1992.08	3841.21	40322.22
Ethanol	**20 %**	390.64	1207.83	1736.73	227.39	21100.03
	40 %	401.17	1323.38	2066.15	1663.32	28988.88

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 not significantly difference. For economic and health considerations the lower concentration of solvent (20% acetone) was recommended to use for extracting the valuable components from the pomace.

Table 3. The average results of the spectrophotometry measurements of the juice samples

	TAC	TPC	FRAP	TEAC	DPPH
CA	0.04	0.37	4.34	0.22	0.73
EA1	12.77	0.39	7.12	0.20	0.62
EA2	21.70	0.44	11.07	0.25	0.86

Based on the results of juices (Table 3.), our desired goal - the enrichment of apple juice - was met, the antioxidant compounds, the FRAP value, polyphenol and anthocyanin content greatly increased by added the pomace extract. Most of the 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.

The enriched apple juices had beneficial sensory properties (Fig.1), the tasters were satisfied with finished products. The most of them are happy to buy the finished products. During product development, the smell and the clearly of the juices should be improved as the participants were the least satisfied with these parameters. According to the sensory analysis, fruit concentrates dilute with only pomace extract without water (EA2 sample) is the most optimum according to evaluators. The results of the

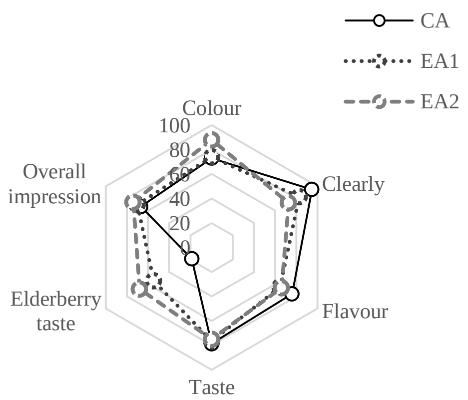

Figure 1. Results of sensory analysis

market research showed that there is a demand for the consumption of enriched juice. Overall, according to market research data, consumers are open to new products, especially if they have a health benefit and are made up of natural materials.

Conclusion

We have successfully recovered and recycled the antioxidant compounds from pomace of elderberry 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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References

[1] A. Mirzaei-Aghsaghali, N. Maheri-Sis, World Journal of Zoology, 3 (2008) 40-46.
[2] K. Kónya, Szarvas, 5-6. (2000) 63-64.
[3] K. Brønnum-Hansen, F. Jacobsen, J. Flink, International Journal of Food Science & Technology 20, (1985) 703–711.
[4] D. Topolska, K. Valachova, P. Rapta S. Silhár, E. Panghyová, A. Horváth, L. Soltés, Chemical Papers 69 (2015) 1202-1210.
[5] E. Mudge, W.L.Applequist, J. Finley, P. Lister, A.K. Townesmith, K.M. Walker, P.N. Brown, Journal of Food Composition and Analysis 47 (2016) 52-59.
[6] I. Ochmian, J. Oszmianski, K. Skupien, Journal of Applied Botany and Food Quality 83 (2009) 64-69.
[7] A. Sidor, A. Gramza-Michalowska, Journal of Functional Foods (2014) 941-958.
[8] M. Oroian, I. Escriche, Food Research International, 74 (2015) 10-36.
[9] C. Chen, X.-M. Xu, Y. Chen, M.-Y. Yu, F.-Y. Wen, H. Zhang, Food Chemistry, 141 (2013) 1573-1579.
[10] C.M. Galanakis, Trends in Food Science & Technology, 26 (2012) 68-87.
[11] C. Eccleston, Y. Baoru, R. Tahvonen, H. Kallio, G.H. Rimbach, A.M. Minihane, The Journal of nutritional biochemistry, 13(6), (2002) 346-354.
[12] B. Yang, H.P. Kallio, J. Agric. Food Chem, 49 (2000) 1939−1947.
[13] I.I.F. Benzie, J.J. Strain, Annalitical Biochemistry, 239. (1966) 70-76
[14] M.S. Blois, Nature, 181 (1958) 1199-1200.
[15] N.J. Miller, C. Rice-evans, M.J. Davies, V. Gopinathan, A. Milner; Clinical Science, 84 (1993) 407-412.
[16] V.L. Singleton, J.A. Rossi, American Journal of Enology and Viticulture, 16 (1965) 144–158.
[17] J. Lee, R. Durst, R. Wrolstad, Journal of AOAC International, 88, (2005) 1269-1278.