THE EFFECT OF DIFFERENT DRYING AND EXTRACTION CONDITIONS ON THE ANTIOXIDANT CONTENT OF ELDERBERRY POMACE

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Abstract

Nowadays waste is a critical subject in every industry, in every household; but in many cases by-products should not be considered as waste. The aim of this study is to explore the best combination of drying and extraction, to achieve the highest antioxidant content in elderberry pomace, which appears as a by-product when making use of the berry. The chosen cultivar, Haschberg is from a pen in Hungary, these elderberries contain high biological activity components, primarily polyphenols, anthocyanins, flavonols which compounds are known to have potential antioxidant properties. In this study, the antioxidant capacity was determined by FRAP (Ferric Reducing Ability of Plasma), Total Polyphenol Content (TPC) and Total Anthocyanin Content (TAC) assays. The optimal drying conditions were previously tested, relying on the results atmospheric drying was executed at 60 and 80 °C. When extracting the antioxidant compounds, ethanol and acetone were used as solvents, applied in different proportions: 1:10, 1:20, 1:30. Based on our result, the highest antioxidant capacity was registered using 20 V/V % acetone in 1:30 ratio, extracted from the pomace, dried at 60°C. Further examination could reveal whether the extracted antioxidant content of the elderberries could be used as bio-preservatives in the food industry.

Introduction

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 [1, 2]. Anthocyanins (specifically cyanidin-3-sambubioside) are responsible for the colour of the berries, as they are the most widespread water-soluble plant pigments [3]. The berries are a good source of protein, amino acids, vitamins and minerals, and contains compounds that show antioxidant effects. Anthocyanins, flavonols, and phenolic acids are bioactive compounds present in the elderberry, which can safely interact with free radicals and even terminate molecule damaging chain reactions [4, 5]. Almost every part of the plant is used somehow, that is why elderberry is known as one of the oldest medical plants [6]. Elderberry's medical properties are associated with polyphenols, antioxidants that play an essential role in preventing several diseases (cardio-vascular, neurodegenerative, etc.) [7]. In the 21st century, industries try to benefit from this plant in many ways. For instance, berries can be utilized as colouring food, jam, or, because of its favorable composition, as dietary supplements [8]. Pomace, which is left when elderberry is processed, could be beneficial to use as animal feed, or to make alcoholic beverages from it. However, when it is well known, that 75-98% of total anthocyanin content can be found in this byproduct, the question arises: shouldn't we take advantage of what we already have, rather than causing more environmental issues? [9]

Our goal, when conducting this experiment, was to find the right extraction method for extracting the antioxidant compounds from black elderberry pomace, which allows further usage in the food industry.

Experimental

The raw material of the study was collected from Nagyenyed, where Haschberg cultivar is grown by a co-operative society called BOTÉSZ. Chemicals used for extraction and antioxidant measurements were provided by Sigma-Aldrich Chemie Ltd.

Elderberries were destemmed, mashed, and then heated to 80°C, to inactivate enzymes. When it was cooled to 35 °C, pectolytic enzyme, Fructozym P was given as a treatment. After an hour, the material was pressed, resulting in juice and pomace. Drying was the next step, using atmospheric dryer at 60°C and 80°C, then the pomace was grinded. It was time, for the extraction, which was performed at room temperature, using two different solvents, acetone and ethanol at different concentrations 20 V/V % and 40 V/V%. We differed one more parameter, the ratio between pomace and extraction solvent, using 1:10, 1:20 and 1:30 proportions. After half-an-hour of extraction, supersonic bath was used for another 30 minutes, to intensify the process. Centrifugation made the phases separate, extracts were further analysed using three methods:

- Antioxidant capacity was determined based on Ferric Reducing Ability of Plasma (FRAP) method, by Benzie and Strain [10]. Antioxidant capacity was defined in ascorbic acid equivalent (mg ascorbic acid equivalent/ 100 g DW).
- Total Polyphenol Content (TPC) was evaluated using a method by Singleton and Rossi [11]. Results were specified in mg gallic acid equivalent/ 100 g DW
- Total Anthocyanin Content (TAC) measurement was based on Lee's pH differential method [12], result are given in mg/ 100 g DW.

Results were calculated, statistical evaluations were performed using Microsoft Excel. Difference between drying at 60°C and 80°C was evaluated by Student t-test at 95% confidence. One-way ANOVA was used for testing the effect of extraction solvents. Data were also evaluated using Pearson's correlation coefficients to identify relationships between phenolic contents, anthocyanin contents and antioxidant activities of elderberry pomace extract.

Results and discussion

Samples, dried at different temperatures, and extracted with two solvents, were measured to define total anthocyanin and polyphenol content, as well as their antioxidant capacity, in order to find the most effective method of extraction to achieve an extract rich in biological activity components.

Figures 1-3. show the average results of the measurements. Elderberry pomace dried at 60 °C showed significantly higher ($p>0.05$) outcome, consequently, lower drying temperature affected the antioxidant compounds in a positive way. Regarding the solvents applied, 20 V/V% concentrated acetone extracted the analyzed components with the best results. More concentrated solvents effected the process negatively, in all cases. Figure 1. draws the attention to the measured FRAP values, which varied between 824.57 and 3529.98 mg ascorbic acid equivalent/ 100 g DW. The highest antioxidant capacity was registered using 20 V/V % acetone in 1:30 ratio with pomace made at 60°C.

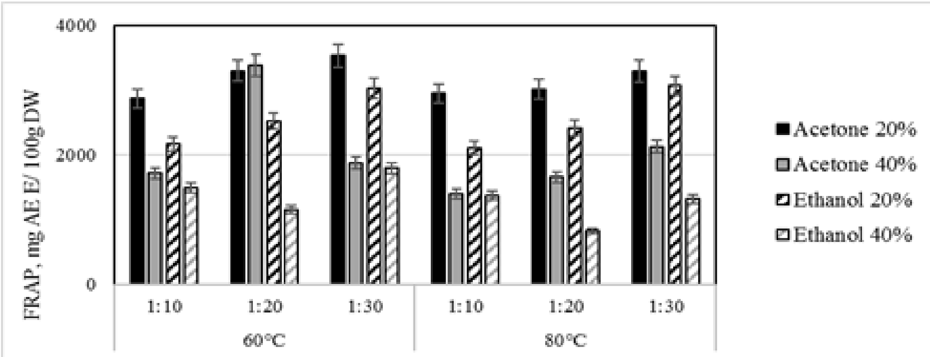

Figure 1. Average values of antioxidant capacities, evaluated using the method relying on Ferric Reducing Ability of Plasma, mg ascorbic acid equivalent/ 100g DW

In Figure 2., total antioxidant content concentrations in mg/ 100 g DW are shown. The highest value (2128.99 mg/ 100 g DW) was reached by the sample which was dried at 60°C, extracted with 20 V/V % acetone, and dosed in 1:30 ratio. This result corresponds with the conclusion of the FRAP method. The sample dried at the same temperature, but treated with 40 V/V% ethanol in 1:20 ratio led to the lowest TAC detected (548.68 mg/ 100 g DW).

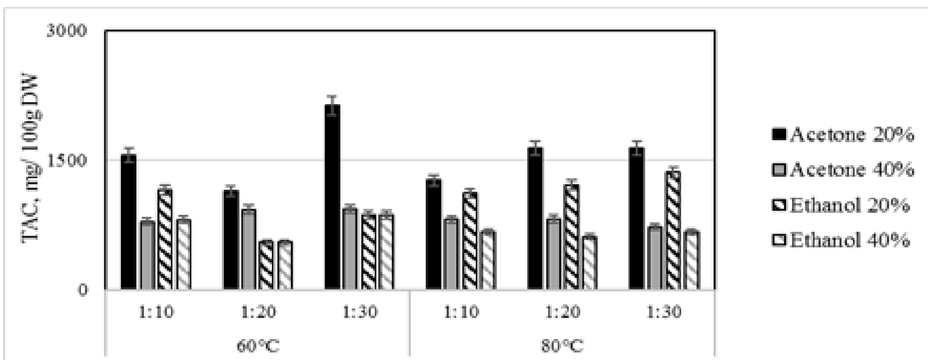

Figure 2. Average values of Total Anthocyanin Content, mg/ 100 g DW

Avarage results, regarding the total polyphenol content of the pomace samples, are presented in Figure 3. All measured polyphenol contents were between 1304.98 and 4318.40 mg gallic acid equivalent/ 100 g DW. Unlike the previous methods, TPC experiment showed the highest value using 20 V/V% ethanol, whereas the second highest was achieved via 20 V/V% acetone (3961.39 mg GAE / 100 g DW, $p>0.05$).

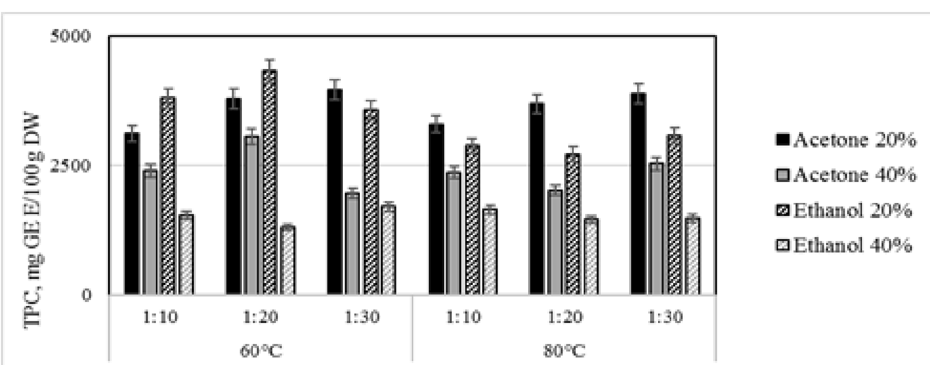

Figure 3. Total polyphenol content (TPC) average results, mg gallic acid equivalent/100g DW

It is well known, that antioxidant potential in plants often correlates with phenolic or anthocyanin compounds [13]. In order to find out which components correlate with antioxidant capacity, Pearson Product Moment Correlation was conducted. We present Person correlation coefficients in Table 1. The highest positive correlation was registered between polyphenol content and antioxidant capacity (R=0.855), there is a weaker correlation (R=0.74) between anthocyanin content and antioxidant capacity.

	FRAP	*TPC*	*TAC*
FRAP	1		
TPC	0.86	1	
TAC	0.74	0.62	1

Table 1. Pearson's correlations among total phenolic content (TPC), total antocianin content (TAC) and antioxidant capacity (FRAP) of elderberry pomace

Conclusion

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. On the other hand, the adequate method widely depends on the treatments applied during the preparation of the samples.

The aim of this study was to extract bioactive compounds from black elderberry pomace, using the optimal extraction-method. According to our results, the most efficient technique is drying at 60°C, using 20% concentrated acetone solvent in 1:30 proportion, for an hour. This combination resulted in outstanding yield, regarding the antioxidant capacity, and the content of both anthocyanins and polyphenols.

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References

[1] D. Topolska, K. Valachova, P. Rapta S. Silhár, E. Panghyová, A. Horváth, L. Soltés, Chemical Papers 69 (2015) 1202-1210.

[2] 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.

[3] I. Ochmian, J. Oszmianski, K. Skupien, Journal of Applied Botany and Food Quality 83 (2009) 64-69.

[4] A. Sidor, A. Gramza-Michalowska, Journal of Functional Foods (2014) 941-958.

[5] M. Oroian, I. Escriche, Food Research International, 74 (2015) 10-36.

[6] A. Simonyi, Z. Chen, J. Jiang, Y. Zong, D.Y. Chuang, Z. Gu, C. Lu, K.L. Fritsche, C.M. Greenlief, G. E. Rottinghaus, A. L. Thomas, D. B. Lubahn, G. Y. Sun, Life Sciences 128 (2015) 30-38.

[7] H.G. Duymus, F. Göger, K. H. C. Baser, Food Chemistry 155 (2014) 112-119

[8]M. M. Stégerné, in B. Sipos, A fekete bodza termesztése, Mezőgazda Kiadó, Budapest, 2010, pp. 82-86.

[9] K. Brønnum-Hansen, F. Jacobsen, J. Flink, International Journal of Food Science & Technology 20, (1985) 703–711.

[10] F. Benzie, J. Strain, Analytical Biochemisrty 239, (1996) 70–76.

[11] V.L. Singleton, J.A. Rossi,American Journal of Enology and Viticulture 16, (1965) 144–158.

[12] J. Lee, R. Durst, R. Wrolstad, Journal of AOAC International, 88, (2005) 1269-1278.

[13] S. Dudonné, X. Vitrac, P.Coutiere, M. Woillez, J.M. Mérillon, Journal of Agricultural and Food Chemistry, 57 (2009), 1768-1774.