PRELIMINARY STUDY OF OPTIMAL EXTRACTION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.) POMACE

Diána Furulyás[1], Rentsendavaa Chagnaadorj[1], Fanni Kis[1], Katalin Bíró[1], Mónika Stéger-Máté[1], Éva Stefanovits-Bányai[2]

[1]*Department of Food Preservation, Szent István University, H-1118 Budapest, Villányi street 29-43., Hungary*
[2]*Department of Applied Chemistry, Szent István University, H-1118 Budapest, Villányi street 29-43., Hungary*
e-mail: furulyas.diana@etk.szie.hu

Abstract
The processing of fruits results in high amounts of waste materials such as peels and seeds which is a critical subject in every industry. The aim of this study is to explore the best combination of drying and extraction method, to achieve the highest antioxidant content in sea buckthorn pomace (SBP), which appears as a by-product when making use of the berries. In the frame of our research the optimal drying conditions were previously tested, relying on the results vacuum drying was executed at 40 and 60 °C furthermore SBP was dried also by atmospheric dryer at 60 and 80°C. Drying curve was also determined. When extracting the antioxidant compounds, 20 and 40 % ethanol and acetone were used as solvents, applied in 1:30 proportion. Amount of total polyphenol content and antioxidant capacity (FRAP) were determined by spectrophotometry methods. Besides regarding amount of valuable components economical aspects must be considered for choosing the optimal drying technology. In case of vacuum drying duration was two times longer than in case of atmospheric technologies. The results show that the highest antioxidant capacity (4958.45 mg AAE/100g dm) was registered using 40% acetone extracted from the pomace, dried at 80°C. Further examination could reveal whether the extracted antioxidant content of the SBP, a by-product of fruit processing technologies, could be used natural food additives as bio-preservatives after appropriate clarification processes.

Introduction
Sea buckthorn (SB) (*Hippophae rhamnoides*) belongs to the *Elaeagnaceae* family [1]. Origin of the plant name is from Greek words: “hippos phao” means brilliant horse. Every part of SB were used in Europe and Central Asia, the fruits, leaves, bark and roots were processed to a food, dietary supplement, feed, firewood, fuel, or even to decorative elements [2]. This plant has a rich history in natural medicine [3], many of the substances that found in sea buckthorn are known to have gained increasing attention in recent years due to their high content of bioactive compounds with health benefits [4]. The SB fruits and seeds are good sources of valuable nutrients: carotenoids, tocopherols, phytosterols, phenolic acids, flavonoids, and proanthocyanidins, demonstrating various useful effects: antioxidant, antimicrobial, anti-inflammatory [5-10]. Processing of SB fruit produces high amount of pomace, which are utilized rather inefficiently or discarded as a waste, so considerable amounts of nutrients are lost [11]. The extraction schemes of pomace were developed and many option of utilization of SBP recently become research topic [12-14]. There is a growing interest in the utilization of antioxidant-rich plant extracts as dietary food supplements [15]. The aim of this study was to

explore the best combination of drying and extraction methods, to achieve the highest antioxidant content in sea buckthorn pomace, which appears as a by-product when making use of the berry and obtain phenolics preparation from sea buckthorn pomace.

Experimental

The "Ascola" SB berries were collected from agricultural plots of Hungary. Chemicals were purchased by Sigma-Aldrich Chemie Ltd. All reagents used were of analytical grade.
SB were destemmed, and then heated to 80°C, to inactivate enzymes. The material was squeezed, resulting in juice and pomace. Drying methods were the next step by Memmert V 200 vacuum dryer (Memmert Gmbh, Schwabach, Germany) at 40°C and 60°C, also by atmospheric dryer (LMIM, Esztergom, Hungary) at 60°C and 80°C. In each case 2 kg of SBP was dried in single layer on three perforated trays until moisture content became lesser than 10%. Water content was determined per every 30 minutes by drying until constant weight at 121 °C using a MAC-50 moisture analyzer (Radwag Waagen GMBH, Hilden, Germany). 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, using two different solvents: acetone and ethanol at different concentrations 20 V/V % and 40 V/V% (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. Samples were further analyzed using two methods:

- The antioxidant capacity of samples was estimated according to the procedure described by Benzie and Strain [16]. Ferric reducing antioxidant power assay (FRAP) was defined in ascorbic acid equivalent (mg ascorbic acid equivalent/ 100 g dm).
- Total polyphenol content (TPC) of the einkorn extract was determined according to the Folin–Ciocalteu spectrophotometric method described by Singleton and Rossi [17]. Results were specified in mg gallic acid equivalent/ 100 g dm.

Results were calculated, statistical evaluations were performed using Microsoft Excel. Independent samples t-test were used for the statistical analyses by Student t-test at 95% confidence.

Results and discussion

Fig. 1 shows the drying curves obtained for SBP dried.

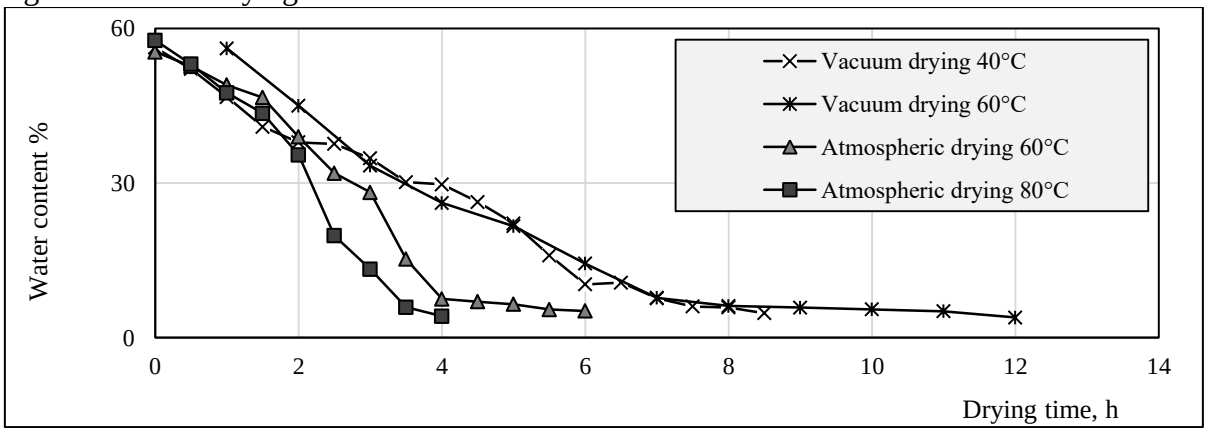

Figure 1. Drying curves

Drying mode (atmospheric or vacuum) and temperature affected dehydration speed. Initial wet content was 57.68% and time needed to reach final wet content (7-10%) was different depending on its drying method and temperature.
In case of atmospheric drying at 60°C and 80°C would require a shorter drying period to reach a moisture content under 10%. On the contrary, when vacuum drying was performed, wet content decreased slowly. The vacuum drying mode at 40°C was the longest process requiring 12 hour instead of atmospheric drying method at 80 °C dried in 4 hour (Fig. 1).

High variation was also observed for the antioxidant activity among the samples (Fig. 2). FRAP, expressed in mgAAE/100 g dm, ranged from 1156.28 to 4958.45 for dried samples.

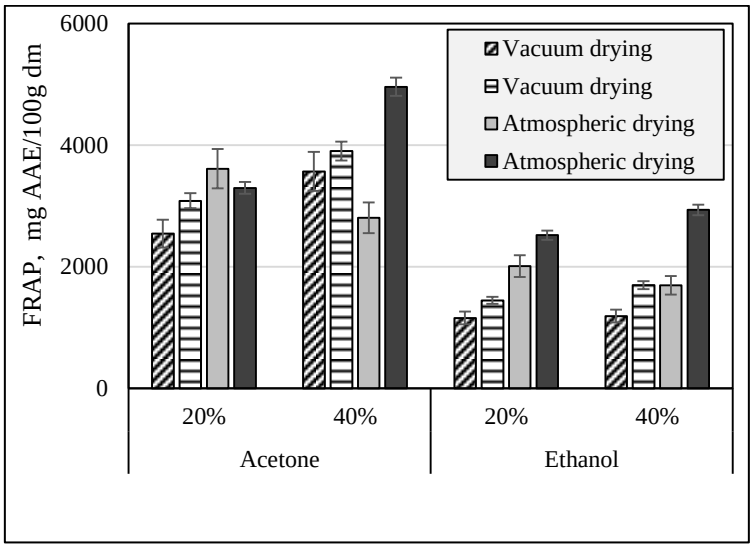

Figure 2. Average values of antioxidant capacities, evaluated using the method relying on Ferric Reducing Ability of Plasma, mg ascorbic acid equivalent/ 100g dm

FRAP value of the atmospheric dried samples at 80°C was the highest among the different drying methods.
Pomace dried at 80 °C showed significantly higher ($p>0.05$) outcome, consequently, lower drying temperature affected the antioxidant compounds in a positive way. Regarding the solvents applied, acetone extracted the analyzed components with the best results. Concentration of ethanol solvent had no significant effect on FRAP value but using different concentration of acetone had significant differences of antioxidant activity yield.

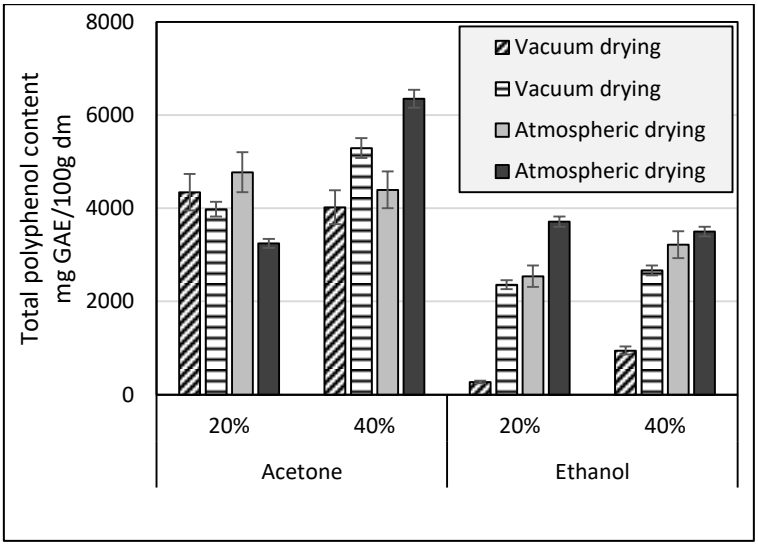

Figure 3. Total polyphenol content (TPC) average results, mg gallic acid equivalent/100g dm

The differences of total polyphenol content of SBP extracts are shown Figure 3. The TPC valeus had a range from 272.28 to 6351.24 mg GAE/100g dm. The highest value was reached by the sample which was dried at 80°C, extracted with 40 V/V % acetone. This result corresponds with the conclusion of the FRAP method.

The results also demonstrate the high polyphenol content of sea buckthorn pomace. The polyphenols are greatly diverse. The changes of polyphenols were influenced by many factors during food processing such as temperature, duration, presence of other components, etc. These effects can be resulted in degradation, transformation or enzymatic browning. Actual polyphenol content depends on the result of these effects [18].

When the samples were drying in atmospheric dryer the presence oxygen increase activity of polyphenol oxidase which play role keys in enzymatic browning because of product of Maillard reaction increasing the polyphenols content was higher at high temperature levels. Instead of during vacuum drying lack of oxygen inhibit these enzymes.

Conclusion

The aim of this research was to set up an environmentally friendly technological process to obtain high-value biologically active extracts from sea buckthorn by-products, thereby helping to reduce waste from the juice industry.

In present study, many pomace extracts was prepared by different drying mode and extraction solvents to find the best method to create pomace extract with high amount of antioxidant components.

Phenolic compounds and antioxidant capacity of SBP were affected by drying temperature (40-60-80 °C) and pressure (atmospheric and vacuum drying). Highest antioxidant capacity and highest amount of phenolic compounds were observed after atmospheric drying at 80 °C, using 40 V/V% acetone extraction solvent. Our results indicated that further experiments are needed for attempt more drying mode and extraction solvent to extract antioxidant components from the pomace and more research are needed for further exploitation on the production of food additives or supplements with high nutritional value.

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