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Diána Pálóczi-Furulyás

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MAGYAR AGRÁR- ÉS
ÉLETTUDOMÁNYI EGYETEM

Hungarian University of Agriculture and Life Sciences

**EXAMINATION OF THE UTILIZATION OF SEA BUCKTHORN AND
BLACK ELDERBERRY POMACE IN FRUIT JUICE SAMPLES**

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Diána Pálóczi-Furulyás

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PhD School/Programme

Name: PhD School of Food Science

Field: Food Science

Head of School: Livia Simon-Sarkadi, DSc
Hungarian University of Agriculture and Life Science University
Institute of Food Science and Technology

Supervisors: Mónika Máté, PhD
Hungarian University of Agriculture and Life Science University
Institute of Food Science and Technology

Éva Stefanovits-Bányai, DSc
Hungarian University of Agriculture and Life Science University
Institute of Food Science and Technology

.....
Livia Simon-Sarkadi
Signature of Head of School

.....
Mónika Máté, Éva Stefanovits-Bányai
Signature of Supervisors

1. Introduction and objectives

A part of the amount of waste produced in the food industry can be directly utilized and sold as a by-product and can also be used as a raw material for other technologies and as an energy carrier, which is important from an environmental and economic point of view.

During fruit and vegetable processing, a large amount of material to be removed is produced, such as pomace - the part of the seed and peel rich in valuable substances produced during juice extraction - which is currently often thrown away as waste, while it contains many valuable substances, so it could be utilized as by-product.

In my thesis, my aim was to examine the by-products from the processing of sea buckthorn (SB) and elderberry (EB) in the food industry, and efficient extraction of biologically active, endogenous compounds from fruit pomace and investigation and utilization of their effect on inhibiting the growth of microorganisms in fruit juice samples.

For all of this, my objectives were:

- to examine the amount of valuable, endogenous compounds of pomace extracts obtained by different drying methods and extraction parameters, and then to select the most effective drying and extraction process for both fruit pomace
- to identify and quantify the polyphenolic components of the pomace extracts produced using the most effective drying and extraction method, as well as to investigate their antimicrobial effect against different microbial strains, focusing on the strains that occur more often in fruit juices;
- to identify the relationships between the antimicrobial effect and endogenous components of the pomace extracts
- to develop a new apple juice product by adding pomace extracts to the apple juice, and to examine their antioxidant content and sensory properties;
- to detect the changes of the new products - apple juices - during storage by determination the amount of endogenous components and their microbiological stability.

2. Material and methods

The fruits were harvested in 2016 and 2018. The sea buckthorn (*Hippophaë rhamnoides* L.) was 'Ascora' variety from Szolnok, while the black elderberry (*Sambucus nigra* L) was 'Haschberg' variety from the Nagyvenyim plantation of BOTÉSZ. The pomace was produced from fruits in laboratory conditions according to the technology used in fruit juices in industrial environment.

The progress of my research work is presented in Figure 1. In my doctoral thesis, the results are evaluated according to the following points:

1. To produce extracts containing high biologically active components content from sea buckthorn and black elderberry pomace, the **drying and extraction methods were optimized.**
2. **The microbial inhibitory effect** of the extracts produced by the selected most effective extraction method was tested against 9 types of microorganisms.
3. Adding the extract to fruit juice, the changes in its valuable components and microbiological characteristics were investigated during an 8-week **storage experiment.**

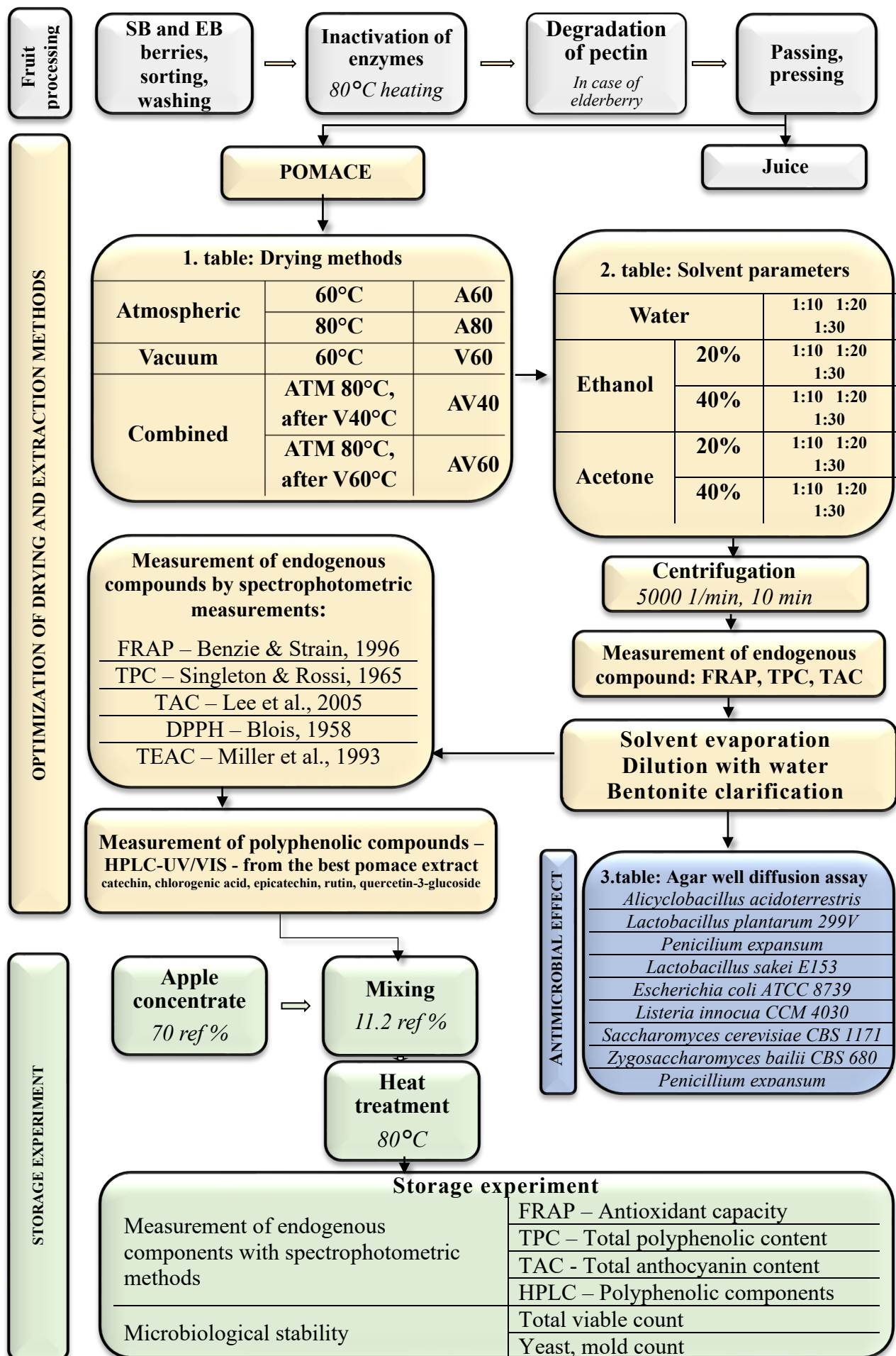


Figure 1: The progress of my research work

2.1. Optimizing the drying and extraction method

In my research, different drying technologies and extraction parameters were investigated during the production of pomace extracts, to determine their effect on the extraction of valuable components. The applied drying and extraction parameters are shown in Table 1-2. (Figure 1.) shows. The drying process was carried out for all samples until a moisture content of 5 (m/m) % was reached. The dried pomace samples were ground before extraction, the extraction process lasted one hour, which also included a half-hour ultrasonic (UH bath) treatment, then, after 10 minutes of centrifugation, I filtered the samples through filter paper with a pore size of 12-15 μm , and then measured the results of TPC, FRAP and in the case of elderberry: TAC.

The evaporation of the solvents was carried out in a drying cabinet at a temperature of 60°C. The effect of solvents on reducing endogenous components was also investigated, by comparing the TPC, FRAP, TEAC, DPPH and TAC measurement results of the extracts before and after evaporation.

In the case of SB and EB pomace extracts produced by the most efficient drying and extraction method, the amount of five polyphenolic compounds was determined by high-pressure liquid chromatography.

2.2. Examination of the microbial inhibitory effect of pomace extracts

After the evaporation of the solvent, extracts of different concentrations were prepared by diluting them with different amounts of water (Table 3), and then filtering with a 0.45 μm syringe filter. The FRAP, TPC and anthocyanin content of the samples was measured, and the antimicrobial effect of the samples against 9 types of microorganisms was determined by agar well diffusion assay.

Table 3: Compositions and codes of pomace extracts used for antimicrobial measurements

Evaporated solvent	Water	Concentration (solvent and water ratio)	Pomace concentration (mg/ml)	Jelölés	
				Sea buckthorn	Elderberry
100 ml	100 ml	1	33	HT-1	FT-1
100 ml	40 ml	2.5	83	HT-2.5	FT-2.5
100 ml	20 ml	5	167	HT-5	FT-5

2.3. Production of apple juices enriched with pomace extract

HT-1 and FT-1 pomace extracts were used to produce apple juice from concentrate. The apple juice extract with 70 Brix% dry matter content provided by Sió-Eckes Kft. was diluted with two different concentrations of pomace extract to reach the 11.2 Brix% value (typical of 100% apple juice). Table 4 contains the composition of apple juices and the codes used to label them.

Table 4: The composition of the apple juices produced in the experiment and their codes

Code		In 100 ml apple juice			Pomace concentration (mg/ml)
Sea Buckthorn	Elderberry	Apple concentrate (ml)	Water (ml)	Pomace extract (ml)	
Control, C		15	85	0	0
HTA-1	BTA-1	15	42.5	42.5	15
HTA-2	BTA-2	15	0	85	30

The apple juice samples were filled in mason jars, then heated to 80°C using a water bath and heat-treated for 15 minutes, and then quickly cooled and stored at 10°C. I performed a sensory examination of the fresh apple juices, and the apple juice samples were stored for 8 weeks, and sampling was carried out every two weeks

The pH, FRAP, TPC and TAC values of the samples were determined. In addition, from the samples taken in the week 0 and 8th week, the amount of 5 polyphenolic compounds (catechin, chlorogenic acid, epicatechin, rutin, quercetin-3-glucoside) were determined by HPLC measurement, as well as the total viable count and yeast/molds count were measured.

3. Results and discussions

3.1. Results of optimizing the drying and extraction method

First, the optimization of the extraction was performed with all the extraction parameters (Figure 2) in case of the pomace samples dried at 80°C (A80).

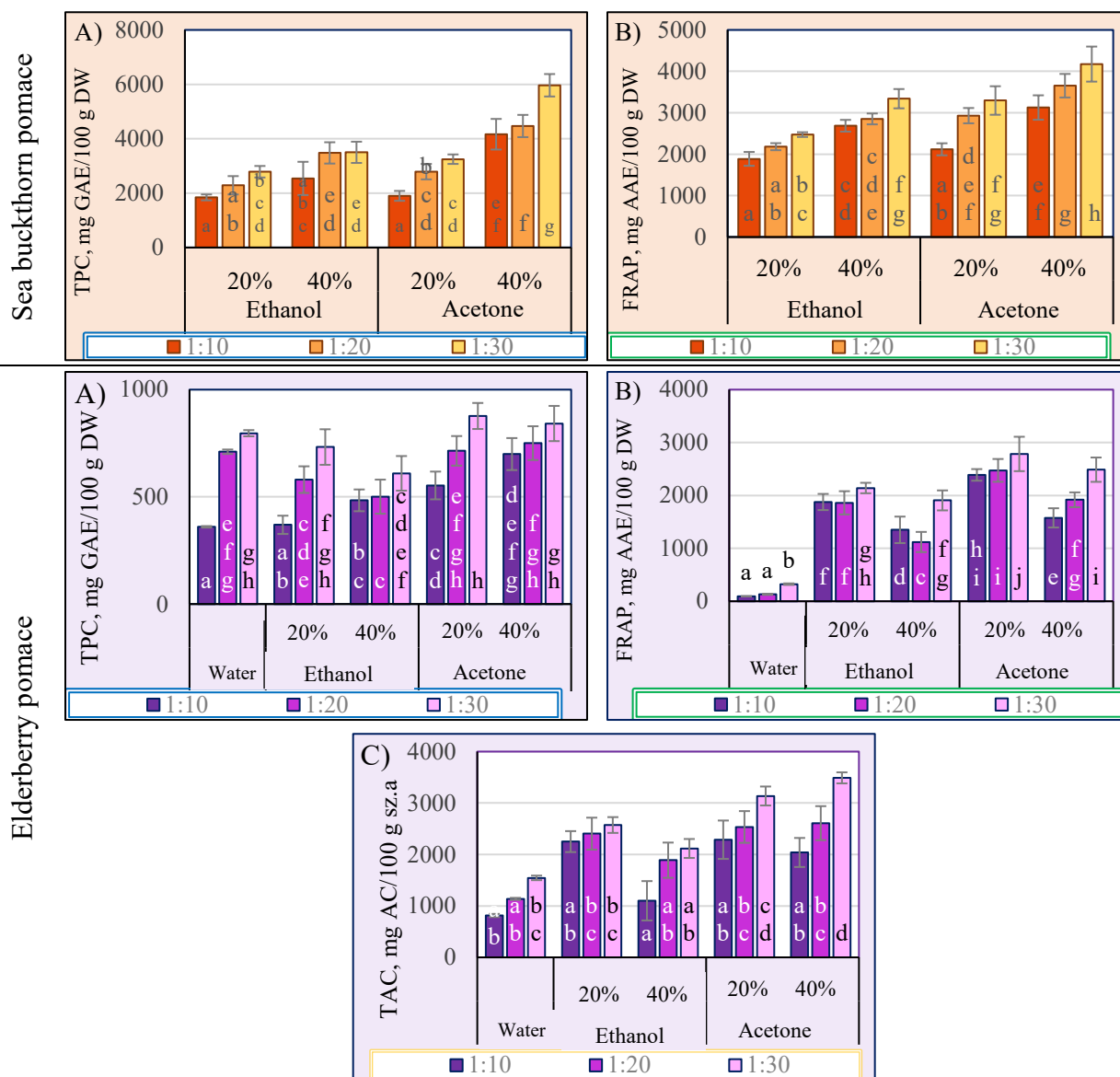


Figure 2: Total polyphenol content (A), FRAP antioxidant capacity (B), and monomeric anthocyanin content (C) of sea buckthorn and elderberry pomace extracts obtained with different extraction solvents and ratio. Different letters on the columns indicate significant differences between the values

The total polyphenol content and FRAP antioxidant capacity results of sea buckthorn and black elderberry pomace - dried at 80°C in an atmospheric dryer (A80) and extracted with 20 and 40

(v/v)% ethanol and acetone - prove that the higher solvent ratio (1:30) provides a more favorable extraction yield for the measured parameters, and in the case of sea buckthorn pomace, a significantly higher polyphenol content and antioxidant capacity can be obtained by using 40 (v/v)% acetone. On the other hand, the black elderberry results showed the highest total polyphenol content and antioxidant capacity (FRAP) value by using 20 (v/v)% acetone. For all monomeric anthocyanin contents, the extraction yields achieved by 20 and 40 (v/v)% acetone were the same.

In the second step, as a continuation of the preliminary experiment, I optimized the drying method, in which I dried sea buckthorn and black elderberry pomace with all the planned drying methods, which were extracted using the previously considered most effective extraction method.

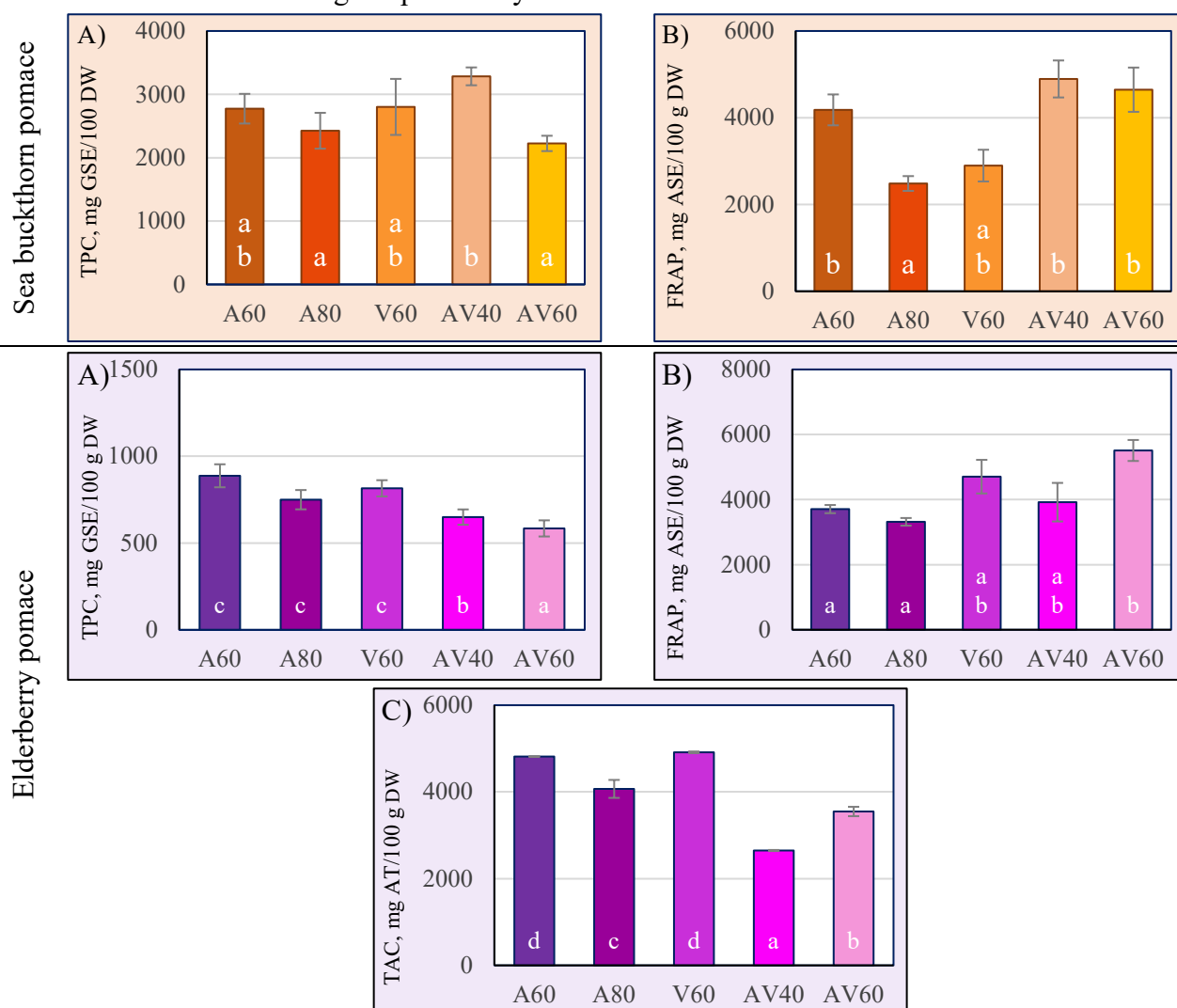
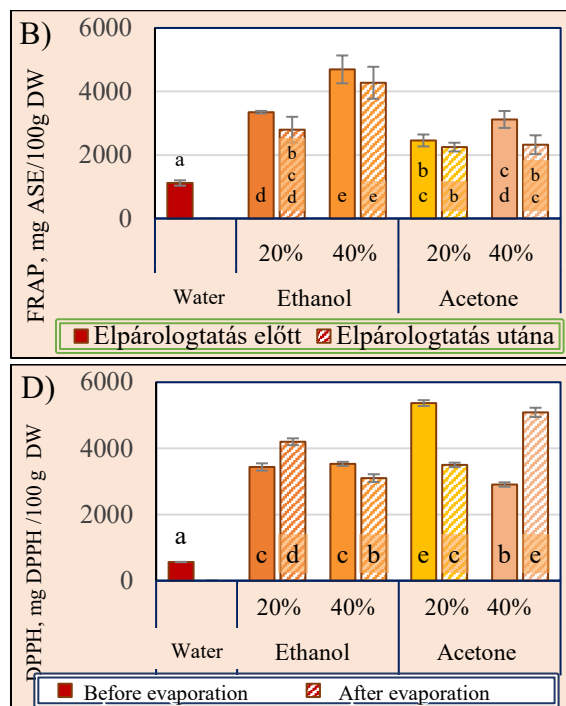
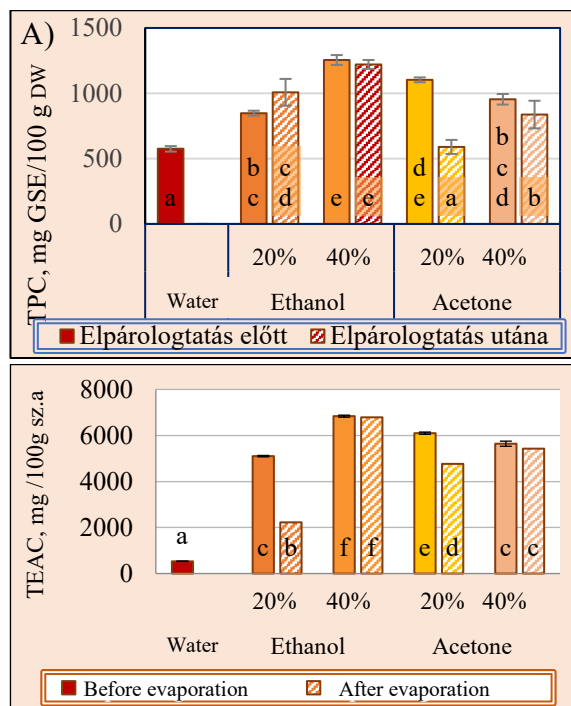


Figure 3: Total polyphenol content - TPC (A), FRAP value (B) and total monomeric anthocyanin content - TAC (C) of sea buckthorn and elderberry extracts dried with different drying methods., Different letters on the columns indicate significant differences between the values

Summarizing the results, the best drying method was in an atmospheric drying cabinet at 60°C, so in the further experiments, I used this drying process to produce pomace extracts.

As the third phase of the preliminary experiment, the changes occurring during the evaporation of the solvents were monitored, with the samples that were dried using the drying method considered to be the most effective.

Sea buckthorn pomace



Elderberry pomace

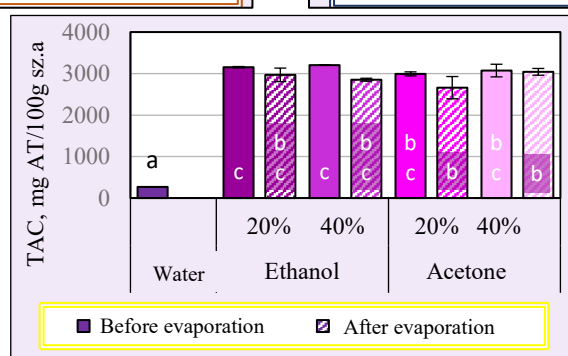
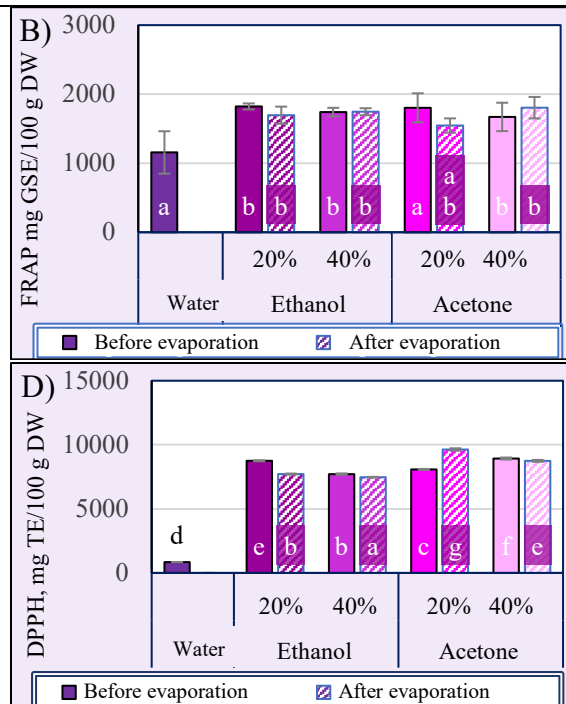
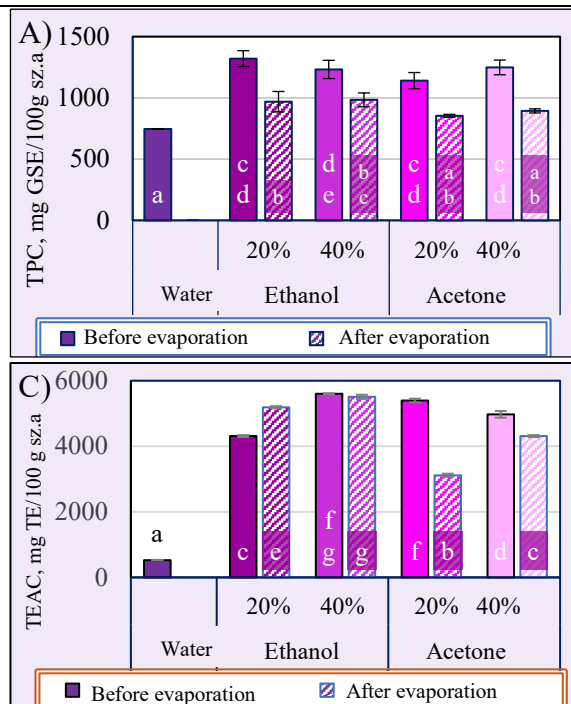


Figure 4: The effect of solvent evaporation of sea buckthorn and elderberry pomace extracts on the results of total polyphenol content – TPC (A), FRAP (B), TEAC (C), DPPH (D) and TAC (E). Different letters on the columns indicate significant differences between the values

During the examination of the polyphenol profile of pomace extracts which were produced by the best drying and extracting methods, UV/VIS chromatograms of the samples measured at wavelengths of 280 and 310 nm were recorded, based on which five polyphenol components were identified in both samples (Figure 5).

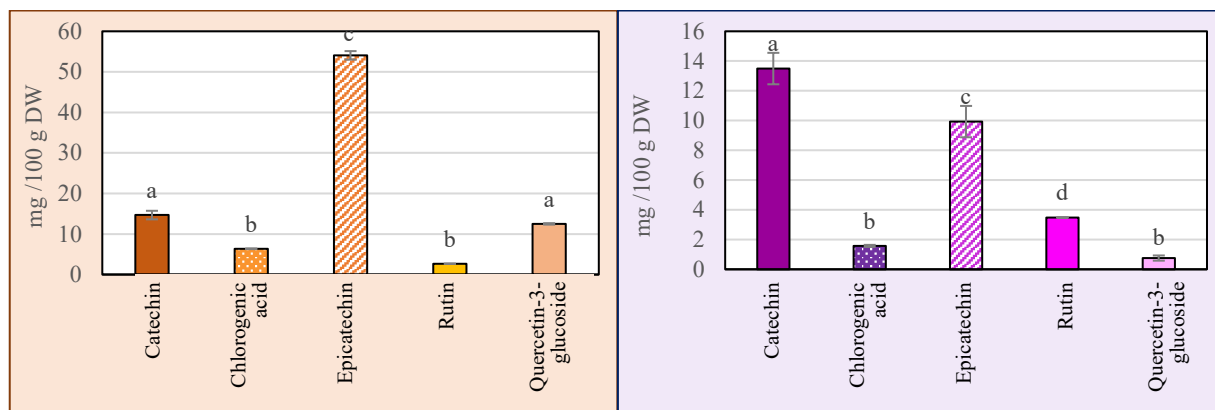


Figure 5: Quantities of identified polyphenolic components of sea buckthorn (left) and elderberry (right) pomace extracts, Different letters on the columns indicate significant differences between the values.

Summing up the HPLC-UV/VIS results, among the identified polyphenol components, epicatechin was measured in sea buckthorn pomace extracts, while catechin and epicatechin were identified in the highest amounts in elderberry pomace extract.

3.2. Examination of the microbial inhibitory effect of pomace extracts

Diluting after the evaporation of the solvents, extracts of different concentrations were produced from the horseradish extracts (extracts condensed one, two and a half times and five times), whose total polyphenol content and antioxidant capacity based on iron-reducing ability, and in the case of black elderberry pomace extracts, their anthocyanin content were measured.

The size of the clearing zones measured during agar well diffusion measurements indicate the antimicrobial inhibitory effect of the samples. I summarize the results obtained in the series of experiments in Table 5.

Table 5: Mean and standard deviation values of TPC, FRAP and TAC measurements of sea buckthorn and elderberry pomace extracts, the different letters indicate the significant difference between the samples, as well as the results of the agar hole diffusion test of the pomace extracts.

	HT-1	HT-2,5	HT-5	BT-1	BT-2,5	BT-5
TPC mg GSE/ 100ml	56,89 ± 0,99a	98,22 ± 2,24b	122,22 ± 2,24c	38,45 ± 1,56a	82,35 ± 7,54b	179,17 ± 14,55c
FRAP mg GSE/ 100ml	106,91 ± 1,39a	253,30 ± 1,96b	405,77 ± 5,88c	66,65 ± 4,45a	117,73 ± 11,24b	357,91 ± 17,37c
TAC mg GSE/ 100ml				89,97 ± 7,08a	227,76 ± 4,89b	442,87 ± 3,38c
<i>E. coli</i>	-	9,5 ± 0,7 mm	12 ± 0,2 mm	-	-	-
<i>L.innocua</i>	8,9 ± 0,3 mm	13,3 ± 1,1 mm	14,8 ± 0,4 mm	-	-	-
<i>L. sakei</i>	9,4 ± 0,8 mm	12,3 ± 0,4 mm	14,3 ± 0,4 mm	-	-	-
<i>A.acidoterrestris</i>	8,1±0,3 mm	9,3±0,4 mm	11,5±0,7 mm	11,8 ± 0,6 mm	16,0 ± 0,7mm	18,8 ± 0,4 mm
<i>S. cerevisiae</i>	8,5 ± 0,6 mm	9,8 ± 0,4 mm	12,8 ± 0,4 mm	-	-	-
<i>P. expansum</i>	-	-	-	-	-	12,8 ± 1,1 mm

Summarizing the agar well diffusion measurement results, it can be said that sea buckthorn extracts have an inhibitory effect against the bacteria *E.coli*, *L.innocua*, *L.sakei*, *A.acidoterrestris* and the yeast *S.cerevisiae*, while black elderberry pomace extracts inhibited the growth of the bacterium *A.acidoterrestris* and the mold *P.expansum* among the tested microorganisms.

3.3. Test results of apple juices enriched with pomace extract

During my experiment, in addition to objective tests, I also examined human subjective perception, in which the consumers' liking for my samples of apple juice enriched with pomace extracts were assessed.

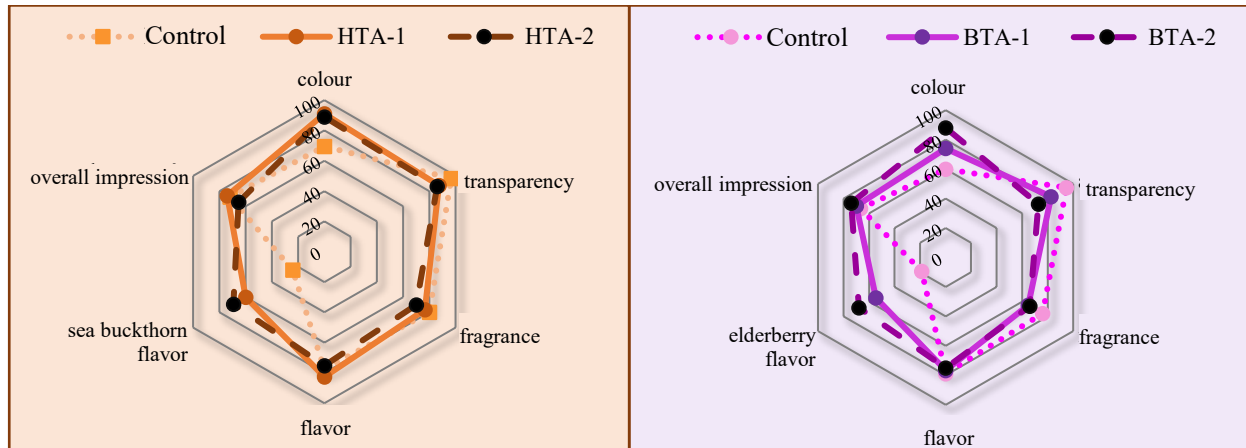


Figure 6: Results of sensory evaluation of apple juices enriched with sea buckthorn (left) and elderberry (right) pomace extracts

Based on the results of the sensory evaluation of sea buckthorn and elderberry pomace (Figure 6), as well as the reviewers' statements, I can say that the enrichment with pomace extract gave the apple juices a pleasant taste and appearance, which were attractive to the reviewers. However, the stronger sour taste of sea buckthorn pomace extract was disturbing when used in larger quantities (HTA-2), but in smaller quantities (HTA-1) it achieved better results in the classification than normal apple juice. Among the apple juices enriched with elderberry pomace extract, the BTA-2 sample was ranked first.

The changes in the results of the parameters measured during storage of apple juices enriched with pomace extracts can be seen in Figure 7.

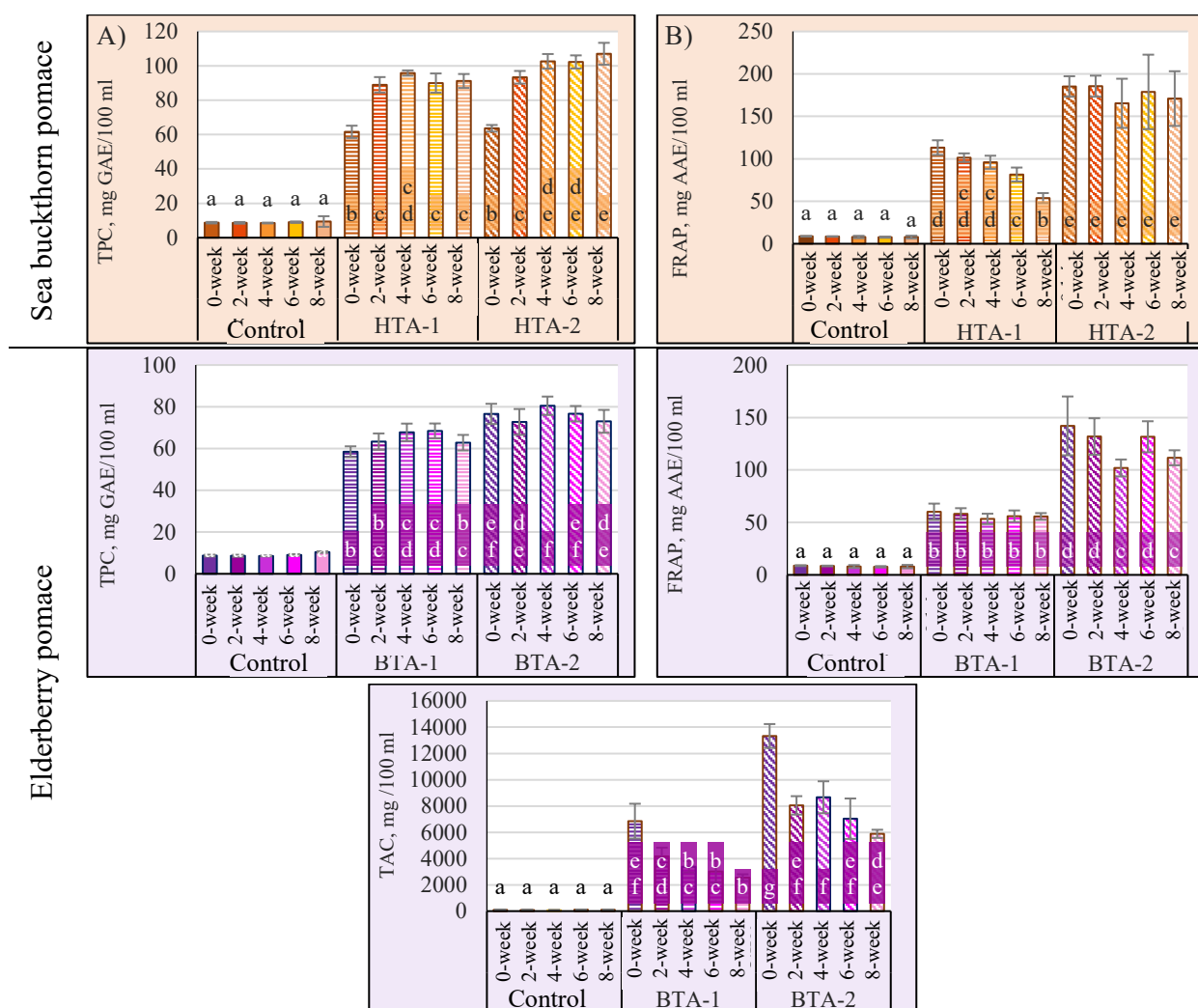


Figure 7: Results of TPC (A), FRAP (B) and TAC (C) of apple juice samples fortified with control and pomace extract. Different letters on the columns indicate significant differences between the values

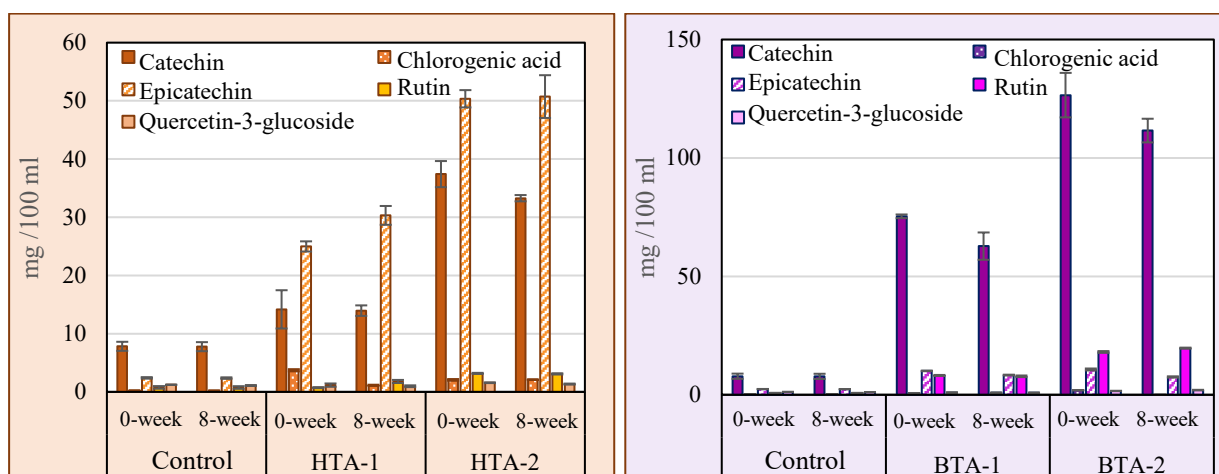


Figure 8: HPLC/VIS measurement results of the polyphenolic components of the control apple juice and the apple juices enriched with pomace extracts

Summarizing the results of the content measurements of the enriched apple juices, it can be said that the enrichment of the apple juices with valuable, biologically active, endogenously effective compounds was successfully achieved.

Using sea buckthorn pomace extract, I measured significantly higher amounts of TPC, FRAP and epicatechin, catechin and chlorogenic acid in the HTA-1 and HTA-2 samples compared to the control apple juice. The total polyphenol content and the amount of polyphenol compounds did not decrease during the 8-week storage.

During the storage of the apple juices enriched with elderberry pomace extract, both the total polyphenol content of the samples and the epicatechin and rutin components measured in significant amounts among the polyphenol compounds remained stable during the 8 weeks of storage. There was a decrease in the measurement results of FRAP, anthocyanin content, and the largest amount of catechin.

Table 7 summarizes the measurement results of total viable counts and mold yeast counts of the 0th day and 8th week samples of the storage experiment of the apple juices enriched with pomace extract.

Table 7: Results of the microbiological examination of the control apple juice and the apple juices enriched with black elderberry pomace extract, expressed as colony-forming unit/100 µl sample

CFU/100 µl	Total viable count		Yeast/molds count	
	0-day	8-week	0-day	8-week
Control	0	48 ± 2	0	6 ± 2
HTA-1	0	41 ± 1	0	5 ± 1
HTA-2	0	3 ± 1	0	1 ± 1
BTA-1	2 ± 1	30 ± 3	0	0
BTA-2	2 ± 1	2 ± 1	0	0

Presumably due to the microbial inhibitory effect of the valuable antioxidant compounds, the microorganisms in apple juices enriched with pomace extract proliferated to a lesser extent during storage than in control apple juices, which is confirmed by the total viable count and the number of molds and yeasts measured in the samples.

4. Conclusions and recommendations

My results support the usefulness of the valuable components remaining in significant quantities in fruit pomace. The spectrophotometric results of my pomace samples dried with different drying methods showed only small differences, which do not justify the use of the more expensive and gentler drying technology (vacuum drying), since it does not cause a significant difference in the amount of antioxidants, so among the drying methods, I considered atmospheric drying at a temperature of 60°C to be the most optimal for sea buckthorn and elderberry pomace.

The different extraction parameters caused larger, significant differences in my measurement results. Based on my results, the most effective solvent for sea buckthorn pomace is 40 (v/v) % acetone. In the case of elderberry pomace, the influence of different extraction parameters is less clear in terms of the extraction of components. Due to the content of the larger polar polyphenolic component, the efficiency of aqueous extraction approaches the efficiency of solvents in the determination of total polyphenol content. The measurement results of the antioxidant capacity and the total monomeric anthocyanin content also show that the use of solvents with a lower concentration and a higher water content {20 (v/v) %} is preferable. Summing up my data, the use of 40 (v/v) % acetone for sea thorn pomace and 20 (v/v) % acetone for elderberry pomace is the most optimal extraction solvent among the tested solvents.

Sea buckthorn pomace extracts showed a stronger and broader antimicrobial inhibitory effect against the tested microbes than black elder pomace extracts. The results indicated an inhibitory effect against the indicator bacteria *Escherichia coli* and *Listeria innocua*, which cause foodborne infections, as well as against *Lactobacillus sakei* lactic acid bacteria, which have a low pH and reproduce at low temperatures, even in the refrigerator (~4°C).

Not only sea buckthorn but also black elder pomace extracts influenced the growth of *Alicyclobacillus acidoterrestris*, a heat-tolerant, spoilage-causing bacterium that often causes problems during fruit juice production, which gives rise to further research into their utilization in fruit juice production. In addition, black elder can inhibit the growth of *Penicillium expansum* mold, which can also be used in several food products. The pomace concentration of the extracts and the measurement results of the antioxidant parameters also closely correlate with the size of the measured clearing zones, so the more concentrated the pomace extracts are in biologically active endogenous compounds, the stronger the inhibitory effect can be measured against microorganisms, which leads to the conclusion that the antimicrobial inhibitory effect one of the root causes is the high antioxidant content of the samples.

Pomace extracts are suitable for enriching apple juices with antioxidants. From the storage tests, it can be concluded that the amount of most measured components remains stable, so no negative changes are expected during storage. The HPLC-UV/VIS results of the polyphenol components also confirm that the caraway extracts greatly increased the amount of valuable endogenous compounds in the apple juice. In the case of sea buckthorn, the increase in the amount of catechin and epicatechin components, while in the case of elderberry, the increase in the amount of catechin, epicatechin and rutin correlates with the percentage of pomace extract in the fortified apple juice samples. According to the tests of total viable counts and total mold and yeast counts in the storage experiment, the cell numbers measured in apple juices containing pomace extracts increased to a much lesser extent at the end of storage than in control samples, which suggests that the pomace extracts help to increase the shelf life of apple juices.

5. New scientific results

1. Based on my results, drying the pomace of the '*Ascora*' sea buckthorn and '*Haschberg*' black elderberry at temperature of 60°C under atmospheric conditions is a gentler method for preserving endogenous compounds than using 80°C. After drying, the 1:30 sample:solvent ratio and the extraction with 40 (v/v) % acetone for sea buckthorn pomace and 20 (v/v) % acetone for black elderberry pomace proved to be the most suitable for extracting endogenous components.
2. I identified epicatechin (54 mg/100g), catechin (15 mg/100g) and quercetin-3-glucoside (13 mg/100g) in the pomace of the sea buckthorn variety '*Ascora*'. The largest amount of catechin (14 mg/100g) and epicatechin (10 mg/100g) was present in the pomace of the black elder variety '*Haschberg*'.
3. I found that the extract of the pomace of the '*Ascora*' sea buckthorn effectively inhibits the growth of *Escherichia coli*, *Listeria innocua*, *Lactobacillus sakei*, and *Alicyclobacillus acidoterrestris* bacteria. The extract of the pomace of the black elderberry variety '*Haschberg*' inhibits the growth of the bacterium *Alicyclobacillus acidoterrestris* and the mold *Penicillium expansum*. Antimicrobial effects are correlated with the concentration of pomace in the extracts, as well as with the amount of endogenous compounds.
4. I found that '*Ascora*' sea buckthorn and '*Haschberg*' black elderberry pomace extracts significantly increase the amount of endogenous components in 100% apple juices enriched with them. The catechin, epicatechin and rutin components were identified in the largest quantities among the tested polyphenolic components in apple juice samples enriched with sea buckthorn pomace extract. The catechin and rutin components were identified in the largest quantities in the apple juice enriched with black elderberry pomace extract. These components remained stable during the 8-week storage at 10°C.
5. I verified that '*Ascora*' sea buckthorn, and '*Haschberg*' black elderberry pomace extract inhibits the growth of microorganisms in enriched apple juice samples during 8 weeks of storage at 10°C.

Publications of the author related to the topic of the dissertation (in the grouping defined by the doctoral school)

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Furulyás, D., Szalóki-Dorkó, L., Máté, M., Stefanovits-Bányai, É. (2024): Enrichment of apple juice with antioxidant-rich elderberry (*Sambucus nigra* L) pomace extract. *Acta Alimentaria*. 53(2):327-335.

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