

**THE THESIS OF THE Ph.D. DISSERTATION**

**CHAGNAADORJ RENTSENDAVAA**

**Gödöllő**

**2021**



**PHYTOCHEMICAL INVESTIGATIONS ON SEA BUCKTHORN JUICE  
ENRICHED WITH POMACE**

**CHAGNAADORJ RENTSSENDÁVÁ**

**Gödöllő**

**2021**

**PhD School/ Program**

**Name:** Food Science Doctoral School

**Field:** Food Science

**Head: Livia Simon-Sarkadi, DSc**

MATE, Institute of Food Science and Engineering  
Department of Nutrition

**Supervisors: Mónika Máté, PhD**

MATE, Institute of Food Science and Engineering  
Department of Fruit and Vegetable Processing Technology

The applicant met the requirement of the PhD regulations of the Hungarian University of  
Agriculture and Life Sciences and the thesis is accepted for the defense process.

.....

Head of Doctoral School

.....

Supervisor

## TABLE OF CONTENTS

<b>TABLE OF CONTENTS</b> .....	1
<b>CHAPTER 1 –INTRODUCTION</b> .....	2
<b>CHAPTER 2 - MATERIAL AND METHODS</b> .....	4
<b>CHAPTER 3 - RESULTS AND DISCUSSION</b> .....	6
<b>3.1 Total polyphenolic content</b> .....	6
<b>3.2. Flavonoids</b> .....	7
3.2.1. Quercetin .....	7
3.2.2. Rutin .....	8
3.2.3. Dihydroxybenzoic acid .....	8
<b>3.3. Antioxidant capacity (FRAP)</b> .....	9
<b>3.4. Beta-carotene</b> .....	10
<b>3.5. Ascorbic acid</b> .....	11
<b>3.6. Total colour difference</b> .....	12
<b>3.7. New scientific results</b> .....	13
<b>CHAPTER 4 - CONCLUSIONS AND RECOMMENDATIONS</b> .....	15
<b>PUBLICATIONS UNDERLYING THE PH.D. THESIS</b> .....	17
.....	
<b>References</b> .....	19

## CHAPTER 1 – INTRODUCTION AND AIMS

According to the FAO of the United Nations (FAO 2014) report, 45% of fruit and vegetable wastes and by-products from the fruit and vegetable processing industry are generated around the world. The no edible 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 (Sharma et al., 2016).

Sea buckthorn (SB) (*Hippophae rhamnoides* L.) excels as an ingredient of functional foods, being outstandingly suitable due to its biologically active compounds. Primarily, it has a positive impact on human health due to its high vitamin C, flavonoid, carotenoid, and tocopherol content. In addition, it is rich in unsaturated fatty acids, proteins, and further vitamins (Suryakumar and Gupta., 2011; Krejcarova et al., 2015). The wound-healing effect of SB is confirmed by most modern studies as well (Upadhyay et al., 2010, Edraki et al., 2014).

SB pomace is a by-product that is produced in large quantities during SB juice extraction, consisting of pulp, seed, and skin. It is a good source of phytochemical compounds like phenolic acids and antioxidants. The pomace is usually dried in order to extend its shelf life for further use, either as a feed supplement or a source of valuable products, e.g. oils extracted from the seeds and the fraction of peel and pulp (Nuernberg et a., 2015).

Along with reducing the wastes, the purpose behind their utilization is also the extraction of beneficial antioxidants. The present study's hypothesis was as follows: the pomace and juice of SB contain phenolic compounds that could add commercial value to these crops. Furthermore, the characterization of the chemical composition and phenolic profile of these SB pomaces would contribute to the further development of natural health products and the native fruit industry in SB.

Exploring such material to utilize in different ways can help in managing and reducing waste. So, the present study aims to explore the potential of selected fruit waste through phytochemical studies, antioxidant assays. These so-called wastes can also be turned into value-added products. The work will also focus on the various aspects by which these by-products can be utilized in the juice industry.

The aim of this doctoral thesis was to investigate the effect of SB pomace for several components of SB juice during storage:

- Difference of chemical compound compared between samples of SB juice enriched with pomace (0.5%, 1% and 2%) and a control sample of SB juice. Samples of enriched and control SB juice were monitored the changes in individual parameters (TPC, FRAP, beta- carotene, flavonoids, vitamin C, pH, colour parameters (L\*, a\*, b\*), and SSC) for 14 months by (18-23°C) storage temperature and the sampling took place every. Each storage sample was stored in a separate container to avoid oxygen and microbial contamination during time sampling.
- The target of the statistical analysis to determine whether the storage time or the different quantities of pomace have an effect on changes of biologically valuable compounds during the test period.

Hence, the present investigation entitled “Phytochemical investigations on SB juice enriched with pomace for storage” was undertaken with the following objectives:

1. Collect and prepare pomace and juice from selected SB berry, prepare the samples.
2. Study of storage stability of the juice sample during the time.
3. Analysing soluble solid content potential and pH.
4. Determination of colour parameters in enriched and SB juice.
5. Determination of total polyphenolic content (TPC) and antioxidant capacity (FRAP) in SB juice.
6. Determination of flavonoid values (rutin, quercetin and hydroxybenzoic acid) in enriched and SB juice.
7. Determination of beta-carotene in SB juice.
8. Determination of ascorbic acid (AAc) in SB juice.

## CHAPTER 2 - MATERIAL AND METHODS

The berries of the SB (*Hippophae rhamnoides* L.) cultivar 'Leikora' was collected from the orchard of Superberry Ltd. (North latitude 47° 10' 29", East longitude 20° 11' 47") near Szolnok located in middle Hungary. Berries were collected during October of 2017 at the stage of commercial maturity, as judged by juiciness and appearance. The separated juice was added to different amounts of dried SB pomace. The treatment was made as control sample (C) of SB juice without pomace, SB juice 99.5% + pomace from SB 0.5% (P0.5), SB juice 99% + pomace of SB 1% (P1) and SB juice 98% + pomace of SB 2% (P2). After heat treatment (90°C and 10min), all samples were cooled with the cool water bath and stored at room temperature for physicochemical analysis at an interval of 2 months for a total period of 14 months (Figure 1).

The parameters examined were as follows:

- Determination of total polyphenol content (Singleton, Rossi, 1965)
- Determination of antioxidant capacity (FRAP) (Benzie, Strain, 1996)
- Analysis of individual flavonoids (Quercetin, Rutin, Dihydroxibenzoic acid) (HPLC instrument)
- Analysis of vitamin C (HPLC instrument)
- Analysis of beta-carotene (HPLC instrument) (Ficzek et al. (2019)
- Analysis of pH (Testo 206 pH measurement)
- Analysis of soluble solid content (SSC) (ATAGO DBX-55 digital refractometer)
- Determination of colour parameters (Konica Minolta CR 410 manual digital colour meter)

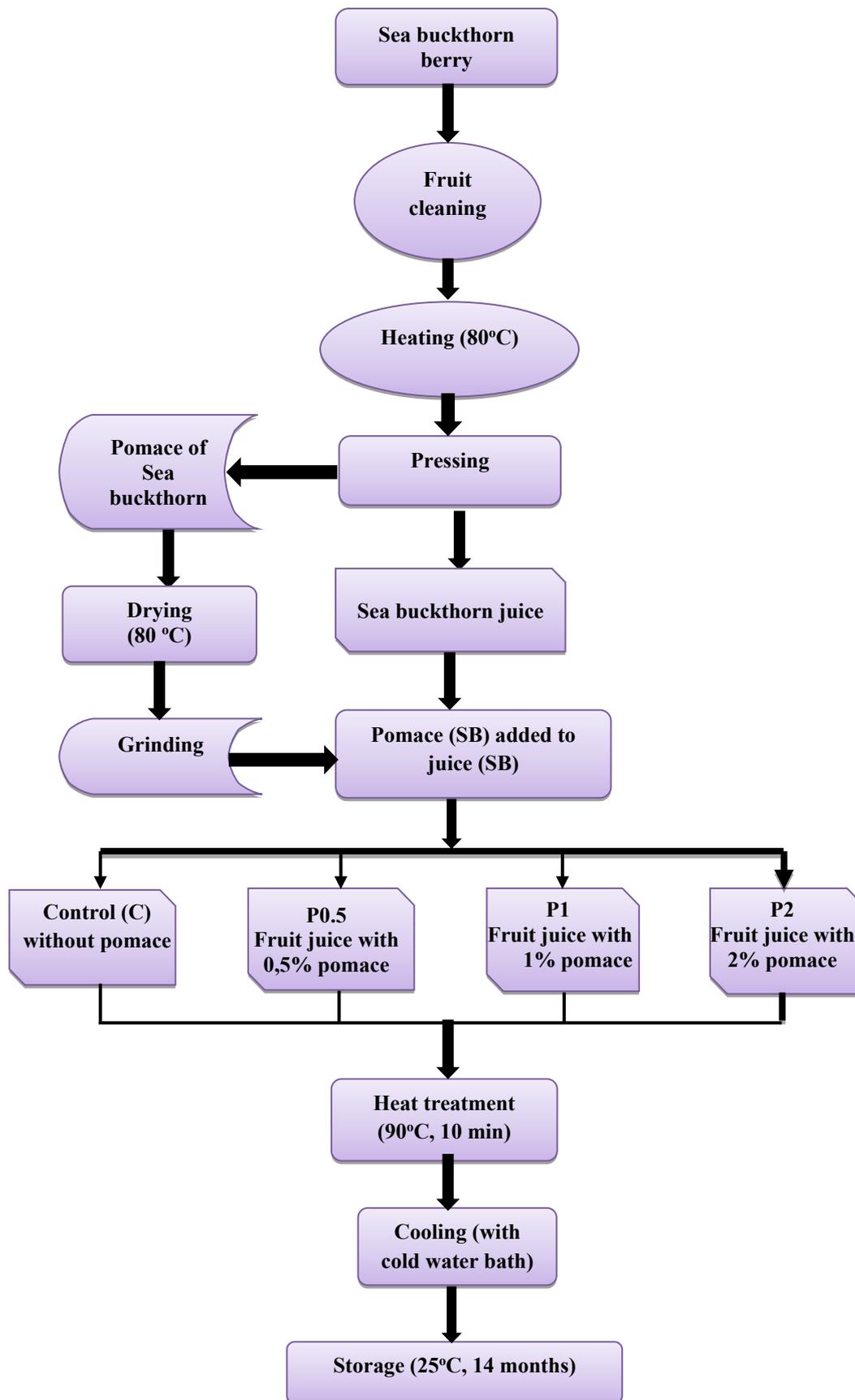


Figure 1. Process flowchart of Sea buckthorn juice extraction

## CHAPTER 3 - RESULTS AND DISCUSSION

### 3.1. Total polyphenolic content (TPC)

Generally, the TPC values in all samples of SB juice (C, P0.5, P1 and P2) decreased during storage. The content of TPC in SB juice ranged from 1400.99  $\mu\text{g GAE mL}^{-1}$  in enriched P2 sample to 1750.59  $\mu\text{g GAE mL}^{-1}$  enriched P0.5 sample.

The TPC content values of the control (C) sample was in the range of 1649.98.6 to 1142.21  $\mu\text{g mL}^{-1}$  end of the experiment (14 months). TPC changes of the control (C) sample and enriched samples (P0.5, P1 and P2) are demonstrated in Table 1.

**Table 1. Effect of pomace treatment and storage time on total polyphenol content of sea buckthorn juice ( $\mu\text{g GAE mL}^{-1}$ )**

Month	Control	P0.5	P1	P2
<b>Initial</b>	1649.98 $\pm$ 165.59 <sup>Aa</sup>	1750.59 $\pm$ 31.02 <sup>Aa</sup>	1439.55 $\pm$ 43.81 <sup>Aa</sup>	1400.99 $\pm$ 37.46 <sup>Ab</sup>
<b>2</b>	1605.45 $\pm$ 40.37 <sup>Aa</sup>	1607.65 $\pm$ 40.96 <sup>Aab</sup>	1302.34 $\pm$ 27.06 <sup>Babc</sup>	1408.53 $\pm$ 25.50 <sup>Bb</sup>
<b>4</b>	1331.93 $\pm$ 33.59 <sup>Ab</sup>	1441.76 $\pm$ 43.21 <sup>Abc</sup>	1049.16 $\pm$ 34.56 <sup>Bd</sup>	1068.88 $\pm$ 65.04 <sup>B</sup>
<b>6</b>	1277.75 $\pm$ 6.55 <sup>Abc</sup>	1143.61 $\pm$ 18.19 <sup>ABde</sup>	1032.53 $\pm$ 23.80 <sup>Bd</sup>	1097.22 $\pm$ 30.69 <sup>Bc</sup>
<b>8</b>	1095.20 $\pm$ 16.85 <sup>Ac</sup>	1083.29 $\pm$ 38.74 <sup>Ae</sup>	1212.57 $\pm$ 28.98 <sup>Bcd</sup>	1320.16 $\pm$ 18.99 <sup>Cbc</sup>
<b>10</b>	1191.12 $\pm$ 32.75 <sup>Abc</sup>	1276.63 $\pm$ 28.70 <sup>Ac d</sup>	1433.27 $\pm$ 43.33 <sup>Bab</sup>	1459.26 $\pm$ 35.74 <sup>Bab</sup>
<b>12</b>	1135.79 $\pm$ 23.57 <sup>Ac</sup>	1286.97 $\pm$ 29.35 <sup>BCcd</sup>	1229.13 $\pm$ 63.85 <sup>ABbcd</sup>	1374.44 $\pm$ 29.05 <sup>Cb</sup>
<b>14</b>	1142.21 $\pm$ 36.11 <sup>Abc</sup>	1207.05 $\pm$ 21.74 <sup>Ade</sup>	1286.18 $\pm$ 105.07 <sup>Aabc</sup>	1624.41 $\pm$ 42.95 <sup>Ba</sup>

Superscripts with small case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means  $\pm$  SD. (Tukey's test,  $p < 0.05$ )

Results showed that storage time and sample juices significantly affected the level of TPC of all samples of SB juice. The lowest (1049.16  $\mu\text{g mL}^{-1}$ ) TPC value was observed for the control (P1) sample in the 4 months. Between 6 and 8 months it comes up to 1212.57  $\mu\text{g mL}^{-1}$ . Therefore, from 8 to 14 months, enriched P2 samples contained the most TPC among all samples of sea buckthorn juice.

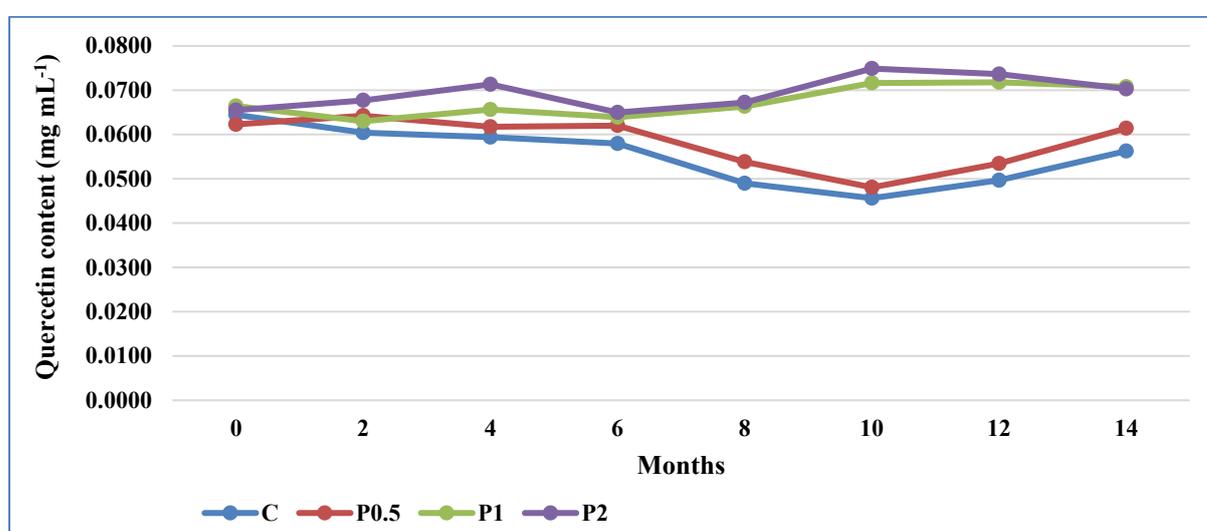
The TPC values of the enriched samples (P0.5, P1 and P2) were in the range of 1750.59  $\mu\text{g mL}^{-1}$  to 1207.05  $\mu\text{g mL}^{-1}$ , 1439.55  $\mu\text{g mL}^{-1}$  to 1286.18  $\mu\text{g mL}^{-1}$  and 1400.99  $\mu\text{g mL}^{-1}$  to 1624.41  $\mu\text{g mL}^{-1}$  on 14 months, respectively. After 14 months, enriched P0.5, P1 and P2 samples retained 5.68%, 12.60%, and 42.27% more TPC compared to control (C) sample.

## 3.2. Flavonoids

### 3.2.1. Quercetin

Different percent content SB pomace slightly compared to quercetin of all samples (C, P0.5, P1 and P2). The values of quercetin in enriched P1 and P2 samples were not significantly higher than in the control (C) sample. At the start of the experiment, enriched P1 and P2 samples retained 3.07% and 1.64% more quercetin, respectively, compared to control (C) sample. Control (C) samples the level of quercetin reduced by 12.66%, during storage.

The quercetin changes of the control (C) sample and enriched samples (P0.5, P1 and P2) are on Figure 2.



**Figure 2. Quercetin content of SB juice during 14 months storage at room temperature**

Between 8 and 10 months, maximum increase was observed in enriched P2 sample (11.38%) followed by enriched P1 sample (7.97%), while between 4 and 6 months, minimum increase was observed in enriched P0.5 sample (0.45%).

During storage time enriched samples of SB juice (P1 and P2) have more stability than other samples (C and P0.5). After 14 months, enriched samples (P0.5, P1 and P2) retained 9.14%, 25.82% and 24.92% more quercetin, respectively, compared to control (C) sample.

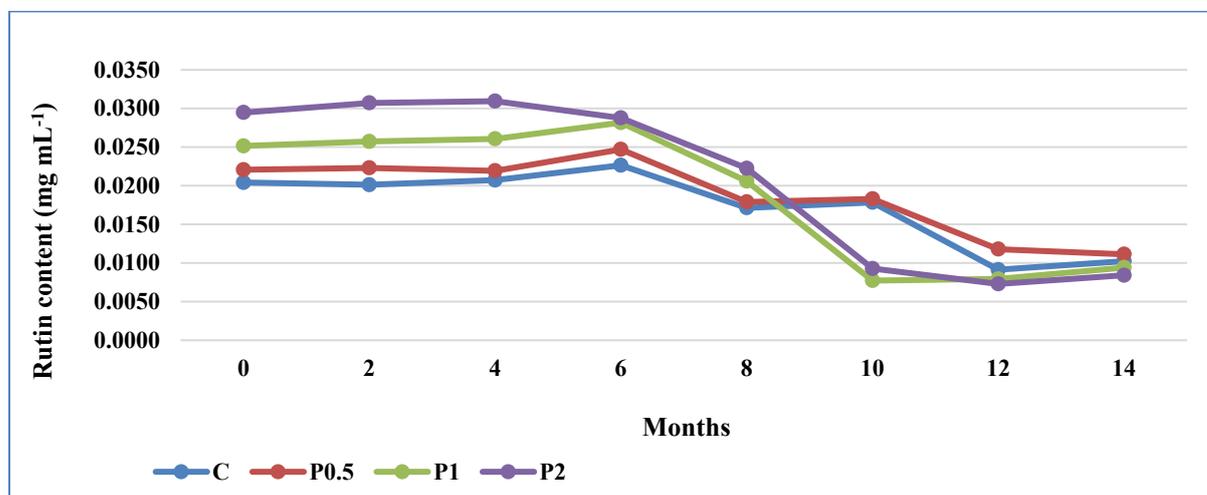
Both pomace treatment and storage time had a significant effect on quercetin values of buckthorn juice. Pomace treatment,  $F(3, 141) = 40.93$ ,  $P < 0.001$  and storage time,  $F(7, 141) = 2.616$ ,  $P < 0.05$ . Moreover, there was a significant interaction between pomace treatment and storage on the quercetin value of the juice,  $F(21, 141) = 3.294$ ,  $P = 0.001$ .

### 3.2.2. Rutin

A different percentage of sea buckthorn pomace improved the value of the rutin in the SB juice. At the beginning of the experiment enriched P0.5, P1 and P2 samples retained 8.08%, 23.18%, and 44.39% more rutin, respectively, compared to control (C) sample (Figure. 3).

In enriched samples P0.5, P1 and P2, the content of rutin is reported to be lower Negi et al. (2013), but not lower than that of control sample Control (C). We are supposing, different results depend on the SB culture, its location (soil and weather) and harvest time.

Maximum increase was observed in enriched P1 sample (12.01%) followed by enriched P0.5 sample (11.96%) and control (C) sample (10.95%).



**Figure 3. Rutin content of sea buckthorn juice during 14 months storage at room temperature**

During storage time, enriched P0.5 samples retained 7.95%, 10.66 and% 24.42% more rutin, respectively, compared to control (C) sample and enriched P1 and P2 samples.

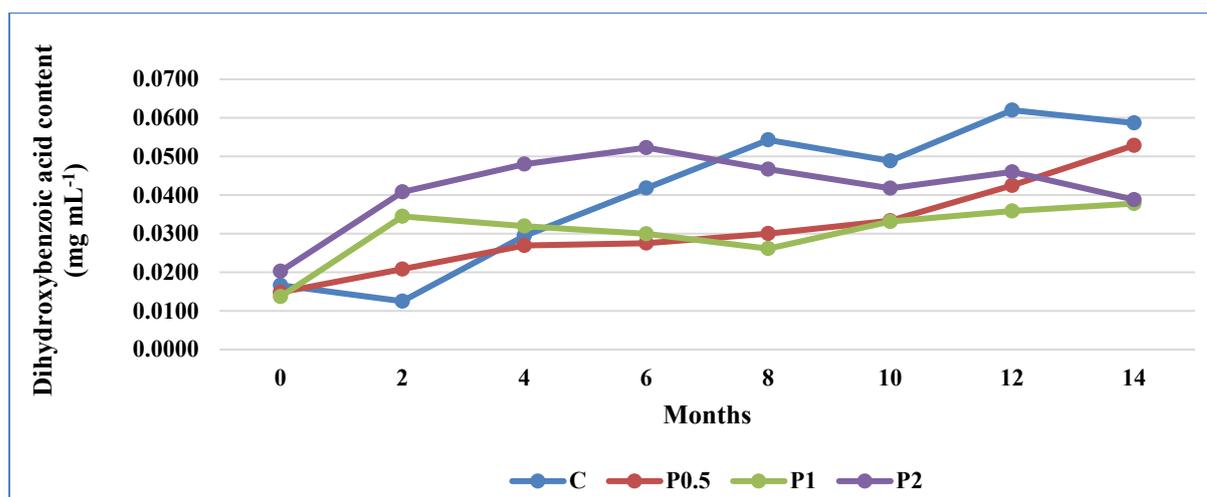
Results showed that enriched P1 and P2 samples retained 8.11% and 17.91% less rutin, respectively, compared to control (C) sample. At the end of experiment, the enriched P0.5 sample retained more rutin than control (C) sample and enriched P1 and P2 samples. However, after 14 months, enriched P1 (62.55%) and P2 (71.53%) samples resulted in much higher rutin degradation than a control (C) sample (50%) and enriched P0.5 (49.57%).

### 3.2.3. Dihydroxybenzoic acid (DHBA)

The DHBA content of all samples (C, P0.5, P1 and P2) are significantly increased compared to the initial day. In an enriched P2 sample a maximum DHBA value 20.3 $\mu$ g mL<sup>-1</sup>

was recorded between 4 and 6 months. Therefore, minimum DHBA value  $13.7\mu\text{g mL}^{-1}$  was observed for the enriched P1 sample.

During storage time constant increase was observed enriched P0.5 sample and more stability than other samples (C, P1 and P2). After 14 months, enriched samples (P0.5, P1 and P2) retained 9.88%, 35.61% and 33.9% less dihydroxybenzoic acid, respectively, compared to control (C) sample (Figure. 4).



**Figure 4. Dihydroxybenzoic acid content of sea buckthorn juice during 14 months storage at room temperature**

### 3.3. Antioxidant capacity (FRAP)

Different percent of SB pomace improved antioxidant capacity FRAP in SB juice on the initial day (P0.5, P1 and P2). The antioxidant capacity (FRAP) in SB juice ranged from  $1066.04\mu\text{g AA mL}^{-1}$  in control (C) sample to  $1202.37\mu\text{g AA mL}^{-1}$  enriched P2 sample in the initial day. Enriched samples (P0.5, P1 and P2) retained 7.07%, 9.26% and 12.79% more antioxidant capacity (FRAP) compared to control (C) sample initial day.

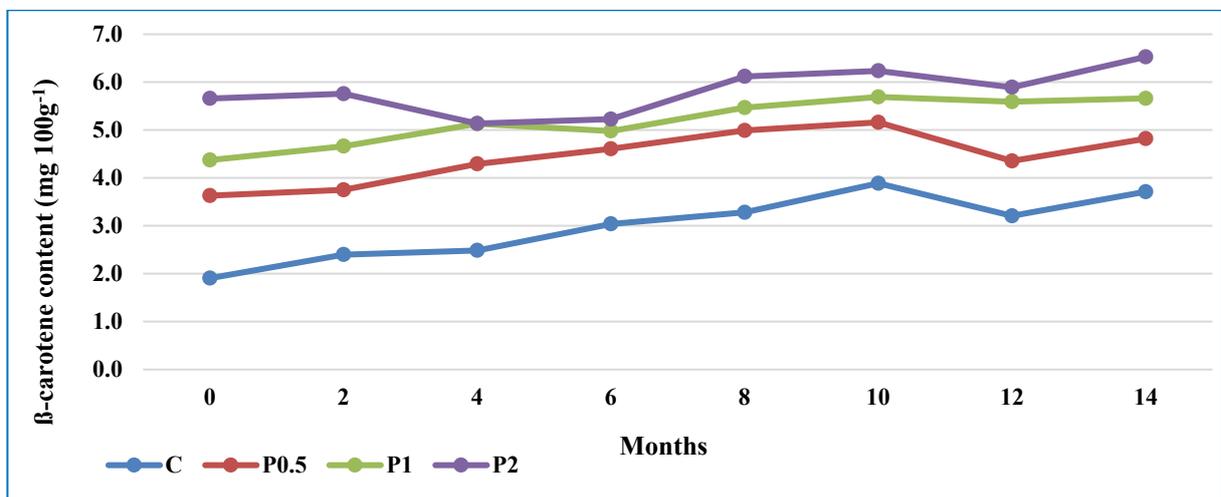
Generally, the antioxidant capacity values in all samples (C, P0.5, P1 and P2) decreased during storage. During storage time, enriched P2 sample had the highest antioxidant activity among other samples (C, P0.5 and P1). Korekar et al. (2014) found considerable differences between the antioxidant capacity (FRAP) values of different Indian SB populations (from to  $180$  to  $1355\mu\text{g mL}^{-1}$ ).

After 14 months, enriched samples (P0.5, P1 and P2) retained 8.31%, 33.92% and 43.60% more antioxidant activity compared to control (C) sample. At end of storage time, all samples (C, P0.5, P1 and P2) had less antioxidant respectively, compared to the initial day (78.12%, 76.70%, 45.31% and 39.90%). Moreover, the enriched P2 sample of FRAP value

was more related with an enriched P1 than enriched P0.5 and control (C) sample. From the enriched samples (P1 and P2), our experiment sees that the SB pomace reduces the loss of FRAP during storage.

### 3.4. Beta-carotene

Different percent of SB pomace improved by beta – carotene of enriched samples(P0.5, P1 and P2). The content of beta-carotene in SB juice ranged from 1.93 mg mL<sup>-1</sup> in control (C) sample to 5.87 mg 100 mL<sup>-1</sup> enriched P2 sample in the initial day. In our experiment enriched samples (P0.5, P1 and P2) retained 90.42%, 129.38% and 196.91% more beta-carotene, respectively, compared to control (C) sample. The beta-carotene changes of the control (C), enriched samples (P0.5, P1 and P2) are illustrated on Figure 5.



**Figure 5. Beta-carotene content of sea buckthorn juice samples during 14 months storage at room temperature**

Therefore, in enriched P0.5, P1 and P2 samples, the content of beta-carotene is reported to be high Eccleston et al. (2002), and higher than that of control sample (C). In carrot juices, the beta-carotene contents ranged from 3.28 to 8.48 mg 100 mL<sup>-1</sup>.

The beta-carotene values of all enriched samples (P0.5, P1 and P2) were significantly higher than control (C) sample for all storage time, thus enriched P2 sample contained the most beta-carotene among all samples (C, P0.5, P1 and P2).

During storage maximum increase was observed control (C) sample (92.23%) followed by enriched P1 sample (25.62%), while minimum increase was observed in enriched P2 sample (11.07%) followed by enriched P0.5 sample (33.52%).

After 14 months, enriched samples (P0.5, P1 and P2) retained 30.35%, 18.23% and 17.32% more beta-carotene, respectively, compared to control (C) sample. During storage time, enriched P1 and P2 samples of beta-carotene are more stable than the control (C) sample. The beta-carotene values of all samples (C, P0.5, P1 and P2) on initial day was 1.93, 3.61, 4.37 and 5.87 mg 100 mL<sup>-1</sup>, which were gradually increased to 3.71, 4.82, 5.49 and 6.52 mg 100 mL<sup>-1</sup> during 14 months of storage, respectively.

### 3.5. Ascorbic acid

In our results, different percent of SB pomace improved by AAC of enriched samples (P0.5, P1, and P2). The enriched P0.5, P1 and P2 samples retained 6.42%, 16.31% and 20.65% more AsA, respectively, compared to control (C) sample in the initial day. The content of AAC in SB juice ranged from 308.57 ± 1.89 mg mL<sup>-1</sup> in control (C) sample to 372.81 ± 1.89 mg 100 mL<sup>-1</sup> enriched P2 sample in the initial day (Table 2).

**Table 2. Effect of pomace treatment and storage time (months) on vitamin C of buckthorn juice (mg 100 g<sup>-1</sup>)**

Month	Control	P0.5	P1	P2
<b>Initial</b>	308.57 ± 1.89 <sup>e,f,A</sup>	328.11 ± 0.44 <sup>f,B</sup>	357.83 ± 0.1.63 <sup>f,C</sup>	372.81 ± 1.89 <sup>e,D</sup>
<b>2</b>	273.30 ± 9.36 <sup>d,e,g,A,B</sup>	258.29 ± 5.48 <sup>e,A</sup>	273.27 ± 5.17 <sup>e,A,B</sup>	299.76 ± 13.59 <sup>b,d,e,f,B</sup>
<b>4</b>	218.72 ± 8.70 <sup>c,d,h,A</sup>	235.46 ± 1.70 <sup>d,e,A</sup>	265.14 ± 5.67 <sup>d,e,B</sup>	266.76 ± 3.35 <sup>c,d,B</sup>
<b>6</b>	192.85 ± 28.53 <sup>b,c,f,g,i,A</sup>	191.24 ± 8.75 <sup>d,A</sup>	230.44 ± 2.70 <sup>d,A</sup>	244.49 ± 9.19 <sup>c,f,g,A</sup>
<b>8</b>	128.70 ± 7.82 <sup>a,b,A</sup>	125.01 ± 4.45 <sup>c,g,h,A</sup>	155.91 ± 1.02 <sup>c,g,h,B</sup>	183.50 ± 0.63 <sup>b,g,h,C</sup>
<b>10</b>	110.63 ± 7.65 <sup>a,i,A</sup>	119.61 ± 1.82 <sup>b,c,i,A,B</sup>	132.27 ± 4.09 <sup>b,c,i,B</sup>	129.60 ± 1.19 <sup>a,A,B</sup>
<b>12</b>	101.22 ± 18.79 <sup>a,h,i,A</sup>	113.98 ± 9.29 <sup>a,b,g,A</sup>	107.63 ± 6.41 <sup>a,b,g,A</sup>	117.66 ± 3.56 <sup>a,A</sup>
<b>14</b>	77.55 ± 3.93 <sup>a,i,A</sup>	77.82 ± 6.14 <sup>a,h,i,A</sup>	97.92 ± 9.40 <sup>a,h,i,A</sup>	98.84 ± 10.42 <sup>a,h,A</sup>

Superscripts with small case letters indicate significance difference by time along the rows. Superscripts with uppercase letters indicate significance difference by treatment along the columns. Presented values are means ± SD. (Tukey's test, p<0.05)

The enriched P2 sample was higher AAC than other enriched samples (P0.5, and P1) and control (C) sample of SB juice. AAC in all samples (C, P0.5, P1, and P2) were continuously declining during the research. During storage maximum decrease was observed enriched P0.5 sample (34.63%) followed by control (C) sample (33.26%), while minimum decrease was observed in enriched P2 sample (29.37%) followed by enriched P1 sample (32.34%).

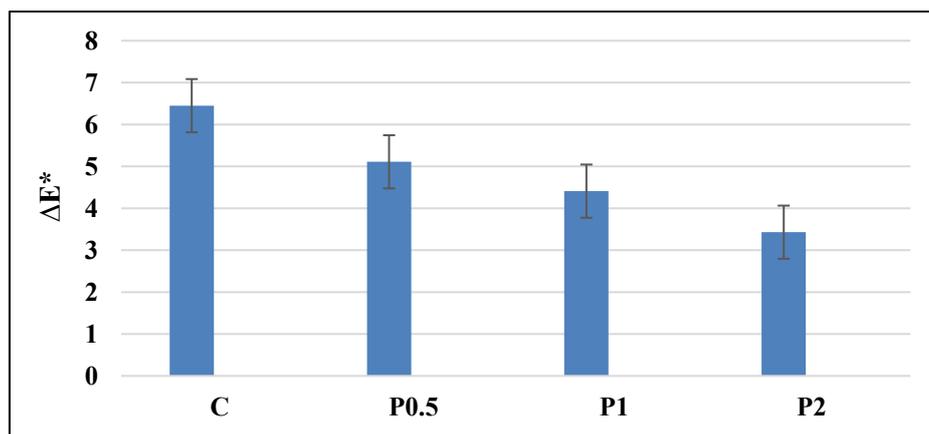
The AAC values of all enriched samples (P0.5, P1 and P2) were significantly higher than control (C) sample for after 14 months. After 14 months, enriched samples (P0.5, P1 and P2)

retained 0.35%, 26.25% and 27.44% more AA, respectively, compared to control (C) sample. Remini et al. (2015) reported that 20% of the initial ascorbic acid was lost after 28 days of storage of pasteurized blood orange juice at 4°C.

### 3.6. Total colour difference

Total colour difference of sea buckthorn juice samples presented Figure 6. The change in the parameters of the sample or its deviation from a given colour pattern can be characterized by the spatial distance between the two-colour points, the total colour difference ( $\Delta E^*$ ).

During these investigations the parameters measured 14 months of all samples were compared with the initial ones, so the  $\Delta E^*$  values were determined with respect to the starting colour coordinates.



**Figure 6. The colour difference parameters ( $\Delta E^*$ ) after the storage period**

Total colour difference increased with time and samples and the increase was significantly different among the samples. The total colour difference value of 2 would be a noticeable difference in visual perception of many products. After 14 months of storage enriched P2 sample, the total colour difference had surpassed 23, while control (C) and enriched P0.5 samples it took about 14 months to exceed 5 (Figure 6).

The colour change of the pomace-containing pulps was 'clearly visible' at the end of storage, since  $\Delta E^*$  values were between 3.0 and 6.0. In the control sample,  $\Delta E^*$  was greater than 6.0, thus the difference was large. As the amount of pomace increased, the colour difference decreased, including a negative correlation ( $r = -0.9681$ ).

### 3.7. New scientific results

1. A significant decrease in the antioxidant activity and TPC were recorded for each sample of sea buckthorn juice during storage time. The added pomace has a protective effect for the antioxidant activity and TPC of the juices. The antioxidant activity decreased to a lesser extent in the pomace-containing juice (P1, 1% pomace added, 45.31%; P2, 2% pomace added, 39.90%), than in the control sample (C, without pomace added, 78.12%). Antioxidant capacity was shown to be directly correlated with TPC.
2. We found that the main flavonoid of the sea buckthorn samples was the quercetin (0.0456 – 0.0748 mg mL<sup>-1</sup>; between 44.64-69.73%), followed by dihydroxybenzoic acid (0.0125 – 0.0620 mg mL<sup>-1</sup>; between 19.11-36.95%) and the rutin (0.0073 – 0.0309 mg mL<sup>-1</sup>; between 11.16-18.41%).
3. After 14 months storage at room temperature, P1 (1% pomace added) (62.55%) and P2 (2% pomace added) (71.53%) resulted in much higher rutin degradation than a control (C, without pomace added) (50%) and P0.5 (0.5% pomace added) (49.57%).
4. A significant increase in the dihydroxybenzoic acid was recorded for each sample of sea buckthorn juice during storage time. After 14 months storage the control (C, without pomace added, 0.0587 mg mL<sup>-1</sup>) sample showed a significant (P<0.05) higher dihydroxybenzoic acid than the P1 (1% pomace added, 0.0378 mg mL<sup>-1</sup>) and P2 (2% pomace added, 0.0388 mg mL<sup>-1</sup>) samples.
5. After 14 months storage, a significant increase in the quercetin was recorded for P1 (1% pomace added) (6.6%) and P2 (2% pomace added) (7.3%), while, in the control (C, without pomace added) (12.6%) and P0.5 (0.5% pomace added) (1.44%) were degraded. During 14 months storage time, the quercetin of sea buckthorn juice was more stable than hydroxy-benzoic acid.
6. A significant increase in the beta-carotene was recorded for each sample of sea buckthorn juice during storage time. After 14 months, enriched samples of sea buckthorn juice (P0.5, P1 and P2) retained 30.35%, 18.23% and 17.32% more beta-carotene, respectively, compared to the control (C) sample.
7. The ascorbic acid content decreased in all samples. The losses of about 74.87%, 76.345%, 72.72% and 73.45% of vitamin C were observed in control samples (C) and enriched samples (P0.5, P1 and P2) after 14 months storage.
8. A significant increase in the pH was recorded for all sea buckthorn juice samples during 14 months of storage. During storage time, the pH values of P2 (2% pomace added) sample were more stable than the control (C, without pomace added), P0.5 (0.5% pomace added)

and P1 (1% pomace added). The soluble solid content of control (C, without pomace added), was significantly lower compared to the other sea buckthorn juice samples (P0.5, P1, P2) after 14 months storage period.

## CHAPTER 4 – CONCLUSION AND SUGGESTION

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. On one hand, these could be retrieved using the proper method, and used again by the food industry. 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.

In our research work the conditions in which the samples were stored were in a cool, dry place. We wanted to represent the normal, average circumstances of a department store during storage. During the experiment, someone measured weekly the ambient temperature and ranged from 18 to 23°C, depending on the season.

In summary, the results from this study indicate that P1 and P2 retained more antioxidant capacity, beta-carotene, TPC, rutin, quercetin, hydroxy-benzoic acid, SSC and ascorbic acid, and less colour parameters compared to the control (C) on the initial day.

P1 and P2 significantly altered chemical parameters such as TPC, FRAP, SSC, beta-carotene, pH-value, quercetin and ascorbic acid. A significant increase in the beta-carotene was recorded for P1 and P2 during storage time. The P1 had quality parameters close of P2 and significantly better than the control (C).

During 14 months storage, the SB control (C) juice lost some of the quercetin, rutin, TPC, FRAP and ascorbic acid content, respectively. We have successfully recovered and recycled the antioxidant compounds from pomace of SB to produce more valuable and SB juice (P0.5, P1, P2). 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.

Experiments have shown that sea buckthorn has a remarkable antioxidant effect, which can neutralize free radicals and helps maintain health. Our results clearly demonstrated that the added pomace amount significantly influenced the phytochemical composition and antioxidant capacity of the enriched samples of sea buckthorn juice studied.

This work in the field of studying the effect of sea buckthorn pomace is only the initial stage, which offers many other possibilities for further research:

- In order to avoid wastage of significant quantities of nutrients by treating the pomace

as waste, it would be worth to use it not only for feeding purposes, but also in functional foods for human consumption.

- In this study, sea buckthorn pomace was used in heat-treated juices, but it would be worthwhile to investigate the effect of pomace on the antioxidant component and shelf life of freshly pressed non-heat-treated juice, as well.
- It would also be worthwhile to study the antimicrobial effect of sea buckthorn pomace in various food products (combine the heat treatment and pomace added) in order to reduce the negative effect of heat treatment. A detailed study is required to investigate the inhibitory effect of sea buckthorn on certain microorganisms.
- Further studies are needed on the use of sea buckthorn pomace in other products, e.g. in jams and jellies. More than 2% pomace content could be used and investigated the antioxidant and rheological properties of jams during storage.
- I consider it necessary to identify additional flavonoid compounds and to monitor their changes in the products due to different technological effects and during storage.

## PUBLICATIONS UNDERLYING THE PH.D. THESIS

### **IF articles (IF value)**

Rentsendavaa Chagnadorj, Dóra Székely, Furulyás Diána, György Végvári, Mónika Stéger-Máté (2020): Stability of carotene and phenols of sea buckthorn (*Hippophae rhamnoides* L.) juice with pomace during storage *Periodica Polytechnica Chemical Engineering* – 65(2): 210 – 218. DOI: [10.3311/PPch.15641](https://doi.org/10.3311/PPch.15641) (IF:1,62)

Ficzek, G. ; Mátravölgyi, G. ; Furulyás, D. ; Rentsendavaa, C. ; Jócsák, I. ; Papp, D. ; Simon, G. ; Végvári, Gy. ; Stéger-Máté, M. (2019): Analysis of bioactive compounds of three sea buckthorn cultivars (*Hippophae rhamnoides* L. ‘Askola’, ‘Leikora’, and ‘Orangeveja’) with HPLC and spectrophotometric methods. *European Journal of Horticultural Science* 84(1): 31-38. DOI: [10.17660/eJHS.2019/84.1.5](https://doi.org/10.17660/eJHS.2019/84.1.5) (IF: 1, 55)

### **Proceeding at international conference**

Rentsendavaa Chagnadorj, Diána Furulyás, Mónika Stéger-Máté, Dóra Babinszki-Székely (2019): Effect of different chemical preservatives on the storage of sea buckthorn (*Hippophae rhamnoides* L.) juice. Biosys Food Eng 2019. 3rd International Conference on Biosystems and Food Engineering. Proceedings. PDF E313 8pp. ISBN 978-963-269-878-6

Furulyás, D., Dobó, V., Székely, D., Chagnadorj, R., Stefanovits-Bányai, Éva, Stéger-Máté, M. (2018): Optimizing of extraction of bioactive components from Sea Buckthorn (*Hippophae rhamnoides* L.) pomace and develop of antioxidant-enriched apple juice. *Analecta Technica Szegedinensia*, 12(1), 1-7. DOI: [10.14232/analecta.2018.1.1-7](https://doi.org/10.14232/analecta.2018.1.1-7)

Chagnadorj Rentsendavaa, Márton Szána, Gitta Ficzek, Lilla Szalóki-Dorkó, Mónika Stéger-Máté, Diána Furulyás (2018): Investigation of nutritional parameters in different parts of Sea buckthorn (*Hippophae rhamnoides* L.) berries. Journal on processing and energy in Agriculture. Book of Proceedings. Vol. 22 (1) p. 10-14. (ISSN 1821-4487) 30<sup>th</sup> Jubilee conference processing and energy in agriculture. Brzece (Kopaonik), Serbia, April 15<sup>th</sup> – 20<sup>th</sup>, 2018. p. 10-14

Furulyás Diána, Rentsendavaa Chagnadorj, Stéger-Máté Mónika, Stefanovitsné BányaiÉva, Mohácsiné Farkas Csilla (2017): Homoktövis (*Hippophae rhamnoides* L.) bogyó és törköly antimikrobiális hatásának vizsgálata három patogén baktériummal szemben. In: Biró Lajos, Gelencsér Éva, Lugasi Andrea, Rurik Imre (szerk.). Magyar Táplálkozástudományi Társaság XLII. Vándorgyűlés program könyve: MTT 1966. 72 p. Siófok, Magyarország, 2017.10.12-2017.10.14. p. 31. 1 p. (ISBN:[978-615-5606-04-5](https://doi.org/10.17174/978-615-5606-04-5))

Rentsendavaa Chagnadorj, Diana Furulyás, Fanni Kis, Katalin Biró, Mónika Stéger-Máté, Éva Stefanovits-Bányai (2017): Preliminary study of optimal extraction of biologically active compounds from Sea buckthorn (*Hippophae rhamnoides* L.) pomace. Book of Abstract 23rd International Symposium on Analytical and Environmental Problems. Szeged Hungary, 2017.10.09-2017.10.10. 2017. p. 392-396. Book of Abstract 23rd International Symposium on Analytical and Environmental Problems. (ISBN 987-963-306-563-1)

Rentsendavaa Chagnaadorj, Diana Furulyás, Gergely Mátravölgyi, Gitta Ficzek, Dávid Papp, Mónika Stéger-Máté (2017): Analysis of the antioxidant properties of Sea buckthorn jams during storage. Book of Abstract 23rd International Symposium on Analytical and Environmental Problems. Szeged Hungary, 2017.10.09-2017.10.10 2017. p. 388-391. Book of Abstract 23rd International Symposium on Analytical and Environmental Problems. (ISBN 987-963-306-563-1)

#### **Abstract at international conference**

Chaagnadorj Rentsendavaa; Nóra, Németh-Kálmándi; Dóra Székely; Mónika Stéger-Máté; Éva Stefanovits-Bányai; Diána Furulyás (2018): Development and production of seasoned beet root chips by microwave vacuum drying. In: István, Dalmadi, László, Baranyai, Quang, Duc Nguyen -Third International Conference on Food Science and Technology Budapest, Magyarország: Szent István Egyetem, Élelmiszertudományi Kar, p. 93-94. Közlemény:30343818

#### **Abstract in Hungarian**

Furulyás Diána, Rentsendavaa Chagnaadorj, Stégerné Máté Mónika, Stefanovitsné Bányai Éva, Mohácsiné Farkas Csilla (2017): Homoktövis (*Hippophae rhamnoides* L.) bogyó és törköly antimikrobás hatásának vizsgálata három patogén baktériummal szemben. In: Biró Lajos et al. (szerk.). Magyar Táplálkozástudományi Társaság XLII. Vándorgyűlésprogramkönyve: MTT 1966. 72 p. Konferenciahelye, ideje: Siófok, Magyarország, 2017.10.12-2017.10.14. Siófok: Magyar Táplálkozástudományi Társaság, 2017. p. 31. 1 p. (ISBN:[978-615-5606-04-5](#))

## REFERENCES

- BENZIE, I. F., STRAIN, J. J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical biochemistry*, 239(1) 70-76. p.
- ECCLESTON, C., BAORU, Y., TAHVONEN, R., KALLIO, H., RIMBACH, G.H. and MINIHAINE, A.M. (2002): Effects of an antioxidant-rich juice (sea buckthorn) on risk factors for coronary heart disease in humans. *J. Nut. Biochem*, 13 346-354. p. [doi.org/10.1016/S0955-2863\(02\)00179-1](https://doi.org/10.1016/S0955-2863(02)00179-1)
- EDRAKI, M., AKBARZADEH, A., HOSSEINZADEH, M., TANIDEH, N., SALEHI, A. and KOOHI-HOSSEINABADI, O. (2014): Healing effect of sea buckthorn, olive oil, and their mixture on full-thickness burn wounds. *Food Chem Toxicol*, 47(6) 1146-53. p. DOI: 10.1016/j.fct.2009.02.002.
- FAO, 2014. Save food – global initiative on food loss and waste reduction. Available from: <http://www.fao.org/docrep/015/i2776e/i2776e00.pdf>.
- FICZEK, G., MÁTRAVÖLGYI, G., FURULYÁS, D., RENTSSENDÁVAA, C., JÓCSÁK, I., PAPP, D., SIMON, G., VÉGVÁRI, GY. and STÉGER-MÁTÉ, M. (2019): Analysis of bioactive compounds of three sea buckthorn cultivars (*Hippophae rhamnoides* L. ‘Askola’, ‘Leikora’, and ‘Orangeveja’) with HPLC and spectrophotometric methods. *European Journal of Horticulture Science*, 84 (1) 31-38. p. <https://doi.org/10.17660/eJHS.2019/84.1.5>
- KOREKAR, G., DOLKAR, P., SINGH, H., SRIVASTAVA, R.B. and STOB DAN, T. (2014): Variability and the genotypic effect on antioxidant activity, total phenolics, carotenoids and ascorbic acid content in seventeen natural population of Sea buckthorn (*Hippophae rhamnoides* L.) from trans-Himalaya. *LWT- Food Science and Technology*, 55. (1). 157-162. p. DOI: [10.1016/j.lwt.2013.09.006](https://doi.org/10.1016/j.lwt.2013.09.006).
- KREJCAROVÁ, J., STRAKOVÁ, E., SUCHÝ, P. and KARÁSKOVÁ, K. (2015): Sea buckthorn (*Hippophae rhamnoides* L.) as a potential source of nutraceuticals and its therapeutic possibilities - A review. *Acta Veterinaria Brno*, 84. (3). 257-268. p.
- NUERNBERG, K., NUERNBERG, G., PRIEPKE, A. and DANNENBERGER, D. (2015). Sea buckthorn pomace supplementation in the finishing diets of pigs – Are there effects on meat quality and muscle fatty acids? *Archives Animal Breeding*. 58 107–113. p. DOI: 10.5194/aab-58-107-2015.
- REMINI, H., MERTZ, C., BELHANI, A., ACHIR, N., DORNIER, M., MADANI, K. (2015): Degradation kinetic modelling of ascorbic acid and colour intensity in pasteurised blood orange juice during storage. *Food Chemistry*, 173 (15). 665-673. p.
- SHARMA, H.S., OBEROI, G.S. and DHILLON, G.C (2016): Fruit and vegetable processing waste: renewable feed stocks for enzyme production. In *Agro-Industrial Wastes as Feedstock for Enzyme Production*. 23-59. p.
- SINGLETON, V. L. and ROSSI, J. A. (1965): Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*. 16(3) 144-158. p.
- SURYAKUMAR, G. and GUPTA, A. (2011): Medicinal and therapeutic potential of Sea buckthorn (*Hippophae rhamnoides* L.). *Journal of Ethnopharmacol*. 138 268-278. p.
- UPADHYAY, N.K., KUMAR, M.S.Y. and GUPTA, A. (2010): Antioxidant, cytoprotective and antibacterial effects of Sea buckthorn (*Hippophae rhamnoides* L.) leaves. *Food Chem Toxicol*, 48 3443-3448. p. <https://doi.org/10.1016/j.fct.2010.09.019>