

ARTICLE

## Effects of food processing technology on valuable compounds in elderberry (*Sambucus nigra* L.) varieties

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**ABSTRACT** Elderberry (*Sambucus nigra* L.) is a potential source of natural food colorants because of its high anthocyanin content. The aim of this work is to reveal which technology step has effect on the valuable components (total anthocyanins, total polyphenols) and on the color parameters in elderberry and in this regard to determine possible differences between elderberry varieties. Based on experiment results concentrate production steps have great effects on the studied parameters in case of two varieties especially in the heating and microfiltration steps but in different ratio. Polyphenolic compounds in 'Samocco' are more stable during the juice production than 'Haschberg'. Color stability test revealed that in case of colored samples 'Samocco' had stronger color intensity in the foods/ models. These differences should be taken into account when selecting a certain variety for industrial utilization.

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### KEY WORDS

food colorant  
technological steps  
anthocyanin  
polyphenol  
color stability

The study of natural colorants is an extensive and active area of investigation since the synthetic colorants have recently been associated with adverse effects in humans. As a result, food producers are trying to replace their synthetic food coloring agents with natural food colorants. There are several permitted pigments in food industry derived from natural sources, which may be used for food coloration. Among them, anthocyanins demonstrate a high potential to be used as natural food colorants due to their attractive orange, red and purple colors and water solubility that allows their incorporation into aqueous food system (Lee and Finn 2007; Del Caro and Piga 2008). Elderberry is a potential source of natural food colorants because of its high amount of anthocyanins and its "easy-to-grow" characteristics. Elderberry pigments are made up of only cyanidin glycosides, from which cyanidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside are the major ones (Dawidowicz et al. 2006). Apart from them, cyanidin-3-*O*-sambubioside-5-*O*-glucoside and cyanidin-3,5-*O*-diglucoside were detected as minor compounds (Hong and Wrolstad 1990). These natural food colorants are generally used in the form of powder, concentrate or extract during food-industrial processes. Hence, the composition of these products is also influenced by the steps of preparing method (pressing, enzymatic treatment, heat treatment etc.). Most of the studies concern the characterization and analysis of these compounds in raw materials before their processing; however,

in our experiments, changes in anthocyanin and polyphenol content and color parameters were investigated during juice production technology at the laboratory circumstances in case of two elderberry varieties.

## Materials and Methods

### Materials

Elderberry fruit samples were harvested in 2012 from Nagyvenyim in Hungary. After the harvest they were processed within 2 hours. One Austrian variety, Haschberg and one Danish variety, Samocco were investigated in our research. 'Haschberg' is the leading European variety and the only Hungarian state-recognized breed. According to our preliminary maturity investigations, 'Samocco' has higher anthocyanin content than the other Danish elderberries grown in Hungary (Szalóki-Dorkó et al. 2013).

Methanol and hydrochloric acid (VWR International Ltd.) were used for extraction and dilution of the samples. Pectinex BE XXL pectolytic enzyme (Novozyme) was used for the treatment of elderberry fruits. Bentonite and ErbiGel were purchased from Kertrade Ltd. to clarify the juice.

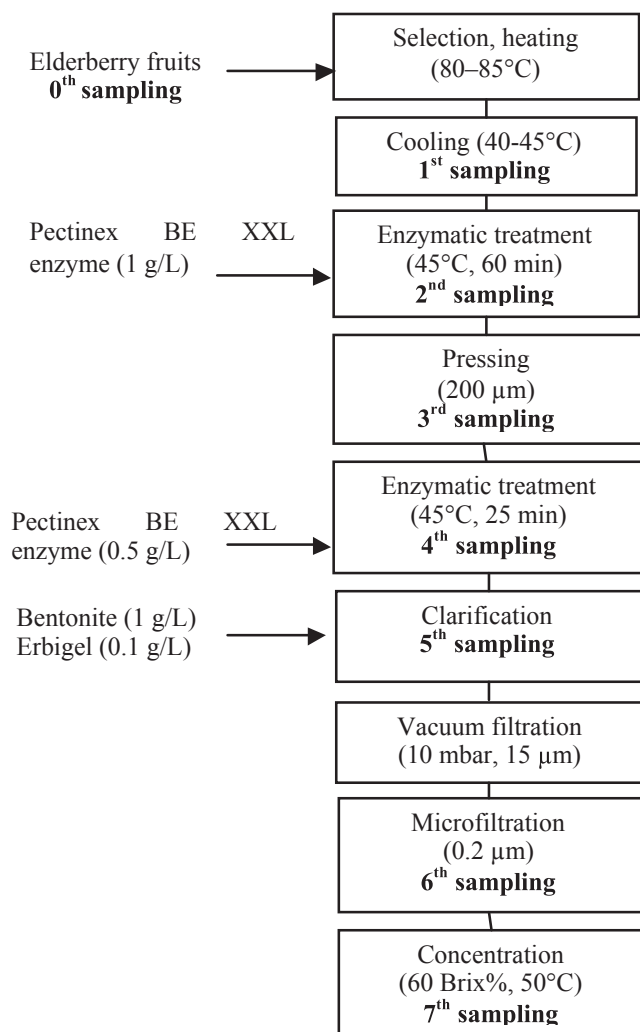
### Methods

#### *Production of elderberry concentrate*

Concentrates of elderberries were prepared under laboratory condition according to the industrial practice using the steps presented in Figure 1.

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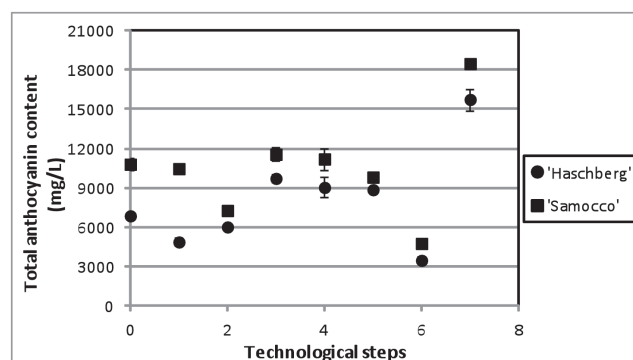
**Figure 1.** Food technological steps for production of elderberry concentrates.

### Sample extraction

After homogenization by commercial food mixer, an amount of 2.5 g sample was weighed into an Falcon-tube and 20 mL of 60.9% aqueous methanol containing 0.1% hydrochloric acid was added. Samples were extracted for 30 min in an ultrasonic bath. After extraction, the treated samples were centrifuged at 4000 rpm for 8 min at room temperature and the supernatant was examined for their total anthocyanin and polyphenol content.

### Analytical methods

Total anthocyanin content was determined by the method of Füleki and Francis (1968). The absorbance (A) of samples was measured at 530 nm with U-2000A Hitachi spectrophotometer. The content of total anthocyanins was expressed in mg cyanidin equivalents (CGE) per L of elderberry samples.



**Figure 2.** Total anthocyanin content during food technology in two elderberry varieties.

Total anthocyanin (TA) content was calculated by the following equation:  $TA \text{ (mg CGE/L)} = A \times 15 \times \text{dilution}$ . Color parameters were measured by digital colorimeter (Konica Minolta CR 410) during which  $L^*$  (lightness factor),  $a^*$  (red-green value) and  $b^*$  (blue-yellow value) parameters were recorded. The most informative values of the color ability of elderberries are  $a^*$  because of its red tone and  $b^*$  because of its blue tone. Total polyphenol content was determined spectrophotometrically according to the method of Singleton and Rossi (1965) at 765 nm, and was calculated after calibration with gallic acid.

### Color stability test

Water, natural yoghurt and jam and juice model samples were used to perform color stability tests. Jam and juice samples were modeled with 60 m/m% and 10 m/m% sugar solutions, respectively. For juice models, sugar solution was supplemented with citric acid to maintain the acidic conditions. After preparation, equal amount of 'Haschberg' and 'Samocco' concentrates were added to the samples and color parameters were measured as described above.

### Statistical analysis

T-test was used to analyze the data derived from total anthocyanin and color parameter measurements. Differences were considered statistically significant when  $P < 0.05$ . All measurements were done in three replicates and standard deviations of mean values were also calculated. Statistical analyses were performed using Statistica 9 (StatSoft Inc., Tulsa, USA) software.

## Results and Discussion

Concentrate production steps have affected the concentration of total anthocyanin in both elderberry varieties (Fig. 2). The highest pigment content was detected in concentrate form of 'Samocco' (18472 mg CGE/L), and the lowest was in

**Table 1.** P values between technology steps in total anthocyanin content in case of two elderberry varieties.

Technology steps	P values	
	Haschberg	Samocco
0-1	0.001*	0.339
1-2	0.006*	0.000*
2-3	0.000*	0.000*
3-4	0.233	0.558
4-5	0.736	0.055
5-6	0.000*	0.000*
6-7	0.000*	0.000*

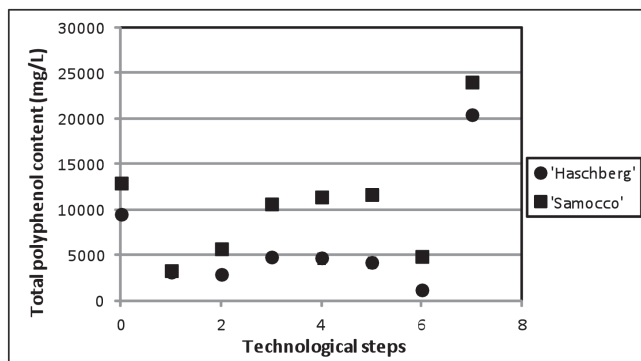
\*representing significant differences in anthocyanin content between technology steps (P <0.05)

'Haschberg' (4768 mg CGE/L) after microfiltration step (6<sup>th</sup> section). There are several technological steps which reduced the total anthocyanin content: cooling after heating (1<sup>st</sup> step), enzymatic treatment (2<sup>nd</sup> and 4<sup>th</sup> steps), clarification (5<sup>th</sup> step) and filtration (6<sup>th</sup> step). Thermal degradation of natural pigment in elderberry is a well-known phenomenon (Sadilova et al. 2006). In our study, elderberry varieties had different heat sensitivity since about 4% reduction could be observed by 'Samocco', while about 29% by 'Haschberg'. Clarification and filtration steps bound and kept back anthocyanin compounds during technology presumably which results the reduction of pigment concentration. This result is in contrast with the black carrot juice because bentonite treatment caused 20% increase in monomeric anthocyanin content (Turkylmaz et al. 2012). Increase in anthocyanin content could be observed during the first enzymatic treatment by the variety of 'Haschberg', which is in contrast to that detected by 'Samocco'. It is probably due to the different pectin composition and anthocyanin profile of varieties. Pigment concentration was increased due to the effect of pressing (3<sup>rd</sup> step) because the molecules were liberated from the shells of berries. The samples were concentrated by evaporation and in this way

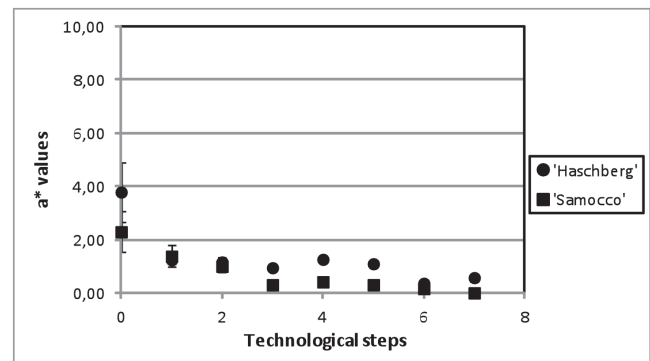
the highest pigment content ('Haschberg' 15744 mg CGE/L; 'Samocco' 18472 mg CGE/L) was presented per unit volume. Before processing 'Samocco' contained higher pigment content (10808 mg CGE/L) than 'Haschberg' (6880 mg CGE/L) which is in contrast to the results of Kaack et al. (2008). This difference did not change during the whole experiment. They documented a maturity test in which 'Haschberg' has higher anthocyanin content than 'Samocco' in Denmark. This difference may arise from the different climate and different pomological technology of the two countries. The differences in total anthocyanin content between the two elderberry varieties were not significant (P>0.05) during the whole technology, but during some technological steps, significant differences were observed (Table 1).

The total polyphenol content (Fig. 3) of elderberry varieties did not change equally during the process. The samples reached the maximum values in concentrate form ('Haschberg' 20995 mg/L, 'Samocco' 23764 mg/L) and the minimum value can be found after the microfiltration step ('Haschberg' 1179 mg/L, 'Samocco' 4862 mg/L). Increase in polyphenol content could be detected after the 2<sup>nd</sup> enzymatic treatment (ca. 7%) and juice clarification (ca. 2.3%) by 'Samocco', while reduction occurred in case of 'Haschberg' (ca. 2% and 11%). Nevertheless, polyphenolic components of 'Samocco' were more stable during pressing, 2<sup>nd</sup> enzymatic treatment and juice clarification than 'Haschberg', which had reduced polyphenol content after these three technological steps. Filtration (6<sup>th</sup> step) and evaporation (7<sup>th</sup> step) sections influenced the polyphenol concentration similarly to that can be observed at anthocyanins.

In color parameter tests, the red-green ratio decreased during the processing. However, after concentration, a slight increase could be observed by the values of 'Haschberg' elderberry variety (Fig. 4). Despite the lower anthocyanin content, 'Haschberg' concentrate showed higher a\* value (0.58) than 'Samocco' (0.01); however, this difference is not visible to the naked eye. Anyway, the initial red-green ratio



**Figure 3.** Total polyphenol content during food technology.



**Figure 4.** The a\* values during food technology in case of two elderberry varieties.

**Table 2.** Color parameters of samples colored by elderberry concentrates.

Samples	Amount of concentrates (g)	Haschberg			Samocco		
		L*	a*	b*	L*	a*	b*
Water	1	22.40 ± 0.22	11.73 ± 0.53	3.21 ± 0.14	19.60 ± 0.20	6.58 ± 0.38	2.32 ± 0.04
Natural yoghurt	0.5	61.41 ± 0.08	14.88 ± 0.05	0.10 ± 0.03	60.21 ± 0.18	14.86 ± 0.09	0.15 ± 0.02
Jam model	1	24.53 ± 1.87	17.67 ± 2.32	8.09 ± 1.45	23.93 ± 2.37	16.32 ± 2.03	6.47 ± 2.30
Juice model	1	24.24 ± 1.03	19.61 ± 1.71	9.15 ± 1.12	23.22 ± 1.62	14.79 ± 1.52	5.60 ± 0.86

Average values of three replicates (n=3) ± standard errors are presented.

**Table 3.** P values between coloring samples in color parameters in case of two elderberry varieties.

Samples	L*	P values	
		a*	b*
Water	0.000	0.000	0.000
Natural yogurt	0.000	0.635	0.000
Jam model	0.669	0.355	0.100
Juice model	0.284	0.001	0.000

had decreased with ca. 85% and ca. 95% by ‘Haschberg’ and ‘Samocco’, respectively, during technological processing.

The color stability test revealed that ‘Samocco’ caused stronger color intensity in the foods/models than ‘Haschberg’ samples (Table 2). This is presumably due to the instability of anthocyanin compounds because their stability is highly variable depending on their structure and the composition of the food matrix (Wrolstad 2000). ‘Samocco’ concentrate had darker blue color in water and the jam / juice models, namely, ‘Haschberg’ concentrates had higher L\*, a\* and b\* values in these samples. Except a\* value of natural yoghurt and jam model, L\* value of jam model and juice model (P>0.05), significant differences were observed between samples colored by the two different elderberry concentrates (P< 0.05) (Table 3.).

## Conclusions

Elderberry fruit is predominantly used for food coloration and varieties with higher anthocyanin content are particularly suitable for commercial applications. Food colorants are usually added as concentrate; however, according to our results, total anthocyanin content, total polyphenol content and color parameters are influenced by the fruit-processing technological steps. ‘Samocco’, the new Danish elderberry variety had higher pigment concentration and color intensity

than the variety of ‘Haschberg’ during processing steps. Food coloring tests revealed that Danish variety may cause darker blue color in certain foods; this property should be considered in industrial applications.

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