The Polyphenols Stability, Enzyme Activity and Physico-Chemical Parameters During Producing Wild Elderberry Concentrated Juice

Ante GALIĆ [1(✉)], Verica DRAGOVIĆ-UZELAC [2], Branka LEVAJ [2], Danijela BURSAĆ KOVAČEVIĆ [2], Stjepan PLIESTIĆ [1], Sabina ARNAUTOVIĆ [2]

Summary

The influence of processing wild elderberry into concentrated juice on polyphenols (total phenols, flavonoids, non-flavonoids, anthocyanins, flavan-3-ols, hydrolysed tannins) stability, activity of polyphenol oxidase (PPO) and peroxidase (POD), and changes of physico-chemical parameters (total and soluble dry matter, total acidity, pH, sugars) were investigated. The amounts of total phenols, flavonoids, non-flavonoids, falvan-3-ols and hydrolysed tannins were analyzed using Folin-Ciocalteu colorimetric method, while the total anthocyanins were determined by bisulphite bleaching method. Total phenols ranged from 25.87 mg/g DM to 38.87 mg/g DM. Total anthocyanins were the most abundant polyphenols in all investigated samples (raw elderberries, elderberries after blanching, elderberry juice after disintegration and pressing, concentrated elderberry juice) and their concentration ranged from 13.12 mg/g DM to 25.67 mg/g DM. Other polyphenols determined in high concentration were hydrolysed tannins, followed by flavan-3-ols, flavonoids and nonfavonoids. After blanching, the concentration of all polyphenols did not decrease significantly. After disintegration of elderberries the concentration of all polyphenols increased, probably due to inactivation of PPO and POD and better isolation of polyphenols from homogenized purée. During processing of elderberry juice into concentrated juice most polyphenols were stable. Total acidity and pH value were not changed during processing, whereas the amounts of total and reducing sugar increased after pressing and additionally after concentration. The obtained results suggest that raw elderberries as well as elderberry concentrated juice are high potential source of polyphenols especially anthocyanins.

Key words

elderberry, polyphenols, anthocyanins, enzymes, physico-chemical parameters

[1] University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia
✉ e-mail: agalic@agr.hr
[2] University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia

Received: November 3, 2008 | Accepted: March 18, 2009

Introduction

Black or common elder (*Sambucus nigra* L.), also called elderberry, is a widespread species, that grows on sunlight-exposed locations in most parts of Europe, Asia, North Africa and the USA. It is a deciduous shrub reaching up to 6 m in height, developing small, white hermaphrodite flowers in large corymbs, and flowering in early summer (Christensen et al., 2007). The mild-flavoured fruits ripen in mild to late summer. Fruit of the elderberry are very dark purple, nearly black. Elderberry is a fruit crop of high quality fruit whose diverse cultivars are already grown in plantations in several developed European countries. The berries are best not eaten row as they are mildly poisonous. The mild toxicity is destroyed by cooking. Chemical compounds from berries have gained interest as functional compounds in food colorants and as potent agents against oxidative stress (Stintzing et al., 2002), which reduce oxidative damage to the human body (Dawidowicz et al., 2006). The industrial processing of elderberries mainly takes place in Northern Europe where the berries are mostly processed into juice and juice concentrate. This production is based on the use of cultivars with a high content of soluble solids and anthocyanins and a delicious favour (Kaack, 1989; Kaack, 1996). Besides juice and concentrates, elderberry fruits may be used for the industrial production of jam, jelly, desserts, wine, cakes, candies and colouring of mixed juices (Jensen et al., 2001). Recently, elderberries have received increased attention due to their high contents of anthocyanins, that are widely used as colour ingredients in various beverages, and that may also provide nutritional benefits. Anthocyanins, as well as other flavonoids, exhibit antioxidant, anti-carcinogenic, immune-stimulating, antibacterial, anti-allergic and antiviral properties; therefore, their consumption may contribute to prevention of several degenerative diseases such as cardiovascular disease, cancer, inflammatory disease and diabetes (Dawidowicz et al., 2006). Anthocyanins found in elderberries are all cyanidin glycosides, comprising the 3-sambubioside, 3-glucoside, 3-sambubioside-5-glucoside and 3,5-diglucoside. Amounts vary between 200 and 1000 mg/100 g fresh weight (FW) (Bronnum-Hansen et al., 1985). Of these pigments, cyanidin 3-sambubioside has been reported as the most stable during processing (Drdak & Daucik, 1990). Elderberry juice also contains many primary metabolites including various sugars and organic acids. Among secondary metabolites, elderberry juice is predominantly characterised by high amounts of the anthocyanins (Lee & Finn, 2007; Kaack & Austed, 1998).

Therefore the aim of these study was to determine the stability of polyphenols (total - phenols (TPC), flavonoids (TF), non-flavonoids (TNF), anthocyanins (TA), flavan-3-ols (FT-ols), hydrolysed tannins (THT)), activity of polyphenol oxidase (PPO) and peroxidase (POD), and changes of physico-chemical parameters (total and soluble dry matter, total acidity, pH, sugars) during production of wild elderberry concentrated juice.

Material and methods

Samples

Selection of berries from the spontaneous wild elderberry population was conducted near Gospić in Lika region (Croatia) at the end of August 2006 in full maturity stage. Immediately after harvesting stalks were removed manually and berries were packed in polyethylene bags, frozen and stored at -18 °C until analysis. Prior to juice processing, the berries were gently defrosted. In the laboratory raw berries were mashed to a pulp using house blender (Mixy, Zepter International) and part of fresh mass was removed and analysed. Rest of the samples were taken after each operation process during production of concentrate juice (after blanching, pressing and concentration). Raw berries were blanched using water vapour (10 min/70 °C) and disintegrated in house blender (Mixy, Zepter International). The mash was collected in a cotton cloth and pressed by increasing the pressure over a period of 10 minutes until no further juice could be obtained. Pressed juice was concentrated by vacuum rota vapour (Büchy) with bath temperature of 40 °C until concentrated juice of 32 °Brix was obtained. All samples were analysed for their total and soluble dry matter, content of carbohydrates (sugars), total acids, pH value, polyphenols and enzymes activity.

Methods

Soluble and total dry matter, pH value, total acidity, and total and reducing sugars were determined according to Regulation (1983). *Soluble dry matter* was measured using a refractometer (Leica 7531 L refractometer) to determine °Brix. *Determination of total dry matter* was conducted with etalonic method by drying at 105 °C until constant mass. *The pH value* of each sample was determined with a pH meter (Iskra MA 5735). *Total acidity* was determined by titrating each sample with standardized 0.1 M NaOH to pH 8.1 using a pH meter. Determination of *directly reducing sugars* by Luff solution was based on the principal that in determined conditions reducing sugars (natural invert) converted $CuSO_4$ from the Luff solution into Cu_2O. Unspent amount of cupric ion was re-titrated with tyosulphate solution. Quantity of sugars was read from the tables; from difference of consumption for blind trail and sample. Unreduced disaccharide (sucrose) first had to be inverted (hydrolyzed) on reducing monosaccharide by acid, and then it was determined by Luff solution. Difference between obtained total invert and natural invert gave quantity of reducing sugar developed by sucrose inversion. All measurements were conducted in duplicates.

Enzymes activity. Activity of polyphenol oxidase (PPO) and peroxidase (POD) were determined using the method described by Gonzales et al. (2000) with some modification. Raw berries, blanched berries, pressed and concentrated juice were analyzed for POD and PPO activities. Stable and highly active POD and PPO extracts were obtained using insoluble polyvinylpolypyrrolidone (PVPP) and Triton X-100 in 0.2 M sodium phosphate, pH 6.5 buffer.

Ten grams of each sample were mixed for 10 min with 25 mL mixture of insoluble polyvinylpolypyrrolidone (PVPP)

and Triton X-100 in 0.2 M sodium phosphate, pH 6.5, buffer using a vortex mixer. After centrifuging at 5500 rpm for 15 min, the supernatant was collected and stored in the refrigerator until analysis. Prepared supernatant was used for analyzing POD and PPO activities.

PPO activity assay. PPO activity was assessed in duplicate using a spectrophotometer (UV-VIS Unicam Helios β) and calculated on the basis of the slope from the linear portion of the curve of $\Delta_A 420$ every 30 sec until absorbance stops to grow. Solution (3 mL, 0.07 M) of dissolved pyrocatechol in 0.05 M sodium phosphate, pH 6.5, buffer, were added to 0.075 mL of PPO extract. Recording of the absorbance was performed immediately. One unit of PPO activity was defined as $0.001 \times \Delta_A 420$ min^{-1} (mL of extract)$^{-1}$.

POD assay. POD activity was evaluated in duplicate and calculated in the same way as described above. A 0.025 mL of enzyme extract was added to 2.7 mL of 0.05 M sodium phosphate buffer (pH 6.5), which contained 0.2 mL of guaiacol (c = 10g/L) and 0.1 mL of hydrogen peroxide (c = 10g/L). Recording of the absorbance was performed immediately. Initial rate of increase in Δ_A 485 was used to measure enzyme activity. One unit of PPO activity was defined as $0.001 \times \Delta_A 420$ min^{-1} (mL of extract)$^{-1}$.

Total phenols, flavonoids and non-flavonoids content. Total phenols content (TPC), flavonoid content (TF), and non-flavonoid content (TNF) were determined using the Folin-Ciocalteu colorimetric method (absorbance measuring at 765 nm) described by Amerine and Ough (1980) and Singleton and Rossi (1965) with some modification. Phenols of the fruits were extracted from 10 g samples using 40 mL 80 % (v/v) aqueous ethanol. The mixture was extracted 20 min in inert atmosphere filtered and through Whatman filter paper using a Buchner funnel. Extraction of the residue was repeated using the same conditions. The filtrates were combined and diluted to 100 mL in volumetric flask with 80 % aqueous ethanol. Obtained extract was used for determination of TPC, TF and TNF. The formaldehyde precipitation was used to determine flavonoids in fruit samples (Kramling and Singleton, 1969). The content of TPC and TNF was measured as follows: 0.5 mL diluted extracts or standard solutions of gallic acid (20-500 mgL^{-1}) was added to a 50 mL volumetric flask containing 30 mL of dd H_20, than 2.5 mL of Folin-Ciocalteu's reagent was added to the mixture and shaken. After 5 min, 7.5 mL of 7 % Na_2C0_3 solution was added with mixing and the solution was immediately diluted to 50 mL with dd H_20. After incubation at room temperature for two hours the optical density of the solution was measured at 765 nm. The results were expressed as mg gallic acid equivalent (GAE)/g of dry matter (DM). The amount of flavonoids was calculated as difference between total phenols and nonflavonoids and expressed as mg gallic acid equivalent (GAE)/g of DM.

Hydrolysed tannins content (THT). Hydrolysed tannins content (THT) from selected samples was determined using method described by Amerine and Ough (1980). Method is based on the principle of hydrolysed tannins precipitation by addition of the cinchonine-sulphate. 5 g of sample was mixed with dd H_20 in a ratio of 1:2. In diluted sample 12.5 mL buffer pH 7.9 and 6.3 mL cinchonine-sulphate solution were added. Mixture was homogenised on magnetic mixer for about 20 minutes and centrifuged at 5500 rpm for 20 min. The obtained supernatant was filtrated into 100 mL volumetric flask. Residue on the filter along with filter paper was rinsed with the 10 mL of 10 % sodium-sulphate solution and centrifuged at 5500 rpm for another 20 minutes. After centrifugation, liquid part was connected with supernatant. pH of that liquid mixture was adjusted to 3.5 by adding 1 M hydrochloric acid and solution was immediately diluted to 100 mL with dd H_20. Residual sediment was dissolved in 25 mL of 1M ethanol's hydrochloric acid solution. Obtained extract was used for THT determination. The results were expressed as mg gallic acid equivalent (GAE)/g of DM.

Flavan-3-ols content. Obtained extract for TPC determination was used for determination of flavan-3-ols (FT-ols) using the method described by Tanner & Brunner (1979). The principle of determining the total flavan-3-ols is based on the specifics of the flavan-3-ols to react with vanillin. Absorbance was measured at 500 nm. The results were expressed as mg (+)-catehin equivalent (CAE)/ g of DM.

Total anthocyanins content. Total anthocyanin content (TA) in extract from selected samples was determined using bisulphite bleaching method (Riberéau-Gayon & Stonestreet, 1965). Anthocyanins of the fruits were extracted from 2 g fresh samples using 2 mL of 0.1 % HCl (v/v) in 96 % ethanol and 40 mL 2 % aqueous HCI (v/v). The mixture was centrifuged at 5500 rpm for 10 min. The obtained supernatant was used for determination of TA. The content of TA was measured as follow: 10 mL of extract was pipet into two test tubes, then 4 mL of 15 % sodium bisulphite was added to one test tube and 4 mL of dd H_20 to the other. After 15 min of incubation at room temperature the absorbance of each mixture was measured at 520 nm. Results were expressed as mg cyanidin-3-glucoside equivalent (Cy-gE)/g of DM.

All spectrophotometric measurements were performed by UV-VIS spectrophotometer UV-Vis Unicam Helios .

Statistical analysis

Linear regression and calculation of standard deviations were done with Excel (Microsoft Office).

Results and discussion

The aim of the present investigation was to determine polyphenols stability, enzyme activity and physico-chemical parameters during production of wild elderberry concentrated juice. Measurements were carried out in duplicate of all the samples taken during production of concentrate juice (raw berries, blanched berries, pressed and concentrate juice). Several studies were made on anthocyanins and other polyphenols content of elderberry cultivars (Kaack et al., 2007; Kaack & Austed, 1998; Bridle & Timberlake, 1996; Lee & Finn, 2007), however, research papers of changes physicochemical composition during elderberry fruit processing are scarce.

Table 1. Physico-chemical parameters during processing wild elderberry into concentrated juice

Parameters		Raw berries	Blanched berries	Pressed juice	Concentrated juice
Dry matter (%)	Total	21.35±0.11	20.48±0.05	15.31±0.09	32.39±0,16
	Soluble	17.00±0.50	15.00±0.00	15.00±0.00	32.00±0,50
pH		4.15±0.00	4.23±0.00	4.21±0.00	4.20±0.00
Total acidity (%)		1.21±0.03	1.20±0.12	1.25±0.07	2.95±0.09
Sugars (%)	Total	6.16±0.09	6.96±0.02	8.54±0.07	20.08±0.12
	Reducing	6.12±0.09	6.92±0.01	8.51±0.08	20.05±0.11
	Sucrose	0.04±0.00	0.04±0.0 1	0.03±0.00	0.03±0.01

Values are means of two replications

Results of chemical analyses of raw berries are in accordance to the available literature (Mratinić & Fotirić, 1998; Vulić et al., 2008; Kaack & Austed, 1998; Kahkonen et al., 1999), so the berries from the wild elderberry population are highly valuable. Total dry matter content was 21.35 %, total acidity content 1.21 %, total sugars content 6.16 %, reducing sugars content 6.12 % and pH value 4.15. Comparisons of those data with the ones in the literature indicated that total dry matter, total acidity and pH value falls within the limits average for non-selected elderberry (Mratinić & Konjić, 1998; Porpaczy & Laszlo, 1984; Lee & Finn, 2007). On the other hand, total sugars content in the raw berries was lover than average for non-selected elderberry (Mratinić & Konjić, 1998; Mratinić & Fotirić, 1998; Vulić et al., 2008). According to the literature reducing sugars are the most common in wild fruits, and they presented about 90 % of the total amount of sugars (Grlić, 1987) what is in accordance with our results. During production pH value is fairly uniform and ranges from 4.15 (raw berries) to 4.23 (blanched berries). Like pH value, total acidity is very uniform and varies from 1.20 % (in blanched berries) to 1.25 % (in pressed juice). Concentrate juice had highest total acidity (2.95 %), probably due to concentrating process where part of organic acids stay trapped in concentrate. In fresh elderberry, as in most wild fruits, reducing sugars are the dominant, while sucrose was represented in a small amount. Reducing sugars were also dominant during all production steps, while sucrose was detected in the lower amounts. Content of total and reducing sugars increased after pressing and additionally after concentration. Due to process of concentration total and reducing sugars content was the highest in concentrated juice with values of 20.08 % and 20.05 % (Table 1).

Table 2 shows the POD and PPO activity in fresh berries and in the samples taken during production. This study demonstrated that blanching time of 10 min and bath temperature of 70 °C was sufficient to inactivate PPO and POD. Both enzymes were completely inactivated after 10 minutes of blanching without residual activity. These results are in accordance with literature showing losses of enzyme activity at the temperature higher than 45 °C (Fenema, 1985)

The content of total phenolics in the samples was determined spectrometrically according to the Folin-Ciocalteu procedure and calculated as gallic acid equivalents (GAE) in milligrams per gram of dry mater. According to Kahkonen et al. (1999) concentration of GAE > 20 mg/g DM presents remarkably high total phenolics content, that means that the raw berries from the wild elderberry population are highly rich sources of these compounds. As in raw berries, in all investigated samples, total phenolics (TPC) were predominant polyphenols. Their amount varied and ranged from 32.22 mg of GAE/g DM in raw berries to 38.87 mg of GAE/g DM in concentrated juice. After blanching, the content of total phenolics were reduced by almost 20 % of the content in fresh berries. High total phenolics content in concentrated juice could be explained by better separation and extraction of bounded phenols from plant tissue after blanching and pressing (Figure 1).

Table 2. The results of polyphenol oxidase (PPO) and peroxidase (POD) activity

	Raw berries	Blanched berries	Pressed juice	Concentrated juice
PPO (activity/g of sample)	0.51	–	–	–
POD (activity/g of sample)	0.66	–	–	–

Values are means of two replications

Among the total phenolic compounds the dominant group were non-flavonoids. Significant amounts were observed in all samples. The lowest concentration of non-flavonoids were detected in blanched berries (18.19 mg GAE/g DM) and the highest in concentrated juice (25.78 mg GAE/g). Significant differences in concentration of non-flavonoids were present between blanched berries and other samples. Non-flavonoids concentration in the blanched berries was approximately 25 % lower.

Compared to concentration of other polyphenols, flavonoids were presented in the lowest amounts in all investigated samples. Quantity changes of flavonoids during processing are shown in Figure 1. Obtained results show that flavonoid concentration constantly grew during production of concentrated juice from 6.50 (GAE)/g DM in raw berries to 12.06 (GAE)/g DM in concentrated juice.

The amount of hydrolysed tannins (THT) increases considerably from 12.50 mg (GAE)/g of DM in row berries to 19.28 mg (GAE)/g of DM in concentrated juice. These increasing are probably a consequence of performed production step.

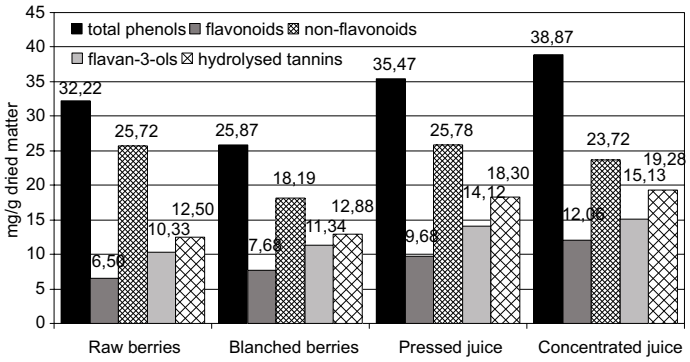

Figure 1. Polyphenolic compounds in samples during processing wild elderberry into concentrated juice

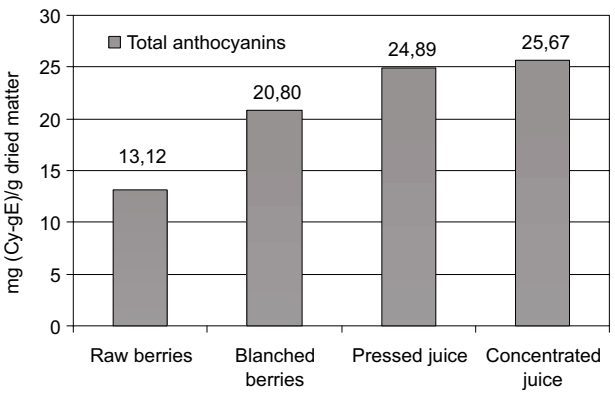

Figure 2. Total anthocyanin content in samples during processing wild elderberry into concentrated juice

Flavan-3-ols (FT-ols) were determined in smaller amounts than TPC, TNF and THT. The FT-ols content in all investigated samples constantly grew during all stages of production. Concentration of flavan-3-ols in fresh berries was 10.33 mg CAE/g of DM while concentrated juice had almost 50 % higher FT-ols content (15.13 CAE/g of DM). The obtained data demonstrate that these compounds are widely distributed in all analyzed samples (Figure 1).

The content of total anthocyanins in the fresh berries has been the subject of many investigations. A great variation in the content of anthocyanins was observed between the elderberry cultivars. Bronnum-Hansen et al. (1985) reported that elderberry anthocyanins are all cyanidin glycosides (cyanidin 3-sambubioside, cyanidin 3-glucoside, cyanidin 3-sambubioside-5-glucoside and cyanidin 3,5-diglucoside) and their total amounts vary between 200 and 1000 mg/100g fresh weight. Kaack and Austed (1998) determined even higher concentration of total anthocyanins in some elderberry cultivars (between 664 and 1816 mg/100g fresh weight). Our results showed that total anthocyanins content of 13.12 mg of Cy-gE/g DM in wild elderberry fruits were in accordance with Kaack and Austed report. During production of concentrated juice every production step led to increased content of total anthocyanins, from 20.80 mg of Cy-gE/g DM in blanched berries to 25.67 mg of Cy-gE/g DM in concentrated juice, probably due to blanching and mashing which leads to degradation of cell walls and releasing anthocyanins into the juice (Landbo et al., 2006) (Figure 2).

The major degradation factors of the anthocyanins are the temperature, the presence of oxygen and light, co-pigmentation, metal ions, enzymes, the pH value, etc. (Jackman et al. 1987). During production of concentrated juice anthocyanin concentration constantly rise and that shows that whole process was performed under optimal conditions. Generally we can conclude that anthocyanins were stable during concentration.

Lastly, considering all analyzed chemical parameters of elderberry fruit quality, it can be concluded that black elderberry fruit falls into the category of biologically high-quality fruits (Mratinić & Fotirić, 1998; Kaack & Austed, 1998; Abuja, 1998), therefore it could be processed into high-quality concentrated juice.

Conclusions

Elderberries were exceptionally rich with the total acids, what presents an important parameter for processing. The amounts of sugar in all investigated samples were very low so it is not the focal chemical compound for technological processing, and it can easily be added to the final product. The obtained results suggest that raw elderberries as well as elderberry concentrated juice are high potential source of polyphenols especially anthocyanins. Total anthocyanins were the most abundant polyphenols in all investigated samples, and they were stable during processing elderberry into concentrated juice.

Besides anthocyanins, other polyphenols were also stable during processing of elderberry juice into concentrated juice. Generally, all of these results show that the row berries and their concentrated juice retain evident content of important bioactive compounds and they can be used as easily accessible source of high value natural components.

The results of these investigations could be useful for the further investigation, and could be contribute to better understanding of influence of processing steps on polyphenols stability.

References

Abuja, P.M., Murkovic, M., Pfannhauser, W. (1998). Antioxidant and prooxidant activities of elderberry (*Sambucus nigra*) extract in low-density lipoprotein oxidation. *J. Agric. Food Chem.* 46: 4091-4096.

Amerine, M. A., Ough, C. S. (1980). Methods for analysis of musts and wine, Jon Wiley & Sons Inc., New York., 205-206.

Bridle, P., Timberlake, F. C. (1996) Anthocyanins as natural food colours – selected aspects. *Food Chemistry* 58: 103-109

Bronnum-Hansen, K., Jacobsen, F. & Flink, J. M. (1985). Anthocyanin colourants from elderbeny (*Sumbucus nigra* L.). 1. Process considerations for production of liquid extract. *J. Fd Technol.*, 20: 703-711.

Christensen, L. P., Knaack, K., & Frette, X. C. (2007). Selection of elderberry (*Sambucus nigra* L.) genotypes best suited for the preparation of elderflower extracts rich in flavonoids and phenolic acids. *European Food Research and Technology*, 227 (1): 293–305.

Dawidowicz, A. L., Wianowska, D., & Baraniak, B. (2006). The antioxidant properties of alcoholic extracts from *Sambucus nigra* L. (antioxidative properties of extracts). *Food Science Technology*, 39(3): 308–315.

Drdak, M., Daucik, P. (1990). Changes of elderberry (*Sambucus nigra*) pigments during the production of pigment concentrates. *Acta Aliment*., 19 (1): 3-7.

Fennema, O.R. (1985) Food Chemistry, Marcel Dekker, Inc., New York, str. 557-564.

Gonzales, E.M., De Ancos, B., Cano, M.P. (2000). Partial Characterization of Peroxidase and Polyphenol Oxidase Activites in Blackberry Fruits. *J. Agric. Food Chem.* 48: 5459-5464.

Grlić, LJ. (1986) Enciklopedija samoniklog jestivog bilja, August Cesarec, Zagreb, str. 292-293.

Jackman, R. L., Yada, R. Y., Tung, M. A., Speers, R. A., (1987). Anthocyanins as food colorants – a review. *Journal of Food Biochemistry* 11: 279–308.

Jensen, K., Christensen, L., Hansen, P., Jørgensen, U., & Kaack, K. (2001). Olfactory and quantitative analysis of volatiles in elderberry (*Sambucus nigra* L.) juice processed from seven cultivars. *Journal of the Science of Food and Agriculture,* 81: 237–244.

Kaack, K. (1989). New varieties of elderberry (*Sambucus nigra* L). *Tidsskr Planteavl* 93: 59-65.

Kaack, K. (1996). "Sampo" and "Samdal", elderberry cultivars for juice concentrates. *Fruit Variety J* 51: 28-31.

Kaack, K. and Austed, T. (1998). Interaction of vitamin C and flavonoids in elderberry (*Sambucus nigra* L.) during juice processing. *Plant Foods Hum Nutr.* 52(3):187–198.

Kaack, K., Fretté X. C., Christensen, L. P. Landbo A. K., Meyer A. S. (2007). Selection of elderberry (*Sambucus nigra* L.) genotypes best suited for the preparation of juice. *Eur Food Res Technol* 226: 843-855.

Kahkonen, M. J.; Hopia, A. I.; Vuorela, H. J.; Rauha, J.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.*, 47: 3954-3962.

Kramling, T. E., Singleton, V. L (1969). An estimate of the non-flavonoid phenols in wines. *Am. J. Enol. Vitic.* 20: 86-92.

Landbo, A.K., Kaack, K., Meyer, A.S. (2006) Statistically designed two step response surface optimization of enzymatic prepress treatment to increase juice yield and lower turbidity of elderberry juice. *Inn. Food Sci. Emerg. Tech.* 8: 135-142.

Lee, J., Finn, C. E. (2007). Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. *Journal of Science of Food and Agriculture*, 87(14): 2665–2675.

Mratinić, E., Fotirić, E. (2007). Selection of black elderberry (*Sambucus nigra* L.) and evaluation of its fruits usability as biologically valuable food. *Genetika*, 39(3): 305-314.

Mratinić, E., Kojić, M. (1998). Samonikle vrste voćaka Srbije, Institut za istraživanja u poljoprivredi, Beograd.

Pravilnik o metodama uzimanja uzoraka te obavljanja kemijskih i fizikalnih analiza radi kontrole kvalitete proizvoda voca i povrća (1983), Sluzbeni list SFRJ br. 29 (Narodne novine 53/91), Zagreb

Porpaczy, A., Laszlo, M. (1984) Evaluation of elderberry (*Sambucus nigra* L.) clones based on the quality of the fruit. *Acta Alimentaria* 13: 109-115.

Riberéau-Gayon, P., Stonestreet, E. (1965) Le dosage des anthocianes dans le vin rouge et détermination de leur structure. *Bulletin de la Societe Chimique de France*. 9(419): 2649-2652.

Singleton, V. L., Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolibdic-phosphotungistic reagents. *American Journal of Enology and Viticulture* 16: 144-158.

Stintzing, F. C., Stintzing, A. S., Carle, R., Frei, B., & Wrolstad, R. E. (2002). Color and antioxidant properties of cyanidin-based anthocyanin pigments. *Journal of Agriculture and Food Chemistry*, 50: 6172–6181.

Tanner, H., Brunner, H. R. (1979). Getränke-analytik. Verlag Heller Chemie und Verwaltungsgesellschaft, Heinz Paris, Schwabisch Hall, 57-61.

Vulić, J. J., Vračar Lj. O., Šumić, Z. M. (2008). Chemical characteristics of cultivated elderberry fruit. *Acta periodica technologica*, 39: 85-90

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