

ORIGINAL ARTICLE

The impact of additives on the quality indices of different black chokeberry products, developed using waste-free processing technology

Inta Krasnova^{1*} , Dalija Seglina¹ , Gunārs Lācis², Aivars Āboltiņš³, Jonas Viškelis⁴

¹ Institute of Horticulture, Unit of Processing and Biochemistry, Dobele/Krimūnu - Latvia

² Institute of Horticulture, Unit of Genetics and Breeding, Dobele/Krimūnu - Latvia

³ Latvian University of Life Science and Technologies, Faculty of Engineering, Jelgava - Latvia

⁴ Lithuanian Research Centre for Agriculture and Forestry Ringgold Standard Institution, Institute of Horticulture, Kaunas/Babtai - Lithuania

*Corresponding Author: Inta Krasnova, Institute of Horticulture, Unit of Processing and Biochemistry, Graudu iela 1, Ceriņi, 3701, Dobele/Krimūnu - Latvia, e-mail: inta.krasnova@llu.lv

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Abstract

The aim of the study was to develop and analyze various black chokeberry (*Aronia melanocarpa* (Michx.)) products using waste-free treatment technology. The chemical composition of black chokeberry berries, and various processed products made from them (juice, pomace, pomace water extract, dried sweetened pomace, and syrup) are described. The effect of activated carbon (AC) on the reduction of proanthocyanidins content in black chokeberry berry juice and water extract and the effect of ascorbic acid additive on the sensory properties of dried sweetened pomace were investigated. Principal component analysis (PCA) resulted in one compact group of products representing the solid products of black chokeberry, and the first two factors of the PC1 (total anthocyanins and flavonoid content) described 98.38% of the total variability. It was concluded that AC allowed for reducing the content of proanthocyanidins in juices but did not affect their taste. However, reduced proanthocyanin content reduces the functionality of the products, and this information must be considered in new product development. The addition of ascorbic acid to dried sweetened black chokeberry pomace did not significantly affect the degree of liking of dried sweetened pomace. Black chokeberry products developed by waste-free technology are rich in biologically active compounds and could be used to produce new functional products.

Keywords: Antioxidant activity; Activated carbon; Pomace; Extracts; Sensory analyses.

Highlights

- Black chokeberries are a suitable raw material for the use of waste-free technology
- The representative indicators in solid chokeberry products are anthocyanins and phenols
- An innovative product – dried sweetened candies from black chokeberries pomace – was developed



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1 Introduction

The importance of fruits and berries in human nutrition is determined by the biologically active compounds they contain, which have a positive effect on the functions of the human body, such as improving metabolism, strengthening immunity, reducing the risk of disease and improving general well-being (Santos-Buelga & Scalbert, 2000; Vinogradova et al., 2020). Among red berries, black chokeberry is given significant attention. Black chokeberry (*Aronia melanocarpa* (Michx.) Elliot) belongs to the Rosaceae family, and it is a deciduous shrub that came to Europe from the eastern part of North America (Vinogradova et al., 2020; Stalažs, 2021). Black chokeberries are rich in biologically active substances, and high in polyphenols, including phenolic acids, flavonoids, flavanols, anthocyanins, proanthocyanidins, which have strong antioxidant properties (Tolić et al., 2017; Denev et al., 2018; Oziembłowski et al., 2022). Ovaskainen et al. (2008) studied 143 different foods, including berries, fruits, vegetables, nuts, grain products, and beverages, and black chokeberry took first place among items in terms of highest total concentrations of polyphenols, including phenolic acids, anthocyanins, other flavonoids and ellagitannins (ETs) (Ovaskainen et al., 2008), as well as they are used in traditional medicine for the prevention of various diseases, including non-communicable ones (Jurendić & Ščetar, 2021).

Nowadays, black chokeberry is widely used in the production of various foods, such as juices, jams, syrups, teas, wines, and food supplements (Denev et al., 2018; Jurendić & Ščetar, 2021). Despite their high antioxidant activity, proanthocyanidins in black chokeberry possess an astringent taste and are detrimental to the taste of fruits and products derived from them (Suomela et al., 2012). Therefore, most of the time they are not consumed in the form of fresh fruit, and are used for processing (Oszmiański et al., 2015). Duffy et al. (2016) concluded that the addition of sweeteners was not sufficient to reduce the bitter taste of the juice and that other solutions are needed, some other limitations, such as the non-involvement of children/young people in sensory analysis, were mentioned.

One of the types of products for the processing of mainly red berries (blueberries, cranberries, rowanberries, and chokeberries) is dried sweetened fruits, consumed and used as a healthier substitute for candy. Such products are high in calories, but they also contain significant amounts of biologically active compounds. For example, dried sweetened cranberry has a remarkable anthocyanin and polyphenol content, which is associated with preparation technology and shelf life (Kovacev et al., 2020). Pietrzyk et al. (2010) studied the quality indicators of candied chokeberry, but there is a lack of data on the chemical composition of dried sweetened chokeberry and its sensory evaluation.

Black chokeberry and its by-products - pomace after obtaining juice - is a valuable raw material not only in the production of new products but also in improving the quality indicators of other foodstuffs. Studies have reported that their use has great potential to increase the colour, flavour, and nutritional value of various products (Jurendić & Ščetar, 2021; Samborska et al., 2019). Black chokeberry pomace is a rich source of polyphenolic compounds, especially anthocyanins, and flavonoids, which makes them worthy of industrial use in the production of food additives, reducing the use of synthetic additives (colorants) (Struck et al., 2016; Vagiri & Jensen, 2017; Trenka et al., 2020). Studies show that different extracts (including water) of various red berry pomace are valuable raw materials with a high antioxidant content and capacity (Hidalgo & Almajano, 2017).

Black chokeberry juice contains a large number of tannins, which affect its taste (He et al., 2015; Pietrzyk et al., 2010). Several studies have reported that the clarification of juices using filtration techniques (membrane, microfiltration, and ultrafiltration) or in combination with clarifying agents (bentonite, polyvinylpolypyrrolidone (PVPP), pectinase + amylase, sago, gelatin, activated carbon (AC), or starch) reduces the presence of tannins (Mazrou et al., 2020; Youn et al., 2004; Pogorzelski, et al., 2006). Activated carbons have long been used in the food and beverage industries where the main purpose is the removal of colour, taste, and odor. Typical examples include the removal of tannins and impurities in the wine industry

(Rodriguez-Reinoso & Silvestre-Albero, 2016). Gunathilake et al. (2018) evaluated the effect of active carbon filtration on the physico-chemical properties of lime juice. Sensory results indicated that natural lime juice showed higher “bitterness” compared to filtered juice. AC filtration and de-sedimentation were effective for minimizing delayed bitterness. But it was found that the phenolic content of the treated juice has been significantly reduced compared with natural lime juice.

Currently, international market reviews indicate a significant market for chokeberry (referred to as Aronia berries), with a projected increase of 8.6% from 2022 to 2027. The market size is forecasted to reach a growth of USD 404.9 million. It is expected that Europe will account for 33% of the global market share by 2023 (Technavio, 2022). With the increasing awareness of the numerous health benefits of chokeberries, the consumption of various chokeberry products, including concentrates, fruits and juices, has already shown a rise. Moreover, science-based clinical trials have the potential to contribute significantly to the growth of the berry market during the forecast period. The development of new chokeberry products can benefit both consumers and producers by enhancing their competitiveness and fostering economic development. It is essential to highlight the significance of the “European Green Deal” strategy in achieving the goal of climate neutrality by 2050, which includes reducing the environmental impact of food processing (European Commission, 2019).

Our research aims to develop various black chokeberry products by testing the effect of additives on their quality and using zero-waste processing technology. The chemical composition of chokeberries and their by-products - juices, pomaces, pomace water extract, and dried sweetened pomace - were studied. The first task was to determine the effect of AC on the reduction of tannin content in chokeberry berry juice and water extract. Our hypothesis was that the added absorbent would decrease the tannin content of chokeberry juice but might affect the product’s taste. Furthermore, assessors of different age groups, including adults and young people, were involved in evaluating the products. In the second task, we conducted a topical study on the taste properties of dried sweetened pomace and syrup, their chemical composition, and sensory acceptance, incorporating ascorbic acid in the production process. Typically, dried sweetened pomaces (or candies) are made from basic raw materials, such as fruits or berries. The product developed in our study is an innovative one that ensures the comprehensive utilization of raw materials. Our hypothesis was that consumers of all ages would appreciate it.

2 Materials and methods

2.1 Materials

Ethanol 96,3% (Kalsnava Distillery, Latvia), Folin–Ciocalteu’s phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), reagent potassium chloride, potassium ferrocyanide, sodium carbonate anhydrous, iron trichloride hexahydrate (Sigma-Aldrich, Germany); 2,2-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,4,6-tripyridyl-s-triazine, tannin acid, ascorbic acid (Fluka, England); aluminum chloride hexahydrate (Alfa Aesar, Thermo Fisher Scientific, Germany); sodium nitrite (VWR, France); sodium acetate trihydrate (AppliChem Chemicals, Germany); (+)-catechin, gallic acid monohydrate, sodium hydroxide, pellets, hydrochloric acid (Merck, USA); AC (Eiro Plus, Ukraine).

2.2 Preparation of samples

The study samples were fruits of black chokeberry harvested at the Institute of Horticulture in Dobeles (Latvia, location: 56°36'39.9"N 23°17'48.8"E) in the stage of full maturity, in August 2019. The fruit was divided into two parts. One part of the fresh fruit was chemically analyzed immediately after harvesting, the other part was put in polyethylene bags (10 kg), frozen, and stored at –18 °C until analysis and product preparation.

2.3 Equipment

Freezer Electrolux EC4200AOW1, (Elektrolux, Sweden), Voran Basket press 60 K (Voran, Austria), water bath Biosan WB-4MS (Biosan, Latvia), shaking incubator GLF 3032 (Labortechnik mbH, Germany), Eppendorf centrifuge 5804R (Eppendorf, Germany), professional B-Master Food dehydrator (Italia), digital refractometer PAL-1 (ATAGO, Japan); spectrophotometer ultraviolet (UV) 1800 (Shimadzu, Japan); High Performance Liquid Chromatography (HPLC) with UV detector system (Shimadzu, Japan).

2.4 Development of products

Three types of products (juices, extracts from pomace, and dried sweetened candies from pomace) were made and tested from black chokeberry using waste-free technology (Figure 1).

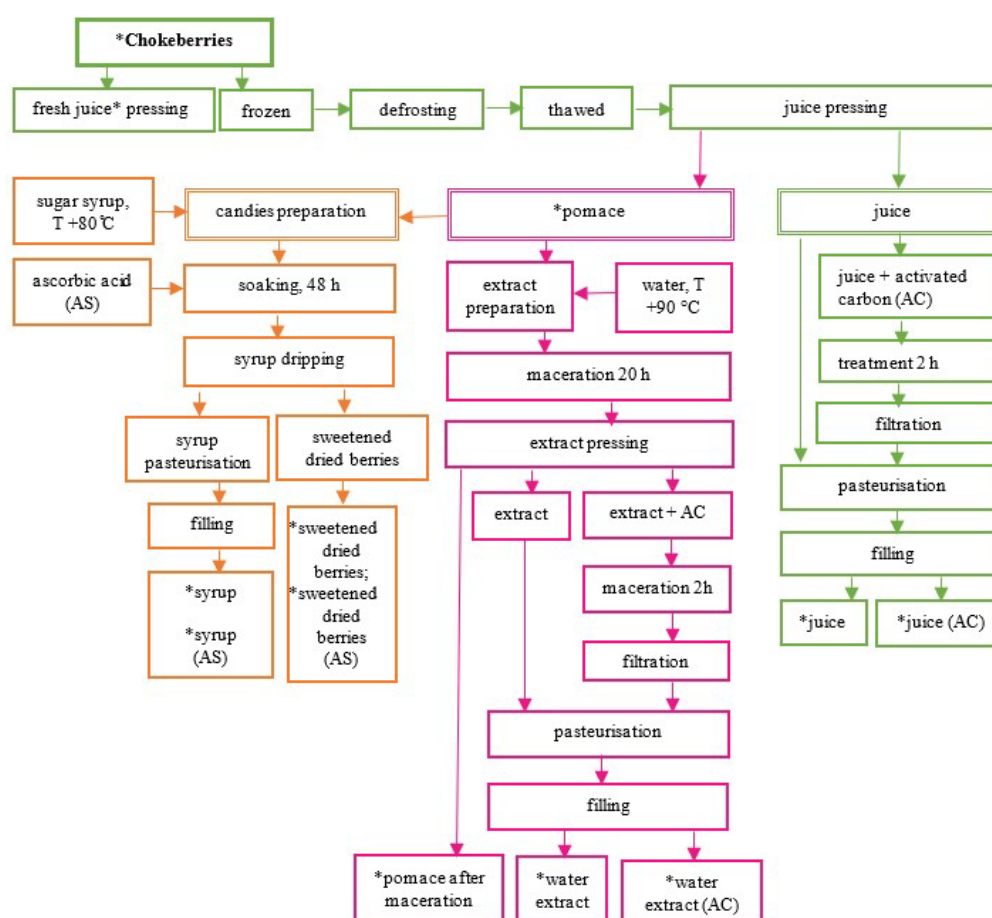


Figure 1. Scheme of the research design (* marked samples were analyzed). AS – ascorbic acid; AC – activated carbon.

2.5 Juice extraction

The juice was extracted from fresh and frozen black chokeberries. Fresh juice was analyzed unpasteurized. Fruits were thawed at room temperature, after which the juice was pressed. Obtained pomace was stored at +4 °C until further processing. The resulting juice was divided into two parts. The first part of the juice was pasteurized at +85 °C (10 min), filled in glass bottles, hermetically sealed. The second part of the juice was poured into beakers and crushed AC (0.3 g per 100 ml) was added. The beakers were placed on a GLF

shaking incubator for 2 hours (140 rpm at + 20 °C), then the juice was centrifuged (5000 rpm for 10 minutes) filtered with Whatman filter paper, pasteurized at +85 °C (10 min), filled in glass bottles, hermetically sealed.

2.6 Pomace extraction

Hot water (+90 °C) was poured onto black chokeberries after freezing the pomace (ratio 1:3 based on our preliminary research), was then stirred well and blended, kept at room temperature for 20 hours, and then squeezed. After extraction the pomaces were collected and stored at +4 °C until further processing, the extract was filtered and divided into two parts. The first part was pasteurized at +85 °C, (10 min) filled in glass bottles, hermetically sealed.

The second part of the filtered extract was poured into beakers and crushed AC (0.3 g per 100 ml) was added. The beakers were placed on a GLF shaking incubator for 2 hours (140 rpm at +20 °C), then the juice was centrifuged (5000 rpm for 10 minutes), filtered with Whatman filter paper, pasteurized at +85 °C (10 min), filled in glass bottles, hermetically sealed.

2.7 Preparation of dried sweetened pomace

For the preparation of dried sweetened pomace (DSP), hot (+80 °C) sugar syrup (water: sugar ratio 1:1) (control sample was poured with) over the frozen black chokeberry pomace. The next sample - dried sweetened pomace with ascorbic acid (DSPAS) 1.5% ascorbic acid (C) - was added during soaking to improve the taste. The pomace was soaked for two days, then drained, and dried at a temperature of $+50 \pm 2$ °C until the moisture content of the dried sweetened pomace was 20%. The syrup of both samples was pasteurized at +85 °C (10 min), filled into glass bottles, hermetically sealed.

2.8 Physical and chemical analyses

The following chemical indices have been determined for black chokeberry (fresh, frozen) and all obtained products: soluble solids, total acid, vitamin C, total proanthocyanidins, anthocyanin, phenolic, flavonoid content, and antioxidant activity (by ABTS^{•+}, DPPH[•], FRAP).

The content of chemical indicators in berries and products was expressed in two ways, depending on the sample: for solid samples (berries, pomace, and dried sweetened pomace) – on dry weight (DW), but for liquid samples (juice, extracts, syrup) – on fresh weight (FW).

Soluble solids content (SSC) °Brix determination in fruits and products was done using a refractometric method.

Total titratable acidity (TTA) of samples was assessed for ten milliliters of water extracts, which was titrated with 0.1 mol/ L NaOH to a pH of 8.1 according to general guidelines on objective tests (OECD, 2018). TTA was expressed as percent citric acid equivalent in fresh/dry weight (FW/DW).

The total phenolic (TP) content of fruits and products was determined by a photometric method using Folin-Ciocalteu reagent described by Singleton et al. (1999). Sample preparation: 1 g of the sample (berries, pomace) or 2 g of liquid (juice, syrup, water extracts) was transferred to a 50 ml flask; 30 ml of 80% ETOH mixture was added, then vortexed and extracted in an ultrasonic bath for 30 minutes, centrifuged and filtered. TP content in the sample was expressed as mg or g Gallic acid equivalent (GAE) 100 g⁻¹ in FW/DW. For the evaluation of antioxidant activity (DPPH[•], ABTS^{•+}, FRAP) the same extracts of the prepared sample for the determination of phenols and flavonoids were used.

Total flavonoids (TF) content of fruit and product extracts was measured by a colorimetric assay method (Yang et al., 2019). The standard curve was measured using catechin and the total flavonoid content was expressed as mg or g catechin equivalents (CE)/100 g in FW/DW).

The total proanthocyanidin (TT) content was estimated by the method of Price and Butler (Paaver et al., 2010). Sample preparation and procedure for measuring: 1 g sample (berries, pomace) or 2 g of juice was transferred to 100 ml flask; 50 ml water was added, and boiled for 30 min. After filtration through a Whatman filter, the solution was transferred to a 100 ml flask and water was added to reach 100 ml mark. 0.5 ml aliquots (or prepared pomace water extract) were finally transferred to vials, 1 ml 1% $K_3Fe(CN)_6$ and 1 ml 1% $FeCl_3$ were added, and water was added to reach 10 ml volume. After five five-minute time period, the solutions were measured spectrophotometrically at 720 nm. The results are expressed as mg or g catechin equivalent (CE) /100 g in FW/DW.

The total anthocyanins (TANC) content was determined by the photometric method and absorbance was measured at 510 and 700 nm described by (Sadowska et al., 2017). Sample preparation: 1 g of the sample (berries, pomace) or 2 g of liquid (juice, syrup, water extracts) was transferred to a 50 ml flask; 30 ml of the mixture (95% ETOH and 1.5 N HCl (85:15)) was added, then vortexed and extracted for 30 min in an ultrasound bath, centrifuged and filtered. The total anthocyanin content was expressed by cyanidin-3-o-glucoside equivalents (CGE) (mg or g/ CGE 100 g in FW/DW).

Vitamin C was determined by HPLC according to standard EN 14130:2003.

DPPH Radical Scavenging Activity Analysis (DPPH•). The antioxidant activity by radical scavenging activity method using 2,2-diphenyl-1-picrylhydrazyl (DPPH• assay) was performed according to a Floegel et al. (2011) with minor modifications. Briefly, the test sample (0.100 mL) was reacted with 2.9 mL of DPPH• solution (0.0039 g DPPH• in 100 mL ethanol). The absorbance of the research sample was done at 515 nm using a spectrophotometer. The radical scavenging activity of the sample was expressed in mmol Trolox equivalents (TE) /100 g in FW/DW.

ABTS Radical Scavenging Activity Analysis (ABTS•+). The antioxidant activity by radical scavenging activity method using ABTS•+ (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay was performed using a spectrophotometric method described by Floegel et al. (2011) with some modifications. The ABTS•+ (ABTS•+) solution was diluted by mixing with buffer solution to obtain an absorbance of 0.700 units at 734 nm. To determine antioxidant scavenging capacity, ABTS•+ reagent (3 ml), was mixed with sample extracts (30 μ l) and absorbance was measured after 6 min. The absorbance results were converted using a calibration curve of the standard. 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid as a standard was used. Results are expressed in mol TE/ 100 g FW/DW.

The ferric reducing antioxidant power assay (FRAP) was done according to Benzie & Strain (1996) with some modifications. The FRAP reagent solution was prepared from 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution. Sample extracts (0.150 mL) reacted with 2.850 mL of the FRAP solution for 10 min in the dark. The changes in absorbance from red to blue were determined at 593 nm. The absorbance results were converted using a calibration curve of the standard and expressed as mmol TE/100 g in FW/DW.

2.9 Sensory analyses

Sensory analyses were performed for the developed products: juice, juice after treatment with AC, pomace extract and dried sweetened pomace. The attributes - appearance (incl. colour), taste, sweetness, aftertaste, and astringency of the products - were assessed using a line scale according to ISO 4121: 2003, Sensory Analysis-Guidelines for the use of quantitative response scales. 25 trained and experienced panelists (from age 20 - 62) from the Institute of Horticulture and 15 students (the average age 17) from the Dobeles State Gymnasium 10A2 class without previous experience in sensory evaluation were involved.

2.10 Statistical analysis

Experimental data processing was carried out by the General Linear Model procedure on the SPSS 25 software package. Descriptive statistical analyses were performed for all obtained results. The closeness of the relationship between the parameters was determined using Pearson correlation coefficient, and Tukey's multiple range test was used in the clarification of significant differences ($p < 0.050$) among the studied samples. Principal component analysis (PCA) analysis was also used in this study. PCA was used in the descriptions of the results to describe the chemical parameters and their amount in all studied samples.

3 Results and discussion

Black chokeberry contains many biologically active substances, but they are rarely used for fresh consumption due to their bitter taste. Mostly they are used for processing (Kapci et al., 2013). Numerous studies have been performed on the chemical composition and antioxidant activity of fresh black chokeberry and its processed products. Our study will complement the existing data range by providing new information on various black chokeberry products that can be prepared using waste-free technology.

3.1 The content of chemical compounds and antiradical activity in the solid product samples

Table 1 summarizes the data for black chokeberry (fresh and frozen) and their processing products (pomace and dried sweetened pomace) chemical parameters and antioxidant activity expressed on DW. The soluble solids content in fresh berries stated 17.5 °Brix, in turn, in juice 18 °Brix (data not shown). Yang et al. (2019) concluded that dark purple black chokeberry SSC was from 13.66 °Brix to 14.67 °Brix, which was affected by many factors, such as climatic conditions and maturity at harvest significant effect on fruit quality (Pogorzelski et al., 2006). The vitamin C content of fresh and frozen berries was accordingly 89.49 mg 100 g⁻¹ and 83.99 mg 100 g⁻¹ DW. Among the analyzed samples, the highest content of vitamin C was determined in the pomace after juice pressing (93.36 mg 100 g⁻¹ DW).

Table 1. The content of titratable acidity, vitamin C, total anthocyanin, total flavonoid, total phenolics, total proanthocyanidin, DPPH[•], ABTS^{•+}, FRAP in the chokeberries and processing products (Dry weight).

Chemical parameters	Fresh berries	Frozen berries	Pomace after juice pressing	Press cake after water extraction	Dried sweetened pomace	Dried sweetened pomace with ascorbic acid
Titratable Acidity, (%)	7.67 ± 0.08 ^e	7.50 ± 0.08 ^d	4.65 ± 0.09 ^c	1.56 ± 0.06 ^b	1.22 ± 0.04 ^a	1.45 ± 0.02 ^b
Vitamin C, mg/100 g	89.49 ± 1.10 ^e	83.99 ± 1.20 ^d	93.36 ± 0.60 ^f	38.04 ± 0.59 ^a	45.88 ± 1.10 ^b	67.80 ± 0.63 ^c
Total anthocyanin content, g/100 g	2.31 ± 0.03 ^e	1.96 ± 0.02 ^d	2.45 ± 0.08 ^f	0.91 ± 0.05 ^a	1.16 ± 0.13 ^b	1.80 ± 0.07 ^c
Total flavonoid, CE g/100 g	1.21 ± 0.02 ^b	1.29 ± 0.02 ^c	1.85 ± 0.03 ^f	0.62 ± 0.01 ^a	1.44 ± 0.05 ^d	1.76 ± 0.06 ^e
Total phenolics, GAE mg/100 g	5.86 ± 0.04 ^e	5.40 ± 0.06 ^b	8.10 ± 0.06 ^c	3.13 ± 0.03 ^a	6.05 ± 0.23 ^d	6.41 ± 0.40 ^d
Total proanthocyanidin, CE g/100 g	2.29 ± 0.11 ^c	1.30 ± 0.00 ^a	3.76 ± 0.03 ^c	2.06 ± 0.02 ^b	2.30 ± 0.13 ^c	2.88 ± 0.07 ^d
DPPH [•] TE mmol/100 g	13.09 ± 0.32 ^d	10.59 ± 0.45 ^c	21.16 ± 0.45 ^e	8.37 ± 0.22 ^b	6.70 ± 0.38 ^a	8.06 ± 0.37 ^b
ABTS ^{•+} , TE mol/100 g	6.92 ± 0.25 ^d	4.68 ± 0.19 ^c	2.48 ± 0.27 ^b	1.31 ± 0.26 ^a	1.08 ± 0.12 ^a	2.53 ± 0.36 ^b
FRAP, TE mmol/100 g	18.15 ± 0.08 ^c	16.89 ± 1.10 ^d	27.22 ± 0.01 ^e	11.17 ± 0.01 ^a	10.92 ± 0.27 ^a	13.87 ± 0.48 ^b

Results are the mean values ± standard deviation from four measurements (n = 4); means in the same row with superscript with different letters (a, b, c, d, e, and h) are significantly different at $p < 0.05$.

It has been determined that 40.7% of vitamin C is still present in the press cake after obtaining the aqueous extract. Slightly more vitamin C is contained in the new pomace-based product – dried sweetened pomace (45.88 mg 100 g⁻¹ DW). The addition of ascorbic acid during the production of DSP increased the vitamin C content to 67.80 mg 100 g⁻¹ DW. Pietrzyk et al. (2010) found that the content of vitamin C in ascorbic acid-enriched black chokeberry candies did not depend on the technology used, and the products contained 82 mg

100 g⁻¹ DW vitamin C on average (Suomela et al., 2012). In contrast, the vitamin C content of candied blackcurrants depended on the enrichment method used.

Black chokeberry and their by-products are excellent raw materials to produce food additives (food supplements and colours), as they contain significant amounts of polyphenolics and anthocyanins (Vinogradova et al., 2020). Total anthocyanin content in fresh berries is slightly higher than in frozen (respectively, 2.31 mg 100 g⁻¹ DW and 1.96 mg 100 g⁻¹ DW). Total phenolic content in fresh berries was 5.86 GAE g/100 g DW, and similar results were obtained (Teleszko & Wojdyło, 2015) in berries (6.35 GAE mg 100 g⁻¹ DW). In addition, the study found that the phenolic content decreased by 7.9% during freezing.

The majority of total proanthocyanidins or condensed tannins are water soluble since these characteristics give fruits and berries a specific bitter taste (Smeriglio et al., 2017). Black chokeberries are particularly rich in these compounds, which makes the berries unfit for fresh consumption. However, total proanthocyanidins have great medical value. Comparing fresh, frozen berries and processed products, we can conclude that pomace contains less proanthocyanidins (1.30 CE g 100 g⁻¹ DW), while in berry (2.29 CE g 100 g⁻¹ DW), and dried sweetened pomace samples (2.30 CE g 100 g⁻¹ DW). The press cake after water extraction had a lower total proanthocyanidin content for 45.2% because a large portion had diffused in the water. In 23 analyzed black chokeberry samples in Bulgaria, the total content of proanthocyanidins was in the range of 522.0 ± 65.2 to 1002.3 ± 51.3 CE 100 g⁻¹ FW (Denev et al., 2018).

The pomace contains significant amounts of various chemical compounds such as total phenolics, flavonoids, and total proanthocyanidins. Data from several studies differs from this study, and total phenolic contents in various black chokeberry pomace from 3.10-6.31 GAE 100 g⁻¹ FW to 9.99 GAE 100 g⁻¹ DW were found (Struck et al., 2016).

Dried sweetened candies made from pomace also contain significant amounts of phenolic compounds (6.05 GAE mg 100 g⁻¹ DW). The additions of ascorbic acid do not significantly affect the phenolic content in DSPAS (6.41 GAE mg 100 g⁻¹ DW). Miletić et al. (2014) investigated the total phenol and antioxidants in different dried fruits (plums, apricots, bilberries, grapes), and the highest total phenolic contents found in a dried black chokeberry 2995.20 ± 42.28 GAE mg 100 g⁻¹ DW.

The antiradical activity of the chokeberries and their processed products used in the study differed between the samples. The DPPH• assay is used to predict antioxidant activities by a mechanism in which antioxidants act to inhibit lipid oxidation, DPPH• radicals are neutralized in the assay and free radical scavenging capacity can be determined (Škrovánková et al., 2012). The DPPH• radical scavenging activity of black chokeberry processing products was highest in the pomace after juice pressing (respectively pomace after juice pressing -POMJ 21.16 TE mmol 100 g⁻¹). The technology of the DSP production method affects the ability of the products to have antiradical activity (6.70 TE mmol 100 g⁻¹), it is decreased as some of the active compounds diffused into the syrup.

ABTS^{•+} radical cation is soluble mostly in water, but also in organic solvents, which allows for determining the antiradical activity of the hydrophilic and lipophilic compounds in samples (Floegel et al., 2011). This includes the effect of water-soluble colour compounds on anthocyanins (which are in high concentration in black chokeberry), as fresh berries had the highest antiradical activity (6.92 mol 100 g⁻¹) of all fresh tested products. Due to the antioxidant properties of vitamin C, black chokeberry DSPAS has 57.3% higher antiradical activity (2.53 TE mol 100 g⁻¹) than without additives.

The ferric reducing antioxidant power (FRAP) allows to measure the reduction of ferric ion (Fe³⁺)-ligand complex to the intensely blue-coloured ferrous (Fe²⁺) complex by antioxidants in an acidic medium (Pandey & Rizvi, 2012).

The results obtained with this method have a similar trend as with DPPH• radicals: the highest antiradical activity (FRAP) was in POMJ (27.22 TE mmol 100 g⁻¹), then fresh berries -FREB and frozen berries -FROB

(respectively 18.15 TE mmol 100 g⁻¹; 16.89 TE mmol 100 g⁻¹), DSPAS (13.87 TE mmol 100 g⁻¹), but the less in water extract from pomace -EPOM (11.17 TE mmol 100 g⁻¹) and DSP (10.92 TE mmol 100 g⁻¹).

Studying various black chokeberry processing products (including industrially produced ones), it was concluded that the highest antiradical activity with ABTS^{•+} is present in dried berry samples (54.4 TE g kg⁻¹ and 74.0 g kg⁻¹ FW), then in pomace – 49.6 TE g kg⁻¹ (Kapci et al., 2013). In addition, similar results were obtained with DPPH[•] radicals, accordingly in dried berries on average 33.4 TE g kg⁻¹, and in pomace – 25.2 TE g kg⁻¹ in the study of Kapci et al. (2013). Antiradical activity in different samples of black chokeberry juice varied from 9.8-10.8 TE g kg⁻¹ (by ABTS^{•+}) to 5.7-6.2 TE g kg⁻¹ (by DPPH[•]).

A strong correlation was found between the content of total phenolic compounds (including TT) and antioxidant activity by various assays (DPPH[•] and FRAP). Likewise, TP found a strong correlation between the TF (R = 0.940) and vitamin C (R = 0.546) and TANC (R = 0.607) content. Scientific studies have shown a strong correlation between total flavonoids and total phenols (R = 0.991) and between total flavonoids and antioxidant activity by the DPPH[•] assay (R = 0.997) and the ABTS^{•+} assay (R = 0.979) in black chokeberry fruit (Floegel et al., 2011).

3.2 Change of chemical parameters and antioxidant activity between black chokeberry solid product samples

To evaluate the chemical composition and antiradical activity of the products developed as a result of black chokeberry waste-free technology, we performed a comparison with the indicators of fresh berries. Statistical data indicated that in all products there was a significant difference between the determined chemical composition indicators and antiradical activity ($p < 0.00$). In comparison to fresh berries, frozen berries showed a small decrease in vitamin C (6.14%), but greater changes in total polyphenols and total proanthocyanidins, as well as a decrease in antioxidant activity (DPPH[•], ABTS^{•+}).

Sadowska et al. (2017) studied the effect of freezing on the black chokeberry and black elderberry total monomeric anthocyanins, vitamin C content, and antioxidant capacity in comparison with fresh berries. They found losses of vitamin C in the black chokeberry and black elderberry (accordingly 9% and 7%) and loss of anthocyanins (accordingly 21% and 2%), which are larger than in our study (Sadowska et al., 2017). The remarkable loss of total phenolic content (31% to 38%) in frozen black chokeberry (*Prunus virginiana* L.) could be caused by dripping during thawing and activation of flavonoid degrading enzymes (Télez-Pérez et al., 2020). On the other hand, convective drying of black chokeberry resulted in a significant loss (43-80%) of anthocyanins because they are very sensitive to temperature and to the presence of oxygen (Samoticha et al., 2016).

Compared to frozen berries, most of the chemical parameters (total phenolic, flavonoids, total proanthocyanidins) in the pomace after juice pressing from frozen berries have increased by 33.3%, 30.5%, and 65.4%, respectively. In turn, total titratable acidity and antiradical activity with ABTS^{•+} decreased (respectively, 38.1%, 47.5%). Red berries, especially black chokeberry, contain large amounts of anthocyanins and polyphenols.

After pressing the juice, the pulp and seeds remain, and with them are parts of antioxidant fractions. These include polyphenolic compounds, such as proanthocyanidins, which are distributed in the berries: 70% in the flesh, 25% in the peel, and 5% in the seeds. Therefore, the total phenol and proanthocyanidin content of black chokeberry pomace is higher than in berries (Struck et al., 2016; Mayer-Miebach et al., 2012).

3.3 The content of chemical compounds and antiradical activity in the liquid product samples

Table 2 summarizes the data on black chokeberry juice (fresh and frozen) and its processing products (extracts and syrups) chemical parameters and antioxidant activity expressed on FW. The soluble solids

content of fresh black chokeberry juice was 18.0 °Brix, while in frozen berry juice, it was 17.8 °Brix (data not shown). Similar and higher values were obtained according to the study by Tolić et al. (2017) for the SSC ranging from 18.15 °Brix to 25.61 °Brix of black chokeberry juices from different growing seasons. Tolić et al. (2015) results indicated that the SSC in the black chokeberry juices purchased on the Croatia market varied from 13.42 °Brix to 21.54 °Brix and the total acid content from 0.29% to 1.32%. In our study, berry juice was found to be more acidic, 1.52% of total acids in fresh berries and 1.35% in frozen ones were determined. The chemical composition of the berries may be related to the time of harvest and the climatic conditions of the growing place.

Table 2. The content of titratable acidity, vitamin C, total anthocyanin, total flavonoid, total phenolics, total proanthocyanidin, DPPH[•], ABTS^{•+}, FRAP in the chokeberries processing products (Fresh weight).

Chemical parameters	Juice from fresh berries	Juice from frozen berries	Juice with activated carbon	Water extract from pomace	Water extract from pomace with activated carbon	Syrup from dried sweetened pomace	Syrup from dried sweetened pomace with ascorbic acid
Titratable Acidity, (%)	1.52 ± 0.01 ^e	1.35 ± 0.02 ^f	1.09 ± 0.02 ^e	0.35 ± 0.02 ^b	0.28 ± 0.05 ^a	0.55 ± 0.02 ^c	0.69 ± 0.01 ^d
Vitamin C, mg/100 g	20.04 ± 0.56 ^e	16.20 ± 0.40 ^b	16.10 ± 0.90 ^b	6.65 ± 0.39 ^a	5.00 ± 0.30 ^a	21.83 ± 1.40 ^e	82.46 ± 1.62 ^d
Total anthocyanin content, mg/100 g	272.58 ± 10.36 ^e	219.81 ± 1.41 ^f	109.74 ± 0.15 ^c	54.85 ± 0.09 ^b	15.94 ± 0.34 ^a	65.24 ± 0.99 ^c	75.11 ± 0.58 ^d
Total flavonoid, CE mg/100 g	207.09 ± 10.79 ^c	248.08 ± 1.93 ^f	163.46 ± 1.92 ^d	86.54 ± 0.00 ^c	67.31 ± 1.93 ^b	55.25 ± 0.85 ^a	57.56 ± 0.85 ^a
Total phenolics, GAE mg/100 g	857.21 ± 2.85 ^e	787.64 ± 2.30 ^f	507.49 ± 16.10 ^d	160.74 ± 1.15 ^b	118.83 ± 4.02 ^a	377.20 ± 17.27 ^c	557.91 ± 24.16 ^c
Total proanthocyanidin, CE mg/100 g	234.25 ± 10.97 ^{cd}	464.06 ± 6.92 ^e	290.78 ± 9.68 ^d	119.17 ± 11.19 ^b	65.40 ± 3.83 ^a	149.52 ± 12.85 ^b	230.40 ± 5.60 ^c
DPPH [•] TE milli M /100 g	2.85 ± 0.08 ^c	2.25 ± 0.05 ^b	1.78 ± 0.05 ^{ab}	0.76 ± 0.08 ^a	0.66 ± 0.05 ^a	1.13 ± 0.04 ^a	1.84 ± 0.05 ^c
ABTS ^{•+} , TE Mol/100 g	1.40 ± 0.09 ^d	1.03 ± 0.05 ^c	0.85 ± 0.04 ^b	0.58 ± 0.03 ^a	0.42 ± 0.02 ^a	0.48 ± 0.01 ^a	0.64 ± 0.04 ^b
FRAP, TE milli M /100 g	2.64 ± 0.06 ^c	1.83 ± 0.03 ^c	1.21 ± 0.06 ^b	1.11 ± 0.05 ^a	0.65 ± 0.04 ^a	1.28 ± 0.06 ^a	1.48 ± 0.05 ^d

Results are the mean values ± standard deviation from four measurements (n = 4); means in the same row with superscript with different letters (a, b, c, d, e, and f) are significantly different at $p < 0.05$.

The vitamin C content of the black chokeberry juice samples after treatment with AC did not differ significantly (fresh berries juice -FREJ 16.2 mg 100 g⁻¹ FW and juice with activated carbon -JAC 16.1 mg 100 g⁻¹ FW). Among all products, the highest content of vitamin C was determined in syrup samples: in the sample with added ascorbic acid 82.46 mg 100 g⁻¹ FW, respectively, without additives – 21.83 mg 100 g⁻¹ FW. Significantly lower amount of vitamin C is also found in aqueous extracts (6.65 mg 100 g⁻¹ FW). The total anthocyanin content of fresh black chokeberry berry juice was 272.58 mg 100 g⁻¹ FW. During processing, the content of anthocyanins in the developed products decreased up to 10 times (in aqueous extracts) and was mostly influenced by factors such as high temperature, presence of oxygen, light, and ambient pH (Kapci et al., 2013). It was reported that in 11 analyzed commercial black chokeberry juice samples total anthocyanin content varied from 154 ± 6 to 1228 mg CGE 100 g⁻¹ FW (Tolić et al., 2015). In addition, a study by Denev et al. (2018) found that increasing the extraction temperature (from 20 °C to 80 °C) led to a gradual increase in both anthocyanin and polyphenol contents of the obtained juices and nectars.

The highest number of total phenols, flavonoids, and proanthocyanidins were found in fresh berry juice (respectively, 857.21 GAE mg 100 g⁻¹, 207.09 CE mg 100 g⁻¹ and 234.25 GAE mg 100 g⁻¹). Studies have shown that the content of proanthocyanidins in black chokeberry juice can vary from 60.0 to 392.6 mg 100 ml⁻¹ (Borowska & Brzóska, 2016). The results of our study indicated that the content of total phenols and flavonoids in black chokeberry syrup samples is 2 to 3 times lower than in juice, but it is still a significant amount. Similar results are reported in the literature, in black chokeberry syrup the amount of total phenol (2.6 GAE g kg⁻¹), total flavonoid (1.0 CE g kg⁻¹), and anthocyanins content (0.1 g kg⁻¹, expressed as grams of cy-3-Glu) was indicated (Kapci et al., 2013).

By analysing the antiradical activity, it was concluded that the results for liquid products with DPPH• was as follows: fresh juice > juice from frozen berries > syrup with ascorbic acid > juice with AC > syrup without additives > pomace water extract > and pomace water extract with AC. Scientific literature reports that the antiradical activity of black chokeberry syrup with ABTS^{•+} and DPPH• was accordingly 3.7 TE g kg⁻¹ and 2.2 TE g kg⁻¹ (Kapci et al., 2013). When studying extracts, it was found that the antioxidant activity of black chokeberry pomace water extract by ABTS^{•+} assay was 1.44 ± 3.90 mmol TE g⁻¹ extract, and DPPH• assay - 0.55 ± 1.51 mmol TE g⁻¹ extract g (Grunovaitė et al., 2016).

The content of hydrophilic compounds in black chokeberry processing products showed several strong positive correlations. Total phenolic was highly correlated with chemical parameters TT, TF, TANC, TTA and DPPH•, ABTS^{•+}, FRAP (R = 0.816, R = 0.774, R = 0.920, R = 0.948, R = 0.980, R = 0.872, R = 0.899). A strong correlation was observed between the antioxidant activity and total polyphenolic content, total flavonoids and total anthocyanins in black chokeberry products (including juice, dried berries and others) available in Croatian markets (Tolić et al., 2015). Nevertheless, to fully understand the effect of processing, research focused on different processing techniques should be done using the same material.

3.4 Change of chemical parameters and antioxidant activity between black chokeberry liquid product samples

The changes in the comparison of chemical parameters and antioxidant activity depending on the treatment technology: the addition of AC to reduce proanthocyanidins in the product and the addition of ascorbic acid to improve the sensory properties of DSP were evaluated. A comparison was made between 1) juices: fresh, frozen and juice with added AC; 2) extracts: water extract and water extract with added AC; 3) syrups: syrup and syrup with added ascorbic acid. The largest changes in the anthocyanin content were found in berry juice, after treatment with AC, anthocyanin content was less than half (from 219.81 to 109.74 mg 100 g⁻¹), but after freezing, the anthocyanin content decreased by 19.36%. Brownmiller et al. (2008) indicated that blueberries can be stored frozen, but anthocyanins and antioxidants are degraded in such storage conditions. In non-clarified juice from frozen blueberries an extensive loss of anthocyanins (28-59%) and antioxidant activity as antioxidant oxygen radical absorbing capacity (43-71%) were determined after pasteurization (Brownmiller et al., 2008).

Other studies have also reported that pre-treatment of black chokeberry by freezing and thawing before juice extraction adversely affected the polyphenol content of the juice. Kobus et al. (2019) found that in a twin gear press it caused a decrease in the total phenolic content by 28% and in a basket press by 22%. Compared to frozen black chokeberry juice, the treatment of AC showed a reduction of all indicators in the range from 0.7% (vitamin C) to 50.1% (TANC). A remarkable reduction of proanthocyanidins (37.3%) and total phenolics (35.6%) was observed. However, it should be noted that reducing the content of proanthocyanidins in the juice reduces the content of anthocyanins, which play important roles for human health. Therefore, this must be considered in the development of new products.

Gunathilake et al. (2018) used AC filters to reduce the bitterness of lime fruit juice. Several indicators were reduced after filtration: tannin content (bitterness), sugar, acid content, including other phenolic compounds (6.13 to 4.7 μ GAE or 26.6%) and DPPH antioxidant activity by 80%–43.86% (Gunathilake et al., 2018). Prommajak et al. (2020) described the negative effects of used agents: many clarifying compounds not only reduce the tannin content but also reduce other useful compound contents in the juice. Several tested clarifiers (casein + bentonite, casein + bentonite + AC, casein + bentonite + polyvinylpolypyrrolidone) caused a significant decrease in the concentration of polyphenolic compounds (Barón et al., 1997). Treatment with AC (using 2.0-3.0 g L⁻¹) reduced the content of phenolic compounds by 20-30% in bush cranberry juice (Velioglu et al., 2006). AC can cause changes in chemical compound concentration due to a high degree of porosity and, consequently, an extended internal surface area, along with physical and chemical adsorption properties. Carbonaceous materials are carbonized after their impregnation with chemical compounds from products during processing (Rimoli et al., 2019). Comparing the syrups formed

during DSP production, we concluded that the ascorbic acid added during the technological process has had a positive effect. The changes indicated that the syrup with ascorbic acid has increased values: total acid, vitamin C, total anthocyanin, total phenol content and antioxidant activity with DPPH[•] and ABTS^{•+} assay (by 13.13% to 73.5%). The presence of acid is required for improved anthocyanin extractability as it has been known the use of acid stabilizes anthocyanins in the flavylum cation form, which is red at low pH (Kapci et al., 2013).

3.5 Sensory evaluation

Sensory properties, especially those with functional properties, are crucial for consumers to make food choices. The sensory attributes (appearance (incl. colour), taste, sweetness, aftertaste and astringency) of black chokeberry pasteurized juice and juice with AC were evaluated (Figure 2). The results indicated that there was a difference of opinion between the groups of panelists involved: trained experts from the Institute of Horticulture (IH) and students from Dobele State Gymnasium (DSG). When evaluating black chokeberry juice samples (without and with AC), significant differences in appearance ($p < 0.001$), sweetness ($p = 0.007$), astringency ($p = 0.013$) and aftertaste ($p = 0.003$) were found between the groups of panelists, but no significant differences were found in taste ($p = 0.053$). Trained experts rated the appearance of the juices with a higher score (respectively, raw juice with 5.5 and AC with 8.2 points), while students rated much lower (4.1 and 6.5 points, respectively).

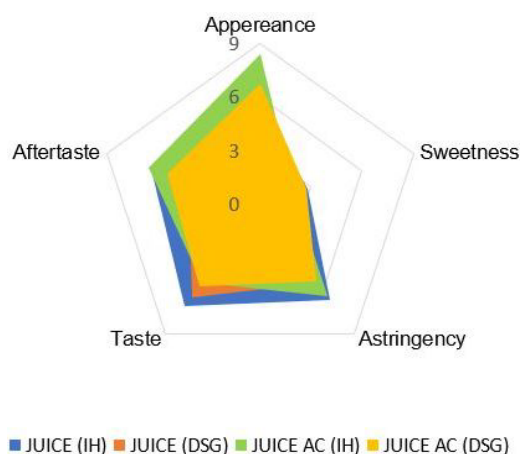


Figure 2. Evaluation of the intensity of sensory attributes of black chokeberries pasteurized juice. JUICE- pasteurized juice; JUICE AC- pasteurized juice with activated carbon; IH- trained panelist; DSG-students.

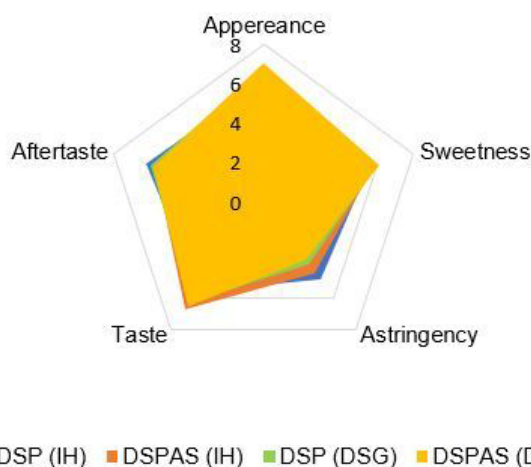


Figure 3. Evaluation of the intensity of sensory attributes of black chokeberries dried sweetened pomace. DSP-dried sweetened pomace; DSPAS-dried sweetened pomace with ascorbic acid; IH-trained panelist; DSG-students.

The panelists had different views on the appearance and taste of the juices. Some experts have said that they like to use raw juice daily, because they like the appearance-saturated colour. It had a good taste and aftertaste, although the bitterness hindered the enjoyment of the product. In turn, some experts have positively evaluated the juice after treatment with AC, indicating that they like the mild aftertaste and appearance of the juice (it is not as dark). Scientific studies have been carried out to improve the sensory properties of black chokeberry juice, its taste and to reduce its bitterness. Gunathilake et al. (2018) studied the bitterness reduction of lime juice and concluded that using filtration with AC minimizes the incidence of delayed bitterness. Duffy et al. (2016) tested the effects of additives on the sweetness and preference of black chokeberry juice to improve the sensory profile acceptability of the juice. They found that the addition of 5% sucrose increased sweetness and shifted weak disliking to above weak liking across all participants. Adding sweet olfactory flavouring, ethyl butyrate, with 3% sucrose maintained the level of liking to nearly that of the 5% added sucrose (Duffy et al., 2016). Opinions on the astringency of dried sweetened black chokeberry pomace differed significantly ($p = 0.039$) between groups of panelists (Figure 3). However, no significant difference was found between the other characteristic indicators: appearance ($p = 0.631$), sweetness ($p = 0.891$), taste ($p = 0.236$) and aftertaste ($p = 0.851$). The DSP evaluation was very similar with slight deviations. Students rated the appearance and sweetness of DSP without and with ascorbic acid additive samples rated by 6.9 points on average, sweetness (respectively, 6.0 and 6.1), but the taste was similar to trained experts' opinion (respectively, 6.3 and 6.6).

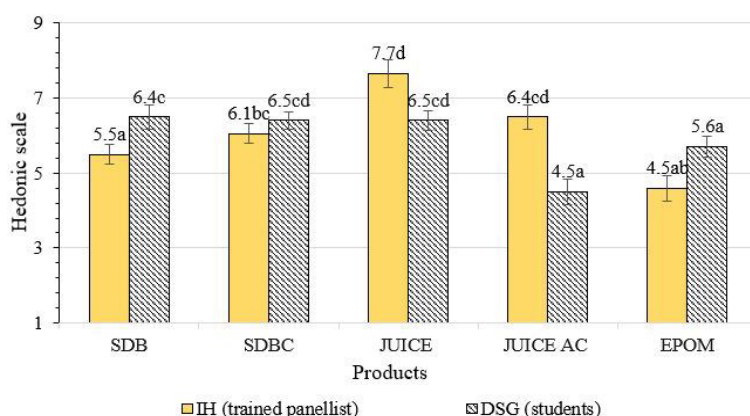


Figure 4. Hedonic evaluation of black chokeberries products. SDB-dried sweetened pomace; SDBC-dried sweetened pomace with ascorbic acid; JUICE- pasteurized juice; JUICE AC- pasteurized juice with activated carbon; EPOM-water extract from pomace. Values in a figure marked with the same letter are not significantly different ($p > 0.05$).

Figure 4 shows the results of products samples evaluation using a 9-point hedonic scale. In general, it can be concluded that the effect of AC treatment on the degree of liking of juice was significant ($p = 0.001$) between both panelist groups. Trained experts rated natural juice higher (6.5), while juice treated with AC was rated higher (7.7). On the other hand, the students rated juice with AC much lower than juice with AC rated by trained experts (4.6), but the natural juice had a similar rating. Some panelists commented that natural juice is better, has a stronger taste, and has the characteristic taste of black chokeberry berries, including astringency. To find out the possibilities of using black chokeberry waste-free technology, the panelists were offered to express their opinion on the taste properties of the pomace water extract. Interestingly, students rated water extract higher (5.7) than trained experts (4.6). In their comments, the young people indicated that the pomace water extract could be the best drink for a hot summer day. Ascorbic acid addition during the preparation of black chokeberry DSP did not significantly affect the degree of liking for DSP ($p = 0.13$). The evaluation concluded that young people rated black chokeberry DSP slightly higher (without ascorbic acid 6.5 and with ascorbic acid 6.4), but the trained panelists were evaluated lower (5.5 and 6.2, respectively). DSP were made from black chokeberry pomace and in the technological process dipped in syrup, seeing that part of the biologically active compounds (including proanthocyanidin) was transferred

to the syrup. Thus, the taste of candied fruit became more pleasant and less astringent, and the amount of ascorbic acid added did not significantly affect the taste. However, some experts noted that DSP with the addition of ascorbic acid is tastier. The taste, including sweet and bitter perception declines with age (Mennella & Bobowski, 2015). Our study found that, compared to young people, adult panelists liked the bitter taste of black chokeberry juice better. In literature, the results of individual studies regarding the association between taste perception qualities and age have been heterogeneous (Barragán et al., 2018).

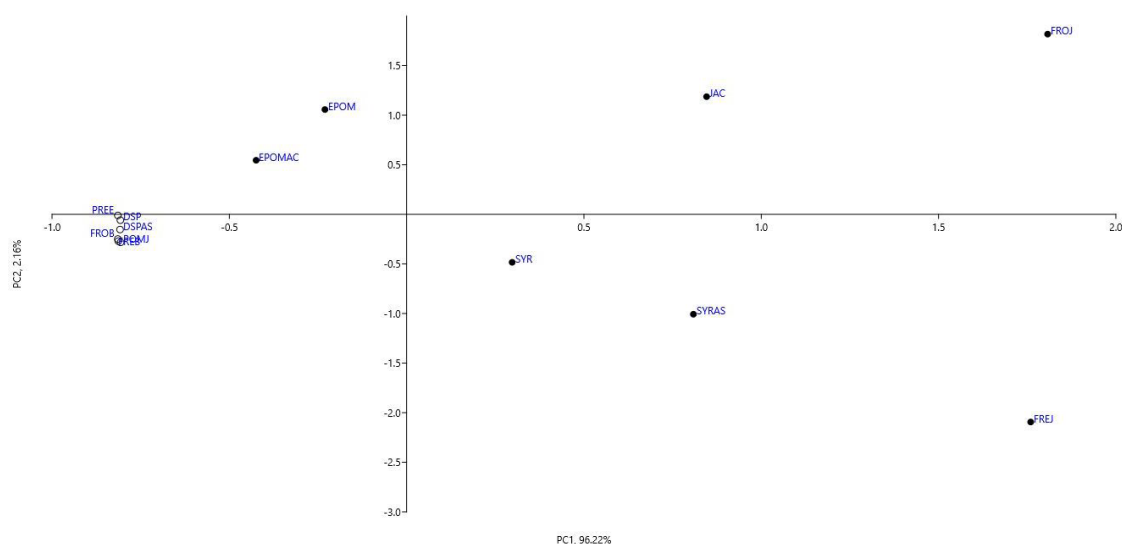


Figure 5. Principal Component Analysis (PCA) of the chemical parameters in dry and liquid black chokeberry products. The vectors indicate the distribution of chemical parameters by the product type. ● – liquid black chokeberry products (expressed on FW); ○ – solid black chokeberry products (expressed on DW).

The new product developed in the study, dried sweetened black chokeberry candies from pomace, can economically contribute to the food industry, especially for small processing companies. Based on the research, other natural acidulants can also be used industrially to improve the taste of candies. Acidic fruit and vegetable juices, such as lemon, lime, rhubarb, pomegranate, etc. will not only help enhance the taste of products but also enrich them with biologically active substances in the raw materials. Similarly, using chokeberry pomace extract in the production of beverages and adding various other berries can improve their taste and value, offering consumers refreshing products.

3.6 Principal component analysis

Raw data of black chokeberry processing products were subjected to PCA, including nine characteristic features and 13 products of two main processing approaches: solid and liquid. PCA resulted in one compact group of products representing the solid products of black chokeberry, which, considering the average values of chemical indicators, are similar in all analysed parameters (Figure 5). The first two factors of PC1 (represented mainly by total anthocyanins and flavonoid content in the samples) describe 98.38% of the total variability, indicating a high representativeness of the PCA results. The following most important parameters that determine the value of the first component are the content of total phenols and proanthocyanidins. A positive correlation was observed as these values increased. The most important indicators of PC2 (representing 2.16% of total variability), characterised by a positive correlation, are the total content of flavonoids and proanthocyanidins. On the other hand, a negative correlation was determined for sample vitamin C content, total phenolics and anthocyanins. PCA shows a significant difference between solid and liquid black chokeberry products. In liquid products (juices, extracts, syrups), significant differences between the results of chemical indicators are determined, which is reflected in PCA in a scattered way.

4 Conclusions

The current study presents comprehensive data on chemical composition and antiradical activity of various developed black chokeberry products using waste-free technology. In comparison with frozen berries, the pomace after juice pressing had a higher content of vitamin C, total phenols, flavonoids and anthocyanidins. The juice treatment with AC showed a reduction of all indicators in the range from 0.7% (vitamin C) to 50.1% (TANC). The remarkable reduction of proanthocyanidins (37.3%) and total phenolics (35.6%) was also observed. The PCA showed that the juice treatment methods did not influence the ranking of solid products of black chokeberry, which, considering the average values of chemical indicators, were similar in all analysed parameters. In accordance with PCA, the most important parameters that determine the value of the first component are total anthocyanins and total flavonoids. Dried sweetened pomaces with ascorbic acid were evaluated between “like slightly” and “like moderately”, and there were no significant differences between groups of panelists of different ages. 100 g of dried sweetened black chokeberry pomace with ascorbic acid contains vitamin C, providing on average 75% of the required daily intake. In general, the results of the study indicate that black chokeberries are a suitable raw material for the use of waste-free technology and the development of functional products.

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