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## CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF BERRY FRUITS FROM *ARONIA MELANOCARPA*, *PRUNUS SPINOSA*, *SAMBUCUS NIGRA* AND *RUBUS FRUTICOSUS*

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**Background.** Berries are a valuable source of minerals, vitamins, and phytochemicals in human nutrition. Due to the growing demand for organic food products enriched with biologically active compounds (BACs), there is a need to assess the levels of these substances in berries of various plant species and to develop optimal methods for processing raw berry materials that preserve their biological value.

The aim of this study was to investigate the content of BACs (total phenolic compounds, flavonoids, anthocyanins, and ascorbic acid) and the antioxidant capacity of fresh and dried berry extracts and berry juices from bush plants distributed in the territory of Ukraine (chokeberry, blackthorn, elderberry, and blackberry).

**Materials and Methods.** The study was conducted using berry fruits of *Aronia melanocarpa* (Michx.) Elliott (chokeberry), *Prunus spinosa* L. (blackthorn), *Sambucus nigra* L. (elderberry), and *Rubus fruticosus* L. (blackberry) growing in natural conditions in the Lviv region. The collecting of plant material, its preparation for analysis, as well as drying of berries, obtaining and pasteurization of berry juices were carried out following conventional methods. Berry extracts were prepared by 90 % ethanol extraction of fresh berries and water-ethanol extraction (in a 1:1 (v/v) ratio) of dry berries. The total content of phenolic compounds and the concentrations of anthocyanins, flavonoids, and ascorbic acid (vitamin C) were determined using generally accepted spectrophotometric



methods. The antioxidant capacity of plant materials was assessed by spectrophotometric analysis using the stable free radical DPPH (1,1-diphenyl-2-picrylhydrazyl). Statistical processing the obtained results was performed using a two-way ANOVA method.

**Results.** Extracts of fresh berries from the studied plant species contained higher concentrations of total phenolic compounds, flavonoids, anthocyanins, and ascorbic acid than berry juices and extracts of dried berries, with the highest content of biologically active substances noted in fresh fruits from *A. melanocarpa*. In particular, chokeberry fruits contained phenolic compounds, anthocyanins, and flavonoids in concentrations of up to 1204 mg GAE (gallic acid equivalents), 643 mg C3GE (cyanidin-3-glucoside equivalents), and 490 mg of QE (quercetin equivalents) per 100 g of sample wet weight, respectively. Pasteurized berry juices had lower concentrations of the indicated compounds than fresh berry extracts, whereas dried berry extracts tended to contain higher concentrations of the mentioned biologically active substances compared to berry juices. Among the plant materials studied, the highest antioxidant capacity measured by the DPPH radical absorption method was found in extracts of fresh chokeberries (93 %), whereas fresh berries of *P. spinosa*, *S. nigra*, and *R. fruticosus* plants showed significantly lower antioxidant potential (83 %, 85 %, and 82 %, respectively). For most of the analyzed parameters (except for the concentration of ascorbic acid), a stable pattern of preservation of biologically active substances in plant materials was observed in descending order: fresh berry extract > dried berry extract > berry juice, which confirmed the advantage of the extraction method compared to direct pressing and juicing for obtaining high levels of biologically active compounds in berry products. At the same time, the obtained results show that both the species characteristics of the berries and the method of their treatment significantly determine the antioxidant profile of berry products.

**Conclusions.** Analysis of the concentrations of total phenolic compounds, flavonoids, anthocyanins and ascorbic acid found in the berries of *A. melanocarpa*, *P. spinosa*, *S. nigra*, and *R. fruticosus* suggests that the fruits of these species are promising sources of natural antioxidants in human nutrition. However, the berry treatment regime significantly affects the content of biologically active substances in berry juices and extracts. The obtained results can be applied in the food industry for the development of functional products with improved antioxidant properties.

**Keywords:** berries, biologically active compounds, antioxidant capacity, phenolic compounds, flavonoids, anthocyanins, functional foods

## INTRODUCTION

Modern trends in nutrition science are increasingly focused on the study and use of functional foods, which can provide the human body not only with essential nutrients, but also with biologically active compounds (BACs) that have additional health-promoting effect (Granato *et al.*, 2020; Oboh *et al.*, 2021; Murugan, 2024). The demand for organic food products enriched with BACs is constantly growing, which actualizes research into natural sources of these substances and methods of their preservation in food products during the processing of raw materials (Granato *et al.*, 2020).

Among the main sources of natural biologically active substances, berry fruits attract special attention, representing a rich source of vitamins, minerals, and phytochemicals such as various groups of phenolic compounds (Häkkinen *et al.*, 1999a,b; Snitynski *et al.*, 1999; Sheng *et al.*, 2009; Zorzi *et al.*, 2020; Djordjević, 2023; Murugan, 2024;

Pedisić *et al.*, 2025). The content and composition of phenolic compounds in berries are currently the subject of active research due to biological activities and potential health benefits of these substances. In particular, this applies to berry fruits of shrub plants belonging to the genera *Aronia* Medik., *Prunus* L., *Sambucus* L., and *Rubus* L., which are widely distributed and cultivated in many countries, including Ukraine (Kim *et al.*, 2021; Merecz-Sadowska *et al.*, 2024; Nisar *et al.*, 2025).

Numerous studies have shown a strong positive correlation between the content of phenolic compounds and the antioxidant activity of berries, as well as of their processed products (Nowak *et al.*, 2018; Zorzi *et al.*, 2020; Kim *et al.*, 2021; Huang *et al.*, 2022; Li *et al.*, 2022; Dobros *et al.*, 2024). In addition, plant phenolic compounds are known to have anti-inflammatory, antimicrobial, cardioprotective, immunomodulatory and potential anticancer effects (Suriyaprom *et al.*, 2022; Djordjević, 2023; Merecz-Sadowska *et al.*, 2024; Stachelska *et al.*, 2025).

Currently, there is growing interest in studying the chemical composition, and antioxidant potential of berries from plants such as *Aronia melanocarpa* (Michx.) Elliott, *Prunus spinosa* L., *Sambucus nigra* L., and *Rubus fruticosus* L. as valuable natural sources of biologically active substances in human nutrition (Liu *et al.*, 2022; Marčetić *et al.*, 2022; Osman *et al.*, 2023; Negrean *et al.*, 2023; Dobros *et al.*, 2024; Nisar *et al.*, 2025). These plant species have a long history of use in folk medicine in European and other countries, and their fruits, juices and extracts are increasingly used in the production of food products (yoghurts, beverages, preserves, jams), food pigments and as part of biologically active food supplements (Kiselova-Kaneva *et al.*, 2022; Nistor *et al.*, 2023; Aguilera & Toledo, 2024).

The presence of different classes of phenolic compounds (flavonoids, anthocyanins, phenolic acids, etc.), which can neutralize free radicals and reduce oxidative stress, thereby preventing the development of chronic diseases, makes the berries valuable objects for scientific research in the fields of nutrigenetics, pharmacology, and nutritional medicine (Zhang *et al.*, 2021; Marčetić *et al.*, 2022; Negrean *et al.*, 2023; Murugan, 2024). Despite numerous research works in this area, the nutritional value and biologically active substances of the fruits of the above-mentioned plant species have not been completely studied. However, the berry fruits from all four mentioned plant species are known to be rich in anthocyanins, which provide the dark color of ripe berries and possess various biological effects (He & Giusti, 2010; Ma *et al.*, 2025). Therefore, it is reasonable to conduct a comparative study of the BAC contents in berries from the mentioned plant species, taking into account their biochemical traits as a criterion. It should also be noted that these berries still remain underutilized in the food industry, which is largely due to the technological difficulties of berry harvesting and processing (Li *et al.*, 2017; Wang *et al.*, 2024).

It has been shown that inappropriate extraction methods, heat treatment, fermentation processes and other types of fruit processing can significantly affect the stability and antioxidant potency of phenolic compounds, vitamin C and other biologically active substances in plant materials (Li *et al.*, 2017; Martinsen *et al.*, 2020; Zia & Alibas, 2021; Mandha *et al.*, 2023; Uribe *et al.*, 2024; Thanasegaran & Mahrar, 2025). At the same time, phenolic compounds in harvested berries are known to be susceptible to oxidation reactions due to their high water content, which may reduce their health benefits (Uribe *et al.*, 2024). Therefore, in the manufacture of functional food products, the use of optimal processing methods that maximally preserve the biological value and nutraceutical properties of plant raw materials is of great importance.

The aim of this study was to investigate the antioxidant capacity and content of biologically active compounds (total phenolic compounds, flavonoids, anthocyanins, and ascorbic acid) in extracts of fresh and dried berries and berry juices from *A. melanocarpa*, *P. spinosa*, *S. nigra*, and *R. fruticosus*.

## MATERIALS AND METHODS

The studies were conducted in 2023–2024 using berry fruits of plants *Aronia melanocarpa* (Michx.) Elliott (chokeberry), *Prunus spinosa* L. (blackthorn), *Sambucus nigra* L. (elderberry), and *Rubus fruticosus* L. (blackberry) growing in natural conditions in the Lviv region of Ukraine. Biochemical studies were conducted in the laboratory of the Department of Ecology at the Lviv National Environmental University and in the Laboratory of Product Quality Control of the Department of Hotel and Restaurant Business and Food Technology at the Ivan Franko National University of Lviv.

The material was obtained from agrarian farms located in the Lviv region. The collecting of plant material and its preparation for analysis were carried out in accordance with conventional methods (State Committee for Technical Regulation and Consumer Policy of Ukraine, 2002, 2004, 2010; SE “UkrNDNC”, 2015). Fresh and ripe berries were picked by hand, cleaned of debris, sorted, and washed. The berries were then placed on trays to drain excess water and stored in a refrigerator at 4 °C until use.

The objects of analysis were extracts of fresh and dried berries obtained by the method of 90 % ethanol and 1:1 (v/v) water-ethanol extraction, respectively, and berry juices.

Spectrophotometric measurements were carried out using a SpectroLab P-1 spectrophotometer (Ukraine) with a wavelength range of 195–1050 nm (an error range is  $\pm 0.5$  nm). All chemical reagents and standards (gallic acid, quercetin, cyanidin-3-glucoside, ascorbic acid) were purchased from Sigma-Aldrich (USA).

**Obtaining fresh berry extracts.** A 50 g sample of fresh berry fruits of each plant species was homogenized in a laboratory blender, then 50 mL of 90 % ethanol was added to 2 g of the fruit mass and mixed for 15 minutes. The mixtures were then centrifuged (6000 g) and the supernatants were used to analyze the content of total phenolic compounds, flavonoids, anthocyanins, and ascorbic acid, as well as to determine antioxidant capacity. Before analysis, additional filtration of the supernatant through membrane filters (0.45  $\mu\text{m}$ ) was used.

**Obtaining dried berry extracts.** Fresh berries were dried in a microwave oven at 50–60 °C until the moisture content reached less than 20 % (Zia & Alibas, 2021). Before extraction, dried berries were crushed to particles smaller than 0.5 mm. Extracts were obtained by infusing 0.5 g of crushed berries in 50 mL of a water-ethanol solution (1:1 (v/v)) at 60 °C for 2 h and at room temperature for 24 h. A water-ethanol solution in a ratio of 1:1 was used in order to obtain a high yield of phenolic compounds from dried berry material (Pudžiuvėlytė & Mačiulskaitė, 2025).

After extraction, samples were filtered through a paper filter (Whatman paper No. 1) and stored at 4 °C until further analysis.

**Obtaining berry juices.** Juices from chokeberry, blackthorn, elderberry, and blackberry fruits were obtained by mechanical pressing of crushed homogeneous pulp in accordance with the requirements of DSTU 7159:2010 (State Committee for Technical Regulation and Consumer Policy of Ukraine, 2010). The obtained juices were clarified

by coarse filtration and pasteurized at a temperature of  $(85 \pm 2) ^\circ\text{C}$  for 5 min. Samples were stored under sterile conditions until analysis. To ensure representativeness, the juices were analyzed after centrifugation and membrane filtration ( $0.45 \mu\text{m}$ ), which facilitated the unification of the corresponding parameters with the supernatants of centrifuged berry extracts.

**Analysis of total phenolic compounds concentration.** The content of total phenolic compounds in the analyzed plant materials was determined by the Folin-Ciocalteu method based on the oxidation of phenols to form a blue-coloured complex, which was analyzed spectrophotometrically at a wavelength of 760 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of sample wet weight, using a gallic acid calibration curve. The calibration curve was constructed using gallic acid solutions with a concentration of 50–500 mg/L (Singleton *et al.*, 1999; Mirdehghan & Rahemi, 2007).

**Determination of total flavonoid content.** To determine the total content of flavonoids (flavonols and other groups of these compounds), the complexation method with aluminum chloride ( $\text{AlCl}_3$ ) was used (Zhishen *et al.*, 1999). The method is based on the formation of a stable flavonoid- $\text{AlCl}_3$  complex with an absorption maximum at  $\lambda = 415 \text{ nm}$ . The results were expressed as milligrams of quercetin equivalents (QE) per 100 g of sample wet weight.

**Determination of anthocyanin content.** Total anthocyanin content was determined by UV-visible spectrophotometry at  $\lambda = 520 \text{ nm}$  in acidic medium (KCl/HCl buffer, pH 1.0) according to the method developed by Giusti and Wrolstad (2001). This method allows for an accurate assessment of the anthocyanin concentration in berry extracts. The concentration of anthocyanins in the samples was calculated using cyanidin-3-glucoside (C3G) as a reference and taking into account the molar extinction coefficient of C3G (449.2 g/mol). The concentration of anthocyanins was expressed as milligrams of cyanidin-3-glucoside equivalents (C3GE) per 100 g of sample wet weight.

**Determination of ascorbic acid concentration.** The content of ascorbic acid (vitamin C) was determined using UV spectrophotometry at a wavelength of 243 nm, at which ascorbic acid exhibits maximum absorption. A calibration curve was constructed using ascorbic acid standard solutions (10–100 mg/L) (Giusti & Wrolstad, 2001). The obtained results were presented as milligrams of ascorbic acid per 100 g of sample wet weight.

**Analysis of antioxidant capacity of plant materials.** The antioxidant capacity of plant materials was assessed by spectrophotometric analysis using the stable free radical DPPH (1,1-diphenyl-2-picrylhydrazyl). The method is based on the ability of analyzed samples to neutralize the DPPH radical, which results in a decrease in absorption intensity at  $\lambda = 517 \text{ nm}$  (Mirdehghan & Rahemi, 2007).

**Data processing.** To assess the influence of berry species (factor A) and form of processing (factor B) on the studied parameters, a two-way ANOVA was used with the main effects and A×B interaction assessment. For comparisons between groups, a post hoc Tukey's HSD test was performed. The level of statistical significance was  $\alpha = 0.05$ . The results are presented as the mean (M)  $\pm$  standard error (SE); the sample size was 5–7 replicates for each group. In **Tables 1–3**, significantly different groups are indicated by different lowercase or uppercase letters (CLD diagram); the same letters indicate no significant differences according to Tukey's test ( $P \geq 0.05$ ).

## RESULTS AND DISCUSSION

Analysis of the chemical composition of berry materials from four plant species (*A. melanocarpa*, *P. spinosa*, *S. nigra* and *R. fruticosus*) revealed significant differences in the content of total phenolic compounds, flavonoids, anthocyanins and ascorbic acid, as well as in antioxidant capacity in different sample forms (namely, fresh and dried berry extracts and berry juices) (Tables 1–3).

As shown in Table 1, the berries of *A. melanocarpa* and *S. nigra* were characterized by high ascorbic acid content ( $51.57 \pm 5.86$  and  $47.71 \pm 4.31$  mg/100 g, respectively), while the concentration of ascorbic acid in *P. spinosa* and *R. fruticosus* berries was noticeably lower ( $34.14 \pm 3.85$  and  $24.86 \pm 2.67$  mg/100 g, respectively). This demonstrates the importance of chokeberry and elderberry fruits as a good source of vitamin C in human nutrition. At the same time, the lower content of ascorbic acid in the fruits of blackthorn and blackberry somewhat limits the role of *P. spinosa* and *R. fruticosus* fruits as potential sources of vitamin C for the human body.

According to the data obtained, the ascorbic acid concentrations were significantly lower in processed products of *A. melanocarpa*, *P. spinosa*, *S. nigra* and *R. fruticosus* berries, such as pasteurized juices and dried berry extracts (Table 1). For ascorbic acid concentration in berry materials, the following descending order was observed: fresh berry extracts > berry juice > dried berry extracts ( $P < 0.05$ ). This can be explained by the heat lability of ascorbic acid and its losses during the processing of plant raw materials (Martinsen et al., 2020; Zia & Alibas, 2021). The obtained results are consistent with the findings of other authors, who point to the adverse effects of technological processes, including drying of berries and juice pasteurization, on the concentration of heat-labile biologically active substances (such as vitamin C) in plant products (Zia & Alibas, 2021; Mandha et al., 2023; Thanasegaran & Mahrar, 2025).

Table 1. Ascorbic acid concentration (mg/100 g) in berries of *Aronia melanocarpa*, *Prunus spinosa*, *Sambucus nigra*, and *Rubus fruticosus* (M ± SE)

Analyzed material	<i>A. melanocarpa</i>	<i>P. spinosa</i>	<i>S. nigra</i>	<i>R. fruticosus</i>
Fresh berry extract	$51.57 \pm 5.86$ aA	$34.14 \pm 3.85$ aB	$47.71 \pm 4.31$ aA	$24.86 \pm 2.67$ aC
Berry juice	$37.29 \pm 4.35$ bA	$29.71 \pm 3.45$ bB	$39.71 \pm 3.55$ bA	$18.29 \pm 2.14$ bC
Dried berry extract	$35.14 \pm 3.93$ cA	$26.00 \pm 3.65$ cB	$36.71 \pm 3.55$ cA	$15.86 \pm 2.79$ cC

**Note:** Values in the same column denoted by the same lowercase letter, or in the same row denoted by the same uppercase letter, are not significantly different ( $P > 0.05$ ) according to Tukey's test

As it is known, vitamin C, despite its pronounced antioxidant properties, is inferior to polyphenolic compounds in providing the overall antioxidant activity of plant foods, including berries. Consequently, the rich profile of phenolic compounds in berry fruits noted by many authors (Marčetić et al., 2022; Djordjević, 2023; Negrean et al., 2023; Dobros et al., 2024; Nisar et al., 2025), primarily determines their antioxidant effectiveness. This particularly concerns flavonoids and anthocyanins, which are more effective than ascorbic acid in their capacity to neutralize free radicals and lipid peroxidation products due to their greater ability to donate electrons or hydrogen atoms (Prochazkova et al., 2011; Leong et al., 2017). On the other hand, ascorbic acid and polyphenols

often exhibit synergistic activity and together define the antioxidant properties of berries and other plant materials (Commisso *et al.*, 2017; Nowak *et al.*, 2018). Some polyphenols can stabilize vitamin C, enhancing its antioxidant effectiveness (Marin *et al.*, 2002); at the same time, ascorbic acid can protect polyphenol molecules from oxidative damage (Katz *et al.*, 2020; Xiao, 2022).

The results of this study regarding the content of phenolic compounds in the berries from *A. melanocarpa*, *P. spinosa*, *S. nigra* and *R. fruticosus* (**Table 2**) suggest their high bioactive potential. This is especially true for fresh chokeberries and blackthorn, which have the highest total phenolic content. The extracts of fresh berries of these two plant species do not differ significantly in the total content of phenolic compounds ( $1203.57 \pm 10.16$  and  $1150.00 \pm 9.16$  mg GAE/100 g, respectively). Elderberry and blackberry have significantly lower total phenolic content than the two berry species mentioned.

**Table 2. Content of total phenolic compounds, anthocyanins and flavonoids in berry materials from *Aronia melanocarpa*, *Prunus spinosa*, *Sambucus nigra*, and *Rubus fruticosus* (M  $\pm$  SE)**

Analyzed material	<i>A. melanocarpa</i>	<i>P. spinosa</i>	<i>S. nigra</i>	<i>R. fruticosus</i>
Total phenolic compounds (mg GAE/100 g)				
Fresh berry extract	1203.57 $\pm$ 10.16 aA	1150.00 $\pm$ 9.16 aA	655.71 $\pm$ 11.52 aB	478.57 $\pm$ 10.33 aC
Berry juice	798.57 $\pm$ 7.30 cA	750.71 $\pm$ 6.77 cB	225.00 $\pm$ 6.27 cC	182.86 $\pm$ 5.22 cD
Dried berry extract	895.57 $\pm$ 9.59 bA	850.86 $\pm$ 7.06 bB	440.71 $\pm$ 9.03 bC	407.86 $\pm$ 7.06 bC
Anthocyanins (mg C3GE/100 g)				
Fresh berry extract	643.00 $\pm$ 13.07 aA	423.30 $\pm$ 10.40 aB	480.00 $\pm$ 9.26 aB	325.90 $\pm$ 9.18 aC
Berry juice	364.65 $\pm$ 9.11 cA	240.71 $\pm$ 11.72 cB	302.86 $\pm$ 6.80 cB	150.86 $\pm$ 4.52 cC
Dried berry extract	550.86 $\pm$ 10.68 bA	320.00 $\pm$ 5.12 bB	417.14 $\pm$ 7.78 bB	222.86 $\pm$ 8.96 bC
Flavonoids (mg QE/100 g)				
Fresh berry extract	490.00 $\pm$ 11.13 aA	323.00 $\pm$ 9.76 aB	245.00 $\pm$ 6.27 aC	220.00 $\pm$ 6.17 aC
Berry juice	401.00 $\pm$ 7.55 cA	248.57 $\pm$ 10.16 cB	150.71 $\pm$ 8.76 cC	98.57 $\pm$ 5.20 cD
Dried berry extract	421.43 $\pm$ 9.86 bA	280.29 $\pm$ 5.71 bB	214.29 $\pm$ 6.12 bC	181.43 $\pm$ 5.95 bC

**Note:** Values in the same column denoted by the same lowercase letter, or in the same row denoted by the same uppercase letter, are not significantly different ( $P > 0.05$ ) according to Tukey's test

It should be noted that the qualitative composition of phenolic compounds varies among the berries of the studied plant species, influencing their biological effects. In particular, this concerns the presence and quantity of phenolic acids, which represent an important group of phenolic compounds. For instance, the fruits of *A. melanocarpa* are characterized by a high content of chlorogenic and neochlorogenic acids (Zielińska *et al.*, 2020), elderberries contain high levels of caffeic and ferulic acids (Osman *et al.*, 2023), while blackberries are rich source of ellagic acid, which has pronounced antioxidant and health-promoting properties (Al-Mugdadi *et al.*, 2019; Wang *et al.*, 2023;

Rahmani *et al.*, 2025; Settakorn *et al.*, 2025). Among the phenolic acids contained in blackthorn berries, 3-caffeoylquinic and 3-*p*-coumaroylquinic acids were found to be the most abundant (Mikulic-Petkovsek *et al.* 2015; Kiselova-Kaneva *et al.*, 2022). These bioactive ingredients largely define the antioxidant potential of berry fruits.

The obtained results (**Table 2**) show that the berries of all studied plant species have a high content of anthocyanins, which are important factors influencing their antioxidant activity. Anthocyanins are water-soluble plant pigments that impart blue, violet, and red colors to plant tissues and account for 18–33 % of the total phenolic compounds in fruit juice (He & Giusti, 2010). Structurally, anthocyanins are glucosides of anthocyanidins, one of the groups of flavonoids formed via the phenylpropanoid pathway. These compounds exhibit pronounced biological activity manifested in antioxidant and other metabolic effects (He & Giusti, 2010; Khoo *et al.*, 2017). The biological activity of anthocyanins is closely related to their structure (*viz.*, aglycone type, the type of glycosylation and the acylation of the glycosyl moieties) (Leong *et al.*, 2017; Luo *et al.*, 2022).

According to the results of this study, the highest anthocyanin content was observed in the extract of fresh chokeberries (**Table 2**). No statistically significant difference was found in the anthocyanin content of fresh blackthorn and elderberries ( $423.30 \pm 10.40$  and  $480.00 \pm 9.26$  mg C3GE/100 g, respectively), allowing them to be combined into one group based on this value, while the anthocyanin content in fresh blackberry extract was significantly lower ( $325.90 \pm 9.18$  mg C3GE/100 g). It is known that anthocyanins in *A. melanocarpa* berries are represented mainly by cyanidin-3-galactoside, which provide high antioxidant activity; other important anthocyanins in chokeberry fruits are cyanidin-3-glucoside, cyanidin-3-xyloside, and cyanidin-3-arabinoside (Liang *et al.*, 2021). Elderberry fruits are quantitatively dominated by cyanidin 3-sambubioside-5-glucoside, which accounts for more than 50 % of the total anthocyanins in the fruits and is relatively stable to processing (Veberic *et al.*, 2009). The predominant anthocyanins in blackthorn berries are cyanidin-3-rutinoside and peonidin-3-glucoside, with cyanidin-3-glucoside present in smaller amounts (Nistor *et al.*, 2023), while cyanidin-3-glucoside is the predominant anthocyanin found in blackberries (Li *et al.*, 2022).

An important group of plant phenolic compounds is represented by flavonoids, which act as powerful antioxidants and possess numerous health benefits (Stachelska *et al.*, 2025). Flavonoids such as quercetin, myricetin, and kaempferol are found in the berries of many plant species and are known to impart high antioxidant activity to the berries (Häkkinen *et al.*, 1999a,b; Suriyaprom *et al.*, 2022). Due to their antioxidant properties, flavonoids exhibit a wide range of biological effects, which ensures a high value of these compounds in human nutrition (Stachelska *et al.*, 2025).

As regards the flavonoid content in fresh berries of the studied plant species, the highest concentration of these compounds measured in quercetin equivalents was found in extracts of chokeberry fruits ( $490.00 \pm 11.13$  mg QE/100 g), while the flavonoid levels were significantly lower in extracts of fresh blackberry, elderberry, and blackthorn fruits (**Table 2**). These results are consistent with data on the high concentration of bioflavonoids in the berries of *A. melanocarpa* plants (Häkkinen *et al.*, 1999a). In terms of flavonoid content, fresh blackthorn berries rank second after chokeberries; fresh elderberries and blackberries rank third, with flavonoid content not significantly different ( $245.00 \pm 6.27$  and  $220.00 \pm 6.17$  mg QE/100 g, respectively).

The results of this study show that the form of plant material (fresh berry extracts, juices, and extracts derived from dried fruits) significantly affects the preservation and

concentration of biologically active substances, such as various groups of polyphenolic compounds (**Table 2**). In most cases, the total phenolic content, as well as concentrations of anthocyanins and flavonoids decreased in the following order: fresh berry extract > dried berry extract > berry juice, according to Tukey's HSD test ( $P < 0.05$ ). This indicates that both species-specific chemical composition and treatment parameters (including the extraction method) influence the final values of the mentioned BACs in the analyzed materials.

Specifically, pasteurized berry juices from all four plant species included in this study had low concentrations of total phenolic compounds (**Table 2**). In particular, the content of phenolic compounds in berry juices from the studied plants decreased by an average of 33.6–65.7 % compared to fresh berries. The highest level of phenolic compound loss was observed in juice derived from *S. nigra* berries (65.7 %), and the lowest level of phenolic compound loss was observed in juice from *A. melanocarpa* berries (33.6 %). Similar trends were observed for the content of anthocyanins and flavonoids in berry juices. Namely, the content of these compounds in pasteurized juices decreased by 36.9–53.7 % and 18.2–55.2 %, respectively, compared to fresh berries, depending on the plant species. Particularly, blackberry juice had the lowest flavonoid content compared to berry juices from other plant species. Analysis of extracts obtained from dried berries of *A. melanocarpa*, *P. spinosa*, *S. nigra*, and *R. fruticosus* plants revealed a higher degree of preservation of phenolic compounds in them compared to berry juices, in most cases with statistically significant differences between these indices in the two mentioned forms of the analyzed berry material (**Table 2**).

The low content of phenolic compounds in berry juices in comparison to that in fresh berries can be explained by the influence of pressing, filtration and heat treatment leading to the degradation of these substances. Furthermore, the low yield of phenolic compounds in berry juices compared to that in extracts of both fresh and dried berries can be explained by the fact that a large portion of phenolic compounds, especially anthocyanins, are concentrated in the berry peel, including the epidermis and tissues located directly underneath it (Herrera-Balandrano *et al.*, 2021; Li *et al.*, 2023). Therefore, a part of these compounds remains in the pomace during the juicing and filtration process.

Comparison of the content of phenolic compounds in extracts of fresh and dried berries showed that the degree of their preservation in dried berry extracts varied depending on the plant species studied. In particular, the least loss of total phenolic compounds was observed in the extracts from *R. fruticosus* dried berries, in which the reduction in phenolic compound content was only 14.8 % compared to fresh fruits. At the same time, the extracts from dried berries of *S. nigra* showed the highest level of loss of total phenolic compounds (32.8 % loss compared to fresh berries). In the dried berry extracts of *A. melanocarpa*, *P. spinosa*, *S. nigra*, and *R. fruticosus* plants, the content of flavonoids and anthocyanins decreased by 12.5–17.5 % and 13.1–31.6 %, respectively, compared to the fresh berry extracts. Analyzing the obtained data, it should be noted that in this case, the differences can be explained not only by the berry processing procedures, but also by the extraction methods and concentrations of extractant used in our experiments.

**Table 3** presents the results of a study of the antioxidant activity of the fresh berry extracts, juices, and dried berry extracts from *A. melanocarpa*, *P. spinosa*, *S. nigra*, and *R. fruticosus* determined by the DPPH radical absorption method. According to the data obtained, the extracts of fresh berries of the studied plant species exhibited the highest antioxidant activity, while berry juices demonstrated the lowest values of this parameter.

During the study, the highest antioxidant capacity was recorded in the fresh berries of *A. melanocarpa* (93 %). The fresh berries of *P. spinosa*, *S. nigra*, and *R. fruticosus* showed virtually the same level of antioxidant activity (83 %, 85 %, and 82%, respectively), which was significantly lower compared to that from *A. melanocarpa* fresh berries. Similarly, almost equal antioxidant activity was observed in the dried berry extracts from *P. spinosa*, *S. nigra* and *R. fruticosus* (79 %, 75 %, and 75 %, respectively).

**Table 3. Antioxidant activity of fresh and dried berry extracts and berry juices from *Aronia melanocarpa*, *Prunus spinosa*, *Sambucus nigra*, and *Rubus fruticosus* assayed by the DPPH radical absorption method (% inhibition, M ± SE)**

Analyzed material	<i>A. melanocarpa</i>	<i>P. spinosa</i>	<i>S. nigra</i>	<i>R. fruticosus</i>
Fresh berry extract	93.00 ± 3.32 aA	83.00 ± 4.28 aB	85.00 ± 3.56 aB	82.00 ± 3.96 aB
Berry juice	79.00 ± 4.76 cA	73.00 ± 6.43 cB	70.00 ± 5.35 cB	65.00 ± 3.96 cC
Dried berry extract	87.00 ± 5.03 bA	79.00 ± 5.86 bB	75.00 ± 4.16 bB	75.00 ± 4.40 bB

**Note:** Values in the same column denoted by the same lowercase letter, or in the same row denoted by the same uppercase letter, are not significantly different ( $P > 0.05$ ) according to Tukey's test

Analysis of the obtained results using a two-way analysis of variance suggests that the free radical scavenging capacity of the analyzed berry material is primarily determined by the form of its treatment (fresh berry extract > dried berry extract > berry juice), while the plant species determines the species-specific level of antioxidant activity in the following order: chokeberry > blackthorn > elderberry > blackberry. Thus, the obtained results show that both the species characteristics of the berries and the method of their processing significantly determine the antioxidant profile of the product.

At the same time, analysis of the obtained data suggests a relationship between the content of phenolic compounds, flavonoids, and anthocyanins in the studied samples and their antioxidant capacity. It is characteristic that the level of antioxidant activity of fresh and dried berry extracts, as well as of berry juices, corresponds to their total phenolic content; accordingly, the analyzed berry materials from *A. melanocarpa* plants enriched with phenolic compounds, including flavonoids and anthocyanins, showed the highest antioxidant activity. A similar trend was observed in the relationship between the content of phenolic compounds and the levels of antioxidant activity in the berry materials from other plant species used in this study. These data are consistent with the important role of phenolic compounds, including flavonoids and anthocyanins, in mediating the antioxidant activity of berry fruits and suggest the health-promoting effects of berries from *Aronia melanocarpa*, *Prunus spinosa*, *Sambucus nigra*, and *Rubus fruticosus* plants.

## CONCLUSIONS

The spectrum of concentrations of phenolic compounds, including flavonoids and anthocyanins, as well as of ascorbic acid found in the berries of *A. melanocarpa*, *P. spinosa*, *S. nigra*, and *R. fruticosus* suggests that the fruits of these species are promising sources of natural antioxidants in human nutrition. The highest content of total phenolic compounds, anthocyanins, and flavonoids was found in the 90 % ethanol extracts of

fresh *A. melanocarpa* berries (up to 1204 mg GAE, 643 mg C3GE, and 490 mg QE per 100 g of sample weight, respectively), which corresponded to the highest level of antioxidant capacity of these samples compared to that from fresh berries of other plant species. However, berry treatment, such as juicing and pasteurization, as well as berry drying, resulted in losses of biologically active substances in the berry products obtained from the studied plants. According to the results of our research, most of the studied parameters (except for the concentration of ascorbic acid) responded similarly to changes in the form of the analyzed material and decreased in the following range: fresh berry extract > dried berry extract > berry juice. Namely, total phenolic content, concentrations of anthocyanins and flavonoids in berry juices from the studied plant species decreased by 33.6–65.7 %, 36.9–53.7 %, and 18.2–55.2 %, respectively, compared to those in the extracts of fresh berries. This is consistent with the reduction in antioxidant capacity (by 12–20.7 %) of pasteurized berry juices compared to fresh berry extracts. At the same time, the decrease in the total phenolic content, as well as in the concentrations of anthocyanins and flavonoids in the water-ethanol extracts of dried berries of the studied plants amounted to 14.8–32.8 %, 13.1–31.6 %, 12.5–17.5 %; the decrease in the antioxidant capacity of the water-ethanol extracts of dried berries was 4.8–11.8 %. The treatment of the obtained results using a two-way analysis of variance confirms that the antioxidant profile of a berry product is determined by a combination of species-specific metabolic characteristics and a form of berry treatment (including extraction method) with a significant decrease in the following order: fresh berry extract > dried berry extract > berry juice ( $P < 0.05$ ). These statistically proven patterns substantiate the need to select a specific type of berry material to create functional products with the desired health properties.

The obtained results provide a basis for further research aimed at developing technologies for processing berries with minimal loss of biologically active substances, as well as for creating innovative health products based on raw berry materials with high antioxidant potential.

## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of Interest:** the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Animal Rights:** this article does not contain any studies with animal subjects performed by any of the authors.

**Human Rights:** this article does not contain any studies with human subjects performed by any of the authors.

## AUTHOR CONTRIBUTIONS

Conceptualization, [B.K.; Y.Z.; H.A.]; methodology, [B.K.; Y.Z.]; validation, [B.K.]; formal analysis, [B.K.; Y.Z.; H.A.]; investigation, [B.K.; Y.Z.; L.F.; I.P.; N.Z.]; resources, [B.K.; M.B.; I.P.]; data curation, [B.K.]; writing – original draft preparation, [B.K.; Y.Z.; L.F.]; writing – review and editing, [B.K.; H.A.]; visualization, [B.K.]; supervision, [B.K.; H.A.]; project administration, [B.K.; M.B.]; funding acquisition, [-].

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## ВМІСТ БІОЛОГІЧНО АКТИВНИХ СПЛУК І АНТИОКСИДАНТНА АКТИВНІСТЬ У ЯГОДАХ РОСЛИН *ARONIA MELANOCARPA*, *PRUNUS SPINOSA*, *SAMBUCUS NIGRA* ТА *RUBUS FRUTICOSUS*

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**Обґрунтування.** Ягоди – багате джерело мінералів, вітамінів і фітохімічних речовин у харчуванні людини. Через зростання попиту на органічні продукти харчування, збагачені біологічно активними сполуками (БАС), є потреба оцінити рівень цих речовин у ягодах різних видів рослин і розробити оптимальні методи переробки ягідної сировини, що дають змогу зберегти її біологічну цінність. Метою роботи було з'ясувати вміст БАС (фенольні сполуки, антоціани, флавоноїди, аскорбінова кислота) і антиоксидантну активність екстрактів свіжих та висушених ягід і ягідних соків чагарникових рослин, поширених на території України (горобина чорноплідна, терен колючий, бузина чорна й ожина кушова).

**Матеріали і методи.** Дослідження проводили з використанням свіжих плодів рослин *Aronia melanocarpa* (Michx.) Elliott (горобина чорноплідна), *Prunus spinosa* L. (терен колючий), *Sambucus nigra* L. (бузина чорна) та *Rubus fruticosus* L. (ожина кушова), що ростуть у природних умовах Львівської області. Збір рослинного матеріалу, його підготовку до аналізу, а також сушіння ягід, отримання та пастеризацію ягідних соків проводили загальноприйнятими методами. Ягідні екстракти готували методом екстракції свіжих ягід 90 % етиловим спиртом і екстракції сухих ягід розчином вода-етанол (у співвідношенні 1:1 (об./об.)). Загальний вміст фенольних сполук і концентрацію антоціанів, флавоноїдів та аскорбінової кислоти (вітаміну С) визначали загальноприйнятими спектрофотометричними методами. Антиоксидантну активність рослинного матеріалу оцінювали за допомогою спектрофотометричного аналізу з використанням стабільного вільного радикала DPPH (1,1-дифеніл-2-пікрілгідразил). Статистичну обробку отриманих результатів проводили методом двофакторного дисперсійного аналізу (ANOVA).

**Результати.** Екстракти свіжих ягід досліджуваних видів рослин містили вищу концентрацію загальних фенольних сполук, флавоноїдів, антоціанів і аскорбінової кислоти, ніж ягідні соки та екстракти сушених ягід, причому найвищий вміст

біологічно активних речовин відзначено у свіжих ягодах *A. melanocarpa*. Зокрема, плоди горобини чорноплідної містили фенольні сполуки, антоціани та флавоноїди в концентраціях відповідно 1204 мг GAE (еквівалентів галової кислоти), 643 мг С3GE (еквівалентів ціанідин-3-глюкозиду) та 490 мг QE (еквівалентів кверцетину) на 100 г сирової маси зразка. У пастеризованих ягідних соках виявлено меншу концентрацію зазначених сполук, ніж у екстрактах свіжих ягід, а екстракти сушених ягід переважно містили вищу концентрацію біологічно активних речовин, порівняно з ягідними соками. Серед досліджених рослинних матеріалів найвищу антиоксидантну активність виявлено в екстрактах свіжих ягід горобини чорноплідної (93 %), а в свіжих ягодах рослин *P. spinosa*, *S. nigra* та *R. fruticosus* виявлено значно нижчий антиоксидантний потенціал (83 %, 85 % та 82 % відповідно).

Для більшості аналізованих параметрів (крім концентрації аскорбінової кислоти) спостерігається така динаміка збереження біологічно активних речовин у досліджуваному матеріалі: екстракт свіжих ягід > екстракт сушених ягід > ягідний сік, що підтверджує перевагу методу екстракції ягід над методом вичавлювання соку для отримання високого рівня біологічно активних сполук у ягідних продуктах. Водночас отримані результати свідчать, що і видові характеристики ягід, і спосіб їхньої обробки суттєво визначають антиоксидантний профіль ягідних продуктів.

**Висновки.** Аналіз концентрацій фенольних сполук, флавоноїдів, антоціанів і аскорбінової кислоти, виявлених у ягодах *A. melanocarpa*, *P. spinosa*, *S. nigra* та *R. fruticosus*, свідчить про те, що плоди цих видів рослин є перспективними джерелами природних антиоксидантів у харчуванні людини. Однак режим обробки ягід суттєво впливає на вміст біологічно активних речовин у ягідних соках та екстрактах. Отримані результати можна застосовувати в харчовій промисловості для розробки функціональних продуктів харчування з посиленими антиоксидантними властивостями.

**Ключові слова:** ягоди, біологічно активні сполуки, антиоксидантна активність, фенольні сполуки, флавоноїди, антоціани, функціональні продукти харчування