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ANTIOXIDANT ACTIVITY, POLYPHENOL AND ANTHOCYANIN CONTENT OF THREE STRAWBERRY SPECIES (*FRAGARIA* X *ANANASSA* DUCHESNE EX ROZIER, *FRAGARIA VESCA* L., AND *FRAGARIA MOSCHATA* WESTON)

Vladimíra Horčinová Sedláčková[1], Olena Vergun[2], Olga Grygorieva[2], Soňa Jankurová[3], Jana Ferencová[3], Eleonóra Krivosudská[3]

Address(es):
[1] Slovak University of Agriculture in Nitra, AgroBioTech Research Centre, Tr. A. Hlinku 2, Nitra, Slovakia, SK-949 76.
[2] M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Sadovo-Botanichna 1, 01014, Kyiv, Ukraine.
[3] Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Plant and Environmental Sciences, Tr. A. Hlinku 2, Nitra, Slovakia, SK-949 76.

*Corresponding author: vladimira.sedlackova@uniag.sk

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ABSTRACT

Fragaria L. species are popularly known as garden-grown (*F.* x *ananassa* Duchesne ex Rozier) or wild (*F. moschata* Weston and *F. vesca* L.) plants with biologically active compounds directly responsible for the high antioxidant potential. This work aimed to determine the total polyphenol compounds, anthocyanins, and antioxidant activity of different extracts of *Fragaria* spp. full ripened fruits and comparative evaluation of plant raw depending on their origin. The berries were collected from Belgium (Namur), Hungary (Kisbér), Poland (Krakow), Slovakia (Bodovka, Ľubochňa, Trenčín, Trenčianske Jastrabie) and studied in 2023. It was conducted the total content of polyphenol compounds (by Folin-Ciocalteu method), anthocyanins (based on reducing the pH value of the extract), and antioxidant activity (by DPPH assay (2.2-diphenyl-1-picrylhydrazyl)) in methyl alcohol, ethyl alcohol, and acetone solutions. Based on experiments the most abundant polyphenols and anthocyanins in the ripening fruits were in both wild species (*F. vesca* – 4505.76 mg.kg^{-1} FW of polyphenols; 258.04 mg.kg^{-1} FW of anthocyanins; *F. moschata* – 5443.01 mg.kg^{-1} FW of polyphenols; 228.69 mg.kg^{-1} FW of anthocyanins) in comparison of garden-grown species (*F.* x *ananassa*) which obtained significantly lower values in all samples. The lowest values in both parameters were measured in the Slovakian sample from Trenčianske Jastrabie (*F.* x *ananassa* – 2427.22 mg.kg^{-1} FW of polyphenols; 86.19 mg.kg^{-1} FW of anthocyanins). Between the lowest and highest values are percentage differences of more than 50% in both analyses. The extracts of *F. vesca and F. moschata* fruits were effective radical scavengers against radicals DPPH (53.92% and 87.17%, respectively) in comparison to *F.* x *ananassa* samples (27.21% 5FE – 52.58% 2FA). Investigated *Fragaria* spp. are a valuable source of antioxidants and experimental results can be used in further biochemical, pharmacological, and nutraceutical research for practice usage.

Keywords: strawberry, *F.* x *ananassa*, *F. vesca*, *F. moschata,* biochemical analyses, antioxidant potential

INTRODUCTION

Regarding the first cultivation of strawberries, we have limited references extending up to the period of Early Romans. Native strawberries were most likely cultivated as early as the time of the Romans, who spent considerable resources importing a wide variety of fruits for their country estates, including apples, peaches, apricots, grapes, cherries, plums, pears, lemons and figs (**Wilhelm and Sagen, 1974**). The first mention of strawberry cultivation in Europe appears in French literature of the 1300s. Ancient records show that King Charles V had over 1000 strawberries planted in the royal gardens of the Louvre in Paris, and strawberries are known to have been grown in four blocks of the gardens of the Dukes of Burgundy (**Darrow, 1966**).

Strawberry is a member of the family Rosaceae and the genus *Fragaria* L. It is known following native strawberries: *F. bucarica* Losink. (Asia, W. Himalayas), *F. daltonia* J. Gay (Asia, Himalayas), *F. nubicola* Lindl. (Asia, Himalayas), *F. gracilis* Losink. (Asia, N. China), *F. mandshurica* Staudt (Asia, N. China), *F. pentaphylla* Losink. (Asia, N. China), *F. corymbose* Losink. (Asia, N. China), *F. moupinensis* (French.) Card (Asia, N. China), *F. gracilis* Losink. (Asia, N.W. China), *F. tibeticia* spec. nov. Staudt (Asia, China), *F. innumae* Makino (Asia, Japan), *F. yezoensis* Hara. (Asia, Japan), *F. nipponica* Lindl. (Asia, Japan), *F. iturupensis* Staudt. (Asia, Iturup Island), *F. nilgerrensis* Schlect. (Asia, S.E. Asia), *F. orientalis* Losink. syn. = *F. corymbose* Losink. (Asia, China, Far Eastern Russia), *F. americana* (Porter) Britton syn. = *F. vesca* (Porter) Staudt. (Europe, Asia, N. America), *F. viridis* Duch. (Europe, Asia), *F. moschata* Duch. (Europe, Asia), *F. chilonensis* (L.) Miller (N. America, S. America, Western N. America, Hawaii, Chile), *F. virginiana* Miller (N. America) (**Hancock *et al.*, 2008; Hummer and Hancock 2009**).

Fragaria vesca L. popularly known as wild strawberry or woodland strawberry grown in temperate and subtropical areas of the northern hemisphere in light forests, on rocky hillsides, in ditches, and meadows from lowlands to mountain areas. Widely spread across Europe, in the temperate zone of Asia, to the east as far as Lake Baikal. It also occurs in Korea, Japan, North America and Canada, South Australia, Tasmania, Java, and New Zealand (**Castroviejo *et al.*, 1998**; **Rousseau-Gueutin *et al.*, 2009**).

Fragaria moschata Weston, the musk strawberry is originally a Central and Eastern European species having spread as an escape from gardens in many areas (**Lee, 1966; Staudt, 2009; Lesemann *et al.*, 2017**). This area extends in the west to central France and England, in the north to southern Scandinavia, in the south to the Apennines, and the north of the Balkan Peninsula, in the east it also extends to western Siberia, even to the upper course of the Yenisei. It also occurs in the Caucasus. In our country, it grows scattered to abundant throughout the territory, with the focus of distribution in the thermophytic and mesophytic, from where it also extends into the lower oreophytic areas. Shrubs, light forests, scrubby slopes, ditches, grassy forest edges, clearings. It is in documents noted that by the late 15th century the musky-flavoured *F. moschata* was also planted in gardens and utilized for its fruit by the Germans, English, and Russians, together with the green strawberry *F. viridis*, which was an ornamental species (**Lesemann *et al.*, 2017**).

Fragaria × *ananassa* Duchesne ex Rozier, the garden strawberry is a cultivated hybrid species in both open fields and in greenhouses throughout the world belongs among the most widely consumed berries in the world not only for its taste and sight but also for its health properties known since ancient times.

Biologically active substances play a decisive role in the preservation of human and animal life, as well as in maintaining the natural balance (**Arellano *et al.*, 2012**). The production of biologically active substances as secondary metabolites is known in all species of organisms, mostly in plants and fungi (up to 80%) and to a lesser extent in animals (**Macholán, 2003**). Plants have proven to contain a wide range of biologically active compounds that can be used to treat various types of diseases, including infectious ones. They act against cancer, microorganisms, and parasites. They are also effective antioxidants, and analgesics and accelerate wound healing (**Chen *et al.*, 2011**).

The major phenolic compounds in strawberries are anthocyanins, ellagitannins, flavan-3-ols, glycosides of quercetin, and kaempferol **(Aaby *et al.*, 2005)**. From flavonols, quercetin had the greatest antioxidant activity, followed by kaempferol, fisetin, their glucuronides and glycosides, and from flavan-3-ols are present

catechin, proanthocyanidin B1, proanthocyanidin B3, proanthocyanidin trimer (**Giamperi *et al*., 2012; Fierascu *et al*., 2020; USDA**). Pelargonidin-based anthocyanins are predominant anthocyanins in cultivated strawberry fruit such as pelargonidin 3-rutinoside, pelargonidin 3-glucoside, and pelargonidin 3-glucoside–succinate in comparison of lower levels of cyanidin-based anthocyanins such as cyanidin 3-glucoside, and cyanidin 3-glucoside–succinate than pelargonidin-based anthocyanins in concentration dependent on the Fragaria species and cultivars (**Gil *et al.*, 1997; Wang and Zheng, 2001; Nowicka *et al.*, 2019; Fierascu *et al.*, 2020**).

D'Urso *et al*. (2016) determined in *F. vesca* from wild and cultivated forms 39 phenolic compounds (including (+) catechin, (−) epicatechin, procyanidin B1 and B2, cyanidin 3-O-glucoside, pelargonidin-3-O-glucoside, pelargonidin-3-O-rutinoside, delphinidin-3-O-glucoside, isoquercetin, gallic acid, p-coumaric acid, phloridzin); composition dependent on the geographical area.

Guerrero-Chavez *et al.* (2015) studied in the fruits *F.* x *ananassa* grown on different altitudes on consecutive years by HPLC DAD and identified following compounds (+)-catechin, (−)-epicatechin, procyanidins, flavonols, anthocyanins (cyanidin3-glucoside, pelargonidin3-glucoside, pelargonidin derivative), hydroxybenzoic acid, p-coumaric acid, other hydroxycinnamic acids; with higher levels recorded at lower altitudes.

Herbal materials applied in traditional medicine are not considered only fruits, but also leaves and roots from wild strawberries due to the rich source of biologically active substances such as phenolic acids, anthocyanins, flavonoids, and tannins (**Mudnic *et al*., 2009; Buendia *et al*., 2010; Buřičová *et al*., 2011; Liberal *et al*., 2014; Ivanov *et al*., 2015**), including vitamins C, E, β-carotene, melatonin, as well as bioactive sugars (**Álvarez-Fernández *et al*., 2014; Rodríguez-Gutiérrez *et al*., 2019**). **Couto *et al.* (2020)** studied wild strawberry young leaves (*F. vesca*) and confirmed the presence of bioactive compounds in this species, which are known for their antiseptic, emollient, and dermatological protection properties. Other authors detail the usage of wild strawberry roots for preparing decoctions and infusions for urinary tract infections, diarrhoea, cough symptoms, gout, and haemorrhoids. These preparations also show anti-dysenteric and antiseptic capacity, diuretic properties, emollients, and dermatologic protectors, or functioning as detoxifiers (**Camejo-Rodrigues *et al*., 2003; Neves *et al*., 2009; Özüdogru *et al*., 2011; Savo *et al*., 2011**).

It is appealing to the human senses of sight and taste due to strawberries' bright red colour, juicy texture, sweetness, and distinct flavour, which is suitable for industrial processing (production of marmalades, jams, and compotes) **(Nuñez-Mancilla *et al*., 2013; Grujić *et al*., 2014; Grujić and Odžaković, 2016)**.

This study has aimed to examine and compare three strawberry species in terms of biochemical composition, such as the content of polyphenols and anthocyanins, determination of antioxidant potential in various fruit extracts and solutions to confirm differences and a more comprehensive view and on three *Fragaria* species from domestic and foreigner sources.

MATERIALS AND METHODS

Plant material

Our research focuses on the 2 wild species (*F. vesca* L. and *F. moschata* Weston) and 1 cultivated garden-grown (*F.* x *ananassa* Duchesne ex Rozier) species from private gardens and commercial stores due to the comparison of biologically active compounds and antioxidant activity of various extracts.

One garden-grown species *F.* x *ananassa* and two wild species *F. vesca* and *F. moschata* in full fruit ripening were chosen for the experiment. From garden-grown form (*F.* x *ananassa*) we used plants grown in the private gardens from Slovakian regions (Trenčín, Trenčianske Jastrabie, and Ľubochňa) and fruits from markets grown in greenhouses (Belgium, Poland, Hungary) purchased in Slovakia. The wild species were grown in the gardens (Trenčín, Bodovka). The fruits were collected at the full maturity stage, frozen at an internal of < -18°C and used for experiments during the summer of 2023. The plant material was analyzed at the Institute of Plant and Environmental Sciences at the Slovak University of Agriculture in Nitra, Slovakia. Basic information about strawberry species used in experiments is summarized in Table 1.

Table 1 Origin of investigated *Fragaria* spp.

Sample No.	Variety	Country	Place	Latitude / Longitude	Meters about sea level
1	*F.* x *ananassa* Duchesne ex Rozier	Slovakia	Trenčín	48.89°N / 18.04°E	210
2	*F.* x *ananassa* Duchesne ex Rozier	Slovakia	Trenčianske Jastrabie	40.80°N / 18.11°E	350
3	*F.* x *ananassa* Duchesne ex Rozier	Slovakia	Ľubochňa	49.12°N / 19.17°E	634
4	*F.* x *ananassa* Duchesne ex Rozier	Belgium	Namur	50.47°N / 4.87°E	147
5	*F.* x *ananassa* Duchesne ex Rozier	Poland	Krakow	50.06°N / 19.94°E	223
6	*F.* x *ananassa* Duchesne ex Rozier	Hungary	Kisbér	47.50°N / 18.04°E	160
7	*F. vesca* L.	Slovakia	Trenčín	48.89°N / 18.04°E	276
8	*F. moschata* Weston	Slovakia	Bodovka	48.82°N / 17.95°E	282

Chemicals

All chemicals were of analytical grade quality and were purchased from Reachem (Slovakia), Centralchem (Slovakia), Sigma-Aldrich (USA, Switzerland), and VWR Chemicals (Belgium). Ethanol (96%; Centralchem s.r.o., Bratislava, Slovakia, p.a.), Methanol (99.5%; Centralchem s.r.o., Bratislava, Slovakia, p.a.), Acetone (≥99.5%; Sigma-Aldrich, USA), Folin-Ciocalteu's phenol reagent (Lowry method, 2N; Sigma-Aldrich, Switzerland), DPPH 2.2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, USA), HCl (≥37%; Centralchem, Slovakia), Na_2CO_3 (99.5%; Centralchem, Slovakia), Na_2HPO_4 (98-102%; Centralchem, Slovakia) and citric acid (100.3%; VWR Chemicals, Belgium).

Instruments

The centrifuge Rotofix 32 A (Hettich, Germany), spectrophotometer Jenway, 6405 UV/Vis (England), vortex shaker (IKA VORTEX 3, Germany), GENESYS 20 Vis Spectrophotometer (Thermo Fisher Scientific Inc., USA) were used in this research.

Determination of polyphenols

The experiment used the spectrophotometric method according to **Lachmann (2003)** using the Folin-Ciocalteu probe. Gallic acid was used as a standard. Weighed 25g of common strawberries (approximately the same size) from all samples, which were homogenized and poured with 50 ml of 70% ethanol. Samples were left on the shaker for 16 hours. After the extraction time, the extract was filtered using filter paper **(Lapornik, 2005)**. 1 ml of the extract was pipetted into a 50 ml volumetric flask and the sample was diluted with distilled water. 2.5 ml of Folin-Ciocalteu test tube was added to the flask and after standing for 3 minutes, 5 ml of 20% aqueous Na_2CO_3 solution was added and mixed. Finally, the volume was topped up with distilled water up to the mark of a volume of 50 ml and the contents of the flask were mixed. The colour complex was formed for two hours. Standard solutions of gallic acid were prepared using a calibration curve (5µm. ml). The absorbance of blue-coloured solutions was measured at a wavelength of 765 nm compared to a blank test spectrophotometrically (JENWAY 6405 UV/Vis spectrophotometer). The results of the total amount of polyphenols in the strawberry samples were obtained based on the calibration curve equation. The results were recalculated and expressed as mg of gallic acid per kg of fresh material (**Lachmann, 2003**).

Determination of the anthocyanins

The analytical method for determining the anthocyanin content was the method by **Lapornik *et al.* (2005)**, which is based on reducing the pH value of the extract to values of 0.5 to 0.8. This pH change ensures the transformation of all anthocyanins into the red-coloured flavylium cation. 1 ml of the extract was pipetted into the test tubes and 1 ml of 0.01% HCl in 80% ethanol was added. 10 ml of 2% aqueous HCl solution was added to the first eight test tubes (A1), and 10 ml of McIlvain's buffer solution with pH = 3.5 was added to the second eight test tubes (A2). McIlvaine's buffer was prepared from 0.2M Na_2HPO_4 and 0.1M citric acid. 1 ml of water and 10 ml of 2% aqueous HCl solution were added to test tube A3. 1 ml of water and 10 ml of McIlvain's buffer solution with pH = 3.5 were added to test tube A4. Test tubes A3 and A4 serve as opposites in the analysis. The absorbance of both samples was measured at a wavelength of 520 nm compared to a blank test spectrophotometrically (JENWAY 6405 UV/Vis spectrophotometer). The total content of anthocyanins was calculated from the differences in absorbance values and expressed in $mg.kg^{-1}$ extract (**Lapornik *et al.*, 2005**).

Determination of antioxidant activity

The antioxidant activity of fresh strawberry fruits was determined in methanolic (FM), ethanolic (FE), and acetone (FA) extracts. The samples 1 g in 25 ml ethyl alcohol (96%) methyl alcohol (99.5%) and acetone (≥99.5%) were mixed for 12 hours and after filtration of samples antioxidant activity was determined. In the frame of antioxidant activity (ability to eliminate the free radicals) was tested the capacity of strawberry fruits to remove DPPH• radicals (2.2-diphenyl-1-picrylhydrazyl) using methods of **Brand-Williams *et al.* (1995)** and of **Sánchez-Moreno *et al.* (1998)**. Absorbance at 515 nm was registered regularly until the reaction equilibrium was reached using the GENESYS 20 Vis Spectrophotometer (Thermo Fisher Scientific Inc., USA). First was measured the DPPH• (Sigma

Aldrich, USA) absorbency without antioxidant substance (control). The inhibition of DPPH• radicals was calculated in the percent of free DPPH• radicals in the samples using the method of **Von Gadow *et al.* (1997)**:

$$\% \text{ of inhibition} = [(AC_0 - AA_t)/AC_0] \times 100;$$

Where: AC_0 is the absorbance of control in time t = 0 min (DPPH• solution), AA_t is the absorbance in the presence of antioxidants in time t min, the result is in % of DPPH• radicals' inhibition.

Number of repeated analyses: All biochemical procedures were conducted in triplicate.

Statistical Analysis

Data were analysed with the ANOVA test and differences between means were compared through the Tukey-Kramer test ($P < 0.05$). Each sample was analysed in triplicate and results are reported as mean concentration ± standard deviation.

RESULTS AND DISCUSSION

Strawberries are a rich source of antioxidant compounds (phenolic acids, flavonoids, and anthocyanins) including vitamins C, E, β-carotene, and melatonin, as well as bioactive sugars studied by **Cerdá *et al.* (2005)**, **Álvarez-Fernández *et al.* (2014), Ariza *et al.* (2016), Rodríguez-Gutiérrez *et al.* (2019),** and others. Plants provide an unlimited source of novel and complex chemical structures produced by secondary metabolism, that are responsible for their biological activity.

Our study aimed to determine polyphenols and anthocyanins content and confirmation of the valuable antioxidant potential of three *Fragaria* species (*F.* x *ananassa*, *F. vesca*, and *F. moschata*) from various countries (Slovakia, Belgium, Poland, and Hungary).

Polyphenols content

In addition to their health benefits, phenolic compounds are directly responsible for fruit quality and contribute to the sensory and organoleptic properties of strawberries. (**Álvarez-Fernández *et al.*, 2014; Morales-Quintana and Ramos, 2019**). Polyphenols as a protective factor in carcinogenesis reduce the bioavailability of carcinogens by interfering with their biotransformation in the liver. **(Kashi *et al.*, 2019; Selvakumar *et al.*, 2020; Li *et al.*, 2023)**.

Based on experiments the most abundant polyphenols in the ripening fruits were in both wild species (*F. vesca* – 4505.76 mg.kg^{-1} FW; *F. moschata* – 5443.01 mg.kg^{-1} FW) in comparison to garden-grown species (*F.* x *ananassa*) which obtained significantly lower values in all samples. The lowest value was measured in the Slovakian sample / Trenčianske Jastrabie (*F.* x *ananassa* – 2427.22 mg.kg^{-1} FW). The percentage difference between the highest (8F) and the lowest (2F) value in the polyphenols content is more than 55%. Results are presented in Figure 1A.

Milošević *et al.* (2016) evaluated strawberries grown in Serbia's conditions and determined the total phenolic content in cultivated strawberries (*F. x ananassa*) in the interval 35.22–323.20 mg GAE.g^{-1} DW, and woodland strawberries (*F. vesca*) 45.75 mg GAE.g^{-1} DW, total flavonoid content in the range 7.21–93.25 mg RUE. g^{-1} (rutin equivalent) DW and 12.10 mg RUE. g^{-1} DW for cultivated and woodland strawberries, respectively. The highest values in all evaluated traits were achieved in blackberries with average values for total phenolic content 110.07 mg AE.g^{-1} DW, for total flavonoid content 51.55 mg RUE.g^{-1} DW.

Another study **(Balasooriya *et al.*, 2016)** investigated the effect of temperature on total polyphenol content. Strawberries grown at a higher temperature had a significantly higher ($P<0.05$) content of total polyphenols (1711 ± 29 mg.kg^{-1}) compared to fruits grown at a lower temperature (939 ± 10 mg.kg^{-1}). Both values of this study are significantly lower than our results. The conclusion from the study **(Balasooriya *et al.*, 2016)** would explain why the Croatian study recorded significantly high values of total polyphenolic compounds in fruits (6379.4 mg.kg^{-1} an average value). Croatia achieves a higher average temperature in comparison to countries from which our samples come.

Decoction extract (39–46 mg GAE.g^{-1}) from strawberry leaves and infusion solution (28–37 mg GAE.g^{-1} DW) represented the highest phenolic content, and these amounts accounted for 10.8%, 7.3% and 6.3% of the dry weight of each extract, respectively **(Ivanov *et al.*, 2015)**. Similar results found in strawberry leaf extracts were reported by **Mudnic *et al.* (2009)** and **Buendia *et al.* (2010)**.

Anthocyanins content

As reported in many research studies, anthocyanins, belonging to the flavonoid group with proven good scavenging activities, demonstrate protection against harmful free radicals, which is directly related to lower incidence and mortality from cancer and cardiovascular diseases, as well as several other health benefits (**Satué-Gracia *et al.*, 1997; Velioglu *et al.*, 1998**; **Lila *et al.*, 2016; Sandoval-Ramírez *et al.*, 2018, 2021**).

Research of three strawberry species and eight various samples confirmed differences between wild and garden-grown species in our experiments. Based on results the most abundant anthocyanins in the ripening fruits were in both wild species (*F. vesca* –258.04 mg.kg^{-1} FW and *F. moschata* –228.69 mg.kg^{-1} FW) in comparison to garden-grown species (*F.* x *ananassa*) which obtained significantly lower values in all samples. The lowest values were measured in the Slovakian sample / Trenčianske Jastrabie (*F.* x *ananassa* – 86.19 mg.kg^{-1} FW) and the Hungarian sample (93.59 mg.kg^{-1} FW). The percentage difference between the highest (7F) and the lowest (2F) value in anthocyanin content is more than 66%. Results are presented in Table 2 and Figure 1B.

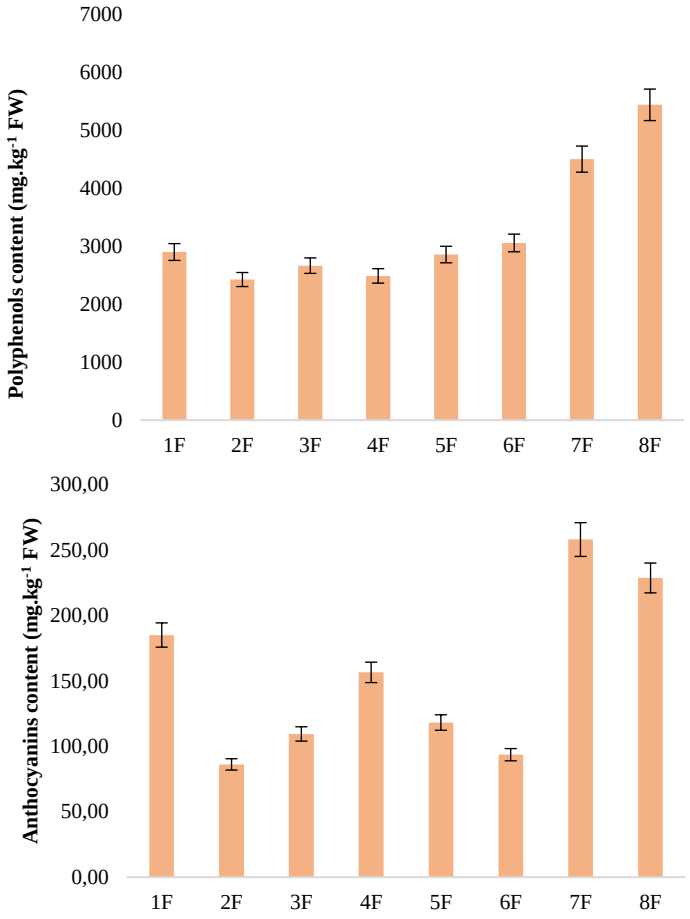

Figure 1 Comparison in contents of polyphenols (1A) and anthocyanins (1B) of *Fragaria* spp. (1F-6F – *F.* x *ananassa*; 7F – *F. vesca*; 8F – *F. moschata*)

Aliman *et al.* (2021) compared 3 different strawberry cultivars (Clery, Marmolada, and Arosa) with wild strawberries in Bosnia and Herzegovina. The results indicate an average value of total anthocyanins in wild strawberries of 237.8±5.4 mg.kg^{-1}. The average content of total anthocyanins in wild strawberries determined by us (240.72 mg.kg^{-1}) is in the same interval. The remaining 3 cultivars from this study contained an average of 179.26 mg.kg^{-1} of anthocyanins. This value is 48% higher compared to the average value of our strawberries of domestic and foreign origin (120.89 mg.kg^{-1}).

Ivanov *et al.* (2015) obtained and determined proanthocyanidins in strawberry leaves collected in May from natural species in the form of infusion (24.9 mg LE/g DW) and decoction (22.3 mg LE/g DW). Extracts obtained from the natural population collected in October reported high concentrations of flavonoids (4.0 and 4.4 mg QE/g DW for the decoction and infusion, respectively). Similar results for the concentration of total proanthocyanidins were reported by **Buendia *et al.* (2010)** and **Ivanov *et al.* (2014)**.

Yildiz *et al.* (2014) studied fifteen wild strawberry accessions (*Fragaria vesca* L.) and one commercial strawberry cultivar *Camarosa* (*Fragaria* × *ananassa* Duch.) sampled in Northeastern Turkey for the purpose determination of some biochemical properties (pH, acidity, total soluble solid content, antioxidant activity) and contents of biological compounds (concentration of anthocyanins, total phenolics, total ellagic acid, and vitamin C). Study demonstrated notable differences among wild strawberries (*Fragaria vesca*) and between wild strawberries and cv. *Camarosa* (*Fragaria* x *ananassa*). The total phenolic content was determined in the interval 138-228 mg GA eq. 100 g^{-1} FW. The total monomeric anthocyanin content was the highest in wild accession 53.51 mg.100 g^{-1} while the lowest was 25.11 mg.100 g^{-1} FW. All wild strawberries exhibited higher antioxidant activity than cv. *Camarosa* by DPPH and FRAP assays. In

general, wild strawberries are an important source of polyphenols, ellagic acid, and antioxidants, which was evidenced by these experiments.
Differences in the biosynthesis of anthocyanins, polyphenols and flavonoids could be explained because these parameters can change according to biotic and abiotic factors (maturity, cultivation technique, type of soil, climate conditions, stress conditions, variety) as described many authors (**Segantini *et al.*, 2015; Saikaew *et al.*, 2018; Jelačić *et al.*, 2020; Becerra *et al.*, 2023**).

Antioxidant activity

Antioxidants play an important role in health protection because they can delay or inhibit the oxidation of lipids or other molecules by inhibiting oxidizing chain reactions (**Velioglu *et al.*, 1998**). Antioxidants in strawberry fruit include phenolic compounds such as phenolic acids and flavonoids, including flavonols and anthocyanins, and vitamin C (**Wang and Lewers, 2007**).
Scavenging of free radicals in our samples was tested using a DPPH (2.2-diphenyl-1-picrylhydrazyl) methylalcohol, ethylalcohol, and acetone solutions of eight samples. Both wild strawberry species (samples 7F and 8F) have the highest antioxidant potential in all solutions compared to strawberry samples (1F–6F). Sample 7F reached more than double the value in all extracts by comparison with samples of *F.* x *ananassa* (Figure 2). The extracts of *F. vesca* and *F. moschata* fruits were effective radical scavengers against radicals DPPH (53.92% 8FE – 87.17% 7FA) against garden-grown *F.* x *ananassa* samples (27.21% 5FE – 52.58% 2FA).

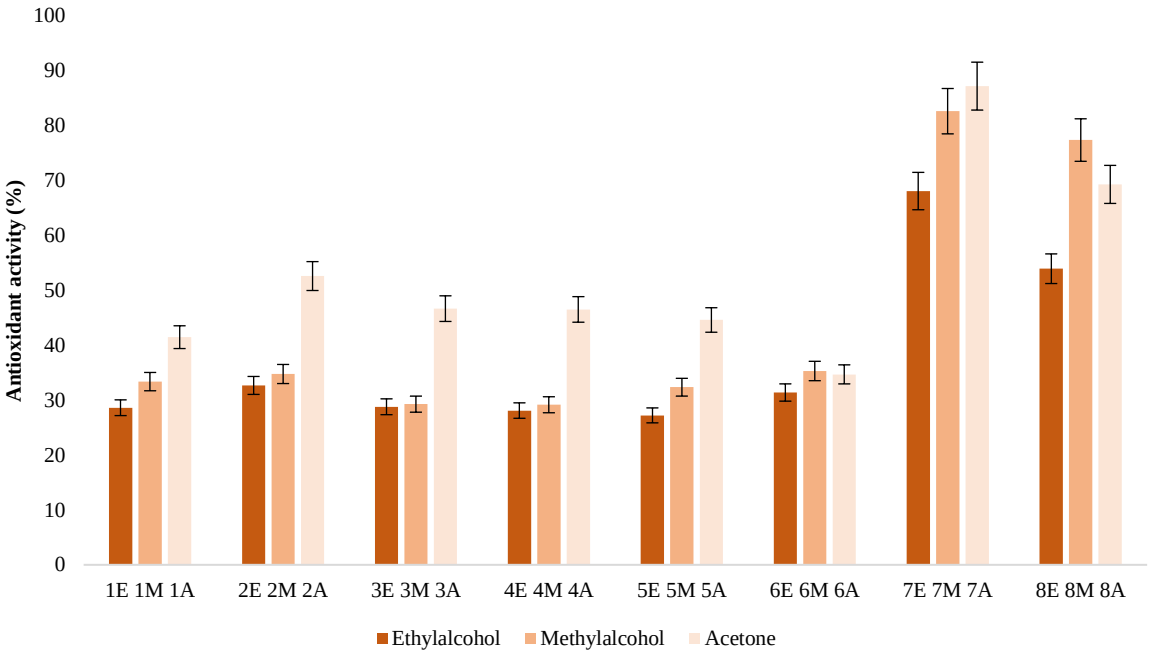

Figure 2 Antioxidant activity of various ethyl alcohol (E), methyl alcohol (M), and acetone (A) extracts of *Fragaria* spp. (Note: 1E, 1M, 1A – 6E, 6M, 6A – *F.* x *ananassa*; 7E, 7M, 7A – *F. vesca*; 8E, 8M, 8A – *F. moschata*)

Olennikov *et al.* (2020) determined the antioxidant potential of *F. viridis*. *F. vesca.* and *F. ananassa* by four assays of ABTS, DPPH, FRAP, and ORAC methods (μM Trolox-eq.g^{-1} of dry weight) in various stages of ripening. The extracts of *F. viridis* fruits in all stages of ripening were effective radical scavengers against both radicals ABTS (35.07–36.22 μM Trolox-eq.g^{-1}) and DPPH (27.53–29.18 μM Trolox-eq.g^{-1}), while the more active scavenger in the ABTS assay gave the extract of ripe fruits and the DPPH assay was the extract of unripe fruits as more active. The extracts of *F. vesca* and *F. ananassa* were less effective in DPPH/ABTS (15.21/19.73 and 9.33/14.67 μM Trolox-eq.g^{-1}, respectively).
Strawberries grown in Serbia's conditions showed high antioxidant protection against free radicals with values in the interval 48.05– 122.80 mg AA. g^{-1} (AAE) DW for cultivar strawberries and 87.12 mg AA. g^{-1} DW for woodland strawberries in comparison of blackberries which achieved the total antioxidant activity 161.88 mg AA. g^{-1} DW **(Milošević *et al.*, 2016)**.
Research of cultivars of *F. ananassa* collected from various countries has shown the following results. Extracts in the DPPH assay showed a wide range of fluctuation of antiradical activity from 9.75–12.83 μM BHT-eq.g^{-1} for Brazil cultivars (**Pineli *et al.*, 2011**) to 3.00–13.15 μM Trolox-eq.g^{-1} for Polish cultivars (**Nowicka *et al.*, 2019**). Similar characteristics were found for ABTS assay data varying from 1.50–2.27 μM Trolox-eq.g^{-1} for Japanese varieties (**Zhu *et al.*, 2015**) to 7.06–29.73 μM Trolox-eq.g^{-1} for Polish cultivars (**Nowicka *et al.*, 2019**).
Dyduch-Siemińska *et al.* (2015) evaluated the content of flavonoids (0.417–0.593 mg.g^{-1} FW and 1.178–1.245 mg.g^{-1} DW), free phenolic acids (1.64–2.84 mg.g^{-1} FW and 4.48–4.98 mg.g^{-1} DW), tannins (2.19–3.40% FW and 4.83–6.09% DW), anthocyanins (90.00–160.50 mg.100g^{-1} FW and 214.61–444.25 mg.100g^{-1} DW), and antioxidant activity (12.40–14.27% FW and 23.40–24.60% DW) using DPPH radical neutralization ability in fresh and air-dried fruits of three wild strawberries cultivars (*F. vesca*).
Other experiments with infusion and decoction of the wild root of *F. vesca* showed higher DPPH scavenging activity, reducing power, and thiobarbituric acid reactive substances inhibition which positive correlated with the highest content of total phenolic compounds (253.42 mg.g^{-1}) mainly due to flavan-3-ols (226.7 mg.g^{-1}) in the infusion and the highest content of total dihydroflavonols (32.97 mg.g^{-1}) in the decoction. This report is example of the great antioxidant potential of *F. vesca* roots that could be displayed directly by consumption in infusions/decoctions or included in pharmaceutical products or pharmaceutical formulations (**Dias *et al.*, 2015**).

CONCLUSION

In general, higher consumption of fruits and vegetables is associated with the prevention of chronic diseases such as diabetes, heart disease, and certain cancers. Apart from essential nutrients, fruits and vegetables also contain a variety of different phytochemicals that can act as antioxidants (flavonoids, phenolic acids, polyphenols), prevent oxidative stress leads to cell damage, and exhibit other bioactive physiological properties. This study demonstrates differences in chemical composition between three *Fragaria* species grown in Belgium, Hungary, Poland, and Slovakia as well as significant differences between garden-grown and wild species. The highest content of polyphenols and anthocyanins were determined in the wild species and results confirmed double values in these parameters in comparison of domestic and foreign garden species. Wild species achieved a significantly higher ability to destroy free radicals in all extracts against garden *Fragaria* x *ananassa*. Natural forms collected from woods and meadows achieved higher values in all examined traits and our experiments confirmed the healthy potential of fruits from natural conditions. Also, wild forms play a prominent role in human nutrition and have health benefits for the consumer in a complex perception. Thus, this study will be useful for obtaining healthy food products and in the pharmacological industry.

Conflicts of interest: All authors declare no conflicts of interest.

Ethical statement: This article doesn't contain any studies that would require an ethical statement.

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