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Heavy Metal Content and Antioxidant Activity of Strawberries (*Fragaria vesca* L.)

Judita Lidiková[1], Natália Čeryová*[1], Olga Grygorieva[2], Jan Brindza[3], Alžbeta Demianová[1], Lukáš Jurčaga[1]

[1] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Nitra, Slovak Republic
[2] M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Department of Fruit Plants Acclimatisation, Kyiv, Ukraine
[3] Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Plant and Environmental Sciences, Nitra, Slovak Republic

Judita Lidiková: https://orcid.org/0000-0001-9922-4300
Natália Čeryová: https://orcid.org/0000-0002-1865-5131
Olga Grygorieva: https://orcid.org/0000-0003-1161-0018
Jan Brindza: https://orcid.org/0000-0001-8388-8233
Alžbeta Demianová: https://orcid.org/0000-0002-3615-5747
Lukáš Jurčaga: https://orcid.org/0000-0001-9693-4796

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Strawberry (*Fragaria vesca* L.) plays a valuable role in human nutrition due to its richness in minerals, vitamins, antioxidants, flavonoids, anthocyanins, and other bioactive compounds that benefit the human body. This study focused on assessing the total polyphenol content (TPC) and antioxidant activity (AA) using UV-Vis spectrophotometry, and levels of selected heavy metals (Pb and Cd) using atomic absorption spectroscopy in strawberries grown at five monitored locations in Slovakia (Hliník, Štitáre, Rišňovce, Sabinov, and Oravská Lesná). TPC values ranged from 1194 to 2389 mg GAE.kg^{-1} FW, while AA values ranged from 2.89 to 3.88 mg TE.kg^{-1} FW. Content of Pb ranged from 0.05–0.97 mg.kg^{-1} FW, and content of Cd ranged from 0.05–0.11 mg.kg^{-1} FW. The measured concentrations of Pb and Cd in strawberries were higher than the limit values set by the European Commission, except for Pb in samples from Hliník, Štitáre, and Rišňovce. Nonetheless, the strawberries from all monitored locations are safe for consumption, as the provisional maximum tolerable daily intake (PMTDI) for cadmium was not exceeded. Overall, strawberries from these locations are a good source of health-beneficial antioxidants and contain relatively low levels of hazardous metals. A negative correlation was observed between Pb levels in strawberry fruits and TPC and AA values.

Keywords: strawberry, heavy metals, polyphenols, antioxidant activity

Introduction

Strawberries are one of the most widely consumed fruits in the Slovak Republic. Known for their rich nutritional profile and appealing sensory qualities, they are often enjoyed raw. Strawberry fruits are low in calories, providing only 32 kcal per 100 g, and have a high water content (over 80%) (Newerli-Guz et al., 2023). They are particularly valued for their bioactive compounds, which include natural antioxidants such as flavonoids, phenolic acids, anthocyanins, and ellagitannins (Ariza et al., 2016). Among the phenolic acids present, strawberries contain hydroxybenzoic and hydroxycinnamic acids. While strawberries are

***Corresponding Author:** Natália Čeryová, Slovak University of Agriculture in Nitra, Tr. Andreja Hlinku 2, 949 76 Nitra, Slovakia
natalia.ceryova@uniag.sk

especially noted for their high vitamin C content, they are also a valuable source of fat-soluble vitamins and folates, contributing to their impressive antioxidant capacity (Miller et al., 2019).

Strawberries contain notable levels of organic acids, including malic, tartaric, citric, and gallic acids, which, along with carbohydrates, significantly influence the fruit's sensory characteristics (Paparozzi et al., 2018). The most prominent group of flavonoids in strawberries are anthocyanins, which exhibit strong antioxidant and anti-inflammatory properties. The primary anthocyanins present are pelargonidin 3-rutinoside and pelargonidin 3-glucoside, both known for their anti-inflammatory effects (Amini et al., 2017). Additionally, strawberries are a rich source of dietary fiber, pectin, and essential minerals. Various strawberry cultivars contain a broad spectrum of minerals, including calcium, magnesium, sodium, potassium, phosphorus, manganese, zinc, copper, and iron (Hossain et al., 2016).

These bioactive compounds can neutralize free radicals, inhibit the expression of inflammatory genes, and protect against oxidative damage. Consequently, they are linked to protective effects against the onset and progression of various chronic diseases, including cardiovascular conditions, certain cancers, Alzheimer's disease, and other related disorders (Atmani et al., 2009; Zunino et al., 2012; Joseph et al., 2014). Ellagic acid in strawberries is noted for its potential in reducing inflammation during acute lung injury (Favarin et al., 2013) and exhibits both antimutagenic and anticarcinogenic properties.

Heavy metal pollution poses a significant threat to agricultural production and can create ecological risks that ultimately impact human health. Strawberries, which can be grown on less fertile, often acidic soils, are particularly susceptible to absorbing higher levels of heavy metals under such conditions. Although strawberries are not considered accumulators of these harmful metals, their potential intake and associated risks warrant closer attention. Therefore, this study aimed to determine the levels of selected risk metals, as well as the polyphenol content and antioxidant activity in strawberries from the various monitored locations.

Materials and methodology

Plant material

The researched samples of strawberries (cultivar Karmen) were obtained from various locations in Slovakia (Hliník, Štitáre, Rišňovce, Sabinov, Oravská Lesná). Samples have been harvested at the stage of complete ripeness.

Cadmium and lead content

Cd and Pb content was determined according to Vollmannova et al. (2014) after mineralization of fresh homogenized sample in a mixture of 5 mL of HNO_3 (Suprapur®, Merck, Darmstadt, Germany) and 5 mL of deionized water (0.054 µS/cm) in the Mars Xpress 5 closed microwave digestion system (CEM Corp., Matthews, NC, USA). Mineralized samples were analyzed by the atomic absorption spectrometer SpectrAA 240Z. The limit of detection of Cd and Pb was 10 $ng.kg^{-1}$, and the limit of quantification was 30 $ng.kg^{-1}$

Extract preparation

25 g of fresh homogenized samples were extracted in 50 ml of 80% methanol for 12 hours and filtered through filtrating paper.

Total polyphenol content

Total polyphenol content was determined by the Folin-Ciocalteu colorimetric method (Lachman et al., 2003). Folin-Ciocalteu phenol reagent (Merck, Germany), 20% Na_2CO_3 (Sigma Aldrich, USA), and distilled water were used. 0.1 mL of extract was pipetted into a 50 mL volumetric flask. 0.85 mL of Folin Ciocalteau reagent was added, and after 3 minutes, 5 mL of 20% Na_2CO_3 was added. The mixture was stirred, and the flask was filled with distilled water to the mark. Flasks were left for 2 hours at laboratory temperature and then measured against a blank solution at 765 nm, using a Shimadzu UV/VIS scanning spectrophotometer. Total polyphenol content was expressed as mg of gallic acid equivalent in 1 kg, based on the calibration curve (R^2 = 0.997)

Antioxidant activity

Antioxidant activity was measured by DPPH radical scavenging assay (Brand Williams et al., 1995). DPPH•+ radical (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich, USA) and methanol (Sigma Aldrich, USA) were used to produce a working DPPH solution. 1 mL of extract was pipetted into 3.9 mL of working DPPH solution, stirred, and left in the dark. After 10 minutes, the solution was measured against a blank solution at nm, using a Shimadzu UV/VIS scanning spectrophotometer. Antioxidant activity was expressed at 130 mmol of Trolox equivalent in 1 kg, based on the calibration curve (R^2 = 0.996).

Statistical analysis

Statistical analysis was performed using XLSTAT (Lumivero, 2024). Kruskal-Wallis test (nonparametric analysis of variance) was conducted to find statistically significant information about differences among the tested samples ($p <0.05$). Spearman correlation was performed to establish the relationship between tested parameters.

Results and discussions

The accumulation of heavy metals in soil and their subsequent entry into the food chain can pose significant health risks for consumers (Bystrická et al., 2015). Table 1 presents the Pb and Cd concentrations measured in strawberry fruits.

Pb levels ranged from 0.05 mg.kg^{-1} FW in samples from Rišňovce to 1.0 mg.kg^{-1} FW in samples from Oravská Lesná. Among the five monitored locations, lead levels exceeded the regulatory limit at two sites, Sabinov and Oravská Lesná, where the limit of 0.1 mg.kg^{-1} FW, as established by Commission Regulation (EU) 2023/915 on maximum limits for contaminants in food, was surpassed. Notably, strawberries from Oravská Lesná displayed a lead concentration ten times higher than the limit. For cadmium, concentrations ranged from 0.05 to 0.11 mg.kg^{-1} FW, exceeding the regulatory limit of 0.03 mg.kg^{-1} FW at all monitored locations.

Similar levels of Pb and Cd in strawberries were reported by Yang et al. (2022), whereas Rusin et al. (2021) observed lower concentrations of these metals in strawberries. Bystrická et al. (2015) reported the content of Cd in strawberry cultivars in the range of 0.01 – 0.04 mg.kg^{-1}, and the content of Pb under the detection limit, despite elevated soil metals level. Pb and Cd are non-essential, toxic elements that are persistent in the environment and challenging to degrade biologically. Cadmium is recognized as a major environmental pollutant with high phytotoxicity. In humans, cadmium exposure has nephrotoxic and hepatotoxic effects and can cause severe damage to the lungs and bones (EFSA, 2012; Rehman et al., 2018). Lead exposure, a longstanding public health concern is associated with neurological issues, including learning disabilities and reduced IQ. Lead also contributes to oxidative stress in the body and disrupts several biochemical processes by binding to functional groups, inhibiting various enzymes, and interfering with vitamin D and calcium metabolism (Thompson, 2018).

Although cadmium levels exceeded established limits at all monitored locations, this exceedance does not necessarily pose a direct threat to human health. According to WHO (2021), the Provisional Maximum Tolerable Daily Intake (PMTDI) for cadmium is set at 25 μg/kg body weight per month, which equates to a weekly intake of 0.437 mg for a 70 kg adult. Our highest recorded cadmium concentration in strawberries was 0.11 mg.kg^{-1} FW. Based on an average weekly strawberry consumption of 23.07 g (Statistical Office of Slovak Republic, 2023), this would result in an intake of 0.0025 mg of cadmium—well below the PMTDI threshold of 0.437 mg. For lead, the WHO has not provided a PTWI value since 2011.

Total polyphenol content and antioxidant activity

In this study, we also measured the total polyphenol content (TPC) and antioxidant activity (AA), as heavy metals are known to influence secondary metabolite production in plants. Table 2 presents TPC and antioxidant activity (AA) values for both fresh and dry matter.

Table 1 Lead and Cadmium content in analyzed samples

Locality	Pb (mg.kg^{-1} FW)	Cd (mg.kg^{-1} FW)	Pb (mg.kg^{-1} DM)	Cd (mg.kg^{-1} DM)
Hliník	0.07 ±0.01^{ab}	0.05 ±0.01^{a}	0.35 ±0.05^{a}	0.25 ±0.05^{a}
Štitáre	0.09 ±0.01^{abc}	0.06 ±0.01^{ab}	0.45 ±0.05^{abc}	0.29 ±0.05^{ab}
Rišňovce	0.05 ±0.01^{a}	0.08 ±0.01^{ab}	0.38 ±0.08^{ab}	0.63 ±0.08^{b}
Sabinov	0.97 ±0.08^{bc}	0.11 ±0.02^{b}	5.23 ±0.43^{bc}	0.59 ±0.11^{ab}
Oravská Lesná	1.00 ±0.11^{c}	0.11 ±0.01^{b}	5.72 ±0.63^{c}	0.63 ±0.06^{b}
Limit value*	0.10	0.03		

Notes: Results are expressed as mean of 4 replications ± standard deviation. Different letters indicate significant differences. * Limit value set by Commission Regulation (EU) 2023/915 on maximum limits for contaminants in food (mg.kg^{-1} FW)

Table 2 Total polyphenol content and antioxidant activity in analyzed samples

Locality	TPC (mg GAE.kg^{-1} FW)	AA (mmol TE.kg^{-1} FW)	TPC (mg GAE.kg^{-1} DM)	AA (mmol TE.kg^{-1} DM)
Hliník	2099 ±41.1^{abc}	3.84 ±0.06^{bc}	10382 ±203^{ab}	19.0 ±0.29^{ab}
Štitáre	2389 ±31.9^{c}	3.88 ±0.05^{c}	11920 ±159^{abc}	19.4 ±0.25^{abc}
Rišňovce	2030 ±19.5^{ab}	3.56 ±0.03^{abc}	15322 ±147^{c}	26.9 ±0.23^{c}
Sabinov	1194 ±16.0^{a}	2.89 ±0.05^{a}	6440 ±86.0^{a}	15.6 ±0.25^{a}
Oravská Lesná	2122 ±33.4^{bc}	3.50 ±0.04^{ab}	12137 ±191^{bc}	20.0 ±0.25^{bc}

Notes: Results are expressed as mean of 4 replications ± standard deviation. Different letters indicate significant differences. TPC – total polyphenol content; GAE – gallic acid equivalent; AA – antioxidant activity; TE – ttrolox equivalent; FW – fresh weight; DM – dry matter.

The highest TPC was observed in strawberries from Štitáre (2389 mg GAE.kg-1 FW), while the lowest was recorded at Sabinov (1194 mg GAE.kg-1 FW). Similar TPC values in strawberries have been reported by Banaś and Korus (2016). The highest TPC content in strawberries, reported by Bojarska et al. (2006), ranged from 497.20 to 787.94 mg GAE.100 g-1 FW. Dzanfezova et al. (2020) reported 1900 – 5700 mg GAE.kg-1 FW in strawberries, depending on genotype and environmental factors. Trebichalský et al. (2015) reported 1262.91–2343.63 mg GAE.kg-1 FW in strawberry cultivars, depending on the cultivar, and soil contamination. TPC in strawberries varies widely due to several factors, including environmental, climatic, and cultivation conditions, as well as temperature, light intensity, variety, soil pH, fertilizer type and amount, water availability, and others (Dzanfezova et al., 2020; Newerli-Guz et al., 2023).

Our study observed a negative correlation between accumulated Pb levels in strawberry fruits and TPC and AA values. However, cadmium did not appear to act as a stress factor in TPC formation, as no positive correlation was found between cadmium content and TPC. According to Trebichalský et al. (2015) TPC and AA of strawberry fruits correlated positively with total content and mobile forms of Pb and Cd in soil.

A positive correlation was observed between TPC and AA. Studies show that the antioxidant activity in strawberries correlates with total polyphenolic content; however, different types of polyphenols, such as anthocyanins, ellagitannins and ellagic acid, contribute variably to this activity (Meyers et al., 2003; Nowicka et al., 2019; Yildiz et al., 2014). According to Chaves et al. (2017) antioxidant activity of strawberries may be related to its content of anthocyanins.

Table 3 Relationships between monitored parameter

	Pb	Cd	TPC	AA
Pb	**1**			
Cd	**0.628**	**1**		
TPC	**-0.511**	0.115	**1**	
AA	**-0.502**	0.288	**0.933**	**1**

Note: Values in bold are statistically significant ($p < 0.05$). TPC – total polyphenol content; AA – antioxidant activity

Conclusion

Strawberries are among the most popular fruits globally and are valued for their health-promoting compounds, such as polyphenols, which exhibit antioxidant properties. Monitoring heavy metals in strawberries is essential to ensure the safety of these widely consumed fruits. In this study, strawberries from Sabinov and Oravská Lesná were found to contain lead levels exceeding the threshold values set by the European Commission (EC). Cadmium levels were above EC thresholds in strawberries from all studied locations. Despite these exceedances, the concentrations of Pb and Cd remain within safe limits for consumption, as determined by the Provisional Maximum Tolerable Daily Intake (PMTDI) values set by the WHO.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical statement

This article does not include any studies necessitating an ethical statement.

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