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Antioxidant Activity and Content of Heavy Metals in Cherry Fruit (*Prunus avium* L.)

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Cherries are highly valued not only for their appealing taste and nutritional benefits but also for their rich profile of phenolic compounds, which contribute significantly to their potential health-promoting properties. Apart from these beneficial substances, cherries may also accumulate traces of heavy metals, which raises important concerns as these metals pose health risks and may interfere with the beneficial actions of phenolic compounds. This study investigates the total polyphenol content (TPC), antioxidant activity (AA), and heavy metal contamination (Cd and Pb) in cherry (*Prunus avium* L.) fruits grown across various localities. TPC ranged from 495.7 to 1198 mg GAE.kg^{-1} FW, and AA varied between 1.85 and 2.99 mmol TE.kg^{-1} FW, with both parameters significantly impacted by the growing location. Heavy metal analysis showed Cd concentrations exceeding the regulatory limit (0.03 mg.kg^{-1} FW) in all samples, while Pb levels surpassed the limit (0.10 mg.kg^{-1} FW) in specific localities. A positive correlation between TPC and AA suggests higher polyphenol levels enhance antioxidant capacity. However, Cd content positively correlated with TPC and AA, whereas Pb exhibited a negative correlation. These findings suggest that environmental factors, including soil and urban pollution, play a crucial role in heavy metal accumulation, which may influence polyphenol synthesis and antioxidant potential. Despite exceeding Cd and Pb limits, the average consumption of cherries is unlikely to pose a significant health risk.

Keywords: *Prunus avium*, polyphenols, antioxidant activity, lead, cadmium

Introduction

Cherry fruits (*Prunus avium* L.) are widely recognized for their vibrant color, refreshing flavor, and health-promoting properties, attributed to a rich array of bioactive compounds. Notably, phenolic compounds – including anthocyanins, flavonoids, and phenolic acids – stand out for their contributions to cherries' high antioxidant activity (Hu et al., 2021). Anthocyanins, particularly cyanidin-3-rutinoside, and cyanidin-3-

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glucoside, are responsible for the characteristic deep red to purple fruit color and exhibit strong antioxidant activity, which helps protect the body's cells from oxidative stress. In addition to anthocyanins, other phenolic compounds such as quercetin, catechins, and chlorogenic acid are present, each adding to the antioxidant capacity and potential health benefits of sweet cherries (Usenik et al., 2008; Covaci, 2024). These antioxidants play a crucial role in neutralizing free radicals, potentially reducing oxidative stress and lowering risks of cardiovascular disease, certain cancers, and other degenerative conditions (Chaudhary et al., 2023). Research shows that the cherry fruit's phenolic content and antioxidant activity can vary significantly. Factors like fruit maturity, genetic differences, and environmental conditions – such as soil composition and altitude – play major roles in this variation (Cosme et al., 2017). For instance, studies indicate that darker-colored cherry cultivars generally contain higher phenolic levels and exhibit greater antioxidant potential than lighter-colored varieties, suggesting that pigmentation intensity may be a marker of bioactive compound content (Pissard et al., 2016). Additionally, polyphenolic content increases as cherries mature, suggesting that harvesting at optimal ripeness may enhance their antioxidant benefits (Mahmood et al., 2013).

Beyond these beneficial compounds, cherries also contain essential minerals like calcium, potassium, and magnesium, as well as trace amounts of heavy metals, which may accumulate due to environmental contamination from soil or water. The heavy metal presence raises health concerns, as excess levels can pose risks and may interfere with the beneficial actions of cherry phenolics. Heavy metals such as lead and cadmium are known to induce oxidative stress in biological systems, potentially counteracting cherries' antioxidant benefits (Goncalves et al., 2022). However, phenolic compounds, particularly anthocyanins and flavonoids, exhibit metal-chelating properties, which allow them to bind to and neutralize certain heavy metals, potentially reducing their harmful effects (Li et al., 2023; Sysak et al., 2023).

This dual role highlights the importance of investigating not only the antioxidant potential of cherries but also how their phenolic compounds interact with any metals present. Therefore, examining the relationship between heavy metal content, phenolic profiles, and antioxidant capacity in cherries is essential. Such insights can help understand how environmental pollutants impact cherry quality and its potential applications in nutraceuticals and functional foods. As cherries are increasingly valued for their health-promoting attributes, understanding these interactions allows for the maximizing potential of their antioxidant benefits while mitigating any risks associated with heavy metal exposure.

Materials and methodology

Plant material

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

Extract preparation

25 g of homogenized cherry fruits were extracted in 50 ml of 80% methanol for 16 hours and filtered through filtrating paper.

Total polyphenol content

Total polyphenol content was determined by the Folin-Ciocalteu colorimetric method according to 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 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 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 according to 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 as mmol of Trolox equivalent in 1 kg, based on the calibration curve (R^2 = 0.996).

Heavy metal content

Cd and Pb contents were determined 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) according to Vollmannova et al. (2014). 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}

Statistical analysis

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

Results and discussions

Total polyphenol content and antioxidant activity

The total polyphenol content and antioxidant activity of cherry fruits are given in Table 1.

Total polyphenol content ranged from 495.7 to 1,198 mg GAE.kg^{-1} FW (2,195–5,618 mg GA.kg^{-1} DM). The locality had a statistically significant impact on the TPC of cherry fruits. These values are roughly within the range of values reported by other authors. Hu et al. (2021) reported 870–1,730 mg GAE.kg^{-1} FW in cherry cultivars grown in Australia. Ceccarelili et al. (2018) reported 785.3–2,680.2 mg GAE.kg^{-1} FW in Italian ancient cherry cultivars. Vavoura et al. (2015) reported 951.4–1,703.5 mg GAE.kg^{-1} FW in cherry cultivars grown in Greece. Melicháčová et al. (2010) reported 278–1,058 mg GAE.kg^{-1} FW in cherries from Slovakia. Mahmood et al. (2013) reported 1,763.8 in unripened, 3,717.1 in semi-ripened, and 6,876.8 mg GAE.kg^{-1} DM in fully ripened cherry fruits. Pissard et al. (2016) reported a wider range of 520–5,880 mg GAE.kg^{-1} FW in cherry fruits.

Antioxidant activity ranged from 1.85 to 2.99 mmol TE.kg^{-1} FM (8.20 – 15.1 mol TE.kg^{-1} DM). The locality had a statistically significant impact on the AA of cherry fruits. These values are roughly within the range reported by Kelebek a Selli (2011) which were 2.08–4.73 mmol TE.kg^{-1} FW in Turkish cherry cultivars. Other authors reported higher values of AA. Pissard et al. (2016) reported the mean antioxidant activity of cherry fruits at 3.69 mmol TE.kg^{-1} FW. Li et al. (2019) reported 3.49 and 4.61 mmol TE.kg^{-1} FW in cherry cultivars Ranier and Bing. Zhaou et al. (2019) reported 4.00 and 3.8 mmol TE.kg^{-1} FW in cherry cultivars Hongdeng and Lapins respectively.

Lead and Cadmium content

Lead and Cadmium contents in analyzed samples are given in Table 2.

Table 1 Total polyphenol content and antioxidant activity of cherry fruits

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	823.6 ±9.22^{abc}	2.24 ±0.03^{abc}	2,927 ±32.8^{ab}	7.96 ±0.11^{a}
Štitáre	1,198 ±19.6^{c}	2.99 ±0.05^{c}	5,618 ±91.9^{c}	14.0 ±0.23^{bc}
Rišňovce	602.2 ±9.13^{ab}	2.09 ±0.05^{ab}	3,291 ±49.9^{abc}	11.4 ±0.27^{abc}
Sabinov	495.7 ±8.51^{a}	1.85 ±0.03^{a}	2,195 ±37.7^{a}	8.20 ±0.13^{ab}
Oravská Lesná	998.3 ±11.3^{bc}	2.96 ±0.03^{bc}	5,099 ±57.9^{bc}	15.1 ±0.15^{c}

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 – Trolox equivalent; FW – fresh weight; DM – dry matter.

Table 2 Lead and Cadmium content in cherry fruits

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); FW – fresh weight; DM – dry matter.

Table 3 Relationships between monitored parameter

Parameters	TPC	AA	Pb	Cd
TPC	**1**			
AA	**0.925**	**1**		
Pb	**-0.633**	**-0.591**	**1**	
Cd	**0.709**	**0.745**	-0.062	**1**

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

The content of Pb in samples ranged from 0.02 to 0.80 $mg.kg^{-1}$ FW (0.10–3.54 $mg.kg^{-1}$ DM). The content of Cd in samples ranged from 0.05 to 0.09 $mg.kg^{-1}$ FM (0.10–0.44 $mg.kg^{-1}$ DM). The locality had a statistically significant impact on the content of Pb and Cd in cherry fruits.

According to Einolghozati et al. (2022) cherry fruits contained 0.175–0.246 mg Pb and 0.012–0.069 mg Cd per kg. Soceanu (2009) reported, that the heavy metal content in cherry fruits varies with the development stage. The content of Cd in ripe cherries was 0.123 $mg.kg^{-1}$ DM, while Pb was not detected. Esposito et al. (2015) reported 0.025 mg $Pb.kg^{-1}$ FW and 0.005 mg $Cd.kg^{-1}$ FW in cherry fruits. Stachowiak et al. (2015) reported 0.02 – 0.05 mg $Cd.kg^{-1}$ DM, while Pb was not detected in the analyzed cultivars. The Cd and Pb accumulation in cherry fruits can be significantly influenced by environmental conditions and urban pollution sources (Khalilnezhad et al., 2024). Factors such as soil composition and terrain can also influence Cd and Pb transfer from soil to the edible parts of plants, affecting contamination levels (de Sousa et al., 2020).

The limit value of Pb (0.10 mg.kg-1 FW) set by the Commission Regulation (EU) 2023/915 was exceeded in the samples from Hliník, Štitáre, and Sabinov. The limit value of Cd (0.03 mg.kg-1 FW) set by the Commission Regulation (EU) 2023/915 was exceeded in all samples. Despite the exceeded amount of cadmium, the average consumption of cherries, which is 400 g per capita per year (Statistical Office of the Slovak Republic, 2023) should not mean a risk for the consumer, considering that it would represent only 0.05–0.18% of the provisional tolerable weekly intake (PTWI). Regarding lead, it is currently not possible to establish a PTWI that would be considered health-protective.

A statistically significant positive correlation between TPC and AA of cherries was observed, which was also reported by Cao et al. (2015), Pissard et al. (2016), Skrzyńsy et al. (2016), and Ceccarelli et al. (2018). Specific polyphenolic compounds in cherries, such as cyanidin derivatives and kaempferol, have been shown to exhibit synergistic effects, enhancing the antioxidant capacity when combined (Kirakosyan et al., 2010). Ahmad et al. (2024) examined 23 fruit species, correlating high total polyphenol content with strong antioxidant activity. This research highlights how fruits with high polyphenol levels can be especially beneficial in combating degenerative diseases.

The positive correlation between Cd and TPC, as well as AA, might be indicative of a plant defence mechanism, where polyphenolic compounds mitigate the oxidative damage induced by Cd exposure. Unlike Cd, Pb showed a negative correlation with TPC and AA, suggesting that areas with higher Pb contamination may see reduced polyphenol content and antioxidant capacity in cherry fruits. This inverse relationship, probably, is the result of the metabolic stress Pb places on the plants, potentially impairing the synthesis of phenolic compounds. Zupka et al. (2015) found a correlation between cadmium content and anthocyanin levels in berries, highlighting that metal accumulation may be linked to variations in polyphenolic compounds, which function as antioxidants to mitigate metal-induced oxidative damage. Additionally, Simek et al. (2016) observed that cadmium exposure in cucumber plants increases the leaf phenolic compound content, likely as a protective response. Esmaeili (2024) reviewed the bioactivity of polyphenols and highlighted that these compounds play a critical role in metal absorption, which may have implications for the antioxidant efficacy of polyphenol-rich fruits in metal-contaminated soils. This study underscores polyphenols' role in managing oxidative stress and their potential interaction with metal ions.

Conclusions

The results of this study underline the significant influence of locality on the polyphenol content, antioxidant activity, and heavy metal contamination in cherry fruits. TPC and AA values fall within ranges reported for various cherry cultivars, confirming their role as important sources of antioxidants. The observed positive correlation between TPC and AA supports the health benefits of cherries, suggesting that higher polyphenol content enhances their antioxidant potential. However, the high Cd levels in all samples, with elevated Pb levels in certain localities, raise food safety concerns due to potential environmental contamination. While the low average consumption of cherries suggests minimal health risks from heavy metal exposure, the lack of a tolerable weekly intake level for Pb remains a concern. The relationship between metal accumulation and polyphenol content indicates that polyphenolic compounds may play a protective role in plants, potentially mitigating metal-induced oxidative stress. These findings highlight the need for monitoring heavy metal contamination in cherry production regions and support further research into the role of polyphenols in countering oxidative damage in metal-rich environments.

Conflict of interest

The authors declare no conflict of interest.

Ethical statement

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

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