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# Chemical composition of *Castanea sativa* Mill. fruits

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Nowadays, especially important is the introduction of new plants into cultivation in connection with global climate change. Acclimatization of new fruit plants in Ukraine contributes to an increase in the biodiversity of flora. *Castanea sativa* Mill. (sweet chestnut) fruits belong to underutilized fruit plants of the forest-steppe of Ukraine, as a promising and economically profitable crop. The aim of the study, which focused on the nutritional value and composition of *C. sativa* fruits of Ukrainian origin, was to strengthen the knowledge about the contents of essential nutrients, fatty and amino acids profiles, and the content of selected elements. Chestnut fruits are distinguished by low lipids content (1.9%), a substantial share of proteins (14.9%) and fructose (19.3 $g.kg^{-1}$), and high β-carotene content (143.1 $mg.kg^{-1}$). The lipid fraction of chestnut fruits is strongly dominated by SFAs, namely palmitic acid (C16:0) 37.93 g.100 $g^{-1}$ of oil, followed by the MUFA oleic acid (C18:1 *9c*) 9.07 g.100 $g^{-1}$ of oil, and PUFA α-linolenic (C18:3 *9c12c15c*) 9.01 g.100 $g^{-1}$ of oil. From 18 determined amino acids (128.1 $g.kg^{-1}$ of DW), glutamic acid was found to be the major component (17.2 $g.kg^{-1}$). Surprisingly essential amino acids/total amino acids ratio amounted to 44%, which according to FAO WHO may be regarded as high-quality protein plant food. Calcium and phosphorus were the most abundant elements (8,213 and 8,155 $mg.kg^{-1}$ of DW respectively), simultaneously with a low Na : K ratio (low amount of Na 9 $mg.kg^{-1}$). Summing up, presented composition and literature data regarding nutrients proved that *C. sativa* fruits are valuable ingredients in a healthy diet.

**Keywords:** sweet chestnut, nutritional value, fatty and amino acids, elements

## Introduction

Nowadays, especially important is the introduction of new plants into cultivation in connection with global climate change (Klymenko et al., 2017; Raza et al., 2019). Acclimatization of new fruit plants in Ukraine contributes to an increase in the biodiversity of flora. *Castanea sativa* Mill. fruits belong to underutilized fruit plants in the forest-steppe of Ukraine, as a promising and economically profitable crop (Klymenko and Grygorieva, 2013; Grygorieva et al., 2017; Klymenko et al., 2017).

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*Castanea sativa* Mill. (chestnut) belongs to Fagaceae Dumort. family. In total, 13 Castanea species are recognized and are native to the temperate zone of the Northern Hemisphere; 5 in East Asia, 7 in North America, and one in Europe (Burnham et al., 1986). The most important of them are: *Castanea sativa* (Europe, Asia Minor, North Africa), *Castanea dentata* (Marsh.) Borkh. (USA), *Castanea mollissima* Blume and *C. crenata* Sieb. et Zucc. (Eastern Asia). However, *Castanea sativa* (sweet chestnut) is the most commonly consumed (Goulão et al., 2001).

*Castanea sativa* fruits have become very important in the human diet due to their nutritional composition and numerous health benefits. Fruits are rich in carbohydrates and can be regarded as a good source of essential fatty acids (Borges et al., 2006), vitamins C and E (Peña-Mendez et al., 2008; Barreira et al., 2009), organic acids (Ribeiro et al., 2007), polyphenols (Neri et al., 2010). *Castanea sativa* fruits are recognized as generally low in fat content, thus supporting a decrease in cholesterol levels. Moreover, fruits contain a high amount of macro- (K, P, Mg, Ca, Na) and micro-nutrients (Mn, Fe, Zn, and Cu) (Poljak et al., 2021). Nuts are predominantly consumed in roasted or boiled form, but also can be used as valuable ingredient in cake and candy manufacturing (Mert et al., 2007). It was stated that cooked *Castanea sativa* is a rich source of phenolic compounds, such as gallic and ellagic acids), and organic acids (mostly citric acid) (Gonçalves et al., 2010).

The study aims to strengthen the knowledge about the nutritional value of *Castanea sativa* Mill. fruits of Ukrainian origin. For that purpose, the contents of most essential nutrients, profiles of fatty and amino acids, and the content of selected elements of *Castanea sativa* were determined.

## Material and methodology

### Sampling

Fruits of *Castanea sativa* Mill. (Figure 1) were collected in July 2022 from trees growing in the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv, Ukraine; 197 m a.s.l.). The biochemical composition of fruits was determined in dried matter.

**Figure 1** *Castanea sativa* Mill.
(A) – tree; (B) – fruits

### Chemicals and reagents

All the chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany), and CentralChem (Slovakia).

### Analysis of proximate composition

Dry matter, ash, and protein content were determined according to CSN-EN 12145 procedures (1997). Total lipid content was determined according to the ISO method (ISO 659; 1998).

### Analysis of sugars

For the determination of sugar content, 1 g of fruits was vigorously shaken with 10 mL of water/ethanol mixture (4 : 1) on a vertical shake table (GFL, Germany). After 1 h of extraction, the mixture was centrifuged at 6,000 rpm for 4 min (EBA 21, Hettich, Germany). The supernatant was filtered through filter paper with 0.45 mm pore size (Labicom, Czech Republic) and filled up to 50 mL in a volumetric flask with ultrapure water.

An HPLC analysis of sugars (fructose, maltose, sucrose, lactose) was performed using an Agilent Infinity 1,260 instrument (Agilent Technologies, USA) equipped with an ELSD detector. Separation of sugars was conducted on a Prevail Carbohydrates ES column (250 × 4.6 mm). Acetonitrile/water (75 : 25 v/v) was used as the mobile phase. The identification of sugars was made by comparing the relative retention times of sample peaks with standards Sigma-Aldrich (Steinheim, Germany). The contents of sugars were expressed as g.kg$^{-1}$ of dry weight.

### β-carotene content

The extraction was performed by the method of Sarker and Oba (2019). Briefly, 1 g of dry chestnut fruits was ground thoroughly in a mortar and pestle with 10 mL of 80% acetone. After removing the supernatant in a volumetric flask, the extract was centrifuged at 10,000 × g for 3–4 min. The final volume was brought up to 20 mL. The absorbance was measured at 480 and 510 nm using a spectrophotometer (UV-VIS spectrophotometer, Jenway Model 6405, England). The content of β-carotene was expressed as mg of β-carotene per kg of dry weight. The following formula was used to calculate β-carotene content:

$$\beta\text{-carotene} = 7.6(\text{abs. at } 480) - 1.49(\text{abs. at } 510) \times \text{final volume}/1{,}000$$

### Elemental analysis

The contents of macro-, microelements, and trace metals were determined by the inductively coupled plasma optical emission spectroscopy (ICP-OES) according to Divis et al. (2015) by using an ICP-OES instrument (Ultima 2, Horiba Scientific, France). Fruits were prepared for analysis after microwave digestion (Milestone 1200, Milestone, Italy), 0.25 g of sample was decomposed in a mixture of nitric acid (6 mL) (Analytika Praha Ltd, Czech Republic) and hydrochloric acid (2 mL) (Analytika Praha Ltd, Czech Republic). After the decomposition sample was filtered through filter paper (0.45 mm pore size) and filled up to 25 mL in a volumetric flask with pure water. The results were expressed as mg.kg$^{-1}$ of dry weight.

### Determination of amino acids

The amino acid profile was determined by ion-exchange chromatography using an AAA-400 Amino Acid Analyzer (Ingos, Czech Republic) and post-column derivatization with ninhydrin and a VIS detector. Separation was provided on a glass column (length 350 mm, inner diameter 3.7 mm) filled with a strong cation exchanger in the LG ANB sodium cycle (Laboratory of Spolchemie) with an average particle size of 12 µM and 8% porosity. The column was heated within the range of 35–95 °C, with the elution of amino acids at 74 °C. A double-channel VIS detector with an inner cell volume of 5 µL was set to 440 and 570 nm. A solution of ninhydrin was prepared in 75% v/v methyl cellosolve and 2% v/v 4 M acetic buffer (pH 5.5). SnCl2 was used as a reducing agent. The solution of ninhydrin was stored in an inert atmosphere ($N_2$) without access of light at 4 °C. The flow rate was 0.25 mL.min$^{-1}$, and the reactor temperature was 120 °C. Individual amino acid values were expressed as g.kg$^{-1}$ of dry fruits.

### Fatty acid composition

Lipids extracted from *Castanea sativa* fruits were converted to fatty acid methyl esters (FAME) to determine fatty acid (FA) composition according to the official method Ce 2-66 (1997). The FAME profile was analyzed by gas chromatography (GC-6890-N, Agilent Technologies, Santa Clara, USA) equipped with capillary column DB-23 (60 m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies, Santa Clara, CA, USA) and FID detector (250 °C; constant flow, hydrogen 40 mL.min$^{-1}$, air 450 mL.min$^{-1}$). A detailed description of the chromatography conditions is presented in the work of Szabóová et al. (2020). Standards of a C4-C24 FAME mixture (Supelco, Bellefonte, PA, USA) were applied to identify FAME peaks. The evaluation was carried out by the ChemStation 10.1 software. The contents of FAs were expressed as g.100.g$^{-1}$ of oil.

### Statistical analysis

The results were subjected to one-way ANOVA followed by the Tukey-Kramer test when the differences between mean values were considered significant at $p < 0.05$. The variability of all parameters was evaluated by descriptive statistics. The results were presented as means with standard error (SE). The PAST 2.17 software was used.

## Results and discussion

Chestnut fruits contain various nutrients (proteins, lipids/fat, free sugars), vitamins (Table 1), and minerals (Table 3) that are important for human health. It was previously stated that chestnut fruits are mainly composed of carbohydrates: primarily starch, ranging from 38.6 up to 67.2 g.100 g$^{-1}$ of dry weight (DW) (de Vasconcelos et al., 2010a). For the commercial quality of chestnut fruits, the presence of monosaccharides and disaccharides (glucose, fructose, sucrose, and maltose) is highly important. Interestingly, saccharose content can reach up to one-third of total sugars. The study of Ciucure et al. (2022) showed that sucrose was the most abundant sugar in chestnut fruits with values ranging from 20.34 up to 154.94 g.kg$^{-1}$ of DW. The sucrose level of chestnut fruits cultivated in Romania was similar to those grown in Turkey (68.20–174.00 g.kg$^{-1}$ of DW) (Mert et al., 2017), but lower than in chestnut fruits cultivated in Italy (2.98–245.09 g.kg$^{-1}$ of DW) (Beccaro et al., 2020) and Portugal (40.30–233.00 g.kg$^{-1}$ of DW) (Barreira et al., 2010), and higher compared with chestnut fruits cultivated in Tenerife (Spain) (31.10–99.40 g.kg$^{-1}$ DW) (Suárez et al., 2012). In our study, the content of fructose was relatively high 19.3 g.kg$^{-1}$ of DW in comparison with other analyzed sugars (Table 1). Research of Ciucure et al. (2022) revealed that fructose content ranged between 1.55–14.35 g.kg$^{-1}$ of DW, and glucose 1.56–14.46 g.kg$^{-1}$ of DW. However, data presented by Ciucure et al. (2022) was higher than those reported by other Authors who found glucose and fructose contents between not detected level and 3.1 g.kg$^{-1}$ of DW for both monosaccharides (Míguelez et al., 2004) or between 0.56–2.40 g.kg$^{-1}$ of DW for fructose and 0.49–1.90 g.kg$^{-1}$ of DW for glucose (Suárez et al., 2012). Mert and Ertürk (2017) indicated fructose content between 1.5–8.0 g.kg$^{-1}$ of DW and glucose between 4.0–11.3 g.kg$^{-1}$ of DW – similar to Ciucure et al. (2022).

**Table 1** Proximate composition of *Castanea sativa* Mill. fruits (means ±SE)

| Component | Content |
|---|---|
| **Proteins (%)** | 14.60 ±0.67 |
| **Lipids (%)** | 1.90 ±0.09 |
| **Fructose (g.kg$^{-1}$)** | 19.30 ±0.48 |
| **Maltose (g.kg$^{-1}$)** | <0.5 |
| **Sucrose (g.kg$^{-1}$)** | <0.5 |
| **Lactose (g.kg$^{-1}$)** | <0.5 |
| **Dry matter (%)** | 92.16 ±3.12 |
| **Ash (%)** | 4.38 ±0.11 |
| **β-carotene (mg.kg$^{-1}$)** | 143.10 ±3.56 |
| **Vitamin A (retinyl acetate) (mg.kg$^{-1}$)** | <0.1 |
| **Vitamin E (α-tocopherol) (mg.kg$^{-1}$)** | 74.50 ±2.19 |

It should be pointed out that *Castanea sativa* fruits contain a relatively low content of lipids (1.9%) and nutritionally important PUFAs (Tables 1 and 2). Lipids extracted from chestnut contained significant levels of saturated fatty acids (SFAs) 48.69 g.100 g$^{-1}$ of oil (Table 1). Other Authors confirmed that chestnut fruits are distinguished by low crude fat content, ranging values between 0.66–3.60%, compared with hazelnuts or almonds (36–77%). Significant differences among the fat levels in chestnut fruits depend on their cultivar, country of cultivation, crop year, and environmental conditions (Borges et al., 2007). Our results support the view that generally fruits are known as poor lipid sources, such as Rosa rugosa pericarp (0.67–0.88%), red raspberries, blackberries, or strawberries (0.25–0.42%) (Tabaszewska et al., 2021). The protein content (14.6%) was recognized as high compared with other cultivated fruits, mostly up to 1%. A study of Ertürk et al. (2006) showed that chestnut fruits may contain only 0.49–2.01 g.100 g$^{-1}$ of DW of lipids and 4.88–10.87 g.100 g$^{-1}$ of DW of proteins. Moreover, chestnut fruits proved to be a rich source of carotenoids, mainly β-carotene (143.1 mg.kg$^{-1}$), whose content was similar to carrots or sweet potato cultivars known for their high amounts.

The application of gas chromatography enabled the detection of 14 fatty acids in lipid fraction extracted from chestnut fruits, which belonged to SFA, MUFAs, and PUFAs. The results of fatty acid composition are presented in Table 2. It was found that chestnut lipid fraction is strongly dominated by SFAs, namely palmitic acid (C16:0) 37.93 g.100 g$^{-1}$ of oil, followed by the oleic acid (C18:1 *9c*) 9.07 g.100 g$^{-1}$ of oil, and α-linolenic (C18:3 *9c12c15c*) 9.01 g.100 g$^{-1}$ of oil. The fatty acid profile is dominated by SFAs, which provide high stability of lipid fraction, however, these fatty acids are considered as not beneficial for the cardiovascular system (Barreira et al., 2009). The obtained results are in accordance with the findings of Zhou et al. (2021).

**Table 2** Fatty acid composition (g.100 $g^{-1}$ of oil) of lipids of *Castanea sativa* Mill. fruits (means ±SE)

| Fatty acid | Content |
|---|---|
| **SFAs** | 48.69 ±0.33 |
| **C12:0** | 0.57 ±0.02 |
| **C14:0** | 1.53 ±0.07 |
| **C16:0** | 37.93 ±1.34 |
| **C17:0** | 0.65 ±0.03 |
| **C18:0** | 5.53 ±0.07 |
| **C20:0** | 0.61 ±0.02 |
| **C22:0** | 1.00 ±0.02 |
| **C24:0** | 0.87 ±0.01 |
| **MUFAs** | 11.38 ±0.05 |
| **C16:1** | 0.76 ±0.02 |
| **C18:1** | 9.07 ±0.13 |
| **C20:1** | 0.69 ±0.02 |
| **C22:1** | 0.86 ±0.03 |
| **PUFAs** | 25.46 ±0.29 |
| **C18:2** | 7.44 ±0.17 |
| **C18:3** | 9.01 ±0.35 |

saturated fatty acids – SFAs; monounsaturated fatty acids – MUFAs; polyunsaturated fatty acids – PUFAs

Even though only two fatty acids from PUFAs were detected, their contents can be regarded as highly important due to their health-promoting properties and significant share in the whole fatty acid profile (25.46 g.100 $g^{-1}$ of oil). Generally, chestnuts may be perceived as a valuable source of essential fatty acids, which are known as important in the regulation of plasma lipid levels, cardiovascular and immune function, insulin action, neuronal development, and visual function (Benatti et al., 2004). Recently consumers have searched for plant sources of PUFAs, especially α-linolenic acid belonging to the n-3 family (a substrate for long-chain PUFAs EPA and DHA synthesis). The consumption of n-3 FAs can slow down the growth of cancer cells and markedly reduce the side effects of chemotherapy (Borges et al., 2007). In turn, oleic acid is the main component of the cell membrane and cell nucleus and also facilitates the dissolution and absorption of fat-soluble vitamins, such as vitamin E (Reboul, 2019). Together with oleic acid, α-linolenic acid is involved in the prevention and treatment of cardiovascular and cerebrovascular diseases, thanks to cholesterol elimination (Visioli and Poli, 2020).

Eighteen amino acids were detected in *Castanea sativa* fruits, nine of them belonged to essential amino acids and nine to non-essential ones (Figure 2). The content of amino acids in chestnut fruits was at the level of 128.1 g.$kg^{-1}$ of DW, while the content of total essential amino acids was 56.3 g.$kg^{-1}$ of DW (amounted to 44%) and 71.8 g.$kg^{-1}$ of DW (56%) for total non-essential amino acids. It should be highlighted that glutamic acid was found to be the major component (17.2 g.$kg^{-1}$),

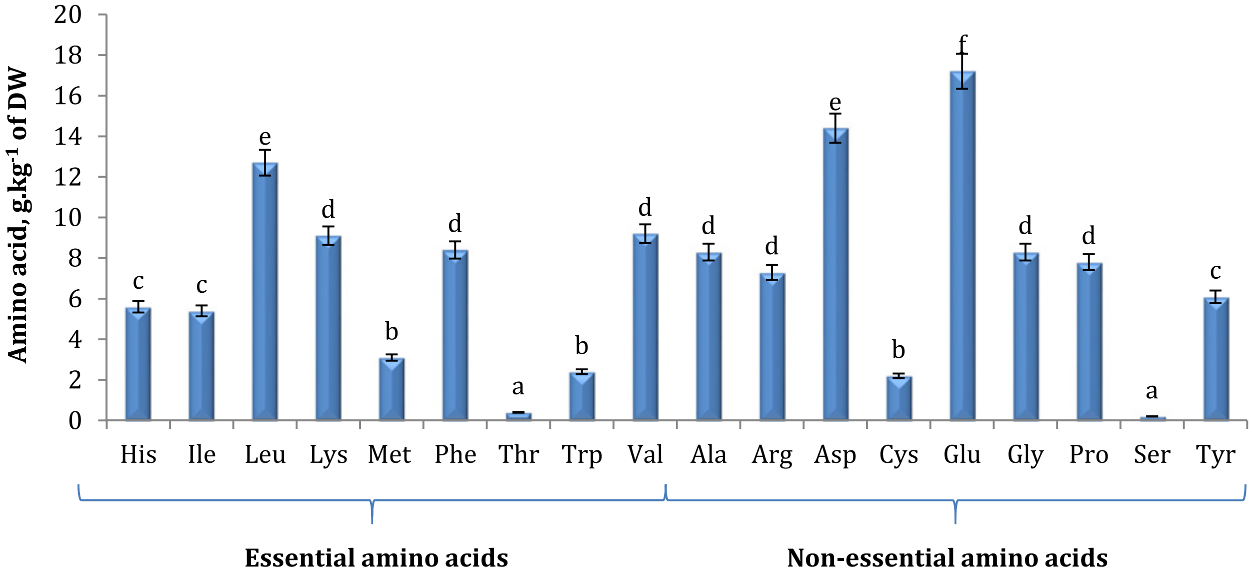

**Figure 2** Amino acid composition (g.$kg^{-1}$ of dry weight; DW) of *Castanea sativa* Mill. fruits
a, b, c, d, e, f – different superscripts indicate the significant differences at $p < 0.05$

followed by aspartic acid (14.4 g.kg$^{-1}$) and leucine (12.7 g.kg$^{-1}$). Generally, chestnut fruits are not considered as a rich source of amino acids. However, it seems worth noting the fact that the essential amino acids/total amino acids ratio amounted to 44%, which suggests that chestnut fruits could be a source of high-quality protein in the human diet. FAO and WHO indicate that foods with a ratio above 40% are a perfect protein source, just like the composition of animal-origin products (Tabaszewska et al., 2021).

The contents of macroelements (K, P, S, Ca, Mg, Na), microelements (Zn, Fe, Cu, Mn, Cr, Se), and metals (Al, As, Cd, Ni, Hg, Pb) in studied samples of chestnut fruits are presented in Table 3. First of all, it should be highlighted that there is still considerable data lacking for the contents of minerals in chestnut fruits. In our study calcium (Ca) and phosphorus (P) were undoubtedly the most abundant elements in *Castanea sativa* fruits (8,213 and 8,155 mg.kg$^{-1}$ of DW respectively), followed by Mg, K, and S. Ertürk et al. (2006) assayed 43–230 mg.100 g$^{-1}$ of Ca, 107–191 mg.100 g$^{-1}$ of P, and 70–160 mg.100 g$^{-1}$ of Mg. Like most vegetables, the Na content in chestnut is very low (9 mg.kg$^{-1}$ of DW). It should be noted that a high intake of Ca, Mg, and K together with a low Na intake is associated with protection against bone demineralization, arterial hypertension, insulin resistance, and cardiovascular risk (Segura et al., 2007). A low Na : K ratio makes chestnuts interesting for diets with a defined electrolytic balance (Borges et al., 2008). In the study of de Vasconcelos et al. (2010b) K and P were predominant and therefore these elements revealed the highest variability. The findings of Ciucure et al. (2022) revealed that K was the most abundant, ranging between 403.56 and 2,652.11 mg.100 g$^{-1}$ DW, followed by Na which ranged between 4.63 and 17.20 mg.100 g$^{-1}$ DW. Calcium contents ranged from 14.01 mg.100 g$^{-1}$ in 'Marsol' to 29.58 mg.100 g$^{-1}$ DW in 'Précoce Migoule', while Mg content varied between 48.62 mg.100 g$^{-1}$ in 'Marsol' and 102.88 mg.100 g$^{-1}$ DW in 'Précoce Migoule'.

Among microelements, the highest content was stated for Mn (712.5 mg.kg$^{-1}$ of DW). For comparison, the content of Mn in different chestnut cultivars was within the range of 2–3.3 mg.100 g$^{-1}$ of DW (de Vasconcelos et al., 2010b), and 0.7–5.5 mg.100 g$^{-1}$ (Ertürk et al., 2006). Chestnut fruits also contained significant amounts of Fe (1.84–3.43 mg.100 g$^{-1}$ of DW) and Cu (0.31–0.98 mg.100 g$^{-1}$ of DW) which are similar to values found in other studies (Ertürk et al., 2006; Poljak et al., 2021)], but lower than those obtained by Borges et al. (2008) and de Vasconcelos et al. (2010b).

**Table 3** Elements composition of *Castanea sativa* Mill. fruits (mg.kg$^{-1}$ of DW) (mean ±SE)

| Element | Content |
|---|---|
| **Macroelements** | |
| **K** | 1,861 ±120 |
| **P** | 8,155 ±232 |
| **Ca** | 8,213 ±221 |
| **S** | 1,597 ±111 |
| **Mg** | 2,114 ±119 |
| **Na** | 9.0 ±0.9 |
| **Microelements** | |
| **Zn** | 26.0 ±1.7 |
| **Fe** | 51.0 ±1.2 |
| **Cu** | 8.0 ±0.5 |
| **Mn** | 712.5 ±67 |
| **Cr** | 4.70 ±0.07 |
| **Se** | <0.2 |
| **Metals** | |
| **Al** | 12.5 ±0.9 |
| **As** | <0.3 |
| **Cd** | 0.147 ±0.002 |
| **Ni** | 3.21 ±0.03 |
| **Hg** | 0.014 ±0.003 |
| **Pb** | 0.30 ±0.002 |

Regarding the presence of metals, the content of aluminum (Al; 12.5 mg.kg$^{-1}$ of DW) dominated among all detected metals in *Castanea sativa* fruits. The content of Ni in chestnut fruits at the level of 3.21 mg.kg$^{-1}$ of DW, does not exceed the maximum permissible value of 67.9 mg.kg$^{-1}$ in vegetables for human consumption (Mensah et al., 2009). However, the content of metals was generally low. The accumulation of trace metals is a normal and essential process for the growth and nurturing of plants (Tabaszewska et al., 2021). Probably low amounts of trace elements resulted from low environmental contamination.

## Conclusions

The presented study strengthens the knowledge about the nutritional value and composition of *Castanea sativa* Mill. fruits of Ukrainian origin. Chestnut fruits are characterized by low lipids content, substantial share of proteins and fructose, and high β-carotene content. The lipid fraction of chestnut fruits is strongly dominated by SFAs, namely palmitic acid, followed by the MUFA oleic acid, and PUFA α-linolenic. Chestnut fruits may be appreciated as plant sources of PUFAs, especially α-linolenic acid belonging to the n-3 family. Among detected 18 amino acids, glutamic acid was found to be the major component. Surprisingly essential amino acids/total amino acids ratio amounted to 44%, which according to FAO WHO may be regarded as high-quality protein plant food. In response to limited information about minerals in chestnut fruits, our study showed that calcium and phosphorus were the most abundant elements, simultaneously with a low Na : K ratio. Summing up, presented composition and literature data regarding nutrients proved that chestnut fruits are valuable ingredients in a healthy diet. In addition, chestnuts are currently used in pediatrics for the treatment of gastroenteritis and as a gluten-free diet in cases of celiac disease.

## Conflict of interest

The authors have no conflicts of interest to declare.

## Ethical statement

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

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