Scientific Papers. Series D. Animal Science. Vol. LXVIII, No. 2, 2025
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

CHARACTERIZATION OF THE PHYSICOCHEMICAL COMPOSITION AND FATTY ACIDS OF WALNUTS

Ioana Roxana ȘOIMUȘAN[1], Adina SABOU[1], Oana-Andreea PECE[1], Anca BECZE[2], Luisa ANDRONIE[1], Aurelia COROIAN[1]

[1]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca,
3-5 Calea Mănăștur Street, 400372, Cluj-Napoca, Romania
[2]INCDO INOE 2000, Research Institute for Analytical Instrumentation Subsidiary,
67 Donath Street, 400293, Cluj-Napoca, Romania

Corresponding author email: aurelia.coroian@usamvcluj.ro

Abstract

Walnuts contain all the major macronutrients: proteins, fats, carbohydrates, bioactive compounds: vegetable proteins, fibers, minerals, tocopherols and phenolic compounds. Walnuts are rich in unsaturated fatty acids, MUFA, PUFA n-3 and n-6. Linolenic acid (18:2n-6) and alpha-linolenic acid (18:3n-3) are two essential acids for the human body and are precursors of C20 and C22 polyunsaturated fatty acids. The average protein content in the studied nuts is 16.85%. The most representative fatty acids were: C18:2 (39.89%), C18:1 (35.56%) and C16:0 (10.58%). The antioxidant capacity of the nuts varied in the range (54.39-57.48 mmol/100 g).

Key words*: antioxidant capacity, ash, fatty acids, moisture, protein.*

INTRODUCTION

Walnut (*Juglans regia* L.) is a plant recognized for its important role in human health and for its valuable economic characteristics (Çağlarırmak, 2003).

The main characteristics of walnuts are: the aroma, which is distinct and can vary depending on the variety and environmental conditions, the color of the walnut kernel is usually yellowish-white to cream, the shell must be hard and intact (Fukasawa et al., 2024).

A key aspect in evaluating the quality of nuts is their microbiological safety, as the presence of pathogenic microorganisms, can form secondar metabolites like mycotoxin such as aflatoxins, that lead to a considerable reduction in the quality of the nuts and can represent a major risk to consumer health (Amaral et al., 2003).

The following factors that can influence the quality of walnuts are: walnut variety and varieties, climatic factors, soil, cultivation techniques, harvesting techniques and storage conditions (Song et al., 2022).

Walnut oil represents a valuable source of lipids because it is rich in monounsaturated fatty acids (MUFA), (primarily oleic acid) and polyunsaturated fatty acids (PUFA), (linoleic and linolenic acids). The fatty acid profile is nutritionally beneficial as unsaturated fatty acids contribute to the regulation of blood lipids, thrombus clearance and immunoregulation (Fernandes & Cabral, 2007).

Studies have shown that walnut consumption improves cognitive performance, memory, has anti-inflammatory and antioxidant effects and reduces the risk of cancer (Chauhan & Chauhan, 2020; Bobeș et al., 2022).

Phenolic compounds in walnuts enhance neurogenesis and have the ability to reduce the oxidative and inflammatory burden of brain cells (Poulose et al., 2014).

Walnuts contain a number of bioactive compounds such as polyphenols and vitamin E, and the inclusion of 30 g/day of walnuts has positive effects on reducing cholesterol levels (Sánchez-González et al., 2017; Tapsell, 2010; Ros, 2010; Banel & Hu, 2009; Sabaté et al., 2010).

Regarding the concept of walnut quality, this is a key aspect that involves both external characteristics, such as the appearance, aroma and color of the walnuts, and internal factors, such as physicochemical composition and microbiological properties. Recently, studies have begun to place greater emphasis on these

aspects, and their awareness is essential for improving walnut-derived products and meeting the demands of the final consumer (Fukasawa et al., 2024).
Nuts must meet food safety standards, so they must be monitored for possible contamination with mycotoxins (aflatoxins) or other pathogens (Sánchez-González et al., 2017).
Walnuts are considered medicinal plants because they contain biologically active substances that have a role in the human body (Sharma et al., 2021).
According to data from the specialized literature, they are some of the most consumed dried fruits, being appreciated by many consumers (Fukasawa et al., 2024).
Walnuts have been appreciated since ancient times, being used by humanity to treat various ailments and disorders of the human body (Zhang et al., 2024).
The medicinal properties of walnuts are given by certain substances that they contain, namely: alkaloids, saponins and polyphenols (Ozkan & Koyuncu, 2005).
Walnuts also act as a functional food due to their vegetable proteins, essential amino acids, omega-3 fatty acids, phenols, vitamins and minerals (Willett, 2012).
Other key benefits to the human body triggered by the introduction of walnut consumption into the daily diet are represented by the anti-inflammatory, antitumor, antioxidant, antihypertensive effects and the role of immunonutrient (Hayes et al., 2016).
In a study conducted in the U.S. on 200 subjects at risk of coronary heart disease, the authors demonstrated the importance of consuming nuts consistently, leading to a decrease in blood cholesterol concentrations (Feldman, 2002).
Walnuts have been attributed many essential benefits for the body, such as reducing the risk of cardiovascular disease, coronary heart disease, type II diabetes, preventing certain types of cancer or improving neurological symptoms (Santos et al., 2017).
Nuts, due to the fiber they contain, act as prebiotics, helping intestinal bacteria have a positive role in the microbiome (Zhang et al., 2024).
Walnut oil (*Juglans regia* L.) contains high levels of tocopherols, phytosterols, polyphenols and a very good antioxidant capacity (Gao et al., 2019; King et al., 2008).
Oxidative stability is an important index for estimating the quality of nut oils and can be used in the process of evaluating the physicochemical indices of nuts (Jin et al., 2017; Prescha et al., 2014).

MATERIALS AND METHODS

In the current context, marked by an increasing interest in beneficial nutrition, the main purpose of this study was to conduct a detailed investigation of the physicochemical composition, fatty acid profile and antioxidant capacity of walnuts, in order to highlight their compositional potential.The walnut samples were collected from Buciumi, Sălaj, Transylvania. The harvest period was September 2024. The walnuts were manually peeled and dried at ambient temperature. The walnuts were stored in paper packages, in the dark until the time of analysis. Sălaj County is influenced by air masses from the west. The characteristics related to the relief (altitude, aspect) and atmospheric circulation lead to the existence of climatic differences between the west and the east of the county and also between the main geomorphological units. The average annual precipitation is 626 mm/year with an uneven distribution, but sufficient for crop productivity. The average annual temperature exceeds 9°C, while the temperature amplitude varies between 19.3 and 27.6°C (Bartha et al., 2024). The relative humidity is 60%, and the altitude does not exceed 1000 meters (the maximum height is 996 m in Măgura Priei, Meseș Mountains) (Pavel & Tudose, 2019; Rusu et al., 2020).

Determination of protein content

Regarding the determination of protein content, we performed it using the Kjeldahl method, the Büchi model.
Mineralization stage of organic substance: the amount of sample to be analyzed is 2 g. The sample for analysis is introduced into the test tube of the mineralization block and then the catalyst (0.5 g copper sulfate + 15 g anhydrous potassium sulfate) and 25 ml sulfuric acid. The sulfuric acid is added gradually, in small quantities in order to entrain any traces of

sample or catalyst. The next stage is to introduce the test tubes into the mineralization block. The mineralization process is carried out at a high temperature (stage 10 of the energy regulator until the water evaporates and then the energy regulator is changed to stage 8). At the end of the mineralization stage, the mineralization block is stopped and the test tubes are removed and then left to cool. The next stage is distillation. The distillation unit is equipped with an apparatus that automatically supplies the quantities of water and sodium hydroxide required for distillation. A volume of 60 ml of boric acid is sucked into the collection vessel, in which the pH meter electrode is also inserted. The distillation process lasts 3 minutes and the nitrogen is collected in the boric acid solution. The titration is carried out with automatic indication of the end point using the pH meter. The ammonia is titrated with 0.1 n sulfuric acid solution to the end point indicated by the pH meter (pH=4.65). In parallel, a blank sample containing about 1 g of sucrose is prepared instead of the sample to be analyzed. Two determinations are performed on the same sample for analysis. A control analysis is carried out by determining the total nitrogen content of acetanilide or tryptophan and adding 1 g of sucrose. The difference between the determinations carried out simultaneously must not exceed 3 g/kg of dry matter. If the repeatability conditions are met, the arithmetic mean of two determinations is taken as the result. The result is expressed with a precision of 0.01 g/100g of product. To determine the crude protein content, the nitrogen content is multiplied by the characteristic factor. The ash was analysed using the calcination process. This lasts approximately six hours.

Following the calcination step, the crucible is placed in a desiccator with anhydrous calcium chloride, weighed, and cooled to room temperature for approximately two hours. The moisture content is determined by drying at a temperature of 105°C for six hours.

Determination of fatty acids

The purpose of this analysis was to identify ester derivatives of fatty acids. The analysis steps were as follows: sample preparation based on weighing and grinding, saponification of the sample with 5Ml saponification solution and subsequent heating of the sample (80°C for 10 minutes) and the last step was gas chromatographic analysis. The reagents used were: saponification solution (3M NaOH), extraction solution 1:1 (v:v) Hexane and MTBE (Methyl-tert-butyl-ether) and methylation reaction 3 M NaOH. The equipment used was 7890 CG-FID Agilent technologies.

The criteria required for the Gas Chromatography analysis were: INNOWAX column (30m x 0.25mm x 0.25µm), temperature program: 50°C (1 min.), 50-300°C (6°C min.1), 300°C (5 min), with a solvent delay of 5 min, injection volume: 1 µL, He flow rate: 1 mL min-1, split ratio 1:20 and the injector temperature to be 200°C and that of the flame ionization detector to be 250°C.

For the determination of fatty acid methyl ester derivatives, we used the following reagents: saponification solution: 3M NaOH, extraction solution: 1:1 (v:v) Hexane and MTBE (Methyl-tert-butyl-ether), and for the methionation reaction we used 3 M NaOH. The first stage was represented by weighing and grinding the sample, the second stage is represented by saponification of the sample which was carried out using 5mL saponification solution followed by heating the sample (80 °C for 10 minutes). The last stage was gas chromatographic analysis.

The equipment used is 7890 CG-FID Agilent technologies.

The main conditions for gas chromatograph analysis are: INNOWAX column (30 m x 0.25 mm x 0.25 µm); temperature program is 50°C (1 min.), 50-300°C (1 min.), 300°C (5 min), with a 5 min. solvent delay; split ratio is 1:20; injection volume is 1µL; He flow rate is 1 mL min^{-1}; injector temperature is 200°C and flame ionization detector temperature is 250°C.

Determination of antioxidant capacity

The sample for analysis was extracted using methanol and through PHOTOCHEM analysis, a process that combines photochemical excitation of free radicals with luminometric detection. Photochemical excitation occurs when the sample is prepared and subsequently introduced into the PHOTOCHEM device. It is exposed to a specific wavelength of UV light. The objective of this step is to generate free radicals within the sample.

Free radical reaction: in the previous step, the generated free radicals react with the antioxidants present in the sample. Antioxidants have the ability to neutralize free radicals by donating an electron and through this process, oxidative damage to the cells is prevented.
Luminometric detection occurs after the free radical reaction phase is completed. The PHOTOCHEM device examines the remaining concentration of free radicals, through a luminometric detection system that measures the intensity of the light transmitted during the reaction.
The results are expressed in ascorbic acid equivalents. For solid samples the following calculation formula is applied:

$$\text{Concentration}\left[\frac{\mu g}{mg}\right] = \frac{Q \times D \times M \times V}{PV \times Mp}$$

Where: Q - nmol of ascorbic acid or trolox read on the device; D - dilution factor; M - molecular mass (trolox = 250.3 ng/nmol); V - extraction volume in ml; PV - volume pipetted into the test tube; Mp - sample mass in g.

RESULTS AND DISCUSSIONS

The analyzed physicochemical components fall within the characteristic values for walnuts, according to our results compared with data from the specialized literature.

Protein, ash and moisture in walnuts

Protein presented an average value of 16.85%, ash 1.98% and moisture 4.02% (Figure 1). Iordănescu et al (2021) studies the physicochemical composition of nuts and observes a lipid content that varied between 56.09% and 66.56%, proteins varied between 12.73% and 20.41%, mineral content had values between 1.31% and 2.49% and water content varied between 1.23% and 5.00%.
The results obtained for protein (Figure 1) were superior to those reported by Iordănescu et al. (2021).
A study conducted in Romania showed lower values for protein (13.81%) and ash (1.7-2.05%) and moisture content had similar values to this study (Leahu et al., 2013). Another study conducted in New Zealand (Savage, 2001) showed the lowest values for protein (13.7-18.1%), lower than those reported by Ozkan & Koyuncu (2005) where the protein level is in the range of 15.17-19.24%, ash 1.26-2.06% and moisture 3.25-3.91%. The results of the protein level in the current study are similar to those reported by Sze-Tao & Sathe (2000).

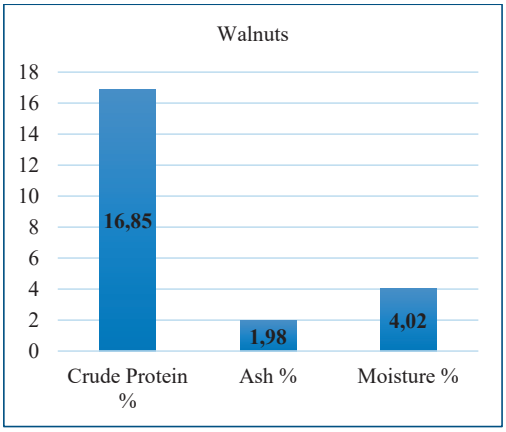

Figure 1. Average protein, ash and moisture content of walnuts

Iordănescu et al. (2021) obtained the highest values for protein content (20.14%).
Chatrabnous et al. (2018) conclude that fresh nuts have higher amounts of protein, water, and minerals compared to dried nuts.
In a study conducted in Turkey, the amount of crude protein in walnuts ranged from 7.05% to 8.10% and the oleic acid content ranged from 20.5% to 26.4%, therefore the authors consider that walnuts are a potential source for obtaining a valuable oil (Ozcan et al., 2010).

Fatty acids from walnuts

Figure 2 shows the average values of fatty acids in walnuts from Transylvania, Romania. The most representative fatty acid is C18:2 (39.89%), followed by C18:1, (35.56%). Patraș & Dorobanțu (2010) analyzed the physicochemical composition of walnuts from the Moldova region and obtained lower values of oleic and palmitic acid and higher values of linoleic acid compared to this study. The most representative amount of C18:2 acid was determined in Serbia with a value of 65.1%, followed by New Zealand (62.3%), China (63.12%), while other studies conducted in Turkey on different walnut samples revealed values that ranged from 43.94% to 60.12 as well as from 49.7% to 55.5% (Rabrenovic et

al., 2008; Zwarts et al., 1999; Gao et al., 2018; Ozkan & Koyuncu, 2005; Özcan et al., 2010).

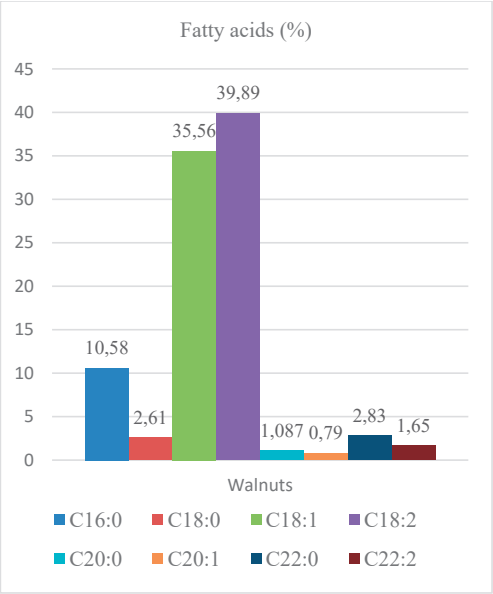

Figure 2. Fatty acids in walnuts

Sandulachi et al. (2012) note that storage for a period of two years has a positive impact on oleic acid, but the qualities of the walnuts are reduced, which is why it is recommended to consume walnuts with a shelf life of one year. Subsequent studies report lower values of C18:1 acid (Gao et al., 2018; Özcan et al., 2010; Zwarts et al., 1999; Savage et al., 1999) and C16:0 acid (Dogan & Akgul, 2005) compared to those obtained in this study.

Walnuts have valuable nutritional qualities, being rich in lipids (between 55% and 76%), proteins (11-25%), carbohydrates (16%), and linoleic acid has a share of 58% of the total polyunsaturated fatty acids (Patraș & Dorobanțu, 2010).

In a study conducted in China, the intrinsic nutritional components of walnuts were investigated, so the authors specified significant differences ($p < 0.05$) between batches regarding color for γ-tocopherol, K and Na, with values between 246.48 and 333.94 mg/kg, 3403.05 to 4409.65 mg/kg and 12.62 to 22.16 mg/kg; the total value regarding amino acids in the study is: (191.91 mg/kg) and respectively the mineral substances: (P: 4165.57 mg/kg, Mn: 75.34 mg/kg) (Qingyang et al., 2022).

An experiment conducted on several varieties of walnut trees (*Juglans regia* L., *Jupiter*, *Sejnov* and *Elit*), from Serbia, obtained the following values regarding the oleic acid content of walnut oils, which ranged between 15.9-23.7% of the total acids, while the linoleic acid content ranged between 57.2-65.1%, and linolenic acid ranged between 9.1-13.6%, therefore the authors specify that the oil extraction process had no significant effect on the fatty acid composition (Rabrenovic et al., 2008).

Antioxidant capacity of walnuts

Figure 3 shows the average values of walnuts under different evaluation methods, in raw and dried state.

The most significant results of antioxidant capacity are noted from dried walnuts. Several studies demonstrate that fresh walnut kernels have a higher antioxidant activity than dried walnut kernels (Açar et al., 2009; Wang et al., 2022; Pellegrini et al., 2006).

Popović et al. (2019) concluded that there are no significant differences in the fatty acid profile between dried and raw walnuts.

The influence of storage period is studied by Amini & Ghoranneviss (2016) and they observe that after 15 and 30 days of storage, the antioxidant capacity of walnuts increases, while the total polyphenol content and antioxidant capacity decrease with the maturation of the walnuts (Pycia et al., 2019).

Gao et al. (2018) observed that roasting walnut kernels at 180°C for 10 and 15 minutes increases antioxidant capacity and oxidative stability, enhances the level of polyphenols (30.08-44.82 mg/kg), phytosterols (1740.91-2048.05 mg kg^{-1}) and tocopherols (470.16-508.68 mg kg^{-1}) and increases fat content (Santos et al., 2017).

The highest values of antioxidant capacity are associated with a high content of phenols or ellagic acid, for example walnuts recorded values ranging between 41 and 823 mg/100 g ellagic acid, followed by pecans (15 and 301 mg/100 g) while chestnuts had lower levels between 0.37 and 149 mg/100 g (Açar et al., 2009).

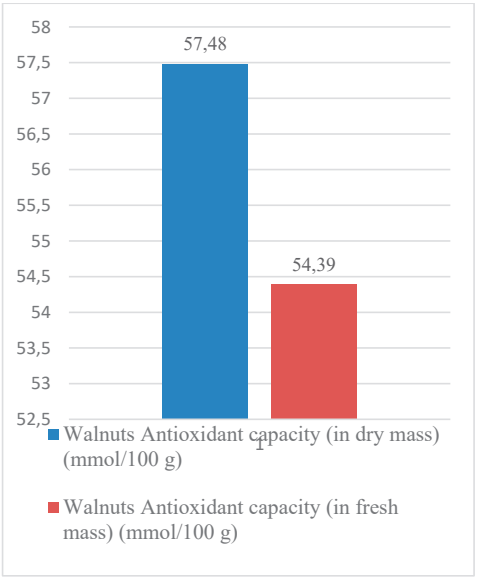

Figure 3. Antioxidant capacity of dried and raw walnut

CONCLUSIONS

The physicochemical characteristics of the walnuts studied from the Transylvania area, Romania are within the standard values.

The most representative fatty acids in quantitative terms are C18:2, C18:1 and C16:0 and the fatty acids with the lowest content are C20:1, C20:0, C22:2, C18:0, C22:0.

The highest level of antioxidant capacity was observed in fresh walnuts.

Therefore, the nutritional value of nuts is due to their kernels and not other components.

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