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PHYSICO-CHEMICAL CHARACTERIZATION OF SOME WALNUT FRUITS COLLECTED IN 2018 FROM UNIVERSITY OF CRAIOVA - S.C.D.P. VÂLCEA, ROMÂNIA

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Abstract
The fruits of common walnut (Juglans regia L.), known also as Carpathian, English or Persian walnut, have been part of the human diet for thousands of years, recently being discovered that they are rich in omega-3 fats and contain higher amounts of antioxidants than most other foods. Among other benefits, they improve brain health, prevent heart disease and cancer, treats cough and stomach ache. The aim of this study was to analyse the chemical composition of the fruits at full maturity from eight walnut cultivars, grown in a trial, at University of Craiova - Fruit Growing Research and Extension Station (S.C.D.P.) Vâlcea, Romania. Walnut varieties with different geographic origins and different agro-biological characteristics were used. The fruits of four Romanian cultivars ('Jupâneşti', 'Sarmis', 'Unival' and 'Valcor'), three French cultivars ('Ferjean', 'Fernor' and 'Franquette') and an American one ('Vina') were analysed. For all varieties, the content of proteins, polyphenols, flavonoids, tannins, carotenoids, water and ash was determined. The walnut cultivars were grown in the same environmental conditions and the same cultural practices were applied, variability in chemical composition of the fruits was observed and depended on their genetic background, agro-biological characteristics and environmental conditions.

Keywords: walnut kernel, polyphenols, flavonoids, tannins, carotenoids

1. INTRODUCTION

The common walnut, known also as Carpathian, English or Persian walnut, is considered king of the nut trees, starting from its Latin name *Juglans regia*. It is grown primarily for its fruits, which are consumed both fresh and processed. Although it grows slowly and produces fruits late, the walnut is a longevive tree living on average about 80-100 years and even much more. It is currently found almost in all countries with temperate climates. Walnut can be found in spontanous and sub-spontanous flora but is also grown in gardens, along roads and in organized orchards. World walnut production was 3,829,626 t and the orchard area harvested reached 1,097,699 ha in 2017 (FAO Stat Database, 2019).

The walnut fruits have been part of the human diet for thousands of years, recently being discovered that they are rich in omega-3 fats and contain higher amounts of antioxidants than most other foods.

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Among other benefits, they improve brain health, prevent heart disease and cancer, treats cough and stomach ache. Due to composition of the fruit, the walnut is classified as a strategic nut crop for human nutrition and is included in the FAO list of priority plants (Gandev, 2007). The seed part of the fruit (kernel) is consumed fresh, toasted, or mixed with other confectionaries. Also, walnut oil can be obtained from kernels.

The aim of this study was to analyse the chemical composition of the fruits at full maturity from eight walnut cultivars, grown in a trial, at University of Craiova - Fruit Growing Research and Extension Station (S.C.D.P.) Vâlcea, Romania. The walnut cultivars have different genetic and geographic origins and exhibit different agro-biological characteristics. The fruits of four Romanian cultivars (‘Jupâneşti’, ‘Sarmis’, ‘Unival’ and ‘Valcor’), three French cultivars (‘Ferjean’, ‘Fernor’ and ‘Franquette’) and an American one (‘Vina’) were analysed. ‘Jupâneşti’, ‘Sarmis’, ‘Unival’, ‘Valcor’ and ‘Franquette’ have terminal bearing, while ‘Ferjean’, ‘Fernor’ and ‘Vina’ present lateral bearing. For all varieties, the content of proteins, polyphenols, flavonoids, tannins, carotenoids, water and ash was determined.

2. MATERIALS AND METHODS

Plant material

The walnut fruits were collected during September and October 2018, depending on the cultivar harvesting time, from University of Craiova - Fruit Growing Research and Extension Station (S.C.D.P.) Vâlcea, Romania. After the harvest, they were immediately dried and stored in the shell at room temperature until the start of the analysis. Bulk samples were selected randomly and the unwanted debrides were removed from bulk sample of walnut nuts. After the walnut fruits were cracked, the walnut kernel was stored at 5˚C prior to experiment. At the analysis moment, the walnut kernel was transformed into a homogeneous mass using an electric grinding machine.

Four Romanian varieties (‘Jupâneşti’, ‘Sarmis’, ‘Unival’ and ‘Valcor’), three French cultivars (‘Ferjean’, ‘Fernor’ and ‘Franquette’) and an American one (‘Vina’) were analysed. All four Romanian varieties are vigorous, have terminal bearing and good productivity. Also, the Romanian varieties have better resistance to low temperatures during winter then the French and American cultivars. The Romanian cultivars analysed and the American cultivar ‘Vina’ mature their nuts in September. ‘Franquette’ cultivar presents a later maturity of the fruit as compared to the other varieties, namely: the second decade of October. More agronomical and genetic information about these walnut cultivars were presented in our previous works (Botu et al., 2001, 2007, 2010, 2017, 2019; Giura et al., 2016a, b).

Chemical substances

Bovine serum albumin, gallic acid, catechin, tannic acid and Folin-Ciocalteu reagent were purchased from Redox Bucharest - Sigma Aldrich, Dako, Epp. Romania. Methanol, ethanol, hexane, acetone, copper sulphate, sodium hydroxide, sodium carbonate, sodium nitrite and aluminium chloride were purchased from Merck Romania SRL.

Chemical analysis and equipment

The water content (moisture) was determined gravimetrically by drying 1 g finely homogenised kernel in an oven at 105-110 °C, until the analysed samples were brought to a constant mass. (AOAC, 1990).

The minerals content (ash) was determined by the calcination of residue after determining the water content from the walnut kernels at 550 °C, until the analysed samples were brought to a constant mass (AOAC, 1990).

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Quantitative determination of proteins was performed using a UV-Vis spectrophotometer PerkinElmer Lambda25, the methodology proposed by Lowry et al., 1951 being respected. The method principle is based on the complexation of the peptide bond with alkaline copper sulphate (biuret reaction) and the reduction of phosphomolybdate and phosphotungstic from the Folin-Ciocalteu reagent by protein tyrosine and tryptophan residues. For analysis, an aqueous extract of homogenised vegetal material with concentration 100 mg/mL was used. The concentration of proteins was calculated using the calibration curve for bovine serum albumin, performed under the same conditions as the samples, using the absorbance values at the maximum absorption, located at 660 nm. 1 mL aqueous extract of vegetal material was added to a 10 mL flask containing 1 mL biuret reagent and 7.5 mL distilled water. After 10 minutes of rest, 0.5 mL Folin-Ciocalteu reagent was added. After 60 minutes of rest, absorbance of the samples was measured and the concentration of proteins was estimated. The blank sample was prepared from 1 mL biuret reagent, 0.5 mL Folin-Ciocalteu reagent and 8.5 mL distilled water. Finally, the content of proteins was expressed as mg bovine serum albumin equivalent/ 100 g vegetal material.

Quantitative determination of polyphenols was performed by spectrophotometric method, the methodology proposed by Singleton and Rossi, 1965 being respected. The method principle is based on forming a blue coloured compound between phosphotungstic acid and polyphenols, in an alkaline medium. For analysis, a methanolic extract of homogenised vegetal material with concentration 100 mg/mL was used. The concentration of polyphenols was calculated using the calibration curve for gallic acid, performed under the same conditions as the samples, using the absorbance values at the maximum absorption, located at 765 nm. 0.5 mL methanolic extract of vegetal material was added to a 10 mL flask containing 7 mL distilled water and 0.5 mL Folin-Ciocalteu reagent. After 5 minutes of rest, 2 mL solution of sodium carbonate 10% was added. After 60 minutes of rest, absorbance of the samples was measured and the concentration of polyphenols was estimated. The blank sample was prepared from 0.5 mL Folin-Ciocalteu reagent, 2 mL solution of sodium carbonate 10% and 7.5 mL distilled water. Finally, the content of polyphenols was expressed as mg gallic acid equivalent/ 100 g vegetal material.

Quantitation of flavonoids was performed by spectrophotometric method. The methodology proposed by Zhishen et al., 1999 was respected. The method principle is based on the formation of a yellow-orange-coloured compound by the reaction of flavonoids and aluminium chloride. For analysis, a methanolic extract of vegetal material with concentration 100 mg/mL was used. The concentration of the flavonoids has been calculated using the calibration curve for catechin, performed under the same conditions as the samples, using the absorbance values of the maximum absorption, located at 510 nm. 1 mL methanolic extract of vegetal material was added to a 10 mL volumetric flask containing 6 mL distilled water and 0.5 mL sodium nitrite 5 %. After 5 minutes of rest, in the volumetric flask was added 0.5 mL aluminium chloride 10%. After 5 minutes, 2 mL solution of sodium hydroxide 1M was added. The absorbance of the solution at 510 nm was measured. The blank sample was prepared from 0.5 mL sodium nitrite 5 %, 0.5 mL aluminium chloride 10%, 2 mL solution of sodium hydroxide 1M and 7 mL distilled water. Finally, the content of flavonoids was expressed as mg catechin equivalent/ 100 g vegetal material.

Quantitative determination of tannins was performed by spectrophotometric method based on the methodology proposed by Makkar et al., 1993 was used. For analysis, an aqueous extract of homogenised vegetal material with concentration 100 mg/mL was used. The concentration of tannins was calculated using the calibration curve for gallic acid, performed under the same conditions as the samples, using the absorbance values at the maximum absorption, located at 760 nm. 1 mL aqueous extract of vegetal material was added to a 10 mL flask containing 6.5 mL

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distilled water and 0.5 mL Folin-Ciocalteu reagent. After 5 minutes of rest, 2 mL solution of sodium carbonate 10% was added. After 60 minutes of rest, absorbance of the samples was measured and the concentration of tannins was estimated. The blank sample was prepared from 0.5 mL Folin-Ciocalteu reagent, 2 mL solution of sodium carbonate 10% and 7.5 mL distilled water. Finally, the content of tannins was expressed as mg gallic acid equivalent/100 g vegetal material.
Quantitative determination of carotenoids (lycopene and β-carotene) was performed by spectrophotometric method based on the methodology proposed by Zechmeister and Polgar, 1943. For extraction of the two compounds, 1 g finely homogenised kernel was used, which was added over 25 mL mixture of solvents (hexane: ethanol: acetone in 2:1:1 volume ratio). The mixture was stirred for 30 minutes at 1500 rpm and then was added 10 mL distilled water and stirring was continued for another 10 minutes. After 15 minutes of rest, the phases were separated. The concentration of carotenoids, expressed as mg lycopene (or β-carotene)/ 100 g vegetal material, was calculated using molar extinction coefficients of 184900 $M^{-1}cm^{-1}$ at 470 nm and 172000 $M^{-1}cm^{-1}$ at 503 nm for lycopene (Rubio-Diaz et al., 2011; DeRitter and Purcell, 1981), respectively 108427 $M^{-1}cm^{-1}$ at 470 nm and 24686 $M^{-1}cm^{-1}$ at 503 nm for β-carotene, in hexane (Zechmeister and Polgar, 1943).

3. RESULTS AND DISCUSSIONS

For all walnut varieties, the moisture and ash content and content in some biologically active compounds, such as proteins, polyphenols, flavonoids, tannins and carotenoids from the walnut kernels, were determined.
Figure 1 presents the results regarding the moisture content of walnut kernel samples.

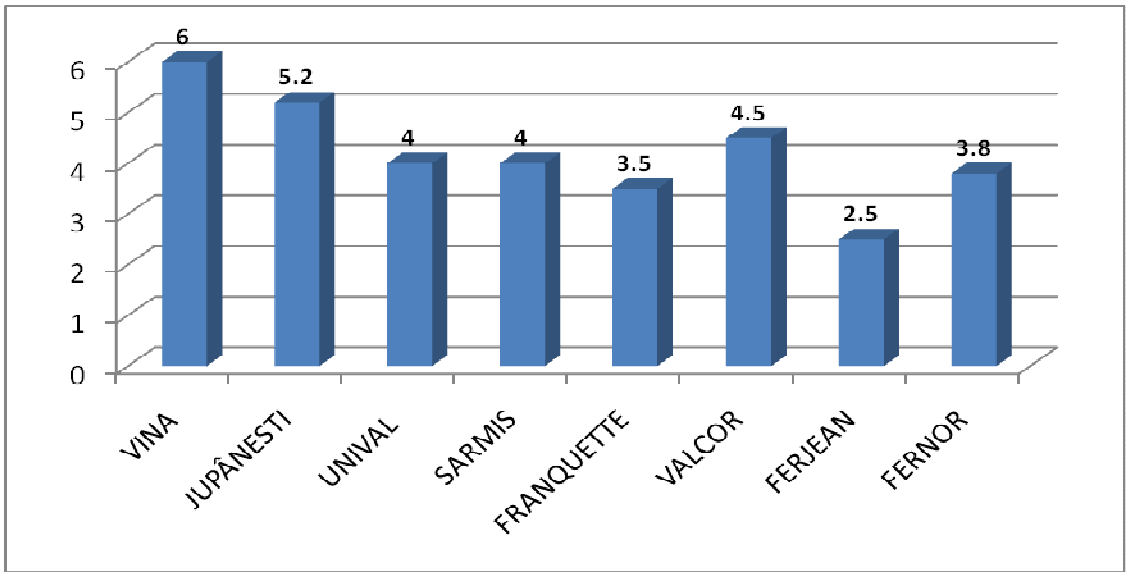

Figure 1. Moisture content

It is noted that the moisture contents for the walnut kernels ranged between 2.5% (‘Ferjean’ variety) and 6% (‘Vina’ variety), values closed to those reported by Khir et al., 2013; Ahad et al., 2017. Our results are in agreement with the FDA regulations for tree nuts define a safe moisture level (moisture content that does not support fungal growth) under 8% (Kader and Thompson, 2002).
Figure 2 presents the results regarding the ash content of walnut kernel samples.

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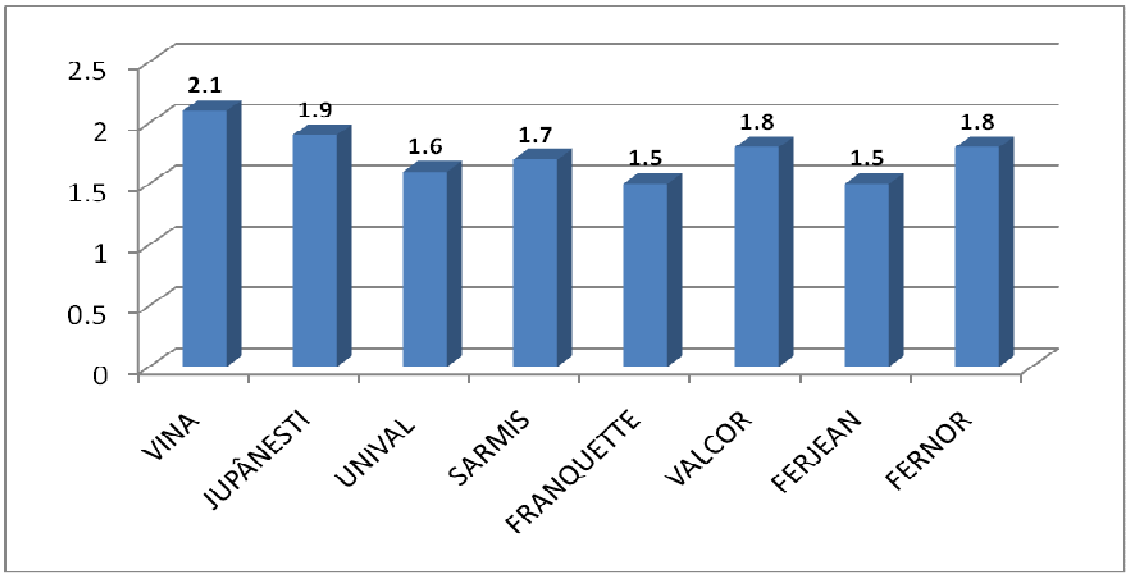

Figure 2. Ash content

The content of mineral substances (ash) in analysed walnut kernels ranged between 1.5% at 'Franquette' and 'Ferjean' varieties and 2.1% in 'Vina' variety. Similar values were presented by Ozkan and Koyuncu, 2005, which reported values between 1.26% and 2.06% for dry matter content in ten walnut genotypes from Turkey.

Literature data arrow that the walnuts have appreciable amounts of proteins (10-24%), carbohydrates (12-18%), dietary fibre (1.5-2%) and minerals (1.7-2%) (Savage, 2001; Amaral et al., 2003; Akca et al., 2005; Ozkan and Koyuncu, 2005; Pereira et al., 2008; Yerlikaya et al., 2012). Proteins content in eight walnut kernels from Vâlcea, Romania is shown in figure 3.

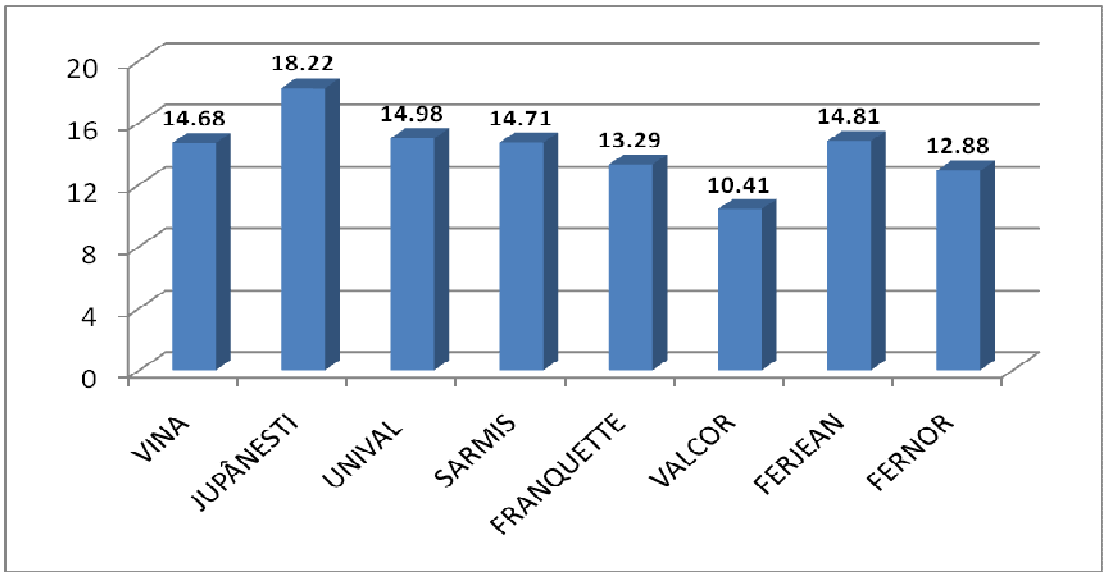

Figure 3. Proteins content (g bovine serum albumin equivalent/100 g vegetal material)

In analysed walnut kernels, the proteins content ranged between 10.41 g/100 g material vegetal, at 'Valcor' variety and 18.22 g/100 g material vegetal, at 'Jupânești' variety. Pereira et al., 2008 have reported that six walnut cultivars from Portugal contained 14.38-18.03% protein and Yerlikaya et

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al., 2012 have determined a content 10.58-18.19% protein for eleven walnut genotypes grown in Turkey.

It is known that the walnuts have antioxidant features due to the presence of some bioactive compounds, such as omega-3 fatty acids (especially alpha-linolenic acid), vitamin E, and dietary fibre (Ros et al, 2004; Bhardwaj et al., 2018; Fatima et al., 2018). In addition to these nutrients, walnuts are rich in plant sterols, especially in polyphenols (Vinson and Cai, 2012; Regueiro et al., 2014; Fatima et al., 2018). Omega-3 fatty acids reduce cardiovascular disease risk, by changing vascular inflammation and improving endothelial dysfunction (Ros et al, 2004; Bhardwaj et al., 2018). Vitamin E, present in walnuts as alpha-tocopherol and gamma-tocopherol, is a strong fat-soluble antioxidant, which protect the mucus and skin cell membranes against the harmful effects of free radicals and to keep their unity (Şen and Karadeniz, 2015). Dietary fibre from walnuts may protect the human body against coronary heart disease through a number of mechanisms (Sabaté and Fraser, 1993; Fraser, 1994). Polyphenolic compounds have beneficial effects over several disease states including: cardiovascular system dysfunction and damage, metabolic syndrome, diabetes, cancer (Li et al., 2011; Murase et al., 2011; Estruch et al., 2013; Tudor-Radu et al., 2016).

Figure 4 presents the results regarding the polyphenols content of walnut kernel samples.

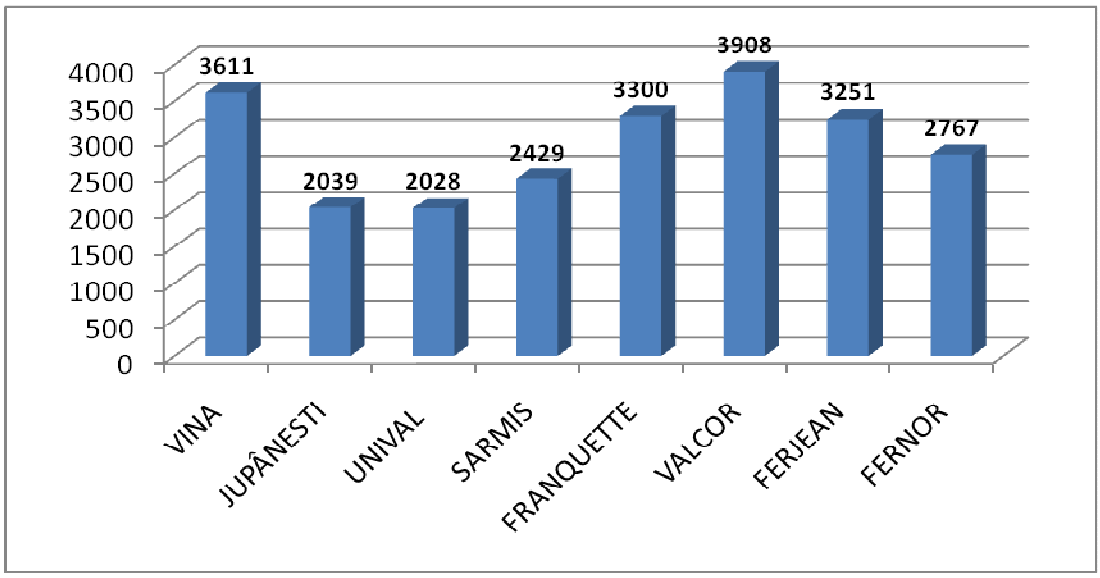

Figure 4. Polyphenols content (mg gallic acid equivalent/100 g vegetal material)

In analysed walnut kernels, the amounts of polyphenolic compounds between ~2030 mg gallic acid equivalent/100 g vegetal material ('Unival' and 'Jupâneşti' varieties) and 3908 mg gallic acid equivalent/100 g vegetal material ('Valcor' variety) were found. As can be seen, the significant differences in the polyphenols content from the analysed walnut kernels were found. Similar results were obtained by Pereira et al., 2008 and Trandafir et al., 2016, who reported the polyphenols content ranged between 5878 mg and 9506 mg gallic acid equivalent/100 g vegetal material, respectively 1131 mg and 2892 mg gallic acid equivalent/100 g vegetal material, for the various genotypes of walnut kernels.

Flavonoids are a large group of hydroxylated phenolic compounds distributed in the green plant kingdom. These phenolic compounds are responsible for the colour and aroma of flowers and fruits, what helps attract pollinating insects. They protect plants from various biotic and abiotic stresses, regulate cell growth and help to filter UV, nitrogen fixation, cell cycle inhibition, as chemical

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messengers (Zhishen et al., 1999; Tudor-Radu et al., 2016). Figure 5 presents the results regarding the flavonoids content of walnut kernel samples.

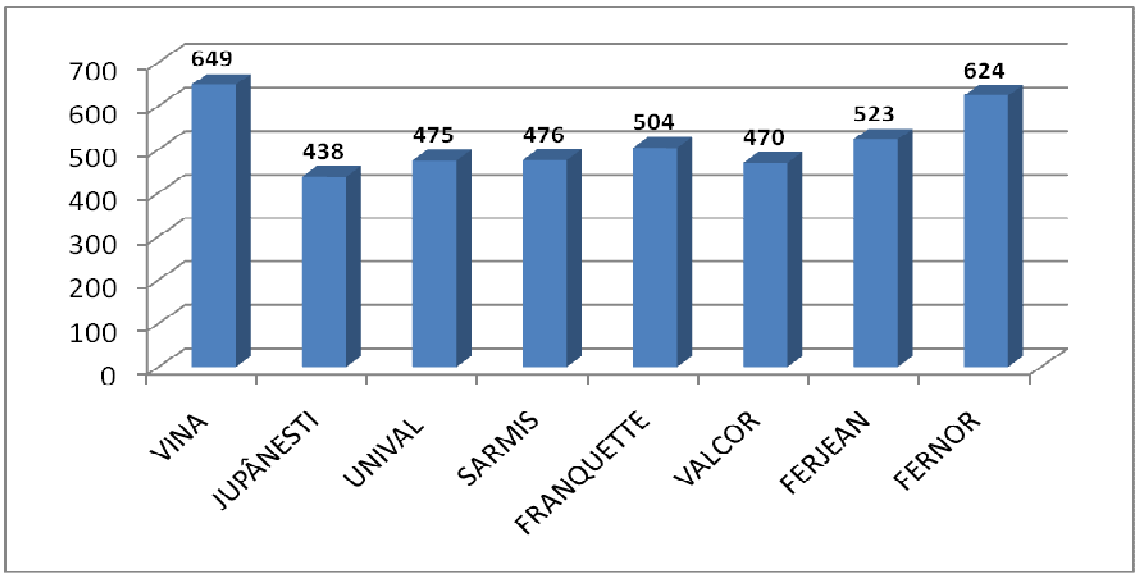

Figure 5. Flavonoids content (mg catechin equivalent/100 g vegetal material)

In analysed walnut kernels, the flavonoids content ranged from 438 mg catechin equivalent/100 g vegetal material (‘Jupâneşti’ variety) to 649 mg catechin equivalent/100 g vegetal material (‘Vina’ variety), our results being in agreement with those of Pereira et al., 2008; Trandafir et al., 2016.
Tannins are water-soluble, astringent substances and secondary metabolites of vegetal materials, with an important biochemical role because they increase the resistance of plants to viruses and microorganisms. These bioactive compounds have antidiarrheal, antifungal and antiseptic action, as a result of the precipitation of bacterial and fungal proteins (Khanbabaee şi van Ree, 2001). Figure 6 presents the results regarding the tannins content of walnut kernel samples.

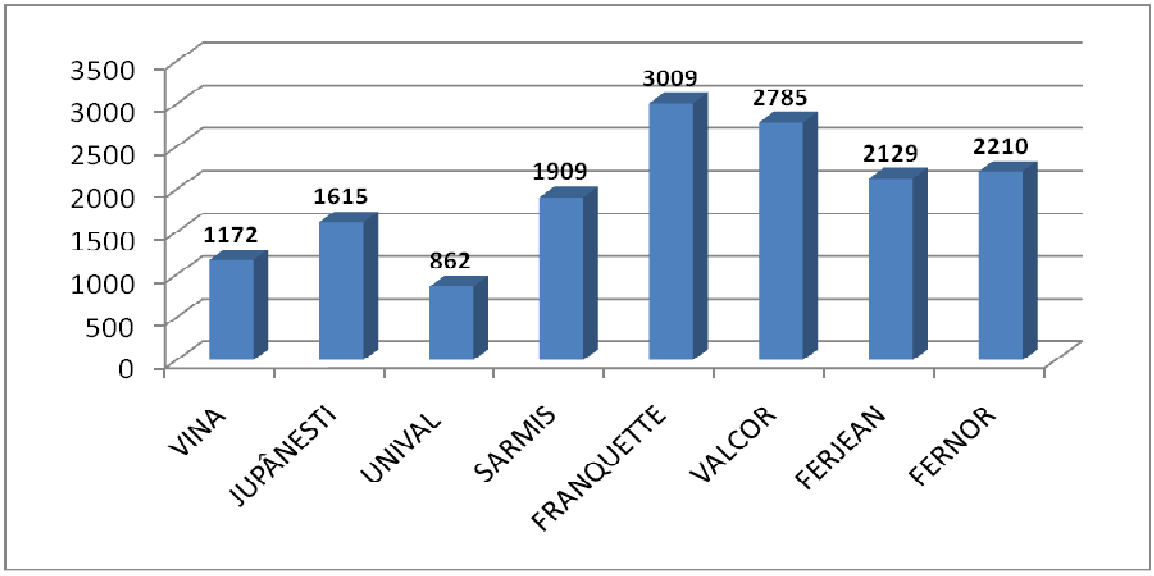

Figure 6. Tannins content (mg gallic acid equivalent/100 g vegetal material)

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In analysed walnut kernels, the tannins content ranged from 862 mg gallic acid equivalent/100 g vegetal material ('Unival' variety) to 3009 mg gallic acid equivalent/100 g vegetal material ('Franquette' variety), these significant differences being determined by the agro-biological characteristics of analysed cultivars.

Carotenoids are natural pigments distinguished by their colour - red, orange and yellow - and their function as photoprotective agents that protect the body from excessive light. Among the most important carotenoids are β-carotene, lycopene, zeaxanthin and lutein. Both animals and humans do not have the ability to synthesize carotenoids, requiring them to be taken from food, living organisms having only the ability to absorb, store and transform carotenoids. Besides being precursors of vitamin A (retinol), which provides the body an antioxidant activity, carotenoids have a protective effect on many diseases due to their ability to neutralize free radicals - molecules that can damage the structure of cells if they are not controlled. Due to this protective effect, carotenoids tend to make the body's immune system stronger and block the progression of precancerous diseases that could affect areas such as the mouth and throat (Zechmeister and Polgar, 1943; Rubio-Diaz et al., 2011; Tudor-Radu et al., 2016).

In investigated walnut kernels, the content of lycopene (figure 7) ranged from ~0.4 mg/ 100 g vegetal material ('Unival', 'Jupâneşti' and 'Fernor' varieties) to 2.49 mg/ 100 g vegetal material ('Ferjean' variety) and the content of β-carotene ranged from 0.02 mg/ 100 g vegetal material ('Fernor' variety) to 1.61 mg/ 100g vegetal material ('Ferjean' variety). A remarkable variety is 'Ferjean' variety, which revealed a high content of lycopene and β-carotene.

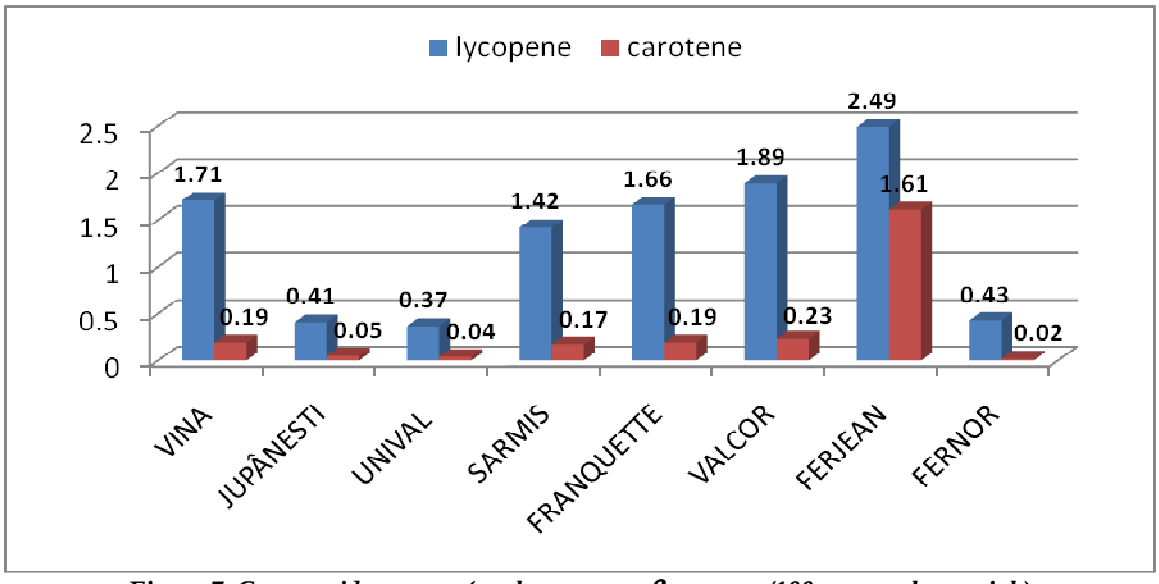

Figure 7. Carotenoids content (mg lycopene or β-carotene/100 g vegetal material)

4. CONCLUSIONS

The walnut kernels have high nutritional value due to the chemical composition, in particular by the content of polyphenols, flavonoids, tannins and carotenoids. The walnut cultivars were grown in the same environmental conditions and the same cultural practices were applied, variability in chemical composition of the fruits was observed and depended on their genetic background, agro-biological characteristics and environmental conditions. Remarkable variety are 'Valcor', 'Vina' and 'Ferjean', which revealed an interesting nutritional composition, showing a high antioxidant status due to its high content of phenolic compounds, flavonoids and lycopene.

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