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ESTIMATION OF BIOCHEMICAL PROPERTIES OF WALNUTS FROM THE REGION OF SUCEAVA - ROMANIA

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Abstract: *The purpose of this paper was to evaluate the biochemical characteristics of walnuts in nine areas of Suceava County. The walnuts have been stored in a cabinet at ambient temperature and we analyzed the physical and chemical parameters of the walnuts: fruit weight, moisture content, ash content, total oil content, protein and total carbohydrates. The predominate component in the kernel, varying between 55.1 and 66.1% was oil, followed by carbohydrate which varied between 12.1 and 24.38 %, and protein with values between 13.81 and 17.22 %. The values obtained from kernel weight varied between 3.42 and 4.84 g, water activity from 0.564 and 0.555, moisture from 3.77 and 4.58 %, ash content from 1.7 and 2.05 %, crude protein. Extracts were prepared /methanol/ water and total polyphenol content (TPC) was in the range of 23.52-17.28 mg GAE/g dry weight walnuts kernel.*

Keywords: *kernel weight, chemical composition, total polyphenol content, antioxidants, total oil;*

1. Introduction

Walnut (*nux juglandes*) is harvested from walnut tree (*Juglans regia*), walnuts are rich sources of nutrients, and excellent sources of phosphorous, magnesium, iron, selenium, zinc and calcium, and walnuts oil is a consistent oil, rich in unsaturated fatty acids.

Sina Cosmulescu, 2009 et al., in analyzing mineral composition in nine walnut cultivars with origin in Romania, has found rich mineral composition, especially potassium, magnesium, calcium.

Walnut kernel contains about 60% lipids, but there may be differences between different varieties of walnut between 50-70 % [2-3], it is a good source of macronutrients and micronutrients and other bioactive. Walnuts also contain 15.8% protein, 13.7% carbohydrate, 4.1% water, and 1.8 % ash [4]. Walnuts contain relevant micronutrients, such as folic acid, niacin and vitamins E and B6, and minerals such as magnesium, copper, zinc, selenium, phosphorus and potassium. [5,6]. Jun Yang, 2009 reported Brazil nuts are unique in that they are the highest known food source of selenium (Se).

The consumption of nuts and nut oil is recommended for those who suffer from hypercholesterolemia and are prone to atherosclerosis [6,7,3]. According to some authors [9] walnuts contain several phytosterols, which have been used as “nutraceuticals” as it appears that they can inhibit intestinal absorption of cholesterol. The high linoleic acid content of walnut oil

makes it undesirable for use in cooking as it is more prone to charring, but walnuts are a perfect ingredient in a variety of breads, muffins, cakes, and biscuits [10].
A. Serrano et al. (2007) evaluated the influence of various cooking methods on the composition and physicochemical characteristics of restructured beef steaks formulated as 20% added walnut. Thus, according to the his research the texture of restructured beef steaks with added walnut was softer and the addition of walnut improved the yield of cooked restructured beef steaks as compared to those formulated with beef fat [11,12]. According B. Olmedilla-Alonso et al. (2008), the consumption of the walnut-enriched meat-based steaks (ca. 19.4 g walnuts/d) over five weeks provoked, on average, decreases in serum total cholesterol and LDL cholesterol concentrations of 4.5% and 5.1%, respectively. Iwamoto M et al. (2002) evaluated the effect of walnut (diet had 12.5% of the energy derived from walnuts (44-58 g/day), comparative with reference and similar decreases were reported in normolipidaemic subjects (cholesterol decreased by 4.4% in men and women and LDL cholesterol by 11% in women). The restructured meat products with added walnuts supplied can be considered functional foods [13]. Secondary products such as green nuts, shells and leaves are used in cosmetics, pharmaceutical and in folk medicine. The *J. regia* leaf extracts can be used as an easily accessible source of natural antioxidants [15]. A. Fernandez-Agullo et al. (2013) analyse the effect of the solvent on the properties of walnut green husk extracts. According to this research, the solvent used resulted to be a significant factor on the total phenols content. The extracts with the highest total phenols content were obtained with 50% ethanol (WEE), followed very closely by 50% methanol and the lowest value was obtained with water. [16]
Different works have characterised the phenolic composition of walnut by-products [6 - 9].
After gathering nuts were dried at room temperature and were subsequently selected two samples for each region studied according to their sensory and size quality characteristics.
In the present work, nine walnuts samples were characterized in respect to their chemical composition and the antioxidant potential.The samples were analyzed for proximate constituents: moisture content, ash content, oil content, protein and total carbohydrates of walnut.

2. Materials and methods

2.1. Samples and storage conditions

In this study the fruit weight, moisture content, ash content, oil content, protein and total carbohydrates of nine walnut (*Juglans regia* L.) samples (Straja-1, Radauti-2, Solca-3, Paltinoasa-4, Campulung Moldovenesc-5, Suceava-6, Falticeni-7, Malini-8 and Dolhasca-9) grown in Suceava were determined.
The samples of the walnuts (200g) used in this work were gathered in September 2011 from the Suceava plateau, an area of Romania. Dried nuts were shelled manually and the parameters were analyzed for each sample.
Since the beginning of the analyses, in April 2012, the walnuts samples were stored in shell in paper bags, in a dark room at approximately 12°C for 5 months.

2.2. Physical analysis

Physical analysis includes kernel properties such kernel weight and kernel ratio.

2.3. Chemical analysis

The determination of moisture and volatile matter content in kernel was effectuated according to the European Standard EN ISO 665/2000 by the drying process in a drying chamber at the temperature of 103 °C.
Water activity plays an important role in the oxidation of walnuts in storage. The water activity of walnuts was evaluated with the device Aqua Lab.
Total ash composition was obtained by calcinations of 5g of sample at 600 °C for 240 min.
Protein content was analyzed by Kjeldahl method.
Oil content was determined in a Soxhlet apparatus, extracting the lipids from 10 g of kernel sample with petroleum ether, followed by extract evaporation to dryness and gravimetric determination according to the European Standard EN ISO 659/1998.
Carbohydrate content was estimated by difference of the other components using the following formula: carbohydrate content = 100% - (% moisture + % protein + % fat + % ash) [9]. Energy was expressed as kilocalories/100g, using the factors mentioned in the Romain Legislation: Energy (kcal) = 4.1x (g protein+g carbohydrate) + 9.3x (g lipid).
The content of total polyphenolic compounds in walnuts methanol extracts diluted 1/10 was determined by Folin-Ciocalteu method. For the preparation of the calibration curve 0.5 mL aliquot of 0.2, 0.3, 0.4, 0.8 and 1.2 μM/mL aqueous gallic acid solution were mixed with 10.0 mL Folin-Ciocalteu reagent (diluted ten-fold) and 1.0 mL sodium carbonate (20.0%) and the volume made up to 10.0 mL with H_2O. The absorption was read after 2 h at 25°C, at 760 nm. All determinations were performed in triplicate. Total phenols were determined as gallic acid equivalents on a dry weight (mg GAE g^{-1} D.W.).

All chemicals used in this study were supplied by Merck companies. Distilled water was used throughout.

2.4. Statistical analysis
Three replications were used to obtain average values and standard deviations for proximate biochemical properties and results are given as averages ± SD.

3. Results and discussion

The results obtained from proximate composition of walnut samples were shown in Table 2.

The kernel weight (g), ranged from 3.42(Falticeni) to 4.84 (Dolhasca) g. The highest positively correlation have been observed in the case of kernel ratio with kernel weight ($r = 0.832$). Significant differences in kernel ratio were found between nine selected walnuts samples kernel weight ($p < 0.01$).

Walnuts also have low water content, with water activity (aw) ranged from: for S 2 Radauti - 0.658 to 0.665 to S1 Straja. The low water content of nuts is related to their relatively easy storage over long periods of time, while the high unsaturated fat content has two major effects: it increases their chances of becoming rancid during cooking at high temperatures, and it also leads to a significant loss of flavor during storage. However, significant differences were not found in water activity of walnuts samples ($p > 0.05$).

Moisture presented the lower values. Moisture content was higher in S9 Dolhasca (4.58%) and lower in S5 Campulung Moldovenesc and S6 Suceava (3.85%). Important to maintain the quality of walnuts is low moisture content, which decreases the likelihood of microbial

growth, premature germination of seeds and late biochemical reactions associated with these processes.

Ash content was higher in S5 Campulung Moldovenesc (2.2%) and lower in S4 Paltinoasa (1.7%). The negatively correlation have been observed in the case of ash with kernel weight (r=-0.784).
If the results were compared with the ones previously obtained by Pereira J. et al. (2008), some differences can be observed namely ash, total oil content (lower in the present work). Its differences could be attributed to the harvesting year and environmental condition, with different temperatures, rainfall and light, which can influence the chemical composition of fruits [17].

The results indicate that walnut consumption conduce to a high input level of energetic value, ranged from 662.95 kcal in sample Falticeni and 726.9 kcal in sample Malini (Table 1).

Protein content of the studied walnuts ranges from 13.81% (Solca) to 17.22% (Suceava) (table 1).
Walnuts are good sources of vegetable protein; they may be used in vegetarian diets. In this case walnuts have been associated with a low cardiovascular risk compared to that of animal protein. The sample with the highest content is sample S4 (Suceava) 17.22 %.
According to Antoanela PATRAŞ, 2010 the proteins content of the Romanian walnut types is between 12% - 24%.

Table 1 Physico-chemical parameters walnut samples

Kernel characteristics	Samples of walnuts								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
Fruit weigh, (g)	11.93± 0.21	13.12± 0.35	13.42± 0.41	14.26± 0.52	12.94± 0.41	13.98± 0.52	12.03± 0.37	13.45± 0.33	14.52± 0.62
Kernel weight (g)	4.03± 0.21	4.71± 0.11	4.75± 0.23	4.78± 0.22	3.75± 0.27	4.21± 0.23	3.42± 0.25	4.35± 0.30	4.84± 0.31
Kernel ratio (%)	43,0924	36,3861	42,8465	52,6395	39,7822	40,1387	41,1929	42,0074	42,368
Water activity (aw %)	0.555	0.565	0.558	0.564	0.561	0.562	0.565	0.556	0.560
Moisture %	4.05± 0.25	3.77± 0.05	3.98± 0.07	4.02± 0.18	3.85± 0.25	3.85± 0.03	4.5± 0.25	4.22± 0.25	4.58± 0.25
Ash %	2.0± 0.13	2.1± 0.13	1.8± 0.11	1.7± 0.09	2.2± 0.13	1.95± 0.012	2.05± 0.015	1.92± 0.13	2.05± 0.13
Crude Protein %	15.2± 0.15	15.59± 0.2	13.81± 0.2	16.12± 0.1	14.81± 0.2	17.22± 0.2	15.26± 0.1	15.06± 0.2	15.26± 0.3
Total oil %	59.9± 1.33	57.85± 1.22	60.07± 1.20	59.07± 1.16	55.1± 0.47	56.68± 0122	53.81± 1.24	65.7± 0.90	66.1± 1.38
Carbohydrate content (CHO) %	20.85	20.69	20.34	19.09	24.04	20.3	24.38	13.1	12.1
Total phenols mg GAE/kg D.W	20.15± 1.02	23.22± 0.92	19.26± 1.27	19.82± 1.18	22.17± 1.25	23.52± 1.30	18.12± 1.09	17.28± 1.20	20.15± 1.28

Values represent mean ± SD [dry weight].

The highest negatively correlation have been observed in the case of total oil with carbohydrate content (r= - 0.992).
The obtained results were compared with the ones previously obtained for fruits collected in the same cultivars and in the same orchard by [17].
Valuable edible nuts produced by walnut trees are well appreciated because they are enriched with unsaturated fat (linoleic, oleic acid) [18]. The percentage values of protein, carbohydrates and lipids, respectively the variation of energetic values (kcal) of walnut samples are presented in figures 1 and 2.

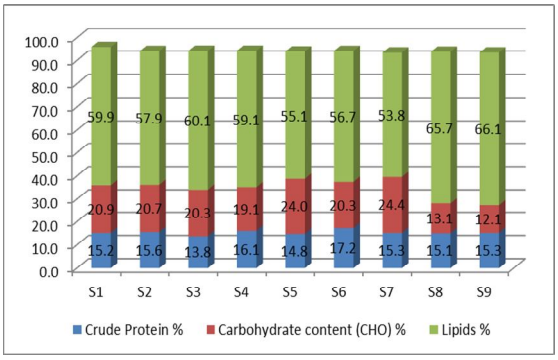

Figure 1. The values of protein (%), carbohydrates (%) and lipids (%)

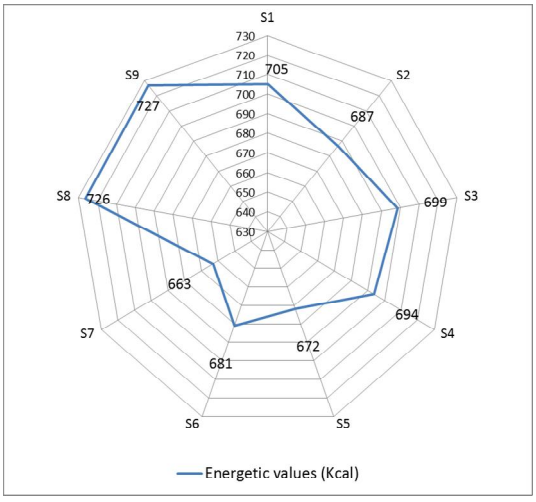

Figure 2. The variation of energetic values of walnut samples

The arithmetic mean of the energetic values (Kcal) for the 9 walnut samples is 694.78 (± 22.23).

Total Oil content

Fat was the highest constitute in all samples, ranging from 53.81% in Falticeni (S7) to 66.1% in Dolhasca (S9).
These values were similar to the results reported in [9], [19].
Oil contents of this study were lower than those reported by other [17]. Pereira et al., 2008, reported total oil contents from 78.83 to 82.4%.
Variation of total oil % in the walnut samples is presented in figure no. 3.

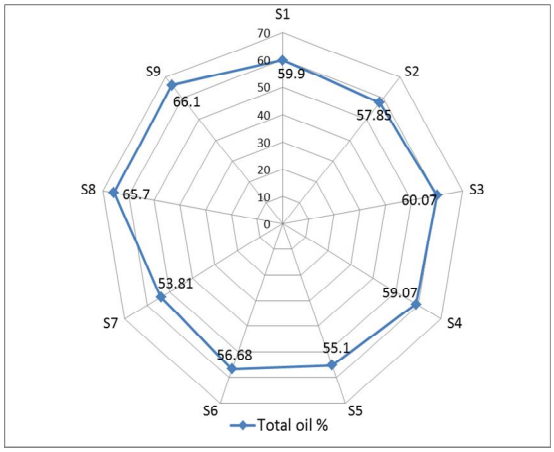

Figure 3. Variation of total oil (%) in the walnut samples

Total phenols

Among all the samples analyzed, walnuts of Suceava contained the highest total phenols 23.52 ± 1.30 mg GAE/kg D.W, followed by Radauti, Campulung Moldovenesc, Straja, Dolhasca, Solca, Paltinoasa, Falticeni and Malini walnuts. Based on the Folin–Ciocalteu method, the total phenolic content mg of GAE per 100 g of nut reported by Jun Yang et al. (2009) was in the order walnuts > pecans peanuts

> pistachios > macadamias > cashews > hazelnuts almonds> pine nuts > Brazil nuts, which shows a different pattern from our data.
However, the results obtained for total phenols content were in accordance with previous studies [19].

4. Conclusions

In conclusion, walnut (*Juglans regia*) is characterized by increased biological and nutritional value.Walnuts have various bioactive components and the consumption of walnuts has many health benefits such as: reducing "bad" cholesterol - LDL in blood and also the triglycerides, they are anti-diabetic and a valuable protective factor against the risks of cardiovascular disease, they also have anti-oxidant and anti-inflammatory effect.
These results demonstrate some differences in chemical and physical properties among the nine studied samples of walnuts from Suceava plateau. These differences could be attributed to environmental and cultivation conditions. The present study revealed that walnut kernels are mainly rich in protein and total oil content.
Total phenols content of walnuts varied from 23.52 mg GAE/g D.W. to 17.28 mg GAE/g D.W.
At drying, the moisture content of kernels varied from 3.85% to 4.58 % (w/w), but the differences among cultivars were not significant.
These data should help in selecting cultivars that are suitable for commercial production of walnut oil in Romania.

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