



Determination of Nutritional Value and Oxidative Stability of Fresh Walnut

Najmeh Chatrabnous, Navid Yazdani*, Kourosh Vahdati

Department of Horticulture, College of Aburaihan, University of Tehran, Tehran, Iran

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ABSTRACT

Walnut (*Juglans regia* L.) is a tree with significant economic value and usage for human health and various food industries. However, fresh walnut kernels are a less widespread product than the dried kernels. This study aimed to determine the fresh walnut kernel properties including, fatty acid composition, proximate composition, total phenolics (TPs), total antioxidant capacity (TAC), acidic, peroxide and saponification values of extracted oil and minerals of fresh walnuts kernels. Green walnut fruits were harvested on commercial maturity. The content of different fatty acids (%) was determined as 16:0; 5.91, 18:1; 77.7, 18:2; 11.13 and 18:3; 2.84. Also, the essential nutritional compounds such as protein content (16 ± 0.67), ash (3 ± 0.32), water (20 ± 0.49), fat (40 ± 0.22) and total carbohydrate (21 ± 0.23) were quantified and reported in percentages. The energy content was 508 ± 0.48 kcal. Acidic values, peroxide values and saponification values in walnut oil were 3 ± 0.14 , 0.3 ± 0.06 and 130 ± 0.54 respectively. Furthermore, the average mineral contents were also determined. In general, it can be claimed that fresh walnuts have significant amounts of protein, oil and minerals, and higher water content in comparison with dry nuts. Nonetheless, our results in comparison with the information available about dry walnuts showed that fresh walnuts contained less oil content compared to dried fruits.

Introduction

Walnuts are one of the oldest planted nut trees in the world (Sze-Tao and Sathe, 2000). They are known to have originated from southeastern Europe, eastern Asia and northern America. Indeed, this fruit is generally consumed as part of the Mediterranean diet and in many communities throughout the world. It has high levels of many nutrients (Abdallah *et al.*, 2015). Moreover, walnut is rich in unsaturated fatty acids, protein, and minerals, which help lower cholesterol levels and keep the brain healthy. The positive effects of walnut consumption for prohibition, management and treatment of diseases related to diet are proven. Walnuts provide

protection from cardiovascular disease (Ros, 2009) and diabetes, reduction of fatness and low grade systemic inflammation and the promotion of blood lipid profile. The healthily effect of walnuts can to be related to their chemical composition (Abdallah *et al.*, 2015).

Dry walnuts are a high-energy food source priming 630 kcal in 100g walnut. Proteins (up to 24%) and lipids (up to 70%) constitute more than 84% of the dry walnut kernel weight (Sze-Tao and Sathe, 2000). Walnuts are rich in fiber, polyunsaturated fatty acids, protein composition rich in essential amino acids and excellent mineral compositions (Tapia *et al.*, 2013a). However,

*Corresponding author: Email: n.yazdani@ut.ac.ir

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the high polyunsaturated fatty acids content make walnuts highly susceptible to oxidative rancidity resulting in off-flavor and discoloration of the oil (Macrae *et al.*, 1993). In addition, the most serious pathogens are fungi such as *Aspergillus flavus* and *A. parasiticus*, which can produce aflatoxins on dry walnut during storage that are both toxic and carcinogenic (Sharma and Sumbali, 2014). Moreover, dry walnuts provide substantial amounts of vitamins and iron (2.5 mg per 100g), magnesium (168.3mg per 100g), potassium (316.7 mg per 100 g) and phosphorus (316.7 mg per 100g) (Wardlaw, 1999) and dry walnut proximate compositions per 100 g walnut sample are as follows: protein 14.1g; total oil 68.0g; total carbohydrates 3.2g; cellulose 9.7g; ash 1.8g and moisture 3.2g (Tonbak *et al.*, 2006). Also, Caglarırmak (2003) investigated the chemical composition of 35 walnut genotypes and reported the following results: moisture 3.2–3.9%; protein 15.5–19.2%; ash 1.2–2.0 and fat 62–71%. However, the information on the composition of fresh walnuts is surprisingly limited. Nuts contain high levels of unsaturated fatty acids which are susceptible to lipid peroxidation when irradiated. Beside irradiation is known to produce free radicals interacting with both lipid and protein molecules. Peroxide value of oil is a valuable index to determine oil quality. Jiang *et al.* (2015) reported that environmental causes such as oxygen concentration, storage temperature and light affect the lipid oxidation. . The supply of antioxidants by way of the food chain is of high consequence for a healthy life. There is no extensive data on antioxidants in the unsaponifiable matter of nuts. Phenolic compounds have attracted substantial attention in the past few years due to their plenty health benefits (Pellegrini *et al.*, 2006). These compounds exhibit antibacterial, antiviral, anti-inflammatory, and vasodilatory actions (Breinholt, 1999; Duthie *et al.*, 2000). Most walnuts have higher TPs and antioxidant capacity than any other nuts

(Pellegrini *et al.*, 2006).m Most of the antioxidant capacity in walnuts results from tocopherols and other phenolic compounds (Arranz *et al.*, 2008). In addition, the TPs concentrations in fresh walnut kernels were higher than kernels that are dried at 36°C and stored at 1°C for 24 hours (Christopoulos and Tsantili, 2012). In addition, TPs reduction arrived at 47% after a three day sun-drying in pistachios Ballisteri *et al.*, (2009) and to 14% in nuts dried at 45°C for 34 hours (Tsantili *et al.*, 2011).

Walnuts are customarily used as dried, but since the 1990s, it has been recognized that fresh walnuts might be more nutritious than dried ones, and the individual flavor of the fresh walnut is appreciated in some countries (Cannella and Dernini, 2004). In addition, the most serious pathogens such as *Aspergillus flavus* and *Penicillium* and *Fusarium* in dry walnuts (Deabas, 2010), are not a risk in fresh walnuts. To date, walnut research has mostly focused on its nutritional composition and lipid profile and, as far as we know, the nutritional values of fresh walnuts have not been extensively studied. In this study, we tried to determine parameters related to quality of fruit and the nutritional value of fresh walnut.

Materials and Methods

Materials

Walnuts (*Juglans regia* L.) were obtained in the late summer when the packing tissue between the kernel halves turned brown. All nuts were harvested from one tree that was considered as a promising genotype from an orchard of the Bavanat, Fars province in Iran. Nuts were hand-picked directly and carried to the laboratory. Then, the husk and hard shell of nuts were manually removed and kernels were stored at 4°C.

Fatty acid analysis

Walnut fat components were extracted by diethyl ether on ice. In order to evaluate fatty acid composition of walnut oil, a 60m × 0.25mm × 320µm film thickness fused-silica capillary column HP-5 was connected to an Agilent-7890A gas chromatograph, equipped with a flame ionization detector (FID) and split/split less injector. The N₂ was used as the carrier gas at a velocity of 23 cm/s and as the make-up gas at a rate of 30 mL/min. A temperature program of 150°C for two minutes rising to 220°C at a rate of 5 °C/min was used. The fatty acid methyl ester (FAME) dissolved in hexane was injected (1 µL) in a split mode of injection at a split ratio of 1:20. The injector and detector temperatures were 230°C and 260°C, respectively. Agilent-7890A integrator was used for recording the peak areas.

Proximate composition

Association of Official Analytical Chemists (AOAC) method was used to determine proximate composition of nuts (AOAC, 1990). Ash (AOAC 20.013), fat (AOAC 22.034), moisture (AOAC 22.003) content were investigated by using standard methods. Nitrogen content was specified by micro-Kjeldahl method, multiplied by 6.25 and reported as protein.

The total carbohydrate was evaluated by subtracting the amount of ash, protein and fat from total dry matter, using the following formula: Carbohydrate content = 100 % - (% moisture + % protein + % fat + % ash). Energy was expressed as kilocalories. Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g lipid) (Pereira *et al.*, 2008).

Mineral analyses

An amount of 0.5 gram of walnut samples was accurately weighed into a Teflon flask. The nut was then mixed with nitric acid and perchloric acid, the concentration of minerals in walnut, such as calcium,

magnesium, potassium, sodium, manganese and iron were calculated according to standard curve, using atomic absorption spectrophotometer (Model Varian 220, Australia). The amount of phosphorus at wavelength of 430 nm with using a spectrophotometer was calculated (Kabas *et al.*, 2007).

Physicochemical properties of walnut oil

The acid value was determined by the standard AOAC method (AOAC, 1990). Peroxide value was calculated in terms of mEq of oxygen per kg of extracted oil (Atares *et al.*, 2011). Saponification number is determined based on standard samples extracted oil by soxhlet. To do this, 1 gram of pure oil free of solvent was entered into an Erlenmeyer flask and was weighed. Potassium hydroxide was added to the flask 15 ml ethanol. The content of the flask was titrated with 1 N hydrochloric acid.

Total phenolic concentration and total antioxidant capacity

Walnuts (500 mg) were crushed to smaller than 0.2 mm in porcelain mortar and were extracted by 80% methanol. For this purpose the mixture was placed on a shaker with 200 rpm for 48 hours at ambient temperature. Then samples were centrifuged at temperatures of 10°C and 2655g. The total phenolics (TPs) concentration was measured by a Folin-Ciocalteu colorimetric method (Singleton *et al.*, 1998). Briefly, 4 mL of diluted extract was added into a tube containing 2.6 mL of deionized water and 1 mL of Folin-Ciocalteu reagent, and the tube was stirred and allowed to stand at room temperature for six minutes. Then, 1 mL of Na₂CO₃ (7%, w/v) was added to the mixture. Absorbance was measured at 750 nm using a spectrophotometer versus a blank after incubation for 90 minutes at room temperature. The results were

expressed as Gallic acid equivalents on a dry weight basis (mg GAE g⁻¹ D.W.).

Total antioxidant capacity (TAC) was evaluated according to DPPH assay (Zhang *et al.*, 2009). The concentration of 4, 6 and 12µL of samples were brought to the final volume 300µL by methanol (80%) into a screw tap tube, then 300µL DPPH solution (2,2-diphenyl- 1-picrylhydrazyl) was added. The decrease in absorbance at 515 nm was recorded versus blank after 30 min incubation at room temperature. The DPPH scavenged based on percent was calculated using the following equation:

$$\text{DPPH Inhibition (\%)} = (A_0 - A / A_0) \times 100$$

(A₀= Blank, solution absorption DPPH, A= sample; solution absorption DPPH after adding the extract).

Also, the IC₅₀ value was defined as the concentration of an antioxidant in the reactive system

necessary to reduce the initial DPPH concentration by 50% and was determined according to the results.

Statistical analysis

In this study, the sampling all experiments were carried out in triplicate and the results depicted as a mean of the three values with the standard error.

Results

The composition of fresh walnut

The composition of the fresh walnuts is shown in Table 1. The protein value of 16.5 percent was found. Moreover, a moisture content of approximately 20 percent was determined. Ash content was about 3 percent, total carbohydrate value was 21 percent and energy was estimated at 508 (kcal per 100g fresh weight).

Table 1. Proximate composition of fresh and dry walnuts, Bavanat genotype

Analysis	Fresh walnuts	Dry walnuts
Ash (%)	3 ± 0.3	3.5 ± 0.1
Protein (%)	16 ± 0.7	19 ± 0.7
Moisture (%)	20 ± 0.5	3 ± 0.2
Fat (%)	40 ± 0.2	51.5 ± 0.8
Total carbohydrate (%)	21 ± 0.2	23 ± 0.6
Energy (kcal/100 g)	508 ± 0.5	592 ± 0.3

Mineral content of walnut

Fresh walnuts contain high levels of minerals that are shown in Table 2. Data illustrates that the studied fresh walnut cultivar contains many necessary dietary minerals, such as potassium (451mg/100g), phosphorus (341mg/100g), magnesium (161 mg/100 g), calcium

(113mg/100g), manganese (3.72mg/100g), iron (3.13 mg/100 g), zinc (3.02mg/100g), copper (1.97mg/100g) and sodium (1.92 mg/100 g),. There was a high level of potassium in the walnut kernel, followed by phosphorus, magnesium and calcium.

Table 2. Mineral contents of fresh walnut (mg/100 g F.W.)

Mineral	Values (mg/100 g)	Mineral	Values (mg/100 g)
Phosphorus (P)	342±0.5	Copper (Cu)	1.72±0.2
Potassium (K)	451±0.2	Iron (Fe)	3.13±0.2
Magnesium (Mg)	161±0.3	Selenium (Se)	3.33±0.1
Zinc (Zn)	3.78±0.4	Sodium (Na)	1.93±0.2
Manganese (Mn)	4.36±0.2	Calcium (Ca)	113±0.7

Fatty acids profile of the walnut cultivar

In this study, fat contents of the kernels were determined (Table 1). In addition, fatty acids profile in the oil of the selected walnut genotype is described in

Table 3 as palmitic (16:0), oleic (18:1), linoleic (18:2), linolenic (C18:3) were detected.

Table 3. Fatty acid composition of oil extracted

Linolenic (C18:3)	Linoleic (C18:2)	Oleic (C18:1)	Palmitic (C16:0)
2.84	11.13	77.7	9.51

Physicochemical properties of walnut oil

Peroxide values (POVs) indicated the oxidation grade of polyunsaturated fatty acid and specified the organoleptic properties of oil. The POVs of fresh walnut kernel were low, 0.3 ± 0.06 meq O_2 /kg oil. Moreover, acid values (AVs) indicated the extent of free fatty acids in the walnut kernel. The initial AVs of fresh walnut kernel were 3.06 ± 0.14 mg g^{-1} fresh weight and also, saponification values (SVs) in the studied fresh walnuts were about 130.13 ± 0.54 percent.

Total phenolics and antioxidant capacity

The average IC₅₀ value was 6.27 ± 0.02 mg. mL^{-1} for sample extracts and scavenging effects based on the percentage which was 95 ± 0.06 . However, the scavenging effect performed by vitamin C (35 μ mol) was 98 percent and the average TPs were 1.54 ± 0.01 mg GAE. g^{-1} DW.

Discussion

The results of proximate composition of fresh and dry walnuts are shown in Table 1. As previously reported, dry walnuts have a high protein level like numerous legumes and cereals (Boye *et al.*, 2010). Therefore, it seems that fresh walnut samples have a valuable protein content which can help human nutrition as well. Caglarirmak (2003) reported proximate amounts of protein (13.8); ash (1.8), moisture (3.0), fat (62.8) and total carbohydrates (18.7) in dry walnuts. In

another study, the proximate composition of different dry Persian walnut cultivars were varied in terms of the amount of fat 62-67, protein 13.8-14.9, ash 2.1-2.2, and total carbohydrates 12.8-16.7 (Gharibzahedi *et al.* (2014)). The same results were also reported by other authors (Tapia *et al.*, 2013b). Drying reduced kernel moisture in 'Franquette' walnut to about 8% (w/w), while the moisture range in different varieties of dried walnut kernel were also reported from 3 to 13% (Kader and Thompson, 2002). Their results illustrated that walnut consumption brings a high input level of energetic value, because walnuts are rich in fat. In addition, genetic factors, soil composition, different geographical origins and maturation can also affect the nutritional composition of nuts (Wakeling *et al.*, 2001). Therefore, some differences between our results and the work of others might be justified by genetic and environmental factors. Moreover, the proximate composition of dry walnuts in Table 1 could be a reference used for comparing the nutritional value of this promising genotype with other dried walnut cultivars.

Our results on the mineral contents of fresh walnut (mg/100 g F.W.) are presented in Table 2. The results obtained in this study were similar to those perceived in dry walnuts from Turkey (Ozcan, 2009). Our results also agree with those reported by Lavedrine *et al.*, (2000) except for magnesium. Walnuts contain high levels of phosphorus (310–510 mg/ 100 g), potassium

(390–700 mg/ 100 g) and magnesium (90–168 mg/ 100 g), but their content in sodium and iron is relatively low (1–15 mg/ 100 g) in contrast, very low levels of sodium, copper, zinc, selenium, manganese and iron may also be present. Moreover, these elements play important roles in human physiology. Our data are also in agreement with earlier results reported by Gharibzahedi *et al.*, (2014) in the case of iron and zinc. They investigated mineral contents of three walnut cultivars in Iran. Furthermore, Tapia *et al.*, (2013) stated that minerals in four cultivars of Persian walnut are mainly magnesium, potassium and calcium, but they reported very low levels of sodium, zinc, copper, manganese and iron. These differences in the minerals content in walnut may be due to the elements available in the soil (Wakeling *et al.*, 2001). Also, the data indicated that magnesium and potassium can possibly improve blood pressure, and it is important to maintain the suitable balance of calcium and potassium in the body (Elin, 1993). Moreover, low levels of magnesium in the diet can contribute to heart assail and hypertension (Tapia *et al.*, 2013b). Based on our results, the levels of these important elements in fresh walnuts are as appropriate in amount as dry walnuts contain likewise, and these could nutritionally benefit consumers.

Often, nut kernels are rich in unsaturated fatty acids with a double bond (Monounsaturated) such as oleic acid, whereas the walnuts are rich in two unsaturated fatty acids, with a few double bonds (making them Polyunsaturated), and contain linoleic acid and linolenic acid (Zwarts, 1999). The ratios of fatty acids are deemed important for their economic and nutritional value (Savage *et al.*, 1999a). Savage *et al.* (1999) conducted experiments on 13 different cultivars of dry walnut and reported that the oleic acid content of the oils ranged from 12.7 to 20.4% of the total fatty acids, while linoleic acid content ranged from 57.0 to 62.5%, and the linolenic acid contents ranged from 10.7 to 16.2% among 13 cultivars. However, our study found oleic

acid to be present in higher amounts. Not only in walnut kernels oil composition, but also its content can be variable depending on the cultivar, location of growth and irrigation rate (Beyhan *et al.*, 1995, Caglarırmak, 2003). Wolf *et al.* (1982) reported that the fatty acid composition was strongly affected by temperature, as a result of which linolenic and linoleic acids declined noticeably. However, oleic acid increased as the temperature increased, but palmate remained unchanged. In our study, polyunsaturated fatty acids (PUFAs) were the principal component of total fats extracted from the fresh walnut due to the high content of oleic acid, and the PUFA/ saturated fatty acids (SFAs) (9.639) was nutritionally suitable in fresh walnuts.

POVs of walnut oils from Toyserkan, Chaboksar and Karaj cultivars were 1.9, 3.1 and 2.2 meq O₂. kg⁻¹ more than POV in our study. Peroxide value depends on a number of factors including the oxidation temperature, the used method for oil extraction, and the sort of fatty acids and natural antioxidants present in the oil (Gharibzahedi *et al.*, 2014). The higher peroxide value in the oil extracted from walnut oil is probably due to the low quantity of bioactive compounds like phenolic content. Jiang *et al.* (2015) reported that the primary peroxide value of fresh walnut kernels were very low, 0.0039±0.0008 meq O₂. kg⁻¹ oil. In the studies carried by Savage *et al.* (1999b), fresh walnut kernels had POVs ranging from 0.15 to 0.29. PUFAs are easily oxidized when in contact with oxygen, heat and light, and oxidized PUFAs can pose threats to human health because they create all kinds of toxic reactions with sugars and proteins in our bodies. According to our findings, fresh walnuts have considerably less POVs than dry walnuts. Moreover, Jiang *et al.* (2015) indicated that the initial AVs of fresh walnut kernel were 3.71 ± 0.02 mg g⁻¹ which are more than the AVs in our study. Meanwhile, AVs of oils were extracted from dry walnuts, and the varieties of Chandler, Franquette,

Hartley, Lara, Mayette, Serr, Sorrento and Tulare exhibited values between 0.05 ± 0.01 to 0.22 ± 0.01 (Martínez and Maestri, 2008), which are less than the values exhibited by fresh walnuts. In addition, in the study of the SVs in dry walnuts, the ranges of SVs were reported to be from 169.1 to 167.6 percent depending on different cultivars (Gharibzahedi *et al.*, 2014). The high SVs displayed that the oils have a smaller molecular weight than other oils with low SVs. The same as POVs and AVs, SVs are also nutritionally better than dry walnuts.

The antioxidant effect indicated by walnut products are derived from phenolic compounds and phytochemicals, which counteract harmful effects of free radicals. In this study, the antioxidant potential of fresh walnut kernel sample was calculated by reducing the scavenging activity power of DPPH radicals. Walnuts contain high contents of α -tocopherol, a vitamin E compound, which has antioxidant activity, being mainly active in the preservation of lipid oxidation (Pereira *et al.*, 2008). Zhang *et al.* (2009) revealed that the IC50 values in walnut oils, which were extracted by different solvents, had values between 0.007 and 20 mg.mL⁻¹. Moreover, Amini and Ghoranneviss (2016) reported that the drying of walnuts could contribute to a reduction of the TPs and antioxidant activity. The reduction rates were 5.1, 4.6, 4.5 and 3.1mg GAE.g⁻¹ DW according to different cultivars, and the same results were obtained for antioxidant activity. However, the antioxidant activities in dried walnuts were 1.5, 7.2, 4.4 and 11.0 μ mol TAE.g⁻¹ DW for Toyeserkan, Taleghan, Mazandaran and Shahmirzad genotypes, respectively. Christopoulos and Tsantili (2012) reported that values of TPs and DPPH assays in the harvested fresh 'Franquette' before storage were 30 mg GAE. g⁻¹ DW and 232 μ mol TAE g⁻¹ DW, respectively, in which the TPs were reduced to 25 mg GAE. g⁻¹ DW in dried walnuts before storage. Furthermore, at harvest, fresh 'Franquette' kernels

showed TAC levels higher than dried ones (Christopoulos *et al.*, 2010). Drying could cause antioxidant losses as a result of the performed methods (Manzocco *et al.*, 2001). These differences could be the result of the different structures of the fruit and also different storage conditions. Indeed, losses of phenols and other antioxidants seem to be unavoidable even under the mild drying conditions (Christopoulos and Tsantili, 2012). Therefore, the higher nutritional value of fresh versus dried walnuts is naturally expected.

The TPs in dry walnuts have been analyzed extensively. However, the reported results were varied. Pereira *et al.* (2008) illustrated those TPs of six different walnut cultivars grown in Portugal ranged between 58.9 and 95.1mg GAE. g⁻¹. In another study, the total polyphenols were assessed in four dry walnut cultivars (*Juglans regia* L. cultivars Serr, Hartley, Chandler and Howard), among which the Howard showed the highest TPs (58.2 mg GAE. g⁻¹) content (Tapia *et al.*, 2013b). In contrast, other observations indicated much lower levels of TPs in dry walnuts ranging from 15.8 to 16.9 mg GAE. g⁻¹ (Anderson *et al.*, 2001, Chen and Blumberg, 2008) or ranging from 10.7 to 16.0 mg GAE. g⁻¹ by Arranz *et al.* (2008) and Anderson *et al.* (2001), respectively. In all studies, the phenolic compounds were more than TPs in our study. These variations in TPs content might be because of having different cultivars or the varied stages of kernel maturity at harvest.

Conclusions

Walnuts contain diverse compounds that increase the nutritional value of the human diet. In this study, chemical composition and the attribute of certain parameters of the kernel and its oil in fresh walnuts were studied. Despite the need for further research, it can be concluded that the fresh walnuts are a richer dietary source due to having the highest protein, carbohydrate, and mineral contents such as Ca, P, K, Mg, Zn, Mn, Fe,

Se and Cu, fatty acid, PUFAs, TPC, oil oxidative stability and antioxidant activity. Therefore, the results support the possible prospect of advertising fresh walnuts as a source of natural antioxidants or for the production of functional foods.

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