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# Nutritive Value of Persian Walnut (*Juglans regia* L.) Orchards

[1]*Sara Aryapak and* [2]*Parisa Ziarati*

[1]Department of Food Sciences and Technology,
Pharmaceutical Sciences Branch (IAUPS), Islamic Azad University, Tehran, Iran
[2]Department of Medicinal Chemistry, Pharmaceutical Sciences Branch (IAUPS),
Islamic Azad University, Tehran, Iran

**Abstract:** *Juglans regia* L. (Persian walnut), is a temperate nut crop and Iran is one of its centers of origin and diversity. According to the statistics provided in 2007 by (FAO), Persian walnut grows in Iran, ranking third globally. As geographical conditions affect the nutritional value of walnuts, the objective of this study was evaluation of protein, crude fiber, fatty acids and some mineral element contents in samples in Tehran and Karaj County farmlands as two economically important provinces. Samples were collected during the 2 years harvest from 12 different distinguished cultivars of trees grown in a replicated trial in an experimental orchard. All trees under the study were of seedlings origin and are growing naturally and treating traditionally. The order depending on the contents of elements (mg/100 g) in *J. regia* samples in Karaj studied regions was Mg> K> Fe > Cu >Ca >Zn> Na, whereas in Tehran farmlands the order is: Mg> K> Fe > Ca >Cu >Zn> Na which shows that high levels of these elements in the soil of area, have a great impact on the highness of calcium and copper in the fruits. Total oil content ranged from 60.9 to 73.1%, while the crude protein ranged from 13.5 to 20.2%. Dietary fiber ranged from 1.0 to 4.3% and starch content made up no more than 2.6% of the remaining portion of the kernel. The main fatty acids of walnut kernel oils were oleic, linoleic, linolenic and palmitic acids. Linoleic acid contents of kernel oils varied between 48.9% and 58.2%. On the other hand, oleic acid contents ranged between 20.0% and 25.3%. As a result, the present study showed the walnut kernels from Iran is a potential source of valuable oil which might be used for edible and other industrial applications.

**Key words:** Walnut kernel · (*Juglans regia* L.) · Food Value · Protein · Mineral elements

## INTRODUCTION

Walnut, whose scientific name is *Juglans regia* L. is a plant from Juglandaceae family [1]. *Juglans regia* L. is the most widespread tree nut in the world. The walnut tree species is native to the old world [2]. Walnut was probably domesticated in Iran and subsequently introduced to other countries [3]. Persian walnut (*Juglans regia* L) is an economically important species cultivated worldwide for its wood and nuts [4]. *Juglans regia* L. (Persian walnut), is a temperate nut crop and Iran is one of its centers of origin and diversity [5]. Therefore Iran plays an important role in walnut production in the world. According to the statistics provided by Food and Agriculture Organization of United Nations (FAO) in 2007, Persian walnut grows on 70,000 ha in Iran, producing 170,000 tons of nuts in shell, ranking third globally[3]. Also in Iran, walnut has a special value in Iranian foods and is very common in traditional Iranian foods for example Fesenjan. In addition walnuts are mostly eaten as Iranian dessert nuts such as Qottab, Baklava, Naan Gerdooee (Persian walnut Cookies) and Ranginak. Optimum geographical conditions in Iran have led to the growth of about 8000 herbal species in the country [6]. Walnut is cultivated in some regions of Iran such as Tehran, Karaj, Hamedan, Khorasan and Fars [6, 7]. Walnut kernels are useful to human health. They have antibacterial, antioxidant and Anticancer activity [2]. Walnuts, the seeds of *Juglans regia* L., are a highly nutritious food [8]. Walnut kernels generally contain about 60% oil, but this can vary from 52 to 70% depending on the cultivar, location grown and irrigation

**Corresponding Author:** Parisa Ziarati, Islamic Azad University, Pharmaceutical Sciences Branch (IAUPS),
Faculty of Pharmacy, Medicinal Chemistry Dept. No 99, Yakhchal, Gholhak, Dr. Shariati, Tehran, Iran.
Tel: +98-21-22633980, Fax: +98-21-22600099

rate [8, 9, 10]. The high protein and oil contents of the kernels of *Juglans regia* L. (Juglandacea) make this fruit in dispensable for human nutrition. Therefore, the walnut is classified as a strategic species for human nutrition and is included in the Food and Agriculture Organization of United Nations (FAO) list of priority plants [11]. The major constituents of the oil are triacylglycerols; free fatty acids, diacylglycerols, monoacylglycerols, sterols, sterol esters and phosphates are all present in only minor quantities [9]. In fact, among vegetable oils walnut oil has one of the highest amounts of PUFAs (up to 78% of the total FA content) [2]. The major fatty acids (FA) found in walnut oil are oleic (18:1 n-9), linoleic (18:2 n-6) and linolenic (18:3 n-3) acids. Therefore, Walnuts have high amount of omega-6 and omega-3 polyunsaturated fatty acids (PUFA), which are essential dietary fatty acids [12]. The Food and Drug Administration (FDA) authorized a health claim indicating that diets including walnuts can reduce the risk of heart disease [8]. The heart benefits of walnuts include lowering cholesterol, reducing inflammation and improving arterial function [1]. Although walnuts are rich in fat, a diet supplemented with walnuts had a beneficial effect on blood lipids, lowering blood cholesterol and lowering the ratio of serum concentrations of low density lipoprotein: high density lipoprotein by 12% [13]. Also Walnuts contained 16.66% protein [14].The content of Albumin, globulin, prolamin and glutelin, respectively accounted for 6.81, 17.57, 5.33 and 70.11% of the total walnut protein [14]. Walnut proteins could be a good source of essential amino acids for adults [8]. Walnut is considered a good source of dietary minerals [15]. Potassium (K), phosphorus (P), magnesium (Mg) and iron (Fe) are found in significant quantities in these nuts [15]. Calcium (Ca), sodium (Na), zinc (Zn) and Copper (Cu) also exist modestly [6, 15]. Iron (Fe) is an element in hemoglobin, myoglobin and a large number of enzymes; therefore it is an essential mineral in daily diet [6, 16]. About 2% of total body weight of human body is consisted of Calcium. Calcium (Ca) is the major component of bone and assists in teeth development [6, 13, 16]. Potassium (K) is the third most abundant mineral in human body which is acting as an electrolyte. This mineral is needed for keeping heart, brain, kidney, muscle tissues and other important organs of human body in good state[6,16].Zinc (Zn), a constituent of enzymes involved in most major metabolic pathways, is an essential element for plants, animals and humans [17]. In addition walnuts have highest levels of polyphenolic antioxidants than any other common edible nuts [6, 18]. They are also good source of flavonoids, vitamins (for example, vitamin A and E), Crude fiber and pectic [1, 2],. It has been reported that there are more than 990000 ha sown to walnut trees in Tehran Province; walnut production in this province is thus economically important in Iran [7]. Water and soil of the cultivation regions have an important role on quality and quantity of micronutrient compounds (such as minerals, vitamins, fatty acids and amino acids) in walnut. The geographical conditions affect the nutritional value of walnuts [6, 16]. The objective of this study was evaluation of protein, crude fiber, fatty acids and some mineral element contents by atomic absorption spectrophotometry in Persian walnut (*Juglans regia* L.) orchards in Tehran and Karaj County Farmlands.

## MATERIAL AND METHODS

**Sampling Methods:** Walnuts (*Juglans regia* L.) were collected during the autumn 2013 and 2014 harvest from 12 different distinguished cultivars of trees grown in a replicated trial in an experimental orchard at Karaj and Tehran county farmlands.

In the areas of Tehran and Alborz province regions (Iran) two main walnut populations were investigated. 100 walnut trees were collected from the Arangeh population in Karaj county, Alborz province (35° 50' 8" N, 51° 0' 37" E ) and 107 collected from Baghestan population, [Baghestan is a city in the Central District of Shahriar County, Tehran Province, Iran (35°38'02"N 51°08'14"E)]. Both regions have been known as favourable areas in Iran to walnut culture and are shown in figure 1. All trees under the study were of seedlings origin and are growing naturally and treating traditionally. The selected genotypes were named based on their location and these names were supplemented with numerical characters. The pomological analyses encompassed 90- 120 nuts per tree in the period of 2 years. The matured nuts samples were collected manually. After harvest, shells of walnut fruit were removed and the kernels of some walnut cultivars were obtained by hand processing from walnuts dried at ambient temperature in air conditions. The fruit were stored in + 4 °C until the analyses. The nut and kernel weights of 100 fruits were determined. The chemical and physical properties (moisture, crude protein, crude oil, crude fiber, ash, relative density, refractive index, free fatty acids, peroxide value, saponification number, unsaponifiable matter) were analyzed according to AOAC methods [19,20]. Nitrogen was established by Kjeldahl analyses, multiplied by 6.25 and determined as protein. The total fat content was determined in accordance with AOAC [22] method.

Fig. 1: Description of Walnut sampling

**Statistical Methods:** For statistical analysis, Microsoft Excel (2010) and XLSTAT-Pro was used (all data were expressed as means ± standard deviations for each sampling and farmland's locations. Data were analyzed by Pearson correlations.

**Moisture Content:** All samples were oven dried at 80°C for 36-48 hours until a constant weight were obtained. The moisture contents were expressed as loss in weights of the wet walnut kernel samples [21-24].

**Crude Fiber:** Five grams of the ground walnut samples were digested in 100 ml of 1.25% $H_2SO_4$. The solutions were boiled for 45 minutes and then were filtered and washed with hot distilled water. The filtrates were digested in 100 ml of 1.25% Sodium Hydroxide solutions. These solutions were heated for 60 minutes, filtered and washed with hot deionized water and over dried. The final oven-dried residues were ignited in a furnace at 550°C. The weights of the left after ignition were measured as the fiber contents and were expressed in term of the weights of the samples before ignition.

**Crude Protein:** The protein nitrogen in one gram of the dried samples were converted to ammonium sulphate by digestion with concentrated $H_2SO_4$ (Merck 96.5%) and in the presence of $CuSO_4$ and $K_2SO_4$ [24, 25]. The solutions were heated and the ammonia evolved were steam distilled into Boric acid 2%. The nitrogen from ammonia were deduced from the titrations of the trapped ammonia with 0.1M HCl with Tashirus indicator (methyl red: methylene blue 2:1) until a purplish pink color were obtained. Crude proteins were calculated by multiplying the valve of the deduced nitrogen by the factor 6.25 mg [24-26].

**Ash Content:** One gram of the oven-dried samples in powder from was placed in acid washed crucible by known weight. They were ignited in a muffle furnace for 4-5 hours at 550 °C. After cooling crucibles they were weighed and the ash contents were expressed in terms of the oven-dried weight of the sample.

**Oil (Lipid) Content:** The lipid contents of five grams of walnut kernel by petroleum ether in a Soxhlet apparatus were extracted. The weight of the lipid obtained after evaporating off the petroleum ether from the extracts gave the weights of the crude fat in the samples [23, 24].

**Carbohydrate Content:** The carbohydrate content of the samples were estimated as the differences obtained after subtracting the values of organic proteins, lipids, ashes and fibers from the total dry matter for both of kernel samples.

**Zinc, Manganese, Copper and Potassium Determination:** For Zinc, Manganese, Copper and Selenium concentration walnut kernel samples were dried in oven for 68-72 hours at a temperature of 85°C. The samples were then ground and sieved through 0.5 mm sieve. The powdered samples then subjected to the acid digestion using nitric acid (65% Merck, Germany), Sulfuric acid (96.5% Merck, Germany) and perchloric acid (70% Sigma- Aldrich). Two gram of air-dried of each homogeneously walnut kernel samples accurately weighed and 20.0 mL of the digestion mixture (3 parts by weight of concentrated nitric acid: 2 parts of concentrated Sulfuric acid & 3 parts by weight concentrated perchloric acid) and heated slowly by an oven and then rise the temperature. The remaining dry inorganic residues were dissolved in 25.0 mL of nitric acid and the solution used for the determination of mineral elements. Blanks and samples were also processed and analyzed simultaneously. All the chemicals used were of analytical grade (AR). Standardized international protocols were followed for the preparation of material and analysis of heavy metals contents [27-33]. The samples were analyzed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame, using at least five standard solutions for each metal and determination of potassium content was followed by FDA Elemental analysis [34]( ORA LABORATORY MANUAL, 2013). Also, periodic testing of standard solutions was performed in order to verify of reliability of the measuring apparatus. The accuracy was checked using quality control test for fungi and their substrate samples to show the degree of agreement between the standard values and measured

values; the difference was less than 5%. The samples were analyzed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene, flame temperature: 2800°C, acetylene pressure: 0.9–1.0 bar, air pressure: 4.5–5 bar, reading time: 1–10 sec (max 60 sec), flow time: 3-4 sec (max 10 sec).

**Iron Determination:** The aliquot was passed through the atomic absorption spectrophotometer to read the iron concentration. Standards were prepared with a standard stock of 10 mg/L using ferrous ammonium sulphate where 3 - 60 ml of iron standard solution (10 mg /L) were placed in stepwise volumes in 100 ml volumetric flasks. 2 ml of hydrochloric acid were added and then brought to the volume with distilled water. The concentration of iron in the aliquot was measured using the atomic absorption spectrophotometer in mg/L. The whole procedure was replicated three times.

**Calcium, Sodium and Magnesium Determination:** The contents of Ca, Mg and Na in walnut kernels were measured by atomic absorption spectrophotometer (AAS) (Model AA-6200 Shimadzu, Japan) according to the method of Hernandez [35]. A 5 g sample was placed in a previously weighed porcelain crucible and heated. The resulting white ash was weighed, dissolved in 12 ml of concentrated nitric acid, percholoric (3:2) and diluted with nitric acid 10% in a 25 ml calibrated flask. The solution then was used to determine Ca, Na and Mg. Standard stock solution of sodium, magnesium and calcium was prepared from AAS grade chemicals (Merck, Germany) by appropriate dilution.

**Selenium Determination:** Stock standard solutions for selenium were 1000 g /mL solution. All reagents and standards were of analytical grade ( Merck, Germany). The palladium matrix modifier solution was prepared by the dilution (10 g/ L) $Pd(NO_3)_2$ and iridium AA standard solution, 1000 g/ mL in 20% HCl, 0.1 % V/V nitric acid prepared by dilution trace pure 65 % nitric acid and 0.1 % Triton X-100 were used. Doubly distilled water was used in all operations. The samples were analyzed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan). The analyze performed according by Analytical Method ATSRD [36].

**Fatty Acid Determination:** Determination of Fatty acid composition for walnut samples were done by using a modified fatty acid methyl ester method as described by *H?s?l* [37, 38]. The oil was extracted three times for 2 g air-dried seed sample by homogenization with petrolium ether. The oil samples (50-100 mg) were converted to its fatty acid methyl esters (FAME). The methyl esters of the fatty acids (1 µl) were analyzed in a gas chromotography (Shimadzu GC-2011) equipped with a flame ionizing detector (FID), a fused silica capillary column (60 m x 0.25 mm i.d.; film thickness 0.20 micrometer).

## RESULTS

The "*ash content"* is a measure of the total amount of minerals present within a food, whereas the "*mineral content"* is a measure of the amount of specific inorganic components present within a food, such as Ca, Na and K. Determination of the ash and mineral content of foods is important for a number of reasons such as nutritional labeling: The concentration and type of minerals present must often be stipulated on the label of a food and quality: The quality of many foods depends on the concentration and type of minerals they contain, including their taste, appearance, texture and stability. The average of moisture content in Tehran kernel samples was 3.11% while it is 2.68 % for Karaj samples which shows that both of them can be preserved for a long time. The ash content of walnut kernels in Tehran and Karaj population were the same. Proximate composition and physicochemichal characteristics of all samples has shown in figure 2.

**Mineral Elements Composition:** Literally, the kernels contain many kinds of essential and mineral elements; magnesium, Potassium and calcium were the major elements. The mean content of mineral elements (mg/100 g DW): Sodium, Selenium, Calcium, Iron, Magnesium, Copper and Zinc in the Persian walnut (*Juglans regia* L.) samples are shown in Table 2. The samples were analyzed by wet digestion method and standardized international protocols were followed for the preparation of material and analysis of heavy metals contents and analyzed by Atomic Absorption Spectrophotometer in Research Laboratory in Pharmaceutical Sciences Branch, Islamic Azad University.

Due to historical surveys walnut is considered as a good source of dietary minerals among common foodstuffs. Walnuts almost contain high quantities of minerals, especially calcium, which is present much higher in Tehran sample walnuts than Karaj population in this study. Potassium, Iron, magnesium, Potassium and Zinc were found in significant quantities in these studied nuts (Fig. 3).

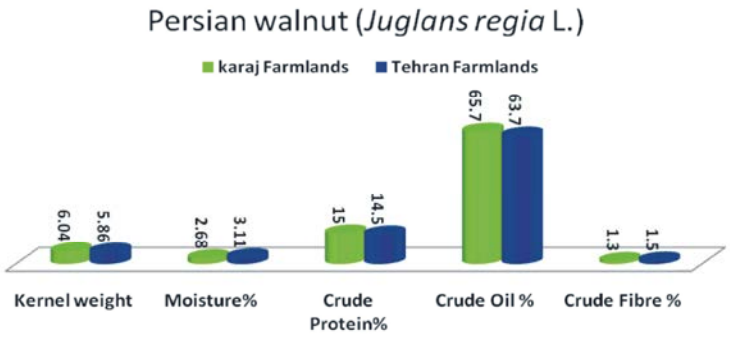


Fig. 2: Proximate composition and physicochemical characteristics of Persian walnut (*Juglans regia* L.) orchards kernel samples.

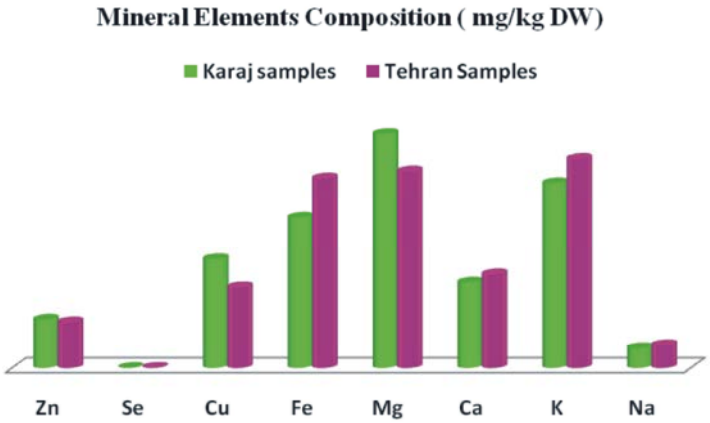


Fig. 3: The mean content of mineral element compositions (mg/kg DW) in two walnut populations (Karaj and Tehran locations)

Table 1: The diversity of nut size and weight of Persian walnuts collected in 2012-2013 in two main walnut populations in the areas of Tehran and Alborz province regions (Iran)

| | | Group | |
|---|---|---|---|
| Traits | | T*(n= 30) | K*(n= 30) |
| Nuts length (mm) | Mean | 31.08 | 43.02 |
| | Min | 24.71 | 39.64 |
| | Max | 36.42 | 45.21 |
| | SD | 3.25 | 1.74 |
| | CV (%) | 10.45 | 4.04 |
| Nuts diameter (mm) | Mean | 28.70 | 33.06 |
| | Min | 24.14 | 30.84 |
| | Max | 33.27 | 36.78 |
| | SD | 2.20 | 1.52 |
| | CV (%) | 7.66 | 4.59 |
| Nuts weight (g) | Mean | 10.20 | 17.14 |
| | Min | 4.12 | 14.73 |
| | Max | 15.23 | 20.16 |
| | SD | 2.95 | 1.76 |
| | CV (%) | 28.92 | 10.26 |
| Kernel weight (g) | Mean | 4.70 | 7.36 |
| | Min | 1.75 | 5.65 |
| | Max | 7.15 | 9.21 |
| | SD | 1.72 | 1.15 |
| | CV (%) | 36.59 | 17.62 |

T*= Tehran; K = Karaj

The oil contents of kernels changed among the location of farmlands to more than about 72% of each. However, because of economical value of the oil, these kernels could be used as potential sources of oils. Arangeh cultivars had more oil and according to variance analyses, differences between walnut cultivars to fatty acid contents were found statistically important at $p < 0.01$ level.

The total oil content is indicated in table 3 and ranged from 60.9 to 73.1% while the crude protein ranged from 13.5 to 20.2%. Dietary fiber ranged from 1.0 to 4.3% and starch content made up no more than 2.6% of the remaining portion of the kernel. Results showed that the oils of both sample population used in this experiment had higher linoleic and oleic acid contents. Linoleic acid contents of kernel oils ranged between 51.4% (Arangeh, Karaj) and 53.2% (Baghestan, Tehran). Stearic and palmitic acids are the main saturated components in all walnut cultivars. Palmitic acid is differed in the different walnut cultivars. Its percentage was found between 6.1% (Arangeh, Karaj) and 6.4% (Baghestan, Tehran). Our results in fatty acid composition are similar to the values in the previous literature [39 - 44].

Table 2: The mean content of mineral elements (mg/100 g DW) in studied Persian walnuts

| Minerals | Mean content of elements in 100 Karaj samples ± SD* (mg/100 g) | Mean content of elements in 100 Tehran samples ± SD* (mg/100 g) | Mean Content of Persian walnut samples ± SD* (mg/100 g) |
|---|---|---|---|
| Na | 58.42 ± 23.168 | 66.54 ± 16.131 | 62.48± 19.650 |
| K | 546.46 ± 13.65 | 617.36 ± 41.73 | 581.91 ± 27.690 |
| Ca | 235.19 ± 15.918 | 276.46 ± 19.362 | 255.82 ± 17.640 |
| Mg | 692.49 ± 20.731 | 580.11 ± 16.423 | 636.30 ± 18.582 |
| Fe | 444.02 ± 45.189 | 559.52 ± 69.461 | 501.77 ± 7.325 |
| Cu | 322.73 ± 20.56 | 239.66 ± 40.744 | 281.20± 30.652 |
| Se | 0.003 ± 0.001 | 0.003 ± 0.002 | 0.003 ± 0.0015 |
| Zn | 142.03 ± 70.33 | 133.92 ± 20.11 | 137.98 ± 45.22 |

*SD = Standard Deviation

Table 3: Fatty acid composition of walnut oils (%)

| | Walnut populations | |
|---|---|---|
| Fatty Acids | Karaj | Tehran |
| Palmitic (C16:0) | 6.1 | 6.4 |
| Stearic C18:0) | 2.7 | 2.6 |
| Oleic (C:18:1) | 25.3 | 21.9 |
| Linoleic (C18:2) | 51.4 | 53.2 |
| Linolenic (C:18:3) | 13.5 | 14.9 |

## CONCLUSION

The order depending on the contents of elements (mg/ 100 g) in *J. regia* samples in Karaj studied regions was Mg> K> Fe > Cu >Ca >Zn> Na whereas in Tehran county farmlands the order is: Mg> K> Fe > Ca >Cu >Zn> Na. Owning to the essential element content determined the soil of Tehran county planting areas is rich in calcium and Karaj farmland's soil is rich in copper and this high levels of these elements in the soil of area, have a great impact on the highness of calcium and copper in the fruits.

A significant positive correlation (0.786) between nut weight and kernel weight was observed in this study which is as reported by other researchers, but no relationship between nut weight and kernel ratio (P ?0.03) was found. Shell thickness showed a negative correlation (-0.611) with kernel ratio, which agrees with the results of *Ghasemi et al*. [5] and Sharma and Sharma [44] and *Arzani et al*. [45] ; however, nut weight showed a positive correlation (+0.375) with shell thickness (P <0.01). This means that heavier nuts have thicker, heavier shells. In conclusion, evaluation of 207 walnut seedling samples reveals that they could be not only very promising raw materials for various industries but also would serves as useful dietary supplements. The high protein and oil values of the kernels and high levels of mineral elements indicate their potentials usefulness in human animal and poultry feed supplements Walnut kernel oil can also be recommended for cosmetics industries due to and for Anti-Aging, Moisturizing, Regenerative & Toning Properties and these kernels should not be overlooked anymore in pharmaceutical technology.

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