International Journal of Food Science and Nutrition
ISSN: 2455-4898, Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 2; Issue 3; May 2017; Page No. 32-35

Kashmir walnuts: Study on fatty acid composition and antioxidant activity

Chilkunda Sannaiah Shivakumara, Dr. Usha Devi C

Department of Food and Nutrition and Research Centre, Smt V.H.D Central Institute of Home science, Bangalore University, Bangalore, Karnataka, India

Abstract
Walnuts are rich source of polyunsaturated fatty acid (PUFA) and highest antioxidant activity which have beneficial activity for reducing the risk of coronary heart diseases. In this study, fatty acid composition and antioxidant activity of two varieties of walnuts were investigated. The fatty acid compositions of Kashmir walnuts were analyzed by Gas chromatography. The total fat content of the sample was assessed by AOCS Official Butt-tube Method Ac 3-44 and Antioxidant activity were analyzed by radical scavenging activity (RSA) toward DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. The PUFA is highest in both the varieties (73.89%) black variety (65.74%) brown variety and MUFA is highest in brown variety (26.21%), black variety (18.17%). Brown variety shows highest radical scavenging activity compare to black variety. The present study showed that Indian Kashmir walnuts are rich in MUFA, PUFA, low in saturated fatty acid and highest antioxidant activity which might be used as a part in our diet which will reduce the risk of coronary artery diseases.

Keywords: walnuts, antioxidant, DPPH, fatty acids

1. Introduction
Walnut, whose scientific name is *Juglans regia* L. is a plant from Juglandaceae family (Ogunmoyole *et al*., 2011) [16]. *Juglans regia L*. is the most widespread tree nut in the world. The walnut tree species is native to the old world (Taha, N, A.M. Al-wadaan., 2011) [26]. Walnut is an important tree nut, which belongs to the angiospermic family Juglandaceae. Every part of the plant has some utility and as such it has carved a special place in socio-religious and economic well-being of the people (Rohini Sharma, Geeta Sumbali., 2014) [22]. Walnut is a high energy food, rich in oil including omega-3 fatty acids, vitamins and minerals, and valued as healthy snack food and bakery ingredients (Rana *et al*., 2007) [20]. Its alpha linolenic acid has substantial cardio protective effects as it increases the ratio of high-density lipoprotein cholesterol to total cholesterol, reducing inflammation and improving arterial function (Hu *et al*., 1999; Diousse *et al*., 2001; Patel, 2005) [9, 5, 19]. It contains 'melatonin' an antioxidant produced by pineal gland and responsible for inducting and regulating sleeps (Reiter *et al*., 2005) [21]. It also reduces the incidence of cancer and, delays neurodegenerative diseases of aging (McGranahan and Leslie, 2012) [13]. India is the 8th largest producer of walnut in the world (Rohini Sharma, Geeta Sumbali., 2014) [22] and Western Himalayan region of India produces high quality walnuts. The Jammu and Kashmir State of India alone accounts for >98% of India's total production with an average productivity of 2.69 metric tonnes/ha from an area of 83613.80 ha and production of 224595.85 metric tonnes (Sharma, 2012) [25]. The major walnut growing states of India are Jammu and Kashmir, Uttarakhand, Himachal Pradesh and Arunachal Pradesh. Among these, Jammu and Kashmir occupies the largest share in total area and production. In J&K, cultivation of walnut is common in Bhaderwah, Poonch, Rajouri, Kupwara, Baramulla, Bandipora, Ganderbal, Budgam, Srinagar, Anantnag and other temperate areas. The kernel is the edible part of the walnut, which is enclosed in a brown seed coat that contains antioxidants to protect the oil rich seed from atmospheric oxygen to prevent rancidity. It is rich in fats, proteins, minerals, vitamins and a substantial quantity of dietary fibers and is therefore, a concentrated source of energy (Rohini Sharma, Geeta Sumbali., 2014) [22].

Walnut as a good source of essential fatty acids and tocopherols contribute to the reduced risk of cancer and coronary heart disease (Miraliakbari and Shahidi 2008) [14]. Nowadays the increased prevalence of diabetes, cancer, cardiovascular problems and other diseases, has raised the awareness in people related to diets rich in antioxidants and polyphenols. Among the different sources of natural antioxidants, walnuts are rich in polyphenols (especially ellagic acid and gallic acid), tocopherol (vitamin E), ellagitannins (tannins) and compounds with potent antioxidant activity, such as melatonin (Tapsell 2010) [27]. The beneficial effects derived from the phenolic compounds, such as their anticarcinogenic, antimutagenic and cardioprotective activities have been attributed to their antioxidant activity (Madhavi *et al*. 1996; Balasundram *et al*. 2006) [12, 3].

Walnut kernels generally contain about 60% oil, but this can vary from 52 to 70% depending on the cultivar, location grown and irrigation rate (Xiaoying, M. *et al*., 2014, Li, X., Y. Zhao, X. Gong and Ch. Zhao, 2014 and Dogan, M. and A. Akgul, 2005) [29, 11, 6]. The high protein and oil contents of the kernels of *Juglans regia* L 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 (Gandev, S., 2007) [7]. 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 (Li, X., Y. Zhao, X. Gong and Ch. Zhao, 2014) [11]. In fact, among vegetable oils walnut oil has one of the highest amounts of PUFA (up to 78% of the total FA content) (Taha, N. and A.M. Al-wadaan., 2011) [26]. 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 (Muradoglu, F., H. *et al*., 2010) [15]. The Food and Drug Administration (FDA) authorized a health claim indicating that diets including walnuts can reduce the risk of heart disease (Xiaoying, M. *et al*., 2014) [29]. The heart benefits of walnuts include lowering cholesterol, reducing inflammation and improving arterial function (Ogunmoyole *et al*., 2011) [16]. 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% (Ozcan, M., 2009) [17]. In addition walnuts have highest levels of polyphenolic antioxidants than any other common edible nuts (Siahnouri *et al*., 2013, Grace *et al*., 2014) [24, 8]. The fatty acid composition of walnut fruits have been investigated in previous works, nevertheless there are several factors such as the cultivar, geographical origin, and agricultural practices that can affect its nutritional composition. (Areias F *et al*., 2000, Parcerisa J *et al*., 1999) [1, 18].

In this study, two varieties of walnuts (brow variety and black variety) grown in Kashmir in India were investigated in respect to their antioxidant activity and fatty acid composition.

2. Materials and Methods

2.1 Sample Collection

Two varieties of walnut sample were collected from wanees distributer of Kashmir walnuts in Bangalore, owned by Sh.Shabirahmed S/o Sh.Abdul Qayoom Wani R/o H. No.45-Gujjar nagar Jammu as proprietor/ partner/ members of HUF/Directors/ Members is a dealer registered under Jammu & Kashmir. Each sample was then subjected to the following analysis.

Fig 1: *Brown variety of walnuts.* **Fig 2:** *Black variety of Walnuts*

2.2 Fatty acid Composition

The fatty acid methyl ester (FAME) of the fat extracted from walnut seeds of two different varieties were prepared by transesterification according to AOCS Official Method (1998) [2]. Analysis was carried out using gas chromatograph (model-GC-15A, Shimadzu Corporation, Kyoto, Japan) equipped with a Flame Ioniztion Detector (FID) and a stainless steel column of 3 m length×0.5 mmID, coated with 15% diethylene glycol succinate on 60–80 mesh chromosorb WAW. The operating conditions were as follows: column temperature 180°C, injector temperature 220°C and detector temperature 230 °C. A reference standard FAME mix (Supelco Inc., Bellefonte, PA, USA) was analyzed under the same operating conditions to determine the peak identity. The fatty acids were expressed as relative area of percentage.

2.3 Total Fat Content

Fat was extracted from walnut seeds according to AOCS Official Butt-tube Method Ac 3–44 (AOCS 1998) [2]. The dried walnut seeds were ground to a fine powder, packed in 26 mm× 60 mm thimbles and extracted with hexane in Soxhlet apparatus. The extracts were desolventized by vacuum flash evaporation (Rotavapor RE 121A, Buchi, Switzerland) at controlled temperature, dried and weighed to get the fat content.

2.4 Radical Scavenging Activity (RSA) toward DPPH Radicals

RSA and the presence of hydrogen donors in the extracted oils were examined by reduction of DPPH radicals in toluene. A toluenic solution of DPPH radical was freshly prepared at a concentration of 10-4 M according to (Bhatnagar *et al*. 2009) [4] with minor modifications. The oil samples (50 ± 1 mg) were placed in test tubes and a 4-ml aliquot of DPPH toluenic solution was added and vortexed for 20 s at ambient temperature. Against a blank of pure toluene without DPPH radicals, the decrease in the absorption at 515 nm was measured in a 1-cm quartz cell after 1, 30, and 60 min of mixing, using a UV-visible spectrophotometer (model UV-1601, Shimadzu corporation, Kyoto, Japan). RSA toward DPPH radicals was estimated from the differences in absorbance of toluenic DPPH solution with or without sample (control) and the inhibition percent was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{absorbance of test sample})}{\text{Absorbance of control}} * 100$$

3. Results and Discussion

Fatty acid composition the analysis of fatty acid composition in two varieties of walnut oils illustrated in table-1, The Polyunsaturated fatty acid (PUFA) is highest in both black and brown varieties of walnuts, in black variety contain 73.92% and brown variety contain 65.744%. Linoleic acid (C18:2) was the most abundant fatty acid in both the varieties, ranging from 59.74% in black variety, 53.87% in brown variety, Oleic acid (C18:1) is the second most abundant fatty acid found in both the varieties 26.21% in brown variety and 18.17% in black variety, Linolenic acid (C18:3) was found in the range of 11.86 % in brown variety and 14.14 % in black variety. The saturated fatty acid was present in both the varieties of walnuts in 8.04% in brow variety, 7.92% in black variety. In remaining fatty acids palmitic acid (C16:0) was found in 5.25%, brown variety, 5.44%, black variety, steric acid (C18:0) 2.79%, brown and 2.48% black variety. Other short chain fatty acids like caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0) and arachidic acid (C20:0) was not found in both the varieties. The black variety of walnut showed low values of MUFA content but high in linoleic, linolenic acids and highest total PUFA (73.92%) compare to brown variety.

Epidemiological and clinical studies indicated that PUFA may have a significant role in the secondary prevention of cardiovascular disease (Lee KW, Lip GY., 2003) [10]. The present study result showed that PUFA was the highest fatty acid found in both varieties of walnuts followed by the monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) receptively. The major fatty acid found in both the

varieties of walnuts are linoleic acid (18:2), Linolenic acid (C18:3) and oleic acid, In our results of fatty acid composition are compare to previously published literature of Persian and Iran walnuts in six different genotype some difference was observed (Sara Aryapak and Parisa Ziarati., 2014 and Vali Akbari *et al*., 2014).

Table 1: Fatty acid composition of oils extracted from walnut kernels of two different varieties.

Fatty acids (g 100 g−1 of oil).	Brown variety	Black variety
C6:0	nd	nd
C8:0	nd	nd
C10:	nd	nd
C12:0	nd	nd
C14:0	nd	nd
C16:0	5.254	5.447
C18:0	2.790	2.481
C18:1	26.21	18.176
C18:2	53.876	59.745
C18:3	11.868	14.149
C20:0	nd	nd
SFA	8.044	7.928
MUFA	26.21	18.176
PUFA	65.744	73.894
Total	99.998	99.998

(Note: nd= not defined)

Total fat content the total fat content of walnut samples depicted table-2, was found 68.25% in brown variety, 65.50% in black variety. The fat content was highest in brown variety compare to black variety with variation in the fatty acid composition. When compare our results to previously published results of Iron cultivator grown walnuts (63.33 to 78.46) our results showed that low in total fat content.

Table 2: Total Fat Content

Fat Content	Brown Variety	Black variety
	68.25 %	65.50 %

Radical Scavenging Activity the radical scavenging activity towards DPPH Radicals (table-3), was expressed as DPPH Scavenging Activity inhibition in different time intervals. The brown variety in 0 minutes 91.53%, black variety 89.84%, 15 minutes brown variety 86.23%, black variety 87.32%, in 30 minutes 85.07% brown variety, 50.37% black variety, 45 minutes brown variety 83.79%, black variety 30.89% and 60 minutes brown variety 50.46%, black variety 15.7%. Our study shows that radical scavenging potential was higher in brown variety compare to black variety in different time intervals.

The radical scavenging activity of the two varieties was determined by DPPH assay as mentioned earlier. From the figure it is observed that the black variety has shown significantly higher RSA, compared to brown variety. This may be attributed to the presence of high amount of poly-phenols and other antioxidant molecules present in it. Although the scavenging activity was similar initially and up to 15 minutes, the activity kept decreasing with respect to black variety when compared to brown one. It clearly demonstrates that over time course the brown variety possess excellent scavenging activity due to potential poly-phenols and other antioxidant molecules present in it.

Table 3: Radical Scavenging Activity (RSA) toward DPPH Radicals

	DPPH scavenging activity (%) Inhibition.	
Time in Minutes	Brown variety	Black Variety
0	91.53	89.84
15	86.23	87.32
30	85.07	50.37
45	83.79	30.89
60	50.46	15.7

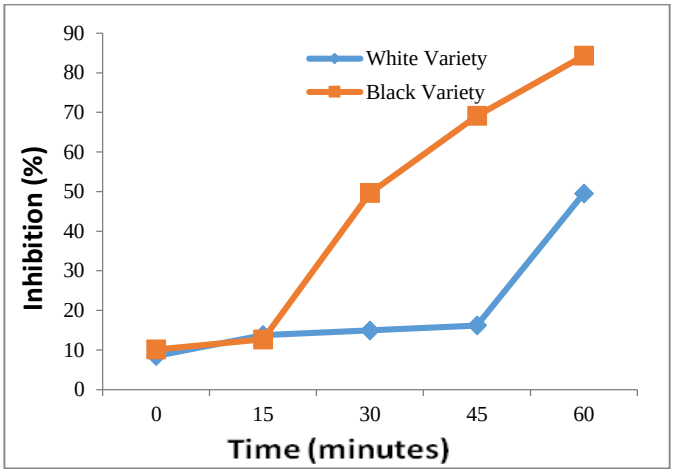

Fig 3: *Radical Scavenging Activity (RSA) toward DPPH Radicals*

4. Conclusion

The fatty acid composition and antioxidant activity of two different varieties of walnuts samples were investigated. PUFA is the most abundant fatty acid present in the both varieties of walnuts followed by MUFA and SFA. The brown variety showed excellent radical scavenging activity towards DPPH Radicals in 30, 45, and 60 minutes the radical scavenging activity kept decreasing with respect to black variety when compared to brown one. The black variety contains highest PUFA compare to brown variety but radical scavenging activity over course time kept decreasing. In brown variety of walnuts showed highest amount of PUFA, MUFA and excellent radical scavenging activity. The present study showed that Indian Kashmir brown variety of walnuts are rich source of PUFA, MUFA, low in saturated fatty acid and highest antioxidant activity which might be used as a part in our diet which will reduce the risk of coronary artery diseases.

5. Reference

1. Areias F, Valentao P, Andrade PB, Ferreres F, Seabra RM. Flavonoids and phenolic acids of sage: influence of some agricultural factors. J Agric Food Chem. 2000; 48(12):6081-4.
2. AOCS official butt-tube method Ac 3–44, AOCS method Nos. Ca 5a–40, AOCS method Nos. American Oil Chemists Society, Champaign, 1998; pp. 8-53.
3. Balasundram N, Sundram K, Sundram S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence and potential uses. Food Chem. 2006; 99:191-203.
4. Bhatnagar AS, Prasanth Kumar PK, Hemavathy J, Gopala Krishna AG. Fatty acid composition, oxidative stability and radical scavenging activity of vegetable oil blends with coconut oil. JAOCS. 2009; 86:991-999.

5. Diousse L, Pankow JS, Eckfeldt JH, Folsom AR, Hopkins PN, Province MA, *et al*. Relation between dietary linoleic acid and coronary artery disease in the National Heart, Lung and Blood Institute Family Heart study. Am J Nutr. 2001; 74:612-619.
6. Dogan M, Akgul A. Fatty acid composition of some walnut (*Juglans regia* L.) cultivars from east Anatolia. Grasas y Aceites. 2005; 56:(Fasc., 4):328-331.
7. Gandev S. Budding and grafting of the walnut (*Juglans regia* L.). And their effectiveness in Bulgaria (Review), Bulgar. J. Agri. Sci. 2007; 13:683-689.
8. Grace MH, Ch W Warlick, Neff SA, Lila MA. Efficient preparative isolation and identification of walnut bioactive components using high-speed counter-current chromatography and LC-ESI-ITTOF-MS. Plants for Human Health Institute, Food Bioprocessing and Nutritional Sciences. 2014; 158:229-238.
9. Hu FB, Stamfer MJ, Manson JE, Rimm EB, Wolk A, Colditz GA, *et al*. Dietary intake of α-linolenic acid and risk of fatal ischemic heart disease among women. Am J Clin Nutr. 1999; 69:890-897.
10. Lee KW, Lip GY. The role of omega-3 fatty acids in the secondary prevention of cardiovascular disease. QJM. 2003; 96(7):465-80.
11. Li X, Zhao Y, Gong X, Ch. Zhao. Quality Evaluation of Walnut Oil through HPLC and in Vitro Antioxidant Activity. Journal of Food and Nutrition Research. 2014; 2:244-249.
12. Madhavi DL, Despande SS, Salunke DK. Food Antioxidants. Technological, Toxicological and Health Perspectives, Marcel Dekker, New York, 1996.
13. McGranahan GH, Leslie C. Walnut. In: Badenes ML, Byrne DH (eds) Fruit breeding. Springer New York Dordrecht Heidelberg London, 2012.
14. Miraliakbari H, Shahidi F. Lipid class compositions, tocopherols and sterols of tree nut oils extracted with different solvents. J. Food Lipids. 2008; 15:81-96.
15. Muradoglu F, Oguz H, Yildiz K, Yilmaz H. Some chemical composition of walnut (*Juglans regia* L.) selections from Eastern Turkey. African Journal of Agricultural Research. 2010; 5:2379-2385.
16. Ogunmoyole T, Kade IJ, Korodele B. *In vitro* antioxidant properties of aqueous and ethanolic extracts of walnut (*Juglans* regia). Journal of Medicinal Plants Research. 2011; 5:6839-6848.
17. Ozcan M. Some Nutritional Characteristics of Fruit and Oil of Walnut (Juglans *regia* L.) Growing in Turkey. Iranian Journal of Chemistry and Chemical Engineering. 2009; 28:57-62.
18. Parcerisa J, Codony R, Boatella J, Rafecas M. Triacylglycerol and phospholipid composition of hazelnut (*Corylus avellana* L.) lipid fraction during fruit development. J Agric Food Chem. 1999; 47(4):1410-5.
19. Patel G. Essential fats in walnuts are good for the heart and diabetes. J Am Diet Assoc. 2005; 105:1096-1097.
20. Rana JC, Singh D, Yadav SK, Verma MK, Kumar K, Predheep K. Genetic diversity collected and observed in Persian walnut (*Juglas regia* L.) in the western himalayan region of India. Plant Genet Resour News Letter. 2007 151:68-73.
21. Reiter RJ, Manchester LC, Tan DX. Melatonin in walnuts: influences on levels of Melatonin and total antioxidant capacity of blood. Int J Appl Basic Nutr Sci. 2005; 21(9):920-924.
22. Rohini Sharma, Geeta Sumbali. Fungal diversity associated with the commercial grades of walnut kernels sold in the markets of Jammu and Kashmir State (India). International Journal of Pharmaceutical Science Invention. ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X; 2014; 3(6):50-57.
23. Sara Aryapak, Parisa Ziarati. Nutritive Value of Persian Walnut (*Juglans regia* L.) Orchards, American-Eurasian J. Agric. & Environ. Sci. 2014; 14(11):1228-1235.
24. Siahnouri Z, Sadeghian M, Salehisormghi M, Qomi M. Determination of Iranian Walnut and Pistachio Mineral Contents. Journal of Basic and Applied Scientific Research. 2013; 3:217-220.
25. Sharma R. Area and production database of fruit crops. Directorate of hortic state department of horticulture, Jammu and Kashmir government, India, 2012.
26. Taha N, Al-wadaan AM. Utility and importance of walnut, *Juglans regia* Linn: A review. African Journal of Microbiology Research. 2011; 5:5796-5805.
27. Tapsell LC. Health benefits of walnut consumption. Acta Horticult. 2010; 86:409-416.
28. Vali Akbari1, Reza Heidari, Rashid Jamei. Fatty acid compositions and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Iran, Advanced Herbal Medicine. 2010; 2014; 1(1):36-41.
29. Xiaoying M, Yufei H, Guogang C. Amino Acid Composition, Molecular Weight Distribution and Gel Electrophoresis of Walnut (Juglans regia L.) Proteins and Protein Fractionations. Int J Mol Sci. 2014; 15(2):2003-2014.