Effect of different ripening stages on walnut kernel quality: antioxidant activities, lipid characterization and antibacterial properties

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Abstract Packing tissue between and around the kernel halves just turning brown (PTB) is a phenological indicator of kernel ripening at harvest in walnuts. The effect of three ripening stages (Pre-PTB, PTB and Post-PTB) on kernel quality characteristics, mineral composition, lipid characterization, sensory analysis, antioxidant and antibacterial activity were investigated in fresh kernels of indigenous numbered walnut selection of Kashmir valley “SKAU-02”. Proximate composition, physical properties and sensory analysis of walnut kernels showed better results for Pre-PTB and PTB while higher mineral content was seen for kernels at Post-PTB stage in comparison to other stages of ripening. Kernels showed significantly higher levels of Omega-3 PUFA (C18:3ⁿ³) and low n6/n3 ratio when harvested at Pre-PTB and PTB stages. The highest phenolic content and antioxidant activity was observed at the first stage of ripening and a steady decrease was observed at later stages. TBARS values increased as ripening advanced but did not show any significant difference in

malonaldehyde formation during early ripening stages whereas it showed marked increase in walnut kernels at post-PTB stage. Walnut extracts inhibited growth of Gram-positive bacteria (*B. cereus*, *B. subtilis*, and *S. aureus*) with respective MICs of 1, 1 and 5 mg/mL and gram negative bacteria (*E. coli*, *P.* and *K. pneumonia*) with MIC of 100 mg/mL. Zone of inhibition obtained against all the bacterial strains from walnut kernel extracts increased with increase in the stage of ripening. It is concluded that Pre-PTB harvest stage with higher antioxidant activities, better fatty acid profile and consumer acceptability could be preferred harvesting stage for obtaining functionally superior walnut kernels.

Keywords Kernel quality · Nutritional value · Fatty acids · Phenolics · Antioxidant properties, antibacterial activity

Introduction

Nuts are good source of essential nutritional and nutraceutical components. During past few years, lipid characterisation and polyphenolic profiling of nuts has gained tremendous interest due to associated health benefits (Banel and Hu 2009). One of the commonly consumed tree nuts includes walnut that belongs to the juglandaceae family. It is one of the oldest grown plants and is distributed worldwide ranking third in nut production after cashews and almonds (Fernandez-Lopez et al. 2000).

Among unsaturated fatty acids, walnuts are reported to contain high amounts of linoleic acid (49.3–62.3%), oleic acid (14.3–26.1%) which play a vital role in alleviating cardiovascular diseases (Tapia et al. 2013). Walnuts are also reported to contain high amounts of vitamin B1, B2, B6, γ -tocopherol and some amounts of lutein/zeaxanthin

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(Stuetz et al. 2017). A combination of unsaturated fatty acids, γ -tocopherols, and other nutrients of walnut extract were assumed to be responsible for anti-cancerous activity (Chung et al. 2016). Regueiro et al. (2013) reported a total of 120 phenolic compounds among which stenophyllanin C, malabathrin A, heterophyllin E, pterocinin B, eucalbanin A, cornusin B, reginin A and alienanin B, reported for the first time. Walnut consumption is also associated to antiatherogenic, anti-inflammatory, antimutagenic and anticancerous properties (Ruijun et al. 2015) and also improves plasma adipokines and endothelial function in people with type 2 diabetes (Djoussé et al. 2016).

Nutritional and nutraceutical content, particularly antioxidant and fatty acid content, determines the shelf life in nuts and is strongly influenced by maturity stage of harvest. Ripening has been reported to affect the phytochemical content in different fruits such as pecan nuts (Bouali et al. 2013), peach (Belhadj et al. 2016) and cashewnuts (Cunha et al. 2017). Sibbett et al. (1974) defined packing tissue between kernel halves an important phenological indicator of degree of nut maturity. However, Kader and Thompson (2002) reported the approximate time period for attainment of maturity as the time when the packing tissue between and around the kernel halves just turn brown. To date walnut harvesting has been done on traditional concept of hull dehiscence and no literature is present that establishes compositional superiority for harvesting at this stage. Although kernel characteristics and other biochemical aspects such as antioxidant and antibacterial properties of walnut have been previously studied but the effect of ripening on these parameters has not been reported so far. Thus, the walnut kernel characteristics, including consumer acceptability on the basis of sensory analysis, in relation to various ripening stages at harvest was the main objective of this investigation. For that we compared various kernel quality parameters, chemical composition, fatty acid characterization, antioxidant activities and antibacterial activities as observed at various development stages of walnut ripening in Kashmir valley—a region that contributes 90% of walnut production in India.

Materials and methods

Plant material and post-harvest handling

Walnut (*Juglans regia* L.) fruits were harvested from mature trees cultivated in the experimental orchard area of Division of Fruit Science in the main campus of Sheri Kashmir university of Agricultural Sciences &

Technology- Kashmir (SKUAST-K) located at Shalimar, Srinagar (lat. 77° 5' N, Long. 38° 5' E, altitude 1646 m), India. On the basis of pilot observational study, approximate time period for possible attainment of PTB stage of maturity i.e. when the packing tissue between and around the kernel halves just turned brown (Kader and Thompson 2002) was determined. Accordingly in three distinct stages of harvesting were considered for this investigation. Date of nuts harvested on 10 September (10/09/16) was deemed as the reference point for initiation of PTB maturity i.e. stage 2. Nuts harvested 10 days earlier to and later than this reference point were designated as pre-PTB i.e. stage-1 (31/08/16) and post-PTB i.e. stage-3 (21/09/16) respectively. Nuts at post-PTB stage is the standard full maturity for harvesting which corresponds to 80% hull dehiscence of green walnuts (Kader and Thompson 2002). Nuts from each tree harvested at three distinct maturity stages were obtained from five trees chosen as replicates. The investigation encompassed 80 nuts per tree per harvesting stage. Immediately after harvest, nuts were transported to laboratory within 2 h. The green husks were peeled off within one day and in-shell nuts were washed with tap water. After cleaning nuts were sun dried for three days under ambient air conditions. Dried nuts were manually but carefully cracked, shelled and only healthy kernels, macroscopically free of disorders and diseases, selected for the experiment were placed in polyethylene bags and stored at 2–4 °C and 65–70% relative humidity for immediate use.

Chemicals & pathogens

1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma–Aldrich (Poole, UK), chloroform, ammonium thiocyanate, potassium iodide, trichloroacetic acid (TCA), sodium thiosulphate, starch indicator, Folin-Ciocalteu reagent, sodium carbonate, potassium ferrocyanide, ferric chloride hexahydrate, phosphate buffer, were purchased from Himedia. Standards such as gallic acid, ascorbic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), α -tocopherol and 1,1,3,3-tetraethoxy-propane (TEP), fatty acid methyl esters of myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid, palmitoleic acid, oleic acid, eicosenoic acid, linoleic acid and linolenic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck Co. (Darmstadt, Germany). Water used for chemical preparation was from Milli-Q water purification system (Millipore, model A10, USA). Pure cultures of *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella Pneumonia* were procured from the Department of

Microbiology, Sheri-e-Kashmir Institute of Medical Sciences, Soura, Jammu & Kashmir, India.

Extract preparation

Sun dried walnut kernels from each maturity stage were ground to a fine powder using a grinder (Sujata Powermatic plus). Ten gram powder was extracted with 250 mL of boiling water for 45 min and filtered through Whatman no. 4 paper. The aqueous extract was frozen, lyophilized and redissolved in water at concentrations of 100 and 10 mg/mL for antibacterial and antioxidant activities assays, respectively.

Physico-proximate composition

Colorimetry

For colorimetry, Hunter Lab spectro-colorimeter (Mini Scan XETM, Hunter Associates Laboratory Inc., Reston, Virginia, USA) was used. The instrument measures color indices, specified by the Commission Internationale de l'Eclairage (CIE). The CIE L^* , a^* , and b^* color indices were measured to determine the kernel color of walnuts. L^* is the lightness component, which ranges from 0 to 100, and parameters a^* (from green to red) and b^* (from blue to yellow) are the two chromatic components which range from -120 to 120 . Whiteness index was calculated according to the equation

$$WI = 100 - \left[(100 - L^*)^2 + (a^*)^2 + (b^*)^2 \right]^{1/2}$$

Browning index

Browning index of walnut kernels was determined according to Lee et al. (2016) with slight modifications. Walnut kernels (20 g) were homogenized in 95% methanol in a laboratory blender for 2 min. The homogenates were centrifuged at 10,000 rpm for 15 min at 4°C and filtered through Whatman No. 2 filter paper. Absorbance was measured immediately at 420 nm. Higher values of absorbance correspond to higher browning of the tissue.

Proximate composition

Moisture, fat, ash and protein contents of ground walnut kernels were determined according to standard AOAC (1998) methods. Carbohydrate content was estimated by difference of the other components. The mineral composition of walnut kernels was determined according to

AOAC (1990). The ash obtained above was digested with 5 mL 6 M HCl in a water bath. After drying, 7 mL 0.1 M HNO_3 was added and the contents were diluted to 100 mL with double deionized water. Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu), and Magnesium (Mg) were determined in an Atomic Absorption Spectrophotometer (Element As AAS4141) whereas, Potassium (K) and sodium (Na) by Flame Photometer (PFP 7 Jenway, England).

Fatty acid composition

Fatty acid composition of walnut kernel oils was determined using fatty acid methyl ester method Hisil (1998). The oil was extracted using petroleum ether as solvent in a Soxhlet apparatus (SocsPlus-SCS06AS) at 70°C for 24 h. The solvent residue was evaporated overnight in an oven at 25°C . This residue was then converted to their fatty acid methyl esters. The methyl esters of the fatty acids were analyzed using gas chromatography (Shimadzu GC-2010) equipped with a flame ionizing detector, a fused silica capillary column SPTM-2380 (60 m $\times$ 0.25 mm, film thickness 0.20 μm). It was operated under the following conditions: oven temperature program, 100°C for 2 min (raised to 200°C at a rate $15^\circ\text{C}/\text{min}$, held at 200°C for 2 min and finally raised to 240°C at $20^\circ\text{C}/\text{min}$ and held for 18 min); both injector and detector at 270°C ; carrier gas, nitrogen at flow rate of 1.51 mL/min. A Standard fatty acid methyl ester mixture (Sigma Chemical Co.) was used to identify sample peaks and the peak area was used to quantify the compounds using WILEY library (8L).

Total phenolic content

The phenolic content was determined according to the method of Shafi et al. (2016) with slight modifications. Sample (1 mL) was mixed with 5 mL of 0.25 N Folin-Ciocalteu reagent. After 3 min incubation, 4 mL of 7.5% Na_2CO_3 was added to the mixture and incubated for 2 h at room temperature. The absorbance was measured at 765 nm. The results were expressed as mg of gallic acid equivalents per gram of lyophilised extract (mg GAE/g).

Antioxidant activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity

DPPH radical scavenging activity was analyzed according to the method described by Baba et al. (2016) with some modifications. Walnut extract (0.3 mL) was mixed with

2.7 mL of DPPH (6×10^{-5} mol/L). The mixture was vortexed and allowed to react in the dark for 30 min. Absorbance was measured at 517 nm and the results were expressed as EC_{50} . The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of percent inhibition against extract concentration. BHA and α -tocopherol were used as standards.

Reducing power

Reducing power was determined according to the method of Nisar et al. (2015) with slight modifications. Briefly, the sample extract (1 mL) was mixed with 2.5 mL of phosphate buffer (pH 6.6, 0.2 M) and 2.5 mL of 1% (w/v) solution of potassium ferrocyanide. The mixture was incubated at 50 °C for 20 min. After cooling the mixture to room temperature, 2.5 mL of 10% (w/v) trichloroacetic acid was added. After vortexing, an aliquot of 2.5 mL was withdrawn and 0.5 mL of 0.1% (w/v) ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) solution was added to it. The absorbance was measured at 700 nm after 2 min. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance registered at 700 nm against the correspondent extract concentration. BHA and α -tocopherol were used as a reference compounds.

Ferric reducing antioxidant power (FRAP)

The FRAP assay was done according to Baba et al. (2016) with some modifications. The stock solutions included 300 mM acetate buffer ($3.1 \text{ g } C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine), solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 μ L acetate buffer, 2.5 μ L TPTZ solution, and 2.5 μ L $FeCl_3 \cdot 6H_2O$ solution. The mixture was warmed at 37 °C and 150 μ L walnut extract was allowed to react with 2850 μ L of the FRAP solution for 30 min in the dark condition. The absorbance was taken at 593 nm. Results are expressed as μ mol Fe^{2+} equivalents/g extract.

Lipid peroxidation

Lipid peroxidation in terms of TBARS was determined according to Baba et al. (2016) with slight modifications. Ground walnut (0.1 g FW) of tissue was extracted with 1 mL of colorimetric reactive TCA (15%)-TBA (0.37%)-HCl (0.25%) heated at 90 °C for 30 min and then cooled. After centrifugation at 11,000 g for 10 min, the absorbance of supernatant was measured at 532 nm. The concentration of MDA was calculated from standard curve using 1,1,3,3-

tetraethoxypropane (TEP) as standard compound. Lipid peroxidation values were expressed as mg of malondialdehyde (MDA) per Kg of sample.

Antibacterial activity

Bacterial strains

The antibacterial activity of the walnut extracts was evaluated using disc diffusion method against gram positive bacteria, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and gram negative bacteria, *Escherichia coli*, & *Klebsiella Pneumonia*.

Disc diffusion

Disc diffusion method was carried out according to Bauer et al. (1966), to assess the presence of antibacterial activities of walnut extracts. A bacterial culture was used to lawn Muller Hinton agar plates evenly using a sterile swab. The plates were dried for 15 min and then used for the sensitivity test. The discs impregnated with a series of various walnut extracts at different minimal inhibitory concentrations were placed on the Mueller–Hinton agar surface. Each test plate consisting of two discs, one disc treated with standard commercial antibiotic (Ampicillin) and the other treated disc with sample extract. The plate was then incubated at 37 °C for 18–24 h depending on the species of bacteria used in the test. After the incubation, the plates were examined for the MIC which is considered to be the lowest concentration of the tested sample able to inhibit the growth of bacteria after 24 h. The diameters of the inhibition zones corresponding to the MICs were measured with an accuracy of 0.01 mm. Each inhibition zone diameter was measured five times (five different plates) and the average was considered for antibacterial activity.

Sensory analysis

Sensory analysis was carried out in well illuminated laboratory (independent booth). A panel of 15 trained panelist (from Department of Food Science and Technology), with sensory evaluation experience, was trained in descriptive evaluation of nuts. Panelists were asked to rinse mouth before each serving. Sensory attributes such as Kernel appearance, cotyledon appearance, flavor, Aroma, sweetness, oily taste were assessed according to a nine-point Hedonic scale (9 = like extremely to 1 = dislike extremely). Average score was worked out on the basis of these individual sensory attributes and designated as “overall consumer acceptability”.

Statistical analysis

Mean values, standard deviation, analysis of variance (ANOVA) were computed using a commercial statistical package (IBM SPSS Statistics 21.Ink). These data were then compared using Duncan's multiple range tests at 5% significance level.

Results and discussion

Physico-proximate composition

Physical parameters

Nut weight and kernel weight increased with advancement in stages of ripening with Pre-PTB showing significantly

lower values than rest of the ripening stages (Table 1). Kernel Colour is the most important parameter for consumer acceptance of walnut. L^* value which represents brightness significantly decreased as ripening advanced. Decrease in L^* value can be due to development of pigments and/or colored compounds due to nonenzymatic browning by oxidized lipid/protein reactions analogous to the Maillard reaction in the pellicle of kernel during ripening. This assumption is further supported because advancement of ripening was associated with an increase in lipid peroxidation during ripening discussed later in this study. The change in L^* value may also be attributed to the change in the spectral characteristics of light reflected from kernel surface. Whiteness index of kernels also followed identical trend as that of the L^* (Table 1). Altuntas (2009) reported L^* value of walnuts decreased with increase in the moisture content of kernels. Advancement of ripening was

Table 1 Proximate composition, mineral content and physical properties of walnut at different maturity stages

Parameters	Stages		
	Pre-PTB	PTB	Post-PTB
Proximate composition			
Moisture (g 100 g ⁻¹ kernel)	7.15 ± 0.21 ^b	6.82 ± 0.23 ^a	6.50 ± 0.23 ^a
Protein (g 100 g ⁻¹ kernel)	12.9 ± 0.31 ^a	13.3 ± 0.42 ^a	15.1 ± 0.45 ^b
Fat (g 100 g ⁻¹ kernel)	51.8 ± 0.71 ^a	54.4 ± 0.72 ^b	58.3 ± 0.65 ^c
Ash (g 100 g ⁻¹ kernel)	1.62 ± 0.01 ^a	1.74 ± 0.02 ^b	1.82 ± 0.05 ^c
Carbohydrate (g/100 gkernel)	25.8 ± 1.25 ^c	22.7 ± 1.03 ^b	19.1 ± 1.01 ^a
Mineral content(mg/100 g kernel weight)			
Ca	160.25 ± 13.00 ^a	160.32 ± 13.25 ^a	160.15 ± 14.10 ^a
Mg	131 ± 9.75 ^a	135 ± 10.50 ^a	13 ± 12.0 ^b
K	325 ± 28.0 ^a	330 ± 30.0 ^a	350 ± 25.0 ^b
Na	2.51 ± 0.8 ^a	2.73 ± 0.9 ^b	3.01 ± 0.5 ^b
Zn	2.22 ± 0.07 ^a	2.15 ± 0.09 ^a	2.40 ± 0.3 ^b
Cu	0.53 ± 0.01 ^a	0.50 ± 0.01 ^a	0.93 ± 0.02 ^b
Mn	1.84 ± 0.12 ^a	1.95 ± 0.15 ^a	2.03 ± 0.2 ^b
Fe	1.60 ± 0.3 ^a	1.53 ± 0.3 ^a	2.11 ± 0.5 ^b
Physical parameters			
Nut weight (g)	10.8 ± 0.80 ^a	11.4 ± 0.73 ^b	11.5 ± 0.80 ^b
Kernel weight (g)	4.85 ± 0.63 ^a	5.58 ± 0.47 ^b	5.62 ± 0.41 ^b
Kernel recovery (%)	44.4 ± 2.89 ^a	47.6 ± 3.25 ^b	49.2 ± 3.32 ^c
Kernel color			
L^*	60.2 ± 3.14 ^c	57.3 ± 3.77 ^b	55.6 ± 3.53 ^a
a^*	1.74 ± 0.08 ^a	2.00 ± 0.12 ^a	2.71 ± 0.13 ^b
b^*	39.9 ± 2.75 ^b	37.9 ± 2.12 ^a	36.2 ± 2.15 ^a
Whiteness index (WI)	44.2 ± 1.20 ^c	43.2 ± 1.28 ^b	42.6 ± 1.15 ^a
Browning index (BI)	0.24 ± 0.01 ^a	0.25 ± 0.01 ^a	0.28 ± 0.01 ^b

Values are mean ± standard deviation (n = 5)

Measurements with different alphabetical superscripts in the same row indicate significant difference among the samples ($p \leq 0.05$)

L^* is a chromatic component measuring lightness while a^* and b^* are two chromatic components ranging from green to red and blue to yellow respectively

accompanied with a decrease in moisture of walnut kernels. Özkan et al. (2003) also found that the surface color characteristics of apricots changed as a result of moisture level change. L^* value of walnut kernels during all the ripening stages were higher than 40, and this ensures good colour quality according to US walnut industry standards (Wang et al. 2007). Advancement of ripening was accompanied with an increase in a^* (redness indicator) value and decrease in b^* (yellowness) value. There was no significant difference in the browning index of Pre-PTB and PTB stages, however, the browning index of Post-PTB stage was found to be significantly higher as compared to other stages of ripening. Browning index showed a significant negative correlation with overall consumer acceptability whereas Whiteness index showed a strong positive correlation with overall acceptability. Being a rich source of fat, browning of kernels may be ascribed to non-enzymatic fat-protein interaction. Nonenzymatic browning by oxidized lipid/protein reactions is associated with the formation of colored low molecular weight compounds and the production of high molecular weight polymers (Hidalgo and Zamora 2000).

Proximate composition

Results of proximate composition of walnut kernels is given in Table 1. Fat was predominant (ranging from 51.8% in pre-PTB stage to 58.3% in post-PTB stage) followed by carbohydrate (19.1% in pre-PTB stage to 25.8% in post-PTB stage) and protein (12.9% in pre-PTB stage to 15.1% in post-PTB stage). Protein and fat accounted for 65, 68 and 73% of kernel weight in pre-PTB, PTB and post-PTB stage respectively, confirming the high energy value of walnuts particularly when harvested at post-PTB stage. Pre-PTB stage showed highest moisture content (7.15%) followed by PTB and post-PTB. Ash content, ranging from 1.62 to 1.82%, showed reverse trend. Proximate composition of walnuts harvested at normal stage of harvesting (i.e. hull split stage equivalent to post-PTB stage in this investigation) is in general agreement with previous studies of several commercial varieties of walnut grown in Spain (Tapia et al. 2013) and Portugal (Amaral et al. 2003). According to the U.S. Department of Agriculture Nutrient Database for Standard Reference (USDA Agriculture Research Service 1999), the composition of English walnuts (*J. regia*) per 100 g edible portion includes 65.2 g of fat and 15.2 g of protein. Proximate composition values of whole walnut in the present study (Table 1) are close to the USDA recorded values as well as those reported by Sze-Tao and Sathe (2000); the existing differences may have originated from differences in the variety, location of cultivation, agronomic conditions and the season of harvest.

Mineral composition showed significant differences ($p \leq 0.05$) among the three ripening stages (Table 1). Kernels of Post-PTB stage showed higher levels of minerals as compared to kernels harvested earlier. Considering Post-PTB stage as normal date of harvest, and the mineral composition of normally harvested walnuts reported by other researchers is in general agreement with this investigation. For example, mineral composition values in the present study are in accordance with the USDA recorded values (USDA 2005) as well as those reported by Sze-Tao and Sathe (2000); the existing differences may have originated from differences in the variety, geographical position and cultural conditions. It has been reported (Lavedrine et al. 2000) that walnuts contain high levels of potassium (390–700 mg 100 g⁻¹), phosphorous (310–510 mg 100 g⁻¹), and magnesium (90–168 mg 100 g⁻¹) and that their content in sodium and iron is quite low (1–15 mg 100 g⁻¹). According to food composition tables (Souci et al. 1994), Zn in walnuts range between 2.0 and 3.2 mg 100 g⁻¹, Cu from 0.3 to 1.4 mg 100 g⁻¹, Mn from 0.7 to 3.2 mg 100 g⁻¹, Fe from 2.1 to 3.1 mg 100 g⁻¹, Mg from 90 to 140 mg 100 g⁻¹, Ca from 61 to 100 mg 100 g⁻¹, Na from 1 to 15 mg 100 g⁻¹, and K from 390 to 700 mg 100 g⁻¹. Mineral composition as noted in this investigation at Post-PTB stage falls around or within the range of these values except for Ca and Zn. Indigenous walnut selection in this study had registered higher levels of Ca (160.25 mg 100 g⁻¹) and lower levels of Zn (2.40 mg 100 g⁻¹). Considering the three ripening stages, kernels at Post-PTB stage were relatively enriched in all the minerals except Ca which remained at more or less at the same level. There was no significant difference in the mineral content of kernels between Pre-PTB stage and PTB stage except in case of Na where it was significantly at higher level at the latter stage than the former.

Fatty acid profile

The fatty acid composition of walnut kernels in three ripening stages is shown in Table 2. PUFA represented about 72–74% of total fatty acids, monounsaturated (MUFA) around 18%, whereas saturated fatty acids did not exceed 10% of total fatty acids. Reports of fatty acid profile of walnuts normally harvested at full maturity in Spain (Tapia et al. 2013), Portugal (Amaral et al. 2003) and Turkey (Koyuncu et al. 2001) were more or less similar to that observed in our study. Considering dynamics of fatty acid profile during developmental stages of fruit maturity, it was found that there was no significant difference among seven different saturated fatty acids (SFA) except that of Pentadecanoic acid (C15:0) which increased marginally,

Table 2 Changes in fatty acids composition (% of total fatty acids) during the maturity of walnut kernels

Sample	PRE-PTB	PTB	POST-PTB
Saturated (SFA)			
C14:0	0.02 ± 0.01 ^a	0.03 ± 0.02 ^a	0.02 ± 0.00 ^a
C15:0	0.01 ± 0.01 ^a	0.02 ± 0.00 ^b	0.02 ± 0.01 ^b
C16:0	6.93 ± 1.51 ^a	7.03 ± 1.39 ^a	7.13 ± 0.72 ^a
C17:0	0.02 ± 0.02 ^a	0.04 ± 0.01 ^a	0.04 ± 0.01 ^a
C18:0	2.50 ± 0.33 ^a	2.53 ± 0.53 ^a	2.56 ± 0.51 ^a
C20:0	0.17 ± 0.03 ^a	0.13 ± 0.03 ^a	0.14 ± 0.02 ^a
C22:0	0.05 ± 0.02 ^a	0.03 ± 0.01 ^a	0.04 ± 0.01 ^a
Total	9.71 ± 1.87 ^a	9.83 ± 1.09 ^a	9.95 ± 1.04 ^a
Monounsaturated (MUFA)			
C16:1 ⁿ⁹	0.15 ± 0.03 ^b	0.11 ± 0.03 ^b	0.07 ± 0.01 ^a
C18:1 ⁿ⁹	17.27 ± 0.83 ^a	17.32 ± 3.01 ^a	17.38 ± 2.82 ^a
C20:1 ⁿ⁹	0.11 ± 0.04 ^a	0.15 ± 0.02 ^{ab}	0.17 ± 0.01 ^b
Total	17.53 ± 0.89 ^a	17.58 ± 3.03 ^a	17.61 ± 2.80 ^a
Polyunsaturated (PUFA)			
C18:2 ⁿ⁶	62.40 ± 2.76 ^b	60.98 ± 5.79 ^{ab}	58.52 ± 5.70 ^a
C18:3 ⁿ³	14.97 ± 1.06 ^b	13.75 ± 2.23 ^b	11.69 ± 1.68 ^a
Total	77.37 ± 3.74 ^b	74.73 ± 7.35 ^b	70.21 ± 7.15 ^a
SFA/PUFA	0.13 ± 0.02 ^a	0.13 ± 0.01 ^a	0.14 ± 0.01 ^a
n6/n3	4.17 ± 0.81 ^a	4.43 ± 0.73 ^a	5.00 ± 0.95 ^b

Values are mean ± standard deviation (n = 5)

Measurements with different alphabetical superscripts in the same row indicate significant difference among the samples ($p \leq 0.05$)

but significantly, at PTB stage and stayed at the same level thereafter. This difference did not impact total saturated fatty acids that varied non statistically during the three development stages of maturity. A nonsignificant variation in the SFA content was reported in pecan nuts (Bouali et al. 2013). Similarly, total MUFA did not differ statistically during various maturity stages despite the fact that one of the components of MUFA, cis-11-eicosonic acid (C20:1^{c-11}) significantly increased at Post-PTB stage as compared to Pre-PTB stage of ripening. PUFA level, either component wise or in their totality, at Pre-PTB stage markedly decreased as ripening advanced to Post-PTB stage. While comparing general trend of our results, in respect of roughly comparable phenology, with development studies in walnut carried out by Greve et al. (1992) and Koyuncu et al. (2001), we found that dynamics of fatty acid profile, as observed in this study are in general agreement with that of Koyuncu et al. (2001) particularly with regard to PUFA, while the trend differs with that of Greve et al. (1992). The disparity can be explained by the fact that the degree of unsaturation of walnut oil may be influenced by various factors such as variety, environment, location etc.

Considering all fatty acids in walnut kernels, relative content of Omega-6 PUFA (C18:2^{n6c}) and Omega-3 PUFA (C18:3ⁿ³) were high in all the maturity stages studied in this investigation and this is in general agreement with those research workers who have made development studies in walnut (Greve et al. 1992; Koyuncu et al. 2001) and three italian hazel nut cultivars (Cristofori et al. 2015). Among tree nuts, walnuts contain a high amount of linoleic acid (C18:2^{n6c}) and linolenic acid (C18:3ⁿ³) compared with other commercially available nuts such as almond, hazelnut, macadamia, peanut and pecan (Kris-Etherton et al. 1999). These components of PUFA are essential dietary fatty acids. Epidemiological and clinical studies suggest that Omega-3 PUFA (C18:3ⁿ³) may have a significant role in the prevention of coronary heart disease and showed that inclusion of walnut consumption in the diet had a significant protective benefit with respect to fatal and nonfatal coronary heart disease events and improvement of blood lipoprotein profile (Banel and Hu 2009). Excessive Omega-6 PUFA relative to Omega-3 PUFA and so a very high n6/n3 ratio promotes pathogenesis of many diseases, including cardiovascular diseases, cancer, and inflammatory and autoimmune diseases, whereas increased levels of Omega-3 PUFA (and so a low n6/n3 ratio) exert suppressive effects. In our studies, n6/n3 ratio values of 4.15 and 4.17 in Pre-PTB stage and PTB stage respectively were significantly low as compared to value of 5.00 at Post-PTB stage. These values are marginally higher than the values reported by Tapia et al. (2013) at full maturity stage of walnut. This implies that consumption of kernels derived from walnuts harvested at Pre-PTB or PTB stage may provide better health benefits than normal stage of harvesting.

Total phenolics and antioxidant activity

The highest content of phenols (80.56 mg GAE/g extract) was observed in the first stage of maturation. A steady decrease was progressively observed at later stages (Table 3). These results in walnut are in fair agreement with the findings of Gawlik-Dziki et al. (2014). Yang et al. (2011) also reported that total phenols in sub-mature fruits of *Morinda citrifolia* was 1.3 times greater than those of ripe fruits. Besides phenolic content that can contribute to the protective effect against oxidative damage to cells, the determination of antioxidant activity allows a more realistic evaluation of the potential protective effect of a food. Results of antioxidant activity accessed by the reducing power assay and FRAP (Ferric reducing antioxidant power), DPPH (2, 2-diphenyl-1-picrylhydrazyl) and TBARS (thiobarbituric acid reactive substances) assay are shown in Table 3. In several reports, the antioxidant

Table 3 Effect of various maturity stages on Total Phenolics and Antioxidant activity of walnut kernels

Stages	DPPH (EC ₅₀)	Reducing Power (EC ₅₀)	FRAP (μM Fe ²⁺ /g extract)	TBARS (mg MDA/Kg)	Total phenolics (mg GAE/g extract)
Pre-PTB	0.19 ± 0.02 ^a	0.20 ± 0.01 ^a	522 ± 13.40 ^b	0.90 ± 0.16 ^a	80.56 ± 2.00 ^c
PTB	0.20 ± 0.03 ^a	0.19 ± 0.01 ^a	512 ± 15.80 ^b	1.05 ± 0.18 ^a	72.87 ± 3.40 ^b
Post-PTB	0.23 ± 0.02 ^b	0.23 ± 0.02 ^b	482 ± 14.42 ^a	1.48 ± 0.23 ^b	65.83 ± 2.28 ^a

Values are mean ± standard deviation (n = 5)

Measurements with different alphabetical superscripts in the same column indicate significant difference among the samples ($p \leq 0.05$)

FRAP ferric reducing antioxidant power, DPPH 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity, TP total phenolics, RP reducing power, TBARS thiobarbituric acid reactive substances

activity of *J. regia* has been described (Christopoulos and Tsantili 2012; Gawlik-Dziki et al. 2014). The DPPH free radical scavenging capacity of walnut kernel extracts varied depending on the stage of maturity (Table 3). Pre-PTB and post-PTB walnut kernels showed a significant free radical scavenging activity with EC₅₀ values of 0.19 ± 0.02 and 0.23 ± 0.02, respectively. The values are expressed as mg/mL. These results are supported by the research findings of Gawlik-Dziki et al. (2014) who reported that tinctures of walnut fruits in advanced stage of maturity showed relatively low antiradical activity as compared to younger fruits. This decrease in free radical scavenging activity during maturation has been reported in peach (Belhadj et al. 2016). There is a close correspondence of DPPH values and reducing power values in terms of EC₅₀ implying that both have homology of concentration dependent antioxidant activities. FRAP assay values, measuring the reduction of Fe³⁺ (ferric ion) to Fe²⁺ (ferrous ion) in presence of antioxidant indicated that walnut fruits at the stage of Post-PTB maturity showed relatively low reducing power as compared to younger fruits. TBARS values expressed as MDA (Table 3) increased as ripening advanced but did not show any significant difference

during early ripening stages whereas it showed marked increase in walnut kernels at post-PTB stage. Increased MDA values are indicative of higher peroxidation levels at later stages of maturity. The correlation coefficients between TPC and antioxidant activity shown in Table 4 showed negative correlation which suggests antioxidant activity can be attributed to non-phenolic compounds such as carotenoids present in walnut. An important implication of this negative correlation is that the health benefits of walnut consumption cannot be solely attributed to its phenolic content. Hence further detailed research should be carried out on non-phenolic compounds present in it.

Antibacterial activity

Walnut kernel extracts belonging to three maturity stages were screened for their antibacterial properties against *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. and*, *K. pneumoniae*. There was a significant increase in the zones of inhibition with advancement in the ripening stages. Zone of inhibition of walnut kernel extract from Post-PTB was significantly greater than zones of inhibition obtained in rest of the samples. The minimal inhibitory concentration

Table 4 Pearson's correlation coefficient of antioxidant activities, total phenolic content, whitening index, browning index and overall acceptability

	FRAP	DPPH	RP	TBARS	TPC	WI	BI
FRAP	1						
DPPH	−0.97**	1					
RP	−0.81**	0.79**	1				
TBARS	−0.25 ^{ns}	−0.27 ^{ns}	−0.50*	1			
TPC	0.93**	−0.89**	−0.62*	−0.19 ^{ns}	1		
WI	0.82**	−0.79**	−0.45*	−0.25 ^{ns}	0.95**	1	
BI	−0.92**	0.96**	0.85**	−0.19 ^{ns}	−0.92**	−0.80**	1
OA	0.85**	−0.81**	−0.55*	−0.20 ^{ns}	0.91**	0.91**	−0.82**

FRAP ferric reducing antioxidant power, DPPH 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity, RP reducing power, TBARS thiobarbituric acid reactive substances, TPC total phenolic content, WI whiteness index, BI browning index, OA overall acceptability, ^{ns} non-significant

* Significant ($p \leq 0.05$), ** highly significant ($p \leq 0.01$)

Table 5 Effect of various maturity stages on Antibacterial activity of walnut kernel

Stages	<i>S. aureus</i>			<i>B. cereus</i>			<i>B. subtilis</i>			<i>K. Pneumoniae</i>			<i>E. coli</i>		
	ZI (mm)	MIC (mg/mL)		ZI (mm)	MIC(mg/mL)		ZI (mm)	MIC(mg/mL)		ZI (mm)	MIC (mg/mL)		ZI (mm)	MIC (mg/mL)	
Pre-PTB	3.60 ± 0.44 ^{a2}	<1		2.71 ± 0.44 ^{a1}	<1		3.66 ± 0.21 ^{a2}	<5		2.89 ± 0.37 ^{a1}	<100		3.28 ± 0.35 ^{a2}	<100	
PTB	5.15 ± 0.36 ^{b2}	<1		3.02 ± 0.23 ^{a1}	<1		5.15 ± 0.48 ^{b2}	<5		2.99 ± 0.37 ^{a1}	<100		3.49 ± 0.38 ^{a1}	<100	
Post-PTB	5.55 ± 0.44 ^{b3}	<1		3.02 ± 0.27 ^{a1}	<1		6.35 ± 0.32 ^{c4}	<5		4.53 ± 0.59 ^{b2}	<100		4.27 ± 0.34 ^{b2}	<100	
Ampicillin	8.61 ± 0.49 ^{bc23}	≤0.00526		8.04 ± 0.31 ^{b1}	≤0.00125		8.68 ± 0.42 ^{d23}	≤0.0127		8.23 ± 0.41 ^{c12}	≤0.00526		8.98 ± 0.27 ^{c3}	≤0.00526	

Values are mean ± standard deviation (n = 5)

ZI zone of inhibition, MIC minimum inhibition concentration (mg/ml)

Measurements with different alphabetical superscripts in the same column and numerical superscripts in a row indicate significant difference among the samples ($p \leq 0.05$)

Table 6 Sensory evaluation of walnut kernels harvested at various stages of ripening

Samples	KA	CA	Flavour	Aroma	Sweetness	Taste	OCA
Pre-PTB	8.93 ± 0.26 ^c	8.86 ± 0.35 ^c	7.86 ± 0.99 ^b	7.80 ± 0.86 ^b	8.20 ± 0.77 ^c	7.66 ± 1.23 ^b	8.22 ± 0.24 ^c
PTB	8.33 ± 0.72 ^b	8.06 ± 0.70 ^b	7.06 ± 0.79 ^a	7.13 ± 0.83 ^a	7.06 ± 0.79 ^b	7.13 ± 0.91 ^b	7.46 ± 0.32 ^b
Post-PTB	7.60 ± 0.63 ^a	7.40 ± 0.74 ^a	7.13 ± 0.64 ^a	6.73 ± 0.88 ^a	6.73 ± 0.88 ^a	6.26 ± 0.79 ^a	6.97 ± 0.48 ^a

Values are mean ± standard deviation (n = 5)

KA kernel appearance, CA cotyledon appearance, OCA overall consumer acceptability

Measurements with different alphabetical superscripts in the same column indicate significant difference among the samples ($p \leq 0.05$)

(MIC) values for the tested bacteria (Table 5) were determined and evaluation of the antibacterial activity was worked out on the basis of average diameters of the inhibition zones corresponding to the MICs of the sample. Despite all the extracts revealed antibacterial activity, the response for each microorganism tested was different. Gram positive bacteria (*B. cereus*, *B. subtilis*, *S. aureus*) were inhibited by walnut extracts of all the maturity stages at very low concentrations, representing MICs of <1 mg/mL for *B. cereus* and *S. aureus* whereas it was <5 mg/mL for *B. subtilis*. Walnut extracts also had antibacterial effect against gram negative bacteria (*E. coli*, *P.* and *K. pneumonia*). Minimum inhibitory concentration was, however, considerably higher (100 mg/mL) to exert antibacterial action in case of all the maturity stages of walnut extracts (Table 5). As maturity advanced antibacterial activity of walnut extracts was found to be stronger for both gram positive and gram negative bacteria. As expected, the standard ampicillin (antibacterial) presented lower MICs than the walnut extracts. Usually, pure active compounds reveal more activity than crude extracts. The minimal inhibitory concentration (MIC) values of walnut extracts as obtained in our studies, in general, were found to be quite low as against those reported by Pereira et al. (2008) against gram positive bacteria, however, MIC was almost in conformity with their results in respect of gram negative bacteria.

Sensory analysis

There was a significant difference in the sensory descriptors between walnuts harvested at different stages of maturity (Table 6). All parameters showed the highest value at Pre PTB stage and a steady decrease of consumer acceptance was seen as the maturity progressed.

Conclusion

The results obtained in this study demonstrate that overall consumer acceptability score of walnut kernels weighed more in favour of earlier ripening stages than Post-PTB stage, however, kernel composition in respect of protein, fat, and mineral content registered an increase, though less marked, as ripening advanced. Lipid characterisation results indicated that the kernels derived from walnuts harvested at Pre-PTB or PTB stage may provide better health benefits especially with regard to Omega-3 PUFA (C18:3ⁿ³). On the basis of the analysis it can be concluded that walnut in the early stages of ripening show higher acceptability and contain more biologically active compounds imparting higher health benefits than in the later

stages of fruit maturity suggesting that harvesting of these walnut kernels should be carried during Pre-PTB or PTB stage of ripening. Walnut kernel extracts belonging to three maturity stages were screened for their antibacterial properties. Inhibition of gram positive and gram negative bacteria by walnut extracts, notably obtained from kernels harvested at PTB or Post-PTB stage, indicates that walnuts can be useful as potential natural antibacterial agents.

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References

- Altuntas E (2009) The effect of moisture content on color characteristics of walnuts. *Int J Food Eng* 5:1–9
- Amaral JS, Casal S, Pereira J, Seabra R, Oliveira B (2003) Determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Portugal. *J Agric Food Chem* 51:7698–7702
- AOAC (1990) Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Arlington
- AOAC (1998) Official Methods of Analysis, 16, 4th Revision edn. Association of Official Analytical Chemists, New York
- Baba WN, Din S, Punoo HA, Wani TA, Ahmad M, Masoodi FA (2016) Comparison of cheese and paneer whey for production of a functional pineapple beverage: nutraceutical properties and shelf life. *J Food Sci Technol* 53:2558–2568
- Banel DK, Hu FB (2009) Effects of walnut consumption on blood lipids and other cardiovascular risk factors: a meta-analysis and systematic review. *Am J Clin Nutr* 90:56–63
- Bauer AW, Kirby WMM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standard single disk method. *Am J Clin Pathol* 36:493–496
- Belhadj F, Somrani I, Aissaoui N, Messaoud C, Boussaid M, Marzouki MN (2016) Bioactive compounds contents, antioxidant and antimicrobial activities during ripening of *Prunus persica* L. varieties from the North West of Tunisia. *Food Chem* 1:29–36
- Bouali I, Trabelsi H, Abdallah Ikram Bou, Albouchi A, Martine L, Gregoire S, Bouzaïen G, Gandour M, Boukhchina S, Berdeaux O (2013) Changes in fatty acid, tocopherol and xanthophyll contents during the development of Tunisian-Grown pecan nuts. *J Am Oil Chem Soc* 90:1869–1876
- Christopoulos MV, Tsantili E (2012) Storage of fresh walnuts (*Juglans regia* L.)—Low temperature and phenolic compounds. *Postharvest Biol Technol* 73:80–88
- Chung J, Kim YS, Lee J, Lee JH, Choi SW, Kim Y (2016) Compositional analysis of walnut lipid extracts and properties as an anti-cancer stem cell regulator via suppression of the self-renewal capacity. *Food Sci Biotechnol* 25:623–629
- Cristoforo V, Bertazza G, Bignami C (2015) Changes in kernel chemical composition during nut development of three Italian hazelnut cultivars. *Fruits* 70:311–322
- Cunha AG, Brito ES, Moura CFH, Ribeiro PRV, Miranda MRA (2017) UPLC–qTOF-MS/MS-based phenolic profile and their biosynthetic enzyme activity used to discriminate between cashew apple (*Anacardium occidentale* L.) maturation stages. *J Chromatogr B* 1051:24–32
- Djoussé L, Lu B, Gaziano MJ (2016) Effects of walnut consumption on endothelial function in people with type 2 diabetes: a randomized pilot trial. *Curr Nutr Rep* 5:1–8

- Fernandez-Lopez J, Aleta N, Alias R (2000) Forest genetic resources conservation of *Juglans regia* L. IPGRI Publishers, Rome
- Gawlik-Dziki U, Durak IA, Pecio IL, Kowalska I (2014). Nutritional Potential of Tinctures from Fruits, Green Husks, and Leaves of *Juglans regia* L. Sci World J vol. 2014, Article ID 501392, 10 p, 2014. doi:[10.1155/2014/501392](https://doi.org/10.1155/2014/501392)
- Greve LC, McGranahan G, Hasey J, Snyder R, Kelley K, Goldhamer D, Labavitch JM (1992) Variation in polyunsaturated fatty acid composition of persian walnuts. J Am Soc Hortic Sci 117:518–522
- Hidalgo FJ, Zamora R (2000) The role of lipids in nonenzymatic browning. Grasas Aceites 51:35–49
- Hisil Y (1998) Instrumental Analysis Techniques. Ege University Engineering Faculty Publication No. 55 Bornova-Izmir. (in Turkish)
- Kader AA, Thompson JE (2002) Postharvest handling systems: tree nuts. In: Kader A (ed) Postharvest Technology of Horticultural Crops. University of California, Division of Agriculture and Natural Resources, Oakland, pp 399–406
- Koyuncu MA, Yarılgac T, Kazankaya A, Koyuncu F, Küçük M, Sen SM (2001) Compositional changes of fatty acids during the development of kernel of yalova-1 and yalova-4 walnut cultivars. Acta Hortic 544:585–589
- Kris-etherton PM, Yu-poth S, Sabate J, Ratcliffe HE, Etherton TD (1999) Nuts and their bioactive constituents: effects on serum lipids and other factors that affect disease risk. Am J Clin Nutr 70:504S–511S
- Lavedrine F, Ravel A, Vilet A, Ducros V, Alary J (2000) Mineral composition of two walnut cultivars originating in France and California. Food Chem 68:347–351
- Lee B, Seo JD, Rhee JK, Kim CY (2016) Heated apple juice supplemented with onion has greatly improved nutritional quality and browning index. Food Chem 201:315–319
- Nisar R, Baba WN, Masoodi FA (2015) Effect of chemical and thermal treatments on quality parameters and anti-oxidant activity of apple (pulp) grown in high Himalayan regions. Cogent Food Agric 1:1–13. doi:[10.1080/23311932.2015.1063797](https://doi.org/10.1080/23311932.2015.1063797)
- Özkan M, Kirca A, Cemeroglu B (2003) Effect of moisture content on CIE color values in dried apricots. Eur Food Res Technol 216:217–219
- Pereira JA, Oliveira I, Sousa A, Ferreira ICFR, Bento A, Estevinho L (2008) Bioactive properties and chemical composition of six walnut (*Juglans regia* L.) cultivars. Food Chem Toxicol 46:2103–2111
- Regueiro J, Sánchez-González C, Vallverdú-Queralt A, Simal-Gándara J, Lamuela-Raventós R, Izquierdo-Pulido M (2013) Comprehensive identification of walnut polyphenols by liquid chromatography coupled to linear ion trap-Orbitrap mass spectrometry. Food Chem 152:340–348
- Ruijun W, Shi W, Yijun X, Mengwuliji T, Lijuan Z, Yumin W (2015) Antitumor effects and immune regulation activities of a purified polysaccharide extracted from *Juglans regia*. Int J Biol Macromol 72:771–775
- Shafi M, Baba WN, Masoodi FA, Bazaz R (2016) Wheat-water chestnut flour blends: effect of baking on antioxidant properties of cookies. J Food Sci Technol 53:4278–4288
- Sibbett GS, Hendricks LC, Carnill G, Olson WH, Jeter R, Ramos DE, Martin GC, Davis CS (1974) Walnut quality and value maximized by harvest management. Calif Agric 28:15–17
- Souci SW, Fachmann W, Kraut H (1994) Food composition and nutrition tables. Medpharm. CRC Press, Stuttgart, pp 955–956
- Stuetz W, Schlörmann W, Glei M (2017) B-vitamins, carotenoids and α - γ -tocopherol in raw and roasted nuts. Food Chem 221:222–227
- Sze-Tao KWC, Sathe S (2000) Walnuts (*Juglans regia* L.): proximate composition, protein solubility, protein amino acid composition and protein and fatty acid composition of *Juglans regia* L in vitro digestibility. J Sci Food Agric 80:1393–1401
- Tapia MI, Sanchez- Morgado JR, Garcia-Parra J, Ramirez R, Hernandez T, Gonzalez-Gomez D (2013) Comparative study of the nutritional and bioactive compounds of four walnut (*Juglans regia* L) cultivars. J Food Compos Anal 31:232–237
- USDA (2005). US department of agriculture, agricultural research service. USDA national nutrient database for standard reference, release 18. Nutrient data laboratory home page. <http://www.ars.usda.gov/ba/bhnrc/ndl>
- USDA Agriculture Research Service. (1999). Release 13. U.S. Department of Agriculture. Agricultural Research Services, Washington, DC. http://www.nal.usda.gov/fnic/cgi-bin/list_nut.pl
- Wang S, Monzon M, Johnson JA, Mitcham EJ, Tang J (2007) Industrial-scale radio frequency treatments for insect control in walnuts: I. Heating uniformity and energy efficiency. Postharvest Biol Technol 45:240–246
- Yang J, Gadi R, Thomson T (2011) Antioxidant capacity, total phenols, and ascorbic acid content of noni (*Morinda citrifolia*) fruits and leaves at various stages of maturity. Micrones Ser 41:167–176