Review

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Walnut (*Juglans regia* L.): genetic resources, chemistry, by-products

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Abstract

Walnut (*Juglans regia* L.) is the most widespread tree nut in the world. There is a great diversity of genotypes differing in forestry, productivity, physical and chemical nut traits. Some of them have been evaluated as promising and may serve as germplasm sources for breeding. The nutritional importance of the nut is related to the seed (kernel). It is a nutrient-dense food mainly owing to its oil content (up to 740 g kg^{-1} in some commercial varieties), which can be extracted easily by screw pressing and consumed without refining. Walnut oil composition is dominated largely by unsaturated fatty acids (mainly linoleic together with lesser amounts of oleic and linolenic acids). Minor components of walnut oil include tocopherols, phospholipids, sphingolipids, sterols, hydrocarbons and volatile compounds. Phenolic compounds, present at high levels in the seed coat but poorly extracted with the oil, have been extensively characterised and found to possess strong antioxidant properties. The oil extraction residue is rich in proteins (unusually high in arginine, glutamic and aspartic acids) and has been employed in the formulation of various functional food products. This review describes current scientific knowledge concerning walnut genetic resources and composition as well as by-product obtainment and characteristics.

Keywords: walnut; genetic resources; chemistry; by-products

INTRODUCTION

Walnut (*Juglans regia* L., Juglandaceae) has been used in human nutrition since ancient times. The walnut tree is native to central Asia, the western Himalayan chain and Kyrgyzstan[1] and was cultivated in Europe as early as 1000 BC.[2] Since then, it has spread and become well adapted to many regions with Mediterranean-type ecosystems throughout the world.

At present, walnut is cultivated commercially throughout southern Europe, northern Africa, eastern Asia, the USA and western South America. World production of whole walnut (with shell) was around 1.5×10^6 t in 2008.[3] China is the leading world producer, followed by the USA, Iran, Turkey, Ukraine, Romania, France and India, but production in other countries such as Chile and Argentina has increased rapidly in recent years.

Walnut is a crop of high economic interest to the food industry: the edible part of the fruit (the seed or kernel) is consumed, fresh or toasted, alone or in other edible products. It is globally popular and valued for its nutritional, health and sensory attributes. The fresh natural kernels are consumed mainly as whole nuts or used in various confectioneries. They are a nutrient-dense food mainly owing to their fat content and protein, vitamin and mineral profiles. Also, walnut kernels serve as a good source of a wide variety of flavonoids, phenolic acids and related polyphenols. Although phenolic compounds have no known nutritional function, they may be important for human health owing to their good antioxidant, antiatherogenic, anti-inflamatory and antimutagenic properties.[4,5] Considering all kernel components, many studies have described various health-beneficial effects of walnut-supplemented diets in comparison with reference diets. Davis *et al*.[6] reported that regular walnut consumption lowered plasma total and low-density lipoprotein (LDL) cholesterol, while Lavedrine *et al*.[7] described a positive effect on blood high-density lipoprotein (HDL) cholesterol and apolipoprotein A1. According to Simopoulos,[8] walnuts are unique because they have a perfect balance of n-6 and n-3 polyunsaturated fatty acids, a ratio of 4 : 1, which has been shown to decrease the incidence of cardiovascular risk. Furthermore, the heart benefits of walnut intake include reducing inflammation and improving arterial function.[9–11] In this sense, walnut phenolics may also have a protective effect on the susceptibility of LDL cholesterol to oxidative modification and, ultimately, on atherosclerosis.[4]

In summary, studies have shown that, although walnuts are considered energy-dense, those who consume them on a regular basis display many health benefits, strong dietary compensation and little change in total energy balance. This article reviews current scientific knowledge concerning walnut genetic resources and composition as well as by-product obtainment and characteristics.

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WALNUT FRUIT CHARACTERISTICS

Recently, the European Forest Genetic Resources Programme (EUROFGENE) has initiated a programme for conservation of the present genetic resource base of walnut. The most promising genotypes are being maintained in various national germplasm collections.[12–14]

The UPOV[15] descriptors have established physical nut and kernel properties of walnut as quality criteria. These properties include nut and kernel weight and dimension, kernel ratio, shell shape and thickness, among others. The relationship between kernel weight (KW) and nut weight (NW), the kernel ratio, is useful as an indicator of good in-shell fruit. A positive correlation between nut and kernel sizes indicates good nut filling. Kernel colour is another walnut quality criterion, with light yellow kernels being preferred.

High variability in the shape, size and weight of fruit, the colour and thickness of shell and the size, weight and external colour of kernel has been reported for walnuts from different regions of the world.[16–23] Most of this variation is attributed to genetic variability and may be useful to breeding programmes desiring high yield or better fruit and kernel quality.

In a recent study, Martínez *et al.*[23] evaluated some commercial walnut varieties cultivated in Argentina. Tulare, Serr and Chandler varieties had the best quality traits: they presented the greatest size and weight of both nuts and kernels and the highest KW/NW ratios. All genotypes evaluated had good colour traits: more than 90% of their kernels fell into the 'extra light' and 'light' categories. Chandler variety had the best performance, since 96% of its kernels were classified as 'extra light'.

Regarding pomological characteristics of native walnut genetic resources, several studies have been carried out in Turkey, which is one of the motherlands of walnut.[20,21,24,25] These works showed a wide variation among genotypes in many fruit characteristics. For example, nut weight varied between 8.15 and 17.2 g and kernel weight between 3.46 and 8.5 g, resulting in KW/NW ratios between 0.23 and 0.57.

On the other hand, Zeneli *et al.*[22] analysed the phenotypic variation in native walnut populations from Albania. Variability in nut weight was between 3.8 and 21.1 g and in kernel weight between 1.85 and 9.8 g, giving KW/NW ratios between 0.32 and 0.64. Some of these physical traits for Macrocarpa, Elongata and Tenera varieties were even higher than those reported for several Californian and French commercial varieties.

Sharma and Sharma[26] studied the genetic variability in some metric nut and kernel characters from walnut seedling trees growing in Himachal Pradesh (India). They found accessions with nut weight as high as 23.6 g and kernel weight up to 9.2 g. Interestingly, they also found that the clustering pattern of walnut genotypes studied did not show parallelism between geographic and genetic diversity.

Díaz *et al.*[27] reported geographic variation for some fruit morphological traits of walnuts from populations of western Spain. The most important pattern of geographic variation was due to latitude.

In summary, other than phenotypic variations attributed to environmental differences, various native walnut genotypes throughout the world have been evaluated as promising with regard to fruit characteristics and can serve as the core of future germplasm collections owing to their high genetic variability in forestry, nut and kernel traits.

Table 1. Oil content and main fatty acids of some commercial walnut varieties from different origins[28–31]

Variety	Oil content (g kg^{-1})	Fatty acids (mg g^{-1})				
		16:0	18:0	18:1n-9	18:2n-6	18:3n-3
Chandler	695–725	66–69	15–17	161–178	565–589	165–186
Franquette	623–724	66–75	19–31	170–284	502–592	117–149
Lara	665–712	63–81	16–28	149–197	579–625	122–152
Mayette	663–732	59–70	18–27	161–223	554–575	130–176
Marbot	663–688	63–71	27–28	163–165	589–597	127–143
Mellanaise	630–697	63–70	26–28	145–171	587–616	125–145
Parisienne	631–715	62–63	24–29	174–195	577–624	96–132
Criolla	676–689	77–78	17–19	173–212	573–578	119–156
Hartley	710–714	68–81	9–13	167–179	584–592	146–160
Serr	711–728	66–67	18–19	204–254	525–563	136–147
Sorrento	716–739	72–76	15–17	172–191	589–593	126–150
Tulare	732–736	61–64	20–22	230–241	559–569	114–120

Fatty acid nomenclature: 16:0, hexadecanoic acid; 18:0, octadecanoic acid; 18:1n-9, *cis*-9-octadecenoic acid; 18:2n-6, *cis*-9,*cis*-12-octadecadienoic acid; 18:3n-3, *cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid.

WALNUT NUTRIENT COMPOSITION

Walnut oil

Walnut is known as a source of high lipid content. Although walnut oil is not described by the current Committee on Fats and Oils of the Codex Alimentarius, small amounts are produced and commercialised in countries such as France, Spain, Chile and Argentina. The oil is used directly (without refining) for edible purposes, mainly as a salad dressing. It is also used in the cosmetic industry as a component of dry skin creams, antiwrinkle and anti-aging products.

The oil content in commercial walnut varieties ranges from 620 to 740 g kg^{-1} kernel (Table 1). Prasad[32] reported a mean value of 690 g kg^{-1} in walnuts from different varieties and geographic origins.

Oil content increases in parallel with kernel development, which describes a double sigmoid curve, with its initial, rapid phase nearly coincident with the beginning of stage III of whole-fruit development.[33,34] The oil content in walnut kernel is determined by the genotype but may also be influenced by environmental conditions and irrigation rate.[32] Minor differences were observed in crop year effects.[28] Chandler, Franquette, Hartley, Lara, Mayette, Serr, Sorrento and Tulare varieties cultivated in Argentina have oil contents exceeding 700 g kg^{-1} kernel. Some of these varieties growing in Portugal have somewhat lower oil contents.[29]

Interestingly, oil yields of various native genotypes are as high as those reported for Californian and French commercial cultivars. Çaglarirmak,[20] Dogan and Akgul[35] and Ozkan and Koyuncu[21] found oil contents of 570–690, 650–700 and 620–710 g kg^{-1} respectively in diverse genotypes from Anatolia (Turkey). In the screening of walnut accessions from Dibra (Albania), Zeneli *et al.*[22] found kernel oil contents varying between 570 and 680 g kg^{-1}, whereas Savage *et al.*[36] determined a narrow range (650–690 g kg^{-1}) in walnut varieties cultivated in New Zealand. In contrast, the oil contents (470–660 g kg^{-1}) reported by Sharma and Sharma[26] for walnut kernels from seedling trees growing in India were wider in range but generally lower than those mentioned previously.

Oil extraction

Two major goals in walnut oil production are to find an appropriate method to recover the oil from the kernels and to improve its oxidative stability. Owing to the high unsaturation level of walnut oil, extreme care needs to be taken to prevent oxidative degradation reactions during storage and processing of fruits and extraction of the oil.

Extraction by solvent was achieved by Demir and Çetin[37] and Crowe *et al*.[38] on a laboratory scale using hexane, methylene chloride or chloroform/methanol. Although this extraction method may remove the total oil mass, it is not suitable on an industrial scale owing to the very high lipid content of the walnut kernel, which requires greater quantities of solvent to extract the oil completely. Martínez ML and Maestri DM (unpublished) determined a ratio of 14 : 1 (solvent mass/kernel mass) to exhaustively extract the oil with hexane using a continuous lixiviation process (described by Labuckas *et al*.[39]) on a pilot plant scale.

Typically, walnut oil extraction is carried out by pressing with either a screw press or a hydraulic press. Although screw pressing will not extensively replace solvent extraction in commodity oilseeds, because it recovers a lower proportion of oil, in the case of new speciality edible oils, screw pressing provides a simple and reliable method of processing small batches of seed. Crews *et al*.[40] extracted walnut oils using a cold press expeller. They found that the volumes of recovered oil varied considerably between samples, apparently as a function of physical characteristics of the kernels, but no indication of oil yields was given.

Martínez *et al*.[41] studied the combined effects of seed moisture content (25, 45 and 75 g kg^{-1}) and temperature (25, 50 and 70 °C) on oil recovery and quality parameters using a pilot-plant-scale screw press. Moistening was more beneficial than heating in terms of oil recovery for the range of conditions used in the study. Highest oil recovery (893 g kg^{-1} kernel) was obtained at 75 g kg^{-1} moisture content and 50 °C temperature. For all conditions tested, the oil quality compared well with that of cold-pressed walnut oil.

Walnut oil extraction employing supercritical carbon dioxide (SC-CO_2) has been investigated by several authors.[41–43] Similarly to the SC-CO_2 extraction of other vegetable oils, the mass of walnut oil extracted is determined initially by the oil solubility in CO_2, and a linear relationship is observed, the slope of which gives the oil solubility in CO_2 under the experimental conditions employed. Thereafter the extraction rate is governed by both solubility and diffusion and decreases continuously with time.

Martínez *et al*.[41] examined the effects of different CO_2 pressures (200 and 400 bar) and temperatures (50 and 70 °C) with regard to oil yield and quality and the time required for oil extraction from pre-pressed walnut kernels. A significant effect of the operating pressure was observed: under isothermal conditions an increase in pressure from 200 to 400 bar caused a notable increase in extraction yield, and the oil recovery was highest (Fig. 1). Similarly, Salgin and Salgin[43] found that the oil extraction yield increased with CO_2 pressure, obtaining the highest yield at 500 bar. SC-CO_2 extraction did not affect the fatty acid (FA) composition, but tocopherol and carotenoid contents were significantly higher than those found in press-extracted walnut oil.[41] Curiously, the oxidative stability of SC-CO_2-extracted oils decreases markedly with respect to press-extracted oils.[41,42,44] An interesting application of SC-CO_2 extraction technology is the obtaining of reduced-fat walnuts.[45]

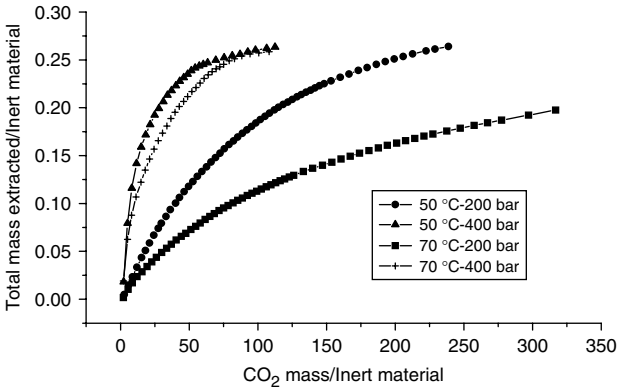

Figure 1. Extraction yield (total mass extracted/inert material) as a function of specific CO_2 mass (CO_2 mass/inert material) during supercritical CO_2 extraction of walnut oil.[41].

Table 2. Physical and chemical characteristics of press-extracted walnut oils[28,30,40,41]

Parameter	Value
Relative density (25 °C)	0.92
Refractive index (n_D^{25})	1.47
Unsaponifiable matter (%)	0.50–0.54
Free FA content (% oleic acid)	0.05–0.50
Peroxide value (meq O_2 kg^{-1})	0.05–0.50
K_{232}	1.0–1.2
K_{270}	0.05–0.1
Saturated FAs (mg g^{-1})	80–110
Monounsaturated FAs (mg g^{-1})	140–285
Polyunsaturated FAs (mg g^{-1})	620–780
Iodine value	150–166
Total tocopherols (mg kg^{-1})	260–600
OSI (Rancimat) (h)	2.6–3.5

FA, fatty acid; K_{232}, conjugated dienes; K_{270}, conjugated trienes; OSI, oxidative stability index.

Chemical composition and oxidative stability

Table 2 shows some physical and chemical characteristics of walnut oil. The major components of walnut oil are triacylglycerols (TAGs; up to 980 g kg^{-1} oil), in which monounsaturated FAs (mainly oleic acid) and polyunsaturated FAs (PUFAs; linoleic and α-linolenic acids) are present in high amounts (Table 1).[28,29,32,40] Nine TAG species have been characterised, of which trilinolein is the most abundant (~37.7%), followed by dilinoleoyl-oleoyl-glycerol (18.5%) and dilinoleoyl-linolenoyl-glycerol (18.4%).[46] The FA composition of walnut oil has been extensively reported for several cultivars from different geographic origins, including Argentina,[28,30] the USA,[40] New Zealand,[36] Portugal,[29,31] Turkey,[20,21,35] Greece[47] and Germany.[48]

In general, the FA composition of walnut oil resembles that of soybean oil, but walnut oil contains a greater concentration of linolenic acid. In fact, among vegetable oils, walnut oil has one of the highest amounts of PUFAs (up to 78% of the total FA content).

Genotype is the major source of variability in FA composition. Only minor differences are attributed to environmental and crop year effects.[28–30] The extraction method (solvent, pressing or SC-CO_2) does not seem to affect the FA composition of the oil.

Table 3. Tocopherol content of some selected varietal walnut oils[51,52]

Variety	Tocopherols (mg kg^{-1})			
	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherols
Franquette	8.9–18.7	189.8–261.6	8.7–40.7	211–297
Lara	11.4–15.7	193.7–229.9	10.4–13.1	223–262
Marbot	10.4–12.6	197.2–262.0	8.2–13.6	220–291
Mayette	11.3–15.6	200.8–258.6	11.4–15.6	229–287
Mellanaise	8.7–10.5	172.6–229.9	8.6–15.2	194–259
Parisienne	11.4–16.5	210.3–252.7	11.2–16.8	240–290
Hartley	10.8–40.5	204.9–264.6	9.8–42.3	236–347

Table 4. Main sterols of walnut oils from different origins[29,40]

Sterol	Content (mg kg^{-1})
Cholesterol	ND–38
Campesterol	44–121
Stigmasterol	ND–16
Clerosterol	11–50
β-Sitosterol	772–2520
Δ^5-Avenasterol	25–153
Δ^5-24-Stigmastadienol	ND–19
Δ^7-Avenasterol	ND–28
Total sterols	902–2833

ND, not detected.

Table 5. Volatile composition of walnut oils from different origins[55,56]

Compound	Content (% NA)
Hydrocarbons	
n-Pentane	8.8–19.5
n-Octane	Tr–5.8
n-Nonane	ND–0.4
Alcohols	
Cyclobutanol	Tr–2.2
1-Pentanol	ND–0.8
1-Hexanol	ND–0.6
1-Heptanol	ND–1.3
1-Octanol	ND–1.1
Furfuryl alcohol	ND–0.8
Aldehydes	
Pentanal	5.4–8.4
Hexanal	3.6–6.7
2-Hexenal	Tr
Heptanal	Tr–4.5
2,4-Heptadienal	ND–4.5
Octanal	Tr–5.6
Nonanal	6.0–9.2
2-Nonenal	ND–1.4
Decanal	ND–0.7
2-Decenal	3.6–6.3
2,4-Decadienal	0.3–9.8
Furan derivatives	
2-Pentylfuran	ND–0.8
2-Octylfuran	ND–0.5
Furancarboxaldehyde	ND–2.6

NA, normalised area; Tr, trace (<0.3%); ND, not detected.

It is important to note that variations in the FA composition of some native genotypes[20,21] are much wider than those observed for commercial varieties. High oleic acid content (up to 400 g kg^{-1}) and consequently comparatively low levels of linoleic and linolenic acids have been reported for native genotypes grown in Turkey.[21] This fact may be of interest for genetic improvement leading to increased oxidative stability and shelf life of the oil.

The polar lipid fraction of walnut oil consists of sphingolipids and phospholipids.[47,49,50] Besides these compounds, tocopherols occur in walnut oil and are important in providing some protection against oxidation.[36,37] The measurement of tocopherol isomers in walnut oil is well documented (Table 3). Significant differences related to genotype, crop year and geographic origin have been observed.[28,36,40,51–53] γ-Tocopherol is the main component, followed by δ- and α-tocopherol. β-Tocopherol is not detected in most cases. Amaral *et al.*[52] report small amounts of γ-tocotrienol.

The presence of other minor unsaponifiable lipid constituents such as sterols, hydrocarbons and volatile compounds is less well documented. It appears that variations in the sterol composition of walnut oil from different countries are higher than variations among genotypes.[28,29,40] Beyond this aspect, there is no doubt that β-sitosterol is the predominant compound of the sterol fraction, followed by campesterol and Δ^5-avenasterol in similar amounts (Table 4). In addition to sterols, small amounts of methylsterols may be detected in walnut oil.[28] A hydrocarbon fraction containing *n*-alkanes and minor quantities of branched and unsaturated hydrocarbons was isolated from press-extracted walnut oil. The *n*-alkane profile was characterised by even-carbon-number components, among which C_{14}–C_{20} compounds predominated.[28] Discrepancies were observed between these data and those of McGill *et al.*,[54] who found that odd-carbon-number *n*-alkanes were the major components.

Walnut oil volatile compounds include low- and medium-molecular-weight (C_4–C_{10}) *n*-alkanes, aliphatic alcohols and aldehydes and furan derivatives (Table 5).[55,56] Saturated and unsaturated aldehydes are the most abundant flavour components. The evidence indicates that short- and medium-chain alkanes and aldehydes in walnut oil are produced from unsaturated FAs (mainly linoleic acid) through oxidative pathways (Fig. 2). The formation of some volatiles (pentanal, 2-heptenal and 2-octenal) may be better explained through an enzymatic pathway rather than a chemical (non-enzymatic) one. In walnuts, as in a wide variety of beans, peas, peanuts and almonds, substantial quantities of lipoxygenases (LOXs) may be present.[57–59] LOXs catalyse the oxidation of PUFAs containing *cis,cis*-1,4-pentadiene units, such as linoleic and linolenic acids. The hydroperoxides produced by LOXs undergo cleavage to give short- and medium-chain hydrocarbons, aldehydes and alcohols (similar to those formed by non-enzymatic autoxidation) that contribute to the headspace volatile flavours.[60]

The slightly astringent flavour of walnut kernel has been associated with the presence of phenolic compounds.[32] Walnut phenolics are found at higher concentration in the seed coat, the skin that covers the pulp. They are principally polyphenolics of the non-flavonoid type and fall into the category of ellagitannins (Fig. 3).[61–63] The levels of phenolic compounds are influenced by various factors. They depend mainly upon genetic factors, but

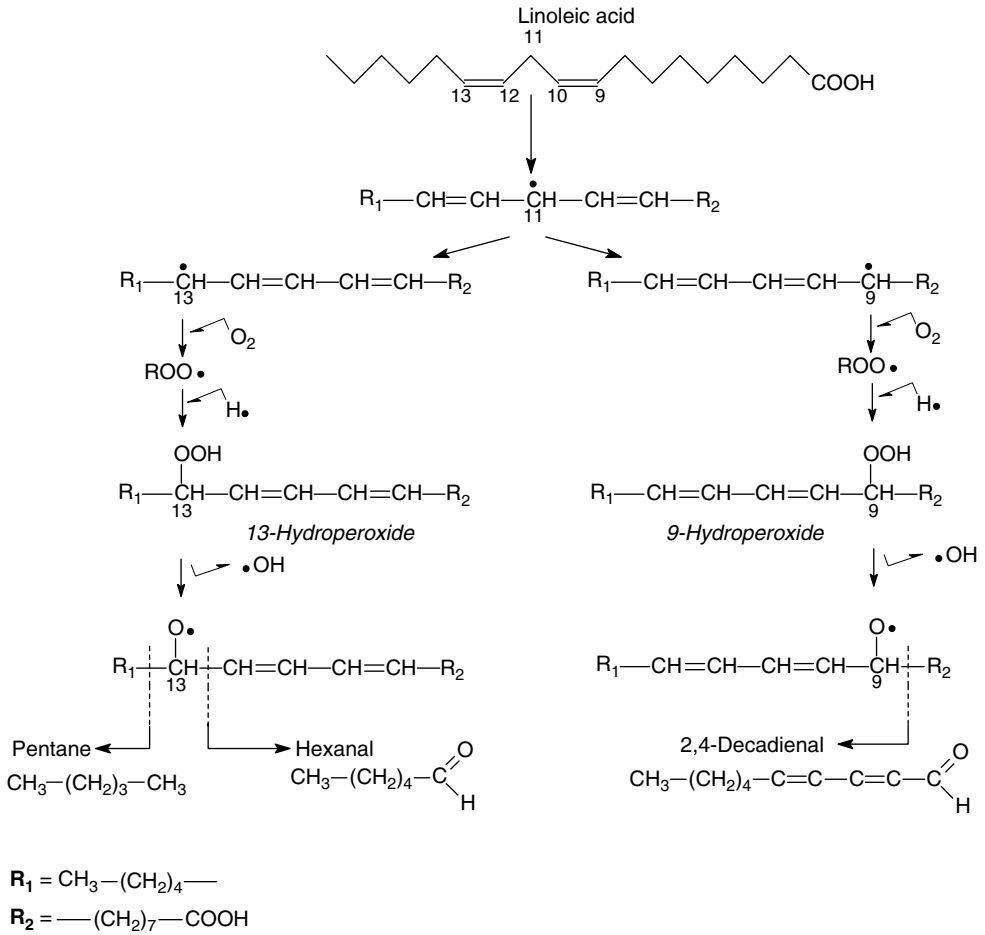

Figure 2. Chemical pathway for generation of major headspace volatile components proceeding from linoleic acid in walnut oil.

environmental conditions, fruit ripeness and storage also affect the composition of walnut phenolics.

Gündüç and El[64] reported that walnut kernels had the greatest total phenolic concentration and the highest antioxidant activity among 25 types of commonly consumed foods. Walnut phenolics were reported to display strong antioxidant and free radical-scavenging capacities.[39,61,62,65,66] Attempts were made to determine the presence of phenolic compounds in press-extracted walnut oil, but results were negative.[30] However, Arranz *et al.*[67] determined small amounts of total polyphenols (0.32 mg g^{-1} oil) in petroleum ether-extracted walnut oil. Nonetheless, because of their low oil solubility, only minor amounts of polyphenolics could be present in the extracted oil. Therefore the activity of phenolics other than tocopherols appears to be negligible in providing some protection against oxidation in walnut oil.

Indeed, walnut oil is highly susceptible to both thermal and photo-oxidative degradation.[30,68] Martínez ML and Maestri DM (unpublished) examined the effect of adding *tert*-butyl hydroquinone (TBHQ), alone or in combination with ascorbyl palmitate (AP), on the photosensitised oxidation of walnut oil exposed to white fluorescent light (800 lx) at room temperature during a 6 month storage period. The results showed that (a) treatments containing 100 or 200 ppm TBHQ were equally effective in retarding the formation of primary oxidation products in the oil and (b) treatments containing TBHQ plus AP were similar to those of TBHQ alone, indicating that AP does not have an additive effect on the antioxidant efficacy of TBHQ. On the other hand, walnut oil supplemented with 100 ppm TBHQ and stored in the dark was well preserved for 6 months, suggesting that it may be stabilised against oxidation by using only half of the maximum concentration (200 ppm) permitted for synthetic antioxidants in edible oils.

Walnut flour

Walnut flour may be obtained from kernel press-cake. It provides appreciable amounts of proteins (450 g kg^{-1} on average). They are mainly composed of glutelins (about 70% of the total seed proteins) together with lesser amounts of globulins (18%), albumins (7%) and prolamins (5%). The majority of total walnut polypeptides have estimated molecular weights in the range 12–67 kDa.[69] Walnut proteins show a typical solubility profile (Fig. 4) with minimum protein solubility at pH 4.[39] Protein solubility increases markedly at pH values below 3 and above 5, reaching a maximum at pH 9–10. This solubility pattern may vary slightly according to the method employed (solvent or pressing) to obtain the walnut flour.

The amino acid (AA) composition of walnut flour is dominated by the acidic AA residues of aspartate and glutamate together with relatively high levels of arginine.[69–71] Walnut proteins contain all essential AAs required for the needs of a human adult. However, compared with the AA requirements of a 2–5-year-old child, lysine is the first limiting AA.[72] The lysine/arginine ratio in walnut

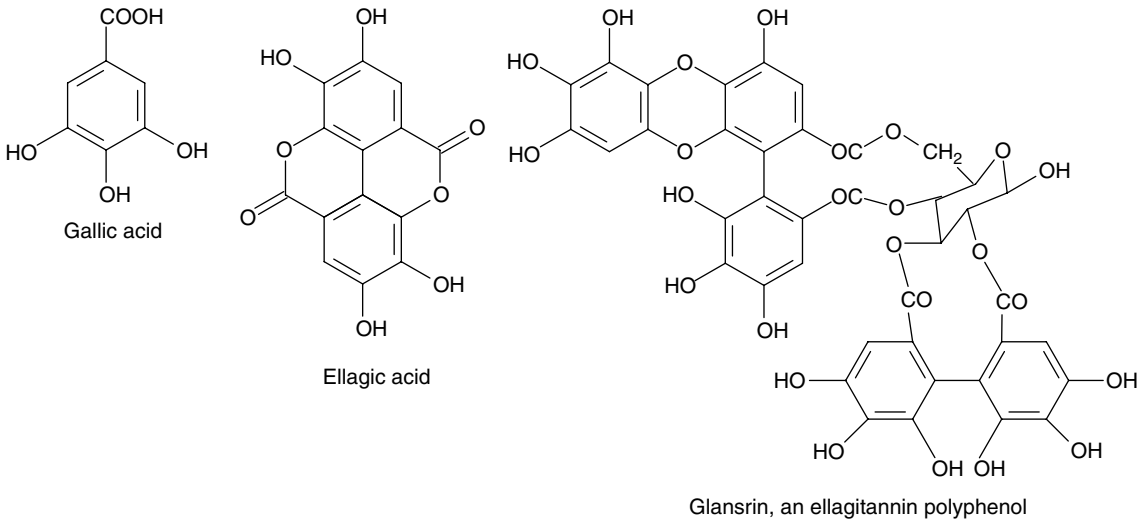

Figure 3. Polyphenolic compounds present in walnut kernel.[61–63].

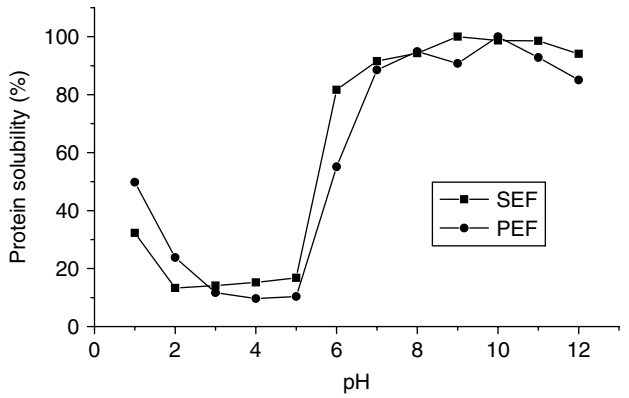

Figure 4. Effect of pH on solubility of walnut proteins obtained from solvent (hexane)-extracted flour (SEF) and press-extracted flour (PEF).[39,69].

proteins is lower than those observed in other common vegetable proteins, and this fact has been identified as a positive feature in the reduction of atherosclerosis development.[73]

Walnut glutelins have been shown to be highly digestible. However, protein solubility and consequently AA bioavailability are adversely influenced by phenolic compounds, especially hydrolysable and non-hydrolysable tannins such as those present in walnut kernels (Fig. 3). When kernels are whole-ground and the oil is extracted, most phenolics remain in the flour, where they may precipitate proteins through various mechanisms such as hydrophobic and ionic interactions and hydrogen and covalent bonding.[74,75] Walnut phenolics may effectively bind to proteins when they are dispersed in aqueous media at neutral pH. Phenolics are mostly present in the hull, and their removal increases protein solubility.[39] Although this procedure is effective in raising protein bioavailability, it is not practical for industrial applications. Instead of such a procedure, extraction of walnut phenolics with aqueous ethanol (700 mL L^{-1}) has been proposed to remove phenolics from the flour without affecting protein availability. Among various solvents tested, aqueous ethanol had the least effect in solubilising walnut seed proteins.[39,76]

Walnut flour has been employed in the formulation of various functional food products such as meat, dairy and bakery products.[77–81] The results of those studies showed that walnut flour components have positive effects on the nutritional, functional and sensory characteristics of the by-products developed. For example, Cofrades *et al.*[81] demonstrated that the incorporation of preheated defatted walnut in the formulation of meat batters improves the thermal gelling ability of myofibrillar proteins, probably because it promotes walnut–muscle protein interactions.

On the other hand, the inclusion of walnut flour in the formulation of food products could serve as a way of incorporating some specific biologically active components present in the flour. Among them, melatonin has received attention because of its beneficial effects on the cardiovascular system[82,83] and its antioxidant and anticarcinogenic properties.[84–86] The level of this compound in walnuts seems to be low (~3.5 ng g^{-1}),[85] but, even so, significant increments in blood melatonin concentration were observed in rats fed a walnut-supplemented diet. This fact, in turn, was correlated with an increase in serum TEAC (Trolox equivalent antioxidant capacity) and FRAP (ferric reducing ability of plasma) values with respect to a control (without walnut) diet. Dietary melatonin from walnut flour also showed inhibitory effects on the growth of a murine breast tumour.[86]

Fibre and minerals are other positive constituents of walnut. Savage[71] reported total dietary fibre contents ranging from 31 to 52 g kg^{-1} kernel (dry matter) in walnut cultivars from New Zealand. Mineral composition was studied by Ravai[87] and Lavedrine *et al.*[88] They reported high levels of potassium (>350 μg kg^{-1} kernel), phosphorus (>300 μg kg^{-1}) and magnesium (>120 μg kg^{-1}) but a relatively low sodium content (<10 μg kg^{-1}).

A number of experiments have been carried out to evaluate storage conditions and shelf life of walnut flour, in-shell walnuts and walnut kernels.[89–92] Vanhanen and Savage[91] found that walnut flour could be preserved from oxidation for up to 26 weeks when stored below 23 °C in polypropylene plastic containers with polyethylene sealing lids. Mexis *et al.*[92] evaluated the effect of packaging and storage conditions on the quality of shelled walnuts. They determined that the effect of the parameters examined followed the sequence temperature > degree of O_2 barrier > lighting conditions. Shelled walnuts retained acceptable quality for at least 12 months in polyethylene terephthalate-SiO_x/polyethylene-N_2 pouches stored at 20 °C.

CONCLUDING REMARKS

There is a great diversity of native and commercial walnut genotypes differing widely in productivity and nut traits. Various native genotypes, especially from Turkey, Albania and India, have been evaluated as promising with regard to some physical and chemical kernel characteristics. They may serve as the cores of germplasm collections and as genetic sources for breeding.

The nutritional importance of walnut is related to its kernel. Walnut kernel mainly contains lipids, among which TAGs are present in very high concentration. Walnut oil composition is primarily dependent on genotype. The composition of triglycerides is largely of unsaturated FAs, mainly linoleic acid together with oleic and linolenic acids in similar amounts. The high oil and essential FA contents of walnut kernel make it a good source for commercial production of edible oil. Walnut oil can be extracted easily by screw pressing. The obtained oil is low in phosphatides and acid and peroxide values, because of which it may be consumed without refining. The elevated unsaturation level, however, may result in poor oxidative stability and shelf life of the oil. Although walnut kernels contain a diverse array of phenolic and polyphenolic compounds with strong antioxidant and radical-scavenging properties, only minor amounts could be present in the extracted oils. Protection against oxidative degradation seems to be limited mainly to tocopherol content. Other minor components characterised in walnut oil include phospholipids, sphingolipids, sterols (principally β-sitosterol), hydrocarbons (particularly C_{14}–C_{20} *n*-alkanes) and volatile components. The majority of the volatile components found in walnut oil are produced by oxidative breakdown of linoleic and linolenic acid hydroperoxides. Considering the sensory attributes characterising such volatiles, they could adversely affect the sensory profile of the raw shelled walnut or its by-products (oil and flour) during handling and storage. Additional work is necessary to evaluate antioxidants as well as packaging and storage conditions aimed towards quality retention.

The oil extraction residue, the walnut flour, is rich in proteins. Walnut proteins are unusually high in arginine and glutamic and aspartic acids and possess good digestibility. Phenolic compounds present in walnut flour may bind to proteins affecting protein solubility and possibly AA bioavailability. Removing phenolics raises protein recovery. This procedure should be considered to attempt successfully the commercial production of good quality walnut flour. Additionally, it may be the source for obtaining natural phenolic antioxidants.

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