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Review

Functional components of walnuts: a review focusing on native and cultivated species

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Walnuts are consumed worldwide as healthy food and are rich in functional components. There are several species of walnuts including Japanese native walnuts. It is well known that native walnuts contain more phytochemicals than cultivated ones. Therefore, the composition of the beneficial components found in native walnuts is of scientific interest. This review aimed to summarize the recent findings and potential uses of native walnuts, including their (1) use as food, (2) functional components (general composition, fatty acids, peptides, phytosterols, tocopherols, polyphenols, and other components), and (3) health-related prospects. Through this review, unexpectedly, we found that even though many are interested in the health benefits of Japanese native walnuts, which have functional components much differ to cultivated species, there is limited information on their contents. This review suggests a need to clarify the detailed amounts of these components.

Keywords: bioactive components, English walnut, health benefits, Hime walnut, Oni walnut, phytochemicals, polyphenols

Introduction

There are various theories about the origin of walnuts, but one of the most popular theories is that they originated in Iran and spread from there to surrounding countries, commonly known by the name "Persian walnut" (Aradhya *et al*., 2017). A study about fossil pollen of walnuts suggests that, after surviving the refugia, walnuts spread to Central Asia, Caucasus, Western Asia, and Eastern Europe, crossbred with other native species in various regions, and were incorporated into their local cuisine (Aradhya *et al*., 2017). It is also estimated that the Oni walnut (*Juglans ailanthifolia* Carr.; native species in Japan), and the English walnut (*Juglans regia* L.; also known as the cultivars Persian walnut and Shinano walnut worldwide) existed on the earth since 13000 B.C. (Taniguchi, 2011; Pollegioni *et al*., 2017).

Several studies have shown that consuming walnuts on a regular basis could modulate a variety of diseases, including chronic inflammatory diseases, cardiovascular diseases, diabetes, cancer, obesity, dementia, and mental disorders (Hardman, 2014; Lokyer *et al*., 2022). Walnuts are regarded as an ideal source of lipids for human health because they contain a 4:1 ratio of ω-6 and ω-3 fatty acids (Chen *et al*., 2014). Furthermore, the total antioxidant content of English walnut was estimated at 21.9 mmol/100 g, higher than other nuts like pistachio (1.7 mmol/100 g), peanut (2.0 mmol/100 g) and grains like buckwheat (1.4 mmol/100 g) (Carlsen *et al*., 2010). Thus, the biological activities of individual phytochemicals (phytosterols, polyphenols, vitamins, and carotenoids, etc.) in walnuts have also gained attention (Ozawa *et al*., 2021; Miyazawa, 2021; Miyazawa *et al*., 2022). However, there is limited information on the components of native walnuts, especially those of Japanese origin. This could be due to its limited cultivation and information.

With this context, this review compares and summarizes

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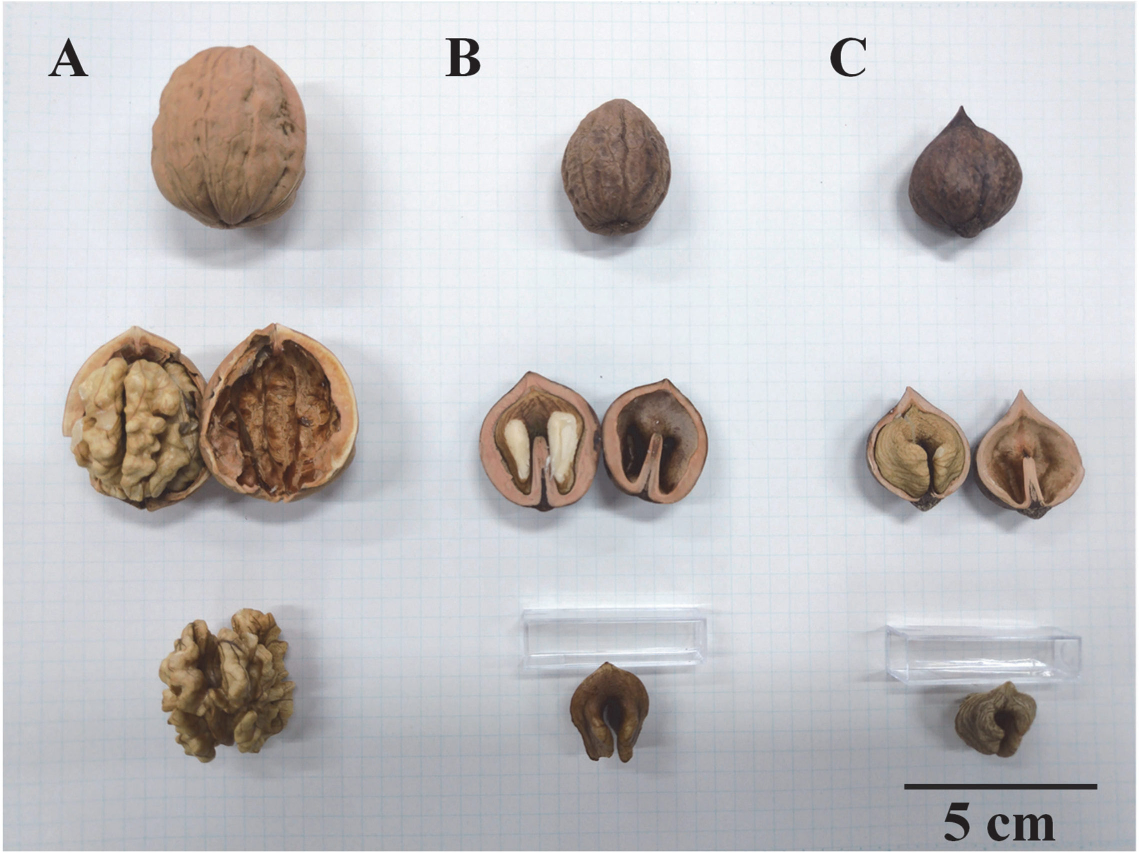

Fig. 1. Photographs of the appearance, shell, and kernel of the walnut species mainly discussed in this review.
A: English walnut (*Juglans regia* L.; cultivar), B: Oni walnut (*Juglans ailanthifolia* Carr.; native species in Japan), C: Hime walnut (*Juglans subcordiformis* Dode.; native species in Japan).

the functional components of the Japanese native walnuts (especially focused on Oni walnut and Hime walnut (*Juglans subcordiformis* Dode.) to the globally cultivated walnut (English walnut) (Fig. 1). This review findings assist in evaluating the value of using native walnuts as functional food to promote health benefits.

Use of walnuts in foods

In 2017, the global production of walnuts was 755 000 tons[i]. Japan produced 151.2 tons, particularly in cold regions (111.3 tons in Nagano, 38.4 tons in Aomori, 1.0 tons in Niigata, and 0.5 tons in Hokkaido)[ii]. The global domestic consumption of walnuts in 2022 was 1 195 000 tons in China, 454 000 tons in the EU, 175 000 tons in the United States, 132 000 tons in Turkey, 75 000 tons in the United Arab Emirates, 66 000 tons in India, 55 000 tons in Japan, 45 000 tons in Kazakhstan, 40 000 tons in South Korea, 30 000 tons in Kyrgyzstan, and 297 500 tons in other countries[iii]. Walnut consumption in Japan increased each year from 2 311 tons in 1985 to 18 796 tons in 2018 to 17 412 tons in 2019 to 18 826 tons in 2020 and to 22 527 tons in 2021[iv]. The following section discusses some of the most important culinary applications of walnuts in various countries.

In the United States and Europe, walnuts are used in cookies (Wakefield, 2011). In Iran, Turkey, and Greece, sweet desserts called baklava (Al-Ismail *et al*., 2020), nazook (Roufs and Roufs, 2014), and baslogh (Vahdati *et al*., 2014) are popular. In Slovenia, potica is eaten (Krizaj *et al*., 2019). In Italy, pasta and meat dishes are seasoned with salsa di noci (walnut paste) (Tomer *et al*., 2020). In India, murabba (Bashir *et al*., 2018), shrikhand (Devshete *et al*., 2020), and lassi (Saha *et al*., 2020) are commonly eaten. China has a wide variety of nut-based foods, including sesame walnut hot cereal, mooncake, tao su, walnut dessert soup, and red dates with walnuts (Shi *et al*., 2014).

In Japanese local cuisine, Oni walnut and Hime walnut are used as cooking ingredients (Takahashi and Uozumi, 2016; Uozumi, 2017). In Tohoku region and Nagano prefecture of Japan, soba noodles served are served with Oni walnut sauce as a dipping sauce, shiso rolls prepared with walnuts, walnut yubeshi, walnut ganzuki, and walnut manju are common. Walnut rice is eaten in Hokkaido and Aomori prefectures, zoni and mamebu soup in Iwate prefecture, walnut agar in Akita prefecture, and seasoning and walnut tofu in Yamagata prefecture[v]. The walnut sauce is used as a bean paste for rice cakes and dumplings in Miyagi and Fukushima prefectures.

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Gohei mochi are eaten in Nagano prefecture, and kakinomoto in Niigata prefecture[vi]. Thus, in Japan and in many parts of the world, walnut-based dishes and sweets are eaten as part of the local culture.

Nutritional and functional components in walnuts

General composition of nutrition facts The general composition of nutrition facts in Oni walnut and Hime walnut (native walnuts in Japan), and English walnut (global cultivar walnut) (Chiba, 2016; Liu *et al.*, 2020; Savage, 2001)[vii][viii] are shown in Fig. 2 and Table 1. Walnuts have a higher fat content than other nuts. For example, almonds, peanuts, cashews, and pistachios have 49.9, 49.2, 46.4, and 47 wt %, respectively (Ros *et al.*, 2021). Briefly, walnuts contain high amounts of fat (English walnut: 70.6 wt %, Oni walnut: 61.5 wt %, Hime walnut: 61.6 wt %) (Fig. 2). Total protein content is higher in Oni walnut (23.75 wt %) and Hime walnut (23.7 wt %), compared to English walnut (12.35 wt %) (Fig. 2). When compared with commercially available foods, the total protein content of Oni walnut and Hime walnut is comparable to that of canned salmon products (23.6 wt %)[ix] and roasted chicken thigh products (24.8 wt %)[x].

Fatty acids The fatty acid content of each walnut species is detailed in Table 2 (Chiba, 2016; Liu *et al.*, 2020;

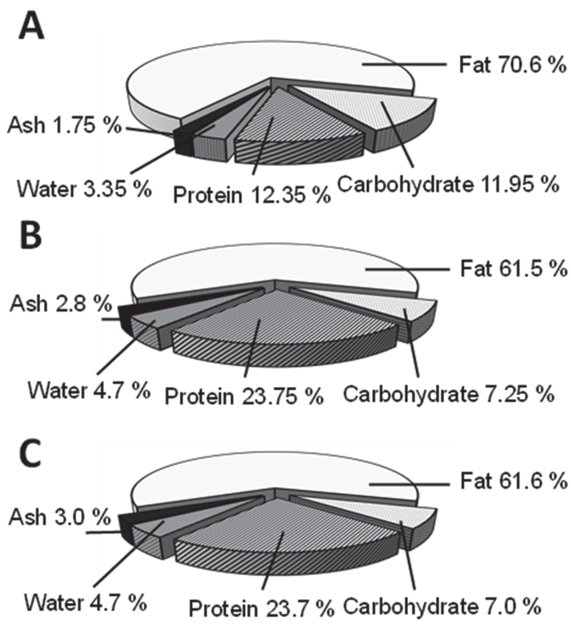

Fig. 2. General composition value (wt %) of walnuts cultivated in Japan.
A: English walnut (*Juglans regia* L.; cultivar), B: Oni walnut (*Juglans ailanthifolia* Carr.; native species in Japan), C: Hime walnut (*Juglans subcordiformis* Dode.; native species in Japan) (Chiba, 2016).

Table 1. General composition of nutrition facts in walnuts (g/100 g).

Species	Water	Protein	Lipid	Carbohydrate	Dietary fiber	Ash	Reference
Oni walnut (Aomori, Japan)	5.1	24.0	59.9	8.0	not measured	3.0	(Chiba, 2016)
Oni walnut (Iwate, Japan)	4.6±0.47	24.2±1.78	61.6±1.73	6.9±0.26	not measured	2.8±0.06	(Chiba, 2016)
Oni walnut (Nagano, Japan)	4.9	21.7	62.8	7.8	not measured	2.8	(Chiba, 2016)
Oni walnut (China)	5.6	24.2	60.3	7.0	not measured	2.9	(Chiba, 2016)
Hime walnut (Nagano, Japan)	4.7	23.7	61.6	7.0	not measured	3.0	(Chiba, 2016)
English walnut (Nagano, Japan)	3.4±0.64	12.4±0.64	70.6±0.57	11.95±0.78	not measured	1.75±0.07	(Chiba, 2016)
English walnut (USA)	4.07	15.2	65.2	13.7	6.7	1.78	(vii)
English walnut (China)	4.07±0.23	19.19±0.89	65.87±2.36	5.91±0.85	not measured	4.96±0.28	(Liu *et al.*, 2020)
English walnut (New Zealand)	6.01±0.56	16.01±1.28	68.0±2.57	1.63±0.26	4.5±0.31	2.04±0.18	(Savage, 2001)
Black walnut (USA)	4.56	24.1	59.3	9.58	6.8	2.47	(viii)

Data were described by mean or mean ± standard deviation (S.D.).
For (Chiba, 2016) and (Savage, 2001), mean and standard deviation were calculated from the data in the references.

Table 2. Total lipid content and fatty acid composition of walnuts (total lipid: wt %; other fatty acids: % of total fatty acids).

Species	Total lipid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	α-Linolenic acid	Others	SFA: MUFA: PUFA ratio	Reference
Oni walnut (Iwate, Japan)	61.6±1.73	2.6±0	0.7±0.06	13.2±1.70	72.0±0.45	9.48±1.82	1.23±0.15	3.5:13.5:81.7	(Chiba, 2016)
Hime walnut (Nagano, Japan)	61.6	2.7	0.8	14.6	73.4	7.3	1.3	3.6:14.8:81.6	(Chiba, 2016)
English walnut (Nagano, Japan)	70.6±0.57	6.7±0	2.7±0	17.45±4.6	62.4±4.24	10.05±0.35	0.75±0.07	9.5:17.6:73.0	(Chiba, 2016)
English walnut (USA)	65.2	6.8	2.6	13.5	58.4	14.0	4.8	9.8:14.2:76.0	(vii)
English walnut (Italy)	64.3±2.07	8.8±0.75	2.7±0.52	14.0±0.63	60.0±1.10	14.0±0.63	0.26±0.05	11.6:14.1:74.4	(Romano *et al.*, 2022)
English walnut (China)	65.87±2.36	5.92±0.38	3.10±0.43	11.59±1.87	69.47±3.65	9.92±1.41	0.0	9.02:11.6:79.4	(Liu *et al.*, 2020)
Black walnut (USA)	59.3	3.3	2.6	25.5	57.0	4.5	7.1	6.4:27.4:66.2	(viii)

Data were described by mean or mean ± standard deviation (S.D.).
SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.
For references (Chiba, 2016) and (Romano *et al.*, 2022), mean and standard deviation were calculated from the data in the references.

Romano *et al.*, 2022)[vii)viii)]. Yan *et al.* reported that kernels of English walnut contained 525 different lipid molecules, of which 207 were glycerolipids, 43 diacylglycerols, 221 glycerophospholipids, 36 sphingolipids, and 18 sphingomyelins, with different composition ratios of individual fatty acids such as oleic acid, linoleic acid, and α-linolenic acid among their subspecies (Yan *et al.*, 2021). Chiba reported that the fatty acid composition of Japanese native Oni walnut and Hime walnut tends to have a relatively high ratio of ω-3 fatty acids, compared to other walnut species (Table 2 in SFA: MUFA: PUFA ratio) (Chiba, 2016).

Candela *et al.* explained that it is important to reduce the daily intake of ω-6 fatty acids as much as possible and to increase the daily intake of ω-3 fatty acids to achieve a healthy diet with 4:1 ratio of ω-6 and ω-3 fatty acids (Candela *et al.*, 2011), and others have considered this as well (Chen *et al.*, 2014). Examples of the recommended daily intake, 10 g of Oni walnut (7.14 g of ω-6 fatty acids and 0.94 g of ω-3 fatty acids), 80 g of one piece of salmon (0.01 g of ω-6 fatty acids and 0.74 g of ω-3 fatty acids), and 8 g of vegetable oil (2.73 g of ω-6 fatty acids and 0.54 g of ω-3 fatty acids), resulting in a total intake of approximately 9.88 g of ω-6 fatty acids and 2.22 g of ω-3 fatty acid, thus the ratio of ω-6 fatty acids to ω-3 fatty acids to be consumed is 4:1 (Chiba, 2016; Kagawa, 2022)[xi)]. Walnut would be a good source to supply α-linolenic acid in diets. Also, walnuts have a high fat content and are said to be moderate hardness to chew for the elderly, allowing them to stay in the stomach for an extended period of time (Singh *et al.*, 2016).

Peptides Peptides are structures consisting of 2–50 amino acids linked by peptide bonds. Walnut peptides, peptides derived from walnuts, have the potential for use in functional foods (Ozawa *et al.*, 2022). Antioxidative properties of peptides have been considered as one of the factors contributing to the biological activities of walnuts (Pan *et al.*, 2020). Since there is still a lack of clinical studies evaluating the function of walnut peptides, this chapter reports on preclinical studies carried out using cultured cells and experimental animals. Chen et al. reported that consumption of walnut meal hydrolysate (single dose at least 333 mg/kg) ameliorated learning and memory impairments in D-galactose-injected cognitive dysfunction model mice (Chen *et al.*, 2015). They further confirmed in a study with rat pheochromocytoma (PC12) cells that the antioxidant activity of walnut peptides, especially those with the sequences Trp-Ser-Arg-Glu-Glu-Gln-Glu-Arg-Glu-Glu (WSREEQEREE) and Ala-Asp-Ile-

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Table 3. Phytosterol content of walnuts (mg/100 g).

Species	Campesterol	β-Sitosterol	Stigmasterol	Total sterol	Reference
English walnut (USA)	5.0	87.0	0.0	92.0	(vii)
English walnut (China)	8.95 ± 2.28	89.56 ± 5.12	1.88 ± 0.74	110.73 ± 9.89	(Gao *et al*., 2016)
Black walnut (USA)	5.0	115.0	1.0	121.0	(viii)
Iron walnut (China)	4.75 ± 1.45	63.08 ± 8.99	1.49 ± 0.52	77.30 ± 11.19	(Gao *et al*., 2016)

Data were described by mean or mean ± standard deviation (S.D.).

For reference (Gao *et al*., 2016), mean and standard deviation were calculated from the data in the reference.

Tyr-Thr-Glu-Glu-Ala-Gly-Arg (ADIYTEEAGR) had an effect on improving cognitive function. Jahanbani *et al*. prepared walnut peptides using hydrolysis of proteins from English walnut with three enzymes (chymotrypsin, trypsin, and proteinase K) and evaluated their antioxidant capacity and anticancer activity (Jahanbani *et al*., 2016). The results showed that the radical scavenging capacity (evaluated by 2,2'-anizo-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method) and ROS scavenging capacity (evaluated by chemiluminescence method) were highest in walnut peptides prepared with chymotrypsin, and also showed growth-inhibitory effects on human breast carcinoma (MDA-MB231) cells and human colorectal adenocarcinoma (HT-29) cells. Wang *et al*. reported on peptides derived from Manchurian Oni walnut cultivated in China, in which they observed that the antioxidative properties of the walnut peptides Leu-Val-Arg-Leu (LVRL) and Leu-Arg-Tyr-Leu (LRYL) from Oni walnut recovered cellular membrane translocation defects of glucose transporter 4 (GLUT4) in human hepatocellular carcinoma (HepG2) cells caused by high glucose (Wang *et al*., 2020).

As mentioned previously, walnut peptides are expected to have various biological activities as functional foods, but there have been few reports examining walnut peptides derived from native walnuts. Native walnuts with their high protein content may have potential peptide sources. However, aspects of walnut peptides such as recommended intake, side effects, allergies, and other biological effects remain unclear and need further study.

Phytochemicals As mentioned in previous sections, walnuts are a rich source of phytochemicals. In this chapter, we discuss the phytochemicals of native and cultivated walnuts in the following order: Phytosterols, Tocopherols, Polyphenols, and Other components.

Phytosterols Phytosterols have been reported to play a major role in the cholesterol-lowering effect of walnuts. The phytosterol content of each walnut species is shown in Table 3 (Gao *et al*., 2016)[vii)viii)]. The major phytosterol in walnuts is β-sitosterol, which is particularly abundant in the kernel (xii). The content of β-sitosterol is higher in a walnut native to the United States (Black walnut: 115 mg/100 g), compared to English walnut (87 mg/100 g) (Table 3). The phytosterol content of Japanese native walnuts (Oni walnut and Hime walnut) is also of interest; however, there are no reports on their quantification. It would be important to determine the phytosterol content of Japanese native walnuts to discuss the effect of their consumption in cholesterol levels.

Tocopherols Nuts are a rich source of a fat-soluble compound, α-tocopherol, which is also known as vitamin E (Miyazawa *et al*., 2019). The tocopherol content of each walnut species is shown in Table 4 (Gao *et al*., 2016; Beyhan *et al*., 2016; Kafkas *et al*., 2017;[vii)viii)xii)]. Unsaturated fatty acids are prone to oxidation, whereas the presence of tocopherols is thought to prevent this reaction (Miyazawa, 2021; Miyazawa, *et al*., 2019; Kafkas *et al*., 2017). γ-Tocopherol is the most abundant tocopherol in walnuts. The content of α-tocopherol, γ-tocopherol, and δ-tocopherol in Japanese native Hime walnut was 0.30 mg/100 g, 21.9 mg/100 g, and 1.6 mg/100 g, respectively; these tend to be lower than in other species (Table 4). Gao *et al*. compared the content of tocopherols in Chinese native Iron walnut and cultivated walnut (English walnut) (Gao *et al*., 2016). Their results showed that α-tocopherol of Iron walnut (3.69 ± 0.28 to 9.24 ± 0.34 mg/100 g) was significantly lower than that of English walnut (11.55 ± 0.9 to 14.42 ± 0.77 mg/100 g), while δ-tocopherol of Iron walnut (7.6 ± 0.53 to 8.85 ± 0.42 mg/100 g) was 3–4 times higher than that of English walnut (2.07 ± 0.16 to 2.8 ± 0.22 mg/100 g). Such differences in tocopherol content among different species have been suggested to be related to genetic and climatic factors (such as relative humidity), but the actual factors have not yet been clearly identified (Gao *et al*., 2016). More detailed evaluations of the impact of the location in which walnuts grow on tocopherol

Table 4. Tocopherol content in walnuts (mg/100 g).

Species	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Reference
Hime Walnut (Japan)	0.30	not measured	21.9	1.60	(xii)
English walnut (USA)	0.70	0.15	20.9	1.89	(vii)
English walnut (Anatolia, Turkey)	1.75±0.346	34.2±4.45 (γ+β)		2.88±0.57	(Beyhan *et al.*, 2016)
English walnut (Turkey)	3.18±0.034	23.6±0.407 (γ+β)		2.64±0.061	(Kafkas *et al.*, 2017)
English walnut (China)	12.86±1.45	0.09±0.025	30.91±3.14	2.34±0.421	(Gao *et al.*, 2016)
Iron Walnut (China)	6.54±2.78	0.05±0.006	24.8±2.478	8.38±0.71	(Gao *et al.*, 2016)
Black walnut (USA)	2.08	0.01	28.8	1.51	(viii)

Data were described by mean or mean ± standard deviation (S.D.).
For references (Gao *et al.*, 2016), (Beyhan *et al.*, 2016), and (Kafkas *et al.*, 2017), mean and standard deviation were calculated from the data in the references.

content are further warranted.

Polyphenols Compared to other nuts such as almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pine nuts, and pistachios, walnuts are known to be rich in polyphenols (Arranz *et al.*, 2008). Okatan et al. identified phenolic compounds in 18 varieties of English walnut in Turkey and showed that the composition of phenolic compounds among each subspecies was different (Okatan *et al.*, 2022). This result suggests that the phenolic compounds contained in different types of walnuts differ from each variety. Arranz et al. reported that most of the antioxidative properties of walnuts are contributed by insoluble tannins, a type of polyphenol (Arranz *et al.*, 2008). Among the insoluble tannins, ellagitannin (1 600 mg/100 g) is the most abundant in walnuts and has been shown to have antioxidant, anti-inflammatory, anti-tumor, and prebiotic effects itself and/or its metabolites (such as ellagic acid and urolithin produced via gut microbiota metabolism) (Ros *et al.*, 2022).

Table 5 shows the total polyphenol content of English walnut and Hime walnut (Romano *et al.*, 2022; 2020; Okatan *et al.*, 2022; Samaranayaka *et al.*, 2008; Fuentealba *et al.*, 2017)[xii]. Interestingly, the total polyphenol content of Hime walnut (1 440.0 mg/100 g) is higher than that of other English walnut varieties from different countries including Canada (1 159.0 ± 168.0 mg/100 g), Italy (1 083.83 ± 109.99 mg/100 g), Chile (985.0 ± 237.31 mg/100 g), and Turkey (937.37 ± 132.96 mg/100 g), suggesting that native walnuts are a rich source of polyphenols.

Table 5. Total polyphenol content of walnuts (mg/100 g).

Species	Total polyphenols	Reference
Hime walnut (Japan)	1 440.0	(xii)
English walnut (Canada)	1 159.0±168.0	(Samaranayaka *et al.*, 2008)
English walnut (Italy)	1 083.83±109.99	(Romano *et al.*, 2022)
English Walnut (Chile)	985.0±237.31	(Fuentealba *et al.*, 2017)
English walnut (Turkey)	937.37±132.96	(Okatan *et al.*, 2022)

Data were described by mean or mean ± standard deviation (S.D.).
For references (Romano *et al.*, 2022), (Okatan *et al.*, 2022), and (Fuentealba *et al.*, 2017), mean and standard deviation were calculated from the data in the references

Other components The content of minerals and vitamins in walnuts are shown in Table 6 and Table 7[vii)viii)xii)], respectively. Hime walnut has more iron 5.04 mg/100 g, magnesium 306 mg/100 g, and potassium 726 mg/100 g, compared to English walnut 2.91 mg/100 g, 158 mg/100 g, 441

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Table 6. Mineral content of walnuts (mg/100 g).

Species	Ca	Fe	Mg	P	K	Na	Zn	Cu	Mn	Se*	Reference
Hime walnut (Japan)	66.4	5.04	306	765	726	not measured	3.62	not measured	1.62	not measured	(xii)
English walnut (Japan)	97.3 ± 18.48	2.40 ± 0.21	149 ± 9.65	355 ± 13.71	453 ± 41.76	not measured	2.15 ± 0.37	not measured	3.73 ± 0.94	not measured	(xii)
English walnut (USA)	98	2.91	158	346	441	2	3.09	1.59	3.41	4.9	(vii)
Black walnut (USA)	61	3.12	201	513	523	2	3.37	1.36	3.9	17	(viii)

Data were described by mean or mean ± standard deviation (S.D.).
* Se is displayed in (µg/100 g). For reference (xii), mean and standard deviation were calculated from the data in the reference.

Table 7. Vitamin content of walnuts (mg/100 g).

Species	Vitamin C	Vitamin B1	Vitamin B2	Niacin	β-Carotene*	Reference
Hime walnut (Japan)	not measured	0.44	0.12	3.73	9	(xii)
English walnut (Japan)	not measured	0.37±0.02	0.12±0.01	0.93±0.27	35±10.13	(xii)
English walnut (USA)	1.3	0.341	0.15	1.12	12	(vii)
Black walnut (USA)	1.7	0.057	0.13	0.47	24	(viii)

Data were described by mean or mean ± standard deviation (S.D.).
* β-Carotene is displayed in (µg/100 g). For reference (xii), mean and standard deviation were calculated from the data in the reference.

mg/100 g, respectively (Table 6). Although limited information is available on the vitamin content of Japanese native walnuts, Hime walnut contains approximately more vitamin B1 (thiamine) 0.44 mg/100 g and niacin 3.73 mg/100 g compared to English walnut 0.34 mg/100 g and 1.12 mg/100 g, respectively.

Health benefits of walnuts

In this section, we discuss cohort studies of walnut health functions reported in the last two years. Guasch-Ferre et al. conducted a large prospective cohort study (The Prevención con Dieta Mediterránea: PREDIMED study) involved 1 833 adults (61–73 years old) with high cardiovascular risk living in Spain. In their comparison between the effects of a walnut-rich diet (10 ± 12 g/day of walnuts, 639 participants) and a diet without walnuts (control diet, 571 participants) using Elastic Net logistic regression analysis (Guasch-Ferre *et al.*, 2021), there was a negative association between the risk of type 2 diabetes and atherosclerosis in the group consuming a diet rich in walnuts. Steffen et al. conducted a 30-year follow-up study of 3 341 young adults (18–30 years old) from the United States who were enrolled in the Coronary Artery Risk Observational Trial (CARDIA study) to investigate the relationship between walnut consumption and cardiac function (Steffen *et al.*, 2021). In an analysis of the first 20 years of the study, the walnut consumption group (average of about 20 g per day, 340 participants) was positively correlated with improvement in cardiac diastolic dysfunction when compared with the nonconsumption group (3 001 participants). Furthermore, by the 25th year of the study, the walnut consumption group had a smaller waist circumference, less abdominal obesity, better cardiac diastolic function scores, and lower pulse pressure and heart rate than the group that did not consume walnuts. Rajaram *et al.* conducted the Walnuts and Healthy Aging (WAHA) study, a two-year randomized controlled trial with 708 healthy older adults (63–79 years old) who added either 0

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g or 30–60 g (about 15 % of their recommended caloric intake) of nuts to their daily diets (Rajaram *et al*., 2021). By the end of the trial, the walnut consumption group had lower levels of serum total cholesterol and LDL cholesterol compared to the control group that did not consume walnuts. Taken together, eating walnuts on a daily basis could lower the risk of obesity and cardiovascular diseases. It is hypothesized that the benefits observed in cohort studies might be related to the α-linolenic acid, polyphenols, and phytosterols found in walnuts, which act as anti-inflammatory and antioxidative agents in the body (Ni *et al*., 2021).

Historically, cultivar walnut varieties have been bred with a greater emphasis towards increasing yield. Native walnuts, on the other hand, are richer in phytochemicals than cultivated walnuts because they have adapted to environmental changes in their growing regions (Berni *et al*., 2018). Polyphenols, a secondary metabolite (a class of phytochemicals) released by plants as a self-defense mechanism, are known to protect the plant body from ultraviolet light, pathogens, and insect damage (Olson *et al*., 2002). It has also been reported that the growth rate of walnut aphid (*Panaphis juglandis*) is different among walnut species (Akkopru *et al*., 2015). In recent years, phytochemical-rich native walnuts have shown their potential health benefits. For example, United States native walnut (Black walnut; *Juglans nigra* L.) has gained much attention (Vu *et al*., 2020). This result was explained by a hypothesis that native walnut, which can be grown in harsher environments, may have more phytochemicals compared to cultivar walnut.

Future prospects in the use of native walnuts for human health

For the last three years since the global pandemic, the coronavirus disease (COVID-19) has become a serious social problem. Galanakis stated that one of the key challenges for the food industry to address in the food system in the post-COVID-19 era is "As consumers are looking to protect themselves and their immune system by adopting healthier diets, the availability of bioactive ingredients of food and functional foods may become critical, as the demand for these products may increase" (Galanakis, 2020). To obtain bioactive compounds such as polyphenols through chemical synthesis approach, concern exists regarding complex synthetic schemes, safety, cost, and environmental concerns. Thus, the production and extraction of beneficial bioactive compounds from plants and fungi are regarded as useful. For example, grains have been used to provide ferulic acid, phytosterols, and vitamin E, while *Bacillus subtilis* has been used to provide small molecules such as bacteriocins and vitamin K (Galanakis, 2022; Miyazawa *et al*., 2023). As detailed in this review, native walnut has higher protein and polyphenol content than cultivated walnut. In the future, native walnuts might become an appealing source of phytochemicals; however, a more detailed quantitative analysis of protein and polyphenol content is required. Furthermore, regular consumption of native walnuts may have the potential to maintain health features. However, while cohort studies show increasing evidence of an association between walnuts and health function, the direct mechanism by which walnut consumption affects health function remains unclear. Further research is warranted to understand the relationship between the consumption of walnuts and various diseases and intestinal bacteria which provides the direct mechanisms related to its function.

Plant-based meat, a term for alternative meats made from plants, is attracting attention as a solution to (1) environmental issues, (2) public health concerns, and (3) health hazards caused by excessive consumption of livestock animal products (Bryant, 2022). Ercoskun et al. confirmed that the use of English walnut paste instead of lard in the preparation of processed meats had no effect on appearance or taste, except for color (Ercoskun and Demirci-Ercoskun, 2010). Canales et al. prepared processed meats containing English walnut paste and conducted a five-week trial with 25 obese adults, who consumed [four steaks (150 g) and one sausage (150 g) with or without 20 wt % English walnut paste, consumed weekly] (Canales *et al*., 2009). Results showed that the group consuming processed meat containing walnut paste had a lower risk of thrombosis than the control group (the group that consumed the meat without walnut paste). In these studies, English walnut was used in pastes for processed meats, but their efficacy might be improved with native walnuts, which have higher protein and polyphenol content.

Addressing food allergies and gluten sensitivity is essential to food safety. Burbano et al. made gluten-free cakes using a blend of English walnut nonfat flour (fat: 55.7 wt %; protein: 24.6 wt %; fiber: 9.4 wt %; ash: 2.7 wt %), cassava flour, cornstarch, and rice flour (Burbano *et al*., 2022). Since Japanese native walnuts are rich in protein [Oni walnut: 23.75 wt %; Hime walnut: 23.7 wt % (Fig. 2)], they have great potential since they can simply be added directly to mixed powder. In this case, the native walnut is expected to have a worse appearance than the English walnut because of its darker skin color, but this could be improved by adding coffee or maple sugar for a better look.

Conclusion

Throughout this review, it was suggested that the content of functional components in native walnuts and cultivated species differed significantly. The general composition of the Oni walnut and Hime walnut is slightly similar to that of the Black walnut native to the United States. These Japanese native walnuts have attracted attention in recent years due to their health benefits. As far as breeding is concerned, they are expected to be more resistant to environmental stresses than the English walnut. Through this review, we found that even though many people are interested in the health benefits of Oni walnut and Hime walnut, which have nutritional values comparable to those of black walnuts, there is limited information on their contents, such as polyphenols, vitamins, and carotenoids. In the future, it will be essential to clarify the amounts of these components in various native walnuts worldwide.

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