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Study of the Main Constituents of Some Authentic Walnut Oils

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This paper describes the composition of walnut oils obtained from nuts collected from seven countries that are major suppliers of walnut oil. Oils were extracted from the nuts using small-scale industry pressing equipment and analyzed using standard methods for fatty acids, fatty acids in the triacylglycerol 2-position, tocopherols and tocotrienols, triacylglycerols, sterols, steradienes, and iodine value. Values for the composition of the sterols, triacylglycerols, fatty acids, iodine value, and tocopherol composition were generally in good agreement with the results of previous similar surveys. Tocotrienols were not detected in any sample. Steradienes (stigmastadiene, campestadiene, stigmastatriene, and campestatriene) were not detected in any oil.

KEYWORDS: Walnut oil; *Juglans regia*; fatty acids; triacylglycerol 2-position; tocopherols; tocotrienols; triacylglycerols; sterols; steradienes; iodine value

INTRODUCTION

World production of walnuts was almost 1 500 000 metric tons in 2003 (*1*). China and the United States were the major producers, with about 25 and 20%, respectively, of the total, and production in these countries has increased rapidly in recent years.

It is well established that consumption of walnuts has benefits to health via the reduction of total plasma cholesterol and low-density-lipoprotein cholesterol (*2*, *3*). Addition of walnuts to the diet has been shown to improve serum lipid profiles (*4*−*6*) and reduce coronary heart disease (*7*−*9*). These properties may be attributed to the fatty acid profile of the oil component, in particular, omega-3 and omega-6 polyunsaturated fatty acids, of which walnut oil is a particularly good source. The contribution of dietary plant sterols and tocopherols to human health has similarly been recognized (*10*, *11*). These components are present in substantial quantity in walnut oil and are likely to remain in the retail product as destructive refining methods are not necessary.

Studies into the composition of edible vegetable oils are required to ensure authenticity and to obtain information on the quality of the oil in terms of storage stability, flavor, taste, and nutrition. Such information can help in assessing dietary intake of beneficial components of the oil.

This paper describes the results for a study of walnut oils obtained from nuts collected in the major producing countries and analyzed by well-established methods using stringent quality control procedures. The Codex Alimentarius Committee on Fats and Oils has published standards for a number of named vegetable oils, which are based on tables of composition (*12*); however, walnut oil is not described in the current Codex standard.

For the survey reported here 30 walnut samples were collected from seven countries chosen to reflect the current market share for walnut oil production. A full audit trail was established to ensure the authenticity of the samples. Oil was extracted under conditions very similar to those used in industry and analyzed using official methods with full quality assurance procedures based on Shewart control charts (*13*).

MATERIALS AND METHODS

Walnuts. Walnuts were collected by the authors from France (eight samples), Hungary (four samples), India (three samples), Italy (four samples), Spain (four samples), and the United States (five samples). Two of the samples from France were collected from the year 2000 harvest and the remainder from 2001. The two samples from China were not collected by the authors but were imported directly to the United Kingdom. The samples are described in **Table 1**.

The nuts were packed, sealed, numbered, and stored at 4 °C in the dark prior to shelling and pressing. The walnuts were shelled by hand and the kernels stored in sealed bags in the dark at 4 °C until transported to the pressing plant.

Walnut oil is obtained principally from cultivars of *Juglans regia* L., the English or Persian walnut; however, the black walnut, *Juglans nigra*, is another possible direct source or rootstock. The walnut tree is native to Europe, Asia Minor, India, and China, although cultivars from the United States are grown worldwide. *J. nigra* is native to the United States but has only limited commercial exploitation and is normally used for direct consumption and confectionery purposes rather than for oil production. All of the samples were identified at source as

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Table 1. Sample Collection Information

country	region	date collected	no.
China	Boading	2000	1[a]
China	Tjanjin	2000	1[b]
France	Montmiral	December 2000	1[c]
France	Dordogne	September/October 2000	1[c]
France	Pillac, Charente	September/October 2001	1[c]
France	Sarthe	October 2001	1[c]
France	Nontrial	October 2001	1[c]
France	Dordogne	October 2001	3[c]
Hungary	Budapest	September/October 2001	4[c]
India	Bombay	2001	3[c]
Italy	Cancello Scala	October 2001	2[c]
Italy	Petralia, Sicily	September/October 2001	1[c]
Italy	Torino	September/October 2001	1[c]
Spain	Catalogne	September 2001	3[c]
Spain	Tarragnia	September/October 2001	1[c]
USA	Stockton, CA	October 2000	1[c]
USA	Stockton, CA	October 2001	4[a]

[a] Kernels. [b] Broken kernel pieces. [c] The remaining samples were whole nut in the shell.

English walnuts. It was not possible to confirm the identity of any nut sample in terms of variety, and it was evident from the size and shape of the nut and the thickness of the shell that various varieties were present. However, each batch of samples appeared to be homogeneous in terms of shell size, shape, and color, and all nuts were in good condition on delivery. Three samples were obtained as kernels; of these, two (one from China and one from the United States) were mostly intact, and the third sample (from China) comprised pieces of kernel.

Walnut Oil. The oil was extracted from the walnut kernels using a cold-press expeller (Statfold Seed Oils, Tamworth, U.K.). The extracted oil was filtered at low pressure but not processed further. Normal cleaning procedures (steam cleaning) were carried out between samples to ensure that there was no adventitious mixing of different samples. The oils were stored in polypropylene bottles under nitrogen at 4 °C in the dark prior to analysis.

Standards. Standards of the sterols stigmasterol (95%) and sitosterol (containing campesterol) and of fatty acid methyl esters were obtained from Sigma, Poole, U.K. The steradienes stigmasta-3,5-diene (69%) with campesta-3,5-diene (13%) and stigmasta-3,5,22-triene (97%) were obtained from Chiron AS, Trondheim, Norway. The internal standard cholesta-3,5-diene (95%) was obtained from Sigma. Tocopherols and tocotrienols (>95%) were obtained from Merk Biosciences, Schwalbach, Germany. The CBR reference sample RM162 (soy and maize oil) was purchased from the Community Bureau of Reference, Brussels, Belgium.

Determination of Sterols. The composition of the sterols (desmethyl sterols) was determined according to the International Standards Organization method (*14*). Gas chromatography (GC) was carried out with a Varian model CP3380 gas chromatograph (Varian Inc., Walton, U.K.) fitted with a JW DB-5 fused silica column (60 m × 0.25 mm i.d. × 0.1 μm film thickness; Fisher Scientific, Loughborough, U.K.). Helium carrier gas was used at a pressure of 30 psi, 1 mL/min. The injector and detector temperatures were 320 °C, and the oven was programmed from 240 to 255 °C at 4 °C/min. The injection volume was 1 μL, with a split ratio of 20:1.

For quality assurance purposes two reference materials were analyzed. These were a mixture of commercial standards comprising sitosterol, campesterol, and stigmasterol and a characterized in-house mixture of equal volumes of sunflower oil and rapeseed oil. Batches for GC comprised a reagent blank, the standard mixture, the reference oil, five samples, and one sample injected in duplicate. Acceptance criteria were that results for the samples injected in duplicate, the standards, and the reference oils had to agree to within ±10% of the mean values.

Determination of Steradienes. The steradienes campesta-3,5-diene, campesta-3,5,22-triene, stigmasta-3,5-diene, and stigmasta-3,5,22-triene and their major isomers were determined by a method based on that according to the European Commission for stigmastadienes in olive oil (*15*). The sensitivity and scope of the official method were increased, as described previously (*16*), by the incorporation of mass spectrometric detection, the use of internal standardization, and the use of calibration graphs.

GC-MS was performed using a Voyager instrument (Thermo-Finnigan, Hemel Hempstead, U.K.) fitted with a JW DB-WAXETR column (30 m × 0.25 mm, Fisher Scientific) with a 0.25 μm phase. The GC used helium carrier gas at a pressure of 15 psi. The injector and transfer line temperatures were 260 °C, and the column oven temperature was held isothermally at 250 °C. Splitless injections of 1 μL were made. The mass spectrometer was operated in selected ion monitoring mode, recording the response for the molecular ions of the steradienes and, for confirmation purposes, the sterol ring fragment at *m*/*z* 255.

For quality assurance purposes an in-house mixture of virgin olive oil with refined olive oil blended to contain ~0.15 mg/kg stigmastadienes was analyzed as a reference material. Extraction batches comprised a reagent blank, the in-house reference mixture, and 6−10 sample oils. Analytical (GC-MS) batches comprised a reagent blank and standards plus one to two of the extraction batches above. Acceptance criteria were that the levels of individual steradienes in the blank did not exceed 0.05 mg/kg and that the stigmastadienes content of the reference oil fell within 0.1−0.2 mg/kg.

Determination of Fatty Acids (FA). The FA composition of the oils was determined according to the International Union of Pure and Applied Chemistry method (*17*−*18*). GC was carried out with a Varian model CP3380 gas chromatograph (Varian Inc.) and a fused silica column (50 m × 0.35 mm i.d.), coated with CP-Sil 88 (0.2 μm film thickness, Varian Inc.). Helium carrier gas was used at a pressure of 10 psi, giving a flow rate of 1 mL/min. The injector and detector temperatures were 250 °C, and the oven was programmed from 160 to 220 °C at 1 °C/min. The injection volume was 1 μL, with a split ratio of 20:1.

For quality assurance purposes a Community Bureau of Reference (CBR) sample RM162 (soy and maize oil) was used. Each analytical batch included a reagent blank, a range of standards, the CBR reference oil, 10−14 samples, 3 samples prepared in duplicate, and 3 samples injected in duplicate. A midrange standard was re-injected at the end of the batch as a back-calibration to check instrument stability. Acceptance criteria for the batch were that the FA concentrations of the CBR and back-calibration standards had to fall between 95 and 110% of the nominal values. Results for the duplicate preparations had to fall within 2% of the mean values for palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) and within 10% of the mean values for myristic acid (C14:0), palmitoleic acid (C16:1), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), and lignoceric acid (C24:0). Results for the samples injected in duplicate had to fall within 2% of the mean values for C16:0, C18:0, C18:1, and C18:2, within 5% of the mean values for C16:1 and C18:3, and within 10% of the mean values for C14:0, C20:0, C20:1, C22:0, and C24:0.

Determination of FA in the Triacylglycerol 2-Position. The composition of FA in the triacylglycerol (TAG) 2-position was determined according to IUPAC method 2.210 (*19*). GC was carried out with a Hewlett-Packard model 5890 gas chromatograph (Agilent Technologie, Waldbronn, Germany) and a fused silica column 60 m × 0.25 mm coated with BPX70 (0.25 μm film thickness, SGE, Darmstadt, Germany). Hydrogen carrier gas was used at a pressure of 21 psi, giving a flow rate of 1.2 mL/min. The injector and detector temperatures were 270 °C, and the oven was programmed from 80 to 120 °C at 20 °C/min, then to 200 °C at 6 °C/min, and then at 4 °C/min to 225 °C and held for 8 min. The injection volume was 1 μL. For quality assurance purposes an in-house reference material (cocoa butter) was analyzed. Each batch included a reagent blank, the cocoa butter reference sample, and up to 20 samples. Two samples in each batch were analyzed in duplicate. Acceptance criteria were that the results for the cocoa butter reference material had to fall within 100 ± 10% of the nominal value and the results for the duplicate preparations had to fall within 2% of the mean values for C16:0, C18:0, C18:1, and C18:2.

Table 2. Sterol Composition of Walnut Oil (Milligrams per 100 g)

sample	China	France	Hungary	India	Italy	Spain	USA
no.	2	8	4	3	4	4	5
cholesterol	0.3–0.41	nd[a]–0.7	nd–0.4	0.8–1.8	0.2–1.4	nd–1.1	0.4–1.6
cholestanol	0.1–0.3	nd–0.3	nd	nd	nd	nd	nd–0.3
brassicasterol	nd	nd–0.2	nd	nd	nd	nd	nd
24-methylenecholesterol	0.4–0.5	nd–0.3	nd	nd	nd	nd	nd–0.4
campesterol	6.7–7.5	6.8–12.1	5.5–6.0	5.5–11.1	4.5–6.9	4.6–7.6	4.4–6.4
campestanol	nd	nd–0.1	nd	nd	nd	nd	nd
stigmasterol	0.7–0.8	nd–0.8	nd–0.3	nd–1.6	nd–0.3	nd–0.5	nd–0.5
Δ7-campesterol	nd	nd	nd	nd	nd	nd	nd
Δ5,23-stigmastadienol	nd	nd–0.7	nd	nd	nd	nd	nd
clerosterol	1.3	1.1–2.9	1.8–2.7	3.4–5.0	1.4–3.5	1.4–4.4	1.3–2.0
β-sitosterol	139.2–141.0	121.3–252.0	105.5–114.3	119.0–151.1	87.7–111.0	88.3–141.0	77.2–102.3
sitostanol	nd	nd	nd	nd	nd	nd	nd
Δ5-avenasterol	13.5–15.3	6.4–14.1	8.2–11.2	11.7–14.8	5.7–7.7	7.5–9.6	5.8–10.7
Δ5,24-stigmastadienol	0.9–1.0	nd–0.6	nd	nd	nd–1.9	nd–1.3	nd–1.3
Δ7-stigmastenol	nd–0.4	nd–0.4	nd–0.8	nd	nd–0.8	nd	nd–0.4
Δ7-avenasterol	nd–0.1	nd–0.3	nd	nd	nd	nd	nd–0.2
total	165.2–166.6	139.9–283.3	122.1–130.3	141.1–184.4	102.0–129.6	103.7–161.4	90.2–123.2

[a] nd, <0.1.

Determination of Tocopherols and Tocotrienols. The tocopherol and tocotrienol composition was determined according to IUPAC method 2.432 (*20*). The HPLC system comprised a Merck-Hitachi model L-6000A pump (Merck, Darmstadt, Germany) and a 250 mm × 4 mm LiChrospher 100 Diol column (particle size 5 μm, Merck, Darmstadt, Germany). The eluent was 4% *tert*-butylmethyl ether in hexane at a flow rate of 2.3 mL/min. The detector was a Hitachi model L-4000 UV operated at 295 nm (Merck). Injections (50 μL) were made using a Bischoff model 728 autosampler (Bischoff Chromatography, Leonberg, Germany). Tocopherol and tocotrienol reference standards were obtained from Calbiochem-Merck Biosciences, Schwalbach, Germany. The reference material was a vapor condensate of mixed vegetable oils enriched in tocopherols and tocotrienols. Each batch included a reagent blank, a reference material, a standard mixture, and up to 20 samples. Two samples in each batch were analyzed in duplicate. Acceptance criteria were that results for the reference sample had to fall within 10% of the characterized value and that results for the duplicate analyses had to agree within 5% of the mean value.

Determination of the Triacylglycerol Carbon Number (TCN). The TCN was determined according to the AOCS method (*21*). GC was performed using a Carlo Erba HRGC 5300 Mega series (CE Instruments, Wigan, U.K.). The GC was fitted with a JW DB-5HT column (15 m × 0.32 mm with a 0.1 μm film thickness, Fisher Scientific) with a helium carrier gas pressure of 12 psi. Cooled on-column injections (1 μL) were made with the oven programmed from 100 °C (held for 1 min) to 310 °C at 49 °C/min and then at 4 °C/min to 390 °C.

For quality assurance purposes a characterized in-house reference mixture of sunflower and rapeseed oils was used. Each batch comprised a reagent blank, a range of standards, the reference oil, 15 samples, and 1 sample analyzed in duplicate. Acceptance criteria were that results for the in-house reference oil and for samples injected in duplicate had to agree to within 10% of the mean values.

Determination of the Iodine Value. The iodine value was calculated from the FA composition according to the American Oil Chemists' Society method (*22*). The data were obtained from the analysis of all samples including the averaged results for three samples prepared in duplicate and the three injected in duplicate. Acceptance criteria were that the iodine values of the reference standard had to fall within 100 ± 10% of those calculated from the FA CBR composition data of the CBR reference material.

Statistical Analysis. The data were examined using the CSL-Metabolab visualization toolbox, a graphical user interface for the Matlab program (The MathWorks Ltd., Cambridge, U.K.) allowing multivariate and classical statistical analyses and methods for rapid integration and spectral visualization. The statistical methods employed were principal components analysis (PCA) for initial data exploration, one-way analysis of variance (ANOVA) to detect whether one or more oils were significantly different from the others for a given factor, and the *t* test for pairwise comparison of different oil types.

RESULTS AND DISCUSSION

Oil Extraction. The volumes of oil returned from the press varied considerably between samples, apparently as a factor of the physical nature of the kernel. They were generally smaller than would be expected from a full-scale oil-pressing plant. This led us to believe that the yield of oil was in most cases not quantitative; no data are presented for the oil content of the samples.

Quality Assurance. For the data reported all results for the replicate injection and analysis samples were within the warning limits of the Shewart control charts, and all results for the reference materials were within the specified limits.

Sterol Composition. The sterol composition of walnut oils expressed in milligrams per 100 g of oil is given in **Table 2**.

The total levels ranged from 90 to 283 mg/100 g with an average value of 141 mg/100 g. One sample from French nuts contained substantially more sterols (283 mg/100 g) than all of the remaining samples (maximum = 184 mg/100 g). The results were in reasonable agreement with the data provided in earlier surveys for a range of countries (*23*−*26*) except for a single report of stigmasterol at ∼6 mg/100 g (*26*).

Levels of cholesterol ranged from <0.1 mg/kg (five samples) to 1.8 mg/kg. This was significantly below the range reported in an earlier significant survey (*23*), which had a maximum of 2.9 mg/100 g. Other workers have reported a single sample from Portuguese nuts with 3.8 mg/100 g (*24*).

The sterol composition expressed in percent m/m of total sterols is given in **Table 3**. Values for the percentage composition were generally in good agreement with the published results (*23*, *27*−*29*).

Steredienе Composition. No steredienes (stigmastadiene, campestadiene, stigmastatriene, and campestatriene) were detected in any walnut oil, subject to a limit of detection of 0.02 mg/kg. There were no reports of the analysis of walnut oils for sterol degradation products in the literature. Steredienes are formed by dehydration of sterols, notably during refining, and would not be expected to be found in oils that had not undergone severe treatment in processing.

FA Composition. The fatty acid composition of the walnut oils is given in **Table 4**.

Table 3. Sterol Composition of Walnut Oil (Percent m/m)

sample	China	France	Hungary	India	Italy	Spain	USA
no.	2	8	4	3	4	4	5
cholesterol	0.2–0.3	nd[a]–0.6	nd–0.3	0.6–0.7	0.2–1.2	nd–1.0	0.4–1.5
cholestanol	nd–0.2	nd–0.2	nd	nd	nd	nd	nd–0.2
brassicasterol	nd	nd–0.1	nd	nd	nd	nd	nd
24-methylenecholesterol	0.3	nd–0.2	nd	nd	nd	nd	nd–0.4
campesterol	4.0–4.5	4.3–5.0	4.3–4.6	4.4–6.1	4.4–5.3	4.5–5.2	4.3–5.2
campestanol	nd	nd–0.1	nd	nd	nd	nd	nd
stigmasterol	0.4–0.5	nd–0.4	nd–0.2	nd	nd–0.3	nd–0.3	nd–0.5
Δ7-campesterol	nd	nd	nd	nd	nd	nd	nd
Δ5,23-stigmastadienol	nd	nd–0.2	nd	nd	nd	nd	nd
clerosterol	0.8	0.7–1.5	1.4–2.2	1.9–2.7	1.2–3.1	0.9–3.9	0.9–2.1
β-sitosterol	83.6–85.4	85.2–89.6	85.0–86.4	81.5–84.4	83.9–86.7	83.8–88.7	83.0–85.6
sitostanol	nd	nd	nd	nd	nd	nd	nd
Δ5-avenasterol	8.2–9.2	4.4–7.7	6.7–8.4	7.9–8.7	5.1–7.1	5.2–7.4	6.4–8.7
Δ5,24-stigmastadienol	0.6	nd–0.4	nd	nd	nd–1.7	nd–1.2	nd–1.2
Δ7-stigmastenol	nd–0.2	nd–0.2	nd–0.6	nd	nd–0.7	nd	nd–0.3
Δ7-avenasterol	nd–0.1	nd–0.2	nd	nd	nd	nd	nd–0.2

[a] nd, <0.1.

Table 4. Fatty Acid Composition of Walnut Oil (Percent m/m)

sample	China	France	Hungary	India	Italy	Spain	USA
no.	2	8	4	3	4	4	5
myristic acid, C14:0	nd[a]	nd	nd	nd	nd	nd	nd
pentadecylic acid, C15:0	nd	nd	nd	nd	nd	nd	nd
palmitic acid, C16:0	5.1–5.4	6.5–7.3	5.8–7.7	5.7–5.8	7.3–8.1	7.1–7.5	6.3–8.0
palmitoleic acid, C16:1	0.1	0.1	0.1	nd–0.1	nd–0.1	nd–0.1	0.1
margaric acid, C17:0	0.1	nd–0.1	nd	nd	nd–0.1	nd–0.1	nd–0.1
heptadecenoic acid, C17:1	nd	nd	nd	nd	nd	nd	nd–0.1
stearic acid, C18:0	2.7–2.9	1.7–2.9	2.1–2.2	2.5–2.6	2.2–2.9	1.9–2.8	2.3–3.1
oleic acid, C18:1	16.9–21.0	15.1–18.9	17.4–22.2	19.8–20.6	14.5–15.3	14.3–19.2	13.4–16.7
linoleic acid, C18:2	60.1–64.1	57.4–64.3	58.3–60.8	57.3–58.1	60.2–63.1	57.6–62.5	59.6–63.1
linolenic acid, C18:3	10.3–10.4	11.3–15.4	10.8–11.6	12.6–13.5	11.8–14.3	12.4–13.2	12.5–15.5
arachidic acid, C20:0	0.1	nd–0.1	nd–0.1	0.1	0.1	0.1	0.1
eicosenoic acid, C20:1	0.1	0.2–0.3	0.2	0.2	0.2	0.2	0.2
behenic acid, C22:0	nd	nd	nd	nd–0.1	nd	nd	nd–0.1
docosenoic acid, C22:1	nd	nd	nd	nd	nd	nd	nd
lignoceric acid, C24:0	nd	nd	nd	nd	nd	nd	nd–0.1

[a] nd, <0.1.

Table 5. Ranges of Saturated, Monounsaturated, and Polyunsaturated Fatty Acids in Walnut Oil

sample	China	France	Hungary	India	Italy	Spain	USA
no.	2	8	4	3	4	4	5
saturated	8.2–8.3	8.5–9.7	8.3–9.9	8.3–8.6	10.2–10.5	9.5–10.2	8.9–10.8
monounsaturated	17.1–21.3	15.4–19.1	17.7–22.5	20.0–20.8	14.8–15.5	14.6–19.5	13.7–17.0
polyunsaturated	70.3–74.6	71.2–75.5	69.1–72.4	70.5–71.3	74.3–74.9	70.7–75.2	72.7–76.3
P/S[a] ratio (mean)	8.4–9.1 (8.8)	7.4–8.0 (7.8)	7.3–8.4 (7.8)	8.3–8.5 (8.4)	7.1–7.3 (7.2)	7.3–7.6 (7.5)	6.7–8.6 (7.7)

[a] P/S, polyunsaturated/saturated.

The values obtained were in very close agreement with the ranges reported elsewhere (*23*, *24*, *26*, *28*, *30*−*35*). Higher proportions of C16:0 and much higher proportions of C18:2 have been reported for an unspecified number of Greek walnut oils (*29*), although those data were skewed by the failure to detect C18:1. A higher range of C16:0 (8−10%) and lower C18:0 (0.9−2.0%) were also reported for an unspecified number of Turkish nuts (*36*). Others have reported similar levels of C16:0 and C18:1 but much different ranges for C18:0 (1.9−15.0%), C18:2 (42.1−49.0%), C18:3 (4.7−7.4%), and C20:0 (3.1−16.0%) in oils from four Turkish nuts (*37*). In cold-pressed oil from six Californian cultivars levels of C16:0 (2.3−4.4%) and C18:0 (0.6−0.8%) were lower (*38*) and levels of C18:1 much lower (one sample at 11.8%, five other samples ranged from 5.75 to 7.7%) but levels of C18:3 higher (16.5−25%).

The proportions of saturated fatty acids are described in **Table 5**. These were low (8−11%) with higher proportions of monounsaturated acids (8−11%, average = 9.4%) and polyunsaturated acids (69−76%, average = 73%), in agreement with published data (*32*, *38*).

FA composition has been reported to differ with variety. In a study of German walnuts (*39*), oils from 30 individual kernels of 10 varieties had proportions of C16:0 and C18:0 within the range reported here, but the average proportion of C16:0 was higher at 7.1%. Proportions of C18:1 were also relatively higher, but those of C18:2 were lower than reported here.

A study describing the composition of a single sample of black walnut, *J. nigra*, suggested that this species had very different proportions of some fatty acids (*32*). The oil contained C16:0 at 3.1%, C18:1 at 58.03%, and C18:3 at 4.9%. Other

Table 6. Iodine Values of Walnut Oil

sample	China	France	Hungary	India	Italy	Spain	USA
no.	2	8	4	3	4	4	5
iodine value	155.2–159.3	156.7–161.2	154.0–156.5	151.4–156.8	159.2–161.0	156.8–160.0	159.1–164.4

Table 7. Fatty Acid Composition at the Triacylglycerol 2-Position of Walnut Oil (Percent m/m)

sample	China	France	Hungary	India	Italy	Spain	USA
no.	2	8	4	3	4	4	5
myristic acid, C14:0	nd[a]	nd	nd–0.1	nd	nd	nd	nd
palmitic acid, C16:0	0.4–0.5	0.4–0.8	0.7–1.0	1.3–1.6	0.7–0.9	0.4–0.6	0.5–0.6
palmitoleic acid, C16:1	0.1	nd–0.1	0.1	0.1–0.2	nd–0.1	nd–0.1	nd–0.1
magaric acid, C17:0	nd	nd	nd	nd	nd	nd	nd
stearic acid, C18:0	0.2	0.1–0.3	0.2–0.3	0.6–0.7	0.2–0.3	0.2	0.2–0.3
oleic acid, C18:1	18.9–22.8	15.8–23.8	19.0–30.9	25.6–33.2	16.2–18.8	18.1–20.0	16.7–20.3
linoleic acid, C18:2	67.3–71.5	65.8–73.5	61.9–69.4	54.1–59.3	69.9–71.5	66.6–71.4	68.4–72.5
linolenic acid, C18:3	8.9–9.0	9.4–13.1	5.7–10.4	9.6–11.6	8.6–12.8	9.2–11.9	9.7–10.4
arachidic acid, C20:0	nd	nd	nd	nd	nd	nd	nd
eicosenoic acid, C20:1	nd	nd	nd	0.1	nd	nd	nd
behenic acid, C22:0	nd	nd	nd	0.1	nd	nd	nd
others	nd–0.1	nd–0.1	nd–0.1	0.6–0.8	nd–0.2	nd–0.1	0.1–0.3

[a] nd, <0.1.

Table 8. Triacylglycerol Carbon Number Composition of Walnut Oil (Percent m/m)

sample	China	France	Hungary	India	Italy	Spain	USA
no.	2	8	4	3	4	4	5
TCN 48	nd[a]	nd	nd	nd	nd	nd	nd
TCN 50	0.3–0.4	0.6–1.4	0.7–1.8	0.7–0.8	0.8–1.7	0.6–1.5	0.6–1.2
TCN 52	15.5	18.4–22.7	19.2–22.8	17.3–17.9	20.5–24.1	20.9–24.6	18.2–23.4
TCN 54	83.0–83.3	76.2–80.0	76.4–80.1	80.6–81.1	74.2–78.2	74.4–77.9	75.7–80.3
TCN 56	0.9–1.1	nd–0.9	nd	0.7–0.9	nd	nd	nd–0.8
TCN 58	nd	nd–0.1	nd	nd	nd	nd	nd
TCN 60	nd	nd	nd	nd	nd	nd	nd

[a] nd, <0.1.

Table 9. Tocopherol and Tocotrienol Composition of Walnut Oil (Milligrams per Kilogram)

sample	China	France	Hungary	India	Italy	Spain	USA
no.	2	8	4	3	4	4	5
α-tocopherol	10	nd[a]–16	nd–19	nd	nd–10	14–17	nd–19
β-tocopherol	nd	nd	nd	nd	nd	nd	nd
γ-tocopherol	276–329	308–447	424–525	319–336	318–445	387–450	247–428
δ-tocopherol	23–27	27–63	20–34	nd–299	12–30	28–40	nd–25
α-tocotrienol	nd	nd	nd	nd	nd	nd	nd
β-tocotrienol	nd	nd	nd	nd	nd	nd	nd
γ-tocotrienol	nd	nd	nd	nd	nd	nd	nd
δ-tocotrienol	nd	nd	nd	nd	nd	nd	nd
total	309–366	383–507	460–569	319–632	358–460	429–505	260–472

[a] nd, <10.

workers reported C16:0 at 3.3%, C18:1 at 30.5%, and C18:3 at 5.1%, again for a single sample (*33*).

Iodine Value. The mean calculated iodine values of walnut oils are given in **Table 6**. The results were comparable to those reported elsewhere (*23*) but with a slightly narrower range.

FA in the Triacylglycerol 2-Position Composition. The triacylglycerol 2-position fatty acid composition of walnut oils is given in **Table 7**. The values are in good agreement with those reported elsewhere (*23*).

The proportions of C16:0 and C18:0 acids were higher in the three Indian samples than in any other, and the values for C18:2 were lower. Low levels of C20:1 and C22:1 FA were found in each of the three Indian samples but in no other oils. However, traces of these acids have been reported in 9 of 13 oils of other countries (*23*).

TCN. The TCN composition of the walnut oils is given in **Table 8**. The results for most triacylglycerols (by carbon number) were very similar to those reported for authentic (*23*) and retail (*40*) walnut oils.

Tocopherol Compostions. The tocopherol composition of walnut oils is given in **Table 9**.

The total tocopherol content ranged from 260 to 632 mg/kg with an average of 446 mg/kg. This was similar to other literature values (*23*, *26*, *33*, *41*−*44*), the levels being well in excess of the very low levels of tocopherols (total of <60 mg/kg) reported for oils extracted with hexane or 2-propanol from an unspecified number of Turkish nuts (*36*).

The major tocol in all samples was γ-tocopherol (range = 247−525 mg/kg, average = 381 mg/kg). This was a narrower and lower range than reported in the literature, range = 252−1096 mg/kg, average = 593 mg/kg (*23*). However, others have reported a considerably narrower and lower range of γ-tocopherol in 13 samples (28−62 mg/kg) (*44*) and in a single sample of unspecified origin at 185 mg/kg (*45*).

The range of α-tocopherol values was relatively small (<10−19 mg/kg) and broadly in agreement with literature values (*23*, *41*, *43*).

β-Tocopherol was not detected in any sample subject to a detection limit of 10 mg/kg. This is in agreement with other results (*23*, *36*, *42*), although it has been reported to be present at levels ranging from 1.0 to 8.2 mg/kg (average = 4.6 mg/kg) in 13 samples from New Zealand, Europe, and the United States (*43*) and at 8 mg/kg in a single sample of commercial oil (*41*).

Δ-Tocopherol was not detected in two samples (one from the United States and one from India). Oil from the two other Indian nuts had much higher levels (135 and 299 mg/kg) compared with those of all of the other samples containing Δ-tocopherol (range = 12−63 mg/kg, average = 43 mg/kg). The results agree with published ranges (*41*−*43*, *46*). The occurrence of odd samples with considerably higher levels has been confirmed elsewhere (*23*). There are no literature reports of the presence of Δ-tocotrienol in walnut oil.

Country of Origin. There are no published data for the composition of oil identified as from Chinese, Hungarian, Indian, or Spanish walnuts and only limited data for other countries; this makes comparison of the effect of geographical location on composition difficult to assess. This survey shows possible differences in the TCN composition of the oils from the Chinese nuts, which had lower TCN50 (0.4 and 0.5%) and TCN52 (15.5%) and higher TCN54 (83%) than any other samples; however, only two samples were collected.

The three oils from Indian nuts were the only samples to contain detectable C20:1 and C22:1 FA in the triacylglycerol 2-position; these samples also contained higher levels of unidentified 2-position fatty acids. Two of the three oils from India had high values of Δ-tocopherol (135 and 299 mg/kg); however, the third sample did not contain any detectable Δ-tocopherol. It is not unusual to find large and erratic differences in the tocopherol content of some oils, and considering the small sample numbers, it is not possible to draw conclusions as to the tocopherol content of Indian nuts in general.

The data were examined using the CSL-Metabolab visualization toolbox, which allowed multivariate and classical statistical analyses and rapid integration and spectral visualization. Separation by country of origin was observed for many determinations (24-methylenecholesterol, sitosterol, FA C18:3, C20:1, 2-position FA C16:0, and triacylglycerols). **Figure 1** shows, for example, the results of an ANOVA for C18:3, where the ranges for samples from China (albeit limited to two samples) and Hungary are separated from all of those from the United States and almost all of those from Spain.

PCA showed there to be good separation in PC scores 3 and 5 between samples from Italy and the pair from China and those from both Spain and France (**Figure 2**).

Factors Affecting the Composition of Walnut Oil. Several factors contribute to measured changes in the composition of vegetable oils. Walnut oil composition is affected by variety, geographical location, climatic effects, and treatment, for example, addition of fertilizer, during growth. The composition is also affected by the maturity of the seed at harvest, its position

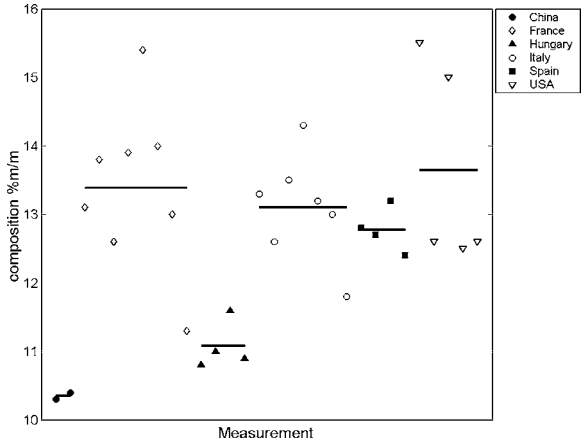

Figure 1. ANOVA for C18:3 fatty acid.

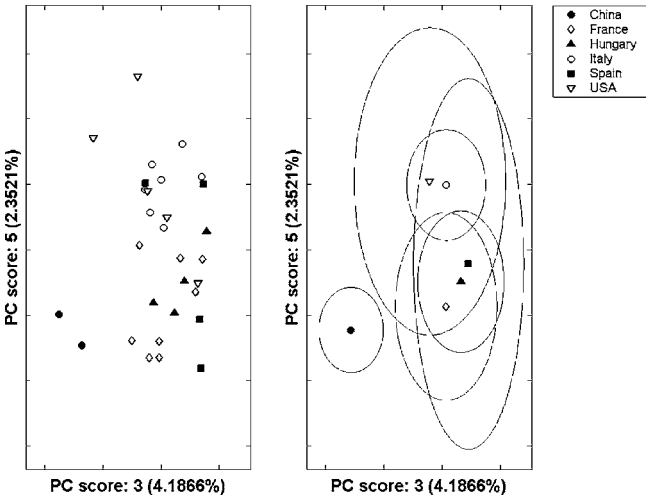

Figure 2. Principal component scores 3 and 5.

on the plant, and its handling after harvest. Postharvest, the storage and transport of the oilseed, the means and conditions of oil extraction, and the storage of the oil sample prior to analysis are all factors that can exert a strong influence on the composition of the oil. Although these factors have been the subject of particular studies, usually based on growth in experimental plots, they are usually beyond the control of agencies collecting samples for the purposes of determining the components of the oil.

Differences in the ranges of particular FA have been linked to cultivar (*38*) and variety, some having slightly higher proportions of oleic acid (C18:1) and low linolenic acid (C18:3). For one variety the linolenic acid was present at almost double the proportion found in some others. The FA composition of European nuts has been reported to be similar to that of nuts from the United States, whereas those of some varieties from New Zealand were much more variable (*34*). The FA composition has been shown to be stable over 4.5 months of storage (*42*) and to change with maturity of the kernel (*31*), with levels of C16:0, C18:2, and C18:3 decreasing as the nut matured with a concomitant increase in C18:1 and the total level of FA remaining constant. Differences have also been found in the tocopherol content of walnuts of different varieties and geographical locations, with the influence of location being dominant (*42*).

Most published papers describe results from oil solvent-extracted from nuts in the laboratory rather than pressed, presumably as the latter procedure is less quantitative and requires expensive apparatus. The solubility of all of the major

analytes is such that it is unlikely that extraction methods affect the oil composition significantly, even with unusual solvent systems. For example, the sterol composition of walnut oil did not differ significantly for oils extracted with supercritical carbon dioxide in place of hexane (*28*).

This paper adds to and updates the available compositional tables for walnut oils. The analyses have been performed using modern equipment and validated methods incorporating stringent quality control measures based on the use of fully characterized reference materials. The geographical origin of the samples has been confirmed by collection from the harvest site and the authenticity of the nuts and oil by the use of an audit trail. Industry conditions for the oil production were followed as closely as possible.

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