Fatty Acid and Tocopherol Contents and Oxidative Stability of Walnut Oils[1]

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ABSTRACT: Walnuts (*Juglans regia* L.) were collected during the 1997 harvest from 13 different cultivars of trees grown in a replicated trial in an experimental orchard at Lincoln University. Two U.S. commercial cultivars (Tehama and Vina), three European commercial cultivars (Esterhazy, G139, G120), and eight New Zealand selections (Rex, Dublin's Glory, Meyric, Stanley, Mckinster, 150, 151, 153) were evaluated. Total lipids were analyzed for fatty acids by capillary gas chromatography, tocopherols by high-performance liquid chromatography, and oxidation stability by Rancimat. The total oil content of the nuts ranged from 64.2 to 68.9% while the stability of the oil ranged from 3.9 to 7.8 h. The oleic acid content of the oils ranged from 12.7 to 20.4% of the total fatty acids, while 18:2 content ranged from 57.0 to 62.5% and the 18:3 contents ranged from 10.7 to 16.2%. Reduced stability of the oil as measured by the Rancimat method appears to be correlated to higher levels of 18:2 in the extracted oil. The total tocopherol contents of these nuts ranged from 268.5 to 436.0 μg/g oil. γ-Tocopherol dominated the profile while α-tocopherol was only 6% of the total content. Peroxide values of the fresh oil were measured spectrophotometrically to give an indication of the overall stability. The levels of total tocopherols when combined with the level of unsaturation in the oil in a multiple regression analysis had a significant relationship (R^2 = 45.2%, $P < 0.001$) with the peroxide value in the oil.

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KEY WORDS: Fatty acids, *Juglans regia* L., lipids, oxidative stability, tocopherol, walnut.

Walnut kernels (*Juglans regia* L.) generally contain about 60% oil (1), but this can vary from 52 to 70% depending on the cultivar, location grown, and irrigation rate (2–4). The major constituents of the oil are triacylglycerols; free fatty acids, diacylglycerols, monoacylglycerols, sterols, sterol esters, and phosphatides are all present in only minor quantities (1). The major fatty acids found in walnut oil are oleic (18:1), linoleic (18:2), and linolenic (18:3) acids. The ratios of these to each other are important to the economic and nutritional value of the nut. Lower linoleic and linolenic acid content oils may have a longer shelf life, and monounsaturated fatty acids may be more desirable because of their potential health benefits (5–7). The high linoleic acid content of walnut oil makes it undesirable for use in cooking as it is more prone to charring, but walnuts are a perfect ingredient in a variety of breads, muffins, cakes, and biscuits (8).

Walnut lipids contain about 70% polyunsaturated fatty acids, and their oxidation is linked to the appearance of unpleasant odors and flavors. Tocopherol isomers provide some protection against oxidation. The measurement of the tocopherol isomers in nut oils is important owing to their antioxidative effect and their positive nutritional effects in human metabolism. Interestingly, the measurement of these isomers in nut oils and walnuts in particular was not well-documented apart from some data on the tocopherol content of walnuts grown in France, the United States (9), and Germany (10). Lavedrine *et al.* (9) identified α-, γ-, and δ-tocopherol in fresh and stored nuts and noted the significant losses that occurred after 3 mon storage at 4°C. They identified γ-tocopherol as the main tocopherol in walnut oil. No β-tocopherol was identified in their samples, but low levels were observed in a mixed commercial sample of walnut oil in Germany (10).

The efficient extraction of fat from walnut samples for analysis must be rapid to prevent loss of tocopherols which are unstable in the presence of unsaturated fats, oxygen, alkali, and metal ions (11). Saponification of the oil or acid hydrolysis did not improve the recovery of the individual vitamin isomers, and direct solvent extraction was both rapid and efficient (9).

The positive nutritional advantages of walnuts in lowering blood cholesterol should not be overlooked (12). These advantages come from the high levels of mono- and polyunsaturated fatty acids and possibly the tocopherol content (6,7). These experiments are unusual as they used specific foods, walnuts and almonds, to lower total plasma and low-density lipoprotein cholesterol, thus reducing the potential risk of coronary heart disease (6,7). The experiments carried out (6,7) showed the positive effect of addition of walnuts to the diet, but neither of these experiments recorded the fatty acid profile of the nuts fed to their experimental subjects. This is important as it was shown that the fatty acid profile of walnut oil varies between cultivars (2). It is important to identify

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these differences in locally grown cultivars and to identify which fatty acids give the best nutritional qualities.

Interestingly, the fatty acid profiles of different walnut cultivars may influence flavor stability during storage. Consumer taste tests showed that locally grown cultivars have markedly differing organoleptic properties after only short-term storage of in-shell nuts in dry cool conditions (13). These differences in flavor stability may depend on the types and amounts of fatty acids present in the different cultivars of walnuts evaluated. However, the major cause of decreased walnut palatability is oxidation of oils during storage and the resulting appearance of unpleasant odors and flavors. An investigation of this phenomenon forms part of an ongoing series of experiments.

The present study is a preliminary investigation of the fat and fatty acid compositions, tocopherols, and stability of 13 of the potentially most useful cultivars of walnuts from Europe and the United States that can be grown in the Canterbury region of New Zealand. These data may help in selecting cultivars that are useful for future commercial production in the region and in identifying suitable cultivars for use in food products.

MATERIALS AND METHODS

Sources of the walnuts. Two replicated walnut variety trials were planted at Lincoln University in 1985 and 1987, respectively, by the Walnut Action Group (Rex Baker Memorial Trial). The trees were grafted clonal material on seedling rootstock (primarily *J. regia* but with some trees on *J. nigra* to obtain adequate rootstock numbers). There were six replicates of 17 cultivars/selections and two replicates of a further five cultivars in total. Two U.S. commercial cultivars (Tehama and Vina), three European commercial cultivars (Esterhazy, G139, G120), and eight New Zealand selections [Dublin's Glory (143), Meyric (1199), Stanley (Ble 300), McKinster, Rex (152), 150, 151, 153] were evaluated. The numbers in parentheses are the Walnut Action Group's Accession Number. The New Zealand seedling selections were made by the Walnut Action Group. The trials were planted in Templeton Silt Loam, and the entire block was surrounded by guard trees to act as pollenizers. Details of the management practices and climate conditions are given elsewhere (14). The harvesting and drying conditions followed the standard methods (14). The walnuts analyzed in this experiment were harvested in April and May 1997 with harvests at 3-d intervals. The walnuts were collected from the ground and were then washed and dried in a forced-air drier at 30°C to a moisture content of *ca.* 9% (15). The nuts were then opened using a standard method (13), and the kernels were vacuum-packed in plastic bags and stored at −70°C until they could be extracted. Chemical analysis was carried out on bulked harvest samples.

Chemical analysis. The total fat content was determined in accordance with AOAC Method 7.061 (16).

Lipid extraction for further analysis. Finely chopped nuts (10 g) were extracted with 30 mL hexane/isopropanol (3:2, vol/vol) at room temperature under vigorous horizontal shaking for 1 h in steel tubes containing four steel balls to facilitate homogenization of the seeds (17,18). The homogenates were filtered through defatted filter papers on a Buchner funnel (Kimble, Vineland, NJ) under vacuum, and the residues were washed twice with 20 mL of the same solvent; thereafter 35 mL of 6.7% sodium sulfate was added, and the upper layer was rotary-evaporated under reduced pressure at 40°C. The pure oil was stored at −20°C until analysis commenced the following day.

Peroxide value. The peroxide value was determined, after calibration with iron chloride solution, following the International Dairy Federation standard Method 74A (19). Freshly extracted oil (0.4 g) was weighed into a 15-mL Kimax (Kimble) tube, and 9.6 mL chloroform/methanol (70:30) was added and mixed. Following this, 0.05 mL ammonium thiocyanate solution (30 g/100 mL) was added and vortexed. The absorbance of this mixture was measured at 500 nm, and after adding 0.05 mL of the iron chloride solution (0.35 g $FeCl_3 \cdot 4H_2O$ in 100 mL distilled water and add 2 mL 10 mol/l HCl) the mixed solution was allowed to stand in the dark for 5 min. The increase in absorbance was measured against a reagent blank (9.9 mL chloroform/methanol mix and 0.05 mL ammonium thiocyanate solution). The peroxide value of the fat was expressed as meq of oxygen/kg fat.

Oxidative stability, Rancimat method. Extracted oil (2.5 g) was weighed into a 25 × 150 mm test tube and connected to a Rancimat 679 (Metrohm, Herisau, Switzerland). Air was passed through the samples at 15 L/min while being heated at 110°C. The gases released during oxidation of the oil sample were carried into a cell containing 60 mL of water. The change in conductivity of the cell was plotted on a graph for 18 h. The oxidative stability was taken as the time corresponding to the point of intersection of the two parts of the graph (the linear part at the beginning of the analysis and the exponential part at the end of the analysis). All analyses were carried out in triplicate.

Analyses of tocopherol by high-pressure liquid chromatography (HPLC). The tocopherol content was determined by direct injection of the oil samples into an HPLC following the method of Dutta *et al.* (20). In brief, oil samples weighing 0.5 g were dissolved in 5.0 mL *n*-heptane, and 25 µL was injected into a guard column (4 × 4 mm) LiChroCART 4-4 coupled to a 25 × 0.4 cm LiChroCART 250-4 packed with Lichrospher 100 NH2, 5-mm particle-size column (Merck KGaA, Darmstadt, Germany). Both columns were fitted onto a Waters 600E solvent delivery system (Waters, Milford, CT). Peaks were detected using a Perkin-Elmer LS-2 filter fluorimeter (Perkin-Elmer, Buckinghamshire, United Kingdom) set at wavelengths of 295 and 320 nm for excitation and emission, respectively. The mobile phase was heptane/*tert*-butylmethylether/tetrahydrofuran/methanol (79:20:0.98:0.02, by vol) at a flow rate of 1.75 mL/min. The relative amounts of each tocopherol were calculated using an external standard method using reference samples of tocopherols (Merck KGaA), and an HP 3396A integrator (Hewlett-Packard, Palo Alto, CA) was used to calculate the peak areas.

Preparation of fatty acid methyl esters (FAME). Approximately 20 mg of extracted oil was treated with 2 mL 0.01 M NaOH in dry methanol at 60°C for 30 min under continuous shaking essentially as described (17). The tubes were cooled under running water, and 2 mL of 10% $NaHSO_4$/25% NaCl in water (1:1), 3 mL of water, and 1 mL hexane were added. The tubes were shaken vigorously and left to stand to allow the layers to separate. The upper hexane layer containing FAME was transferred to a small tube and stored at –20°C for later analysis by capillary-column gas–liquid chromatography (GC).

FAME analysis by GC. For this purpose, a 50 × 0.22 mm, 0.25 µm film thickness fused-silica WCOT capillary column BPX70 (SGE, Austin, TX) was connected to a Varian 3700 gas chromatograph (Palo Alto, CA) equipped with a flame-ionization detector and split/splitless injector. Helium was used as the carrier gas at a velocity of 23 cm/s, and as the make-up gas at a rate of 30 mL/min. A temperature program of 158°C for 5 min rising to 220°C at a rate of 2°C/min was used. The FAME dissolved in hexane was injected (1 µL) in a split mode of injection at a split ratio of 40:1. The injector and detector temperatures were 230 and 250°C, respectively. A Varian 4270 integrator was used for recording the peak areas. No response factors were applied in calculating fatty acid composition, since the GC temperature program showed almost equal responses for different FAME standard mixtures. All analyses were carried out in duplicate.

Data analysis. The data were analyzed using multiple regression and General Linear Model analysis of variance in Minitab for Windows in which all parameters were included in a stepwise fashion in all combinations. The program then selected the best combination of factors which maximized the adjusted R^2 value.

RESULTS AND DISCUSSION

The total oil content of walnut kernels ranged from 64.2 to 68.9%, while the stability of the freshly extracted oil ranged from 3.9 to 7.8 h (Table 1). Walnut oil is more unstable in the Rancimat test when compared to hazelnut oil (21) because walnut oil contains much higher levels of polyunsaturated fatty acids. Interestingly, the stability of the oils extracted from individual cultivars ranged widely, and more unstable oils such as those extracted from the European-sourced nuts (Esterhazy and G139) did not have higher levels of linoleic (18:2) and linolenic (18:3) acids compared to more stable oils extracted from Vina, G120, and Stanley. Overall the Rancimat values for the samples of fresh oil were higher than those observed by Matthäus (10) for a mixed sample of oil which may have been stored for some time prior to analysis. There was also some difference in the conditions used between the two Rancimat tests. The results from this experiment and from Matthäus (10) confirm that walnut oil is relatively unstable compared to other common plant oils. The peroxide values of the oil ranged from 1.0 to 5.4 meq oxygen/kg fat. The freshly extracted oil from the New Zealand selected cultivars contained both the highest and lowest peroxide values of all the nuts tested. All the nuts had a very acceptable taste, and no rancidity could be detected organoleptically.

The major fatty acids in walnuts, as determined by capillary-column GC (Table 1), were palmitic (16:0), oleic (18:1), linoleic (18:2), and linolenic (18:3). The polyunsaturated fatty acids made up between 69.6 and 78.7% of the total fatty acids; and analysis of the data shows that the 18:2 and 18:3 fatty acids increased together in particular samples, and at the same time the contents of the 14:0, 16:0, 16:1, and 18:0 were

TABLE 1
Total Oil, Rancimat Value (h), Peroxide Value (meq O_2/kg fat) and Fatty Acid Composition (%) of 13 Different Cultivars of Walnuts Grown at Lincoln, Canterbury, New Zealand[a]

Selections and origins	Total oil	Peroxide value	Rancimat value	16:0	18:0	18:1	$18:1^{\Delta 11}$	18:2	18:3	20:1
Europe and United States										
Esterhazy	64.2	2.2	3.9	7.47	1.63	17.44	0.68	58.83	13.54	0.12
G139	64.2	4.1	3.9	6.65	1.40	16.46	0.71	61.98	12.71	0.14
G120	65.3	4.6	7.5	7.73	2.05	19.58	0.69	57.09	12.45	0.12
Tehama	67.6	2.0	6.1	7.61	1.35	19.54	0.81	57.88	12.38	0.14
Vina	66.9	3.3	7.5	6.46	1.43	17.94	0.68	58.03	15.07	0.11
New Zealand										
Rex	67.7	2.9	4.8	6.59	0.07	12.66	0.81	62.48	16.17	0.11
Dublin's Glory	66.2	1.0	4.6	7.76	0.08	18.95	0.85	57.01	13.10	0.12
Meyric	68.9	5.1	5.4	7.30	0.08	18.09	0.85	58.43	13.31	0.11
Stanley	64.9	5.1	7.8	6.72	0.08	20.36	0.63	59.24	11.18	0.11
McKinster	66.4	2.1	5.9	6.22	0.06	18.71	0.77	61.31	10.65	0.06
150	65.2	5.4	4.9	7.15	0.06	17.39	0.74	60.45	12.65	0.12
151	64.5	4.9	4.2	6.75	0.06	16.20	0.71	61.72	12.71	0.14
153	67.6	2.8	4.9	6.84	0.06	14.35	0.58	61.64	15.21	0.10
SE mean	0.5	0.2	0.1	0.15	0.08	0.48	0.40	0.52	0.23	0.02
Significance	***	***	***	***	***	***	*	***	***	NS

[a]Means of duplicate analyses. Trace amounts of 14:0, 16:1, 20:0, and 22:0 were present in all cultivars; these fatty acids made up <0.2% of the total fatty acids. $P <$ * = 0.05, *** = 0.001.

TABLE 2
Total and Individual Tocopherol Contents (μg/g oil) of the 13 Different Cultivars of Walnuts Grown at Lincoln, Canterbury, New Zealand[a]

Selections and origins	α	β	γ	δ	Total
European and United States					
Esterhazy	25.5	5.2	343.2	62.1	436.0
G139	18.1	3.5	288.3	32.4	342.4
G120	25.2	5.8	327.2	45.0	403.2
Tehama	26.1	5.3	273.6	37.3	342.3
Vina	24.0	3.5	300.9	48.5	376.9
New Zealand					
Rex	14.8	3.7	233.9	37.8	290.2
Dublin's Glory	28.7	4.2	355.0	47.0	434.8
Meyric	27.7	4.7	304.9	48.2	385.6
Stanley	28.5	1.0	328.6	44.8	402.8
McKinster	23.3	4.2	265.2	29.6	322.2
150	20.2	3.1	206.9	38.3	268.5
151	20.1	8.2	284.6	42.1	355.0
153	20.6	6.8	267.2	28.0	322.6
SE mean	1.8	2.1	6.9	4.2	9.6
Significance	**	NS	***	**	***

[a]Means of duplicate analysis. $P <$ ** = 0.01, *** = 0.001.

reduced together. The profile of fatty acids found in this experiment is comparable to data previously reported in the literature (2–4,11,22) except that this is the first study to report the presence of *cis*-vaccenic acid ($18:1^{\Delta 11}$), which ranged from 0.6 to 0.9% in walnut oil (Table 1). Some variation in the fatty acid composition between the different cultivars of walnuts grown at Lincoln can be seen. Interestingly, while the fatty acid compositions of the European- and United States-sourced nuts were comparable, the New Zealand selections showed considerable variations. For instance, in the case of 16:0, 18:1, 18:2, and 18:3, the New Zealand selections showed both the highest and the lowest values for each of these fatty acids.

The total tocopherol content of New Zealand nuts ranged from 290 to 435 μg/g oil (Table 2) with the New Zealand cultivars generally showing an increased variability indicated by the higher SD values, when compared to European- and United States-sourced material. The New Zealand selected cultivar Rex had the lowest total tocopherol content while Dublin's Glory had the highest level of all the cultivars. The proportion of the individual tocopherols remained constant in all the nuts. The tocopherol content of walnuts in this study is comparable to the mixed commercial sample of oil measured by Matthäus (10) and one sample of walnut oil measured by Coors (23), except that they did not observe β-tocopherol in their samples. This is the first study to accurately record the β-tocopherol content of named cultivars of walnuts. As anticipated by Lavedrine *et al*. (9), β-tocopherol is present at low levels in walnut oil. Previous studies were unable to separate β- and γ-tocopherols in walnut oil (9). These studies confirm that γ-tocopherol is the predominant homolog. The tocopherol values observed in the samples of fresh oil in this study are variable between different cultivars, but overall the values are higher than the values recorded for Hartley and Franquette by Lavedrine *et al*. (9). Notably, however, their nuts were stored at +4°C for 3 mon prior to analysis. They went on to show that significant losses of γ-tocopherol occurred if the nuts were stored 5 and then 14 mon at the same temperature.

The data in Table 3 show the best statistically determined regression retationships for Rancimat and peroxide values based on the tocopherol and fatty acid composition of the nut oil. The regression analysis shows that the Rancimat value was negatively associated with the 18:2, 20:1, and $18.1^{\Delta 11}$ contents. In contrast the peroxide value of the freshly extracted oil was positively associated with the total tocopherol and 20:1 contents. This is the first report of the possibility that some of the minor oil components may adversely affect oil stability as previous reports did not look at these components. Surprisingly the statistical analysis did not indicate strong relationships between 18:3 contents and the Rancimat and peroxide values. There is thus the possibility of breeding or selecting for low levels of these products to enhance storage, as a substantial range was found in different cultivars (Table 1).

TABLE 3
Most Significant Regression Relationships Derived Between Measured Stability Values of the Extracted Oil and Its Tocopherol and Fatty Acid Concentrations

	R^2_{adj}	P Intercept	P	SS	P	SS	P	SS	P overall
			[18:2]		[20:1]		[$18:1^{\Delta 11}$]		
Rancimat value	45.2	***	**	10.1	*	8.0	**	7.3	***
			[total tocopherol]		[20:1]				
Peroxide value	44.5	***	***	22.0	*	7.0	—	—	***

Regression equations
Rancimat value[a] = 36.6 – 0.0377 [18:2] – 23.3 [20:1] – 8.04 [$18.1^{\Delta 11}$][c]
Peroxide value[b] = 8.49 – 0.0202 × [total tocopherol] + 21.7 [20:1]

[a]Hours.
[b]meq oxygen/kg fat.
[c]Fatty acids as g/100 g oil, tocopherol as μg/g oil. $P <$ * = 0.05, ** = 0.01, *** = 0.001. SS = sums of squares for regression component.

Further verification of the possible involvement of these fatty acids is important in the understanding of the causes of rancidification and breakdown of walnut oils during storage.

The present study is the first to record the total and individual tocopherol content of fresh oil extracted from named walnut cultivars. Studies are under way to determine the stability of the polyunsaturated fatty acids and the tocopherol content when stored as whole nuts under commercial conditions for an extended time.

Owing to the high commercial value of both whole walnuts and cold-pressed walnut oil, extreme care needs to be taken to prevent oxidation of the unstable polyunsaturated fatty acids in the oil. These studies showed that a considerable range in stability to the Rancimat test occurs between the different cultivars of walnuts. The tocopherol content of the oil extracted from different cultivars of walnuts all grown under the same environmental conditions is also quite variable. It is important to maintain high levels of freshness to prevent the development of rancidity and off-flavors in these nutritious high-value products.

The results of the experiment presented here show that New Zealand-grown walnuts have a distinctive fatty acid and tocopherol profile. The variable composition of New Zealand-selected cultivars of walnuts might provide a better range of nuts for different end uses and selection compared to the more uniform composition of nuts commonly grown in the United States and Europe.

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