Application of a Rapid Transesterification Method for Identification of Individual Fatty Acids by Gas Chromatography on Three Different Nut Oils

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The lack of uniformity of analytical techniques employed for quantification of fatty acids led to the successful implementation of a rapid transesterification method using tetramethylammonium hydroxide to determine and compare the total fatty acid content of almond, pecan and macadamia oils. Palmitic, oleic and linoleic acids comprised the largest part of the total fatty acid content in almond and pecan oils. Although oleic acid was also the main constituent of macadamia oil, its concentration was substantially lower than in the other oils.

Methyl esters are still the most popular derivatives for gas chromatographic analyses of fatty acids (1), and several methods are available for their preparation (2). Usually they are prepared by saponification followed by acid-catalyzed methanolysis. The most popular preparation techniques for methyl esters utilize boron trifluoride, diazomethane, hydrochloric acid/methanol, sulfuric acid/methanol and perchloric acid/methanol. Most of the above techniques include a time consuming extraction step, and therefore there is always a need for a rapid and reproducible method to determine the wide range of fatty acids (3).

Methyl esters may also be prepared by transesterification, but base-catalyzed transesterification, using sodium methoxide in methanol, is not suitable for preparations of fatty acids methyl esters for GC-analyses. The technique does not convert any present free fatty acids to esters and requires a number of extra, time consuming, manipulative steps (4).

A new technique has been developed to overcome both of these disadvantages (1). Tetramethylammonium hydroxide in methanol, a very strong organic base, can be employed as a catalyst for the transesterification of triglyceride fatty acids to methyl esters. Free fatty acids present are also converted to tetramethylammonium hydroxide soaps that are easily pyrolyzed in the injection port of the gas chromatograph to yield methyl esters. Total fatty acid composition can also be determined with this method by adding an additional neutralization step, followed by a solvent modification to obtain a homogeneous solution. There are also other organic bases available, but tetramethylammonium hydroxide is more readily obtainable (1).

This transesterification method was previously and successfully utilized for almond oil, but with a different quaternary ammonium hydroxide (5). The method was also compared with other procedures for vegetable oils and rapid, highly sensitive, quantitative results were obtained (6). This rapid method appears to be an attractive alternative for gas chromatographic analyses of fatty acids (2).

Unsaturated fatty acids contribute 70-90% of total fatty acids present in the samples in almond oil. Saturated fatty acids were found in relatively low concentration (7-23.6%) (7). Iranian almond oil had lower content of myristic (trace) and stearic (0.4-1.4%) acids, and higher content of palmitic (6.0-8.1%), oleic (67.6-80.8%) and linoleic (11.9-24.4%) acids. Palmitoleic acid (0.4-1.9%) was also detected, but no other fatty acids were reported (8).

Eight different fatty acids were found in macadamia oil, namely myristic (0.6%), palmitic (8.7%), palmitoleic (22.1%), stearic (3.6%), oleic (59.1%), linoleic (1.8%), arachidic (2.2%) and eicosenoic (1.6%) acids. Behenic, docosenoic and lignoceric acids were also detected (9). The fatty acid composition of macadamia tree leaves differs from nut kernel composition. Caprylic, capric and lauric acid were found, but leaves contained no palmitoleic, arachidic or eicosenoic acid (10).

Oleic and linoleic acids account for about 90% of the total fatty acid content of pecan oil (11). It contains oleic (71-79%), linoleic (16-25%), stearic (2%) and palmitic (3%) acids (12). Small quantities of myristic (0.04%) and no linolenic acids occur (13). Twenty-three fatty acids were also positively identified in pecan oil, of which 13 contributed less than 0.2% of the total fatty acid content (14). Linolenic, arachidic, eicosenoic and palmitoleic acids were positively identified in small quantities (9).

However, it is difficult to compare data concerning fatty acid composition of different nuts from various sources, mainly because of a lack of uniformity of analytical techniques used for quantification. The cultivars used can also affect nut composition, and variations may occur. These variations in fatty acid composition may further influence the nutritional value and storage ability of the nuts (9).

The objectives of this study were to quantitatively determine the individual fatty acid content of specific almond, pecan and macadamia oils by using the same analytical procedure. These results then enabled us to make an objective comparison between the composition of the different nut oils.

EXPERIMENTAL PROCEDURES

Material. Shelled almond (Davey), pecan (Moore) and macadamia (Nelmar) kernels were defatted with petroleum ether (boiling point below 40°C) by Soxhlet extraction. The nut oils thus obtained were used for determinations.

Method. The transesterification method described by Metcalfe and Wang (1) was used to determine total fatty acid content, and gas chromatographic conditions were

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modified according to the column used. Gas-liquid chromatography was performed with a Hewlett-Packard 5831 A gas chromatograph equipped with a flame ionization detector and an electronic integrator. Glass columns (2 m × 3 mm i.d.) were packed with 15% diethylene glycol succinate with chromosorb W (80-100 mesh) as carrier material. Nitrogen was used as carrier gas at 15 ml/min. Hydrogen and air were supplied to the detector at 42.5 and 255 ml/min, respectively. Oven temperature was isothermally held at 185°C for 40 min. The temperature was thereafter elevated by 2°C/per min until 230°C was reached. Subsequent injections were done at 80 min intervals. Detector temperature was 230°C and temperature of injection block was 225°C.

Preparation of internal standard. Heptadecanoic acid (0.64131 g) was diluted with diethyl ether to 25 ml. The internal standard (200 μl) was placed in screw cap glass vials (8 ml) and evaporated to dryness over calcium chloride under reduced pressure at room temperature.

Preparation of standards. Fatty acid standards (Sigma Chemical Company, St. Louis, MO), namely myristic (14:0), myristoleic (14:1), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0) and eicosenoic (20:1) acids were accurately (ca. 10 mg) weighed into glass vials containing internal standards.

Preparation of experimental samples. Nine replications (100 mg) of the oils were weighed into glass vials containing internal standards. Diethyl ether (3 ml) and 20% (m/v) tetramethylammonium hydroxide in methanol (200 μl) were added to the vials containing standards and experimental samples. Vials were shaken for one min and layers allowed to settle. One drop 0.1% (m/v) thymol blue in methanol was added and 0.2 M hydrochloric acid in methanol was added dropwise until the indicator turned yellow. Methanol (0.5 ml) was then added to make a homogenous solution. Aliquots of these (3 ml) were quantitatively transferred to septum-sealed vials by means of a syringe. Samples were injected into the gas chromatograph with an automatic injection system (ca. 1.5 μl, reproducible). The syringe was washed with hexane between injections. Quantification was done with an electronic integrator relative to the area of the internal standard.

RESULTS AND DISCUSSION

This transesterification method was successfully implemented on the different nut oils. The method was thoroughly tested with the available standards and good reproducible results were obtained. This enabled us to compare the quantitative results obtained for total fatty acids of different nut oils by a uniform determination technique in the same study. Although very little quantitative data for nuts analyzed by means of this method were available, the different fatty acids identified by means of this method showed good correspondence with those obtained by other techniques stated in literature.

Heptadecanoic acid, which does not undergo β-oxidation and is not normally present in plant oils, was used as internal standard. Preliminary tests proved that no heptadecanoic acid was present in any of the nut oils.

Good gas chromatographic separations were obtained with the temperature program used. An elevation of the temperature was required at the end of the analysis to ensure that the long-chain (20 and more carbon atoms) fatty acids were separated. If the complete analysis was done isothermally at 185°C, fatty acids accumulated on the column and separation of arachidic and eicosenoic acids was impossible. Isothermal conditions prolonged the total analysis period, but had the advantage that good separation of stearic, oleic and linoleic acids was assured.

The total fatty acid composition of the three different nut types identified by this method are listed in Table 1. Traces of myristoleic acid was positively identified in almonds, although Mehran and Filsoof (8) and Beuchat and Worthington (9) did not report its presence. As stated in the available literature, no linolenic, arachidic and eicosenoic acids were identified. Previously, only Butte (5) has applied this specific method to nuts. He reported the same fatty acid distribution except for myristic and myristoleic acids. Another difference was that he utilized a different transesterification catalyst—namely trimethyl (m-trifluorotolyl) ammonium hydroxide.

Although Eckey (13) could not detect linolenic acid in pecan oils, small quantities were identified by this method. This confirmed the results of Beuchat and Worthington (9). No myristoleic, palmitoleic or arachidic

TABLE 1

Mean Fatty Acid content of Oils Extracted from Three Types of Nut Kernels

Fatty acids	Fatty acid content (g/100 g oil)[a] of:		
	Almond	Pecan	Macadamia
14:0	0,04 (0,00)	0,03 (0,01)	0,52 (0,53)
14:1	0,12 (0,09)	—	—
16:0	6,33 (0,22)	5,57 (0,09)	7,88 (0,26)
16:1	0,59 (0,02)	—	15,76 (0, 69)
18:0	0,97 (0,05)	1,51 (0,05)	3,35 (0,13)
18:1	58,36 (1,68)	57,66(1,18)	44,99 (1,60)
18:2	24,24 (0,98)	18,64 (0,44)	2,01 (0,17)
18:3	—	0,93 (0,06)	—
20:0	—	—	2,23 (0,18)
20:1	—	0,11 (0,03)	1,53 (0,37)

[a]Standard deviation in brackets.

acids were identified, although Beuchat and Worthington (9) detected palmitoleic and arachidic acids. Pecan oils had more or less the same fatty acid composition as almonds, except that the lower unsaturated fatty acids (myristoleic and palmitoleic acids) were absent. However, they contained linolenic and eicosenoic acids, which were not present in almonds.

The fatty acid composition of macadamia oils differed from those of the other nut types. Myristoleic and linolenic acids were not detected and only a small amount of myristic acid occurred. Although oleic acid was also the main constituent of macadamia oils, its concentration was substantially lower than in the other oils. Macadamia oils also contained considerable amounts of arachidic and eicosenoic acids. These different fatty acids were also positively identified by other authors, although not always in the same proportions. Beuchat and Worthington (9) also detected behenic, docosenoic and lignoceric acids, but they were not included in our study.

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