GC Analysis of Fatty Acids in Walnuts and Peanuts

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Nuts are considered nutrient dense foods and their consumption has been associated with reduced risk of coronary heart disease. The health benefits of nuts are partially attributable to their high content of unsaturated fatty acids. For example, α-linolenic acid, or "ALA", is an unsaturated fatty acid found in flaxseeds and walnuts.[1] ALA is a precursor to the formation within the body of the essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[2]

Established methods from AOCS® and AOAC® can be used to determine the fatty acid composition of nuts. This work demonstrates the application of aspects of these methods in the determination of the fatty acid composition of walnuts and peanuts. We will present data showing analysis on GC capillary columns of three different selectivities: Omegawax®, SP™-2560 and SLB®-IL111.

Column Selectivity for Fatty Acid Methyl Esters

The moderately polar Omegawax is made with a polyethylene glycol (PEG) based phase. It elutes fatty acid methyl esters (FAMEs) by degree of unsaturation, with minimal overlap between different carbon chain lengths. It is used in applications requiring analysis of saturated, mono and polyunsaturated fatty acids (PUFAs). However, it cannot provide optimal resolution of *cis* and *trans* isomer groups. For analysis of *cis/trans* FAMEs, a more polar column is required. The SP-2560 is a highly polar cyanosilicone column. The selectivity of this phase enables it to resolve *cis* and *trans* isomers, along with providing positional geometric isomer separations. The SLB-IL111 is an ionic liquid column and has higher polarity than the SP-2560. In the analysis of *cis/trans* FAMEs, the SLB-IL111 has demonstrated elution patterns which are complimentary to the SP-2560. We used the Omegawax in the profiling of the fatty acids (including PUFAs) present in walnuts and peanuts. The SP-2560 and SLB-IL111 columns were then used to determine C18:1 *cis/trans* isomers.

Experimental

1 g samples of walnuts (shelled and chopped) and peanuts (dry roasted and unsalted) were prepared using acid digestion/alkali hydrolysis followed by methylation as described in AOCS Official Method Ce 1k-09.[3] BHT was added as an antioxidant prior to extraction. All samples were concentrated to 1 mL prior to GC analysis. The GC columns used for the analysis were as follows:

1. Omegawax, 30 m x 0.25 mm I.D., 0.25 µm
2. SP-2560, 100 m x 0.25 mm I.D., 0.20 µm
3. SLB-IL111, 100 m x 0.25 mm I.D., 0.20 µm

The GC analysis conditions were from AOCS Ce 1i-07 (Omegawax column) and AOCS Ce 1h-05 (SP-2560 column).[4,5] Peak identifications were done by retention time matching to the Supelco® 37-Component FAME Mix, run under the same GC conditions. This work was previously published.[6,7]

Results and Discussion

Analysis on the Omegawax Column

Chromatograms of walnut and peanut extracts analyzed on the Omegawax column are presented in **Figures 1 and 2**. This column would be used if determining saturated and *cis*-unsaturated fatty acids per AOCS method Ce-1i-07. The relative percentages of the fatty acids detected in the extracts were calculated as percent of total fatty acid methyl ester (FAME) area. **Table 1** shows the percentages calculated compared to the expected fatty acid compositions for walnut and peanut oils.

Table 1. Fatty Acid Compositions: Published Data for Nut Oils vs. Experimentally Determined from Whole Nuts

	Walnut Oil: Published Data[8]	Walnut Extract: Experimentally Determined	Peanut Oil: Published Data[8]	Peanut Extract: Experimentally Determined
C14:0	—	—	0–0.1%	0.1%
C16:0	7–8%	9.0%	8.3–14%	12.7%
C16:1	0.1–0.2%	0.1%	0–0.2%	0.1%
C18:0	1.8–2.2%	2.7%	1.9–4.4%	2.9%
C18:1 *cis*	17–19%	14.3%	36.4–67.1%	54.8%
C18:2	56–60%	59.4%	14.0–43.0%	23.9%
C18:3	13–14%	14.0%	0–0.1%	0.1%
C20:0	0.1%	0.1%	1.1–1.7%	1.1%
C20:1	0.2%	0.2%	0.7–1.7%	0.9%
C22:0	0.1%	0.1%	2.1–4.4%	1.9%
C22:1	—	—	0–0.3%	0.2%
C24:0	—	—	1.1–2.2%	1.1%
C24:1	—	—	0–0.3%	0.4%

The fatty acid profiles obtained were in good agreement with the published data.[8] In walnuts, as expected, linoleic (C18:2n6c) was the most abundant fatty acid, followed by oleic (C18:1n9c), ALA (C18:3n3) and palmitic (C16:0). Walnuts are considered a significant plant source of ALA. Both ALA and linoleic acid are the predominant essential fatty acids in humans.[2] For peanuts, the most abundant fatty acid was oleic, followed by linoleic and palmitic. The health benefits of peanuts and peanut oil are associated with their high oleic acid content. Oleic acid is believed to help lower cholesterol and reduce the risk of heart disease. Oleic acid, also found in olive oil, is a major contributor to the health benefits associated with the "Mediterranean Diet."[9]

Analysis on SP-2560 and SLB-IL111 Columns

The FAME composition of vegetable oils is normally determined using AOCS Method Ce 1h-05, which designates the use of a highly polar cyanosilicone column.[5] The SP-2560 column is specifically mentioned in this method. The use of a highly polar column enables simultaneous determination of *trans* isomers of unsaturated fatty acids along with saturated and *cis*-unsaturated. Since our sample extracts were obtained from whole nuts, no *trans* fats were expected to be present. This was confirmed by analysis on both the SP-2560 and SLB-IL111 columns for walnuts (**Figures 3 and 4**) and peanuts

Figure 1. FAMEs in Walnut Extract on the Omegawax

column: Omegawax, 30 m x 0.25 mm I.D., 0.25 µm (24136)
oven: 170 °C, 1 °C/min to 225 °C
inj. temp.: 250 °C
detector: FID, 260 °C
carrier gas: helium, 1.2 mL/min constant flow
injection: 1 µL, 100:1 split
liner: 4 mm I.D., split/splitless type, wool packed single taper FocusLiner™ design
sample: extract of walnuts

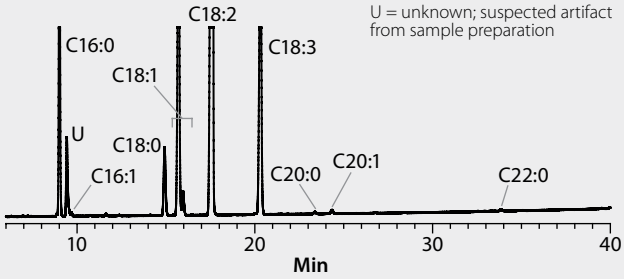


Figure 2. FAMEs in Peanut Extract on the Omegawax

sample: Extract of peanuts
All other conditions the same as Figure 1

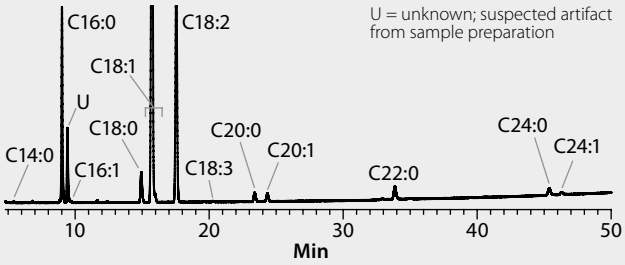


Figure 3. FAMEs in Walnut Extract on the SP-2560

column: SP-2560, 100 m x 0.25 mm I.D., 0.20 µm (24056)
oven: 180 °C isothermal
inj. temp.: 250 °C
detector: FID, 260 °C
carrier gas: helium, 1.0 mL/min
injection: 1 µL, 100:1 split
liner: 4 mm I.D., split/splitless type, wool packed single taper FocusLiner™ design
sample: extract of walnuts

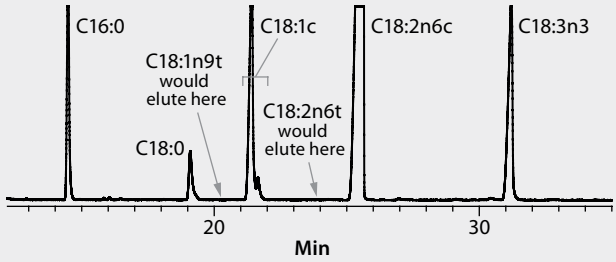
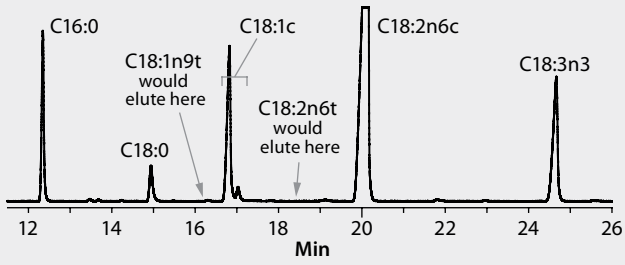


Figure 4. FAMEs in Walnut Extract on the SLB-IL111

column: SLB-IL111, 100 m x 0.25 mm I.D., 0.20 µm (29647-U)
oven: 168 °C isothermal
All other conditions the same as Figure 3



(not shown). For maximum sensitivity, the undiluted sample extract was injected under the conditions indicated in the method. As indicated, no *trans* isomers of the C18:1n9 or C18:2n6 FAMEs were detected using either column.

Conclusion

The data presented here demonstrates the high PUFA content found in both walnuts and peanuts. The Omegawax provided the selectivity necessary to elute the FAMEs found in the nut extracts by chain length and degree of unsaturation. Confirmation of the absence of *trans* fatty acids was achieved through use of the highly polar SP-2560 and confirmed with the extremely polar SLB-IL111.

References

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Description	Cat. No.
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SLB-IL111, 100 m x 0.25 mm I.D., 0.20 µm	29647-U

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