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# Dual Analysis of Triglycerides from Certain Common Lipids and Seed Extracts

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**ABSTRACT:** A number of reference oils, two commercial oils, and several oil extracts from seeds of *Nicotiana* species were analyzed for the fatty acid content and also for triglyceride composition. The seed oils were obtained using an accelerated solvent extraction procedure, which was proven to be very efficient and reproducible. The fatty acids were analyzed after the hydrolysis of the oils, using trimethylsilylation and gas chromatography/mass spectrometry (GC/MS) analysis. The levels of sixteen molecular species of triglycerides in the oils were measured after GC separation using MS for identification and flame ionization detection (FID) for quantitation. The results for the fatty acids and those for triglycerides were combined to generate uniform information regarding the composition of the analyzed oils. For a number of oils, the individual triglyceride quantitation and mass spectra were reported for the first time. The study showed that in some cases, oils with similar fatty acid content do not have the same triglycerides profile. The fatty acids and triglycerides profile for selected *Nicotiana* species were described for the first time in the literature.

**KEYWORDS:** Triglycerides, fatty acids, seed oil, *Nicotiana* species

## INTRODUCTION

Analysis of triglycerides is an important objective related to various food products, beverages, cosmetics, flavors and fragrances, paints and varnishes, and more recently to biodiesel fuel. Since in triglycerides the acids that esterify the three OH groups of glycerin can vary, a considerable diversity of this class of compounds exists. Related to their importance, numerous procedures were reported in the literature for the analysis of triglycerides. Their analyses typically follow two complementary paths, one being the analysis of fatty acids that esterify the glycerin molecule, and the other the analysis of triglycerides as whole molecules. Some studies on triglycerides are limited to only one type of such path, either analysis of fatty acids[1−5] or only of triglycerides,[6−9] although many other studies were performed following both paths.[10−21] However, even the studies performing both paths of analysis, typically do not attempt to corroborate the quantitative results from the two procedures. The distribution of fatty acids in triglycerides is described in some reports using the 1,3-random, 2-random theory of fatty acid distribution.[22,23] However, this theory was proven to lead to erroneous results in certain instances.[24,25] The present study describes this distribution for several common lipids and tobacco seed extracts.

There are numerous techniques recommended in the literature for the analysis of fatty acids from triglycerides. The first step for this type of analysis is the hydrolysis of the triglyceride. This can be done using procedures such as enzymatic hydrolysis[9,12] or hydrolysis using a strong base.[4,9] The free fatty acids resulting from hydrolysis can be analyzed after derivatization as methyl esters which are obtained using diazomethane[11] or more commonly using methanol in the presence of a strong Lewis acid such as $BF_3$.[4] Standards for the quantitation of methyl esters of fatty acids are readily available (e.g., Larodan standards, Malmö, Sweden). Another derivatization procedure for the free fatty acid analysis is silylation to generate trimethylsilyl (TMS) esters.[26] The methyl or TMS esters are usually analyzed by GC or by GC/MS. In the present study, free fatty acids were analyzed by GC/MS as trimethylsilyl derivatives, the quantitation being done using standards. High pressure liquid chromatography (HPLC) was also used for the analysis of fatty acids, e.g., after derivatization with 2-bromoacetophenone.[3]

Analyses of whole triglyceride molecules by various techniques were also reported in the literature. Such techniques can use GC,[6−10] HPLC,[16−18] TLC,[12] or a combination of those.[12,19] Most GC techniques use a flame ionization detector (FID), while the HPLC techniques most frequently use evaporative light scattering detectors (ELSD)[19] and more recently MS detectors.[27,28] Particular interest was given in the analysis of fatty acids to the separation and identification of positional and configurational isomers.[29−31] Most techniques dedicated to this subject use argentation chromatography applied either with thin layer chromatography (TLC) or HPLC.

In the present study, triglycerides were measured using a GC separation followed by MS peak identification and FID detection for quantitation. The quantitative results obtained for the fatty acids and for individual triglycerides were combined to generate uniform information regarding the composition of several common natural triglycerides and for the seed oil extracts from several *Nicotiana* species.

## MATERIALS AND METHODS

**Reagents.** Three types of samples were used in this study: a set of reference oils, two commercial oils, and several oil extracts from seeds of *Nicotiana* species. Reference oils were obtained from Supelco (Bellefonte, PA 16823-0048, USA). Triglyceride standards with identical fatty acids

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esterifying the three OH groups of glycerin were also obtained from Supelco. The purity of each standard varied between 99.3% and 99.9% (as indicated on individual certificates of analysis). The triglyceride standards with different fatty acids esterifying the three OH groups were obtained from Indofine Chemicals (Hillsborough, NJ 08844, USA). The purity of these standards was indicated as higher than 99%. Two commercial oils were obtained from the US market. Fatty acid standards were obtained at reagent grade from Aldrich (Aldrich, Milwaukee, WI 53201, USA). Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was obtained from UCT (Bristol, PA 19007, USA), of reagent grade. Diatomaceous earth was obtained from Acros Organics (Geel, Belgium). All other chemicals were also obtained from Aldrich and were of reagent grade.

**Seed Extraction.** Seed extraction was performed on an accelerated solvent extraction instrument, ASE 350 (Dionex Corporation, Sunnyvale, CA, USA). For the extraction, 0.200 ± 0.001 g of seeds and ~2.5 g of diatomaceous earth were ground using a mortar and pestle. The samples were transferred to 22-mL stainless-steel extraction cells in which the remaining void space was filled with 3-mm glass beads. The extraction was performed in the following conditions using hexane as a solvent: oven temperature 105 °C, pressure between 1400 and 1700 psig, static time 10 min, rinse volume 100%, purge time 90 s, and 3 static cycles. After the extraction, the vials (previously weighed) containing the extract were placed in a TurboVap II (Zymark Corporation, Horsham, PA, USA) to evaporate all the solvent using dry nitrogen for 90 min. The oil yield was measured by weighing.

**Analysis of Fatty Acids.** The analysis of fatty acids started with the hydrolysis of the triglycerides. For this purpose, 0.3 to 0.5 mg of oil (precisely weighed) was treated with a 50 μL solution of 2 M KOH in ethanol. The mixture was heated in a 1.5 mL capped vial for 30 min at 78 °C in a heating block, to generate potassium salts of the fatty acids. After that, the cap of the vial was removed and the ethanol evaporated. Complete evaporation of ethanol, which takes 3−5 min, is necessary to avoid the formation of small proportions of ethyl esters when HCl is further added. To the vial, a 25 μL solution of 6 M HCl was added to neutralize the base and change the organic acid potassium salts into free acids. Then, 750 μL of *n*-nonane was added to extract the free acids. The nonane solution was treated in the vial with about 0.2 g of anhydrous $Na_2SO_4$ for drying. From the dry nonane solution, 500 μL was taken in a separate 1.5 mL vial, treated with 25 μL of pyridine, 100 μL of dimethylformamide (DMF) that contains 400 μg/mL of *tert*-butylhydroquinone (TBHQ), and with 300 μL BSTFA with 1% TMCS. TBHQ is used as a chromatographic standard. The vials with the samples were heated at 78 °C for 30 min, followed by GC/MS analysis. The analysis of the samples was performed using a GC/MS instrument (Agilent 7890/5975 system, Wilmington, DE, USA) equipped with a Zebron ZB-50 column (Phenomenex, Torrence, CA 90501-1430, USA) that was 60 m long, of 0.25 mm i.d., and of 0.50 μm film thickness. The parameters for the GC/MS analysis were initial oven temperature 50 °C, initial time 0.5 min, first oven ramp rate 10 °C/min, final oven temperature first ramp 200 °C, final time first ramp 0 min, second oven ramp rate 3 °C/min, final oven temperature second ramp 250 °C, final time second ramp 0 min, third oven ramp rate 20 °C/min, oven final temperature 300 °C, final time 2 min, total run time 36.66 min, inlet temperature 300 °C, inlet mode split, injection volume 0.5 μL, carrier gas $H_2$, constant flow, flow rate 0.71 mL/min, initial pressure 12.05 psi, split ratio 1:20, split flow 14.20 mL/min, MS detector (MSD), MSD transfer line temperature 300 °C, ion source temperature 230 °C, quadrupole temperature 150 °C, MSD EM gain 2.0, MSD solvent delay 8.0 min, MSD mass range 33−550 amu.. The peak identification was performed using both standards (when available) and mass spectra library searches (on NIST 08 library). The chromatography allows excellent separation of acids in the range C6 to C27 and allows one to differentiate isomers such as oleic and elaidic acid.

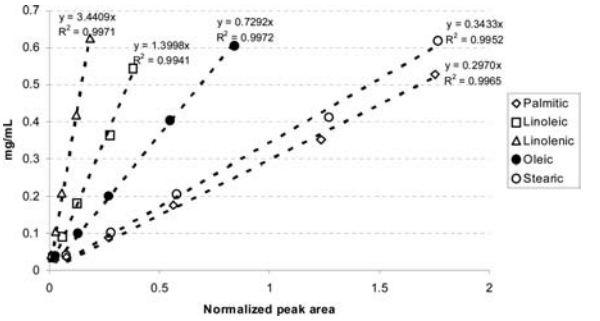



**Figure 1.** Calibration curves for the quantitation of free fatty acids using specific extracted ions.

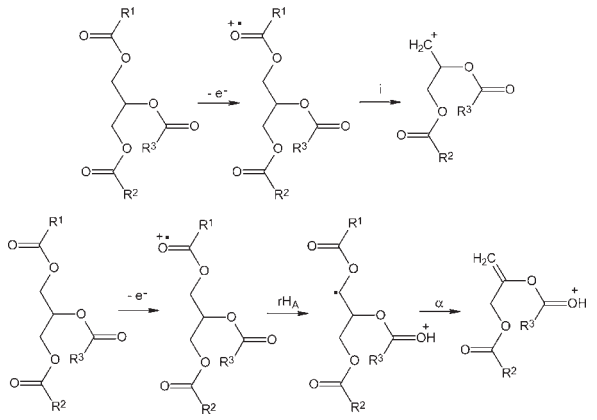
The quantitation of fatty acids was obtained only for palmitic, oleic, linoleic, linolenic, and stearic acids. Solutions of free acids in nonane were used as samples and processed by the procedure previously described. The quantitation was obtained using calibration curves for standards with five concentrations in the range 35 μg/mL to 650 μg/mL (in the injected solution). The graph showing the calibration curves representing mg/mL acid as a function of normalized area counts of the peak area is shown in Figure 1. The peak areas were measured for specific extracted ions of each acid. The ions used for the calibration were $m/z$ = 313 for palmitic acid, $m/z$ = 341 for stearic acid, $m/z$ = 339 for oleic acid, $m/z$ = 337 for linoleic acid, and $m/z$ = 335 for linolenic acid. As seen from Figure 1, the $R^2$ coefficients for all calibrations are above 0.994. The dependences shown in Figure 1 also indicate that the sensitivity of the signal for the four acids with the same number of carbon atoms (C18) is decreasing as the number of double bonds in the molecule increases. Also, palmitic acid shows better sensitivity than stearic acid.

The upper concentrations range for good linearity for the free acids was limited to 650 μg/mL since at concentrations higher than 1 mg/mL the response was not linear anymore. The LOD and LOQ values for the analysis were not of interest since, typically, a sufficient amount of oil is available for analysis.

This analytical procedure for fatty acid analysis is simple and can be easily applied to very small quantities of oil, as compared, for example, with the typical procedure of analysis of fatty acids using methyl ester formation.[4] Also, the GC separation is very good and can be achieved in a shorter time than typically required for the separation of the methyl esters of larger fatty acids (e.g., C24:1).

**Analysis of Triglycerides as Intact Molecules.** For the analysis of triglycerides as an intact molecule, a solution containing about 0.5 mg/mL oil in *n*-nonane (bp 151 °C) was made from each sample. This solution was analyzed directly by GC, using the following conditions: initial oven temperature 130 °C, initial time 1.0 min, first oven ramp temperature 30 °C/min, final temperature first ramp 300 °C, final time first ramp 0.0 min, oven temperature rate second ramp 4.0 °C/min, final temperature second ramp 365 °C, final time 7.0 min, total run time 29.92 min, inlet type cold on column, inlet mode ramped, inlet initial temperature 130 °C, initial time 0.1 min, inlet temperature rate 150 °C/min, final inlet temperature 300 °C, injection volume 0.2 μL, carrier gas $H_2$, constant flow mode, and flow rate 0.8 mL/min. The GC was equipped with a Rtx-65TG column, 30 m × 0.25 mm, with 0.1 μm film thickness (Restek, Bellefonte, PA 16823, USA). Similar separation was obtained using a CP-Tap column, 25 m × 0.25 mm, 0.1 μm film (Varian, Walnut Creek, CA 94598, USA) in the same conditions. The GC used MS detection for peak identification and FID for quantitation purposes. The conditions for the MSD were transfer line temperature 300 °C, ion source temperature 230 °C, EM gain 2.0, solvent delay 3.0 min, operating mode scan EI+, and mass range 50−800 amu. The conditions for the FID were detector temperature 300 °C, $H_2$ flow 30 mL/min, air flow 400 mL/min, and make up flow $N_2$ 25 mL/min.

The identification of triglyceride peaks in the chromatograms was obtained either using the mass spectra or both mass spectra and standards (when standards were available). For a number of triglycerides with different fatty acids esterifying the three OH groups, standard mass spectra were not available in common mass spectral libraries (e.g., NIST 08). Spectral assignment can be easily done for many triglycerides on the basis of typical fragments in the spectrum.[32,33] Characteristic ions are generated by the elimination of either one of the fatty acid moieties by reactions of the type:



(where i indicates inductive cleavage, $rH_A$ indicates radical site rearrangement with $\gamma$-H migration, and $\alpha$ indicates radical site $\alpha$-cleavage). As an example, the mass spectrum obtained in EI+ mode of palmito linoleo olein or (palmitoyloxy)-(linoleyloxy)-propyl-octadecenoate is shown in Figure 2 (the correct position of each fatty acid residue on the glycerin molecule cannot be determined from the mass spectrum, and therefore, the position of substituents is unknown). The structures of some of the diagnostic ions are shown in Figure 3.

An attempt to obtain molecular ions in the mass spectra of triglycerides by using CI+ ionization mode in the presence of methane did not succeed. Even at relatively high pressure of the CI gas and at lower ion source temperature, the triglyceride molecules were fragmented, and only higher abundance (as compared to the EI+ spectra) for the ions of larger fragments were obtained in the spectra but not the molecular ion.

Quantitation for several common triglycerides present in the analyzed oils was obtained using calibration curves representing triglyceride concentration as a function of peak areas generated using FID response. The use of MS detection was limited to the identification of triglyceride molecular species. Better reproducibility of peak areas was obtained using FID detection, which was chosen for quantitation. The response factors used for the calculation of the concentration based on the chromatographic peak areas are given in Table 1. As seen from Table 1, the quantitation response factors are reported only for 19 triglycerides, which are common in the oils evaluated in this study. Besides the common triglycerides listed in Table 1, some oils have other triglycerides in their composition, as is the case for linseed, palm, coconut, and menhaden oils.

The linearity for the reported calibrations was verified in the range 0.05 mg/mL to 2.0 mg/mL triglyceride. However, not all triglycerides identified in the analyzed oils were available as standards. For these triglycerides, the response factors were estimated using interpolation (extrapolation for dilinolein linolenin). The response factors showed dependence on both the number of double bonds, as well as the number of carbon atoms in the molecule. Figure 4 shows the graph (and equation) used for the estimation of the response factor for triglycerides with 57 carbon atoms and different numbers of double bonds, when the

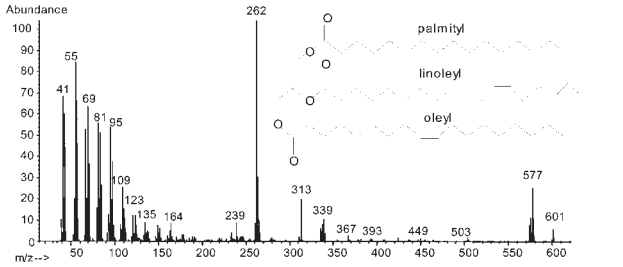
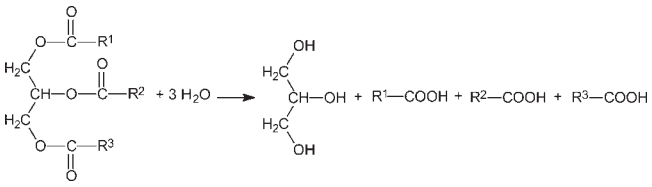



**Figure 2.** Positive ion EI mass spectrum of a triglyceride molecular species that contains palmityl, linoleyl, and oleiyl chains in the molecule (the correct position of fatty acid chains is unknown).

standards were not available. The points in the graph show compounds for which standards were available.

**Fatty Acid and Triglyceride Composition Corroboration.** Triglycerides generate fatty acids in a reaction of the following type:



On the basis of the stoichiometry of this reaction, the amount of free acids generated from each triglyceride can be easily calculated when the amount of triglyceride is known. The level of each free acid calculated as a sum from the amount of individual triglycerides in the sample should be in good agreement with the level directly measured as free acids following sample hydrolysis and analysis.

## RESULTS AND DISCUSSION

**Seed Extraction Efficiency.** Accelerated solvent extraction has been applied for the oil extraction from the seeds of different *Nicotiana* species. The results are indicated in Table 2. The re-extraction of the remaining material, after one extraction was completed, generated less than 0.1% oil. The low RDS% shown in Table 2 and the re-extraction results indicated that accelerated solvent extraction is an efficient and reproducible method of oil extraction. The results are also in good agreement with results previously reported regarding the glyceride content of tobacco seeds.[34−36]

**Free Fatty Acid Results for Oil Samples.** A typical total ion chromatogram (TIC) for the fatty acids from a commercial cooking oil hydrolysate sample is shown in Figure 5. The peak identification obtained using mass spectra is given in Table 3 as a function of peak retention times. The table also shows the peak area % for one total ion chromatogram. The same procedure used for the analysis of fatty acids in cooking oil was applied to a number of reference oils and for the oil extracts from the seeds of *Nicotiana* species. Peak area % from Table 3 represents only a semiquantitative indication of the amount of a particular acid in the hydrolyzed sample. True quantitative results were obtained on the basis of calibrations only for five selected fatty acids, namely, palmitic, linoleic, linolenic, oleic, and stearic. For most of the analyzed samples, these five acids account for more than 90% of fatty acids content. The measurements were performed in duplicate and are shown in % weight free acid reported to the weight of the oil sample. The results are given in Table 4. All of

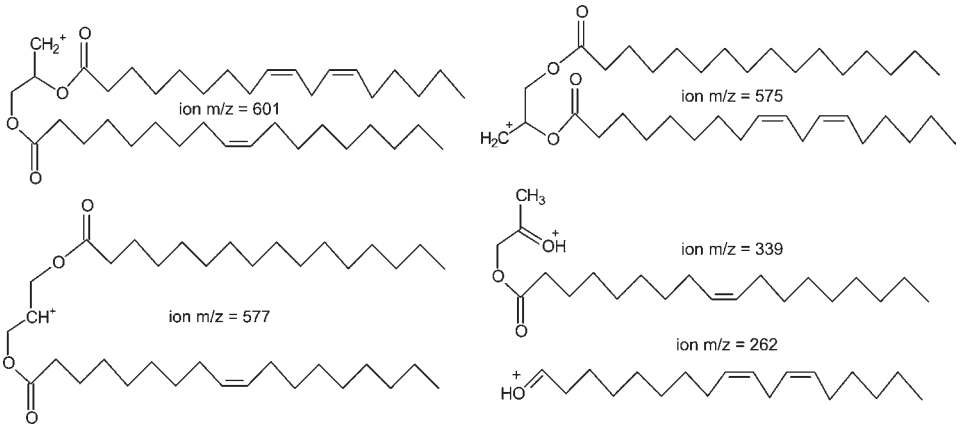



**Figure 3.** Structures of several diagnostic ions in the spectrum of a triglyceride species that contains palmityl, linoleyl, and oleyl fatty acid chains in the molecule.

**Table 1. Response Factors for the Triglyceride Concentration as a Function of Chromatographic Peak Areas Generated Using FID Response**

| | compound | Ret time | response factor | ratio to tripalmitin | $R^2$ |
|---|---|---|---|---|---|
| 1 | tripalmitin | 15.44 | $1.36432 \times 10^{-8}$ | 1.000 | 0.9727 |
| 2 | tripalmitolein | 16.12 | $1.52742 \times 10^{-8}$ | 1.120 | 0.9997 |
| 3 | dipalmito olein | 16.34 | estimated | 1.334 | |
| 4 | dipalmito linolein | 16.60 | estimated | 1.517 | |
| 5 | palmito stearo olein | 17.55 | $1.93733 \times 10^{-8}$ | 1.420 | 0.9987 |
| 6 | palmito diolein | 17.73 | $2.20474 \times 10^{-8}$ | 1.616 | 0.9878 |
| 7 | palmito stearo linolein | 17.81 | estimated | 1.616 | |
| 8 | palmito oleino linolein | 18.00 | estimated | 1.853 | |
| 9 | palmito dilinolein | 18.28 | $2.90368 \times 10^{-8}$ | 2.128 | 0.9765 |
| 10 | tristearin | 18.49 | $1.78586 \times 10^{-8}$ | 1.309 | 0.9908 |
| 11 | palmito linoleo linolenin | 18.61 | estimated | 2.450 | |
| 12 | linoleo distearin | 18.93 | estimated | 1.715 | |
| 13 | triolein | 19.13 | $2.72045 \times 10^{-8}$ | 1.994 | 0.9985 |
| 14 | distearo olein | 19.23 | $2.05466 \times 10^{-8}$ | 1.506 | 0.9874 |
| 15 | dioleino linolein | 19.44 | $3.55268 \times 10^{-8}$ | 2.604 | 0.9889 |
| 16 | stearo oleino linolein | 19.53 | estimated | 1.994 | |
| 17 | dilinoleo olein | 19.77 | estimated | 3.115 | |
| 18 | trilinolein | 20.11 | $5.17076 \times 10^{-8}$ | 3.790 | 0.9983 |
| 19 | dilinoleo linolenin | 20.53 | estimated | 4.592 | |

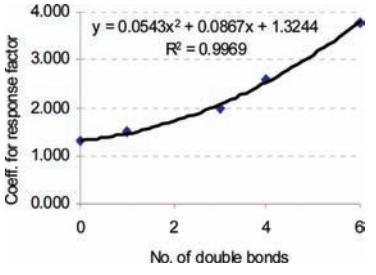



**Figure 4.** Graph (and equation) used for the estimation of the response factor for triglycerides with 57 carbon atoms and different numbers of double bonds.

**Table 2. Seed Oil Extraction Yield (%) and Relative Standard Deviation of the Measurement (RSD%)**

| tobacco type | average | RSD% |
|---|---|---|
| *Nicotiana tabacum*, flue-cured | 38.10% | 0.48% |
| *Nicotiana tabacum*, burley | 39.20% | 3.39% |
| *Nicotiana tabacum*, oriental | 39.45% | 0.78% |
| *N. glutinosa* | 36.95% | 0.21% |
| *N. alata* | 30.73% | 0.39% |
| *N. langsdorfii* | 29.47% | 0.18% |
| *N. sylvestris* | 38.08% | 0.81% |
| *N. suaveolens* | 36.83% | 0.60% |
| *N. rustica* | 29.20% | 2.40% |

the results were obtained with a relative standard deviation (RSD %) lower than 6%, the majority of RSD % values being around 2−3%. The results for the fatty acid content in the analyzed samples of common oils is in excellent agreement with data reported in the literature (see, e.g., refs 37−39). For the seeds of

*Nicotiana* species, all the oils except for those from *N. suaveolens* had a composition somewhat similar to that of safflower oil and grape seed oil. The oils from *N. suaveolens* seeds had a higer level of linolenic acid.

The results given in Table 4 do not give a total of 100% for the five acids analyzed in this study. The difference to 100% is caused in part by the inherent inexactness of the analytical measurements, but it is mainly the result of the presence of other fatty acids in the triglycerides, that were not quantiatated. Larger differences from 100% were seen in particular for coconut and menhaden oil. Detailed results including peak identification and the peak area % for the fatty acids from the coconut oil hydrolysate are given in Table 5 and for the fatty acids from a menhaden oil hydrolysate are given in Table 6. All the peaks corresponding to a fatty acid in the chromatogram of coconut oil hydrolysate were easily identified as TMS derivatives. The spectrum of each acid showed the characteristic molecular ion ($M^+$) and also the ion M-15$^+$. An unknown peak (at 14.61 min) was also present in the chromatograms of other oil hydrolysates, and it probably resulted from the reagents.

The identification of most of the peaks in the hydrolysate of menhaden oil was also possible, the acids displaying the typical $M^+$ and M-15$^+$ ions, as shown in Table 6. However, as the molecular weight of the acid increases and as the number of double bonds in the molecule is higher, the $M^+$ and M-15$^+$ ions are smaller and smaller, and uncharacteristic ions with $m/z$ = 145, 129, 117, 75, and 73 containing the Si ion are more intense. Some of the TMS derivatives of a few acids with more than 23 carbon atoms were only tentatively identified.

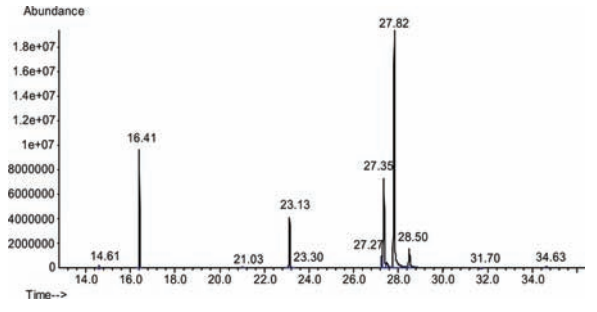



**Figure 5.** GC/MS chromatogram of TMS derivatives of fatty acids from a commercial cooking oil hydrolysate sample. Peak identification can be obtained using the data from Table 3.

**Results for Triglycerides As Intact Molecules in the Oils.** The analysis of triglycerides as intact molecules using the chromatographic procedure described in the Materials and Methods section, allows the separation of individual components, as shown in Figures 6 and 7 for a commercial cooking oil. The chromatogram shown in Figure 6 was generated using the

**Table 4. Quantitative Results Regarding the % Weight of Free Fatty Acids Reported to the Weight of Oil Sample**

| oil | palmitic | linoleic | linolenic | oleic | stearic | total |
|---|---|---|---|---|---|---|
| olive | 11.8 | 12.27 | | 66.46 | 3.16 | 93.69 |
| safflower | 5.50 | 74.04 | | 11.78 | 2.15 | 93.47 |
| sunflower | 6.15 | 56.24 | | 26.98 | 2.46 | 91.83 |
| corn | 11.07 | 49.11 | 1.21 | 31.44 | 2.08 | 94.91 |
| soybean | 9.66 | 55.76 | 7.37 | 18.14 | 2.77 | 93.70 |
| canola | 6.14 | 20.93 | 5.12 | 58.76 | 2.75 | 93.72 |
| linseed | 4.54 | 13.44 | 62.19 | 11.77 | 1.76 | 93.70 |
| palm | 36.88 | 12.88 | | 39.8 | 4.83 | 94.39 |
| cottonseed | 15.73 | 54.76 | 0.02 | 19.96 | 2.32 | 92.79 |
| coconut | 6.23 | 2.90 | | 5.87 | 1.69 | 16.69 |
| lard | 19.84 | 21.33 | 1.35 | 37.65 | 7.14 | 87.31 |
| peanut | 13.80 | 17.61 | 0.87 | 44.48 | 2.70 | 79.46 |
| menhaden | 12.75 | 0.17 | 0.36 | 6.77 | 1.76 | 21.81 |
| commercial cooking | 8.71 | 51.47 | 5.21 | 24.26 | 4.04 | 93.69 |
| commercial grape seed | 6.88 | 69.12 | 0.98 | 14.55 | 3.60 | 95.13 |
| *N. tabacum*, flue-cured (USA) | 8.63 | 69.71 | 0.36 | 12.68 | 2.12 | 93.50 |
| *N. tabacum*, flue-cured (India) | 10.73 | 62.86 | 1.00 | 15.53 | 3.59 | 93.71 |
| *N. tabacum*, burley | 9.12 | 65.46 | 1.82 | 13.70 | 2.71 | 92.81 |
| *N. tabacum*, oriental | 8.77 | 67.88 | 0.24 | 13.99 | 2.81 | 93.69 |
| *N. glutinosa* | 7.99 | 65.01 | 0.17 | 18.02 | 2.42 | 93.61 |
| *N. alata* | 7.83 | 68.89 | 1.92 | 11.97 | 3.09 | 93.70 |
| *N. langsdorfii* | 8.15 | 67.90 | 1.72 | 13.43 | 2.49 | 93.69 |
| *N. sylvestris* | 8.35 | 66.54 | 3.82 | 12.88 | 2.10 | 93.69 |
| *N. suaveolens* | 8.26 | 31.30 | 27.88 | 23.42 | 2.84 | 93.71 |
| *N. rustica* | 9.24 | 68.96 | 4.75 | 10.02 | 1.73 | 94.70 |

**Table 3. Peak Identification and Relative Peak Area for the MS Chromatogram of TMS Derivatives of Fatty Acids from a Commercial Cooking Oil Hydrolysate Sample**

| no. | compound | Ret. time | MW | identifying ions | formula | symbol | area % |
|---|---|---|---|---|---|---|---|
| 1 | glycerin 3TMS | 10.74 | 308.64 | 205, 218 | C12H32O3Si3 | | 0.15 |
| 2 | unknown | 14.61 | ? | 192, 163 | ? | | 0.24 |
| 3 | internal standard (I.S.) | 16.41 | | | | | |
| 4 | column bleed | 21.03 | | | | | |
| 5 | palmitic acid TMS | 23.13 | 328.613 | 313, 328 | C19H40O2Si | C16:0 | 9.52 |
| 6 | palmitoleic acid TMS | 23.30 | 326.597 | 311, 326 | C19H38O2Si | C16:1 Z-9 | 0.07 |
| 7 | stearic acid TMS | 27.27 | 356.667 | 341, 356 | C21H44O2Si | C18:0 | 2.56 |
| 8 | oleic acid TMS | 27.35 | 354.651 | 339, 354 | C21H42O2Si | C18:1 Z-9 | 20.33 |
| 9 | elaidic acid TMS (trans-9-C18:1) | 27.49 | 354.651 | 339, 354 | C21H42O2Si | C18:1 $\times$ $10^{-9}$ | 2.09 |
| 10 | linoleic acid TMS | 27.82 | 352.635 | 337, 352 | C21H40O2Si | C18:2 Z,Z-9,12 | 59.72 |
| 11 | linolenic acid TMS | 28.50 | 350.62 | 335, 350 | C21H38O2Si | C18:3 Z,Z,Z-6,9,12 | 4.99 |
| 12 | arachidic acid TMS | 31.70 | 384.721 | 369, 384 | C23H48O2Si | C20:0 | 0.13 |
| 13 | 11-eicosenoic acid TMS | 31.80 | 382.705 | 367, 382 | C23H46O2Si | C20:1 Z-11 | 0.06 |
| 14 | docosanoic acid TMS (behenic) | 34.63 | 412.78 | 397, 412 | C25H52O2Si | C22:0 | 0.14 |


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**Table 5. Peak Identification and Relative Peak Area for the MS Chromatogram of TMS Derivatives of Fatty Acids from Coconut Oil Hydrolysate Sample**

| no. | compound | Ret. time | MW | identifying ions | formula | symbol | area % |
|---|---|---|---|---|---|---|---|
| 1 | hexanoic acid (caproic) | 8.98 | 188.342 | 173, 188 | C9H20O2Si | C6:0 | 0.41 |
| 2 | glycerin 3TMS | 10.74 | 308.64 | 205, 218 | C12H32O3Si3 | | 0.11 |
| 3 | octanoic acid TMS (caprylic) | 11.79 | 216.397 | 201, 216 | C11H24O2Si | C8:0 | 5.81 |
| 4 | deacnoic acid TMS (capric) | 14.34 | 244.451 | 229, 244 | C13H28O2Si | C10:0 | 4.12 |
| 5 | unknown | 14.61 | ? | 192, 163 | ? | | 0.18 |
| 6 | internal standard (I.S.) | 16.41 | | | | | |
| 7 | dodecanoic acid TMS (lauric) | 16.75 | 272.505 | 257, 272 | C15H32O2Si | C12:0 | 49.80 |
| 8 | tetradecanoic acid TMS (myristic) | 19.59 | 300.559 | 285, 300 | C17H36O2Si | C14:0 | 19.10 |
| 9 | column bleed | 21.03 | | | | | |
| 10 | palmitic acid TMS | 23.13 | 328.613 | 313, 328 | C19H40O2Si | C16:0 | 9.27 |
| 11 | stearic acid TMS | 27.27 | 356.667 | 341, 356 | C21H44O2Si | C18:0 | 2.39 |
| 12 | oleic acid TMS | 27.35 | 354.651 | 339, 354 | C21H42O2Si | C18:1 Z-9 | 7.74 |
| 13 | linoleic acid TMS | 27.82 | 352.635 | 337, 352 | C21H40O2Si | C18:2 Z,Z-9,12 | 1.07 |

**Table 6. Peak Identification and Relative Peak Area for the MS Chromatogram of TMS Derivatives of Fatty Acids from Menhaden Oil Hydrolysate Sample[a]**

| no. | compound | Ret. time | MW | identifying ions | formula | symbol | area % |
|---|---|---|---|---|---|---|---|
| 1 | glycerin 3TMS | 10.74 | 308.64 | 205, 218 | C12H32O3Si3 | | 0.11 |
| 2 | unknown | 14.61 | ? | 192, 163 | ? | | 0.20 |
| 3 | internal standard (I.S.) | 16.41 | | | | | |
| 4 | tetradecanoic acid TMS | 19.59 | 300.559 | 285, 300 | C17H36O2Si | C14:0 | 8.56 |
| 5 | *n*-pentadecanoic acid TMS ? | 21.24 | 314.586 | 299, 314 | C18H38O2Si | C15:0 | 0.29 |
| 6 | palmitic acid TMS | 23.13 | 328.613 | 313, 328 | C19H40O2Si | C16:0 | 26.18 |
| 7 | palmitoleic acid TMS | 23.30 | 326.597 | 311, 326 | C19H38O2Si | C16:1 Z-9 | 14.18 |
| 8 | hexadecadienoic acid | 23.85 | 324.581 | 309, 324 | C19H36O2Si | C16:2 | 0.47 |
| 9 | hexadecatrienoic acid | 23.96 | 322.565 | 307, 322 | C19H34O2Si | C16:3 | 0.41 |
| 10 | heptadecanoic acid | 25.09 | 342.640 | 327, 342 | C20H42O2Si | C17:0 | 0.21 |
| 11 | stearic acid TMS | 27.27 | 356.667 | 341, 356 | C21H44O2Si | C18:0 | 3.44 |
| 12 | oleic acid TMS | 27.35 | 354.651 | 339, 354 | C21H42O2Si | C18:1 Z-9 | 9.00 |
| 13 | elaidic acid TMS (trans-9-C18:1) | 27.49 | 354.651 | 339, 354 | C21H42O2Si | C18:1 $\times 10^{-9}$ | 3.97 |
| 14 | linoleic acid TMS | 27.82 | 352.635 | 337, 352 | C21H40O2Si | C18:2 Z,Z-9,12 | 1.09 |
| 15 | linolenic acid TMS | 28.50 | 350.62 | 335, 350 | C21H38O2Si | C18:3 Z,Z,Z-6,9,12 | 0.40 |
| 16 | stearidonic acid | 28.55 | 348.603 | 333, 348 | C21H36O2Si | C18:4 Z,Z,Z,Z-6,9,12,15 | 2.00 |
| 17 | 11-eicosenoic acid TMS | 31.80 | 382.705 | 367, 382 | C23H46O2Si | C20:1 Z-11 | 1.19 |
| 18 | eicosapentaenoic acid TMS | 32.31 | 376.658 | 361, 376 | C23H36O2Si | C20:5 ? | 0.20 |
| 19 | eicosapentaenoic acid TMS (EPA) | 32.94 | 376.658 | 361, 376 | C23H36O2Si | C20:5 Z,Z,Z,Z,Z-5,8,11,14,17 | 12.37 |
| 20 | docosadienoic acid TMS | 33.94 | 408.743 | 393, 408 | C25H48O2Si | C22:2 ? | 0.24 |
| 21 | docosadienoic acid TMS | 33.94 | 408.743 | 393, 408 | C25H48O2Si | C22:2 ? | 0.11 |
| 22 | docosenoic acid TMS (erucic) | 34.62 | 410.759 | 395, 410 | C25H50O2Si | C22:1 Z-13(?) | 1.69 |
| 23 | docosadienoic acid TMS | 34.75 | 408.743 | 393, 408 | C25H48O2Si | C22:2 ? | 0.20 |
| 24 | unknown acid TMS | 34.98 | | 387, 73, 75 | | | 0.12 |
| 25 | docosahexaenoic acid TMS (DHA) | 35.33 | 398.663 | 383, 73,75 | C25H38O2Si | C22:6 Z,Z,Z,Z,Z,Z-4,7,10,13,16,19 | 10.43 |
| 26 | unknown | 35.76 | | | | | 0.86 |
| 27 | tetracosadienoic acid TMS? | 36.08 | 436.797 | 421, 73 | C27H52O2Si | C24:2 ? | 1.64 |
| 28 | tetracosenoic acid TMS | 36.65 | 438.813 | 423, 75 | C27H54O2Si | C24:1 Z-15 | 0.34 |
| 29 | hexacosapentaenoic acid TMS? | 39.10 | 458.803 | 458, 443 | C29 H50O2Si | C26:5 | 0.10 |

[a] Note: ? indicates tentative identification.

MS detection and the one in Figure 7 using FID detection. The identification of the chromatographic peaks shown in Figure 6 is given in Table 7. Peak identification was performed either by comparing the retention time and mass spectra with that of standards (when available) or by basing it only on the diagnostic ions generated by the loss of a fatty acid residue from the triglyceride molecule, as explained for palmito linoleo olein in Materials and Methods. Information on diagnostic ions in the mass spectra of selected triglycerides was obtained and reported for the first time in the literature. The slight difference in the

retention times between the corresponding peaks in Figures 6 and 7 is not surprising, considering the differences in the experimental setup.

The calculation of the level of triglyceride based on the FID peak area and the quantitation response factor given in Table 1 are given in Table 7. The areas were measured using the electronic data processing capability of the GC instrument, although manual integration was necessary in certain instances, such as integration of the peaks for palmito linoleo linolenin and for dilinoleo linolenin.

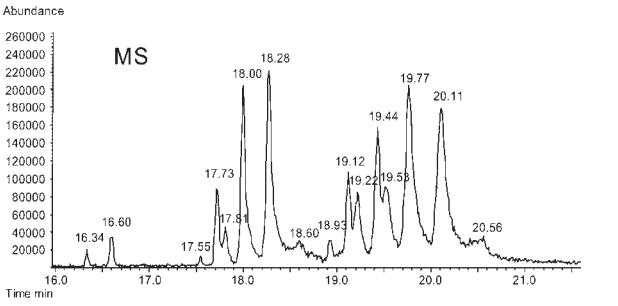


**Figure 6.** Chromatogram of a commercial cooking oil generated using MS detection (total ion chromatogram or TIC). Peak identification following retention times as given in Table 7.

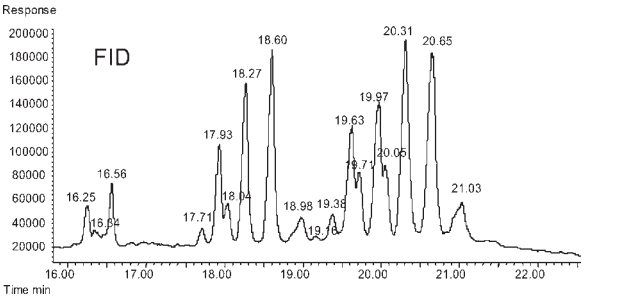


**Figure 7.** Chromatogram of a commercial cooking oil generated using FID detection.

Several other oils have a composition qualitatively similar to that of the commercial cooking oil. These oils were analyzed only for the triglycerides listed in Table 7 that were adequate for a good description of their composition. The results are given in Table 8. All the results reported in Table 8 were obtained from duplicate measurements. The precision of the results indicated as RSD % showed differences from one triglyceride to another and also from oil to oil. The RSD % values were excellent (within 3 to 4%) for triglycerides that gave well-defined peaks in the chromatogram, but much higher RSD % values were obtained for palmito linoleo linolenin, linoleo distearin, and particularly for dilinoleo linolenin. The RSD % for dilinoleo linolenin was as high as 25%.

The results for the seeds of *Nicotiana* species showed that these oils have a similar composition regarding the triglyceride profile. One exception is that of the oil from *N. suaveolens*, which is considerably different from that of the seeds of other *Nicotiana* species, as it also showed the fatty acid content. The triglyceride profile of tobacco seed oils were also similar to that of grape seed oil, as expected from the similarity of fatty acid content. However, although similar in the fatty acid content with safflower oil, the tobacco seed oils did not have a similar profile regarding the triglycerides. This finding indicates that fatty acid content provides only partial information regarding the composition of an oil, and two oils, although similar in fatty acid content, can have significant differences in triglyceride profile.

The composition of most oils evaluated in this study were well described by the 16 triglycerides listed in Table 8. Small deviations from 100% account of triglyceride weight can be explained by analytical variability and small levels of other triglycerides. However, several oils had a different triglyceride composition. From the analysis of free fatty acids, coconut oil and menhaden oil were expected to contain different triglycerides compared to those reported in Table 8. As an example, the TIC for the coconut oil is given in Figure 8. The peak

**Table 7. Peak Identification, Relative Peak Area for the MS Chromatogram and % Triglyceride from FID Measurement for a Commercial Cooking Oil[a]**

| | compound | formula | Ret. time in MS | MW | identifying ions | area % from MS | triglyc. % from FID |
|---|---|---|---|---|---|---|---|
| 1 | dipalmito olein | C53H100O6 | 16.34 | 833.380 | **551**, **577**, 339 | 0.51 | 0.27 |
| 2 | dipalmito linolein | C53H98O6 | 16.60 | 831.364 | **551**, **575**, 335 | 1.42 | 0.85 |
| 3 | palmito stearo olein | C55H104O6 | 17.55 | 861.434 | **579**, **577**, **605**, 341 | 0.34 | 0.19 |
| 4 | palmito diolein | C55H102O6 | 17.73 | 859.418 | **577**, **603**, 339 | 4.14 | 2.65 |
| 5 | palmito stearo linolein | C55H102O6 | 17.81 | 859.418 | **579**, **575**, **603**, 341 | 2.14 | 1.37 |
| 6 | palmito oleino linolein | C55H100O6 | 18.00 | 857.402 | **577**, **575**, **601**, 339 | 12.51 | 9.19 |
| 7 | palmito dilinolein | C55H98O6 | 18.28 | 855.386 | **575**, **599**, 337 | 15.93 | 13.44 |
| 8 | palmito linoleo linolenin? | C55H96O6 | 18.61 | 853.370 | **597**, **573**, **575**, 335, | 1.40 | 1.36 |
| 9 | linoleo distearin | C57H106O6 | 18.93 | 887.472 | **607**, **603**, 341, 264 | 1.03 | 0.70 |
| 10 | triolein | C57H104O6 | 19.13 | 885.456 | **603**, 339, 264 | 5.34 | 4.56 |
| 11 | distearo olein | C57H108O6 | 19.23 | 889.488 | **607**, **605**, 341, 262 | 5.31 | 3.17 |
| 12 | dioleino linolein | C57H102O6 | 19.44 | 883.440 | **603**, **601**, 339, 262 | 8.36 | 8.63 |
| 13 | stearo oleino linolein | C57H104O6 | 19.53 | 885.456 | **601**, **603**, **605**, 341 | 8.68 | 6.87 |
| 14 | dilinoleo olein | C57H100O6 | 19.77 | 881.424 | **601**, **599**, 339, 262 | 16.37 | 20.21 |
| 15 | trilinolein | C57H98O6 | 20.11 | 879.408 | **599**, 337,262 | 12.90 | 19.39 |
| 16 | dilinoleo linolenin | C57H96O6 | 20.53 | 877.392 | **597**, **599**, 337 | 3.62 | 6.59 |

[a] Note: ions with $m/z$ values in bold result from the loss of a fatty acid residue from the triglyceride molecule.

**Table 8. Triglyceride Composition % of Several Standard Oils and Extracts of Seeds of *Nicotiana* Species**

| | compound | olive | safflower | sunflower | corn | soybean | canola |
|---|---|---|---|---|---|---|---|
| 1 | dipalmito olein | 2.27 | 0.01 | 0.03 | 0.27 | 0.22 | 0.64 |
| 2 | dipalmito linolein | 0.75 | 0.11 | 0.24 | 0.89 | 1.47 | 0.35 |
| 3 | palmito stearo olein | 0.12 | 0.04 | 0.07 | 0.10 | 0.22 | 0.15 |
| 4 | palmito diolein | 1.38 | 0.35 | 0.65 | 2.41 | 1.50 | 7.65 |
| 5 | palmito stearo linolein | 0.14 | 0.10 | 0.34 | 0.45 | 1.23 | 2.46 |
| 6 | palmito oleino linolein | 29.18 | 2.48 | 5.05 | 11.86 | 8.50 | 3.93 |
| 7 | palmito dilinolein | 3.08 | 9.84 | 9.36 | 14.31 | 13.83 | 0.51 |
| 8 | palmito linoleo linolenin? | 0.00 | 0.21 | 0.12 | 0.45 | 1.58 | 0.00 |
| 9 | linoleo distearin | 0.00 | 0.20 | 0.33 | 0.32 | 0.52 | 1.43 |
| 10 | triolein | 47.22 | 1.42 | 5.26 | 4.18 | 1.65 | 32.82 |
| 11 | distearo olein | 3.18 | 0.84 | 1.89 | 1.28 | 1.88 | 1.36 |
| 12 | dioleino linolein | 6.72 | 3.00 | 12.37 | 17.17 | 7.07 | 29.96 |
| 13 | stearo oleino linolein | 1.31 | 2.63 | 3.89 | 2.10 | 3.63 | 3.63 |
| 14 | dilinoleo olein | 1.56 | 18.34 | 32.44 | 27.36 | 20.55 | 7.83 |
| 15 | trilinolein | 0.86 | 56.11 | 24.03 | 13.36 | 19.42 | 0.82 |
| 16 | dilinoleo linolenin | 0.35 | 0.82 | 1.15 | 1.95 | 12.14 | 4.78 |
| | total | 98.12 | 96.51 | 97.21 | 98.44 | 95.42 | 98.31 |

| | compound | linseed | palm | cotton | lard | peanut | grape |
|---|---|---|---|---|---|---|---|
| 1 | dipalmito olein | 0.08 | 19.25 | 0.67 | 9.16 | 0.91 | 0.26 |
| 2 | dipalmito linolein | 0.04 | 12.90 | 2.54 | 8.16 | 1.26 | 0.74 |
| 3 | palmito stearo olein | 0.08 | 2.82 | 0.18 | 3.62 | 0.69 | 1.38 |
| 4 | palmito diolein | 1.30 | 19.62 | 1.69 | 14.42 | 12.13 | 0.49 |
| 5 | palmito stearo linolein | 0.36 | 3.27 | 1.28 | 4.23 | 3.90 | 1.57 |
| 6 | palmito oleino linolein | 1.84 | 12.13 | 21.80 | 6.70 | 12.19 | 7.86 |
| 7 | palmito dilinolein | 2.37 | 0.63 | 25.51 | 3.91 | 4.51 | 6.11 |
| 8 | palmito linoleo linolenin? | 5.11 | 0.33 | 1.76 | 1.04 | 0.30 | 0.04 |
| 9 | linoleo distearin | 3.37 | 1.01 | 0.21 | 2.34 | 1.71 | 0.62 |
| 10 | triolein | 5.46 | 4.35 | 1.97 | 7.18 | 23.39 | 0.70 |
| 11 | distearo olein | 0.23 | 1.20 | 0.55 | 4.53 | 2.60 | 0.91 |
| 12 | dioleino linolein | 3.55 | 1.49 | 4.23 | 14.79 | 12.66 | 8.33 |
| 13 | stearo oleino linolein | 1.13 | 0.06 | 1.64 | 2.95 | 4.70 | 1.19 |
| 14 | dilinoleo olein | 3.18 | 0.32 | 15.58 | 7.09 | 5.24 | 19.33 |
| 15 | trilinolein | 1.76 | 0.30 | 22.45 | 3.59 | 3.58 | 46.93 |
| 16 | dilinoleo linolenin | 9.11 | 0.14 | 0.14 | 1.10 | 1.46 | 0.67 |
| | total | 38.98 | 79.81 | 102.20 | 94.83 | 91.22 | 97.13 |

| | compound | flue-cured | India | burley | oriental | *glutinosa* |
|---|---|---|---|---|---|---|
| 1 | dipalmito olein | 0.28 | 0.30 | 0.16 | 0.13 | 0.29 |
| 2 | dipalmito linolein | 1.56 | 1.72 | 1.50 | 1.33 | 1.66 |
| 3 | palmito stearo olein | 0.11 | 0.12 | 0.02 | 0.15 | 0.12 |
| 4 | palmito diolein | 0.63 | 0.69 | 0.51 | 0.44 | 0.67 |
| 5 | palmito stearo linolein | 0.81 | 0.89 | 0.77 | 0.74 | 0.86 |
| 6 | palmito oleino linolein | 6.10 | 6.72 | 5.70 | 5.05 | 6.49 |
| 7 | palmito dilinolein | 17.70 | 17.31 | 14.81 | 20.04 | 16.72 |
| 8 | palmito linoleo linolenin? | 0.45 | 0.50 | 0.42 | 0.25 | 0.48 |
| 9 | linoleo distearin | 0.09 | 0.10 | 0.23 | 0.33 | 0.10 |
| 10 | triolein | 0.59 | 0.65 | 0.21 | 0.68 | 0.63 |
| 11 | distearo olein | 0.84 | 0.92 | 0.90 | 0.62 | 0.89 |
| 12 | dioleino linolein | 5.25 | 5.78 | 5.40 | 4.75 | 6.10 |
| 13 | stearo oleino linolein | 3.64 | 4.00 | 4.33 | 3.21 | 3.87 |
| 14 | dilinoleo olein | 19.37 | 18.13 | 22.14 | 19.83 | 20.59 |
| 15 | trilinolein | 41.82 | 38.29 | 38.78 | 40.74 | 36.97 |

**Table 8. Continued**

| | compound | flue-cured | India | burley | oriental | *glutinosa* |
|---|---|---|---|---|---|---|
| 16 | dilinoleo linolenin | 0.65 | 0.71 | 1.26 | 0.71 | 0.69 |
| | total | 99.89 | 96.86 | 97.13 | 98.99 | 97.12 |

| | compound | *alata* | *langsdorfii* | *sylvestris* | *suaveo-lens* | *rustica* |
|---|---|---|---|---|---|---|
| 1 | dipalmito olein | 0.32 | 0.13 | 0.23 | 0.39 | 0.34 |
| 2 | dipalmito linolein | 1.79 | 1.09 | 2.11 | 1.09 | 1.50 |
| 3 | palmito stearo olein | 0.13 | 0.11 | 0.04 | 0.40 | 0.18 |
| 4 | palmito diolein | 0.72 | 0.18 | 0.57 | 1.65 | 0.72 |
| 5 | palmito stearo linolein | 0.93 | 0.76 | 0.80 | 0.89 | 0.83 |
| 6 | palmito oleino linolein | 7.00 | 4.44 | 6.17 | 5.64 | 5.09 |
| 7 | palmito dilinolein | 18.03 | 19.65 | 16.82 | 10.19 | 16.45 |
| 8 | palmito linoleo linolenin? | 0.52 | 0.38 | 1.56 | 4.90 | 0.24 |
| 9 | linoleo distearin | 0.11 | 0.20 | 0.02 | 0.27 | 0.30 |
| 10 | triolein | 0.68 | 0.76 | 0.55 | 3.44 | 0.34 |
| 11 | distearo olein | 0.96 | 0.62 | 0.32 | 1.25 | 1.06 |
| 12 | dioleino linolein | 4.63 | 3.75 | 4.53 | 5.33 | 3.28 |
| 13 | stearo oleino linolein | 4.17 | 3.57 | 3.43 | 6.55 | 2.85 |
| 14 | dilinoleo olein | 15.56 | 18.55 | 14.70 | 18.14 | 17.42 |
| 15 | trilinolein | 39.86 | 38.93 | 41.19 | 8.34 | 45.64 |
| 16 | dilinoleo linolenin | 0.74 | 3.06 | 4.06 | 25.65 | 1.65 |
| | total | 96.13 | 96.18 | 97.11 | 94.11 | 97.89 |

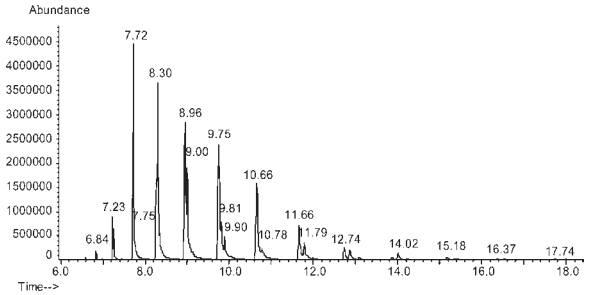


**Figure 8.** Chromatogram of coconut oil generated using MS detection (total ion chromatogram or TIC). Peak identification following retention times as given in Table 9.

identification based on mass spectra is described in Table 9. Specific triglycerides from this oil can be identified on the basis of their mass spectra that contain characteristic ions formed by fragmentations similar to those described for palmito linoleo olein and shown in Figure 3 (each diagnostic ion for triglycerides identified in coconut oil are shown in Table 9, e.g., elimination of the fragment C8:0 from dioctanoyloxypropyl dodecanoate leads to ion $m/z$ = 383).

Larger deviations from 100% can also be seen in Table 8 for palm oil and in particular for linseed oil. Both of these oils contain other triglycerides besides those listed in Table 8. For example, palm oil contains considerable levels of tripalmitin and also traces of triglycerides derived from myristic and lauric acids. Linseed oil contains triglycerides derived from linolenic acid not included in the list from Table 8. The TIC trace for a sample of linseed oil generated in the same conditions as the chromatogram from Figure 6 is given in Figure 9. Many peaks from this chromatogram are identical to those described in Table 8. However, a few additional triglycerides were identified

**Table 9. Peak Identification and Relative Peak Area for the MS Chromatogram of Coconut Oil**

| | esterifyng acids | Ret. time | identifying ions resulting by an acid fragment loss | Rel area % |
|---|---|---|---|---|
| 1 | C8:0, C8:0, C12:0 | 6.84 | 383, 327, 327 | 0.52 |
| 2 | C8:0, C10:0, C12:0 | 7.23 | 411, 383, 355 | 1.29 |
| 3 | C6:0, C12:0, C12:0 | 7.26 | 429, 355, 355 | 1.09 |
| 4 | C8:0, C12:0, C12:0 | 7.72 | 439, 383, 383 | 10.87 |
| 5 | C6:0, C12:0, C14:0 | 7.75 | 467, 383, 355 | 3.19 |
| 6 | C10:0, C10:0, C12:0 | 8.27 | 439, 439, 411 | 5.23 |
| 7 | C8:0, C12:0, C14:0 | 8.30 | 467, 411, 483 | 9.18 |
| 8 | C6:0, C12:0, C16:0 | 8.34 | 495, 411, 355 | 2.46 |
| 9 | C12:0, C12:0, C12:0 | 8.96 | 439, 439, 439 | 11.07 |
| 10 | C8:0, C12:0, C16:0 | 9.00 | 495, 439, 383 | 9.24 |
| 11 | C12:0, C12:0, C14:0 | 9.75 | 467, 467, 439 | 11.96 |
| 12 | C8:0, C12:0, C18:0 | 9.81 | 523, 467, 383 | 3.97 |
| 13 | C8:0, C12:0, C18:1 | 9.90 | 521, 465, 383 | 3.34 |
| 14 | C12:0, C14:0, C14:0 | 10.60 | 495, 467, 467 | 6.25 |
| 15 | C12:0, C12:0, C16:0 | 10.66 | 495, 495, 439 | 4.27 |
| 16 | C10:0, C12:0, C18:1 | 10.78 | 521, 493, 411 | 2.83 |
| 17 | C12:0, C14:0, C16:0 | 11.66 | 523, 495, 467 | 4.93 |
| 18 | C12:0, C12:0, C18:1 | 11.79 | 521, 521, 439 | 1.96 |
| 19 | C8:0, C16:0, C18:1 | 11.85 | 577, 465, 439 | 0.84 |
| 20 | C12:0, C14:0, C18:0 | 12.74 | 551, 523, 467 | 0.84 |
| 21 | C14:0, C14:0, C16:0 | 12.74 | 523, 523, 495 | 0.64 |
| 22 | C12:0, C14:0, C18:1 | 12.87 | 549, 521, 467 | 1.63 |
| 23 | C12:0, C14:1 C18:2 | 13.10 | 546, 519, 465 | 0.36 |
| 24 | C12:0, C16:0, C18:0 | 13.87 | 579, 523, 495 | 0.28 |
| 25 | C12:0, C16:0, C18:1 | 14.02 | 577, 521, 495 | 0.89 |
| 26 | C14:0, C16:0, C18:1 | 15.18 | 577, 548, 523 | 0.43 |
| 27 | C12:0, C18:1, C18:1 | 15.36 | 603, 521, 521 | 0.12 |
| 28 | C16:0, C16:0, C18:1 | 16.37 | 577, 577, 551 | 0.19 |
| 29 | C16:0, C18:1, C18:1 | 17.74 | 603, 577, 577 | 0.14 |

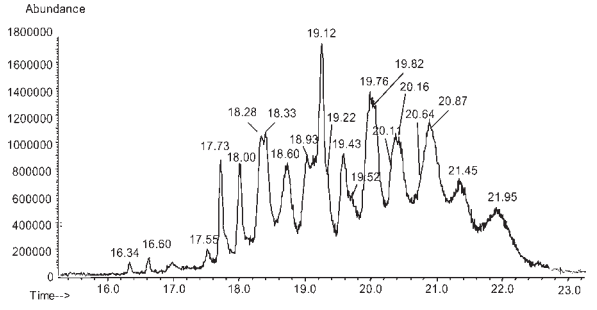


**Figure 9.** Chromatogram of linseed oil generated using MS detection (total ion chromatogram or TIC). Peak identification following retention times as given in Table 7 and in Table 10.

(some tentatively) in linseed oil, and they are given in Table 10. The presence of other triglycerides besides those quantitated in linseed oil explains the deviation from 100% in Table 8. Also, some triglycerides with even higher unsaturation (such as trilinolenin) may not be seen in the chromatogram if they decompose before being eluted.

**Corroboration of Results for Fatty Acids and Triglyceride Composition.** The present study provides unique information by attempting to follow two paths of triglycerides analysis and generating quantitative results for both fatty acids and whole triglyceride molecules that are further corroborated. The level of each free acid was calculated as a sum from the amount of individual triglycerides analysis in each sample. For most samples, a very good agreement was obtained between the level directly measured as free acids following sample hydrolysis and analysis as given in Table 4 and the levels calculated from the triglyceride content, which is given in Table 11.

One exception to the good agreement between the data from Table 11 and Table 4 is linseed oil, where the level of linolenic acid is much lower based on the calculation, as compared to the level obtained from the direct analysis. This indicated once more that the linseed oil composition is not well described by the 16 triglycerides listed in Table 8. Direct measurement of triglycerides esterified with more than one linolenic acid is not possible in the chromatographic conditions described in this study. The high level of linolenic acid detected by the analysis of linseed oil hydrolysate shows that this oil contains a high level of such triglycerides and that they were not captured in the results from Table 11. Considering that the results from Table 11 were calculated and not directly measured, the values for the fatty acid composition given in Table 4, which were experimentally measured, should be considered closer to the correct values. The good agreement between the levels of fatty acids measured and those calculated from the triglyceride content is additional proof that the oil composition as measured for whole triglycerides is correct.

**Table 10. Peak Identification for Extra Peaks in the MS Chromatogram for Linseed Oil[a]**

| | compound | formula | Ret. time in MS | MW | identifying ions |
|---|---|---|---|---|---|
| 1 | palmito oleino linolenin | C55H98O6 | 18.33 | 855.386 | **577**, **599**, **573** |
| 2 | dioleino linolenin? | C57H100O6 | 19.82 | 881.424 | **604**, **599**, 339 |
| 3 | stearo linoleo linolenin? | C57H100O6 | 20.28 | 881.424 | **603**, **601**, **597** |
| 4 | oleino linoleo linolenin? | C57H98O6 | 21.45 | 879.408 | **597**, **599**, **601** |
| 5 | oleino dilinolenin | C57H96O6 | 21.95 | 877.392 | **595**, **599**, 335 |

[a] Note: Ions with $m/z$ values in bold result from the loss of a fatty acid residue from the triglyceride molecule.

**Table 11. Calculated Levels of Fatty Acid % Weight Reported to the Weight of Oil Sample**

| oil | palmitic | linoleic | linolenic | oleic | stearic | total |
|---|---|---|---|---|---|---|
| olive | 12.00 | 16.45 | 0.11 | 62.74 | 2.54 | 93.84 |
| safflower | 3.98 | 75.13 | 0.33 | 11.32 | 1.56 | 92.32 |
| sunflower | 4.84 | 57.65 | 0.40 | 27.30 | 2.80 | 92.99 |
| corn | 9.57 | 51.51 | 0.76 | 30.44 | 1.87 | 94.15 |
| soybean | 9.09 | 56.24 | 4.37 | 18.38 | 3.18 | 91.26 |
| canola | 5.00 | 22.47 | 1.52 | 61.25 | 3.81 | 94.05 |
| linseed | 3.39 | 16.05 | 4.56 | 10.47 | 2.82 | 37.29 |
| palm | 31.39 | 11.30 | 0.15 | 29.96 | 3.45 | 76.25 |
| cottonseed | 17.62 | 59.12 | 0.62 | 18.86 | 1.49 | 97.71 |
| lard | 20.81 | 24.26 | 0.69 | 36.96 | 7.94 | 90.66 |
| peanut | 11.41 | 22.48 | 0.56 | 47.01 | 5.79 | 87.25 |
| commercial cooking | 9.14 | 53.76 | 2.54 | 24.48 | 5.20 | 95.12 |
| commercial grape seed | 5.84 | 68.20 | 0.22 | 16.31 | 2.34 | 92.91 |
| *N. tabacum*, flue-cured (USA) | 8.86 | 70.14 | 0.35 | 14.11 | 2.07 | 95.53 |
| *N tabacum*, flue-cured (India) | 9.11 | 66.35 | 0.39 | 14.52 | 2.28 | 92.65 |
| *N. tabacum*, burley | 7.68 | 67.62 | 0.54 | 14.69 | 2.38 | 92.91 |
| *N. tabacum*, oriental | 8.89 | 70.24 | 0.31 | 13.31 | 1.93 | 94.68 |
| *N. glutinosa* | 8.79 | 66.19 | 0.37 | 15.33 | 2.20 | 92.88 |
| *N. alata* | 9.48 | 66.52 | 0.40 | 13.16 | 2.37 | 91.93 |
| *N. langsdorfii* | 8.40 | 68.47 | 1.10 | 12.06 | 1.96 | 91.99 |
| *N. sylvestris* | 9.22 | 68.42 | 1.80 | 11.84 | 1.60 | 92.88 |
| *N. suaveolens* | 8.01 | 50.57 | 9.74 | 18.20 | 3.50 | 90.01 |
| *N. rustica* | 8.17 | 71.15 | 0.60 | 11.58 | 2.12 | 93.62 |

## AUTHOR INFORMATION


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