Optimization of the Mass Spectrometric Analysis of Triacylglycerols Using Negative-Ion Chemical Ionization with Ammonia

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ABSTRACT: Conditions for the mass spectrometric analysis of triacylglycerols, *via* direct exposure probe, with ammonia negative-ion chemical ionization were optimized. Triacylglycerols were most favorably ionized, using the reactant gas pressure of approximately 8500 mtorr at the ion source temperature of 200°C with the instrumentation used. Abundant $[M - H]^-$ ions were produced under these conditions without the formation of $[M + 35]^-$ cluster ions, which would interfere with the molecular weight region of triacylglycerols in the spectra. A rapid desorption of the sample from the probe wire is recommended, using a relatively high heating rate (approximately 40 mA s^{-1}), to minimize thermal degradation of unsaturated molecules and the reducing effect of double bonds on the mass spectrometric response of triacylglycerols. Furthermore, the abundance of $[M - H]^-$ ion was enhanced by rapid heating, which we found to be important to improve the sensitivity. The appropriate amount of sample applied to the rhenium wire was in the region of 50–300 ng for one determination, i.e., only a few nanograms of a single triacylglycerol is required for production of a reliable spectrum. The reproducibility of the method was good as demonstrated with standards and a raspberry seed oil sample. The described mass spectrometric method is a fast and potentially useful tool for semiquantitative determination of triacylglycerol mixtures of various fats and oils. The discrimination, caused by differences in molecular size and unsaturation of triacylglycerols with 50 to 56 acyl carbons, was negligible under our optimized ionization conditions, thus, no correction factors were needed. These findings have not yet been verified with instruments in other laboratories. However, the present study shows how the analysis of triacylglycerols can be improved, regardless of the instrument, by optimization of the analytical conditions.

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The first mass spectrometric studies of triacylglycerols (TAG) in the 1960s were performed with electron ionization (EI) impact (1,2). In addition to fragment ions, the spectra consisted of $M^{+\bullet}$ and $[M - 18]^+$ ions at low abundance, which were used to determine the molecular weight distribution of TAG of several vegetable oils (3,4). As compared with EI spectra, a greater abundance of ions, consisting of the intact molecule, and less fragmentation are obtained by soft ionization methods, chemical ionization (CI) being used most often (5,6). TAG have been ionized using methane, isobutane, or ammonia as a reactant gas in the positive-ion mode to produce $[M + H]^+$ or adduct ions. Murata and Takahashi (7,8) ionized TAG with ammonia, which resulted in the formation of abundant $[M + NH_4]^+$ ions and $[M + H - RCO_2H]^+$ fragment ions useful for the determination of both molecular weights and combinations of fatty acids in TAG molecular species. Kuksis and colleagues (9,10) introduced the formation of prominent $[M + H]^+$ and $[M + H - RCO_2H]^+$ ions when TAG were separated by reversed-phase high-performance liquid chromatography (HPLC) combined with mass spectrometry (MS) using acetonitrile and propionitrile for elution and as reactant gases. The detection sensitivity was greatly improved by using negative-ion CI with dichloromethane, which yielded solely $[M + Cl]^-$ ions (11,12).

It has been shown recently by Kallio and Currie (13,14) that MS utilizing ammonia negative-ion CI is an efficient tool for the analysis of TAG. NH_2^- ion is a strong Brønsted base which is capable of deprotonating most organic molecules, including ketones and esters (15,16). TAG will be ionized to form deprotonated ions using ammonia as the reactant gas in negative ion mode. The *m/z* values of $[M - H]^-$ ions define unambiguously the number of acyl carbons and the number of double bonds present in the TAG of most fats and oils, and the abundance of $[M - H]^-$ ions determines the proportions of different molecular weight species. Similar data cannot be achieved with a single mode of either gas chromatography (GC) or HPLC, which are the most widely utilized techniques in lipid research. In addition, more detailed information on molecular species is obtained by collision-induced dissociation of a selected $[M - H]^-$ ion. The daughter-ion spectrum provides information on the fatty acid constituents and their distribution between *sn*-2 and *sn*-1,3-positions in TAG.

In this research, the conditions for mass spectrometric analysis of TAG of seed oils using ammonia negative-ion CI were optimized. Samples were introduced *via* a direct exposure probe into the ion source without chromatographic separation before MS. First of all, the effects of ion source temperature and reactant gas pressure, as well as the rate of probe heating on the quality of ammonia negative-ion CI mass spec-

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Abbreviations: CI, chemical ionization; EI, electron ionization; GC, gas chromatography; HPLC, high-performance liquid chromatography; MS, mass spectrometry; TAG, triacylglycerols.

tra of TAG, were studied. In addition, the reproducibility of the method and its applicability for quantitative analysis of TAG were investigated.

EXPERIMENTAL PROCEDURES

TAG standards and samples. Standards of both saturated and unsaturated TAG, listed in Table 1, were purchased from Sigma (St. Louis, MO). A stock solution of each compound (approximately 0.2–0.4 mg mL^{-1}) was prepared by dissolving the saturated TAG in dichloromethane and the unsaturated ones in hexane containing 0.02% (wt/vol) butylated hydroxytoluene (Sigma) to prevent oxidation. Individual components or mixtures of several standards were dissolved in hexane for mass spectrometric determinations. Lipids in the seeds of black currant (*Ribes nigrum*), lingonberry (*Vaccinium vitis-idaea*), and raspberry (*Rubus idaeus*), grown wild in Finland, were extracted with chloroform/methanol (2:1, vol/vol) using a modified Folch procedure (17) followed by purification of the TAG by elution from a short column of Florisil™ (Fluka Chemie AG, Buchs, Switzerland) with 10 mL hexane/diethyl ether (4:1, vol/vol) as the mobile phase. TAG were dissolved in hexane (approximately 1 mg mL^{-1}) for MS determinations. All solvents used were HPLC-grade supplied by Rathburn (Walkerburn, Scotland) and Merck (Darmstadt, Germany).

Mass spectrometric analysis. The mass spectrometric determinations were conducted with a Finnigan MAT TSQ-700 triple quadrupole instrument (San Jose, CA) with an ICIS II data system (Finnigan MAT). TAG were analyzed using negative-ion CI with ammonia (≥99.998%; Prax Air, Oevel, Belgium). The optimization of the ion source conditions was carried out with a mixture of four TAG standards at ammonia pressures of 3000, 5000, 7000, 8500, and 10000 mtorr at ion source temperatures of 120, 150, 180, 200, and 220°C. The ammonia pressure was monitored with a Convectron gauge (Phillips Company, Granville, OH) which was situated in the gas line before the flow-controller into the ion source. A volume of 0.5 μL of the sample was applied to the rhenium wire loop of the direct exposure probe (Finnigan MAT). The probe was introduced into the ion source after the solvent was evaporated, and the heating of the probe wire with the current was started to vaporize the sample. The desorption of TAG from the rhenium wire was optimized by testing heating rates between 2.5 and 100 mA s^{-1} both with mixtures of TAG standards and seed oil samples. The effect of concentration on the mass spectrometric response of TAG was tested with a mixture of 14 standards (mixture A, Table 1) with concentrations varying from 2.6 to 379.6 μmol L^{-1} of each compound. The concentration of the internal standard, *rac*-18:0-18:0-14:0, was constant (24.7 μmol L^{-1}) in each dilution. The repeatability and reproducibility of the mass spectrometric method was determined with TAG standards (mixture B, Table 1) and raspberry seed oil. Electron energy was 70 eV and filament current 400 μA throughout the experiment. The *m/z* range scanned was 250–950 during the optimization of the ion source conditions and that of the probe heating rate, and 700–1100 during the concentration, repeatability, and reproducibility experiments. Scans containing ions representing deprotonated TAG were averaged in each analysis. The abundances of the ions were corrected by ^{13}C isotope content, when necessary, before expressing the results as the mean of four analyses.

RESULTS AND DISCUSSION

Ammonia negative-ion CI of TAG yielded very simple mass spectra containing abundant $[M - H]^-$ ions. The *m/z* values and abundances of $[M - H]^-$ ions define the number of acyl

TABLE 1
Reference Triacylglycerols and Their Abbreviations Used in This Study

Triacylglycerols	Abbreviation	ACN:*n*[a]	Mixture A (ng μL^{-1})[b]	Mixture B (ng μL^{-1})[b]
Saturated				
Tripalmitoylglycerol	16:0-16:0-16:0	48:0	210	420
rac-1,2-Distearoyl-3-myristoyl-*sn*-glycerol	*rac*-18:0-18:0-14:0	50:0	–[c]	412
Triheptadecanoylglycerol	17:0-17:0-17:0	51:0	212	424
rac-1,2-Distearoyl-3-palmitoyl-*sn*-glycerol	*rac*-18:0-18:0-16:0	52:0	202	404
Tristearoylglycerol	18:0-18:0-18:0	54:0	212	424
Trinonadecanoylglycerol	19:0-19:0-19:0	57:0	210	420
Trieicosanoylglycerol	20:0-20:0-20:0	60:0	206	412
Unsaturated				
rac-1-Palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol	*rac*-16:0-18:1-18:0	52:1	200	200
rac-1,2-Dioleoyl-3-palmitoyl-*sn*-glycerol	*rac*-18:1-18:1-16:0	52:2	326	652
rac-1,2-Distearoyl-3-oleoyl-*sn*-glycerol	*rac*-18:0-18:0-18:1	54:1	194	388
rac-1,2-Dioleoyl-3-stearoyl-*sn*-glycerol	*rac*-18:1-18:1-18:0	54:2	287	1148
Trioleoylglycerol	18:1-18:1-18:1	54:3	212	424
rac-1,2-Dilinoleoyl-3-oleoyl-*sn*-glycerol	*rac*-18:2-18:2-18:1	54:5	206	412
Trilinoleoylglycerol	18:2-18:2-18:2	54:6	236	472
Trilinolenoylglycerol	18:3-18:3-18:3	54:9	188	752

[a]ACN is the combined number of acyl carbons in a triacylglycerol, *n* is the combined number of double bonds in the three acyl chains of a triacylglycerol.
[b]Composition of the stock solution.
[c]Internal standard (20.6 ng μL^{-1}) added to each dilution in concentration experiments.

carbons and double bonds in TAG and the proportions of different molecular weight species. During this optimization study, more detailed information on the effects of different parameters on the analysis of TAG was considered important in order to expand the applicability of the method. The samples were introduced *via* a direct exposure probe into the ion source. The evaporation of different TAG does not occur simultaneously from the rhenium wire (18); therefore, the ion abundances obtained from the averaged spectra were used for further calculations.

Optimization of the ion source conditions. It is well known that ion source conditions may have a significant influence on the composition of the reactive CI gas plasma and, consequently, on the resulting mass spectra of sample molecules (16). The aim of the optimization was to find ion source conditions which will yield maximum sensitivity of deprotonated ions of TAG at minimum ion source pressure without formation of interfering cluster ions. The test mixture consisted of *rac*-1,2-distearoyl-3-palmitoyl-*sn*-glycerol, tristearoylglycerol, *rac*-dilinoleoyl-3-oleoyl-*sn*-glycerol, and trilinolenoylglycerol, each compound in the concentration of 0.15–0.17 $\mu g\ \mu L^{-1}$ in hexane, representing typical components in the seed oils of various plants, especially in the seeds of Northern wild berries. TAG containing 52, 54, and 56 acyl carbons with 1–9 double bonds are the most abundant constituents of several seed oils.

Ammonia negative-ion CI of TAG resulted in a spectrum which contained $[M - H]^-$ and $[M + 35]^-$ ions at various proportions, depending on the CI conditions in the ion source. In addition, only abundant RCO_2^- ions were produced with little structural significance and, therefore, are not discussed here. Figure 1A shows the abundance of $[M - H]^-$ ions originated from trilinolenoylglycerol at different ion source conditions. The ionization of *rac*-1,2-distearoyl-3-palmitoyl-*sn*-glycerol, tristearoylglycerol, and *rac*-1,2-dilinoleoyl-3-oleoyl-*sn*-glycerol were similar to that presented in Figure 1A. In general, the ionization efficiency decreased with increasing ion source temperature at constant CI gas pressure. At constant ion source temperature, the ionization efficiency of TAG increased with increasing reactant gas pressure up to 8500 mtorr. Some day-to-day variation existed in the ionization of the TAG, e.g., sometimes the ionization efficiency at 8500 and 10000 mtorr was nearly the same.

The proportion of the abundance of the cluster ion $[M + 35]^-$ from that of the corresponding $[M - H]^-$ ion originated from trilinolenoylglycerol is shown in Figure 1B. The formation of cluster ions during the analyses of *rac*-1,2-distearoyl-3-palmitoyl-*sn*-glycerol, tristearoylglycerol, and *rac*-1,2-dilinoleoyl-3-oleoyl-*sn*-glycerol at different ion source conditions resembled that shown in Figure 1B. Typically, the contribution of cluster ions decreased with increasing reactant gas pressure and increasing ion source temperature. For example, the proportions of cluster ions were more than 10% of the corresponding $[M - H]^-$ ion at the pressure of 3000 and 5000 mtorr at the ion source temperature of 120°C for each TAG studied. On the other hand, no cluster ions were observed at temperatures of 200 and 220°C when the intensities of the ions of interest were derived from the averaged scans.

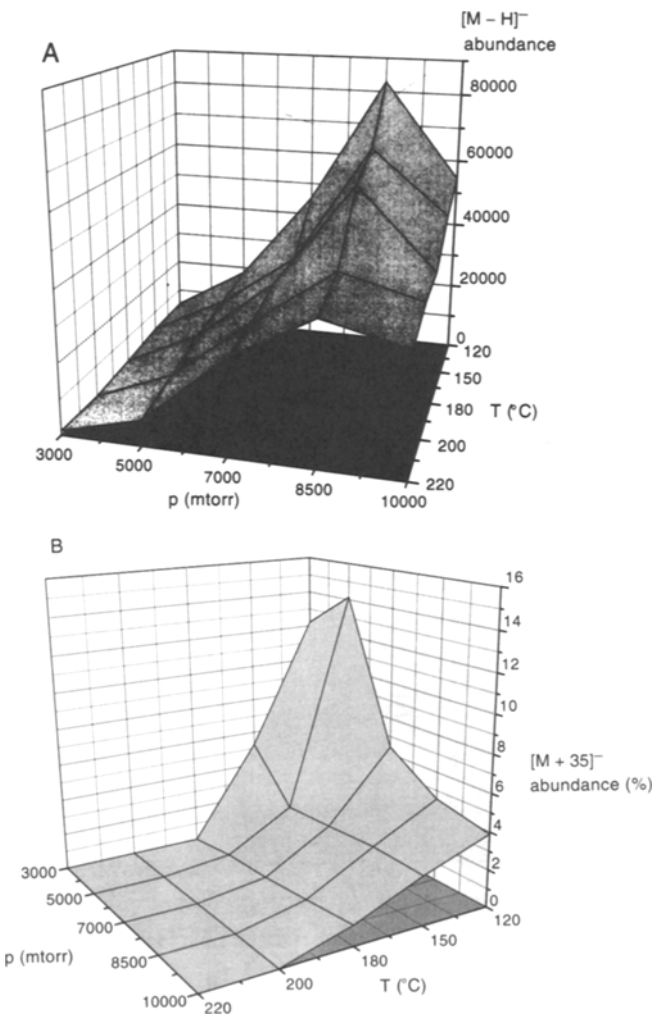


FIG. 1. A: The abundance of $[M - H]^-$ ion at different ion source conditions. B: The proportion of the abundance of the cluster ion, $[M + 35]^-$, from that of the corresponding deprotonated molecular ion at different ion source conditions. Sample: trilinolenoylglycerol; T, temperature; p, pressure.

Based on the ionization efficiency of TAG, the reactant gas pressure should be high and the ion source temperature low for maximal ionization. On the contrary, the formation of cluster ions is favored at low temperatures. Cluster ions disturb the molecular weight area of TAG in the spectra; thus, the formation of clusters should be eliminated with high enough ion source temperatures. TAG were most favorably ionized using the reactant gas pressure of approximately 8500 mtorr at the ion source temperature of 200°C with the instrument used, for the determination of molecular weight distribution of fats and oils. These conditions may serve as a guideline for optimization of the CI conditions for TAG, but, it may be necessary to make adjustments for these conditions depending on the sample and instrumentation. All experimental measurements described below were carried out at the optimized CI conditions.

Optimization of the probe heating rate. The effect of the heating rate of the rhenium wire of the direct exposure probe was studied with single standards (tristearoylglycerol, *rac*-1,2-dilinoleoyl-3-oleoyl-*sn*-glycerol, and trilinolenoylglycerol), a fifteen-component reference mixture (mixture B, diluted 1:9 with hexane, Table 1), black currant, and lingonberry seed oils. Unsaturated TAG are the most abundant constituents in most seed oils, containing even more than nine double bonds in their acyl chains. Therefore, it is of great significance to reduce thermal degradation or polymerization of these unsaturated molecular species during their desorption from the rhenium wire in the ion source. Heating rates from 2.5 to 100 mA s^{-1} were tested in this study. As an example, the influence of the probe heating rate on the proportions of RCO_2^-, $[M - RCO_2]^-$, and $[M - H]^-$ ions of the reference compound *rac*-1,2-dilinoleoyl-3-oleoyl-*sn*-glycerol is shown in Figure 2. We assumed that the proportions of RCO_2^- and $[M - RCO_2]^-$ ions would indicate the degree of thermal degradation of TAG. Surprisingly, the intensities of fragments resulting from the acyl loss of reference TAG, $[M - RCO_2]^-$, were very low, if found at all. The proportions of RCO_2^- and $[M - H]^-$ did not change significantly according to the rate of probe heating. However, the higher the heating rate the higher the abundance of $[M - H]^-$ ions. Such enhanced signal of the $[M - H]^-$ ions is essential to improve sensitivity. Very high heating rates resulted in bad peak shapes and high standard deviations because few data points only were acquired.

The effect of probe heating rate on the proportions of TAG of the fifteen-component reference mixture, according to the intensities of $[M - H]^-$ ions, is presented in Figure 3A. The proportions of unsaturated TAG with more than three double bonds were underestimated with a low heating rate of the probe. However, similar effect was not determined with the analyses of TAG of black currant seed oil (Fig. 3B) and lingonberry seed oil (Fig. 3C), although they contained around 62 and 82% TAG with six or more double bonds, respectively. An increase in the heating rate resulted in slightly reduced proportions of larger TAG, whereas those of smaller molecules increased. Overall, the heating rate of the probe did not significantly change the TAG distribution of the seed oils studied. It appears most appropriate to use a heating rate of around 40 mA s^{-1} of the direct exposure probe wire for the vaporization of TAG to obtain good sensitivity and reproducibility of the analysis and to minimize the discrimination of polyunsaturated TAG.

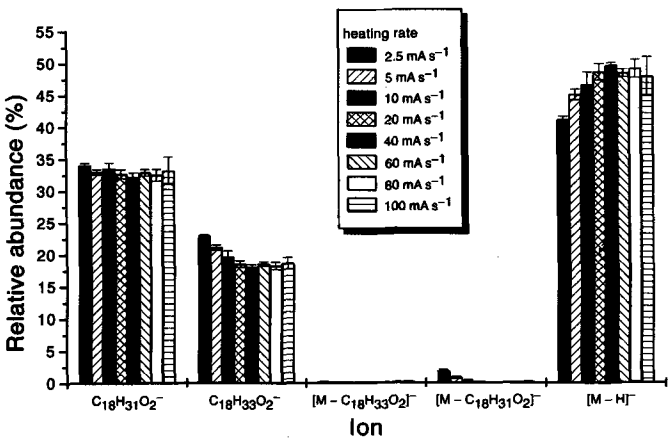


FIG. 2. The effect of the heating rate of the direct exposure probe on the proportions of RCO_2^-, $[M - RCO_2]^-$, and $[M - H]^-$ ions of *rac*-1,2-dilinoleoyl-3-oleoyl-*sn*-glycerol. Reactant gas pressure, 8500 mtorr; and ion source temperature, 200°C.

Effect of the sample amount. The effect of sample amount on the mass spectrometric response of TAG was tested with several dilutions of a mixture of reference components (mixture A, Table 1) concentrations varying from 2.6 to 379.6 μmol L^{-1} of each compound. A constant volume of a sample (0.5 μL) was applied to the rhenium wire; therefore, the amount of sample analyzed was changed by varying the concentration of the sample. An internal standard, *rac*-18:0-18:0-14:0, was added to each dilution (24.7 μmol L^{-1}) to eliminate inaccuracies in injection volumes.

The mass spectrometric response of the $[M - H]^-$ ions of TAG varied a lot as a function of the molecular size, with a slow probe heating (Fig. 4A). The response decreased with increasing molecular size, i.e., the total number of acyl carbons in TAG. As expected, the double bonds had a diminishing effect on the mass spectrometric response of unsaturated TAG (Fig. 4B). In addition to concentration, the effect of probe heating rate on the proportions of TAG was studied using slow (4 mA s^{-1}) and rapid (80 mA s^{-1}) heating (Fig. 5). Correction factors for each standard were calculated in relation to tripalmitoylglycerol (48:0) to determine the potential of this method for quantitative purposes (Table 2). When the sample was vaporized slowly, the proportions of unsaturated TAG were underestimated, being lower the more dilute the sample was. This resulted in large correction factors for unsaturated components (Table 2). The discrimination of polyunsaturated TAG can be eliminated by using rapid heating of a sample and a small sample amount. In this case, the correction factors of both saturated and unsaturated TAG were close to 1.0 (Table 2), i.e., the measured distribution of TAG was close to that of the composition of the mixture. The analysis of high-concentration samples using rapid heating of the probe resulted in increased proportions of smaller TAG and decreased proportions of larger molecules, as well as unsaturated components. Minor inaccuracies in the correction factors might be caused by sample preparation and impurities of the standards.

A few studies on the quantitative analysis of TAG with direct introduction inlet mass spectrometry have been published utilizing either EI (3) or ammonia CI (8,18). In these studies, the mass spectrometric response of TAG was reported to depend on the molecular structure, i.e., number of double bonds and molecular size; thus, correction factors had to be used for quantitative measurements. The results of the present study clearly show that the variation in the mass spectrometric response of TAG caused by differences in unsaturation and molecular size could be reduced, and nearly eliminated, by heating the probe wire very rapidly (Table 2), if the sample

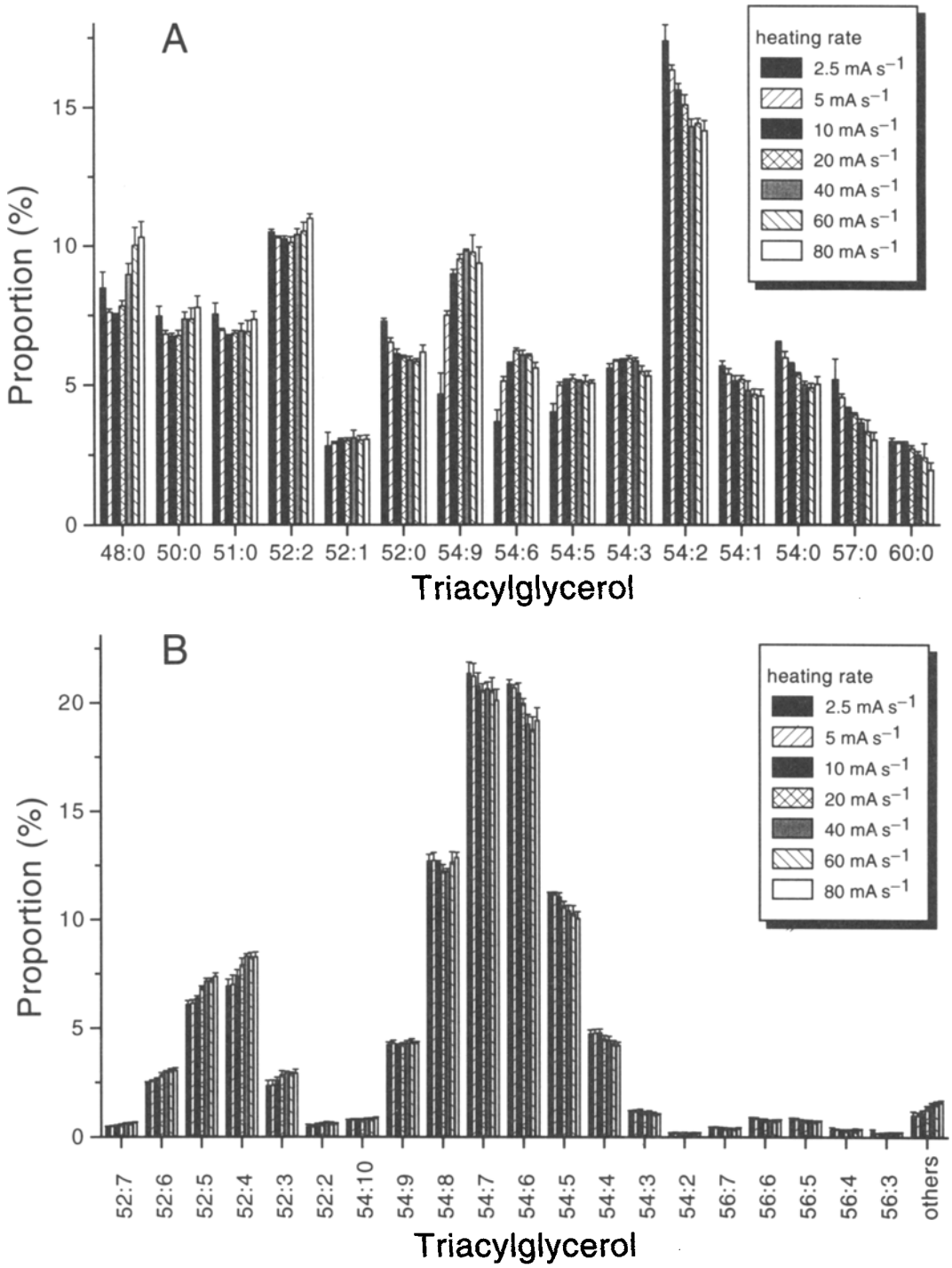


FIG. 3. The effect of the heating rate of the direct exposure probe on the distribution of triacylglycerols: A, reference mixture B (see Table 1), diluted 1:9 with hexane; B, black currant seed oil; and C, lingonberry seed oil. The proportions of triacylglycerols are based on the ^{13}C corrected abundances of $[M - H]^-$ ions of four analyses. Reactant gas pressure, 8500 mtorr; and ion source temperature, 200°C.

amount was small enough. Too small an amount of sample resulted in unacceptable standard deviation of the analysis. Based on the analysis of the TAG reference mixture, the appropriate concentration of the sample was approximately 100–600 ng μL^{-1}, i.e., 50–300 ng TAG per determination. In other words, only a few nanograms of a single TAG is required for production of a reliable spectrum. It is necessary to adjust the concentration of the sample to a range suitable for the analysis in order to avoid too large sample amounts. To compare TAG distributions, the concentration of each sample

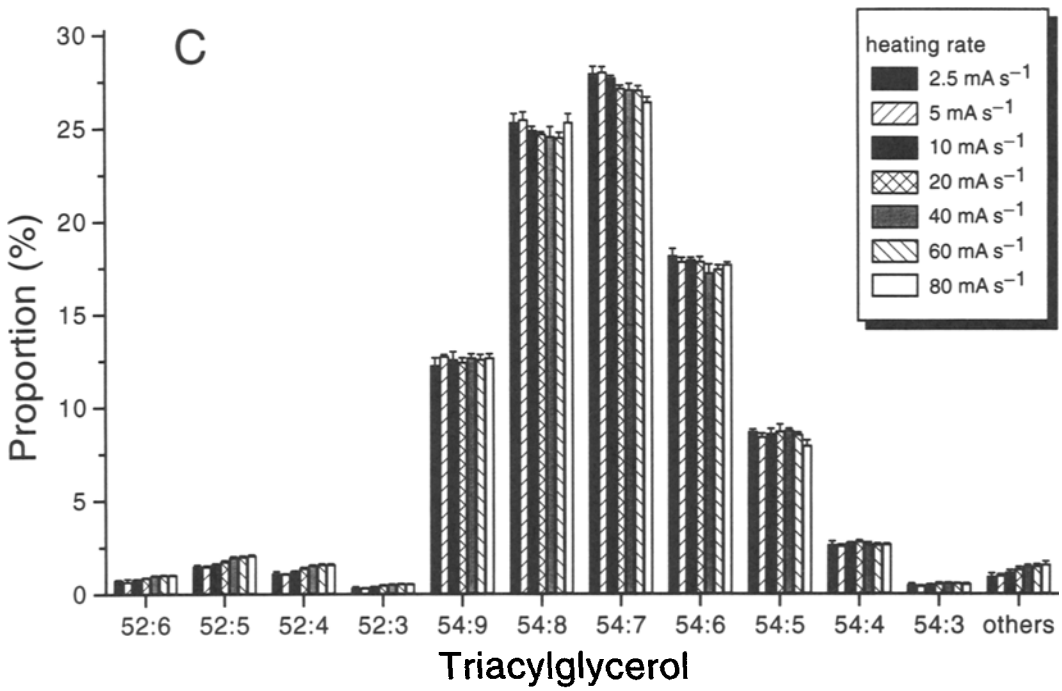
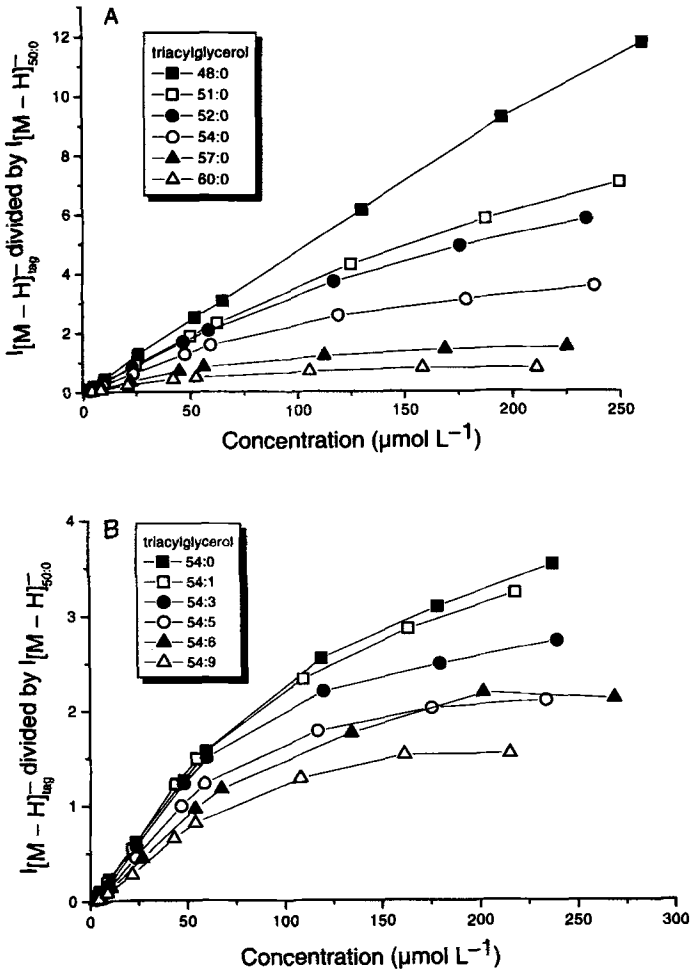


FIG. 3. (continued).



should be approximately the same to prevent mistakes in interpretation of the results caused by concentration differences. Under well-controlled conditions, the method is capable for semiquantitative determination of TAG mixtures of most fats and oils consisting of TAG with 50–56 acyl carbons without the use of correction factors.

Reproducibility of the analysis. The reproducibility of the mass spectrometric determination was studied with a mixture of fifteen TAG standards (mixture B, Table 1, Fig. 6A) and with the TAG isolated from the seeds of raspberry (Fig. 6B). The results are expressed as the mean of four analyses with error bars indicating the standard deviations. The coefficient of variation was typically less than 10% for the components comprising 1% or more of the total amount of TAG. In addition, the reproducibility of the determination from day to day and over longer periods was tested at different points of time. No major changes were observed in the molecular weight distribution of the TAG based on the averaged abundances of $[M - H]^-$ ions. The differences were more obvious with the fifteen-component mixture; nevertheless, reasonable reproducibility of the analysis was achieved in the *m/z* range, which covers the molecular weight area of TAG present in

FIG. 4. Response curves for the $[M - H]^-$ ions of A, the saturated triacylglycerols; and B, the unsaturated triacylglycerols having 54 acyl carbons (reference mixture A, see Table 1) with the addition of 24.7 μmol L^{-1} of *rac*-1,2-distearoyl-3-myristoyl-*sn*-glycerol as internal standard. A volume of 0.5 μL of the sample was applied to the rhenium wire of the direct exposure probe. Reactant gas pressure, 8500 mtorr; and ion source temperature, 200°C. The heating rate of the direct exposure probe was 4 mA s^{-1}; I = intensity; tag = triacylglycerol.

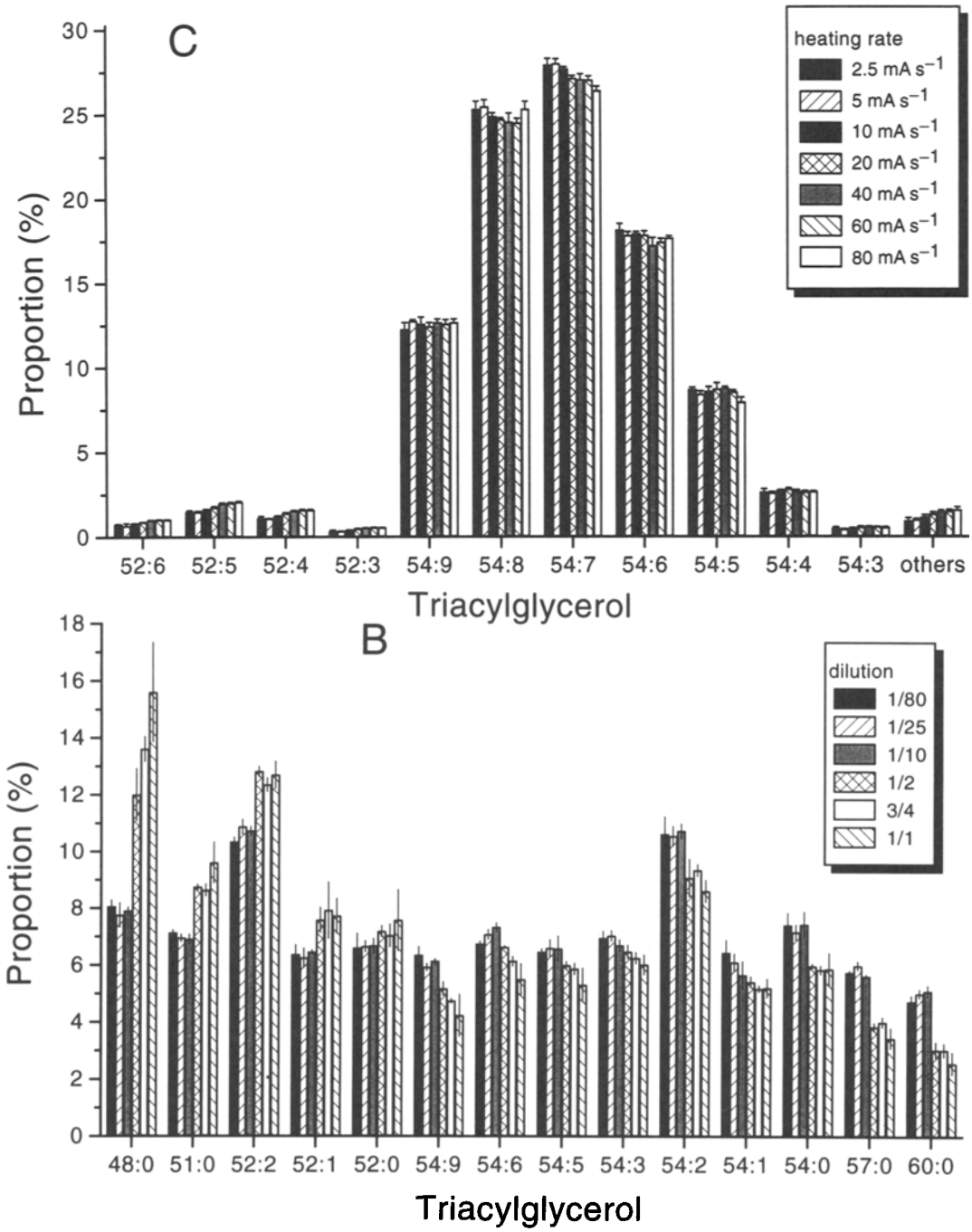


FIG. 5. The effect of concentration on the proportions of triacylglycerols of the reference mixture A (see Table 1) using A, a low (4 mA s^{-1}) and B, a high (80 mA s^{-1}) heating rate of the direct exposure probe. A volume of 0.5 µL of sample was applied to the rhenium wire of the direct exposure probe. The proportions of triacylglycerols are based on the ^{13}C corrected intensities of $[M - H]^-$ ions of four analyses. Reactant gas pressure, 8500 mtorr; and ion source temperature, 200°C.

most seed oils. Minor differences in the distribution may be explained by changes occurring in the sample as well as how the instrument has been tuned.

Mass spectrometric analysis of TAG using ammonia negative-ion CI is a fast and useful method to determine the molecular weight distribution of TAG. Information is obtained on both the number of acyl carbons and double bonds in TAG and the proportions of different molecular weight species. Although we have optimized the analytical conditions for the determination of TAG present in berry seed oils, the method can also be applied to other samples containing mainly TAG with 52, 54, and 56 acyl carbons. The analysis of more complex samples containing either a wide range of TAG with different molecular sizes, such as milk fat, or highly unsaturated

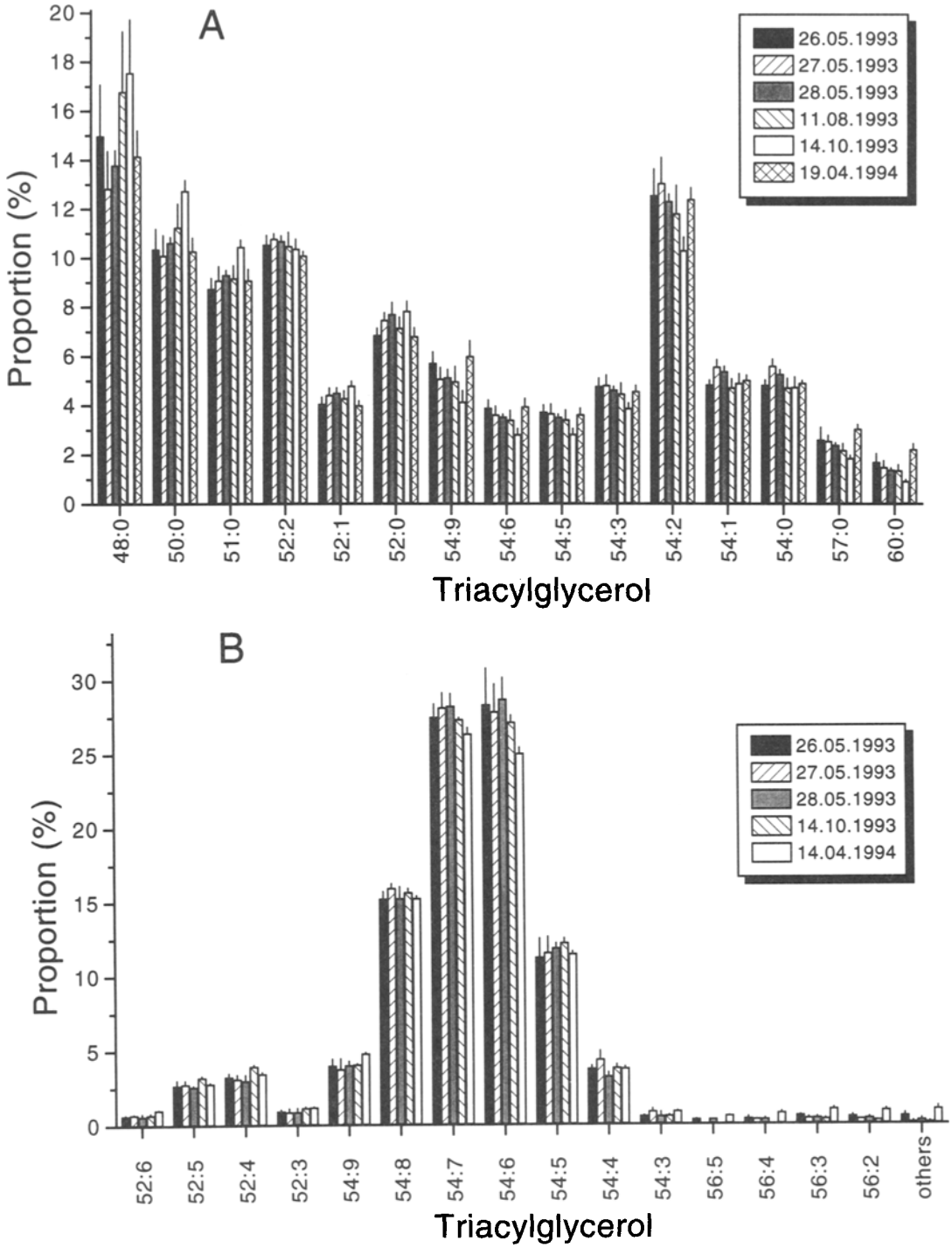


FIG. 6. The repeatability and reproducibility of the mass spectrometric determination for the molecular weight distribution of triacylglycerols: A, reference mixture B (see Table 1), and B, raspberry seed oil. The proportions of triacylglycerols are based on the ^{13}C corrected abundances of $[M - H]^-$ ions of four analyses. Reactant gas pressure, 8500 mtorr; and ion source temperature, 200°C.

components, such as fish oils, may require further optimization of the conditions. In the case of complex samples, it may be advantageous to separate or fractionate the samples, e.g., by chromatography prior to MS determination. This would facilitate the interpretation of the results. Compared with MS, unit resolution of TAG cannot be achieved with either GC or HPLC; GC is not suitable for the analysis of highly unsaturated TAG because of their thermal degradation and polymer-

TABLE 2
Correction Factors for Triacylglycerols in Relation to Tripalmitoylglycerol Using a Slow and Rapid Heating of the Direct Exposure Probe

	Correction factor[a]					
	Dilution[b]					
Triacylglycerol	1/80	1/25	1/10	1/2	3/4	1/1
Slow heating (4 mA s^{-1})						
48:0	1.00	1.00	1.00	1.00	1.00	1.00
51:0	1.22	1.08	1.12	1.25	1.22	1.17
52:0	1.17	1.10	1.06	1.33	1.30	1.27
52:1	1.17	1.12	1.13	1.31	1.33	1.29
52:2	1.49	1.26	1.24	1.39	1.46	1.44
54:0	1.24	0.98	1.14	1.54	1.61	1.63
54:1	1.33	1.09	1.12	1.58	1.68	1.70
54:2	1.43	1.15	1.17	1.45	1.58	1.59
54:3	2.09	1.47	1.38	1.63	1.83	1.93
54:5	3.14	1.85	1.81	1.91	2.22	2.34
54:6	5.04	2.80	2.18	2.21	2.57	2.75
54:9	8.03	4.51	2.85	2.53	2.95	3.18
57:0	1.68	1.36	1.40	2.20	2.61	2.88
60:0	2.22	1.64	1.52	2.65	3.47	4.09
Rapid heating (80 mA s^{-1})						
48:0	1.00	1.00	1.00	1.00	1.00	1.00
51:0	1.08	1.07	1.10	1.32	1.51	1.56
52:0	1.10	1.05	1.07	1.50	1.74	1.85
52:1	1.13	1.11	1.09	1.41	1.53	1.80
52:2	1.14	1.04	1.07	1.37	1.61	1.79
54:0	0.99	0.99	0.97	1.83	2.13	2.43
54:1	1.05	1.06	1.17	1.86	2.20	2.51
54:2	0.94	0.91	0.91	1.64	1.81	2.25
54:3	1.07	1.01	1.09	1.71	2.00	2.39
54:5	1.12	1.05	1.08	1.80	2.08	2.64
54:6	1.23	1.13	1.11	1.86	2.29	2.93
54:9	1.05	1.08	1.07	1.92	2.37	3.05
57:0	1.21	1.12	1.21	2.70	2.94	3.92
60:0	1.38	1.25	1.26	3.20	3.64	4.93

[a]The ^{13}C corrected abundances of $[M-H]^-$ ions were corrected to correspond an equimolar amount of each triacylglycerol before calculating the correction factors as the abundance of $[M-H]^-$ ion of 48:0 divided by the abundance of $[M-H]^-$ ion of the analyte.
[b]Different dilutions (vol_{stock}/vol_{tot}) of the reference mixture A (see Table 1).

ization at the temperatures needed for analysis; and a problem with HPLC is the lack of universal detector without sample discrimination, although light-scattering detectors are useful for many applications. In addition, the mass spectrometric analysis of TAG *via* a direct exposure probe is a very fast method—one analysis takes only 2–3 min. In this study, we have demonstrated that the analysis of TAG, without discrimination according to molecular size or unsaturation, is possible under well controlled mass spectrometric conditions (optimized CI conditions, small enough sample amount). These findings have not yet been verified with other instruments in other laboratories. However, similar results should be achievable elsewhere with comparable instruments after optimization of the analysis of TAG. This technique allows rapid determination of TAG profile, which is very informative in addition to the fatty acid composition of the sample, for example for the needs of quality control in the food industry. Molecular species information could be valuable to improve technological properties of various products or to design products for special purposes, e.g., milk substitutes for infants. This technique may also allow rapid determination of adulterations of various fats and oils, if there are enough differences in the native TAG compositions of the different raw materials.

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