Gas Chromatography–Chemical Ionization–Mass Spectrometric Fatty Acid Analysis of a Commercial Supercritical Carbon Dioxide Lipid Extract from New Zealand Green-lipped Mussel (*Perna canaliculus*)

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ABSTRACT: Supercritical fluid extracts of New Zealand green-lipped mussels (NZGLM) have been suggested to have therapeutic properties related to their oil components. The large number of minor FA in NZGLM extract was characterized by a GC–CIMS/MS method that excels at identification of double-bond positions in FAME. The extract contained five major lipid classes: sterol esters, TAG, FFA, sterols, and polar lipids. The total FA content of the lipid extract was 0.664 g/mL. Fifty-three unsaturated FA (UFA) were fully identified, of which 26 were PUFA, and a further 21 UFA were detected for which concentrations were too low for assignment of double-bond positions. There were 17 saturated FA, with 14:0, 16:0, and 18:0 present in the greatest concentration. The ten n-3 PUFA detected included 20:5n-3 and 22:6n-3, the two main n-3 FA; n-3 PUFA at low concentration were 18:3, 18:4, 20:3, 20:4, 21:5, 22:5, 24:6, and 28:8. There were 43 UFA from the n-4, n-5, n-6, n-7, n-9, and n-10 families, with 16:2n-4, 16:1n-5, 18:1n-5, 18:2n-6, 20:4n-6, 16:1n-7, 20:1n-7, 16:1n-9, 18:1n-9, and 20:1n-9 being the most abundant. In general, we estimated that FAME concentrations greater than 0.05% (w/w) were sufficient to assign double-bond position. In total, 91 FA were detected in an extract of the NZGLM, whereas previous studies of fresh flesh from the NZGLM had reported identification of 42 FA. These data demonstrate a remarkable diversity of NZGLM FA.

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The New Zealand green-lipped mussel (NZGLM), *Perna canaliculus*, is a bivalve native marine mussel from the mollusc family Mytilidae that is found in the deep-sea beds in the Hauraki Gulf in New Zealand's North Island waters (1). It is distinguishable from other bivalve species by the presence of a bright green stripe around the posterior ventral margin of the shell and its distinctive green lip, which is visible on the inside of the shell.

For a number of years, it has been claimed that preparations from the NZGLM have therapeutic value in the treatment of inflammatory conditions including arthritis (2) and asthma (3). Animal studies suggest a reduction in adjuvant-induced polyarthritis, collagen-induced arthritis, or carageenan-induced footpad edema when treated with extracts from the NZGLM (4,5). Two commercial products from the NZGLM have been produced. One is a stabilized freeze-dried powder extract known as Seatone® (MacLab, Abbotsford, Victoria, Australia); the other is a supercritical carbon dioxide oil extract from the freeze-dried stabilized powder, sold in capsules after the addition of olive oil and vitamin E, that is known as Lyprinol® (Pharmalink International, Queensland, Australia).

A recently developed CI–tandem MS (CI–MS/MS) method using acetonitrile reagent gas enables identification of the number and position of double bonds in FAME (6,7). In this method, acetonitrile self-reacts to produce the reagent (1-methyleneimino)-1-ethenylium of mass 54 *via* a previously described mechanism (8). The CI reaction yields diagnostic ions of $[M + 54]^+$, MH^-, $[MH - 32]^-$, and $[MH - 32 - 18]^-$, which are used to identify carbon chain length and number of double bonds. The $[M + 54]^+$ ion is isolated for MS^2 analysis, where fragmentation patterns vary depending on the number of double bonds. In monoenes, cleavage takes place at bonds allylic to the double bond; dienes exhibit fragmentation vinylic to the double bonds. For higher-order polyenes, cleavage is at sites vinylic to the second double bond from each end of the carbon chain. The two diagnostic ions, previously designated α (ester-containing) and ω (terminal methyl-containing), were used to identify the location of the double bonds within the FAME (7). Since full identification is possible using the highly predictable fragments, FA standards are not needed, and less common FA are readily identified. The advantages of this method have been discussed previously (7). This identification method has been used in several studies identifying FA within a sample, including those present at less than 1% (w/w), in bovine milk fat (9), mouse liver, primate brain (7), and other marine oils (10). The aim of the present study was to examine the FA in the main lipids found in the lipid extract from the NZGLM.

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Abbreviations: NZGLM, New Zealand green-lipped mussel; PL, polar lipid; SE, sterol ester; ST, sterol; UFA, unsaturated FA.

MATERIALS AND METHODS

The extract from NZGLM was obtained from McFarlane Marketing (Melbourne, Australia). Briefly, to obtain the lipid extract, mussels were harvested, crushed and stabilized, and centrifuged; the supernatant extract from the mussels was frozen and then freeze dried. The lipids from the freeze-dried powder were extracted in commercial quantities by supercritical CO_2.

TLC. TLC was used to separate individual neutral lipid classes [phospholipids, TAG, FFA, sterols (ST), and sterol esters (SE)] using a solvent system of hexane/diethyl ether/glacial acetic acid (60:17:0.1). Individual fractions from TLC separation were transmethylated with BF_3 (14%) for 10 min at 100°∞C. A complete oil sample plus an internal standard of tricosanoic acid (23:0) was treated similarly to the TLC fractions. Following methylation, heptane and a saturated NaCl solution were then added to the mixture. The organic layer containing FAME was then removed for analysis.
Instrumentation. In all analyses, a Varian Saturn 2000 ion trap mass spectrometer coupled to a Varian 3400 gas chromatograph (GC) equipped with a Varian 8200 autoinjector (Varian, Walnut Creek, CA) was used. The column was an SGE Chromatography Supplies (Austin, TX) BPX-70 (0.32 mm × 0.25 μm × 60 m). The GC oven temperature was ramped from 60 to 170°C at 50°C/min, held for 5 min, ramped to 200°C at 4°C/min, held for 5 min, ramped to 255°C at 49°C/min, and held for 9.18 min.

Analysis to determine FA identities was conducted using CI–MS with acetonitrile as the CI reagent. The "CI Auto" option in the software with the following CI parameters was used: CI storage level = 22.0 *m/z*, ejection amplitude = 8.0 V, background mass = 65 *m/z*, maximum ionization time = 2500 μs, maximum reaction time = 100 ms, target total ion chromatogram (TIC) = 5000 counts, and prescan ionization time = 100 μs. Additional mass spectrometer parameters were: emission current = 20 μA; mass defect = 116 mmu/100 u; trap temperature = 175°C; manifold temperature = 45°C; transfer line temperature = 200°C; axial modulation = 3.6 V. Through CI–MS, the chain length and number of double bonds were determined. In CI–MS/MS with acetonitrile, the $[M + 54]^+$ ion is trapped and then forms two diagnostic ions used to determine the location of the double bond (7).

Percent composition and concentrations of FA were determined through EI-MS. Samples were run in triplicate and peaks were integrated using Varian Saturn GC–MS Workstation software version 5.2.1. In these runs, each FA had a different ionization efficiency, resulting in peak areas not proportional to their concentration. To compensate for this, an equal-weight FAME mixture, 68A (Nu-Chek-Prep, Elysian, MN), was run along with samples to generate instrumental response factors. Response factors for all FA in the sample were based upon comparable FA in the 68A mixture. For FA not included in 68A, the response factor was interpolated from those directly calculated. Factors used in interpolation include: number of carbons, number of double bonds, and location of double bond relative to the end of the chain. All factors were normalized relative to 16:0. The raw peak area was multiplied by the appropriate response factor to generate peak areas proportional to the amount of FA in the sample. The adjusted peak areas were used to determine the percent composition of the FA in the sample. Concentrations were determined based on the known concentration of the internal standard added to the original sample.

RESULTS

The lipid extract contained five major fractions as separated by TLC, which corresponded to SE, TAG, FFA, ST, and polar lipids (PL). The total FA content of the lipid extract, derived from the analysis of the total FA, amounted to 664 mg/mL of the sample; SE was present at 41.4% (230 mg/mL); TAG, 7.7% (43 mg/mL); FFA, 46.8% (260 mg/mL); and PL, 4.1% (23 mg/mL). The identity of the bands between ST and PL has not been established, however they may be partial glycerides. In contrast to this commercial extract, the lipid extract of fresh NZGLM contains 57–62% phospholipids (11).

Table 1 shows all FA revealed from analysis, including 70 fully identified FA, and 21 for which it was not possible to assign the identification of the position of the double bonds. Sixteen unresolved FA pairs were present. Seventeen saturated FA were identified, the main ones being 14:0, 16:0, and 18:0. There were 10 n-3 PUFA identified, including EPA and DHA as the two main n-3 PUFA. Other n-3 PUFA included 18:3, 18:4, 20:3, 20:4, 21:5, 22:5, 24:6, and 28:8.

Thirty-nine UFA from the n-4, n-5, n-6, n-7, n-9, and n-10 families were also identified, with 16:2n-4, 16:1n-5, 18:1n-5, 18:2n-6, 20:4n-6, 16:1n-7, 20:1n-7, 16:1n-9, 18:1n-9, and 20:1n-9 being the most abundant FA in these six families.
Sixteen FA pairs could not be separated by this technique. The two most abundant were 20:5/20:4n-3 and 22:2/22:4. Fourteen other FA were present, and for these it was not possible to assign the position of the double bonds. The reason for this was their low proportion, in general less than 0.05% (w/w), in the total mixture. The 20:5 present in the unresolved peak was an unidentified isomer, separate from the major, fully resolved component, 20:5n-3.

Of the 91 FA reported, only 16 represented more than 1% of the total FA. In decreasing order of abundance, these were EPA, 16:0, DHA, 14:0, 16:1n-7, 18:0, 18:1n-5, 18:4n-3, 18:2n-6, 20:4n-6, 18:3n-3, 16:1n-5, 20:1n-9, 18:1n-9, 15:0, and 16:1n-9.

The FA of the four main FA-containing fractions were also analyzed, as shown in Table 2.**<<T2>>** The proportion of the main FA in these fractions was not the same, with the most obvious differences being for 14:0, which varied from 5.29% of PL FA to 8.72% of FFA; 16:0, which varied from 12.27% of PL FA to 23.04% of FFA; 20:5n-3, which varied from 16.89% for TAG to 26.91% for PL FA; and 22:6n-3, which varied from 11.40% for TAG to 18.44% for PL FA. In terms of FA composition, the FFA and TAG FA were the most similar of the four fractions.

TABLE 1
FA Composition and Concentration of the Total Lipids from a Lipid Extract of the New Zealand Green-Lipped Mussel[a]

FA	Weight %	mg/mL oil	FA	Weight %	mg/mL oil
12:0	0.07 ± 0.01	0.45 ± 0.03	15:1n-9	0.01 ± 0.01	0.08 ± 0.07
14:0	0.04 ± 0.00	0.25 ± 0.02	18:1n-9	1.24 ± 0.23	8.18 ± 1.50
14:0	0.00 ± 0.00	0.01 ± 0.01	20:1n-9	1.60 ± 0.09	10.6 ± 0.6
14:0	8.42 ± 0.14	55.7 ± 1.1	22:1n-9	0.01 ± 0.00	0.07 ± 0.01
15:0	1.05 ± 0.01	6.96 ± 0.09			
16:0	18.4 ± 0.2	122 ± 2	16:1n-10	0.43 ± 0.01	2.82 ± 0.06
17:0	0.13 ± 0.03	0.84 ± 0.16			
17:0	0.78 ± 0.02	5.17 ± 0.08	14:1n-7/15:0	0.07 ± 0.01	0.43 ± 0.05
18:0	3.34 ± 0.05	22.1 ± 0.3	15:0/15:1	0.08 ± 0.00	0.54 ± 0.02
20:0	0.05 ± 0.00	0.35 ± 0.02	15:1n-6/16:0	0.11 ± 0.00	0.74 ± 0.01
22:0	0.04 ± 0.00	0.25 ± 0.03	16:1n-9,7,5	10.3 ± 0.2	68.1 ± 1.6
			17:1n-9,11/16:2n-7	0.00 ± 0.00	0.00 ± 0.00
18:3n-3	1.61 ± 0.04	10.7 ± 0.2	16:2n-6/17:0	0.02 ± 0.01	0.16 ± 0.06
18:4n-3	2.70 ± 0.17	17.8 ± 1.1	17:1n-7,5	0.01 ± 0.00	0.08 ± 0.01
20:3n-3	0.05 ± 0.01	0.35 ± 0.09	17:1n-7,8	0.03 ± 0.00	0.16 ± 0.02
20:5n-3	21.2 ± 0.4	140 ± 3	18:0/17:1n-6	0.19 ± 0.00	1.23 ± 0.02
21:5n-3	0.52 ± 0.02	3.45 ± 0.10	16:3n-4/17:1n-4	0.18 ± 0.01	1.19 ± 0.04
22:5n-3	0.70 ± 0.02	4.64 ± 0.13	18:1n-11,9	0.14 ± 0.01	0.94 ± 0.04
22:6n-3	13.1 ± 0.1	86.5 ± 0.8	18:2n-6/19:1	1.74 ± 0.03	11.5 ± 0.1
24:6n-3	0.25 ± 0.02	1.66 ± 0.13	19:1n-9,8/18:3n-6	0.14 ± 0.00	0.92 ± 0.01
28:8n-3	0.18 ± 0.03	1.20 ± 0.17	20:2/19:4n-5	0.07 ± 0.01	0.43 ± 0.04
			20:3/21:1	0.18 ± 0.00	1.19 ± 0.03
16:2n-4	0.87 ± 0.00	5.75 ± 0.03	20:5/20:4n-3	0.43 ± 0.02	2.85 ± 0.12
18:2n-4	0.36 ± 0.01	2.38 ± 0.08	22:2/22:4	0.40 ± 0.02	2.64 ± 0.10
18:3n-4	0.25 ± 0.01	1.64 ± 0.03	22:5n-6/24:0	0.18 ± 0.01	1.16 ± 0.08
18:4n-4	0.00 ± 0.00	0.01 ± 0.00			
			12:1	0.01 ± 0.00	0.07 ± 0.03
14:1n-5	0.02 ± 0.00	0.10 ± 0.02	14:1	0.03 ± 0.00	0.18 ± 0.01
18:1n-5	3.15 ± 0.03	20.9 ± 0.3	15:1	0.00 ± 0.00	0.00 ± 0.00
			18:1	0.01 ± 0.00	0.05 ± 0.02
20:2n-6	0.37 ± 0.01	2.46 ± 0.06	18:1	0.04 ± 0.00	0.27 ± 0.01
20:3n-6	0.19 ± 0.01	1.22 ± 0.03	20:2	0.60 ± 0.04	4.00 ± 0.22
20:4n-6	1.73 ± 0.09	11.5 ± 0.6	22:1	0.00 ± 0.00	0.03 ± 0.00
22:4n-6	0.16 ± 0.01	1.03 ± 0.09	22:3	0.20 ± 0.01	1.35 ± 0.03
			22:3	0.37 ± 0.02	2.45 ± 0.10
18:1n-7	0.13 ± 0.00	0.86 ± 0.02	24:1	0.04 ± 0.04	0.26 ± 0.29
18:2n-7	0.03 ± 0.01	0.22 ± 0.07	22:5	0.03 ± 0.00	0.16 ± 0.01
19:1n-7	0.26 ± 0.01	1.69 ± 0.06	24:4	0.01 ± 0.01	0.09 ± 0.03
20:1n-7	0.90 ± 0.03	5.93 ± 0.18	24:5	0.10 ± 0.03	0.68 ± 0.17

[a]Results shown as mean ± SD of triplicate determinations.

DISCUSSION

The gas phase derivatization reaction developed in our laboratory (7) allowed for the more detailed FA profile. Previous analysis of fresh NZGLM flesh by nonpolar GC identified 42 FA, of which 16 accounted for greater than 1% of total FA (11). In the current analysis, no FA unique to the NZGLM were found. However, several less common FA were observed in the sample, including 14:1n-5, 14:1n-7, 15:1n-9, 16:2n-4, 16:3n-4, 17:1n-4, 17:1n-5, 17:1n-7, 17:1n-9, 17:1n-11, 18:2n-4, 18:3n-4, 18:4n-4, 19:1n-7, 19:4n-5, and 21:5n-3. The presence of these particular FA is consistent with previous analyses of FA in marine life, including the mussel species *Mytilus galloprovincialis* (12,13). There is no record of these FA appearing in mammals or other terrestrial species. As with previous occurrences of these FA, they appeared as a minor fraction of the total FA found and were present at less than 1% of the total lipid composition. Other samples have also been analyzed using this method to find minor FA components. Samples from golden algae (*Schizochytrium* sp.), primate brain white matter, and mouse liver were examined, and all showed a number of minor components (7).

In the analysis of saturated FA, four were found to appear in multiple peaks; 14:0 (3 peaks), 15:0 (3 peaks), 16:0 (3 peaks), 17:0 (3 peaks). This occurrence is most likely the result of branched-chain FA including isoprenoid FA, with different isomers being resolved in the chromatographic analysis. Our method applies only to UFA since reaction with a double bond is required, thus chain branching in saturated FA cannot be established. Murphy *et al.* (11) reported three branched-chain FA in fresh samples of the NZGLM (4,8,12–trimethyl tetradecanoic acid, i17:0, i18:0).

Omega-3 (n-3) FA made up a significant amount of the FA in the sample, with 40% of all FA being in this category. DHA and EPA accounted for 84% of the n-3 PUFA. EPA and DHA are also present in high amounts in other species, including

TABLE 2
FA Composition (%) of the Different Fractions from the TLC-Fractionated Lipid Extract from the New Zealand Green-Lipped Mussel[a]

FA	Phospholipids	FFA	TAG	Sterol esters
12:0	0.17 ± 0.00	0.09 ± 0.01	0.07 ± 0.02	0.19 ± 0.01
14:0	0.06 ± 0.00	0.03 ± 0.00	0.02 ± 0.01	0.07 ± 0.00
14:0	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.00
14:0	5.34 ± 0.21	8.80 ± 0.24	7.18 ± 0.10	7.44 ± 0.19
15:0	0.59 ± 0.00	1.11 ± 0.04	0.83 ± 0.01	0.79 ± 0.00
16:0	12.37 ± 0.02	23.24 ± 0.41	20.90 ± 0.26	15.86 ± 0.13
17:0	0.11 ± 0.01	0.11 ± 0.00	0.14 ± 0.00	0.11 ± 0.01
17:0	0.38 ± 0.01	0.74 ± 0.03	0.86 ± 0.01	0.52 ± 0.00
18:0	2.16 ± 0.02	4.00 ± 0.03	5.67 ± 0.06	3.11 ± 0.05
20:0	0.04 ± 0.00	0.04 ± 0.00	0.10 ± 0.00	0.05 ± 0.00
22:0	0.05 ± 0.00	0.02 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
18:3n-3	1.94 ± 0.03	1.59 ± 0.01	1.26 ± 0.07	1.71 ± 0.01
18:4n-3	4.38 ± 0.04	2.50 ± 0.11	2.03 ± 0.07	3.69 ± 0.08
20:3n-3	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.02 ± 0.00
20:5n-3	27.13 ± 0.03	19.26 ± 0.28	16.69 ± 0.20	22.13 ± 0.19
21:5n-3	0.73 ± 0.02	0.45 ± 0.00	0.41 ± 0.01	0.56 ± 0.02
22:5n-3	0.88 ± 0.02	0.65 ± 0.01	0.71 ± 0.01	0.77 ± 0.01
22:6n-3	18.60 ± 0.17	11.94 ± 0.09	11.27 ± 0.27	14.66 ± 0.14
24:6n-3	0.13 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.16 ± 0.01
28:8n-3	0.10 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	0.07 ± 0.01
16:2n-4	0.87 ± 0.01	0.78 ± 0.03	0.64 ± 0.02	0.75 ± 0.00
18:2n-4	0.34 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.37 ± 0.00
18:3n-4	0.19 ± 0.00	0.17 ± 0.01	0.05 ± 0.01	0.16 ± 0.01
18:4n-4	0.03 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
14:1n-5	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
18:1n-5	2.06 ± 0.03	2.31 ± 0.07	2.43 ± 0.01	2.51 ± 0.04
20:2n-6	0.34 ± 0.01	0.37 ± 0.01	0.46 ± 0.00	0.42 ± 0.01
20:3n-6	0.22 ± 0.00	0.18 ± 0.01	0.24 ± 0.01	0.22 ± 0.00
20:4n-6	1.87 ± 0.02	1.86 ± 0.02	1.54 ± 0.04	1.74 ± 0.03
22:4n-6	0.16 ± 0.00	0.16 ± 0.00	0.20 ± 0.01	0.18 ± 0.01
18:1n-7	0.10 ± 0.00	0.13 ± 0.00	0.06 ± 0.02	0.13 ± 0.00
18:2n-7	0.02 ± 0.00	0.01 ± 0.00	1.11 ± 0.09	0.01 ± 0.00
19:1n-7	0.09 ± 0.15	0.00 ± 0.00	0.13 ± 0.11	0.00 ± 0.00
20:1n-7	0.69 ± 0.03	0.79 ± 0.03	1.16 ± 0.04	0.93 ± 0.03
15:1n-9	0.05 ± 0.01	0.02 ± 0.00	0.12 ± 0.01	0.19 ± 0.01
18:1n-9	1.06 ± 0.01	0.94 ± 0.05	1.35 ± 0.05	1.43 ± 0.01
20:1n-9	0.97 ± 0.03	1.29 ± 0.05	2.11 ± 0.08	1.47 ± 0.03
22:1n-9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
16:1n-10	0.48 ± 0.02	0.42 ± 0.01	0.66 ± 0.01	0.63 ± 0.01
14:1n-7/15:0	0.06 ± 0.00	0.07 ± 0.01	0.07 ± 0.00	0.06 ± 0.01
15:0/15:1	0.06 ± 0.00	0.08 ± 0.00	0.10 ± 0.00	0.07 ± 0.00
15:1n-6/16:0	0.07 ± 0.01	0.11 ± 0.00	0.10 ± 0.01	0.08 ± 0.00
16:1n-9,7,5	10.54 ± 0.09	10.62 ± 0.21	11.88 ± 0.30	11.63 ± 0.09
17:1n-9,11/16:2n-7	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.05 ± 0.00
16:2n-6/17:0	0.01 ± 0.00	0.01 ± 0.00	0.79 ± 0.01	0.00 ± 0.00
17:1n-7,5	0.01 ± 0.00	0.01 ± 0.00	0.29 ± 0.00	0.01 ± 0.00
17:1n-7,8	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
18:0/17:1n-6	0.16 ± 0.00	0.14 ± 0.01	0.24 ± 0.01	0.17 ± 0.00

(continued)

the bivalves *Ostrea lutaria* and *Crassostrea gigas*; in another New Zealand mollusc, *M. canaliculus*, DHA and EPA account for 85% of n-3 FA (14).

The very long chain FA 28:8n-3 was also found in this sample. This FA was discovered in dinoflagellate oils (10,15), as well as the in the Tasmanian blue mussel (*M. edulis)* (11). It

TABLE 2 ***(continued)***

16:3n-4/17:1n-4	0.18 ± 0.01	0.14 ± 0.00	0.15 ± 0.01	0.17 ± 0.00
18:1n-11,9	0.09 ± 0.00	0.12 ± 0.01	0.21 ± 0.00	0.15 ± 0.00
18:2n-6/19:1	1.73 ± 0.02	1.73 ± 0.03	1.72 ± 0.07	1.75 ± 0.02
19:1n-9,8/18:3n-6	0.18 ± 0.00	0.17 ± 0.01	0.19 ± 0.01	0.17 ± 0.01
20:2/19:4n-5	0.05 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.21 ± 0.00
20:3/21:1	0.11 ± 0.00	0.12 ± 0.00	0.11 ± 0.01	0.11 ± 0.00
20:5/20:4n-3	0.38 ± 0.01	0.28 ± 0.01	0.23 ± 0.00	0.28 ± 0.00
22:2/22:4	0.31 ± 0.01	0.37 ± 0.01	1.05 ± 0.02	0.68 ± 0.01
22:5n-6/24:0	0.13 ± 0.01	0.12 ± 0.00	0.12 ± 0.01	0.13 ± 0.00
12:1	0.03 ± 0.00	0.04 ± 0.00	0.02 ± 0.01	0.04 ± 0.00
14:1	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.01 ± 0.01
15:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:1	0.02 ± 0.00	0.03 ± 0.00	0.01 ± 0.01	0.03 ± 0.00
20:2	0.46 ± 0.01	0.56 ± 0.02	0.83 ± 0.03	0.78 ± 0.00
22:1	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:3	0.04 ± 0.01	0.39 ± 0.01	0.60 ± 0.01	0.03 ± 0.02
22:3	0.45 ± 0.02	0.18 ± 0.16	0.14 ± 0.12	0.11 ± 0.19
24:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
22:5	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
24:4	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
24:5	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01

[a]Results shown as mean ± SD of triplicate determinations.

has been suggested that the presence of 28:8n-3 in the mussel species is dietary in origin, with algae being a source (15). Since its recent discovery, it has been detected in marine oils but not in tissues of terrestrial mammals. Because it is 4-5 desaturated, 28:8n-3 is a structural analog of 22:6n-3; however, no physiological properties have yet been reported for this FA in mammals.

The technique used for this analysis allowed for a more detailed FA profile of the analyte. Previously, FA were quantified using GC–FID, and the GC–MS method used here was only used for qualitative determination of FA. Through the use of EI–MS, the amount of the various FA was determined. Response factors for the various FA were more difficult to determine. However, this method is advantageous also in that a smaller sample amount is required for analysis. In decreasing the sample concentration, GC resolution increased, permitting the observation of those FA present in small amounts, especially those present at less than 1% of the whole sample. Full identification was still not possible for some FA present in low concentrations owing to inefficient ionization or reaction with the CI reagent. In these cases, the amount of $[M + 54]^+$ ion formed was not enough to allow for proper MS–MS analysis.

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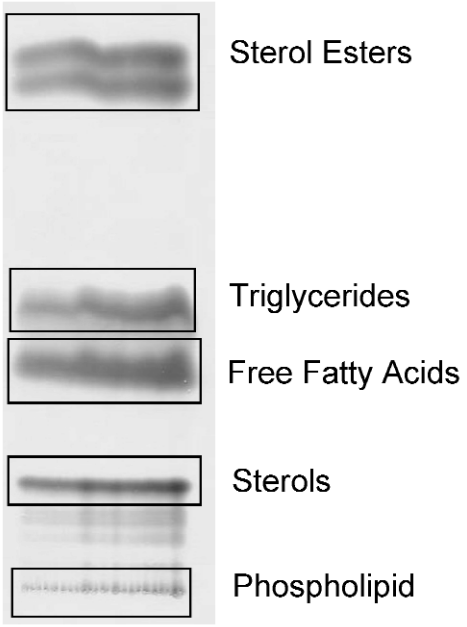

FIG. 1. Image of TLC plate with separated components from the lipid extract of the New Zealand green-lipped mussel.