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Regiospecific Analyses of Triacylglycerols of Hoki (*Macruronus novaezelandiae*) and Greenshell™ Mussel (*Perna canaliculus*)

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Abstract The lipid profiles of the two most important New Zealand marine oil sources were investigated, with particular attention to the regioisomeric compositions of triacylglycerides (TAG), using ^{13}C-nuclear magnetic resonance analysis. Oils from hoki (*Macruronus novaezelandiae*) and Greenshell™ mussel (*Perna canaliculus*) (GSM) were analyzed for their lipid content, lipid class and fatty acid profile. The regiospecific distribution of long chain (C ≥ 20) polyunsaturated fatty acids (LC-PUFA) between the *sn*-1,3 and *sn*-2 glycerol positions was calculated from ^{13}C responses in the carbonyl region in the triacylglycerol fraction. Rendered hoki oil (RHO) produced from the viscera and filleting discards, had a similar lipid profile to that of hoki liver oil (HLO) confirming that the liver is the major source of oil in RHO. The regioisomeric distribution of fatty acids showed differences between the two oil sources. Docosahexaenoic acid (DHA) had a regioisomeric distributional preference to the *sn*-2 position in TAG from all the oils (59.2% HLO, 54.3% RHO and 63.4% GSM). Eicosapentaenoic acid (EPA) had a more even distribution along the triacylglycerol backbone in hoki TAG (29.1% HLO, 33.6% RHO) while there was a slight *sn*-2 positional preference in the GSM TAG (37.6%). This regioisomeric information is vital to distinguish LC-PUFA-rich marine oils from other marine sources for authentication purposes.

Keywords Phospholipids · Triacylglycerols · Fatty acid composition · ^{13}C NMR · Eicosapentaenoic acid · Docosahexaenoic acid · Lipid molecular species

Abbreviations

ANOVA	1-way analysis of variance
CMO	Commercial mussel oil
DAGE	Diacylglyceryl ether
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid(s)
FALD	Dimethylacetals of aliphatic aldehyde(s)
FAME	Fatty acid(s) methyl ester
GSM	Greenshell™ mussel
HLO	Hoki liver oil
LC	Long chain (C ≥ 20) omega
MUFA	Monounsaturated fatty acid(s)
NMI	Non-methylene interrupted PUFA
n-3	Omega 3
n-6	Omega 6
PUFA	Polyunsaturated fatty acid(s)
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phophatidylinositol
PL	Polar lipid(s)
PS	Phosphatidylserine
RHO	Rendered hoki oil
SFA	Saturated fatty acid(s)
TAG	Triacylglycerol(s)
TLE	Total lipid extract(s)
tr	Trace amounts

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Introduction

There are many studies on the health benefits of increased consumption of long chain (C ≥ 20) omega 3 polyunsaturated fatty acids (n-3 LC-PUFA) from marine oils, especially docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3). It is now thought that health benefits of n-3 LC-PUFA, in particular DHA, are not only related to the amount consumed in the diet, but also to the positional distribution within the triacylglycerols (TAG) [1]. Fatty acids (FA) in the *sn*-2, "middle" position of the glycerol backbone are the most bio-available for human digestion [1]. Therefore, marine oils with large proportions of n-3 LC-PUFA in the *sn*-2 position are nutritionally more desirable.

Full regiospecific analysis of natural mixtures of TAG is a major challenge. The traditional method for analysis of FA (which destroys the regiospecific information on the TAG by trans-esterification to methyl esters) shows that marine oils contain complex mixtures of FA. For example, hoki liver oil (see below, Table 1) contains at least 12 different FA at >1% [2], which could give rise to many TAG with different permutations of composition and position. Some progress has been made on High performance liquid chromatography (HPLC) separation of complex natural TAG mixtures with regiospecific analyses by MS–MS, but better chromatographic resolution is needed [3]. ^{13}C-nuclear magnetic resonance (^{13}C NMR) spectroscopy offers an alternative approach, using natural mixtures of TAG analyzed without further separation. Aursand and co-workers have shown that in the ^{13}C-NMR spectra of TAG the carbonyl signals of the chief PUFA can be resolved, with distinct signals for *sn*-2 and *sn*-1,3 FA [4]. This has been confirmed by NMR analyses of synthetic structured TAG [5]. The relatively high price of fish oils with high LC-PUFA levels and differences in quality of oils from different origins and species has led to adulteration and mislabeling. DNA methods of identification are not possible in oils, as DNA is only present in trace concentrations, so ^{13}C-NMR carbonyl profiles along with total fatty acid analyses have been suggested for authentication of marine oils [6].

The major marine oils produced in New Zealand are from the fin-fish species hoki (*Macruronus novaezelandiae* Hector 1871, family Merlucciidae) and the Greenshell™ mussel (GSM, *Perna canaliculus* Gmelin 1791, family Mytilidae). Hoki is a hake species. It is known as blue grenadier in Australia, Langschwanz-Seehecht in Germany, nasello azzurro in Italy and merluza azul in Spain. Hoki fishing is one of the most important fisheries in New Zealand and has Marine Stewardship Council certification as being a well managed species. The quota varies

Table 1 Fatty acid content and lipid class composition (g/100 g oil) of rendered hoki oil (RHO) and hoki liver oil (HLO)

Fatty acid (g/100 g)	RHO	HLO	*f*
14:0	3.3 ± 0.1b	2.9 ± 0.1a	19.9
16:0	15.5 ± 0.4	13.9 ± 0.4	
18:0	2.4 ± 0.0b	1.8 ± 0.0a	539
Other SFA[a]	1.4 ± 0.0b	1.2 ± 0.0a	16.4
Total SFA	23.6 ± 0.4	21.8 ± 0.5	
16:1n-7	5.4 ± 0.1	5.6 ± 0.2	
18:1n-7	4.0 ± 0.0b	4.4 ± 0.1a	9.4
18:1n-9 OA	22.2 ± 0.2a	26.1 ± 0.3b	142
20:1n-9	10.6 ± 0.2a	12.2 ± 0.5b	13.6
22:1n-11	4.2 ± 0.1b	3.7 ± 0.1a	14.7
22:1n-9	1.3 ± 0.0	1.5 ± 0.1	
24:1n-9	1.4 ± 0.0b	1.1 ± 0.0a	22.6
Other MUFA[b]	1.9 ± 0.1	2.1 ± 0.1	
Total MUFA	51 ± 0.2a	56.8 ± 1.0b	53.4
18:3n-3 ALA	0.7 ± 0.0	0.6 ± 0.0	
18:4n-3 SDA	0.9 ± 0.0b	0.8 ± 0.0a	9.6
20:4n-3	1.7 ± 0.0	1.5 ± 0 1	
20:5n-3 EPA	6.0 ± 0.1	5.3 ± 0.4	
22:5n-3 DPA	1.7 ± 0.0	1.5 ± 0.0	
22:6n-3 DHA	12.4 ± 0.2b	10.5 ± 0.6a	9.5
Other n-3[c]	0.1 ± 0.0	0 ± 0.1	
Total n-3	23.3 ± 0.4b	20.2 ± 1.2a	8.7
Total n-3 LCPUFA	20.2 ± 0.3b	17.3 ± 1.0a	12.4
18:2n-6 LA	1.1 ± 0.0b	0.9 ± 0.0a	33.9
20:4n-6 AA	0.4 ± 0.0b	0.3 ± 0.0a	18.7
22:4n-6	0.2 ± 0.0	0.2 ± 0.1	
Other n-6[d]	0.7 ± 0.0a	0.9 ± 0.0b	60.4
Total n-6	2.4 ± 0.1	2.4 ± 0.1	
Other PUFA[e]	0.7 ± 0.1	0.9 ± 0.1	
Total PUFA	26.4 ± 0.4	23.4 ± 1.3	
Lipid class (g/100 g)[f]			
WE	2.0 ± 0.4	1.7 ± 0.3	
TAG	97.1 ± 0.4	97.8 ± 0.3	
FFA	0.1 ± 0.0	0.0 ± 0.0	
ST	0.7 ± 0.1b	0.3 ± 0.1a	12.4
PL	0.2 ± 0.1	0.1 ± 0.0	

Values are mean ± SEM, for five RHO and three HLO, each analyzed three times, so $n = 24$. Means in a row with different letters differ significantly as determined by Tukey–Kramer HSD, $P < 0.01$. *f* mean sum of squares

SFA saturated fatty acids; *MUFA* monounsaturated fatty acids; *PUFA* polyunsaturated fatty acids; *DHA* docosahexaenoic acid; *DPA* docosapentaenoic acid; *EPA* eicosapentaenoic acid; *SDA* stearidonic acid; *LA* linoleic acid; *ALA* α-linolenic acid; *AA* arachidonic acid; *WE* wax ester; *TAG* triacylglycerol; *FFA* free fatty acid; *ST* sterol; *PL* polar lipid

[a] Includes 15:0, 17:0, 20:0, 22:0 and 24:0

[b] Includes 16:1n-9, 16:1n-5, 18:1n-5, 20:1n-7, 22:1n-7, 22:1n-11 and 24:1n-11

[c] Includes 21:5n-3 and 24:6n-3

[d] Includes 16:2n-6, 18:3n-6, 20:2n-6, 22:2n-6 and 24:5n-6

[e] Includes 16:2n-4, 16:3n-4 and 18:2n-9

[f] Determined by TLC–FID

depending on stocking information and was set at 110,010 metric tonnes for the 2009/10 fishing season [7]. Hoki liver oils have been reported to have total PUFA concentrations of 19–24 g/100 g, with 9–12 g/100 g DHA and 5–6 g/100 g EPA [2]. Fish oil from rendered hoki offal is sold either as TAG, or as enriched n-3 LC-PUFA ethyl esters by trans-esterification and molecular fractionation. The lipid class profile and regioisomeric distribution of the n-3 LC-PUFA in hoki oils have not been reported previously.

GSM is native to New Zealand and is an important aquaculture species. It was estimated to be worth $NZ 175 million in exports in 2007 [8]. GSM are largely exported as frozen or live whole foods but are also sold in the form of lipid extracts, e.g. Lyprinol®, and mussel powders, e.g. Seatone®. GSM oil is recognized for its anti-inflammatory properties including benefits against arthritis [9]. The lipid class profile of GSM has been reported as 57.1–61.7 g/100 g polar lipids (PL) and 17.8–25.3 g/100 g TAG, with the remainder being 5.5–6.8 g/100 g sterols, 9.6–14.9 g/100 g free fatty acids and trace amounts of wax esters [10]. A separate study found that GSM lipids contained total PUFA concentrations of 19–27 g/100 g, with 6–11 g/100 g DHA and 8–10 g/100 g EPA [2]. As with hoki, there is no published information on the regioisomeric distribution of these FA in GSM oils.

This study assessed lipid class, fatty acid profile and regioisomeric distribution of LC-PUFA in hoki and GSM oils. Rendered hoki oil (RHO rendered from whole offal) was compared with oil extracted from hoki liver (HLO) to assess the role the lipid-rich liver has on the overall lipid profile. GSM solvent-extracted oil was compared with commercial mussel oil (CMO, Lyprinol®). This is the first study looking at Southern Hemisphere marine species by regiospecific analysis of LC-PUFA. This assessment of commercial and extracted oils will provide vital information to distinguish between sources of marine oils for authentication purposes.

Experimental Procedures

Sampling

GSM were harvested from the Marlborough Sounds in August and September 2009. After transport and dry storage, mussels were rehydrated for 24 h in 100-L bins supplied with 10 L/min filtered seawater on a flow-through basis with auxiliary aeration. Three replicate batches of 20 mussels each were collected and shelled, the weight of mussel meat was measured, then each composite sample was homogenized and the oil extracted immediately.

Fresh hoki livers and rendered oils were sourced from Sealord Group Ltd (Nelson, New Zealand). Five rendered hoki oils (RHO), produced by crushing hoki offal (waste, frames and guts) and separating the oils, were obtained from the Sealord rendering plant on separate days between the 5th and 11th of August 2009. Three batches of fresh hoki livers were obtained from the Sealord filleting line over the same period. These samples came from commercial catches of fish from different locations off the west coast of New Zealand. Capsules of commercial mussel oil (CMO, Lyprinol®, Batch C8016, Pharmalink International Ltd, Auckland, New Zealand) were purchased from a local retail outlet. Each capsule was stated to contain 50 mg of GSM extract, 100 mg of olive oil and 0.225 mg of vitamin E (alpha-tocopherol).

Lipid Extraction, Fractionation and Fatty Acid Analysis

GSM oils were extracted by a modified Bligh and Dyer protocol [11]. A single phase extraction, $CHCl_3$/MeOH/H_2O (1:1:0.9 by vol), was used to yield a total lipid extract (TLE). Hoki livers were homogenized then centrifuged at 10,000 *g* for 10 min, then hoki liver oils (HLOs) were decanted off from the solid and aqueous phases.

Lipid classes were analyzed with an Iatroscan MK V thin-layer chromatography–flame ionization detector (TLC–FID) analyzer (Iatron Laboratories, Japan). Samples were spotted onto silica gel SIII Chromarods (5 μm particles size) and developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the lipid separation was hexane: diethyl ether: acetic acid (60:17:0.1, v/v/v). After development for 25 min, the Chromarods were oven-dried and analyzed immediately to minimize adsorption of atmospheric contaminants. Lipid classes were quantified by DAPA software (Kalamunda, W.A., Australia). The FID was calibrated for each compound class with phosphatidylcholine; cholesterol; cholesteryl ester; oleic acid; hydrocarbon (squalene); wax ester (WE) (derived from fish oil); triacylglycerol (TAG, derived from fish oil); and diacylglyceryl ether (DAGE, purified from shark liver oil).

An aliquot of TLE and each lipid fraction was transmethylated in methanol: chloroform: hydrochloric acid (10:1:1, v/v/v) for 1 h at 100 °C. After addition of water the mixture was extracted three times with hexane: chloroform (4:1, v/v) to obtain fatty acid methyl esters (FAME). Samples were made up to 1 mL with an internal injection standard (23:0 or 19:0 FAME) and analyzed by gas chromatography (GC) performed using a Shimadzu 2010 GC with an Restek GTx silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness), and mass spectroscopy (MS) on a Shimadzu 2010 QP GC–MS. Samples (1 μL) were injected via a splitless injector at 220 °C. The column temperature program was: 60 °C at 0 min; 40 °C min^{-1} to 100 °C; then 10 °C min^{-1} to 170 °C;

then 5 °C min^{-1} to 185 °C; 2 min hold; then 3 °C min^{-1} to 197 °C; then 0.5 °C min^{-1} to 199 °C; 1 min hold; then 5 °C min to 230 °C; 3 min hold; then 5 °C min^{-1} to 250 °C; 5 min hold. Helium was the carrier gas. GC results were typically repeatable to within ±5% of the individual component area for replicate analyses.

Lipid classes were fractionated by solid phase extraction (SPE) silica column chromatography. TLE was applied to a 500 mg silica SPE column (Alltech, Deerfield, Illinois, USA) and separated into neutral lipids, glycolipids and polar lipids in a stepwise elution of chloroform (10 mL), acetone (20 mL) and methanol (10 mL), respectively [12].

Regiospecific Analysis of Triacylglycerols using ^{13}C-NMR Spectroscopy

TAG were stored at −20 °C until immediately prior to sample preparation. Only one CMO sample was tested as all the capsules came from the same batch. Approximately 120 mg of each TAG sample was transferred to a 5-mm NMR tube and dissolved in $CDCl_3$ (700 μL). ^{13}C-NMR spectra were obtained using a Varian 500 MHz VNMRS system operating at 125.7 MHz for carbon, using a 1H-19F/15N-31P 5 mm one NMR^{TM} probe, and the following parameters: 64 K data points, ^{13}C excitation pulse 60°: sweep width −10 to +200 ppm; acquisition time 1.23 s; relaxation delay 0.5 s; 12,000 scans; 0.2 Hz exponential line broadening. Carbonyl signal assignments were made according to Aursand [13], after referencing to the singlet arising from C1 of 22:6n-3 in the *sn*-2 position of TAG at 172.13 ppm, with the following integration regions: 173.29–173.21, other *sn*-1,3; 173.21–173.17, 20:4n-3 *sn*-1,3; 173.17–173.13, 22:5n-3 *sn*-1,3; 173.07–173.04, 18:4n-3 *sn*-1,3; 173.01–172.98, 20:5n-3 *sn*-1,3; 172.88–172.81, other *sn*-2; 172.80–172.77, 20:4n-3 *sn*-2; 172.76–172.73, 22:5n-3 *sn*-2; 172.67–172.64, 18:4n-3 *sn*-2; 172.61–172.59, 20:5n-3 *sn*-2; 172.54–172.51, 22:6n-3 *sn*-1,3; 172.15–172.12, 22:6n-3 *sn*-2. Three sub-samples of one RHO TAG sample were analyzed at the start, middle and end of analyzing the other TAG samples, showing repeatability to within ±5% relative standard deviation for the proportions of the resolved fatty acid carbonyl signals.

Statistical Analysis

Mean values were reported plus or minus standard error of the mean. Percentage data were arcsin transformed prior to analysis. Normality and homogeneity of variance were confirmed and a comparison between means was achieved by 1-way analysis of variance (ANOVA). Multiple comparisons were achieved by Tukey–Kramer HSD (honestly significant difference). Significance was accepted as probabilities of 0.05 or less. Statistical analysis was performed using SPSS® statistics 17.0 software.

Results and Discussion

Hoki Oil Fatty Acid and Lipid Class Analyses

The hoki livers had an average oil content of 68.5 ± 1.1 g/100 g liver tissue ($n = 3$). The fish were caught betwen the 5th and 11th August (late winter) and their liver oil contents were in the range previously reported for livers of female hoki caught in September [2].

The three HLOs and five RHOs were analyzed for individual fatty acids by GC and for lipid class composition by TLC–FID (Table 1). The HLOs had very similar fatty acid profiles to those previously reported [2]. There were no major ($P < 0.01$) significant differences in the fatty acid profile of the RHOs from the commercial rendering plant over five days, even though it is most probable that the hoki were caught from different locations around New Zealand (there is no GPS information on catch sites). When results for the five RHOs were combined, slight but significant differences were shown when compared with HLOs (Table 1). The key differences were in the total monounsaturated fatty acids (MUFA) (51.0 g/100 g RHO compared with 56.8 g/100 g HLO) and in the n-3 PUFA (23.3 g/100 g RHO compared with 20.2 g/100 g HLO). The slightly higher omega 3 content of the rendered oils may be due to inclusion of omega 3 rich organs such as brains and eyes. Both types of hoki oils in this study have substantial concentrations of EPA (5.3–6.0 g/100 g), DHA (10.5–12.4 g/100 g), and n-3 LC-PUFA (18.9–21.7 g/100 g).

There were only very minor differences in the lipid class profiles of RHOs and HLOs, indicating that the bulk of the rendered oils were most likely derived from the liver (Table 1). Both of the oils were predominantly TAG (97.1–97.8 g/100 g) with only minor amounts of other lipid classes present. The only significant difference in the lipid class profile was in the sterols (ST): 0.7 g/100 g RHO compared with 0.3 g/100 g HLO which may indicate that the sterol concentration is greater in the viscera than just in the liver. However, sterols in both RHO and HLO are a relatively minor lipid class (<1% of all lipid).

Mussel Oil Fatty Acid and Lipid Class Analyses

The average weight of the individual mussels in the three GSM composite samples was 52.9 ± 1.7 g with a condition factor of 0.31 ± 0.10. GSM produced 3.1 ± 0.4 g oil/100 g mussel meat via solvent extraction. This lipid content is higher than previously reported by Murphy et al.

[10], who found 1.79 ± 0.44 g oil/100 g or by McLean and Bulling [2], who found a seasonal range from 1.0 to 1.8 g oil/100 g. It is uncertain why there is an increase in oil content. The increase could be due to the extraction method or other factors such as season, temperature, sexual state or diet.

The GSM oils (TLEs) contained large amounts of n-3 LC-PUFA (37.8 g/100 g), predominantly EPA and DHA (Table 2). This is in agreement with the previously published analyses of GSM TLEs, which also found palmitic acid as the other main fatty acid [2, 10]. These GSM oils contained similar levels of TAG and polar lipids, with minor amounts of sterols and FFA (Table 2). By contrast, Murphy et al. [10] reported higher levels of phospholipids (around 60 g/100 g) than TAG (around 22 g/100 g) in their GSM oil samples. Seasonal, location, temperature, diet, storage time of samples and sexual maturation factors will all effect the ratio of TAG and PL, and any combination of these factors will affect the lipid class composition of GSM.

Not previously reported is the distribution of fatty acids in the different lipid classes of GSM. EPA levels were higher in TAG than in PL, but DHA levels did not differ significantly between these classes (Table 2). Consequently, n-3 LC PUFA levels were significantly higher in GSM TAG (39.4 g/100 g) than in PLs (33.3 g/100 g). This suggests that the mussel is storing these important FA as TAG rather than incorporating them into the membrane PL. Levels of non-methylene interrupted PUFA 20:2 NMI and 22:2 NMI were much higher in PL than in TAG. NMI are indicative of consumption of red algae and some zooplanktonic pteropods, and similar concentrations have been reported in gastropods and other molluscs [10, 14]. However, in the green abalone, *Haliotis fulgens*, there is some evidence that the 20:2 and 22:2 NMI may be metabolic products of desaturation of LC-MUFA [14] and therefore may be endogenously produced by the mussel.

The GSM polar lipid fraction contained a noteworthy amount (15.0 g/100 g) of dimethylacetals of aliphatic aldehydes (FALD), primarily 18:0 with minor components of 16:0, 18:1 and 20:0 (Table 2). These dimethylacetals are formed during the acidic methylation in preparation for GC–MS analyses, when the vinyl ether bond of plasmalogens is broken and aldehydes are generated. The presence of FALD was noted previously in GSM TLE, but levels were not given [10].

The CMO was Lyprinol®, which is a supercritical CO_2 extract of freeze-dried GSM powder with added olive oil and vitamin E [15]. The high level of olive oil (100 mg in 150 mg) in the CMO results in a high level of TAG with the FA profile dominated by oleic acid (18:1n-9, OA) and with a moderate amount of linolenic acid (18:2n-6 LA, Table 2). As expected from the published results on a supercritical CO_2 extract of GSM [15] the relative levels of the other lipid classes in CMO were FFA > ST > PL (Table 2). The high amount of olive oil clearly reduced the n-3 LC PUFA level in the CMO (9.7 g/100 g), with EPA (6.3 g/100 g) and DHA (3.0 g/100 g) still the dominant fractions. As expected, the composition of GSM TAG is more similar to that of the CMO than the PL fraction, due to TAG is more readily extracted by supercritical CO_2 [15].

Triacylglycerol Regiospecific Analyses by NMR

The TAG fractions from the three HLO, five RHO and three GSM oils were analyzed for their *sn*-1,3 to *sn*-2 ratios of important PUFA by ^{13}C-NMR spectroscopy (the PUFA levels in the CMO were too low to give reliable quantitation under the standard NMR conditions). Carbonyl peak assignments were made according to Aursand [13] and were checked by comparing the relative proportions (area of *sn*-1,3 plus *sn*-2 peaks of individual PUFA/total area of all area of *sn*-1,3 plus *sn*-2 carbonyl peaks) with results from GC–MS analyses of FAME from the same TAG fractions. The two methods showed good correlation for EPA ($r^2 = 0.98$) but not for DHA ($r^2 = 0.04$), as shown in Fig. 1. We do not know why this was, as close inspection of the NMR spectra did not suggest any overlapping signals. Sacchi et al. [16] and Aursand et al. [17] previously reported better agreement between NMR and GC methods for total fatty acid composition of tuna and salmon lipids, but only for one sample of each. The results are summarized in Table 3. If an FA is 33.3% in the *sn*-2 position, there is a random distribution of that FA across the three positions of the glycerol molecule rather than some regiospecificity in the biosynthesis.

These first regiospecific analyses of hoki fish oils demonstrated that DHA was preferentially located at the *sn*-2 position in both RHO (59.2%) and HLO (54.3%) (Table 3). The preference of EPA for the *sn*-2 position was lower or close to random in these same fish oils (RHO 29.1% and HLO 33.6%). The preference of the other FA in hoki oils, SFA and MUFA, for the *sn*-2 position was also lower or close to random (Table 3).

This preference of DHA for the *sn*-2 position in hoki oils was also found in all other reported regiospecific analyses of fish TAG: Atlantic salmon (76% *sn*-2), mackerel (75%), herring (>90%) [6]; cod liver (74%) [18]; and tuna (66%) [16]. Conversely, in these studies EPA had a reduced preference for the *sn*-2 position, with 47, 30 and 68% for salmon, mackerel and herring, respectively [6]. Atlantic salmon is the most widely reported marine oil analyzed regiospecifically by ^{13}C-NMR analysis. Similar profiles have been found for different tissues, farmed and wild fish, and from salmon grown at elevated temperatures [6, 17, 19–21]. As TAG are broken down by lipases in the fish gut,

Table 2 Fatty acid content and lipid class composition (g/100 g) of total lipid extract (TLE), triacylglycerol (TAG) and polar lipid (PL) fractions of Greenshell™ mussel (GSM) oil and commercial mussel oil (CMO)

Fatty acids (g/100 g)	GSM TLE	GSM TAG	GSM PL	CMO TLE	CMO TAG	CMO PL	*f*
14:0	4.8 ± 0.4b	6.0 ± 0.3b	1.4 ± 0.1a	1.7 ± 0.0a	1.5 ± 0.0a	5.4 ± 0.4b	67.7
16:0	15.70 ± 7b	15.5 ± 0.7b	15.5 ± 0.9b	11.9 ± 0.0a	11.4 ± 0.0a	19.1 ± 1.2	8.0
18:0	4.0 ± 0.1b,c	3.4 ± 0.1a,b	4.1 ± 0.3b,c	3.0 ± 0.0a	2.9 ± 0.0a	4.3 ± 0.1c	8.2
Other SFA[a]	2.9 ± 0.0b	1.9 ± 0.0a,b	6.6 ± 0.1c	0.6 ± 0.0a	0.6 ± 0.0a	1.8 ± 0.0a,b	29.7
Sum SFA	27.4 ± 1.0b	26.9 ± 0.8b	27.7 ± 1.2b	17.2 ± 0.1a	16.4 ± 0.1a	36.6 ± 1.5c	24.8
16:1n-7	9.0 ± 0.4b	11.8 ± 0.4c	2.6 ± 0.4a	4.1 ± 0.0a	4.0 ± 0.0a	8.2 ± 0.6b	115.1
18:1n-7c	2.6 ± 0.1b	2.9 ± 0.2b	1.1 ± 0.1a	4.0 ± 0.1c	4.0 ± 0.1c	4.1 ± 0.2c	116.5
18:1n-7t	0.5 ± 0.0a,b	0.6 ± 0.0b	0.2 ± 0.0a	0.4 ± 0.2a,b	0.4 ± 0.2a,b	0.3 ± 0.1a	8.9
18:1n-9 OA	1.1 ± 0.1a	1.3 ± 0.1a	1.1 ± 0.4a	55.4 ± 0.3c	55.9 ± 0.3c	27.8 ± 0.5b	9117.9
20:1n-9	2.4 ± 0.1b	2.3 ± 0.2b	2.4 ± 0.1b	0.9 ± 0.0a	0.8 ± 0.0a	1.4 ± 0.2a	26.7
20:1n-7	1.5 ± 0.2b	1.7 ± 0.1b	0.8 ± 0.0a	0.4 ± 0.0a	0.4 ± 0.0a	0.8 ± 0.1a	22.4
Other MUFA[b]	0.8 ± 0.0	0.5 ± 0.0	1.4 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	
Sum MUFA	17.9 ± 0.4b	21.1 ± 0.6c	9.5 ± 0.6a	65.4 ± 0.3e	65.6 ± 0.3e	43.0 ± 0.5d	1306.0
18:3n-3 ALA	0.9 ± 0.0b,c	1.1 ± 0.0a,b,c	0.2 ± 0.1a	0.8 ± 0.2b	1.1 ± 0.2c,d	1.4 ± 0.1d	35.4
18:4n-3 SDA	2.8 ± 0.1c	3.2 ± 0.2c	1.0 ± 0.1a	0.7 ± 0.0a	0.7 ± 0.0a	1.9 ± 0.0b	101.2
20:4n-3 + 20:2 NMI	1.0 ± 0.0b	0.9 ± 0.0b	1.5 ± 0.1c	0.2 ± 0.0a	0.2 ± 0.0a	0.4 ± 0.1a	116.2
20:5n-3 EPA	23.5 ± 1.3d	26.8 ± 1.0d	16.8 ± 0.4c	6.3 ± 0.1a	6.3 ± 0.0a	11.5 ± 0.8b	82.7
22:5n-3 DPA	1.4 ± 0.1b	1.2 ± 0.1b	2.2 ± 0.1c	0.2 ± 0.0a	0.2 ± 0.0a	0.3 ± 0.0a	94.0
22:6n-3 DHA	11.8 ± 0.7b	10.6 ± 0.9b	12.8 ± 1.1b	3.0 ± 0.0a	3.1 ± 0.0a	5.1 ± 0.4a	23.1
Sum n-3 PUFA[c]	41.5 ± 1.3d	43.8 ± 1.5d	34.6 ± 1.2c	11.2 ± 0.4a	11.6 ± 0.3a	20.6 ± 1.8b	115.2
Sum n-3 LC PUFA[c]	37.8 ± 1.1d	39.4 ± 1.2d	33.3 ± 0.8c	9.7 ± 0.2a	9.8 ± 0.2a	17.3 ± 0.8b	134.2
18:2n-6 LA	1.4 ± 0.1b	1.7 ± 0.1c	0.5 ± 0.0a	4.8 ± 0.0e	4.9 ± 0.0e	3.2 ± 0.0d	849.0
20:2n-6 AA	0.3 ± 0.0b	0.3 ± 0.0b	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.3 ± 0.0b	22.4
20:3n-6	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	
20:4n-6	1.1 ± 0.1c	0.8 ± 0.1b,c	1.7 ± 0.1d	0.2 ± 0.0a	0.2 ± 0.0a	0.3 ± 0.0a,b	39.3
Other n-6[d]	0.8 ± 0.0a	0.7 ± 0.0a	1.9 ± 0.2a	0.3 ± 0.0b	0.3 ± 0.0a	0.4 ± 0.0a	11.9
Sum n-6 PUFA	3.7 ± 0.3a	3.6 ± 0.3a	4.4 ± 0.4a,b	5.5 ± 0.0c	5.6 ± 0.0c	4.4 ± 0.1a,b	6.8
20:2NMI	1.2 ± 0.1b	0.5 ± 0.0a	3.3 ± 0.3c	0.1 ± 0.0a	0.2 ± 0.0a	0.3 ± 0.0a	95.5
22:2NMI	1.3 ± 0.1c	0.7 ± 0.1b	2.2 ± 0.1d	0.1 ± 0.0a	0.1 ± 0.0a	0.2 ± 0.1a	82.4
Other PUFA[e]	1.4 ± 0.0b	1.3 ± 0.0b	1.7 ± 0.1b	0.2 ± 0.0a	0.2 ± 0.0a	0.4 ± 0.0a	32.9
Sum PUFA	49.1 ± 1.2c	49.9 ± 1.4c	46.2 ± 1.3c	17.1 ± 0.4a	17.7 ± 0.4a	25.8 ± 1.9b	99.3
Other FA							
4,8,12 TMTD	1.0 ± 0.1a,b	1.0 ± 0.1a,b	1.5 ± 0.4b	0.2 ± 0.0a	0.2 ± 0.0a	0.3 ± 0.0a	8.7
16:0 OH	0.5 ± 0.1b,c	0.7 ± 0.1c	0.1 ± 0.0a,b	0.0 ± 0.0a	0.0 ± 0.0a	0.1 ± 0.0a,b	16.6
18:0FALD	3.6 ± 0.1b	0.3 ± 0.0a	12.6 ± 0.7c	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	247.8
Other FALD[g]	0.7 ± 0.0b	0.1 ± 0.0a	2.4 ± 0.1c	0.0 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	102.4
Total FALD	4.3 ± 0.1c	0.4 ± 0.0a	15.0 ± 0.4c	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	154.0
Lipid class (g/100 g)[f]							
TAG	49.2 ± 3.0a			93.1 ± 2.2b			972.5
FFA	2.9 ± 0.6a			5.0 ± 2.6b			34.2
ST	5.4 ± 0.8b			1.2 ± 0.8a			42.7
PL	42.5 ± 2.9b			0.6 ± 0.2a			789.9

Values are mean ± SEM, for three each of GSM samples and one each of CMO samples, each analysed three times, so $n = 36$. Means in a row with different letters differ significantly as determined by Tukey–Kramer HSD, $P < 0.01$. Significance was calculated by Chi squared *t* test. *f* mean sum of squares

[a] Includes 15:0, 17:0, 20:0, 22:0 and 24:0

[b] Includes 16:1n-9, 16:1n-5, 18:1n-5, 20:1n-7, 22:1n-7, 22:1n-11 and 24:1n-11

[c] Includes 21:5n-3 and 24:6n-3

[d] Includes 16:2n-6, 18:3n-6, 20:2n-6, 22:2n-6 and 24:5n-6

[e] Includes 16:2n-4, 16:3n-4 and 18:2n-9

[f] Determined by TLC–FID

[g] Includes C17:0 FALD and C18:1 FALD

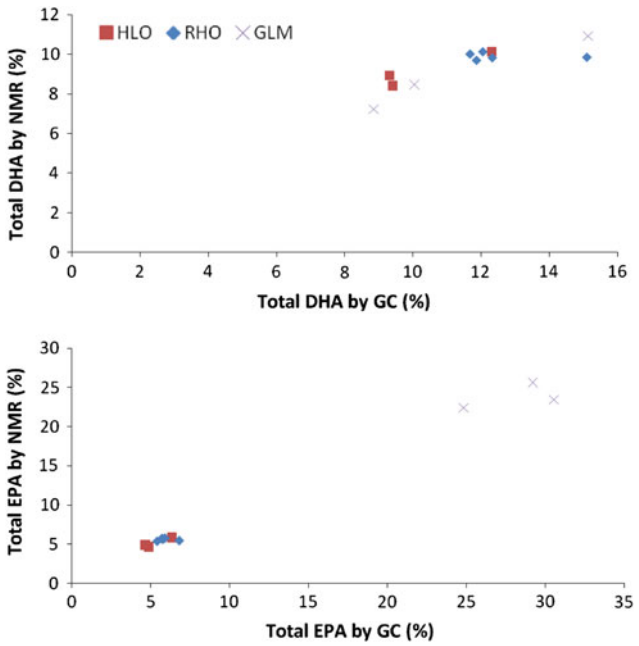

Fig. 1 Total (*sn*-1,3 plus *sn*-2) proportions of the LC-PUFA, DHA and EPA in the triacylglycerol fractions from rendered hoki oils (RHO), hoki liver oils (HLO) and Greenshell™ mussel (GSM) oils determined by GC and by ^{13}C-NMR spectroscopy

Table 3 Percentage distribution of fatty acid in the *sn*-2 position in the triacylglycerol fractions from rendered hoki oils (RHO), hoki liver oils (HLO) and Greenshell™ mussel (GSM) oils determined by ^{13}C-NMR spectroscopy

Fatty acid	HLO	RHO	GSM	*f*
22:6n-3 DHA	59.2 ± 1.1a	54.3 ± 2.1a	65.4 ± 1.1b	15.2
20:5n-3 EPA	29.1 ± 0.5	33.6 ± 1.0	37.6 ± 4.9	
18:4n-3 SDA	39.5 ± 1.3a	46.0 ± 1.4b	42.2 ± 1.1a,b	7.6
20:4n-6 AA	23.1 ± 1.4a	30.8 ± 0.8b	21.4 ± 2.8a	9.9
22:5n-3 DPA	48.8 ± 1.9	46.4 ± 4.1	39.1 ± 5.3	
Others	30.6 ± 0.2b	30.9 ± 0.1b	27.7 ± 1.6a	5.5

Values are mean ± SEM, for three HLO, five RHO and three GSM, so $n = 11$. Means in a row with superscripts without a common letter differ as determined by Tukey–Kramer HSD, $P < 0.05$

the structural rearrangement of FA on the glycerol backbone is achieved de novo by the fish. The analytical technique has been used to correlate (for authentication purposes) fish and/or oil capsules to the geographical region, country, and farm from where it was produced [22]. Regiospecificity would be an ideal method to identify adulteration of fish oil with that from marine mammals such as seal and whale. These oils have a contrasting profile to oil from fish, with DHA predominantly in the *sn*-1 and *sn*-3 positions [23].

We believe our regiospecific analyses of TAG from GSM oils to be the first for any molluscs. There was a preference for DHA at the *sn*-2 position (65.4%), whereas EPA distribution was close to random in GSM TAG (37.6%, Table 3). This *sn*-2 position preference of DHA and reduced preference in EPA is similar to fish oils [6, 16, 18, 21]. Due to the large size of the gonad in mussels and it being a good source of lipid, sexual development may play a significant role in FA distribution. It has been shown that GSM oil content is increased over summer and autumn seasons as they prepare to spawn [2]. Lipid stores for GML over the winter months are utilized for gonad production and stores will increase in mussels prior to spawning. Further work looking at the timing of spawning and the oil content and quality, in particular the regioisomeric distribution of n-3 LC PUFA, may be important to lipid content (in particular the n-3 LC PUFA), lipid class and regiospecific nature of oil from GSM.

Conclusion

An increased public and scientific knowledge of the beneficial properties of omega 3 and the resultant increase in nutraceutical supplementation, combined with an increase in price of fish oil (due to a combination of an increase in demand and a limit on resources), has raised the possibility of adulteration of premium n-3 LC PUFA-rich marine oils with oil from less expensive sources. It has been argued that for authentication purposes, regioisomeric information may be a useful tool to identify the adulteration of fish oils, as this characteristic is more difficult to manipulate than pure fatty acid composition [6]. Our results on hoki and GSM oils add to the database of regioisomeric TAG profiles for commercial marine species. In addition there needs to be an assessment of natural variability for each commercial species and an investigation into how factors such as sex, diet, environment, location and season affect the regioisomeric distribution of FA in the TAG.

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