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ORIGINAL PAPER

Transesterification of Fish Oil to Produce Fatty Acid Ethyl Esters Using Ultrasonic Energy

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Abstract This study evaluated the production of fatty acid ethyl esters from fish oil using ultrasonic energy and alkaline catalysts dissolved in ethanol. The feasibility of fatty acid ethyl ester production was determined using an ultrasonic bath and probe, and between 0.5 and 1% KOH (added to the fish oil). Furthermore, factors such as ultrasonic device (bath and probe), catalyst (KOH and C_2H_5ONa), temperature (20 and 60 °C), and duration of exposure (10–90 min) were assessed. Sodium ethoxide was found to be a more efficient catalyst than KOH when transesterifying fish oil. Ultrasonic energy applied for greater than 30 min at 60 °C using 0.8% of C_2H_5ONa as a catalyst transesterified over 98% fish oil triglycerides to fatty acid ethyl esters. It is reasonable to conclude that the yield of fatty acid ethyl esters produced by applying ultrasonic energy to fish oil is related to the sonication time. Due to increases in the surface area contact between the reactants and the catalyst, ultrasonic energy has the potential to reduce the production time required by a conventional large-scale commercial transesterification method that uses agitation as a way of mixing.

Keywords Transesterification · Ultrasonic energy · Fish oil · Polyunsaturated fatty acids · Base catalysts · Fatty acid ethyl esters

Introduction

Biodiesel refers to the fatty acid alkyl esters that are produced from vegetable oils or animal fats in the presence of an alcohol and a catalyst, and the most common method used to produce it is through transesterification of oil [1, 2]. It has been proposed that the transesterification occurs in three steps: (1) reaction of triglycerides (TG) and alcohol leading to diglycerides (DG) and one monoester; (2) DG react with alcohol and produce monoglycerides (MG) and monoester, and (3) MG react with alcohol and produce glycerol and monoester [3–5]. The complete mechanism to convert TG to glycerol and monoesters has complicated kinetics since the process comprises not less than twelve reaction equilibria [6].

Transesterification can be catalyzed by enzymes, acid, or base. However, both enzyme and acid catalysis are generally slower than the more commonly used base catalysts [7]. On the commercial scale, the common alkali catalysts are NaOH and KOH because of their low cost [6]. Stoichiometrically, transesterification of TG requires a 3:1 molar ratio of alcohol:oil. However, the transesterification reaction is an equilibrium reaction, thus an excess of alcohol is the most convenient approach to push the reaction towards the products [1, 6].

One particular technique used for the transesterification of vegetable oil is low frequency ultrasound instead of mixing and heating [6, 8]. Stavarache et al. [6] used various alcohols in a molar ratio of 6:1 of methanol:oil, and various concentrations of NaOH and KOH, and found that ultrasonics can accelerate the transesterification process by

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forming tiny droplets that increase the surface area for contact between the two phases (alcohol and oil). Colucci et al. [8] established that it is feasible to produce biodiesel from soybean oil using ultrasonic mixing, reporting a >99% conversion to fatty acid methyl esters (FAME) when using ultrasonic energy (using a probe) for more than 15 min at 40 °C and 1.5% KOH as a catalyst.

Studies of the ultrasonically assisted transesterification process of TG have been executed mainly on vegetable oils [1, 4, 6–8] and never on fish oils. In fish oil, more than 60 different fatty acids have been identified, and more than 80–85% of them are represented by four groups of fatty acids: (1) C14:0 and C16:0, (2) C16:1 and C:18:1, (3) C20:1 and C22:1, and (4) C20:5, C22:5 and C22:6 [9]. Furthermore, fish oil is rich in the ω-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which represent more than 90% of all polyunsaturated fatty acids (PUFA) in fish oil [10]. EPA and DHA have been reported to provide beneficial human health effects [11]. Fatty acid ethyl esters (FAEE) can be used for food applications and our study assesses their possible production from fish oil by ultrasonic means, which could have a potential application in the production of high-purity ω-3 free fatty acids such as EPA and DHA. The aim of this research was to evaluate the feasibility of producing FAEE from fish oil by using ultrasonic energy and a base catalyst. Furthermore, this study evaluated the effect of ultrasonic devices, and factors such as catalyst, temperature and sonication time, on the transesterification of fish oil with ethanol.

Experimental Procedures

Materials

Deodorized fish oil 1812 TG (18 and 12% of EPA and DHA, respectively) was manufactured at Ocean Nutrition Canada (ONC), Mulgrave, NS, Canada. This fish oil has 0% of FAEE and approximately 100% of TG. Also, ONC distilled fish FAEE fractions (>99% FAEE) (Mulgrave, NS, Canada), produced by transesterifying fish oil via conventional means (using mechanical stirring and a temperature above 60 °C) were used for comparison to all samples generated from this study (using ultrasonic energy). All solvents and catalysts were obtained from Sigma-Aldrich (St Louis, MO) unless stated otherwise.

Characterization of Oil

The molecular weight of fish oil was calculated through a GC analysis performed using a Varian gas chromatograph 3600 equipped with a 0.20 μm film thickness fused silica capillary column 100 m × 0.25 mm (SP-2560, Supelco/Sigma-Aldrich, St Louis, MO). Oligomers (polymers) and conjugated dienes were performed following AOCS official methods Cd 22-91 and Ti 1a-64, respectively [12]. The polyene index [ratio of PUFA percentage (sum of EPA and DHA) to saturated fatty acids (C16:0) percentage] was determined. Furthermore, the milligrams of KOH required to neutralize free fatty acids in 1 g of oil (acid value) were determined following the AOCS official method Cd 3d-63 [12].

Feasibility of Using Ultrasonic Energy to Produce FAEE from Fish Oil

For both ultrasonic bath (U/S) and probe (U/S–P), a kinetic controlled transesterification reaction was performed using a 6:1 molar ratio of ethanol:fish oil, and either 0.5, 0.75 or 1% KOH with respect to the fish oil weight. Fish oil (45 g) (approximately 0.05 mol) was placed in three 250 mL Erlenmeyer flasks for the U/S, and round bottom flasks for the U/S–P experiments. Each flask, containing the fish oil, was placed in the U/S or subjected to an U/S–P as indicated in Fig. 1. The solution of KOH in ethanol (0.225, 0.338 and 0.45 g of KOH dissolved in 17.25 mL of ethanol) was added to the fish oil and sonicated for 30 min (indirect sonication at a frequency of 35 kHz for the U/S and direct sonication at a frequency of 20 kHz for the U/S–P) at 20 °C. Eighty milliliters of a 2% citric acid solution were added and mixed gently for 30 min to neutralize the catalyst. The reaction mixture was transferred to a 100 mL graduated cylinder, the glycerol was allowed to separate (2 h), then the top layer (FAEE) was washed with 20 mL of water, letting the sample sit (1 h) after washing. The FAAE layer recovered was then analyzed by HPLC and GC.

In addition, the feasibility of using a two-stage procedure was tested. Half of the ethanol/KOH stock solution (17.25 mL of ethanol or 0.3 moles of ethanol with either 0.5, 0.75 or 1% KOH with respect to the fish oil) was added to the fish oil and sonication applied for 15 min. In the second stage, the other half of the ethanol/KOH stock solution was added to the mix, and another 15 min of sonication applied.

TLC Assessment of the Ultrasonically Assisted Transesterification

The TLC procedure was performed by first drying a 20 × 10 cm silica gel 60 F254 glass TLC plate (Merck, Gibbstown, NJ) in a forced air-drying oven for 1 h at

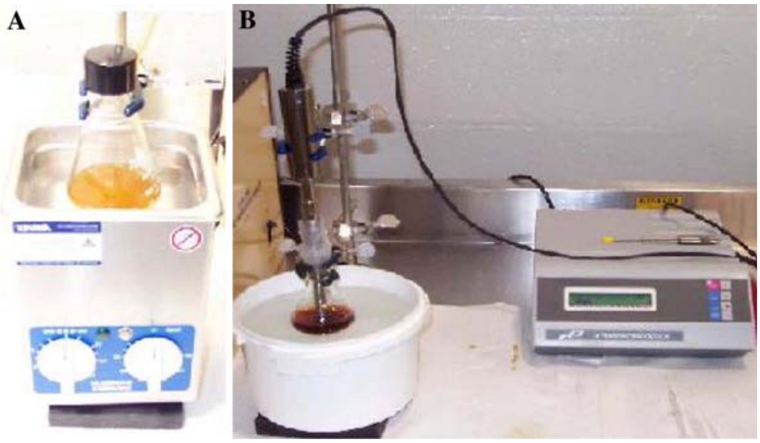

Fig. 1 US (**a**) and U/S–P (**b**) set-up used to produce FAEE

120 °C and cooled in a desiccator under vacuum for approximately 10 min. Six microliters of each sample was spotted on the TLC plate using a CAMAG Automatic TLC Sampler 4; the plate was developed in petroleum ether:chloroform (15:85) and sprayed with a solution of phosphomolybdic acid in ethanol.

Assessment of Different Ultrasonic Treatments

A 6:1 molar ratio of ethanol:fish oil, and either 1% KOH or 0.8% of C_2H_5ONa with respect to the fish oil weight, were used for both U/S and U/S–P. The experiments used 1% KOH at 20 °C, 1% KOH at 60 °C, 0.8% C_2H_5ONa at 20 °C, and 0.8% C_2H_5ONa at 60 °C. Fish oil (90 g) was placed in a flask and just before applying ultrasonic energy, 34.5 mL of either the KOH or C_2H_5ONa in ethanol solution was added. Ultrasonic energy was applied for 90 min (35 kHz of indirect sonication for the U/S, and 25 kHz of direct sonication for the U/S–P) at either 20 or 60 °C. Samples (5 mL) were taken at six sampling points: 10, 20, 30, 45, 60 and 90 min. Nine milliliters of a 2% citric acid solution were added and mixed gently for 30 min to neutralize the catalyst. The FAEE layer of each sample was left to separate (2 h), and then washed with 2 mL of water, letting the sample sit (1 h) after washing. The FAEE layer recovered was analyzed by HPLC to determine percentages of lipid classes.

HPLC and GC Analysis of FAEE

An Agilent HPLC with evaporative light scattering detection (ELSD) detector was used to analyze the transesterification levels (by lipid classes) of the ultrasonic transesterified fish oil (FAEE, TG, DG and MG). This instrument was equipped with a Waters Spherisorb S3CN 150 × 2 mm column (Waters, Milford, MA). A flow rate of 0.6 mL/min during the 10 min of analysis at 30 °C was used. The mobile phase gradient employed was hexane:methyl *tert*-butyl ether (98:2) for the first 5 min, then ramped to 80:20 over the next 2 min, and finally ramped to 0:100 for the remaining 3 min.

Ultrasonically transesterified samples were analyzed by GC to determine EPA and DHA contents. Area percentages were reported in relation to an internal standard of C19:0 (Nu-Chek Prep. Inc., Elysian, MN). A flow of 5 mL/min hydrogen was used in an Agilent 6890 GC System equipped with a flame ionization detector (FID), and a FAMEWAX Column 0.32 mm ID × 30 m length with a film thickness of 0.25 µm (Restek, Bellefonte, PA).

Statistical Analysis

Percentages of lipids (FAEE, TG, DG, MG, DHA ethyl ester and EPA ethyl ester) produced from all transesterification treatments using ultrasonic energy were analyzed statistically using a general linear model procedure present in the software package SAS 9 (SAS Institute Inc., Cary, NC). Differences amongst individual percentages means were deemed to be significant at $p < 0.05$.

Results and Discussion

Characterization of Oil

A molecular weight of 903.01 g/mol was calculated using data of methylation and GC analysis of the fish oil (Table 1). The polyene index (ratio of EPA + DHA/C16:0) of all ultrasonically transesterified samples was 1.83–1.86 which suggests that this low value was not due to oxidation but more probably due to impurities in the fish oil mixture resulting in gravimetric errors. Oligomer levels were small (<0.6%) and not significantly different ($p < 0.05$) between

Table 1 Fatty acid composition of 1812 TG fish oil for calculation of average molecular weight

Fatty Acid	Molecular mass (g/mol)	Percentage	TG mass (g/mol)	Average mass (g/mol)
C14	228.4	5.70	723.2	45.25
C15	242.4	0.39	765.2	2.96
C16	256.4	15.90	807.2	128.34
C18	284.4	3.32	891.2	29.59
C16:1	254.4	7.06	801.2	56.59
C17:1	268.4	0.83	843.2	7.00
C18:1	282.4	12.11	885.2	107.20
C20:1	310.5	1.81	969.5	17.50
C22:1	338.6	2.16	1,053.8	22.77
C24:1	366.6	0.76	1,137.8	8.65
C16:2	252.4	0.54	795.2	4.33
C18:2	280.4	3.46	879.2	30.46
C18:3	278.4	1.37	873.2	11.96
C18:4	276.4	3.70	867.2	32.09
C20:4	304.5	1.84	951.5	17.51
C22:4	332.6	0.74	1,035.8	7.69
C20:5	302.5	18.09	945.5	171.04
C22:5	330.6	2.76	1,029.8	28.42
C22:6	328.6	15.80	1,023.8	172.00
Unknown	278.4	0.65	831.2	5.68
		100	Calculated fish oil average molecular mass	903.01

samples. Also, ultrasonically transesterified samples had levels equivalent to <0.8% of conjugated dienes. Polyene index, and levels of oligomers and conjugated dienes were well within specifications for FAEE concentrates produced by ONC. Furthermore, the fish oil had a low acid value (0.1), which indicates a very low amount of free fatty acids, thus catalyst consumption by soap formation was expected to be minimal [13].

Feasibility of Using Ultrasonic Energy to Produce FAEE from Fish Oil

Potassium hydroxide was used as a catalyst due to its low price and relative ease of handling in both a laboratory and production setting. KOH percentages over 1% were not tested since these may be considered as excessive and may actually promote the formation of potassium soap and other side reactions. For the experiments developed using an U/S, 1% KOH as a catalyst produced 92.3% of FAEE. The first fatty acids to be transesterified are mostly saturated fatty acids bound to the external 1 and 3-positions of the glycerol backbone, while fatty acids in the 2-position require more energy and are thus not easily transesterified [13]. In the majority of fish oil triglycerides, PUFAs are bound to the 2-position of the glycerol backbone [10]. This may explain why when a reduced amount of catalyst (0.5% KOH) was used, the resulting mix of fish oil glycerides and FAEE solidified after 1 h at 4 °C (only producing 76.3% of FAEE). The other two samples using 0.75 and 1% KOH did not solidify under the same conditions. Approximately 5 h after ultrasonics application was completed, the glycerol layer was still not separated in the experiments using 0.5 and 0.75% KOH, possibly because low degree of transesterification was achieved, hence, less glycerol was produced, or because some of the fatty acids formed soap that acted as a phase transfer catalyst [6]. Another reason may relate to the formation of a strong emulsion of glycerol within the FAEE layer. This happened only when the U/S–P was used.

The transesterification of fish oil glycerides using the two-stage treatment technique produced more intense spots of fish oil glycerides (untransesterified glycerides) (Fig. 2). Less intense spots were observed in the continuous 30 min ultrasonic treatments, thus more fish oil glycerides were transesterified, especially when 1% KOH was used. FAEE samples from ONC did not show fish oil glycerides because these samples were purified by distillation.

When 1% KOH was used there was no significant difference in the production percentages of lipid classes between the U/S and U/S–P ($p < 0.05$) (Fig. 3). The U/S may offer better results for transesterification in a batch system. Although U/S can be designed for use in a continuous transesterification system for fish oil, where FAEE can be gradually removed while new fish oil is placed into the reactor without shutting down the ultrasonic energy, an U/S–P may be easier to design [14].

The two-stage ultrasonic treatment technique (two 15 min applications of ultrasonics) produced only approximately 52% (as area percentages) of FAEE, and particularly high area percentages of MG (15.7%). Furthermore, in the two-stage treatments, high percentages of TG remained intact, which may be due to the addition of only half (8.63 mL) of the ethanol/KOH stock solution for the first 15 min of sonication, thus less alcohol and catalyst were available for the transesterification of TG to proceed to FAEE.

Concentrations of >5% TG may degrade a GC column due to their strong retention, thus only samples of FAEE fractions containing <5% of TG (Fig. 3) were analyzed for DHA and EPA ethyl esters content percentages by GC-FID (Fig. 4). The ONC FAEE sample contained 99% FAEE, while the most efficient treatments of ultrasonic energy within the feasibility study (U/S–30 min–1% KOH and U/S–P–30 min–1% KOH) produced 92.4% of FAEE (Fig. 3). The ONC FAEE (99% FAEE) and the FAEE produced with U/S–30 min–1% KOH and U/S–P–30 min–1% KOH

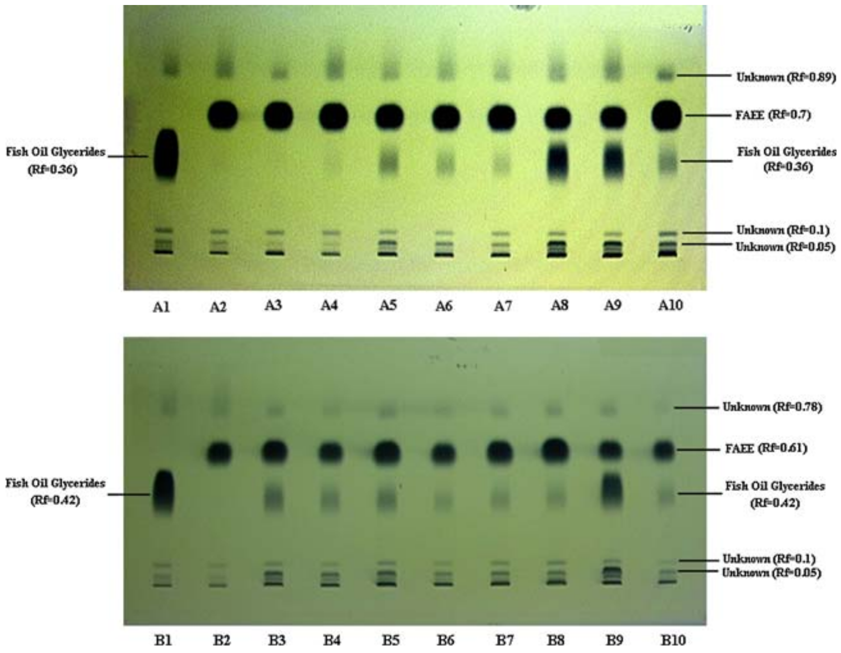

Fig. 2 TLC of FAEE: fish oil (*A1* and *B1*); ONC FAEE (*A2*, *A3*, *A4* and *B2*); U/S for 30 min with 0.5, 0.75 and 1% KOH (*A5*, *A6* and *A7*, respectively); U/S for 30 min (two 15 min periods adding half of catalyst at 0 and 15 min) with 0.5, 0.75 and 1% KOH (*A8*, *A9* and *A10*, respectively); U/S–P for 15 min with 0.5, 0.75 and 1% KOH (*B3*, *B5* and *B7*, respectively); U/S–P for 30 min with 0.5, 0.75 and 1% KOH (*B4*, *B6* and *B8*, respectively); U/S–P for 15 and 30 min (*B9* and *B10*, respectively) with half of 1% KOH added at 0 and 15 min

(92.4% FAEE) were significantly different ($p < 0.05$). However, EPA and DHA ethyl esters percentages were the same for ONC FAEE (16% of EPA and 14.9% of DHA) and the two most efficient ultrasonic treatments mentioned above (16% of EPA and 14.4% of DHA) (Fig. 4). This indicates that distinct levels of ultrasonic energy may have the potential to transesterify EPA and DHA selectively. Also, Fig. 4 shows that EPA and DHA ethyl ester percentages increased in relation to the percentage of KOH used, which may indicate that with 1% KOH there was more catalyst available to transesterify PUFA located in the 3-position of the glycerol backbone, as mostly saturated fatty acids are the first to be transesterified [13].

Assessment of Different Ultrasonic Treatments

Five treatments produced the highest percentages of FAEE ($p < 0.05$) (97.6–98.2%) (treatments 24, 42, 44, 46 and 48 of Table 2). All the latter treatments used 0.8% C_2H_5ONa as a catalyst at 60 °C (Table 2). The yield of FAEE increased with the reaction time and temperature. It is possible that ultrasonically produced bubbles at high temperatures may not collapse, or that their collapse is cushioned by the alcohol vapors trapped inside the reaction flask, resulting in a decrease in the effectiveness of mixing via sonication [14]. However, FAEE production did seem not to be affected at 60 °C, possibly due to the solubility of

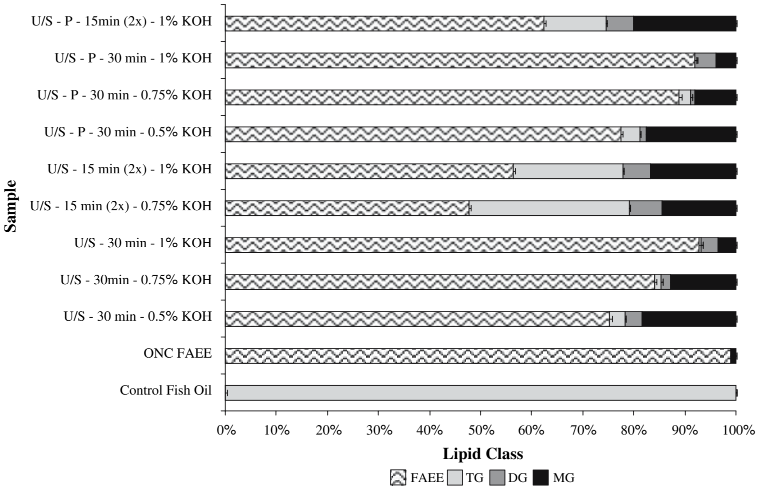

Fig. 3 HPLC analysis of FAEE, TG, DG and MG from different treatments of fish oil using ultrasonic energy (2× indicates that half of catalyst solution was added at the beginning of each 15 min period within a two-stage procedure) ($n = 3$)

oil in ethanol (reaction transferred into the homogenous stage) and the mixing effect of ultrasonic energy.

In just 10 min of sonication (U/S and U/S–P) approximately 92% of FAEE were consistently produced, and only a small gain of FAEE (approximately 6%) was observed when an additional 80 min of ultrasonic energy was applied, indicating that almost the entire transesterification process occurred within the first 10 min of treatment.

In terms of TG percentage depletion it is easy to observe that the most efficient catalyst used was C_2H_5ONa ($p < 0.05$) as it is possible that TG are not being converted entirely to FAEE but also to other lipid fractions such as DG and MG (Table 2). This indicates that distinct levels of ultrasonic energies may have the potential to direct the conversion of TG to specific lipid fractions by affecting the reaction equilibriums involved in the mechanism of TG conversion to glycerol. A previous study [6] indicated that the complete transesterification of TG (esterified with the same fatty acid) to glycerol and ester could involve at least 12 equilibrium reactions. Also, C_2H_5ONa may have improved the transesterification of fish oil because it is a stronger base than KOH.

Although the frequencies of sonication used differed between ultrasonic devices (35 and 20 kHz for U/S and U/S–P, respectively), a significant difference in any lipid class percentages ($p < 0.05$) between ultrasonic devices was not observed (Table 2). When using an U/S, the ultrasonic energy travels upwards, thus the ultrasonic energy is attenuated by a glass wall (of flask that contains the oil and the ethanol/KOH stock solution) and the oil before it can make the ethanol (top layer) cavitate. The probe in the other hand focuses ultrasonic energy downward, therefore the layer of ethanol/KOH stock solution cavitates first with no attenuation of the ultrasonic energy by either the glass wall or the oil. This may have compensated for the frequency difference of 15 kHz between the U/S and the U/S–P, thus differences in transesterification levels (FAEE, TG, DG and MG content percentages) between the two ultrasonic devices were not observed. A previous study [15] found that when using an U/S, 40 kHz of indirect sonication produced better results of transesterification of vegetable oil than 28 kHz. However, the same study did not compare the effect of frequencies between U/S and U/S–P devices.

The maximum conversion of fish oil TG to FAEE was 98.1%, slightly below the 99.4% of FAME production reported in a previous study of vegetable oil [8]. However, a direct comparison is limited because the 99.4% conversion rate is of FAME and not FAEE. Also, vegetable oil is more stable and has a different lipid composition, a factor that may affect the outcome of transesterification process. For example, the main four groups of fatty acids in fish oil are (1) C14:0 and C16:0, (2) C16:1 and C18:1, (3) C20:1 and C22:1, and (4) C20:5, C22:5 and C22:6 [9], whereas in soybean oil the main groups are C16:0, C18:0, C18:1, C18:2 and C18:3 [10]. More importantly, fish oil is much richer than the soybean oil in unsaturated fatty acids [10].

The main rationale of using ultrasonic energy to aid the transesterification of fish oil was the benefit of a reduction in the time taken (of at least 1.5 h) to achieve high levels of transesterification (>98%) when compared to a conventional transesterification method. This may represent a commercial advantage over a conventional transesterification method that requires an initial heating of the oil to 70–80 °C, mechanical stirring and significantly longer reaction times (2 h just for the transesterification reaction) to produce >98% of FAEE [3, 5]. Moreover, the use of ultrasonic energy did not affect the quality of the final product in terms of polyene index (1.83–1.86), and production of unwanted oligomers (<0.6%) and conjugated dienes (<0.8%). This study showed that ultrasonic energy could be used to efficiently transesterify fish oil, a technology that may be applicable for the production of EPA and DHA.

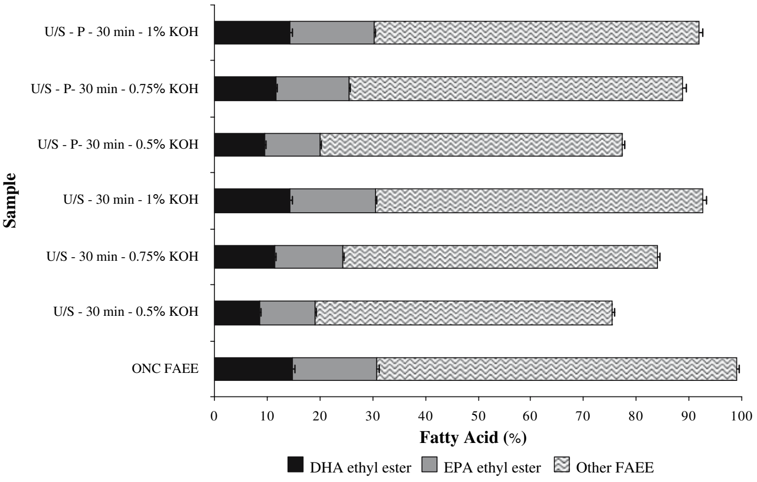

Fig. 4 GC analysis of DHA and EPA from the FAEE fraction ($n = 3$)

Table 2 Lipid classes (%) produced by transesterifying fish oil using ultrasonics

No.	Ultrasonic device	Treatment	FAEE	TG	DG	MG
1	Bath	1% KOH–20 °C (10 min)	89.1^{a}	6.8^{d}	2.6^{b}	1.5
2	Bath	1% KOH–60 °C (10 min)	94.3^{b}	2.3^{b}	1.7^{a}	1.7
3	Bath	1% KOH–20 °C (20 min)	94.2^{b}	3^{c}	1.5^{a}	1.3
4	Bath	1% KOH–60 °C (20 min)	94.9^{b}	1.7^{b}	1.6^{a}	1.8
5	Bath	1% KOH–20 °C (30 min)	93.7^{b}	2.8^{b}	1.8^{a}	1.7
6	Bath	1% KOH–60 °C (30 min)	95.5^{b}	1.4^{b}	1.5^{a}	1.6
7	Bath	1% KOH–20 °C (45 min)	95.6^{b}	1.9^{b}	1.3^{a}	1.2
8	Bath	1% KOH–60 °C (45 min)	96^{c}	1.3^{b}	1.3^{a}	1.4
9	Bath	1% KOH–20 °C (60 min)	95.5^{c}	1.8^{b}	1.4^{a}	1.3
10	Bath	1% KOH–60 °C (60 min)	96.8^{c}	1^{b}	1.1^{a}	1.1
11	Bath	1% KOH–20 °C (90 min)	96.4^{c}	1.5^{b}	1.0^{a}	1.1
12	Bath	1% KOH–60 °C (90 min)	96.3^{c}	1.2^{b}	1.2^{a}	1.3
13	Bath	0.8% C_2H_5ONa–20 °C (10 min)	87.7^{a}	7^{d}	3.5^{b}	1.8
14	Bath	0.8% C_2H_5ONa–60 °C (10 min)	95.4^{c}	1.5^{b}	1.5^{a}	1.6
15	Bath	0.8% C_2H_5ONa–20 °C (20 min)	93.3^{b}	2.9^{c}	2.2^{b}	1.6
16	Bath	0.8% C_2H_5ONa–60 °C (20 min)	96.2^{c}	1^{b}	1.2^{a}	1.6
17	Bath	0.8% C_2H_5ONa–20 °C (30 min)	94.7^{c}	2.2^{b}	1.7^{a}	1.5
18	Bath	0.8% C_2H_5ONa–60 °C (30 min)	96.2^{c}	1.1^{b}	1.3^{a}	1.4
19	Bath	0.8% C_2H_5ONa–20 °C (45 min)	96^{c}	1.5^{b}	1.3^{a}	1.2
20	Bath	0.8% C_2H_5ONa–60 °C (45 min)	96.5^{c}	0.8^{a}	1.2^{a}	1.5
21	Bath	0.8% C_2H_5ONa–20 °C (60 min)	96.3^{c}	1.2^{b}	1.3^{a}	1.2
22	Bath	0.8% C_2H_5ONa–60 °C (60 min)	97^{c}	0.7^{a}	0.9^{a}	1.4
23	Bath	0.8% C_2H_5ONa–20 °C (90 min)	96.8^{c}	0.9^{b}	1.1^{a}	1.2
24	Bath	0.8% C_2H_5ONa–60 °C (90 min)	98.1^{d}	0.6^{a}	ND	1.3
25	Probe	1% KOH–20 °C (10 min)	87.2^{a}	7.6^{d}	3.2^{b}	2
26	Probe	1% KOH–60 °C (10 min)	95.5^{c}	1.4^{b}	1.2^{a}	1.9
27	Probe	1% KOH–20 °C (20 min)	92.9^{b}	3.9^{c}	1.9^{a}	1.3
28	Probe	1% KOH–60 °C (20 min)	96^{c}	1.1^{b}	1.2^{a}	1.7
29	Probe	1% KOH–20 °C (30 min)	93.4^{b}	3.2^{c}	1.8^{a}	1.6
30	Probe	1% KOH–60 °C (30 min)	95.8^{c}	1.1^{b}	1.3^{a}	1.8
31	Probe	1% KOH–20 °C (45 min)	95^{c}	2.3^{b}	1.4^{a}	1.3
32	Probe	1% KOH–60 °C (45 min)	96.4^{c}	0.9^{b}	1^{a}	1.7
33	Probe	1% KOH–20 °C (60 min)	95.3^{c}	2^{b}	1.4^{a}	1.3
34	Probe	1% KOH–60 °C (60 min)	96.2^{c}	0.9^{b}	1.2^{a}	1.7
35	Probe	1% KOH–20 °C (90 min)	96.2^{c}	1.5^{b}	1.1^{a}	1.2
36	Probe	1% KOH–60 °C (90 min)	97.3^{c}	1^{b}	ND	1.7
37	Probe	0.8% C_2H_5ONa–20 °C (10 min)	92.5^{b}	3.3^{c}	2.6^{b}	1.6
38	Probe	0.8% C_2H_5ONa–60 °C (10 min)	96.2^{c}	1^{b}	1.3^{a}	1.5
39	Probe	0.8% C_2H_5ONa–20 °C (20 min)	95.3^{c}	1.5^{b}	1.7^{a}	1.5
40	Probe	0.8% C_2H_5ONa–60 °C (20 min)	96.6^{c}	0.8^{a}	1.1^{a}	1.5
41	Probe	0.8% C_2H_5ONa–20 °C (30 min)	96.1^{c}	1.2^{b}	1.4^{a}	1.3
42	Probe	0.8% C_2H_5ONa–60 °C (30 min)	97.6^{d}	0.8^{a}	ND	1.6
43	Probe	0.8% C_2H_5ONa–20 °C (45 min)	96.5^{c}	1.2^{b}	1.1^{a}	1.2
44	Probe	0.8% C_2H_5ONa–60 °C (45 min)	98^{d}	0.6^{a}	ND	1.4
45	Probe	0.8% C_2H_5ONa–20 °C (60 min)	96^{c}	1.2^{b}	1.5^{a}	1.3
46	Probe	0.8% C_2H_5ONa–60 °C (60 min)	98.2^{d}	0.5^{a}	ND	1.3
47	Probe	0.8% C_2H_5ONa–20 °C (90 min)	96.6^{c}	1.1^{b}	1.1^{a}	1.2
48	Probe	0.8% C_2H_5ONa–60 °C (90 min)	98.2^{d}	0.6^{a}	ND	1.2

Fish oil (control) contains 0% FAEE, ≈100% TG and <0.5% of free fatty acids; the MG fraction did not show a significant trend (increasing or diminishing) regarding the sonication time; sum of FAEE, TG, DG and MG is 100%; *ND* = not detected

Values in FAEE, TG and DG columns with different letter of superscript are significantly different ($p < 0.05$, $n = 3$)

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References

1. Miao X, Wu Q (2006) Biodiesel production from heterotrophic microalgal oil. Bioresour Tech 97:841–846
2. Stern R, Hillion G, Gateau P, Guibet J (1987) Process for manufacturing a composition of fatty acid esters useful as gas oil substitute motor fuel with hydrated ethyl alcohol and the resultant esters composition, U.S. Patent 4,695,411
3. Ma F, Hanna MA (1999) Biodiesel production: a review. Bioresour Tech 70:1–15
4. Darnoko D, Cheryan M (2000) Continuous production of palm methyl esters. J Am Oil Chem Soc 77:1269–1272
5. Freedman B, Butterfield RO, Pryde EH (1986) Transesterification kinetics of soybean oil. J Am Oil Chem Soc 63:1375–1380
6. Stavarache C, Vinatoru M, Nishimura R, Maeda Y (2005) Fatty acids methyl esters from vegetable oil by means of ultrasonic energy. Ultras Sonochem 12:367–372
7. Zhang Y, Dubé MA, McLean DD, Kates M (2003) Biodiesel production from waste cooking oil: 1. process design and technical assessment. Bioresour Tech 89:1–16
8. Colucci JA, Borrero EE, Alape F (2005) Biodiesel from an alkaline transesterification reaction of soybean oil using ultrasonic mixing. J Am Oil Chem Soc 82:525–530
9. Tucker BW, Pigott GM (1993) Fish oils, composition and properties, in encyclopedia of food science, food technology and nutrition. Academic, London, p 1896
10. Bockisch M (1993) Fats and oils handbook, in vegetable fats and oils. AOCS, Chicago, pp 225–226
11. Covington MB (2004) Omega-3 fatty acids. Am Fam Phys 70:133–140
12. Firestone D (ed) (1998) Official methods and recommended practices of the American oil chemists' society, 5th edn. AOCS, Champaign
13. Stavarache C, Vinatoru M, Maeda Y (2007) Aspects of ultrasonically assisted transesterification of various vegetable oils with methanol. Ultras Sonochem 14:380–386
14. Mason TJ, Lorimer JP (2002) Introduction to applied ultrasonics, in applied sonochemistry. Wiley-VCH Verlag GmbH, Weinheim, pp 1–24
15. Stavarache C, Vinatoru M, Nishimura R, Maeda Y (2003) Conversion of vegetable oil to biodiesel using ultrasonic irradiation. Chem Lett 8:716–717