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Chemical transesterification of tuna oil to enriched omega-3 polyunsaturated fatty acids

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Abstract

The effect of reaction conditions on transesterification of tuna oil with $n-3$ fatty acid methyl ester ($n-3$ FAME) using sodium methoxide as catalyst was studied. The lipid and fatty acid composition of the reaction products were analyzed. The time course of the reaction indicated that the highest incorporation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) occurred after 5 h, and did not change significantly during the next 20 h ($P \geqslant 0.05$). The catalyst concentration of 1.5% w/w of reactants showed the highest incorporation of EPA and DHA. Incorporation of EPA and DHA were increased with increasing reaction temperature ($P < 0.05$) and the highest incorporation was observed at 80 °C. The highest EPA and DHA incorporation occurred at a mole ratio of tuna oil and $n-3$ FAME of 1:4. The recovery of triglyceride was decreased with the increasing of all reaction factors. Tuna oil enriched with $n-3$ fatty acids was successfully produced by chemical interesterification.

Keywords: Tuna oil; Transesterification; *n*- 3 fatty acids; Fatty acid methylester

1. Introduction

Fish oils are rich in $n-3$ polyunsaturated fatty acids ($n-3$ PUFAs), especially eicosapentaenoic acid (EPA) (eicosapentaenoic acid, C20:5) and docosahexaenoic acid (DHA) (docosahexaenoic acid, C22:6) that have been shown to be important for prevention of a range of human diseases and disorders (Uauy & Valenzuela, 2000; Wanasundara & Shahidi, 1998). Generally, fish oils have complex mixtures of fatty acids with varying chain lengths and degree of unsaturation. Overconsumption of fish oils to obtain $n-3$ PUFAs may increase the intake of cholesterol and other saturated fatty acids by consumers (Haagsma, Gent, Luten, Jong, & Doorn, 1982; Shahidi & Wanasundara, 1998). Therefore, concentration or enrichment of fish oil in $n-3$ PUFAs could help avoid such a concern.

The triglyceride (TG) form of $n-3$ PUFAs is considered to be nutritionally more favorable than methyl or ethyl esters of fatty acids as seen from experimental results indicating impaired intestinal absorption of methyl or ethyl esters of $n-3$ PUFAs in animals (Shahidi & Wanasundara, 1998). Thus, TG of $n-3$ PUFAs is often promoted as being more natural than other fatty acid derivatives.

Interesterification is a reaction that exchanges carbonyl groups of fatty acids within and between TG molecules and it is used to modify the structure and composition of oils to improve the physical and nutritional properties of TG (Basheer, Mogi, & Nakajima, 1995). There are three reactions associated with interesterification: acidolysis, alcoholysis and transesterification (Marangoni & Rousseau, 1995). Presently, there are two types of interesterification in use, i.e. enzymatic and chemical interesterification. These has been numerous research of fish oil enriched with $n-3$ PUFAs using enzymatic interesterification (Adachi, Okumura, Ota, & Mankura, 1993; Myynes, Barstad, Olsen, & Elvevoll, 1995; Yamane, SuZuki, & Hoshino, 1993; Zuyi & Ward, 1993). Enrichment by chemical interesterification has not been reported. Chemical interesterification

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has some advantages over enzymatic modification, which include lower catalyst cost, use of existing and more available industrial procedures and equipment, and shorter reaction times (Konishi, Neff, & Mounts, 1993).

In this study, the fatty acid composition of fish oil (tuna oil) was chemically modified to enrich $n-3$ PUFAs, primarily EPA and DHA, by using sodium methoxide ($NaOCH_3$) as catalyst. The effects of reaction time and temperature, catalyst concentration, and reactants mole ratio were studied. Lipid and fatty acid composition of tuna oil were analyzed before and after transesterification.

2. Materials and methods

2.1. Materials

Crude tuna oil was obtained from Chotiwat Manufacturing Co., Ltd. (Songkhla, Thailand). The degumming, refining, bleaching, winterization and deodorization of the oil were carried out according to Rungsilp (1998). Fatty acid methyl ester standards were purchased from Aldrich Chemical Co, Inc. (Milwaukee, WI). Sodium methoxide was obtained from Fluka Chemie (Buchs, Switzerland). All other chemicals used in this study were analytical grade.

2.2. Methods

2.2.1. Preparation of free fatty acids from tuna oil

Preparation of free fatty acids from tuna oil was carried out according to Senanayake and Shahidi (1999) and Wanasundara and Shahidi (1999) with slight modification. Tuna oil (25 g, treated with 200 ppm butylhydroquinone, BTHQ) was saponified using a mixture of KOH (5.75 g), water (11 ml) and 95% aqueous ethanol (66 ml) for 1 h at 62 °C under nitrogen condition. After saponification, distilled water (50 ml) was added to the saponified mixture and the unsaponifiable matter was extracted with 2×100 ml of hexane and discarded. The saponifiable matter in the aqueous layer was acidified to pH 1.0 with 6 N HCl and the free fatty acids were extracted into 50 ml of hexane. The hexane layer containing free fatty acids was then dried over anhydrous sodium sulfate and the solvent was removed using a vacuum rotary evaporator at 40 °C. The free fatty acids were stored at −20 °C under nitrogen until use.

2.2.2. Preparation of $n-3$ fatty acids concentrates from tuna oil by urea complexation

The separation of $n-3$ fatty acids from the hydrolyzed fatty acids mixture of tuna oil was carried out using urea-fatty acid adduct formation according to Senanayake and Shahidi (1999) and Wanasundara and Shahidi (1999). Free fatty acids (10 g) were added under constant stirring to a hot (60 °C) solution of 30 g urea in 150 ml of 95% aqueous ethanol. The solution was heated and stirred until clear, then it was allowed to crystallize at room temperature for 5 h and then kept at 4 °C for 24 h for further crystallization. The formed crystals were separated from the liquid by vacuum filtration. The filtrate was diluted with an equal volume of water and acidified to pH 4–5 with 6 N HCl and equal volume of hexane was subsequently added and the mixture was stirred for 1 h, then transferred to a separatory funnel. The hexane layer containing free fatty acids was separated from the aqueous layer and washed with distilled water to remove any remaining urea and then dried over anhydrous sodium sulfate. The solvent was subsequently removed at 40 °C using a vacuum rotary evaporator.

2.2.3. Preparation of $n-3$ fatty acids methyl ester

Preparation of $n-3$ fatty acids methyl ester was carried out according to Jham, Teles, and Campos (1982) with modification for scale up. The $n-3$ fatty acids (25 g) were hydrolyzed with 500 ml of KOH in methanol (0.5 M) at 100 °C for 5 min in tightly capped glass bottles. After hydrolysis, the mixture was esterified with 200 ml of HCl in methanol (4:1 vol/vol) and then heated in an oil bath for 15 min at 100 °C. The mixture was cooled and then 1000 ml of distilled water was added. The fatty acid methyl ester was extracted with 2×500 ml of hexane. The hexane layer was dried quickly over anhydrous sodium sulfate and the solvent was removed using a vacuum rotary evaporator at 40 °C. The $n-3$ fatty acids methyl ester was stored at −20 °C in nitrogen until use. The fatty acid composition of this sample was determined using gas chromatography (GC).

2.2.4. Transesterification

Sodium methoxide-catalyzed transesterification was carried out in screw capped tube and gently stirred on a magnetic-stirrer hot plate. The reactants, tuna oil and $n-3$ fatty acids methyl ester were dried in test tube at 60 °C under vacuum of 30 cmHg for 15 min. The mole ratio of tuna oil to $n-3$ fatty acids methyl ester was changed using different amount of $n-3$ fatty acids methyl ester. After the temperature of reactants had reached the reaction temperature (60, 70 and 80 °C), sodium methoxide powder (0.5%, 1.0% and 1.5% w/w of reactants) was added. After 5 h, the reaction was stopped with the addition of 2 ml of dilute citric acid (4% w/v) then washed with three volume of 2 ml of distilled water. The oil phase (reaction product) was dried using anhydrous sodium sulfate. The lipid class of oil was analyzed by thin-layer chromatography/flame ionization detector analyzer (TLC/FID) and the fatty acid composition was analyzed by GC.

2.2.5. Gas chromatography analysis

The reaction products were analyzed by thin-layer chromatography (TLC) on silica gel 60 plates (Willis & Marangoni, 1999) using a two-step development system. In the first step, the plates were developed for 8 cm with *n*-hexane:diethyl ether:formic acid (50:20:0.3, vol/vol/vol). The second development (10 cm) was made with *n*-hexane:benzene (50:50, vol/vol). The bands were visualized in iodine tank and the bands that corresponded to triglyceride (TAG) were scraped from the TLC plates and methylated to fatty acid methyl ester using the procedure according to Jham et al. (1982). The gas chromatography was carried out using a Perkin–Elmer Autosystem XL (The Perkin–Elmer Corporation, Norwalk, CT) equipped with a PERMABOND-FFAP DF-0.25 fused-silica capillary column 25 m × 0.25 mm id (MACHEREY-NAGEL, Germany) and FID detector, and operated in a split mode with split ratio 100:1. The injector and detector temperatures were 245 and 250 °C, respectively. The column temperature was held at 150 °C for 0.5 min, then programmed to 170 °C at 4 °C/min, further programmed to 195 °C at 5 °C/min, the final temperature was 215 °C at 10 °C/min from 195 °C and held for 7.5 min. The carrier gas used was helium set at a flow rate of 0.5 ml/min (15 psi). The content of fatty acid methyl ester as mg/g oil was calculated with 19:0 as internal standard according to Joseph and Ackman (1992).

2.2.6. TLC-FID analysis

The lipid composition of the oils was performed on silica-gel-coated quartz rods (Chromarod-SIII, Iatron Laboratories, Inc., Japan) using a two-step development system. The first development (8 cm) was made with *n*-hexane:diethyl ether:formic acid (50:20:0.3, vol/vol/vol). After development, the solvent was removed by flushing the rods with a stream of nitrogen and drying in air for ≈5 min. The rods were then placed in the second solvent system, and developed for 10 cm with *n*-hexane:benzene (50:50, vol/vol). The lipid composition was determined on an Iatroscan Model TH-10 analyzer equipped with a flame ionization detector (Iatron Laboratories, Inc., Japan).

2.2.7. Statistical analysis

The design of the experiments was three-way factorial, with the variables of reaction temperature (60, 70 and 80 °C), mole ratio of tuna oil to $n-3$ fatty acids methyl ester (1:2, 1:3 and 1:4) and $NaOCH_3$ concentration (0.5%, 1.0% and 1.5% w/w of reactants). The data were subjected to the analysis of variance (ANOVA) univariate method ($P < 0.05$). Comparison of means after the ANOVA test was performed using the Duncan's multiple range test.

3. Results and discussion

3.1. Effect of reaction time on recovery of TG and incorporation of n − 3 PUFA into tuna oil

The lipid composition of refined tuna oil and $n-3$ FAME are shown in Table 1. The main component was

Table 1
Lipid composition (% peak area) of refined tuna oil and $n-3$ fatty acids methyl ester ($n-3$ FAME)[a]

Lipid composition[b]	Refined tuna oil	$n-3$ FAME
FAME	ND[c]	98.80 ± 0.20
TG	99.19 ± 0.05	ND
FFA	0.22 ± 0.16	0.46 ± 0.45
DG	0.14 ± 0.12	0.13 ± 0.16
MG	0.45 ± 0.22	0.62 ± 0.46

[a] Mean ± SD from duplicate determination.
[b] Lipid composition; FAME, fatty acid methyl ester; TG, triglyceride; FFA, free fatty acid; DG, diglyceride; MG, monoglyceride.
[c] ND, not detected.

Table 2
Fatty acid composition (mg/g oil) in refined tuna oil, $n-3$ fatty acids methyl ester ($n-3$ FAME) and transesterified tuna oil[a]

Fatty acids	Refined tuna oil	$n-3$ FAME	Transesterified tuna oil[c]
C14:0	23.35 ± 0.34	0.81 ± 0.05	9.81 ± 0.88
C16:0	161.74 ± 0.68	ND[b]	70.11 ± 1.03[b]
C16:1	49.17 ± 0.01	29.57 ± 0.26	26.44 ± 0.96
C18:0	37.05 ± 1.44	ND	19.48 ± 1.27
C18:1	131.72 ± 0.04	0.75 ± 0.30	61.28 ± 1.65
C18:2	22.50 ± 0.13	8.73 ± 1.84	8.14 ± 0.35
C20:4	15.97 ± 0.11	32.32 ± 0.40	14.70 ± 0.43
C20:5	41.09 ± 0.30	74.75 ± 1.07	60.39 ± 0.70
C22:1	15.92 ± 1.59	ND	12.28 ± 0.17
C22:4	23.91 ± 0.09	56.01 ± 0.70	25.29 ± 0.72
C22:6	266.12 ± 0.91	565.94 ± 0.11	388.14 ± 1.57
Others	211.46 ± 0.66	231.12 ± 0.40	303.94 ± 0.88

[a] Mean ± SD from duplicate determination.
[b] ND, not detected.
[c] Transesterification was carried out at 80 °C for 5 h, the mole ratio of refined tuna oil per $n-3$ FAME was 1:4 and $NaOCH_3$ amount was 1.0% by weight of reactants.

TG for refined tuna oil and FAME for $n-3$ FAME with 99.2% and 98.8%, respectively. It was clearly shown that the purification and methylation methods used in this study were lightly efficient. The major fatty acids present in tuna oil were C16:0, C18:1, C20:5 and C22:6 (Table 2). As a result of urea complexation, C16:0 and 18:1 were eliminated almost completely while C20:5 and C22:6 were enriched from 41.1 to 74.8 and 266.1 to 565.9 mg/g oil, respectively. Haagsma et al. (1982) and Wanasundara and Shahidi (1999) have reported similar results for urea complexation experiments carried out for cod liver and seal blubber oils, respectively.

The dependence of reaction rate of transesterification on time was studied at 80 °C for 24 h in the presence of 1.0% w/w of sodium methoxide ($NaOCH_3$). The yield of TG, in term of relative amount comparing to the initial amount, recovered from transesterified products is shown in Fig. 1. When the reaction time increased, the TG yield was decreased ($P < 0.05$), showing that after 24 h the TG was only 59% of the initial value. However, the TG yield did not significantly decrease from 3 to 8 h of reaction time ($P \geqslant 0.05$). The decrease of TG was due to the formation of di- and monoglycerides (Konishi et al., 1993) and saponification producing soaps and methyl esters (Marangoni & Rousseau, 1995; Sreenivasan, 1978).

The fatty acid composition and EPA and DHA concentration in TG of transesterified tuna oil is given in Table 2 and Fig. 2, respectively. The fatty acid composition of tuna oil before transesterification was

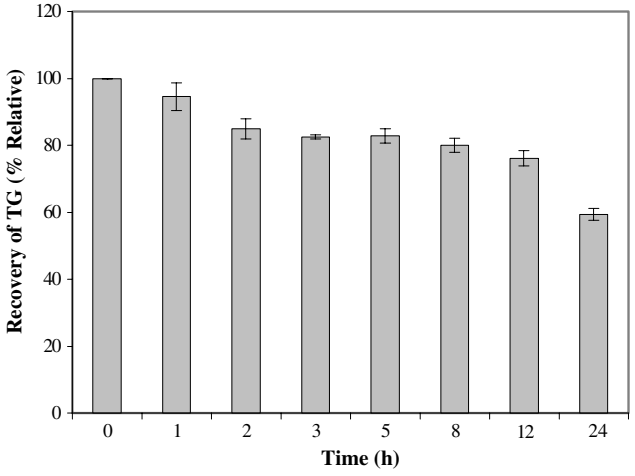

Fig. 1. Effect of reaction time on recovery of TG. Transesterification was carried out at 80 °C for 24 h, the mole ratio of tuna oil to $n-3$ FAME was 1:4 and $NaOCH_3$ amount was 1.0% by weight of reactants.

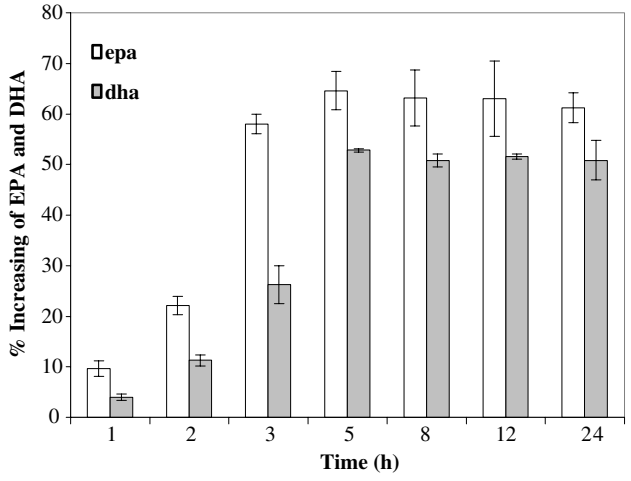

Fig. 2. Effect of reaction time on incorporation of EPA and DHA in to tuna oil with transesterification. Transesterification was carried out at 80 °C for 24 h, the mole ratio of tuna oil to $n-3$ FAME was 1:4 and $NaOCH_3$ amount was 1.0% by weight of reactants.

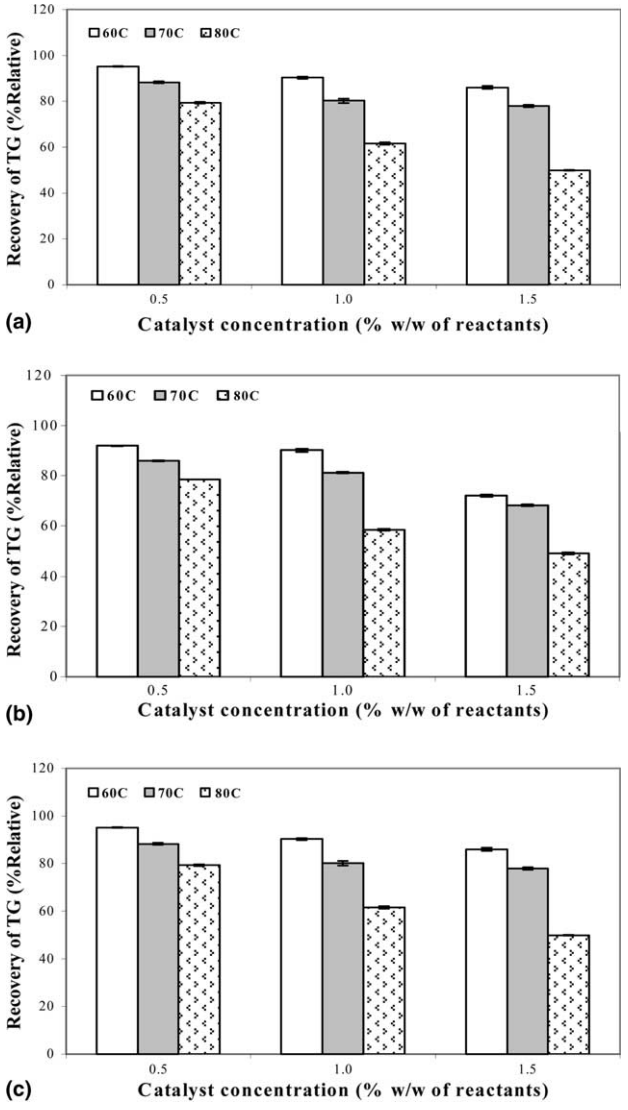

Fig. 3. Effect of reaction conditions on TG recovery of tuna oil after transesterification with the mole ratios of tuna oil to $n-3$ FAME were 1:2 (a), 1:3 (b) and 1:4 (c) at 60, 70 and 80 °C. The amount of catalyst ($NaOCH_3$) were 0.5%, 1.0% and 1.5% weight of reactants.

Table 3
Linear regression coefficient of full factorial design-effects for response variables (TG recovery, EPA and DHA) on transesterification of tuna oil

Reaction conditions	TG recovery (%)	EPA increasing (%)	DHA increasing (%)
Catalyst concentration	−0.718**	0.796**	0.817**
Reaction temperature	−0.545**	0.315*	0.369**
Mole ratio of reactants	−0.256*	0.381**	0.206

* $P < 0.05$.
** $P < 0.01$.

different from fatty acid composition after transesterification. After transesterification for 5 h, EPA and DHA were increased from 41.1 to 60.4 and 266.1 to 388.1 mg/g oil, respectively. The further the ester interchange progressed, the more EPA and DHA methyl esters were consumed and the more EPA and DHA were incorporated into TG. Thus, the EPA and DHA concentrations in TG were increased with increasing reaction time until 5 h ($P < 0.05$, Fig. 2). After 5 h, EPA and DHA concentration were not significantly increased upto 24 h ($P \geqslant 0.05$), indicating that the reaction was probably complete. Konishi et al. (1993) found that the ester interchange between TG and methyl stearate took place rapidly at 60 °C, i.e. ≈2 h. It has been reported that if the reaction mixture has a sufficient concentration of catalyst, the actual interesterification reaction is extremely fast (Marangoni & Rousseau, 1995). Previous research has shown that the time of reaction varies widely (5 min to 6 h) depending on reaction conditions, such as the catalyst level, temperature and solubility of the catalyst in the reactants (Marangoni & Rousseau, 1995; Sreenivasan, 1978). Konishi et al. (1993) revealed that in certain cases, the interesterification can take as long as 24 h, even with catalyst pre-activation. Moreover, it is impossible to predict the onset of the reaction and it was very difficult to obtain only a partial interesterification, so most reactions were conducted until equilibrium was reached (Marangoni & Rousseau, 1995). Therefore, time of the transesterification for 5 h was required under our reaction conditions.

3.2. Effect of reaction conditions on recovery of TG and incorporation of EPA and DHA

The effect of reaction conditions including catalyst concentration, reaction temperature and mole ratio of reactants on the recovery of TG was given in Fig. 3. All factors showed the same responses in TG recovery, the TG level were significantly decreased ($P < 0.05$) when the reaction conditions increased. From Table 3, the catalyst concentration and reaction temperature better correlated with the recovery of TG than the mole ratio of reactants. The loss of TG in tuna oil after transesterification was described previously in Section 3.1.

The incorporation of EPA and DHA into TG of tuna oil increases their net concentration compared with those in the original tuna oil. Incorporation of EPA and DHA increased with increasing concentration of $NaOCH_3$ ($P < 0.05$) independently of reaction temperature (Figs. 4 and 5). Increased incorporation of stearic acid into soybean oil with increasing amount of $NaOCH_3$

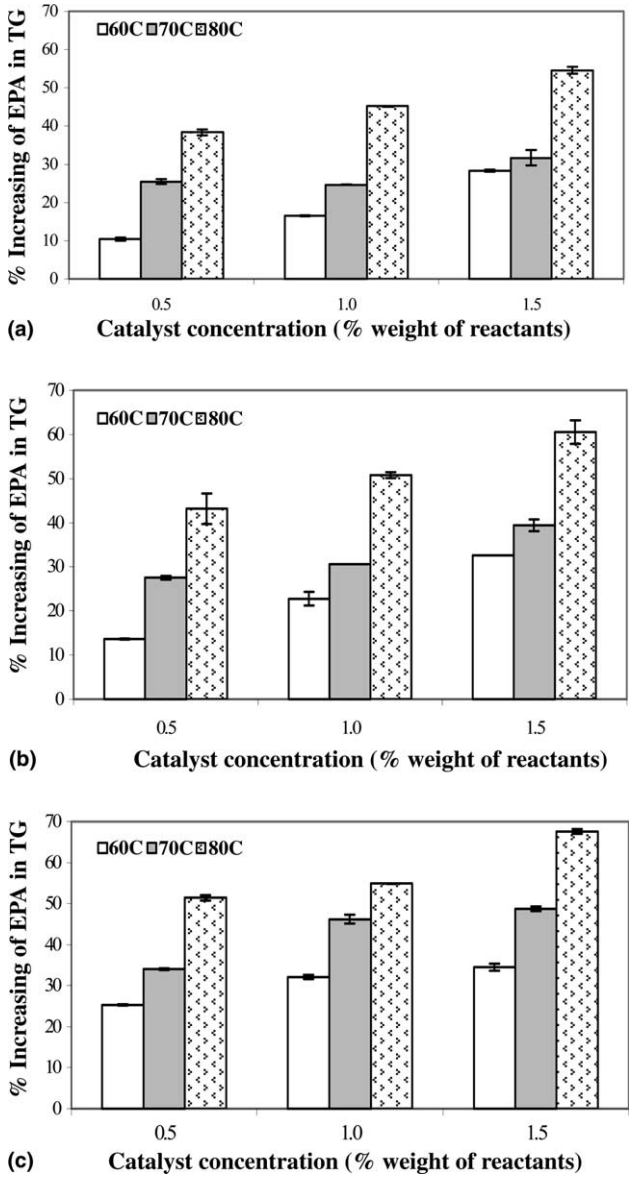

Fig. 4. Effect of reaction condition on incorporation of EPA into TG of tuna oil after transesterification. The mole ratios of tuna oil to $n-3$ FAME were 1:2 (a), 1:3 (b) and 1:4 (c) at 60, 70 and 80 °C. The amount of catalyst ($NaOCH_3$) were 0.5%, 1.0% and 1.5% weight of reactants.

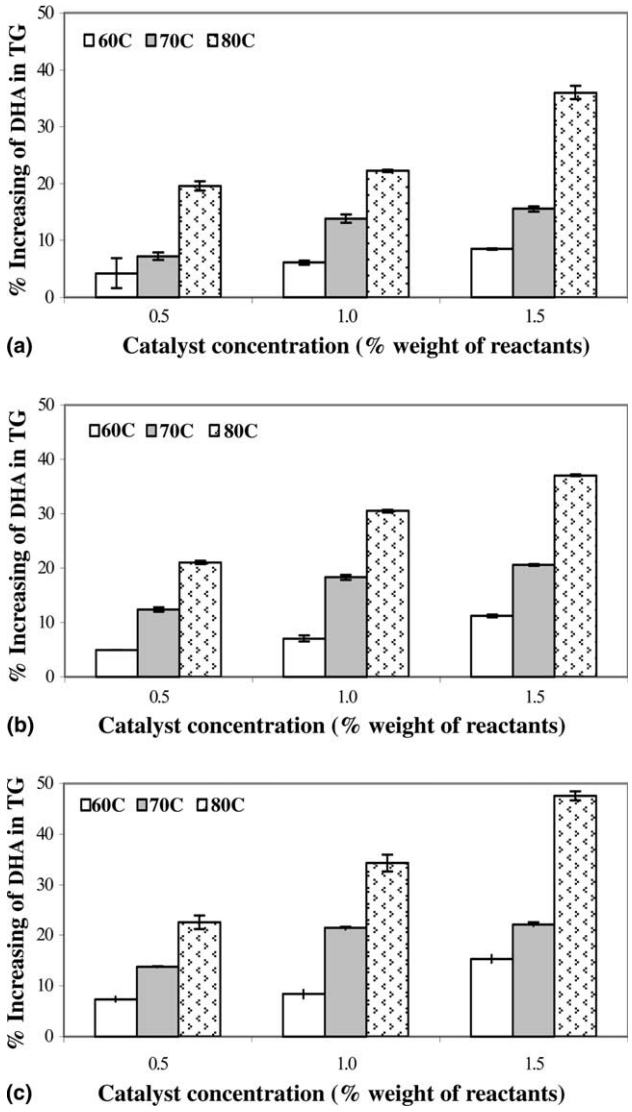

Fig. 5. Effect of reaction condition on incorporation of DHA into TG of tuna oil after transesterification. The mole ratios of tuna oil to $n-3$ FAME were 1:2 (a), 1:3 (b) and 1:4 (c) at 60, 70 and 80 °C. The amount of catalyst ($NaOCH_3$) were 0.5%, 1.0% and 1.5% weight of reactants.

has been reported to result in improved ester interchange (Konishi et al., 1993). The oil quality and the efficiency of the drying system used play critical roles in determining the dosage of $NaOCH_3$ (Liu & Lampert, 1999). In general, $NaOCH_3$ can be used in low levels, (0.2–0.5% w/w), if the starting oil is well refined and dry (Kimoto, Endo, & Fujimoto, 1994; Schmidt, Hurtova, Zemanovic, Sekretar, Simon, & Ainsworth, 1996; Willis & Marangoni, 1999). In this study, more $NaOCH_3$ was used due to the presence of moisture, free fatty acids and peroxide substances, which might impair catalyst performance (Liu & Lampert, 1999; Marangoni & Rousseau, 1995; Sreenivasan, 1978).

Temperature affects the transesterification kinetics. The dependence of incorporated EPA and DHA on temperature was studied at 60, 70 and 80 °C (Figs. 4 and 5). The incorporation of EPA and DHA increased with increasing temperature ($P < 0.05$), due to higher ester interchange. Konishi et al. (1993) reported similar results in the case of transesterification of soy bean oil with methyl stearate. Interesterification can be carried out at 0–240 °C depending on the type of catalyst used (Haumanm, 1994). In general, sodium methoxide ($NaOCH_3$) is active at temperature around 50–90 °C (Marangoni & Rousseau, 1995) but interestrification can be initiated at 70–120 °C by 0.05–0.5% $NaOCH_3$ (Liu & Lampert, 1999). Addition of antioxidants or an inert atmosphere treatment should also be considered for the case of high reaction temperature in order to prevent oxidative deterioration of oil especially fish oil due to the high level of polyunsaturated fatty acids.

Figs. 4 and 5 show the incorporation of EPA and DHA into tuna oil at various $n-3$ FAME to tuna oil molar ratios. With $n-3$ FAME as the acyl donor, EPA and DHA incorporation into TG of tuna oil increased as the molar ratio increased ($P < 0.05$) and highest at 1:4 molar ratio of tuna oil to $n-3$ FAME. Akoh, Jennings, and Lillard (1995) studied the total incorporation of EPA and DHA into trilinolein at various $n-3$ FAME to trilinolein molar ratio. They found that, the incorporation of EPA did not increase beyond a molar ratio of EPA ethyl ester/trilinolein of 4, the incorporation of DHA increased as the molar ratio increased up to 5. Schuchardt, Sercheli, and Vargas (1998) revealed that alcoholysis reaction requires 1 mol of a TG and 3 mol of alcohol, which the similar reaction pattern as in this study (transesterification of tuna oil requires 1 mol of oil and 3 mol of FAME). However, some excess level of the FAME should be used to increase the incorporation of fatty acids. This is limited by the economic implication since it increases the cost. For this study a mole ratio of tuna oil to $n-3$ FAME of 1:4 was found necessary.

4. Conclusion

Tuna oil was successfully chemically transesterified. The lipid and fatty acid composition were measured to help and understand the effect of reaction conditions on the recovery of TG and the incorporation of EPA and DHA. It was found that chemical transesterification could enrich $n-3$ PUFA, especially EPA and DHA, in tuna oil. However, loss of TG between transesterification lead to lower TG yields, which would be an interesting area for further study.

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