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Incorporation of docosahexaenoic acid (DHA) into evening primrose (*Oenothera biennis* L.) oil via lipase-catalyzed transesterification

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Abstract

Six commercial lipases (Novozym 435 from *Candida antarctica*, Lipozyme IM from *Mucor miehei*, PS-30 from *Pseudomonas* sp., AP-12 from *Aspergillus niger*, AY-30 from *Candida rugosa*, and Novozym-677BG from *Thermomyces lanuginosus*) were tested for their ability to incorporate docosahexaenoic acid (DHA; 22:6ω3) into evening primrose oil. Among the enzymes examined, Novozym 435 from *Candida antarctica* was chosen over the other enzymes to catalyse the transesterification reaction owing to higher incorporation of DHA. As enzyme concentration, substrate mole ratio and incubation time increased, incorporation of DHA also increased. For the time course reaction, incorporation of DHA increased up to 25.2% after 24 h. The highest DHA incorporation (37.4%) occurred at a substrate mole ratio of 1:3 (evening primrose oil: DHA). The positional distribution of the various fatty acids, between the *sn*-1,3 and *sn*-2 positions on the glycerol backbone, was also determined. Evening primrose oil containing DHA was successfully produced and may be useful in certain food and nutraceutical applications.

Keywords: Evening primrose oil; Docosahexaenoic acid; Novozym 435; *Candida antarctica*; Transesterification

1. Introduction

Evening primrose (*Oenothera biennis* L.) is a biennial plant belonging to the family of Onagraceae and is a common weed that is native to North America. Interest in evening primrose oil (EPO) has intensified in recent years because of its γ-linolenic acid (GLA; 18:3ω6) content (Senanayake & Shahidi, 1999a). At present, EPO is one of the most important sources of GLA, which is in growing demand for its clinical and pharmaceutical applications (Hudson, 1984).

The oil content of evening primrose seeds is 17–25% (Beaubaire & Simon, 1987; Wolf, Kleiman, & England, 1983), of which 7–10% is GLA (Fieldsend, 1996; Gibson, Lines, & Neumann, 1992). The total GLA content of the seeds is approximately 2.5% (Wolf, Kleiman, & England, 1983). The oil, as marketed, is made up of 97–98% triacylglycerols (TAG), 1.5–2.0% unsaponifiable matter and 0.5–1.0% polar lipids (Hudson, 1984). The EPO is generally obtained by mechanical pressing followed by extraction with hexane (Helme, 1996). There are preliminary indications that EPO may be better, in some of its physiological effects, than other oils in which GLA occurs. One possible explanation is that GLA is present in EPO almost entirely as molecular species of TAG in which one GLA is combined with two linoleic acid (LA; 18:2ω6) molecules (Fieldsend, 1996). Another possibility is that minor components of EPO, not GLA, are responsible for some of the effects. GLA from other oils (borage, blackcurrant and fungal) may also be biologically less effective than that from EPO, partly because of the other fatty acids present and partly because of the different stereospecific distribution of fatty acids in the TAG of oils (Horrobin, 1990).

GLA is biosynthesized from linoleic acid (18:2ω6) by Δ6 desaturase in the metabolic pathway of ω6 fatty acids. GLA is also an intermediate precursor of certain eicosanoids. These eicosanoids are important for various physiological functions in the human body. Impairment of the Δ6 desaturase activity may result in

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the functional deficiency of long-chain polyunsaturated ω6 metabolites of linoleic acid which would lead to pathogenesis associated with inflammatory, autoimmune and neoplastic diseases (Wu & Meydani, 1996). It has been claimed that GLA is effective in reducing inflammation and treating atopic eczema, diabetic neuropathy and certain cancers (McDonald & Fitzpatrick, 1998). There is also scientific evidence that GLA-rich oils such as EPO, are effective in the treatment of cardiovascular disease, age-related diseases and alcoholism (Broadhurst & Winther, 2000).

Within the ω3 family, docosahexaenoic acid (DHA; 22:6ω3) is of particular interest because it has been shown to be essential for optimal brain and eye function (Kim & Edsall, 1999). DHA is an important building block of the brain, nerves and eyes. The brain itself is made up of 60% lipid (Kinsella, 1993), and DHA is the most abundant fatty acid in both the brain and the retina (Senanayake & Shahidi, 2000a).

Transesterification reactions, that are catalyzed by enzymes or chemical catalysts produce modified oils by changing the fatty acid profile and/or positional distribution of fatty acids in the triacylglycerol molecule (Senanayake & Shahidi, 2000b, 2001). Previously, the enzyme-catalyzed incorporation of EPA ethyl ester into evening primrose oil has been reported (Akoh, Jennings, & Lillard, 1996). In this study, fatty acid profile and stereospecific distribution of evening primrose oil were modified to contain DHA, using lipase from *Candida antarctica* as the biocatalyst. The effects of different enzymes, enzyme concentration, incubation time, substrate mole ratio and the solvent type on percent incorporation of DHA into evening primrose oil were also examined.

2. Materials and methods

2.1. Materials and solvents

Evening primrose oil was obtained from Efamol, Inc. (Kentville, NS, Canada). Algal oil containing DHA (DHASCO®; 47.4% DHA) was from Martek Biosciences Corporation (Columbia, MD). Lipases, Novozym-435 (*Candida antarctica*), Lipozyme-IM (*Mucor miehei*) and Novozym-677BG (*Thermomyces lanuginosus*) were acquired from Novo Nordisk (Franklinton, NC). Lipases PS-30 (*Pseudomonas* sp.), AP-12 (*Aspergillus niger*), and AY-30 (*Candida rugosa*) were obtained from Amano Enzyme USA Co. Ltd. (Troy, VA). Lipozyme-IM is a *sn*-1,3-specific lipase from *Mucor miehei*, immobilized on a macroporous anion–exchange resin. Novozym-435 from *Candida antarctica* was immobilized on a macroporous acrylic resin. However, lipases from *Pseudomonas* sp., *Aspergillus niger*, *Candida rugosa* and *Thermomyces lanuginosus* were not immobilized. Solvents (*n*-hexane, isooctane, toluene, benzene, acetone, ethyl acetate and petroleum ether) were purchased from Fisher Scientific (Nepean, ON, Canada). All other chemicals were American Chemical Society (ACS) grade or better.

2.2. Assay of lipase activity

Lipase activity was determined as described by Senanayake and Shahidi (1999a). Lipase activity was measured by assaying fatty acids produced by the hydrolysis of triacylglycerols. Enzyme activities of microbial lipases tested are shown in Table 1.

2.3. Enzymatic transesterification

Unless otherwise stated, transesterification reactions were carried out in screw-capped test tubes containing evening primrose oil (297 mg), DHA (120 mg), lipase (30–200 enzyme activity units), water (2% by weight of substrates plus enzyme) and hexane (3 ml). The mixture was incubated in a thermostatted water bath at 20–55 °C and agitated at 250 rpm with magnetic stirring. Individual sample vials were removed and analyzed at different time periods (12–24 h).

2.4. Screening of lipases

Six commercial enzymes, namely, lipases from *Candida antarctica*, *Mucor miehei*, *Pseudomonas* sp., *Aspergillus niger*, *Candida rugosa* and *Thermomyces lanuginosus,* were used in this study. Evening primrose oil (500 mg) was mixed with DHA (194 mg), then lipase (500 enzyme activity units) and water (2% by weight of substrates plus enzyme) were added in hexane (3 ml). The reaction mixture was incubated at 37 °C for 24 h in an orbital shaking water bath at 250 rpm.

2.5. Separation of acylglycerols after transesterification

The enzymes were removed by passing the reaction mixture through a bed of anhydrous sodium sulphate. Samples were placed in 250 ml conical flasks and 20 ml of a mixture of acetone/ethanol (1:1, v/v) were added. The reaction mixture was titrated with 0.5 N NaOH to a phenolphthalein endpoint. The mixture was transferred to a separatory funnel and thoroughly mixed with 25 ml hexane. The lower aqueous layer was separated and discarded. The upper hexane layer, containing acylglycerols, was passed through a bed of anhydrous sodium sulphate. The acylglycerol fraction was subsequently recovered following hexane removal at 45 °C, using a rotary evaporator. The composition of the acylglycerol fraction was determined by thin layer chromatography-flame ionization detection (TLC-FID). The acylglycerols were methylated with 2 ml of 6% H_2SO_4 in methanol at

Table 1
Effect of different lipases on DHA incorporation (%) into evening primrose oil (EPO)[a]

Enzyme	Source	Enzyme activity (units)	DHA incorporation (%)
Novozym-435	Candida antarctica	554	28.7±0.5
Lipozyme-IM	Mucor miehei	13,613	20.1±0.4
PS-30	*Pseudomonas* sp.	11,936	24.2±1.3
AP-12	*Aspergillus niger*	8142	14.0±0.8
AY-30	*Candida rugosa*	38,707	7.53±0.2
Novozym-677BG	*Thermomyces lanuginosus*	7658	2.62±0.4

[a] The reaction mixture contained 500 mg oil, 194 mg DHA, 500 units of enzyme and 3 ml hexane. The reaction mixture was incubated at 37 °C for 24 h in an orbital shaking water bath at 250 rpm. Results are means of triplicate determinations from different experiments.

60 °C for 14 h, extracted with hexane (2 ml) and concentrated under nitrogen.

2.6. Gas chromatography analysis

Fatty acids were analyzed with a Hewlett Packard 5890 Series II gas chromatograph (Hewlett Packard, Toronto, ON) equipped with a SUPELCOWAX-10 column (0.25 mm diameter, 30 m length, 0.25 μm film thickness; Supelco Canada Ltd., Oakville, ON) and flame-ionization detector (FID). The oven temperature was initially set at 220 °C for 10.25 min and then ramped to 240 °C at 30 °C/min and then held there for 9 min. The injector and detector temperatures were both at 270 °C. UHP helium was used as a carrier gas (15 ml/min). HP 3365 Series II ChemStation software (Hewlett Packard, Toronto, ON) was used for data handling. The fatty acid methyl esters (FAMEs) were tentatively identified by comparison of their retention times with those of authentic standard mixtures (GLC-461; Nu-Check; Elysian, MN). The area under each peak was calculated on a weight percentage basis, using methyl tricosanoate ($C_{23:0}$) as an internal standard.

2.7. Hydrolysis of modified oils by pancreatic lipase

Hydrolysis of modified EPO triacylglycerols by pancreatic lipase was carried out according to the method described by Christie (1982). Tris-hydrochloric buffer (5 ml; 1.0 M, pH 8.0), 0.5 ml of calcium chloride (2.2%, w/v) and 1.25 ml of sodium taurocholate (0.05%, w/v) were added to 25 mg of oil in a glass test tube. The whole mixture was allowed to equilibrate at 40 °C in a water bath for 1 min and, subsequently, 5.0 mg of porcine pancreatic lipase (EC. 3.1.1.3, Sigma) were added to it. The mixture was then placed in a Gyrotory water bath shaker (Model G76, New Brunswick Scientific Co. Inc., New Brunswick, NJ) at 200 rpm under nitrogen for 8–10 min at 40 °C. Ethanol (5 ml) was added to stop the enzymatic hydrolysis followed by the addition of 5.0 ml of 6.0 N HCl. The hydrolytic products were extracted three times with 50 ml of diethyl ether and the ether layer was washed twice with distilled water and dried over anhydrous sodium sulphate. After removal of the solvent under vacuum at 30 °C, the hydrolytic products were separated on silica gel thin-layer chromatography (TLC) plates (20 × 20 cm; 60 Å mean pore diameter, 2–25 μm mean particle size, 500 μm thickness, with dichlorofluorescein, Sigma) impregnated with 5% (w/v) boric acid. The plates were developed using hexane/diethyl ether/acetic acid (70:30:1, v/v/v). After drying, the bands were located by viewing under short (254 nm) and long (356 nm) wavelength UV lights (Spectraline, Model ENF-240C, Spectronics Co., Westbury, NY). The bands were scraped off and their lipids extracted into chloroform/methanol (1:1, v/v) or diethyl ether and subsequently used for fatty acid analysis by the gas chromatographic procedure described above.

2.8. Determination of tocopherols in oils

The content of tocopherols in modified and unmodified evening primrose oils was determined by high-performance liquid chromatography (HPLC). Tocopherols in the oils were separated using a Lichrosorb Si-60 (3.2 × 20 mm, 5 μ; Merck, Darmstadt, Germany) analytical column by employing diethyl ether-hexane (5:95, v/v) as the mobile phase (Thompson & Hatina, 1979). The apparatus used for the analysis was a Shimadzu HPLC equipped with two LC-6A pumps, SPD-6AV UV–vis spectrophotometric detector and C-R4A Chromatopac for data handling. A 20-μl sample (1 g of oil was dissolved in 10 ml of mobile phase) and a flow rate of 1 ml/min were used for the analysis. The tocopherols in the oils were detected using a UV detector set at 295 nm and components identified by comparing their retention times with those of known tocopherol standards.

3. Results and discussion

3.1. Enzyme screening

Six commercial lipases from *Candida antarctica* (Novozym 435), *Mucor miehei* (Lipozyme IM), *Pseudomonas* sp. (PS-30), *Candida rugosa* (AY-30), *Aspergillus*

niger (AP-12) and *Thermomyces lanuginosus* (Novozym-677BG) were screened for their ability to incorporate DHA into evening primrose oil (EPO) at 37 °C in hexane. As shown in Table 1, the extent of DHA incorporation with various lipases was in the order of *Candida antarctica* > *Pseudomonas* sp. > *Mucor miehei* > *Aspergillus niger* > *Candida rugosa* > *Thermomyces lanuginosus*. The lipases from *Candida antarctica*, *Mucor miehei* and *Pseudomonas* sp. and, to a limited extent, lipases from *Aspergillus niger* and *Candida rugosa,* incorporated DHA into EPO. However, lipase from *Thermomyces lanuginosus* was not efficient in incorporating DHA into EPO. Lipase from *Candida antarctica* was used further in this study because it afforded the highest DHA incorporation into EPO (28.7%, after 24 h). Since lipases from *Candida antarctica* and *Mucor miehei* were used in the immobilised form, it should be noted that their activity might have been affected by the immobilisation process. In a study on the transesterification of borage oil and DHA by lipases from different sources, it was observed that Novozym 435 from *Candida antarctica* showed a stronger preference toward incorporation of DHA into the oil (Senanayake & Shahidi, 1999b)

3.2. Effect of enzyme concentration

The effect of enzyme concentration (lipase from *Candida antarctica*) on the incorporation of DHA (%) into EPO is shown in Fig. 1. The extent of DHA incorporation increased noticeably with the increase of lipase concentration. However, when the enzyme was present at a level greater than 150 units, the degree of DHA incorporation remained virtually constant. Thus, 150 units of enzyme concentration were sufficient to saturate the reaction system. For the enzyme load reaction, the highest DHA incorporation (26.2%) after 24 h incubation was achieved at 150 enzyme activity units.

3.3. Time course

The changes in fatty acid profile of EPO, after transesterification with DHA using lipase from *Candida antarctica,* are listed in Table 2. After 24 h reaction at 37 °C, the content of DHA incorporated into this oil was 25.2%. The main fatty acid found in EPO before enzymatic modification was linoleic acid (18:2ω6; 72.6%). The content of γ-linolenic acid (GLA; 18:3ω6) found in this oil was 9.1%. The amounts of monounsaturated, saturated and total ω6 fatty acids decreased upon DHA incorporation into the oil. The modified EPO had an ω3/ω6 ratio of 0.16–0.4 (Table 2). Sridhar and Lakshminarayana (1992) were able to effectively modify groundnut oil by incorporating eicosapentaenoic acid (EPA; 20:5ω3) and DHA using a *sn*-1,3-specific lipase from *Mucor miehei* as the biocatalyst.

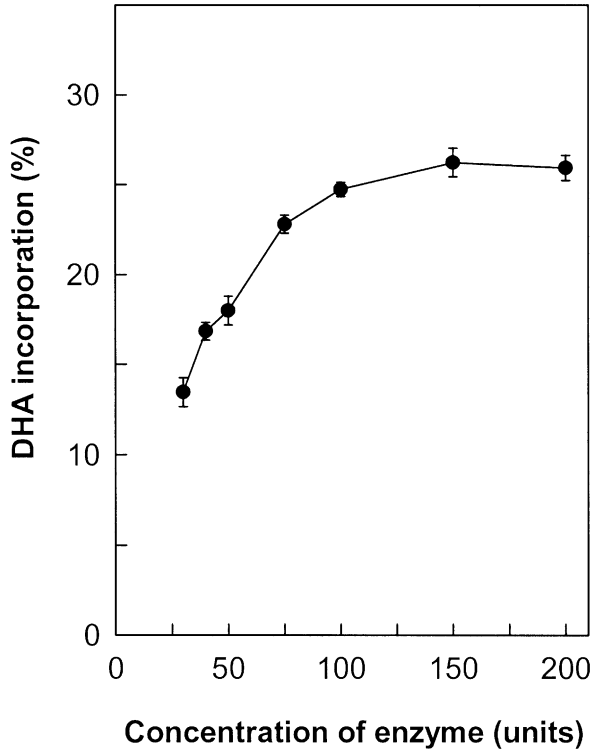

Fig. 1. Effect of enzyme concentration on the incorporation of DHA into evening primrose oil. The reaction mixture contained 297 mg oil, 120 mg DHA, 30–200 units of *Candida antarctica* lipase preparation and 3 ml hexane. The reaction mixture was incubated at 37 °C for 24 h in an orbital shaking water bath at 250 rpm.

Table 2
Changes in fatty acid profile of evening primrose oil (EPO) during lipase-catalyzed transesterification with DHA[a] (% incorporated)

Fatty acid	Duration of transesterification (h)	
	0	24
14:0	0.04±0.01	ND[b]
16:0	6.17±0.09	4.70±0.50
16:1	0.04±0.02	ND[b]
17:0	0.08±0.01	ND[b]
18:0	1.75±0.12	0.69±0.07
18:1	8.65±0.56	4.21±0.50
18:2ω6	72.6±0.91	54.3±1.26
18:3ω6	9.12±0.38	7.6±0.45
18:3ω3	0.16±0.03	ND[b]
20:0	0.34±0.05	ND[b]
20:1	0.29±0.07	ND[b]
20:2	0.05±0.05	ND[b]
22:0	0.14±0.05	0.11±0.04
22:1	0.12±0.01	ND[b]
22:6ω3	ND[b]	25.2±0.10
ω3/ω6 Ratio	0.001	0.40

[a] The reaction mixture contained 297 mg evening primrose oil, 120 mg DHA, 150 units of *Candida antarctica* and 3 ml hexane. The reaction mixture was incubated at 37 °C in an orbital shaking water bath at 250 rpm. Experimental results are means of triplicate determinations.

[b] Not detected,

The resultant contents of EPA and DHA of the modified oil were 9.5 and 8.0%, respectively. Haraldsson, Höskuldsson, and Sigurdsson (1989) succeeded in preparing EPA-enriched triacylglycerols (40% EPA and 25% DHA) as well as DHA-enriched triacylglycerols (TAG) of 48% DHA and 12% EPA, using appropriate EPA or DHA concentrates, respectively. *Mucor miehei* lipase-catalysed interesterification of cod liver oil, with ω3 PUFA concentrates, was used in the latter study.

3.4. Effect of temperature on the incorporation of DHA into EPO

Fig. 2 illustrates the effect of reaction temperature on lipase-catalyzed transesterification of EPO with DHA by an immobilized *Candida antarctica* lipase. The temperature range examined was 20–55 °C. DHA incorporation increased as the temperature was increased up to 45 °C. When the temperature increased further, the degree of DHA incorporation remained constant. The optimum temperature range for this reaction was 45–55 °C. Thus, higher temperatures, up to 55 °C, seemed more suitable for better performance of *Candida antarctica* lipase. This finding lends further support to those reported by Akoh and Huang (1995). The higher temperature optimum for *Candida antarctica* enzyme was probably partly due to the fact that immobilisation conferred greater thermostability on this enzyme. Kosugi and Azuma (1994) used an immobilized lipase from *Candida antarctica* for production of pure TAG from EPA or DHA with glycerol. The TAG formation was reported to be faster at 60 °C, even though the TAG yield was the same at 40 and 60 °C. Recently, Ikeda and Kurokawa (2001) used the same enzyme for esterification of geraniol and acetic acid. For this reaction, *Candida antarctica* lipase showed an increase in activity with increasing temperature with maximum activity at 60 °C.

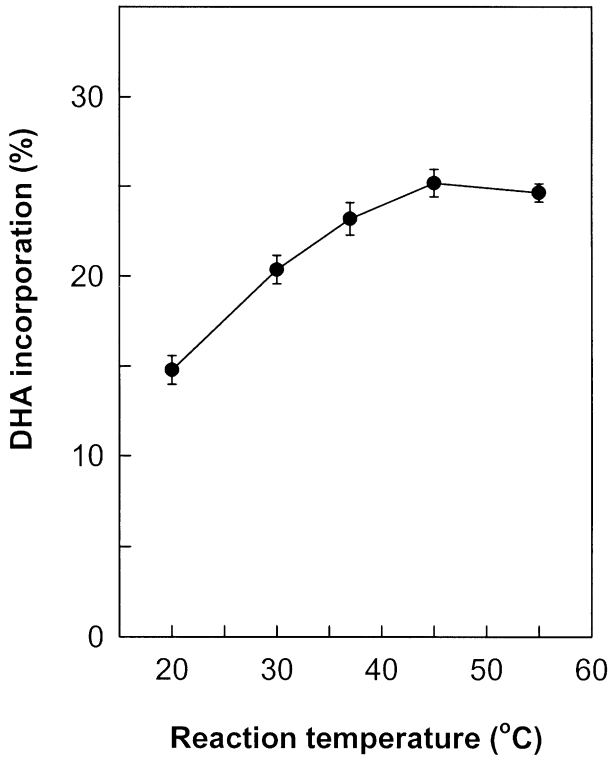

Fig. 2. Effect of temperature on DHA incorporation into evening primrose oil. The reaction mixture contained 297 mg oil, 120 mg DHA, 150 units of *Candida antarctica* lipase preparation and 3 ml hexane. The reaction mixture was incubated at different temperatures (20–55 °C) for 24 h in an orbital shaking water bath at 250 rpm.

3.5. Reactions in organic solvents and solvent-free media

The effects of various solvents on enzymatic behaviour have been studied. Attempts have been made to correlate these effects with the hydrophobicity of solvents (Laane, Boeren, & Vos, 1985). Solvent hydrophobicity is generally measured by log P (the logarithm of partition coefficient between water and octanol). To select the most suitable solvent for transesterification of EPO with DHA by lipase from *Candida antarctica*, the effect of the presence of various organic solvents (isooctane; log $P = 4.5$, hexane; log $P = 3.5$, toluene; log $P = 2.5$, benzene; log $P = 2.0$, acetone; log $P = -0.23$, ethyl acetate; log $P = 0.68$ and petroleum ether; no log P value was reported) in the reaction medium were examined (Fig. 3). Although the use of solvent-free reactions were not the aim of this work, these systems were tested to demonstrate the advantages of solvents for tailor-made synthesis of modified oils. The reactions carried out in *n*-hexane showed the highest degree of DHA incorporation (25.5%) in EPO. Organic solvents, such as *n*-hexane, have several functions, including increasing the solubility of nonpolar substrates and shifting the reaction towards synthesis rather than hydrolysis (Klibanov, 1986). However, satisfactory incorporation of 18.1% of DHA in EPO was achieved in a solvent-free system.

3.6. Effect of mole ratio of substrates on the incorporation of DHA into EPO

Table 3 shows the effect of increasing substrate mole ratio (from 1:1 to 1:3) on DHA incorporation into EPO. As the mole ratio of EPO to DHA was increased from 1:1 to 1:3, incorporation of DHA increased. Incorporation of DHA in EPO was increased up to 37.4% at a mole ratio of 1:3. Although the substrate mole ratio can be manipulated to achieve the desired level of DHA incorporation, economically there is no advantage of using excess DHA, which is expensive. Higher concentration of DHA (free acid) in the reaction medium may also result in the substrate inhibition of the lipase. Desorption of water by the fatty acid substrate may also be a secondary mode of inhibition.

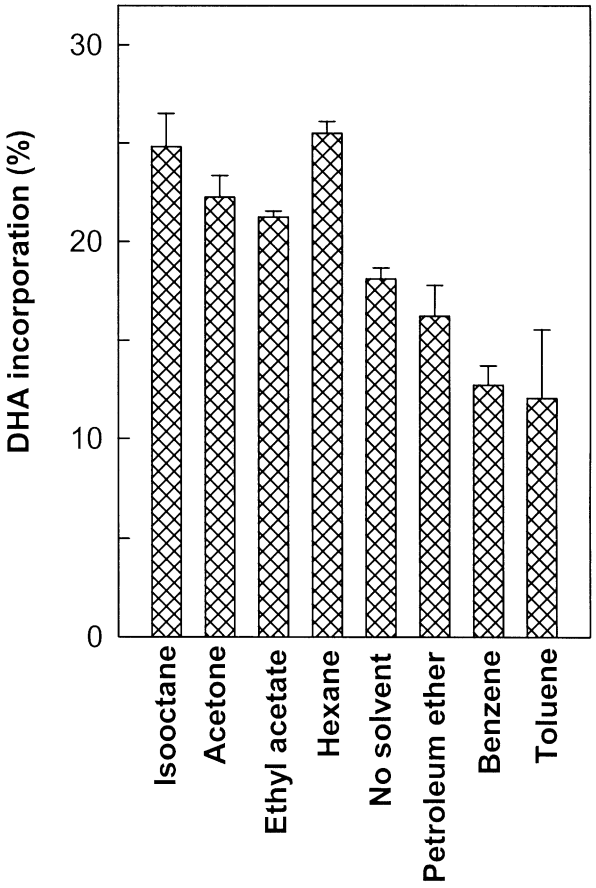

Fig. 3. Effect of different organic solvents on the incorporation of DHA into evening primrose oil. The reaction mixture contained 297 mg oil, 120 mg DHA, 150 units of *Candida antarctica* lipase preparation and 3 ml hexane. The reaction mixture was incubated at 37 °C for 24 h in an orbital shaking water bath at 250 rpm.

Table 3
Effect of mole ratio of substrates on DHA incorporation% into evening primrose oil (EPO)[a]

Major fatty acids	Mole ratio		
	1:1	1:2	1:3
16:0	4.53±0.50	3.72±0.17	2.81±0.23
18:0	0.22±0.07	0.21±0.05	0.20±0.03
18:1	4.81±0.50	4.33±0.24	3.84±0.52
18:2ω6	53.3±1.26	50.6±1.60	47.3±1.34
18:3ω6	7.61±0.45	7.40±0.21	6.21±0.25
22:6ω3	27.2±0.10	31.1±1.80	37.4±0.82

[a] Mole ratios of evening primrose oil TAG to DHA were varied from 1:1 to 1:3. Reactions were carried out at 37 °C for 24 h in an orbital shaking water bath at 250 rpm.

3.7. Composition of the products of transesterification catalysed by Candida antarctica lipase

The products formed by transesterification of EPO and DHA contained TAG, DAG and MAG. The free fatty acids (FFA) were not detected by thin layer chromatography-flame ionization detection (TLC-FID) since they were removed by NaOH after the enzymatic reaction. The transesterification yielded relatively large proportions (89.1%) of TAG (Table 4). The transesterification of EPO and DHA yielded much lower proportions of hydrolysis products i.e. 2.0% DAG and 8.9% MAG after 24 h at 37 °C. The TAG fraction contained 7.6% GLA and 27.3% DHA. The DAG fraction also had 4.3% GLA and 24.6% DHA. Meanwhile, the MAG fraction contained corresponding values of 9.1 and 20.2%, respectively (Table 4).

3.8. Positional distribution of fatty acids in modified oil

Pancreatic lipase hydrolysis was performed in this study to determine the fatty acid composition of the *sn*-2 and *sn*-1,3 positions of modified EPO. The results showed that high amounts of 16:0, 18:0 and 18:1 were incorporated with high selectivity in the *sn*-1,3 positions of modified EPO (Table 5). GLA was randomly distributed over the *sn*-1,3 and *sn*-2 positions of TAG. However, linoleic acid was mainly attached to *sn*-1,3 positions of TAG. As desired, in the *sn*-2 position, 38.2% DHA was esterified (Table 5). Based on the percent fatty acid distribution of total TAG located on the *sn*-2 position, DHA represents 45.3%, indicating that 54.7% of these fatty acid residues were attached to primary positions of modified EPO (Table 5).

3.9. Effect of enzymatic transesterification on the tocopherol content of oil

Tocopherols are one of the most important group of natural antioxidants present in edible oils and serve as free radical scavengers and singlet oxygen quenchers. γ-Tocopherol and δ-tocopherol have frequently been found superior to α-tocopherol in terms of their antioxidant activities (Elmadfa & Wagner, 1997). These compounds can retard lipid autoxidation, mainly by donating a hydrogen atom to a lipid peroxyl radical, forming a hydroperoxide and a tocopheroxyl radical, and subsequent reaction of tocopheroxyl radical with a second lipid peroxyl radical to peroxy tocopherones (Kamal-Eldin, & Appelqvist, 1996). Individual tocopherol contents of unmodified and modified evening primrose oils were determined (Table 6). Unmodified evening primrose oil contained α-, γ- and δ-tocopherol contents of 680, 222 and 218 ppm, respectively. However, their corresponding contents were reduced to 534, 0 and 197 ppm, respectively, after enzyme-catalyzed transesterification reactions, suggesting possible losses during the enzymatic reaction and subsequent processing. The exposure to light and heat during processing may be contributing factors to these tocopherol losses. Therefore, this finding suggests that modified oils, prepared by enzymatic reactions, need to be supplemented with appropriate antioxidants

Table 4
Fatty acid composition of acylglycerol components of enzymatically modified evening primrose oil (EPO) separated after transesterification by *Candida antarctica* lipase[a]

Major fatty acids	Lipid component (%)		
	TAG[b] (89.1)	DAG (2.0)	MAG (8.9)
16:0	3.52±0.41	2.69±0.02	3.78±0.61
18:1	4.53±0.25	4.16±0.50	5.45±0.35
18:2ω6	54.4±1.18	61.1±0.91	57.0±1.41
18:3ω6	7.60±0.57	4.32±0.61	9.10±0.62
22:6ω3	27.3±0.65	24.6±0.78	20.2±0.54

[a] The reaction mixture contained 297 mg evening primrose oil, 120 mg DHA, 150 units of *Candida antarctica* and 3 ml hexane. The reaction mixture was incubated at 37 °C for 24 h in an orbital shaking water bath at 250 rpm.
[b] TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols.

Table 5
Composition and positional distribution of fatty acids in enzymatically modified evening primrose oil triacylglycerols

Fatty acid	Composition (w/w%)	Positional distribution	
		sn-2 position	*sn*-1 + *sn*-3 positions
16:0	7.6±0.17	3.7±0.40 (16.2)[a]	9.0±0.35 (83.8*)[b]
18:0	2.3±0.42	1.5±0.26 (21.7)	3.0±0.57 (78.3*)
18:1	4.6±0.35	1.5±0.54 (10.9)	6.3±0.16 (89.1*)
18:2ω6	48.4±0.64	45.0±0.60 (31.0)	43.0±1.0 (69.0*)
18:3ω6	7.1±0.15	7.5±0.82 (35.2)	6.8±0.56 (64.8)
22:6ω3	28.1±0.70	38.2±0.52 (45.3)	29.1±0.72 (54.7)

Values in parentheses indicate percent fatty acid distribution of total triacylglycerols located on *sn*-2, and *sn*-1 plus *sn*-3 positions. * = indicates predominance of fatty acid in a given position.
[a] (% fatty acid at the sn-2 position /% fatty acid in triacylglycerols × 3) × 100.
[b] 100—product of[a].
[c] *Indicater predominance of fatty acid in a given position.

Table 6
Tocopherol contents of unmodified and modified evening primrose oils

Sample	Tocopherols (ppm)			
	α	γ	δ	Total
Unmodified evening primrose oil	680	222	218	1120
Modified evening primrose oil	534	ND[a]	197	730

[a] Not detected.

(natural or synthetic) before incorporation into food, use as nutraceuticals or in other applications.

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