Separation of Eicosapentaenoic Acid and Docosahexaenoic Acid in Fish Oil by Kinetic Resolution Using Lipase

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ABSTRACT: The objective of this study was to investigate the use of lipases as catalysts for separating eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish oil by kinetic resolution. Transesterification of various fish oil triglycerides with a stoichiometric amount of ethanol by immobilized *Rhizomucor miehei* lipase under anhydrous solvent-free conditions resulted in a good separation. When free fatty acids from the various fish oils were directly esterified with ethanol under similar conditions, greatly improved results were obtained. By this modification, complications related to regioselectivity of the lipase and nonhomogeneous distribution of EPA and DHA into the various positions of the triglycerides were avoided. As an example, when tuna oil comprising 6% EPA and 23% DHA was transesterified with ethanol, 65% conversion into ethyl esters was obtained after 24 h. The residual glyceride mixture contained 49% DHA and 6% EPA (8:1), with 90% DHA recovery into the glyceride mixture and 60% EPA recovery into the ethyl ester product. When the corresponding tuna oil free fatty acids were directly esterified with ethanol, 68% conversion was obtained after only 8 h. The residual free fatty acids comprised 74% DHA and only 3% EPA (25:1). The recovery of both DHA into the residual free fatty acid fraction and EPA into the ethyl ester product remained very high, 83 and 87%, respectively.
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KEY WORDS: DHA, EPA, esterification, ethanolysis, fish oil, kinetic resolution, lipase, Lipozyme IM, n-3 fatty acids, *Rhizomucor miehei* lipase.

There is a constantly growing demand for concentrates of the long-chain polyunsaturated n-3 type fatty acids characteristic of marine lipids, especially *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA), as a consequence of their beneficial health effects (1). Recently, we described a highly efficient process to concentrate both EPA and DHA by lipase-catalyzed ethanolysis of fish oil (2). There are numerous reports in the literature involving biotransformations of n-3 polyunsaturated fatty acids from marine oil by lipases (2–17). They reveal that many of the commercially available lipases discriminate against n-3 polyunsaturated fatty acids, and that lipases that display any significant activity toward n-3 fatty acids usually prefer EPA to DHA as a substrate. There are several reports on the direct esterification of polyunsaturated free fatty acids (FFA) from fish oil using simple alcohols to bring about DHA concentration (5–8). Very recently Shimada and co-workers reported a highly efficient concentration of DHA by a direct esterification of the FFA from tuna oil with various longer-chain alcohols using lipases (9,10). The preparation of homogeneous triacylglycerols containing either pure EPA or DHA also has been described by direct esterification of glycerol (11). Besides their wide range in fatty acid selectivity, lipases are ideally suited for esterification processes involving the highly labile long-chain n-3 type polyunsaturated fatty acids due to the mildness they offer (4). Their all-*cis* n-3 structure is prone to a partial destruction by oxidation, *cis-trans* isomerization, or double-bond migrations, and they also are susceptible to polymerization because of the pH and high temperature required in conventional chemical processes. Finally, lipase-promoted hydrolysis of fish oil triacylglycerols has also been commonly practiced, usually with an emphasis on DHA enrichment for infant formula development (12–17).

In our above-mentioned EPA and DHA enrichment work, lipases from two strains of bacteria [*Pseudomonas fluorescens* and *Pseudomonas* species lipases from Amano (Milton Keynes, England)] were observed to convert the bulk of the unwanted saturated and monounsaturated fatty acids of the fish oil triacylglycerols into ethyl esters. The n-3 polyunsaturated fatty acids including both EPA and DHA, on the other hand, remained attached to the residual acylglycerols, mainly as mono- and diacylglycerols, but also triacylglycerols, depending upon the extent of conversion. No solvents were required and only stoichiometric amounts of ethanol were required. By that method, it became easy to obtain concentration levels of 50% EPA + DHA in the residual glyceride mixture in very high EPA and DHA recovery. In the continuation of the work we became interested in investigating the possibility of discriminating between EPA and DHA in fish oil instead of concentrating both EPA and DHA by lipase-promoted kinetic resolution. Thus, different requirements for the lipase were made and a lipase strongly discriminating between EPA and DHA was needed. In order to avoid complications related to possible regioselectivity of the lipase toward triacylglycerols (18) and nonrandom distribution of EPA and DHA into various positions of the glycerol backbone (19), as well as a possible triacylglycerol selectivity of the lipase (15), a different approach was required. It was decided

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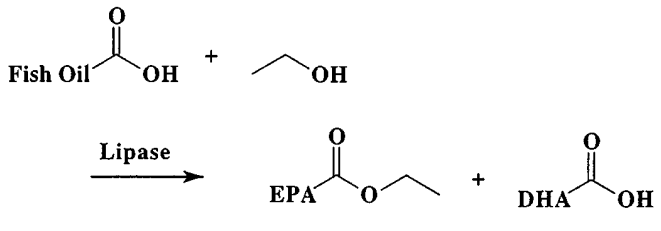
to base that approach on the direct esterification of FFA from fish oil. This is demonstrated in Scheme 1 and the results are described in this report.



SCHEME 1

EXPERIMENTAL PROCEDURES

The fungal lipase from *Rhizomucor miehei,* provided as Lipozyme IM, was supplied immobilized by Novo Nordisk A.S. (Bagsvaerd, Denmark). It was used directly as supplied with 10% moisture content. *Candida rugosa* and *Rhizopus delemar* lipases were supplied by Amano Enzyme Europe Ltd. and employed directly as powder, without any preadjustment or optimization of pH. Refined sardine oil, comprising 18% EPA and 12% DHA; herring oil, comprising 5.5% EPA and 8.0% DHA; and tuna oil, comprising 6% EPA and 23% DHA, were provided by Pronova Biocare A.S. (Bergen, Norway). They were used without any further refinement. A concentrate of 77% EPA and 10% DHA was supplied by Pronova Biocare A.S. and was converted into FFA by a previously described procedure (11). All solvents were analytical grade and purchased from Merck AG (Darmstadt, Germany). Fatty acid analyses were performed on methyl esters employing a Perkin-Elmer 8140 gas chromatograph using a 30-m capillary column, DB-225 30 N 0.25 mm with hydrogen as carrier gas, according to our previously described procedure (20). Preparative thin-layer chromatography (TLC) was conducted on silica gel plates from Merck (Art. 5721) after washing with a 50:50 mixture of chloroform/methanol and heating at 110°C for 30 min. Elution was performed with an 80:20:1 mixture of petroleum ether/diethyl ether/acetic acid. Rhodamine 6G (Merck) was used to visualize the bands, which subsequently were scraped off and the fat methylated for fatty acid analyses. Methyl esters of $C_{19:0}$ or $C_{21:0}$, purchased from Sigma, were added to the samples as internal standards before injection. When calculating the weight percentage of ethyl esters, the ethyl moiety attached to the fatty acid was counted and corrected for by a calculated conversion parameter, from the gas chromatography (GC) area percentages of the methyl esters. The parameter used was 1.047.

Hydrolysis of fish oils. Fish oil triglycerides (100 g, approximately 0.34 mol; molecular weight approximately 882 g/mol) were added to a solution of sodium hydroxide (27.2 g, 0.68 mol) in water (122 mL) and ethanol (96%, 122 mL). The mixture was refluxed under nitrogen and the reaction monitored by analytical TLC (silica gel; eluted with 1:9 ethyl acetate/petroleum ether). The reaction was completed in less than 2 h. The mixture was allowed to cool to room temperature and then carefully acidified by adding an aqueous solution of 2–4 M HCl until slightly acidic. The temperature was not allowed to rise significantly; if necessary, ice was added to cool the mixture. The resulting FFA were then extracted two times with a 1:1 mixture of petroleum ether/diethyl ether. The combined organic solvents were washed with water until the washings were almost neutral in pH, and then dried over anhydrous magnesium sulfate. The organic solvent was removed *in vacuo* on a rotary evaporator to extract the free fatty acids.

Lipase-catalyzed ethanolysis. The ethanolysis reactions were performed in a manner identical to a previously described procedure (2).

Lipase-catalyzed esterification. In a typical procedure the immobilized lipase preparation (0.5 g) was added to a mixture of the fish oil FFA (5.64 g, approximately 20 mmol; molecular wt. approx. 282 g/mol) and absolute ethanol (2.76 g, 40 mmol). The resulting enzyme suspension was gently agitated at room temperature under nitrogen. Small samples (100–200 mg) were withdrawn by the aid of a Pasteur pipette at appropriate times. Enzyme particles were separated by filtering the sample through a cotton wool plug located inside a second Pasteur pipette. The ethanol was removed *in vacuo* on a rotary evaporator. Approximately 50 mg of the residual oil was accurately weighed and dissolved in isopropanol (25 mL). The resulting mixture was titrated with an aqueous 0.0100 M sodium hydroxide solution, using phenolphthalein as an indicator. Blank titrations of the isopropanol were performed on a regular basis. Preparative TLC was applied to separate the ethyl esters and the residual FFA, each of which was subsequently methylated and subjected to gas–liquid chromatography (GLC) to reveal their fatty acid composition.

Lipase activity. According to the supplier, the activity of the Lipozyme IM preparation was 8 BAUN/g. The BAUN (Batch Acidolysis Units Novo) method was developed by Novo Nordisk for measuring the catalytic activity of immobilized lipase and is based on incorporation of decanoic acid into high-oleic sunflower oil in a nonsolvent-based assay. The incorporation was followed by GLC analyses of methyl esters derived from the triacylglycerols. 1 BAUN is equal to an incorporation of approximately 2.4% decanoic acid per h per g Lipozyme (dry wt) at standard conditions (10% water content as based on weight of Lipozyme, at 70°C). Full description of the method can be provided by the supplier.

RESULTS AND DISCUSSION

Separation of EPA and DHA from a mixture by kinetic resolution (21) by lipase is based on different rate constants, k, for EPA and DHA in their lipase-catalyzed biotransformations, where some substrates, S, of EPA and DHA will be converted into the corresponding products, P. Kinetic resolution occurs if k_{EPA} is not equal to k_{DHA} and the reaction is terminated at some stage between 0 and 100% conversion. Maximal separation can be expected when k_{EPA} is far higher than k_{DHA} or vice versa for k_{DHA}. As the term indicates, fast processes are favorable for kinetic resolution, and any sort of an equilib-

TABLE 1
Ethanolysis of Sardine Oil[a]

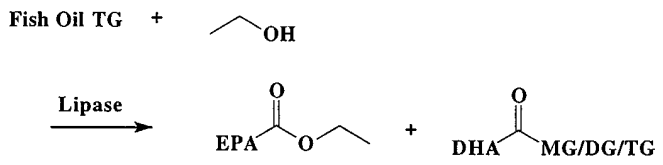
		Ethyl dsters			Residual glycerides		
Time (h)	Conv.%	EPA%	DHA%	EPA rec.%	EPA%	DHA%	DHA rec.%
2	5.2	7.2	1.8	2.0	18.7	12.0	99.2
5	13.8	7.7	1.0	5.8	20.4	13.9	99.0
8	35.0	10.0	0.6	22.9	21.5	16.5	97.8
12	53.6	16.4	0.6	28.2	22.2	18.6	96.6
24	67.4	16.4	1.1	64.2	19.0	23.2	91.0
48	73.7	17.8	1.7	74.8	16.9	27.8	85.5

[a]Conv.%, conversion—weight percentage of produced ethyl esters based on total weight of fat; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; EPA% or DHA%, area percentage of EPA or DHA as provided by gas–liquid chromatography (GLC) fatty acid analysis; EPA rec.% or DHA rec.%, recovery (or yield) of EPA and DHA into ethyl esters or residual glycerides, defined as proportion of their total weight in the initial fish oil.

rium must be avoided in order to get optimal results. Possible types of processes include a direct esterification of FFA, transesterification of esters, or hydrolysis of esters.

In our previously described ethanolysis process to concentrate EPA together with DHA in fish oil, the *Pseudomonas* lipases were observed to display a significant preference for DHA as compared to EPA. This is most unusual, since lipases normally prefer EPA to DHA as a substrate. In order to separate EPA and DHA in fish oil, a different lipase was therefore required. Besides a good tolerance toward fish oil triacylglycerols and the ethanolic reaction conditions a strong discrimination between EPA and DHA was required. Lipozyme IM is an immobilized 1,3-regiospecific *R. miehei* fungal lipase commercially available from Novo Nordisk. It is known to display a good discrimination between EPA and DHA in favor of EPA (22), and became the lipase of choice.

Ethanolysis of fish oil. When sardine oil was treated under the previously described ethanolysis conditions with Lipozyme at 20°C the results exhibited in Table 1 were obtained. The sardine oil comprised 18% EPA and 12% DHA as initial composition. Like before, a stoichiometric amount of absolute ethanol was used, which means three molar equivalents of ethanol as based on glycerol, or one equivalent as based on the number of ester functions present in the glycerol backbone. This process is demonstrated in Scheme 2.



SCHEME 2

The weight percentageof the produced ethyl esters was used directly to monitor the progress of the reaction in terms of conversion. The area percentage provided by GLC fatty acid analysis of the ethyl ester product and the residual acylglycerol mixture, which was not further fractionated, directly designated the extent of enrichment of EPA or DHA into both fractions as the ethanolysis reaction proceeded. The recovery or yield of EPA into the ethyl esters or DHA into the acylglycerols is defined as their wt% denoting the proportion of their total weight in the initial fish oil. Promising results were obtained (Table 1) and a high extent of conversion into ethyl esters was observed. High recovery of EPA into the ethyl esters was obtained as well as a very favorable ratio of EPA to DHA (10:1) as based on area percentage after 48 h. Very high recovery of DHA was obtained in the residual acylglycerol mixture. However, high DHA to EPA ratios were not obtained as a result of unfavorable DHA to EPA ratio in the initial oil.

That situation changed when tuna oil was treated under the same conditions, as shown in Table 2. The tuna oil contained 6% EPA and 23% DHA. Again, high extent of conversion into ethyl esters was obtained. At 65% conversion, after 24 h reaction time, the residual acylglycerol mixture contained 49% DHA and its recovery remained higher than 90%. A much more favorable ratio of DHA to EPA, 8:1, was obtained for the tuna oil than for to the sardine oil. At a higher conversion the DHA content of the acylglycerols continued to increase, but at the cost of lower recovery. This behavior clearly demonstrates that a compromise must be maintained between the extent of conversion and composition of the acylglycerols, as well as the EPA and DHA recovery (2).

Esterification of fish oil FFA. In order to avoid complications related to regioselectivity of the lipase and the fact that EPA and DHA are usually unevenly distributed among the various positions of fish oil triacylglycerols (19), a different approach was proposed. It was decided to base this approach on the direct esterification of FFA obtained from fish oil hydrolysis with ethanol according to Scheme 1. A lipase such as Lipozyme was expected to convert the bulk of the fatty acids present, including EPA, into ethyl esters, leaving the more reluctant DHA untouched in the residual FFA. The reaction conditions for the esterification reactions were similar to those in the ethanolysis reactions. The reactions were conducted at room temperature under nitrogen atmosphere, without a solvent, using a twofold molar excess of absolute ethanol. A 10% dosage of immobilized lipase was used as based on the weight of fatty acids, leaving the water content of the reaction medium below 1%. It became crucial to use only stoichiometric amounts of ethanol, since a more excessive use of ethanol resulted in dramatic drops in ac-

TABLE 2
Ethanolysis of Tuna Oil[a]

		Ethyl esters			Residual glycerides		
Time (h)	Conv.%	EPA%	DHA%	EPA rec.%	EPA%	DHA%	DHA rec.%
2	5.0	2.2	0.0	1.6	7.1	28.2	100
5	11.6	2.7	0.0	5.7	7.7	30.9	100
8	33.0	3.3	0.0	14.7	8.2	36.1	100
12	51.8	3.9	1.1	31.5	9.1	43.0	97.2
24	64.5	7.4	2.9	60.2	6.1	49.1	90.2
48	70.2	7.6	6.6	74.8	6.1	53.7	77.6

[a]See Table 1 for abbreviations.

tivity and performance of the lipase. This is not surprising, since not many lipases were expected to tolerate the polar conditions involving FFA and ethanol (23).

When sardine oil FFA were treated under the direct esterification reactions with Lipozyme the results shown in Table 3 were obtained. As can be noticed the reaction rates were much faster for the esterification than the ethanolysis process, which serves the kinetic resolution very well. More than 50% extent of conversion into ethyl esters had been reached after less than 2 h. Recoveries of EPA into ethyl esters and DHA into the residual FFA were also very high. The area percentage ratios between EPA and DHA reached 7:1 in the ethyl esters and 6:1 between DHA and EPA in the FFA at 82% conversion after 24 h, where 50% DHA composition was obtained in nearly 80% DHA recovery, despite the unfavorable initial DHA to EPA ratio in the sardine oil.

The results were even more dramatic when tuna oil FFA were treated under the esterification reaction conditions with ethanol, as can be seen in Table 4. At 70% conversion after 11 h, area percentage ratios as high as 30:1 were obtained in the residual FFA, which comprised 77% DHA in high recoveries. The EPA recovery into the ethyl esters also remained extremely high, 90%. These results are similar to those obtained by Shimada and co-workers for the direct esterification of tuna oil FFA with lauryl alcohol by *R. delemar* lipase (9,10). That lipase was reported not to tolerate the ethanolic conditions used in the present work, as we confirmed and also established for the *C. rugosa* lipase, which is also known to discriminate strongly between EPA and DHA (15–17).

Table 5 displays the results when FFA from herring oil were treated under the direct esterification conditions with Lipozyme. Herring oil is a relatively inexpensive fish oil with relatively low EPA and DHA, 5.5 and 8%, respectively. It is easy to obtain a highly favorable DHA composition in excellent DHA recovery from herring oil (Table 5). At a higher degree of conversion it became possible to achieve DHA levels higher than 50% from herring oil FFA in good DHA recovery (not included in the table).

Table 6 shows the results of treating an FFA concentrate of 77% EPA and 10% DHA under the direct esterification re-

TABLE 3
Esterification of Sardine Oil Fatty Acids[a]

		Ethyl esters			Residual FFA		
Time (h)	Conv.%	EPA%	DHA%	EPA rec.%	EPA%	DHA%	DHA rec.%
1	32.4	7.3	0.0	13.7	22.0	16.8	100
2	56.4	11.0	0.0	33.1	28.7	27.1	100
5	72.7	19.1	1.1	73.2	18.6	37.3	92.7
8	76.2	21.0	1.6	84.0	12.8	44.5	89.7
11	78.9	21.9	3.0	89.8	9.3	49.6	84.4
24	82.0	20.5	3.0	92.0	8.1	49.6	78.4

[a]Conv.%, conversion—defined as mol% of produced ethyl esters as based on mol of initial free fatty acids (FFA). See Table 1 for other abbreviations.

TABLE 4
Esterification of Tuna Oil Fatty Acids[a]

		Ethyl esters			Residual FFA		
Time (h)	Conv.%	EPA%	DHA%	EPA rec.%	EPA%	DHA%	DHA rec.%
1	43.0	3.4	1.3	22.2	9.0	47.0	97.9
3	61.5	5.9	1.7	50.1	8.7	68.3	96.4
5	65.0	7.9	3.2	53.5	5.7	70.2	91.5
8	67.6	10.0	5.1	86.5	3.2	73.6	82.7
11	70.1	9.1	9.1	89.1	2.4	77.2	78.3
24	72.7	8.8	11.6	91.6	2.1	73.9	70.6

[a]See Table 3 for Conv.%; see Tables 1 and 3 for other abbreviations.

TABLE 5
Esterification of Herring Oil Fatty Acids[a]

		Ethyl esters			Residual FFA		
Time (h)	Conv.%	EPA%	DHA%	EPA rec.%	EPA%	DHA%	DHA rec.%
1	17.6	1.2	0.0	3.7	6.7	10.4	100
3	52.3	2.4	0.0	19.4	10.9	17.7	100
6	69.9	3.4	0.4	38.9	12.4	24.4	96.4
8	74.4	3.7	0.4	48.5	11.4	25.8	95.7
12	80.0	6.9	1.0	81.7	6.2	36.6	90.1
24	82.1	7.6	1.6	90.2	6.6	37.3	83.6

[a]See Table 3 for Conv.%; see Tables 1 and 3 for other abbreviations.

TABLE 6
Esterification of 77% EPA and 10% DHA Fatty Acid Concentrate[a]

		Ethyl esters			Residual FFA		
Time (h)	Conv.%	EPA%	DHA%	EPA rec.%	EPA%	DHA%	DHA rec.%
1	27.8	91.5	0.0	44.1	74.1	14.3	100
2	49.2	90.5	0.0	57.5	67.7	16.1	100
3	61.8	90.5	1.0	87.1	60.2	23.5	93.6
5	75.7	90.8	1.5	87.1	42.0	34.8	88.2
8	82.3	88.6	2.0	92.1	35.4	43.2	82.3
11	84.1	87.6	2.7	92.9	35.4	45.4	76.1

[a]See Table 3 for Conv.%; see Tables 1 and 3 for other abbreviations.

TABLE 7
Esterification of Sardine Oil Fatty Acids with Various Alcohols[a]

		Ethyl esters			Residual FFA		
Alcohol	Conv.%	EPA%	DHA%	EPA rec.%	EPA%	DHA%	DHA rec.%
Ethanol	78.7	18.9	1.4	86.1	11.3	53.9	91.2
n-Butanol	87.5	22.2	2.8	98.2	1.9	69.4	78.0
n-Hexanol	86.2	22.9	2.3	97.6	3.5	62.3	82.3
Isopropanol	54.0	10.7	0.8	29.3	30.3	27.3	96.7

[a]See Table 3 for Conv.%; see Tables 1 and 3 for other abbreviations.

action conditions. The concentrate was supplied as ethyl esters by Pronova Biocare. After 2 h at 50% conversion no DHA was detected in the ethyl ester fraction, which comprised over 90% EPA, free of DHA. This demonstrates that lipase can be used to prepare concentrates enriched with either EPA or DHA. Despite the highly unfavorable initial DHA to EPA ratio in the concentrate, DHA exceeded EPA in the residual fatty acid fraction. This was after only 8 h reaction time at 82% conversion, when 92% of EPA had reacted, with 82% of the initial DHA remaining in the residual FFA fraction.

The above results reveal that the reaction rate of direct esterification is much higher than the ethanolysis reaction. This was also observed for the direct esterification of pure EPA and DHA with glycerol, which was much faster than the corresponding interesterification of pure EPA and DHA as ethyl esters with tributyrin to generate homogenous triacylglycerols of EPA and DHA (11). The much higher reaction rate serves the kinetic resolution very well indeed. The direct esterification is superior to the ethanolysis reaction and offers a highly efficient separation between EPA and DHA in fish oils. Concentrates highly enriched with EPA as ethyl esters and DHA as FFA can be prepared from fish oil in a single step with very high to excellent recovery of both EPA and DHA.

The effect of varying the alcohol type. In order to investigate the effect of varying the type of alcohol in the direct esterification, FFA from sardine oil were treated with ethanol, *n*-butanol, *n*-hexanol, and isopropanol for 69 h. A tenfold molar excess of the alcohols was used. The results are displayed in Table 7. It is interesting to notice that both *n*-butanol and *n*-hexanol offered more favorable results than ethanol in terms of both reaction rate and composition. It should be pointed out that using a tenfold excess is not the optimal condition for ethanol. As had been anticipated, the secondary alcohol is a far less favorable substrate for the 1,3-regiospecific lipase. A 70% DHA content in the residual FFA fraction for *n*-butanol, with only 2% EPA present, still in very high DHA recovery, is an extremely promising result. Also, the favorable EPA to DHA ratio of 10:1 in the ethyl esters in extremely high EPA recovery is noticeable for the butanol and hexanol cases, and may imply their possible superiority over ethanol.

Stepwise enzymatic treatment. It is believed that a stepwise enzymatic treatment of FFA from fish oil may aid optimal concentration as well as recovery of both EPA and DHA. At lower

conversion the recovery of DHA in the residual fatty acids is still very high with minimal losses. Similarly, the ethyl ester fraction is virtually free of DHA at lower conversion. In order to provide some insight into this, a double-step treatment of FFA from fish oil with an unfavorable DHA to EPA ratio was investigated. It was demonstrated that this strategy can be applied to obtain highly enriched DHA in good recovery from sardine oil FFA. In a first step at 77% conversion, FFA of 52% DHA and 13% EPA were obtained in 92% DHA recovery. The ethyl ester fraction comprised 19% EPA and 1.3% DHA, with 83% EPA recovery. The fractions were separated by an aqueous alkaline treatment and when the resulting DHA-enriched FFA fraction was subjected to a second esterification reaction, the residual FFA contained 70.3% DHA and only 1.3% EPA at 50% conversion after only 3 h. The overall yield of DHA was 73%. This strategy may be developed into a powerful technique for separating EPA and DHA by a two-step or a multistep approach, where a careful selection of fish oil and control of the extent of conversion are the most important parameters.

In conclusion, a highly efficient esterification process is described in which Lipozyme fulfilled the criteria of separating EPA and DHA by kinetic resolution, and tolerated the ethanolic conditions very well. Based on these results there are reasons to believe that, in terms of separating EPA and DHA, lipase can be used as a powerful alternative to traditional chemical separation techniques. The considerable reduction in bulkiness related to solvent-free conditions renders this technique ideally suited for industrialization. The type of fish oil and extent of conversion are highly important parameters in controlling the degree of concentration. Finally, multi-step processes may offer highly enriched or pure EPA or DHA concentrates by carefully controlling the choice of starting oil and extent of conversion.

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