Lipase-Assisted Concentration of n-3 Polyunsaturated Fatty Acids in Acylglycerols from Marine Oils

Udaya N. Wanasundara and Fereidoon Shahidi*

Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9

ABSTRACT: Preparation of n-3 polyunsaturated fatty acid (PUFA) concentrates from seal blubber oil (SBO) and menhaden oil (MHO) in the form of acylglycerols was carried out by hydrolysis with a number of commercial microbial lipases. The lipases tested were *Aspergillus niger*, *Candida cylindracea* (CC), *Chromobacterium viscosum*, *Geotrichum candidum*, *Mucor miehei*, *Pseudomonas* sp., *Rhizopus oryzae*, and *Rhizopus niveus*. After lipase-assisted hydrolysis of oils, free fatty acids were removed, and fatty acid composition of the mixture containing mono-, di-, and triacylglycerols was determined. All lipases were effective in increasing the n-3 PUFA content of the remaining acylglycerols of both SBO and MHO. The highest concentration of n-3 PUFA was provided by CC lipase; 43.5% in SBO [9.75% eicosapentaenoic acid (EPA), 8.61% docosapentaenoic acid (DPA), and 24.0% docosahexaenoic acid (DHA)] and 44.1% in MHO (18.5% EPA, 3.62% DPA, and 17.3% DHA) after 40 h of hydrolysis. Thus, CC lipase appears to be most suitable for preparation of n-3 PUFA in the acylglycerol form from marine oils.

JAOCS 75, 945–951 (1998).

KEY WORDS: Acylglycerols, marine oils, menhaden oil, microbial lipase, n-3 polyunsaturated fatty acid concentrates, seal blubber oil.

The importance of marine oils in human nutrition and disease prevention was scientifically recognized three decades ago. Epidemiological studies in the early 1970s postulated that the low incidence of coronary heart disease among Greenland Eskimo might be related to their distinctive dietary habit and use of marine lipids, which are rich in polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA; $C_{20:5n-3}$) and docosahexaenoic acid (DHA; $C_{22:6n-3}$) (1,2). Several sources of information suggest that humans originally consumed a diet with a ratio of n-6 to n-3 fatty acids of about 1:1, whereas today this ratio ranges from 10:1 to 20–25:1 in affluent Western societies. Therefore, the Western diets are deficient in n-3 fatty acids, compared with the diet on which humans were evolved and their genetic patterns established (3).

The beneficial effects of PUFA have been ascribed to their ability to lower serum triacylglycerol (TAG) and cholesterol levels and enhance their excretion, to increase membrane fluidity, and, by conversion to eicosanoids, to reduce thrombosis (4,5). The n-3 fatty acids are considered essential for normal growth and development throughout the life cycle and may play an important role in the prevention and treatment of coronary artery disease, hypertension, arthritis, other inflammatory and autoimmune disorders, and cancer (4,6). Therefore, consumption of appropriate amounts and proportions of n-6 and n-3 fatty acids needs to be considered.

It has been suggested that PUFA concentrates, devoid of more saturated fatty acids, are much better than marine oils themselves because they allow daily intake of total lipids to remain as low as possible. With the growing public awareness of the nutritional benefits of consuming PUFA concentrates, the market for these products is expected to grow. In response to this demand, pharmaceutical industries have used different methods for preparing PUFA concentrates from marine oils. Chromatographic separation, fractional distillation, low-temperature crystallization, supercritical fluid extraction, and urea complexation are currently practiced for preparation of PUFA concentrates from marine oils. However, most of these methods produce PUFA concentrates in the form of free fatty acids or their corresponding alkyl esters.

The acylglycerol form of PUFA is considered to be nutritionally more favorable than methyl or ethyl esters of fatty acids due to the impaired intestinal absorption of alkyl esters of n-3 fatty acids observed in laboratory animals (7–9). It has also been shown that methyl and ethyl esters of unsaturated fatty acids hydrolyze at a slower rate than their corresponding acylglycerols (10). From a marketing point of view, mono-, di-, and triacylglycerols are often promoted as being more "natural" than free fatty acids and their methyl or ethyl esters (11).

Research in recent years has given much attention to microbial lipases to produce n-3 fatty acid concentrates in the form of acylglycerols by hydrolysis of marine oils (12–16). Bottino *et al.* (17) have illustrated the mechanism of resistivity of lipases toward the long-chain n-3 PUFA in marine oils. The presence of carbon-carbon *cis* double bonds in the fatty acids results in bending of the chains. Therefore, the terminal methyl group of the fatty acid lies close to the ester bond, which may cause a steric hindrance effect on lipases. The high bending effect of EPA and DHA due to the presence of five and six double bonds, respectively, enhances the steric

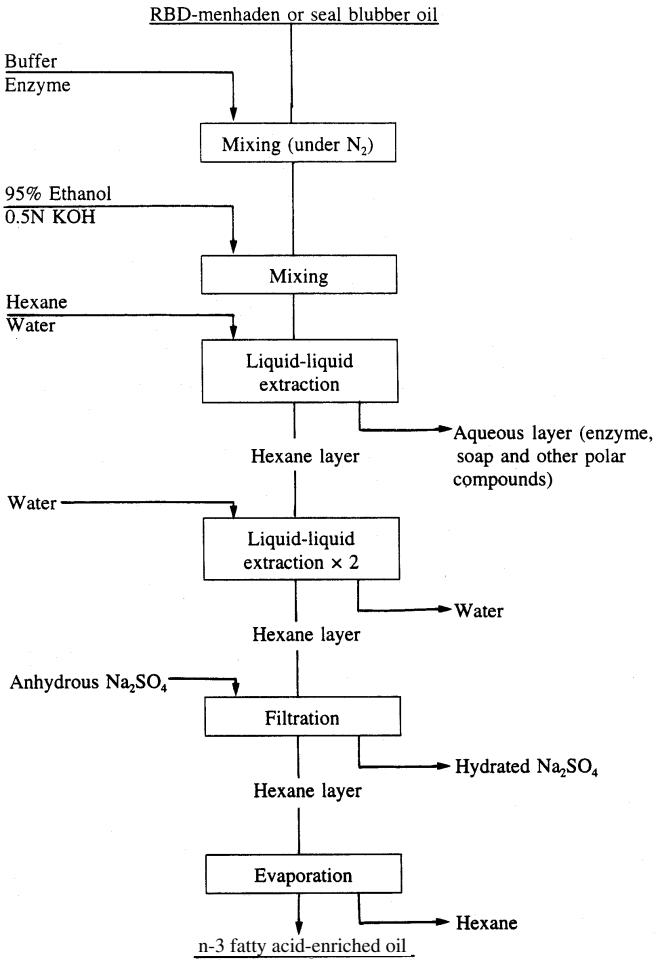
*To whom correspondence should be addressed.
E-mail: fshahidi@morgan.ucs.mun.ca

hindrance effect; therefore, lipases cannot reach the ester linkage of these fatty acids and glycerol. However, saturated or monounsaturated fatty acids do not present any barriers to lipase and they can be easily hydrolyzed. Therefore, fatty acid selectivity of a lipase for EPA and DHA has allowed separation of these fatty acids from the remaining fatty acids in marine oils, which is important in the production of n-3 fatty acid concentrates.

In this study, preparation of n-3 PUFA-enriched acylglycerols from seal blubber oil (SBO) and menhaden oil (MHO) *via* enzymatic hydrolysis was attempted. Several microbial lipases were used to screen suitable enzyme(s) for enriching n-3 PUFA in both oils.

MATERIALS AND METHODS

Materials. Freshly prepared, refined, bleached, and deodorized (RBD) MHO, devoid of any additives, was obtained from Zapata Protein (USA) Inc. (Reedville, VA). Blubber of harp seal was obtained from local sources in Newfoundland. The extraction, refining, bleaching, and deodorization of the oil were carried out as described elsewhere (18,19). Microbial lipases (*Aspergillus niger,* AN; *Mucor miehei,* MM; *Rhizopus oryzae,* RO; *Rhizopus niveus,* RN; *Candida cylindracea,* CC; *Chromobacterium viscosum,* CV; *Geotrichum candidum,* GC; *Pseudomonas* sp., PS) were provided by different manufacturers as shown in Table 1. Hydrolytic activities of enzymes were measured according to the method described elsewhere (20). Fatty acid methyl ester (FAME) standards were purchased from either Supelco (Oakville, Ontario, Canada) or Nu-Chek-Prep (Elysian, MN) companies. All other chemicals used in this study were of American Chemical Society (ACS) grade or better.



SCHEME 1

Enzymatic hydrolysis of SBO and MHO. Hydrolysis of SBO and MHO (oils were stabilized with 200 ppm of butylated hydroxyanisole) by microbial lipases and separation of the n-3-enriched fraction was carried out according to Scheme 1. Oil (4 g) and phosphate buffer (6.0 mL of a 0.1 M solution; pH 6.0 or 7.0) with 800 units (200 U/g oil) of lipase were placed in a glass container (4 cm diameter and 7 cm height). The container was flushed with nitrogen and sealed with a rubber cap and Parafilm. Containers were then placed in a Gyrotory water bath shaker at 35 ± 1°C and 200 rpm. Hydrolyzed samples were removed periodically (a separate sample container each time) to determine the percentage of hydrolysis. Lipolytic activity was quenched by introducing 2 mL methanol to the mixture.

TABLE 1
Microbial Lipases Used and Their Characteristics

Origin	Manufacturer	Optimal temperature (°C)	Optimal pH	Positional specificity	Activity (U/g)[a]
Aspergillus niger	Amano Enzyme[b]	30–40	5.0–7.0	1-, 3- ≫ 2-	15,500
Mucor miehei	Novo Nordisk[c]	30–45	6.5–7.5	1-, 3- ⋙ 2-	10,000
Rhizopus oryzae	Amano Enzyme	30–45	5.0–8.0	1-, 3- ⋙ 2-	82,000
Rhizopus niveus	Amano Enzyme	30–45	5.0–8.0	1-, 3- ⋙ 2-	10,000
Candida cylindracea	Amano Enzyme	30_50	5.0–8.0	Random	22,500
Chromobacterium viscosum	Asahi Chemicals[d]	—	—	Random	90,000
Geotrichum candidum	Amano Enzyme	30–45	6.0–8.0	Random	3,000
Pseudomonas sp.	Amano Enzyme	40–60	5.0–9.0	Random	35,000

[a]Enzyme activity was determined according to the method described elsewhere (20). Substrate was tributyrin.
[b]Lombard, IL.
[c]Bagsvaerd, Denmark.
[d]Shizuoka-Ken, Japan.

Determination of hydrolysis percentage. Unhydrolyzed oil (SBO and MHO), hydrolyzed acylglycerols, and free fatty acids were extracted into hexane and used to determine the acid value according to the AOCS method (21; method number Cd 3a-63). The hydrolysis percentage of the oils, after enzyme treatment, was calculated as:

$$\text{Hydrolysis (\%)} = \frac{\text{Acid value}_{(\text{hydrolyzed oil})} - \text{Acid value}_{(\text{unhydrolyzed oil})}}{\text{Saponification value}_{(\text{unhydrolyzed oil})} - \text{Acid value}_{(\text{unhydrolyzed oil})}} \times 100 \quad [1]$$

where acid value is expressed as the number of mg of KOH required to neutralize free fatty acids present in 1 g of oil; the saponification value (21; method number Cd 3-25) is defined as the number of mg of KOH required to saponify 1 g of oil.

Separation of acylglycerols and free fatty acids after enzymatic hydrolysis. After adding the required amount of 0.5 N KOH to neutralize fatty acids released during hydrolysis (the required amount of KOH was determined by the acid value), the mixture was transferred into a separatory funnel and mixed thoroughly with 100 mL hexane and 50 mL distilled water (Scheme 1). The lower aqueous layer was separated and discarded. The upper layer (hexane), containing tri-, di- and monoacylglycerols, was washed two times with 50 mL distilled water and then passed through a bed of anhydrous sodium sulfate. The acylglycerols were subsequently recovered after hexane removal at 45°C in a rotary evaporator, and the fatty acid composition was determined as described in the following section. All enzymes listed in Table 1 were screened for their efficacy in concentrating n-3 fatty acids of both SBO and MHO.

Determination of fatty acid composition of unhydrolyzed fraction of oils. Fatty acid composition of the unhydrolyzed fraction of oil was determined by its conversion to methyl esters (22). About 10 mg of each oil was weighed into a 6-mL, well-cleaned, Teflon-lined, screw-capped conical vial. Transmethylation reagent (2 mL, freshly prepared 6 mL of concentrated sulfuric acid made up to 100 mL with spectral-grade methanol and 15 mg hydroquinone as an antioxidant) was added to the sample vial and mixed by vortexing. The mixture was incubated overnight at 60°C and subsequently cooled. Distilled water (1 mL) was added to the mixture and, after thorough mixing, extracted three times with 1.5 mL of high-performance liquid chromatography (HPLC)-grade hexane. A few crystals of hydroquinone were added to each vial prior to extraction with hexane. Hexane layers were separated, combined, and transferred to a clean tube, and then washed two times with 1.5 mL distilled water. In the first wash, the aqueous layer was removed, and in the second wash, the hexane layer was separated and evaporated under a stream of nitrogen. FAME were then dissolved in 1 mL carbon disulfide and used in subsequent gas-chromatographic analysis.

A Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Mississauga, Ontario, Canada), equipped with a flame-ionization detector and split/splitless injector, was used for analyzing FAME. A Supelcowax 10 column (0.25 mm × 60 m; 0.25 μm film thickness, Supelco) was used for separation of FAME. Chromatographic parameters were set as follows: injector and detector temperatures, 250°C; oven temperature programming: held 10.25 min at 220°C, then ramped to 240°C at 30°C/min, followed by a hold period of 9 min. Total run time was 19.92 min. Helium was used as a carrier gas. FAME were identified by comparison of their retention times with those of the reference standards. The content of fatty acids was calculated from their corresponding integration data.

RESULTS AND DISCUSSION

Fatty acid composition of oils used for hydrolysis by microbial lipases showed that MHO had a higher amount of EPA (13.2%) and DHA (10.1%) than SBO, but the latter had a higher content of DPA (4.7%), which is less abundant in fish oils (2.4% in MHO) (Table 2). Furthermore, SBO contained a markedly higher amount of monoenes (59.9%) than MHO (27.3%). DPA is an important intermediate in the conversion of EPA to DHA.

Hydrolysis percentages of both oils as a function of time are shown in Figure 1. All microbial lipases tested were able to hydrolyze fatty acids in both oils, but at different rates. Among the lipases tested, CC lipase gave the highest degree of hydrolysis of SBO, followed by RO lipase. However, in MHO, RO lipase gave the highest degree of hydrolysis. Other

TABLE 2
Fatty Acid Composition (w/w%) of Refined, Bleached, and Deodorized Seal Blubber and Menhaden Oils[a]

Fatty acid	Seal blubber oil	Menhaden oil
14:0	3.73 ± 0.08	8.32 ± 0.12
14:1n-9	1.09 ± 0.04	0.38 ± 0.01
15:0	0.23 ± 0.00	0.71 ± 0.02
16:0	5.98 ± 0.03	17.12 ± 0.25
16:1n-7	18.02 ± 0.04	11.36 ± 0.14
17:0	0.92 ± 0.00	2.45 ± 0.12
17:1	0.55 ± 0.02	1.86 ± 0.03
18:0	0.88 ± 0.00	3.33 ± 0.02
18:1n-9	20.83 ± 0.06	6.68 ± 0.12
18:1n-11	5.22 ± 0.03	3.46 ± 0.07
18:2n-6	1.51 ± 0.02	1.42 ± 0.09
18:3n-3	0.59 ± 0.00	1.82 ± 0.00
18:4n-3	1.00 ± 0.02	2.90 ± 0.05
20:0	0.11 ± 0.00	0.20 ± 0.01
20:1n-9	12.16 ± 0.02	1.44 ± 0.06
20:2n-6	0.16 ± 0.00	0.21 ± 0.00
20:3n-6	0.14 ± 0.00	0.46 ± 0.03
20:4n-6	0.46 ± 0.01	0.83 ± 0.02
20:5n-3	6.41 ± 0.08	13.23 ± 0.18
22:0	—	0.12 ± 0.00
22:1n-11	2.01 ± 0.04	0.12 ± 0.05
22:2	—	0.02 ± 0.00
22:4n-6	0.11 ± 0.01	0.19 ± 0.03
22:5n-3	4.66 ± 0.01	2.40 ± 0.03
22:6n-3	7.58 ± 0.02	10.06 ± 0.11

[a]All values are mean of three replicates ± standard deviation.

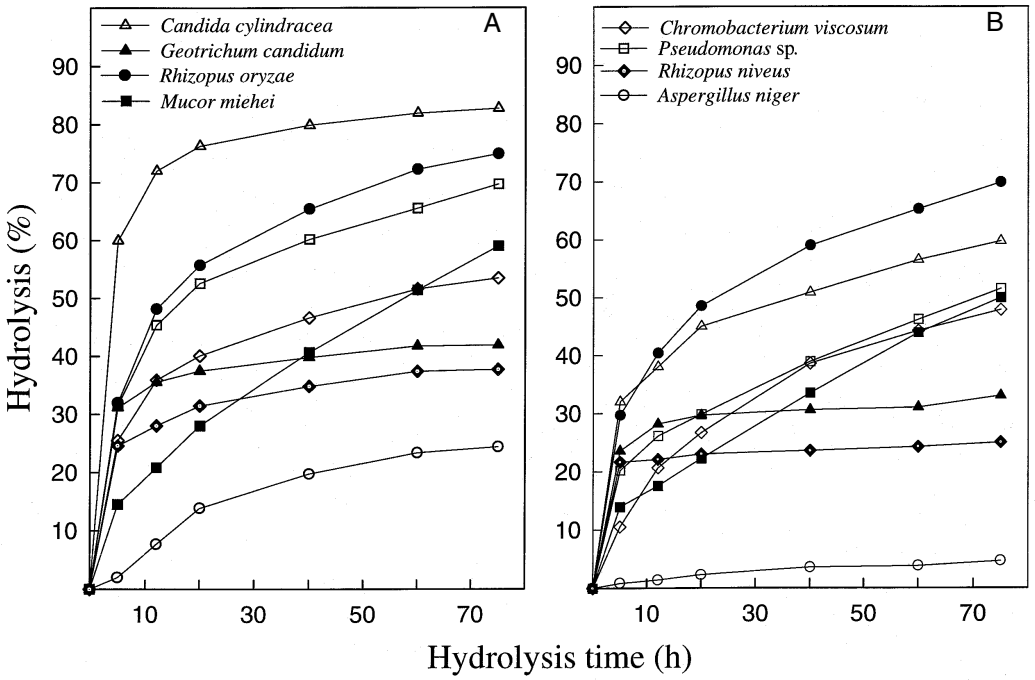


FIG. 1. Time courses of hydrolysis of (A) seal blubber oil (SBO) and (B) menhaden oil (MHO) by different microbial lipases.

lipases studied gave lower degrees of hydrolysis than CC and RO in both oils. At a given time, all lipases rendered considerably higher degrees of hydrolysis in SBO than in MHO. This difference may be due to the presence of higher amounts of PUFA in MHO than SBO, especially EPA and DHA, which exhibit resistance to enzymatic hydrolysis. The degree of hydrolysis of SBO after 9 h in the presence of CC lipase was over 70%, and the same degree of hydrolysis was achieved after 60 h when RO lipase was used. However, none of the lipases was able to afford over 70% hydrolysis in MHO during a 75-h period.

Figures 2–4 show the changes in the content of total n-3 fatty acids, EPA and DHA, in the nonhydrolyzed fractions (acylglycerols) of both oils upon enzymatic (lipase) hydrolysis. Among the lipases tested, CC lipase significantly ($P < 0.05$) increased the total n-3 fatty acids as well as the EPA and DHA contents of SBO as the hydrolysis reaction progressed. However, at 80% hydrolysis, the same lipase resulted in a decrease in the content of EPA (Fig. 3). After 12 h of hydrolysis, CC lipase gave a twofold increase of total n-3 fatty acids in SBO. But in MHO, this lipase was able to increase the content of total n-3 fatty acids by only 11% (from 30% in original oil to 41% after hydrolysis) during the same hydrolysis period. In MHO, the highest contents of total n-3 fatty acids and DHA were obtained by RO lipase-assisted hydrolysis. However, when RO lipase was used, the EPA content of both oils was decreased during the course of the reaction (Fig. 3). This may be due to the fact that RO lipase selectively hydrolyzes EPA in the oils by exhibiting acyl-chain specificity. Tanaka *et al.* (12) have also reported that EPA content of tuna oil was decreased upon hydrolysis by lipase from *Rhizopus* species, namely *R. delemar* (RD) and *R. javanicus* (RJ). Kotting and Elbe (23) have reported that lipases from *Rhizopus* species are 1,3-position-specific. The EPA content of SBO was decreased from 6.4 to 4.3% during a 75-h RO lipase-assisted hydrolysis. However, upon hydrolysis of MHO by RO lipase, EPA content was increased up to 9 h, after which it began to decline. During 75 h of hydrolysis, the content of EPA in MHO decreased from 13.2 to 12.5%. Therefore, the rate of hydrolysis of EPA from SBO was much higher than that from MHO. This may be due to the cumulative effect of both 1,3-positional and acyl-chain specificity of RO lipase. EPA was located mainly in the *sn*-1 and *sn*-3 positions of the TAG in SBO. However, EPA in MHO was equally distributed over the *sn*-2 and *sn*-3 positions and present only in small amounts in the *sn*-1 position (24). Therefore, the higher hydrolysis rate of EPA from SBO may reflect the abundance of this fatty acid in the *sn*-1 and *sn*-3 positions, which are highly vulnerable to 1,3-specific RO lipase hydrolysis.

In MHO, the total n-3 fatty acid content increased from 30% (original oil) to 44.6, 44.1, and 41.7% after 40-h hydrolysis by RO, CC and GC lipases, respectively. The corresponding increase in DHA content in this oil was from 10.1 to 23.5, 17.3, and 14.8%, respectively. In SBO, the maximum increase of total n-3 fatty acids, from 20.2 to 45.0%, was reached by employing CC lipase under similar experimental conditions. Other lipases were less effective in increasing the content of n-3 fatty acids in the oil and gave maxima of 33.2, 30.6, 29.3, 26.1, 25.5, 25.3, and 24.6% for RO, GC, MM, PS, CV, RN and AN lipases, respectively, during the course of hydrolysis. The lipase from *Pseudomonas* sp. showed a slightly higher degree of hydrolysis for both oils than lipases from CV, GC, RN, MM and AN. Although enrichment of total n-3 fatty acids and DHA in the oils was small, the content of

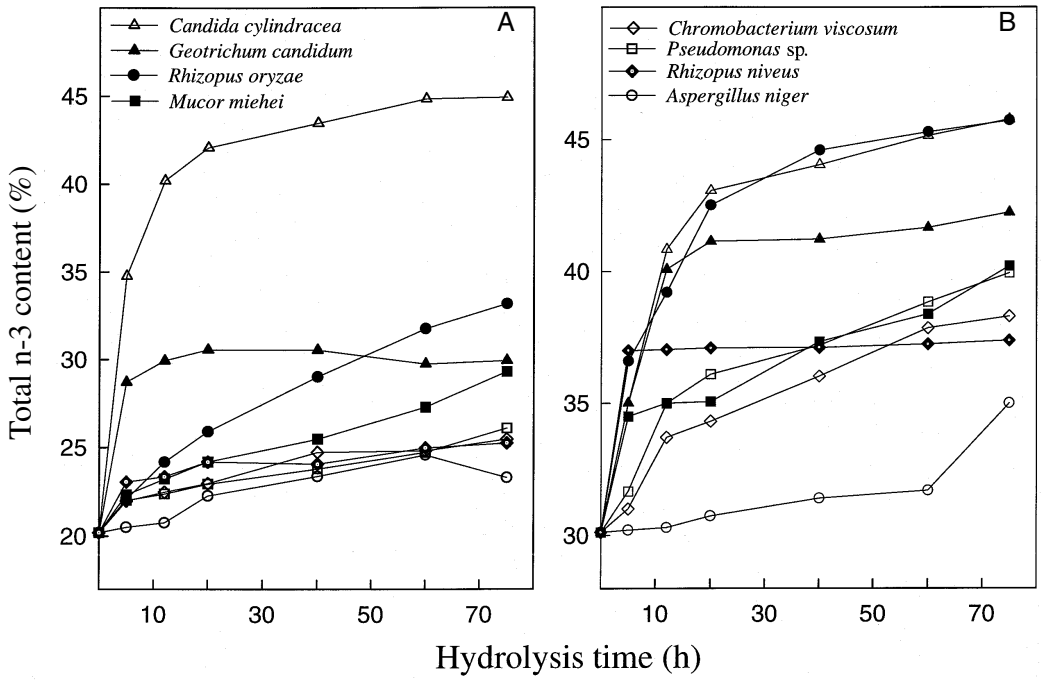


FIG. 2. Changes of total n-3-fatty acid content during hydrolysis of (A) SBO and (B) MHO by different microbial lipases. See Figure 1 for abbreviations.

EPA was increased from 6.4 to 10.5% in SBO and from 13.2 to 20.1% in MHO.

Among the lipases tested in this experiment, AN lipase gave the lowest total n-3 fatty acids in the nonhydrolyzed fraction of both oils. Even though this lipase is 1,3-specific, it was not able to hydrolyze much of the saturated fatty acids present in the *sn*-1 position of MHO (about 43.3% of saturated fatty acids are present in the *sn*-1 position of MHO). Similarly, 1,3-specific MM lipase was not able to hydrolyze much of the saturated fatty acids in both marine oils. Therefore, no correlation was found between positional specificity and ease of hydrolysis of fatty acids for both oils in this study.

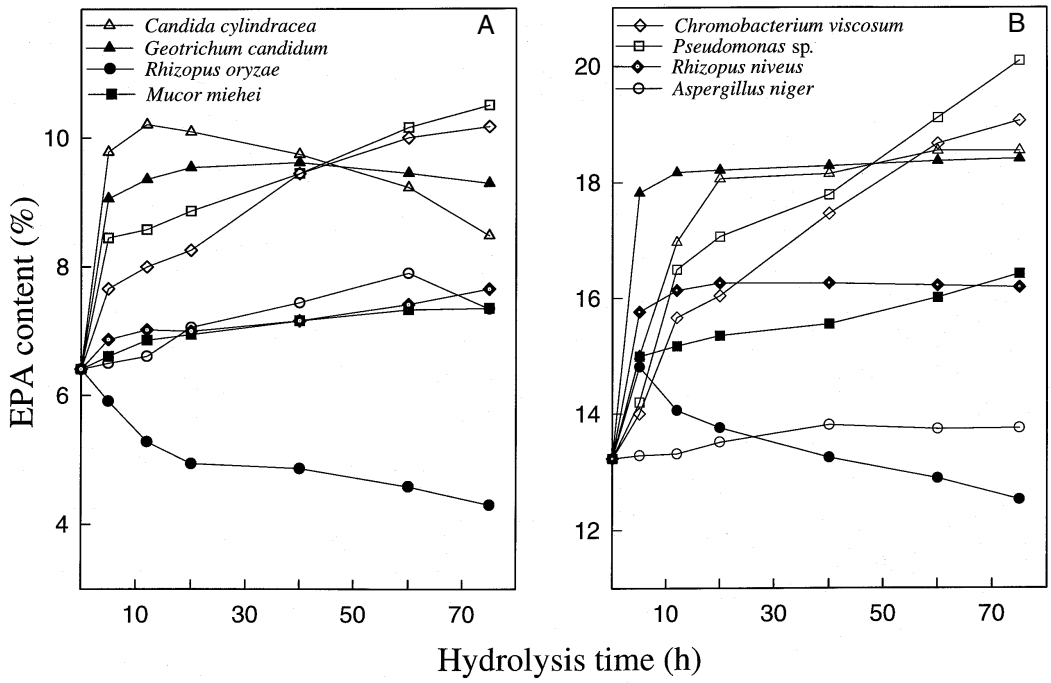


FIG. 3. Changes of eicosapentaenoic acid (EPA) content during hydrolysis of (A) SBO and (B) MHO by different microbial lipases. See Figure 1 for abbreviations.

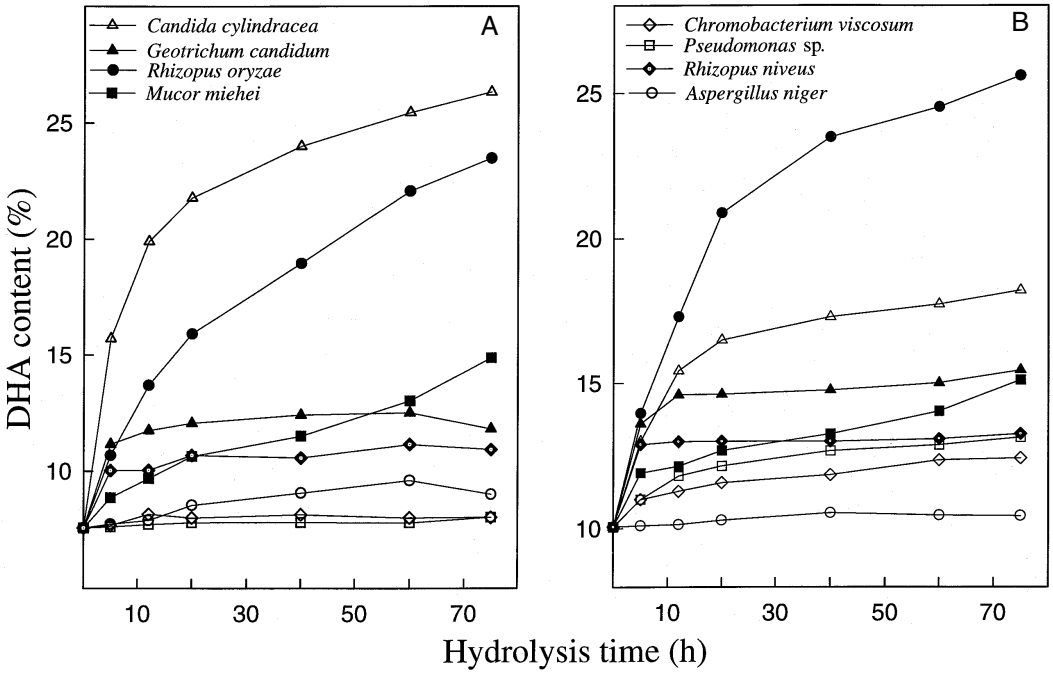


FIG. 4. Changes of docosahexaenoic acid (DHA) content during hydrolysis of (A) SBO and (B) MHO by different microbial lipases. See Figure 1 for abbreviations.

This implies that the concept of positional specificity of lipases alone cannot be used to explain the observed hydrolysis differences of both oils. Hoshino *et al*. (13) have shown that the course of hydrolysis of marine oils by lipases is dictated by cumulative effects of various factors, such as differences in substrate specificities, including fatty acid and positional specificity of lipase, differences in the rate of the reverse reaction, which occurs during hydrolysis, differences in fatty acid composition of the oil, and reactivity of each lipase toward partial acylglycerols (mono- and diacylglycerols).

Among the lipases tested, CC lipase seems to be the most active one in increasing the contents of total n-3 fatty acids, EPA and DHA, in the nonhydrolyzed fraction of both SBO and MHO. RO lipase was also effective in enriching the total n-3 fatty acids and DHA in both oils, but this lipase selectively hydrolyzed EPA from the TAG of both oils. Therefore, the contents of EPA in the final products were lowered. Tanaka *et al*. (12) have used six types of microbial lipases (AN, CC, CV, RD, RJ and PS) to hydrolyze tuna oil and found that CC lipase was the most effective in increasing the DHA content in the concentrates. CC lipase increased the DHA content in the nonhydrolyzed fraction in tuna oil to three times that in the original sample. However, other lipases did not increase the DHA content in the oil. It has been suggested that hydrolysis by CC lipase takes place in two steps; first, TAG molecules devoid of DHA are hydrolyzed, and this is followed by the hydrolysis of TAG that contain DHA (12,25). Therefore, CC lipase recognizes the whole molecular structure and not only its ester bonds. Hoshino *et al.* (13) have also used several lipases for selective hydrolysis of cod liver and sardine oils. The best hydrolysis results were obtained for the nonregiospecific CC and 1,3-specific AN lipases, but none of the lipases could raise the EPA content of the acylglycerols considerably. However, the total content of n-3 fatty acids exceeded 50% when these two lipases were employed.

Use of enzymes to produce n-3 fatty acid concentrates has the advantage over traditional methods (chromatographic separation, molecular distillation, etc.) of concentration because such procedures involve extremes of pH and high temperature, which may partially destroy the natural all-*cis* n-3 PUFA by oxidation and by *cis-trans* isomerization or double bond migration. Therefore, the mild conditions (temperature less than 50°C, pH 6–8, and less chemicals) used in enzymatic hydrolysis provide a promising alternative that could also save energy and increase product selectivity. In addition, the enzymatic hydrolysis method produces n-3 fatty acids in the acylglycerol form, which is considered to be nutritionally favorable.

ACKNOWLEDGMENTS

This work was supported, in part, by a research grant from the Natural Science and Engineering Research Council (NSERC) of Canada to the corresponding author. The authors are grateful to Zapata Protein (USA) Inc. for providing samples of refined, bleached, and deodorized menhaden oil, and to Amano Enzymes (USA) Co. Ltd., Asahi Chemcals (Japan), and Novo Nordisk (Denmark) for their generous supply of microbial lipases.

REFERENCES

1. Bang, H.O., J. Dyerberg, and N. Hjorne, The Composition of Food Consumed by Greenland Eskimos, *Acta. Med. Scand. 200*:59–73 (1976).
2. Dyerberg, J., Linolinate-Derived Polyunsaturated Fatty Acids

and Prevention of Atherosclerosis, *J. Nutr. Rev. 44*:125–134 (1986).
3. Simopoulos, A.P., Omega-3 Fatty Acids in Health and Disease and Growth and Development, *Am. J. Clin. Nutr. 54*:438–463 (1991).
4. Kinsella, J.E., Food Components with Potential Therapeutic Benefits: The n-3 Polyunsaturated Fatty Acids of Fish Oils, *Food Technol. 40*: 89–97 (1986).
5. Singh, G., and R.K. Chandra, Biochemical and Cellular Effects of Fish Oils, *Prog. Food Nutr. Sci. 12*:371–419 (1988).
6. Branden, L.M., and K.K. Carroll, Dietary Polyunsaturated Fats in Relation to Mammary Carcinogenesis in Rats, *Lipids 21*:285–288 (1986).
7. Hamazaki, T., A. Hirai, T. Terano, J. Sajiki, S. Kondo, T. Fujita, Y. Tamura, and A. Kumagai, Effects of Orally Administrated Ethyl Ester of Eicosapentaenoic Acid on Pig-Like Substance Production by Rat Aorta, *Prostaglandins 23*:557–567 (1982).
8. El-Boustani, S., C. Colette, L. Monnier, B. Descomps, C.A. Paulet, and F. Mendey, Enternal Absorption in Man of Eicosapentaenoic Acid in Different Chemical Forms, *Lipids 22*:711–714 (1987).
9. Lawson, L.D., and B.G. Hughes, Human Absorption of Fish Oil Fatty Acids as Triacylglycerols, Free Fatty Acids or Ethyl Esters, *Biochem. Biophys. Res. Comm. 152*:328–335 (1988).
10. Yang, L.Y., A. Kuksis, and J.J. Myher, Lumenal Hydrolysis of Menhaden and Rapeseed Oil and Their Fatty Acid Methyl and Ethyl Esters in the Rats, *Biochem. Cell Biol. 67*:192–204 (1989).
11. Haraldsson, G.G., and P.A. Hoskuldsson, The Preparation of Triglycerides Highly Enriched with ω3 Polyunsaturated Fatty Acids *via* Lipase Catalysed Interesterification, *Tetrahed. Lett. 30*:1671–1674 (1989).
12. Tanaka, Y., J. Hirano, and T. Funada, Concentration of Docosahexaenoic Acid in Glyceride by Hydrolysis of Fish Oil with *Candida cylindracea* Lipase, *J. Am. Oil Chem. Soc. 69*: 1210–1214 (1992).
13. Hoshino, T., T. Yamane, and S. Shimuzu, Selective Hydrolysis of Fish Oil by Lipase to Concentrate ω3 Polyunsaturated Fatty Acids, *Agric. Biol. Chem. 54*:1459–1467 (1990).
14. Shimada, Y., K. Maruyama, S. Okazaki, M. Nakamura, A. Sugihara, and Y. Tominaga, Enrichment of Polyunsaturated Fatty Acids with *Geotrichum candidum* Lipase, *J. Am. Oil. Chem. Soc. 71*:951–954 (1994).
15. Yadwad, V.B., O.P. Ward, and L.C. Noronha, Application of Lipase to Concentrate the Docosahexaenoic Acid (DHA) Fraction of Fish Oil, *Biotech. Bioeng. 38*:956–959 (1991).
16. Maehr, H., G. Zenchoff, and D.L. Coffen, Enzymic Enhancement of n-3 Fatty Acid Content in Fish Oils, *J. Am. Oil Chem. Soc. 71*:463–467 (1994).
17. Bottino, N.R., G.A. Vandenburg, and R. Reiser, Resistance of Certain Long-Chain Polyunsaturated Fatty Acids of Marine Oils to Pancreatic Lipase Hydrolysis, *Lipids 2*:489–493 (1967).
18. Shahidi, F., and U.N. Wanasundara, Oxidative Stability of Encapsulated Seal Blubber Oil, in *Flavor Technology, Physical Chemistry, Modification and Process*, edited by C.-T. Ho, C.-T. Tan, and C.-H. Tong, ACS Symposium Series No. 610, American Chemical Society, Washington DC, 1995, pp. 139–151.
19. Wanasundara, U.N., and F. Shahidi, Stabilization of Seal Blubber and Menhaden Oils with Green Tea Catechins, *J. Am. Oil Chem. Soc. 73*:1183–1190 (1996).
20. Wanasundara, U.N., Marine Oils, Stabilization, Structural Characterization and Omega-3 Fatty Acid Concentration, Ph.D. thesis, Memorial University of Newfoundland, St. John's, Canada, 1996.
21. *Official Methods and Recommended Practices of the American Oil Chemists' Society,* 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1990.
22. Shahidi, F., U.N. Wanasundara, and N. Brunet, Oxidative Stability of Oil from Blubber of Harp Seal (*Phoca groenlandica*) as Assessed by NMR and Standard Procedures, *Food Res. Inter. 27*:555–562 (1994).
23. Kotting, J., and H. Elbe, Lipases and Phospholipases in Organic Synthesis, in *Lipases*, edited by P. Woolley and S.B. Petersen, Cambridge University Press, Cambridge, 1994, pp. 289–313.
24. Wanasundara, U.N., and F. Shahidi, Positional Distribution of Fatty Acids in Triacylglycerols of Seal Blubber Oil, *J. Food Lipids 4*:51–64 (1997).
25. Lie, O., and G. Lambertsen, Fatty Acid Specificity of *Candida cylindracea* Lipase, *Fette Seifen Anstrichm. 88*:365–368 (1986).

[Received August 4, 1997; accepted March 3, 1998]