Lipase-Catalyzed Modification of Borage Oil: Incorporation of Capric and Eicosapentaenoic Acids to Form Structured Lipids

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ABSTRACT: Two immobilized lipases, nonspecific SP435 from *Candida antarctica* and *sn*-1,3 specific IM60 from *Rhizomucor miehei*, were used as biocatalysts for the restructuring of borage oil (*Borago officinalis* L.) to incorporate capric acid (10:0, medium-chain fatty acid) and eicosapentaenoic acid (20:5n-3) with the free fatty acids as acyl donors. Transesterification (acidolysis) reactions were carried out in hexane, and the products were analyzed by gas–liquid chromatography. The fatty acid profiles of the modified borage oil were different from that of unmodified borage oil. Higher incorporation of 20:5n-3 (10.2%) and 10:0 (26.3%) was obtained with IM60 lipase, compared to 8.8 and 15.5%, respectively, with SP435 lipase. However, SP435 lipase was able to incorporate both 10:0 and 20:5n-3 fatty acids at the *sn*-2 position, but the IM60 lipase did not. Solvents with log *P* values between 3.5 and 4.5 supported the acidolysis reaction better than those with log *P* values between –0.33 and 3.0.

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KEY WORDS: Borage oil, *Candida antarctica*, lipase, medium-chain fatty acid, n-3 PUFA, *Rhizomucor miehei*, triacylglycerol.

Borage oil is one of the most important and commercially available sources of γ-linolenic acid or GLA (18:3n-6), an essential fatty acid (1,2). In recent years, research has been directed toward the production of GLA for applications in curing skin-related and other diseases (3). In humans and other mammals, GLA is the first metabolite formed during the bioconversion of linoleic acid (18:2n-6) to prostaglandins by δ-6 desaturase (1,2). The excess n-6 polyunsaturated fatty acids (PUFA) function as precursors for the biosynthesis of prostaglandins (PG), leukotrienes (LT) of the 2 and 4 series (4,5), which under certain circumstances can enhance vasoconstriction (6), platelet aggregation (7), immunosuppression (8), and monokines depression (9). The n-3 PUFA, such as 20:5n-3, influence series-2 eicosanoid concentrations by altering the pool of arachidonic acid metabolites in tissues, competing for the desaturases that synthesize tissue fatty acids (10), and by competitively inhibiting the breakdown of n-6 fatty acids by cyclooxygenase. The n-3 PUFA are considered antiinflammatory, and previous studies have shown that diets rich in n-3 PUFA may lower incidence of cardiovascular diseases (11).

Structured lipids (SL), triacylglycerols (TAG) that contain mixtures of short-chain or medium-chain, or both, and long-chain fatty acids on glycerol molecules, are thought to be effective means of delivering TAG with desired fatty acids as "nutraceuticals, functional lipids, and medical or pharmalipids" to target specific diseases and metabolic conditions, or for optimal nutrition (12). SL may have effects on immune response, eicosanoid synthesis, and inflammation events. Medium-chain fatty acids (MCFA) are more rapidly cleared from the blood because of their smaller molecular size and greater solubility (13); they are also rapidly hydrolyzed in the intestinal lumen and are more rapidly oxidized in the liver for quick energy. Severely catabolic patients may benefit from this rapidly available and high-energy fuel because MCFA are less likely to be deposited in adipose tissue but more likely to be oxidized in tissues (14).

Lipase-catalyzed reactions, such as transesterification of vegetable oils and n-3 PUFA (15–17), have been successfully used for restructuring lipids. In the present study, an attempt to enzymatically restructure borage oil to form SL that contain 18:3n-6, 20:5n-3, and 10:0 was investigated with two immobilized lipases, IM60 and SP435. The effect of molar ratio, time course, enzyme load, water, and solvent type were also studied.

MATERIALS AND METHODS

Materials. Borage oil, porcine pancreatic lipase, and capric acid (10:0, 99% pure) were obtained from Sigma Chemical Co. (St. Louis, MO). Immobilized *sn*-1,3-specific lipase, IM60, and nonspecific lipase, SP435, were provided by Novo Nordisk Biochem, North America, Inc. (Franklinton, NC). Eicosapentaenoic acid, 20:5n-3 or EPA (45% pure), was supplied by Callanish Ltd. (Scotland, United Kingdom). All solvents were of analytical grade and obtained from Aldrich Chemical Co. (Milwaukee, WI).

Enzymatic modification reaction. Unless otherwise speci-

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fied, for general modification of borage oil, the reaction mixtures for acidolysis consisted of borage oil (100 mg), 20:5n-3 (76 mg), 10:0 (40 mg) (molar ratio 1:2:2, respectively) with 10% of enzyme (w/w of substrates) and 5% water in 3 mL hexane. The resulting suspension was agitated in an orbital shaker at 55°C for 24 h at 200 revolutions/min. Molecular sieves 4Å were added after 2 h. All reactions were in duplicate.

Analysis of products. The enzyme was filtered through an anhydrous sodium sulfate column. The TAG were isolated from the modified borage oil by preparative thin-layer chromatography (TLC) on silica gel 60 plates with petroleum ether/ethyl ether/acetic acid (90/10/1, vol/vol/vol) as developing solvent. The bands were visualized under ultraviolet radiation after spraying with 0.2% dichlorofluorescein in methanol. Bands corresponding to TAG were scraped from the TLC plate and methylated with 3 mL of 6% HCl in methanol at 70–80°C for 2 h. The fatty acid methyl esters (FAME) were extracted twice with 2 mL hexane, dried over sodium sulfate, and concentrated under nitrogen. An HP 5890 Series II gas–liquid chromatograph (GLC; Hewlett-Packard, Avondale, PA), equipped with a DB-225 fused-silica capillary column 30 m × 0.25 mm i.d. (J&W Scientific, Folsom, CA) and FID detector, was used for the analysis of fatty acid composition. The injector and detector temperatures were 250 and 260°C, respectively. The column temperature was held at 190°C for 5 min and then programmed to 215°C at 20°C/min. Helium was the carrier gas, and the total gas flow was 23 mL/min. The relative content of FAME as mol% was quantitated by an on-line computer with heptadecanoic acid (17:0) as internal standard.

Fatty acids at the sn*-2 position.* The distribution of fatty acids at the *sn*-2 position of borage oil TAG was determined by a modified method of Luddy *et al.* (18). TAG of borage oil were isolated from TLC and hydrolyzed with porcine pancreatic lipase, and the resulting 2-monoacylglycerols after developing the TLC plate with hexane/ethyl ether/acetic acid (50:50:1, vol/vol/vol) were isolated, methylated, and analyzed as FAME by GLC.

RESULTS AND DISCUSSION

Transesterification (acidolysis) reactions, catalyzed by *sn*-1,3-specific IM60 and nonspecific SP435 lipases, were carried out with a 1:2:2 molar mixture of borage oil, 20:5n-3, and 10:0 in hexane for 24 h. The fatty acid compositions of borage oil and modified products at various substrate molar ratios are given in Table 1. Results show that incorporation of 20:5n-3 was similar in both reactions, 10.2% with IM60 and 8.8% with SP435, but the incorporation of 10:0 was higher with IM60 (26.3%) than with SP435 (15.5%). According to previous studies in our laboratory, better incorporation of 20:5n-3 into vegetable oils was obtained with 20:5n-3 ethyl ester (EEPA) than with the free fatty acid (EPA, 45%) with SP435 as the biocatalyst (16). SP435 gave higher 20:5n-3 incorporation into melonseed oil, compared to an *sn*-1,3-specific immobilized enzyme, IM60, when EEPA was the acyl donor (15). The lower incorporation of 20:5n-3 by IM60 could also be attributed in part to the purity (45% pure) of EPA free fatty acid.

After transesterification, the total n-6 PUFA fatty acids content of borage oil TAG decreased by 21.5 and 14.7%, respectively, with IM60 and SP435, while saturated fatty acids increased, respectively. Foglia and Sonnet (19) reported that reactions with enzymes such as SP435, which are not acyl or positionally selective, are complicated by acyl migration, causing loss of the desired acid residue. Overall, both reactions led to reduction in the n-6 PUFA, an increase in saturated fatty acids, and incorporation of 20:5n-3 and medium-chain fatty acids (10:0).

Time course is important in monitoring the progress of enzymatic reactions by determining the shortest time necessary to obtain good yields and for minimizing the overall production cost for the process. The rates of transesterification, given in Figure 1, show that, as incubation time increased, 20:5n-3 and 10:0 incorporation into TAG of borage oil increased. EPA and capric acid incorporation increased rapidly at an early stage of the reaction, between 10 and 16 h for both enzymes, but the optimal incorporation occurred at around 40 h.

Molar ratio of substrates (TAG:acyl donors, 20:5n-3 and

TABLE 1
Fatty Acid Profile (mol%) of Unmodified and Modified Borage Oil, Catalyzed by IM60 and SP435 Lipases at Different Substrate Molar Ratios[a]

Substrate		Fatty acids								Total	Total	Total
molar ratio	Lipase	10:0	16:0	18:0	18:1n-9	18:2n-6	18:3n-6	20:1n-9	20:5n-3	n-6	n-3	saturated
1:1:1	IM60	21.5	8.3	3.2	16.5	28.7	17.1	ND[b]	4.7	45.8	4.7	33.0
	SP435	12.7	13.4	3.6	16.8	32.7	17.7	ND	3.1	50.4	3.1	29.7
1:2:2	IM60	26.3	7.6	2.4	16.3	20.7	16.5	ND	10.2	37.2	10.2	36.3
	SP435	15.5	10.4	3.5	17.8	26.9	17.1	ND	8.8	44.0	8.8	29.4
1:3:3	IM60	31.6	7.2	2.1	15.4	32.3	14.0	ND	11.4	46.3	11.4	40.9
	SP435	14.7	9.8	2.0	15.0	34.4	15.1	ND	9.0	49.5	9.0	26.5
Unmodified borage oil		ND	14.6	4.3	19.2	38.6	20.1	3.2	ND	58.7	ND	18.9

[a]Molar ratio of borage oil:20:5n-3:10:0. The reaction was performed in the presence of 5% added water and 10% (w/w of substrates) lipase. Incubation was at 55°C for 24 h.
[b]ND = not detectable.

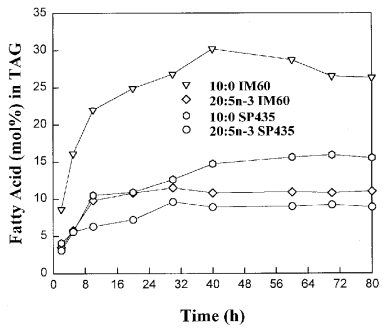

FIG. 1. Time course of incorporation of 20:5n-3 and 10:0 into borage oil triacylglycerols (TAG) by IM60 and SP435 lipase-catalyzed transesterification in 3 mL hexane. Reaction mixture was incubated at 55°C in an orbital shaking water bath for 24 h at 200 rpm. Molar ratio of borage oil, 20:5n-3, and 10:0 was 1:2:2.

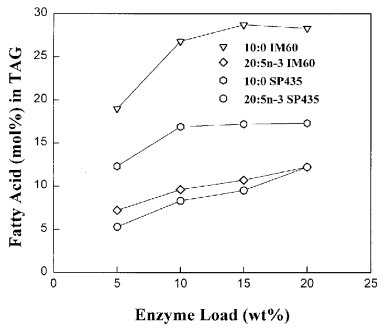

FIG. 2. Effect of IM60 and SP435 lipase load on incorporation of 20:5n-3 and 10:0 into borage oil TAG. See Figure 1 for reaction conditions and symbols.

10:0) and enzyme load also affected mol% incorporation of 20:5n-3 and 10:0. Mol% incorporation increased as the molar ratio (Table 1) and enzyme load (Fig. 2) were increased. For IM60 lipase, the largest increase in 10:0 (5.3%) occurred between a molar ratio of 1:2:2 and 1:3:3 while the 20:5n-3 (5.5%) increase occurred between 1:1:1 and 1:2:2. We also performed the reaction in other solvents, such as isooctane, pentane, hexane, toluene, acetone and acetonitrile, and found higher incorporation of 10:0 and 20:5n-3 with hexane and isooctane with both enzymes (Table 2). These two solvents with high log *P* values (20), defined as the partition coefficient between water and octanol, showed higher biocatalytic activity than solvents with medium log *P* values, such as pentane and toluene, and solvents with low log *P* values, such as acetone and acetonitrile. Incorporation of 20:5n-3 and 10:0 decreased with a decrease in log *P* value or an increase in polarity.

It has been reported that some amount of water is necessary for maintaining the three-dimensional structure of enzymes; however, excess water usually leads to hydrolysis (21). Huang *et al.* (15) reported that excess water decreased the incorporation of 20:5n-3 into vegetable oil when SP435 was the biocatalyst. Figure 3 shows that the incorporation of 20:5n-3 and 10:0 decreased when water was added to the reaction mixture that contained SP435 and increased when added to IM60. Thus, IM60 lipase tolerated more water during this acidolysis reaction than SP435 lipase.

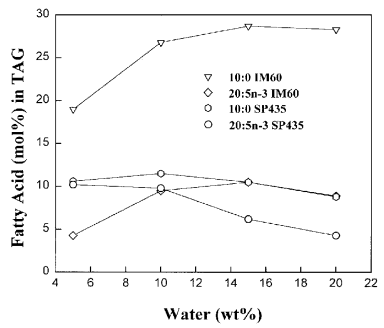

FIG. 3. Effect of added water on incorporation of 20:5n-3 and 10:0 into borage oil TAG by IM60 and SP435 lipases. See Figure 1 for reaction conditions and symbols.

The use of an *sn*-1,3-specific lipase supposedly ensures modification of the acyl composition of TAG exclusively at the *sn*-1 and *sn*-3 positions to yield products that cannot be obtained by conventional transesterification with chemical catalysts. It also appears that TAG having unusual structures

TABLE 2
Effect of Organic Solvent on Mol% Incorporation of 20:5n-3 and 10:0 into Borage Oil[a]

		Log *P* value					
		Hexane	Isooctane	Pentane	Toluene	Acetone	Acetonitrile
Fatty acid	Lipase	3.5	4.5	3.0	2.5	–0.23	–0.33
10:0	IM60	26.3	27.1	12.3	11.0	5.3	3.4
	SP435	15.5	14.2	6.5	6.1	2.4	2.0
20:5n-3	IM60	10.2	9.8	6.7	5.2	3.9	3.5
	SP435	8.8	6.7	3.5	4.3	3.0	1.8

[a]Molar ratio of borage oil:20:5n-3:10:0 = 1:2:2. See Table 1 for reaction conditions.

TABLE 3
Analysis of Fatty Acids at the *sn*-2 Position of Unmodified and Lipase-Modified Borage Oil[a]

	Fatty acids at *sn*-2 position (mol%)								Total	Total	Total
	10:0	16:0	18:0	18:1n-9	18:2n-6	18:3n-6	20:1n-9	20:5n-3	n-6	n-3	saturated
Unmodified borage oil	ND[b]	3.9	2.1	23.1	46.7	20.2	ND	ND	66.9	ND	6.0
IM60-modified borage oil	ND	5.2	1.7	22.1	49.1	21.9	ND	ND	71.0	ND	6.9
SP435-modified borage oil	7.5	6.0	1.9	15.2	49.7	14.5	ND	5.2	64.2	5.2	15.0

[a]Molar ratio of borage oil:20:5n-3:10:0 = 1:2:2.

[b]ND = not detectable.

seldom occur in nature and can only be prepared by transesterification of common fats and oils using lipases (22). For instance, TAG containing 20:5n-3 and 10:0 at the *sn*-1,3 positions and 18:3n-6 at the *sn-2* position using IM60 and 20:5n-3, 18:3n-6, and 10:0 at the *sn*-2 position using SP435, which rarely occur in nature can easily be obtained by transesterification of MCFA and n-3 PUFA (Table 3). Such TAG could be of interest as dietetic products, because fatty acids, such as 20:5n-3 and 10:0, at the *sn*-1,3 positions would be rapidly released by the pancreatic lipase of most mammalian origins, and 20:5n-3 would be available as an essential fatty acid in addition to 18:2n-6 (23). The absence of 20:5n-3 and 10:0 at the *sn*-2 position of modified borage oil catalyzed by IM60 demonstrates that, under our assay conditions, this enzyme acted as an *sn*-1,3-specific lipase. On the other hand, SP435 acted as a nonspecific lipase, which led to the incorporation of some 20:5n-3 and 10:0 at the *sn*-2 position with a concomitant decrease in 18:3n-6 at this position. Presence of 10:0 at the *sn*-2 position may be useful in the design of SL and for studies of lymphatic absorption of MCFA as 2-monoacylglycerols. The design of such lipids is of interest in understanding the metabolism of SL.

We have demonstrated here that borage oil, rich in 18:3n-6 and poor in n-3 PUFA, can be modified to incorporate both 20:5n-3 and 10:0 in the glycerol molecules. This modified or restructured borage oil may be useful in the treatment of certain clinical disorders, which at present involves use of individual sources of 18:3n-6, 20:5n-3, and MCFA, or physical mixtures. SL that are enzymatically obtained from borage oil may help ameliorate inflammatory response and modulate eicosanoid biosynthesis. They may provide essential fatty acids (18:2n-6, 18:3n-6, and 20:5n-3) as well as 10:0 for quick energy and should be considered as possible dietary fats in treatment of lipid or TAG malabsorption disorders.

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REFERENCES

1. Gunstone, F.D., Gamma-Linolenic Acid Occurrence and Physical and Chemical Properties, *Prog. Lipid Res. 31*:145–163 (1992).
2. Horrobin, D.F., Nutritional and Medical Importance of Gamma-Linolenic Acid, *Ibid. 31*:163–194 (1992).
3. Horrobin, D.F., in *Antioxidants, Free Radicals and Polyunsaturated Fatty Acids in Biology and Medicine,* edited by A.T. Diplock, J.M.C. Gutterridge, and V.K.S. Shukla, International Food Science Centre A/S, Lystrup, 1993, pp. 181–198.
4. Moncada, S., and J.R. Vane, Arachidonic Acid Metabolites and the Interactions Between Platelets and Blood-Vessel Walls, *N. Engl. J. Med. 17*:1142–1147 (1979).
5. Moncada, S., and J.R. Vane, Pharmacology and Endogenous Roles of Prostaglandin Endoperoxides, Thromboxane A_2 and Prostacyclin, *Pharmacol Rev. 30*:293–331 (1979).
6. Hechtman, H.B., W.V. Huval, M.A. Mathieson, R. Valeri, and D. Shepro, Prostaglandin and Thromboxane Mediation of Cardiopulmonary Failure, *Surg. Clin. North Am. 63*:263–283 (1983).
7. Rietschel, E.T., U. Schade, and H. Luederitz, Prostaglandins in Endotoxicosis, in *Microbiology*, edited by D. Shlessinger, American Society for Microbiology, Washington, DC, 1980, pp. 66–72.
8. Goodwin, J., and J. Ceuppens, Regulation of the Immune Response by Prostaglandins, *J. Clin. Immunol. 3*:295–315 (1983).
9. Knudsen, P.J., C.A. Dinarello, and T.B. Strom, Prostaglandins Post-Transcriptionally Inhibit Monocyte Expression of Interleukin 1 Activity by Increasing Intracellular Cyclic Adenosine Monophosphate, *J. Immunol. 137*:3189–3194 (1986).
10. Mohrhauer, H., and R.T. Holman, The Effect of Dose Level of Essential Fatty Acids upon Fatty Acids Composition of the Rat Liver, *J. Lipid Res. 4*:151–159 (1963).
11. Kromhault, D., E.B. Bosscheiter, and C.C. de Lezenne, The Inverse Relationship Between Fish Consumption and 20-year Mortality from Coronary Heart Disease, *N. Engl. J. Med. 312*:1205–1209 (1985).
12. Akoh, C.C., Structured Lipids—Enzymatic Approach, *INFORM 9*:1055–1061 (1995).
13. Babayan,V.K., Medium-Chain Triglycerides and Structured Lipids, *Nutr. Sup. Serv. 6*:26–29 (1987).
14. Mascioli, E.A., B.R. Bistrian, V.K. Babayan, and G.L. Blackburn, Medium-Chain Triglycerides and Structured Lipids as Unique Nonglucose Energy Sources in Hyperalimentation, *Lipids 22*:421–423 (1987).
15. Huang, K.-H, C.C. Akoh, and M.C. Erickson, Enzymatic Modification of Melon Seed Oil: Incorporation of Eicosapentaenoic Acid, *J. Agric. Food Chem. 42*:2646–2648 (1994).
16. Huang, K.H., and C.C. Akoh, Lipase-Catalyzed Incorporation of n-3 Polyunsaturated Fatty Acids into Vegetable Oils, *J. Am. Oil Chem. Soc. 71*:1277–1280 (1994).
17. Akoh, C.C., and R.V. Sista, Enzymatic Modification of Borage Oil: Incorporation of Eicosapentaenoic Acid, *J. Food Lipids 2*: 231–238 (1995).
18. Luddy, F.E., R.A. Barford, S.F. Herb, P. Magidman, and R.W. Riemenschneider, Pancreatic Lipase Hydrolysis of Triglycerides by a Semimicro Technique, *J. Am. Oil Chem. Soc. 51*:693–696 (1964).

19. Foglia, T.A., and P.E. Sonnet, Fatty Acid Selectivity of Lipases: Gamma-Linolenic Acid from Borage Oil, *Ibid. S4*:417–420 (1995).
20. Laane, C., S. Boeren, K. Vos, and C. Veeger, Rules for Optimization of Biocatalysis in Organic Solvents, *Biotechnol. Bioeng*. *30*:81–87 (1987).
21. Macrae, A.R., in *Biotechnology for the Oils and Fats Industry*, edited by C. Ratledge, P. Dawson, and J. Rattray, American Oil Chemists' Society, Champaign, 1984, pp.189–193.
22. Dordick, J.S., Enzymatic Catalysis in Monophasic Organic Solvents, *Enzyme Microb. Technol*. *11*:194–211 (1989).
23. Ricardo, S., and K.D. Mukherjee, Interesterification of Lipids Using an Immobilized *sn*-1,3 Specific Triacylglycerol Lipase, *J. Agric. Food Chem. 35*:1005–1008 (1987).

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