Solvent-Free Enzymatic Synthesis of Structured Lipids Containing CLA from Coconut Oil and Tricaprylin

Alejandro Rocha-Uribe* and Ernesto Hernandez

Food Protein R&D Center, Texas A&M University, College Station, 77843-2476

ABSTRACT: Lipase-catalyzed acidolysis of different TAG with CLA was performed to produce structured lipids (SL) containing CLA. An immobilized lipase from *Mucor miehei* (Lipozyme IM, Novo Nordisk Inc., Franklinton, NC) was used as the biocatalyst in a solvent-free system. Coconut oil and tricaprylin, which are sources of medium-chain FA, were the starting substrates, and a mixture of FFA (MFFA) containing 73% CLA was the donor of the acyl groups. For each TAG, four different ratios of TAG/MFFA were blended to prepare about 500 g of mixture containing 10, 20, 30, and 40% CLA (w/w). Each blend was reacted with 5% lipase at 65°C for 48 h under nitrogen. Over the range of TAG/MFFA ratios examined, CLA was incorporated effectively by the enzyme. Lipozyme IM exhibited no special preference for any particular FA, since the incorporation of FA was proportional to their concentration in the system. FFA, PV, *p*-anisidine value (*p*-AV), iodine value (IV), and saponification number (SN) were evaluated for all the SL. FFA, PV, and *p*-AV depended on the purification process and showed no significant deterioration of SL with respect to the original TAG, whereas IV and SN depended on the composition of the SL, mainly the CLA content.

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KEY WORDS: Coconut oil, conjugated linoleic acid, lipases, solvent-free acidolysis, structured lipids, tricaprylin.

CLA is a nutraceutical FA that has been reported to show a wide range of biological effects, such as inhibition of carcinogenic tumor growth (1), reduction of atherosclerotic risk (2), and reduction of body fat (3). The term CLA refers to a mixture of geometrical and positional isomers of linoleic acid (18:2) containing conjugated double bonds. The *cis*-9,*trans*-11-CLA and the *trans*-10,*cis*-12 isomers of CLA are believed to be primarily responsible for the beneficial physiological effects of CLA.

CLA occurs naturally, mainly in animal fats (milk and meat), at low concentrations. It is formed primarily as an intermediate during the biohydrogenation of linoleate by rumen bacteria. Consequently, many efforts have been directed toward finding mechanisms to produce more CLA and incorporate it into several kinds of edible fats to achieve prophylactic or therapeutic effects. Reports about the enrichment of edible fats with synthetic CLA involving the lipase-catalyzed incorporation of CLA have shown the feasibility of enzymatic incorporation of CLA into different kinds of TAG (4–9).

Incorporation of CLA into medium-chain TAG to produce new types of healthful fats and oils (i.e., structured lipids, or SL) seems a viable means of supplying CLA in the diet. SL have been receiving increasing attention in the food area, since they may be a good vehicle for providing nutraceutical FA to consumers. SL containing mixtures of either short-chain ($<C_8$), medium-chain (C_8–C_{12}), both short- and medium-chain, and long-chain ($>C_{12}$) FA in the same glycerol molecule have been reported (10). By structuring TAG in this way, the health benefits of the long-chain FA (i.e., PUFA) are enhanced by the nutritional and metabolic advantages of the medium-chain FA, resulting in a combination that can provide special health properties. The development of nutraceutical fats consisting of SL containing CLA, which could provide the important physiological properties of CLA enhanced by the metabolic benefits of medium-chain FA, is reported in this paper.

Kim and cowriters (11,12) reported the synthesis of SL containing CLA. They tested several commercially available lipases to transesterify mixtures of CLA-ethyl ester and tricaprylin in hexane at 55°C. In such studies, Lipozyme IM (immobilized lipase from *Mucor miehei*) and lipase *PS-C* (from *Pseudomonas cepacia*) showed high activity in incorporating CLA into the *sn*-1,3 positions of TAG. Lipozyme IM also has been used in the absence of organic solvent to synthesize SL from peanut oil and caprylic acid (13). The main objective of this work was to investigate the synthesis of SL containing CLA by incorporating different levels of CLA, as FFA, into two TAG that are good sources of medium-chain FA (i.e., coconut oil and tricaprylin) by enzymatic acidolysis in a solvent-free system and by using Lipozyme IM as a biocatalyst. The physicochemical properties of such SL were evaluated, as were the effects on their physicochemical properties of purifying the SL.

MATERIALS AND METHODS

Materials. Safflower oil and coconut oil were purchased at a local supermarket. Lipozyme IM (immobilized lipase from *M. miehe*) was obtained from Novo Nordisk Inc. (Franklinton, NC). Tricaprylin, all other reagents, and all standards were obtained from Sigma Chemical Company (St. Louis, MO). Solvents were obtained from VWR-Scientific Products (McGaw Park, IL).

Preparation of CLA. CLA FFA were prepared by alkali-catalyzed isomerization of safflower oil using sodium hydroxide

*To whom correspondence should be addressed at Dr. Manuel Nava 6, Zona Universitaria, San Luis Potosi, S.L.P., 78210, Mexico.
E-mail: arochau@uaslp.mx

at 180°C, following a method reported by Ip *et al.* (14). The procedure was modified by the use of propylene glycol instead of ethylene glycol to produce high yields of the two major active CLA isomers (i.e., *cis*-9-,*trans*-11-, and *trans*-10,*cis*-12-CLA isomers). A mixture of FFA (MFFA) containing about 73% CLA resulted from the isomerization reaction and was used in the acidolysis process.

Enzymatic acidolysis of TAGs with CLA. Coconut oil and tricaprylin were the starting substrates, and the MFFA obtained from the isomerization process including CLA was the acyl group donor. For each type of TAG, four different ratios of TAG/MFFA were blended to reach predetermined levels of CLA (i.e., 10, 20, 30, and 40%, w/w). Five hundred grams of each blend was reacted with 5% lipase (Lipozyme IM, with a 3.9% moisture content) at 65°C for 48 h under N_2, and with constant agitation provided by a magnetic stirrer.

Purification of SL. After acidolysis, the reaction mixture was filtered through anhydrous sodium sulfate to remove the enzymes and any residual water. The excess FFA present in the mixture was then removed using a 2″ Pope short-path wiped-film distillation unit (Pope Scientific, Menomonee Falls, WI). The mixtures were passed through the short-path distillation system to obtain an FFA concentration, as oleic acid, of less than 2%. FFA were removed from the SL under a vacuum of 0.07 Torr, with the evaporator set at 190°C, the condenser temperature at 36°C, the wiper speed at 20% forward, and the flow rate at 6–8 mL/min. The resulting SL from the short-path still were further purified by alkali refining with 20% NaOH solution (wt/vol) at 65°C, followed by centrifugation (22,500 × *g* for 20 min), then bleached in vacuum at 98°C with 0.2% silica gel and 0.4% bleaching clay. A filter aid (0.2%) was added to the mixture, and it was filtered through Whatman no. 41 filter paper. The final samples were cooled, placed in plastic bottles, and stored in a freezer (−20°C) under inert atmosphere until analysis.

FA composition. The FA composition, including the concentration of major isomers of CLA and total CLA, was determined by GC of the methyl ester derivatives. The TAG methylation process consisted of reacting 10 mg of oil with 0.5 mL of 5% sodium methoxide in methanol at 65°C for 20 min. The reaction was stopped by the addition of 0.5 mL of saturated NaCl. The methyl esters were extracted with 1 mL of hexane (HPLC grade). The methylation procedure for the MFFA consisted of adding 3 mL of 0.5% HCl in methanol to 10 mg of sample, then heating at 70°C for 2 min to minimize artifact formation (15). The methyl esters were extracted with 1 mL of hexane (HPLC grade). One microliter of methyl ester solution in hexane was injected onto a 100-m, 0.25-mm i.d., 0.25-mm film Stabilwax capillary GC column (Restek Corp, Bellefonte, PA) using hydrogen as the carrier gas. The column oven was raised from 150 to 200°C at a rate of 10°C/min, then from 200 to 250°C at a rate of 3°C/min and held at 250°C for 20 min. A Varian Model 3400 GC system in split injection mode (250°C) and fitted with an FID (300°C) was used. All the FA were identified by comparison of their retention times with those of authentic standards.

Characterization of SL. FFA, PV, *p*-anisidine value (*p*-AV), iodine value (IV), and saponification number (SN) were determined using AOCS Official Methods Ca 5a-40, Cd 8-53, Cd 18-90, Ja 14-91, and Cd 3-25, respectively (16). All tests were performed in duplicate. Average values and SD are reported herein.

Experimental design. The experimental design explored two main factors: the concentration of CLA in the blend before acidolysis (at four levels: 10, 20, 30, and 40%) and the type of TAG used as substrate (coconut oil and tricaprylin). The 4 × 2 treatments were replicated twice.

Statistical analysis. ANOVA was performed using STATISTICA software, version 6.1 (StatSoft, Inc., Tulsa, OK). Significant effects on physical properties attributable to the main factors (i.e., level of CLA and substrate) were determined.

RESULTS AND DISCUSSION

Production of CLA. The initial FA compositions of the safflower oil and MFFA containing CLA are given in Table 1. The main change during isomerization was the conversion of linoleic acid to CLA. The content of CLA reached in the final MFFA was 72.8%, meaning that almost 98% conversion was reached under the experimental conditions of this work. The GC system used to analyze the FA profile was adequate to resolve the two major isomers produced during isomerization (i.e., the *cis*-9,*trans*-11- and *trans*-10,*cis*-12 CLA isomers). These two isomers accounted for 97.5% of the total CLA present in the MFFA, and they were present in about equal amounts, as reported previously (15).

FA composition. The FA compositions of the two oils before and after lipase-catalyzed acidolysis are given in Tables 2 and 3, respectively. These tables show the effect of acidolysis of the starting substrates with increasing amounts of MFFA on the content of CLA and all other FA present in the system. The CLA content of coconut oil-based SL increased from 9.38 to

TABLE 1
FA Composition of Safflower Oil (before alkali-catalyzed isomerization) and a Mixture of FFA (MFFA) Containing CLA (after alkali-catalyzed isomerization)[a]

	FA % (w/w)						
					CLA		
	16:0	18:0	18:1	18:2	*c*9,*t*11	*t*10,*c*12	Other
Safflower oil	6.7 ± 0.2	2.6 ± 0.1	15.4 ± 0.9	74.5 ± 2.2	ND	ND	ND
MFFA	6.7 ± 0.64	2.5 ± 0.8	15.4 ± 1.0	1.7 ± 0.1	35.1 ± 1.0	35.9 ± 1.0	1.8 ± 1.0

[a]Mean ± SD of 12 samples. ND, not detected.

TABLE 2
FA Composition of Coconut Oil, MFFA Containing CLA, and Coconut Oil-Based Structured Lipids (SL) with Different Levels of CLA[a]

			Coconut oil-based SL with CLA (%)			
FA	Coconut oil	MFFA	10	20	30	40
8:0	9.9 ± 0.3	ND	6.8 ± 0.3	6.1 ± 0.4	5.3 ± 0.9	4.5 ± 0.3
10:0	6.9 ± 0.1	ND	5.4 ± 0.2	4.7 ± 0.2	3.9 ± 0.4	3.1 ± 0.2
12:0	47.9 ± 1.1	ND	42.7 ± 0.2	37.4 ± 0.5	29.4 ± 0.9	21.6 ± 1.3
14:0	17.8 ± 0.7	0.1 ± 0.1	16.1 ± 0.1	13.2 ± 0.2	9.8 ± 1.3	8.0 ± 0.5
16:0	8.2 ± 0.6	6.7 ± 0.6	8.3 ± 0.15	7.6 ± 0.1	7.2 ± 0.4	7.2 ± 0.1
18:0	2.4 ± 0.1	2.6 ± 0.3	2.5 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.5 ± 0.0
18:1	5.4 ± 0.3	15.5 ± 0.3	7.2 ± 0.1	8.2 ± 0.2	9.7 ± 0.3	11.0 ± 0.3
18:2	1.4 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
CLA[b]	ND	72.8 ± 1.1	9.4 ± 0.2	18.2 ± 0.6	30.6 ± 2.1	39.9 ± 2.0
Major[c]	ND	95.2 ± 0.2	95.9 ± 1.1	94.9 ± 0.6	95.3 ± 0.2	95.3 ± 0.2

[a]Mean ± SD of two replicates each with a duplicate analysis.
[b]Total CLA.
[c]Fraction of the major isomers of CLA with respect to total CLA. For other abbreviations see Table 1.

TABLE 3
FA Composition of Tricaprylin, MFFA Containing CLA, and Tricaprylin-Based SL with Different Levels of CLA[a]

			Tricaprylin-based SL with CLA (%)			
FA	Tricaprylin	MFFA	10	20	30	40
8:0	99.5 ± 0.1	ND	83.6 ± 1.3	70.1 ± 0.6	53.3 ± 0.9	40.6 ± 1.2
10:0	0.5 ± 0.1	ND	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0
16:0	ND	6.7 ± 0.6	1.2 ± 0.1	2.3 ± 0.2	3.4 ± 0.2	4.1 ± 0.2
18:0	ND	2.6 ± 0.3	0.5 ± 0.1	1.0 ± 0.1	1.5 ± 0.1	1.6 ± 0.1
18:1	ND	15.5 ± 0.3	2.6 ± 0.1	5.2 ± 0.2	7.9 ± 0.4	9.8 ± 0.3
18:2	ND	1.7 ± 0.1	0.2 ± 0.04	0.5 ± 0.1	0.8 ± 0.1	1.0 ± 0.1
CLA[b]	ND	72.8 ± 1.1	11.3 ± 0.1	21.9 ± 0.1	32.5 ± 1.9	40.6 ± 0.5
Major[c]	ND	95.2 ± 0.2	95.1 ± 0.8	95.5 ± 0.6	95.5 ± 0.2	96.2 ± 0.2

[a]Mean ± SD of two replicates each with a duplicate analysis.
[b]Total CLA.
[c]Fraction of the major isomers of CLA with respect to total CLA. For other abbreviations see Table 1.

39.93% with increasing amounts of MFFA. The CLA content of tricaprylin-based SL increased from 11.34 to 40.6% with increasing FFA in the acidolysis reaction. A nonsignificant difference was found in CLA contents between coconut oil-based and tricaprylin-based SL for the same CLA level ($P > 0.05$). The CLA content of the final SL was very close to the initial concentration of CLA in each blend before acidolysis (i.e., 10, 20, 30, and 40%). The same effect was observed for all the other FA present. Thus, the net effect of acidolysis was an increase in the concentrations of the larger and more unsaturated FA, mainly due to the incorporation of CLA and oleic acid (18:1). The use of lipase from *M. miehei* (Lipozyme IM), an *sn*-1,3-specific enzyme, has been well documented. Previous reports have indicated that Lipozyme IM can be used to interchange different classes of FA into the *sn*-1 and *sn*-3-positions of the glycerol backbone of TAG to produce different classes of SL (15,17,18). Under the conditions of this study, the lipase from *M. miehei* displayed no preference for any specific FA. The results found here for coconut oil SL differed from those reported by Reena *et al.* (19), who reported that the overall preference shown by the enzyme for incorporation of FA was 18:0 > 18:2 > 22:0 > 18:1, 18:3, 14:0, 20:4, 22:6 > 16:0 > 12:0 >> 10:0. However, they carried out the acidolysis reaction in hexane and at 37°C, which could explain the observed differences. These differences may indicate an important effect of temperature and choice of solvent on enzyme activity selectivity for FFA activity. Also, no selectivity for either of the two major isomers of CLA was observed. Similar results were reported by McNeill *et al.* (15), who enriched palm oil with a mixture of *cis*-9,*trans*-11- and *trans*-10,*cis*-12-CLA isomers by using Lipozyme IM.

Characterization of SL. The physicochemical qualities of the starting materials, coconut oil-based SL, and tricaprylin-based SL are summarized in Table 4. In general, low levels of FFA, PV, and *p*-AV were observed for all the samples, regardless of the level of CLA. Important physicochemical changes (i.e., the breakdown of TAG and oxidation) have been observed in the production of other SL during interesterification and molecular distillation (20). However, in the present study, the levels of FFA, PV, and *p*-AV were no higher than those of the fresh TAG, with the exception of slightly higher FFA values for tricapylin-based SL with respect to the fresh TAG. This result could be due to the higher hydrolytic susceptibility observed by esters of the shorter-chain FA concentrate of caprylic acid

TABLE 4
Physicochemical Quality of the Original Safflower Oil, Coconut Oil, Tricaprylin, Coconut Oil-Based SL (COSL), and Tricaprylin-Based SL (TCSL) with Different Levels of CLA[a]

	Characteristics[b]				
Sample	FFA	PV	*p*-AV	IV	SN
Safflower oil	0.15 ± 0.06	0.90 ± 0.11	1.94 ± 0.06	142.67 ± 1.26	184.2 ± 0.16
Coconut oil	0.25 ± 0.11	0.86 ± 0.12	0.95 ± 0.16	8.69 ± 0.23	247.9 ± 1.10
COSL (CLA%)					
10	0.15 ± 0.01	0.35 ± 0.07	0.93 ± 0.16	21.82 ± 1.81	234.58 ± 0.56
20	0.23 ± 0.01	0.5 ± 0.14	0.98 ± 0.10	37.66 ± 0.26	226.43 ± 3.05
30	0.25 ± 0.02	0.55 ± 0.29	1.22 ± 0.13	56.24 ± 0.32	217.44 ± 1.77
40	0.14 ± 0.1	0.33 ± 0.18	1.23 ± 0.21	70.29 ± 3.39	207.84 ± 3.05
Tricaprylin	0.17 ± 0.7	0.46 ± 0.02	0.97 ± 0.16	0	343.25 ± 1.1
TCSL (CLA%)					
10	0.34 ± 0.12	0.41 ± 0.07	0.31 ± 0.2	20.69 ± 1.81	303 ± 1.23
20	0.36 ± 0.09	0.59 ± 0.14	0.83 ± 0.09	36.87 ± 0.26	286.28 ± 2.35
30	0.28 ± 0.04	1.09 ± 0.19	0.78 ± 0.21	53.51 ± 0.32	259.28 ± 1.41
40	0.24 ± 0.17	0.20 ± 0.08	0.79 ± 0.08	70.72 ± 3.39	234.18 ± 3.05

[a]Mean ± SD of two replicates each with a duplicated analysis.
[b]*p*-AV, *para*-anisidine value; IV, iodine value; SN, saponification number; for other abbreviations see Tables 1 and 2.

(8:0). In spite of such FFA values in tricaprylin-based SL, one can generalize that coconut oil- and tricaprylin-based SL showed minimal deterioration during the processing and purification steps, since nonsignificant differences were found in physicochemical quality between the SL with different levels of CLA ($P < 0.05$) or from different TAG sources ($P < 0.05$). The use of nitrogen during the processing and purification steps probably helped to prevent deterioration. Also, the purification steps following molecular distillation (i.e., refining and bleaching) may have contributed to reduced values of FFA, PV, and *p*-AV.

On the other hand, SN and IV, which are related to the molecular characteristics of SL, depended on their FA composition (Table 4). Decreased SN and increased IV were observed with increasing amounts of MFFA incorporated into the SL during acidolysis. Such effects were the result of the greater incorporation of larger and more unsaturated FA (i.e., CLA and oleic acid, 18:1) with increasing amounts of MFFA in the acidolysis reaction. SN of coconut oil-based SL were lower than those of tricaprylin-based SL for the same level of CLA ($P < 0.05$) owing to the higher M.W. of the FA in the original coconut oil. In contrast, no significant difference in IV was found between SL originating from either coconut oil or tricaprylin but bearing the same CLA level since both original TAG had low unsaturation levels (the IV for coconut oil and tricaprylin equaled 8.7 and 0, respectively; Table 4).

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REFERENCES

1. Ip, C., J.A. Scimeca, and H.J. Thompson, Conjugated Linoleic Acid. A Powerful Anticarcinogen from Animal Fat Sources, *Cancer 74*:1050–1054 (1994).
2. Lee, K.N., D. Kritchevsky, and M.W. Pariza, Conjugated Linoleic Acid and Atherosclerosis in Rabbits, *Atherosclerosis 108*:19–25 (1994).
3. Park, Y., K.J. Albright, W. Liu, J.M. Storkson, M.E. Cook, and M.W. Pariza, Effect of Conjugated Linoleic Acid on Body Composition in Mice, *Lipids 32*:853–858 (1997).
4. Garcia, H.S., J.M. Storkson, M.W. Pariza, and C.G. Hill, Enrichment of Butteroil with Conjugated Linoleic Acid *via* Enzymatic Interesterification (acidolysis) Reactions, *Biotechnol. Lett. 20*:393–395 (1998).
5. Arcos, J.A., C. Otero, and C.G. Hill, Rapid Enzymatic Production of Acylglycerols from Conjugated Linoleic Acid and Glycerol in a Solvent-Free System, *Ibid. 30*:617–621 (1998).
6. Martinez, C.E., J.C. Vinay, M.R. Brieva, C.G. Hill, and H.S. Garcia, Lipase-Catalyzed Interesterification (acidolysis) of Corn Oil and Conjugated Linoleic Acid in Organic Solvents, *Food Biotechnol. 13*:183–193 (1999).
7. Garcia, H.S., J.A. Arcos, D.J. Ward, and C.G. Hill, Jr., Synthesis of Glycerides Containing n-3 Fatty Acids and Conjugated Linoleic Acid by Solvent-Free Acidolysis of Fish Oil, *Biotechnol. Bioeng. 70*:587–591 (2000).
8. Torres, C.F., E. Barrios, and C.G. Hill, Jr., Lipase-Catalyzed Acidolysis of Menhaden Oil with CLA: Optimization by Factorial Design, *J. Am. Oil Chem. Soc. 79*:457–466 (2002).
9. Torres, C.F., F. Munir, L.P. Lessard, and C.G. Hill, Jr., Lipase-Mediated Acidolysis of Tristearin with CLA in a Packed-Bed Reactor: A Kinetic Study, *Ibid. 79*:655–661 (2002).
10. Akoh, C.C., Structured Lipids—Enzymatic Approach, *inform 6*:1055–1061 (1995).
11. Kim, I.H., C.S. Yoon, and K.W. Lee, Transesterification of Conjugated Linoleic Acid and Tricaprylin by Lipases in Organic Solvent, *Food Res. Int. 34*:301–306 (2001).
12. Kim, I.-H., C.-S. Yoon, S.-H. Cho, K.-W. Lee., S.-H. Chung, and B.-S Tae, Lipase Catalyzed Incorporation of Conjugated Linoleic Acid into Tricaprylin, *J. Am. Oil Chem. Soc. 78*:547–551 (2001).
13. Lee, K., and C.C. Akoh, Solvent-Free Enzymatic Synthesis of

Structured Lipids from Peanut Oil and Caprylic Acid in a Stirred Tank Batch Reactor, *Ibid. 75*:1533–1537 (1998).
14. Ip, C., S.F. Chin, J.A. Scimeca, and M.W. Pariza, Mammary Cancer Prevention by Conjugated Dienoic Derivatives of Linoleic Acid, *Cancer Res. 51*:6118–6124 (1991).
15. McNeill, G.P., C. Rawlins, and A.C. Peilow, Enzymatic Enrichment of Conjugated Linoleic Acid Isomers and Incorporation into Triglycerides, *J. Am. Oil Chem. Soc. 76*:1265–1268 (1999).
16. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, edited by D. Firestone, AOCS, Champaign, 1998, Methods Ca 5a-40, Cd 8-53, Cd 18-90, Ja 14-91, Cd 3-25.
17. 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).
18. Yankah, V.V., and C.C. Akoh, Lipase-Catalyzed Acidolysis of Tristearin with Oleic or Caprylic Acids to Produce Structured Lipids, *Ibid. 77*:495–500 (2000).
19. Reena, R., S. Divakar, and B.R. Lokesh, Plackett–Burman Design for Determining the Preference of *Rhizomucor miehei* Lipase for FA in Acidolysis Reactions with Coconut Oil, *Ibid. 79*:555–560 (2002).
20. Akoh, C.C., and C.O. Moussata, Characterization and Oxidative Stability of Enzymatically Produced Fish and Canola Oil-Based Structured Lipids, *Ibid. 78*:25–30 (2001).

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