Enzymatic Acidolysis of an Arachidonic Acid Single-Cell Oil with Capric Acid

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ABSTRACT: Incorporation of capric acid (CA) into arachidonic acid (AA) single-cell oil, using five commercial lipases, indicated that lipase PS-30 from *Pseudomonas* sp. was most effective. The optimal conditions included an oil-to-CA mole ratio of 1:3, a temperature of 45°C, incubation time of 24 h, 4% lipase from *Pseudomonas* sp., and a 2% (w/w) water content. Examination of positional distribution of FA on the glycerol backbone of modified AA single-cell oil with CA showed that 89.7% of CA was concentrated in the *sn*-1,3 positions of the TAG molecules. AA was mainly located at the *sn*-2 position of the modified AA single-cell oil. Enzymatically modified AA single-cell oil had a higher conjugated dienes (CD) value than its unmodified counterpart. TBARS values of both modified and unmodified AA single-cell oils increased progressively during the entire storage period, but no significant difference existed between TBARS values of both oils. Thus, enzymatically modified oil was more susceptible to oxidation than its unmodified counterpart, when considering both CD and TBARS values. Removal of natural antioxidants during oil modification might play a significant role in rapid oxidative deterioration of enzymatically modified oils. This possibility was confirmed when starting materials were subjected to the same reaction process in the absence of any enzyme, as the resultant oil was indeed significantly less stable than the control oil.

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KEY WORDS: Acidolysis, arachidonic acid single-cell oil, capric acid (C10:0), conjugated dienes, lipase, medium-chain fatty acids, oxidative stability, positional distribution, structured lipids, thiobarbituric acid-reactive substances (TBARS).

Structured lipids (SL) are TAG in which FA are located in specific locations on the glycerol backbone as a result of chemical or enzymatic means. Much attention has been paid to SL because of their potential biological functions and nutritional properties, including reduction in serum TAG, LDL cholesterol, and total cholesterol (1); improvement of immune function and protection against thrombosis (2); reduction of protein breakdown (3); improvement of absorption of other fats (1); lower caloric value and preservation of reticuloendothelial system function (4,5); improvement of nitrogen balance (2); and reduction in the risk of cancer (6).

Lipase-catalyzed interesterification offers many advantages over chemically assisted interesterification. It produces fats or oils with a defined structure because it incorporates a specific FA at a specific position of the glycerol moiety. It requires mild experimental conditions without the potential for side reactions, consumes less energy, reduces heat damage to reactants, and produces easily purified products (7). These SL may be produced *via* direct esterification, acidolysis, alcoholysis, or interesterification reactions. However, the ordinary methods cited in the literature for production of SL are based on reactions between two TAG molecules (interesterification) or between a TAG and an acid (acidolysis).

Medium-chain FA (MCFA) are saturated molecules with a carbon chain length ranging from 6 to 12; they are prepared from oils of tropical fruits such as coconut and palm kernel (5). Medium-chain TAG (MCT) exhibit unique structural and physiological features. They are easily absorbed, they are metabolized as quickly as glucose, and they are rapidly cleared from the blood (3). Preparation of SL containing an essential or specific FA is very desirable. Arachidonic acid (AA) single-cell oil is a rich source of arachidonic acid (AA: 40–50%) with only small amounts of other long-chain PUFA being present. AA single-cell oil is produced by the microfungus *Mortierella alpina* (8). The research reported here used AA single-cell oil for production of SL. An SL containing a mixture of MCFA for rapid energy release and the long-chain FA, AA, as a source of conditionally essential FA would be useful in the alleviation of malabsorption syndromes. AA-enriched MAG are expected to be nearly completely absorbed. The position of FA in the TAG molecules (*sn*-1, -2, and -3) would have a significant impact on their metabolism in the body. In general, FA at the *sn*-1 and *sn*-3 positions are hydrolyzed by pancreatic lipase and absorbed, whereas those at the *sn*-2 position remain unchanged and are used in the synthesis of new TAG.

In examining the oxidative stability of modified and unmodified AA single-cell oil, a number of stability tests using chemical and instrumental techniques are usually used (9,10). The conjugated dienes (CD) test is a simple and rapid method to evaluate primary products of lipid oxidation, whereas the 2-thiobarbituric acid (TBA) test is used to examine secondary products of lipid oxidation.

The objectives of this study were (i) to incorporate a MCFA (capric acid), as a rapid source of energy, into the glycerol backbone of AA single-cell oil, (ii) to optimize reaction conditions for the preparation of AA single-cell oil-based SL, (iii) to determine the positional distribution of FA in the enzymatically modified AA single-cell oil, and (iv) to evaluate the oxidative stability of the SL so produced.

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MATERIALS AND METHODS

Materials. Two lipases, from *Candida antarctica* (Novozyme 435) and *Mucor miehei,* were acquired from Novo Nordisk (Franklinton, NC). Other lipases, namely, *Pseudomonas* sp. (PS-30), *Aspergillus niger* (AP-12), and *C. rugosa* (AY-30), were obtained from Amano Enzyme (Troy, VA). All lipases used in this work, including AP-12 and AY-30, were obtained in the immobilized form. AA single-cell oil (ARASCO™) containing 40–50% AA was obtained from Martek Bioscience Corporation (Columbia, MD). FAME (GLC-461) were purchased from Nu-Chek-Prep (Elysian, MN). Porcine pancreatic lipase (EC 3.11.3), sodium taurocholate, silica gel TLC plates (20 × 20 cm; 60 Å mean pore diameter, 2–25 μm mean particle size, 500 μm thickness, containing dichlorofluorescein) were purchased from Sigma Chemical Co. (St. Louis, MO). All solvents used in these experiments were of analytical grade and were purchased from Fisher Scientific (Nepean, Ontario, Canada).

Acidolysis reaction. In general, AA single-cell oil (500 mg) was mixed with CA at oil-to-CA mole ratios ranging from 1 to 3 in a screw-capped test tube, and then lipase (2–10% by weight of substrates) and water (1–2.5% by weight of substrates and enzyme) were added in hexane (3.0 mL). Samples were flushed with nitrogen and the containers capped and incubated for different periods (12 to 48 h) in an orbital shaker at 250 rpm at 25–55°C. Experiments for different time periods and replicates were carried out in separate containers.

Separation of acylglycerols after acidolysis. After a given time, a mixture of acetone and ethanol (20 mL; 1:1, vol/vol) was added to stop the reaction. To neutralize FFA, the reaction mixture was titrated against a 0.5 M NaOH solution (using a phenolphthalein indicator) until the color of the solution turned pink. Hexane (25 mL) was added to the mixture to extract the acylglycerols. The mixture was transferred into a separatory funnel and thoroughly mixed. The two layers (water, hexane) were allowed to separate, and the lower aqueous layer was discarded. The hexane layer was passed through a bed of anhydrous sodium sulfate to remove any residual water. The hexane was evaporated using a rotary evaporator at 45°C, and the acylglycerol fraction was recovered and transferred to special transmethylation vials.

FA composition of products. (i) Preparation of FAME. FA profiles of products were determined following their conversion to methyl esters. Transmethylation reagent (2.0 mL, freshly prepared 6.0 mL of concentrated sulfuric acid made up to 100 mL with methanol and 15 mg of hydroquinone as an antioxidant) was added to the sample vial, followed by vortexing. The mixture was incubated at 60°C for 24 h and subsequently cooled to room temperature. Distilled water (1 mL) was added to the mixture and after thorough mixing, a few crystals of hydroquinone were added to each vial to prevent oxidation; lipids were extracted three times, each with 1.5 mL of pesticide-grade hexane. The hexane layers were separated, combined, and transferred to a clean test tube and then washed two times, each with 1.5 mL of distilled water. The hexane layer (the upper layer) was separated from the aqueous layer and evaporated under a stream of nitrogen. FAME were then dissolved in 1.0 mL of carbon disulfide and used for subsequent GC analysis.

(ii) Analysis of FAME by GC. The FAME were analyzed using a Hewlett-Packard 5890 Series II gas chromatograph (Agilent, Palo Alto, CA) equipped with a Supelcowax-10 column (30 m length, 0.25 mm diameter, 0.25 μm film thickness; Supelco Canada Ltd., Oakville, Ontario). The oven temperature was first set at 220°C for 10.25 min and then raised to 240°C at 30°C/min and held there for 15 min. The injector (flame ionization) and detector (FID) temperatures were both set at 250°C. Ultra high purity helium was used as a carrier gas at a flow rate of 15 mL/min. Data were treated using Hewlett-Packard 3365 Series II Chem Station Software (Agilent). The FAME were identified by comparing their retention times with those of authentic standard mixtures (GLC-461; Nu-Chek-Prep), and the results were presented as percent by weight (% w/w).

(iii) Hydrolysis by pancreatic lipase. Hydrolysis of modified oil by pancreatic lipase was performed according to the method described by Christie (11) with a slight modification. Tris-HCl buffer (5.0 mL; 1.0 M, pH 8.0), 0.5 mL of calcium chloride (2.2%, wt/vol) and 1.25 mL of sodium taurocholate (0.05) were added to 25 mg of modified oil in a glass test tube. The mixture was allowed to stand at 40°C in a water bath for 1.0 min, followed by the addition of 5.0 mg of porcine pancreatic lipase (EC 3.11.3; Sigma). The mixture was subsequently placed in a gyrorotatory water bath shaker at 250 rpm under nitrogen for 1 h at 40°C. Ethanol (5.0 mL) was added to the mixture to stop the enzymatic reaction, followed by the addition of 5.0 mL of 6.0 M HCl. The hydrolytic products were extracted three times with 50.0 mL of methanol/chloroform (1:1, vol/vol), and the upper layer was removed, washed twice with distilled water, and passed through a bed of anhydrous sodium sulfate. The solvent containing hydrolytic products was evaporated under a stream of nitrogen. Silica gel TLC plates were evenly sprayed with 5% (wt/vol) boric acid and dried at 100°C for 1 h prior to use. The hydrolytic products were then separated on silica gel TLC plates. The plates were developed using hexane/diethyl ether/acetic acid (70:30:1, by vol) for 40–50 min and then allowed to dry in air. The bands were located by viewing under a short (254 nm) and a long (365 nm) wavelength light (Spectroline, Co., Westbury, NY). The bands (FFA and MAG) were scraped off and their lipids extracted into methanol/chloroform (1:1, vol/vol). FA profiles of lipids were analyzed by the GC method described in an earlier section.

(iv) Oxidative stability tests. The SL produced from AA single-cell oil *via* acidolysis with CA as well as the original oil were kept under accelerated oxidation conditions at 60°C in a Schaal oven for 3 d. Each day (24 h) of storage of oils under Schaal oven conditions at 60°C is equal to 1 mon of storage at room temperature (12). Oils (0.4–0.5 g) were placed in loosely capped test tubes (10 mm diameter and 4.0 cm height) and stored at 60°C in a forced-air oven (Thelco, Model 2; Precision Scientific Co., Chicago, IL). Samples were removed from the oven at 0, 6, 12, 24, 36, 48, and 72 h,

cooled to room temperature, flushed with nitrogen, capped, and stored at −20°C until analyzed. The experiments were carried out in triplicate.

CD. CD in the oils were determined according to IUPAC method 20505 (13). Oil samples (0.02–0.04 g) were weighed into 50-mL volumetric flasks, dissolved in iso-octane (2,2,4-trimethylpentane), and made up to the mark with the same solvent. The contents were thoroughly mixed, and the absorbance was read at 234 nm using a Hewlett-Packard model 845LA diode array spectrophotometer (Agilent). Pure iso-octane was used as the blank. CD concentrations were calculated using the following formula:

$$CD = A/(c \times d) \quad [1]$$

where A = absorbance of the solution at 234 nm, c = concentration of the solution in g/mL, and d = length of the cell in cm.

TBARS determination. The determination of TBARS was carried out as described by AOCS method Cd 19-90 (14). Oil samples (0.05–0.10 g) were weighed into 25-mL volumetric flasks, dissolved in a small volume of 1-butanol, and made up to the mark with the same solvent. An aliquot of 5 mL of the mixture (oil and solvent) was transferred into a dry screw-capped test tube, and then a volume of 5 mL of freshly prepared TBA reagent (0.5 g TBA in 250 mL 1-butanol) was added. The constituents of the mixture were thoroughly mixed, and the test tube was placed in a water bath at 95°C for 2 h. Heated samples were cooled in an ice bath, and the absorbance of the resulting colored complex was read at 532 nm using a Hewlett-Packard diode array Model 8452 A spectrophotometer (Agilent). The number of micromoles of malonaldehyde (MA) equivalents per gram of oil, expressed as TBARS values, was calculated using the formula

$$C = (0.415 \cdot A_{532})/w \quad [2]$$

where the factor 0.415 is obtained from a standard regression line produced using 1,1,3,3-tetramethoxypropane as a precursor of MA. In this formula C is the concentration of MA; A, the absorbance of the colored complex at 532 nm; and w, the mass of the oil.

Statistical analysis. All experiments were performed in triplicate. Data are reported as mean ± SD. Normality was tested using SigmaStat (Richmond, CA). ANOVA and Tukey's test were carried out at a level of $P < 0.05$ to assess the significance of differences between mean values.

RESULTS AND DISCUSSION

The FA profile of AA single-cell oil before and after modification with CA by *Pseudomonas* sp. is shown in Table 1. The major unsaturated FA found in AA single-cell oil before enzymatic incorporation were arachidonic (39.0%), oleic (34.1%), and linoleic (5.1%) acids. Furthermore, this oil also contained saturated FA such as stearic (7.3%) and palmitic (4.3%) acid. The proportions of different FA of AA single-cell oil found in this work were different from those reported by Arterburn *et al.* (8), who found that AA single-cell oil derived from microfungus *M. alpina* contained arachidonic (51.4%), oleic (8.3%), linoleic (6.3%), γ-linolenic (3.6%), palmitic (9.4%), and stearic (10.3%) acids. The differences between the FA profiles presented here and those reported by Arterburn *et al.* (8) might represent variation in the source and batch of the oil, method of preparation, and fermentation conditions employed.

TABLE 1
FA Composition of AA Single-Cell Oil TAG Before and After Modification with CA[a]

FA	Before modification (wt%)	After modification[b] (wt%)
10:0	Trace	19.3 ± 0.58
14:0	0.2 ± 0.01	0.2 ± 0.01
16:0	4.3 ± 0.04	2.7 ± 0.03
18:0	7.3 ± 0.2	5.0 ± 0.07
18:1n-9	34.1 ± 0.6	23.4 ± 0.29
18:2n-6	5.1 ± 0.09	4.0 ± 0.04
18:3n-6	2.1 ± 0.19	1.5 ± 0.02
20:3n-6	1.3 ± 0.02	1.1 ± 0.02
20:4n-6	39.0 ± 0.56	36.5 ± 0.46
20:5n-3	1.7 ± 0.03	1.3 ± 0.01
22:5n-6	2.0 ± 0.21	2.3 ± 0.17
24:0	0.2 ± 0.02	ND
Others	2.8	1.7

[a]Values are mean values of triplicate determinations ± SD.
[b]The reaction mixture contained 500 mg arachidonic acid (AA) single-cell oil, 315 mg capric acid (CA), 4% (w/w) *Pseudomonas* sp. enzyme, and 3.0 mL hexane. The reaction mixture was incubated at 45°C for 24 h in an orbital shaker at 250 rpm.

Five lipases were screened for their ability to incorporate CA into AA single-cell oil (Table 2). The degree of CA incorporation into AA single-cell oil with these five lipases was in the order of *Pseudomonas* sp. > *M. miehei* > *C. rugosa* > *A. niger* > *C. antarctica.* The incorporation of CA into AA single-cell oil was catalyzed effectively by only two of the five lipases tested. However, there was no significant difference ($P > 0.05$) in the CA incorporation into AA single-cell oil when lipases from *C. antarctica* and *A. niger* were employed. *Pseudomonas* sp. gave the highest degree of incorporation of CA into AA single-cell oil (24%, after 24 h). The results reported here agree with the findings of Zhou *et al.* (15), who

TABLE 2
Effect of Lipase Source on the Percent Incorporation of CA into AA Single-Cell Oil

Enzyme	Incorporation[a] (%)
Candida antarctica	2.4 ± 0.21[a]
Mucor miehei	21.7 ± 0.33[d]
Pseudomonas sp.	24.0 ± 0.47[e]
Candida rugosa	3.9 ± 0.19[c]
Aspergillus niger	3.0 ± 0.21[b,a]

[a]Values are means of triplicate determinations ± SD. Values with different superscripts are different ($P < 0.05$) from one another. For abbreviations see Table 1.

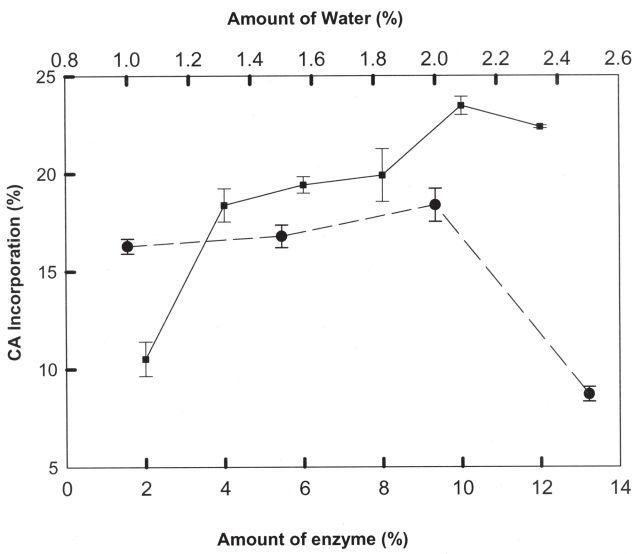

FIG. 1. The effect of enzyme load (■) and amount of added water (●) on the percent incorporation of capric acid (CA) into arachidonic acid (AA) single-cell oil. Data are presented as mean ± SD.

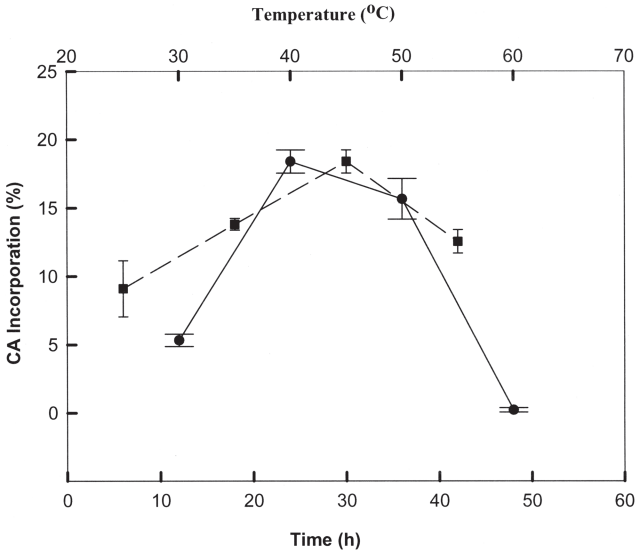

FIG. 2. The effect of reaction temperature (■) and time (●) on the percent incorporation of CA into AA single-cell oil. Data are presented as mean ± SD. For abbreviations see Figure 1.

reported that lipase from *P. fluoresescens* gave the highest incorporation of caprylic acid (8:0) into fish oil. Because acidolysis of AA single-cell oil with CA was best with lipase from *Pseudomonas* sp., this enzyme was selected for subsequent experiments.

As the number of moles of CA increased from 1 to 3, its incorporation into AA single-cell oil also increased. The optimal incorporation of CA into AA single-cell oil (23.3%) may be obtained at an oil-to-CA mole ratio of 1:3 because TAG molecules can incorporate a maximum of three FA in their backbone (data not shown). However, in enzymatic reactions, this ratio is not always limited to 1:3.

The effect of enzyme load (%) on the incorporation of CA into AA single-cell oil is shown in Figure 1. As the enzyme load increased from 2 to 10%, the incorporation of CA into AA single-cell oil increased gradually. Above the 10% enzyme load, there was a slight decrease in incorporation of CA into AA single-cell oil, possibly owing to factors such as deficiency of available water for hydration of the enzyme. As the amount of lipase in the reaction mixture was raised, the amount of added water remained constant at 2% (w/w). The highest CA incorporation into AA single-cell oil (23.5%) was achieved at an enzyme load of 10%. Results of this work agree with those of Jennings and Akoh (16), who reported that the maximum incorporation of CA (41.4%) into fish oil was achieved with an enzyme load of 10%. Furthermore, the present results agree with previous findings (5) for acidolysis of triolein with caprylic acid and when incorporating CA into seal blubber oil (17). Although a better incorporation of CA into the oils was obtained with a 10% enzyme load, we chose an enzyme load of 4% for subsequent experiments because this amount of enzyme was appropriate when considering the marginal increase in the yield at a higher enzyme level and hence the overall economy of the process.

Figure 2 shows the time course of this reaction using PS-30 from *Pseudomonas* sp. Between 12 and 24 h, the percent CA incorporated into AA single-cell oil increased significantly and reached a maximum at 24 h but then decreased, especially after 36 h. The decrease of CA incorporation into AA single-cell oil after 48 h may be due to the prolonged incubation time at high temperatures, resulting in denaturation of the lipase and thus loss of its 3-D structure and hence its activity. Another possible explanation may be the occurrence of the reverse (hydrolysis) reaction. Jennings and Akoh (18) successfully incorporated CA (up to 27%) into rice bran oil in 72 h by using immobilized lipase from *Rhizomucor miehei*. The results reported here are in contrast with the findings of Jennings and Akoh (16), who reported that the highest incorporation (41.2 mol %) into fish oil occurred at 48 h, and those of Senanayake and Shahidi (17), who found that as the reaction time of acidolysis of seal blubber oil with CA increased from 24 to 72 h, the CA incorporation increased from 25.4 to 29.6%.

The incorporation of CA into AA single-cell oil gradually increased as the reaction temperature increased from 25 to 45°C (Fig. 2), reaching an optimal incorporation of 18.3% at 45°C after 24 h of incubation. Low CA incorporation into AA single-cell oil was observed at lower temperatures (25–35°C). When the temperature rose above 45°C, CA incorporation into AA single-cell oil declined. In general, a 10°C increase in temperature results in doubling of the reaction rate for a nonenzymatic reaction. However, a higher incorporation of CA is expected at high temperatures but below the denaturation temperature for the enzyme. Senanayake and Shahidi (17) observed that as the reaction temperature increased from 30 to 50°C, incorporation of CA into seal blubber oil was increased and reached the highest level (26.9%) at 45°C, thus lending support to the findings in this study. The results presented here showed that *Pseudomonas* sp. lipase is more reactive at

TABLE 3
Positional Distribution[a] (*sn*-2 and *sn*-1 + *sn*-3) of FA in Unmodified AA Single-Cell Oil

FA	*sn*-2	*sn*-1 + *sn*-3
10:0	0.9 ± 0.53 (31.0)	0.8 ± 0.99 (69.0)
14:0	2.0 ± 0.92 (3.4)	0.4 ± 0.08 (96.3)
16:0	3.3 ± 0.66 (27.3)	5.6 ± 0.47 (72.7)
18:0	4.8 ± 1.4 (21.9)	8.8 ± 0.9 (78.1)
18:1	18.3 ± 5.6 (17.9)	29.9 ± 5.9 (82.1)
18:2n-6	3.6 ± 0.4 (23.3)	5.0 ± 1.2(76.7)
18:3n-6	1.3 ± 0.9 (20.3)	2.0 ± 0.7 (79.7)
20:4n-6	22.1 ± 8.7 (18.8)	23.1 ± 3.4 (81.2)

[a]Values indicate percent FA distribution of total TAG present at the (*sn*-1 + *sn*-3) and the *sn*-2 positions. Values in parentheses are (% FA at the *sn*-2 position/% FA in TAG × 3) × 100; for (*sn*-1 + *sn*-3) = 100 − *sn*-2.

45°C than at 25–35°C. Therefore, a reaction temperature of 45°C was used in the remaining experiments.

Figure 1 shows the effect of water (1–2.5%) on the incorporation of CA into AA single-cell oil. As the amount of water increased from 1 to 2%, incorporation of CA into AA single-cell oil increased significantly ($P < 0.05$). The highest incorporation of CA into AA single-cell oil (18.4%) was recorded at a 2% (w/w) water content. Increase in the water content of above 2% led to a decline in CA incorporation into AA single-cell oil, possibly due to the presence of excess water, which usually leads to hydrolysis. Thus, the presence of a small amount of water on the surface of the enzyme is required to maintain its 3-D structure without leading to hydrolysis.

The positional distribution of FA on the glycerol backbone of unmodified AA single-cell oil is summarized in Table 3. AA was mainly located at the *sn*-2 position (22.1%) of the TAG molecules. Table 4 shows the FA constituents at the *sn*-2 and (*sn*-1 + *sn*-3) positions of the modified AA single-cell oil with CA used pancreatic lipase-assisted hydrolysis. CA (89.7%) was mostly esterified to the *sn*-1,3 positions. No attempt was made here to use Grignard reagent to find the proportion of FA in each of the *sn*-1 and *sn*-3 positions. Thus, lipase from *Pseudomonas* sp. shows some regiospecificity and may preferentially incorporate CA at the *sn*-1 + *sn*-3 positions of TAG. The possibility also exists that the resulting TAG structure corresponds to the lowest Gibbs free energy state.

TABLE 4
Positional Distribution (*sn*-2 and *sn*-1 + *sn*-3) of FA in Modified AA Single-Cell Oil with CA

FA	*sn*-2	*sn*-1 + *sn*-3
10:0	6.0 ± 2.32 (10.3)	14.8 ± 2.96 (89.7)
14:0	1.5 ± 0.48 (2.5)	0.9 ± 0.51(97.5)
16:0	3.9 ± 0.25 (47.8)	3.5± 0.12 (52.2)
18:0	6.0 ± 0.61 (40.1)	8.9 ± 4.61 (59.9)
18:1	2.0 ± 2.86 (28.3)	21.8 ± 2.02 (71.7)
18:2n-6	3.5 ± 0.64 (29.2)	3.5 ± 0.80 (70.8)
18:3n-6	1.3 ± 0.29 (28.9)	1.5 ± 0.42 (71.1)
20:4n-6	40 ± 4.96 (36.2)	31.5 ± 10.1 (63.8)
20:5n-3	1.6 ± 0.18 (41.0)	1.0 ± 0.25 (59.0)

[a]Values indicate percent FA distribution of total TAG present at the (*sn*-1 + *sn*-3) and *sn*-2 positions. Values in parentheses are (% FA at the *sn*-2 position/% FA in TAG × 3) × 100; for (*sn*-1 + *sn*-3) = 100 − *sn*-2.

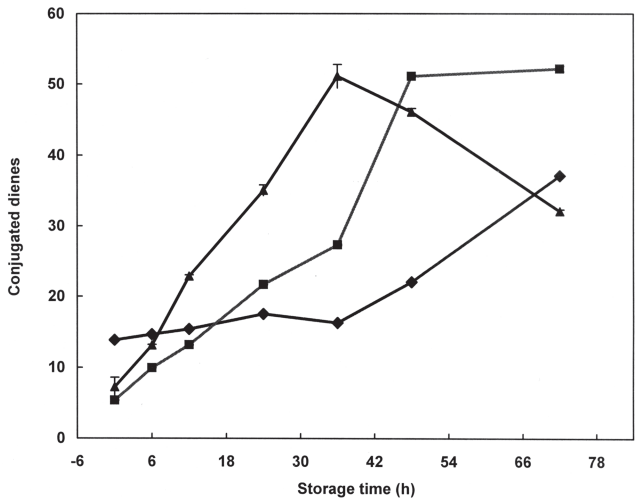

FIG. 3. Conjugated diene values of (■) modified AA single-cell oil and (▲) starting materials subjected to reaction steps without any lipase as well as (◆) the control (unmodified oil) stored under Schaal oven conditions at 60°C. Data are presented as mean ± SD. For abbreviation see Figure 1.

The CD values of the modified AA single-cell oil with CA, the original oil, and the starting materials subjected to the same reaction process, in the absence of any enzyme, to the same process steps are shown in Figure 3. The control (original) oil was stable under oxidative conditions from 6 to 36 h of storage, hence reflecting a longer induction period. As the storage time increased from 36 to 72 h, the CD values of the original AA single-cell oil increased steadily and reached their maximum value (37.1) at 72 h. The CD of modified AA single-cell oil increased with storage time (6 to 48 h) under Schaal oven conditions at 60°C. The highest CD value (51.1) was obtained at 48 h, after which the CD value reached a

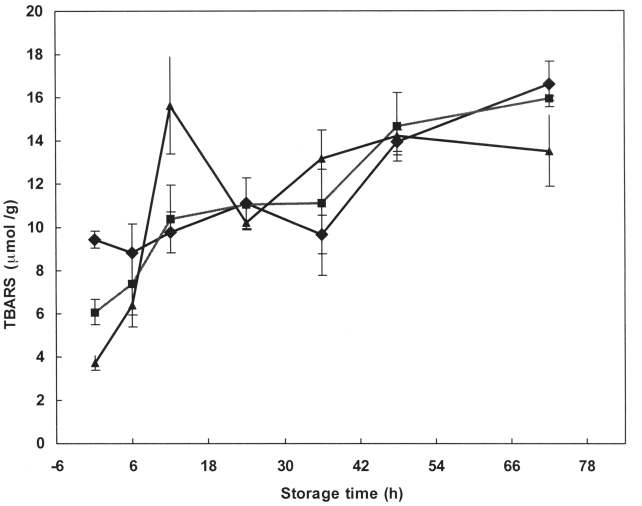

FIG. 4. TBARS values (μmol/g) of (■) modified AA single-cell oil and (▲) starting materials subjected to reaction steps without any lipase as well as (◆) the control (unmodified oil) stored under Schaal oven conditions at 60°C. Data are presented as mean ± SD. For abbreviation see Figure 1.

plateau. CD values were higher in AA single-cell oil subjected to the same reaction process in the absence of any enzyme over the entire storage period. CD values were higher in modified AA single-cell oil than the original oil from 24 to 72 h, thus reflecting a shorter induction period for AA single-cell oil. The results showed that modified AA single-cell oil was less stable than the original oil. This was demonstrated to be due in part to the loss of natural antioxidants during the preparation of SL, as shown here (see Fig. 3 for data when reactants were subjected to process steps in the absence of any enzyme) and as supported by the findings of Akoh and Moussata (18), who reported a significant loss of tocopherols in fish- and canola-based SL. Theoretically, incorporation of CA (saturated FA) into highly unsaturated oils should have enhanced their oxidative stability.

Figure 4 shows TBARS values of AA single-cell oil modified in the presence of lipase and without lipase as well as the control unmodified oils. The TBARS values of both oils increased steadily over the entire storage period. There was no difference ($P > 0.05$) between the modified and unmodified oils. Furthermore, there was no difference ($P > 0.05$) between enzymatically modified AA single-cell oil and the mixture subjected to the same reaction condition but in the absence of any enzyme. The results reported here indicate that modification of AA single-cell oil with CA had little effect on TBARS values of the oil. TBARS are secondary oxidation products resulting from degradation of hydroperoxides. Hence, their appearance depends on the rate of breakdown of the hydroperoxides involved. However, this may be influenced by the presence of antioxidants in these systems. Nonetheless, it is not apparent how this effect would influence the breakdown of hydroperoxides and their subsequent neutralization.

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