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Enzymatic Synthesis of Structured Triacylglycerols Containing CLA Isomers Starting from *sn*-1,3-Diacylglycerols

Silvia Maurelli · Francesca Blasi · Lina Cossignani · Ancilla Bosi · Maria Stella Simonetti · Pietro Damiani

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Abstract The synthesis of structured triacylglycerols (TAG) by the enzymatic reaction between *sn*-1,3-diacylglycerols (*sn*-1,3-DAG) and conjugated linoleic acid (CLA) isomers was studied. Both the substrates of the reaction were produced from vegetable oils, the *sn*-1,3-DAG from extra virgin olive oil and the CLA isomers from sunflower oil. The enzymatic reactions between these substrates were catalyzed for 96 h by an immobilized lipase from *Rhizomucor miehei* (Lipozyme IM) and the reactions carried out in solvent were monitored every 24 h by using high-performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD). The enzymatic reactions were carried out in different reaction media (hexane, isooctane and solvent free) and with different CLA/*sn*-1,3-DAG ratios. Total % acidic composition and structural analysis data were evaluated to verify the presence of CLA isomers in *sn*-2- position of synthesized TAG. The results showed good levels of CLA incorporation in *sn*-1,3-DAG, from 19.2% of TAG synthesized in solvent free conditions with a 0.5:1 substrate ratio, to 47.5% of TAG synthesized in isooctane with a 2:1 substrate ratio. It was observed that for all the reaction media, the best *sn*-2- acylic specificity was obtained with a 0.5:1 substrate ratio.

Keywords CLA · *sn*-1,3-DAG · Lipozyme IM · Structured lipids

S. Maurelli · F. Blasi · L. Cossignani · A. Bosi · M. S. Simonetti · P. Damiani (✉)
Dipartimento di Scienze Economico-Estimative e degli Alimenti, Sezione di Chimica Bromatologica, Università di Perugia, Via Romana, Interno Orto Botanico, 06126 Perugia, Italy
e-mail: chimbrom@unipg.it

Introduction

The term conjugated linoleic acid (CLA) refers generically to a class of positional and geometrical isomers of conjugated octadecadienoic acids, two of which (*cis*9, *trans*11 and *trans*10, *cis*12 CLA) are known to possess biological activity [1].

Many studies have shown beneficial effects of CLA isomers on human and animal health; it has been demonstrated that the main CLA isomers protect against tumorigenesis [2], stimulate the immune system [3], prevent diabetes [4], reduce the incidence of atherosclerosis in rabbits [5, 6] and hamsters [7]. Moreover dietary CLA reduce body fat mass and affect gene expression of proteins regulating energy metabolism in mice [8].

Endogenous production of CLA isomers by humans is very limited, therefore, a very large proportion of CLA found in the body tissue is from dietary origin [9].

At present, there is a great interest in CLA as nutritional complements and different products are now commercially available [10] as free fatty acids (FA) or alkyl ester mixtures.

Many studies have been carried out on the synthesis of structured triacylglycerols (TAG) containing CLA isomers by lipase-catalyzed reactions, among which esterification, transesterification or acidolysis reactions [11–13]. McNeill et al. [14] developed a method for the enzymatic enrichment of *cis*9, *trans*11 and *trans*10, *cis*12 CLA isomers and for the incorporation of each enriched fraction into palm oil TAG with *Rhizomucor miehei* lipase. Another piece of research dealt with the preparation of regioisomers of structured TAG containing 1 mol of CLA and 2 mol of caprylic acid [15]. Villeneuve et al. [16] proposed a chemoenzymatic synthesis of structured TAG containing CLA in the central position and lauric acid at the external

ones. The enzymatic approach in structured lipids synthesis is attractive since the reaction conditions are mild and a control of the FA distribution is possible even if the main drawback is represented by the occurrence of an acyl migration phenomena, in particular regarding the intermediate diacylglycerols (DAG) [17]. It has been demonstrated that absorption and metabolism of FA depend on the esterified position on the TAG backbone; in fact, it is well known that the ones located at the central *sn*-2- position are rapidly absorbed; in fact, pancreatic lipase quickly hydrolyzes the *sn*-1- and *sn*-3-positions to give *sn*-2-monoacylglycerols (MAG), easily absorbed [18].

This research was carried out with the objective of synthesizing structured TAG containing CLA isomers starting from *sn*-1,3-DAG, obtained from extra virgin olive oil (EVO) and CLA, obtained from sunflower oil. The *sn*-1,3-regiospecific immobilized lipase, Lipozyme IM, from *R. miehei* was used; different CLA/*sn*-1,3-DAG ratios and different reaction media (hexane, isooctane and solvent free) were studied to obtain high incorporation of CLA isomers into the free *sn*-2- position of *sn*-1,3-DAG. The reactions carried out in solvent were monitored using high-performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD). The regiospecific analysis of the structured TAG was carried out using the α-MAG procedure.

Materials and Methods

Materials

The linoleic acid conjugated methyl esters mixture (*cis*9, *trans*11 and *trans*10, *cis*12-octadecadienoic acid methyl esters, containing <1% linoleic acid methyl ester; catalog number O5632) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Lipase from *R. miehei* (Lipozyme IM), immobilized on an anionic exchange resin, 42 U/g, was obtained from Fluka (Chemika, Buchs, Switzerland).

Solvents and reagents were of analytical grade, purchased either from Sigma-Aldrich or Fluka; the HPLC grade solvents were from Carlo Erba Reagents (Milan, Italy) or J.T. Baker (Mallinckrodt Baker B.V., Deventer, Holland).

Methods

Production and Isolation of sn-1,3-DAG

The production of *sn*-1,3-DAG was carried out as reported in a previous work [19]. The typical FA mol% composition of the *sn*-1,3-DAG was: palmitic acid (C16:0; 12.2%), palmitoleic acid (C16:1n-7 + n-9 isomer; 0.8%), stearic acid (C18:0; 1.9%), oleic acid (C18:1n-9 + n-7 isomer; 78.7%), linoleic acid (C18:2n-6; 5.0%), linolenic acid (C18:3n-3; 0.5%) and arachidic acid (C20:0; 0.3%).

Preparation of CLA Isomers from Sunflower Oil

The alkaline hydrolysis of sunflower oil, the LA purification with urea and the alkaline isomerization of LA to CLA isomers were carried out following the procedures reported in a previous work [20]; the phase of LA purification was carried out three times. The FA mol% composition of the obtained CLA isomer mixture was determined after methylation reaction [21] and high-resolution gas chromatography (HRGC) analysis. CLA isomers were identified in comparison with CLA standard mixture.

A typical CLA mixture FA mol% composition was: C18:1n-9 + n-7 (1.2%), C18:2n-6 (0.3%), and total CLA (98.5%) with the following isomeric distribution: *c*9,*t*11 (48.1% isomer/total CLA), *t*10,*c*11 (47.9% isomer/total CLA), *t*,*t* isomers (2.1% isomer/total CLA) and other CLA isomers (1.9% isomer/total CLA).

Production and Isolation of TAG

The substrates *sn*-1,3-DAG and CLA isomers, in different CLA/*sn*-1,3-DAG molar ratios (0.5:1; 1:1; 2:1), were reacted with Lipozyme IM, 15% by weight of substrates. The used enzyme was previously dried: it was washed three times with fresh dry ethanol, filtered under vacuum, oven-dried at 50 °C and then stored in an anhydrous ambient [22]. The reaction was stirred (100 rpm) for 96 h, in an amber closed vial in a dark place, at 40 °C in solvent free conditions and in two different organic solvents, hexane and isooctane (1 ml/70 mg by weight of total substrates). The reactions carried out in solvent were monitored as follows: every 24 h an aliquot (20 µl of the total reaction volume) was withdrawn and stopped by filtering (0.2 µm nylon membrane filter, Corning Incorporated, Corning, Germany). The mixture was dried under a nitrogen stream. The residue was dissolved in chloroform/methanol (2:1, v/v); 10 µl were analyzed by HPLC-ELSD. The analyzes were carried out using a gradient pump, Models 305 and 307 (Gilson, Middletown, WI, USA), a Lichrosorb Si-60 column (5 µm, 250 × 4.0 mm i.d., Merck, Darmstadt, Germany) and an ELSD (Sedex 55, S.E.D.E.RE., France), operating at 40 °C and a nitrogen pressure of 240 kPa. The chromatograms were acquired and the data handled using the Class-VP software (Shimadzu, Kyoto, Japan). The samples were analyzed by gradient elution: the mixture hexane/isopropyl alcohol

(95:5, v/v) was maintained for the first 6 min at a flow rate of 0.7 ml/min, then it was changed to the mixture hexane/isopropyl alcohol (80:20, v/v) at a flow rate of 1.0 ml/min, that was held for 20 min; then the column was reconditioned with hexane/isopropyl alcohol (95:5, v/v) with a flow of 0.7 ml/min.

At the end of the reaction (96 h), the mixture was washed with hexane and filtered. The TAG synthesis products were subjected to thin layer chromatography (TLC) to isolate the TAG fraction from trace amounts of reaction reagents and by-products (MAG and DAG fractions), using silica gel plates (SIL G-25, 20 × 20 cm, 0.25 mm, Macherey-Nagel, Germany). A mixture of petroleum ether/diethyl ether/formic acid (70:30:1, v/v/v) was used as the developing solvent for the TLC. The TAG fraction (Rf ≅ 0.75) was scraped off and extracted from silica by a mixture of hexane/diethyl ether (50:50, v/v; 10 ml × 3); the organic extracts were pooled and the solvent was evaporated using a nitrogen stream. The TAG fraction was analyzed to obtain total and positional FA mol% compositions.

Analysis of Total FA Composition (% mol) of the Synthesized TAG

To evaluate the total FA composition of the synthesized TAG, 3 mg were dissolved in 1 ml of hexane and then 0.2 ml of 2 N methanolic KOH were added; after 3 min, water was added and the upper organic phase, containing the FA methyl esters (FAME) was dried over anhydrous Na_2SO_4 and then concentrated under nitrogen stream for HRGC analysis. The apparatus and the chromatographic conditions for HRGC analyzes of FAME were the same as reported in a previous work [19].

Structural Analysis of the Synthesized TAG: Chemical Procedure using α-MAG

To carry out the structural analysis, TAG were subjected to partial chemical hydrolysis with the Grignard reaction as reported by Turon et al. [23]. The obtained α-MAG were transesterified and the FAME analyzed by HRGC as described for total TAG.

The acidic composition (% mol) of the *sn*-2-position was calculated applying the following formula

$$A_2 = 3 \times A_t - 2 \times A_{1(3)}$$

where

A_2 % intrapositional composition of FA esterified in *sn*-2-position

A_t % total composition of FA esterified in all the three *sn*-positions of TAG

$A_{1(3)}$ % intrapositional composition of FA esterified in *sn*-1(3)-positions.

Statistical Analysis

Three samples for each reaction medium were analyzed and the results were reported as mean values ± standard deviations (SD).

Results and Discussion

The enzymatic esterification of the *sn*-2- position of *sn*-1,3-DAG with CLA isomers showed many advantages over chemical procedure [24]: mild reaction conditions, fewer side products, the reaction by filtration is easily stopped, easy recovery of the enzyme giving an opportunity for its reuse. Anhydrous Lipozyme IM was found to give the best yield of esterification with respect to not anhydrous Lipozyme IM and to the other screened lipases, both *sn*-1,3-specific and aspecific (data not shown) and, therefore, was selected for use in the subsequent studies.

All the reactions carried out in a solvent were monitored every 24 h for 96 h by using HPLC-ELSD; the Figs. 1 and 2 show the % composition of the acylglycerol species TAG, *sn*-1,3-DAG and *sn*-1,2-DAG for the reactions carried out in isooctane and in hexane, respectively, at three different CLA/*sn*-1,3-DAG ratios (0.5:1, 1:1, 2:1; A, B and C, respectively). The values were reported as % area, calculated by area normalization without considering the FA area. The MAG % composition was not represented because it was always <2.7%.

The best TAG% production after 24 h was obtained in isooctane (42%) with a 1:1 CLA/*sn*-1,3-DAG ratio, even if for all the reactions the highest TAG% was reached after 96 h, from 67.2% for the reaction carried out in hexane with a 0.5:1 ratio to 87.3% for the one carried out in isooctane with a 1:1 ratio. The *sn*-1,2-DAG % composition was always <12.1, for all the considered reactions; their presence in the reaction medium could be due to hydrolytic phenomena catalyzed by Lipozyme IM, both on *sn*-1,3-DAG and on synthesized TAG or could be due to intramolecular isomerization of *sn*-1,3-DAG, even if the *sn*-1,3-DAG are more stable than *sn*-1,2-DAG [25]. The reactions performed with a 0.5:1 CLA/*sn*-1,3-DAG ratio were mainly subjected to these phenomena.

No particular differences were observed by the comparison of the composition trends of the reactions carried out in the two different solvents; however, all the enzymatic syntheses, catalyzed by anhydrous Lipozyme IM, gave good results.

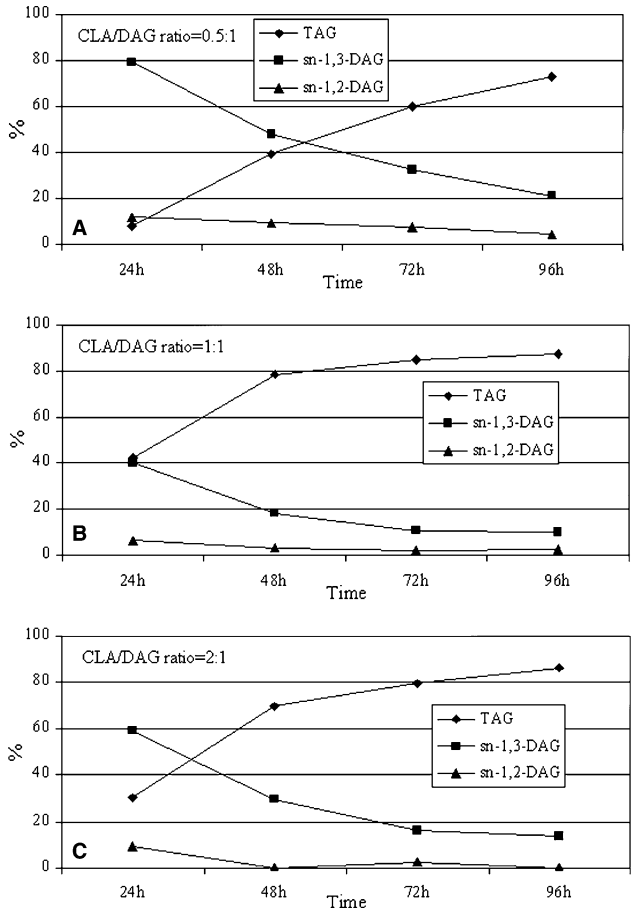

Fig. 1 Composition (% area) of enzymatic reactions in isooctane, catalyzed at 40 °C by anhydrous Lipozyme IM. CLA/*sn*-1,3-DAG ratio: 0.5:1, 1:1, 2:1; A, B and C, respectively

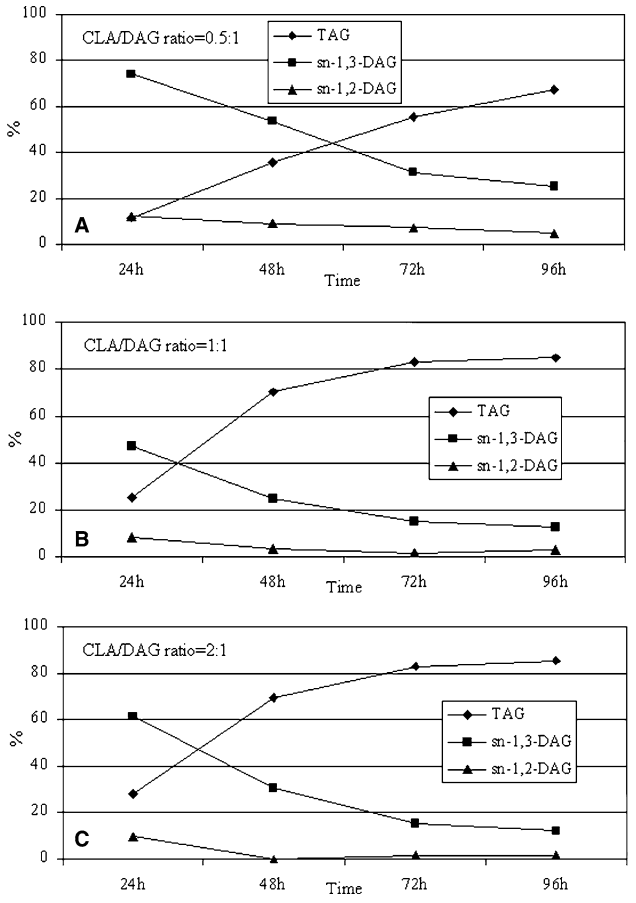

Fig. 2 Composition (% area) of enzymatic reactions in hexane, catalyzed at 40 °C by anhydrous Lipozyme IM. CLA/*sn*-1,3-DAG ratio: 0.5:1, 1:1, 2:1; A, B and C, respectively

After 96 h the reactions were stopped and the TAG obtained were analyzed to determine their total FA mol% composition and subjected to structural analysis in order to verify the FA regiodistribution in the glycerol backbone. Tables 1, 2, and 3 show the FA% compositions of structured TAG, α-MAG and *sn*-2-position, obtained after 96 h with three different CLA/*sn*-1,3-DAG ratios (0.5:1, 1:1, 2:1, respectively) and in three different media: (1) isooctane, (2) hexane, (3) solvent free.

The % incorporation of CLA isomers in *sn*-1,3-DAG increased by increasing the CLA/*sn*-1,3-DAG ratio; the lowest value was 19.2% in solvent free conditions with a 0.5:1 CLA/*sn*-1,3-DAG ratio, while the highest one was 47.5% in isooctane when the ratio was 2:1. The CLA isomer % contents in TAG obtained at the final reaction time were generally higher than those reported in the literature [15, 16]. Obviously the values higher than 33.3% could be indicative of the occurrence of acyl migrations. It should be noted that these percentages were mainly represented by *c*9,*t*11- and *t*10,*c*12-CLA isomers, reported as primarily responsible of the beneficial physiological effects of CLA family, while the *t*,*t*-CLA isomers, which could be formed from *c*9,*t*11- and *t*10,*c*12-isomers because of isomerization processes, were present in low percentages (about 2.5%); this value was, in any case, higher than that one obtained with the chemical procedure [20], perhaps because the enzymatic reaction temperature was higher.

The composition, mol%, of the other FA esterified in the structured TAG was similar to that one of EVO used for the enzymatic production of *sn*-1,3-DAG.

To confirm the hypothesis of the occurrence of acyl migrations and to verify the regiodistribution of CLA isomers in the synthesized TAG, a procedure based on chemical deacylation of TAG was used; this method utilized the α-MAG class, so that the FA mol% composition of the *sn*-2- position was indirectly obtained by calculation. The use of this structural procedure was suggested from the results of a previous work [24], which reported better results when compared with the enzymatic one (pancreatic lipase hydrolysis), which may suffer from acylic specificity.

Table 1 Structured TAG (0.5:1 CLA/*sn*-1,3-DAG ratio): total, α-MAG and *sn*-2- position FA compositions

FA	TAG[a]	TAG[b]	TAG[c]	α-MAG[a]	α-MAG[b]	α-MAG[c]	*sn*-2-[a]	*sn*-2-[b]	*sn*-2-[c]
C16:0	8.9 ± 0.1	9.4 ± 0.3	9.3 ± 0.3	8.9 ± 0.2	9.4 ± 0.2	10.6 ± 0.3	8.7 ± 0.6	9.5 ± 0.6	6.7 ± 0.4
C16:1n-9+n-7	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.8 ± 0.0	0.5 ± 0.0	1.3 ± 0.0	0.0 ± 0.0	0.8 ± 0.2	−0.9 ± 0.2
C18:0	1.6 ± 0.2	1.7 ± 0.1	1.7 ± 0.2	2.1 ± 0.2	1.7 ± 0.0	2.2 ± 0.1	0.5 ± 0.0	1.6 ± 0.1	0.8 ± 0.0
C18:1n-9+n-7	60.3 ± 4.1	63.5 ± 3.7	64.2 ± 3.8	65.8 ± 0.6	66.9 ± 0.1	65.2 ± 2.0	49.1 ± 0.4	56.7 ± 0.3	62.2 ± 0.4
C18:2n-6	4.0 ± 0.1	4.4 ± 0.4	4.2 ± 0.2	4.3 ± 0.2	4.2 ± 0.0	4.1 ± 0.2	3.3 ± 0.0	4.8 ± 0.0	4.3 ± 0.0
C18:3n-3	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.8 ± 0.1	1.1 ± 0.0	0.8 ± 0.0
C20:0	0.4 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.6 ± 0.0	0.8 ± 0.1	0.7 ± 0.0
*c*9,*t*11-CLA	11.3 ± 0.7	8.9 ± 0.5	8.2 ± 0.5	8.1 ± 0.2	7.6 ± 0.1	7.5 ± 0.3	17.7 ± 0.4	11.5 ± 0.2	9.5 ± 0.2
*t*10,*c*12-CLA	11.2 ± 0.4	8.6 ± 0.5	8.4 ± 0.4	8.0 ± 0.2	7.6 ± 0.1	7.6 ± 0.2	17.7 ± 0.0	10.5 ± 0.1	10.1 ± 0.1
t,*t*-CLA	0.7 ± 0.2	1.1 ± 0.1	1.4 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	1.5 ± 0.3	3.2 ± 0.5
Other CLA isomers	0.6 ± 0.1	0.7 ± 0.0	1.2 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.9 ± 0.1	1.2 ± 0.1	2.7 ± 0.3
Total CLA isomers	23.8	19.3	19.2	17.4	16.5	16.1	36.9	24.7	25.5

Mean values, mol% ± SD, $n = 3$

[a] Isooctane

[b] Hexane

[c] Solvent free

Table 2 Structured TAG (1:1 CLA/*sn*-1,3-DAG ratio): total, α-MAG and *sn*-2- position FA compositions

FA	TAG[a]	TAG[b]	TAG[c]	α-MAG[a]	α-MAG[b]	α-MAG[c]	*sn*-2-[a]	*sn*-2-[b]	*sn*-2-[c]
C16:0	7.9 ± 0.1	7.9 ± 0.3	9.1 ± 0.3	8.7 ± 0.2	10.6 ± 0.3	8.9 ± 0.3	6.1 ± 0.4	2.4 ± 0.1	9.4 ± 0.6
C16:1n-9+n-7	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1
C18:0	1.3 ± 0.2	1.2 ± 0.1	1.3 ± 0.1	1.6 ± 0.0	1.7 ± 0.0	1.7 ± 0.1	0.6 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
C18:1n-9+n-7	53.4 ± 3.6	51.7 ± 3.0	54.0 ± 3.2	57.4 ± 0.2	56.0 ± 0.1	57.4 ± 1.7	45.4 ± 0.3	43.1 ± 0.2	47.2 ± 0.3
C18:2n-6	3.6 ± 0.1	3.6 ± 0.3	3.8 ± 0.2	3.9 ± 0.0	4.3 ± 0.0	3.8 ± 0.2	3.0 ± 0.0	2.2 ± 0.0	3.7 ± 0.0
C18:3n-3	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.3 ± 0.0
C20:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	0.1 ± 0.0
*c*9,*t*11-CLA	16.0 ± 1.0	16.1 ± 1.0	14.7 ± 1.0	12.9 ± 0.2	13.4 ± 0.2	11.8 ± 0.4	22.2 ± 0.5	21.5 ± 0.4	20.3 ± 0.4
*t*10,*c*12-CLA	15.3 ± 0.5	16.2 ± 0.9	14.6 ± 0.7	12.6 ± 0.0	12.2 ± 0.1	11.3 ± 0.3	20.7 ± 0.0	24.0 ± 0.3	21.2 ± 0.2
t,*t*-CLA	0.8 ± 0.2	1.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.5 ± 0.0	2.9 ± 0.2	0.4 ± 0.1	2.3 ± 0.5	−3.1 ± 0.5
Other CLA isomers	0.7 ± 0.1	1.1 ± 0.0	0.5 ± 0.1	0.7 ± 0.1	0.3 ± 0.0	0.9 ± 0.0	0.7 ± 0.1	2.6 ± 0.3	−0.1 ± 0.0
Total CLA isomers	32.8	34.5	30.7	27.1	26.4	26.9	44.0	50.4	38.3

Mean values, mol% ± SD, $n = 3$

[a] Isooctane

[b] Hexane

[c] Solvent free

The structural analysis showed that CLA isomers were also esterified in *sn*-1(3)- positions, occurring because of interesterification reactions catalyzed by the enzyme in these positions and because of isomerization processes of acylglycerol species. The percentage of CLA isomers in the *sn*-1(3)- positions increased by increasing the CLA/*sn*-1,3-DAG ratio; the lowest value was 16.1% in solvent free conditions with a 0.5:1 CLA/*sn*-1,3-DAG ratio, while the highest one was 44.9% in hexane when the ratio was 2:1. The reactions carried out in isooctane showed the highest incorporation of CLA isomers in the *sn*-1(3)-positions, for each considered ratio.

The best incorporation of CLA isomers in the free *sn*-2-position of *sn*-1,3-DAG was obtained when the CLA/*sn*-1,3-DAG ratio was 2:1, in particular when the reactions were carried out both in isooctane and in hexane. When the ratio was 1:1, high values (54.4%) were reached in the presence of hexane.

It can be concluded that all the enzymatic syntheses catalyzed by anhydrous Lipozyme IM permitted to produce

Table 3 Structured TAG (2:1 CLA/*sn*-1,3-DAG ratio): total, α-MAG and *sn*-2- position FA compositions

FA	TAG[a]	TAG[b]	TAG[c]	α-MAG[a]	α-MAG[b]	α-MAG[c]	*sn*-2-[a]	*sn*-2-[b]	*sn*-2-[c]
C16:0	6.6 ± 0.1	6.6 ± 0.2	6.8 ± 0.2	7.0 ± 0.2	7.0 ± 0.2	7.0 ± 0.2	5.8 ± 0.4	5.7 ± 0.3	6.3 ± 0.4
C16:1n-9+n-7	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.1
C18:0	1.1 ± 0.2	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.1	0.8 ± 0.0
C18:1n-9+n-7	40.9 ± 2.8	41.1 ± 2.4	43.5 ± 2.6	42.8 ± 0.1	42.8 ± 0.1	43.8 ± 1.3	37.0 ± 0.3	37.8 ± 0.2	42.8 ± 0.3
C18:2n-6	3.0 ± 0.0	2.9 ± 0.3	3.0 ± 0.2	3.1 ± 0.0	3.0 ± 0.0	3.0 ± 0.1	2.7 ± 0.0	2.7 ± 0.0	3.1 ± 0.0
C18:3n-3	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
C20:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
*c*9,*t*11-CLA	22.8 ± 1.4	22.7 ± 1.4	20.9 ± 1.4	21.4 ± 0.3	21.2 ± 0.3	20.8 ± 0.8	25.6 ± 0.5	25.6 ± 0.5	21.0 ± 0.4
*t*10,*c*12-CLA	22.1 ± 0.7	22.2 ± 1.2	20.3 ± 0.9	21.2 ± 0.0	21.0 ± 0.2	20.4 ± 0.6	23.8 ± 0.0	24.7 ± 0.3	20.1 ± 0.2
t,*t*-CLA	1.6 ± 0.4	1.4 ± 0.1	2.5 ± 0.3	1.4 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	2.1 ± 0.3	0.8 ± 0.2	4.0 ± 0.7
Other CLA isomers	1.0 ± 0.2	1.1 ± 0.0	1.1 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	1.0 ± 0.1
Total CLA isomers	47.5	47.4	44.8	44.8	44.9	44.0	52.8	52.3	46.1

Mean values, mol% ± SD, $n = 3$

[a] Isooctane

[b] Hexane

[c] Solvent free

structured TAG with good levels of CLA isomers in the *sn*-2- position; in particular the reactions carried out with a 0.5:1 CLA/*sn*-1,3-DAG ratio gave the lowest degree of acyl migration. The results of the structural analysis, obtained with the described procedure, showed that the reactions carried out with a 2:1 CLA/*sn*-1,3-DAG ratio gave the best CLA incorporation in the*sn*-2- position in all the media that were considered, in particular in ones performed in organic solvents.

References

1. Pariza MW, Park Y, Cook ME (2001) The biologically active isomers of conjugated linoleic acid. Prog Lipid Res 40:283–298
2. Ochoa JJ, Farquharson AJ, Grant I, Moffat LE, Heys SD, Wahle KWJ (2004) Conjugated linoleic acids (CLAs) decrease prostate cancer cell proliferation: different molecular mechanism for *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers. Carcinogenesis 25: 1185–1191
3. Cook ME, Miller CC, Park Y, Pariza M (1993) Immune modulation by altered nutrient metabolism: nutritional control of immune induced growth depression. Poult Sci 72:1301–1305
4. Houseknecht KL, Vandenheuvel JP, Moya-Camarena SY, Portocarrero CP, Peck LW, Nickel KP, Belury MA (1998) Dietary conjugated linolenic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty *fa/fa* rat. Biochem Biophys Res Commun 244:678–682
5. Arbonés-Mainar JM, Navarro MA, Guzmán MA, Arnal C, Surra JC, Acín S, Carnicer R, Osada J, Roche HM (2006) Selective effect of conjugated linoleic acid isomers on atherosclerotic lesion development in apolipoprotein E knockout mice. Atherosclerosis 186:318–327
6. Lee KN, Kritchevsky D, Pariza MW (1994) Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 108: 19–25
7. Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca JA, Huth PJ (1997) Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery 22:266–277
8. Takahashi Y, Kushiro M, Shinohara K, Ide T (2002) Dietary conjugated linoleic acid reduces body fat mass and affects gene expression of proteins regulating energy metabolism in mice. Comp Biochem Physiol B 133:395–404
9. Salminem I, Mutanen M, Jauhiainem M, Aro A (1998) Dietary trans fatty acids increase conjugated linoleic acids levels in human serum. J Nutr Biochem 9:93–98
10. Saebo A (2003) Commercial synthesis of conjugated linoleic acid. In: Nelson GJ (ed) Advances in conjugated linoleic acid research, vol 1. AOCS Press, Champaign, pp 71–81
11. Hirose T, Yamauchi-Sato Y, Arai Y, Negishi S (2006) Synthesis of triacylglycerol containing conjugated linoleic acid by esterification using two blended lipase. J Am Oil Chem Soc 83:35–38
12. Kim IH, Yoon CS, Lee KW (2001) Transesterification of conjugated linoleic acid and tricaprylin by lipases in organic solvents. Food Res Int 34:301–306
13. Garcia HS, Arcos JA, Keough KJ, Hill CG (2001) Immobilized lipase-mediated acidolysis of butter oil with conjugated linoleic acid: batch reactor and packed bed reactor studies. J Mol Catal B: Enzym 11:623–632
14. McNeill GP, Rawlins C, Peilow AC (1999) Enzymatic enrichment of conjugated linoleic acid isomers and incorporation into triglycerides. J Am Oil Chem Soc 76:1265–1268
15. Kawashima A, Nagao T, Watanabe Y, Kobayashi T, Ikeda I, Tominaga Y, Shimada Y (2004) Preparation of regioisomers of structured TAG consisting of one mole of CLA and two moles of caprylic acid. J Am Oil Chem Soc 81:1013–1020
16. Villeneuve P, Barouh N, Barea B, Piombo G, Figueroa-Espinoza MC, Turon F, Pina M, Lago R (2007) Chemoenzymatic synthesis of structured triacylglycerols with conjugated linoleic acids (CLA) in central position. Food Chem 100:1443–1452
17. Xu X (2000) Enzymatic production of structured lipids: process reactions and acyl migration. Information 11:1121–1131
18. Brindley DN (1984) Digestion, absorption and transport of fats: general principles. In: Wiseman J (ed) Fats in animal nutrition. Butterworths, London, pp 85–103

19. Blasi F, Cossignani L, Simonetti MS, Damiani P (2007) Biocatalysed synthesis of sn-1, 3-diacylglycerol oil from extra virgin olive oil. Enzyme Microb Technol 41:727–732
20. Cossignani L, Simonetti MS, Damiani P (2005) Biocatalyzed acidolysis of olive oil triacylglycerols with 9c, 11t and 10t, 12c isomers of conjugated linoleic acid. Eur Food Res Technol 220:267–271
21. Christie WW (2003) Lipid analysis, 3rd edn. The Oily Press, Bridgwater
22. Piyatheerawong W, Iwasaki Y, Xu X, Yamane T (2004) Dependency of water concentration on ethanolysis of trioleoylglycerol by lipases. J Mol Catal B Enzym 28:19–24
23. Turon F, Bachain P, Caro Y, Pina M, Graille J (2002) A direct method for regiospecific analysis of TAG using α-MAG. Lipids 37:817–821
24. Blasi F, Cossignani L, Bosi A, Maurelli S, D'Arco G, Fiorini D, Simonetti MS, Damiani P (2008) Synthesis and structural analysis of structured triacylglycerols with CLA isomers in *sn*-2-position. J Am Oil Chem Soc 85:613–619
25. Laszlo JA, Compton DL, Vermillion KE (2008) Acyl migration kinetics of vegetable oil 1, 2-diacylglycerols. J Am Oil Chem Soc 85:307–312