J Am Oil Chem Soc (2008) 85:613–619
DOI 10.1007/s11746-008-1240-z

ORIGINAL PAPER

Synthesis and Structural Analysis of Structured Triacylglycerols with CLA Isomers in the *sn*-2- Position

Francesca Blasi · Lina Cossignani · Ancilla Bosi · Silvia Maurelli · Gilda D'Arco · Dennis Fiorini · Maria Stella Simonetti · Pietro Damiani

Received: 2 October 2007 / Revised: 10 April 2008 / Accepted: 14 April 2008 / Published online: 6 May 2008

Abstract The present research deals with the chemical esterification of the *sn*-2- position of *sn*-1,3-diacylglycerol (*sn*-1,3-DAG) with 9*cis*,11*trans* (*c*9,*t*11) and 10*trans*,12*cis* (*t*10,*c*12) conjugated linoleic acid (CLA) isomers to obtain structured triacylglycerols (TAG); the *sn*-1,3-DAG substrates were produced from extra virgin olive oil by means of enzymatic reactions while CLA isomers were obtained using a three-step procedure based on alkaline hydrolysis of sunflower oil, urea purification of linoleic acid (LA) and alkaline isomerization of LA. The results showed good levels of CLA incorporation in structured TAG at the tested temperatures: 37.5% at 4 °C and 39.1% at 14 °C. To evaluate the incorporation of CLA isomers in *sn*-2- position of *sn*-1,3-DAG structural analysis of the newly synthesized TAG was carried out using an enzymatic and a chemical method. The results of the structural analysis also showed up the occurrence of acyl migration. The pancreatic lipase method allowed the direct determination of the fatty acid composition of TAG *sn*-2- position but this enzymatic method showed different results ($p < 0.05$) in respect to the chemical one; this occurrence could be due to an acylic specificity of the lipase. High incorporation of CLA isomers in *sn*-2- position of TAG was observed, 77.0% at 4 °C and 81.5% at 14 °C, considering the results of the chemical procedure.

Keywords CLA · *sn*-1,3-DAG · Structured TAG · Structural analysis

F. Blasi · L. Cossignani · A. Bosi · S. Maurelli · G. D'Arco · 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

D. Fiorini
Dipartimento di Scienze Chimiche, Università di Camerino, Via S.Agostino 1, 62032 Camerino, Italy

Introduction

The interest in structured triacylglycerols (TAG) containing specific fatty acids (FA), as conjugated linoleic acid (CLA) isomers, in definite *sn*-positions of the glycerol backbone, has recently increased. Different synthetic methods, both enzymatic and chemical, have been developed in order to produce particular TAG molecular species. It is well known that absorption and metabolism of FA depend on the position esterified on the TAG backbone and in particular it is well documented that the FA located at the *sn*-2- position are physiologically very important, because the pancreatic lipase, regiospecific for the external positions of TAG, hydrolyzes the *sn*-1- and *sn*-3- positions to give *sn*-2-monoacylglycerols (MAG), which are efficiently absorbed [1–3].

Conjugated linoleic acid represents a family of many isomers of linoleic acid (LA), with different position and *cis*(*c*)/*trans*(*t*) configuration of their conjugated double bonds. Many studies on CLA have shown beneficial effects on human and animal health [4], in fact anticarcinogenesis [5], immunomodulation [6], body composition modification [7] and antiatherosclerosis [8] effects have been observed. Anyway these effects are principally attributed to the *c*9,*t*11 and *t*10,*c*12 CLA isomers.

Much work has been done to perform TAG structural analysis, using both enzymatic and chemical procedures. The determination of the regiospecific distribution of FA in

TAG backbone is generally based on a deacylation technique which produces "representative" partial acylglycerols. Usually the chemical methods (Grignard deacylation of TAG), in comparison with enzymatic procedures, show no significant selectivity with respect to chain length, unsaturation of acyl groups and double bond configuration, so a representative pool of partial acylglycerol is formed [9]. However the principal difficulties are due to the occurrence of acyl migration of partial acylglycerols, considering both diacylglycerol (DAG) and MAG classes [10, 11]; undesirable acyl migrations may occur during these reactions and a contamination caused from isomerization can be observed [12]. In any case, some procedures for TAG structural analysis have been successfully developed, procedures based on the use of all isomeric DAG classes [13] and of *sn*-1(3)-MAG or *sn*-2-MAG [14, 15].

The present research was undertaken in order to obtain TAG containing a high percentage of bioactive CLA isomers in the *sn*-2- position, structured lipids with nutritional advantages due to the real bioavailability of the considered isomers. The CLA isomers, produced starting from sunflower oil as reported in a previous work [16], were esterified into the free *sn*-2- position of the *sn*-1,3-DAG [17] using a chemical method. Another objective of the research was the structural analysis of the synthesized TAG, in particular the determination of FA% composition of the *sn*-2- position.

To this end, an indirect chemical procedure (procedure A), based on the analysis of *sn*-1(3)-MAG classes obtained from TAG deacylation, was carried out; moreover an enzymatic method (procedure B), which allowed to obtain directly the FA composition of TAG *sn*-2- position from the *sn*-2-MAG produced from TAG hydrolysis with pancreatic lipase, was used. The comparison between the results of FA% composition of TAG *sn*-2- position obtained using the procedures A and B allowed us to evaluate eventual enzymatic specificity toward particular FA, in particular the CLA isomers.

Materials and Methods

Materials

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

N,N′-dicyclohexylcarbodiimide (DCCD) and 4-dimethylaminopyridine (DMAP), ≥99.0%, were from Fluka (Chemika, Buchs, Switzerland).

Solvents and reagents used were of analytical grade, purchased either from Sigma-Aldrich or Fluka; the solvents for chromatography were high-performance liquid chromatographic (HPLC) grade, purchased 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 [17]. Briefly, the procedure was the following: extra virgin olive oil was treated with ethanol 96° and Novozym 435, overnight at 45 °C, under magnetic stirring. The products of ethanolysis reaction were reacted for 48 h with anhydrous Lipozyme IM at 25 °C, under magnetic stirring in an amber opened vial, in a solvent-free system under vacuum. The work up of the reaction and the isolation of the *sn*-1,3-DAG were carried out as reported in the cited work; the *sn*-1,3-DAG yield was 60% and this fraction resulted isomerically pure. An aliquot (5 mg) was transesterified and the fatty acid methyl esters (FAME) analyzed by high-resolution gas chromatography (HRGC); the other part was used to synthesize structured TAG. The typical FA% composition of the *sn*-1,3-DAG was: palmitic acid (C16:0; 13.0%), palmitoleic acid (C16:1n−9 + n−7; 0.8%), stearic acid (C18:0; 1.8%), oleic acid (C18:1n−9 + n−7isomer; 78.1%), linoleic acid (C18:2n−6; 5.2%), linolenic acid (C18:3n−3; 0.6%) 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 procedure reported in a previous work [16]. Briefly, sunflower oil was reacted for 1 h at 70 °C with potassium hydroxide, water, ethanol 95% and butylated hydroxytoluene. The isolated FA were subjected to urea purification using FA, urea and methanol in the following ratio, 1.0 g:1.5 g:4.5 ml; the mixture was maintained at 5 °C overnight and then subjected to filtration under vacuum. This step was carried out two times. The purified LA was subjected to the isomerization reaction to CLA isomers with 1-butanol and potassium hydroxide, heating to reflux for 12 h to 140 °C.

The FA% composition of the products of the three steps of the preparation of CLA isomers was determined after methylation [18] and HRGC analysis; in Fig. 1a–c the HRGC profiles of alkaline hydrolysis of sunflower oil, LA purification with urea and alkaline isomerization of fraction enriched in LA are shown. CLA isomers were identified in comparison with a CLA standard mixture. The FA% composition of the CLA mixture was: C18:1n−9 + n−7

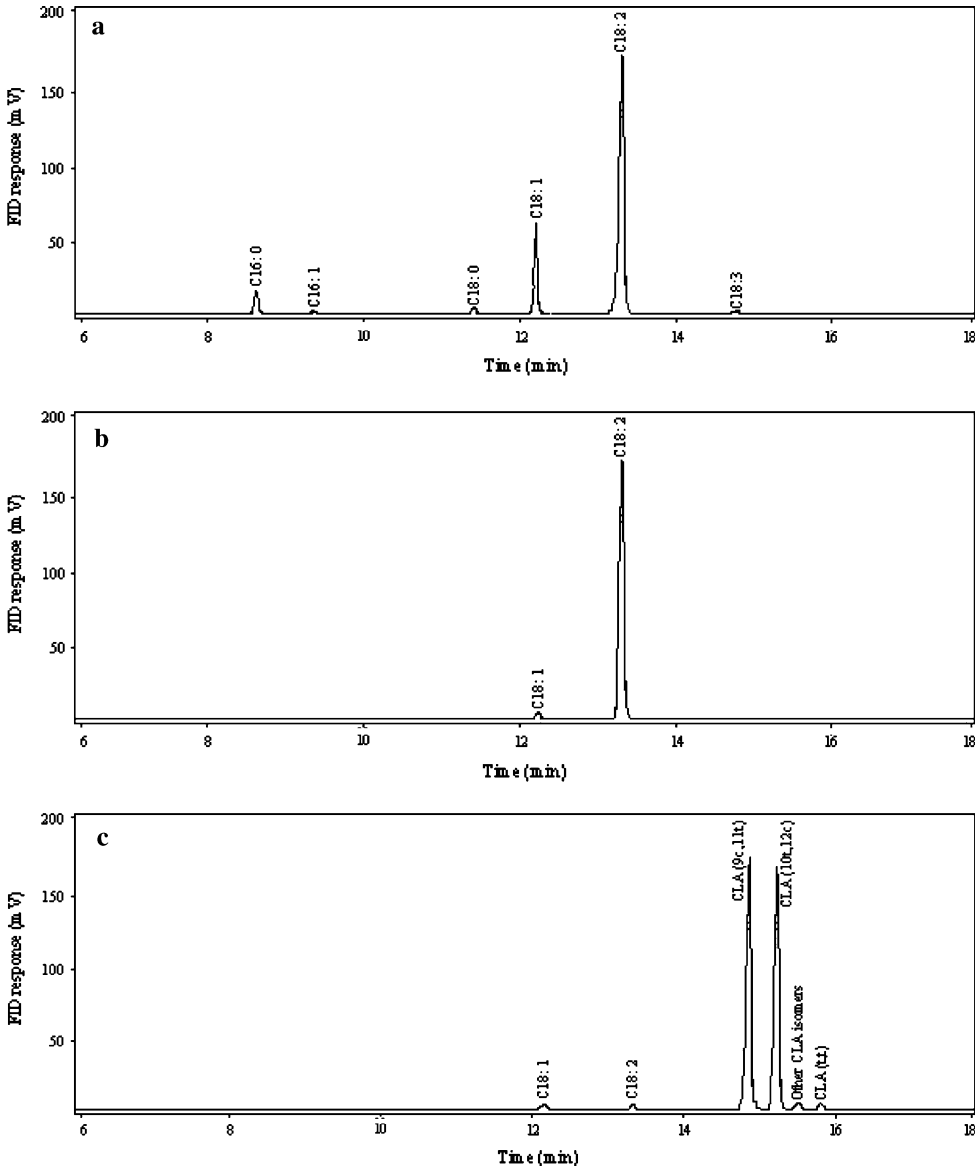

Fig. 1 High-resolution gas chromatography profiles of alkaline hydrolysis of sunflower oil (**a**), LA purification with urea (**b**) and alkaline isomerization of fraction enriched in LA (**c**)

(1.5%), C18:2n−6 (0.2%) and total CLA (98.3%) with the following isomers distribution: *c*9,*t*11 (47.8% isomer/total CLA), *t*10,*c*11 (47.3% isomer/total CLA), *t*,*t* isomers (2.6% isomer/total CLA) and other CLA isomers (2.2% isomer/total CLA).

Production and Isolation of TAG

A solution of *sn*-1,3-DAG (0.5 mmol), CLA isomers (0.75 mmol), DCCD (2.0 mmol) and DMAP (2.0 mmol) in 10 ml of dichloromethane was stirred for 24 h, in an amber closed vial in a dark place, at two different temperatures, 4 and 14 °C. Finally, the mixture was washed two times with water; the aqueous phase was collected and washed with hexane. The organic phase was added to the dichloromethane solution, then the solvents were dried over anhydrous Na_2SO_4, filtered (0.2 μm nylon membrane filter; Corning Incorporated, Corning, Germany) and evaporated by a nitrogen stream. The reaction products were dissolved in 1 ml of the mixture chloroform/methanol (2:1, v/v).

An aliquot (20 μl) was filtered and analyzed by an HPLC-evaporative light scattering detector (ELSD) to monitor the reaction. The HPLC-ELSD analyses were carried out using a gradient pump, Models 305 and 307 (Gilson, Middletown, WI, USA), a Lichrosorb Si-60 column (5 μm, 250 mm × 4.0 mm i.d.; Merck, Darmstadt, Germany) and an ELSD (Sedex 55, S.E.D.E.RE., France), operating at 40 °C and 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 flow rate of 0.7 ml/min, then it changed to the mixture hexane/isopropyl alcohol (80:20, v/v) at 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), flow 0.7 ml/min. At the end of the reaction, the HPLC profile showed the TAG fraction (90%) and trace amounts of DAG and MAG fractions.

The remaining part of the TAG synthesis products was subjected to 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) was used as developing solvent for the thin layer chromatography (TLC). The TAG fraction (Rf ≅ 0.75) was scraped off and extracted from silica by the mixture hexane/diethyl ether (50:50, v/v; 10 ml × 3); the organic extracts were pooled and the solvent was evaporated by nitrogen stream. The obtained TAG fraction was weighted (80% yield) and analyzed to obtain total and positional FA% compositions.

Structural Analysis of the Synthesized TAG

Chemical procedure using α-MAG (procedure A): The TAG fraction was subjected to partial chemical hydrolysis with Grignard reaction as reported by Turon et al. [14]. The *sn*-1(3)-MAG obtained were transesterified and the FAME analyzed by HRGC.

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

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

where A_2 is % intrapositional composition of FA esterified in *sn*-2- position, A_t is % total composition of FA esterified in all the three *sn*- positions of TAG, $A_{1(3)}$ is % intrapositional composition of FA esterified in *sn*-1(3)- positions.

Enzymatic procedure (procedure B): The TAG fraction was subjected to hydrolysis with hog pancreas lipase as described by the NGD method [15]. The *sn*-2-MAG obtained were transesterified and the FAME analyzed by HRGC.

Preparation of FAME: TAG, *sn*-2-MAG and *sn*-1(3)-MAG were transesterified as follows: about 3 mg was 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 was dried over anhydrous Na_2SO_4 and then concentrated under a nitrogen stream for HRGC analysis.

HRGC analysis: A DANI 1000DPC gas-chromatograph (Norwalk, CT, USA), equipped with a split–splitless injector and with a flame-ionization detector was used. The fused silica WCOT capillary column CP-Select CB for FAME (50 m × 0.25 mm i.d., 0.25 μm f.t.; Varian, Superchrom, Milan, Italy) was used. The chromatograms were acquired and processed using Clarity integration software. The chromatographic conditions were the following: the injector and detector temperature was 250 °C; the oven temperature was 130 °C, then increased to 250 °C at 3 °C/min; the final temperature was held for 10 min. The carrier gas (He) flow rate was 1 ml/min and the split ratio was 1:70.

Statistical Analysis

Four samples for each temperature were analyzed and the results were reported as mean values ± standard deviations (SD).

Student's *t*-test, calculated using Excel 1997 (Microsoft Corporation, Redmond, WA, USA), was used to evaluate the differences between FA compositions of TAG (total and positional) of the different samples (type: 3, heteroscedastic; tails: 2).

Results and Discussion

The chemical approach for the esterification of *sn*-2-position of *sn*-1,3-DAG with CLA isomers showed many advantages, as simplicity, quickness and low cost; moreover the reaction, carried out overnight using DCCD as coupling-condensation agent and DMAP as catalyst, gave almost complete conversion, both at 4 and at 14 °C. In Fig. 2a, b the HRGC profiles of *sn*-1,3-DAG, starting compounds for the synthesis, and of TAG synthetized at 4 °C, are showed; the profile was the same if the reaction temperature was 14 °C. The FA% compositions of the TAG, synthetized at 4 and 14 °C, are reported in Table 1; the percentages of total CLA isomers were 37.5 and 39.1%, respectively, at 4 and 14 °C. It can be noted that these percentages were mainly represented from *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 very low percentages (≤0.7%). Little but significant differences ($p < 0.05$) were observed between the percent contents, both for *c*9,*t*11- and *t*10,*c*12- CLA isomer, in TAG synthesized at 4 and 14 °C. The others FA esterified in the structured TAG were typical of extra virgin olive oil used for the enzymatic production of *sn*-1,3-DAG.

Considering that the theoretical maximum incorporation of CLA isomers would be 33.3%, corresponding to a 100% esterification yield of the internal position of TAG, the differences observed between the values obtained and the theoretical ones could be explained by the occurrence of

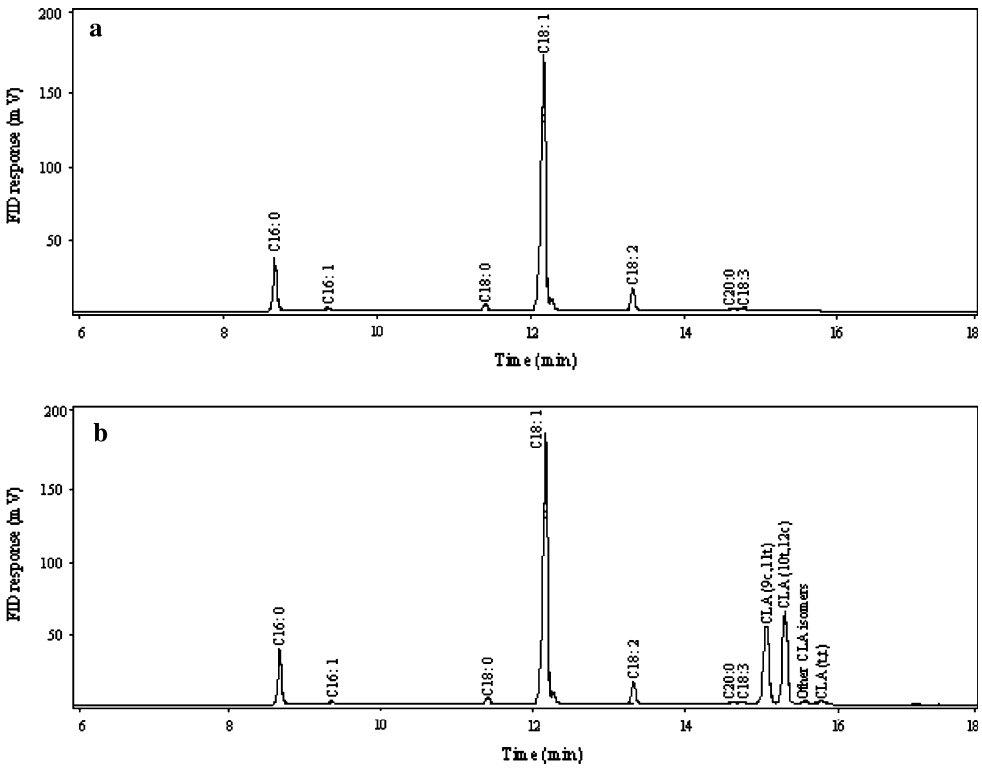

Fig. 2 High-resolution gas chromatography profiles of FAME from *sn*-1,3-DAG (**a**) and synthesized TAG (**b**)

Table 1 Fatty acid composition (mean values mol% ± SD, $n = 4$) of TAG synthesized at 4 and 14 °C

FA	TAG 4 °C	TAG 14 °C
C16:0	7.5 ± 0.1[a]	6.8 ± 0.2[b]
C16:1n−9 + n−7	0.6 ± 0.1[a]	0.5 ± 0.1[a]
C18:0	1.2 ± 0.1[a]	1.2 ± 0.1[a]
C18:1n−9 + n−7	48.8 ± 0.2[a]	48.5 ± 0.5[a]
C18:2n−6	3.8 ± 0.3[a]	3.4 ± 0.2[b]
C18:3n−3	0.3 ± 0.1[a]	0.4 ± 0.1[a]
C20:0	0.1 ± 0.1[a]	0.2 ± 0.1[b]
*c*9,*t*11-CLA	18.2 ± 0.2[a]	18.8 ± 0.2[b]
*t*10,*c*12-CLA	18.1 ± 0.3[a]	18.9 ± 0.2[b]
t,*t*-CLA	0.6 ± 0.1[a]	0.7 ± 0.1[a]
Other CLA isomers	0.6 ± 0.1[a]	0.7 ± 0.1[a]
Total CLA isomers	37.5	39.1

Data with the same superscript letters within rows are not significantly ($p < 0.05$) different

acyl migrations. To confirm this hypothesis and to verify the regiodistribution of CLA isomers in the synthesized TAG, two different procedures were used, one involving the chemical deacylation of TAG, the second based on TAG enzymatic hydrolysis by pancreatic lipase. Using the *sn*-1(3)-MAG method (procedure A) the FA% composition of the *sn*-2- position was indirectly obtained by calculation, while in the case of the enzymatic method (procedure B) the same data were directly obtained from the analysis of *sn*-2-MAG.

In Table 2, the FA compositions of the *sn*-1(3)- positions, directly obtained from analysis of *sn*-1(3)-MAG, have been reported; it can be noted that CLA isomers were also esterified in *sn*-1(3)- positions, occurrence due to isomerization processes of acylglycerol species, as previously indicated. As indicated in Table 2, no significant differences ($p > 0.05$) were observed in *sn*-1(3)-MAG obtained from TAG synthesized at 4 and 14 °C, for both the CLA isomers.

The comparison between the acidic % compositions of the *sn*-2- position obtained with the two different procedures has been reported in Tables 3 and 4, respectively, for TAG synthesized at 4 and 14 °C. It can be noted that a good incorporation of CLA was obtained at the tested temperatures, in fact the two CLA isomers *c*9,*t*11- and *t*10,*c*12- were the most abundant FA esterified in TAG central position; the percentages of the *t*,*t*-isomers of CLA were low both at 4 and 14 °C (not higher than 1.6%). As already observed, a process of acyl migration of FA initially esterified in *sn*-1- and *sn*-3- positions occurred; in particular oleic acid, the most abundant FA in *sn*-1,3-DAG, was obviously the most involved FA and it was detected in TAG *sn*-2- position in relevant percentages. The comparison between the percent compositions of *c*9,*t*11- and *t*10,*c*12- CLA isomers obtained from the chemical procedure (procedure A) and the enzymatic one (procedure B) showed little but significant differences ($p < 0.05$) both at 4 °C and at 14 °C. This occurrence could be due to a selectivity of lipase for chain length or unsaturation degree

Table 2 Fatty acid composition (mean values mol% ± SD, $n = 4$) of *sn*-1(3)-MAG, obtained from TAG synthesized at 4 and 14 °C

FA	*sn*-1(3)-MAG 4 °C	*sn*-1(3)-MAG 14 °C
C16:0	9.5 ± 0.7[a]	9.4 ± 1.6[a]
C16:1n−9 + n−7	0.5 ± 0.1[a]	0.5 ± 0.1[a]
C18:0	2.0 ± 0.2[a]	2.0 ± 0.5[a]
C18:1n−9 + n−7	63.7 ± 1.7[a]	64.0 ± 1.3[a]
C18:2n−6	5.2 ± 1.2[a]	5.9 ± 2.0[b]
C18:3n−3	0.2 ± 0.1[a]	0.5 ± 0.2[b]
C20:0	0.9 ± 0.6[a]	0.2 ± 0.1[b]
*c*9,*t*11-CLA	8.5 ± 0.1[a]	8.2 ± 1.2[a]
*t*10,*c*12-CLA	8.6 ± 0.4[a]	8.4 ± 1.2[a]
t,*t*-CLA	0.5 ± 0.1[a]	0.2 ± 0.2[a]
Other CLA isomers	0.3 ± 0.1[a]	0.6 ± 0.2[a]
Total CLA isomers	17.9	17.4

Data with the same superscript letters within rows are not significantly ($p < 0.05$) different

Table 3 Fatty acid composition (mean values mol% ± SD, $n = 4$) of the *sn*-2- position of TAG synthesized at 4 °C, obtained with the chemical (A) and enzymatic (B) method

FA	*sn*-2-A 4 °C	*sn*-2-B
C16:0	3.4 ± 1.1[a]	3.2 ± 0.2[a]
C16:1n−9 + n−7	0.8 ± 0.4[a]	0.2 ± 0.1[b]
C18:0	−0.3 ± 0.1[a]	0.6 ± 0.1[b]
C18:1n−9 + n−7	19.0 ± 4.2[a]	24.3 ± 0.6[b]
C18:2n−6	1.0 ± 1.4[a]	1.8 ± 0.1[a]
C18:3n−3	0.5 ± 0.1[a]	0.1 ± 0.1[b]
C20:0	−1.3 ± 1.4[c]	0.1 ± 0.1[d]
*c*9,*t*11-CLA	37.8 ± 0.5[a]	32.6 ± 0.2[b]
*t*10,*c*12-CLA	37.1 ± 1.8[a]	32.9 ± 0.1[b]
t,*t*-CLA	0.8 ± 0.2[a]	1.3 ± 0.1[b]
Other CLA isomers	1.3 ± 0.4[a]	1.2 ± 0.2[a]
Total CLA isomers	77.0	68.0

Data with the same superscript letters within rows are not significantly ($p < 0.05$) different

of the FA, in particular of CLA isomers. Considering the differences between the *sn*-2- intrapositional CLA percentages of TAG obtained at 4 and 14 °C, it should be noted that slightly higher incorporation was obtained at 14 °C with respect to 4 °C, in particular when the data of the procedure A are compared ($p < 0.05$ for both *c*9,*t*11- and *t*10,*c*12- CLA isomers). In the same Table 3 it can be observed that using the chemical procedure 77.0% of total CLA isomers was obtained in TAG *sn*-2- position while this value was 68.0% if the enzymatic procedure was used, for TAG synthesized at 4 °C; slightly higher values of CLA incorporation in the *sn*-2- position were obtained at

Table 4 Fatty acid composition (mean values mol% ± SD, $n = 4$) of the *sn*-2- position of TAG synthesized at 14 °C, obtained with the chemical (A) and enzymatic (B) method

FA	14 °C	
	sn-2-A	*sn*-2-B
C16:0	2.0 ± 0.6[a]	3.4 ± 0.0[a]
C16:1n−9 + n−7	0.4 ± 0.2[a]	0.2 ± 0.0[a]
C18:0	−0.6 ± 0.5[a]	0.6 ± 0.0[b]
C18:1n−9 + n−7	18.5 ± 1.6[a]	25.7 ± 0.1[a]
C18:2n−6	−2.1 ± 0.7[a]	1.8 ± 0.0[a]
C18:3n−3	0.0 ± 0.3[a]	0.2 ± 0.0[a]
C20:0	0.2 ± 0.1[a]	0.1 ± 0.0[a]
*c*9,*t*11-CLA	39.6 ± 2.0[a]	33.1 ± 0.4[b]
*t*10,*c*12-CLA	39.4 ± 2.1[a]	33.8 ± 0.3[b]
t,*t*-CLA	1.6 ± 0.4[a]	1.3 ± 0.1[a]
Other CLA isomers	0.9 ± 0.3[a]	1.4 ± 0.0[b]
Total CLA isomers	81.5	69.6

Data with the same superscript letters within rows are not significantly ($p < 0.05$) different

14 °C, for both chemical (81.5%) and enzymatic procedures (69.6%).

It can be concluded that the chemical synthesis, carried out under the described experimental conditions, permits us to produce structured TAG with good levels of CLA isomers in the *sn*-2- position. The results of the structural analysis obtained with the described procedures showed little differences in the *sn*-2- intrapositional composition of TAG containing CLA isomers; this could suggest that the chemical procedure gives better results when compared with the enzymatic one, that may suffer from acylic specificity.

References

1. Brindley DN (1984) Digestion, absorption and transport of fats: general principles. In: Wiseman J (ed) Fats in animal nutrition. Butterworths, London, pp 85–103
2. Christensen MS, Hoy CE, Becker CC, Redgrave TJ (1995) Intestinal absorption and lymphatic transport of eicosapentaenoic (EPA), docosahexaenoic (DHA) and decanoic acids: dependence on intramolecular triacylglycerol structure. Am J Clin Nutr 65:56–61
3. Sadou H, Leger CL, Descomps B, Barjon JN, Monnier L, Crastes de Paulet A (1995) Differential incorporation of fish oil eicosapentaenoate and docosahexaenoate into lipids of lipoprotein fractions as related to their glycerol esterification: a short-term (postprandial) and long-term study in healthy humans. Am J Clin Nutr 62:1193–2000
4. Whigham LD, Cook ME, Atkinson RL (2000) Conjugated linoleic acid: implications for human health. Pharm Res 42:503–510
5. 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
6. Luongo D, Bergamo P, Rossi M (2003) Effects of conjugated linoleic acid on growth and cytokine expression in Jurkat T cells. Immunol Lett 90:195–201
7. Hargrave KM, Azain MJ, Miner JL (2005) Dietary coconut oil increases conjugated linoleic acid-induced body fat loss in mice independent of essential fatty acid deficiency. Biochim Biophys Acta 1737:52–60
8. 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
9. Becker CC, Rosenquist A, Holmer G (1993) Regiospecific analysis of triacylglycerol using allyl magnesium bromide. Lipids 28:147–149
10. Brockerhoff H (1971) Stereospecific analysis of triglycerides. Lipids 6:942–956
11. Serdarevich B (1967) Glyceride isomerization in lipid chemistry. J Am Oil Chem Soc 44:381–393
12. Turon F, Bonnot F, Caro Y, Pina M, Graille J (2003) Acyl migration incidence on accuracy of a triacylglycerol regioanalysis—a theoretical evaluation. Chem Phys Lipids 125:41–48
13. Christie WW, Nikolova-Damyanova B, Laakso P, Herslof B (1991) Stereospecific analysis of triacyl-*sn*-glycerols *via* resolution of diastereomeric diacylglycerol derivatives by high performance liquid chromatography on silica. J Am Oil Chem Soc 68:695–701
14. 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
15. NGD, Norme Italiane per il Controllo dei Grassi e Derivati (1989) Official Method C46, Stazione sperimentale per le industrie degli olii e dei grassi, Milano, Italy, pp 1–3
16. Cossignani L, Simonetti MS, Damiani P (2005) Biocatalyzed acidolysis of olive oil triacylglycerols with 9*c*,11*t* and 10*t*,12*c* isomers of conjugated linoleic acid. Eur Food Res Technol 220:267–271
17. 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
18. Christie WW (2003) Lipid analysis. 3rd edn. The Oily Press, Bridgwater