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ORIGINAL PAPER

Study of Some Experimental Parameters in the Synthesis of Triacylglycerols with CLA Isomers and Structural Analysis

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Abstract The present research deals with the synthesis of structured triacylglycerols (TAG) by enzymatic treatment of *sn*-1,3-diacylglycerol (*sn*-1,3-DAG) with conjugated linoleic acid (CLA) isomers using the immobilized lipase from *Rhizomucor miehei* (Lipozyme® IM) under different experimental conditions. In particular, the influence of reaction parameters, such as temperature, enzymatic load, reaction time and DAG/CLA ratio has been evaluated using an experimental design software with a screening objective. Two responses have been selected, they are the percentage of CLA isomers in total TAG and in the *sn*-2-position and a three-level-4-factor fractional factorial experimental design was used to screen the variables. The results showed that the selected experimental variables have an influence on the enzymatic reaction, in particular, the DAG/CLA substrate ratio and the temperature, both of which inversely correlated with CLA incorporation, but also the enzymatic load and the reaction time, both directly correlated with CLA incorporation. The best results for CLA isomer % content both in total TAG (46.3%) and in the *sn*-2- position (52.2%) were obtained at 40 °C for 96 h, with 20% enzymatic load and a 0.5 reactive ratio.

Keywords CLA · *sn*-1,3-DAG · Structured TAG · Experimental design

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Introduction

Enzymatic modification of fats and oils for the production of specific structured lipids has been of great interest for a long time [1, 2]. Structured lipids are tailor-made fats and oils with improved nutritional or physical properties because of modifications to incorporate fatty acids (FA) or to change the position of existing FA on the glycerol backbone. Polyunsaturated FA, among which those of the n-3 series, have been added to natural lipids with the objective of improving their nutritional value [3–5]. Also conjugated linoleic acid (CLA) isomers have been used for designing structured lipids in order to obtain "added value" lipids [6–10], in fact, some CLA isomers have been shown to exhibit beneficial effects on animals, such as antiadipogenic, anticarcinogenic, antiatherogenic, antidiabetogenic and antiinflammatory properties [11–15]. Few papers have tested the effects of CLA isomers on humans and long-term randomized clinical trials, controlled with placebo, need to be made using large samples of patients to evaluate the efficacy and safety of CLA isomers [16].

The enzymatic modification of lipids, if compared with chemical catalysis, is very attractive since the reaction conditions are mild and a control of the FA distribution is possible, even if the occurrence of acyl migration of intermediate partial acylglycerols [17] could compromise the isomeric purity of the products.

Numerous experimental parameters, among which, temperature, enzymatic load, time, reaction medium, water activity [18] affect the enzymatic reactions carried out in order to obtain structured lipids, with the objective of improving the nutritional or functional characteristics of natural lipids. Statistical design strategy is useful for evaluating the influence of reaction parameters on structured lipids production and for optimizing the conditions of

the synthetic reactions. The application of experimental design in food technology has been successfully demonstrated through many publications [19–21].

The importance of the different reaction variables can be determined statistically by conducting a screening experiment. Screening designs are typically used in the early stages of experimental work when many factors need to be investigated, and it is likely that some of these factors have little or no effect on the responses measured. Factors that are identified as important by screening experiment design can be investigated more thoroughly in subsequent optimization experiments.

This research has been carried out with the objective of evaluating the influence of some reaction parameters, such as time, enzymatic load, temperature and DAG/CLA ratio, in the enzymatic synthesis of structured TAG containing CLA isomers. The substrates were represented by *sn*-1,3-DAG, obtained from extra virgin olive oil and CLA, synthesized starting from sunflower oil. The immobilized lipase from *Rhizomucor miehei*, Lipozyme® IM, has been used because it was found to give the best yield of esterification with respect to other screened lipases, both *sn*-1,3-specific and aspecific [22]. The regiospecific analysis of the structured TAG has been obtained using the chemical deacylation of TAG, the analysis of the obtained *sn*-1(3)-monoacylglycerols (MAG) and the calculation of the FA% composition of TAG *sn*-2- position.

The influence of the reaction parameters has been evaluated using an experimental design software, purchased from Umetrics AB, MODDE 5.0™.

Materials and Methods

Materials

Linoleic acid conjugated methyl ester (*cis*- and *trans*-9,11- and -10,12-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 purchased from Fluka (Buchs, Switzerland).

Solvents and reagents used were of analytical grade, purchased either from Sigma-Aldrich or Fluka.

Modde 5.0™ was purchased by Umetrics AB (Umeå, Sweden).

Methods

Production and Isolation of sn-1,3-DAG

The products of the enzymatic ethanolysis reaction of olive oil were treated for 48 h with anhydrous Lipozyme® IM at 25 °C under magnetic stirring in an amber-colored opened vial, in a solvent-free system under vacuum [23]. The *sn*-1,3-DAG were isolated by TLC, then an aliquot (5 mg) was derivatized and analyzed by high resolution gas chromatography (HRGC).

The FA composition (mol%) of the *sn*-1,3-DAG, as mean values of two determinations, was: palmitic acid (C16:0; 12.7%), palmitoleic acid (C16:1 n-7 + n-9 isomer; 0.8%), stearic acid (C18:0; 1.8%), oleic acid (C18:1 n-9 + n-7 isomer; 77.9%), linoleic acid (C18:2 n-6; 5.6%), linolenic acid (C18:3 n-3; 0.7%) and arachidic acid (C20:0; 0.4%).

Preparation of CLA Isomers

CLA isomer production was carried out starting from sunflower oil. The oil was subjected to alkaline hydrolysis in order to obtain FA; then the linoleic acid purification with urea and the alkaline isomerization of linoleic acid to CLA isomers were carried out following the procedure reported in a previous work [24]; in this research, the linoleic acid purification was carried out three times instead of two. The CLA isomer mixture was stored at −20 °C.

The FA composition (mol%) of the obtained CLA mixture was: C18:1 n-9 + n-7 (1.2%), C18:2 n-6 (0.3%), and total CLA (98.5%) with the following isomeric distribution: 9*c*,11*t* (48.1% isomer/total CLA), 10*t*,12*c* (47.9% isomer/total CLA), *t*,*t* (2.1% isomer/total CLA) and other CLA isomers (1.9% isomer/total CLA).

Experimental Design

The influence of the reaction parameters on the synthesis of TAG containing CLA isomers has been studied by using the Statistical Design Package MODDE 5.0™. Four factors, temperature, time, DAG/CLA ratio, enzymatic load were considered as variables in the following ranges:

- Temperature, 40–80 °C
- Enzymatic load, 4–20% by weight of substrates
- Molar ratio, DAG/CLA 0.5–2.0
- Reaction time, 24–96 h.

Two responses, % incorporation of CLA in total TAG and in the *sn*-2- position, were selected.

A three-level, 4-factor fractional factorial IV resolution design with a total of 11 experiments, including three replicated center points, was employed. The reactions were conducted in random order and in the experimental conditions indicated in the worksheet (Table 1).

The model was then fitted using multiple linear regression (MRL) analysis.

Table 1 Worksheet and responses for fractional factorial IV resolution design

Exp. no.	Run order	Factors				Responses	
		T^a (°C)	E.l.[b] (%)	R.r.[c]	Time (h)	Total CLA (% mol)	*sn*-2-CLA (% mol)
1	3	40	4	0.5	24	27.7	26.0
2	10	80	4	0.5	96	9.9	7.6
3	6	40	20	0.5	96	46.3	52.2
4	4	80	20	0.5	24	12.4	16.9
5	8	40	4	2.0	96	9.4	9.7
6	11	80	4	2.0	24	2.4	3.7
7	7	40	20	2.0	24	12.1	7.3
8	9	80	20	2.0	96	19.8	20.4
9	5	60	12	1.25	60	27.2	27.6
10	2	60	12	1.25	60	26.9	27.3
11	1	60	12	1.25	60	27.8	32.0

[a] Temperature

[b] Enzymatic load

[c] Reactive ratio (DAG/CLA ratio)

Production and Isolation of Structured TAG

A solution of *sn*-1,3-DAG and CLA isomers in hexane (1 mL/70 mg by weight of total substrates) was reacted under magnetic stirring (100 rpm) in the presence of anhydrous Lipozyme® IM, in an amber screw-capped vial, in different experimental conditions described in Table 1. The enzyme had been previously washed three times with fresh dry ethanol, filtered under vacuum, oven-dried at 50 °C and then stored in anhydrous ambient.

At the end of these reactions, the samples were filtered (0.2 μm nylon membrane filter, Corning Incorporated, Corning, Germany), then the hexane was dried over anhydrous Na_2SO_4 and evaporated by a nitrogen stream. Products were subjected to TLC analysis to isolate the TAG fraction using silica gel plates (SIL G-25, 20 × 20 cm, 0.25 mm, Macherey-Nagel, Germany), with the mixture petroleum ether:diethyl ether:formic acid (70:30:1, v/v) as developing solvent. The TAG fraction (Rf ≈ 0.75) was scraped off and extracted (10 mL × 3) from the silica by hexane-diethyl ether (50:50, v/v); the organic extracts were pooled and the solvent was evaporated using a nitrogen stream. The obtained TAG were derivatized and analyzed to determine the total and positional FA% molar compositions.

FA Composition (mol%) Analysis of Structured TAG

TAG were transesterified in order to obtain the respective fatty acid methyl esters (FAME) as follows: about 10 mg were dissolved in 3 mL of hexane and then 0.5 mL of 2N methanolic KOH were added; after stirring for 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.

The analyses were carried out using a DANI 1000DPC gas-chromatograph (Norwalk, CT, USA), equipped with a split–splitless injector and with a flame ionization detector. 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 temperatures was 250 °C; the oven temperature was 180 °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.

The reported data correspond to the mean values of three determinations.

Structural Analysis of the Synthesized TAG

A chemical procedure, based on TAG partial hydrolysis with the Grignard reaction, was used, as reported by Turon et al. [25]. The obtained α-MAG isolated by TLC (Rf ≈ 0.26) were derivatized and analyzed by HRGC, as described in the previous section for TAG. The acidic mol% 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 the % intrapositional composition of FA esterified in *sn*-2- position, A_t is the % total composition of FA esterified in all the three *sn*- positions of TAG, $A_{1(3)}$ is the % intrapositional composition of FA esterified in *sn*-1(3)- positions.

Results and Discussion

To study the influence of the selected variables on CLA isomers incorporation in *sn*-1,3-DAG substrates, the experimental design was carried out, as previously reported, by entering the four factors (temperature, time, DAG/

CLA ratio, enzymatic load) and the two responses (% incorporation of CLA in total TAG and in *sn*-2- position), by selecting the screening objective and by choosing the three-level-4-factor fractional factorial experimental design to screen the variables.

Then, the software generated the model, the design and the worksheet. In Table 1, the experimental conditions for the enzymatic reactions have been reported, together with the results of the analytical determinations, that are the responses shown in the last two columns. Obviously the best results are those showing the highest mol% of CLA isomers in total TAG.

However, the control of the FA positional % distribution in total TAG is also important to study the structure of the synthesized TAG and also to value the nutritional significance of new lipid matrix. In fact, it is known that absorption and metabolism of FA depend on the position occupied in the TAG molecules and that FA located in TAG *sn*-2- position are well absorbed as *sn*-2-MAG [23]. The results reported in Table 1 show considerable differences for CLA % in total TAG and in *sn*-2- position among the experiments. It can be observed that the experiment No. 3 (40 °C, enzymatic load 20% by weight of all substrates, DAG/CLA 0.5, 96 h) gave the highest incorporation of CLA isomers both in the total (46.3%) and in *sn*-2- position (52.2%), while the No. 6 (80 °C, enzymatic load 4% by weight of all substrates, DAG/CLA 2, 24 h) gave the lowest incorporation of CLA isomers both in the total (2.4%) and in *sn*-2- position (3.7%). Careful consideration should be reserved to the similarity of the above reported values of the two responses for each experiment; this occurrence suggest that a randomization of FA in TAG molecules could have happened. To confirm this hypothesis, the evaluation of the FA% compositions of the *sn*-1- and *sn*-3-MAG, obtained from TAG by chemical deacylation, and used to calculate the FA% compositions of the *sn*-2- position, could be useful. For all the experiments carried out, the results show similar FA% compositions for total TAG, *sn*-1(3)-MAG and TAG *sn*-2- position (data not shown). As an example, in Table 2 the results have been reported, as mean values and standard deviations (SD), for the three centre points (experiments No. 9–11, carried out at 60 °C, for 60 h, with a substrate ratio of 1.25 and an enzymatic load of 12%). The FA% compositions shown in Table 2 are very similar and show the presence of characteristic FA of olive oil, mainly oleic acid, besides the CLA isomers.

Moreover, it should be observed that the two CLA isomers are almost equally represented in total and positional TAG compositions and that minor CLA isomers (*t,t* and others not identified) are present in very low percentages. The minor CLA isomer % contents were only slightly higher with respect to the starting CLA isomer mixture.

Table 2 FA % compositions (mean values mol% ± SD, $n = 3$) of total TAG, *sn*-1(3)- and *sn*-2- position for TAG synthesized in the conditions of centre point

FA	TAG	*sn*-1(3)-	*sn*-2-
C 16:0	9.3 ± 0.3	10.0 ± 0.2	8.1 ± 1.4
C 16:1 (n-9 + n-7)	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.1
C 18:0	1.4 ± 0.1	1.9 ± 0.5	0.4 ± 1.1
C 18:1 (n-9 + n-7)	56.7 ± 0.3	56.4 ± 0.5	57.4 ± 1.5
C 18:2 (n-6)	3.9 ± 0.0	3.9 ± 0.1	4.1 ± 0.1
C 18:3 (n-3)	0.5 ± 0.0	0.5 ± 0.1	0.3 ± 0.2
C 20:0	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.1
9*c*,11*t*-CLA	13.4 ± 0.4	12.7 ± 0.3	14.6 ± 1.6
10*t*,12*c*-CLA	12.7 ± 0.1	12.3 ± 0.4	13.6 ± 0.9
t,t-CLA	0.7 ± 0.0	0.8 ± 0.1	0.4 ± 0.3
Other CLA isomers	0.5 ± 0.1	0.6 ± 0.0	0.3 ± 0.3

The results obtained as responses of the experiments carried out in this research should be discussed considering the enzymatic activity of Lipozyme® IM on the *sn*-1,3-DAG, in the presence of CLA isomers. First of all, it is known that the immobilized lipase from *R. miehei* exhibits *sn*-1,3- regiospecificity, but the lack of regiocontrol has been observed [21], in particular for the occurrence of isomerization processes concerning the DAG intermediates. Considering the substrates of the reported enzymatic reactions, the Lipozyme® IM could have catalyzed esterification reactions as well as acidolysis ones.

Concerning the analysis of the results with the Modde 5.0™ software, the model was fit to the data using MLR analysis. The best summary of the fit of the model is given by R^2 and Q^2 values, which represent, respectively, the fraction of variation of the responses explained by the model and the fraction of variation of the responses that can be predicted by the model. At first, poor R^2 and Q^2 values were obtained (R^2/Q^2: 0.644/−0.658 for total %CLA and 0.605/−0.876 for *sn*-2- %CLA), for this reason, interaction terms have been added to the model and a much better fit was obtained (R^2/Q^2: 0.999/0.995 for total %CLA and 0.995/−0.533 for *sn*-2- %CLA).

In Fig. 1a and b, the coefficient plots of the considered factors (temperature, *T*; enzymatic load, E.l.; DAG/CLA molar reactive ratio, R.r.; reaction time, time; and interaction terms) are reported for total %CLA and *sn*-2- %CLA, respectively. It can be observed that both the responses are positively influenced by enzymatic load, time and their interaction term while DAG/CLA molar ratio,

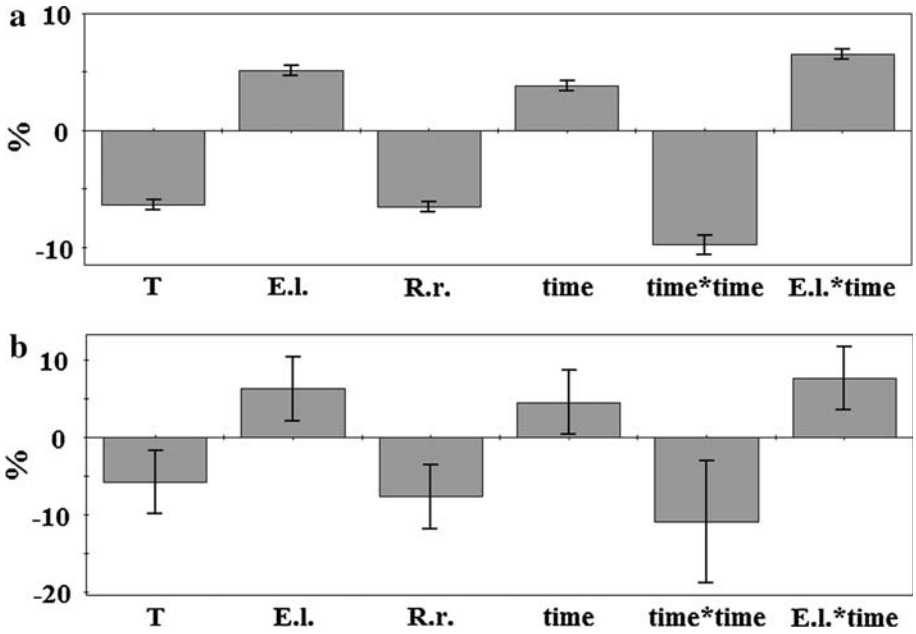

Fig. 1 Coefficient plots of the selected factors for total %CLA (**a**) and *sn*-2- %CLA (**b**) responses (for the abbreviations see footnotes in Table 1)

temperature and the interaction term time × time exhibit an inverse correlation with the responses.

In Fig. 2a and b, the two-dimensional contour plots showing total %CLA and *sn*-2- %CLA as a function of DAG/CLA ratio and temperature, respectively, have been reported. It can be noted that the best CLA isomer incorporation occurred for the lowest reactive ratio and temperature for both the considered responses.

Finally, in Fig. 3a and b, the three-dimensional response surfaces corresponding to total %CLA and *sn*-2- %CLA as a function of the enzymatic load and time (temperature and DAG/CLA molar ratio were fixed at 60 °C and 1.25) are respectively shown. It is possible to observe that the best CLA isomers incorporation occurred for a time of about 80 h and for the highest enzymatic load (20% by weight of substrates).

The results obtained show that better CLA incorporation could be achieved by enlarging the ranges for some parameters, for example, by setting lower values for the temperature and reactive ratio or higher values for the enzymatic load. Nevertheless, it should not forgotten that some conditions, such as low temperatures, could be not applicable in enzymatic reactions, or be difficult or not economical enough to realize on a large scale, as a very high enzymatic load.

In conclusion, the results of this investigation have shown that all the tested parameters (time, temperature, enzymatic load and reactive ratio) have an influence on the synthesis of TAG containing CLA isomers and that some interaction terms improved the goodness of the fit of the model. The mol% of total FA and positional compositions of synthesized TAG have shown that, in addition to the

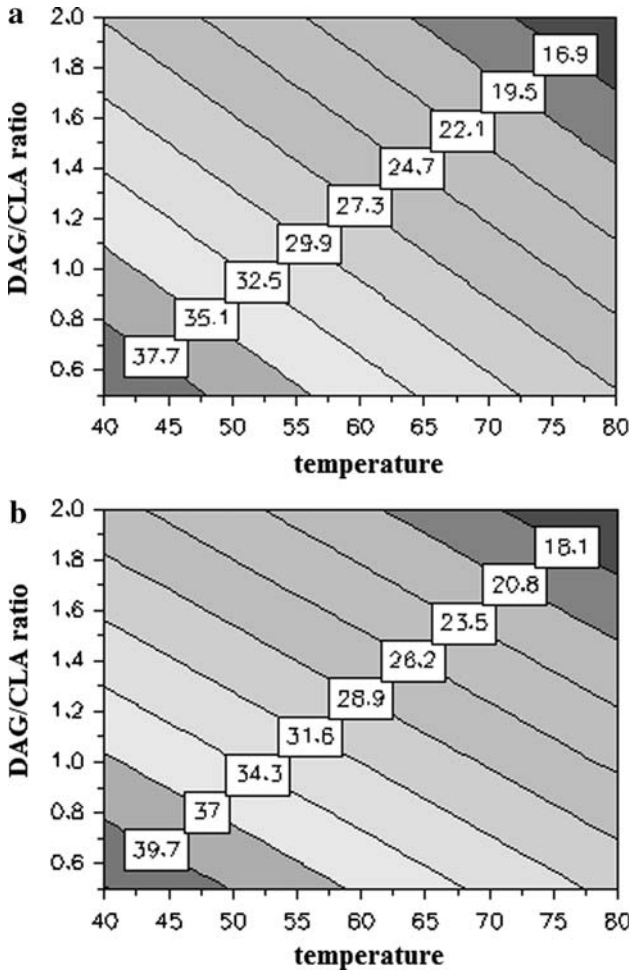

Fig. 2 Two-dimensional contour plots showing total %CLA (**a**) and *sn*-2- %CLA (**b**) as a function of DAG/CLA ratio and temperature, when time was fixed at 60 h and enzymatic load at 12%

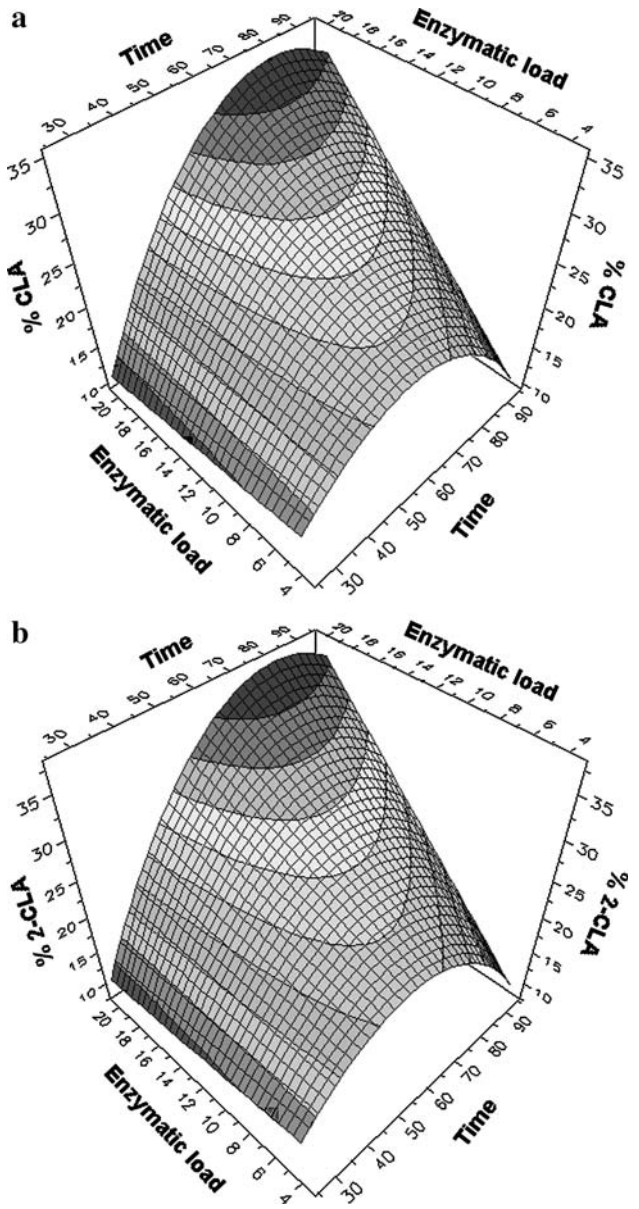

Fig. 3 Response surfaces corresponding to total %CLA (**a**) and *sn*-2-%CLA (**b**) as a function of the enzymatic load and time, when temperature and DAG/CLA molar ratio were fixed at 60 °C and 1.25

enzymatic reactions of esterification and acidolysis, the isomerization processes should also be taken into account to explain the structure of the TAG obtained.

References

1. Macrae R, Hammond RC (1985) Present and future applications of lipases. Biotechnol Genet Eng Rev 3:193–217
2. Macrae R (1989) Tailored triacylglycerols and esters. Biochem Soc Trans 17:1146–1148
3. Senanayake SPJN, Shahidi F (2002) Structured lipids: acidolysis of gamma-linolenic acid-rich oils with n-3 polyunsaturated fatty acids. J Food Lipids 9:309–323
4. Hamam F, Shahidi F (2006) Synthesis of structured lipids containing medium-chain and omega-3 fatty acids. J Agric Food Chem 54:4390–4396
5. Sahin N, Akoh CC, Karaali A (2006) Human milk fat substitutes containing omega-3 fatty acids. J Agric Food Chem 54:3717–3722
6. 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 the *sn*-2- position. J Am Oil Chem Soc 85:613–619
7. Villeneuve P, Barouth N, Baréa 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
8. Kim IH, Lee SM (2006) Synthesis of diacylglycerols containing CLA by lipase-catalyzed esterification. J Food Sci 71:C378–C382
9. Rocha-Uribe A, Hernandez E (2004) Solvent-free enzymatic synthesis of structured lipids containing CLA from coconut oil and tricaprylin. J Am Oil Chem Soc 81:685–689
10. 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
11. Park Y, Storkson JM, Albright KJ, Liu W, Pariza MW (1999) Evidence that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids 34:235–241
12. Ip C, Banni S, Angioni E, Carta G, McGinley J, Henry JT, Barbano D, Bauman D (1999) Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. J Nutr 129:2135–2142
13. Koba K, Akahoshi A, Yamasaki M, Tanaka K, Yamada K, Iwata T, Kamegai T, Tsutsumi K, Sugano M (2002) Dietary conjugated linolenic acid in relation to CLA differently modifies body fat mass and serum and liver lipid levels in rats. Lipids 37:343–350
14. Ryder JW, Portocarrero CP, Song XM, Cui L, Yu M, Combatsiaris T, Galuska D, Bauman DE, Barbano DM, Charron MJ, Zierath JR, Houseknecht KL (2001) Isomer-specific antidiabetic properties of conjugated linoleic acid. Improved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. Diabetes 50:1149–1157
15. Yu Y, Correll PH, Vanden Heuvel JP (2002) Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: evidence for a PPAR gamma-dependent mechanism. Biochim Biophys Acta 1581:89–99
16. Salas-Salvadó J, Márquez-Sandoval F, Bulló M (2006) Conjugated linoleic acid intake in humans: a systematic review focusing on its effect on body composition, glucose, and lipid metabolism. Crit Rev Food Sci Nutr 46:479–488
17. Xu X (2000) Production of specific-structured triacylglycerols by lipase-catalyzed reactions: a review. Eur J Lipid Sci Technol 102:287–303
18. Van Camp J, Huyghebaert A, Goeman P (1998) Enzymatic synthesis of structured modified fats. In: Christofe AB (ed) Structural modified food fats: synthesis, biochemistry and use. AOCS PRESS, Champaign, pp 20–45
19. Kim BH, Akoh CC (2005) Modeling of lipase-catalyzed acidolysis of sesame oil and caprylic acid by response surface methodology: optimization of reaction conditions by considering both acyl incorporation and migration. J Agric Food Chem 53(20): 8033–8037
20. Senanayake SPJN, Shahidi F (2002) Lipase-catalyzed incorporation of docosahexaenoic acid (DHA) into borage oil: optimization using response surface methodology. Food Chem 77: 115–123

21. Ferreira-Dias S, Correia AC, Baptista FO, da Fonseca MMR (2001) Contribution of response surface design to the development of glycerolysis systems catalyzed by commercial immobilized lipases. J Mol Catal B Enzym 11:699–711
22. Maurelli S, Blasi F, Cossignani L, Bosi A, Simonetti MS, Damiani P (2009) Enzymatic synthesis of structured triacylglycerols containing CLA isomers starting from *sn*-1,3-diacylglycerols. J Am Oil Chem Soc 86:127–133
23. Blasi F, Cossignani L, Simonetti MS, Damiani P (2007) Biocatalysed synthesis of *sn*-1,3-diacylglycerol from extra virgin olive oil. Enzyme Microb Technol 41:727–732
24. 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
25. 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