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Biocatalyzed acidolysis of soybean oil triacylglycerols to increase oleic acid content☆

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Abstract

Lipase catalyzed acidolysis of triacylglycerols (TAG) of soybean oil with oleic acid in organic solvent was studied; immobilized lipase from *Rhizomucor miehei* was used and the effects of reaction time, incubation temperature and enzyme load on TAG total and positional fatty acid (FA) percentage compositions were investigated. The results show that oleic acid incorporation was high after 24 and 48 h, while after 72 h a lower level of oleic acid in TAG was observed. Moreover, for the reactions carried out at 30 and 40 °C, it was observed that the oleic acid level was about 46.5% while ligtly higher values (about 49%) were observed at 50 °C; however, under this last condition, the modification of *sn*-2 position FA composition was higher. Finally, the variable enzyme load resulted also important on the incorporation of oleic acid; in particular, even if the value of 10% (w/w) of enzyme load resulted in a lightly lower incorporation of oleic acid in soybean oil TAG (about 45%), in this situation a minimal modification of *sn*-2 position FA composition was obtained.

Keywords: Soybean triacylglycerols; Lipase catalyzed acidolysis

1. Introduction

The modification of fatty acid (FA) composition of oilseeds has been practised for many years in order to control three quality aspects: oxidative stability, nutritional value and functional properties [1]. Regular and unhydrogenated soybean oil contains about 11% palmitic acid, 4% stearic, 23% oleic, 54% linoleic and 8% linolenic; with this composition, soybean oil has a good nutritional profile because of its high proportion of unsaturated FA; for the same reason, and in particular for the levels of polyunsaturated linoleic and linolenic acids, the oil has a poor oxidative stability.

To improve oxidative stability and to increase the saturate level of soybean oil, hydrogenation is often used, but the process is expensive and has limited capacity and direction for oil modification (e.g., it cannot decrease the degree of saturation). Furthermore, hydrogenation produces *trans* FA, which have been shown to have adverse health implications [2].

During the past three decades, significant progress has been made in modifying soybean oil composition through plant breeding and genetic engineering. Genetic modification and breeding of soybeans have been used not only to decrease linoleic and linolenic acids, but also to decrease saturated FA, to increase oleic acid, palmitic acid and stearic acid [3–5].

A different way to obtain modifications of FA composition of lipids is the production of structured lipids, which can be defined as triacylglycerols (TAG) modified chemically or enzymatically to change the FA composition and/or the positional distribution in the glycerol backbone [6–11]; although chemical interesterification is simple and inexpensive, it is

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Table 1
Soybean TAG modified samples and values of considered variables

Sample	Time (h)	Temperature (°C)	Enzyme load (%, w/w)
A 24-30-20	24	30	20
B 48-30-20	48	30	20
C 72-30-20	72	30	20
D 24-40-20	24	40	20
E 24-50-20	24	50	20
F 24-30-10	24	30	10
G 24-30-30	24	30	30

not capable of modifing specific positions due to the random nature of the reactions. The reactions catalyzed by lipases are characterized by mild conditions, specificity of enzymatic action and better acceptability. In this paper, the ability of a *sn*-1,3 regiospecific lipase, Lipozyme IM from *Rhizomucor miehei*, to catalyze the acidolysis of soybean oil with oleic acid was explored. Effects of reaction time, incubation temperature and enzyme load on the degree of incorporation of oleic acid in soybean oil TAG were also investigated.

2. Experimental

2.1. Chemicals and materials

The starting soybean oil was purchased from a local foodstore. The lipase, Lipozyme IM, was gently donated by Novo Nordisk (Bagsvaerd, Denmark); this enzyme, derived from *Rhizomucor miehei*, is immobilized on a macroporous anion exchange resin and has 1,3-positional specificity. All chemicals and solvents were of ACS purity. The TAG fraction of oils, before and after the acidolysis reactions, were analyzed following the in Sections 2.3–2.5 steps.

All the experimental steps were carried out at least twice and the reported results represent the average values.

2.2. Acidolysis of soybean oil with oleic acid

In general, soybean TAG (50 mg) were mixed with oleic acid at a mole ratio of 1:2, in hexane (2 mL); then the lipase Lipozyme IM was added and the reactions were carried out for different times (24, 48 and 72 h), at different incubation temperatures (30, 40 and 50 °C) and with different enzyme loads (10, 20 and 30%, w/w). At the end of the enzymatic reaction, the lipase was separated by filtration and the TAG fraction was isolated by TLC, as described in Section 2.3.

In Table 1, the considered samples are reported, together with the values of the studied variables.

2.3. Analysis of TAG % FA composition

The TAG fraction was purified by TLC (silica gel precoated plates, 20 cm × 20 cm, 250 μm) using hexane–diethyl ether–formic acid (80:20:2, v/v/v) as the developing solvent [12]. The TAG fraction (R_f = 0.64) was extracted from silica with diethyl ether (3 mL × 2 mL) and the organic extracts were pooled.

An aliquot of TAG was dissolved with 2 mL of hexane and then 0.4 mL of 2 M KOH in anhydrous methanol were added [13]; after 3 min, 3 mL of water were added. The organic layer, separated by centrifugation, was dried over anhydrous sodium sulfate, then concentrated with a N_2 stream to ∼0.5 mL for HRGC analysis of fatty acid methyl esters (FAME). A Perkin-Elmer Autosystem gas chromatograph (Norwalk, CT, USA), equipped with a split-splitless injector and a flame ionization detection (FID) system, was used; the separation of FAME was carried out using a Supelcowax 10 column (30 m × 0.25 mm i.d., d.f. = 0.25 μm) (Supelco, Milan, Italy). The temperature of the oven was maintained at 165 °C for 3 min, then it was raised at 3 °C/min to 240 °C. Helium was the carrier gas. The PE Turbochrom Navigator 4.1 was the software used to acquire and to handle the chromatograms.

2.4. Analysis of % FA composition of TAG sn-2 position

The TAG fraction was subjected to pancreatic lipase hydrolysis to determine the FA % composition of TAG *sn*-2 position [14]; the *sn*-2-monoacylglycerols were extracted from scraped TLC bands and were methylated for HRGC analysis (as described in Section 2.3).

2.5. Stereospecific analysis of TAG by sn-1,2-diacilglycerolkinase method

The FA % positional compositions of TAG were obtained using the *sn*-1,2-diacilglycerolkinase procedure, as reported in a previous paper [12]; in brief, the TAG fraction was treated with methyl magnesium bromide in dry diethyl ether and then the hydrolysis mixture was applied to TLC plates of silica impregnated with boric acid to isolate the *sn*-1,2(2,3)-diacylglycerol fraction. This fraction was extracted with diethyl ether, then the solvent was evaporated and 0.1 mL of cardiolipin in methanol (5 mg/mL, Fluka, Bucks, Switzerland) were added to the solution, with subsequent ultrasonication for 1–2 min and solvent removal with N_2 stream. To the residue were added: 20 μL of *sn*-1,2-diacylglycerol kinase buffered solution (from *E. coli*, 1 mg/mL in 10 mM phosphate buffer pH 7.0, 20% glycerol, 2 mM β-mercaptoethanol, with defined specific activity – this specimen: 10.6 U/mg, Calbiochem, La Jolla, CA, USA), 1 mL of buffer Tris (pH 6.6) and 100 μL of Na_2ATP aqueous solution (8 mM). After incubation at 40 °C for 90 min under constant stirring, the required products were extracted with chloroform–methanol (1:1, v/v) and the *sn*-1,2-phosphatidic acids were isolated by TLC. The phosphatidic acids were transesterified and the obtained FAMEs were analyzed by HRGC (as described in Section 2.3).

The results were then used to obtain the *sn*-1, *sn*-2 and *sn*-3 FA compositions according to [12].

Table 2
Total and positional FA % composition of soybean oil TAG[a]

FA	Soybean oil			
	TAG	*sn*-1	*sn*-2	*sn*-3
C 14:0	0.1 ± 0.0	0.1 ± 0.1	–	0.1 ± 0.1
C 16:0	11.8 ± 0.2	20.7 ± 0.7	0.5 ± 0.1	14.2 ± 1.5
C 16:1 (*n*-9)	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	–
C 18:0	3.9 ± 0.1	7.5 ± 0.1	0.1 ± 0.1	4.0 ± 0.1
C 18:1 (*n*-9)	20.2 ± 0.4	19.4 ± 0.8	19.5 ± 1.1	21.6 ± 0.8
C 18:2 (*n*-6)	54.4 ± 0.4	43.0 ± 0.6	71.8 ± 0.7	48.3 ± 0.7
C 18:3 (*n*-3)	8.7 ± 0.3	8.8 ± 0.3	7.9 ± 0.7	9.6 ± 0.4
C 20:0	0.3 ± 0.1	–[b]	–	0.9 ± 0.1
C 20:1 (*n*-11)	0.3 ± 0.0	–	–	0.8 ± 0.1
C 22:0	0.2 ± 0.0	0.1 ± 0.1	–	0.5 ± 0.1

[a] Average values and standard deviations of four replicates.
[b] Lower than 0.1%.

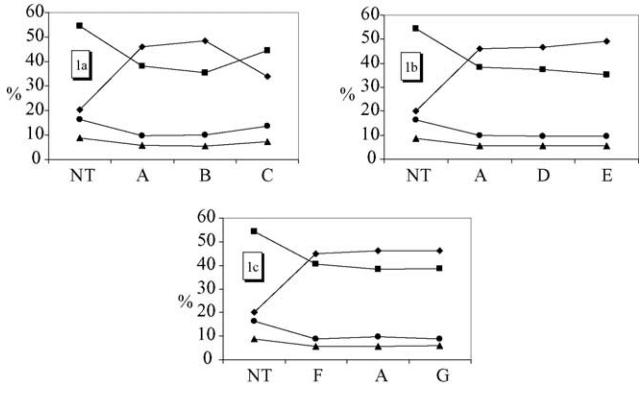

Fig. 1. Trends of % contents of C 18:1^{9c} (♦),C 18:2^{9c,12c} (■),C 18:3^{9c,12c,15c} (▲), sum of saturated FA (●) in total TAG of not treated sample (NT) and samples treated for different times (1a), at different incubation temperatures (1b) and with different enzyme loads (1c).

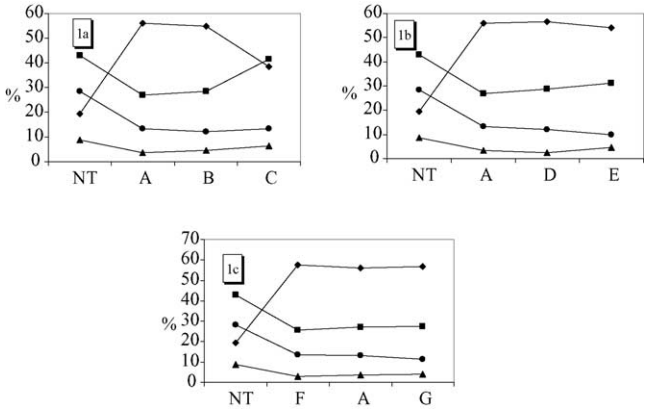

Fig. 2. Trends of % contents of C 18:1^{9c} (♦),C 18:2^{9c,12c} (■),C 18:3^{9c,12c,15c} (▲), sum of saturated FA (●) in TAG *sn*-1 position of not treated sample (NT) and samples treated for different times (1a), at different incubation temperatures (1b) and with different enzyme loads (1c).

3. Results and discussion

The results relative to the total and positional % FA compositions of the starting soybean oil TAG, reported in Table 2, show that the most abundant FA is linoleic acid which is greatly represented in the *sn*-2 position but also in outer positions of TAG; oleic and linolenic acids show no preference for the three positions of glycerolic moiety while the saturated FA are esterified mainly in the *sn*-1- and *sn*-3 positions of TAG.

Concerning the results for the treated samples, the trends of the most significant FA in total TAG are reported in Fig. 1; they are relative to the non treated sample (NT) and the samples treated for different times (A–C) Fig. 1a, at different incubation temperatures (A, D and E) Fig. 1b and with different enzyme loads (F, A and G) Fig. 1c.

With regard to the effect of reaction time, high levels of oleic acid were incorporated after 24 and 48 h while after 72 h a lower level of oleic acid was measured in soybean modified TAG; the results of stereospecific analysis (Figs. 2–4) show that oleic acid was incorporated mainly in the *sn*-1- and *sn*-3 positions of TAG. This occurrence was expected because a lipase 1,3-regiospecific was used as the catalyst in order to address oleic acid mainly in *sn*-1- and *sn*-3 positions and to obtain the lesser modifications of FA composition in TAG *sn*-2 position; in fact, from a nutritional point of view, high

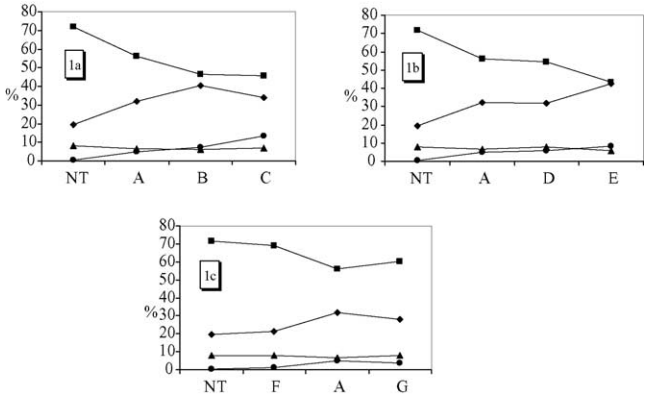

Fig. 3. Trends of % contents of C 18:1^{9c} (♦),C 18:2^{9c,12c} (■),C 18:3^{9c,12c,15c} (▲), sum of saturated FA (●) in TAG *sn*-2 position of not treated sample (NT) and samples treated for different times (1a), at different incubation temperatures (1b) and with different enzyme loads (1c).

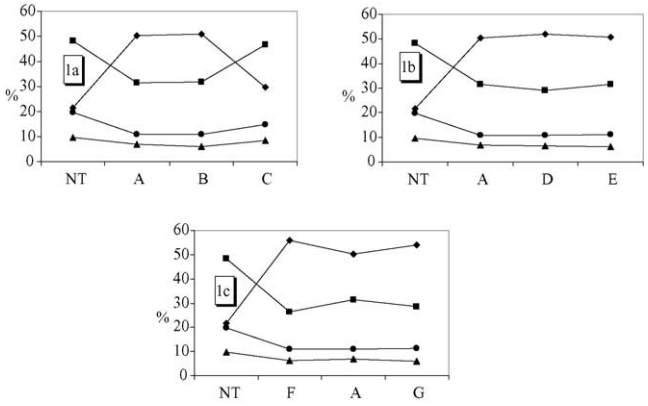

Fig. 4. Trends of % contents of C 18:1^{9c} (♦),C 18:2^{9c,12c} (■),C 18:3^{9c,12c,15c} (▲), sum of saturated FA (●) in TAG *sn*-3 position of not treated sample (NT) and samples treated for different times (1a), at different incubation temperatures (1b) and with different enzyme loads (1c).

levels of linoleic acid in this position confer positive feature to food lipids as the FA esterified in TAG *sn*-2 position are characterized by better intestinal absorption [15,16].

The results obtained for the reactions carried out for different times show that after 48 h the oleic acid incorporation was slightly higher than after 24 h but higher modifications of FA composition of *sn*-2 position occurred.

Obviously, as a consequence of the incorporation of oleic acid, lower % contents were observed for linoleic, linolenic and saturated FA in soybean TAG. The light increase of oleic acid % content in *sn*-2 position of TAG after the acidolysis reactions is a consequence of occurred isomerization processes [17].

The results obtained for the samples treated at different temperatures show that at 50 °C a better incorporation of oleic acid was obtained in regard to the samples treated at 30 and 40 °C; however, the higher temperature caused higher modification of *sn*-2 position FA composition.

Finally, it was observed that the variable enzyme load was also important on acidolysis reaction between soybean TAG and oleic acid; at this regard, the most interesting result was represented by the value of 10% (w/w) enzyme load. In fact, with this value of enzyme load, even if a lightly lower incorporation of oleic acid in soybean oil TAG was obtained, minimal modifications of *sn*-2 position FA composition occurred (Figs. 1c, 3).

4. Conclusions

It can be concluded that the acidolysis reactions carried out in this study are effective to increase the oleic acid content in soybean oil TAG under the described experimental conditions; the best values for the considered variables are: time 24 h, temperature 30 °C and enzyme load 10% (w/w), considering that the degree of incorporation of oleic acid in soybean oil TAG was acceptable and that the lesser modifications of TAG *sn*-2 position % FA composition occurred.

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