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Chemical and Enzymatic Interesterification of a Blend of Palm Stearin: Soybean Oil for Low *trans*-Margarine Formulation

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Abstract A blend of palm stearin and soybean oil (70/30, wt%) was modified by *chemical interesterification* (CIE) and *enzymatic interesterification* (EIE), the latter batch-wise (B-EIE) and in continuous (C-EIE). Better oil quality, mainly in terms of acidity, free tocopherol and partial acylglycerol content, was obtained after EIE. The clear melting point after any interesterification process was similar and about 9 °C lower as result of the modification in the TAG profile, which approaches the calculated random distribution. Interesterification changed the SFC profile significantly. For the fully refined interesterified blends, the SFC profile was similar and clearly different from the starting blend. Interesterification decreased the content of solids at temperatures >15 °C and increased the content of solids at temperatures <15 °C. This increase was less remarkable after C-EIE, suggesting that full randomization was not achieved in the used conditions, probably caused by a too short residence time of the oil in the enzymatic bed. During B-EIE, variations in SFC with time, principally at low temperatures, were still observed although the TAG composition was stable. At low temperatures, the reaction rate calculated from SFC was very low, confirming an important effect of the acyl migration on this parameter.

Keywords Chemical interesterification · Batch-wise and continuous packed bed enzymatic interesterification · "Low *trans*" margarine formulation · Lipozyme® TL IM

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Introduction

Interesterification as such or used in combination with dry fractionation has received increasing interest lately as an alternative to partial hydrogenation [1] for the production of "low *trans*" hard fat suitable for shortenings, stick or tub-type margarines and confectionary fat production [2]. In this context, palm stearin, a hard fraction obtained by palm oil dry fractionation, is a suitable alternative. It is added to improve tolerance to high temperatures, and for crystal morphology and stability [3]. However, blending with polyunsaturated soft oils (like sunflower, soybean or rapeseed oils) remains necessary in order to impart plasticity to the final interesterified product [4]. A wide range of consumer table margarines and spreads, bakery margarines and frying shortenings can be formulated by mixing interesterified blends and native oils in adequate proportions [5].

The solid fat content (SFC) profile is critical in the evaluation of suitability for shortening or margarine formulation: the values of $SFC_{10\ °C}$, $SFC_{20\ °C}$ and $SFC_{35\ °C}$ are important as related to the rheological behavior of fats at storage, packaging and utilization of bakery margarines, respectively [5]. The $SFC_{10\ °C}$ will determine the hardness of the final product at refrigerator conditions. $SFC_{10\ °C}$ and $SFC_{20\ °C}$ are important parameters for determining the feasibility of the use of a blend in the production of bakery

margarines [6]. The $SFC_{35\ °C}$ is particularly important in margarine manufacture, since it is related to the extent of melting in the mouth. In interesterified blends, this parameter must be as low as possible to prevent a sandy and coarse texture of the margarine [5].

Interesterification can be conducted chemically or enzymatically. Chemical interesterification (CIE) is usually random and produces complete positional randomization of the acyl groups on the glycerol backbone [7]. Depending from enzyme regioselectivity, enzymatic interesterification (EIE) can be random or specific. Intermediate specificity can also be obtained simply by adjusting the residence time of the enzymatic process. Nevertheless, a full conversion seems to be preferred: Zhang et al. [8] observed that margarine storage stability increases with an increasing conversion degree of the interesterified blend. Random interesterification (CIE or EIE) is mainly used for the production of commodity fats with the main purposes to modify the overall melting properties, to increase compatibility within the solid phase (not achievable by simple blending) and to enhance plasticity of the final product. A regioselective enzyme is strictly required for specific interesterification (EIE); in that case, acyl exchange is limited to the *sn*-1,3 positions of the glycerol backbone. Specific interesterification is mainly used in the production of specialty fats (like cocoa butter equivalents, infant formulation products, low calorie fats or easily absorbable oils). Chemical interesterification is usually conducted batch-wise, allowing the production of a large number of (small) batches with a low degree of cross-contamination. Technology has improved greatly over the last few decades: less catalyst consumption, less side reactions and less oil losses can be guarantied. Enzymatic interesterification is preferably conducted continuously by using immobilized enzyme in a fixed bed configuration; this set-up is less suitable in the case of many stock changes and is mainly used for the production of larger batches of "bulk" oil. However, enzymatic interesterification requires milder reaction conditions compared to chemical interesterification, leading to fewer side reactions, less post-treatment and in consequence fewer oil losses.

EIE for the production of margarine fats is well described in the literature. Studies have been carried out on different blends (in % weight): palm stearin/coconut oil and soybean oil/fully hydrogenated soybean oil, both in different ratios [9], butterfat/rapeseed oil 70/30 [10], palm stearin/palm kernel olein in different ratios [3, 11, 12], palm stearin/soybean oil 55/45 [6], palm stearin/coconut oil 75/25 [7, 13] and 70/30 [8, 14, 15], palm stearin/palm kernel oil/sunflower oil 55/25/20 [5], palm stearin/sunflower oil 40/60 [4], high melting palm stearin with different liquid oils (sunflower, soybean and rice bran) 40/60 [16], palm stearin/mustard oil 70/30 [17], low erucic rapeseed oil/tallow 60/40 [18], lard/high oleic sunflower oil in different ratios [19]. Most of these studies were done either batch-wise [3, 4, 9, 11, 12, 15, 16, 19], or both batch-wise and continuously, the latter either in a continuous packed bed reactor [5, 10] or in a continuous fluidized-bed reactor [6]. Only few studies have been carried out to compare the product quality after CIE and EIE: Zhang et al. [7, 13] studied a blend of palm stearin and coconut oil (75/25, wt%) in terms of TAG profile, DAG content, residual acidity and SFC profile. Batch-EIE was used to investigate parameters such as lipase load, water content, temperature and reaction time, and to examine the effect of these on product quality and reusability of the enzyme. Two different enzymes were investigated in terms of regioselectivity; final products were compared with purely randomized blends from chemical interesterification in terms of TAG compositions and SFC profiles. Some years later, the same authors [8, 14] investigated the storage stability of similar margarines (based on interesterified palm stearin and coconut oil (70/30, wt%), blended with sunflower oil in 50-50 ratio) produced by EIE and CIE. Physical and chemical properties were monitored and compared. Margarine produced from EIE fat had physical properties similar to the margarine produced from the CIE fat in terms of color, hardness, dropping point and crystal form. Sensory panel evaluations did not show any clear difference between the margarines However, the oxidative stability of the enzymatically interesterified produced margarine was better when stored at 25 °C; on the other hand, taste and smell remained similar compared to chemically interesterified produced margarine. Ledóchowska and Wilczyńska [18] compared oxidative stability of chemically and enzymatically interesterified fats based on a blend of low erucic rapeseed oil and tallow (60/40, wt%). Stability of the enzymatically interesterified product was similar to the one of the native oil; in contrast, inferior oxidative stability was observed for the chemically interesterified product. All these studies clearly suggest that EIE produced *trans*-free fats can meet industrial demands for the production of margarine fats [8] and for this reason can be used as alternatives to partially hydrogenated types [20].

In this paper, a blend of palm stearin and soybean oil (70/30, wt.%) targeted for the production of hard stock used in "low *trans*" stick margarine or puff pastry margarine formulations was investigated. Solid fat content profile, TAG distribution and corresponding calculated degree of interesterification were used to monitor the conversion during the batch-wise enzymatic reaction with Lipozyme® TL IM. A continuous enzymatic process was implemented under fixed bed conditions. A chemically interesterified blend was chosen as reference for comparison of melting

properties (SFC profile, melting point) and product quality (partial acylglycerol content, residual acidity, color and tocopherol content) of the interesterified products, before and after final deodorization.

Experimental Procedures

Materials

Refined, bleached, and deodorized palm stearin (PS) [clear melting point (CMP) 55.5 °C, iodine value 34.8] was obtained from Unimills, The Netherlands. RBD soybean oil (SBO) was purchased in a local supermarket (O'Cool, Belgium). Lipozyme® TL IM, a commercial, silica-granulated lipase from *Thermomyces lanuginosa*, which rearranges the fatty acids with a certain *sn*-1,3 specificity, was obtained from Novozymes A/S (Bagsvaerd, Denmark). Sodium methoxide was provided by Degussa-Hüls, Niederkassel, Germany. All the chemicals and reagents used for the analyses were of analytical grade.

Experimental Methods

Laboratory-Scale Batch Chemical Interesterification (CIE)

The fat blend (350 g) was dried while stirring under vacuum for 60 min at 120 °C. After lowering the temperature to 90 °C, 0.1% sodium methoxide (powder dissolved in oil) was added as the catalyst. Interesterification was conducted under a vacuum at 90 °C for 30 min after the appearance of the characteristic dark 'brownish' color. After completion of the reaction, the vacuum was broken and a 20% citric acid solution was added to inactivate the catalyst, while the mixture was stirred for a further 15 min. Post-bleaching was performed with 0.75% of activated bleaching earth (Tonsil 210 FF) under vacuum for 30 min at 90 °C. Filtration was done over a preheated Buchner filter.

Laboratory-Scale Batch Enzymatic Interesterification (B-EIE)

Fresh Lipozyme® TL IM has an equilibrium water content of approximately 5% (wt.%); to avoid by-products formation caused by hydrolysis of the fat, this content needs to be reduced prior to carrying out the experiments. But before water removal, the enzyme needs to be de-aerated. De-aeration was performed by contacting the enzyme with preheated liquid oil (70 °C) in a glass pear-shaped flask (relative enzyme vs. oil quantity: 4% (wt.%)). The pear was connected to the rotavapor and vacuum was applied to the mixture, while agitating gently by swirling at 70 °C until no air was released from the granules (approx. 20 min). The rotating rate was adjusted in order to guarantee a good suspension of the enzyme in the oil. The agitation was stopped and the particles were allowed to settle; the oil was removed by decanting.

Free water removal was carried out in a rotavapor at 70 °C. Three volumes of pre-heated liquid oil (600 g each) were interesterified for 30 min at 70 °C to reduce the water content of Lipozyme® TL IM (relative enzyme vs. oil quantity: 4% (wt.%)), consuming this free water in hydrolytic side reactions. After four oil pre-treatments, most of the free water was removed. An enzymatic rearrangement must contain a certain amount of water because the reaction takes place at the water/oil boundary phase and because the water is required for the maintenance of an active hydrated state; however, too much water will discourage the esterification and instead promote hydrolysis.

The Lipozyme® TL IM was quickly washed with the blend to be studied in order to remove the liquid oil. 600 g of the blend was melted in an oven before use and transferred into the reactor. When the oil blend reached the set temperature (70 °C), the immobilized lipase was added into the system at a dose of 4% of substrate to start the reaction, and distributed evenly by an impeller stirrer set at 200 rpm. Sampling was performed every hour during a maximum period of 8 h; stirring was stopped for 1 min before each sampling. The lipase was allowed to fully settle to the bottom, where it remained while products were withdrawn from the top of the reactor and subsequently filtered over a Buchner filter before analysis.

Laboratory-Scale Continuous Packed-Bed Enzymatic Interesterification (C-EIE)

Prior to C-EIE, the immobilized enzyme was conditioned by sending liquid oil through the columns, in order to remove air and water (see above). FFA of the interesterified liquid oil was followed and conditioning was stopped when FFA was stable.

For the C-EIE reaction, a packed-bed reactor was used. This reactor consists of two columns (length 20 cm; inner diameter 2.5 cm) placed in series and each filled with 45 g immobilized enzyme, and submerged in a thermostated water bath for temperature control. The blend was pumped into the reactor at a specified flow rate of 300 g/h which gives an oil flow rate/enzyme-ratio of 3.3 and a residence time of 27 min through both columns. The reaction temperature was 70 °C. The interesterified blend was collected after 5 enzyme bed volumes, to avoid cross contamination with the previous liquid oil used for conditioning.

Batch Deodorization

CIE and EIE fats were deodorized at lab-scale under the following conditions: 240 °C, 3 mbar, 1.5% sparge steam and 60 min residence time.

Analytical Determinations

Fatty Acid Composition

Preparation of fatty acid methyl esters was done according to the AOCS Official Method Ce 2-66 (alternate method for fats and oils with acid value <2) [21]. GC determination was based on the AOCS Official Method Ce 1e-91 [21]. The FAME were separated on a 6890N gas chromatograph from Agilent Technologies, equipped with a flame ionization detector and a BPX 70 capillary column (60 m length × 0.10 mm internal diameter) (Supelco, Bellefonte, PN, USA). Initial column- temperature was set at 60 °C and increased at a rate of 10 °C/min to 150 °C, then further to 175 °C at 5 °C/min and held isothermally for 45 min at 175 °C. Injector and detector were maintained at 250 °C. Helium was used as the carrier gas, flowing at 0.3 mL/min. The flow rates of hydrogen and air were, respectively, 30 and 400 mL/min. The injection volume was 0.5 μL in hexane. Determination was carried out in duplicate.

Color

Color was determined at 70 °C by means of an automatic Lovibond PFX 880/P instrument mounted with a heater.

Solid Fat Content

The solid fat content (SFC) was analyzed using low field pulsed Nuclear Magnetic Resonance (p-NMR) with a Bruker Minispec mq 20 (Bruker, Germany), according to the standard IUPAC method 2.150 [22]. Serial methods, with and without tempering, were applied. Data were reported as averages of two measurements.

Clear Melting Point

The clear melting point (CMP) was determined with a Mettler FP 90 central processor and a Mettler FP81HT capillary melting point unit, supplied by Mettler Toledo and especially designed for it, connected to a recorder for the registration of the melting curve. The procedure recommended by Mettler for edible oils and fats (heating rate: 1 °C/min, starting temperature: at least 10–15 °C below the expected melting point) was used. After reaching the end temperature, the CMP resulted from the interpolation on the recorded chart. Triplicate analyses were performed.

Iodine Value

The iodine value (IV) was determined by the Wijs method according to the AOCS Official recommended method Cd 1b-87 [21].

Free Tocopherol Content

The free tocopherol content was analyzed by HPLC (Hewlett Packard 1050 Series Chromatograph equipped with 4 modules (injector, Hewlett Packard 1100 Series FLD-detector, online degasser and pump)), based on the AOCS Official Method Ce 8-89 [21]. The column was an Alltima Silica U 5 μm: 250 mm length × 4.6 mm internal diameter. A fluorescence detector was used and mobile phase in the chromatographic system was hexane/isopropanol (9:1, v:v). The flow rate of the pump was set to be constant at 1.5 mL/min. The injection volume was 20 μL. The peaks in the experimental samples were identified by comparison with elution times of standards. Determinations were carried out in duplicate.

Free Fatty Acids Content

The free fatty acid (FFA) content of the oil samples was determined according to the AOCS Official Method Ca 5a-40 [21]. An average molecular weight of 256 of the oil blend was used for calculation. Data were reported as averages of two measurements.

Partial Acylglycerol and Triacylglycerol Contents

Partial acylglycerol and triacylglycerol profiles were analyzed by reversed-phase HPLC (RP-HPLC), based on the AOCS Official Method Ce 5b-89 [21]. Minor practical adjustments to the flow rate and of the mobile phase composition were made in order to improve acylglycerol separation, in compliance with the above-mentioned official method. All equipment—pump, column, auto-sampler and detector—was supplied by Waters (Zellik, Belgium). A 2-column Nova-Pak® C18 (4 μm, 3.9 × 150 mm) system was used with acetonitrile-acetone (37.5:62.5, v:v) as mobile phase at a flow rate of 1.2 mL/min. Samples were dissolved in chloroform/methanol (5:5, v:v); a differential refractometer was utilized for the detection. The chromatograms were processed using Empower Pro software, with a generic Apex Track® method for integration.

The equivalent carbon number (ECN) was applied to predict the elution order. ECN = CN-2(DB), where CN is the total carbon number and DB is the total number of

Table 1 Physical and chemical characteristics (including TAG distribution and degree of interesterification (DI) based on TAG composition) of initial and CIE blend (70/30 PS/SBO, wt.%)

	FDS	CIE
%FFA (as C16:0)	0.03 ± 0.002	0.3 ± 0.006
Color (Lovibond $5^{1/4''}$)	24Y/2.4R	21Y/1.8R
α-Tocopherol (ppm)	77 ± 2.1	38 ± 2.5
α-Tocotrienol (ppm)	37 ± 0.7	17 ± 1.5
γ-Tocopherol (ppm)	212 ± 12.0	163 ± 3.5
γ-Tocotrienol (ppm)	65 ± 3.6	52 ± 2.5
δ-Tocopherol (ppm)	61 ± 0.6	57 ± 0.6
δ-Tocotrienol (ppm)	10 ± 0.6	10 ± 0.6
Total tocos (ppm)	462 ± 19.6	337 ± 11.2
Fatty acid composition (wt%)		
SFA	51.8 ± 0.07	51.8 ± 0.07
MUFA	26.4 ± 0.0	26.5 ± 0.01
PUFA	21.8 ± 0.07	21.7 ± 0.14
Total *trans*	0.11 ± 0.01	0.11 ± 0.01
Clear melting point (°C)	53.6 ± 0.06	44.5 ± 0.06

Solid fat content[a] (%)	FDS (NT)	FDS (T)	CIE (NT)	CIE (T)
0 °C	57.0 ± 0.3	53.0 ± 0.5	60.4 ± 0.3	63.7 ± 0.4
5 °C	59.7 ± 0.3	49.2 ± 0.0	64.2 ± 0.3	60.2 ± 0.1
10 °C	57.1 ± 0.1	44.1 ± 0.1	61.3 ± 0.1	52.5 ± 0.1
15 °C	52.2 ± 0.1	38.2 ± 0.1	52.5 ± 0.1	40.0 ± 0.1
20 °C	45.2 ± 0.1	35.8 ± 0.1	41.6 ± 0.1	27.4 ± 0.1
25 °C	38.1 ± 0.1	34.1 ± 0.4	30.9 ± 0.1	23.4 ± 0.3
30 °C	31.2 ± 0.1	32.3 ± 0.1	21.6 ± 0.1	20.6 ± 0.0
35 °C	25.2 ± 0.1	25.8 ± 0.1	15.4 ± 0.1	15.1 ± 0.3
40 °C	19.7 ± 0.3	20.1 ± 0.2	10.7 ± 0.2	10.2 ± 0.2
45 °C	14.1 ± 0.2	14.4 ± 0.1	5.5 ± 0.1	4.7 ± 0.1
50 °C	6.2 ± 0.1	6.7 ± 0.2	0.0 ± 0.3	0.0 ± 0.0

TAG composition[b] (wt%)	ECN	FDS	CIE	RAND
LnLL	40	2.28 ± 0.02	0.25 ± 0.01	0.21
LLL (+OLLn) [TAG A]	42	7.04 ± 0.02	1.42 ± 0.03	1.27
LLnP	42	1.01 ± 0.01	1.49 ± 0.03	1.03
OOLn	44	0.0 ± 0.0	0.32 ± 0.01	0.36
LLO	44	5.61 ± 0.02	3.17 ± 0.03	2.85
PLL (+OLM + OLnP)	44	5.68 ± 0.02	8.23 ± 0.08	7.05
PPLn (+MLP)	44	0.32 ± 0.01	2.4 ± 0.05	1.91
OOL	46	3.52 ± 0.03	4.19 ± 0.03	4.28
OLP	46	7.95 ± 0.06	15.14 ± 0.08	14.08
PPL (+OOM + MPO) [TAG B]	46	6.02 ± 0.04	14.15 ± 0.05	14.29
MPP	46	1.22 ± 0.02	0.73 ± 0.02	0.79
OOO (+OLS)	48	2.42 ± 0.04	1.72 ± 0.03	3.04
POO (+PSL)	48	10.92 ± 0.09	10.55 ± 0.07	11.89
POP	48	20.83 ± 0.15	18.88 ± 0.13	17.36
PPP	48	16.61 ± 0.02	9.62 ± 0.03	10.87
SOO	50	1.04 ± 0.05	0.67 ± 0.02	0.92
PSO	50	3.59 ± 0.03	3.42 ± 0.04	3.46
SPP	50	3.3 ± 0.02	2.88 ± 0.03	3.25

Table 1 continued

TAG composition[b] (wt%)	ECN	FDS	CIE	RAND
SOS (+PArO)	52	0.27 ± 0.01	0.24 ± 0.01	0.47
PPAr (+SSP)	52	0.37 ± 0.06	0.54 ± 0.08	0.61
SSS		21.5 ± 0.12	13.77 ± 0.16	15.52
SUS		31.03 ± 0.24	39.09 ± 0.28	37.49
SUU		26.6 ± 0.23	36.08 ± 0.28	34.97
UUU		20.87 ± 0.13	11.07 ± 0.14	12.01
Partial acylglycerols (%)		3.6 ± 0.05	5.1 ± 0.08	/
DI based on TAG composition (%)		0.0	98.9	100

FDS feedstock, *CIE* chemically interesterified blend, *RAND* theoretical random triacylglycerol composition, calculated from the fatty acid composition, *ECN* equivalent carbon number, *SSS* tri-saturated triacylglycerols, *SUS* di-saturated triacylglycerols, *SUU* di-unsaturated triacylglycerols, *UUU* tri-unsaturated triacylglycerols, [*TAG A*] TAG or group of TAG that decreases the most during interesterification, [*TAG B*] TAG or group of TAG that increases the most during interesterification, *M* myristic acid, *L* linoleic acid, *Ln* linolenic acid, *P* palmitic acid, *S* stearic acid, *O* oleic acid, *Ar* arachidic acid

[a] Determined by NMR according to IUPAC serial method 2.150: *NT* non tempered method and *T* tempered method

[b] Between brackets, TAG possibly formed during interesterification

Table 2 Groups of TAGs (%) before and after CIE (%)

	FDS	CIE
Group 1 (>10%)	PPP	POP
	POP	PPP
	POO	POO
		PPL
		OLP
Group 2 (5–10%)	PPL	PLL
	OLP	
	PLL	
	LLO	
	LLL	
Group 3 (<5%)	PSO, OOL, PPAr, LnLL, OOO, SOO, MPP, LLnP, PPLn, SOS, SPP and OOLn.	PSO, OOL, PPAr, LnLL, OOO, SOO, MPP, LLnP, PPLn, SOS, SPP, OOLn, LLL and LLO

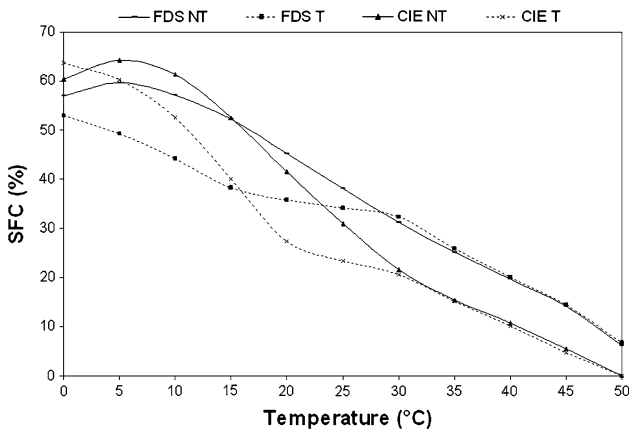

Fig. 1 Solid fat content profiles (*T* tempered and *NT* non tempered serial method) of PS/SBO blend (70/30, wt%) before and after chemical interesterification (CIE). *FDS* feedstock

double bonds on the fatty acids. Individual peaks were identified by comparing the retention time with that of references. The values are the means of 3 analyses, reported with standard deviations.

Evaluation Methods of Interesterification Degree

Two methods were used to evaluate the degree of interesterification (DI): the first based on the TAG composition and the second on the SFC profile.

The method based on TAG composition consists in comparing the theoretical random TAG composition with the experimentally determined TAG compositions before and after interesterification. Theoretical random TAG composition was calculated based on FA composition (mol%) of the fat blend by applying the law of probability (assuming 100% randomization) (Desmet Ballestra Internal Programme). DI was calculated as described in the following Eq. 1:

$$\mathrm{DI}\ (\%) = 100 * [(\mathrm{A/B})_{\mathrm{FDS}} - (\mathrm{A/B})_{\mathrm{CIE\ or\ EIE}}] / [(\mathrm{A/B})_{\mathrm{FDS}} - (\mathrm{A/B})_{\mathrm{RAND}}]$$

where DI is the degree of interesterification, A is the TAG or group of TAG that decreases the most during interesterification, B is the TAG or group of TAG that increases the most during interesterification, FDS is the feedstock,

Table 3 Physical and chemical characteristics (including TAG distribution and degree of interesterification (DI) based on TAG composition) of initial and B-EIE blends (70/30 PS/SBO, wt%)

	FDS	B-EIE		
		1 h	2 h	3 h
%FFA (as C16:0)	0.03 ± 0.002	/	/	/
Color (Lovibond $5^{1/4''}$)	24Y/2.4R	/	/	/
α-Tocopherol (ppm)	77 ± 2.1	76 ± 0.9	74 ± 1.3	73 ± 2.0
α-Tocotrienol (ppm)	37 ± 0.7	37 ± 3.6	38 ± 4.2	38 ± 5.5
γ-Tocopherol (ppm)	212 ± 12.0	212 ± 7.2	208 ± 7.8	199 ± 9.4
γ-Tocotrienol (ppm)	65 ± 3.6	62 ± 2.7	61 ± 2.4	62 ± 2.0
δ-Tocopherol (ppm)	61 ± 0.6	62 ± 0.3	60 ± 2.2	58 ± 1.2
δ-Tocotrienol (ppm)	10 ± 0.6	9 ± 0.2	9 ± 0.5	9 ± 0.5
Total tocos (ppm)	462 ± 19.6	458 ± 14.9	450 ± 18.4	439 ± 20.6
Fatty acid composition (wt%)				
SFA	51.8 ± 0.07	/	/	/
MUFA	26.4 ± 0.0			
PUFA	21.8 ± 0.07			
Total *trans*	0.11 ± 0.01			
Clear melting point (°C)	53.6 ± 0.06	50.7 ± 0.07	44.8 ± 0.0	44.4 ± 0.07

Solid fat content[a] (%)	K^k (h^{-1})	FDS (NT)	FDS (T)	1 h (NT)	1 h (T)	2 h (NT)	2 h (T)	3 h (NT)	3 h (T)
0 °C	0.21	57.0 ± 0.3	53.0 ± 0.5	55.0 ± 0.3	51.8 ± 0.2	53.9 ± 0.1	55.7 ± 0.3	53.8 ± 0.3	59.0 ± 0.1
5 °C	0.24	59.7 ± 0.3	49.2 ± 0.0	59.7 ± 0.1	48.0 ± 0.1	59.5 ± 0.3	52.0 ± 0.1	60.0 ± 0.3	55.4 ± 0.2
10 °C	0.26	57.1 ± 0.1	44.1 ± 0.1	57.8 ± 0.1	40.6 ± 0.1	57.9 ± 0.1	47.0 ± 0.1	58.5 ± 0.0	48.5 ± 0.2
15 °C	/	52.2 ± 0.1	38.2 ± 0.1	50.4 ± 0.1	33.9 ± 0.1	49.1 ± 0.1	39.3 ± 0.2	50.0 ± 0.0	40.4 ± 0.0
20 °C	/	45.2 ± 0.1	35.8 ± 0.1	42.1 ± 0.5	28.8 ± 0.2	39.7 ± 0.3	29.3 ± 0.1	40.8 ± 0.0	30.2 ± 0.1
25 °C	1.60	38.1 ± 0.1	34.1 ± 0.4	32.4 ± 0.1	26.7 ± 0.3	30.1 ± 0.1	26.0 ± 0.3	30.6 ± 0.3	25.4 ± 0.2
30 °C	1.19	31.2 ± 0.1	32.3 ± 0.1	24.6 ± 0.2	25.0 ± 0.1	22.0 ± 0.1	23.1 ± 0.2	22.4 ± 0.2	22.3 ± 0.2
35 °C	0.98	25.2 ± 0.1	25.8 ± 0.1	18.7 ± 0.0	19.2 ± 0.2	16.4 ± 0.1	16.4 ± 0.3	15.2 ± 0.2	19.1 ± 0.3
40 °C	1.37	19.7 ± 0.3	20.1 ± 0.2	13.6 ± 0.1	12.8 ± 0.1	10.4 ± 0.2	11.1 ± 0.1	10.5 ± 0.4	10.4 ± 0.1
45 °C	1.24	14.1 ± 0.2	14.4 ± 0.1	8.6 ± 0.1	7.7 ± 0.1	6.1 ± 0.3	6.2 ± 0.1	5.7 ± 0.3	5.6 ± 0.1
50 °C	/	6.2 ± 0.1	6.7 ± 0.2	0.0 ± 0.0	1.4 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.5 ± 0.1

TAG composition[b] (wt%)	FDS	B-EIE		
		1 h	2 h	3 h
LnLL	2.28 ± 0.02	1.15 ± 0.02	0.63 ± 0.01	0.43 ± 0.02
LLL (+OLLn) [TAG A]	7.04 ± 0.02	3.89 ± 0.01	2.5 ± 0.01	1.89 ± 0.01
LLnP	1.01 ± 0.01	1.44 ± 0.02	1.53 ± 0.01	1.59 ± 0.01
OOLn	0.0 ± 0.0	0.13 ± 0.01	0.22 ± 0.01	0.26 ± 0.01
LLO	5.61 ± 0.02	4.2 ± 0.02	3.6 ± 0.01	3.29 ± 0.01

Table 3 continued

TAG composition[b] (wt%)	FDS	B-EIE		
		1 h	2 h	3 h
PLL (+OLM + OLnP)	5.68 ± 0.02	7.49 ± 0.02	8.25 ± 0.01	8.37 ± 0.01
PPLn (+MLP)	0.32 ± 0.01	1.32 ± 0.01	1.96 ± 0.01	2.14 ± 0.01
OOL	3.52 ± 0.03	3.69 ± 0.02	3.96 ± 0.01	3.88 ± 0.01
OLP	7.95 ± 0.06	11.89 ± 0.04	13.9 ± 0.05	14.75 ± 0.02
PPL (+OOM + MPO) [TAG B]	6.02 ± 0.04	10.47 ± 0.05	12.65 ± 0.08	13.84 ± 0.06
MPP	1.22 ± 0.02	0.88 ± 0.03	0.8 ± 0.01	0.61 ± 0.01
OOO (+OLS)	2.42 ± 0.04	1.89 ± 0.02	1.76 ± 0.01	1.56 ± 0.02
POO (+PSL)	10.92 ± 0.09	10.9 ± 0.08	10.74 ± 0.08	10.53 ± 0.06
POP	20.83 ± 0.15	20.26 ± 0.13	19.64 ± 0.1	19.62 ± 0.13
PPP	16.61 ± 0.02	12.37 ± 0.09	10.29 ± 0.07	9.72 ± 0.09
SOO	1.04 ± 0.05	0.83 ± 0.02	0.71 ± 0.01	0.65 ± 0.01
PSO	3.59 ± 0.03	3.67 ± 0.02	3.55 ± 0.04	3.62 ± 0.04
SPP	3.3 ± 0.02	2.88 ± 0.02	2.66 ± 0.02	2.68 ± 0.02
SOS (+PArO)	0.27 ± 0.01	0.26 ± 0.02	0.25 ± 0.01	0.26 ± 0.01
PPAr (+SSP)	0.37 ± 0.06	0.39 ± 0.01	0.39 ± 0.11	0.31 ± 0.1
SSS	21.5 ± 0.12	16.52 ± 0.15	14.14 ± 0.21	13.32 ± 0.22
SUS	31.03 ± 0.24	35.98 ± 0.23	38.05 ± 0.24	39.48 ± 0.25
SUU	26.6 ± 0.23	32.55 ± 0.18	35.13 ± 0.16	35.89 ± 0.11
UUU	20.87 ± 0.13	14.95 ± 0.1	12.67 ± 0.06	11.31 ± 0.08
Partial acylglycerols (%)	3.6 ± 0.05	4.5 ± 0.05	4.0 ± 0.08	4.6 ± 0.06
DI based on TAG composition (%)	0.0	73.8	89.9	95.6

	B-EIE				
	4 h	5 h	6 h	7 h	8 h
%FFA (as C16:0)	/	/	/	/	/
Color (Lovibond $5^{1/4''}$)	/	/	/	/	29Y/2.7R
α-Tocopherol (ppm)	73 ± 1.8	71 ± 0.7	71 ± 1.0	71 ± 1.2	72 ± 2.5
α-Tocotrienol (ppm)	37 ± 4.8	37 ± 4.6	37 ± 5.6	36 ± 4.6	37 ± 5.7
γ-Tocopherol (ppm)	203 ± 8.2	199 ± 7.9	198 ± 9.3	197 ± 8.2	200 ± 7.8
γ-Tocotrienol (ppm)	62 ± 2.7	62 ± 2.6	60 ± 3.4	60 ± 2.4	60 ± 3.6
δ-Tocopherol (ppm)	58 ± 0.8	57 ± 3.9	55 ± 3.4	55 ± 1.8	55 ± 2.5
δ-Tocotrienol (ppm)	9 ± 0.3	9 ± 0.4	9 ± 0.5	8 ± 0.8	8 ± 2.1
Total tocols (ppm)	442 ± 18.6	435 ± 20.1	430 ± 23.2	427 ± 19.0	432 ± 24.2

Table 3 continued

	B-EIE				
	4 h	5 h	6 h	7 h	8 h
Fatty acid composition (wt%)					
SFA	/	/	/	/	51.5 ± 0.14
MUFA					26.4 ± 0.01
PUFA					22.0 ± 0.07
Total *trans*					0.11 ± 0.0
Clear melting point (°C)	44.2 ± 0.07	44.2 ± 0.07	44.2 ± 0.0	44.1 ± 0.0	44.4 ± 0.07

Solid fat content[a] (%)	K^k (h^{-1})	4 h (NT)	4 h (T)	5 h (NT)	5 h (T)	6 h (NT)	6 h (T)	7 h (NT)	7 h (T)	8 h (NT)	8 h (T)
0 °C	0.21	55.2 ± 0.2	60.3 ± 0.3	56.7 ± 0.2	61.2 ± 0.5	56.0 ± 0.3	62.1 ± 0.3	57.4 ± 0.4	61.7 ± 0.3	57.8 ± 0.2	62.5 ± 0.1
5 °C	0.24	61.5 ± 0.2	56.5 ± 0.1	62.9 ± 0.2	58.4 ± 0.1	62.4 ± 0.5	58.4 ± 0.1	62.9 ± 0.4	59.0 ± 0.1	63.2 ± 0.2	57.9 ± 0.1
10 °C	0.26	59.5 ± 0.2	50.0 ± 0.1	60.6 ± 0.1	51.4 ± 0.1	60.7 ± 0.1	51.2 ± 0.1	60.9 ± 0.1	51.7 ± 0.1	60.8 ± 0.1	50.9 ± 0.1
15 °C	/	51.2 ± 0.1	40.2 ± 0.0	51.8 ± 0.1	41.5 ± 0.1	51.5 ± 0.2	40.2 ± 0.0	52.1 ± 0.2	40.7 ± 0.0	52.4 ± 0.1	39.2 ± 0.0
20 °C	/	42.0 ± 0.0	28.7 ± 0.1	42.8 ± 0.2	30.7 ± 0.2	41.4 ± 0.0	29.8 ± 0.1	42.0 ± 0.1	29.7 ± 0.1	42.8 ± 0.1	27.9 ± 0.1
25 °C	1.60	31.7 ± 0.0	24.6 ± 0.4	32.4 ± 0.1	25.7 ± 0.3	31.0 ± 0.1	25.1 ± 0.1	31.7 ± 0.1	25.6 ± 0.3	32.1 ± 0.1	24.0 ± 0.0
30 °C	1.19	22.5 ± 0.1	21.3 ± 0.1	23.1 ± 0.1	22.5 ± 0.1	22.0 ± 0.1	22.0 ± 0.2	22.4 ± 0.2	22.2 ± 0.2	22.7 ± 0.0	21.6 ± 0.3
35 °C	0.98	15.4 ± 0.1	15.1 ± 0.1	15.4 ± 0.2	15.5 ± 0.2	15.3 ± 0.0	15.9 ± 0.3	15.2 ± 0.1	15.5 ± 0.2	15.9 ± 0.1	15.5 ± 0.4
40 °C	1.37	10.7 ± 0.5	10.1 ± 0.1	9.9 ± 0.3	10.2 ± 0.2	10.5 ± 0.5	10.3 ± 0.2	10.4 ± 0.1	9.9 ± 0.1	10.3 ± 0.1	10.6 ± 0.1
45 °C	1.24	5.3 ± 0.0	4.7 ± 0.2	5.1 ± 0.1	5.1 ± 0.1	5.1 ± 0.4	5.3 ± 0.2	5.1 ± 0.2	5.4 ± 0.3	5.4 ± 0.0	5.1 ± 0.2
50 °C	/	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.4	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.1	0.0 ± 0.3	0.1 ± 0.1	0.0 ± 0.1	0.3 ± 0.0

TAG composition[b] (wt%)	B-EIE					
	4 h	5 h	6 h	7 h	8 h	RAND
LnLL	0.33 ± 0.02	0.29 ± 0.01	0.27 ± 0.02	0.29 ± 0.02	0.26 ± 0.02	0.21
LLL (+OLLn) [TAG A]	1.6 ± 0.02	1.49 ± 0.01	1.51 ± 0.02	1.47 ± 0.02	1.5 ± 0.02	1.27
LLnP	1.56 ± 0.05	1.59 ± 0.03	1.54 ± 0.02	1.5 ± 0.03	1.53 ± 0.02	1.03
OOLn	0.28 ± 0.02	0.32 ± 0.02	0.31 ± 0.01	0.29 ± 0.02	0.29 ± 0.02	0.36
LLO	3.16 ± 0.01	3.18 ± 0.02	3.19 ± 0.02	3.17 ± 0.01	3.22 ± 0.01	2.85
PLL (+OLM + OLnP)	8.38 ± 0.05	8.45 ± 0.03	8.33 ± 0.02	8.22 ± 0.03	8.35 ± 0.02	7.05
PPLn (+MLP)	2.2 ± 0.02	2.4 ± 0.04	2.41 ± 0.01	2.47 ± 0.01	2.4 ± 0.01	1.91
OOL	4.02 ± 0.01	4.18 ± 0.02	4.2 ± 0.02	4.27 ± 0.02	4.28 ± 0.02	4.28
OLP	15.05 ± 0.1	15.23 ± 0.09	15.16 ± 0.08	15.1 ± 0.06	15.42 ± 0.04	14.08
PPL (+OOM + MPO) [TAG B]	14.24 ± 0.08	14.27 ± 0.08	14.33 ± 0.07	14.31 ± 0.09	14.29 ± 0.08	14.29
MPP	0.66 ± 0.01	0.74 ± 0.01	0.66 ± 0.01	0.77 ± 0.01	0.69 ± 0.01	0.79
OOO (+OLS)	1.6 ± 0.04	1.72 ± 0.03	1.6 ± 0.08	1.73 ± 0.02	1.66 ± 0.02	3.04
POO (+PSL)	10.52 ± 0.14	10.58 ± 0.13	10.39 ± 0.09	10.48 ± 0.12	10.53 ± 0.09	11.89
POP	19.32 ± 0.11	18.99 ± 0.13	19.06 ± 0.1	18.97 ± 0.13	18.76 ± 0.24	17.36
PPP	9.55 ± 0.01	9.29 ± 0.01	9.46 ± 0.01	9.52 ± 0.01	9.37 ± 0.01	10.87

Table 3 continued

TAG composition[b] (wt%)	B-EIE					RAND
	4 h	5 h	6 h	7 h	8 h	
SOO	0.66 ± 0.04	0.67 ± 0.02	0.67 ± 0.03	0.68 ± 0.02	0.72 ± 0.01	0.92
PSO	3.53 ± 0.03	3.4 ± 0.04	3.53 ± 0.04	3.45 ± 0.04	3.47 ± 0.08	3.46
SPP	2.63 ± 0.01	2.59 ± 0.01	2.74 ± 0.01	2.65 ± 0.01	2.64 ± 0.01	3.25
SOS (+PArO)	0.31 ± 0.0	0.24 ± 0.01	0.28 ± 0.02	0.23 ± 0.01	0.27 ± 0.01	0.47
PPAr (+SSP)	0.35 ± 0.1	0.39 ± 0.09	0.33 ± 0.08	0.41 ± 0.04	0.33 ± 0.02	0.61
SSS	13.19 ± 0.13	13.01 ± 0.12	13.19 ± 0.11	13.35 ± 0.07	13.03 ± 0.05	15.52
SUS	39.66 ± 0.24	39.3 ± 0.3	39.61 ± 0.24	39.43 ± 0.28	39.19 ± 0.42	37.49
SUU	36.17 ± 0.29	36.52 ± 0.3	36.09 ± 0.24	35.98 ± 0.26	36.55 ± 0.18	34.97
UUU	10.99 ± 0.13	11.18 ± 0.11	11.08 ± 0.17	11.22 ± 0.11	11.21 ± 0.11	12.01
Partial acylglycerols (%)	4.8 ± 0.07	4.0 ± 0.04	5.0 ± 0.07	4.7 ± 0.06	4.3 ± 0.04	/
DI based on TAG composition (%)	97.8	98.6	98.5	98.7	98.5	100

FDS feedstock, *RAND* theoretical random triacylglycerol composition, calculated from the fatty acid composition, [*TAG A*] TAG or group of TAG that decreases the most during interesterification, [*TAG B*] TAG or group of TAG that increases the most during interesterification, *SSS* tri-saturated triacylglycerols, *SUS* di-saturated triacylglycerols, *SUU* di-unsaturated triacylglycerols, *UUU* tri-unsaturated triacylglycerols, *B-EIE* batch enzymatically interesterified blend, *k* reaction rate based on %SFC at different temperatures

CIE is the chemically interesterified blend, EIE is the enzymatically interesterified blend, and RAND is the theoretical random TAG composition.

During B-EIE, the degree of interesterification was also calculated with regards to % SFC at different temperatures [23] as defined by Eq. 2:

$$\mathrm{DI}(\%) = 100 * [(\mathrm{SFC}_0 - \mathrm{SFC})/= (\mathrm{SFC}_0 - \mathrm{SFC}_\infty)] \quad (2)$$

where DI is the conversion degree, SFC_0 is the SFC at time 0 (in the FDS), SFC is the SFC at batch reaction time t and SFC_∞ is the SFC at the equilibrium stage (after CIE).

Reaction rate k was derived from both approaches (TAG and SFC) following Eq. 3:

$$\mathrm{DI}(\%) = 100 * (1 - \mathrm{e}^{-kt}) \quad (3)$$

Results and Discussion

Chemical Interesterification (CIE)

A blend of palm stearin and soybean oil (70/30, wt.%) was chemically interesterified according to what is described in "Experimental Procedures".

The initial blend and the chemically interesterified product were analyzed and compared for fatty acid composition, triacylglycerol distribution, clear melting point, solid fat content profile (tempered and non tempered methods), acidity, diacylglycerol content, free tocopherols and color (Table 1).

Fatty acid composition of the initial blend was about 52% saturated, 26% mono-unsaturated and 22% poly-unsaturated; the composition was not affected by the interesterification process neither the *trans* content which remained around 0.1%.

TAGs of the blend were classified into three main groups (Table 2). The main TAG components (POP, PPP and POO) were arbitrarily put in group 1 (>10% wt.%); group 2 (5–10% wt%) was made of OLP, LLL, PPL, PLL and LLO and group 3 (>5% wt.%) of PSO, OOL, PPAr, LnLL, OOO, SOO, MPP, LLnP, PPLn, SOS, SPP and OOLn.

Within group 1, POO was practically untouched after CIE while PPP was seriously decreased; POP was only decreased by less than 10%. POP, PPP and POO still belonged to the same group after CIE.

In group 2, PPL, OLP and PLL were increased while LLL and LLO were decreased. PPL and OLP went up as part of group 1 while LLL and LLO went down to group 3; PLL stayed in group 2. All the TAG components classified in group 3 stayed in this group after CIE. PPL [TAG B] (that was moving up to group 1) and LLL [TAG A] (that was moving down to group 3) were observed to

be the most varying TAG components during the chemical interesterification process. They were followed and compared with the theoretical composition (RAND, supposed to be fully randomized) by using the composition of the initial blend (FDS) as reference in order to calculate the degree of interesterification (DI). The oil was assumed to be fully randomized with a DI close to 100% (98.9%). The main result of modification of TAG profile consecutive to CIE was a decrease in the clear melting point of the final product of about 9 °C (from 53.6 to 44.5 °C).

The solid fat content profile (non-tempered method) of the interesterified product was higher than the one of the initial blend but only below 15 °C; at higher temperatures, the interesterified product was softer (3–4% in SFC). The same behavior was observed by using the tempered method but to a larger extent (8–11% in SFC) (Fig. 1). Similar results were observed in other studies [13, 24]. According to Zhang et al. [13], in a blend of palm stearin/coconut oil, SFC was effectively decreased within the temperature range of 35–40 °C and increased at temperatures lower than 30 °C. Ahmadi et al. [24] indicated that the SFC of CIE blends was higher compared to the non interesterified samples at low temperatures (0–40 °C) and lower at high temperatures (20–70 °C), in blends of high-oleic sunflower and fully hydrogenated canola oils. Other studies reported a decrease in SFC upon interesterification [1, 5, 18, 20].

The acidity (expressed in C16:0) of the CIE product went up to 0.3% and the observed partial acylglycerol content was significantly increased. No soaps were detected.

The free tocopherol content of the chemically interesterified oil was highly affected as the result of esterification of the hydroxyl group; it is interesting to note that mainly alpha and gamma tocopherols and tocotrienols seem to be esterified while delta components are protected. Decrease of alpha (the most biologically active) and gamma (the most antioxidant) tocopherols and tocotrienols at the benefit of esterified analogues are both detrimental for the oxidative stability of the final product.

The color of the chemically interesterified oil was slightly decreased as the result of the bleaching.

Batch Enzymatic Interesterification (B-EIE)

Sampling was carried out every hour and the main quality parameters (triacylglycerol distribution, fatty acid composition, clear melting point, solid fat content profile, acidity, partial acylglycerol content, free tocopherols and color) were evaluated and compared with the initial blend (Table 3).

Variation of TAG distribution (EIE) was followed and compared with the theoretical composition (RAND, supposed to be fully randomized) by using the composition of the initial blend (FDS) as reference. PPL [TAG B] that was increasing the most during the CIE (see above) and LLL [TAG A] that was decreasing the most during CIE (see above) were taken into consideration to calculate the degree of interesterification (DI) with respect of time. This DI was increasing substantially during the first 4 h and reached equilibrium at around 100% during the next 4 h. In this final period, the degree of interesterification (and hence the TAG distribution) became very close to the value calculated for CIE meaning that a complete random redistribution was apparently obtained. SSS and UUU contents were decreasing very equally, the same as SUS and SUU which were increasing also very similarly (Fig. 2). This finding is consistent with previous studies [1, 24]. Petrauskaite et al. [1] observed a decrease in SSS and UUU and an increase of SUU after chemical interesterification of blends of palm stearin/soybean oil and fully hydrogenated soybean oil/soybean oil. Ahmadi et al. [24] studied chemical and enzymatic interesterification of tristearin/triolein-rich blends. They found that interesterification resulted in a decrease of UUU and SSS and an increase of SUU and SUS components.

The modifications in solid fat content profile appeared to be different below and above 15 °C (non tempered SFC profile) (Fig. 3a). As already mentioned above, the interesterified product became harder below 15 °C and softer at higher temperatures. However, in the first 2 h, the solid fat content profile decreased first at low temperature (below 5 °C) and gradually exceeded the solid fat content of the initial blend later on; variations were still observed

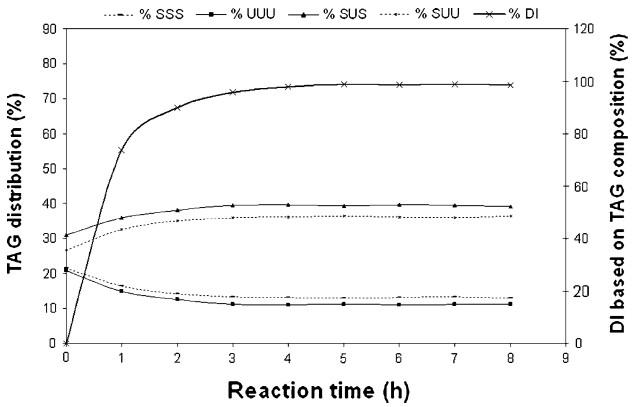

Fig. 2 Evolution of the TAG distribution and of the degree of interesterification (DI) calculated from TAG with reaction time during the B-EIE. *SSS* tri-saturated triacylglycerols, *SUS* di-saturated triacylglycerols, *SUU* di-unsaturated triacylglycerols, *UUU* tri-unsaturated triacylglycerols

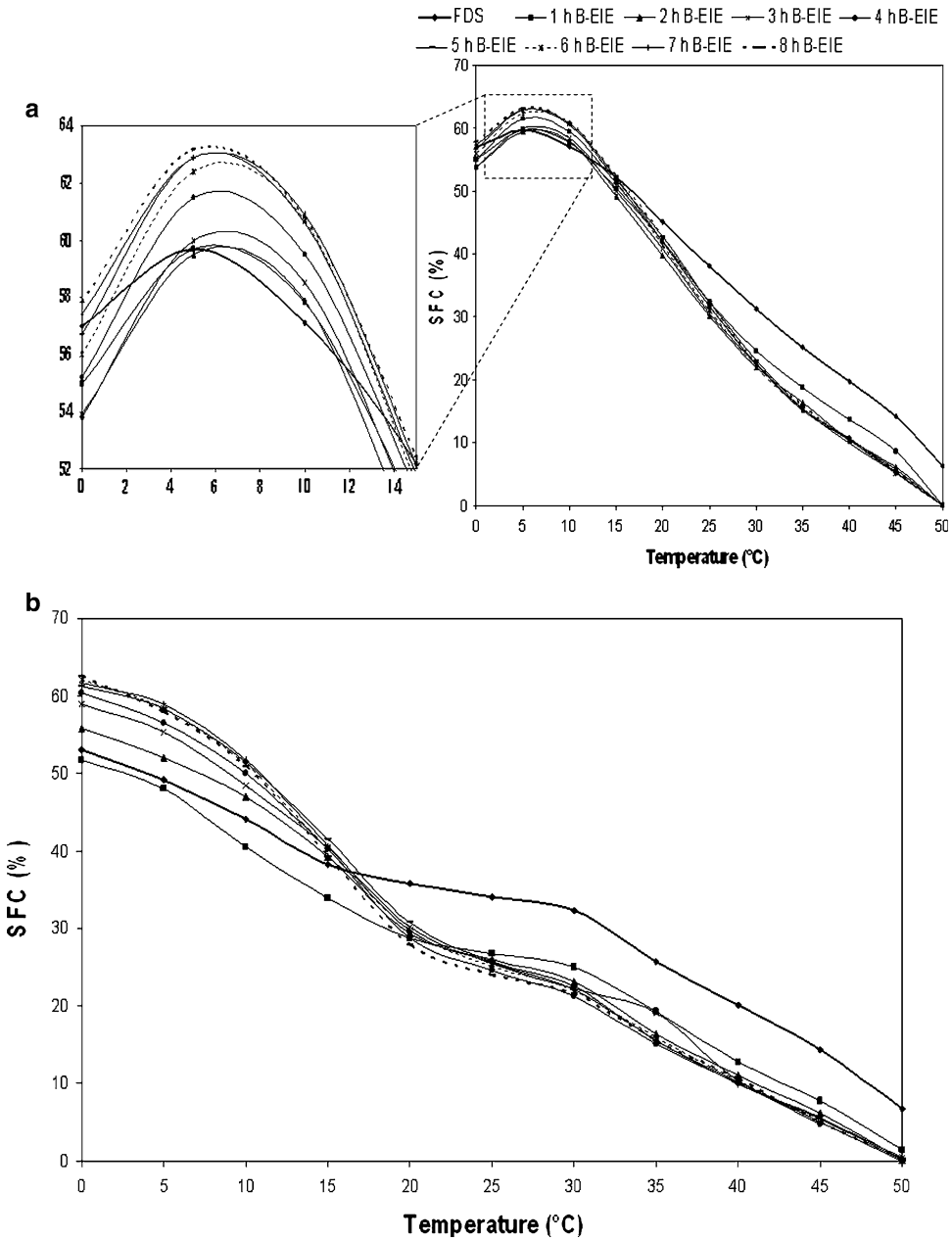

Fig. 3 Evolution with time of the SFC profile during batch enzymatic interesterification (B-EIE), non tempered (NT) (**a**) and tempered (T) (**b**) SFC methods. The inset in Fig. 3a is an enlarged part of the time range of 0–15 °C. *FDS* feedstock

between 4 and 8 h. Stabilization was clearly faster in the SFC profile above 15 °C. This behavior was also clearly observed on the tempered solid fat content profile (Fig. 3b). On the other hand, the clear melting point decreased to a stable value already after 4 h, in correlation with the variation of the solid fat content profile at temperatures above 15 °C.

Variations in SFC, principally below 15 °C, were still observed although the degree of interesterification calculated from TAG was stable, testifying that fat composition was still being modified after the 4-h reaction time. The degree of interesterification was also calculated according to the formula of Xu et al. [23] based on SFC at different temperatures (Fig. 4). The solid fat content profile of the chemically interesterified product was used as reference (equilibrium). The tempered method was selected for the calculation of the degree of interesterification. Considering the SFC profile above 15 °C, a degree of interesterification of 100% was already achieved after 4 h while a longer time was necessary to stabilize the SFC below 15 °C and achieve a degree of interesterification close to 100%. The degree of interesterification calculated from

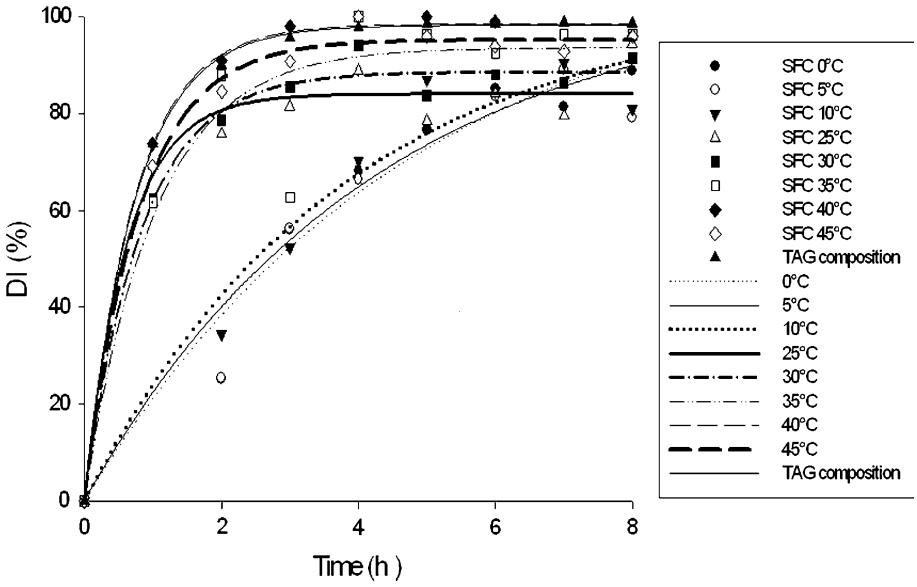

Fig. 4 Evolution of the degree of interesterification (DI) with time during B-EIE based on TAG composition and %SFC at different temperature (tempered method). For all the curves plotted, the probability value (*P*) was lower than 0.05

Table 4 Physical and chemical characteristics (including TAG distribution and degree of interesterification (DI) based on TAG composition) of initial and C-EIE blend (70/30 PS/SBO, wt%)

	FDS	C-EIE
%FFA (as C16:0)	0.03 ± 0.002	0.2 ± 0.005
Color (Lovibond $5^{1/4''}$)	24Y/2.4R	26Y/2.5R
α-Tocopherol (ppm)	77 ± 2.1	78 ± 0.9
α-Tocotrienol (ppm)	37 ± 0.7	37 ± 0.3
γ-Tocopherol (ppm)	212 ± 12.0	210 ± 3.6
γ-Tocotrienol (ppm)	65 ± 3.6	61 ± 4.4
δ-Tocopherol (ppm)	61 ± 0.6	59 ± 2.7
δ-Tocotrienol (ppm)	10 ± 0.6	9 ± 4.4
Total tocos (ppm)	462 ± 19.6	454 ± 16.3
Fatty acid composition (wt%)		
SFA	51.8 ± 0.07	50.7 ± 0.14
MUFA	26.4 ± 0.0	26.5 ± 0.01
PUFA	21.8 ± 0.07	22.6 ± 0.07
Total *trans*	0.11 ± 0.01	0.10 ± 0.0
Clear melting point (°C)	53.6 ± 0.06	44.3 ± 0.07

Solid fat content[a] (%)	FDS (NT)	FDS (T)	C-EIE (NT)	C-EIE (T)
0 °C	57.0 ± 0.3	53.0 ± 0.5	52.9 ± 0.3	61.8 ± 0.1
5 °C	59.7 ± 0.3	49.2 ± 0.0	60.6 ± 0.3	58.1 ± 0.1
10 °C	57.1 ± 0.1	44.1 ± 0.1	58.7 ± 0.1	50.9 ± 0.0
15 °C	52.2 ± 0.1	38.2 ± 0.1	50.9 ± 0.2	41.4 ± 0.0
20 °C	45.2 ± 0.1	35.8 ± 0.1	41.8 ± 0.1	29.7 ± 0.1
25 °C	38.1 ± 0.1	34.1 ± 0.4	31.8 ± 0.1	23.8 ± 0.3
30 °C	31.2 ± 0.1	32.3 ± 0.1	22.6 ± 0.1	21.0 ± 0.1
35 °C	25.2 ± 0.1	25.8 ± 0.1	15.0 ± 0.1	15.5 ± 0.1
40 °C	19.7 ± 0.3	20.1 ± 0.2	10.6 ± 0.1	10.3 ± 0.1

Table 4 continued

Solid fat content[a] (%)	FDS (NT)	FDS (T)	C-EIE (NT)	C-EIE (T)
45 °C	14.1 ± 0.2	14.4 ± 0.1	5.2 ± 0.1	5.2 ± 0.2
50 °C	6.2 ± 0.1	6.7 ± 0.2	0.0 ± 0.2	0.1 ± 0.1

TAG composition[b] (wt%)	ECN	FDS	C-EIE	RAND
LnLL	40	2.28 ± 0.02	0.31 ± 0.01	0.21
LLL (+OLLn) [TAG A]	42	7.04 ± 0.02	1.64 ± 0.02	1.27
LLnP	42	1.01 ± 0.01	1.62 ± 0.03	1.03
OOLn	44	0.0 ± 0.0	0.34 ± 0.02	0.36
LLO	44	5.61 ± 0.02	3.43 ± 0.05	2.85
PLL (+OLM + OLnP)	44	5.68 ± 0.02	8.74 ± 0.03	7.05
PPLn (+MLP)	44	0.32 ± 0.01	2.56 ± 0.01	1.91
OOL	46	3.52 ± 0.03	4.38 ± 0.02	4.28
OLP	46	7.95 ± 0.06	15.24 ± 0.05	14.08
PPL (+OOM + MPO) [TAG B]	46	6.02 ± 0.04	14.12 ± 0.09	14.29
MPP	46	1.22 ± 0.02	0.78 ± 0.02	0.79
OOO (+OLS)	48	2.42 ± 0.04	1.8 ± 0.03	3.04
POO (+PSL)	48	10.92 ± 0.09	10.57 ± 0.08	11.89
POP	48	20.83 ± 0.15	18.51 ± 0.12	17.36
PPP	48	16.61 ± 0.02	8.92 ± 0.03	10.87
SOO	50	1.04 ± 0.05	0.66 ± 0.02	0.92
PSO	50	3.59 ± 0.03	3.25 ± 0.03	3.46
SPP	50	3.3 ± 0.02	2.47 ± 0.02	3.25
SOS (PArO)	52	0.27 ± 0.01	0.23 ± 0.01	0.47
PPAr (+SSP)	52	0.37 ± 0.06	0.44 ± 0.04	0.61
SSS		21.5 ± 0.12	12.61 ± 0.11	15.52
SUS		31.03 ± 0.24	38.67 ± 0.26	37.49
SUU		26.6 ± 0.23	36.83 ± 0.21	34.97
UUU		20.87 ± 0.13	11.9 ± 0.15	12.01
Partial acylglycerols (%)		3.6 ± 0.05	3.6 ± 0.06	/
DI based on TAG composition (%)		0.0	97.5	100

FDS feedstock, *RAND* theoretical random triacylglycerol composition, calculated from the fatty acid composition, *ECN* equivalent carbon number, *SSS* tri-saturated triacylglycerols, *SUS* di-saturated triacylglycerols, *SUU* di-unsaturated triacylglycerols, *UUU* tri-unsaturated triacylglycerols, [*TAG A*] TAG or group of TAG that decreases the most during interesterification, [*TAG B*] TAG or group of TAG that increases the most during interesterification, *C-EIE* continuous enzymatically interesterified blend

TAG followed closely the one calculated from SFC above 15 °C. After a 4-h reaction time, equilibrium based on %SFC below 15 °C was not achieved while it was not reflected by the TAG distribution but well on the SCF profile above 15 °C. Modifications in the SFC profile still observable after 4 h reaction time below 15 °C could be attributed to intramolecular rearrangements (acyl migration) occurring within the SUS/SUU fraction, considering that SUS/SUU components mainly contribute to the low temperature SFC profile. Total SUS/SUU fraction not increasing anymore after 4 h; position isomers would be formed during this last period not reflected in the TAG profile. Interesterification should not be evaluated exclusively in terms of the changes occurring in particular TAGs.

The reaction rate is calculated based on %SFC at different temperatures confirmed the fact that the effect of acyl migration was sensitive at low temperature SFC (Table 3) where the reaction rate was lower (about 0.2–0.3 h^{-1}). This reaction rate increased at higher temperature SFC (1.2–1.6 h^{-1}), with a maximum of 1.6 h^{-1} at 25 °C.

A lower amount of partial acylglycerols was formed at the end of B-EIE compared to CIE. This amount only depends on the quantity of water initially present in the system.

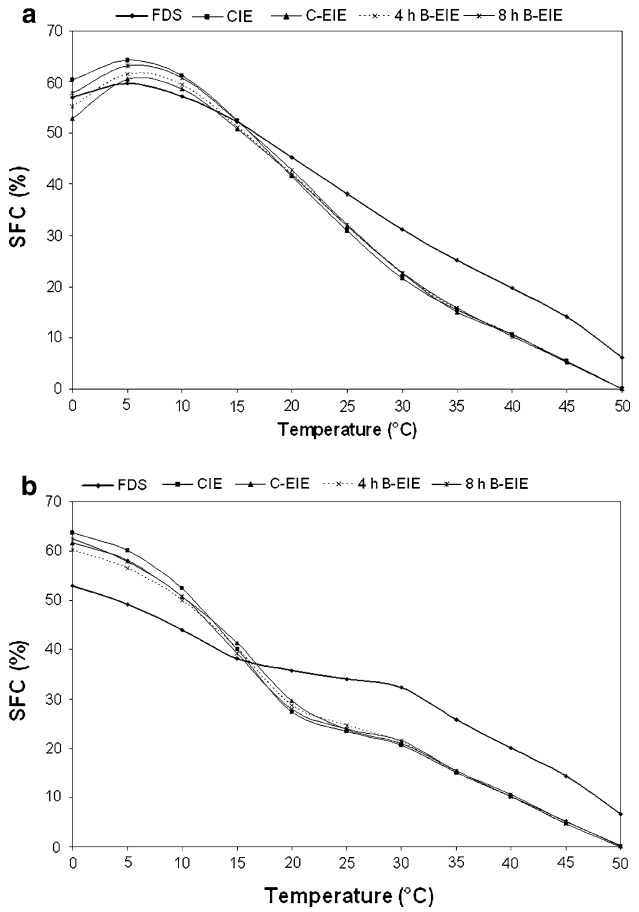

Fig. 5 Solid fat content profiles of PS/SBO (70/30, wt%) blends before and after interesterification, non tempered (NT) (**a**) and tempered (T) (**b**) SFC methods. *FDS* feedstock, *CIE* chemically interesterified blend, *B-EIE* batch enzymatically interesterified blend, *C-EIE* continuous enzymatically interesterified blend

Unlike chemical interesterification, the free tocopherol content of the interesterified oil was not greatly affected by the enzymatic treatment. This preservation is highly beneficial for the oxidative stability and vitamin properties of the oil.

At the end of the batch enzymatic interesterification, the color expressed in Lovibond was similar to the one of the feedstock. No bleaching was necessary to restore the color.

Continuous Enzymatic Interesterification (C-EIE)

Continuous enzymatic interesterification was conducted at 300 g/h, which is an acceptable flow rate with respect to industrial applications. In such conditions, the degree of interesterification calculated from TAG was close to 100% (97.5%). Quality parameters were also evaluated and compared with the initial blend (Table 4).

Table 5 Physical and chemical characteristics of fully refined CIE, C-EIE and 8 h B-EIE blends (70/30 PS/SBO, wt%)

	RBD CIE	RBD C-EIE	RBD 8 h B-EIE
%FFA (as C16:0)	0.02 ± 0.004	0.02 ± 0.005	0.03 ± 0.002
α-Tocopherol (ppm)	30 ± 1.2	60 ± 1.5	54 ± 0.8
α-Tocotrienol (ppm)	16 ± 4.2	30 ± 3.8	28 ± 4.6
γ-Tocopherol (ppm)	85 ± 8.4	151 ± 7.8	136 ± 9.4
γ-Tocotrienol (ppm)	31 ± 2.4	47 ± 3.6	42 ± 2.0
δ-Tocopherol (ppm)	13 ± 0.6	30 ± 1.2	23 ± 0.8
δ-Tocotrienol (ppm)	0 ± 0.0	3 ± 0.2	2 ± 0.3
Total tocos (ppm)	175 ± 16.8	321 ± 18.1	285 ± 17.9
Fatty Acid Composition (wt%)			
SFA	51.9 ± 0.07	51.1 ± 0.14	51.7 ± 0.07
MUFA	26.5 ± 0.0	26.5 ± 0.01	26.5 ± 0.07
PUFA	21.6 ± 0.07	22.5 ± 0.14	21.8 ± 0.01
Total *trans*	0.16 ± 0.0	0.15 ± 0.1	0.14 ± 0.0
Partial acylglycerols (%)	4.8 ± 0.7	3.5 ± 0.4	4.2 ± 0.7
Clear melting point (°C)	44.5 ± 0.07	44.1 ± 0.07	44.5 ± 0.07
Solid fat content[a] (%)			
0 °C	62.0 ± 0.2	57.3 ± 0.2	61.0 ± 0.3
5 °C	66.0 ± 0.2	63.1 ± 0.2	66.0 ± 0.3
10 °C	63.0 ± 0.1	60.0 ± 0.1	62.8 ± 0.1
15 °C	53.8 ± 0.1	51.2 ± 0.0	53.4 ± 0.1
20 °C	42.9 ± 0.3	41.5 ± 0.0	43.3 ± 0.1
25 °C	32.1 ± 0.1	30.9 ± 0.2	32.4 ± 0.1
30 °C	22.4 ± 0.2	21.8 ± 0.1	23.0 ± 0.1
35 °C	15.6 ± 0.1	15.1 ± 0.1	16.1 ± 0.1
40 °C	10.7 ± 0.1	10.3 ± 0.4	10.7 ± 0.3
45 °C	5.6 ± 0.3	5.1 ± 0.2	5.4 ± 0.3
50 °C	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.2

CIE chemically interesterified blend, *C-EIE* continuous enzymatically interesterified blend, *B-EIE* batch enzymatically interesterified blend

[a] Determined by NMR according to IUPAC method 2.150 (non-tempered method)

The solid fat content profile of the C-EIE oil was similar to the one after CIE and to the one of the B-EIE, for temperatures above 15 °C (Fig. 5a, b). The clear melting point after C-EIE was also similar to the one after CIE and after 8-h B-EIE. In contrast, below 15 °C, the SFC profile was lower than the one of the CIE (supposed to be fully randomized) and close to the one obtained after 4 h of B-EIE. We could conclude that the continuously interesterified oil was not fully randomized, probably as the result of a too short residence time in the enzymatic packed-bed which did not allow final acyl migration to occur.

Quality parameters such as tocopherol content, final acidity, partial acylglycerol and color, were slightly better

during the C-EIE than during the B-EIE, because of the shorter oil–enzyme contact time in the continuous fixed bed process compared to the batch reaction. This justifies the industrial employ of continuous operation in EIE. After any of those enzymatic processes, the resulting oil presented better quality parameters compared to the chemically interesterified oil.

If we consider products issued from oil hydrolysis (FFA and partial acylglycerols), lower contents were found after C-EIE compared to CIE. The percentage of these hydrolytic products is an important quality criterion, because they cause low yield of the final product since, as volatile compounds, they will be removed during deodorization. Ledóchowska and Wilczyńska [18] showed that the presence of a non-TAG fraction in the interesterification products lowers their resistance to oxidation, because of their pro-oxidative properties, contribute to the deterioration of product quality by rancidity [25]. Moreover, low content of DAG shows earlier crystallization onset, faster and improved crystallization rate, higher SFC which enables the addition of more liquid oil, improved post hardening process and better baking performance [26]. It is then important to keep this non-TAG fraction as low as possible. On the other hand, it was shown that DAG [26] can be regarded as beneficial since they can stabilize β-polymorphic crystals in margarine-containing hydrogenated rapeseed and soybean oils.

Refining of Interesterified Blends

CIE, B-EIE (8 h) and C-EIE oils were deodorized according to the procedure described above. Interesterified products were compared (Table 5).

The free fatty acid content was lowered and reverted to normal values.

A slight increase in the *trans*-fatty acids during post-deodorization was observed, from 0.11% in the feedstock to 0.14–0.15%, which is acceptable after deodorization.

No significant reduction of partial acylglycerols was observed, assuming that mainly DAGs were formed during the interesterification process.

Degradation of free tocopherols after post-deodorization was more pronounced with CIE compared to the deodorized EIE. Risk of oxidative damage after EIE is less than after CIE, which is in accordance with Chu et al. [3] observations.

In terms of SFC profile (non tempered method), the removal of FFA during deodorization made the interesterified fat slightly harder at low temperature, whatever the interesterification process.

Besides the better quality of the EIE with regard to the CIE, the fats produced by lipase-catalyzed continuous interesterification are slightly different from the chemically randomized products, mainly at low temperature SFC (Fig. 6).

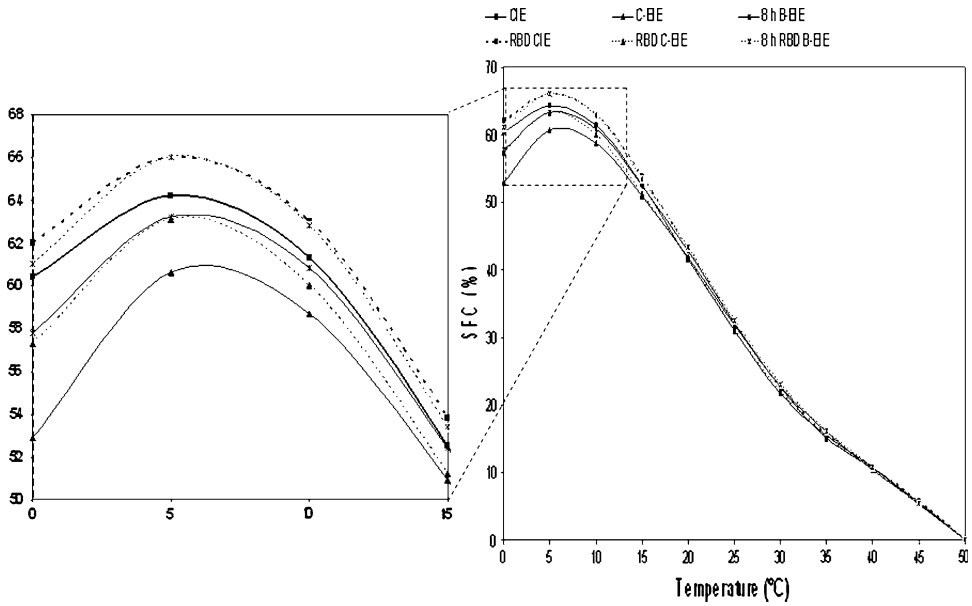

Fig. 6 Solid fat content profiles of interesterified PS/SBO (70/30, wt.%) blends before and after deodorization (non tempered SFC method). The inset is an enlarged part of the time range of 0–15 °C. *CIE* chemically interesterified blend, *B-EIE* batch enzymatically interesterified blend, *C-EIE* continuous enzymatically interesterified blend

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