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Enzymatic interesterification to produce zero-*trans* and dialkylketones-free fats from rapeseed oil

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Abstract – This study aims to evaluate the potential of applying enzymatic interesterification (EIE) to produce new healthy zero-*trans* high vitaminic content margarine fats, based on rapeseed oil (RO) only, with a specific focus on process-induced contaminants: the dialkylketones. Three blends made of RO and fully hydrogenated rapeseed oil (60:40, 70:30 and 80:20 wt:wt%) were considered. Compositional and melting properties, polymorphic behavior, appearance and textural characteristics were compared before and after interesterification. Interesterification improved both functional and textural characteristics. Enzymatic interesterification produced fats which presented similar triacylglycerol profile and physicochemical properties as the chemically interesterified one (CIE), however, preserving more tocopherols and without forming dialkylketones (process-induced contaminants). On the contrary, it was shown that after CIE, dialkylketones were formed: C18:1–C18:1 and C18:1–C18 chain-lengths being the major ones, and interestingly, their amount increased with the unsaturation in the blends. Our results indicate the great potential of enzymatic interesterification to produce new healthy fats entirely based on rapeseed oil. This study provides valuable information for the food industry, showing EIE as cutting-edge sustainable technology.

Keywords: enzymatic interesterification / rapeseed-based margarine / zero-trans / tocopherols / dialkylketones

Résumé – **Interestérification enzymatique pour produire des graisses sans isomères *trans* et sans dialkyl-cétones à partir d'huile de colza.** Cette étude vise à évaluer le potentiel de l'interestérification enzymatique (IEE) pour produire de nouvelles matières grasses pouvant être utilisées dans la formulation de margarines avec une teneur élevée en vitamines et dépourvues d'acides gras *trans*, à partir d'huile de colza. Un accent particulier a été mis sur des contaminants induits par le processus : les dialkyl-cétones. Trois mélanges d'huile de colza et d'huile de colza totalement hydrogénée (60:40, 70:30 et 80:20 p:p %) ont été considérés. Les compositions triglycéridiques des mélanges, leurs propriétés de fusion, comportement polymorphique, apparence et caractéristiques texturales ont été comparés avant et après interestérification (IE). L'IE a amélioré les caractéristiques fonctionnelles et texturales des trois mélanges considérés. L'IEE a produit des graisses présentant un profil en triacylglycérols et des propriétés physicochimiques similaires à ceux des produits issus de l'interestérification chimique (IEC), mais en préservant plus de tocophérols et sans former de dialkyl-cétones. Au contraire, il a été montré que les dialkyl-cétones sont induites par l'IEC : les longueurs de chaînes C18:1–C18:1 et C18:1–C18 étant les plus importantes, et de manière intéressante, leur quantité augmente avec l'insaturation dans les mélanges de départ. Nos résultats indiquent donc le grand potentiel de l'IEE pour produire de nouvelles graisses saines entièrement basées sur l'huile de colza. Cette étude fournit des informations précieuses pour l'industrie alimentaire, montrant l'IEE comme une technologie de pointe au caractère durable.

Mots clés : interestérification enzymatique / margarine à base de colza / zéro-trans / tocophérols / dialkyl-cétones

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Highlight

- Formation of new triacylglycerols giving the required melting properties.
- Similar properties achieved whatever the technology used: chemical or enzymatic.
- No process-induced contaminants (dialkylketones) with the enzymatic process.
- Enzymatic interesterification of rapeseed oil: a healthy alternative.

1 Introduction

Fat modifications such as fractionation, interesterification or hydrogenation make it possible to modify the fingerprint of an oil in order to broaden its use in the food industry. Fractionation is a physical process based on crystallization; interesterification and hydrogenation are chemical processes using catalysts. In the past, the food industry widely utilized partial hydrogenation to produce base-stocks for margarine formulation. Partial hydrogenation creates, however, high amounts of *trans*-fatty acids (TFA). The formation of *trans*-isomers was previously considered as an advantage because they crystallize faster, have higher melting points and better oxidative stability than *cis*-isomers. Partial hydrogenation is now banned in most countries since a direct relationship between TFA and increased cardiovascular risks has been recognized (Mozaffarian *et al.*, 2009). A study in Denmark, showed the correlation between the reduction of the TFA content in food, as a consequence of the law that initially limited TFA content, and the lower cardiovascular disease (CVD) mortality (Wilczeka *et al.*, 2017). Partially hydrogenated oils are no longer "generally recognized as safe" (GRAS) by the Food and Drug Administration (FDA), and on May 2018, the World Health Organization called for the elimination of industrially-produced *trans*-fats from the global food supply by 2023 (WHO, 2018). Fractionation and interesterification processes are now largely admitted as the main industrial alternatives (Gibon, 2012). Interesterification (IE) redistributes the FA on the glycerol by ester-ester exchanges. As a result of these exchanges, IE modifies the triglyceride (TAG) profile without changing the saturation degree, creating new physicochemical properties (Gibon and Kellens, 2014). This technology has great potential for modifying lipids for their use as commercial products and is now commonly used in the food industry (Gibon, 2021). The literature also indicates the great potential of IE to produce *trans*-free margarine fats from different edible lipid sources (Nekrasov *et al.*, 2020). Some examples have been reported by Zhao *et al.* (2014), Ruan *et al.* (2014), Shi *et al.* (2015), Wirkowska-Wojdyła *et al.* (2016), Li *et al.* (2018), Yu *et al.* (2018), Zhang *et al.* (2018), Pang *et al.* (2019), Simone Dos Santos *et al.* (2020), Segura and Jachmanián (2020) and Cui *et al.* (2022). All these reports indicate that the intreseterified fats may potentially be used in margarines preparation, as IE generally broadens the plastic range and modifies the polymorphic stability. The development of lipids with improved nutritional properties is now of increasing interest and in this context, other sources of lipids with essential fatty acids and/or bioactive components worth to be investigated for making versatile products with good technofunctional and nutritional properties by interesterification (Sivakanthan and Madhujith, 2020). It has been proven that polyunsaturated fatty acids, and specifically linoleic (L) and linolenic (Ln) acids, two essential FA, are effective in the prevention and treatment of coronary heart diseases (CHD) (Wijendran and Hayes, 2004). Rapeseed oil (RO), which is now the third most consumed vegetable oil worldwide (Statistica, 2021), has a high oleic (O) acid content and a well-balanced un-saturated FA composition (L:Ln ratio of 2:1) (Gunstone and Gunstone, 2006). In this context, and in the attempt to develop new healthy zero-*trans* margarine-fats totally based on RO, fully hydrogenated rapeseed oil (FHRO) was selected as hard fat and blended with RO before being interesterified. RO and FHRO have a low palmitic (P) content; FHRO has a very high stearic (S) acid content (>90%) which has no effect on the cholesterol blood level because it is quickly transformed into oleic acid in the liver (Stanley, 2009). Substituting palmitic acid with stearic acid lowers LDL-cholesterol concentrations, which is a well-known risk factor for CHD (van Rooijen and Mensink, 2020).

IE can be catalyzed by a chemical, such as sodium methoxide (chemical interesterification [CIE]) or by a lipase (enzymatic interesterification [EIE]). CIE has been commonly practiced in the industry for a long time; EIE is now receiving more attention as an emerging technology for the commodity and the specialty fats industry (Zhang *et al.*, 2020). Compared to CIE, EIE offers several advantages: milder conditions, lower oil losses and better oil quality (Gibon and Kellens, 2014; Sivakanthan and Madhujith, 2020).

Moreover, in 2006 CIE was associated with the formation of underinvestigated process-induced contaminants, *i.e.* dialkylketones (DAK) (Verhe *et al.*, 2006). DAK are a class of foreign substances which are potentially toxic. Literature regarding this class of components remains however very scarce. The possible formation of long-chain DAK as by-products of the CIE process was described by Verhe *et al.* (2006), and a formation mechanism during CIE, involving a β-keto-ester was tentatively proposed. Salgarella *et al.* (2021) recently reported the formation of DAK during deodorization: the DAK content increased with temperature, and the processes appeared to be significant above 240 °C. The chemical structure of DAK was elucidated both by GC-MS and LC-HRMS by Santoro *et al.* (2018). DAK are ketones deriving from FA and having two straight alkyl chains ranging from C10 and C24. The alkyl groups may be the same or different (chain length and unsaturation). A few studies reported the determination and quantification of DAK using GC-FID (Kato and Watanabe, 2022) and further identification using a GC-MS system (Mariani and Bellan, 2011; Mascrez *et al.*, 2021).

Up to now, very little is known about the long-chain DAK, and their potential impact on human health remains unclear; they are generally considered as a health concern. This is the reason why the German Federal Institute for Risk Assessment set a limit for their migration from packaging (BfR, 2017): it should not exceed 5 mg/kg in food stuff. FDA (2018) states that alkylketene dimers and their hydrolysis products (DAK) should not exceed 0.4 wt% of the paper or paperboard. However, so far, no legal limits are set for food due to a lack of both occurrence and toxicological data. Nevertheless, the importance to avoid their formation in food is highlighted on a patent for the process production of infant formula (T'Zand *et al.*, 2009)

To the best of our knowledge, there is no report on using the interesterification process on RO-FHRO blends to produce new healthy trans-free fats suitable for margarine production, especially regarding the possible formation of DAK. The present study was thus designed to fill these gaps by evaluating the potential of applying EIE to produce zero-*trans* and high vitaminic content margarine fats using RO only, with a specific focus on DAK occurrence. The TAG compositions (by RP-HPLC-RI), the solid fat content profiles (by p-NMR), the melting curves (by DSC), the polymorphism (by powder X-ray diffraction) and the hardness (by penetrometry) were examined and compared before and after EIE and CIE. The DAK content was assessed using SPE and GC-FID after microwave-assisted saponification. The DSC melting curves and the TAG compositions were analyzed as a function of the batch-EIE reaction time; they were used to highlight the modifications in melting properties and to quantify their kinetics.

2 Material and methods

2.1 Materials

Refined RO and FHRO were obtained from Cargill (Hamburg, Germany and Izegem, Belgium). Lipozyme TLIM®, a *Thermomyces lanuginosa* lipase, immobilized on silica carrier, which rearranges the fatty acids on the glycerol preferentially in a random way, was kindly supplied by Novozymes A/S (Bagsvaerd, Denmark). Three blends were considered, containing 60% (blend-1), 70% (blend-2) and 80% (blend-3) of RO (wt%), blended with FHRO. Commercial cooking and spreadable margarines were purchased from local supermarkets. Their fat phases were used as references. To recover the fat phase, the margarines were first melted at 70 °C to ensure complete melting, the upper lipid phase was separated and centrifuged for 15 min at 33 000 g. Anhydrous sodium sulfate was added to trap residual water traces and filtered under vacuum at 70 °C.

2.2 Methods

2.2.1 Batch-chemical interesterification

Before performing the CIE at lab-scale, each blend (FFA $< 0.05\%$, PV < 1.0 meq O_2/kg) was dried under vacuum (50 mbar, 120 °C, 30 min) to reach a water content <100 ppm. 0.5 wt% of sodium-methoxide (Merck) was added as a powder to 500 g of dried oil and CIE was performed for 30 min after the appearance of the characteristic dark "brownish" color at 90 °C, under vacuum (25 mbar). The reaction was stopped by adding citric acid as a 20% solution after the vacuum was broken; it was further mixed with the oil for 15 min. Afterwards, 1 wt% activated bleaching earth (Süd-Chemie, Tonsil Optimum 210 FF) was added to remove colored matters and impurities (30 min, 90 °C, 25 mbar); the CIE-blends were then vacuum filtered over a Büchner, using Whatman paper filter n° 1 (De Clercq *et al.*, 2012; Danthine *et al.*, 2014a). The CIE-blends were not post-deodorized.

2.2.2 Batch-enzymatic interesterification

Batch-EIE was conducted at a lab-scale using a double jacketed reactor (900 g). Fresh immobilized Lipozyme TLIM® was added as catalyst. The dry enzyme was first conditioned: de-aerated and dehydrated, as recommended by the supplier (Costales-Rodríguez *et al.*, 2009). Batch-EIE was conducted at 70 °C for 8 h, with a load of 4 wt% of lipase. The stirrer was set at 200 rpm to ensure an even distribution of the particles. Sampling (5 mL) was performed every hour. The product was withdrawn from the top of the reactor using preheated (80 °C) material (pipette and filter cloth (porosity 100 µm [03-100/44, Sefar Nitex, Switzerland]) to ensure the removal of the enzyme and avoid solidification (Danthine *et al.*, 2014a, 2014b). The EIE-blends were not post-deodorized.

2.2.3 Physicochemical properties

2.2.3.1 FA composition

FA composition was determined by GC-FID according to the AOCS C2 2-66 and Cc 1a-13 methods (AOCS, 2017). Data are reported as averages of two independent measurements.

2.2.3.2 TAG composition

The TAG composition was analyzed by reverse-phase HPLC-RI based on the official AOCS method Ce 5b-89 (AOCS, 2017). Minor practical adjustments were made in order to improve TAG separation, all in compliance with the above-mentioned official method. The analysis was performed on a Waters system equipped with two stainless steel Nova-Pak C18 columns (4 mm, 3.9 × 150 mm). The mobile phase was an isocratic mixture of acetone and acetonitrile (62.5:37.5 v:v) with a flow rate of 1.2 mL min^{-1}; the injection volume was 20 µL. The samples were dissolved in methanol:chloroform (1:1 v:v); a differential refractometer was utilized for the detection. Peak areas were correlated with the quantities of TAG in the sample. Peaks were integrated with Empower Pro "Apex Track" algorithm; peak areas below 4000 area counts (equivalent to approximately 0.04% of the total peak area) were not taken into account. Data are reported as averages of two independent measurements.

2.2.3.3 Tocopherols content

The tocopherol content (α-, Y- and δ-tocopherols) was determined by HPLC-FLD based on the AOCS Ce 8-89 method (AOCS, 2017). The analysis was performed with a Hewlett Packard 1050 Series HPLC equipped with 4 modules (auto-injector, FLD-detector (excitation wavelength = 290 nm and emission wavelength = 330 nm), online degasser and pump), using Alltech Alltima Silica 5 µm (250 mm length × 4.6 mm internal diameter) non-polar column. Isocratic elution was applied with a flow rate of 1.5 mL min^{-1} with hexane: isopropanol (99.5:0.5 v:v) as mobile phase. The samples (0.1–0.2 g) were first dissolved in 25 mL of hexane and 20 µL were injected. Peak identification was made by comparison with retention times of calibrated standards. Data are reported as averages of two independent measurements.

2.2.3.4 Dialkylketones (DAK)

The DAK content was determined using microwave-assisted saponification (MAS) using an Ethos-X microwave system equipped with a high-pressure SR 12 rotor from Milestone Srl (Milan, Italy), followed by a solid-phase extraction (SPE) and GC-FID (Thermo Fischer Trace Ultra 1300) and GC-MS (Shimadzu GCMS-TQ8050 NX,) determination (Mascrez *et al.*, 2021). The chromatographic separation was performed on a 30 m × 0.25 mm i.d. × 0.5 μm df SLB-5 ms capillary column (silphenylene polymer, practically equivalent in polarity to poly [5% diphenyl/95% methylsiloxane]) kindly obtained from Millipore-Sigma (Belfonte, PA, USA). Briefly, MAS was performed on 2.5 g of fat added of 25 mL of KOH/EtOH/water 2 M. The unsaponifable extract was purified by a lab-made SPE to isolate DAK from the remaining fat components. Finally, GC-FID for quantification and GC-MS for confirmation purposes were used. Data are reported as averages of two independent measurements.

2.2.3.5 Melting behavior by pulsed Nuclear Magnetic Resonance (p-NMR)

The solid fat content (SFC) melting profiles were recorded using p-NMR spectrometer (Minispec-mq20, Bruker, Karlsruhe, Germany), according to the IUPAC 2.150 a serial method (1 h at 0 °C, 30 min at each temperature) (IUPAC, 1987) and according to the method described by Ribeiro *et al.* (2009), which consists in a parallel method with a longer period at 0 °C (2 h) followed by 1 h at each temperature.

2.2.3.6 Melting behavior by differential scanning calorimetry (DSC)

The DSC analyses were carried out using a Q-2000 DSC (TA Instruments, New Castle, USA), using hermetic pans. Calibration was made with indium (m.p. 156.6 °C; ΔH = 28.71 J/g) and eicosane (m.p. 36.8 °C; ΔH = 247.4 J/g) standards. Nitrogen was used as purge gas in order to prevent condensation in the cells. An empty pan was used as a reference. The melting profiles were recorded using AOCS cj1-94 method (AOCS, 2017). The integration and peak temperature measurements were performed using the Universal Analysis Software version 4.2 (TA Instruments, New Castle, USA).

2.2.3.7 Textural properties (cone penetrometry)

Samples were prepared and analyzed as described by Danthine (2012). Briefly, the fats were melted for 15 min at 80 °C to erase the thermal memory, poured into plastic vessels (40 g) to produce "shortenings" by statically crystallizing them at −20 °C for 30 min, in order to mimic at lab-scale an industrial scraped-surface heat exchanger (Danthine and Deroanne, 2003). Samples were subsequently stored at 15 ± 0.5 °C for 48 h. Texture measurements were carried out after this "tempering": samples were first held at 20 ± 0.5 °C for 1 h, before measuring them in a controlled temperature cabinet using a SMS TA.XT2i/5 texturometer (Stable Micro System, Surrey, UK). Penetration tests were carried out with a cone probe (P/45C) penetrating the product in its container to a distance of 5 mm at a controlled speed of 0.5 mm/s, trigger force was 0.5 g (Lefébure *et al.*, 2013). The maximum penetration force was recorded, corresponding to the hardness of the samples (Detry *et al.*, 2021). Data are reported as the mean of five replicates.

2.2.3.8 Polymorphism

The polymorphim of samples – prepared as for textural evaluation – was investigated by powder X-ray diffraction using a Bruker D8-Advance Diffractometer (Bruker, Germany) (λ Cu = 1.54060 Å, 40 kV, 30 mA) equipped with a temperature control system (TTK450 & TCU 110, Anton-Paar, Graz, Austria). Long- (1–10 °2θ) and short-spacing runs (15–27 °2θ) were recorded using a Lynx-eye detector; data were analyzed with the Diffrac.Eva software (Bruker, Germany).

2.2.3.9 Evaluation of the degree of interesterification (DI)

The DI was determined based on a comparison between the actual TAG composition (before and after IE) and a theoretical random TAG calculation (RAN). RAN composition was calculated based on the FA composition (% mol) following the method described by Costales-Rodríguez *et al.* (2009). DI was calculated according to Equation (1).

$$DI(\%) = 100 \times \left((A/B)_{NIE} - (A/B)_{IE}\right)/\left((A/B)_{NIE} - (A/B)_{RAN}\right), \quad (1)$$

with A = the TAG that decreased the most during the IE reaction (in this case SSS), B = the TAG that increased the most during the IE reaction (in this case = SOO), NIE = before IE, RAN = random calculated.

2.2.3.10 Statistics

Data were statistically analyzed using Minitab 17 software (Minitab LLC, State College, Pennsylvania, USA). One-way analysis of variance (ANOVA) was implemented to identify statistically significant differences between means. All assays were conducted in triplicate unless otherwise specified. Means and standard deviations were calculated and differences between means were determined with a significance level of $\alpha = 0.05$.

3 Results and discussion

3.1 Compositional properties

The FA composition of RO, FHRO and the three blends containing respectively 60 (blend-1), 70 (blend-2) and 80% (blend-3) of RO (wt%), expressed as the mean of two replications, are presented in Table 1. For the RO, the predominant FA were oleic (64.1%), linoleic (18.5%) and linolenic (8.5%) types; the TFA content was 0.37%. As expected, the stearic acid content in FHRO was high (92.1%), followed by palmitic acid (5.5%). The total saturated fatty acids content of FHRO was 99.9%, demonstrating that the hydrogenation process was complete (only 0.1% of C18:2cis remained). The average TFA contents were 0.17%, 0.18% and

Table 1. FA relative compositions* (%) of RO, FHRO and the three investigated blends (blend-1 = RO:FHRO 60:40; blend-2 = RO:FHRO 70:30; blend-3 = RO:FHRO 80:20 wt:wt%).

FA relative composition	RO	FHRO	Blend-1	Blend-2	Blend-3
C14:0	0.04	0.05	0.04	0.04	0.02
C16:0	4.40	5.50	4.83	4.68	4.56
C16:1t	0.03	N.D.	0.01	N.D.	N.D.
C16:1c	0.20	N.D.	0.11	0.13	0.14
C17:0	0.10	N.D.	0.11	0.11	0.11
C17:1	0.06	N.D.	0.04	0.04	0.05
C17:2	0.07	N.D.	0.04	0.05	0.05
C18:0	1.60	92.10	38.08	29.00	19.90
C18:1t	N.D.	N.D.	N.D.	N.D.	N.D.
C18:1c	64.10	N.D.	38.49	45.00	51.50
C18:2t	0.04	N.D.	N.D.	N.D.	N.D.
C18:2c	18.50	0.10	11.08	12.95	14.80
C18:3t	0.30	N.D.	0.16	0.18	0.22
C18:3c	8.50	N.D.	5.04	5.87	6.73
C20:0	0.50	1.70	0.98	0.83	0.70
C20:1	1.10	N.D.	0.65	0.74	0.84
C22:0	0.20	0.40	0.29	0.27	0.25
C22:1	0.14	N.D.	0.06	0.09	0.10
Total TFA	0.37	N.D.	0.17	0.18	0.22

N.D.: not detectable; c: cis; t: *trans*; TFA: trans fatty acids.
* Average value between two independent measurements.

0.22% for blend-1, −2 and −3 respectively. They are all suitable to produce margarines, as, since 2019, the maximum TFA authorized amount in processed food products is 2% on a fat basis (UE 2019/649, 2019). Due to their high RO content, the blends also had a favorable C18:2n-6/C18:3n-3 ratio of 2.2; this is of considerable importance since the goal was to formulate new healthy foods.

The three blends were both CIE and EIE and compared to NIE materials. As expected, no changes occurred in the FA profiles since IE does not affect the FA composition. On the contrary, analyzing the TAG profile is highly indicative for monitoring the randomization occurring during IE, thanks to the degree of interesterification (DI). The TAG composition of the NIE, the actual TAG composition of the CIE blends and the calculated theoretical random composition (RAN) are presented in Table 2. SSS, OOO, OOL, and LLO were the major TAGs in the NIE blends. The highest SSS content (32.9%) was seen in blend-1 due to the highest FHRO content, while OOO, OOL and LLO contents were directly linked to the amount of RO. As expected, the TAG composition was significantly modified after IE. A DI of 100% was obtained after CIE, indicating complete randomization; CIE blend-1 (CIE-1), -2 (CIE-2) and -3 (CIE-3) TAG compositions closely fit with the RAN corresponding ones (Tab. 2). The TAG diversity increased after IE: randomization resulted in the formation of new TAG species such as SOS, due to the high stearic and oleic contents in the original blends, and POS + SSL; the percentages of SOO, POL + SLL + SOLn and POO + SOL increased significantly while SSS, OOO, OOL and PSS contents decreased. After 8 h of enzymatic reaction, the TAG composition was identical to CIE, with a DI of 100% for the three blends. Figure 1 summarizes the TAG modifications due to IE. It is obvious that the tri-unsaturated TAG (UUU), like OOO, and the tri-saturated TAG (StStSt) like SSS, were reduced while the di-unsaturated TAGs (StUU), like SOO, and the di-saturated TAGs (StUSt), like SOS, POS and POP, were increased (St = saturated, U = unsaturated). These modifications were positive regarding the targeted food applications. Indeed, being (semi-)solid at ambient temperature but (semi-) liquid at body temperature, StUSt usually improve the structural plasticity and gloss of products, while StUU favor lubricity and spreadability.

Regarding the EIE reaction kinetics, the TAG composition was already drastically modified after one hour of reaction (DI > 94%) for the 3 blends (Tab. 3a–c). The DI approached 100% after 6 h of reaction, and at 8 h reached an identical TAG composition as for CIE.

3.2 Physicochemical properties

3.2.1 Melting profiles

3.2.1.1 Differential scanning calorimetry

As shown in Figure 2, the DSC melting profiles of the three NIE blends presented two sharp endothermic peaks: a low melting (LM) peak at about −11 °C and a very high melting (VHM) peak at 62/65 °C. The LM peak was due to RO and the VHM peak to FHRO. Both CIE and EIE lead to drastic changes in the melting behaviors thanks to the better TAG compatibility. As a result, the LM peak observed for the NIE-blends was shifted to higher temperatures as a broader LM endothermic area (shoulders at 2/−6/−8 °C) and a well-resolved medium melting (MM) peak (5/18 °C, depending on the blends); contrarily, the VHM was shifted to lower temperatures, as a broader endothermic high melting (HM) peak (49/45/40 °C, depending on the blends).

Table 2. TAG compositions[*] (%) of the three investigated blends: NIE, RAN (calculated), CIE, EIE (8 h) and DI (%). (blend-1 = RO:FHRO 60:40; blend-2 = RO:FHRO 70:30; blend-3 = RO:FHRO 80:20 wt:wt%).

	Blend-1				Blend-2				Blend-3			
	NIE	RAN	CIE	EIE (8 h)	NIE	RAN	CIE	EIE (8 h)	NIE	RAN	CIE	EIE (8 h)
TAG	HPLC	–	HPLC	HPLC	HPLC	–	HPLC	HPLC	HPLC	–	HPLC	HPLC
OLLn	4.5	1.4	1.8	1.8	5.2	2.3	2.9	2.7	5.9	3.4	4.1	4.0
LnLnS	0.6	0.3	0.7	0.7	0.7	0.3	0.9	0.8	0.9	0.3	0.8	0.8
OOLn	4.3	1.5	1.7	1.7	5.0	2.4	2.5	2.6	5.8	3.6	3.9	3.9
LLO	7.3	2.5	2.7	2.8	8.5	3.9	4.3	4.3	9.7	5.9	6.3	6.3
PLL + POLn + SLLn	1.4	2.3	2.6	2.6	1.6	2.6	2.9	2.7	1.8	2.8	2.9	2.8
OOL	13.5	5.2	5.4	5.6	15.8	8.4	8.4	8.7	18.0	12.4	12.7	12.8
POL + SLL + SOLn	3.0	7.8	8.2	8.3	3.5	8.5	8.8	8.6	4.0	8.3	8.5	8.5
PPL + PSLn	0.3	0.8	0.8	0.9	0.3	0.7	0.7	0.7	0.4	0.6	0.6	0.7
OOO	17.6	6.0	5.9	6.0	20.5	9.6	9.2	9.5	23.4	14.3	13.9	14.0
POO + SOL	3.9	12.7	12.8	13.0	4.5	14.0	13.7	13.7	5.2	13.7	13.6	13.5
POP + SLnS + PLS	0.4	4.1	4.1	4.2	0.4	3.2	3.2	3.2	0.5	2.2	2.3	2.3
PPP	0.2	N.D.	N.D.	0.1	0.2	N.D.	0.1	0.1	0.2	N.D.	0.1	0.1
OOG	0.7	0.3	0.2	0.2	0.8	0.4	0.4	0.4	0.9	0.6	0.5	0.6
SOO	1.4	17.7	17.3	17.4	1.7	18.5	17.5	17.5	1.9	16.4	15.8	15.7
POS + SSL	0.1	9.9	9.7	9.6	0.1	7.6	7.4	7.2	0.1	6.6	4.8	4.7
PPS	0.7	0.3	0.3	0.3	0.6	0.2	0.2	0.2	0.5	0.2	0.2	0.1
OOA + OGS	0.5	1.0	1.2	1.3	0.6	1.0	1.4	1.3	0.7	1.0	1.4	1.2
SOS	0.1	17.4	16.5	16.3	0.1	11.8	11.0	10.9	0.1	6.3	5.9	5.9
PSS	6.0	2.4	2.0	1.8	4.6	1.3	1.1	1.3	3.2	0.6	0.5	0.5
SSG + SAO	N.D.	0.3	1.4	1.3	N.D.	0.2	1.0	1.0	N.D.	0.1	0.3	0.6
SSS	32.9	5.7	4.2	4.0	24.7	2.5	2.0	2.2	16.4	0.8	0.7	0.8
SSA	0.8	0.4	0.3	0.1	0.6	0.2	0.3	0.3	0.4	0.1	0.3	0.2
DI (%)	–	–	100	100	–	–	100	100	–	–	100	100

P: palmitic acid; S: stearic acid; O: oleic acid; L: linoleic acid; Ln: linolenic acid; A: arachidic acid; G: gondoic acid; N.D.: not detectable.

[*] Average value between two independent measurements.

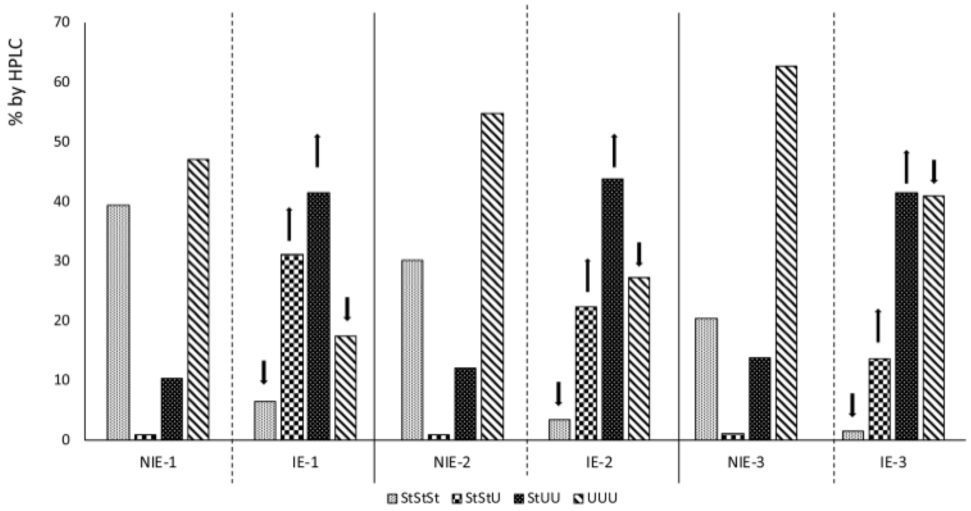

Fig. 1. Summary of the TAG modifications due to IE, StStSt: tri-saturated TAGs; StStU: di-saturated TAGs; StUU: mono-saturated TAGs; UUU: tri-unsaturated TAGs (blend-1 = RO:FHRO 60:40; blend-2 = RO:FHRO 70:30; blend-3 = RO:FHRO 80:20 wt:wt%; NIE = non-interesterified; IE = interesterified).

Table 3a. TAG compositions* (%) of blend-1 (RO:FHRO 60:40 wt:wt%), NIE and EIE as a function of time and the corresponding degree of interesterification: DI (%).

	NIE 1	EIE 1 h	EIE 2 h	EIE 3 h	EIE 4 h	EIE 5 h	EIE 6 h	EIE 7 h	EIE 8 h
OLLn	4.5	3.6	2.4	2.2	1.9	1.9	1.8	1.8	1.8
LnLnS	0.6	0.8	0.7	0.7	0.7	0.6	0.6	0.7	0.7
OOLn	4.3	3.3	2.4	2.1	1.9	1.9	1.8	1.7	1.7
LLO	7.3	5.9	4.0	3.5	3.0	2.9	2.9	2.9	2.8
PLL + POLn + SLLn	1.4	2.4	2.4	2.6	2.4	2.6	2.6	2.6	2.6
OOL	13.5	11.2	8.1	7.0	6.3	6.0	5.9	5.7	5.6
POL + SLL + SOLn	3.0	6.7	7.1	7.9	7.8	8.1	8.3	8.3	8.3
PPL + PSLn	0.3	0.7	0.7	0.8	0.8	0.9	0.9	0.9	0.9
OOO	17.6	13.4	9.5	7.8	6.9	6.5	6.3	6.2	6.0
POO + SOL	3.9	9.8	11.1	12.1	12.4	12.7	12.9	12.9	13.0
POP + SLnS + PLS	0.4	2.6	3.3	3.8	4.0	4.1	4.2	4.2	4.2
PPP	0.2	0.1	0.1	0.1	0.1	N.D.	0.1	0.1	0.1
OOG	0.7	0.4	0.3	0.3	0.3	0.2	0.2	0.2	0.2
SOO	1.4	10.5	13.4	15.4	16.2	16.7	17.2	17.0	17.4
POS + SLS	0.1	5.3	7.3	8.4	9.0	9.3	9.4	9.5	9.6
PPS	0.7	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3
OOA + OGS	0.5	0.9	1.0	1.1	1.2	1.2	1.2	1.2	1.3
SOS	0.1	9.8	13.1	14.8	15.6	16.0	16.2	16.2	16.3
PSS	6.0	2.8	2.8	2.0	2.4	1.9	1.9	1.8	1.8
SSG + SAO	N.D.	0.8	1.0	1.2	1.2	1.3	1.3	1.3	1.3
SSS	32.9	8.5	8.7	5.7	5.5	4.4	4.1	4.2	4.0
SSA	0.8	N.D.	0.2	0.2	0.3	0.4	0.1	0.4	0.1
DI (%)	0.0	97.9	98.6	99.8	99.9	100	100	100	100

P: palmitic acid; S: stearic acid; O: oleic acid; L: linoleic acid; Ln: linolenic acid; A: arachidic acid; G: gondoic acid; N.D.: not detectable.
* Average value between two independent measurements.

Table 3b. TAG compositions* (%) of blend-2 (RO:FHRO 70:30 wt:wt%), NIE and EIE as a function of time and the corresponding degree of interesterification: DI (%).

	NIE 2	EIE 1 h	EIE 2 h	EIE 3 h	EIE 4 h	EIE 5 h	EIE 6 h	EIE 7 h	EIE 8 h
OLLn	5.2	4.4	3.5	3.4	2.9	2.9	2.9	2.9	2.7
LnLnS	0.7	0.8	0.9	0.9	0.8	0.8	0.8	0.8	0.8
OOLn	5.0	4.4	3.4	3.0	2.9	2.7	2.7	2.5	2.6
LLO	8.5	7.4	5.7	5.3	4.7	4.7	4.4	4.5	4.3
PLL + POLn + SLLn	1.6	2.5	2.6	2.7	2.7	2.9	2.9	2.8	2.7
OOL	15.8	13.9	11.3	10.4	9.6	9.3	9.0	8.9	8.7
POL + SLL + SOLn	3.5	6.7	7.5	8.2	8.3	8.7	8.8	8.8	8.6
PPL + PSLn	0.3	0.6	0.7	0.7	0.8	0.7	0.7	0.8	0.7
OOO	20.5	16.5	13.0	11.5	10.4	9.9	9.6	9.5	9.5
POO + SOL	4.5	9.7	11.4	12.3	12.9	13.4	13.6	13.8	13.7
POP + SLnS + PLS	0.4	1.9	2.5	2.8	3.0	3.1	3.2	3.2	3.2
PPP	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1
OOG	0.8	0.5	0.5	0.5	0.4	0.5	0.4	0.3	0.4
SOO	1.7	9.5	13.0	14.5	16.0	16.6	17.1	17.2	17.5
POS + SLS	0.1	3.5	5.3	5.6	6.6	6.5	7.1	6.8	7.2
PPS	0.6	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2
OOA + OGS	0.6	1.0	1.1	1.1	1.2	1.3	1.3	1.3	1.3
SOS	0.1	6.4	9.0	9.7	10.6	10.7	10.5	10.8	10.9
PSS	4.6	2.4	2.0	1.5	1.4	1.2	1.1	1.1	1.3
SSG + SAO	N.D.	0.5	0.7	0.8	1.0	0.9	0.9	1.0	1.0
SSS	24.7	7.0	5.5	4.6	3.5	2.5	2.3	2.2	2.2
SSA	0.6	N.D.	N.D.	0.1	0.2	0.3	0.3	0.4	0.3
DI (%)	0.0	95.8	98.0	98.7	99.4	99.9	100	100	100

P: palmitic acid; S: stearic acid; O: oleic acid; L: linoleic acid; Ln: linolenic acid; A: arachidic acid; G: gondoic acid; N.D.: not detectable.
* Average value between two independent measurements.

Table 3c. TAG compositions* (%) of blend-3 (RO:FHRO 80:20 wt:wt%), NIE and EIE as a function of time and the corresponding degree of interesterification: DI (%).

	NIE 3	EIE 1 h	EIE 2 h	EIE 3 h	EIE 4 h	EIE 5 h	EIE 6 h	EIE 7 h	EIE 8 h
OLLn	5.9	5.2	4.3	4.4	3.9	4.1	4.2	3.9	4.0
LnLnS	0.9	0.9	0.8	0.9	0.8	0.9	0.9	0.8	0.8
OOLn	5.8	4.9	4.3	4.1	3.9	3.9	3.7	3.9	3.9
LLO	9.7	8.6	7.4	7.0	6.7	6.5	6.6	6.5	6.3
PLL + POLn + SLLn	1.8	2.5	2.6	2.8	2.8	2.8	2.9	2.9	2.8
OOL	18.0	16.4	14.7	13.9	13.4	13.3	12.9	12.9	12.8
POL + SLL + SOLn	4.0	6.6	7.5	8.0	8.2	8.5	8.5	8.5	8.5
PPL + PSLn	0.4	0.6	0.6	0.6	0.6	0.7	0.6	0.7	0.7
OOO	23.4	19.4	16.5	15.1	14.6	13.9	13.9	13.9	14
POO + SOL	5.2	9.6	11.2	12.1	12.8	13.0	13.2	13.3	13.5
POP + SLnS + PLS	0.5	1.5	1.9	2.0	2.2	2.2	2.2	2.2	2.3
PPP	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.1
OOG	0.9	0.8	0.7	0.6	0.6	0.6	0.6	0.7	0.6
SOO	1.9	8.6	11.7	13.4	14.6	14.9	15.3	15.5	15.7
POS + SLS	0.1	2.3	3.6	4.2	4.6	4.6	4.7	4.6	4.7
PPS	0.5	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1
OOA + OGS	0.7	1.0	1.2	1.1	1.3	1.3	1.2	1.2	1.2
SOS	0.1	4.0	5.4	5.6	5.9	5.9	5.8	5.9	5.9
PSS	3.2	1.5	1.2	0.8	0.7	0.6	0.5	0.6	0.5
SSG + SAO	N.D.	0.3	0.5	0.6	0.6	0.6	0.6	0.6	0.6
SSS	16.4	5.0	3.7	2.3	1.5	1.1	0.9	0.8	0.8
SSA	0.4	N.D.	N.D.	0.2	0.2	0.2	0.2	0.2	0.2
DI (%)	0.0	93.8	96.9	98.6	99.4	99.7	99.9	100.0	100.0

P: palmitic acid; S: stearic acid; O: oleic acid; L: linoleic acid; Ln: linolenic acid; A: arachidic acid; G: gondoic acid; N.D.: not detectable.
* Average value between two independent measurements.

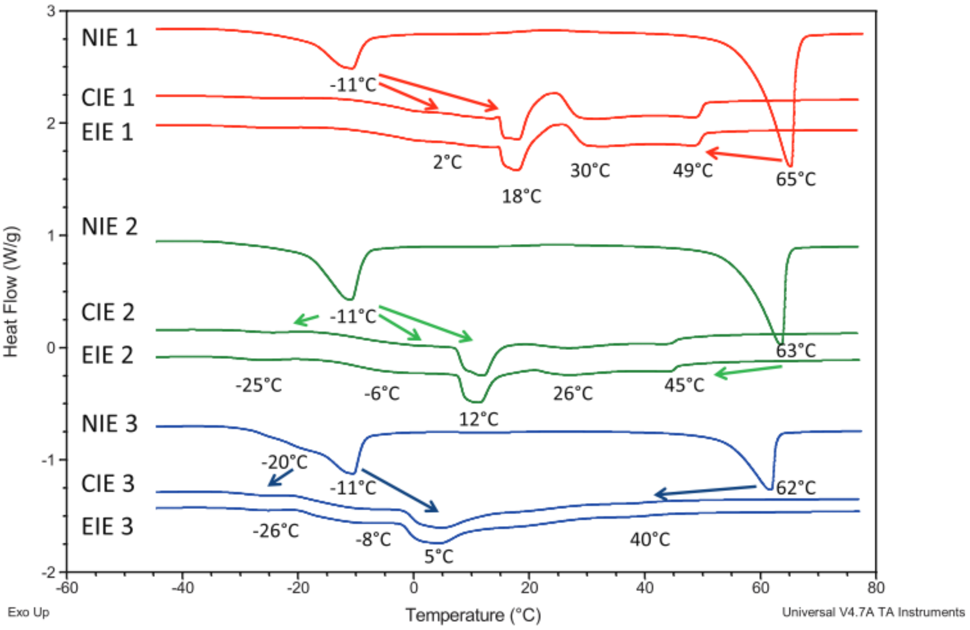

Fig. 2. DSC melting curves of the non-interesterified (NIE), chemically (CIE) and enzymatically intereterified (EIE) blends (blend-1 = RO:FHRO 60:40; blend-2 = RO:FHRO 70:30; blend-3 = RO:FHRO 80:20 wt:wt%).