

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

Sabine Danthine^{1,*} , Sébastien Closset¹, Jeroen Maes², Steven Mascrez³ , Christophe Blecker¹, Giorgia Purcaro³  and Véronique Gibon²

¹ Food Science and Formulation, Gembloux Agro-Bio Tech, ULiège, 5030 Gembloux, Belgium

² Desmet Ballestra Group, R&D Center, Zaventem, Belgium

³ Analytical Chemistry, Gembloux Agro-Bio Tech, ULiège, 5030 Gembloux, Belgium

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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 – **Intérestérification 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'intérestérification 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 polymorphe, apparence et caractéristiques texturales ont été comparés avant et après intérestérification (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'intérestérification 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 : intérestérification enzymatique / margarine à base de colza / zéro-*trans* / tocophérols / dialkyl-cétones

*Corresponding author: sabine.danthine@uliege.be

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 interesterified 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 O₂/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-, γ- 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 (Mascres *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 unsaponifiable 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 cjl-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 polymorphism 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).

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

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 α=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 $62/65^{\circ}\text{C}$. 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 $2/-6/-8^{\circ}\text{C}$) and a well-resolved medium melting (MM) peak ($5/18^{\circ}\text{C}$, depending on the blends); contrarily, the VHM was shifted to lower temperatures, as a broader endothermic high melting (HM) peak ($49/45/40^{\circ}\text{C}$, 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%).

TAG	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
SSS	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.

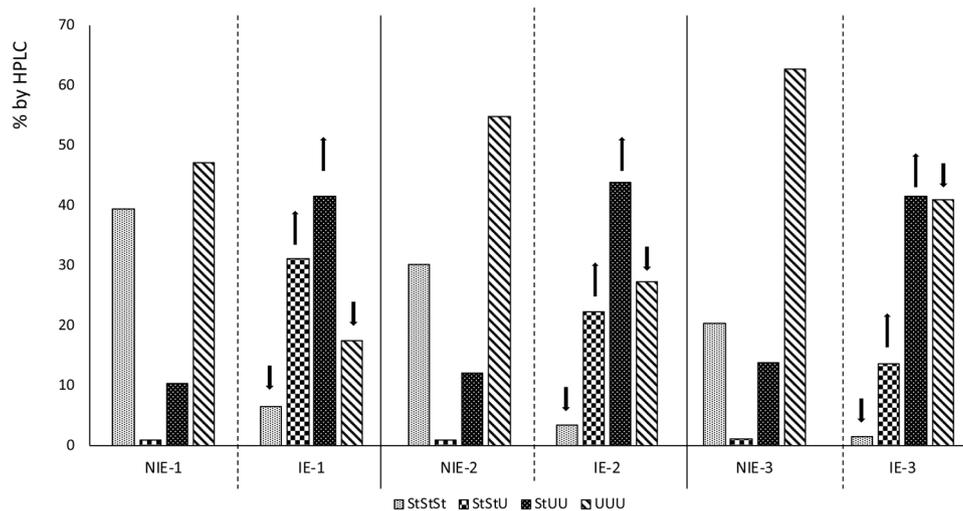


Fig. 1. Summary of the TAG modifications due to IE, StStSt: tri-saturated TAGs; StStU: di-saturated TAGs; StUU: mono-saturated TAGs; UUU: tri-unsaturated TAGs (blend-1 = RO:FHRO 60:40; blend-2 = RO:FHRO 70:30; blend-3 = RO:FHRO 80:20 wt:wt%; NIE = non-interesterified; IE = interesterified).

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.

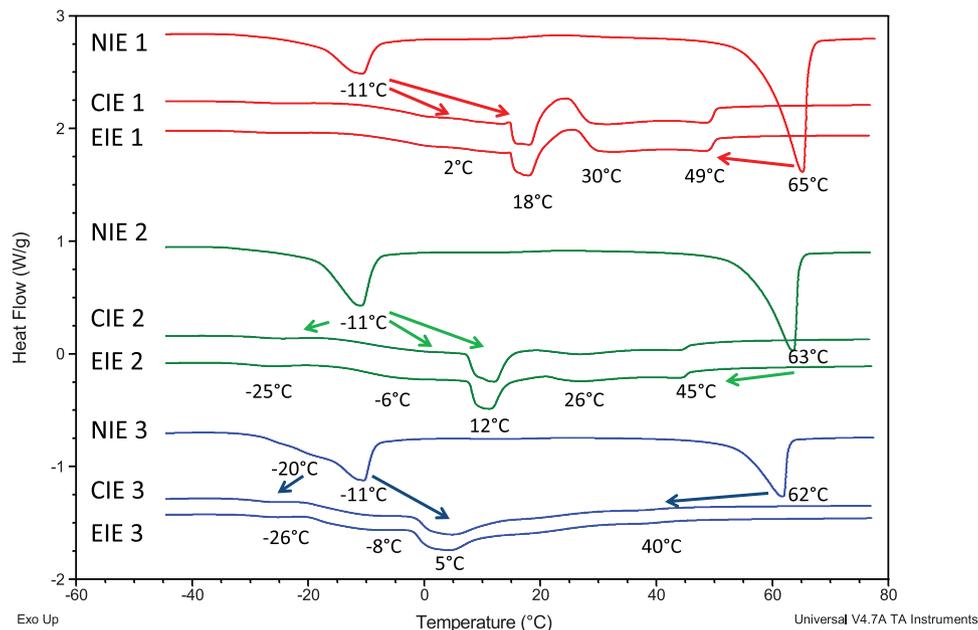


Fig. 2. DSC melting curves of the non-interesterified (NIE), chemically (CIE) and enzymatically interesterified (EIE) blends (blend-1 = RO:FHRO 60:40; blend-2 = RO:FHRO 70:30; blend-3 = RO:FHRO 80:20 wt:wt%).

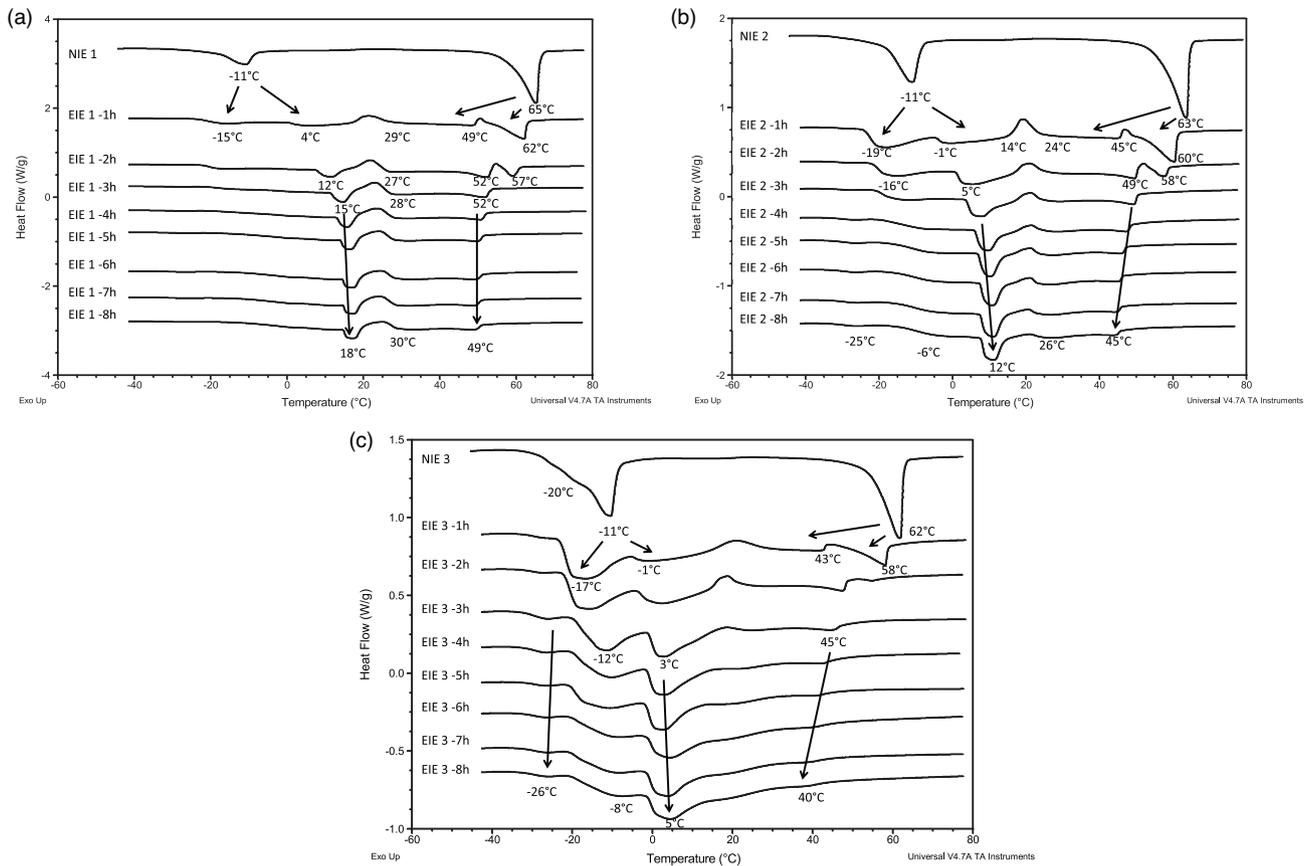


Fig. 3. a–c: Evolution of the DSC melting profiles as a function of the enzymatic interesterification reaction time; a: blend-1; b: blend-2; c: blend-3 (blend-1 = RO:FHRO 60:40; blend-2 = RO:FHRO 70:30; blend-3 = RO:FHRO 80:20 wt:wt%).

DSC curves of samples withdrawn every hour during the enzymatic reaction are presented in Figure 3a–c. After only one hour, the melting profile changed drastically whatever the blend, as for the TAG composition: the LM peak observed in the NIE-blends was split into two broad areas on either side of the original peak. It was then progressively transformed into a well-resolved MM peak which moved to reach about 18 °C for blend-1, 12 °C for blend-2 and 5 °C for blend-3. This resulted from the decrease of the low melting point UUU-TAG content and the formation of new medium melting point TAG (StUU and StUSt). At the same time, the HM peak was shifted to lower values and reached 49 °C for blend-1 and 45 °C for blend-2, and about 40 °C for blend-3 after 8 h of reaction. This resulted from the decrease of the content in high melting point StStSt and the increase of StUU and StUSt (Fig. 1). The new patterns remained quite similar from 6 h to 8 h of reaction, as it was for the TAG composition (Tab. 3a–c).

3.2.1.2 Solid fat content

Melting profile and melting point (MP) are parameters of significant importance for characterizing and developing edible fat. SFC greatly influences the suitability of fats for a particular application. The amount of fat crystals is responsible for many product characteristics, including general appearance, ease of packing, organoleptic properties, ease of

spreading, and oil exudation (Karabulut *et al.*, 2004). The SFC melting profiles (obtained using IUPAC 2.150 a serial method) of the NIE-blends, the CIE and the EIE-blends, are presented in Figure 4a–c. The SFC profiles of all IE-blends were significantly different from the non-interestered ones ($P < 0.05$).

Due to the presence of FHRO, all the NIE were completely melted at about 65 °C, with a higher SFC and a steeper melting profile for blend-1 compared to blends-2 and -3. After IE, they were all softer at all temperatures with MP shifted to about 55 °C, 50 °C and 40 °C, respectively; the SFC melting profiles were also steeper at all compositions. This lower MP can be explained by the substantial reduction of the SSS content (whose melting point is 65 °C) from 32.9, 24.7 and 16.4% to 4.0, 2.2 and <1.0% respectively. Figure 4a–c shows that CIE and EIE-blends had similar profiles but significantly different from the NIE-blends; it is evident that IE lead to an important reduction of the SFC for the 3 blends, at all temperatures. This was especially true for temperatures higher than 30 °C. For example, at 40 °C, SFC ranged from about 14% to about 31% for the NIE blends, while, after IE, they decreased to about 1–9% only. It is important to notice that the SFC melting profiles of all the IE blends had an unusual shape with a decrease around 5 °C for blend-2 and blend-3 and 10 °C for blend-1 (see circles on Fig. 4a–c). This behavior is linked to the new TAG composition, which induced slower crystallization kinetics.

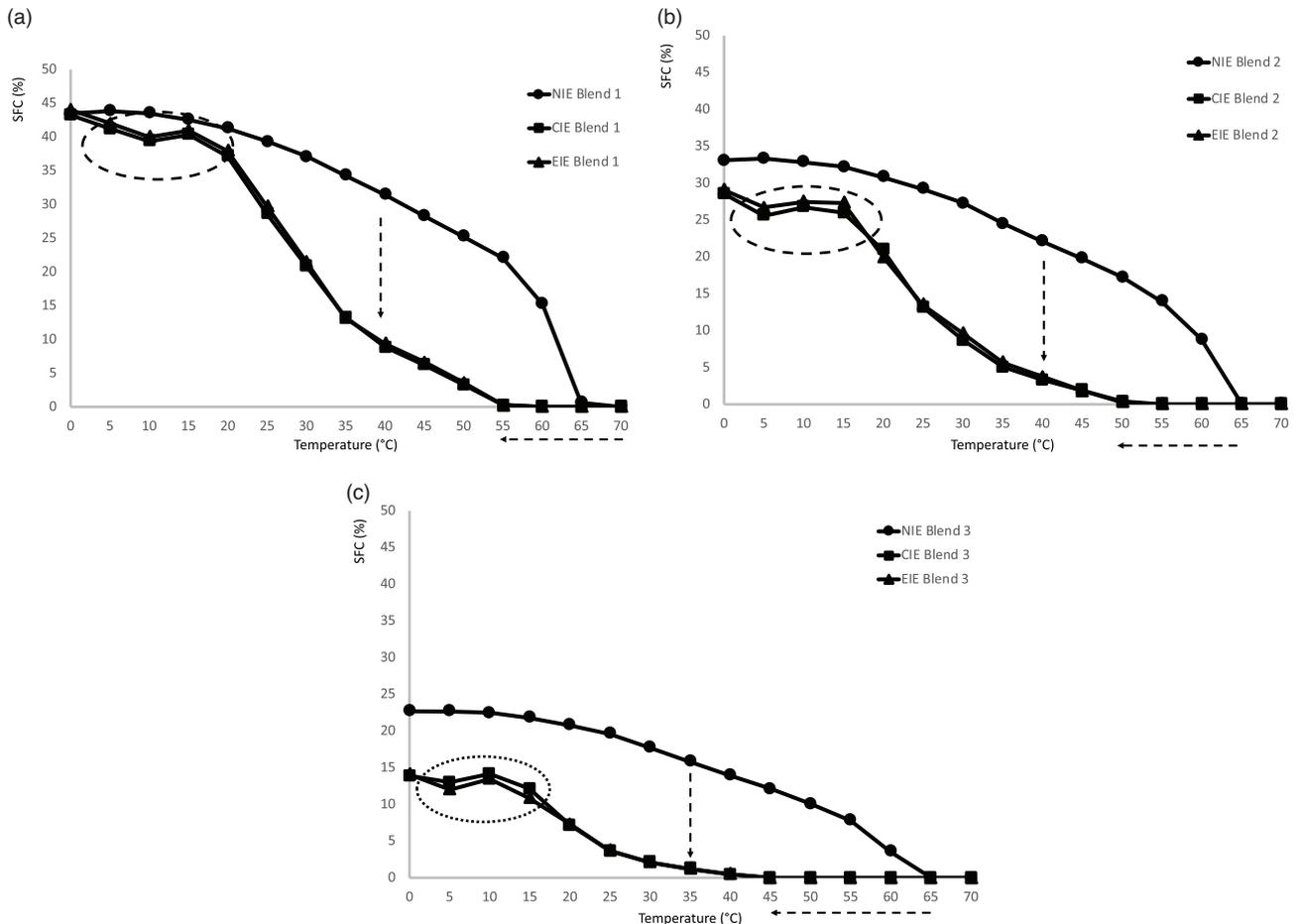


Fig. 4. a–c: Solid fat content melting curves obtained using the IUPAC 2.150 a non-tempered serial method, before (NIE) and after chemical (CIE) or enzymatic (EIE) interesterification; a: blend-1; b: blend-2; c: blend-3 (blend-1 = RO:FHRO 60:40; blend-2 = RO:FHRO 70:30; blend-3 = RO:FHRO 80:20 wt:wt%). The circles highlight the unusual shape with a SFC decrease at around 5 °C (blend-2 and blend-3) or 10 °C (blend-1).

Ribeiro *et al.* (2009) reported a similar atypical behavior for blends made of SO and FHRO; to better visualize the slower crystallization, they developed a SFC method including a tempering of 2 h at 0 °C and 1 h at each other temperatures. The results obtained for the IE blends with this modified method compared to the results obtained using the IUPAC 2.150 a serial method are presented in Figure 5a–c. The unusual shape observed at around 5–10 °C disappeared by using the modified one (arrows). This demonstrates that a longer tempering might be needed to reach equilibrium, which is typical of StUSt based fats.

3.3 Food applications

As the compositions and SFC profiles of CIE and EIE fats were similar, for clarity, only the properties of the EIE-blends are presented and discussed.

After EIE, a broader range of medium melting TAG was formed, as aforementioned (Tab. 3a–c, Fig. 1). This should create an optimal melting temperature range for applications such as margarines. On one hand, SSO and POS + SSL formation and increase in SOL + LLS + POL and SOO contents will endow lubricity, aeration, clarity and gloss to

the fat. On the other hand, PSS and SSS usually act as structuring agents and moisture barriers; however, a low amount of SSS is essential to avoid unwanted waxy mouthfeel. SFC melting profiles are often used to determine whether a fat blend is suitable for an application or not. For example, SFC values at temperatures between 4 and 10 °C give an idea regarding the ease of spreadability of the product when removed from the fridge; SFC should be lower than 32% at 10 °C to ensure good spreadability (Karabulut *et al.*, 2004); EIE-blends-3 (EIE-3) and -2 (EIE-2) fitted these requirements. Plastic fats should have not only plasticity and spreadability at low temperature, but also maintain their appearance, without any oil diffusion, at room temperature: SFC at 20–22 °C determines this product's stability; SFC of about 10% is essential to prevent oiling off. Finally, SFC at body temperature directly influences sensorial and organoleptic properties of the products. Fat melting should happen in this temperature range to release aroma and avoid a waxy feeling in the mouth, no (or very low) SFC at 40 °C is required, as fats exhibit their slip melting point when the SFC is reduced to 5% (Noor Lida and Ali, 1998); this was the case of EIE-2 and EIE-3. SFC melting profiles were further compared to the melting profile of fats extracted from commercial products: spreadable

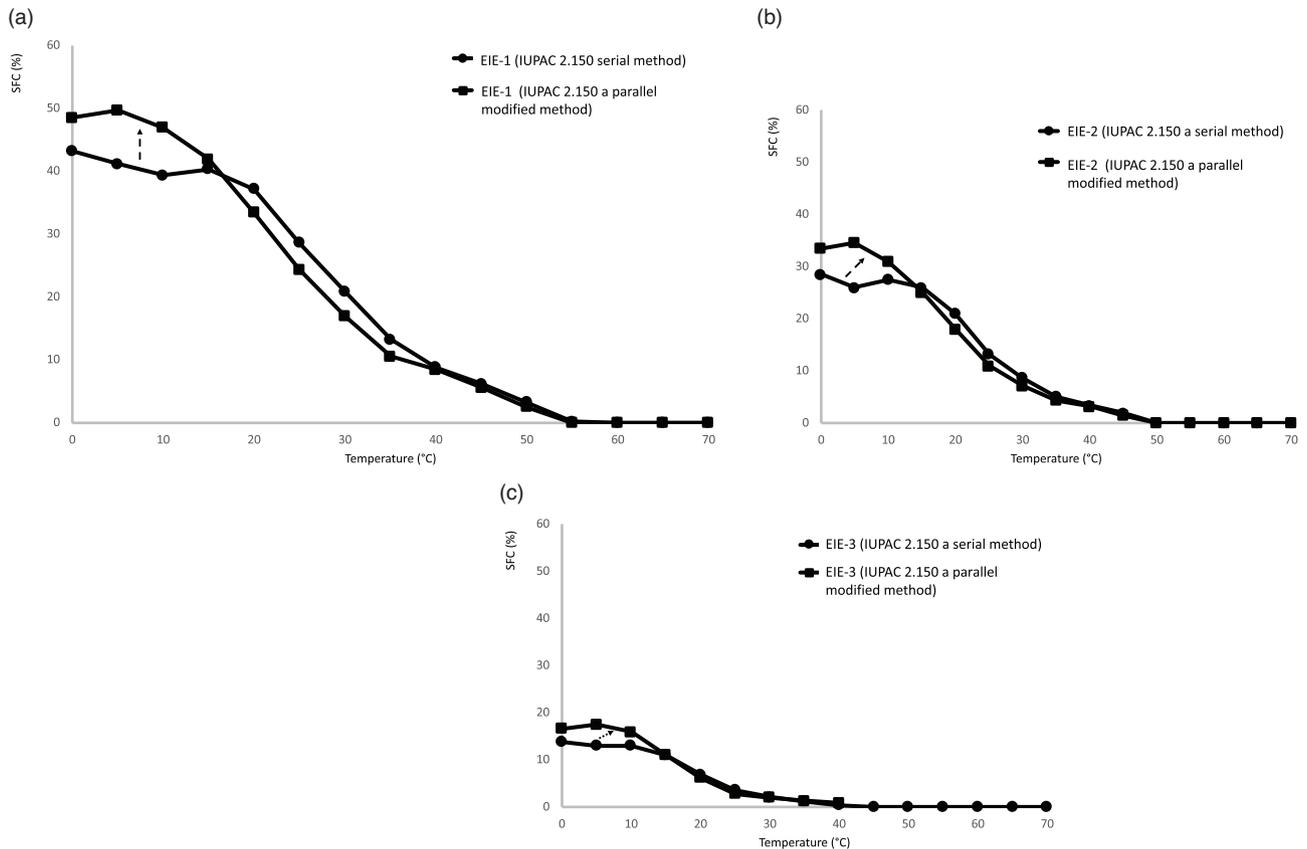


Fig. 5. a–c: Comparison of the solid fat content melting curves obtained for the enzymatically interesterified blends; a: blend-1; b: blend-2; c: blend-3 (EIE-1 = RO:FHRO 60:40; EIE-2 = RO:FHRO 70:30; EIE-3 = RO:FHRO 80:20 wt:wt%), using the IUPAC 2.150 a non-tempered serial method (1 h at 0 °C, 30 min at each temperature) and using a modified method (2 h at 0 °C and 1 h at each temperature).

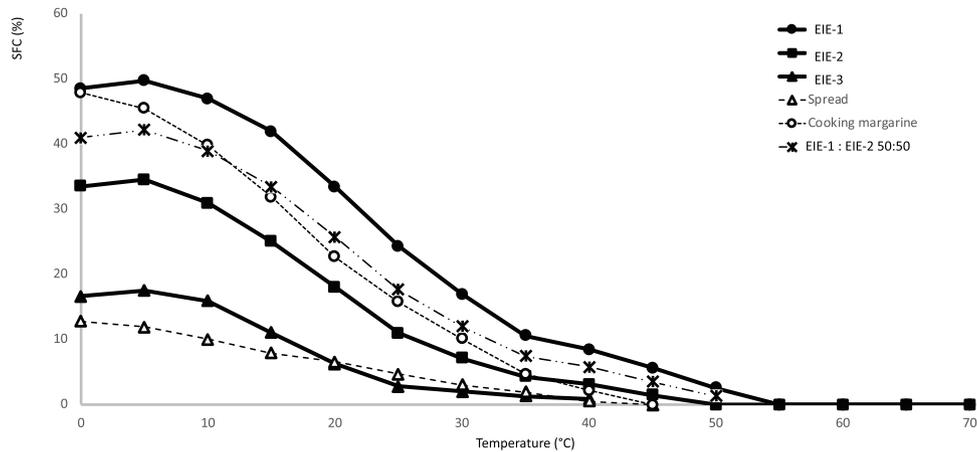


Fig. 6. Comparison of the solid fat content melting curves obtained for the enzymatically interesterified blends (EIE-1 = RO:FHRO 60:40; EIE-2 = RO:FHRO 70:30; EIE-3 = RO:FHRO 80:20 wt:wt%) and fat extracted from commercial reference margarines.

and cooking margarines. The melting profile of EIE-3 resembled the melting profile of the fat phase extracted from the spreadable margarine (Fig. 6). The melting profile of the fat phase extracted from the cooking margarine was intermediate between the melting profiles of EIE-1 and EIE-2: it was similar to the melting profile of a mixture of 50:50 wt% of EIE-1 and EIE-2. These results indicated the great potential of EIE to produce *trans*-free margarine fats fully based on RO.

Finally, the hardness of the EIE-fats was evaluated by cone penetrometry and compared to the same commercial references (Tab. 4). It is obvious that all the EIE-fats were softer than the corresponding NIE ones. The hardness of the reference cooking margarine was very close to a 50:50% blend of EIE-1 and EIE-2 while the spread was very close to EIE-3. The macroscopic appearance of the blends was also modified after EIE (Fig. 7). NIE-blends had a hard and brittle structure,

Table 4. Hardness* (N) of the crystallized fats (blend-1 = RO:FHRO 60:40, blend-2 = RO:FHRO 70:30, blend-3 = RO:FHRO 80:20 wt:wt%).

Blend	Hardness (N)
NIE-1	6.167 ± 0.539
NIE-2	2.398 ± 0.213
NIE-3	0.255 ± 0.539**
EIE-1	3.858 ± 0.208
EIE-2	1.075 ± 0.069
EIE-3	0.421 ± 0.033
EIE-1 : EIE-2 (50:50 wt:wt%)	2.060 ± 0.110
Spreadable margarine	<0.010
Cooking margarine	1.964 ± 0.099

NIE: non Interesterified; IE: interesterified.

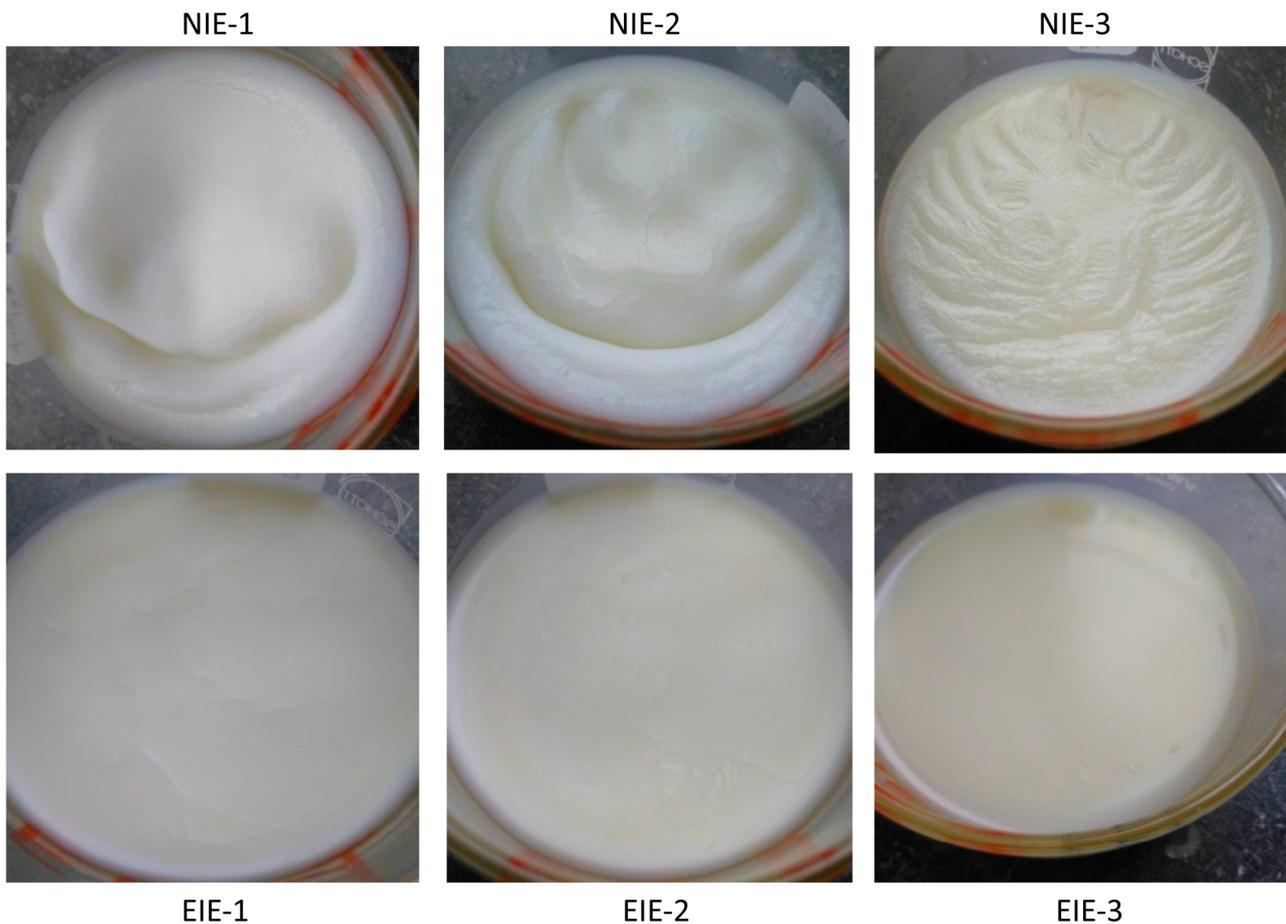
* Mean of at least five replications.

** Huge coefficient of variation due to irregularities in the surface sample, see [Figure 7](#).

Table 5. Tocopherol content* (mg/kg) of the interesterified blend-2 (RO:FHRO 70:30 wt:wt%).

	α -tocopherol [mg/kg]	γ -tocopherol [mg/kg]	δ -tocopherol [mg/kg]	Total [mg/kg]
EIE-2	138	277	6	421
CIE-2	17	162	5	184

* Average value between two independent measurements.

**Fig. 7.** Macroscopic appearance of the three blends before (NIE) and after enzymatic interesterification (EIE) (blend-1 = RO:FHRO 60:40; blend-2 = RO:FHRO 70:30; blend-3 = RO:FHRO 80:20 wt:wt%).

with an irregular surface and it can be noticed that they were crystallized under the β -form, with a double packing (2L) ([Fig. 8](#)). On the contrary, the EIE-blends, mostly crystallized under β' -2L-form, with a small proportion of β -2L-form ([Fig. 8](#)). EIE of the investigated blends not only reduced the content of the high-melting point TAG but also significantly

increased the number of different TAGs, which in turn, favored crystallization into a β' -form, which is the most desired form for margarines. The new *trans*-free fats obtained by EIE were more homogeneous, plastic and glossy compared to the initial blends ([Fig. 7](#)) and are therefore suitable for margarines production.

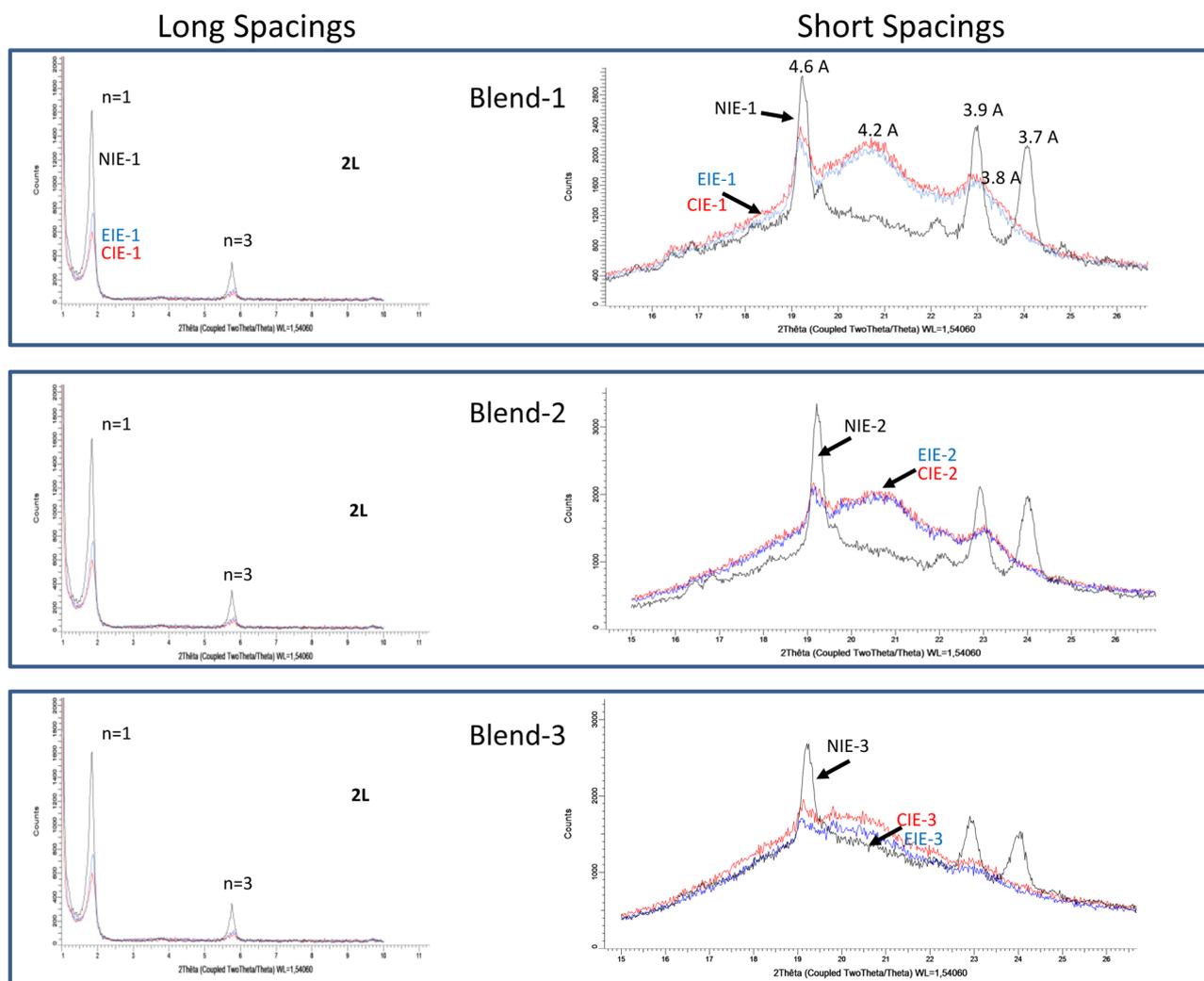


Fig. 8. Powder X-ray diffractogram (short and long spacings) obtained before (NIE) and after chemical (CIE) or enzymatic interesterification (EIE) (blend-1 = RO:FHRO 60:40; blend-2 = RO:FHRO 70:30; blend-3 = RO:FHRO 80:20 wt:wt%).

Table 6. Dialkylketones (DAK) content* (mg/kg) of the NIE, CIE and EIE blends (blend-1 = RO:FHRO 60:40; blend-2 = RO:FHRO 70:30; blend-3 = RO:FHRO 80:20 wt:wt%).

DAK	Blend-1			Blend-2			Blend-3		
	NIE	CIE	EIE	NIE	CIE	EIE	NIE	CIE	EIE
C16–C16	N.D.	0.9	N.D.	N.D.	4.1	N.D.	N.D.	1.5	N.D.
C16–C18:1	N.D.	7.0	N.D.	N.D.	8.1	N.D.	N.D.	31.5	N.D.
C16–C18	N.D.	5.4	N.D.	N.D.	3.3	N.D.	N.D.	9.4	N.D.
C18:1–C18:1	N.D.	31.4	N.D.	N.D.	57.7	N.D.	N.D.	204.1	N.D.
C18:1–C18	N.D.	49.8	N.D.	N.D.	45.0	N.D.	N.D.	136.9	N.D.
C18–C18	N.D.	19.5	N.D.	N.D.	9.4	N.D.	N.D.	31.8	N.D.
C18:1–C20	N.D.	2.1	N.D.	N.D.	<LOQ	N.D.	N.D.	5.7	N.D.
C18–C20	N.D.	1.1	N.D.	N.D.	<LOQ	N.D.	N.D.	0.9	N.D.
Total		117			128			422	

N.D.: not detectable; LOQ: limit of quantification.

* Average value between duplicate samples injection.

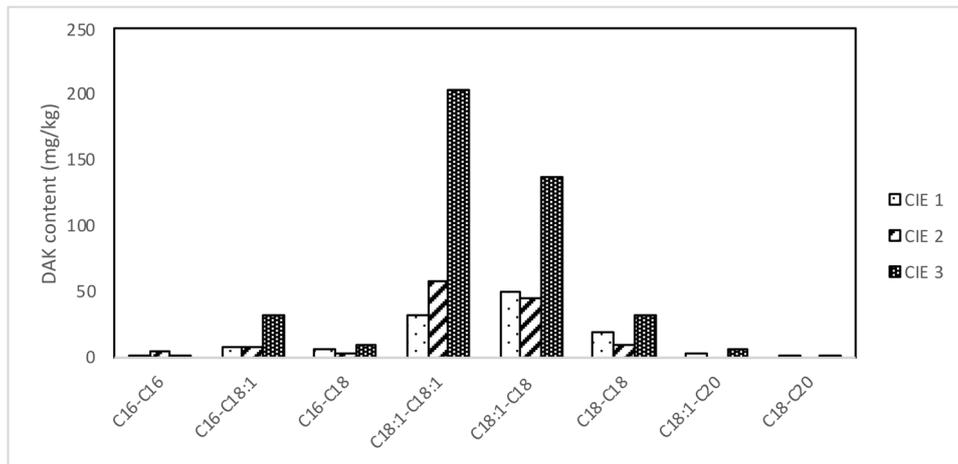


Fig. 9. Dialkylketones (DAK) content (mg/kg) of the three chemically interesterified (CIE) fat blends (CIE-1 = RO:FHRO 60:40; CIE-2 = RO:FHRO 70:30; CIE-3 = RO:FHRO 80:20 wt:wt%).

3.4 Minor components

Besides changing the TAG composition of fats and oils, IE processes can impact the content in minor components. Tocopherols are minor components naturally present in the oil as different isomers: α , β , γ and δ tocopherols. They have vitaminic activities ($\alpha > \beta > \gamma > \delta$) and antioxidative properties ($\delta > \gamma > \beta > \alpha$), so that their content should be preserved as much as possible. Tocopherol content of RO is high, γ - and α - forms being the dominant ones (Piravi-Vanak, 2020).

Results obtained for blend-2 showed that the total tocopherol content was highly affected by CIE, which led to a significantly stronger reduction of the total tocopherol content compared to EIE (Tab. 5). This is consistent with data reported by Costales-Rodríguez *et al.* (2009) for CIE of a blend of palm stearin–soybean oil. The vitaminic activity was better preserved thanks to the milder conditions of the enzymatic process.

3.5 Dialkylketones

Table 6 shows that CIE produced potentially toxic DAK, while EIE did not. Regarding the type and amount formed during the CIE, C18:1–C18:1 and C18:1–C18 were dominant in the three CIE-blends, followed by C18–C18 and C18:1–C16. This was obviously linked to the FA composition of the initial blends as both C18:1 and C18 were the major FA (Tab. 1). Moreover, interestingly, the type and amount of DAK formed during CIE depended on the initial fat composition, and especially the unsaturation degree: the highest amount was found in blend-3 (422 mg/kg), then blend-2 (128 mg/kg), then blend-1 (117 mg/kg), meaning that the amount of DAK formed decreased with the saturation degree. Blend-3, which was the most unsaturated, contained the highest amount of C18:1–C18:1, C18:1–C18 and C18:1–C16 (Fig. 9).

4 Conclusion

This study showed that using FHRO as hard fat and RO as soft oil is a suitable alternative to produce high-quality zero-*trans* margarines by interesterification. The optimal

omega 3/omega 6 ratio of 2.2 is retained in the interesterified products. The interesterified blends have melting characteristics and hardness scales that can simulate commercial margarines, thus IE of RO and FHRO can potentially be used to produce high-quality zero-*trans* fats. However, the triglyceridic composition of the new fats is such that their crystallization rate is slowed down. Regarding DAK formation during CIE, it was shown that the type and amount depended on the initial fat composition, and especially the unsaturation degree. Our results indicate the great potential of IE to produce new fats fully based on RO, however, EIE is preferred to CIE for the preparation of healthy *trans*-free margarine fats for two reasons: (i) better preservation of tocopherols: vitamin supply and preserved oxidative stability and (ii) absence of process-induced contaminants (*i.e.*, DAK). As a consequence EIE can be viewed as cutting-edge sustainable technology for the food industry.

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