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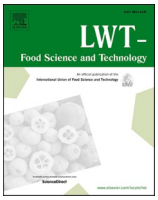

The effect of enzymatic interesterification on the high oleic-high stearic sunflower oil fractionation and the physico-chemical properties of stearins

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ABSTRACT

Oil fractionation processes typically involve the crystallization of the most saturated triacylglycerols to facilitate the separation of the liquid phase. However, the fractionation of new high stearic oils derived from sunflower (HOHS) is not as efficient as that of tropical fats. In the present work we studied the effect of enzymatic inter esterification (EIE) on the HOHS sunflower oil fractionation. EIE altered the properties and melting point of the HOHS oil, so it eased fractionation allowing operation at lower temperatures (8 °C). These fractionations produced stearins with high saturated fatty acid contents at higher yields than the non-EIE oil (28.3% vs 18.7%). The melting profile of the stearins were also characterized. EIE stearins displayed broader melting profiles with higher crystallization onsets (from 26.3 °C to 34 °C). Fractionations proved to be reproducible at pilot plant scale. The resulting stearins exhibited a solid content of 30% at 20 °C and displayed solidification curves similar to palm-based filling fats. The results indicate that changes in fatty acid distribution in the TAG backbone critically affect TAG crystallization, which was faster and more reproducible, not requiring prior dewaxing and seeding. Moreover, the fractionation products differed, yielding stearins with higher levels of solids.

1. Introduction

Most vegetable seed oils are rich in unsaturated fatty acids, mainly oleic, linoleic and linolenic acids. By contrast, tropical fats and oils often contain high levels of saturated fatty acids, which may be short (lauric and myristic) or long chain (palmitic and stearic) fatty acids (Gunstone, 2002). Liquid unsaturated oils are often used, in retailing, frying and sauce formulations. They are also important part of many shortenings and margarines, where they bring desired softness and spreadability characteristics. Tropical oils are usually solid or semi-solid at room temperature, and although some of these products are traded directly, they are generally processed to produce fractions in which saturated fatty acids are concentrated. These fractions serve as a base for the elaboration of many specialty fats and structured lipids, that are used specifically in confectionary, bakery, spreads, ice cream and filling products (Salas et al., 2009). The process of palm oil fractionation is especially remarkable, both in terms of its dimension and complexity.

Palm oil is obtained from the pulp of oil palm fruit and it is an oil rich in palmitic, oleic and linoleic fatty acids. The melting curve of palm oil is complex, with a long melting range from −20 to 50 °C. Thus, at room temperature, it is solid or semisolid that limits its uses. Accordingly, The majority of palm oil production undergoes a commonly employed dry fractionation process, involving the successive crystallization and separation of fractions enriched in triacylglycerols (TAGs) with different degrees of saturation, without adding any solvents. This process is performed in multiple steps, involving crystallization, separation of the solid (stearin) and liquid (olein) phases by filtration, and stearin squeezing at high pressure to expel the olein and augment the proportion of saturated fatty acids. The resulting fractions are palm stearins rich in palmitic acid and tripalmitin TAGs, intermediate fractions like palm mid-fractions rich in mono- and disaturated TAGs, and liquid fractions like oleins and superoleins (Kellens et al., 2007). Palm stearins are generally used as hardstock to formulate lipids of different compositions and melting profiles through enzymatic interesterification (EIE) of liquid oils and palm fractions. The palm mid-fractions are used in confectionary and specialty product formulation. Tropical oils rich in lauric acid, like palm kernel and coconut, are also dry fractionated to produce stearins enriched in lauric acid, which are used to formulate

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Table 1
Percentage of the composition of fatty acids and saturated distribution index (SDI) of HOHS (S15, S15/EIE and S16/EIE) oils.

	Fatty acid						Total saturated	SDI
	16:0	18:0	18:1	18:2	20:0	22:0		
S15	4.5	14.6	76.3	2.4	1.0	1.2	21.3	11.8
S15/EIE								105.8
S16/EIE	4.7	16.3	72.1	4.2	1.2	1.6	23.7	100.5

Data correspond to means of 3 technical replicates. The variation due to the method of determination was below 5% for the fatty acid composition and below 10% for the SDI determination.

cocoa butter substitutes (Dian et al., 2017; Gibon, 2006).

In the past decades, common seed oil species have been modified by mutagenesis or genetic engineering to produce higher levels of saturated fatty acids. This is the case of species like sunflower, canola or soybean (Rahman et al., 1995; Zarhloul et al., 2006; Fernández-Moya et al., 2005). Among these modified oil crops, special attention has been paid to HOHS strains of sunflower that produce oils with more stearic acid on a high oleic acid background, and that could represent a non-GMO alternative to tropical fats for production in temperate climates (Salas et al., 2021). However, the enzymatic machinery of sunflower limits the disaturated TAGs content of HOHS sunflower oil, favoring the accumulation of monosaturated and triunsaturated TAGs (Martínez-Force et al., 2004). In practice HOHS hybrids grown at different locations with distinct climates produce oils with stearate content between 16 and 20% (Salas et al., 2021). HOHS oils with such a saturated fatty acid content are very stable liquid oils with higher cloud points than regular sunflower or high oleic sunflower oils, although they still do not display the melting curves required for use as a plastic or confectionary fat.

The fractionation of HOHS oils has been investigated (Bootello et al., 2011; Salas et al., 2011; Salas et al., 2021) and those can be solvent fractionated to produce stearins with high levels of solids that can be used to formulate cocoa butter equivalents and chocolate compounds with interesting properties. Dry fractionation of this oil is also possible, although it is not as easy as that of palm oil. HOHS oil does not crystallize as fast or as readily as palm oil, and it requires some seeding to ensure the process is controlled and reproducible. Moreover, wax esters (WEs) naturally present in sunflower oil slow and even hinder the crystallization process, also clogging the filtration cloths used in press filters to separate and concentrate the crystallized TAGs. Therefore, a partial dewaxing step is commonly required prior to fractionation (Bootello et al., 2011). The product of this fractionation is a stearin enriched in disaturated TAGs, with potential applications as a plastic fat. Olein is rich in monosaturated TAGs and thus, being a liquid oil especially stable in frying. In all these fractions saturated fatty acids are almost exclusively found on the external *sn*-1,3 positions of the TAGs, giving rise to the so-called symmetrical disaturated TAGs. This can be explained biochemically as oil crops growing in temperate climates have enzymatic machinery that avoids the transfer of saturated fatty acids to the *sn*-2 positions of glycerolipids (Browse & Somerville, 1991; Stymne & Stobart, 1987). Rearranging the internal fatty acid distribution of fats and oils is a common step in their processing, and it can be achieved using either an alkaline chemical catalyzer like sodium methoxide, or enzymatically with immobilized enzymes such as Lipozymes (Gibon, 2011; Holm & Cowan, 2008). Therefore, it should be possible to change the symmetrical distribution of other high saturated oilseeds to obtain distributions closer to those of fats like palm oil to be fractionated in a similar way.

The main objective of this work is to establish an effective process for the production of high melting point stearins from HOHS sunflower.

Table 2
Percentage of the composition of triacylglycerol (TAG) and TAG class composition of the oils used in this study.

Oil sample	Triacylglycerols																			TAG Class		
	PStP	POP	PLP	PStSt	POSt	POO	PLSt	POL	PLL	StStSt	StOSt	SOO	StLSt	OOO	StOL	OOL	SLL	OLL	StOA	OOA	OLA	StOB	OOB	SSS	SUS	SUU	UUU
S15	0.0	0.4	0.0	0.0	2.5	8.6	0.1	0.4	0.2	0.1	3.5	32.7	0.0	41.5	1.3	2.4	0.2	0.2	0.5	2.2	0.0	0.5	2.7	0.1	7.5	48.3	44.1
S15/IE	0.1	0.4	0.1	0.2	2.4	6.5	0.3	0.4	0.2	0.4	4.9	25.1	0.0	44.7	1.9	4.1	0.0	0.2	1.1	2.0	0.2	1.3	3.6	0.7	10.5	39.8	49.0
S16/EIE	0.1	0.5	0.1	0.4	3.3	7.5	0.4	0.9	0.2	0.5	5.9	24.1	1.4	38.3	3.4	6.1	0.3	0.5	0.9	2.0	0.0	1.0	1.9	1.0	13.9	39.9	45.2

Triacylglycerols were named using 3 letters corresponding to the fatty acids: P, palmitic; St, stearic; O, oleic; L, linoleic; A, arachidic; B, behenic. Within the triacylglycerol classes, S represents a saturated fatty acid and U an unsaturated fatty acid. Data represents the means of three technical replicates, the variation due to the method of determination was below 5%. The order given to the letters in all species and classes do not imply any information about their distribution. SSS: trisaturated TAGs; SUS: disaturated TAGs, SUU: monosaturated TAGs, UUU: triunsaturated TAGs.

Table 3
Percentage of the composition of triacylglycerol class of the stearins and oleins obtained from fractionation of the S15 oil at different temperatures.

		Trisaturated SSS			Disaturated SUS			Monosaturated SUU			Triunsaturated UUU		
Stearins	S15	0.1			7.4			48.1			44.4		
	18 °C	0.25	±	0.01 [a]	27.7	±	1.2 [a]	38.8	±	0.7 [a]	33.2	±	0.5 [a]
	16 °C	0.20	±	0.04 [a]	27.4	±	0.4 [a]	38.9	±	0.1 [a]	33.6	±	0.2 [a]
	14 °C	0.01	±	0.02 [b]	27.7	±	1.4 [a]	40.3	±	1.6 [ab]	32.0	±	2.6 [a]
	12 °C	0.10	±	0.01 [b]	21.0	±	1.3 [b]	41.8	±	0.8 [b]	37.1	±	0.5 [b]
	10 °C	0.07	±	0.02 [b]	9.0	±	3.1 [c]	50.2	±	1.0 [c]	40.7	±	4.1 [b]
Oleins													
	18 °C	0.0	±	0.0	6.5	±	0.2 [a]	49.3	±	0.1 [a]	44.2	±	0.2 [a]
	16 °C	0.0	±	0.0	6.2	±	0.6 [ab]	47.7	±	2.3 [a]	46.1	±	1.9 [a]
	14 °C	0.0	±	0.0	5.8	±	0.2 [b]	49.6	±	0.2 [a]	44.56	±	0.04 [a]
	12 °C	0.0	±	0.0	4.6	±	0.6 [b]	50.0	±	0.3 [a]	45.4	±	0.3 [a]
	10 °C	0.0	±	0.0	5.5	±	0.2 [b]	48.5	±	0.4 [a]	46.0	±	0.5 [a]

Data correspond to the average ± SD of 3 independent fractionation experiments. The means of each series were analyzed by using the final fractionation temperature as the treatment, applying a one-way ANOVA with post hoc Tukey test (P value < 0.05). Superscript letters indicate equivalence groups. TAG composition of fractions is shown in Supplementary Table S1.

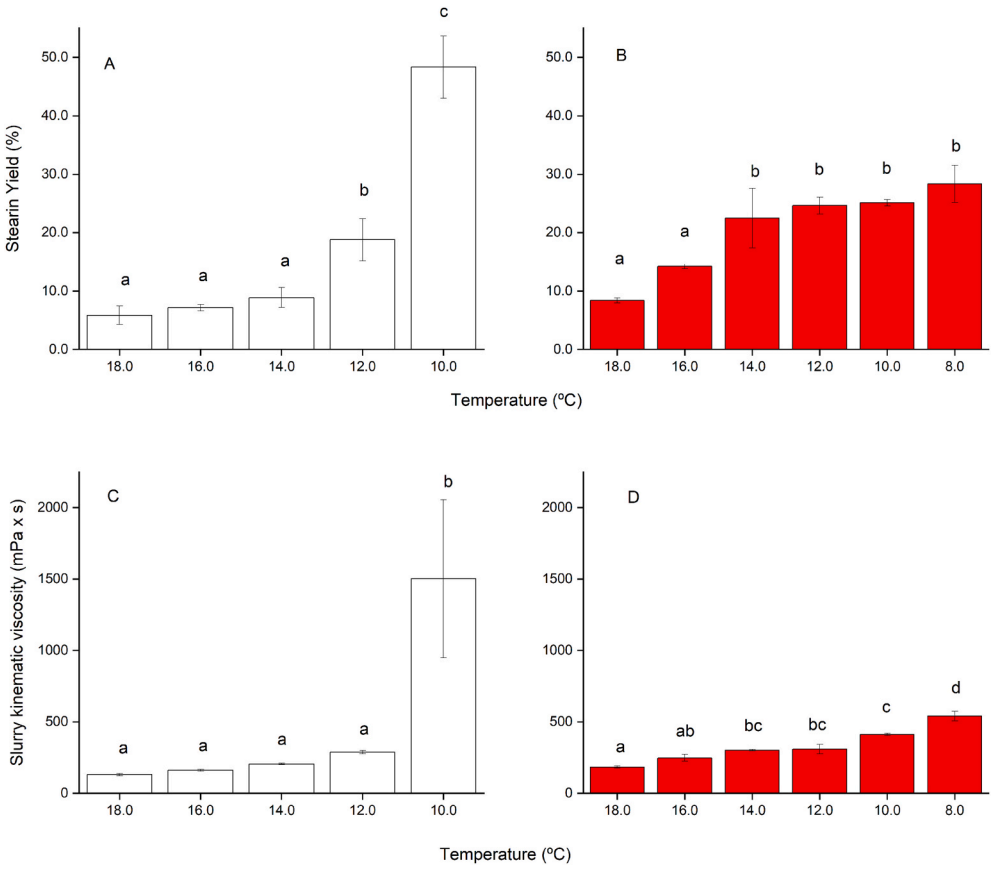

Fig. 1. The stearin yield (A, B) and final slurry viscosity (C, D) following fractionation of the high oleic high stearic S15 (white bars) and S15/EIE (red bars) oils at different temperatures. Data correspond to the average ± SD of 3 independent fractionation experiments. The means of each series were analyzed by using the final fractionation temperature as the treatment applying a one-way ANOVA with post hoc Tukey test (P value < 0.05). Letters on bars indicate equivalence groups within each series. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

These would be stearic and oleic based fats which have been classified as healthier than palmitic based fats (Bonanome & Grundy, 1988) and are an alternative to palm, a crop that has caused controversy among consumers for health and sustainability reasons.

Consequently, the influence of the saturated fatty acid distribution on the fractionation of HOHS sunflower oil has been studied, defining an index to evaluate the distribution of saturated fatty acids in TAGs. This can be established by lipase hydrolysis followed by analyzing the fatty acid composition at the *sn*-2 position of the TAGs that form the oil or fat, relating this to the total saturated fatty acid composition. How this saturated distribution index (SDI) relates to the crystallization profiles of fats and oils, and how they can be fractionated was assessed by comparing fractionation patterns. Moreover, the fraction composition was studied, comparing the results obtained with the fractionation of non-interesterified HOHS sunflower oils and the same oils submitted to enzymatic interesterification (EIE). Pilot plant scale trials were carried out which indicated that the process can be scaled up without significantly modifying the results observed in previous trials.

2. Experimental procedures

2.1. Oils used

Three HOHS sunflower oils were used in this work: a non-interesterified (non-EIE) refined, bleached and deodorized (RBD) oil that contains 14.6% stearic acid and that was named S15; the same oil

Table 4
Percentage of the composition Fatty acids of the stearins and oleins obtained from fractionation of the S15 oil at different temperatures.

		16:0			18:0			18:1			18:2			20:0			22:0			Total SAT			SDI		
	S15	4.5			14.6			76.3			2.4			1.0			1.2			21.3			11.8		
Stearins	18 °C	5.2	±	0.1^{a}	23.1	±	0.3^{a}	66.3	±	0.9^{a}	1.78	±	0.03^{a}	1.5	±	0.2^{a}	2.1	±	0.4^{a}	31.9	±	0.9^{a}	11.3	±	0.7^{a}
	16 °C	5.4	±	0.2^{a}	22.8	±	0.5^{a}	67.0	±	0.5^{a}	1.76	±	0.03^{a}	1.3	±	0.2^{a}	1.73	±	0.03^{a}	31.3	±	0.6^{a}	8.4	±	0.3^{b}
	14 °C	5.39	±	0.01^{a}	22.6	±	0.5^{a}	66.8	±	0.6^{a}	1.77	±	0.03^{a}	1.5	±	0.1^{a}	2.0	±	0.1^{a}	31.4	±	0.6^{a}	9.9	±	0.2^{c}
	12 °C	5.3	±	0.1^{a}	20.0	±	0.6^{b}	69.5	±	0.7^{b}	1.9	±	0.1^{b}	1.3	±	0.04^{a}	1.9	±	0.1^{a}	28.5	±	0.7^{b}	9.5	±	0.7^{ab}
	10 °C	4.34	±	0.04^{b}	17.4	±	0.1^{c}	73.1	±	0.6^{c}	2.1	±	0.1^{c}	1.3	±	0.1^{a}	1.7	±	0.6^{a}	24.8	±	0.6^{c}	8.8	±	1.8^{ab}
Oleins	18 °C	4.4	±	0.2^{a}	14.2	±	0.2^{a}	77.6	±	0.6^{a}	2.2	±	0.1^{a}	0.8	±	0.1^{ab}	0.8	±	0.1^{a}	20.2	±	0.5^{a}	nd		
	16 °C	4.56	±	0.02^{a}	14.1	±	0.1^{a}	77.6	±	0.2^{a}	2.29	±	0.1^{ab}	0.7	±	0.1^{a}	0.8	±	0.1^{a}	20.2	±	0.2^{a}	nd		
	14 °C	4.5	±	0.1^{a}	13.90	±	0.02^{a}	77.4	±	0.2^{a}	2.29	±	0.02^{ab}	1.0	±	0.2^{b}	0.9	±	0.2^{a}	20.4	±	0.2^{a}	nd		
	12 °C	4.4	±	0.1^{a}	13.4	±	0.2^{a}	78.0	±	0.3^{a}	2.41	±	0.01^{ab}	0.9	±	0.1^{ab}	0.8	±	0.1^{a}	19.6	±	0.2^{a}	nd		
	10 °C	4.34	±	0.03^{a}	12.3	±	0.6^{b}	79.2	±	0.7^{b}	2.5	±	0.1^{b}	0.9	±	0.1^{ab}	0.8	±	0.1^{a}	18.3	±	0.6^{b}	nd		

Data correspond to the average ± SD of 3 independent fractionation experiments. The means of each series were analyzed by using the final fractionation temperature as the treatment, applying a one-way ANOVA with post hoc Tukey test (P value < 0.05). Superscript letters indicate equivalence groups. SAT, saturated fatty acids; SDI, saturated distribution index.

being enzymatically interesterified, which was named S15/EIE and an EIE (see below) RBD oil containing 16.3% stearic acid, named S16/EIE (both supplied by Bunge Loders Croklaan BV). The S15 oil was used for laboratory scale trials and the S16/EIE oil for the pilot plant scale trials. The reactions of enzymatic interesterifications were carried out with Lipozyme TL (Novozymes, Copenhagen, Denmark) applying a procedure depicted ahead. A palm-based commercial filling fat was used as reference for comparison of solid fat content profiles of pilot plant scale stearins.

2.2. Fatty acid composition

The fatty acid composition of the lipid and oil fractions was determined by transmethylation of the fatty acids to produce the corresponding fatty acid methyl esters (FAMEs). Transmethylation was carried out at 80 °C by treating 5–10 mg of the sample with a 2 mL volume of 2% H_2SO_4/10% toluene in methanol. The resulting FAMEs were extracted with 2 mL heptane and analyzed by gas chromatography (GC) on an Agilent 7890 GC system (Palo Alto, USA) coupled to a Supelco SP-2380 column (30 m × 0.25 mm, 0.2 μm liquid phase: Bellefonte, USA) that was maintained at 180 °C. A pressure ramp from 80 to 100 kPa was applied at a rate of 10 kPa/min, the carrier gas was hydrogen and a split ratio of 40:1 was used. The retention times of the different fatty acids were determined by injecting the corresponding standards. The standards used were commercial methyl esters, purchased from Merk (Darmstat, Germany).

2.3. Determination of triacylglycerol (TAG) composition

The TAG determination was performed by GC using a similar system to that reported previously (Bootello et al., 2011), into which the oils (conveniently diluted in hexane) were injected directly. The Agilent 8890 GC system (Palo Alto, CA, USA) was equipped with a Quadrex Aluminium-Clad 400-65HT column (30 m × 0.25 mm i. d., 0.1 μm film thickness: Woodbridge, CT, USA), using hydrogen as the carrier gas. The injector and detector temperatures were both 350 °C, the oven temperature was 320 °C, a head pressure gradient from 70 to 120 kPa was applied during analysis and a 40:1 split ratio was used. The different TAG species were identified and quantified according to Fernández-Moya et al. (2000), applying the correction factors reported by Carelli and Cert (1993). When necessary, commercial TAG standards purchased to Larodan (Solna, Sweden) were used.

2.4. Determination of the saturated distribution index (SDI)

The SDI of a given oil or fraction was defined as the ratio in percentage of the composition of saturated fatty acids in the *sn*-2 position ($SAFA_{sn\text{-}2}$) against the total saturated fatty composition of the oil or fat ($SAFA_{Total}$).

$$SDI = \frac{SAFA_{sn-2}}{SAFA_{Total}} \times 100$$

The total proportion of saturated fatty acids is given through the fatty acid composition of the fat and the composition at the *sn*-2 position can be determined by pancreatic lipase analysis. The method applied was a modification of the standard Method ISO 6800, 1997 method. The oil or fat (100 mg) was transferred to a tube and a 2 mL volume of 0.1 M Tris pH 8.5/0.1% sodium cholate was added ($C_{24}H_{39}NaO_5$; CAS: 361-09-1), homogenizing the mixture at 80 °C. The resulting emulsions were added 1–5 units of pancreatic lipase (Sigma-Aldrich, San Luis, Misuri, Estados Unidos) and vortexed for 2 min. The reaction was then stopped by adding 0.5 mL 5 M HCl and the lipids were extracted with 2 mL ethyl ether. The lipids resulting from hydrolysis were separated by TLC using a 20 × 20 plate of silica gel, which was developed with hexane/ethyl ether/acetic acid (50:50:1 by volume) as the solvent. The band corresponding to monoacylglycerols was scrapped off from the plate and

Table 5
Percentage of the composition of triacylglycerol class of the stearins and oleins obtained from fractionation of the S15/EIE oil at different temperatures.

		Trisaturated SSS			Disaturated SUS			Monosaturated SUU			Triunsaturated UUU		
	S15/EIE	0.1			7.5			48.1			44.3		
Stearins	18 °C	9.8	±	0.1[a]	23.0	±	0.4[ab]	31.5	±	0.4[a]	35.8	±	0.2[a]
	16 °C	5.6	±	0.4[b]	24.3	±	0.8[a]	33.4	±	0.2[b]	36.8	±	0.9[ab]
	14 °C	4.6	±	0.9[bc]	21.2	±	1.5[ab]	35.1	±	0.8[c]	39.2	±	1.6[b]
	12 °C	4.0	±	0.1[c]	20.8	±	1.6[ab]	36.1	±	0.3[cd]	39.1	±	1.5[b]
	10 °C	3.5	±	0.4[c]	20.1	±	0.6[b]	36.7	±	0.2[d]	39.7	±	0.7[b]
	8 °C	3.3	±	0.3[c]	19.4	±	0.2[b]	37.5	±	0.3[d]	39.8	±	0.2[b]
Oleins	18 °C	0.2	±	0.1[a]	9.3	±	2.3[a]	40.0	±	0.90[a]	50.5	±	1.62[a]
	16 °C	0.0	±	0.0[b]	7.9	±	0.2[b]	41.0	±	0.13[b]	51.0	±	0.14[ab]
	14 °C	0.0	±	0.0[b]	7.3	±	0.7[bc]	40.9	±	0.07[b]	51.8	±	0.69[bc]
	12 °C	0.0	±	0.0[b]	6.9	±	0.5[bc]	41.2	±	0.26[b]	51.9	±	0.27[bc]
	10 °C	0.0	±	0.0[b]	7.0	±	0.3[bc]	40.9	±	0.06[b]	52.1	±	0.19[c]
	8 °C	0.0	±	0.0[b]	6.6	±	0.3[d]	41.0	±	0.28[b]	52.3	±	0.42[c]

Data correspond to the average ± SD of 3 independent fractionation experiments. The means of each series were analyzed by using the final fractionation temperature as the treatment, applying a one-way ANOVA with post hoc Tukey test (P value < 0.05). Superscript letters indicate equivalence groups. TAG composition of fractions is shown in Supplementary Table S2.

transmethylated. The resulting FAMEs were analyzed by GC as described above and considered to be the fatty acid composition at the *sn*-2 position of the fat. With this definition, oils presenting a symmetrical distribution of saturated fatty acids will have them esterified the Sn-1 and Sn-3 positions, showing SDI values close to zero. When the distribution is random, the composition of the 3 positions will be identical, so the SDI value will be 100.

2.5. Enzymatic interesterification of oils

Immobilized lipozyme TL (Novozymes, Bagsværd, Denmark) was used for oil EIE. Prior to performing the reaction, the enzyme was conditioned with the same oil, maintaining 50 g of the enzyme with 500 mL of the HOHS sunflower oil for 30 min at 70 °C, applying vacuum and stirring in a rotavapor for 3 times. The enzyme was then filtered in a Buchner funnel and fresh oil was added. The conditioned oil was then used in EIE reactions that were carried out at 70 °C under nitrogen for 24 h in a 1 L reactor, and with sufficient stirring to keep the enzyme suspended in the oil. The enzyme used (0.5% by weight) was added to the reaction when the oil reached the reaction temperature and after the reaction, the EIE oil was filtered in a Buchner funnel and the enzyme reutilized or stored at 5 °C (see Supplementary Fig. S1). In these conditions the EIE reaction was complete and the free fatty acid content of the oil did not increase more than one unit. In the case that it rose 1.0 units or more, the oil was submitted to neutralization.

2.6. Dry fractionation of the oils

Oils were fractionated at laboratory scale in a 1 L crystallizer. The general procedure involved loading the oil and destroying the previous crystalline structure by heating at 60 °C, followed by cooling it to 35 °C at the maximum cooling capacity of the bath. The temperature of the oil was then decreased to 18 °C by applying a 2 h ramp. The oil was kept at that temperature for 3 h before applying a ramp to the final crystallization temperature of 2 h. The slurry was then maintained at that temperature for at least 16 h. Crystal seeding was employed to fractionate non-IE oils using a slurry produced with the same oil crystalized at 18 °C for 5 days and kept at that temperature. At least 1% of seeding slurry was used to induce crystallization and stirring was kept as low as possible to maintain the temperature homogeneous within the crystallizer (about 30 rpm). Prior to phase separation, the viscosity of the slurry was measured with a Nahita model 802 viscosimeter (Navarra, Spain). A 50 mL volume of the crystalized slurry was filtered under vacuum in a Buchner funnel precooled on ice and using two layers of Miracloth tissue (Merk, Darmstadt, Germany) as the filtering medium. Stearin was then washed with 100 mL of ice-cooled hexane to remove the liquid olein trapped in it. In general, all fractionations involved 3 slurry filtrations to get an average of the compositions. The phase separation method was consistent and the composition within the same crystallization did not usually vary more than 5%. The solvent was then removed from the resulting fractions and they were characterized.

Some fractionations were carried out on a mini pilot plant scale using a Desmet Ballestra (Brussels, Belgium) fractionation miniplant. The fractionation set-up and procedure was similar to that describe by Calliauw et al. (2007). fractionations were performed on a 5–10 L scale, with a stirring and temperature program similar to that described above. In this case, filtration of the slurry was performed with a membrane press filter, increasing the pressure inside the crystallizer up to 5 bar. Once filled, the connections to the crystallizer were closed and the stearin cake was squeezed, steadily applying pressure within the filtration unit until it reached 25–30 bar. The stearin was finally obtained by releasing the pressure and dismounting the filter.

2.7. Differential scanning calorimetry

The melting and crystallization profiles of the oil fractions obtained were studied by differential scanning calorimetry (DSC) in a Q2000 V23.5 scanner (TA instruments, New Castle, DE, USA), calibrated prior to use with indium, azobenzene and undecane. The samples were studied by loading approximately 6–7 mg of the oil or fat sample into the Tzero aluminum pans, which were then sealed, and using an empty pan as a reference. The weight of the pans and the sample was measured in an electronic microbalance (Sartorius M2P: Sartorius AG, Goettingen, Germany) and the melting curves were then obtained by destroying the crystalline structure of the sample by heating at 80 °C for 5 min. The oil was then cooled to −80 °C by applying a ramp of −10 °C/min and it was kept at that temperature for 5 min. Subsequently, a heating ramp to 90 °C was applied at a rate of 5 °C/min, during which heat flow was monitored. The crystallization profile was obtained by destroying the structure at 90 °C for 5 min and applying a cooling ramp to −80 °C during which the heat flow in the sample was recorded. Nitrogen was used to purge the system after each analysis and the results were processed using the TA Universal Analysis software provided by the manufacturer.

2.8. Solid fat content by p-NMR

The solid fat content was determined by pulsed nuclear magnetic resonance using a Bruker Minispec unit, equipped with on-board software for data processing (Bruker, Milton, Ontario, Canada). Nuclear

Table 6
Percentage of the composition of fatty acids of the stearins and oleins resulting from fractionation of S15/EIE oil at different temperatures.

		16:0			18:0			18:1			18:2			20:0			22:0			Total SAT			SDI		
	S15/EIE	4.5			14.6			76.3			2.4			1.0			1.2			21.3			105.8		
Stearins	18 °C	6.5	±	0.2^{a}	24.6	±	0.5^{a}	63.4	±	0.8^{a}	1.91	±	0.01^{a}	1.60	±	0.02^{ab}	2.0	±	0.2^{ab}	34.7	±	0.8^{a}	107.4	±	0.6^{a}
	16 °C	6.0	±	0.1^{b}	22.7	±	0.4^{ab}	66.3	±	0.5^{ab}	1.98	±	0.04^{a}	1.41	±	0.02^{bc}	1.7	±	0.2^{ab}	31.8	±	0.6^{b}	102.7	±	1.5^{b}
	14 °C	5.3	±	0.1^{c}	21.6	±	1.7^{bc}	67.6	±	3.0^{bc}	1.4	±	0.9^{a}	1.8	±	0.3^{a}	2.4	±	0.7^{a}	31.1	±	2.1^{bc}	100.6	±	4.9^{bc}
	12 °C	4.1	±	0.1^{d}	21.7	±	0.9^{bc}	68.3	±	1.0^{bc}	1.87	±	0.04^{a}	1.6	±	0.1^{ab}	2.4	±	0.10^{a}	29.9	±	1.1^{bcd}	97.0	±	2.1^{c}
	10 °C	5.2	±	0.1^{c}	20.4	±	0.2^{c}	69.8	±	0.3^{c}	1.83	±	0.02^{a}	1.22	±	0.02^{cd}	1.66	±	0.03^{ab}	28.4	±	0.3^{cd}	99.0	±	2.2^{c}
	8 °C	5.24	±	0.02^{c}	20.0	±	0.3^{c}	70.5	±	0.3^{c}	1.82	±	0.01^{a}	1.04	±	0.04^{d}	1.40	±	0.02^{b}	27.6	±	0.3^{d}	94.4	±	3.1^{c}
Oleins	18 °C	4.6	±	0.1^{a}	13.0	±	0.1^{a}	78.0	±	0.2^{a}	2.41	±	0.04^{a}	0.80	±	0.03^{a}	1.12	±	0.07^{a}	19.6	±	0.18^{ab}	nd		
	16 °C	4.5	±	0.1^{b}	12.5	±	0.1^{ab}	78.9	±	0.1^{bc}	2.46	±	0.02^{a}	0.73	±	0.04^{a}	0.85	±	0.14^{b}	18.6	±	0.09^{c}	nd		
	14 °C	4.45	±	0.02^{bc}	12.4	±	0.3^{bc}	79.4	±	0.5^{c}	2.26	±	0.3^{a}	0.7	±	0.4^{a}	0.80	±	0.10^{b}	20.0	±	0.12^{b}	nd		
	12 °C	4.35	±	0.01^{c}	12.4	±	0.2^{bc}	78.5	±	0.2^{ab}	2.35	±	0.08^{a}	0.93	±	0.02^{a}	1.47	±	0.03^{c}	19.1	±	0.23^{bc}	nd		
	10 °C	4.04	±	0.01^{d}	12.4	±	0.1^{bc}	79.9	±	0.1^{de}	2.21	±	0.02^{a}	0.60	±	0.02^{a}	0.88	±	0.03^{ab}	17.9	±	0.10^{d}	nd		
	8 °C	4.0	±	0.1^{c}	11.9	±	0.2^{c}	80.5	±	0.4^{e}	2.21	±	0.02^{a}	0.59	±	0.05^{a}	0.82	±	0.10^{a}	17.3	±	0.38^{d}	nd		

Data correspond to the average ± SD of 3 independent fractionation experiments. The means of each series were analyzed by using the final fractionation temperature as the treatment, applying a one-way ANOVA with post hoc Tukey test (P value < 0.05). Superscript letters indicate equivalence groups. SAT, saturated fatty acids; SDI, saturated distribution index.

magnetic resonance tubes with a 10 mm diameter were filled with approximately 2.0–2.5 g of completely melted fat. Samples were measured according to Method ISO 8292-1, 2008 official method (non-stabilized, parallel).

2.9. Statistical analysis

Analysis were made in triplicate and compositions of initial oils corresponded to the average of that 3 technical replicates. Fractionations were carried out in triplicate, so data concerning them were expressed as mean ± standard deviations of compositions obtained in independent trials, this includes slurry viscosities, stearin yield, SDI values and fraction composition. The means comparison was carried out using the final fractionation temperature as the treatment, applying a one-way ANOVA with post hoc Tukey test (P value < 0.05). The studies of DSC were also made in triplicate, being the data supplied the mean of the parameters corresponding to stearin samples coming from different trials ± standard deviations. This concerned to data of melting peaks, crystallization onsets and melting intervals. The statistical treatments were carried out using the program IBM SPSS Statistics 27.

3. Results

3.1. Feedstock characterization

Three HOHS oils (namely S15, S15/EIE and S16/EIE) were used in this work having different stearate content. Fatty acid compositions are presented in Table 1. S15 and S15/EIE oils share the same fatty acid composition but different SDI value since interesterification rearranges fatty acid within TAG backbone but does not change fatty acid composition. Oils S15, S15/EIE and S16/EIE contained 14.6 and 16.3% of stearic acid, low linoleic acid content (2.4 and 4.2%, respectively) with a total saturated fatty acid content of 21.3 (S15) and 23.7% (S16/EIE) (Table 1). The saturated fatty acids were mostly bound at the 1,3 external position of the TAGs in the non-EIE S15 oil, with a SDI of 11.8. By contrast, both enzymatically interesterified HOHS oils had SDIs around 100, which indicated that the reaction was complete and the saturated fatty acids were distributed equally among all the TAG positions, the TAG compositions of both these oils are shown in Table 2. The initial S15 oil had a disaturated TAG content of 7.5%, with only traces of trisaturated TAGs (0.1%). The predominant TAGs were those of the monosaturated class with 48.3% content. The EIE reaction increased both the trisaturated and disaturated TAG content in S15 to values of 0.7% and 10.5%, respectively, at the expense of monosaturated class TAGs. An increase in triunsaturated TAGs was also observed after the EIE reaction (Table 2). The TAG composition of the S16/EIE oil experienced similar changes in composition as those expected for an EIE oil of this class, with slightly more disaturated and trisaturated TAGs than in the S15 oil.

3.2. Fractionation of S15 HOHS sunflower oil

The first set of fractionation trials involved the S15 HOHS sunflower oil, applying final fractionation temperatures from 18 to 10 °C. The composition of the resulting stearin and olein fractions were analyzed after phase separation (Table 3). The disaturated TAG content of stearins increased between 29.7 and 21% in the 18 to 12 °C range, with only traces of trisaturated TAGs. A higher disaturated content was obtained at higher final fractionation temperatures, while at 10 °C the increase in disaturated TAGs was low with respect to the initial oil, reaching 9.0%. The disaturated TAG composition of oleins decreased as the temperature dropped from 18 to 10 °C, ranging from 6.5 to 4.6%, which represented an increase in the recovery of high melting point TAGs at the lower temperatures in that range. At 10 °C the disaturated content of the olein was 5.5%. The stearin yield and the viscosity of the crystalized slurry increased progressively from 18 to 12 °C, reaching levels close to 20%

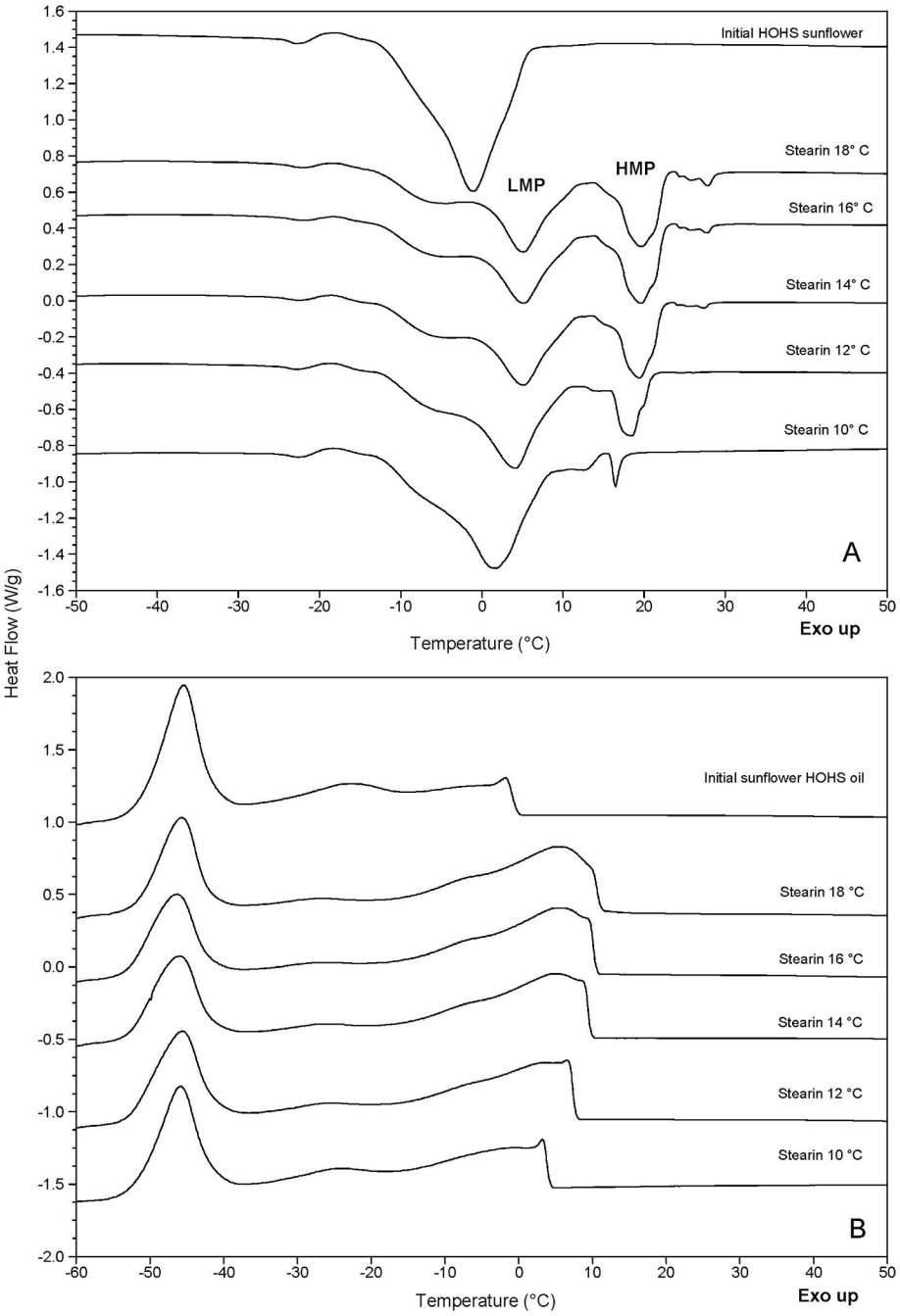

Fig. 2. Melting (A) and crystallization (B) curves of the high oleic high stearic S15 oil and the stearins obtained from its fractionations at different temperatures. VLMP: very low meting point peaks, LMP: low melting point peaks, HMP: high melting peaks.

and 250 mPa × s at 12 °C, respectively. However, at 10 °C the viscosity of the slurry was considerably higher, reaching values around 1400 mPa × s (Fig. 1). A much larger amount of stearin resulted from this fractionation, although its disaturated and saturated fatty acid content was not high (Table 2, Fig. 1). Both the fatty acid composition and saturated fatty acid distribution of the stearins was determined too (Table 4), with all stearins displaying SDI values around 10 that were similar to the initial oil. No fractionation was possible at temperatures below 10 °C.

The same series of trials were run for the EIE S15 oil (S15/EIE). The stearins resulting from this series of fractionations contained both trisaturated and disaturated TAGs (Table 5). The trisaturated TAG content decreased with the final fractionation temperature, varying from 9.8 to 3.3% from 18 to 8 °C. The disaturated level varied less with the fractionation temperature, remaining in the range between 23 and 19% at the temperatures studied. Only traces of trisaturated TAGs or none at all were detected in oleins and the disaturated content varied from 9.3 to 6.6% (see Table 6 for the saturated fatty acid content and distribution). The stearic acid content of stearins was between 22.9 and 19.8%, which was similar to that in stearins obtained from non-IE oils (Tables 4 and 6). The SDI value remained around 100, although it tended to decrease in the stearins obtained at lower temperatures. A higher stearin yield was obtained at lower fractionation temperature, in a range between 7.4 and 29% at the temperatures assayed (Fig. 1A). The slurry was always easy to filter, with viscosity values between 184 and 540 mPa × s.

3.3. DSC characterization of oils and stearins

The melting and crystallization curves of the stearins resulting from

Table 7
Data obtained from the melting and crystallization curves by differential scanning calorimetry of the initial oils and the different stearins obtained from non-interesterified (not IE) or interesterified sunflower HOHS oils.

	Fraction	VLMP (°C)			LMP (°C)			HMP (°C)						Crystallization onset (°C)		
Not IE	S15 Oil		–		−1.2	±	0.0		–			–		0.2	±	0.3
	Stearin 18 °C		–		5.0	±	0.1	19.6	±	0.1		–		11.3	±	0.7
	Stearin 16 °C		–		4.8	±	0.6	19.4	±	0.6		–		10.3	±	1.1
	Stearin 14 °C		–		5.0	±	0.1	19.5	±	0.0		–		10.6	±	0.3
	Stearin 12 °C		–		3.9	±	0.5	18.4	±	0.5		–		8.2	±	0.8
	Stearin 10 °C		–		1.2	±	0.4	14.4	±	1.8		–		3.9	±	1.1
Interesterified	EIE S15 Oil	−9.9	±	0.1	9.8	±	0.2		–			–		17.9	±	0.8
	Stearin 18 °C	−8.6	±	0.1	7.9	±	1.3	26.6	±	0.5	45.8	±	0.3	34	±	1.1
	Stearin 16 °C	−8.0	±	0.2	9.1	±	0.1	25.5	±	0.7	43.4	±	0.4	29.4	±	0.5
	Stearin 14 °C	−10.5	±	0.6	8.5	±	0.8	28.2	±	1.0	39.2	±	0.6	29.9	±	1.5
	Stearin 12 °C	−10.3	±	0.4	8.3	±	0.6	26.2	±	0.3	37.6	±	0.5	27.4	±	1.2
	Stearin 10 °C	−9.8	±	0.9	8.4	±	0.3	25.7	±	0.9	37.5	±	0.7	26.3	±	0.7
	Stearin 8 °C	−10.7	±	0.4	8.3	±	0.3	26.0	±	0.2	37.1	±	0.2	26.3	±	0.5

Data correspond to the average ± SD of 3 independent fractionation experiments. VLMP: very low meting point peaks, LMP: low melting point peaks, HMP: high melting peaks.

the two series of fractionations of S15 and S15/EIE oils were studied by DSC. Results corresponding to S15 oil and stearins are shown in Fig. 2 and Table 7. S15 oil displayed a melting interval of ΔT = 39.8 °C (ranging from −24.8 to 15.0 °C), with a major endothermic peak at −1.2 °C. The stearins displayed several heat transfer peaks and more extended intervals, from 44.7 to 57 °C (Fig. 2A). Thermograms for S15 stearins exhibited low-melting and high-melting peaks and displayed wider melting intervals embracing from ΔT = 44.7 to 57 °C (Fig. 2A). The stearins obtained at 18 to 12 °C displayed similar curves, whereas those obtained as a result of the fractionation at 10 °C had a curve similar to that of the initial oil. Small endothermic peaks were also observed at approximately 28 °C. With regards to the crystallization curves, the onsets of stearin crystallization was higher than that of the initial oil (0.2 °C), between 8.2 and 11.3 °C, whereas the onset of crystallization of the stearins obtained at 10 °C began at 3.9 °C. The data corresponding to the S15/EIE oil and the S15/EIE stearins resulting from its fractionation at different temperatures are shown in Fig. 3 and Table 7. The S15/EIE oil displayed a melting profile wider than the S15 oil, embracing from −27.7 to 38.5 °C (ΔT = 66.2 °C). S15/EIE oil showed a major very-low-melting peak at −9.9 °C but, unlike S15 oil, there were some shoulders in the low-melting-point region (0–20 °C). S15/EIE stearins also displayed wider melting intervals with 3 different peak regions (very-low-melting, low-melting and high-melting). The melting intervals varied from ΔT = 69.2 to 76.6 °C, being broader for the S15/EIE stearins having higher trisaturated TAGs content. The crystallization onsets were also higher for the S15/EIE oil and stearins as compared with their non-interesterifed homologous (Fig. 3B). Thus, the crystallization onset of the S15/EIE oil was 17.9 °C, whereas those from the stearins varied from 26.3 to 34.0 °C.

3.4. Fractionation at pilot plant scale

Fractionation trials were run at pilot plant scale using press filtration and squeezing to separate the stearins. The IE oil used in this experiment was slightly different (S16/EIE), with a higher level of stearic acid (16.3%) and linoleic acid (4.2%: Table 1). The oil was fractionated at 10 and 8 °C and the stearins obtained at those temperatures were very similar (Tables 8 and 9), displaying around 27% of stearate, with trisaturated and disaturated contents around 3.5 and 34%, respectively. The SDI index was also the same for both stearins with values of 92.2 and, as expected from bench trial results, close to 100. The trials produced similar amounts of stearins, with yields of being around 27% of the initial oil.

3.5. p-NMR characterization of stearins

The solid fat content (SFC) of the stearins obtained at pilot plant scale by fractionation of S16/EIE was determined by p-NMR in a range of temperatures embracing from 10 to 40 °C. The curves of solids of both fats were similar, decreasing steadily along those temperatures (Fig. 4). When compared with a commercial palm-based filling fat the HOHS sunflower stearins displayed less solid content at 10 °C but higher at 20 °C. At higher temperatures all the 3 fats displayed similar SFC values.

4. Discussion

HOHS sunflower oil can be dry fractionated to produce stearins with higher levels of saturated fatty acids, as shown previously (Bootello et al., 2011). This process is limited by several factors, such as the small range of temperatures that can be used and the presence of WEs, which restricts the possible uses of this oil. Here we studied how rearranging the internal distribution of saturated fatty acids by means of an EIE reaction affects the fractionation of this oil, with the idea of obtaining a simpler, more repeatable and better performing process. We fractionated the S15 oil at different temperatures, which allowed us to determine the window of suitable temperatures for fractionation and the composition of the resulting fractions. The general procedure of fractionation involved crystallization of the oil at 18 °C, a temperature at which well-shaped crystals with higher levels of saturated fatty acids were formed. This was followed by maintaining this final fractionation temperature or ramping it down, which induced the growth of the crystals initially formed. This method gives reproducible and reliable results that are easily scalable to establish an industrial process.

The use of EIE is quite widespread in the production of specialty oils and fats. Most of these works involved the interesterification of palm and palm kernel fractions with other oils to get fats with the appropriate melting profiles (Khatoon et al., 2012; Sivakanthan & Madhujith, 2020). Therefore, EIE is used with stearins obtained from fractionation and is not usually applied prior that process. Other combination of these two operations takes place in the production of cocoa butter equivalents from EIE between high oleic oils and stearic acid using 1,3-specific lipozyme RM (Ray et al., 2014, 2022). In this case EIE is associated to solvent fractionation to produce stearins with very high contents of desaturated type TAGs. Palm oil and palm kernel oil are not usually interesterified prior fractionation due they have a near-random fatty acid distribution. Therefore, as far as we know, the effect of EIE on the fractionation of saturated-rich and symmetrically distributed oils had not been investigated to date.

The cost of the enzyme could be a limiting factor for the development

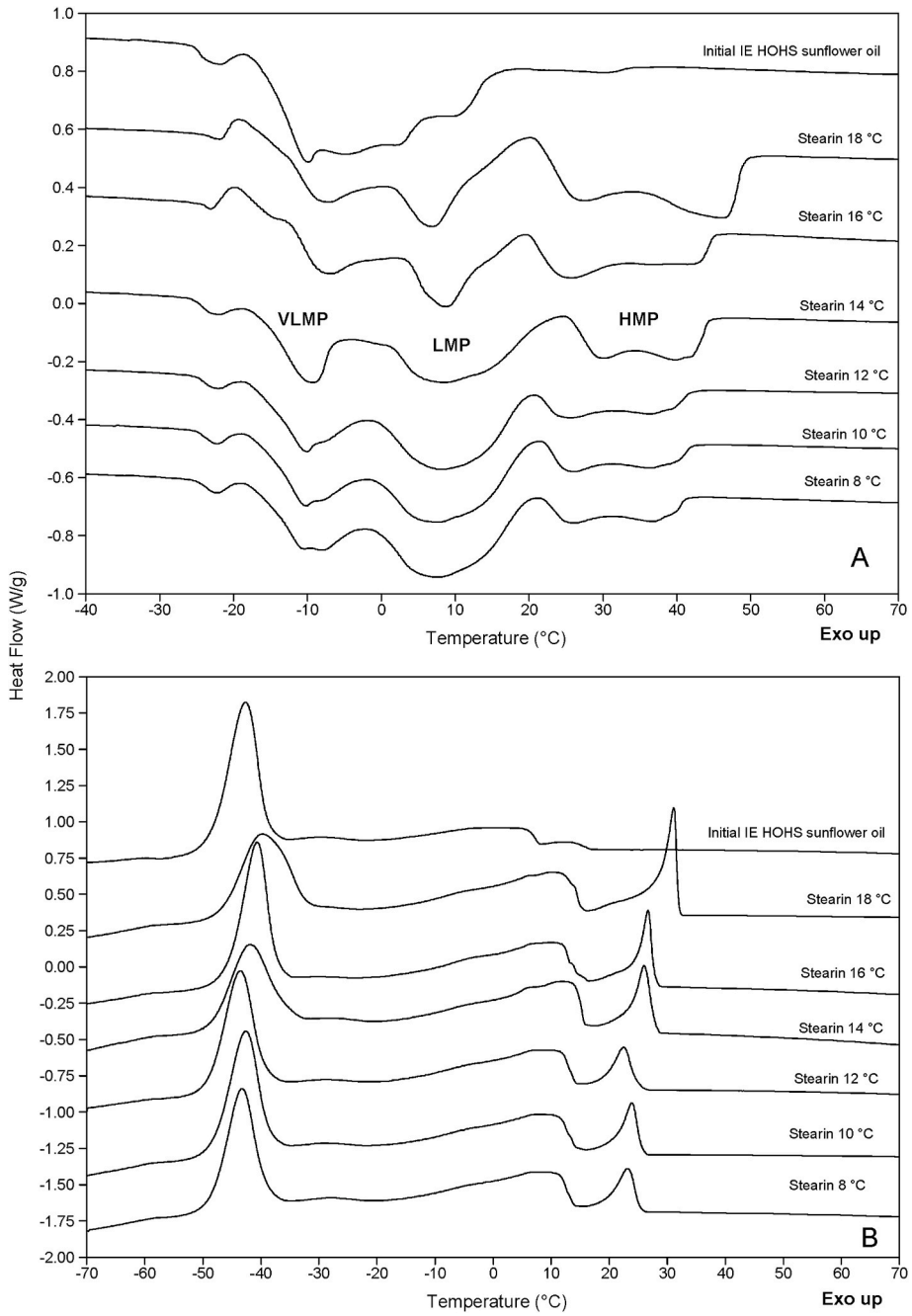

Fig. 3. Melting (A) and crystallization (B) curves of the interesterified high oleic high stearic S15 oil (S15/EIE), and the stearins obtained from it when fractionated at different temperatures. VLMP: very low meting point peaks, LMP: low melting point peaks, HMP: high melting peaks, EIE: enzymatically interesterified.

of this process. However, we must bear in mind that the prices of these enzymes are becoming increasingly competitive and that in the case of the lipozyme-TL used in this work, the enzyme, being immobilized, can be reused a large number of times, considerably reducing the cost of interesterification. In addition, if the reaction is carried out in a continuous process without cooling the catalyst, the increase in acidity experienced by the oil is minimized, thus avoiding subsequent neutralization stages, which makes the EIE highly competitive in comparison with chemical interesterification (Dijkstra, 2015).

When non-EIE HOHS sunflower oil was used it was necessary to perform partial dewaxing and crystal seeding for reliable and reproducible results, as seen elsewhere (Bootello et al., 2011). Sunflower WEs are long chain saturated species that tend to crystallize before TAGs, then interacting with the larger TAG crystals that form and clogging the filtration media. If seeding is not employed nucleation takes longer and the subsequent crystal growth occurs too fast, making the process less reproducible. A slurry was used for seeding that was prepared from the same, partially dewaxed oil, and it was left to crystallize in a chamber at 18 °C for at least 1 week. This slurry contained large, well-shaped and stable crystals that were good for seeding.

When the saturated fatty acid distribution in this oil was assessed, there was a predominance of symmetrically disaturated TAGs of the 1,3-distearoyl-2-oleoy-glycerol (StOSt) type with a SDI value of 11.8, and only traces of trisaturated TAGs (Table 1). The oil was fractionated in a range of final temperatures from 18 to 10 °C, yielding stearins enriched in TAGs of the symmetrical disaturated type. Examining the composition and stearin yield led us to conclude that the level of disaturated TAGs in stearins reached a plateau in operations performed between 18 and

Table 8
Percentage of the composition of fatty acids of the stearins and oleins resulting from fractionation of interesterified S16/EIE oil at 8 and 10 °C at pilot plant scale applying pressure filtration and squeezing. Yield of stearin.

	16:0			18:0			18:1			18:2			20:0			22:0			Total SAT	Yield (%)	SDI
EIE S16	4.7			16.3			72.1			4.2			1.2			1.6					
Stearins																					
10 °C	6.73	±	0.04	27.9	±	1.3	56.7	±	1.8	3.3	±	0.2	2.3	±	0.2	3.3	±	0.5	40.1 ± 2.0	26.8 ± 0.6	92.2 ± 2.2
8 °C	6.5	±	0.1*	27.0	±	0.3	57.6	±	0.6	3.1	±	0.2	2.6	±	0.8	3.1	±	0.1	39.1 ± 0.4	27.4 ± 1.5	92.2 ± 0.6
Oleins																					
10 °C	4.2	±	0.1	12.2	±	0.1	76.57	±	0.01	4.6	±	0.2	0.96	±	0.03	1.5	±	0.14	18.8 ± 0.2	–	
8 °C	4.4	±	0.1	12.7	±	0.0	75.7	±	0.9	4.9	±	0.4	0.95	±	0.01	1.43	±	0.03	19.4 ± 0.5	–	

Data correspond to the average ± SD of 3 independent fractionation experiments. The means of each series were analyzed by using the final fractionation temperature as the treatment, applying a one-way ANOVA with post hoc Tukey test (P value < 0.05). Asterisk indicates significant differences. SAT, saturated fatty acids; SDI, saturated distribution index.

Table 9
Percentage of the composition of triacylglycerol class of the stearins and oleins resulting from the fractionation of interesterified S16/EIE oil at 8 and 10 °C at pilot plant scale by pressure filtration and squeezing.

	Trisaturated SSS			Disaturated SUS			Monosaturated SUU			Triunsaturated UUU		
S16/EIE	1.3			14.1			39.8			44.9		
Stearins												
10 °C	3.4	±	0.1	34.2	±	1.6	36.1	±	0.3	26.3	±	1.4
8 °C	3.5	±	0.2	33.3	±	0.9	35.5	±	1.4	27.7	±	0.5
Oleins												
10 °C	0.0			5.8	±	0.2	41.7	±	0.1	52.5	±	0.1
8 °C	0.0			6.2	±	1.0	43.8	±	2.7	49.9	±	1.7

Data correspond to the average ± SD of 3 independent fractionation experiments. The means of each series were analyzed by using the final fractionation temperature as the treatment, applying a one-way ANOVA with post hoc Tukey test (P value < 0.05). No significant differences were found between treatments in any component. TAG composition of fractions is shown in Supplementary Table S3.

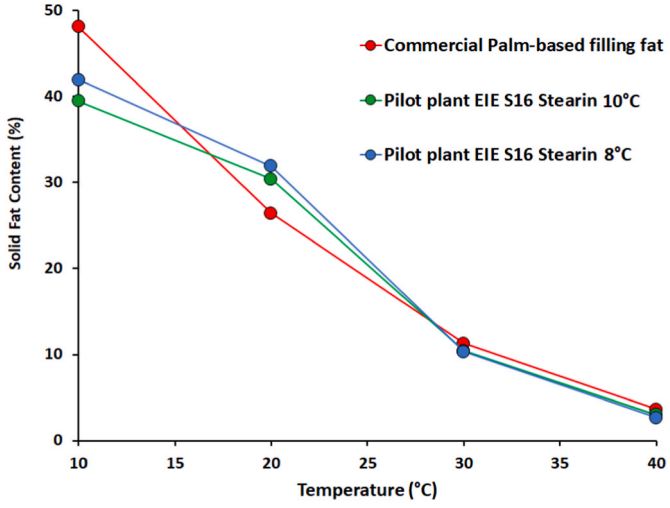

Fig. 4. Solid fat content measured by p-NMR at different temperatures of the stearins obtained by fractionation at different temperatures of the oil S16/EIE. A filling fat based on palm stearin was included as a control. EIE: enzymatically interesterified.

12 °C, with the solid fractions increasing in yield from 5.8 to 18.7% of the initial oil and with lower levels of saturated fatty acids in the corresponding oleins (Fig. 1, Tables 3 and 4). In these fractionations the slurry was moderately viscous (from 131 to 287 mPa × s), which allowed easy and rapid filtration of the stearin. It is important to remark that all filtrations of non-EIE feedstock were done using a vacuum filtration setup at bench scale. When fractionation was performed at 10 °C the slurries were much more viscous and they were more resistant to filtration due to the higher proportion of smaller crystals (Fig. 1). The enrichment of disaturated TAGs in the stearins was also significantly lower under these conditions, indicating that this temperature marks the limit for effective fractionation of this oil to obtain stearins enriched in disaturated TAGs suitable as plastic fats. As expected, the saturated fatty acid distribution in the TAGs of the stearins resulting from this oil was very similar to that in the initial oil, with a predominance of the symmetrical disaturated TAGs (Table 4), as can be deducted from the low SDI values. The level of trisaturated TAGs in the stearins increased with increasing fractionation temperatures but they always stayed below 0.3% since the non-EIE feedstock did not contain trisaturated TAGs and the SDI value of the stearins remained low.

A similar series of fractionations was carried out using the same oil previously interesterified (S15/EIE). As the SDI reached a value around 100 (Table 1), the EIE reaction approaches completion and the saturated fatty acids were randomly distributed at all TAG positions. The reaction changed the TAG composition of the oil, significantly increasing the high melting point TAG content (trisaturated and disaturated classes) at the expense of monosaturated TAG. There was also an increase in triunsaturated TAGs (Table 2). All these changes favor better fractionation as they increased the proportion of easily crystallizable TAGs, with no need to perform seeding and giving rise to slurries that were easily filtered at all final temperatures assayed, without a need for even partial dewaxing.

The composition of the fractions obtained from S15/EIE included stearins with higher trisaturated TAG content (from 9.8 to 3.3%, Tables 5 and 6). The proportion of disaturated TAGs remained more stable, varying from 23% at 18 °C to 19% at 8 °C. The oleins were virtually free

of trisaturated TAGs and they were enriched in monosaturated and triunsaturated TAGs. In terms of the fatty acid composition and distribution, S15/EIE stearins had a similar total saturated fatty acid content from 27.3 to 32.2% (Table 6). These saturated fatty acids remained randomly distributed in the stearins, which all displayed SDI values around 100, although they tended to diminish in stearins obtained at lower temperatures. The yield and slurry viscosity indicated that fractionation was effective over the temperature range assayed, with yields increasing as the final fractionation temperature decreased, reaching values of 28% at 8 °C (Fig. 1). The slurry remained moderately viscous (below 600 mPa × s), allowing fast and effective filtration of the stearins at all the temperatures assayed. Hence, the window of fractionation of IE HOHS oil was considerably wider than that of non-IE oils, making the process simpler, more reproducible and producing higher stearin yields and better saturated fatty acid recovery from the initial oil.

The melting and crystallization curves of the stearin fractions obtained from both series of fractionations were characterized by DSC (Figs. 2 and 3, Table 7). Melting of the initial HOHS oil produced a single peak with a melting interval of $\Delta T = 39.79$ °C. Unlike palm oil, where two main groups of low-melting and high-melting peaks are evident in its melting curve (Braipson Dathine & Gibon, 2007), HSHO sunflower oil exhibited a single low-melting peak, making the dry fractionation operation more challenging since most of TAG species displayed similar melting properties. The stearins obtained from fractionation of both oils displayed broader melting intervals, from $\Delta T = 44$ to 57 °C, and they displayed melting peaks matching the different TAG classes they can contain trisaturated, disaturated, monosaturated, and triunsaturated. Furthermore, small peaks around 28 °C were detected in some of the S15 stearins melting curves (Fig. 2A). While these peaks could be related to traces of trisaturated TAGs, this temperature is lower than expected for this type of high melting point TAGs type. This was also confirmed by the lower values for the onset of crystallization curve (Fig. 2B) and the absence of isolated exothermic peaks at higher temperature typical of trisaturated TAGs (Fig. 3B). We hypothesize this could be a polymorphic transition from β'_1 to most stable β polymorph according to the work reported by Rincón-Cardona et al. (2013) for the polymorphic behavior of HSHO sunflower soft stearins. S15 stearins were plastic fats at room temperature, with an onset of crystallization from 3.9 to 11.3 °C in the DSC system used, similar to those detected previously (Bootello et al., 2011). The lower crystallization onset (0.2 °C) of S15 oil explains the need of applying high melting point crystals seeding to promote nucleation during dry fractionation operation. The EIE reaction considerably changed the melting curve of the initial oil, making it significantly broader, with a melting interval of 66 °C and an onset of crystallization at 17 °C. This shift was caused by the increase in high-melting point trisaturated and disaturated TAGs at the expense of monosaturated TAGs that melted closer to the temperature of the triunsaturated TAGs. This also explains the rapid and easy crystallization observed during fractionation. The stearins from IE HOHS oils had very long melting intervals, with a range of values that reached 74 °C in some cases. Their profiles also contained 4 peaks, although those corresponding to high-melting point TAGs were in much higher proportions. The exothermic peaks observed below the very-low-melting region most probably corresponds to the polymorphic transformation of unstable polymorphs when applied the heating rate (5 °C/min) used in the melting curve determination. These recrystallizations also occurred between the low-melting-point and high-melting-point regions, being more exothermic at increasing trisaturated content in the S15/EIE stearins. All S15/EIE stearins showed a separate exothermic peak above 20 °C and before the bulk crystallization occurring at lower temperatures. These peaks were not present in S15 stearin crystallization curves (Fig. 2B) and they are due to the crystallization of the high-melting trisaturated TAGs. It can be concluded that trisaturated TAGs together with the mixture of symmetric and asymmetric disaturated TAGs (SUS-type/SSU-type) will impart different physical properties and functionality to S15/EIE stearins. Trisaturated TAGs are known to accelerate crystallization by acting as seeds during nucleation stage. They also could contribute to the occurrence of the exothermic peaks observed above the base line during DSC measurements. For most of the S15/EIE stearins, one peak and one shoulder can be observed in the thermogram at around 20 °C as a result of disaturated and trisaturated intersoluble TAGs interaction (Fig. 3A). These fractions were all solid at room temperature and based on their stearic and oleic acid content, they could be employed as fats for bakery or margarine formulations. The stearins obtained at higher temperatures tended to have broader melting intervals and a higher onset of crystallization, which was as high as 34 °C.

Finally, the promising results obtained in laboratory studies were reproduced at pilot plant scale. The main difference between the two procedures, other than the size of the crystallization unit, was the process of slurry filtration and the removal of the entrapped olein. As indicated in the experimental section, filtration was carried out under vacuum in the laboratory trials and followed by washing with ice-cool hexane. The pilot plant allowed pressure filtration by filling a membrane filter with the crystallized slurry. The olein will drain easily if the slurry crystallizes correctly, forming a compressible stearin cake. Once the filter is filled it is closed, and the stearin cake can be squeezed at high pressure to expel most of the entrapped olein. This procedure was assayed at pilot plant scale with an oil (S16/EIE) that contained slightly more stearic acid than S15. This oil was enzymatically interesterified in a 40 L reactor and its composition assessed, with a slightly higher proportion of trisaturated and disaturated TAGs in S16/EIE but still within a similar range. Consequently, we used similar conditions of fractionation. The more interesting issues assayed were those corresponding to lower temperatures, as they yielded a higher proportion of stearin and could be more appropriate conditions, potentially giving rise to more viscous slurries that are difficult to filter. Fractionation at both 8 and 10 °C produced slurries that were easily filtered in the membrane filters, and the stearin cakes obtained were quite compressible and could be squeezed to 30 bar while still yielding consistent stearins. The stearin fraction has a disaturated content ranging from 33 to 34%, with levels of trisaturated TAGs around 3% (Tables 8 and 9). Indeed, a higher proportion of high melting point TAGs was produced at pilot plant scale than on a laboratory scale, indicating that industrial methods make the process even more effective. In both cases the stearin yield was around 25–27%, which is notable for oils with this saturated fatty acid content. Fig. 4 shows solid fat content profiles of S16/EIE pilot plant stearins produced at 8 and 10 °C compared with a commercial palm-based filling fat. Sunflower stearins matched the solid content at higher temperatures (30 and 40 °C) while having a steeper melting profile between 20 and 30 °C. The steepness of the solid fat content curve is related to the coolness (cooling sensation when the fat melts) and flavor release (Talbot, 2009). In this regard, S16/EIE pilot plant stearins could be used as ingredient for bakery or confectionery fillings when a cool-melting effect is required without using palm or fully hydrogenated lauric fats.

5. Conclusions

The studies carried out here indicate that rearranging the distribution of saturated fatty acids in high saturated oils like HOHS sunflower oil improves the performance of dry fractionation process. This oil tends to have a symmetrical distribution of saturated fatty acids in the TAG backbone, which makes it more difficult to fractionate, with a low onset of crystallization and narrow window of fractionation temperature. The rearrangement of the TAGs achieved with the Lipozyme TL reaction changed the TAG composition of the oil, giving rise to trisaturated TAGs and asymmetrical disaturated TAGs of the SSU type, favoring fractionation, and making it more reliable and reproducible. Moreover, these oils had a broader temperature window of fractionation, which allowed stearins to be recovered with high levels of solids at temperatures as low as 8 °C, and with notable yields. The results of this work could be very important in the future for the fractionation of high saturated oil crops

(sunflower and others) that are being developed in different breeding programs, or by biotechnology approaches, making them a real and profitable alternative to tropical fats like palm and palm kernel.

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CRediT authorship contribution statement

JoaquínJ. Salas: Conceptualization, Supervision, Writing – original draft, Investigation. **Miguel A. Bootello:** Conceptualization, Investigation, Writing – review & editing. **Eija Piispa:** Conceptualization, Writing – review & editing. **László Hornyák:** Conceptualization, Investigation, Writing – review & editing. **Mónica Venegas-Calerón:** Project administration, Formal analysis, Methodology. **Enrique Martínez-Force:** Methodology, Visualization, Formal analysis. **Rafael Garcés:** Conceptualization, Supervision, Project administration, Methodology, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115042.

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