



Effect of solvents on the fractionation of high oleic–high stearic sunflower oil



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ABSTRACT

Solvent fractionation of high oleic–high stearic (HOHS) sunflower oil was studied to determine the best solvent to use (hexane or acetone) in terms of the operational parameters and properties of the final stearins. Acetone fractionation on two types of HOHS sunflower oils (N17 and N20) was carried out at temperatures from 5 to 10 °C using micelles with different oil/solvent ratios. Acetone was more suitable than hexane as a solvent for HSHO sunflower oil fractionation because it allowed the oil to be fractionated at higher temperatures and at lower supercooling degrees. Likewise, a sunflower soft stearin obtained by dry fractionation of HOHS sunflower oil was also used to produce high-melting point stearins by acetone or hexane fractionation. The fractionation of these stearins could be performed at higher temperatures and gave higher yields. The combination of dry and solvent fractionation to obtain tailor-made stearins is discussed.

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1. Introduction

The term fractionation refers to fractional crystallization, a physical process that involves the separation of a multi-component mixture into solid and liquid phases with different physicochemical properties, as applied to triacylglycerols (TAGs) (Krisnamurthy & Kellens, 1996). Fractionation is the result of two processes: a crystallization step, where solid crystals are produced from the bulk oil, and a separation or filtration step where the solid phase (crystals) is isolated from the liquid phase. When considering TAGs, the liquid phase is called 'olein', and it is enriched in triunsaturated (UUU) and monosaturated triacylglycerols (SUU). By contrast, disaturated (SUS) and trisaturated triacylglycerols (SSS) concentrate in the solid 'stearin' phase. The basis of such fractionation resides in the solubility of the TAG species in the liquid phase at controlled temperatures, which is dependent on the molecular weight and degree of unsaturation (Gibon, 2006). There are currently two types of industrial-scale fractionation processes: dry and solvent fractionations. In the latter, the crystallization of the TAGs takes place in micelles where the oil is diluted in an organic solvent, which is followed by a vacuum filtration step and cake washing with fresh solvent to remove the entrapped olein from the filtered stearin (Timms, 1997). The main advantage of solvent fractionation is the high efficiency of separation and the purity of

the resulting stearins that can be achieved in a single step. However, due to the high investment and operational costs of solvent fractionation, it is usually only applied to produce valuable specialty fats and oils, such as those used to formulate cocoa butter equivalents (CBEs) and other confectionary products (Kang, Jeon, Kim, & Kim, 2013; Kang, Kim, Kim, Lee, & Kim, 2013).

As stated above, crystallization takes place in micelles with different oil/solvent (O/S) ratios in function with the type of solvent and processing conditions. Hexane and acetone are the solvents commonly used in the fat and oil industry, however acetone is more selective for TAG separation due to its smaller molecular volume (Hamm, 1986; Timms, 1983). The choice of solvent also affects the separation of polar and non-polar lipids. Non-polar solvents like hexane concentrate polar compounds in the stearin phase (free fatty acids, monoacylglycerols and diacylglycerols), whereas with a more polar solvent like acetone such compounds tend to stay in the olein (Timms, 2003). Since stearin is usually the most valuable fraction, the use of a polar solvent is generally preferred as it reduces the presence of these polar compounds in the final product.

In addition to the polarity of the solvent, other properties influence the phase behaviour and crystallization kinetics of TAGs during fractionation, such as chemical nature, oil solubility or viscosity (Hartel, 1992; Wellner, Garti, & Sarig, 1981). The addition of a solvent to the oil dramatically lowers its viscosity, thereby increasing the diffusion of TAG molecules and also the probability of them attaching to the surface of the growing crystals. As a result, bigger

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and more perfect crystals are formed, reducing the tendency to make co-crystals and dislocation in the crystal lattice (Illingworth, 2002). The solubility of the crystallizing fat in the solvent also influences the fractionation process. Thus, the crystallization temperature is generally lower when the oil is mixed with a solvent due to an increase in TAG solubility in the resulting micelle (Liu, Biliaderis, Przybylski, & Eskin, 1995). In fact, hexane (with a Snyder polarity index 0.1 p') is a better solvent for fats than acetone (which has a Snyder polarity index 5.1 p') and it tends to dissolve crystallizing TAGs (Wright, Hartel, Narine, & Marangoni, 2000). It has been reported that the fractionation yield of squid viscera stearin increased with increasing solvent polarity (Yang, Chang, & Chen, 1992). The effect of solvents on the viscosity during fractionation has also been studied on cottonseed oil (Kapseu, Kayem, Balesdent, & Schuffenecker, 1991), where hexane reduces the viscosity of the O/S mixture more than acetone. This decrease in viscosity of the micelles favours molecular diffusion and speeds up crystallization kinetics, although other factors like the solubility of the solvent or temperature must be taken into account.

Recently, oil seed biotechnology has made it possible to produce new lines of mutant sunflowers with altered fatty acid compositions, combining breeding techniques and mutagenesis (Fernández-Moya, Martínez-Force, & Garcés, 2005). Amongst such lines, high oleic–high stearic (HOHS) sunflower oil is of special interest due to its high stearic acid content (ranging from 17% to 22% of the total fatty acids) and levels of oleic acid. This fatty acid composition makes it possible to use HOHS sunflower oil as a source of stearic-rich fats for baking and confectionary formulations. However, since the HOHS sunflower oil does not contain enough solids at high temperature for these applications, it is necessary to concentrate the saturated fatty acids by means of fat fractionation. The dry fractionation and crystallization kinetics of HOHS sunflower oil have been studied (Bootello, Garcés, Martínez-Force, & Salas, 2011), and show that this technique produces soft stearins with a disaturated TAG content between 25% and 40%, and with a melting profile appropriate for plastic fats, structured lipids, fillings and shortenings. However, these dry fractionation stearins cannot be used for CBE formulation due to their low solid fat content. In this regard, solvent fractionation could be a more suitable method to produce high-melting point sunflower stearins with high levels of SUS (Bootello, Hartel, Garcés, Martínez-Force, & Salas, 2012; Bootello et al., 2013).

Although hexane fractionation of HOHS sunflower oil to produce stearate-rich stearins has been studied (Salas, Bootello, Martínez-Force, & Garcés, 2011), the acetone fractionation of this oil has not yet been described. Thus, the aim of the present work was to gain more insight into the solvent fractionation of HOHS sunflower oil and elucidate which solvent is more appropriate for the production of high-melting point stearins, hexane or acetone.

2. Materials and methods

2.1. Materials

Two refined, bleached and deodorised HOHS sunflower oils (N17 and N20), and a sunflower dry fractionated stearin (S35) with distinct stearic acid contents, were studied in solvent fractionation experiments. HOHS sunflower oils were provided by Nutrisun Business (Mar del Plata, Argentina), while S35 was obtained from HOHS sunflowers as reported elsewhere (Bootello et al., 2011). Acetone (purity 99.5%) and hexane (alkane mixtures, purity 95%) were purchased from Panreac (Barcelona, Spain). All other reagents were of analytical grade and were obtained from Panreac (Barcelona, Spain) or Sigma–Aldrich (Madrid, Spain).

2.2. Oil fractionation

Solvent fractionation of HOHS sunflower oils and S35 were performed on a laboratory scale, using two routes for the production of solvent stearins: (a) direct fractionation from N17 and N20 HOHS sunflower oils; or (b) double fractionation from the S35 dry fractionation stearin obtained previously by dry fractionation of the N17 and N20 oils. These oils and fractions were melted at 80 °C to remove any prior structure, and then mixed with different amounts of solvent: acetone or hexane. The resulting oil micelles were transferred to hermetic flasks and stored at 5 and 10 °C without stirring in a controlled-temperature water bath (P-Selecta, Barcelona, Spain). Fractionation was monitored by periodically taking aliquots of the liquid supernatant in order to analyse the TAG composition. Vacuum filtration of the precipitated stearins was carried out when the composition of the supernatant was constant over time. This step was performed in a cold room at the fractionation temperature using a portable vacuum pump (Neuberg Laboport, Freiburg, Germany), a Kitasato flask and a Buchner funnel. The Buchner funnel was precooled to the fractionation temperature to avoid the partial melting of the stearins. Stearins were washed with 100 ml of fresh solvent at the fractionation temperature in order to reduce the entrained liquid oil in the cake. Stearins and oleins were then distilled in a rotavapor (IKA, Staufen, Germany) and stripped by bubbling nitrogen to remove the last traces of solvent.

2.3. TAG analysis by GLC

Approximately 5 mg of oil was dissolved in 1.8 ml of heptane in glass vials and the TAGs were analysed according to Fernández-Moya, Martínez-Force, and Garcés (2000). The analysis of TAGs was carried out by injecting 1 μ l aliquots of these solutions into the GC system, an Agilent 6890 gas chromatography apparatus (Palo Alto, CA, USA), using hydrogen as the carrier gas. The injector and detector temperatures were both 370 °C, the oven temperature was 335 °C, and a head pressure gradient from 70 to 120 kPa was applied. The gas chromatography column was a Quadrex Aluminium-Clad 400-65HT (30 m length, 0.25 mm i.d., 0.1 μ m film thickness: Woodbridge, CT, USA), and a linear gas rate of 50 cm/s, a split ratio 1:80 and a flame ionisation detector (FID) were used. The TAG species were identified according to Fernández-Moya et al. (2000) and quantified by applying the correction factors reported Carelli and Cert (1993).

2.4. Analysis of fatty acid methyl esters

The fatty acid moieties of TAGs were transmethylated into fatty acid methyl esters by treating 10 mg of oil samples with 3 ml of methanol/toluene/sulphuric acid (88/10/2 v/v/v) for 1 h at 80 °C (Garcés & Mancha, 1993). The resulting methyl esters were then extracted twice with 1 ml of heptane and analysed by GC in a Hewlett–Packard 6890 gas chromatography apparatus (Palo Alto, CA, USA). The column used was a Supelco SP-2380 fused silica capillary column (30 m length; 0.25 mm i.d.; 0.20 μ m film thickness: Bellefonte, PA, USA) with hydrogen as the carrier gas at 28 cm s⁻¹. The detector and oven were maintained at a temperature of 200 °C and 170 °C, respectively. The different methyl esters were identified by comparing their retention times with those of known standards. A Supelco 37 component FAME Mix (Sigma–Aldrich, Madrid, Spain) was used as the analytical standard with the following retention times: methyl palmitate (2.7 min), methyl stearate (3.7 min), methyl oleate (4.1 min), methyl linoleate (4.7 min), methyl arachidate (5.4 min) and methyl behenate (7.6 min).

2.5. Thermal analysis by DSC

The melting and crystallization profiles of solvent stearins were determined by differential scanning calorimetry (DSC) in a Q2000 V23.5 scanner (TA instruments, New Castle, DE, USA). The instrument was calibrated prior to use with indium, azobenzene and undecane (Sigma–Aldrich, Madrid, Spain), and nitrogen was used to purge the system. Samples were prepared by transferring approximately 7 mg of the melted oils into aluminium pans that were then hermetically sealed, with an empty pan serving as reference. The exact weight of the pans and the sample was determined in an electronic microbalance, Sartorius M2P (Sartorius AG, Goettingen, Germany). The results were processed using the TA Universal Analysis software provided by the manufacturer.

Melting profiles were determined by three different temperature programs. For non-tempered samples, Program A was used: heating to 90 °C, holding for 5 min, cooling at 10 °C/min to –60 °C, holding for 20 min and finally heating to 90 °C at a rate of 5 °C/min. Program B was used for samples tempered outside the calorimeter, with the aluminium pans containing oil samples tempered according to the AOCS official method Cd 16–81, and stored for 40 h prior to DSC measurement. For these samples the following DSC program was used: cooling at 10 °C/min down to –60 °C, holding for 20 min and heating to 90 °C at 5 °C/min. The second DSC method for tempered samples, Program C, aimed to avoid the formation of exothermic peaks that were observed in the melting profile in some of the non-tempered samples. This method is based on the melting of the metastable crystals formed using the onset temperature of the exothermic peak (T_{on-exo}), defined as the temperature where the metastable form begins to recrystallize during the melting stage. This program was performed with the following cooling/reheating process: heating to 90 °C, holding for 5 min, cooling at 10 °C/min to –60 °C, holding for 20 min, heating to T_{on-exo} at a rate of 10 °C/min, holding for 5 min, cooling at 10 °C/min to –60 °C, holding for 40 min and finally heating to 90 °C at a rate of 5 °C/min. The T_{on-exo} was determined after running Program A (as indicated by the arrows in Fig. 3A). Moreover, the crystallization profile was also obtained by completely melting the oils at 90 °C for 5 min and decreasing the temperature to –60 °C at a rate of 10 °C/min.

2.6. Statistical analysis

Fractionation experiments and kinetic studies were carried out in duplicate and all the fractionation results presented correspond to the average of two independent determinations. The standard deviations of the fatty acid and triacylglycerol composition are given as Supplementary data. All DSC experiments were conducted in triplicate and the mean values are reported.

3. Results and discussion

3.1. Acetone fractionation of N17 and N20 HOHS sunflower oils

Acetone is a more polar solvent than hexane, in which trisaturated and disaturated TAGs will exhibit lower miscibility. Acetone is commonly used in the solvent fractionation of oils due to its ability to crystallize SUS, such as StOSt and POST, the most abundant TAG species in CBEs. The production of stearate-rich butters by the hexane fractionation of N17 and N20 HOHS sunflower oils has been studied previously (Salas et al., 2011), where fractionating both oils at 0 and 5 °C using O/S ratios ranging from 1/3 to 3/1 was utilised. In the present work, acetone fractionation was performed at different temperatures, between 5 and 10 °C and O/S

ratios varying from 1/1 to 1/4 (all these conditions and the corresponding codes for each fraction are summarized in Table 1).

The kinetics of acetone fractionation was monitored by analysing the TAG composition of the supernatant. The variation in concentration of StOSt in the olein was adopted as the preferred parameter to study, given that this specific TAG species will be the most important component of the resulting stearins. The acetone fractionation of N17 HOHS sunflower oil (Fig. 1A and B) precipitated StOSt more rapidly when compared with the data obtained previously for hexane fractionation (Salas et al., 2011). All micelles reached a constant concentration of StOSt after 48 h. The StOSt content in the final olein fraction was lower than that obtained by hexane fractionation, ranging from 0.9% to 1.2% at 5 °C (Fig. 1A) and from 1.6% to 1.9% at 10 °C (Fig. 1B). Acetone fractionation of N20 HOHS sunflower oil (Fig. 1C and D) was quicker than that of the N17 oil, reaching a constant StOSt concentration in the olein after 24 h at both fractionation temperatures. The final StOSt concentration in the oleins was lower for N20 oil than for N17 oil, ranging from 0.7% to 0.9% at 5 °C (Fig. 1C) and from 1.3% to 1.6% at 10 °C (Fig. 1D). Hence, the influence of the O/S ratio on the final concentration of StOSt was weaker in N20 oil than in N17 oil. From the kinetic point of view, disaturated TAGs are less soluble in acetone and tend to form nuclei which accelerates the crystallization process. Conversely, the formation of nascent crystals is hampered in hexane by the higher solubility of TAGs in this solvent (Wright et al., 2000). Since crystallization in acetone was less specific than in hexane, slower cooling ramps should be used with this solvent to avoid the rapid, non-specific crystallization of TAGs, which would in turn lead to stearins with a less saturated fatty acid content. These results showed that fractionation of HOHS oil with acetone takes place at higher temperatures and is a faster process than using hexane.

When the final compositions of the stearins obtained by acetone fractionation of N17 and N20 HSHO sunflower oil were assessed, there was an enrichment in stearic acid and in disaturated TAGs with regards the native oils (Tables 2 and 3). In terms of the fatty acid composition of N17 and N20 stearins, there was an increase in the total saturated content at higher crystallization temperatures (Table 2), which was mainly due to the contribution of stearic, arachidic and behenic acid. Palmitic acid remained almost constant. Furthermore, the total saturated content of the N20 stearins obtained at 10 °C (N20A4 to N20A7) was similar to that of the N17 stearins obtained at 5 °C (N17A1 to N17A3), suggesting that oils with a higher saturated fatty acid content could be fractionated at higher temperatures. The TAG composition of these fractions also indicated that the N17 and N20 stearins obtained at 10 °C contained more SUS, although they were produced at lower yields (Table 3). Moreover, there was little influence from the O/S ratios on the TAG composition and yield

Table 1

Codes corresponding to the stearins obtained by solvent fractionation of N17 and N20 high oleic–high stearic sunflower oils and S35 sunflower dry stearin.

Code	N17		Code	N20		Code	S35	
	O/S	T (°C)		O/S	T (°C)		O/S	T (°C)
N17A1	1/1	5	N20A1	1/1	5	S35H1	1/1	5
N17A2	1/2	5	N20A2	1/2	5	S35H2	1/1	10
N17A3	1/3	5	N20A3	1/3	5	S35H3	1/2	5
N17A4	1/1	10	N20A4	1/1	10	S35H4	1/2	10
N17A5	1/2	10	N20A5	1/2	10	S35A1	1/2	5
N17A6	1/3	10	N20A6	1/3	10	S35A2	1/2	10
N17A7	1/4	10	N20A7	1/4	10	S35A3	1/4	5
						S35A4	1/4	10

The first three characters indicate the initial oil, the fourth is the type of solvent (A = acetone, H = hexane) and the last one denotes the fractionation conditions (O/S ratio and temperature) as indicated in the adjacent columns.

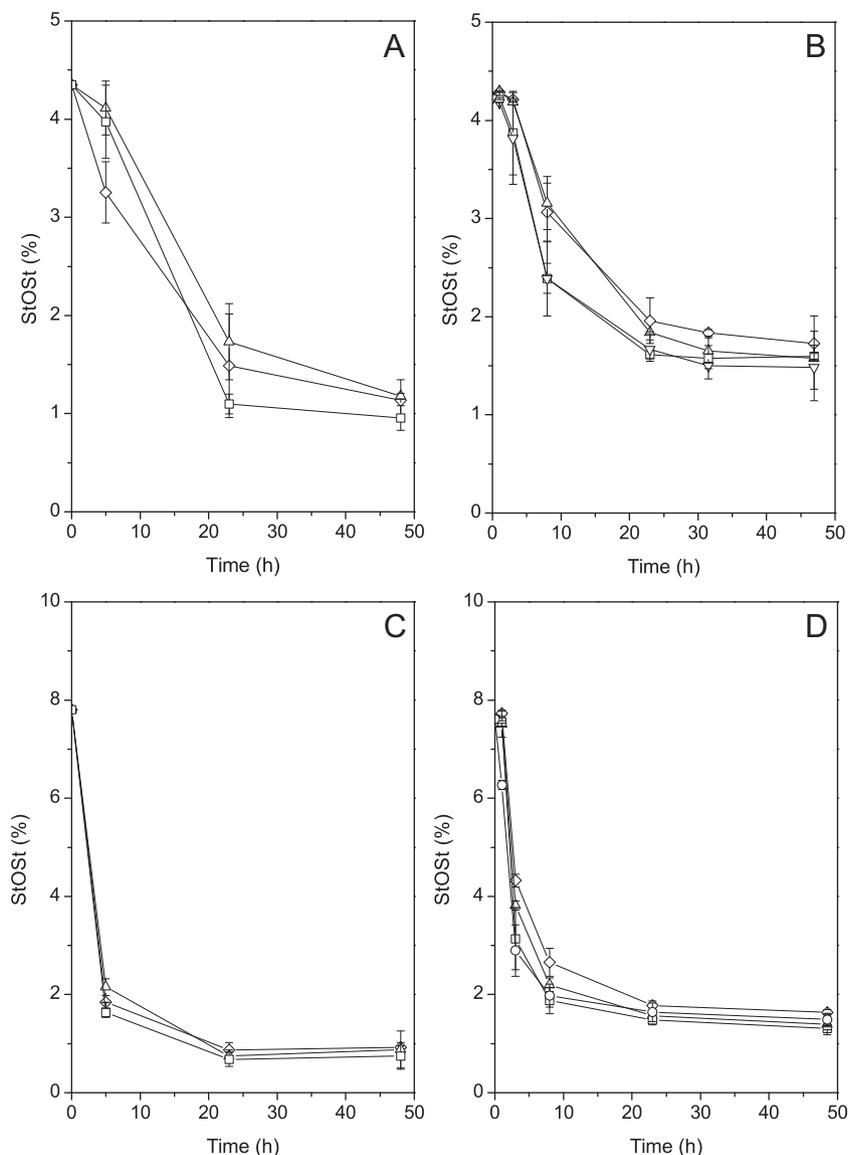


Fig. 1. Course of acetone fractionation of N17 and N20 high oleic–high stearic sunflower oils and the StOSt content of the olein fractions. Graphs (A) and (B) correspond to the fractionation of the N17 oil at 5 and 10 °C, respectively. Graphs (C) and (D) correspond to the fractionation of the N20 oil at 5 and 10 °C, respectively. Different O/S ratios (v/v) were used in each experiment: 1/1 (◇), 1/2 (△), 1/3 (□), 1/4 (▽).

of stearin, within a set of experiments at the same temperature. This contrasted with the results of hexane fractionation, in which fractionation temperature and O/S ratio had a strong influence (Salas et al., 2011). Acetone exhibited weaker selectivity than hexane in the crystallization of TAGs, giving rise to stearins with lower stearic acid and SUS content when the fractionation assays were performed under the same conditions but with the different solvents. Furthermore, acetone was more selective for the crystallization of symmetric disaturated TAGs (SUS) with respect to non-symmetric disaturated TAGs (SSU) when fractionating palm oil. Whereas both types of TAGs tended to co-crystallize together in hexane (Hashimoto, Nezu, Arakawa, Ito, & Maruzeni, 2001). Since N17 and N20 HOHS sunflower oils contain virtually no saturated fatty acids in the *sn*-2 position (Fernández-Moya et al., 2005), this effect would not have any influence on the fractionation of HOHS oils and their fractions.

3.2. Solvent fractionation of a sunflower soft stearin

Sunflower soft stearins obtained by dry fractionation of N17 or N20 HOHS sunflower oils can be used as intermediates in

the production of high-melting point stearins by solvent fractionation. The higher disaturated TAG content of these stearins (25–40% SUS), when compared to N17 or N20 HOHS sunflower oils (8–15% SUS), allows solvent fractionation to occur at higher temperatures. However, the high production costs of a solvent fractionation plant would make it interesting to combine both types of fractionation, using part of the dry stearins for the production of solvent stearins. Since the average yield of stearin in dry fractionation is about 15%, a smaller capacity solvent plant could be designed for this purpose. Thus, the combination of dry and solvent fractionation would mean an important saving in solvent compared with direct solvent fractionation, considerably reducing operation costs.

We determined the fatty acid and TAG composition of stearins obtained by solvent fractionation of a sunflower dry stearin containing 35% of total saturated acid (S35; Tables 2 and 3). Hexane fractionation produced stearins (S35H1 to S35H4) richer in stearic acid and SUS than acetone fractionation (S35A1 to S35A4). The higher solubility of TAGs in hexane made it possible to fractionate the soft stearin using higher O/S ratios (Table 1), which would mean less equipment and expense in terms of the purchasing of

Table 2

Fatty acid composition of stearins obtained by acetone and hexane fractionation of N17 and N20 high oleic–high stearic sunflower oils, and S35 sunflower dry stearin. The composition of the initial N17, N20 and S35 oils is shown for reference, and the fractionation conditions for each fraction are described in Table 1.

	Fatty acids (% w/w)						Total saturated
	16:0	18:0	18:1	18:2	20:0	22:0	
<i>Initial oils</i>							
N17 Oil	4.0	16.8	72.0	4.4	1.2	1.5	23.5
N20 Oil	5.4	20.4	66.3	3.3	1.7	2.8	30.3
S35	5.4	25.3	62.3	3.0	1.7	2.3	34.7
<i>Acetone</i>							
N17A1	7.5	36.2	49.0	2.2	2.3	2.8	48.8
N17A2	7.8	38.5	46.3	1.9	2.4	3.1	51.8
N17A3	7.7	39.1	45.7	1.8	2.4	3.3	52.5
N17A4	6.0	45.4	41.3	1.3	2.7	3.1	57.2
N17A5	6.1	48.2	38.4	1.0	2.9	3.4	60.6
N17A6	6.1	49.5	37.1	0.8	2.9	3.5	62.0
N17A7	6.0	49.3	37.2	0.8	3.0	3.7	62.0
N20A1	7.2	29.9	55.4	2.1	2.1	3.3	42.5
N20A2	7.3	30.8	54.2	2.0	2.2	3.5	43.8
N20A3	8.1	34.1	50.2	1.7	2.3	3.6	48.1
N20A4	7.1	37.6	47.7	1.5	2.5	3.6	50.8
N20A5	7.5	41.5	43.4	1.2	2.7	3.8	55.5
N20A6	7.3	40.6	44.0	1.2	2.8	4.2	54.9
N20A7	7.8	43.7	40.7	0.9	2.8	4.1	58.4
S35A1	6.1	35.2	51.6	2.0	2.2	2.8	46.4
S35A2	5.5	34.0	53.1	2.0	2.3	3.2	44.9
S35A3	6.7	42.1	44.1	1.3	2.6	3.2	54.6
S35A4	6.1	44.8	41.5	1.0	2.8	3.7	57.5
<i>Hexane</i>							
S35H1	6.0	42.7	44.3	1.7	2.3	3.0	54.0
S35H2	5.0	46.5	41.7	1.3	2.5	3.0	57.0
S35H3	5.5	54.0	34.6	0.0	2.6	3.3	65.4
S35H4	3.6	54.1	36.5	0.0	2.7	3.2	63.6

Peaks accounting for less than 0.1% of the total fatty acids were not integrated. The data represents the average of two independent determinations. Standard deviations can be found as [Supplementary material](#).

solvents. For the same O/S ratio, more SUS were present in S35H3 stearin obtained at 5 °C than from S35H4 obtained at 10 °C (Table 3). This was due to the fact that more POST crystallized at 5 °C. Although StOSt is the main disaturated TAG present in these hard stearins, we must bear in mind that other SUS species like POST, StOA and StOB may have a different crystallization pattern. Acetone fractionation experiments produced stearins with lower levels of stearic acid and SUS but at higher yields (Table 3). In the same fractionation conditions (5 °C, O/S ratio = 1/2), hexane fractionation produced 94.5% SUS stearin (S35H3) and acetone fractionation 58.3% SUS stearin (S35A1), yet with an almost 3-fold higher yield. This was due to the rapid and non-specific crystallization kinetics of TAGs in acetone at lower temperatures. For this reason, it is advisable to fractionate at higher temperatures, especially when the initial oil has an SUS content above 25%, as is the case for sunflower soft stearins. Conversely, higher yields of stearin were obtained from acetone fractionation of S35 than from acetone fractionation of N17 and N20 HOHS sunflower oils (Table 3). This result could be relevant in designing an industrial process for the production of high melting point stearins from HSHO as it shows that sunflower soft stearins can be fractionated to obtain fractions enriched in SUS in a way similar to that found for the HOHS oils N17 and N20. This could be used to organise a two-step fractionation process involving dry and solvent fractionation of these oils for the production of high stearate butters, appropriate for CBE formulations. The possibility for a previous step of dry fractionation would avoid the preparation of large amounts of micelle with the initial HOHS oil, and only fractionate with solvents for soft stearin,

which accounts over 15% of the initial oil. This would allow the same amount and quality of hard stearin to be obtained with much less solvent, considerably reducing production costs of the process.

3.3. Distribution of disaturated TAGs in the solvent fractionation of HOHS sunflower oils

To gain an overview of the solvent fractionation of these HOHS sunflower oils and fractions, a distribution diagram of disaturated TAGs (SUS) in olein and stearin was generated (Fig. 2). This diagram was obtained from data corresponding to a large number of acetone and hexane fractionations, for both N17 and N20 HOHS sunflower oils, and sunflower dry stearins containing 25–40% of SUS. To compare both types of solvent fractionation, a maximum operation time of 48 h was established. On the basis of this data (Fig. 2), the SUS content of final oleins and stearins could be estimated as a function of the fractionation conditions (temperature, O/S ratio). It is also possible to calculate the yield of stearin through a graphical approach, using the lever rule, according to Eq. (1):

$$\text{Yield of stearin (\%)} = 100 \cdot \frac{\%SUS_0 - \%SUS_{\text{olein}}}{\%SUS_{\text{stearin}} - \%SUS_{\text{olein}}} \quad (1)$$

Where %SUS₀, %SUS_{stearin} and %SUS_{olein} are the percentages of SUS in the initial oil, stearin and olein, respectively. Thus, given the concentration of SUS in the initial oil, we can estimate the yield of stearin as the temperature and the O/S ratio varies in the distribution diagram. The O/S ratio influenced the final SUS concentration in stearins for acetone fractionation (Fig. 2), although in some cases the deviations, indicated by the error bars, suggested that there were equivalent amounts of SUS in the stearin area, especially at 10 °C. When acetone was used as the solvent, the highest levels of SUS were reached at a higher temperature (15 °C) and lower O/S ratios (1/4). These conditions favoured the specific crystallization of disaturated TAGs, since crystallization was slower at higher temperatures. Furthermore, at lower O/S ratios more oil is diluted in the micelle, promoting the crystallization of a higher proportion of disaturated TAGs than SUU and SSS (Salas et al., 2011). The SUS content in the olein fractions varied in a narrower range for the different O/S ratios (Fig. 2), with the SUS content tending to increase at higher fractionation temperatures. In fact, oleins from acetone fractionation could be dry fractionated or recirculated to generate an initial SUS-enriched oil. Conversely, hexane fractionation occurred at lower temperatures (between 0 and 10 °C), yielding stearins with a very high SUS content (up to 96%). However, the oleins from hexane fractionation had more disaturated TAGs (up to 30% SUS at 10 °C and an O/S ratio 1/2), which is not desirable since the aim was to concentrate this class of TAG in the solid fraction. Nevertheless, the information displayed in Fig. 2 is essential to set the conditions of HOHS fractionation as it provides the temperature and O/S ratio required to obtain a given level of SUS in the stearin, from the view of the starting material, the solvent and the expected yield of the process.

3.4. Thermal analysis by DSC

The thermal analysis of three solvent stearins with the same disaturated TAG content (around 70% SUS) obtained under different conditions was determined by DSC (Fig. 3). These DSC experiments were carried out to determine whether the solvent and the type of initial oil, influence the thermal behaviour of these stearins. N20A6 stearin was produced from HOHS N20 oil by acetone fractionation, while the S35H1 and S35A4 stearins were obtained from S35 dry stearin by hexane and acetone fractionation, respectively. Despite the similar fatty acid and TAG composition, these stearins had different melting and crystallization profiles and accordingly, for temperature Program A (Fig. 3A), both the

Table 3

Triacylglycerol composition and yields of stearins obtained by acetone and hexane fractionation for N17 and N20 high oleic–high stearic sunflower oils, and S35 sunflower dry stearin. The composition of the initial N17, N20 and S35 oils is shown for reference and the fractionation conditions for each fraction are described in Table 1.

	Triacylglycerols (% w/w)												Triacylglycerol class (% w/w)			Yield of stearin (% w/w)	
	POP	POST	POO	POL	StOSt	StOO	OOO	StOL	OOL	StOA	OOA	StOB	OOB	SUS	SUU		UUU
<i>Initial oils</i>																	
N17 Oil	0.3	2.2	7.2	1.0	4.2	32.5	31.9	5.2	6.3	0.7	2.8	0.7	4.2	8.1	52.9	38.2	–
N20 Oil	0.6	4.4	8.7	0.8	7.5	35.8	23.5	3.9	3.5	1.5	3.1	1.4	4.9	15.4	57.2	27.0	–
S35	0.5	5.5	5.5	0.7	18.3	26.2	23.3	4.0	4.6	2.8	2.2	3.1	3.3	30.1	42.0	27.9	–
<i>Acetone</i>																	
N17A1	1.1	14.5	3.1	0.3	36.6	17.8	11.7	1.7	1.5	5.1	1.1	4.4	1.3	61.6	25.2	13.1	9.1 ± 4.6
N17A2	1.1	15.6	2.5	0.3	39.7	16.3	9.5	1.5	1.1	5.6	0.9	4.7	1.1	66.8	22.7	10.5	9.1 ± 3.0
N17A3	1.1	15.3	2.7	0.3	39.3	15.9	9.9	1.3	1.2	5.8	1.0	5.0	1.2	66.4	22.5	11.1	9.4 ± 1.0
N17A4	0.7	11.6	1.5	0.3	51.8	9.4	5.9	0.8	1.1	7.3	0.9	7.5	1.1	78.9	14.2	6.8	3.3 ± 0.8
N17A5	0.7	12.3	1.0	0.4	54.8	7.3	3.9	0.7	1.0	7.9	0.8	8.1	1.3	83.7	11.4	4.9	3.9 ± 0.2
N17A6	0.7	12.4	0.7	0.6	56.3	6.7	2.8	0.6	0.4	8.1	0.7	8.4	1.5	85.9	10.9	3.2	4.2 ± 0.3
N17A7	0.7	12.5	0.8	0.4	56.4	6.6	2.8	0.6	0.5	8.2	0.8	8.7	1.1	86.5	10.3	3.3	4.2 ± 0.1
<i>N20</i>																	
N20A1	1.1	11.7	6.2	0.4	23.5	26.8	15.1	2.2	1.7	3.7	1.9	3.2	2.5	43.2	40.0	16.8	16.1 ± 1.4
N20A2	1.1	12.2	5.7	0.3	24.3	26.2	14.3	2.2	1.5	3.9	1.9	3.5	2.7	45.1	39.1	15.8	16.1 ± 0.2
N20A3	1.3	14.6	4.3	0.3	30.2	22.2	10.5	1.7	1.1	5.0	1.7	4.6	2.5	55.7	32.7	11.6	12.7 ± 0.9
N20A4	0.9	12.3	3.8	0.4	36.5	17.4	9.6	1.3	1.3	6.2	1.4	6.7	2.2	62.6	26.7	10.7	17.3 ± 2.0
N20A5	1.0	14.0	2.5	0.5	43.0	13.0	6.4	0.9	1.1	7.3	1.0	7.9	1.6	73.1	19.7	7.2	14.8 ± 1.0
N20A6	1.0	13.8	2.8	0.4	41.1	14.2	7.1	1.0	1.0	7.1	1.1	7.6	1.7	70.6	21.5	7.9	15.5 ± 0.1
N20A7	1.0	15.1	1.8	0.5	46.3	10.7	4.5	0.6	0.7	8.0	0.8	8.7	1.2	79.0	15.8	5.2	13.0 ± 0.5
<i>S35</i>																	
S35A1	0.7	9.3	3.3	0.3	35.8	16.0	15.2	1.1	1.8	5.7	1.5	6.7	2.4	58.3	24.7	17.1	45.6 ± 4.4
S35A2	0.5	7.8	3.8	0.3	32.1	18.2	17.7	1.5	2.5	5.1	1.4	6.2	2.7	51.8	28.0	20.2	51.4 ± 2.9
S35A3	0.7	11.5	1.8	0.1	46.0	10.6	8.2	0.7	1.5	7.4	0.9	8.6	1.9	74.3	16.0	9.7	36.5 ± 1.5
S35A4	0.6	10.0	2.0	0.2	45.6	11.0	9.2	1.1	1.7	7.3	1.2	8.7	1.4	72.1	17.0	10.9	36.7 ± 1.0
<i>Hexane</i>																	
S35H1	0.6	9.9	1.9	0.1	46.4	11.8	8.5	1.3	1.3	6.5	1.1	7.8	3.0	71.0	19.2	9.8	29.0 ± 3.5
S35H2	0.5	9.4	1.8	0.1	55.3	9.2	7.1	0.8	0.0	6.6	0.8	7.2	1.2	78.0	13.8	8.1	17.3 ± 2.1
S35H3	0.4	10.0	0.3	0.1	65.6	3.4	1.2	0.0	0.0	8.4	0.2	10.0	0.3	94.5	4.3	1.2	17.3 ± 2.4
S35H4	0.3	7.2	0.7	0.0	68.1	3.9	2.9	0.4	0.0	7.5	0.1	8.4	0.4	91.5	5.2	3.3	6.6 ± 2.0

Peaks accounting for less than 0.1% of the total triacylglycerols were not integrated. The data represents the averages of two independent determinations. Standard deviations can be found as [Supplementary material](#). Triacylglycerols were named with 3 letters: P, palmitic; O, oleic; St, stearic; L, linoleic; A, arachidic; B, behenic. SUS, disaturated triacylglycerols; SUU, monosaturated triacylglycerols; UUU, triunsaturated triacylglycerols.

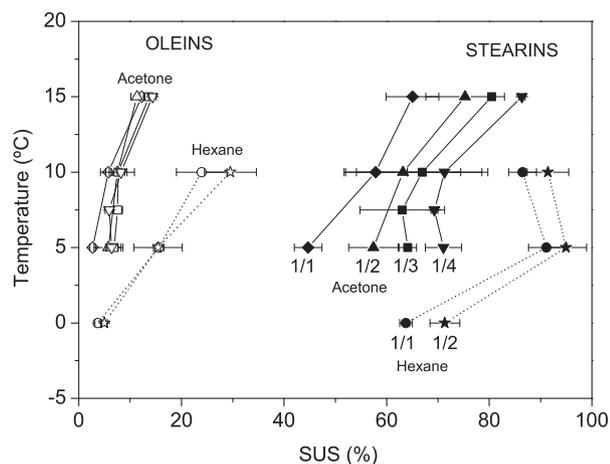


Fig. 2. Distribution diagram of the disaturated TAGs in the solvent fractionation of HOHS sunflower oils. O/S ratios (v/v) used in acetone fractionation: 1/1 (◇- olein, ◆- stearin), 1/2 (△- olein, ▲- stearin), 1/3 (□- olein, ■- stearin), 1/4 (▽- olein, ▼- stearin). O/S ratios (v/v) used in hexane fractionation: 1/1 (○- olein, ●- stearin), 1/2 (◊- olein, ★- stearin).

N20A6 and S35H1 stearins had an exothermic peak (indicated by the arrows). This phenomenon can be attributed to the formation of metastable crystals in the α polymorph, provoked by the rapid cooling rate during this DSC method. The recrystallization of these metastable polymorphs into a more stable polymorph (β' or β) is responsible for the observed exothermic peak (Márquez, Pérez, &

Wagner, 2013). However, the S35A4 stearin did not exhibit an exothermic peak and, even though this stearin is richer in UUU (OOO and OOL) compared to the N20A6 and S35H1 stearins, no endothermic peaks (like peaks a or c) were found in the temperature interval between -15 and 6 °C. Other stearins obtained with the same solvent and initial oil (e.g. S35A3) exhibited the same thermal behaviour (data not shown). The polymorphic behaviour of HOHS stearins has been studied using synchrotron X-ray scattering measurements and DSC (Rincón-Cardona, Martini, Candal, & Herrera, 2013). Up to five polymorphic forms have been reported for an HSHO solvent stearin containing 81.4% of SUS: α , β_2 and β_1 (under isothermal crystallization), and β_2 and β_1 (after long term storage). According to those results, the β_2 crystals from a HOHS solvent stearin exhibited a melting temperature of 29.8 °C. Thus, the large endothermic peak obtained around 27 °C in our DSC experiments (Fig. 3A, peaks b (27.0 ± 0.2), d (27.5 ± 0.2) and e (26.3 ± 0.1)) for each stearin probably corresponds to the β_2 polymorph.

Since the formation of metastable crystals is not desirable during the processing or storage of fat products, a tempering method (temperature Program C) was applied to the N20A6 and S35H1 stearins (Fig. 3B). The $T_{\text{on-exo}}$ used in Program C (as defined in the Section 2) was 6.4 ± 0.4 °C for N20A6 stearin and 6.0 ± 0.6 °C for S35H1 stearin. After running this DSC method, only one endothermic peak was observed that corresponded to the β_2 polymorph (Fig. 3B, peaks b (26.9 ± 0.6) and d (27.2 ± 0.3)). For both stearins this peak-melting temperature was virtually the same as that obtained with Program A (peak b (27.0 ± 0.2)). However, in some applications, like chocolate manufacture, the β_2 polymorph is required. For this reason, these three stearins were tempered using

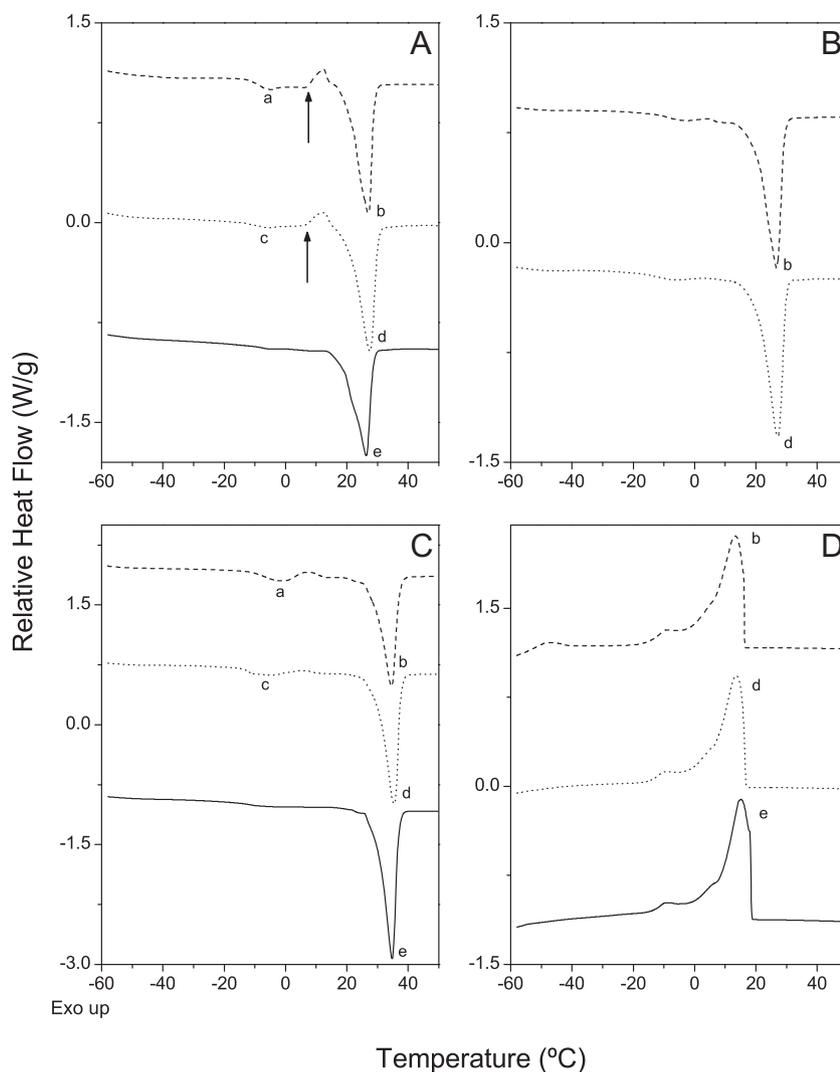


Fig. 3. Melting (A–C) and crystallization profiles (D) of the N20A6 (dashed line), S35H1 (dotted line) and S35A4 (solid line) stearins. The temperature program A (graph (A)), temperature program B (graph (C)) and temperature program C (graph (B)) were applied to the aforementioned stearins. See Section 2 for a description of the temperature programs.

Program B (Fig. 3C), a program based in a long tempering outside the calorimeter where the samples are allowed to crystallize into the most stable polymorph. In these conditions (as shown in Fig. 3C), all stearins crystallized mainly into the β_2 polymorph (Rincón-Cardona et al., 2013), the preferred form of cocoa butter in chocolate products to avoid bloom (Afoakwa, Paterson, Fowler, & Vieira, 2009). For the N20A6 and S35H1 stearins, a mixture of a metastable polymorph (peaks a and c) and β_2 (peaks b (34.7 ± 0.5) and d (35.4 ± 0.2)) was obtained. Again, S35A4 stearin exhibited a different behaviour, only crystallizing into the β_2 polymorph (peak e, 34.8 ± 0.1) after the tempering process. Each of the three fractions showed a similar pattern in terms of the crystallization profiles (Fig. 3D). However, the S35A4 stearin crystallized at higher temperature (15.2 ± 0.1 °C) than the other fractions (peak b (13.4 ± 0.2), peak d (13.6 ± 0.4)).

4. Conclusions

Solvent fractionation is a technique suitable for the production of a large variety of high-melting point stearins from N17 or N20 HOHS sunflower oils. By dry fractionation of these sunflower oils it is possible to obtain soft stearins with a maximum disaturated

TAG content of about 35–40%. Although these dry fractions show good properties as filling fats or shortenings, they can also be used as an intermediate step in the production of high-melting point sunflower stearins by solvent fractionation. Combining both types of fractionation make it possible to obtain tailor-made stearins with different melting points, hardness and disaturated TAG content (up to 96%) according to processing conditions (temperature, type of solvent, ratio oil/solvent).

The choice of solvent (hexane or acetone) in the fractionation process is a matter of great importance. Parameters like the crystallization rate, fractionation temperature or even the melting profile of the resulting stearins were influenced by the type of solvent. In general, acetone was more suitable than hexane for solvent fractionation of HSHO sunflower oil and its dry stearins. For the production of a solvent stearin with certain disaturated TAG content, acetone permitted fractionation at a higher temperature and with a lower degree of supercooling, leading to faster crystallization kinetics and higher yields of stearin. On the other hand, hexane fractionation could be of interest to obtain solvent stearins with a very high SUS content, although it must be taken into account that a large amount of disaturated TAGs will remain in the olein, which is not desirable.

The present work contains information useful for the design of a process of solvent fractionation for HOHS oil. It points to acetone as the solvent of choice in terms of time of processing, amount of solvent used and processing yield. In the fractionation of fractions enriched in stearic acid, it was also possible to adjust the conditions for the fractionation (temperature and O/S) ratio, which opened the possibility of combining dry and solvent fractionation for reduced production costs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2014.09.136>.

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