

Fractionation of *Trans* from Partially Hydrogenated Soybean Oil Fatty Acids

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Received: August 25, 2011

Accepted: September 8, 2011

Published: February 1, 2012

doi:10.5539/jfr.v1n1p157

URL: <http://dx.doi.org/10.5539/jfr.v1n1p157>

Abstract

A multi-stage, low temperature solvent fractional crystallization process was developed in this work for the removal of *trans* fatty acids (TFA) and saturated fatty acids in free fatty acids (FFAs) obtained from hydrolysis of partially hydrogenated soybean oil (PHSO). The effects of host solvents, concentration of FAs, crystallization temperature and time on separation of TFA and SFA were studied. Among all the three solvents examined namely acetone, hexane and ethanol, acetone proved to be promising solvent. By employing acetone as a solvent TFA can be reduced to 11.6% from an initial content of 19.5% and SFA can be drop down to 3% from an initial content of 20%, by applying stepwise crystallization temperatures from 5°C to -20°C. Crystallization temperatures lower than -30°C such as -35°C and -40°C can further lower the TFA content in liquid fraction down to 5-8% by employing acetone as a solvent. TFA content can be reduced down to 12.2% and 14%; and SFA content could be reduced to 3.3% and 3.1% from an initial content of 20% when hexane and ethanol were employed as solvents, respectively. Optimum FAs to solvent ratio was 1:5 (w/v) and with a time period of 12 h for each step of crystallization. The crystallization behavior of TFA strongly depends on the type of solvents employed and crystallization temperature, while SFA crystallization depends mostly upon temperature rather than nature of solvent. Even though total removal of TFA could not be achieved in the present study, a road map has been developed for a fractionation of TFA and SFA from partially hydrogenated oil FFAs.

Keywords: *Trans* fatty acids, Elaidic acid, Partially hydrogenated soybean oil (PHSO), Fractional crystallization

1. Introduction

Trans fatty acids (TFAs) have been reported to be risk factors involved in cardiovascular diseases as well as many other negative effects for human (Buckle K, 2010; Valenzuela A & Morgado N, 1999; Semma M, 2002). It is well accepted that TFAs should be removed or minimized from our food, where the labeling of TFA content is well ruled in many parts of the world. TFAs arise in our foods mainly from industrial partial hydrogenation and natural ruminant sources. For the industrially produced TFAs, a broad mixture of TFAs is formed with elaidic acid (9*t*-18:1) as the major isomer (European Food Safety Authority [EFSA], 2004; Stender S, Dyerberg J & Astrup A, 2006). In general TFAs are present in different amounts in a broad range of foods, including most foods made with partially hydrogenated oils, while partial hydrogenation is traditionally a key technology for oil modification in frying oil and plastic fat industries.

Even though the main mode now is to rule out the use of partial hydrogenation, the possible removal of TFAs from partially hydrogenated soybean oil (PHSO) is still of interest for part of the industrial sectors as well as for academic documentation since this has not been conducted before. Initial attempts had been made to decrease the TFAs directly from PHSO TG by means of fractional crystallization as did for palm oil (Timms R.E, 2005, 1983; Hamm W, 1986; Zaliha O *et al*, 2004; Nissim G & Kiyotaka S, 2001; Kiyotaka S, 2001). Due to the randomized

distribution of TFAs in PHSO, the fractionation of PHSO triglycerides could result in little difference between the fractions in terms of TFA content (data not shown). As part of a package strategy including enzymatic selective hydrolysis and following further fractionation, the fractionation of fatty acid mixture is in central concern for this study. Therefore, the objective of the present study was to separate TFAs from PHSO fatty acids, a mixture was formed during the *trans* selective hydrolysis of PHSO.

2. Experimental

2.1 Materials

PHSO was provided by Archer Daniels Midland Company (ADM, Decatur, IL). *Candida Antarctica A*, immobilized on resin, was obtained from Codexis, Inc., (Pasadena, CA, USA) with marked assayed activity of transesterification of ethyl laurate with 1-butanol in isooctane (7.0 U/g). Fatty acid methyl ester standard was purchased from Sigma Aldrich Chemie GmbH, Steinheim, Germany. All the solvents and chemicals used were of analytical grade.

2.2 Methods

2.2.1 Preparation of fatty acid mixture from PHSO

The mixture was prepared by means of PHSO hydrolysis with *Candida Antarctica A* (5 wt % of oil weight) under conditions, viz. 100 mM *tris*-HCl buffer [pH 7.0, with 1:1 ratio (w/v, based on oil weight)] which contains 10 mM CaCl₂, and the contents magnetically stirred at 40°C for 24 h, where the fatty acids were recovered by short path distillation under conditions namely feed temperature, 160-180°C; evaporator temperature, 200-220°C; condenser temperature, 40°C; heat exchange temperature, 80°C and vacuum less than 0.001 mbar. The mixture has purity of ~98%. The fatty acid composition of the fatty acid mixture used is as follows, Palmitic, 13.6%; Stearic, 6.4%; TFA (*t*-18:1) 16.3%; CFA (*c*-18:1), 35.6%; TFA (*t*-18:2), 2.7%; CFA (*c*-18:2), 22.4%; TFA (*t*-18:3), 0.5% and CFA (*c*-18:3), 2.5%, where TFA, CFA indicates *trans* fatty acids and *cis* fatty acids, respectively.

2.2.2 Fractional crystallization of PHSO fatty acids

A series of crystallizations were conducted by employing solvents namely acetone, hexane and ethanol. The cooler used for the crystallization was a COMFORT Heto Chill Master (Holm & Holby, Copenhagen, Denmark). The fatty acid mixture was dissolved in the solvent in separate glass vials and heated for 30 min at 35-40°C with vigorous shaking under N₂ atmosphere until all the fatty acids were dissolved homogeneously. The solutions were cooled to room temperature. The crystallization process was carried out by changing temperature from 5°C to -20°C by decreasing 5°C each step. Each step of crystallization was carried out for 12 h and crystals were separated by means of a Buchner funnel and vacuum based filtration at the same temperature. The crystals were washed twice with pre-cooled fresh solvent at the same temperature as the crystallization. After each crystallization, the resulting liquid fraction was employed to perform the next step of the crystallization process. Hence, the sample was fractionated into 5-6 consecutive fractions. If no crystallization was found in the solution, the same solution was continuing to the next temperature step. After completion of each step of crystallization, aliquots were collected from the liquid and crystal parts for fatty acid composition analysis.

2.2.3 Gas chromatographic analysis

The AOCS Official Method Ce 1h-05 was employed to analyze the fatty acid composition of the samples (AOCS, 2005). The samples were methylated into methyl esters as follows. To 100 mg sample, 1 mL BF₃-methanol solution was added; the resulting solution was vigorously shaken for 2 min and kept for 30 min under mild heating. Subsequently, 3 mL n-heptane were added and the reaction mixture again shaken for 1 min and then set aside for 1-2 min to achieve phase separation. The upper phase was dried with anhydrous sodium sulfate and used for GC analysis. The analysis was carried out on a Thermo Fisher Scientific Gas Chromatograph system (TRACE GC Ultra, Pittsburgh, USA), equipped with a FID detector and capillary column (SLB-IL 100, 60 m X 0.25 mm X 0.2 µm film thickness). The following optimal GLC conditions were set up for the sample analysis by gas chromatograph, a split ratio adjusted at 1:100, the injector and detectors were maintained at 250 and 275 °C. The oven temperature was maintained at 170°C for 40 min run, and the flow rate of carrier gas (N₂) was 1.5 mL/min. The area percentage was recorded with a Thermo Fisher data system and the fatty acids were identified with standards. The responses were calibrated with standard as well. The analysis was conducted in duplicates and the average was used for evaluation.

3. Results and Discussion

3.1 Determination of operation mode: crystallization equilibration and multi-step operation

Crystallization consists of nucleation and crystal growth stages. The time to reach equilibration is a basic parameter to examine before investigation of effects of other variables. As expected, the crystallization of TFAs from solution continuously progressed with time from 4 h to 12 h (Figure 1). Starting from 19.5% in PHSO, TFA content could be reduced to 11.6%, 12.2% and 14.0% with acetone, hexane and ethanol as solvent, respectively. Nevertheless, prolonging crystallization time from 12 h to 24 h did not result in significant reduction of TFA content in solution, with $\leq 0.3\%$ reduction for all the three solvent systems. Therefore, 12 h could be regarded as the time period in which equilibrium of the fractional crystallization of TFA was essentially reached. As a result, 12 h was adopted as an optimum for the operation time of fractional crystallization.

The crystallization process is governed by thermodynamic and kinetic factors. In principle, the crystallization equilibrium could be broken by removing crystals (Glynn P.D & Reardon E.J, 1990). However, due to complex composition of the samples, interference effects may exist among the various fatty acid components. As presented in Table 1, a single step of the run at 12 h, 24 h and 36 h crystallization time period conducted in parallel, resulted in similar fatty acid composition for both liquid and crystal fractions, which agreed well with the observation in Figure 1. However, with stepwise removal of crystals, the fatty acid composition for crystal fraction significantly changed. For instance in the crystal fractions, the content of *trans*-18:1 was 26.3% (step 1), 37.6% (step 2) and 30.0% (step 3); the content of 18:0 was 21.0% (step1), 4.6% (step 2) and 5.2% (step 3); and oleic acid 6.0%, 18.3 and 28.2%, respectively.

A general observation should be noticed. In the resulting crystal fractions obtained from the stepwise operation, all the *cis* fatty acid content continuously increased (from step 1, 15.1% to step 2, 39%), which means the *cis* fatty acids were significantly lost with further stepwise operations. The removal of total TFAs from liquid fractions was only improved with 4.9%. A compromise between the goal of TFAs removal and recovery of the rest should be made.

(Table 1 & Figure 1)

3.2 Effect of temperature on fractional crystallization of TFAs

Lower temperature provides the means to form the crystallization driving force – super saturation of the solute. The cooling profile is an important operation variable to manipulate crystallization pattern. Figure 2 shows fatty acid composition changes in liquid fractions with the temperature varied from 5°C to -40°C. Until the temperature reached -5°C there was no decrease in TFA while less than 50% TFA can be reduced even by -20°C. On the other hand, saturated fatty acid content reduced more significantly. At a temperature of -20°C, saturated fatty acid content could be decreased to 3%. Even by -40°C, the TFA and saturated fatty acid levels could decrease to 6.0% and 0.8%, respectively. Therefore, TFA is very challenging to be completely removed in the current system. However, a certain reduction can be achieved with the better reduction of saturated fatty acids.

(Figure 2)

3.3 Effects of solute/solvent ratio on fractional crystallization of TFAs

At a given temperature, the degree of super saturation depends on the concentration of solute in solution. For the fractionation purposes, the concentration of the solute mixture also influences the crystallization rate and packing lattice of the crystals which affects separation. As shown in Table 2, whenever the experiments were carried out with 1:3 and 1:4 (w/v) ratios with all the three solvents, none of the solvents tested gave very good fractionation. For instance, at the ratios of 1:3 and 1:4, the content of *trans* 18:1 decreased from 16.3% to 14.3% and 13.5%, respectively. On the other hand, with 1:5 and 1:10 (w/v) ratios, all the solvents exhibited significant improvement in separation. The possible reason could be, a meta-stable solution occurs at higher concentration of fatty acids, in which spontaneous crystal growth is favored. High solute concentration might produce supersaturated solutions where nucleation was favored against crystal growth, thus decreasing the efficiency of fractionation. Among all the solvents tested, acetone gave the best results, while the ratio of 1:5 is recommended considering the cost and practicability.

A further examination is made for the three solvents. Table 3 presented more results for this purpose. Compared with hexane and ethanol, acetone proved to be a better solvent in general as shown in Table 2. On the other hand, we can see saturated fatty acids can be almost equally separated from all the three solvents while TFAs can be better separated by employing acetone as a hosting solvent.

3.4 Fractional crystallization of saturated fatty acids vs TFA

Figure 3A is drawn on crystal fractions for the 3 solvents used. This confirms the conclusion that acetone shows better performance. As a general observation in the data presented in Table 1-3, and Figure 1, the crystallization of saturated fatty acids occurred more readily than TFAs. To have a quantitative understanding, we plotted the fractionation efficiency of the crystallization process for the removal of saturated fatty acids vs TFA in acetone, hexane and ethanol (Figure 3B). As can be seen, the plot strongly deviated from non-selective crystallization to the saturated fatty acids. Until nearly 50% removal of saturated fatty acids, there was no significant reduction in the content of TFA in liquid fractions. The maximum removal of TFAs achieved were 40% by acetone at -20°C, meanwhile up to 85% of saturated fatty acids had been reduced. This is understandable as the higher m.p. fatty acids start to crystallize first. Pure stearic acid has a m.p. about 69°C while *trans* 18:1 has a m.p. about 46°C. Due to the intermediate m.p. of TFAs, they hardly crystallized in the presence of higher concentrations of saturated fatty acids (Benjamin M.O, Michael S.K, Nikova A.T & Schwartz D. K, 2002). Furthermore, unsaturated fatty acids have higher solubilities in hydrophilic solvents than saturated fatty acids, which enable easier separation of saturated fatty acids.

4. Conclusions

In general, this initial study shows that the reduction of TFA from the fatty acid mixture is possible in certain extent, but with high challenge to remove further below 10%. Significant low temperature has to be used to drive further low level so that makes the practical operation uneconomically favorable. From the study, the crystallization behavior of TFA strongly depends on the type of solvent employed and crystallization temperature, conversely the crystallization behavior of SFA is mainly temperature dependent and independent of solvent used. Fatty acid to solvent ratio also influences the efficiency of fractional crystallization and requires an optimum ratio (1:5, w/v) to give better fractionation. Stepwise crystallization is preferable rather than single step separation. Crystallization time at each step is important and an optimum time period (12 h) is required to stabilize crystal formation. It is certainly true that the crystallization process examined in the present study has to be refined and optimized; however, the current study may provide a roadmap for the future studies.

Acknowledgments

The authors gratefully acknowledge Flemming Lund Sorensen for his kind technical assistance.

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Table 1. Fatty Acid Composition Change of Liquid and Crystal Fractions in Multi-Step Operations

Fatty Acid Composition (w %)								
Sample	16:0	18:0	18:1, TFA	18:1, CFA	18:2, TFA	18:2, CFA	18:3, TFA	18:3, CFA
L F-1	3.20 ±0.06	1.10 ±0.02	11.50±0.23	44.00±0.88	2.50 ±0.05	32.60 ±0.65	0.60 ±0.01	4.50 ±0.09
C F-1	35.40 ±0.70	21.00±0.42	26.30±0.53	6.00 ±0.12	1.90 ±0.04	5.80±0.12	0.40±0.00	3.20 ±0.06
L F-2	2.00 ±0.04	0.50 ±0.01	9.20 ±0.18	45.40 ±0.90	0.90 ±0.02	35.20±0.70	0.50 ±0.01	6.30±0.13
C F-2	17.00 ±0.34	4.60 ±0.09	37.60±0.75	18.30 ±0.37	4.30 ±0.09	13.20 ±0.26	1.30±0.03	3.70 ±0.07
L F-3	1.70 ±0.03	0.30 ±0.00	8.20±0.16	46.00±0.92	0.20 ±0.00	37.90±0.76	0.30 ±0.00	5.40 ±0.11
C F-3	15.70 ±0.31	5.20 ±0.10	30.00 ±0.60	28.20 ±0.56	6.70 ±0.13	9.50 ±0.19	3.40 ±0.07	1.30 ±0.03
LF-24 h	2.70 ±0.05	0.90 ±0.02	10.90±0.22	46.30±0.93	2.40±0.05	31.40±0.63	0.60 ±0.01	4.80±0.10
CF-24 h	36.10 ±0.72	21.60±0.43	26.90 ±0.54	5.10 ±0.10	2.10±0.04	4.70 ±0.09	0.50±0.01	3.00±0.06
LF-36 h	2.60 ±0.05	0.80±0.02	10.90 ±0.22	46.90±0.94	2.20 ±0.04	31.90 ±0.64	0.60 ±0.01	4.10±0.08
CF-36 h	36.40 ±0.73	21.90±0.44	26.60±0.53	4.90±0.10	2.30 ±0.05	4.50 ±0.09	0.60±0.01	2.80±0.06

Abbreviations: TFA, *trans* fatty acid; CFA, *cis* fatty acid; LF-1, 2, 3 indicates liquid fraction of 1, 2 and 3 steps; CF-1, 2, 3 indicates crystal fraction of 1, 2 and 3 steps; solvent, acetone; sample to solvent ratio, 1:5 (w/v); each crystallization step carried out for 12 h; crystallization temperatures were maintained at -35°C.

Table 2. Fatty Acid Compositions of Liquid Fractions by Fractional Crystallization at Different Sample/Solvent Ratios

Solvent	Ratio (w/v)	Fatty Acid Composition (w %)							
		16:0	18:0	18:1, TFA	18:1, CFA	18:2, TFA	18:2, CFA	18:3, TFA	18:3, CFA
Original		13.6	6.4	16.3	35.6	2.7	22.4	0.5	2.5
Acetone	1:3	11.50±0.23	4.40±0.09	14.30±0.29	40.60±0.81	2.50±0.05	23.50±0.47	0.50±0.01	2.70±0.05
Acetone	1:4	8.50±0.17	2.50±0.05	13.50±0.27	42.30±0.85	1.50±0.03	28.10±0.56	0.40±0.01	3.20±0.06
Acetone	1:5	2.70±0.05	0.30±0.00	8.90 ±0.18	45.00±0.90	1.90±0.04	36.70±0.73	0.30±0.01	4.20±0.08
Acetone	1:10	2.10±0.04	0.20±0.00	8.40 ±0.17	45.00±0.90	1.90±0.04	37.40±0.75	0.30±0.01	4.70±0.09
Hexane	1:3	12.50±0.25	4.70±0.09	15.70±0.31	37.60±0.75	2.80±0.06	23.10±0.46	0.80±0.02	2.80±0.06
Hexane	1:4	7.60 ±0.15	1.70±0.03	12.70±0.25	41.80±0.84	2.90±0.06	29.10±0.58	0.90±0.02	3.30±0.07
Hexane	1:5	2.90 ±0.06	0.40±0.00	9.10 ±0.18	44.90±0.90	2.30±0.05	36.00±0.72	0.80±0.02	3.60±0.07
Hexane	1:10	2.40 ±0.05	0.40±0.00	8.90 ±0.18	44.80±0.90	1.90±0.04	36.40±0.73	0.60±0.01	4.60±0.09
Ethanol	1:3	10.50±0.21	5.40±0.11	15.30±0.31	39.60±0.79	3.50±0.07	22.60±0.45	0.50±0.01	2.60±0.05
Ethanol	1:4	4.80 ±0.10	2.50±0.05	14.70±0.29	40.90±0.82	2.70±0.05	30.60±0.61	0.60±0.01	3.20±0.06
Ethanol	1:5	2.60 ±0.05	0.50±0.01	11.10±0.22	44.10±0.88	2.50±0.05	35.70±0.71	0.40±0.01	3.10±0.06
Ethanol	1:10	2.50 ±0.05	0.50±0.01	10.90±0.22	43.90±0.88	2.70±0.05	36.80±0.74	0.50±0.01	2.20±0.04

Abbreviations: TFA, *trans* fatty acid; CFA, *cis* fatty acid; Operation conditions: temperature, -20°C; crystallization time period, 12 h.

Table 3. Fatty Acid Compositions of Liquid Fractions by Fractional Crystallization in Different Solvents

Temp (°C)	Acetone			Hexane			Ethanol		
	SFA	TFA	CFA	SFA	TFA	CFA	SFA	TFA	CFA
Original	20.0	19.5	60.5	20.0	19.5	60.5	20.0	19.5	60.5
5	12.90±0.26	19.50±0.39	67.60±1.35	13.00±0.26	19.50±0.39	67.50±1.35	13.80±0.28	19.50±0.39	66.70±1.33
0	10.90±0.22	19.50±0.39	69.60±1.39	10.00±0.20	19.50±0.39	70.50±1.41	10.00±0.20	19.50±0.39	70.50±1.41
-5	7.80±0.16	17.50±0.35	74.70±1.49	7.80±0.16	18.50±0.37	73.70±1.47	7.80±0.16	18.50±0.37	73.70±1.47
-10	5.50±0.11	16.30±0.33	78.20±1.56	4.00±0.08	17.00±0.34	79.00±1.58	4.10±0.08	17.50±0.02	78.40±1.57
-15	3.50±0.07	12.40±0.25	84.10±1.68	3.50±0.07	14.00±0.28	82.50±1.65	3.20±0.06	16.10±0.32	80.70±1.61
-20	3.00±0.06	11.60±0.23	85.40±1.71	3.30±0.07	12.20±0.24	84.50±1.69	3.10±0.06	14.00±0.28	82.90±1.66

Abbreviations: TFA, *trans* fatty acid; CFA, *cis* fatty acid; Operation conditions: temperatures varied from 5°C to -20°C; sample to solvent ratio, 1:5 (w/v); crystallization time period is 12 h.

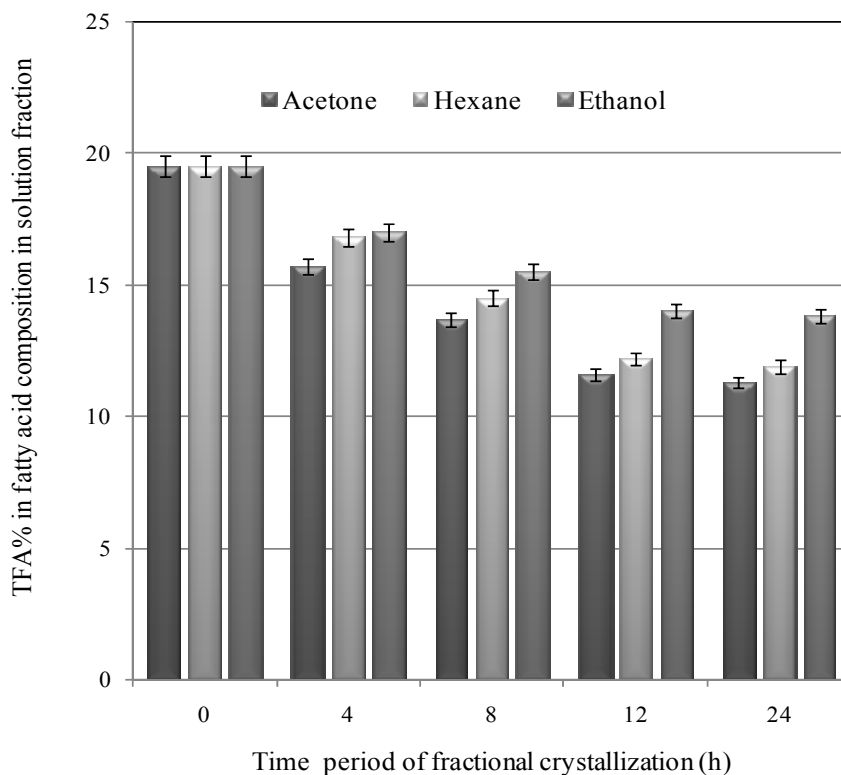


Figure 1. Fractional Crystallization of TFA with Different Time Periods. Operation conditions: sample to solvent ratio, 1:5 (w/v); and temperature maintained at -20°C. Abbreviations: TFA-*trans* fatty acid

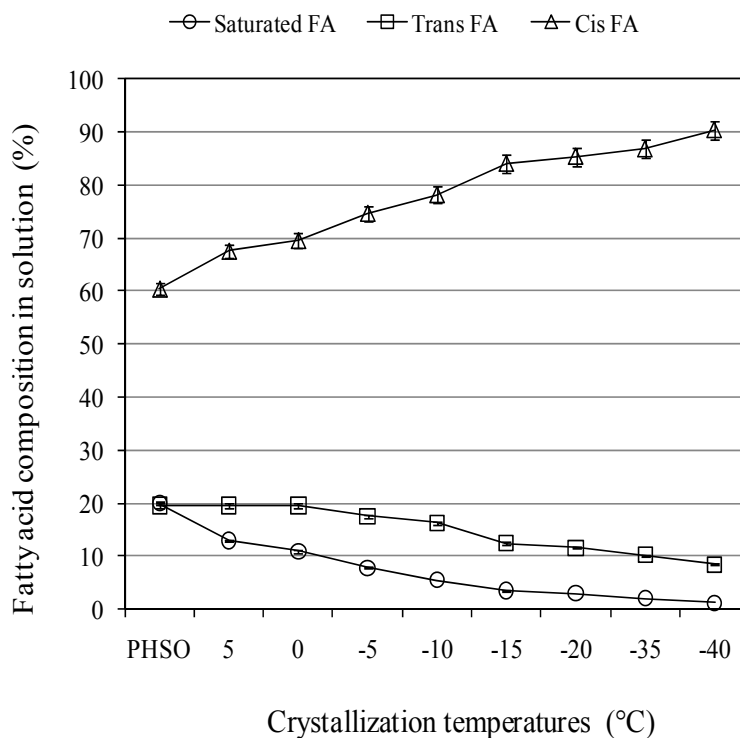


Figure 2. Effects of Temperatures on Fractional Crystallization of TFA in Acetone

Operation conditions: temperature varied from 5 to -40°C, sample to solvent ratio, 1:5 (w/v); each step of crystallization carried out for 12 h. Abbreviations: FA-fatty acid, TFA-*trans* fatty acid, PHSO-partially hydrogenated soybean oil.

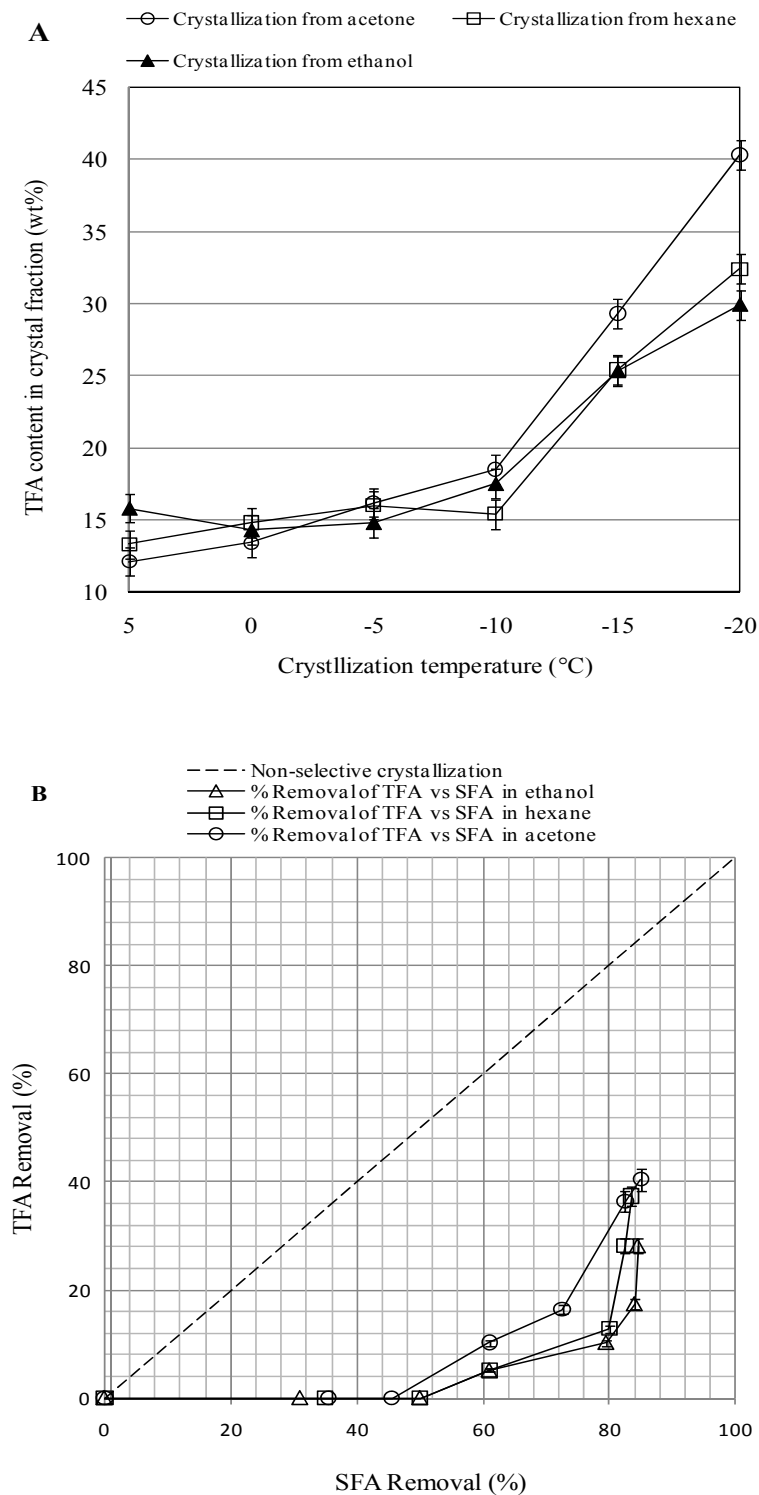


Figure 3. Solvent Dependency of Fractional Crystallization of SFA vs TFA

(A) TFA Percentage in Crystal Fractions from Different Solvents; (B) Relative Removal Content of SFA vs TFA in Different Solutions. The relative removal content of SFA or TFA was calculated as the percentage of fatty acids removed from solution of its original content (SFAs, 20% and TFAs, 19.5%). Operation conditions: temperature varied from 5 to -20°C, each crystallization step carried out for 12 h; sample to solvent ratio, 1:5 (w/v). The plotted points in Fig. 3B corresponding to operation temperature in the order of 5, 0, -5, -10, -15 and -20°C, respectively. Abbreviations: SFA, saturated fatty acid; TFA, *trans* fatty acid.