Physical Properties of Interesterified Fat Blends

M.A.M. Zeitoun[a], **W.E. Neff**[b], **G.R. List**[b] **and T.L. Mounts**[b,*]
[a]Alexandria University, Faculty of Agriculture (Saba Basha), Department of Food Science, Alexandria, Egypt and [b]Food Quality and Safety Research, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois

Fat blends, formulated by mixing fully hydrogenated soybean oil with nine different commonly used vegetable oils in a ratio of 1:1 (w/w), were subjected to interesterification (also commonly referred to as rearrangement or randomization) with sodium methoxide catalyst. Fatty acid composition and triacylglycerol molecular species of each fat blend and the interesterified product were determined and correlated with the following physical properties: melting, crystallization characteristics and solid fat content. The differences in the endothermic and exothermic peak temperatures, total heat of fusion and crystallization (β and β' crystalline content) and solid fat content among the fat blends clearly showed the effect of the composition of each oil on the physical properties. Oils that contained a considerable amount of palmitic acid had a favorable influence on the crystallization and polymorphic form of interesterified fat blends.

KEY WORDS: Fatty acids, interesterification, margarine, physical properties, polymorphism, triacylglycerol.

Interesterification of fully hydrogenated fats and natural oils can produce a fat blend with optimum characteristics. Rearrangement or randomization of acyl residues in triacylglycerols has provided fats or oils with new physical properties (1–5). Interesterification can be controlled by selection of the raw material and the processing parameters to obtain a fat with melting characteristics and consistency that are "tailor-made" for specific end uses.

Basestocks used for formulation of North American margarines have *trans* fatty acid contents ranging from 33.1 to 45.0% for stick margarines and from 22.4 to 30.1% for soft margarines (6,7).

Interest in the development of a zero-*trans* solid fat with physical properties suitable for margarines has been heightened due to recent reports of unfavorable effects of dietary *trans* fatty acids on low-density and high-density lipoprotein-cholesterol levels (8).

Interesterification of fully hydrogenated soybean oil (FHSBO) with liquid soybean oil (SBO) has been used as an alternative to partial hydrogenation to produce a zero-*trans* solid fat with the desired physical properties (9).

Polymorphic behavior of fully hydrogenated soybean, rapeseed, cottonseed and palm oils and their mixture with liquid oil was studied by deMan *et al.* (10). FHSBO (under the conditions employed by deMan) was exclusively in the β form, and the others were mixtures of α, β and β' polymorphic forms. Crystallization characteristics and polymorphic stability of hydrogenated canola oil (CAO) were affected by addition of palm oil (PO) (11,12).

We have examined the effect on the physical properties of fat products formed by the interesterification of nine commonly used edible oils with fully hydrogenated soybean oil to assist in the proper choice of oils to produce a hard-fat phase with optimum texture and melting characteristics for use in margarines.

EXPERIMENTAL PROCEDURES

Materials. Vegetable oils: SBO, CAO, sunflower (SFO), cottonseed (CSO), corn (CO), PO, peanut (PNO), safflower (SO) and coconut (CNO) were obtained either from local markets or industrial sources as finished edible oils. FHSBO was obtained from an industrial source. High-performance liquid chromatography (HPLC)-grade solvents acetonitrile and methylene chloride were filtered through a 45-micron disc and degassed before use. Sodium methoxide was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI).

Interesterification procedures. One-hundred grams of each oil and fat blend was charged into a 500-mL round-bottom flask, placed on a rotary evaporator and heated for 60 min at 95°C under vacuum to remove traces of moisture from the oil. The mixture was cooled to 80°C, 0.3% sodium methoxide was added as catalyst and the mixture was stirred at 80–90°C. After heating for 5–8 min, the color of the mixture became brownish due to formation of active catalyst, which is a reaction complex between the catalyst and the triacylglycerols (TAG). After 50 min reaction, the mixture was cooled to *ca.* 60°C, and 5% citric acid (4% conc.) was added to neutralize the catalyst while the mixture was stirred mechanically at 60 r.p.m. If necessary, the mixture was heated to 75–80°C to separate the aqueous layer. The reaction mixture was washed two times with distilled water until neutral. Filter aid (Celite analytical filter Aid 300 G, Fisher Scientific Co., Pittsburgh, PA) (2%) was added, mixed well and the mixture was filtered through a Buchner filter. The filtrate was dried under vacuum in a rotary evaporator and then heated to 85°C under vacuum. Then, 1.5% activated clay was added, and bleaching was continued under vacuum for 25 min at 95°C. After filtration through a Buchner filter covered with filter aid, the interesterified fat was steam deodorized (1–6 mm Hg, 220°C, 3 h).

Analytical procedures. Fatty acid methyl esters (FAME) were prepared and analyzed by gas chromatography (GC) as described previously (13). Pure TAGs were obtained from interesterified and noninteresterified blends of FHSBO with each of the nine vegetable oils by solid-phase extraction chromatography (SE) (13). The TAGs were then resolved by gradient reverse-phase high-performance liquid chromatography with flame-ionization detection (GRP-HPLC-FID) (13). The suitability of the GRP-HPLC-FID system for TAG molecular species (TAGMS) separation was proved by running TAGMS standards that contained saturated fatty acids of chainlength from 6–16 carbons (tripalmitin) and 18 carbons (tristearin). The HPLC-FID area percent was within 0.1 to 1.7% of the wt% of the TAGMS standard components.

The polymorphic forms of the fat crystals in the blends of FHSBO and the nine vegetable oils studied, before and after interesterification, were evaluated with a General Electric XRD-6 Diffractometer, emitting Cu-Kα radiation

---

[*] To whom correspondence should be addressed at 1815 N. University Street, Peoria, IL 61604.

Copyright © 1993 by the American Oil Chemists' Society

($\lambda = 1.5418$ Å). The instrument had been retrofitted with a Nicolet I system. Data were collected from 3 to 33°θ with a step width of 0.05° and a step time of 5 s, operating at room temperature. X-ray data were processed by a computer programmed to calculate absorption intensity-background, intensity/maximum intensity and peak width, in degrees (2°θ), for each crystalline form and the relative content of β and β' crystals. The β was calculated from the intensity of the short spacing of 4.6Å. The β' fraction was calculated from the intensities of the short spacing of 3.8 and 4.2Å. Prior to measurement of x-ray diffraction, the samples were subjected to temperature cycling, initially at 5°C for one day, followed by two days at 20°C, one day at 5°C, two days at 20°C and finally at 5°C for fourteen days (6).

Differential scanning calorimetry (DSC) was performed in a Perkin-Elmer (Norwalk, CT) DSC-7 Thermal Analysis System, with ΔH calculated by the instrument software. Analysis was begun at a sample temperature of 5°C and the sample was heated at a rate of 5°C/min to 90°C, and then the sample was cooled at 5°C/min to 0–5°C, with dry ice as a coolant. Prior to DSC, the samples were stored at 5°C for 36 d.

Solid-fat content (SFC) was determined with a pulsed nuclear magnetic resonance (NMR) spectrometer (Minispec PC 120, Brucker, Germany). Prior to analysis, samples were heated to completely liquify to assure homogeneity, and then the heating was stopped and the samples were cooled and held at 0°C for 15 min, tempered at 27°C for 30 min and cooled again and held at 0°C for 30 min. Samples were then equilibrated at the lowest temperature in the temperature range of interest for at least 30 min, the solids content was measured by pulsed NMR, and then they were equilibrated at the next highest temperature. Sample melting point was determined according to the AOCS Official Method CC 1-25 (14).

RESULTS AND DISCUSSION

Fatty acid compositions and melting points of the interesterified fat blends are listed in Table 1. Differences in the fatty acid compositions were due to the different vegetable oils used in each blend. Though the total saturated fatty acid levels show differences among the blends, except for the blend with CNO, there is little difference in the melting point. Interestingly, the interesterified blend with the largest amount of saturated fatty acids exhibits the lowest melting point.

TAG. The TAGMS of the blends, before and after interesterification, as determined by GRP-HPLC-FID, are presented in Tables 2 and 3. These results show that substantial changes in TAGMS occur due to the interesterification reaction. Triunsaturated (U_3) and trisaturated (S_3) fatty acid-containing TAGs significantly decreased and diunsaturated-saturated (U_2S) and unsaturated-disaturated (US_2) fatty acid-containing TAGs significantly increased for all the vegetable oil blends, except for PO, after interesterification (Table 2). For example, in the SBO blend after interesterification, U_3 and S_3 were decreased by one-fourth, U_2S was about doubled, and US_2 increased twentyfold. Contents of the individual TAGMS are presented for the highly saturated CNO blend in Table 3. After interesterification, most S_3-containing di-C_{14} or higher-carbon-number fatty acids were increased. However, dipalmitin-stearin and tristearin were significantly decreased. Further, TAGs containing two fatty acids having a lower carbon number, such as caprylic, capric or lauric, were decreased. Similar to the US_2 in the blends listed in Table 2, the content of distearic-oleic-containing TAGs increased after interesterification. Such considerable increase or decrease in the amount of specific TAGMS, observed in all the oils evaluated, was due to the random rearrangement of the fatty acids during interesterification.

DSC Analysis. The DSC heating and cooling curves in the constant-temperature time studies of the interesterified blends stored 36 d at 5°C mainly exhibited more than one peak in all blends. The DSC melting thermograms exhibited four endothermic peaks (Table 4) for interesterified blends of FHSBO with PO, PNO, CO and SBO. Blends with CSO, SO and CAO exhibited five endothermic peaks; SFO, six peaks; and CNO, three peaks. The DSC crystallizing thermograms for each blend exhibited three peaks. The melting point of each blend was close to the temperature of the last melting peak of its heating curve. There was considerable difference among the interesterified blends in the endothermic and exothermic peak temperatures, total ΔH of crystallization and the supercooling temperature (Table 4). These differences indicate the effect of each vegetable oil on the melting and crystalliza-

TABLE 1

Fatty Acid Composition and Melting Points (°C)[a] of Interesterified Blends (1:1, wt%) of Fully Hydrogenated Soybean Oil with Nine Vegetable Oils

Vegetable oil[b]	Fatty acid composition (area %)									Melting point
	10:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:1	
PO	—	—	—	26.6	46.3	22.4	4.7	—	—	48.2
CNO	1.6	19.2	9.0	10.8	59.0	2.4	—	—	—	41.0
CSO	—	—	—	17.3	46.1	10.7	25.9	—	—	50.0
PNO	—	—	—	11.4	46.0	25.8	15.5	0.8	0.5	51.0
SBO	—	—	—	10.2	46.9	12.3	26.6	4.0	—	52.0
CO	—	—	—	10.7	45.0	14.1	29.5	0.7	—	52.0
SFO	—	—	—	8.2	46.9	10.0	34.9	—	—	51.0
SO	—	—	—	8.4	45.3	8.8	37.2	0.3	—	51.0
CAO	—	—	—	6.8	42.8	34.6	10.7	4.4	0.7	50.0

[a] Melting point determined by capillary tube method.

[b] PO, palm oil; CNO, coconut oil; CSO, cottonseed oil; PNO, peanut oil; SBO, soybean oil; CO, corn oil; SFO, sunflower oil; SO, safflower oil; and CAO, canola oil.

TABLE 2

Triacylglycerol Composition[a] of Blends of Vegetable Oil with Fully Hydrogenated Soybean Oil (1:1, wt%), Before (B) and After (I) Interesterification

Vegetable oil[c]	Triacylglycerol[b] (area %)							
	U_3		U_2S		US_2		S_3	
	B	I	B	I	B	I	B	I
PO	9.5	2.3	20.5	13.7	13.3	43.4	56.7	40.6
CSO	15.7	9.8	24.3	31.2	8.5	33.9	51.5	25.1
PNO	29.0	6.2	15.3	31.1	2.4	47.3	53.3	15.4
SBO	31.0	9.2	17.1	32.1	1.5	46.4	50.4	12.3
CO	31.8	9.0	15.3	35.2	1.1	45.9	51.8	9.9
SFO	37.6	8.3	11.3	34.3	0.3	46.9	50.8	10.5
SO	37.4	17.2	11.5	38.9	0.4	34.0	50.7	9.9
CAO	39.1	9.2	8.5	37.1	0.7	44.2	51.7	9.5

[a] Determined by reverse-phase high-performance liquid chromatography with flame-ionization detector.
[b] Fatty acids in triacylglycerol: U = unsaturated, S = saturated. Other abbreviations as in Table 1.
[c] Vegetable oil in blend with fully hydrogenated SBO.

TABLE 3

Triacylglycerol Composition[a] of Blend of Coconut Oil with Fully Hydrogenated Soybean Oil (1:1, wt%), Before (B) and After (I) Interesterification

TAGMS[b]	Area %	
	B	I
LaCC	1.0	0.9
LaLaC	5.3	1.1
LaLaCa	7.6	1.8
LaLaLa	9.4	3.3
LaLaM	8.4	5.9
MLaM	5.3	6.1
PLaM	3.5	12.8
SLaM	2.0	11.8
SPLa	1.3	10.7
SPM	0.9	20.0
SOS	1.6	7.7
PPS	15.2	6.8
SSS	38.5	11.1

[a] Determined by reverse-phase high-performance liquid chromatography with flame-ionization detector.
[b] Fatty acids in triacylglycerol: C, caprylic; Ca, capric; La, lauric; M, myristic; P, palmitic; S, stearic; O, oleic. TAGMS, triacylglycerol molecular species.

tion behavior of the interesterified blend. This effect is related to the differences in TAGMS (Tables 2 and 3). For example, deMan *et al.* (10) studied the DSC melting characteristics of fully hydrogenated fats blended with CAO. They reported only one endothermic peak (71.5°C), which was close to the last melting peak of FHSBO. In the current study, the interesterified blend with CAO exhibited five endothermic peaks, clearly showing the effect of interesterification on melting characteristics.

DSC is a useful technique for investigation of the transition of crystal forms during melting of fats (15). It is recognized that when fat is heated it can exhibit multiple melting phases. Each recrystallization step represents a transition of the polymorph from its less stable form to a more stable form (16,17). Because the more stable crystal form has a higher melting point, the transition peak temperature can serve as an important indicator of the polymorphic form of the crystals.

X-ray diffraction analysis. Quantitation of the relative intensities of the short spacing at 4.6Å (β) and 4.2Å and 3.8Å (β'), as measured with spectra for the blends at constant temperature, is presented in Table 5. The β' form of crystals is desired for favorable molecular packing of the

TABLE 4

Differential Scanning Calorimetry Melting and Crystallization Characteristics of Interesterified Blends of Vegetable Oil with Fully Hydrogenated Soybean Oil (1:1, wt%)

Vegetable oil	Peak temperature (°C)		Total ΔH (J/g)		Supercooling[a] temperature (°C)
	Endothermic	Exothermic	Fusion	Crystal	
PO	12, 17, 37, 47.3	35, 17, 14	88.0	67.5	13
CNO	18, 36, 40	29, 22, 16	86.8	32.2	13
CSO	11, 18.5, 36, 45, 50.5	34, 17, 15	79.3	55.8	16
PNO	12.5, 18, 39, 52	34, 17, 15	73.2	53.8	18
SBO	14, 21, 42, 54	34, 17, 14.3	64.0	47.5	18
CO	12, 19, 41, 52	32, 16, 13	59.8	32.2	20
SFO	16, 21, 29, 35, 43, 52	31, 16.5, 14	58.8	38.5	21
SO	12.7, 18, 35, 48, 51	30, 16, 14	49.9	35.8	22
CAO	11.6, 18, 25, 37, 51	31, 16, 13	49.8	32.0	19

[a] Temperature difference between melting point and crystallization temperature. Abbreviations as in Table 1.

TABLE 5

β and β′ Crystallization Form Content[a] in Blends of Vegetable Oil with Fully Hydrogenated Soybean Oil (1:1, wt%) Before and After Interesterification

Vegetable oil	Before (%)		After (%)	
	β	β′	β	β′
PO	55.0	45.0	28.0	72.0
CNO	53.0	47.0	25.3	74.7
CSO	62.4	37.6	41.0	59.0
PNO	59.0	41.0	48.0	52.0
SBO	75.3	24.7	54.0	46.0
CO	77.0	23.0	52.0	48.0
SFO	100.0	0.0	63.0	37.0
SO	81.2	18.8	62.0	38.0
CAO	77.4	22.6	55.5	44.5

[a]Determined by x-ray diffraction at 23°C; β′ strong short spacing of 4.6Å; β′ strong short spacing of 3.8 and 4.2Å. Abbreviations as in Table 1.

fatty acid chains of a solid fat used for production of margarine or table spread (18). The blends before interesterification exhibited the β form more than the β′ form, whereas after interesterification the β′ form was greater. That may be due to the randomization and rearrangement occurring during the interesterification reaction (3,4,9). The highest content of β′ form was found in the interesterified blends of FHSBO with CNO, PO and CSO, and the lowest content of β′ was found with SFO and SO.

The differences in the polymorphic state of the interesterified blends could be due to the variation in TAGMS composition of each blend. For example, palmitic acid-containing TAGMS content differs among the blends (Tables 2 and 3). Thus, the diversity of acyl chainlength may contribute to observed differences in the polymorphic state. D'Souzo *et al.* (7) concluded that the difference between β and β′ margarines could be related to the 16C fatty acid and 54C TAG of the high-melting fractions. They also noted that in the β-tending margarines the 16C fatty acid content was below 11%, but it was above 17% in the β′-tending margarines. Hernqvist *et al.* (19) concluded that hydrogenated low-erucic acid rapeseed oil containing a high amount (11%) of palmitic acid must be considered as a β′-stable fat.

The liquid oil composition also affects the polymorphic state of the solid blend (Table 5). Interesterified blends containing SFO, SO and CAO as the liquid portion exhibited mostly β crystals, whereas using PO and CNO as the liquid portion showed mostly β′ crystals. deMan *et al.* (10) found that 50% FHSBO in CAO was exclusively in the β form and a fully hydrogenated CSO and PO in rapeseed oil blends were mixtures of β and β′ forms. Therefore, it may be concluded that the differences in crystal form observed in the current study arise from the liquid oil portion of the blends.

SFC determination. The SFC of the interesterified blends is presented in Table 6. Blends with high content of saturated fatty acid-containing TAGMS, like CNO and PO, had the highest SFC among all the blends, and the SFC of the PO blend was nearly twice as great as that of all the other blends, except for the CNO blend. Where the SFC curves of all of the blends, except PO and CNO, had the same slope, the actual SFCs were different. This may be due to the differences in the saturated fatty acid-TAGMS composition of each oil.

The SFC of an interesterified blend of PO (75%) and FHSBO (25%) is also presented in Table 6. The hardness of this blend was similar to that of the blend of FHSBO with CSO at a ratio of 1:1 (w/w). This indicates an advantage to the use of PO because more palmitic and less stearic acid was incorporated, while at the same time, desirable melting and crystallization behavior required for margarines was obtained.

The results of these investigations indicate that the physical properties of interesterified fat blends are significantly affected by the TAGMS composition of the liquid oil portion of the blend. Zero-*trans* margarines may be produced by interesterification with a variety of oils. Oils that contain high content of palmitic acid-containing TAGMS contribute to a product with favorable melting characteristics and crystallization behavior. Therefore, plant breeders should consider obtaining oilseeds that yield oils with a high content (15–25%) of palmitic acid as a more suitable composition to produce margarines and table spreads.

TABLE 6

Solid Fat Content[a] of Interesterified Blends of Vegetable Oil with Fully Hydrogenated Soybean Oil (1:1, wt%)

Vegetable oil[b]	% Solid fat at °C										
	0	10	21	27	33	38	40	43	46	49	54
PO[c]	63.4	55.9	43.2	38.3	23.0	17.2	13.3	7.1	2.2	1.1	0.0
PO	86.2	80.5	73.3	71.5	54.2	44.8	37.2	28.9	21.2	14.0	0.0
CNO	97.0	89.6	73.4	65.4	40.6	28.1	19.6	11.9	7.5	4.6	0.3
CSO	69.0	58.8	43.0	30.4	24.8	22.0	20.4	15.6	10.6	5.7	1.9
PNO	65.1	55.7	40.6	38.3	26.9	21.0	17.2	12.2	7.4	6.3	1.0
SBO	63.0	53.3	38.5	33.8	23.8	19.4	16.3	13.7	9.8	7.5	3.8
CO	59.7	48.9	33.1	30.4	19.7	17.5	14.0	10.5	8.2	6.2	1.7
SFO	60.9	51.0	36.3	30.1	21.3	17.6	14.9	11.9	9.4	7.2	3.0
SO	58.7	48.8	34.8	28.0	19.5	16.2	13.5	10.6	8.4	6.2	2.5
CAO	53.8	43.2	32.1	30.1	17.9	14.9	10.8	8.0	6.8	5.6	1.1

[a]Determined by pulsed NMR spectrometry as described in the Experimental Section.
[b]Abbreviations as in Table 1.
[c]Blend of PO (75%) and fully hydrogenated soybean oil (25%).

ACKNOWLEDGMENTS

The authors gratefully acknowledge J. Hagemann, NCAUR, for differential scanning calorimetry, A. French, SRRL, New Orleans, LA, for x-ray diffractometry and J. Finely, Nabisco Food Co., Hanover, NJ, for pulsed NMR analyses.

REFERENCES

1. Sreeniwasan, B., *J. Am. Oil Chem. Soc. 55:*796 (1978).
2. Lanning, S.J., *Ibid. 62:*400 (1985).
3. Hustedt, H.H., *Ibid. 53:*390 (1976).
4. Lo, Y.C., and A.P. Handel, *Ibid. 60:*815 (1983).
5. Young, F.V.K., *Ibid. 62:*373 (1985).
6. Postmus, E., L. deMan and J.M. deMan, *Can. Inst. Food Sci. Technol. J. 22:*481 (1989).
7. D'Souza, V., L. deMan and J.M. deMan, *J. Am. Oil Chem. Soc. 68:*153 (1991).
8. Mensink, R.P., and M.B. Katan, *N. Eng. J. Med. 323:*439 (1990).
9. List, G.R., E.A. Emken, W.F. Kwolek, T.D. Simpson and H.J. Dutton, *J. Am. Oil Chem. Soc. 54:*408 (1977).
10. deMan, L., J.M. deMan and B. Blackman, *Ibid. 66:*1777 (1989).
11. Yap, P.H., J.M. deMan and L. deMan, *Ibid. 66:*1792 (1989).
12. Yap, P.H., J.M. deMan and L. deMan, *Ibid. 66:*1784 (1989).
13. Zeitoun, M.A.M., W.E. Neff, E. Selke and T.L. Mounts, *J. Liquid Chrom. 14:*2685 (1991).
14. *Official Methods and Recommended Practices of the American Oil Chemists' Society,* 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1989, Method Cc 1-25.
15. Yap, P.H., J.M. deMan and L. deMan, *J. Am. Oil Chem. Soc. 66:*693 (1989).
16. Hoerr, C.W., and F.R. Paulicka, *Ibid. 45:*793 (1968).
17. Wilton, I., and G. Wode, *Ibid. 40:*707 (1963).
18. Weiderman, L.H., *Ibid. 55:*823 (1978).
19. Hernqvist, L., O. Leissner and B. Petersson, *Fat. Sci. Technol. 89:*190 (1987).

[Received May 21, 1992; accepted February 1, 1993]