Potential Margarine Oils from Genetically Modified Soybeans[1]

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ABSTRACT: Genetically modified soybeans were processed into finished, refined, bleached, and deodorized oils. Fatty acid composition was determined by gas–liquid chromatography. Glyceride structure was characterized according to degree of unsaturation by high-performance liquid chromatography, lipase hydrolysis, and gas–liquid chromatography. Compared to common varieties with 15% saturated acids, genetically modified soybeans yielded oils containing 24–40% saturated acids. Several varieties were examined, including the Pioneer A-90, Hartz HS-1, and Iowa State A-6 lines. Pioneer A-90 contained 17% stearic acid, had a solid fat index (SFI) of 6.0 at 10°C (50°F) and zero from 21.1 to 40°C (70 to 104°F), and therefore lacked sufficient solids for tub-type margarine. To improve its plastic range, the Pioneer oil was blended with palm oil, randomized palm oil, or interesterified palm/soy trisaturate basestock. After blending with 10–40% of these components, the high-stearic acid oil had an SFI profile suitable for soft tube margarine. The A-6 varieties, 32–38% saturates, showed SFI profiles with sufficient solids at 10°C (50°F) and 21.1°C (70°F) to qualify as a stick-type margarine oil, but lacked sufficient solids at 33.3°C (92°F); however, after small amounts (2–3%) of cottonseed or soybean hardstocks were added, the A-6 oils qualified as stick margarine oil. The HS-1 variety, when blended with small amounts (2–3%) of hardstock, possessed sufficient solids at 10°–33.3°C (50–92°F) to prepare soft tub margarine oil.
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KEY WORDS: Dropping points, gas–liquid chromatography, genetically modified soybeans, high-performance liquid chromatography, interesterified triacylglycerols, margarine oils, palm oil, randomized palm oil, solid fat index, stearic acid.

During the past decade, much progress has been made in plant breeding technology to develop soybean genotypes with altered fatty acid composition. One approach has produced oils with decreased linolenic acid contents that have superior end-use properties as frying or cooking oils (1,2). Research work, most notably at Iowa State University, has resulted in the development of the A-6 variety, which contains high contents of stearic acid (3–5). Wilson and Bulton (4) have reported that, while the commercial value of high-stearic acid soybeans is unknown, feeding studies (4) have shown that the oil does not raise serum cholesterol levels, and therefore, might find outlets in novel food applications. We report here the formulation of potential margarine oils from genetically modified soybeans with elevated amounts of stearic acid.

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EXPERIMENTAL PROCEDURES

The high-stearic acid soybeans were supplied by (i) Iowa State University (Ames, IA) (A-6); two bushels from the 1989 crop year were processed as follows: one bag (Sample 1, Table 1) was processed as a single lot, while the second bag was divided into two equal portions prior to processing (Samples 2–3, Table 1); (ii) Jacob Hartz Seed Co. (Stuttgart, AR) (HS-1; Sample 4, Table 1) from the 1993 crop year; and (iii) Pioneer Hybrid International, Inc. (Waterloo, IA) (A-90; 143073; Sample 5, Table 1) from the 1992 crop year.

Seed preparation, oil extraction, and processing were carried out as described previously (2). Refined, bleached, and deodorized palm oil (PO) was obtained from PVO International (Granite City, IL). Randomized palm oil and interesterified palm/soy trisaturate (80:20) (IPS) were prepared according to previously described procedures (6). The soybean and cottonseed oil hardstocks were obtained from Riceland Foods (Stuttgart, AR) and Bunge Foods (Bradley, IL), respectively. Fatty acid compositions were determined in duplicate by capillary gas–liquid chromatography (7). Triacylglycerols (TAG) were analyzed in duplicate by reversed-phase high-performance liquid chromatography (7). Solid fat index (SFI) and dropping points were determined by the American Oil Chemists' Society official methods (8). Lipase hydrolysis of TAG was carried out as described previously (9). SFI determinations were conducted in triplicate, and values reported are the means. Dropping points are the means of duplicate determinations. Agreement between SFI and dropping point determinations were in the ranges reported as acceptable by the methods.

RESULTS AND DISCUSSION

The fatty acid and TAG composition of the high-stearic acid soybean oils (SBO) are given in Table 1, along with their SFI and dropping point data. The effects of genetic breeding are evident from the fatty acid composition data, in that total sat-

TABLE 1
Composition and Properties of Soybean Oil from Genetically Modified Beans

			Fatty acid composition (%)[a]						Triacylglycerols (%)[b]				Solid fat index @ °C[c]					Drop point
Sample	Variety	Source	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Calc. IV	UUU	UUS	SUS	SSS	10	21.1	24.7	33.3	40	(°C)[d]
1	A-6	Iowa State	9.0	23.9	18.9	40.9	7.2	105.9	22.1	47.8	26.3	2.3	11.2	0	0	0	0	16.4
2	A-6	Iowa State	8.4	33.0	16.6	36.2	6.6	94.2	15.9	39.5	40.8	2.5	22.8	12.1	0	0	0	18.7
3	A-6	Iowa State	9.4	27.1	16.8	38.5	6.5	98.1	18.1	41.0	37.5	2.3	18.7	7.9	0	0	0	14.2
4	HSI	Hartz Seed	8.9	20.6	18.8	43.5	5.5	105.7	28.8	42.6	23.9	2.5	12.7	1.5	0	0	0	17.6
5	A-90	Pioneer	8.0	17.2	16.6	47.0	10.1	121.3	36.0	45.5	17.2	1.5	6.0	0	0	0	0	14.2
	Hardin	RBD soy	9.9	4.1	24.9	52.8	7.2	131.7	58.9	28.5	12.7	0.4	0	0	0	0	0	—

[a]By gas–liquid chromatography; Iowa State University (Ames, IA); Hartz Seed (Jacob Hartz Seed Co., Stuttgart, AR); Pioneer (Pioneer Hybrid International, Inc., Waterloo, IA). [b]By reversed-phase high-performance liquid chromatography; U, unsaturated; S, saturated. [c]By AOCS Method Cd 10-57 (Ref. 8). [d]By AOCS Method Cc 18-80 (Ref.8).

urated acids (palmitic and stearic) were increased from about 15% in common soybean oils to 22–36%. High-performance liquid-chromatographic data permit the TAG to be grouped with respect to unsaturation, i.e., UUU, UUS, USS, and SSS. Ordinary SBO contains about 28% TAG with only one saturated acid/TAG, about 13% with two saturated acids/TAG (UUS), and nearly 60% containing no saturated acids/TAG (UUU). SSS TAG are essentially absent in SBO.

In contrast, genetically modified SBO contained 16–36% UUU, and 64–84% of the TAG contained at least one saturated acid per TAG molecule; 18.7–43.3% of the TAG contained two or more saturated acids per TAG molecule. Because of its highly unsaturated TAG content, ordinary SBO contains no solid fat at the temperature ranges required for margarine oils, i.e., 10–33.3°C. All of the genetically modified oils contained appreciable solids at 10°C, ranging from 6.0 to 22.8. At 21.1°C, only two of the five genetically modified oils possessed enough solids for margarine, whereas at 26.7 and 33.3°C none of the oils contained any solid fat. Based on the data given in Table 1, it is apparent that, if such oils are to be incorporated into margarine basestock, additional, harder components would be required to furnish 21.1, 26.7, and 33.3°C solids. Logical sources for such harder components are PO, RPO, or an IPS basestock (6). The SFI profiles of these oils over a temperature range of 10–40°C are shown in Figure 1.

The Pioneer A-90, A-6, and HS-1 high-stearic acid SBO were subjected to lipase hydrolysis, and the results are given in Table 2, along with the data for the original TAG and the 2-monoglyceride. Normal soybean TAG are characterized by the almost total absence of saturated acids in the 2-position, random distribution of oleic and linolenic acid on all glycerol positions, and the high proportion of linoleic acid in the 2-position (10). These observations are based on lipase hydrolysis data and suggest that the same biosynthetic pathways are operative in both high-stearic and normal soybean varieties.

The SFI profiles of the Pioneer A-90 high-stearic oil blended with PO, RPO, or an IPS (80% PO:20% SBO) are presented in Table 3. The results showed that, as the PO content increased, the SFI values increased at all temperatures. Based on typical SFI profiles of soft tub margarines (10°C = 10–12; 21.1°C, 4.5–6; 33.38C, 1.6–3.0), the optimum level of PO in a blend with a high-stearic oil is estimated to be 35–40%. Similar results were obtained with an RPO blend, except the 10°C end of the SFI curve was flatter than that of the PO blend (Fig. 1). Utilization of PO in margarine formulations has been reviewed by Duns (11). PO crystallizes slowly, which can lead to texture problems, including graininess. Slow crystallization is attributed to the presence of partial glycerides and symmetrical SUS TAG.

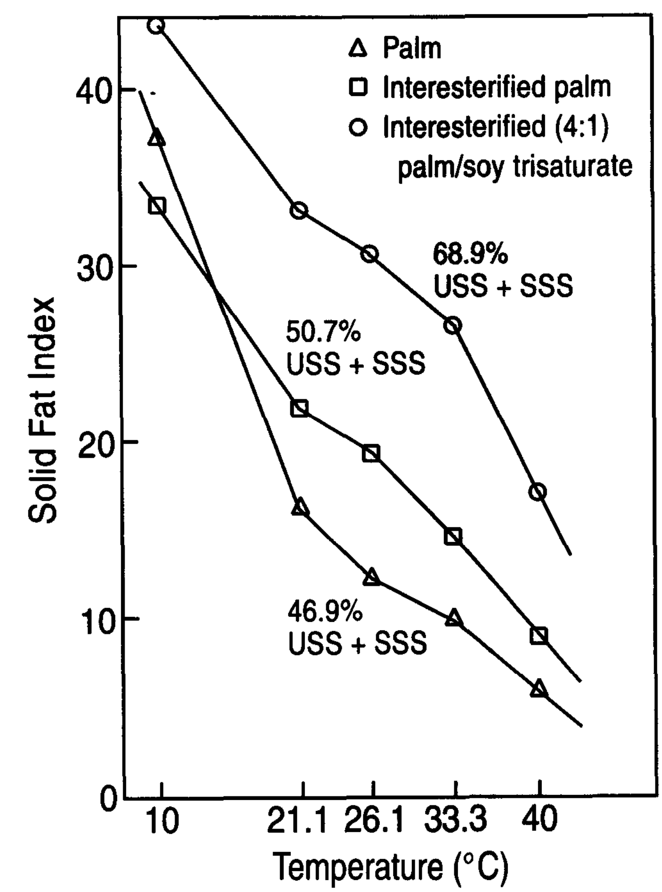

FIG. 1. Solid fat index profiles of palm oil, randomized palm oil, and interesterified palm/soy trisaturate basestock (80:20) over the range 10–40°C (50–104°F); S, saturated; U, unsaturated.

TABLE 2
Fatty Acid Composition of High-Stearic Triacylglycerols and 2-Monogylcerols After Lipase Hydrolysis[a]

Sample	Fatty acid composition (%)				
	16:0	18:0	18:1	18:2	18:3
Triacylglycerols					
Pioneer A-90	8.0	17.2	16.6	47.0	10.1
Hartz HSI TAG	8.0	21.2	19.2	43.3	5.1
Iowa State A-6[b]	8.2	23.3	18.9	40.1	6.3
Iowa State A-6[b]	7.7	30.3	16.4	36.2	5.8
Iowa State A-6[d]	8.1	28.2	17.3	37.3	6.0
2-Monoacylglycerols					
Pioneer A-90	0.4	0.7	16.1	71.8	11.0
Hartz HSI	0.7	1.2	25.7	67.1	5.2
Iowa State A-6[b]	0.6	1.3	24.8	65.4	7.9
Iowa State A-6[c]	0.9	2.3	24.8	64.6	7.3
Iowa State A-6[d]	0.7	1.9	24.7	64.8	7.6

[a]See Table 1 for company sources.
[b]See Table 1, Sample 1.
[c]See Table 1, Sample 2.
[d]See Table 1, Sample 3.

Another disadvantage to utilizing PO in soft margarines is known as "post hardening," in which the consistency of the finished product increases for up to several weeks after manufacture. Post hardening reportedly results from the crystallization of the symmetrical TAG (SUS and SSS) content of the oil. Interesterification can alleviate the problem of post hardening but limits the use of rearranged PO in soft margarine to about 15% (11). Thus, use of an IPS basestock may be more advantageous than PO or RPO in blends with high-stearic SBO, because only 10% IPS is required compared to 35–40% PO or RPO. SFI profiles, determined for blends of PO or RPO with ordinary soybean oil, showed elevated 10°C values compared to the Pioneer A-90 and HS-1 oils, while the lower end of the curves (i.e., 21.1–33.3°C) were similar. Thus, spreadability at refrigerator temperatures may be influenced more than lubricity at room or body temperatures when high-stearic oils are formulated into soft margarine basestocks. Use of these high-stearic oils would be most beneficial when considering spreadability directly out of the refrigerator.

The A-6 varieties contained the highest levels of saturated acids when compared to the Pioneer A-90 and Hartz HS-1 (genotypes). Only 18–22% of the TAG were free of saturated acids, whereas nearly 40% contained two or more saturated acids/TAG molecule (Table 1). The SFI profiles (Table 1) showed that Samples 2 and 3 contained enough solids at 10 and 21.1°C for stick-type margarines, but lacked sufficient 33.3°C solids. To provide additional solids at 33.3°C, Samples 2 and 3 oils were blended with small amounts (2–3%) of cottonseed and soybean hardstock (IV 9.2 and 0.0, respectively). The SFI profiles of these oils are shown in Table 4. The addition of hardfat (2–3%) provided sufficient solids at 33.3°C required for stick margarine. Some typical hydrogenated products showed SFI ranges of 23–27 at 10°C, 15–16 at 21.1—C, and 3–5 at 33.3°C. The other A-6 sample (sample 1, Table 1) would probably be suitable for soft tube margarine when blended with PO, RPO, or IPS.

The SFI profiles of the HS-1 oil, blended with 2–3% of cottonseed or soybean hardstocks, are presented in Table 4. The HS-1 oil possesses enough solids at 10°C for soft tub margarine but lacks 21.1–33.3°C solids. Incorporation of 2–3% cottonseed or soybean flakes provides enough solids at these temperatures to qualify as a soft tub margarine oil.

The term "lubricity," in food systems containing vegetable oil applies to the ease of handling (pourability) or mouthfeel (12). Mouthfeel can apply to both the ability to form an oily film and/or how well a solid fat melts in the mouth to give a pleasant, cooling effect. Lubricity can be measured at four temperature ranges, and based on work of Cochrane, as reviewed by Bessler and Orthoefer (12), triglycerides are broken down into four groups. Group 1 consists of triglycerides melting from −13.3 to 1.1°C and consist primarily of UUU and USS types, which are important nutritionally and will re-

TABLE 3
Solid Fat Index Profiles of Pioneer[a] High-Stearic Soy Oil–Palm Oil Blends

% A-90	% Hard component	Solid fat index at temperature (°C)					Drop point (°C)
		10.0	21.1	26.7	33.3	40.0	
100	0	6.0	0.0	0.0	0.0	0.0	—
75	25 Palm	7.5	2.6	1.7	0.6	0.3	—
70	30 Palm	7.9	3.1	2.1	1.5	0.3	—
65	35 Palm	8.6	3.6	2.6	1.9	0.3	—
60	40 Palm	10	4.7	3.4	2.7	0.6	31.9
75	25 Palm-Int.	4.7	4.4	1.7	1.7	1.4	—
70	30 Palm-Int.	5.6	4.6	3.1	2.1	1.4	—
65	35 Palm-Int.	6.8	5.4	2.8	2.8	0.9	—
60	40 Palm-Int.	8.7	7.3	4.1	4.1	2.2	34.4
92	8 Palm–Soy Int.	5.2	2.6	1.5	1.0	0.8	—
90	10 Palm–Soy Int.	7.5	4.7	4.1	2.7	0.6	29.4
88	12 Palm–Soy Int.	5.7	3.8	2.9	2.7	0.5	—
85	15 Palm–Soy Int.	6.0	4.3	3.4	1.8	0.4	—
80	20 Palm–Soy Int.	6.4	5.4	4.4	2.7	0.2	—
Tub margarine oil	Soy/hydrogenated soy	10–12	4.5–6	3.0–3.5	1.6–3.0	0.1–1.3	31.7–34.4

[a]Pioneer A-90; see Table 1 for company source.

TABLE 4
Solid Fat Index Profiles of Hardstock Blends with A-6 and HS-1 Soybean Oils

% High stearic	Hardstock		Solid fat index (°C)			Drop point
Soy A-6	%	Type	10	21.1	33.3	(°C)
100[a]	0	—	22.8	12.1	0.0	18.3
98	2	Soy	25.0	16.3	2.3	38.2
97	3	Soy	27.2	16.7	4.4	42.6
98	2	Cotton	25.4	15.1	2.2	—
97	3	Cotton	26.4	15.8	3.0	40.2
100[b]	0	—	18.7	7.0	0.0	19.9
98	2	Soy	24.4	13.6	3.0	50.7
97	3	Soy	24.9	14.7	4.8	38.0
98	2	Cotton	23.6	13.8	2.9	—
97	3	Cotton	24.2	16.6	3.7	40.0
Hydrogenated soy/soy[c]	—	—	23	16.6	4.2	49.4
HS-1[d]						
100	0	—	12.1	1.5	0.0	17.6
98	2	Soy	12.1	4.7	2.6	38.0
97	2	Soy	12.8	5.8	1.6	43.3
98	2	Cotton	15.1	5.5	1.6	36.4
97	3	Cotton	16.1	5.8	2.9	39.1
Soy/hydrogenated soy[c]	—	—	14.1	7.9	2.5	32.2
Soy/hydrogenated soy[c]	—	—	11.3	5.4	3.0	31.7
Soy/hydrogenated soy[c]	—	—	9.7	4.4	1.6	30.5

[a]Sample 2, Table 1. [b]Sample 3, Table 1. [c]Commercial margarine oil. [d]Sample 4, Table 1.

main liquid and pourable at refrigerator temperatures. Group 2 triglycerides melt from 5.6 to 22.8°C and are important because these triglycerides will remain liquid at room or ambient temperature. They consist mainly of triolein and palmito and stearo dioleins. Groups 3 and 4 triglycerides melt at 10.6 to 41.7°C and 56.1° to 65°C, and consist entirely of disaturated and trisaturated glycerides. These glycerides are important to margarine fats because they influence mouthfeel and structure at room (25°C) temperatures. The genetically modified soybean oils contained high concentrations of the Group 3 and 4 triglycerides (Table 1) and should lend themselves well to margarine formulations.

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