inhibitor activities and tannins and higher protein digestibility would be readily used in animal feed.

Karanja seeds also are known to contain antinutritional factors, including karanjin, a furanoflavonoid (1.25%, $C_{18}H_{12}O_4$, m.p. 158.5 C); pongamol, a diketone (0.85%, $C_{18}H_{14}O_4$, m.p. 128 C); and glabrin, a complex amino acid (trace, $C_{21}H_{42}O_{12}N_3$, m.p. 290 C decomp) (25). Toxicity of these antinutritional factors and that of seed meal was extensively investigated (16,18). Destruction of these toxic compounds by acid hydrolysis processing was chemically verified (18). Earlier reports (16,18) of the merits and disadvantages of this processing method showed that nutrient composition and amino acid profile of karanja seed meal remained almost unaltered after processing. The present results show that this processing also reduced the levels of trypsin and chymotrypsin inhibitors and improved the protein digestibility significantly.

For the first time, this study reports the presence of trypsin and chymotrypsin inhibitors in the defatted seed meals of akashmoni and karanja and also demonstrates that suitable processing can reduce these protease inhibitor activities and improve the protein digestibilities of these seed meals.

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♣Effect of Triglycerides Containing 9,10-Dihydroxystearic Acid on the Solidification Properties of Sal *(Shorea robusta)* Fat

S. YELLA REDDY and **J.V. PRABHAKAR,*** Discipline of Lipid Technology, Central Food Technological Research Institute, Mysore 570 013, India

ABSTRACT

Components affecting solidification properties of sal *(Shorea robusta)* fat have been studied. Triglycerides containing 9,10-dihydroxystearic acid (DHS-TGs) present to about 3% have been found to affect the supercooling property of sal fat at as low a level as 2%. The DHS-TGs were composed of 57.5% stearic, 5.8% arachidic, 6% palmitic and 30.5% 9,10-dihydroxystearic acids. As DHS-TGs are soluble in acetone, solvent fractionation using acetone improved the supercooling capacity of stearin while that of the olein fraction was not affected. When the fat was subjected to dry fractionation at 35 C, DHS-TGs, due to their high melting nature, were removed to a greater extent in the form of stearin, thereby improving the supercooling capacity of the olein.

INTRODUCTION

Sal fat is one of the commercially important vegetable fats used in the manufacture of cocoa butter substitutes. It is similar to Borneo tallow in physical properties and is rich in 2-oleyl disaturated glycerides. In our studies on preparation of cocoa butter extenders, we noticed variations in the solidification properties of commercial sal fat samples (Fig. 1). This presented problems in maintaining uniform quality in sal fat fractions obtained from different batches of fats using one set of fractionation conditions. Preliminary studies indicated that some minor components present in refined and bleached sal fat affected its solidification properties. In this paper, the effect of one of the minor components, triglycerides containing 9,10-dihydroxystearic acid, on the solidification properties of sal fat is reported.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Refined and bleached sal fat received from M/s Specialty Fats Pvt. Ltd., Khamgaon, was used for this study. The fat (Type 2) had the cooling characteristics shown in Figure 1 curve a.

Solvent Fractionation

The flow diagrams of the different fractionation procedures used are given in Figure 2. Sal fat (200 g) was dissolved in 400 ml of acetone by warming it to about 45 C. The solution was cooled gradually to 20 C and held at this temperature for about 3 hr. Then it was filtered, and the solid (stearin) was washed once with 20 ml of chilled acetone at 20 C. The stearin (Fraction 1) and olein (yield 80%, Fraction 2) fractions were desolventized in a rotary flash evaporator under reduced pressure.

In another experiment, 200 g of sal fat were dissolved in 400 ml of acetone by warming the solution to about 45 C. The solution was cooled gradually to 15 C and held at this temperature for 2 hr. Then it was filtered, and stearin was washed once with 20 ml of chilled acetone at 15 C. The solvent from stearin (yield 90%, Fraction 3) and olein (Fraction 4) was removed under vacuum as before.

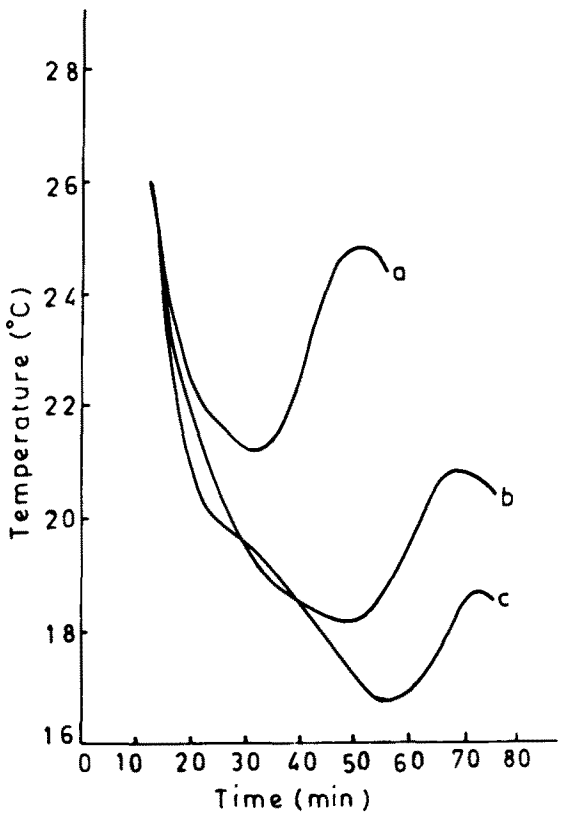

FIG. 1. Cooling curves of cocoa butter (b), and sal fat (c) type 1 and (a) type 2.

Dry Fractionation

Sal fat (200 g) was heated to about 55 C to obtain a clear liquid, then cooled to 35 C and held at this temperature for about 6 hr. Then it was filtered using a Buchner funnel, to obtain stearin (Fraction 5) and olein (yield 90%, Fraction 6).

Purification of Triglycerides of Sal Fat

Sal fat triglycerides, free from partial glycerides, free fatty acids and other polar components and triglycerides containing epoxy and 9,10-dihydroxystearic acids, were obtained by the following procedure:

To the fat (200 g) dissolved in hexane (400 ml), 100 g of silica gel (100-200 mesh, activated at 110 C for 3 hr) was added; the mixture was stirred with a magnetic stirrer for 2 hr and filtered. The silica gel residue was washed twice with 25 ml hexane. The washings and the filtrate were pooled, and the solvent was removed in a rotary flash evaporator under reduced pressure to obtain a normal triglycerides (TGs) fraction (Fraction 7, yield 90%). The purity of the TGs was checked by TLC.

Isolation of Triglycerides Containing 9,10-Dihydroxystearic Acid

The triglycerides containing 9,10-dihydroxystearic acid (DHS-TGs) were isolated from the polar components of sal fat adsorbed on silica gel. The silica gel residue was shaken with 3 × 100 ml of a mixture of chloroform/methanol (3:1) and filtered. The extract after desolventizing in a rotary flash evaporator (20 g) was put in 50 ml of petroleum ether (60-80 C) and warmed on a waterbath until dissolved. The solution was allowed to stand at ambient temperature (24-26 C) for about 2 hr, and the separated crystals (Fraction 8) were removed by filtration and washed with petroleum ether. The yield of Fraction 8 was 4.8 g. The purity of the fraction was checked by TLC. The flow diagram of the procedure used for isolation of DHS-TGs is given in Figure 3.

Preparation of Fatty Acid Methyl Esters

The samples were saponified with 1N alcoholic KOH, the unsaponifiable matter was removed by extracting with diethyl ether, neutralized with 1N HCl, and the liberated fatty acids were extracted with diethyl ether and converted into methyl esters using diazomethane (1).

Isolation of Methyl 9,10-Dihydroxystearate from Mixed Methyl Esters of Fatty Acids

The mixed fatty acid methyl esters (8 g) of fraction 8 were dissolved in 30 ml petroleum ether (60-80 C) by warming on a waterbath. The solution was allowed to stand at ambient temperature (24-26 C) for about 2 hr. The separated

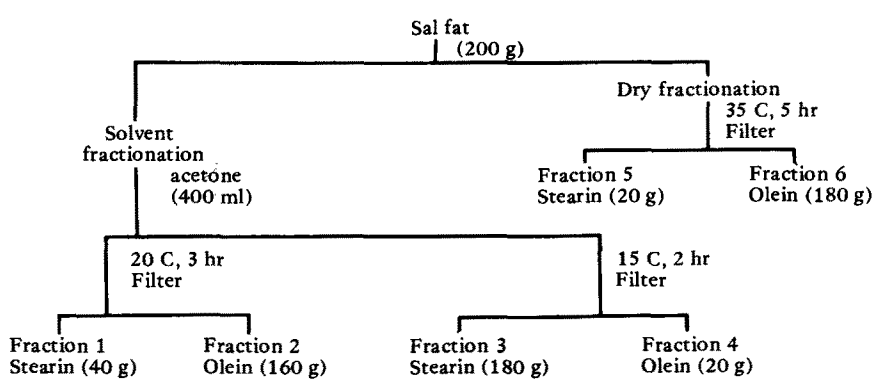

FIG. 2. Flow diagram of solvent and dry fractionation of sal fat.

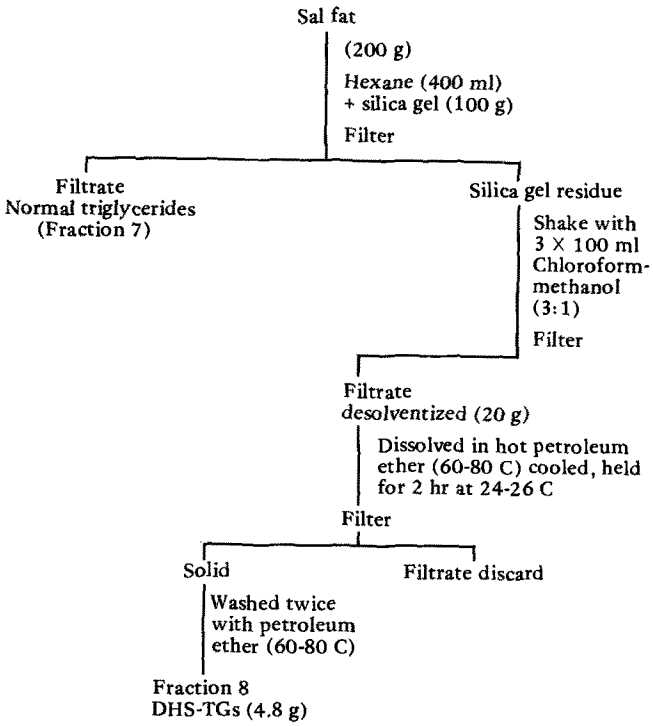

FIG. 3. Flow diagram for isolation of DHS-TGs.

crystals (Fraction 9) were removed by filtration, washed with petroleum ether and recrystallized from the same solvent (yield, 1 g). The purity of these crystals was checked by TLC and GLC.

Thin Layer Chromatography

The DHS-TGs and other minor constituents present in sal fat and its fractions were estimated by TLC-densitometry. The chloroform-methanol extract of the polar components recovered from silica gel residue was dried under reduced pressure and dissolved in 3 ml of chloroform. 5-10 μl of this solution was quantitatively spotted on pre-activated (at 110 C for 1 hr) 300 μ-thick silica gel G plates (20 x 20 cm). The plates were developed using a petroleum ether/diethyl ether/acetic acid (60:40:1, v/v/v) solvent system. The spots were visualized by spraying the plates with 50% H_2SO_4 followed by charring at 160 C for 3 hr. The chromatoplates were scanned using a Camag T Scanner, equipped with lamp 110-852, primary filter B(7-60), secondary filter 110-823, density 10%, and range selector at 3. The relative percentage of individual spots was calculated from the peak area.

Preparation of TMS Derivatives

The trimethylsilyl (TMS) derivatives of Fraction 9 and of authentic methyl 9,10-dihydroxystearate were prepared according to the procedure described by Belwadi et al (2).

About 2-3 mg of the sample was dissolved in 0.2 ml of dry pyridine in a 10 ml stoppered flask; 0.05 ml of hexamethyl disilane and 0.01 ml of chlorotrimethyl silane were added and incubated at 40 C for 1 hr. The silyl ethers were injected directly into the GLC.

Gas Liquid Chromatography

A CIC gas chromatograph equipped with a flame ionization detector operating under the following conditions was used for the analyses of the fatty acid methyl esters and TMS derivatives of methyl 9,10-dihydroxystearate: Column, 2.4 M x 0.3 CM SS column packed with chromosorb W (60-80 mesh) coated with 15% diethylene glycol succinate; column temperature 180 C isothermal; carrier gas, nitrogen 15 ml/min and hydrogen 20 ml/min. The peaks were identified by comparing the retention times with those of authentic samples. The area of the peaks was measured by triangulation, and the results were expressed as proportional wt% of the components in the sample.

Infrared (IR) Spectra

The potassium bromide pellet technique was used to record IR spectra of Fraction 9 and authentic methyl 9,10-dihydroxystearate on a Jasco spectrometer.

Cooling Curves

Cooling curves of sal fat and its fractions were obtained using Shukoff's flask on 25 g sample following the procedure described by Wilton and Wode (3). This procedure was preferred to Jensen's method, as it was sensitive enough to detect even small differences among samples. In the interpretation of the cooling curves the following terminology was used.

Supercooling capacity. A fat with a high minimum temperature of perhaps 21.1 C (Fig. 1a) on the cooling curve prior to a rise in temperature has a low or reduced supercooling capacity compared to a fat with a lower minimum temperature, say 16.7 (Fig. 1c).

Dilatometry

Dilatation studies were carried out according to the procedure given in British Standard Method 2 (4).

RESULTS AND DISCUSSION

Effect of Fractionation on the Solidification Properties of Sal Fat

The cooling behavior of two types of refined and bleached sal fat is given in Figure 1. Sal fat Type 1 had a higher supercooling capacity (Fig. 1c) than cocoa butter and Type 2 sal fat. Such fats cause delays and need increased cooling. Type 2 sal fat had a lower supercooling capacity (Fig. 1a) than cocoa butter. It is known that such fats with low supercooling capacity have a tendency to separate from the melt early (5). Hence, both types of sal fat required fractionation prior to use.

Important to the present study was the fact that refined and bleached sal fats having batch to batch variations in their cooling behavior could not be fractionated to a consistently uniform product using a single set of fractionation conditions. The following discussion will, therefore, be limited to the assessment of factors affecting the cooling behavior of sal fat.

When sal fat (Type 2) was solvent fractionated at 20 C, as much as 20% of stearin was removed (Fig. 2). The solid fat content (SFC) of the olein fraction (Fraction 2), as expected, decreased at all temperatures (Table II), but its cooling behavior was not appreciably different from that of the original fat (Fig. 4). On the other hand, when the fat was subjected to dry fractionation at 35 C, removing in the process about 10% of the stearin, there was a remarkable improvement in the supercooling capacity of the olein fraction (Fraction 6) compared to the original fat (Fig. 4). However, the SFC of Fraction 6 (Table II) was not affected, probably because dry fractionated stearin always contains considerable occluded olein and the amount of the harder fraction removed constitutes only a small portion of the stearin. Also, during dry fractionation a considerable amount of DHS-TGs, which lower the SFC of the fat (6), were removed in the stearin fraction (Fraction 5, Table I).

TABLE I

Minor Components and Normal Triglycerides Content of Sal Fat and its Fractions[a]

Fraction no.	Sample	DHS-TGs	DGS	FFA	Unidentified[b]	TGs
	Sal fat	3.4	2.7	0.5	2.0	91.4
1.	Stearin, solvent fractionated at 20 C	2.6	2.3	1.3	1.9	92.0
2.	Olein, solvent fractionated at 20 C	3.6	4.8	1.2	–	90.0
3.	Stearin, solvent fractionated at 15 C	2.3	1.6	1.2	1.8	93.0
4.	Olein, solvent fractionated at 15 C	3.0	5.7	0.8	2.0	88.5
5.	Stearin, dry fractionated at 35 C	30.2	19.7	–	6.5	43.6
6.	Olein, dry fractionated at 35 C	1.6	2.4	0.8	1.8	93.4
7.	Normal triglycerides	–	–	–	–	100.0
8.	Purified DHS-TGs	100	–	–	–	–

[a]Values are relative percentages.

[b]Probably triglycerides containing epoxystearic acid.

This probably resulted in improvement of the SFC of the olein fraction.

When sal fat was solvent fractionated at 15 C instead of 20 C, 90% of it separated out as stearin (Fig. 2). This fraction (Fraction 3), containing all the high melting glycerides, had a higher SFC than the original fat but showed considerable supercooling capacity (Fig. 4) compared to both the original fat and Fraction 2. From this it was inferred that the high-melting trisaturated glycerides present in Type 2 sal fat perhaps were not entirely responsible for the low supercooling capacity. The fact that removal of as little as 10% olein by low temperature solvent fractionation (Fig. 2) improved supercooling capacity indicated that the components responsible for reducing the supercooling capacity of the fat were soluble in acetone. Also, improvement in the supercooling capacity after removal of 10% of the stearin by dry fractionation revealed that the component(s) responsible for reducing sal fat's supercooling capacity, besides being acetone soluble, had high melting points.

Isolation of Components Affecting Supercooling Capacity of Sal Fat

To find out the component(s) affecting the supercooling capacity of sal fat, dry fractionated stearin (Fraction 5, Fig. 2) was subjected to TLC. This showed the presence of diglycerides (DGs), TGs, one spot at Rf 0.70 (probably triglycerides containing epoxystearic acid) and one dense spot at Rf 0.26 just below DGs. Fraction 5 (20 g) was dissolved in 40 ml of hot petroleum ether (60-80 C) and allowed to stand at ambient temperature (24-26 C) for about 2 hr. Then it was filtered, and the crystals were washed with petroleum ether (yield 4 g). TLC of the crystals showed only one dense spot at Rf 0.26 similar to that of Fraction 8 isolated from the polar fraction of sal fat. The fatty acids obtained after saponification of Fraction 8 were dissolved in hot diethyl ether and allowed to stand at ambient temperature for about 10 min. The separated crystals were removed by filtration and washed with ether. The melting point of the crystals was 93 C, identical to that for DL-threo 9,10-dihydroxystearic acid.

TABLE II

Solid Fat Content of Sal Fat and its Fractions

Fraction no.	Sample	% solids at ° C				
		20	30	32.5	35	37
	Sal fat	73.4	64.0	50.0	18.8	1.4
2	Olein, solvent fractionated at 20 C	65.2	55.2	39.4	8.4	1.0
3	Stearin, solvent fractionated at 15 C	76.0	74.0	59.0	21.0	2.0
6	Olein, dry fractionated at 35 C	73.0	64.4	50.8	22.2	1.0
7	Normal triglycerides	75.0	67.0	53.0	19.6	–

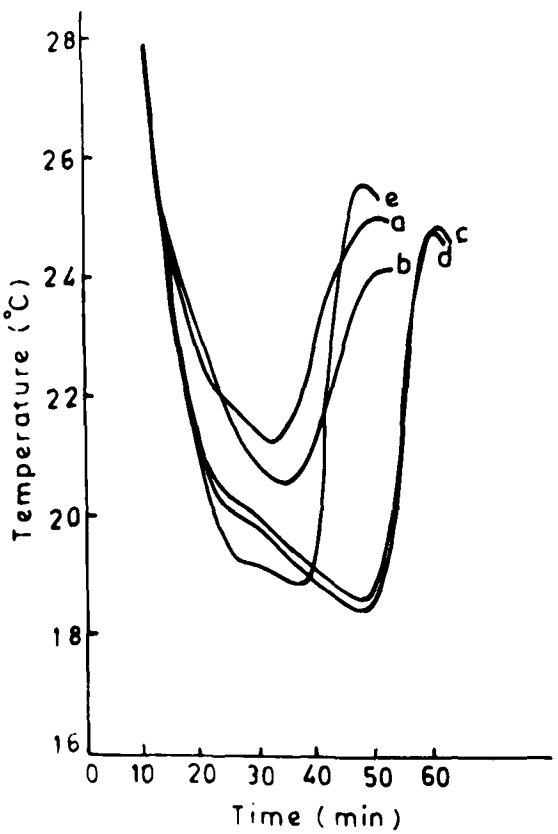

FIG. 4. Cooling curves of (a) sal fat (type 2); and its fractions– (b) solvent fractionated olein at 20 C (Fraction 2); (c) solvent fractionated stearin at 15 C (Fraction 3); (d) dry fractionated olein at 35 C (Fraction 6), and (e) normal triglycerides (Fraction 7).

TLC of the fatty acid methyl esters of Fraction 8 showed two spots, one corresponding to those for methyl esters of normal fatty acids and the other corresponding to that for authentic methyl 9,10-dihydroxystearate. The fatty acid methyl esters of Fraction 8 were dissolved in hot petroleum ether and allowed to stand at ambient temperature for 2 hr. The separated crystals were removed by filtration, washed with petroleum ether and recrystallized from the same solvent (Fraction 9). TLC of Fraction 9 showed only one spot corresponding to that for authentic methyl 9,10-dihydroxystearate. The identity of Fraction 9 was further established by co-chromatography, melting point, GLC of silyl ethers and comparison of IR spectra with that of authentic methyl 9,10-dihydroxystearate.

GLC of TMS derivatives of fatty acid methyl esters of Fraction 8 showed the presence of stearic (57.5%), palmitic (6.0%), arachidic (5.8%) and 9,10-dihydroxystearic (30.5%) acids. This indicated that Fraction 8 separated from sal fat by silica gel adsorption or from Fraction 5 consisted of triglycerides containing 9,10-dihydroxystearic acid and straight chain saturated fatty acids. Triglycerides containing 9,10-epoxy and 9,10-dihydroxystearic acids are known to be present in sal fat to the extent of 6-12% (2,7).

Distribution of DHS-TGs between Stearin and Olein Fractions

The data in Table I show that the content of the DHS-TGs in the olein fraction (Fraction 2) separated by acetone fractionation at 20 C was similar to that of the original fat, whereas their content in the olein fraction (Fraction 6) separated by dry fractionation and also in the stearin fraction (Fraction 3) separated by acetone fractionation at 15 C were much lower. The data also show that diglyceride content increased slightly in Fraction 2 and decreased slightly in Fractions 3 and 6, compared to the original fat. There was not much difference in the content of other minor constituents of the original fat and its fractions. This indicated that the reduced supercooling property of sal fat or some of its fractions probably was due to the presence of DHS-TGs.

Effect of DHS-TGs on Solidification Properties of Sal Fat

The effect of DHS-TGs on solidification properties of sal fat TGs was studied by mixing the DHS-TGs (Fraction 8) with the purified normal triglycerides (Fraction 7) and determining the cooling characteristics. Figure 5 shows that DHS-TGs reduced the supercooling property of sal fat TGs even at the 2% level. Also, the inflection point or the formation of α-crystals (3.8) diminished with an increase in the level of DHS-TGs (Fig. 5).

DHS-TGs probably had the effect we observed on the supercooling capacity of sal fat because they crystallized out first from the melt, due to their high melting nature, and the crystals acted as nuclei for the supercooled fat, resulting in its early crystallization.

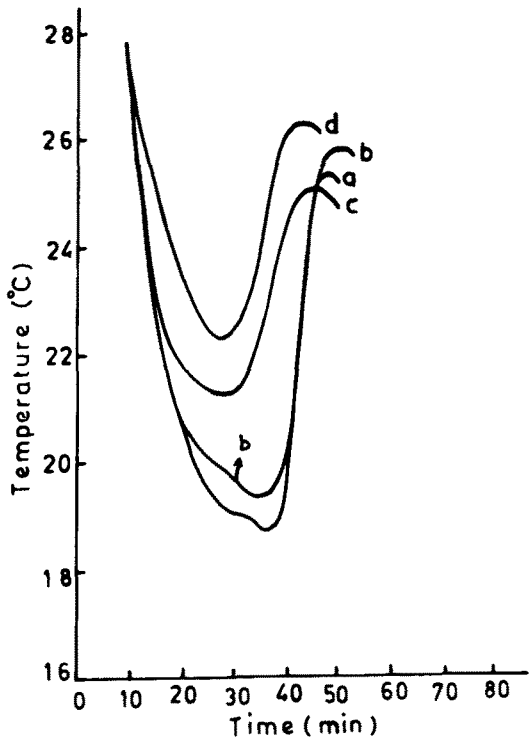

FIG. 5. Cooling curves of (a) normal triglycerides of sal fat, and (b), (c) and (d) with added 9,10-dihydroxystearic acid containing triglycerides at 1,2 and 3%, respectively.

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