ent, which is an interesting feature of cyanolipid-containing sapindaceous seed oils. Argentation TLC of the methyl esters prepared from cyanolipid I showed a high percentage of C_{20} monoene. Such an observation was not made in the case of cyanolipid III.

GLC analyses of methyl esters derived from *C. anacardioides* show the following fatty acid composition: 11.7 (16:0), 8.2 (16:1), 6.2 (18:0), 9.6 (18:1), 15.6 (18:2), 2.0 (20:0) and 46.0 (20:1).

ACKNOWLEDGMENTS

M. S. Ahmad provided necessary facilities for this work, and CSIR, New Delhi, gave financial support to J. Mustafa and A. Gupta.

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[Received September 23, 1985]

Study on the Polymorphism of Normal Triglycerides of Sal *(Shorea robusta)* Fat by DSC. I. Effect of Diglycerides

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The effect of diglycerides (DG) on the phase transition of various polymorphic forms of normal triglycerides (TG) of sal fat was investigated by differential scanning calorimetry. Three levels of DG, 5, 10 and 15%, were used. DG delayed the phase transition of lower melting crystal forms to higher forms of TG when the samples were brought to a congealed state by rapid cooling (20 C/min) and heated at rates ranging from 1.25 to 10 C/min; the extent depended on the level of DG and the rate of heating. As the level of DG and the rate of heating increased, the delay in phase transition of crystal forms I → II → III was more pronounced. The phase transition of crystal forms I, II and III to form IV was delayed at 5 and 10% levels of DG, while at the 15% level the phase transition of form I to higher forms was completely stopped when the samples were tempered at 0 C for 18 hr and heated at 10 C/min. DG at 10 and 15% levels retarded the phase transition of form IV to the most stable (V) form of TG when the samples were tempered at 0 C for 1 hr followed by 3 hr at 26 C.

Sal *(Shorea robusta)* belongs to the family Dipterocarpaceae as does *Shorea stenoptera*, the source of the commonly known borneo tallow. It is found mostly in Northeast and Central India. The winged seeds contain dark green kernels (72%) which yield 13–15% of dark greenish hard fat (1). Sal fat occupies an important position in its contribution to the total potential of fats of tree origin with an estimated potential of about 700,000 tons (2). Two-thirds of sal fat triglycerides comprise the symmetrical, monounsaturated, disaturated (GS_2U) type, mostly (about 50%) 2-oleodistearin (3). For this reason, and due to its attractive price, the demand for sal fat has been increasing steadily for use in cocoa butter extenders and confectionery fat formulations.

One major problem with commercial refined and bleached sal fats is inconsistency in their solidification properties. This presents problems in obtaining consistently uniform fat fractions from different lots of sal fats using a set of fractionation conditions (unpublished data). Certain minor components (Table 1) like diglycerides (DG) and triglycerides containing 9,10-dihydroxystearic acid (DHS-TG) have been found to be responsible for this inconsistent behavior of sal fat. The DHS-TG have been shown to affect the solidification properties of sal fat by accelerating the onset of crystallization and reducing the supercooling capacity (4). The present paper describes the effect of the minor component-diglycerides on the polymorphic transition of sal fat as measured by differential scanning calorimetry (DSC).

TABLE 1

Minor Components and Normal Triglycerides of Two Sal Fat Samples[a]

Sample	DHS-TG	DG	FFA	Unidentified[b]	TG
Sal fat I	1.4	7.2	1.5	1.4	89.0
Sal fat II	3.3	2.2	0.3	2.0	92.0

[a]Values are relative percentages.
[b]Probably triglycerides containing epoxystearic acid (11).

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MATERIALS AND METHODS

Refined and bleached sal fat was procured from M/s K.N. Oil Industries, Mahasamund, M.P., India and from M/s Specialty Fats P. Ltd., Khamgaon, India. The normal triglycerides (TG) of sal fat were purified by silica gel adsorption technique as described earlier (4). To 100 g of sal fat dissolved in 200 ml of hexane, 50 g of silica gel (100–200 mesh, activated at 110 C for 3 hr) were added. The mixture was stirred for about 2 hr and filtered. The silica gel residue (A) was washed twice with 25 ml of hexane. The filtrate and the washings were pooled and desolventized to get pure normal TG. The purified TG did not contain any other components as revealed by TLC.

The diglycerides were isolated from the silica gel adsorbed material as follows: The silica gel residue (A) was shaken successively three times with 100 ml of chloroform/methanol (3:1) and filtered, and the pooled filtrate was desolventized to get an extract (B). About 15 g of this extract (B) was dissolved in chloroform (15 ml) and taken on a column (2.5 × 35 cm) of silica gel (110–200 mesh adjusted to 5% moisture), and the diglycerides were separated by a slight modification of the procedure described in AOAC (5).

First normal TG were removed by eluting with 200 ml of a mixture of petroleum ether/benzene (1:1). The DG were eluted with 400 ml benzene followed by 400 ml of 5% diethyl ether in benzene. The fractions containing DG were pooled and rechromatographed on another silica gel column to obtain pure DG. The purity of DG was checked by TLC.

DG consisted of 31% 1,2 and 69% 1,3 isomers and had 9.4% palmitic, 40.5% stearic, 1.7% arachidic and 48.5% oleic acids. The minor components in two sal fat samples were estimated by TLC-densitometry according to the procedure described earlier (4).

Differential scanning calorimetry (DSC). A Mettler TA-3000 differential scanning calorimeter (DSC) was used in this study. The heat flow of the instrument was calibrated using indium. The PT-100 sensor was calibrated using indium, zinc and lead. About 3 mg of each sample was weighed accurate to 0.1 mg into standard aluminum pans and the covers crimped in place. An empty aluminum pan with pierced lid was used as a reference. The sample and reference pans were placed in the DSC cell and, after holding at 60 C for 10 min to destroy all crystal nuclei, the thermograms were recorded under the following experimental conditions:

- Rapidly cooled sample: The sample was rapidly chilled to −30 C at 20 C/min to get the sample in a congealed state or in the least stable form. Then the sample was heated at 10 C/min and the thermogram recorded. Similarly, thermograms of rapidly chilled samples were recorded for heating rates of 5, 2.5 and 1.25 C/min.
- Sample tempered at 0 C: The sample was chilled to 0 C and held for 18 hr in a refrigerator, then quickly transferred to the DSC cell and the thermogram recorded at a heating rate of 10 C/min.
- Sample tempered at 0 C and 26 C: The sample was chilled to 0 C and held for 1 hr, then held at ambient temperature (26–28 C) for 3 hr and transferred to the DSC cell. The thermograms of the crystals generated were recorded at a heating rate of 10 C/min.

The enthalpies or heats of fusion (ΔH) of various crystalline forms were either recorded directly by the in-

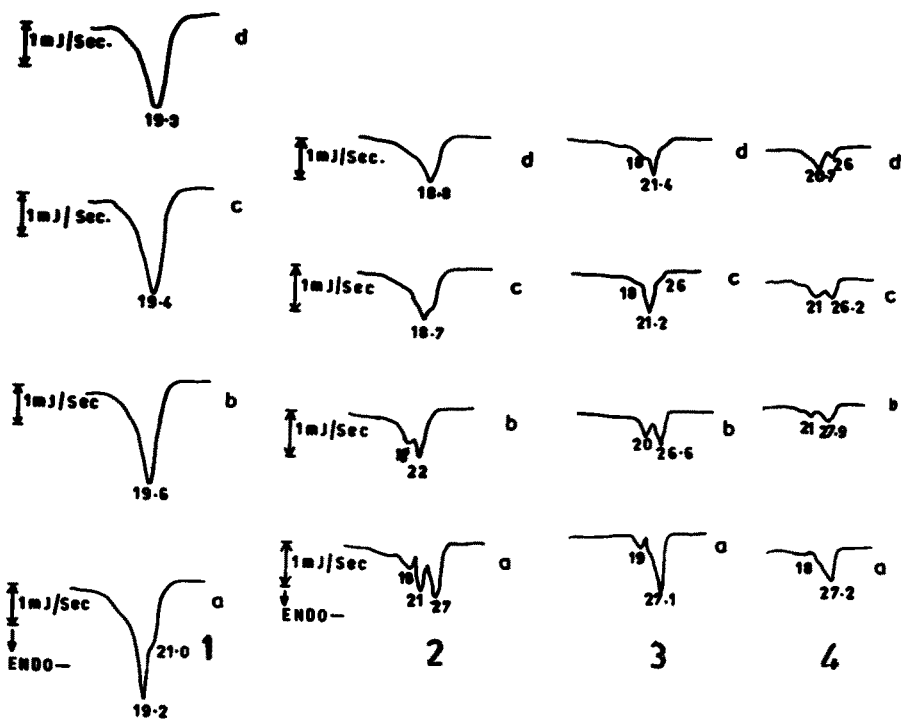

FIG 1. DSC heating curves of rapidly cooled (20 C/min) sal fat triglycerides (TG) and TG containing diglycerides (DG). Heating rates (I) 10 C/min; (II) 5 C/min; (III) 2.5 C/min, and (IV) 1.25 C/min. a, b, c, d, respectively, indicate the curves of TG and TG containing DG at 5, 10 and 15%.

strument or obtained by calculating the area under each curve.

The different crystal forms of sal fat TG were designated as I, II, III, IV, etc., with increasing order of melting temperature.

RESULTS AND DISCUSSION

Rapidly cooled samples. The fusion enthalpy curves of rapidly (20 C/min) cooled samples of TG and mixtures of TG and DG are shown in Figure 1. When the rapidly cooled TG were supplied heat at a high rate of 10 C/min, a fusion peak of crystal form I at 19.2 C with a small shoulder of form II at 21 C was observed (Fig. 1-Ia). When the rate of heating was reduced to 5 C/min, three fusion peaks at 18, 21 and 27 C corresponding to crystal forms I, II and III, respectively, were noticed (Fig. 1-IIa). At the heating rate of 2.5 C/min, the peak corresponding to form II disappeared and a major peak of form III at 27.1 C and a small peak of form I at 19 C were observed (Fig. 1-IIIa). With further lowering of the rate of heating to 1.25 C/min, a major peak of form III at 27.2 and a small hump of form I at 18 C were noticed (Fig. 1-IVa). These results indicated that when the rate of heat input was rapid, TG showed mostly the peak of form I. As the rate of heating was reduced the transition of forms I → II → III became rapid, and most of the crystals transformed to form III at the heating rate of 1.25 C/min.

In the presence of DG at 5, 10 and 15%, heating at the rate of 10 C/min resulted in a fusion peak corresponding to form I. There was no peak or shoulder corresponding to form II as observed in pure TG (Fig. 1-I). When the rate of heating was reduced to 5 C/min, the effect of DG on the phase transition of forms I → II → III was clearly seen; the extent depended on the level of DG (Fig. 1-II). At the 5% level, the peak of form I was small, the peak corresponding to form II was predominant, and there was no peak of form III as observed in TG (Fig. 1-IIb and Table 2). At the 10% level, the form I at 18.7 C was predominant, while there was only a shoulder corresponding to form II at 21 C and the peak of form III was totally absent (Fig. 1-IIc). At the 15% level, only one peak corresponding to form I at 18.8 C was noticed, and there were no peaks or shoulders of forms II or III (Fig. 1-IId). This was unlike the TG. When the rate of heating was reduced to 2.5 C/min, DG at the 5% level showed two peaks corresponding to forms II and III (Fig. 1-IIIb). At 10 and 15% levels of DG, a major peak of form II with small shoulders of forms I and III was observed (Fig. 1-IIIc and d).

When the rate of heating was further reduced to 1.25 C/min, addition of DG at 5 and 10% levels showed two peaks corresponding to forms II and III with a small hump of form I (Fig. 1-IVb and c), while at the 15% level the peak of form II was predominant and that of form III was small (Fig. 1-IVd and Table 2). Thus, the peak

TABLE 2

Changes in Heats of Fusion (ΔH) of Polymorphic Forms of Normal Triglycerides of Sal Fat after Addition of Diglycerides

	Rates of heating C/min															
	Rapidly cooled samples											Sample tempered at 0 C 18 hr			Tempered at 0 C 1 hr followed by 25 C 3 hr	
	10		5			2.5			1.25			10			10	
	I	II	I	II	III	I	II	III	I	II	III	I	III	IV	IV	V
Normal triglycerides (TG)	19.2	21.0[a]	18.0	21.0	27.0	19.0	—	27.1	18.0	—	27.2	18.0[a]	27.1	32.8	—	35.0
Peak Temp. C																
ΔH (J/g)	53.0	—	7.8	17.4	30.8	5.6	—	57.3	3.4	—	80.6	—	24.0	32.0	—	111.0
TG + 5% DG																
Peak Temp. C	19.6		18.0	22.0	—	—	20.0	26.6	18.0	21.3	27.9	19.0[a]	26.8	32.0	31.0[a]	34.8
ΔH (J/g)	49.0		16.0	39.0	—	—	16.4	34.0	3.6	14.2	33.7	—	33.2	6.24	—	65.5
TG + 10% DG																
Peak Temp. C	19.4		18.7	21.0[a]	—	18.0[a]	21.2	26.0[a]	18.0[a]	21.0	26.2	17.8[b]	27.0	31.0	30.5	—
ΔH (J/g)	47.6		46.7	—	—	—	44.5	—	—	29.4	34.5	75.0	26.0	—	36.0	—
TG + 15% DG																
Peak Temp. C	19.3		18.8	—	—	18.0[a]	21.4	—	—	20.7	26.0	19.2	—	—	29.9	—
ΔH (J/g)	48.4		52.5	—	—	—	38.2	—	—	41.7	6.0	45.0	—	—	31.0	—

[a]Only humps.

[b]Also a small hump of form II at 21 C.

J/g, Joules/gram.

corresponding to form II, which was not observed in TG, became more and more predominant, and that of form III became less distinct with the increase in DG levels (Fig. 1-III and IV). In this way DG delayed the phase transition of forms I → II → III of rapidly cooled TG, the effect being pronounced both with the increase of the heating rate and the increase in DG levels.

Sample tempered at 0 C for 18 hr. The heating curves of samples tempered at 0 C for 18 hr are shown in Figure 2. TG showed a small peak of form I and two major peaks corresponding to forms III and IV, the latter being dominant (Fig. 2a and Table 2). The appearance of form IV, which was not observed in the heating curves of rapidly cooled TG, indicated that the transformation to form IV occurred even at supercooled (0 C) temperature. On tempering of TG after addition of DG at 5%, two major peaks of forms III and IV and a small peak of form I were observed as with pure TGs, but the dominant peak was that of form III and not of form IV, observed in pure TG (Fig. 2b and Table 2). Increasing the level of DG to 10% resulted in dominance of form I at 17.8 C, while the peaks corresponding to forms III and IV were very small, unlike at the lower level of DG (Fig. 2c). With a further increase in DG concentration to the 15% level, only one peak corresponding to form I at 19.2 C was noticed, and the peaks of forms II, III or IV were conspicuously absent (Fig. 2d). These results indicated that the DG delayed the phase transition of forms I → II → III → IV of TG of sal fat at supercooled temperature (0 C), and the effect increased with the level of DG.

Samples Tempered at 0 C for 1 hr following 3 hr at 26 C. Thermograms of the samples tempered at 0 C for 1 hr following 3 hr at 26 C recorded using a heating rate of 10 C/min showed that TG were completely transformed to the stable crystal form (form V) as shown by the sharp single peak at 35 C (Fig. 3a). With DG at the 5% level, a major peak of form V at 35 C showed up with a shoulder of form IV at 30 C indicating incomplete transition of form IV to V (Fig. 3b). When the concentration of DG was increased to 10 or 15%, there was only one peak of form IV, and the peak corresponding to form V was totally absent (Fig. 3c and d), showing the inhibitory effect of DG on the phase transition of form IV to V.

DG are known to delay the $\alpha \rightarrow \beta'$-phase transition in

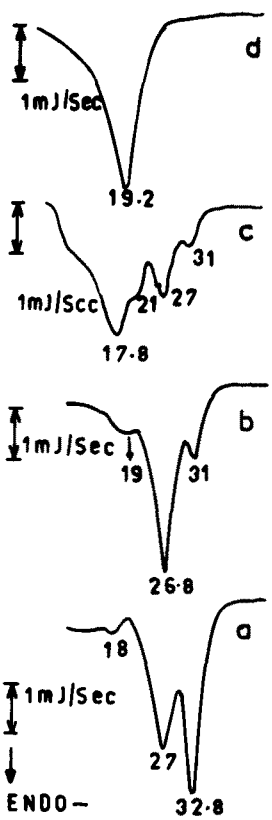

FIG. 2. DSC heating curves (10 C/min) of samples tempered at 0 C for 18 hr (a) TG; b, c and d are of TG containing DG at 5, 10 and 15%, respectively.

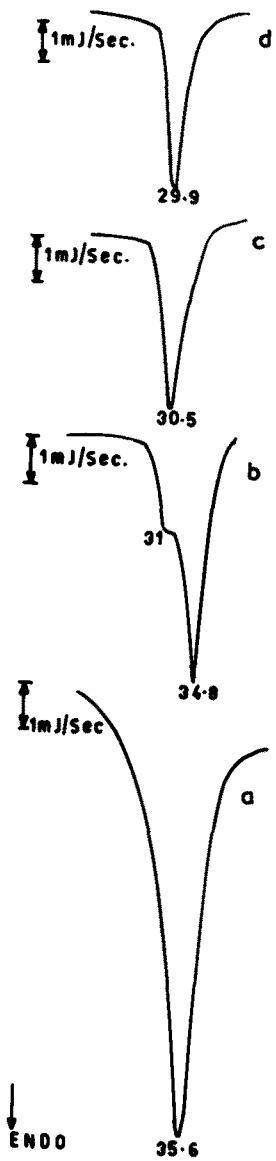

FIG. 3. DSC heating curves (10 C/min) of samples tempered at 0 C for 1 hr followed by 3 hr at 26 C (a) TG; b, c and d are of TG containing DG at 5, 10 and 15%, respectively.

palm oil (6-8) and also found to stabilize β′-form in hydrogenated rapeseed oil (9,10). The literature on their effect in other fats, especially confectionery hard butters, is scanty.

The above results show that diglycerides of sal fat either inhibited or delayed the phase transition of all the crystal forms of TG from their lower melting crystal forms to the next higher melting forms. The delay in phase transition and stability of lower melting crystal forms was more pronounced with the increase in DG level from 5 to 15%. The effect of DG in delaying the phase transition of forms I → II → III was more pronounced at higher rates of heating. DG at 5 and 10% levels also were found to delay the solid-solid transition of lower melting crystal forms (I, II, III) to higher melting crystal form IV at supercooled temperatures (0 C), while at the 15% level, they virtually inhibited the phase transition of form I to higher polymorphs of TG. DG also delayed the transition of form IV to V even after tempering. DG, therefore, could have a beneficial effect in producing a smooth consistency in margarine, whereas they have a somewhat deleterious effect in the manufacture of chocolate because they affect the quality of the product.

ACKNOWLEDGMENT

B. L. Amla, Director of the Central Food Technological Research Institute, has shown keen interest in this work.

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[Received March 20, 1985]

Radiolysis of Lipids in Monolayers. I. Saturated Fatty Acids

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In order to study the effect of molecular orientation on the behavior of lipids when exposed to high energy radiation, model systems of palmitic acid or ethyl palmitate adsorbed as monolayers on silica were irradiated with ^{60}Co at 25 Mrad under vacuum, and the volatile products compared with those of control samples irradiated in bulk. Major quantitative differences were observed. More of the C_{n-1} alkane relative to the shorter-chain members of the homologous series were formed in bulk samples as compared to samples in monolayer. The C_{n-2} alkene and C_n aldehyde also were formed in greater quantities in bulk. These observations are explained on the basis of a reduced preferential cleavage near the carbonyl group and a restricted mobility of free radical intermediates, in the case of the monolayers.

The effects of irradiation on fatty acids, esters, triacylglycerols, natural fats and fat-containing foods have been reported earlier (1-4). General mechanisms were deduced largely from irradiation of bulk lipids in the liquid phase where the molecules are much less organized than in the solid state or in biological membranes. It has been proposed that the primary event in the radiolysis of oxygen-containing compounds is the loss of a non-bonding electron from an oxygen atom, with the result that the unpaired electron is highly localized on the oxygen atom (5). The products which arise from irradiation of the lipids in complex foods are qualitatively similar to those formed from the irradiation of natural fats in bulk (6).

In biological systems, the lipid molecules usually exist with a high degree of order as, for example, the bilayer arrangement in cell membranes. The question arises whether products resulting from irradiation of bulk liquids differ qualitatively or quantitatively from those in the ordered state. An understanding of the specific mechanisms by which these differences may arise would be necessary in the extrapolation of results from one situation to another. Since the study of ordered lipid molecules as they exist in biological membranes is extremely complex, pure fatty acid and fatty acid esters adsorbed on silica were used as a model system to investigate the effect of molecular orientation on the radiolysis of lipids. The nature of adsorption of lipid molecules on silica has been reported in an earlier publication (7).

MATERIALS AND METHODS

Materials. The substrates palmitic acid and ethyl palmitate were purchased in the highest available purity from Sigma Chemical Co., St. Louis, Missouri. These were used without further purification. Silica gel G was purchased from Applied Science Laboratories, State College, Pennsylvania. It had a particle size of 10-40 μ.

Preparation of monolayers. Lipid monolayers were prepared according to the procedures described by Porter and coworkers (8), with minor modifications. More than

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