

# Effect of Triglycerides Containing 9,10-dihydroxystearic Acid on Polymorphism of Sal (*Shorea robusta*) Fat

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The effect of the triglycerides containing 9,10-dihydroxystearic acid (DHS-TG) on the phase transition of polymorphic forms of normal triglycerides (TG) of sal fat was investigated by differential scanning calorimetry (DSC) under different cooling and heating modes. Four levels of DHS-TG, 2, 5, 8 and 10%, were used. DHS-TG accelerated the phase transition of lower melting crystal forms I → II → III of TG obtained under rapid (20°C/min) or slow (2°C/min) rates of cooling. They delayed the phase transition of crystal form III to IV of TG at 0°C. Also, DHS-TG reduced the heat of fusion ( $\Delta H$ ) of the stable form (V) of TG obtained after tempering at 0°C and at 26°C.

Sal (*Shorea robusta*) fat comprising mostly 2-oleodisaturated triglycerides has good potential for use in chocolate and confectionery. One major problem with commercial sal fats is their inconsistency in solidification properties. The solidification properties of commercial refined and bleached sal fat have been found to be affected by components such as diglycerides (DG) and the triglycerides containing 9,10-dihydroxystearic acid (DHS-TG) (1,2). Besides, these components also affect fractionation of the fat during preparation of cocoa butter extenders (unpublished data). Therefore, the effect of these components on the polymorphic phase transition of various crystal forms of purified normal triglycerides (TG) of sal fat is of interest. In an earlier communication (3), the effect of DG on the phase transition of various crystal forms of TG was reported. The present paper describes the effect of DHS-TG on the phase transition of various crystal forms of sal fat TG as measured by differential scanning calorimetry (DSC).

## MATERIALS AND METHODS

Refined and bleached sal fat was procured from M/s Specialty Fats P. Ltd., Khamgaon, India.

The normal TG of sal fat were purified by silica gel adsorption, and DHS-TG were isolated by the same procedure as described earlier (1).

The fat solution in hexane was treated with silica gel (100–200 mesh) and filtered. The filtrate was desolventized to get pure normal TG of sal fat. The silica gel residue was treated with chloroform/methanol (3:1, v/v) and filtered. The extract after desolventizing was dissolved in hot petroleum ether (60–80) and held at ambient temperature (25°C) for about one hr. The separated crystals were removed by filtration and washed with petroleum ether to obtain pure DHS-TG. The purities of TG and DHS-TG were checked by TLC. DHS-TG had a m.p. of 72°C and consisted of 30.5% DHS, 59.5% stearic acid, 6.0% palmitic acid and 5.8% arachidic acid (4). DHS-TG were mixed with TG at 2, 5, 8 and 10% levels.

## Differential Scanning Calorimetry (DSC)

A Mettler TA-3000 system was used in the study. The heat flow of the instrument was calibrated using indium, zinc and lead. About 3 mg of sample was accurately weighed into a standard aluminum pan and the cover crimped in place. An empty aluminum pan with a pierced lid was used as a reference.

The thermograms were obtained under the same experimental conditions as described earlier (3). After holding the sample at 60°C for 10 min to destroy all crystal nuclei, the thermograms were recorded under conditions of rapid cooling, slow cooling, tempering at 0°C and tempering at 0°C and 26°C.

**Rapid cooling.** To find out the effect of DHS-TG on the formation and rate of transition of the least stable crystal form of sal fat TG, the samples were cooled rapidly at 20°C/min to –30°C. The crystals thus generated were heated immediately at rates of 10, 5, 2.5 and 1.25°C/min successively to obtain fusion enthalpy curves.

**Slow cooling.** Slow cooling of fat usually results in the formation of higher stable crystal forms than rapid cooling. Hence, the samples were cooled slowly at 2°C/min to –30°C to assess the effect of DHS-TG on phase transition of the higher melting crystal forms of TG; the fusion enthalpy curves of the crystals generated were recorded at a heating rate of 10°C/min.

**Tempering at 0°C.** To study the effect of DHS-TG on phase transition of various crystal forms in the solid state, the samples were cooled to 0°C and held at 0°C for 18 hr. The samples were transferred immediately into the DSC cell and heated at 10°C/min to obtain enthalpy curves of the crystals generated.

**Tempering at 0°C and 26°C.** To assess the effect of DHS-TG on the formation of the most stable crystal form of TG, the samples were chilled to 0°C and held at 0°C for one hr and then at 26°C for three hr. The samples were then transferred to the DSC cell and heated at 10°C/min, to record the heating curves of the crystals generated.

The heats of fusion ( $\Delta H$ ) of various crystal forms were recorded directly by means of a Mettler TC-10 data processor. The nomenclature, I, II, III, IV, V, was used to denote the crystal forms of sal fat TG with increasing melting points.

## RESULTS AND DISCUSSION

The effect of DHS-TG on phase transition of various crystal forms of sal fat TG was studied under different experimental conditions.

**Rapid cooling.** The fusion enthalpy curves of rapidly (20°C/min) cooled (to –30°C) samples of sal fat TG and their mixtures with DHS-TG obtained at different heating rates are shown in Figures 1. The samples showed peaks corresponding to different crystal forms depending on the rate of heating. At a high

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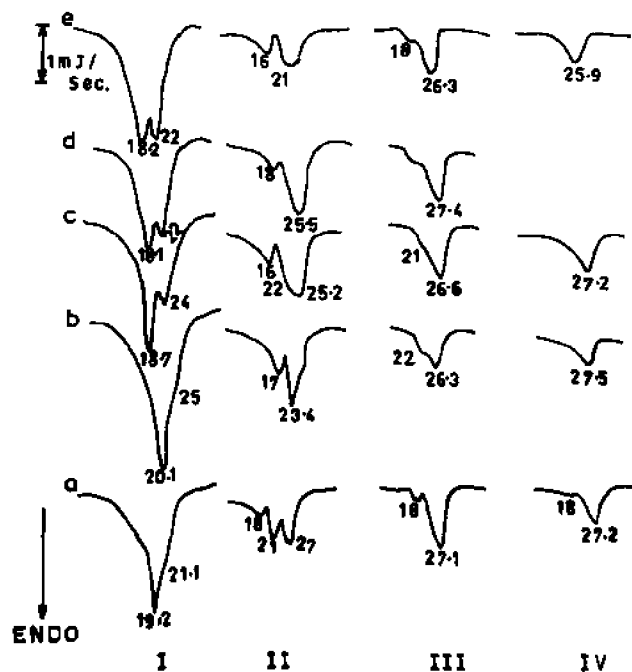


FIG. 1. DSC fusion enthalpy curves of rapidly cooled (20°C/min) sal fat triglycerides (TG) and TG containing DHS-TG. Heating rates: I, 10°C/min; II, 5°C/min; III, 2.5°C/min, and IV, 1.25°C/min. a, b, c, d, e, respectively, indicate the curves of TG and TG containing DHS-TG at 2, 5, 8 and 10% levels.

heating rate of 10°C/min, the crystals of TG were mostly in polymorphic form I, as indicated by a peak at 19.2°C, with only a small shoulder at 21°C corresponding to

form II (Fig. 1-1a). Reduction in the rate of heating resulted in phase transition of form I to higher melting crystal forms. Form II that showed up only as a shoulder and form III that was absent at a heating rate of 10°C/min, were prominent (Fig. 1-11a) at a heating rate of 5°C/min. With further lowering of the rate of heating to 2.5 and 1.25°C/min, a major peak of form III with only a small peak of form I were observed (Fig. 1-IIIa, and Table 1). These results indicated that as the rate of heating was reduced there was a rapid transition of crystal forms I → II → III and at low heating rates of 2.5 or 1.25°C/min, the transformation to form III of TG was almost complete. When DHS-TG were added to TG prior to rapid cooling, the phase transition of the above three crystal forms of TG varied depending on the level of DHS-TG and on the rates of heating. At low heating rates of 2.5 and 1.25°C/min, the addition of DHS-TG had no significant effect on the phase transition of crystal forms I → II → III and to higher forms of TG (Fig. 1-III and IV).

Thus, at a heating rate of 2.5°C/min, when DHS-TG were added at 2 and 5% levels, a major peak of form III with a shoulder corresponding to form II was observed, and when the level of DHS-TG increased to 8% and 10%, the peaks obtained were similar to those obtained for pure TG (Fig. 1-III and Table 1). Also, at a heating rate of 1.25°C/min, the peaks obtained after addition of DHS-TG were similar to those obtained for pure TG (Fig. 1-IV). On the other hand, as the rate of heating was increased to 5°C/min and 10°C/min, the addition of DHS-TG accelerated the phase transition of forms I → II → III of TG, the effect increasing with the level of DHS-TG (Fig. 1-I and II). At the heating rate of 10°C/min the peak of form II,

TABLE 1.

Effect of Triglycerides Containing Dihydroxystearic Acid (DHS-TG) on Heats of Fusion ( $\Delta H$ ) of Polymorphic Forms of Normal Triglycerides (TG) of Sal Fat<sup>a</sup>

Polymorphs	Rate of heating (C/min)										
	10		5			2.5			1.25		
	I	II	I	II	III	I	II	III	I	II	III
1. TG											
Peak temp (°C)	19.2	21.0	18.0	21.0	27.0	19.0	—	27.1	18.0	—	27.2
$\Delta H$ (Joule/g)	53.0	*	7.8	17.4	30.8	5.6	—	57.3	3.4	—	80.6
2. TG + 2% DHS-TG											
Peak temp (°C)	20.1	24.0	17.5	23.5	26.0	—	22.0	26.3	—	—	27.5
$\Delta H$ (Joule/g)	58.0	*	*	51.0	*	—	*	52.0	—	—	68.0
3. TG + 5% DHS-TG											
Peak temp (°C)	18.7	24.0	—	22.0	25.2	—	21.0	26.6	—	—	27.2
$\Delta H$ (Joule/g)	49.0	*	—	*	59.5	—	*	59.8	—	—	80.5
4. TG + 8% DHS-TG											
Peak temp (°C)	18.1	22.0	17.0	—	25.5	18.0	—	27.4	—	—	—
$\Delta H$ (Joule/g)	—	—	*	—	46.5	*	—	65.2	—	—	—
5. TG + 10% DHS-TG											
Peak temp (°C)	18.2	22.5	16.0	22.0	26.9	18.0	—	26.3	—	—	25.9
$\Delta H$ (Joule/g)	—	—	—	—	—	*	—	64.0	—	—	62.7

<sup>a</sup>Molten fat at 60°C cooled at 20°C/min to -30°C and subjected to different rates of heating.

\*Shoulders.

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which appeared only as a shoulder in the case of pure TG, became distinct and bigger as the level of DHS-TG increased (Fig. 1—I). Similarly, at 5°C/min, DHS-TG accelerated the phase transition of form II to III of TG (Fig. 1—II). However, at the 2% level, DHS-TG had no significant effect on phase transition of the above three crystal forms of TG at any of the heating rates.

**Slow cooling.** Slow cooling is known to generate higher melting polymorphs than rapid cooling. However, slow cooling (2°C/min) of sal fat TG from 60°C to -30°C showed mostly crystal form I as shown by a major peak at 18.7°C, with a small hump corresponding to form II (Fig. 2—Ia, Table 2). Under these experimental conditions, both slow and rapid cooling of TG produced mostly crystal form I. However, addition of DHS-TG caused a significant change in the transformation of lower melting crystal forms to the next higher forms of TG. DHS-TG accelerated the phase transition of crystal forms I → II → III, the effect increasing with the level of DHS-TG (Fig. 2—II, and Table 2). It is evident from Figure 2—II that most of the crystals of TG were transformed to form III by addition of DHS-TG at 5% and above. These results indicated that under a slow rate of cooling, the conditions that prevail in chocolate making, DHS-TG accelerated the phase transition of crystal forms I → II → III of sal fat TG.

**Tempering at 0°C.** The fusion enthalpy curves of the samples tempered at 0°C for 18 hr are shown in Figure 3A. Addition of DHS-TG to TG prior to tempering resulted in delayed phase transition of crystal form III to IV of TG. At all levels of DHS-TG only a single peak of form III was observed, as against an additional peak of 32.8°C corresponding to form IV (Fig. 3A) in the case of pure TG. This delay in phase

transition of higher melting crystal form may be advantageous in margarine manufacture, where smaller size crystals of lower stability are preferred for better texture.

**Tempering at 0°C and 26°C.** The fusion enthalpy curves of samples tempered at 0°C for one hr and at 26°C for three hr are shown in Figure 3B. Addition of DHS-TG to TG prior to tempering did not affect the formation of the most stable form (V) of TG. However, DHS-TG reduced the heat of fusion ( $\Delta H$ ) of form V of TG (Table 2).

These results indicated that DHS-TG accelerated the phase transition of lower melting crystal forms I → II → III of sal fat TG obtained under rapid or slow rates of cooling, whereas they delayed the phase transition of form III to IV at 0°C. Also, DHS-TG reduced the  $\Delta H$  of the stable form V of TG. These results explain our earlier findings of the effects of DHS-TG on the solidification properties of sal fat TG as observed in Shukoff's cooling curve (1). Because DHS-TG accelerated the phase transition of form I → II → III, the TG containing DHS-TG exhibited accelerated onset of crystallization and reduced supercooling property on the cooling curve. Also, as DHS-TG delayed the phase transition of form III to IV and reduced  $\Delta H$  of the stable form of TG, possibly due to steric hindrance, they reduced the temperature difference between maximum and minimum points on the cooling curve.

From these results it can be concluded that DHS-TG have a somewhat deleterious effect on the quality of sal fat for use in chocolate, as their presence may affect the manufacturing conditions and quality of the product. However, they may be advantageous in margarine manufacture because they delay the formation of intermediate crystal forms.

TABLE 2

Effect of Triglycerides Containing Dihydroxystearic Acid (DHS-TG) on Various Polymorphic Forms and Their Heats of Fusion of Normal Triglycerides (TG) of Sal Fat

Polymorphs	Rate of heating 10°C/min							
	Chilled fat <sup>a</sup>			Tempered <sup>b</sup> at 0°C 18 hr				Tempered <sup>b</sup> 0°C 1 hr and 26°C 3 hr
	I	II	III	I	II	III	IV	V
1. TG								
Peak temp (°C)	18.7	21.0	—	18.0	—	27.1	32.8	35.0
$\Delta H$ (Joule/g)	52.0	*	—	*	—	24.0	32.0	111.0
2. TG + 2% DHS-TG								
Peak temp (°C)	18.2	23.0	—	—	—	27.9	—	—
$\Delta H$ (Joule/g)	—	—	—	—	—	62.0	—	—
3. TG + 5% DHS-TG								
Peak temp (°C)	—	22.5	26.4	—	—	27.9	—	35.3
$\Delta H$ (Joule/g)	—	*	77.8	—	—	54.8	—	92.7
4. TG + 8% DHS-TG								
Peak temp (°C)	—	22.5	27.7	—	23.0	27.6	—	—
$\Delta H$ (Joule/g)	—	*	67.8	—	*	47.3	—	—
5. TG + 10% DHS-TG								
Peak temp (°C)	—	—	26.5	—	—	28.0	—	33.4
$\Delta H$ (Joule/g)	—	—	76.0	—	—	60.6	—	69.8

<sup>a</sup>Molten fat at 60°C cooled at 2°C/min to -30°C and heated at 10°C/min.

<sup>b</sup>Molten fat at 60°C allowed to cool to 0°C and tempered prior to heating at 10°C/min.

\*Shoulders.

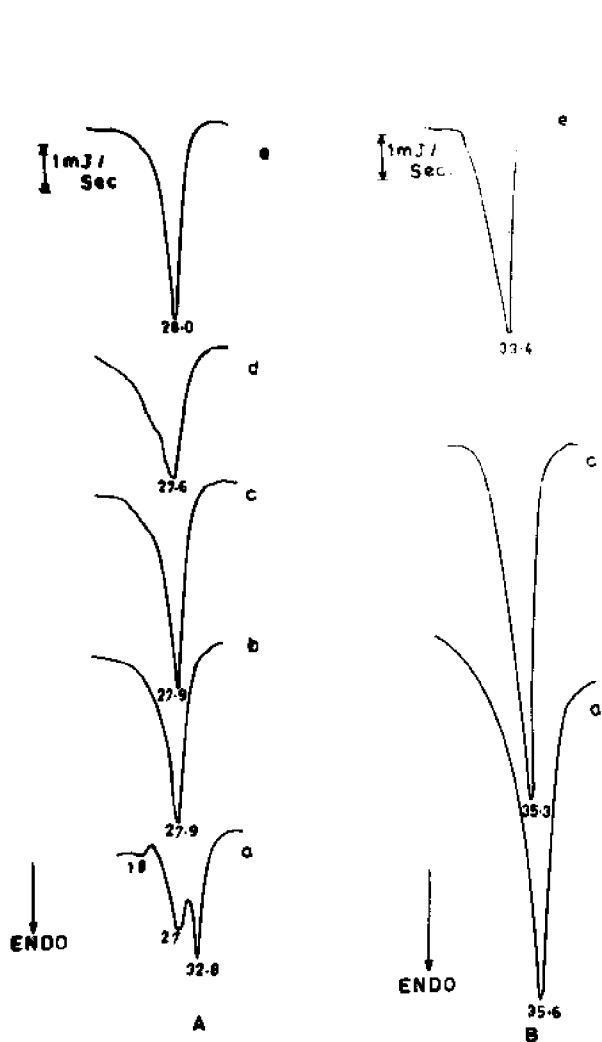


FIG. 2. I, DSC cooling curves at the rate of  $2^{\circ}\text{C}/\text{min}$ . II, DSC heating curves ( $10^{\circ}\text{C}/\text{min}$ ) after cooling at  $2^{\circ}\text{C}/\text{min}$ . a, b, c, d, and e, respectively, indicate the curves of TG and TG containing DHS-TG at 2, 5, 8 and 10%.

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#### REFERENCES

1. Yella Reddy, S., and J.V. Prabhakar, *J. Am. Oil Chem. Soc.* 62:1126 (1985).

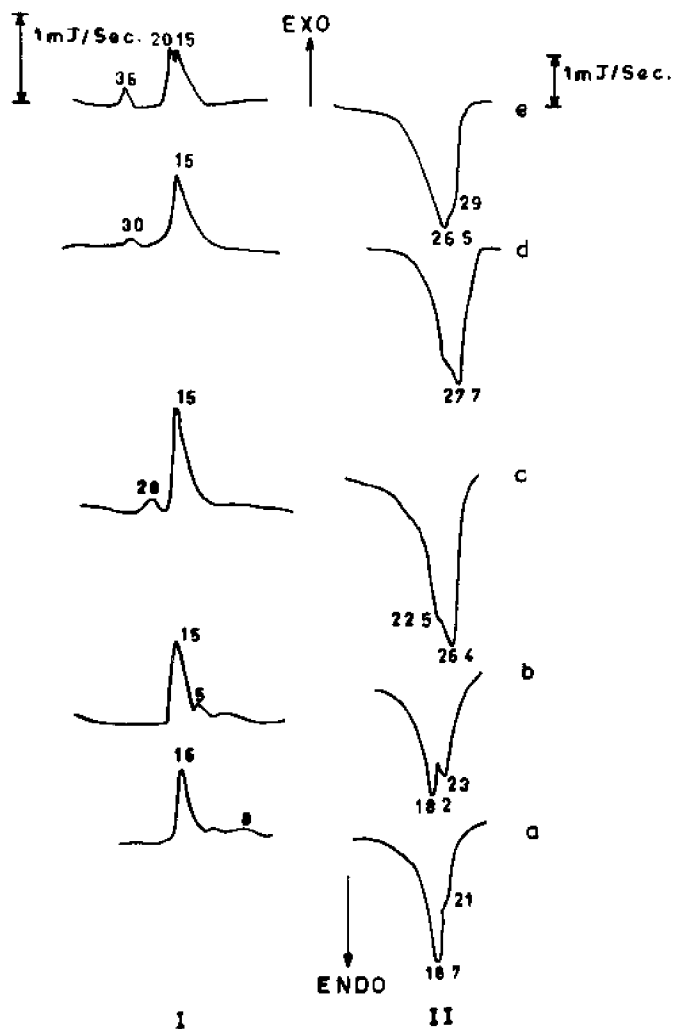


FIG. 3. A, DSC heating curves ( $10^{\circ}\text{C}/\text{min}$ ) of samples tempered at  $0^{\circ}\text{C}$  for 18 hr. B, DSC heating curves ( $10^{\circ}\text{C}/\text{min}$ ) of samples tempered at  $0^{\circ}\text{C}$  for one hr and at  $26^{\circ}\text{C}$  for 3 hr. a, b, c, d, e, respectively indicate the curves of TG and TG containing DHS-TG at 2, 5, 8 and 10% levels.

2. Yella Reddy, S., and J.V. Prabhakar, *Fat Sci. Technol.* 89:394 (1987).
3. Yella Reddy, S., and J.V. Prabhakar, *J. Am. Oil Chem. Soc.* 63:672 (1986).
4. Yella Reddy, S., and J.V. Prabhakar, *Ibid.* 64:97 (1987).

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