Organogelation of Plant Oils and Hydrocarbons by Long-Chain Saturated FA, Fatty Alcohols, Wax Esters, and Dicarboxylic Acids

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ABSTRACT: Conversion of oils into gels generally involves altering the chemical characteristics of the liquid. We describe here the gelling of vegetable oils, essential oils, and hydrocarbons at ambient temperature, without changing the chemical characteristics of the liquids, using saturated FA having carbon chain lengths of 10 to 31. The gelling ability of the added FA increased linearly with their chain lengths. Structure–function studies demonstrated that the carboxyl group, position of an additional hydroxyl group, and acyl chain length played an important role in gelation. Long-chain saturated fatty alcohols, wax esters, and dicarboxylic acids also had the ability to gel plant oils and hydrocarbons.

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Hydrogenation is the method currently used to convert various oils into gels; it involves altering the chemical characteristics of the oil (1). Hydrogenation of polyunsaturated TAG is an expensive process, and the resultant products not only have high levels of saturates but also have *trans* FA, which have been implicated in an elevated risk of coronary heart disease (2). Organogelation is an alternative method that can be used to modify the physical properties of oils without altering their chemical characteristics.

Organogelators are capable of forming gels by self-assembling into filaments through noncovalent interactions, such as hydrogen bonding (3), π–π stacking (4), coulombic interactions (5), and van der Waals forces (6). These filaments then entangle to form a 3-D network that prevents the solvent from flowing at the macroscopic level, whereas the liquid phase often prevents the collapse of the network. Most gelators developed so far are based on hydroxy FA (3,7,8), saccharides (9), surfactants (5,10), steroids (6), bis-ureas (11), amides (12), and lecithins (13). Many of these organic compounds are synthetic and require lengthy protocols for their synthesis. In spite of the many chemical compositions and physical properties of gels that are known (3–9), the structural requirements for a molecule to gel an organic liquid are still not clearly understood.

The hardness of kokum (*Garcinia indica*) fat is due to the presence of free stearic acid. This observation led us to investigate new chemical entities with similar structures. In the present study, we have focused mainly on long-chain saturated FA and their derivatives as naturally abundant organogelators for vegetable oils and hydrocarbons.

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EXPERIMENTAL PROCEDURES

Plant materials and biochemicals. Garcinia indica Choisy (kokum) trees growing in the mountainous forests of Karnataka state on the western coast of India were the source of kokum seeds. The collected seeds were frozen in liquid nitrogen before storing at −80°C. GC–MS analysis revealed that the FA from kokum seed TAG were composed of stearic acid (59.7%), oleic acid (35.5%), and palmitic acid (4.8%). Commercially available oils were purified by silica column chromatography prior to use. Sunflower, lavender, castor, rapeseed, and peanut oils were obtained from Oleogen (Manamadurai, India). The FA composition of sunflower oil was palmitic acid (7.1%), stearic acid (4.2%), oleic acid (15.8%), and linoleic acid (72.9%). The chemical composition of lavender [*Lavandula angustifolia* (syn. *L. officinalis*)] oil was linalool (47.8%), lavendulyl acetate (38.4%), β-caryophyllene (9.2%), and ocimene (4.6%). TAG and all the FA and their derivatives were obtained from Sigma Chemical Company (St. Louis, MO). The petrol and diesel used in this study were from Bharat Petroleum (Mumbai, India).

Lipid analysis. A 10-g quantity of mature seeds was frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. Lipids were extracted from the powder using 20 mL of boiling isopropanol. The mixture was then centrifuged briefly, the supernatant was removed, and the extraction was repeated. The pooled isopropanol extracts were brought to dryness with a rotary evaporator. The tissue residue was then re-extracted twice with 38 mL of chloroform/methanol/10% acetic acid (1:2:0.8, vol/vol). After centrifugation, the supernatant was added to the isopropanol extract, and 20 mL each of chloroform and water was added to the mixture. The biphasic system was vortexed and centrifuged. The lower chloroform phase was dried in a rotary evaporator. The lipid residue was dissolved in chloroform/methanol (1:1, vol/vol) and stored at −20°C.

Silica for column chromatography (100 g; 60–120 mesh) was packed in hexane, and the total lipid extract (1 g) was loaded onto the silica column and eluted with 10% ethyl acetate in hexane to remove FFA, MAG, and DAG, yielding pure TAG. The purified TAG were converted to FAME by

heating at 75°C for 60 min in 4.5 mL of methanolic HCl (0.6 N), prepared by diluting 5 mL of concentrated HCl with 90 mL methanol (14). The FAME were analyzed under GC–EI–MS conditions using a VG AutoSpecM mass spectrometer equipped with an HP 5890 series II gas chromatograph fitted with an HP-5 capillary column (30 m × 0.32 mm i.d.) (Hewlett-Packard, Palo Alto, CA). Helium was used as a carrier gas at a flow velocity of 26 cm s^{-1}. The chromatograph was programmed for an initial temperature of 100°C for 5 min followed by a 10°C min^{-1} ramp to 220°C. The final temperature was maintained for 10 min (15).

Gelation experiments. In a typical gelling experiment, an appropriate amount of FA or its derivative and oil (2 mL) were put into a test tube (70 × 27 mm) and sealed with a thin Teflon disk screw cap (Wheaton; Millville, NJ). The tubes were heated to 60 to 65°C with shaking (75–100 rpm) until all the solid material had dissolved. The solution was allowed to cool at room temperature. Gelation was considered to have occurred when a homogeneous substance was obtained that exhibited no gravitational flow. The melting temperature of the gel was recorded from the point when melting began to the optical clarity end point.

Phase contrast light microscope. Phase contrast light microscopy observations were made with a confocal laser-scanning microscope (TCS SP; Leica, Heidelberg, Germany) using a 40× objective lens and phase contrast optics.

Spectral analysis. All NMR measurements were carried out in a solution of chloroform-d using a Bruker DRX 500 spectrometer (Karlsruhe, Germany) operating at 500.13 MHz. Chemical shifts were measured on the δ (ppm) scale. FTIR spectra were obtained on a PerkinElmer Spectrum 1000 FTIR spectrometer (Norwalk, CT), wherein the neat samples were loaded directly onto the loading cell.

RESULTS AND DISCUSSION

Initial experiments revealed that when solid kokum fat was mixed with purified sunflower oil (20%, wt/vol), the mixture gelled in 12 h at room temperature and in 1 h at 4°C (Table 1). It was also observed that the purified TAG from kokum retained the gelling activity when tested with other vegetable oils (castor, peanut, and rapeseed). It was hypothesized that the kokum lipids could be true organogels or that solids could have trapped the liquids in the process. Saturated fatty acyl glycerol esters (from plant or synthetic origin) showed a similar gelling ability, and the minimum amount required for gelling decreased with increasing chain length (Table 1).

The most abundant FA in kokum, stearic acid, was sufficient to induce gelation of sunflower oil by itself. On further analysis, we found that all saturated FA of chain lengths 10:0 to 31:0 (representing the number of carbon atoms and the number of double bonds, separated by a colon) were also capable of gelling vegetable oils. Of these, 2% stearic acid (18:0) was sufficient to induce solidification of various vegetable oils by itself. Sunflower oil was mixed with stearic acid (2%), and the resultant product was viewed under a confocal laser-scanning microscope. The micrographs revealed the presence of tightly packed spherical (mostly multilamellar) vesicles, 50–200 nm in diameter (Fig. 1). A similar observation was also made with gemini surfactants (10). However, further structural analyses are required to prove the presence of multilamellar vesicles in the gel. This experiment indicated that the saturated FA was capable of gelling sunflower oil. When tried with other FA, the minimum amount of FA required for gelation of sunflower oil decreased with an increase in chain length (60% for decanoic acid, 15% for dodecanoic acid, 6% for tetradecanoic acid, 4% for hexadecanoic acid, and 2% for octadecanoic acid) and remained almost constant with further increases in chain length. The melting points of the gels increased from 30 to 70°C as chain lengths of the added FA increased (Fig. 2A). Interestingly, a similar observation was made in the case of essential oils such as lavender, rose, and lemongrass. FA longer than 26:0 did not show any further increase in the solidification ability of essential oils (Fig. 2A). This solidification phenomenon has potential for use in the food, health, and transportation industries. This finding was extended to hydrocarbons, and to our surprise, a similar observation was also made with them (Fig. 2B).

TABLE 1
Organogelation of Purified Sunflower Oil by Glycerol Esters of Saturated FA[a]

Gelator	Minimum quantity (%, w/w)	Melting temperature (°C)
Kokum fat	20.0 ± 0.7	26–29
Trilaurin	36.0 ± 2.1	25–28
Trimyristin	31.0 ± 1.8	26–30
Tripalmitin	27.0 ± 1.5	29–32
Tristearin	22.0 ± 0.7	34–37

[a]Gelation was carried out at 25°C. Values are mean ± SD of four determinations.

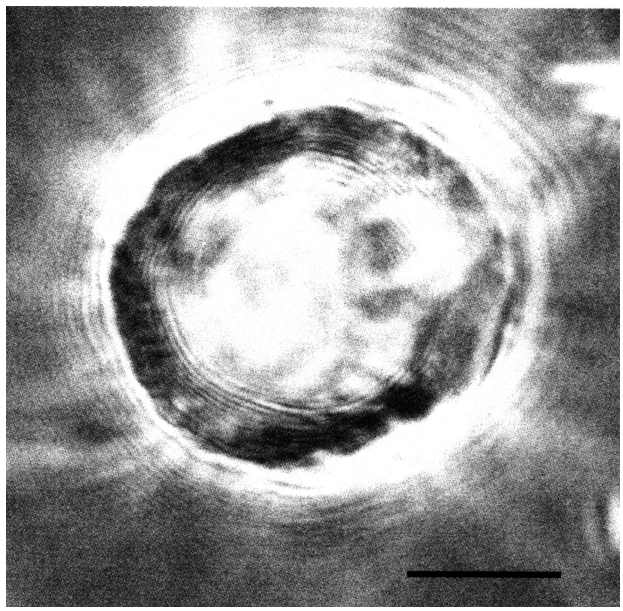

FIG. 1. Micrograph of sunflower oil gelled with the addition of 2% stearic acid. The presence of multilamellar vesicles is evident. Bar: 200 nm.

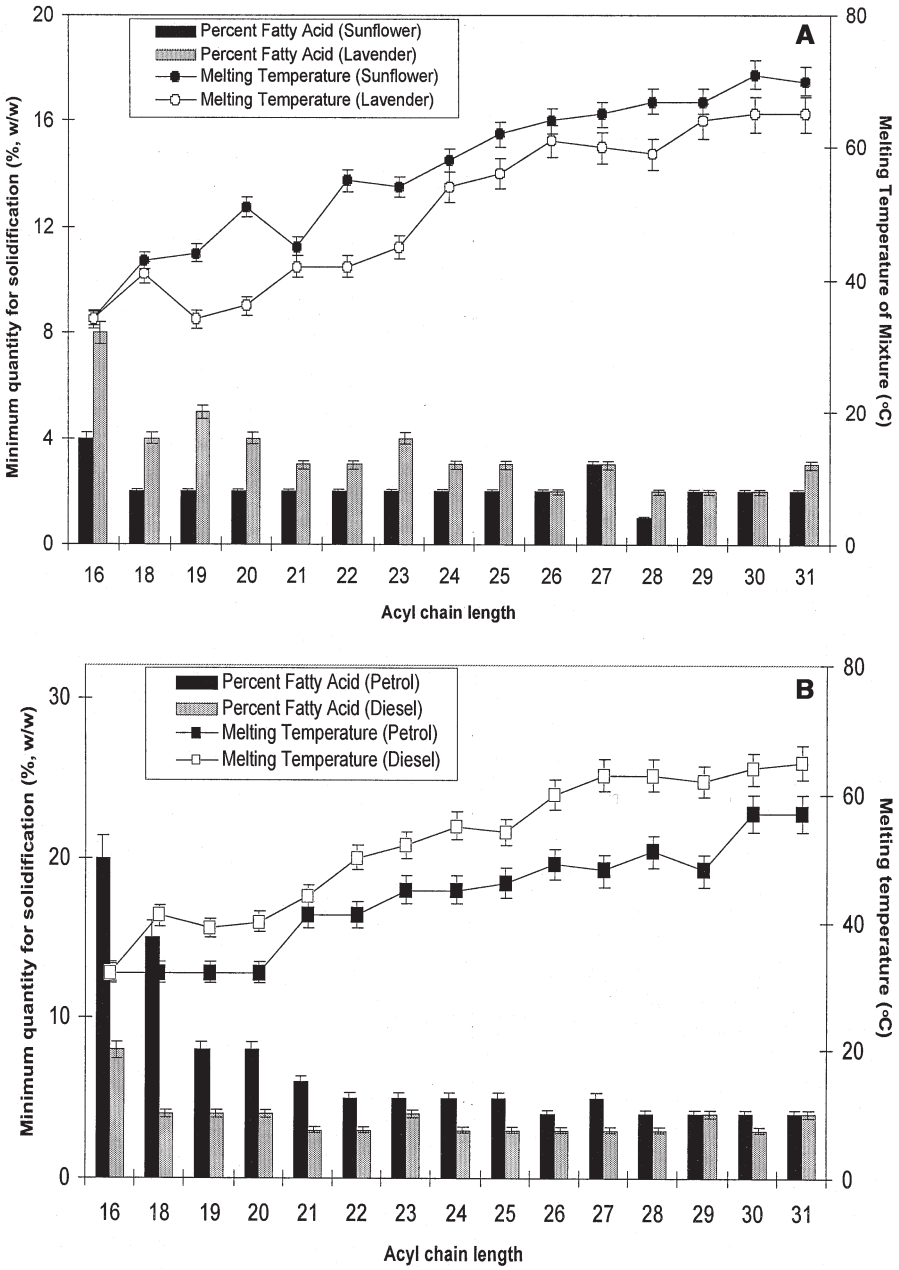

FIG. 2. Organogelation of sunflower and lavender oils and petrol and diesel fuels with saturated FA. (A) The minimum amounts of FA required to gel sunflower and lavender oils are represented by a bar graph, and the melting temperatures of gelled sunflower and lavender oils are represented by a line graph. (B) The minimum amounts of FA required to gel petrol and diesel are represented by a bar graph, and the melting temperatures of gelled petrol and diesel fuel are represented by a line graph. The indicated amounts of saturated FA (16:0 to 31:0) were used. Error bars on the line graphs indicate the mid-value of the melting temperature from the time the gel begins to melt until the optical clarity end point, with the lower and upper values indicated, respectively. Error bars on the bar graphs represent the SE of the percent minimum quantity of FA required for solidification.

To elucidate the role of various functional groups on FA in promoting organogelation, we used FA derivatives for gelling various oils. A hydroxyl group in the middle of the acyl chain of an 18:0 FA (12-hydroxyoctadecanoic acid) was found to improve the efficiency of the FA as an organogelator. The amount of hydroxy FA required to gel plant oils and hydrocarbons was half that of stearic acid (Fig. 3A). However, a fivefold higher amount of 12-methyloctadecanoic acid was required to gel oils compared to the FA.

Dihydroxyoctadecanoic acid with two hydroxyls adjacent to each other in *threo* or *erythro* conformation near the middle of the acyl chain was capable of gelling plant oils but at a higher concentration than that required for the monohydroxy FA. Methylation of the carbonyl hydroxyl of docosanoic acid (22:0) greatly reduced its gelling ability. On the contrary, hexacosanoic acid (26:0) and triacontanoic acid (30:0) did not show any decrease in organogelation ability upon methylation (Fig. 3A).

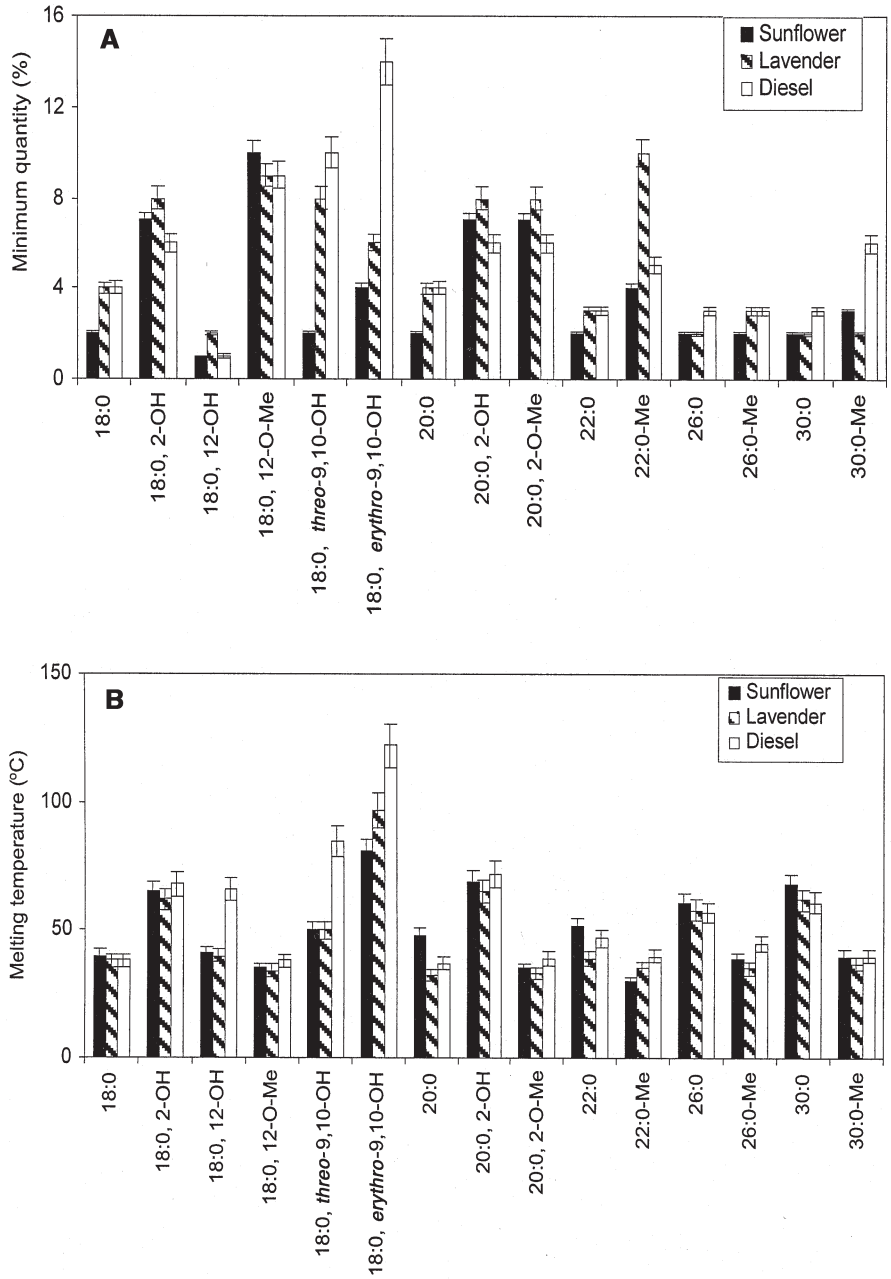

FIG. 3. Gelation of sunflower oil, lavender oil, and diesel fuel by modified saturated FA. (A) The minimum quantity of FA required to form gels of sunflower oil, lavender oil, and diesel fuel; (B) melting temperatures of the gelled sunflower oil, lavender oil, and diesel fuel. Error bars indicate the mid-value of the melting temperature from the time the gel begins to melt until the optical clarity end point, with the lower and upper values indicated, respectively.

Saturated fatty alcohols showed lower efficiencies compared to saturated FA of the same chain lengths in their ability to gel various oils (Fig. 3A and Table 2). Saturated wax esters, with the exception of stearoyl behenate (22:0/18:0 ester), showed organogelling efficiencies similar to those of the FA.

In addition, dicarboxylic acids (adipic, suberic, and sebacic acids) were capable of gelling plant oils at efficiencies that were many-fold higher than monocarboxylic acids of the same chain lengths (Fig. 3A and Table 2). However, dicarboxylic FA were not able to gel hydrocarbons.

To determine whether organogelation by FA caused any chemical modifications in the oils, sunflower oil gelled with stearic acid was analyzed by GC–MS, and triolein gelled with stearic acid was analyzed by ^{1}H NMR and FTIR. GC–MS analysis of the gelled sunflower oil revealed no alteration in the chemical composition apart from the change in stearic acid level due to exogenous addition of stearic acid (data not shown). ^{1}H NMR analysis in a chloroform solution of pure triolein and triolein gelled with stearic acid revealed that the spectra of the two compounds were identical, indicating that there were no alter-

TABLE 2
Organogelation[a] of Sunflower Oil, Lavender Oil, and Diesel Fuel by Long-Chain Saturated Fatty Alcohols, Wax Esters[b], and Dicarboxylic Acids

	Sunflower		Lavender		Diesel	
Gelator	Minimum (%)	m.p. (°C)	Minimum (%)	m.p. (°C)	Minimum (%)	m.p. (°C)
20:0 alcohol	3 ± 0.2	40–45	4 ± 0.6	49–52	4 ± 0.5	59–62
22:0 alcohol	3 ± 0.2	43–47	3 ± 0.1	50–53	3 ± 0.4	42–44
26:0 alcohol	3 ± 0.5	45–48	2 ± 0.2	39–43	2 ± 0.3	50–52
30:0 alcohol	4 ± 0.5	55–60	2 ± 0.2	59–63	3 ± 0.2	62–65
22:0/14:0-ester	2 ± 0.1	35–38	3 ± 0.3	59–62	5 ± 0.4	60–64
22:0/16:0-ester	2 ± 0.1	36–40	3 ± 0.4	42–46	5 ± 0.3	45–48
22:0/18:0-ester	1 ± 0.1	42–45	2 ± 0.2	48–52	2 ± 0.2	41–44
22:0/20:0-ester	2 ± 0.3	42–45	3 ± 0.2	49–52	2 ± 0.1	41–43
22:0/22:0-ester	3 ± 0.2	63–66	5 ± 0.5	65–68	5 ± 0.4	63–66
Adipic acid	4 ± 0.3	140–145	3 ± 0.4	89–92	—	—
Suberic acid	4 ± 0.4	80–85	5 ± 0.6	77–80	—	—
Sebacic acid	4 ± 0.3	80–84	5 ± 0.4	76–81	—	—

[a]Gelation was carried out as described in the Experimental Procedures section. Mean values ± SD are indicated for three independent determinations. —, no gelation observed.
[b]The wax esters are represented as behenic acid (22:0)/alcohol ester.

ations in the chemical structure of gelled triolein (data not shown). FTIR analysis of the pure triolein and the triolein gelled with stearic acid revealed no alterations in the chemical structure of either the added FA or triolein (data not shown). These data suggest that the physical properties of various oils can be modified without changing their chemical composition and properties by adding the appropriate type and quantity of FA. The gelled oils were stable at ambient temperature for a minimum of 3 mon, suggesting the robust nature of such gels.

One possible mechanism of gelation is that the FA, by virtue of having hydrophobic and charged hydrophilic components, align themselves head-to-tail in a linear fashion. The linear structures arrange themselves to create a lattice structure with sufficient spacing for TAG to become embedded in the lattice and form a gel or a solid. There is a possibility that the acyl chains form an irregular network, stabilized by intermolecular hydrogen bonding, which could entrap organic liquids. Alternatively, they could form a large spherical monolayer that entraps the TAG in the interior hydrophobic environment, resulting in gelation of the oil.

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