The Potential of Soapstock-Derived Film: Cottonseed and Safflower

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ABSTRACT: Oilseed soapstock is seldom used today for the recovery of fatty acids, but it is often added to oilseed meal. The energy value of oilseed meal is marginally increased by the addition of soapstock. To find alternative uses for oilseed by-products, cottonseed and safflower soapstock samples from industrial plants were characterized using American Oil Chemists' Society recommended and modified methods. The characterization included moisture and volatiles, phosphorus and nitrogen, neutral oil, total fatty acid amount and individual fatty acid profile, and total gossypol for cottonseed soapstock samples. The characterization indicated that cottonseed soapstock samples contained a slightly larger amount of neutral oil than safflower. These soapstock samples were frozen to –40°C at 40 mm Hg for more than 8 h, thawed, and the low-boiling compounds were removed by evaporation under reduced pressure. The freeze-dried soapstocks were mechanically pulverized in an inert atmosphere until able to pass through a 50-mesh screen. When these freeze-dried soapstock particles were rehydrated with deionized water, the formation of a gel phase was observed. Casting of this gel phase onto a substrate and subsequent drying without heating resulted in a thin film, a liposome-like material, with a uniform thickness of about 0.01″. The lamination capability of freeze-dried oilseed soapstocks by rehydration may be attributed to the formation of multiple bilayer lamellae by phospholipids from the oilseed soapstock. Due to its biodegradable nature, the use of soapstock-derived film as a composite or by itself as an encapsulating agent is highly attractive. The potential of this liposome-like material as a chemical carrier is discussed.

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KEY WORDS: Biodegradable, by-products, characterization, lamellae, oilseed, soapstock.

Vegetable oil extraction aims to produce oilseed meal and edible oil. Free fatty acids (FFA) and other minor oilseed components, which are simultaneously extracted with the main vegetable oil components (triacylglycerols), are usually segregated from crude vegetable oil by various refining techniques. In general, caustics are used to remove FFA from crude vegetable oil. In caustic refining, phosphorus lipids and hydratable and nonsaponifiable compounds, including plant sterols and long-chain hydrocarbons, are removed in addition to FFA . In cottonseed, gossypol is also known to be removed by a caustic (1). In decades past, most soapstocks were acidulated to recover FFA, which were in turn used as the raw materials for industrial soaps. Such fatty acid recovery is no longer practiced because of poor economics and environmental concerns in discharging effluent water (2). Instead soapstock is either added to oilseed meal or disposed of with little economic compensation.

In soybean oil processing, hydratable phosphatides are recovered by a water-washing step prior to caustic refining and then are used as the valuable raw material for lecithin production (3). However, water-washing recovery of phospholipids has not often been practiced in other edible-oil producing processes, such as cottonseed or safflower, due to economic reasons or inferior quality of the by-products (1). Nevertheless, it is no longer prudent to dispose of these valuable oilseed by-products without significant economic compensation.

Kim *et al.* (4) recently attempted to convert plant sterols in oilseed soapstocks *via* biocatalysts to yield pharmaceutical intermediates. More investigations on utilization and recovery of plant sterols are expected. Other than plant sterols, oilseed soapstocks contain essential phospholipids which possess the important physical property of "mesomorphism," a property necessary for structural facilitation into a bilayer lamella (5). The potential use of soapstocks as a raw material to produce a composite or an encapsulating agent is considered in this paper.

Because of its zwitterionic properties derived from oilseed phospholipids, lamellae derived from oilseed soapstock should have a wide spectrum of applications for chemical encapsulation or targeted delivery in animals and humans. To test its laminating capability, the initial screening of cottonseed and safflower soapstocks from industrial sources was conducted. The chemical analyses of the soapstocks and the initial results for the lamination capability are presented.

METHODS AND MATERIALS

Cottonseed and safflower soapstock samples from industrial sources (J.G. Boswell Co., Corcoran, CA; Chickashay Cotton Oil Mill, Lamesa, TX; Yazoo Valley Oil Mill, Greenwood, MS) were used in this study. Scheme 1 represents the simplified process diagram by which the cottonseed/safflower soapstock samples were produced. PBSY oil represents prime,

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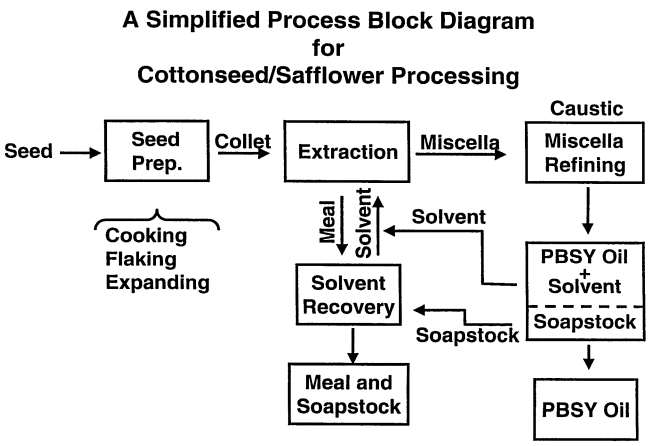

SCHEME 1

bleachable summer yellow oil. Safflower seed was alternatively processed with cottonseed at the oilseed plant, so that the oil- and soapstock-producing steps were invariant for both types of oilseed. After seed preparation steps, which included cooking, flaking, and passing through expanders, the oilseed collets were extracted with commercial-grade hexane. As indicated in the diagram, the soapstock samples were produced by miscella refining with caustics, then separated from the oil-bearing solvent phase by centrifugation.

The following methods were used to characterize the soapstock samples: (i) neutral oil and total fatty acids by AOCS methods G5-40 and G3-53, respectively (6); (ii) total gossypol by AOCS method Ba 8-78 (6); (iii) elemental analyses for nitrogen and phosphorus by ASTM D5373/E258 (7) and induction-coupled plasma (ICP)/Kjeldahl methods, respectively; and (iv) moisture and volatiles by a modified AOCS method Aa 3-38 (6). In addition to these assays, the composition analyses by capillary gas chromatography (GC) after trimethylsilyl (TMS) derivatization was performed. The details of these GC analyses were presented elsewhere (8). The results of these analyses are summarized in Table 1. As a supplementary analysis, the amount FFA in crude oil samples was determined by AOCS method Ca 5a-40 (6). Cottonseed crude oil contained 2 to 3% FFA, and safflower about 0.5%.

The soapstock samples contained a considerable amount of meal fines suspended in extraction solvent, water, and other volatile components. The volatile components, including solvent, water, and other low-boiling compounds, were removed by use of a freeze-dryer (Virtis model 20src-x; Gardiner, NY). The details of this freeze-drying operation were as follows. A few batches of samples, about 100 g, spread thinly in a large glass dish, were frozen to −40°C overnight under a steady vacuum at 40 mm Hg. After completing the freezing cycle for more than 8 h, the samples underwent a drying cycle. This thawing–drying cycle started from −40°C, raising the temperature by 10° at a time, while the vacuum pressure in the drying chamber was maintained at a steady value for approximately 1 h. The final drying was done at room temperature (25°C) for 1 to 2 h under equilibrium, usually at 35 mm Hg.

After the freeze-drying cycle, the samples were placed in a chamber filled with an inert gas, either nitrogen or carbon dioxide, followed by mechanical pulverization to produce fine soapstock particles passable through a 50-mesh sieve. The collection of pulverized soapstock raw material was tested in various polar and nonpolar solvents [acetone, ethanol, isopropyl alcohol (IPA), *n*-hexane, chloroform, glycerol, and deionized water] for solubility, consistency, viscosity, and lamination capability. The lamination capability was tested by dissolving the freeze-dried soapstock powder in the test solvents. The dissolved material was cast at room temperature on a glass or a polymeric support surface, dried in inert atmosphere, and separated from the substrate surface.

RESULTS AND DISCUSSION

Typical capillary gas chromatograms given in Figures 1 and 2 show the compositional analysis of these soapstocks. One may note from these chromatograms that gossypol was the unique component shown only in the cottonseed soapstock sample. Safflower samples showed a large amount of sucrose and glycerophosphates compared to cottonseed samples. The following major classes of lipids in these chromatograms included sodium soaps of saturated fatty acids (myristic, palmitic, stearic, and arachidic) and unsaturated fatty acids (palmitoleic, linoleic, and oleic); glycerophosphates; mono-, di-, and triacylglycols (with carbon numbers between 48 and 58); saccharides (sucrose, raffinose, and stachyose); and sterols (campesterol, stigmasterol, and β-sitosterol). Glycerol was the resulting product from the hydrolysis of the lipids catalyzed by added caustics. The summary of the major lipid composition is presented in Table 2.

In addition to lipids, the soapstock samples contained a considerable amount of entrapped extraction solvent and water. Oilseed lipids, such as aliphatic sodium soaps, sterols

TABLE 1
Elemental and Chemical Analysis of Cottonseed and Safflower Soapstocks

Soapstock type	Volatiles (%)	Elements (%)		Fatty acid and oil[a] (%)		Gossypol[a] (%)
		Nitrogen	Phosphorus	Total FA	Neutral oil	
Cottonseed	46–66	0.49–0.64	0.53–0.64	50–55	15–22	1.6–2.2
Safflower	55–65	0.77–0.99	0.90–0.98	50–63	11–15	ND

[a]Determined by the AOCS official methods (Ref. 6). FA, fatty acid; ND, not detected.

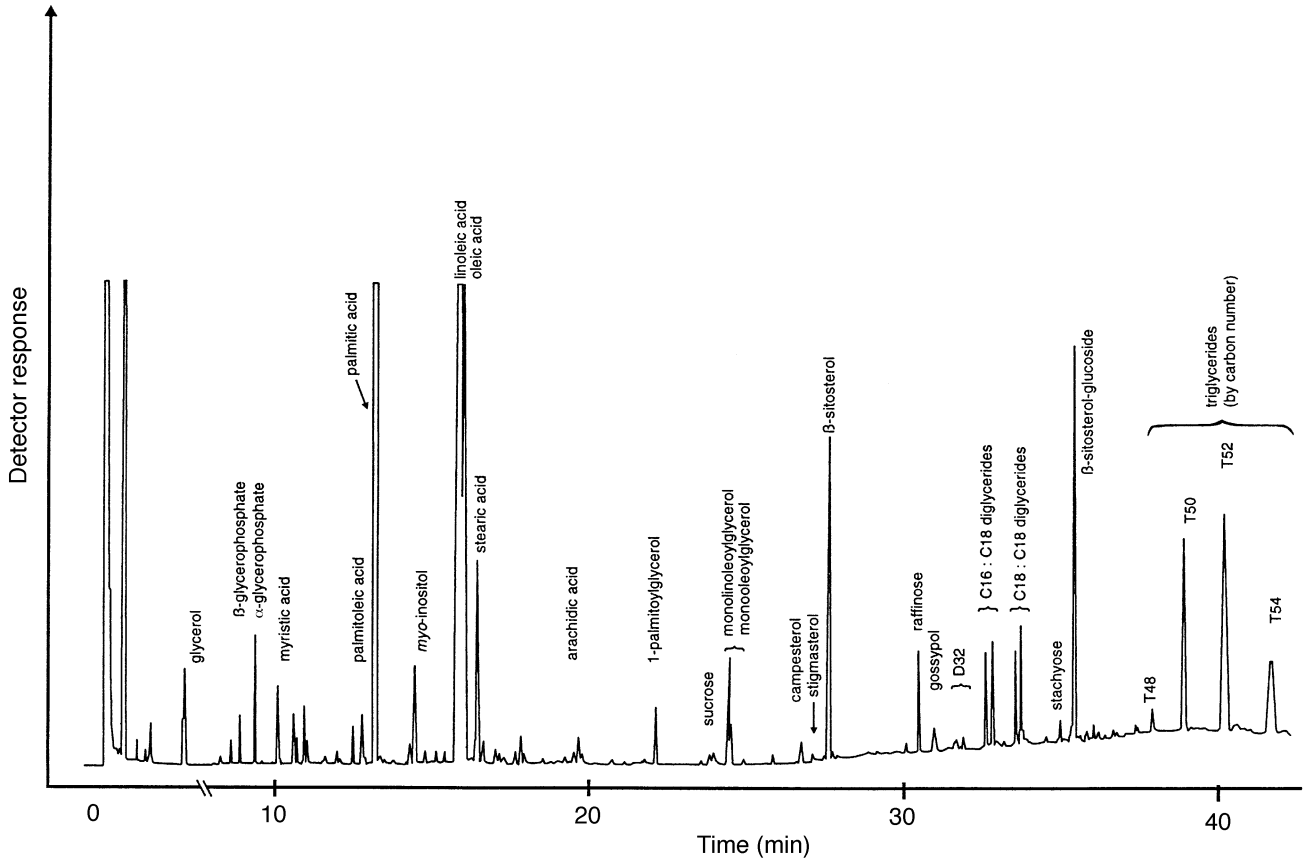

FIG. 1. A gas chromatogram of trimethylsilyl (TMS)-derivatized cottonseed soapstock.

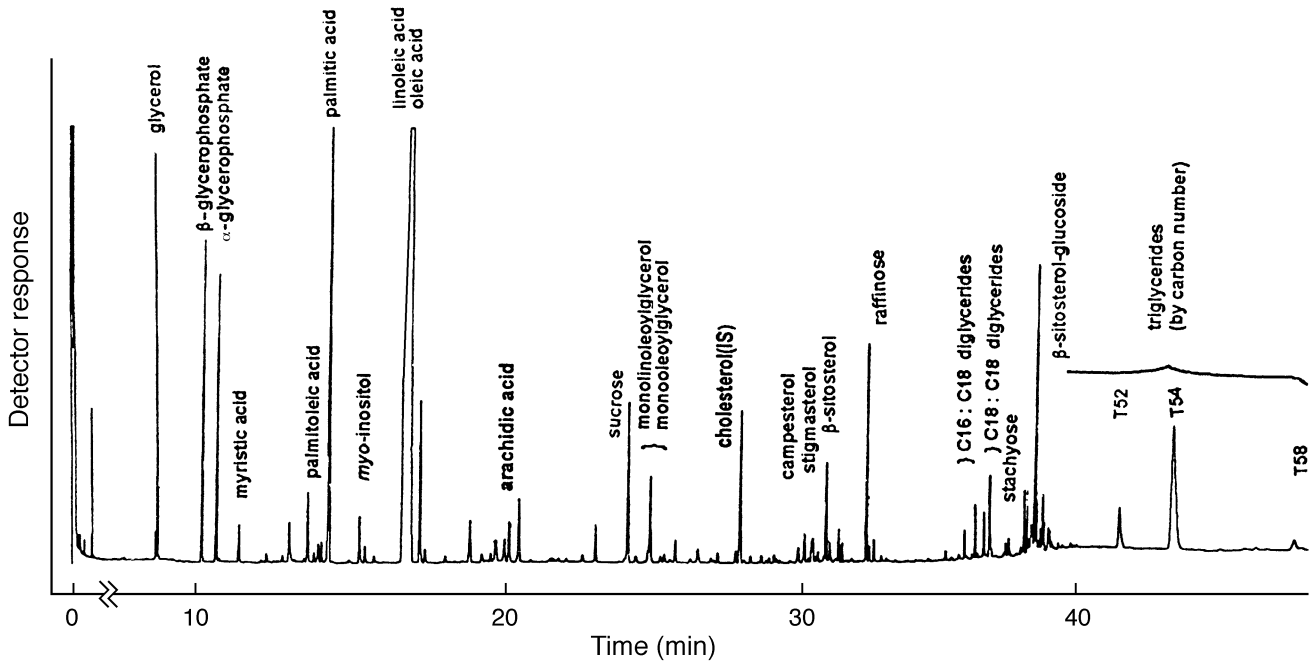

FIG. 2. A gas chromatogram of TMS-derivatized safflower soapstock. IS, internal standard; see Figure 1 for other abbreviation.

TABLE 2
Typical Chemical Composition of Cottonseed and Safflower Soapstock[a]

	Fatty acids[b]		Saccharides[c]		Sterols[d]	Glycerides		
Soapstock	Sat.	Unsat.	Sucrose	Oligosacch.	β-Sitosterol	Mono	Di	Tri
Cottonseed	20.9	40.7	Trace	0.8	2.9	1.8	2.6	11.5
Safflower	12.1	50.3	1.9	1.2	2.0	1.3	1.5	10.5

[a]Units in percentage.
[b]Saturated fatty acids included myristic, palmitic, stearic, and arachidic acids. Unsaturated fatty acids included palmitoleic, oleic, and linoleic acids.
[c]Oligosaccharides included raffinose and stachyose.
[d]β-Sitosterol included campesterol and stigmasterol (these were minor sterols).

and phospholipids in their pure chemical state, take various crystalline forms at a lower temperature (5). A few reports have recently shown that even a mixture of phospholipids forms a lamella at low temperatures (9,10), which leads to the production of liposome materials. The sodium soaps and phospholipids in the soapstock samples, which were randomly mixed in a slurry of entrapped free oil, solvent, and water, tended to stratify but did not form a planar surface or an ordered structure.

Oilseed lipids have an important physical property, however—the capability to form a bilayer lamella in an aqueous system. To test the lamination capability of the lipids, it was necessary to produce a batch of uniformly mixed and dried lipids in particulate form. To produce a homogeneous raw material with little entrapped water, solvent, free oil, or other degenerated lipid species with low boiling points, three typical drying techniques were tested. These were freeze-drying, spray-drying, and oven-drying under reduced pressure. Because the spray-drying treatment generally produces the raw material in a particulate form without additional mechanical pulverization, it was considered to have an advantage over freeze-drying. Experimental spray-drying treatment was not satisfactory, however, because the high operating temperatures required for most spray-dryers (above 200°C) caused the oilseed lipids to oxidize severely, even with some carbonization, in the presence of atomized hot oil. Oven-drying of soapstocks at 100°C also caused severe discoloration and some carbonization. Oven-drying at 70°C under reduced pressure partially discolored the samples. This is consistent with a report that oilseed phosphatides with sugars and amines underwent browning reactions at temperatures above 70°C (11). Carbonized lipids were not soluble in the selected solvents.

Treating the soapstock samples by freeze-drying at –40°C and 40 mm Hg completely removed water, solvents, and some glycerol. The weight loss by freeze-drying, however, amounted to about 60 to 80% of the raw material, indicating that deep-freezing, thawing, and equilibrium evaporation in a high vacuum was more effective in removing the moisture and volatile material than heating. Note that volatiles (Table 1) ranged from 46 to 66% for cottonseed, and 55 to 65% for safflower. Recently, this technique of lipid freeze-drying and thawing was termed "cryo-preservation" by a group of liposome investigators (12,13). It appeared in this investigation that freezing and thawing have preserved the physicochemical properties of the oilseed lipids, especially mesomorphism and hydration.

By using freeze-dried soapstocks, a quick screening test for solubilization was conducted with six preselected solvents: chloroform, hexane, acetone, ethanol, isopropanol, and water. Primarily, the test aimed at finding which solvent was required in the least amount to make a homogeneous and uniform phase with the freeze-dried soapstock material. We have examined whether the test solvent facilitated forming a solid structure of dissolved material after drying the mixture by passive evaporation.

Water, chloroform, and hexane mixed with the freeze-dried soapstocks worked most effectively with about 2 to 3 g solvent to 1 g raw material. Each formed a uniform phase easily spread on a planar surface. IPA, ethanol, and acetone tended to form separated phases and required more solvent to dissolve the raw material. Although hexane dissolved the raw material well, the solid material produced after drying was too brittle to form a planar surface of significant size. It is apparent that hexane is a good solvent, but not suitable for the lamination of lipids. Chloroform was excellent for dissolving and laminating the lipids, but owing to the possible environmental concerns of large-scale production, the film produced *via* chloroform was not further examined. These soapstock lipids, including phospholipids, sterols, glycerides, and even saturated and unsaturated sodium soaps, are known to exhibit mesomorphism, i.e., the capability to form a highly ordered liquid crystal structure before solidifying in an aqueous system (5).

The lipids' ability to form an ordered liquid structure appeared to be preserved to some extent after freeze-drying. Upon rehydration, the freeze-dried oilseed lipids formed a gel phase. Drying the gel, which was cast upon a smooth planar surface, resulted in a thin film of uniform thickness and with a smooth surface. This film is shown in Figure 3. It was stored in a clear glass container at room temperature for 6 mon. The physical structure and strength of the film were intact after the storage (the same test for determining the film strength was conducted prior to and after storage). The film thickness was variable depending upon casting technique.

The film in Figure 3 had a uniform thickness of 0.01″ and a dimension of 3″ × 3″. It had the strength to withstand about 0.01 psi without breaking. Film strength was tested by apply-

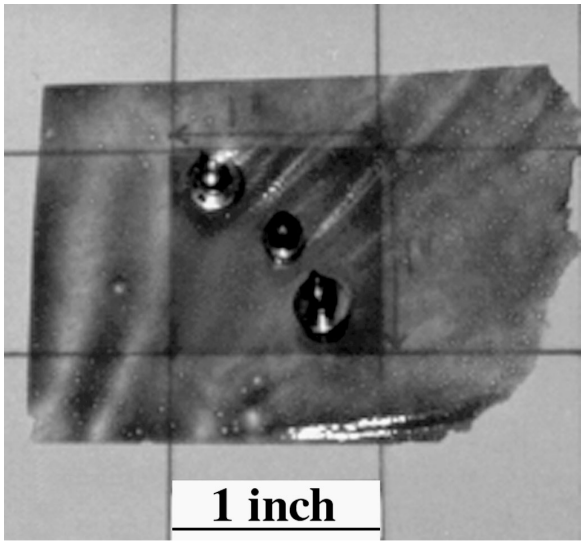

FIG. 3. A thin film derived from the freeze-dried safflower soapstock.

ing known weights per unit area. This film may have a physical property similar to liposome (14), which was imparted by water-insoluble, but water-swelling amphiphiles (5), such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and monoglyceride. From the phosphorus content (Table 1), one may postulate that 25–30% of phosphatides in the soapstocks contributed a significant physical property to the mixture of freeze-dried lipids. [A multiplying factor of 25 is generally used to estimate the amount of phosphatides from the phosphorus content (15).]

The phospholipids in the soapstock samples should have a similar makeup to those reported in the literature (16,17), consisting of 25–35% phosphatidylcholine, 20–30% phosphatidylethanolamine, 15–25% phosphatidylserine, 5–10% phosphatidylinositol, and small amounts of degenerated species, i.e., lysophosphatides. It is highly possible that PC, PE, and monoglycerides in the freeze-dried oilseed formed lamellar surfaces by hydration and formed thin film structures. A few nonpolar lipids, such as esters of fatty acids, which were possibly formed by hydrolysis of lipids by caustics, are known to hinder the lamellar layer formation (5). It is also probable that removal of short-chain fatty acid esters derived from glycerides and phosphatides by freeze-drying promoted the formation of lamellae.

The film produced from the oilseed soapstocks may have a variety of applications as a carrier and encapsulating agent of chemicals and biochemicals. Although there are numerous applications for encapsulation using biodegradable film, a particular application for drug delivery was considered. Since the film is made of amphiphiles, applications may be possible either in a lipophilic or hydrophilic environment. Therefore, it is highly probable that this oilseed-derived film may be useful for encapsulating and delivering drugs and other biomaterials in animal and human applications.

Previous reports (7) cited the use of modified liposomes for targeted drug deliveries in human applications. Since the film produced from the oilseed soapstocks has a similar lipid composition, one can see the potential for commercial utilization of the film. Because the film is also soluble in water and forms a mesophase, use of this film may range from parenteral, dermal drug formulations to cosmetic applications.

For cottonseed soapstock, the presence of gossypol may pose a problem in drug delivery or other applications. Gossypol removal from the oilseed soapstock is technically feasible and has been demonstrated by Pons *et al.* (18) by extracting with methyl ethyl ketone or a similar solvent system. Therefore, it is obvious that after removing gossypol by Pons' method (18), the potential applications of cottonseed soapstock-derived film should be as good as those for safflower soapstock-derived film.

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