TABLE II

Methyl Ester Composition (%) of Fractions Obtained in Experiment 3.

Methyl esters	Fraction A (31.2)[a]	B (27.7)	C (32.1)
Oleate	99.7	0.7	0.4
Linoleate	0.0	98.5	1.0
Linolenate	0.0	0.0	92.0
Unknown	0.3	0.8	6.4

[a] Figures in parentheses indicate the weight of the fraction in mg.

TABLE III

Analysis of Fractions Obtained in Experiment 5

Methyl ester % GLC	Fraction A[a] (2.6)[b]	B (72.7)	C (1.0)[c]	D (21.0)
Monoene	.....	100[c]	.....	93
Diene	.....	0	.....	6
Triene	.....	0	.....	1

[a] Fraction A was crystalline and is presumed to be methyl stearate.
[b] Figures in parentheses indicate the weight of fractions in mg.
[c] I.R. analysis indicated 90% *trans*-double bonds in fraction B, 35% in fraction C, and 0% in fraction D. Fraction C also contained a considerable amount of conjugated diene.

Conclusions

Column chromatography using silica impregnated with silver nitrate can be successfully applied for separations of i) *cis*- and *trans*-isomers of fatty acid methyl esters and ii) fatty acid esters according to degree of unsaturation.

In contrast to separations by means of Hg(II) acetate no chemical operations preceding and following the above-mentioned chromatographic procedure have to be carried out.

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Suspensions of High-Melting Triglycerides[1,2]

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Abstract

A method for preparing stable oil/water suspensions of cottonseed stearine, tristearin, tripalmitin, trimyristin, methyl stearate, and palmitic acid in concentrations up to 10% with minimum concentrations of stabilizing agents is described. Using 2.5% of polyethylene glycol 400 monostearate (based on weight of hard fat), 0.1% of Pluronic F 68, and 0.2–0.25% of Carbopol 934 (the concentrations of these two agents are based on the weight of the aqueous phase), suspensions of the hard fats were prepared by simple stirring, were stable for at least one month at room temperature, and could be sterilized. The size of the dispersed fat particles was 20–40 μ. Apparent viscosities of cottonseed stearine suspensions at 2, 5, and 10% concentrations were 3.59, 5.95, and 6.62 poises at 25C, respectively. Suspensions as described should have utility in those areas of investigation in which solid fatty materials in the form of stable dispersions are desirable.

Introduction

MANY INVESTIGATIONS have been conducted in the general field of specificity and rates of enzymatic hydrolysis of liquid fatty materials. Among the investigations which may be cited are those on the specificity of pancreatic lipase (9,10,11) and on the enzymatic hydrolysis of vegetable and animal fats (8,5,3). For such investigations the liquid fatty materials are dispersed in aqueous media and stabilized by suitable emulsifying agents, or by gels (6), and enzymatic activities are then determined at the usually-employed temperature of 37C, although 45C has been used in order to have a liquid system (7). Higher temperatures for obtaining liquid systems would be expected to destroy enzymatic activity.

Relatively few such enzymatic investigations have been applied to normally solid fatty materials because of the difficulty in maintaining stable dispersions of hard fats. Some portion of an emulsifier molecule may be soluble in a high-melting triglyceride above its melting point and an excellent dispersion may form, but upon cooling and solidification of the fat, solubility of the emulsifier apparently decreases and the emulsifier no longer is effective in maintaining stability of the system, and phase separation results. The few reported systems of dispersed hard fat contain relatively low concentrations of the fatty material; Weber and King emulsified the higher fatty acid monoglycerides with sodium cholate, with 4×10^{-6} moles of substrate in 50 cc (14); tristearin was emulsified by Balls and Matlock, who used a mixture of bile and glycerol and obtained a final concentration of 0.1% (1). Dissolving a high-melting fat in a carrier such as a liquid vegetable oil is of limited value because the solubility is less than 1% at room temperature (13), and the oil carrier would thus be in such large concentration as to interfere with the determination of hydrolysis of the dissolved fat.

The purpose of the present investigation was to develop a system in which high-melting fats could be dispersed and stabilized in concentrations up to 10% with a minimum amount of added stabilizing agents, and which systems could be sterilized if need be. Such systems should have utility in those areas of investi-

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gation in which such fatty materials in the form of stable dispersions are desirable, for example the previously mentioned enzymatic studies. The systems described in this report are considered to be suspensions of solid fat in a thickened aqueous phase, rather than emulsions. The products used to stabilize these suspensions normally are considered to be surface active agents, and to some extent may function as such. However, in this report they have been designated as stabilizing agents, rather than the former.

Materials and Methods

High-Melting Fatty Materials. Cottonseed oil hydrogenated to an iodine value (I.V.) of 0.4 (cottonseed stearine) was used to determine the minimum concentrations of stabilizing agents required for a satisfactory suspension. Additional fatty materials used were tristearin, tripalmitin, trimyristin, methyl stearate, glycerol monostearate, and palmitic acid. With the exception of the cottonseed stearine and methyl stearate, these were commercial products, used as received.

Triolein, an unsaturated liquid triglyceride, was used for comparison with the high-melting materials.

Stabilizing Agents. The suspensions were stabilized with polyethylene glycol 400 monostearate (Glyco Chemicals, Division of Chas. L. Huisking & Co.); Pluronic F 68 (Wyandotte Chemical Corp.); and Carbopol 934 (B. F. Goodrich Chemical Co.). Polyethylene glycol 400 monostearate (to be identified as PGS) and Pluronic F 68 are nonionic surface active agents, and previously have been used in the preparation of sterilizable emulsions containing 15% of cottonseed oil (12). Carbopol 934 is a synthetic gum, whose function was to increase the viscosity of the various suspensions. Each of the 3 stabilizing agents were used as received from the manufacturer.

The only hydrolyzable fatty acid moieties in this stabilizer system were the acyl groups of the PGS.

Preparation of Suspensions. The concentration of each stabilizing agent required for producing a stable suspension of the cottonseed stearine was determined by systematically varying the amounts of each in a system containing 2% of the stearine and an aqueous phase. These concentrations were then applied to systems containing 5 and 10% of stearine, and to the other fatty materials in concentrations of 5%, and adjusted if required. The weight of PGS was based on the weight of fatty material; the weights of Pluronic F 68 and Carbopol 934 were based on the weight of the aqueous phase.

Pluronic F 68 and Carbopol 934 in aqueous solution were heated to 65C on a thermostated hot plate equipped with magnetic stirrer. The fatty material and PGS were weighed into beaker, heated to 60–65C, and slowly added to the hot aqueous phase with vigorous stirring to disperse the fat particles. After 5 min, during which the temperature was maintained, the suspension was neutralized with 1N NaOH for gelation of the Carbopol 934, and cooled to 25C with continuous stirring. Each suspension was observed for visual appearance and phase separation.

Order of addition of the 2 phases was not important, as suspensions prepared by either method appeared to be identical. Carbopol 934 solution could not be neutralized prior to its addition to the system without adversely affecting stability of the suspension.

All of the suspensions prepared were of the O/W (oil-in-water) type, since they were easily diluted with water.

Particle Size. Size of the dispersed fat particles in representative 2, 5, and 10% suspensions were determined microscopically at 950×, using a calibrated eyepiece scale. Each suspension was diluted with distilled water so as to contain 1% of the fat prior to particle size determination.

Viscosity. The apparent viscosity and relative flow characteristics of the 2, 5, and 10% stearine suspensions were determined at 25C with a Stormer Viscosimeter.

Stability. Suspensions were allowed to stand undisturbed at room temperature, and any separation of phases noted.

Heat stability was determined by autoclaving a sealed bottle of suspension for 15 min at 121C.

Results and Discussion

Stearine Suspensions. The formulations with cottonseed stearine at concentrations of 2% and varying amounts of stabilizing agents are given in Table I. Those systems containing only 1 stabilizing agent (1–3) separated into 2 phases while cooling, with no apparent differences in rate of separation. Systems 4–10 (with the exception of 6) also separated into 2 phases, separation occurring at somewhat different rates. Systems 4 and 5, with no PGS, were visibly separated about 1 hr after stirring ended; systems 7, 8, and 9 separated very shortly after stirring ended; system 10 separated on standing overnight. System 6 solidified as a homogenous solid product, because of the high content of Carbopol 934. Systems 11 and 12 did not separate, but contained relatively large particles of solidified stearine, 150–200 μ in diameter.

Systems 13–16, with PGS concentrations of 5% and 4 concentrations of Pluronic F 68 and Carbopol, were poor to satisfactory suspensions, system 15 being the best. A majority of the particles of the latter system were 20 μ in diameter, with the largest particle 40 μ. The particles of system 16 were 40–60 μ, and 60–100 μ in system 14. System 13 separated into 2 phases after about 1 hr.

The appearances of typical systems are represented in Figure 1. From left to right, respectively, are shown rapid phase separation (within 1 hr), slow phase separation (approximately 24 hr), and a stable dispersion (stable at least 1 month).

The concentration of PGS could be reduced to 2.5%, as in system 19, and maintain particle size

TABLE I

Systems Containing 2% of Cottonseed Stearine and Varying Amounts of Dispersants

System No.	Dispersants			Appearance of system
	PGS[a]	F 68[b]	934[b]	
	%	%	%	
1	0	0	0.25	Separated
2	0	0.3	0	Separated
3	3	0	0	Separated
4	0	0.3	0.1	Separated on standing
5	0	0.3	0.2	Separated on standing
6	0	0.3	0.3	Solidified
7	1	0	0.1	Separated
8	2	0	0.1	Separated
9	4	0	0.1	Separated
10	8	0	0.1	Separated on standing
11	10	0	0.1	Very coarse
12	15	0	0.2	Very coarse
13	5	0.3	0	Separated
14	5	0.3	0.2	Coarse
15	5	0.1	0.2	Good
16	5	0.1	0.15	Slightly coarse
17	2.5	0.1	0.1	Separated on standing
18	2.5	0.1	0.15	Coarse
19	2.5	0.1	0.2	Good
20	2.0	0.1	0.2	Coarse

[a] Wt % polyethylene glycol monostearate based on wt of fat phase.
[b] Wt % of Pluronic F 68 and Carbopol 934 based on wt of water phase.

equal to system 15. This concentration of PGS (25 mg per g of stearine) appeared to be minimum, as system 20 with 2% of PGS was poor. The probable effect of the PGS is to enable the aqueous phase to "wet" the dispersed solid fat particles (2). King found that gel-stabilized systems are improved by such agents (4).

System 19, containing a minimum concentration of stabilizing agents, had no phase separation during 1 month of storage at room temperature. After autoclaving this system, a very slight separation was noticeable, but the separated material was easily resuspended by shaking. Particle size did not increase.

At both 5 and 2.5% concentration of PGS, the required ratio of Pluronic F 68:Carbopol 934 was 1:2. Ratios other than this did not provide good suspensions, as the dispersed fat particles were grainy.

Systems containing 5 and 10% of stearine were prepared with the same concentrations of PGS and Pluronic F 68 as in systems with 2% stearine content, but with the concentration of Carbopol 934 increased to 0.25%. Particles sizes in these systems were the same as in system 19.

Viscosity. The apparent viscosities of stable suspensions of 2, 5, and 10% stearine content were 3.59, 5.95, and 6.62 poises, respectively, at 25C. In each of these suspensions the concentrations of PGS and F 68 were 2.5 and 0.1%, respectively; the Carbopol 934 content of the 2% stearine dispersion was 0.2%, and of the other two dispersions 0.25%. The apparent viscosities of 0.2 and 0.25% solutions of Carbopol 934 were 0.9 and 2.1 poises, respectively, at 25C.

The increasing apparent viscosities of suspensions with increasing stearine content were not proportional to stearine content.

Each of these suspensions flowed easily in the manner of liquid systems. The fluidity characteristics are indicated by the consistency data in Table II, which represent the rates of flow of the suspensions as functions of the applied force, at 25C. Data for glycerol have been included for comparison.

Other Fatty Materials. With stabilizing systems containing 2.5% of PGS, 0.1% of Pluronic F 68, and 0.2% of Carbopol 934, suspensions of tripalmitin, trimyristin, and methyl stearate at concentrations of 5% were good, particle size was 20–40 μ, and they were stable at room temperature for more than 1 month. A suspension of tristearin at 5% concentration was equally good with the concentration of Carbopol 934 increased to 0.25%.

The palmitic acid system formed soaps upon neutralizing the Carbopol 934 present, and while completely homogeneous, the system solidified.

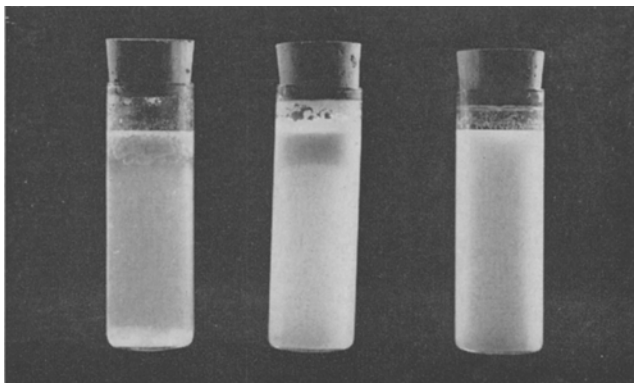

FIG. 1. Systems of high-melting triglycerides representing (left to right) rapid phase separation (within 1 hr), slow phase separation (approximately 24 hr), and a stable dispersion (stable for at least 1 month).

TABLE II

Fluidity Characteristics of Suspensions of Cottonseed Stearine at 25C

Driving force	Rate of flow			Glycerol
	Suspensions, stearine content			
	2%	5%	10%	
g	*rev/sec*	*rev/sec*	*rev/sec*	
20	1.85	1.26	1.24	0.56
50	3.52	2.15	1.92	1.34
75	4.59	3.31	3.05	1.92
100	5.88	4.20	3.94	2.40
125	6.85	4.95	4.59	2.87
150	.....	6.02	5.32	3.32
175	.....	6.85	5.88	3.73
200	.....	.....	6.49	4.10
225	.....	.....	7.81	4.42

Glycerol monostearate precipitated from the system upon addition to the water phase, and could not be suspended.

Triolein, liquid at room temperature, could not be suspended at a 5% concentration with the same concentrations of stabilizing agents used for the high-melting triglycerides. The triolein systems separated into two layers when stirring was completed. This indicates the considerable effect of emulsifier solubility and surface phenomena involved in emulsions or dispersions, and the inability of applying one system of stabilizing agents to materials of widely different physical properties.

Conclusion

The three stabilizing agents PGS, Pluronic F 68, and Carbopol 934 could be used to stabilize suspensions of a variety of hard fatty materials, with small adjustments in concentration of the Carbopol 934 in the range 0.2–0.3%. The concentration of PGS seems adequate at 2.5%, based on the weight of fat; the required ratio of Pluronic F 68:Carbopol 934 seems to be 1:2. The probable effect of the PGS is to enable the aqueous phase to "wet" the dispersed solid fat particles, but only in the presence of Pluronic F 68.

Suspensions of high-melting fat in which the concentrations of stabilizing agents were at a minimum could be prepared only with all three present. They do not function with liquid fats in the same manner as they do with solid fats, nor with glycerol monostearate.

While this investigation has not employed the multitude of stabilizing agents which might provide stable suspensions of high-melting fats, it has described a system in which solid fatty materials in the form of stable dispersions in aqueous media are available.

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