Modification of Vegetable Oils

II. A Cocoa Butter Substitute From Cottonseed Oil[1]

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Introduction

The vegetable butters or solid seed fats include cocoa butter, Borneo tallow, shea butter, Mowrah butter, Chinese vegetable tallow, and several other fats of lesser importance. Of these, cocoa butter, the fat of seeds or "beans" of the tree *Theobroma cacao*, is produced in by far the greatest volume, and is the only member of the group which is of commercial importance in the United States.

The distinguishing feature of the vegetable butters is their very short plastic range. Cocoa butter, for example, is hard and brittle at temperatures below about 80° F. Above this temperature, however, it softens rapidly, and at about 90° F. it becomes completely liquid. The behavior of cocoa butter with heating and cooling is in sharp contrast to that of animal fats and hydrogenated oils, which soften and melt much more gradually, and are plastic over a considerable temperature range.

Because of the fact that they remain hard and free from greasiness at ordinary room temperatures, but melt completely in the mouth, cocoa butter and certain other hard vegetable butters are particularly desirable coating or enrobing fats, for the production of confections, coated sweet biscuits and similar food materials.

Complete statistics relative to the production and consumption of cocoa butter in the United States are not available. However, in 1939 the production exclusive of that used in chocolate coating compositions amounted to 57 million pounds (13). In 1940, 729 million pounds of cocoa beans were imported into the United States, from the following sources (12):

	Million pounds
Gold coast	271.5
Nigeria	138.2
Other Eastern Hemisphere Countries	17.2
Brazil	180.3
Dominican Republic	46.9
Venezuela	24.4
Ecuador	20.1
Other Western Hemisphere Countries	30.4
Total	729.0

The fat content of cocoa beans is slightly greater than 50 percent.

Most of the imported beans are processed into chocolate, without separation of the fat. Considerable quantities, however, are pressed and partially defatted, in the manufacture of beverage cocoa. Some beans are also solvent extracted, to produce fat-free residues suitable for the extraction of theobromine and the preparation of concentrates for "cola" type soft drinks.

Aside from its use as an edible fat, cocoa butter finds a limited but important use in the manufacture of pharmaceutical products. Its physical characteristics make it a particularly desirable base for suppositories and other products which are required to melt at body temperature.

All of the cocoa butter consumed in the United States is imported. The investigation reported here was carried out with the object of developing a suitable substitute for this fat from purely domestic raw materials.

Composition of Cocoa Butter

The fatty acids of cocoa butter are the common C_{16} and C_{18} acids: palmitic, stearic, oleic and linoleic. The relative proportions of the various acids are not greatly different from the proportions prevailing in mutton tallow or other hard animal fats, and are approximately as follows: by weight (8):

	Percent
Palmitic acid	25
Stearic acid	35
Oleic acid	38
Linoleic acid	2

The distinctive physical properties of cocoa butter are due to the manner in which its saturated and unsaturated fatty acids are grouped in the glyceride molecules. According to Hilditch and Stainsby (8), the glycerides of cocoa butter are approximately as follows:

	Percent
Tri-saturated glycerides	2.5
Di-saturated, mono-unsaturated	76.8
Mono-saturated, di-unsaturated	20.7
Tri-unsaturated	0.0

The low melting point of cocoa butter is undoubtedly due to its negligible content of fully saturated glycerides. Its brittleness or lack of plasticity at ordinary temperatures may be attributed to the predominance of di-saturated glycerides and the absence of tri-unsaturated glycerides. Tallow or other animal fats of equivalent fatty acid composition contain substantial proportions of tri-saturated glycerides and corresponding amounts of di-unsaturated and possibly tri-unsaturated glycerides.

Theory of the Process

It is not possible to approach the composition of cocoa butter by hydrogenation of an ordinary vegetable oil, due to the excessive quantities of fully saturated glycerides which are formed during the reaction. Although the hydrogenation process may be made quite selective with respect to the limited formation of saturated acids, there is at present no means known for repressing the formation of saturated glycerides.

It would appear, however, that it should be possible to produce a fat of approximately the desired composition by hydrogenating a vegetable oil to a low iodine value and then removing the fully saturated glycerides from the hydrogenated product by solvent crystallization. Hilditch and Jones (6) have estimated

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the proportions of different glycerides in fats resulting from the selective hydrogenation of cottonseed oil (Table 1). According to these investigators, triunsaturated glycerides disappear from the oil at a relatively early stage in the hydrogenation. A hydrogenated fat with its saturated glycerides removed should consist, therefore, only of di- and mono-saturated glycerides. By choosing a hydrogenated fat of the proper iodine value as the starting material, it should be possible to obtain any desired ratio between di- and mono-saturated glycerides in the finished product.

TABLE 1

The Glyceride Composition of Cottonseed Oil, Hydrogenated to Various Iodine Values (According to Data of Hilditch and Jones)

	Iodine value	Glycerides, percent				Ratio, di-sat. to mono-sat. glycerides
		Tri-sat.	Di-sat.	Mono-sat.	Tri-unsat.	
Analyses by Hilditch and Jones	13.2	65.3	27.4	7.3	0.0	3.8
	20.1	44.0	46.3	9.7	0.0	4.8
Analyses by interpolation	21	44	44	12	0	3.7
	22	44	42	14	0	3.0
	23	43	41	16	0	2.6
	24	42	40	18	0	2.2
	25	41	38	21	0	1.8
	26	39	37	24	0	1.5
	27	38	36	26	0	1.4
	28	37	35	28	0	1.3
	29	36	34	30	0	1.1
Analyses reported by Hilditch and Jones	29.8	33.5	33.2	33.2	0.0	1.0
	42.8	15.9	24.8	59.3	0.0	0.4

The factor which might be expected most seriously to affect the preceding calculations is the unavoidable production, during hydrogenation, of certain isomeric forms of oleic acid. Since these "iso-oleic" acids are higher in melting point than the normal or naturally occurring form of oleic acid, an artificial product with the same ratio of di- and mono-saturated glycerides as cocoa butter (about 3.7 to 1) would probably soften and melt at an unduly high temperature. In order to compensate for the presence of iso-oleic acid, a starting material with a somewhat lower ratio of di- to mono-saturated glycerides would be indicated.

It is also possible that hydrogenation might produce a different configuration within a given class of glycerides from that in the natural fat; e.g., the hydrogenated mono-unsaturated glycerides might have their unsaturated fatty acid in the α-position, whereas in naturally occurring fats it might be in the β-position. There appears to be little information in the literature to indicate either the probability or possible effect of such an occurrence.

Preparation of the Products

The cottonseed oil used in the investigation was a lot of commercially refined and bleached oil. It had a saponification value of 195.9, an iodine value of 108.3, and a thiocyanogen value (with 0.2 N reagent) of 65.9. On the assumption that the thiocyanogen value of oleic acid is 89.3 [2] and the value for linoleic acid is 96.7,[2] the composition of its fatty acids was calculated to be as follows: saturated acids, 27.1 percent; oleic acid, 20.5 percent; and linoleic acid, 52.4 percent.

Three batches of oil were hydrogenated. The conditions of hydrogenation were varied in such a manner as to produce a low content of iso-oleic acids in one case, a medium content of iso-oleic acids in another case, and a high content of these acids in the third case. From each batch, portions of hydrogenated oil were withdrawn, at iodine values within the range of 20 to 30 units. The operating data relative to the hydrogenation, and also comparative analytical data on hydrogenated samples from each batch, are recorded in Table 2.

TABLE 2

Hydrogenation of Cottonseed Oil for the Production of a Cocoa Butter Substitute: Operating and Analytical Data

Operating data:			
Run No.	CO-45	CO-43	CO-44
Catalyst used	No. 1	No. 1	No. 2
Temperature, °F.	250	225	375
Percent nickel (catalyst)	0.1	0.1	0.3
Hydrogen pressure, p.s.i.	50	70	10
Stirring speed, r.p.m.	408	520	290
Pounds of oil	12	12	16
Time to I.V. of 25, min.	169	172	330
Analysis of hydrogenated oil:			
Glycerides			
Iodine value	24.1	25.2	24.1
Thiocyanogen value	21.5	23.6	20.9
Fatty acids:			
Iodine value	25.1	26.1	25.1
Thiocyanogen value	23.4	24.4	22.1
Composition, percentage (calculated from I.V. and T.V.)			
Saturated	73.8	72.7	75.3
Oleic	24.3	25.4	21.6
Linoleic	1.9	1.9	3.1
Solid acids, percent (Twitchell method)	73.1	74.4	80.0
Iodine value of solid acids	7.9	8.6	12.1
Iso-oleic acids, percent (calculated from yield and iodine value of solid acids)	6.4	7.1	10.8
Saturated acids, percent (Bertram method)	71.9	70.5	71.4

Solvent crystallization of the hydrogenated oil was in all cases conducted in two stages, using commercial petroleum naphtha (Skellysolve B) as the solvent, in a ratio of 4 parts by weight of solvent to 1 part of fat.

The first crystallization was carried out by allowing the mixture of melted fat and solvent to stand for 18 hours at 74 to 76° F. The separated crystalline portion was removed by decantation through a paper filter; the filtrate was then recrystallized and filtered at a lower temperature in the apparatus described in a previous communication (2). It was found that the rate of cooling, crystallization time, etc., in the second crystallization were not critical, and could be varied within wide limits without effect on the characteristics of the product.

The two fractions resulting from the first crystallization in each experiment are hereafter designated as the first hard and first soft fractions; the two fractions resulting from the second crystallization are referred to as the second hard and soft fractions. The second soft fraction is, of course, the final product.

Removal of the bulk of the solvent from the various fractions was accomplished by ordinary distillation. The last traces of solvent were removed by bubbling a current of hydrogen through the oil while it was maintained at 120°C., under reduced pressure. In some cases, the fat was finally converted to a bland, tasteless, and odorless product by steam deodorization at 450° F. under a pressure of 1 to 2 mm. of mercury.

The conditions under which the various crystallization experiments were carried out, and the yields of the various fractions are listed in Table 3.

Examination of the Products

For evaluation of the finished products, reliance was placed primarily upon a micro-penetration method, in

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[2] Tentative constants recommended by the Fat Analysis Committee of the A. O. C. S. for 0.2 N thiocyanogen reagent.

TABLE 3

Fractional Solvent Crystallization of Hydrogenated Cottonseed Oils to Produce a Cocoa Butter Substitute: Operating Details and Yields of the Various Fractions

Run No.	81	83	86	88	90	93
Hydrogenated oil used	CO-41	CO-43	CO-44	CO-43	CO-43	CO-45
Iodine value of the oil	30.0	25.0	24.1	22.5	25.0	24.1
Temp. of 1st crystallization, °F.	74-76	74-76	74-76	74-76	74-76	74-76
Temp. of 2nd crystallization, °F.	35	35	35	35	40	35
Yields:						
Original mixture:						
Fat, gms.	400	403	400	400	400	400
Solvent, gms.	1600	1612	1600	1600	1600	1600
First crystallization:						
Hard fraction—						
Fat, gms.	28.4	111.9	135.3	206.6	128.1	150.1
Solvent, gms.	40.4	18.2	89.6	380.3	28.4	38.5
Soft fraction—						
Fat, gms.	374.0	288.1	269.0	187.4	270.3	249.8
Solvent, gms.	1557.0	1590.3	1501.1	1219.8	1569.8	1554.4
Second crystallization:						
Hard fraction—						
Fat, gms.	199.7	170.1	183.4	104.1	133.2	136.3
Solvent, gms.	278.1	226.9	182.8	156.2	169.5	182.4
Soft fraction—						
Fat, gms.	165.2	117.1	80.7	87.3	135.2	111.5
Solvent, gms.	1287.8	1362.9	1323.1	1062.8	1402.0	1373.5
Yield, percent	41.3	29.0	20.2	21.8	33.8	27.9
Max. theoretical yield, percent*	51.3	34.2	24.1	31.7	38.5	32.3

* Assuming a perfect separation of liquid and solid fractions after each crystallization.

which the consistency of the fat at specific temperatures is indicated by the depth to which it is penetrated by a standard needle, dropped from a definite height. The method used was a modification of one first proposed by Freyer (5) and will be described elsewhere by Feuge and Bailey (4). It is well adapted to indicating the extent to which fats exhibit the sharp softening point which is the distinctive characteristic of cocoa butter. The great difference between cocoa butter and an ordinary hydrogenated vegetable oil (margarine oil) is evident from the micro-penetration curves of Figure 1. Micro-penetration curves of some of the modified fats are reproduced in Figure 2.

As another means of judging the new products, the sample which most closely resembled cocoa butter in consistency (No. 93) was subjected to a solidification test, employing the technique recommended by Jensen (9). In this test a 75 gm. sample of the fat, in a 19 x 3.5 cm. test tube, is brought to an initial temperature of 31° C., and is then solidified in a 16° C. air bath. The sample is slowly stirred at the rate of six strokes a minute, with a wire-loop stirrer, after the first crystals appear. The results of this test, in comparison with a similar test on cocoa butter, are shown in graphic form in Figure 3.

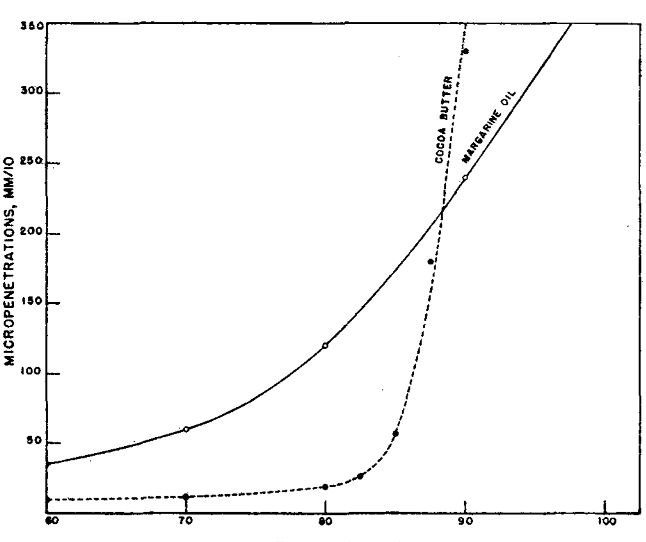

FIG. 1. Consistency of cocoa butter in comparison with ordinary hydrogenated vegetable oil (margarine oil).

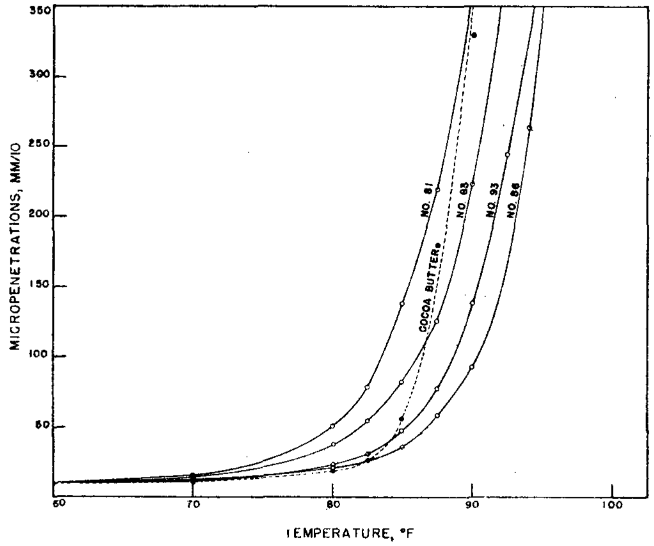

FIG. 2. Consistency of certain of the modified fats, in comparison with cocoa butter.

The volume changes associated with melting of cocoa butter and modified fat No. 93 were investigated by means of the dilatometer (Figure 4). In the dilatometric tests, the dried and degassed sample was solidified by rapid chilling under water and after standing overnight, its temperature was raised stepwise, in increments of 2° and 3° C. After each increase in temperature, the sample was held at the new temperature until its volume had attained equilibrium, as evidenced by constancy in the dilatometer readings over a period of one hour.

The cocoa butter used in the different tests was purchased in a retail drug store, and was a product of a leading American manufacturer of this material.

Analytical data on the finished products obtained in the various experiments are contained in Table 4. In Table 5 is a more detailed analysis of the fractions resulting from a single preparation of fat (No. 93).

The official methods of the American Oil Chemists' Society were used for the determination of saponifica-

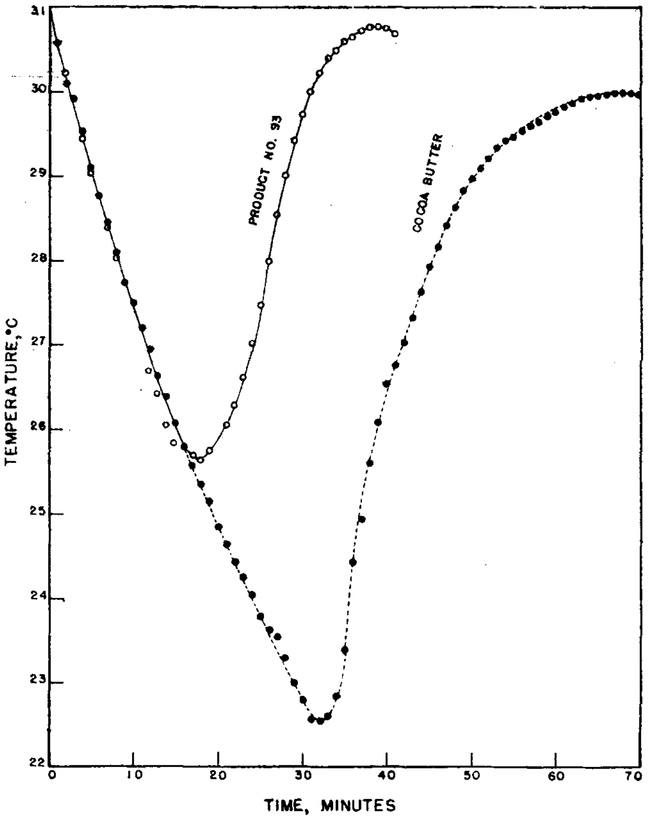

FIG. 3. Solidification test curves, modified fat No. 93 and cocoa butter.

tion values and iodine values, and for the separation of solid and liquid acids by means of their lead salts. Since the hydrogenated oil samples could not be dissolved in the official A. O. C. S. thiocyanogen reagent, the following modification was used in the determination of their thiocyanogen values: The weighed samples were dissolved in a measured volume of carbon tetrachloride, and an equal volume of the official A. O. C. S. 0.2 N thiocyanogen reagent was added, thus giving an effective reagent concentration of 0.1 N. Thiocyanogen values for oleic acid of 89.4, and for linoleic acid of 93.9 were assumed in calculating the compositions of fatty acids from their iodine and thiocyanogen values.[3] For the determination of saturated acids by oxidation, the Pelikan and Von Mikusch modification (11) of the original Bertram method was employed.

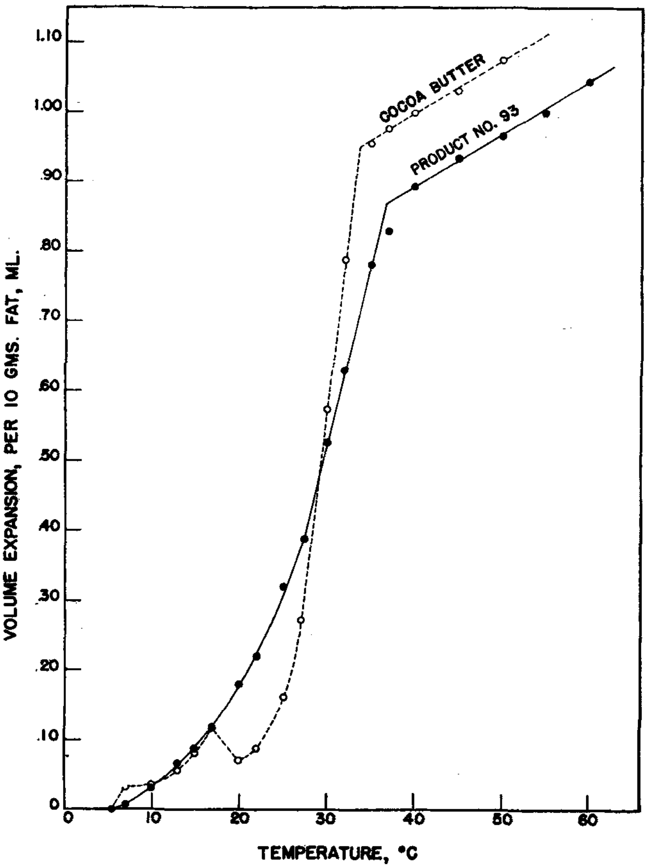

FIG. 4. Dilatometric tests, modified fat No. 93 and cocoa butter.

Discussion of the Results

The new, modified products were in most respects very similar to true vegetable butters. They crystallized to a fine-grained mass, fractured with the "snap" characteristic of good hard butters, and in most cases melted either completely or with practical completeness at 98° F.

Careful examination of the modified products by both chemical and physical methods revealed minor differences between these fats and cocoa butter. Because of these differences, the modified fats appear to be slightly inferior to cocoa butter in some respects, although in other respects the characteristics of the new fats might be expected to prove advantageous. It does not appear to be possible to duplicate the composition and characteristics of cocoa butter exactly by the present process, because of the unavoidable presence in the products of iso-oleic acids.

TABLE 4

Cocoa Butter and Cocoa Butter Substitutes: Analytical Data

Sample No.		81	83	86	88	90	93	Cocoa butter
Iodine value		53.9	47.5	47.3	44.8	45.5	45.1	39.2
Micropenetration, mm/10	60.0° F.	11	11	10	9	8	9	11
	70.0°	16	15	13	13	13	13	12
	80.0°	51	38	22	27	21	24	19
	82.5°	78	54	26	32	27	31	26
	85.0°	138	79	37	42	39	48	57
	87.5°	220	127	59	68	55	77	180
	90.0°	362	224	93	116	90	138	330
	92.5°	400	390	175	266	195	245	400
	95.0°	....	400	370	391	293	400	....
Solidification point, °C.		....	....	....	....	....	30.75	30.00
Temp. rise in solidification test, °C.		....	....	....	....	....	5.10	7.45
Volume change, 20° to 40° C. (cc. per 10 gms. fat)		....	....	....	....	....	.71	.92

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[3] Tentative constants recommended by the Fat Analysis Committee of the A. O. C. S. for 0.1 N thiocyanogen reagent.

TABLE 5

Analysis of the Various Fractions Obtained in the Preparation of Cocoa Butter Substitute No. 93

Fraction	Original hydrog. oil	First hard	First soft	Second hard	Second soft
Iodine value, glycerides	24.1	8.1	33.5	23.8	45.1
Thiocyanogen value, glycerides	21.5	6.9	31.2	21.1	42.0
Iodine value, fatty acids	25.1	8.5	34.9	25.0	47.3
Thiocyanogen value, fatty acids	23.4	8.1	32.7	23.5	44.1
Composition of fatty acids, percentage (calculated from I.V. and T.V.):					
Saturated	73.8	90.9	63.3	73.6	50.6
Oleic	24.3	8.7	34.3	24.9	46.0
Linoleic	1.9	0.4	2.4	1.5	3.4
Solid acids, percent (Twitchell method)	73.1	88.2	69.2	76.1	56.3
Iodine value of solid acids	7.85	2.87	15.05	9.90	20.3
Iso-oleic acids, percent (calculated from I.V. of solid acids)	6.4	2.8	11.6	8.4	12.7
Saturated acids, percent (Bertram method)	71.9	88.8	59.7	70.3	47.7

The modified fats are more unsaturated than cocoa butter; their iodine values varied from 44.8 to 53.9, as compared with a value of 39.2 for cocoa butter. They do not soften and melt quite as sharply as cocoa butter (cf. Figure 2). As a consequence, products which are as brittle as cocoa butter at ordinary temperatures are somewhat higher than this fat in melting point, and products of the same melting point as cocoa butter are softer at lower temperatures. The higher melting point of some of the new products may, however, be advantageous in some respects, as pure cocoa butter is not as resistant to melting at ordinary summer temperatures as might be desired.

The tendency of the modified fats to soften somewhat more gradually than cocoa butter is also evident in the dilatometric tests (Figure 4). The slope of the dilatometric curve for product No. 93 within the range of 25° to 35° is indicative of the more gradual transformation of its glycerides from the solid to the liquid form between these two temperatures.

The modified products do not supercool to the same extent as cocoa butter (cf. Figure 3). In the solidification test one of the new fats exhibited a temperature rise of 5.10° C. as compared with 7.45° for cocoa butter. Jensen (9) considers this rise in temperature significant, and states that fats with a rise of less than 3° are unsuitable for chocolate molding. He gives the following values for the temperature rise in representative samples of different confectioner's fats: cocoa butter, 6.4°; Borneo tallow, 6.1°; palm kernel oil stearine, 2.6°; coconut oil stearine, 1.3°.

The modified fats also do not exhibit the phenomenon of polymorphism to the same pronounced degree as cocoa butter. The polymorphism of cocoa butter was very evident in the dilatometric tests, wherein the temperature of quickly chilled samples of fat was raised in increments of 2° and 3° over the complete range of temperature within which softening and melting occurs. The transformation of a fat from a lower to a higher melting crystal modification is accompanied by a contraction of the material, so that when the temperature of the fat is raised such transformation is evidenced by a slow decrease in the volume of the fat following the rapid increase in volume due to thermal expansion and the melting of fat crystals. Nearly 10 hours were required for cocoa butter to attain a constant volume after its temperature was raised from 17° to 20° C. Under the same conditions a sample of modified fat gave no evidence of any such slow transformation. Polymorphic changes are also indicated by the irregularities in the dilatometric curve for cocoa butter (Figure 4). At 17° and below, cocoa butter contained so large a proportion of low-melting, unstable crystal forms that it actually decreased in volume upon being heated from 17° to 20°.

The significance of the differences in behavior of the two fats upon heating and cooling is not obvious, but it would appear that the tendency of the modified fat to crystallize principally in a stable form might be an advantage in some respects.

Cocoa butter expands and contracts somewhat more than the modified fat upon melting and solidifying. The change in volume of cocoa butter between 20° and 40° C. is 0.92 ml. per 10 gms. of fat (Figure 4). The change in volume of modified fat No. 93, between the same temperatures, is 0.71 ml. It is probable, however, that under rapid conditions of cooling the difference in contraction between the two fats would be less than that indicated in Figure 4, due to the tendency of the cocoa butter to crystallize in low-melting, unstable, forms of high specific volume.

It is apparent from the experimental results that the best products, in the largest yields, are obtained from oil which has been hydrogenated under conditions which produce no more than moderate amounts of iso-oleic acid. The actual yields obtained in the laboratory might be slightly increased in commercial operation, through more efficient separation of the filtrates from the hard fractions. It is possible to calculate, from the percentage of solvent remaining in the hard fractions, the theoretical yields which would result from a perfect separation of the filtrate from these fractions, by washing the separated crystals. These theoretical yields, for the various experiments, are listed in Table 3. It would probably be impossible to attain such yields in practice, but they are of interest, inasmuch as they represent the maximum recovery possible from the process.

The residue of hard fat remaining after separation of the cocoa butter-like fraction is of course suitable for use in the manufacture of blended-type shortenings, etc.

The values reported for iso-oleic acid contents, in Tables 2 and 5, must be regarded as relative rather than absolute, due to the present lack of a reliable method for the estimation of these acids in highly hydrogenated oils. It is evident that the separations of iso-oleic acid must have been variable, inasmuch as a poor materials balance with respect to this acid is obtained amongst the various fractions. It has been noted previously, by various investigators (3, 7, 10) that the Twitchell method gives lower values for saturated acids than are obtained by other methods. In the present case, the official Twitchell method apparently fails to effect even a near quantitative separation of the saturated acids, inasmuch as estimations of saturated acids by this method are much lower than those calculated from iodine and thiocyanogen values, or determined by the Bertram oxidation method.

The iodine and thiocyanogen values of the various products indicate the presence of small percentages of linoleic acid. It is uncertain whether these indicated percentages are real or apparent only, and due to the *limitations* of the thiocyanogen method, as applied to highly hydrogenated fats. If the Bertram method is assumed to represent the true saturated acid contents of the samples, calculation upon this basis shows no linoleic acid. Common experience with the hydrogenation process would indicate the presence of an appreciable linoleic acid content in cottonseed oil hydrogenated to an iodine value of 25 to be improbable.

Summary

1. Selective hydrogenation, followed by fractional crystallization from a solvent, has been used to prepare from cottonseed oil a hard butter, very similar to cocoa butter.

2. The new product differs somewhat in composition from cocoa butter, due to an unavoidable content of iso-oleic acid.

3. Examination of the new product by a micropenetration technique, by a standard solidification test, and by means of the dilatometer, reveals minor physical differences between it and cocoa butter.

4. The new fat has a slightly longer plastic range than cocoa butter. It supercools less strongly and contracts slightly less upon solidification. It exhibits the phenomenon of polymorphism to a less pronounced degree than cocoa butter.

5. A relatively low yield of the new fat is inherent in the process used for preparing it. In the case of the product most closely resembling cocoa butter, a yield of approximately 28 percent was obtained in the laboratory. The residual 72 percent is suitable for use as a hardening agent in shortening and similar products.

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The Application of the Ferric Thiocyanate Method to the Determination of Incipient Rancidity in Fats and Oils[1]

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A recent paper from this laboratory (1) describes a colorimetric method for determining fat-peroxides in whole milk powder. The method is based on the oxidation of ferrous to ferric iron by the peroxides present and the colorimetric determination of the ferric iron as ferric thiocyanate.

Subsequently the procedure was modified for use in the development of a practical antioxidant for lard and shortening (5). The details of the method, as applied to fats and oils, are given in this paper, and the results of a comparative study with other methods are also presented.

Description of the Method

1. *Preparation of the Reagent:* The reagent consists of a solution of 0.1% ferrous ammonium sulphate and 0.4% of ammonium thiocyanate in 96% acetone. The ammonium thiocyanate is weighed into a volumetric flask and an amount of distilled water equivalent to 4% of the final volume is added. The salt is dissolved, the solution allowed to stand for 5 to 10 minutes, and then brought almost to volume with anhydrous acetone and thoroughly mixed. (Neglect of this last operation may result in a cloudy solution when the final ingredient is added.) The ferrous ammonium sulphate is added, the solution diluted to the mark with acetone, and thoroughly shaken. The reagent is kept in the dark for two hours, shaking frequently during the interim. After filtering through an acetone washed filter paper, or decanting from the undissolved ferrous salt, the reagent is ready for use.

Acetone which has not already been used in the test must be carefully purified by distilling it from a small amount of ferric chloride, drying over calcium chloride, and redistilling before use. Spent solvent does not require any addition of ferric chloride since ferric-ions are already present, but it must be carefully dried and redistilled since the water content is critical.

2. *Preparation of the Calibration Curve:* A standard of reference curve (1) is prepared from readings obtained with standard solutions of ferric chloride (0.2 to 14.0 μg. per ml.) in purified anhydrous acetone. A 1 ml. aliquot of the solution is added to 9 ml. of reagent consisting of 0.4% ammonium thiocyanate in 96% acetone. The intensity of the color is measured with a Coleman spectrophotometer set at 485 mu. The color, which develops without heating, fades

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[1] Macdonald College Journal Series No. 186.