3. In survival studies 14 out of 18 rats fed 20% MCT were alive after 2 years; of their controls fed 20% lard, 10 out of 19 survived.
4. Reproduction studies in females gave equally poor results on unsupplemented low-fat, MCT, and LCT diets regarding implantation, birth weight, and survival rate. The weaning weights of the young on MCT were however the highest. With 2% LA weaning weights were equally high with LCT and MCT but lower with low-fat diet.
5. In animals fed low-fat diets not supplemented with LA, low serum cholesterol was associated with high liver cholesterol. With MCT, serum values were higher and liver values were significantly lower. With unsupplemented LCT, serum and liver values were high. When the three diets were supplemented with 2% LA, there were no longer any differences in the serum levels and in the liver levels. Whether or not the presence of some oleate in the MCT and LCT influenced the cholesterol results is not certain.
6. The differences in the effects of MCT and LCT are discussed.

Acknowledgments

We are indebted to William Scott for the U. V. analysis and to S. F. Herb for the gas chromatography of the MCT. These analyses were carried out at the Eastern Regional Research Laboratory of the U. S. Department of Agriculture.

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[Received December 18, 1958]

Glyceride Structure of Vegetable Oils by Countercurrent Distribution. IV. Cocoa Butter[1,2]

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COCOA BUTTER is a fat of unusual physical properties. It melts completely at 33°C. or slightly below body temperature; it is a hard brittle solid at normal room temperatures. Cocoa butter is used in confectionery products largely because its physical properties contribute to glossy coatings, absence of stickiness, and favorable volume changes in the molding operation. Because of the demand for the properties cocoa butter imparts, large quantities of the bean are imported even when domestic fats are in plentiful supply.

The unique melting characteristics of cocoa butter are a consequence of the arrangement of the fatty acids in its glycerides. This fact is illustrated by comparison of cocoa butter with mutton tallow, which is similar to it in fatty acid composition but different in physical properties and unsuitable for use as a confectionery fat. Recent work has shown that oleic acid is predominately in the 2 position in cocoa butter (3, 11, 12, 15) and that palmito-oleo-stearin is the main glyceride.

The glyceride composition of cocoa butter has been investigated by Hilditch and Stainsby (8) and by Meara (13), using fractional crystallization techniques. Recently countercurrent distribution has been shown to be an effective technique for the fractionation of glyceride oils (5, 16, 17). Experimental results obtained with this technique and described and discussed in this paper, are in general agreement with previous data of Hilditch (8) and of Meara (13).

Experimental

Iodine values were determined by the Wijs procedure (1), which was scaled down to allow for the small samples available. Linoleic acid was determined by the 45-min. alkali isomerization method of Brice *et al.* (2). Oleic acid content was calculated from the iodine value and the linoleic acid content, and saturated acids are reported as the difference between the sum of the unsaturated acids and 100%. Stearic acid was determined by a modification of the Nijkamp chromatographic procedure (14). Palmitic acid was

[1] Presented at Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Exposition of Modern Laboratory Equipment, Pittsburgh, Pa., March 3, 1958.

[2] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned number 991 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

[3] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

taken as the difference between total saturated acids and stearic acid.

The cocoa butter used in these experiments is typical of cocoa butter generally (7). It had an iodine value of 37.6. Fatty acids derived from it were composed of 3.6% linoleic acid, 35.5% oleic acid, and 60.9% total saturated acids (made up of 34.6% stearic acid and 26.3% palmitic acid).

Countercurrent distribution was carried out in an automatic 200-tube apparatus in a manner similar to the procedure used to fractionate linseed, soybean, and safflower oils (5, 16, 17). The solvent system consisted of 3.8 liters of furfural and 3.8 liters of nitroethane equilibrated with 10 liters of pentane-hexane. Each tube of the apparatus required 40 ml. of the lower nitroethane-furfural phase. The pump was adjusted to deliver 6 ml. of upper phase to tube 0 at each decantation stage.

Cocoa butter was prepared for introduction in the apparatus by dissolving 15 g. in 5 ml. of the upper phase. Because of the changes in interface position resulting from the use of so large a sample, two transfer stages and two equilibrations were carried out in graduated separatory funnels, using 6-ml. portions of upper phase and adjusting the lower layers each time to 40 ml. by adding the appropriate solvent layer. The solutions were then transferred to the first three tubes of the apparatus, and distribution was continued automatically. Minor shifts in the interface continued for several more stages; these were corrected by removing lower phase and adding upper phase ahead of the samples as previously described (5, 16, 17).

Because cocoa butter glycerides are separated with greater difficulty than linseed or soybean glycerides, it was necessary to apply a greater number of transfers. This was possible by using recycling procedures (4). Preliminary distributions had shown that 750 transfers could be applied before the more rapidly moving components would overtake and mix with the more slowly moving components. Accordingly 750 transfers were applied, using the recycling procedure, and then 400 more transfers were applied to remove effluent fractions in the automatic fraction collector.

Selected fractions were washed three times with equal volumes of 75% ethanol to remove small amounts of nonvolatile solvents and then transferred to tared flasks. Volatile hydrocarbon solvent was removed under vacuum at room temperature. The weight of the fractions is plotted *versus* transfer number in Figure 1.

Results and Discussion

The weight curve for cocoa butter does not show a clear-cut separation into glyceride groups containing the same number of double bonds as was achieved with linseed and soybean oils. This results partly from the closeness of partition coefficients of cocoa butter glycerides and partly from complications arising out of the large amount of palmitic acid present. Palmito-glycerides move through the countercurrent distribution apparatus less rapidly than do stearo-glycerides. In this respect palmitic acid resembles oleic acid so that the observed fractionation results from both differences in unsaturation and chain length. Even though it is not readily apparent from the weight curve, fractionation has taken place, as shown by the variation in the composition of the fractions.

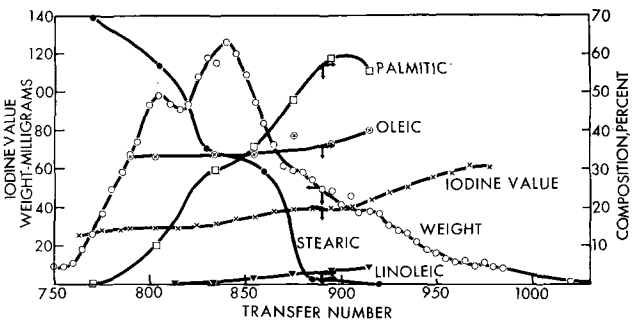

FIG. 1. Countercurrent fractionation of cocoa butter glycerides with a pentane-hexane, furfural, nitroethane solvent system.

The weight curve in Figure 1 is composed of two partially separated major peaks; the long shoulder on the right is indicative of incomplete separation of several minor components. Large fractions in the center, which make up most of the weight, have iodine values near the 28.5 to 30.5 values of stearo- and palmitomono-oleins, respectively. However iodine values of smaller fractions range from 25.2 on the left side of the graph to 64.6 on the right. The low iodine value indicates a small amount of trisaturates in the less polar glycerides on the left; the high iodine value indicates glycerides containing at least three double bonds on the right portion of the curve. Stearic acid is concentrated at the left of the curve in the first fractions which emerge from the instrument, whereas the highest values for palmitic acid are found in the shoulder on the right of the weight curve. The highest value observed for stearic acid is 70.5%. If the saturated acids above 67% in the small fractions at the left are assigned to tristearin, it amounts to only about 0.16% of the total cocoa butter glycerides. All fractions containing stearic acid in excess of 33% must contain oleodistearin. This figure may be calculated from the excess stearic acid, that is, the amount not attributed to tristearin. If the remainder of the stearic acid above 33% is assigned to oleodistearin, a value of 22% is obtained.

In the same way palmitic acid values above 33% may be assigned to oleodipalmitin and linoleodipalmitin. However, since the linoleic acid content is small in these fractions, the linoleodipalmitin has been neglected. Then the oleodipalmitin calculated from the palmitic acid is equivalent to 12%.

The area of the curve between the portions assigned to oleodistearin and oleodipalmitin is equivalent to 51% of the glycerides. This area centers around the largest fractions in the weight curve near transfer 845. The equimolar portions of stearic, palmitic, and oleic acids indicate that these fractions must be mainly oleopalmitostearin. If a correction for two-double-bond glycerides is made, based on iodine value, there is left a value of 41% for oleopalmitostearin. The fractionation obtained was not adequate to permit an estimation of other glycerides. However iodine value and fatty acid composition of the fractions indicate that monounsaturated glycerides containing linoleic acid as well as diunsaturated glycerides must be present.

The cocoa butter samples analyzed by Hilditch and Stainsby and by Meara have fatty acid compositions quite similar to our sample. A comparison of our data on glyceride composition with their results as summarized by Meara is shown in Tables I and II.

TABLE I

Comparison of Cocoa Butter Samples

	Hilditch and Stainsby		Meara	Present work
	1927 Sample	1935 Sample		
Iodine value	37.1	36.7	36.7	37.6
Stearic acid, weight %	34.5	35.4	35.4	34.6
Palmitic acid, weight %	24.4	24.4	24.4	26.3
Oleic acid, weight %	39.1	38.1	38.1	35.5
Linoleic acid, weight %	2.0	2.1	2.1	3.6

TABLE II

Glyceride Composition of Cocoa Butter (%)

	Hilditch and Stainsby	Meara	Present work
SSS	.....	.....	0.16
Trisaturates	2.5	2.6	.....
SSO	18.4	22.0	22.0
PPO	6.5	3.7	12.0
SOP	51.9	57.0	41.0
SOO	12.0	5.8	.....
POO	8.4	7.4	.....
OOO	.....	1.1	.....

The chief difference seems to be the somewhat larger oleodipalmitin and lower oleopalmitostearin contents found in our work.

A number of patterns have been proposed to describe the glyceride composition of fats, and it is of interest to compare the values shown in Table II with the composition predicted by some of the proposals. Table III elaborates the glyceride composition of our sample of cocoa butter calculated for a random, strict even, and Kartha partial random distribution, and compares these values with our experimental results. From these data it is clearly evident that cocoa butter does not follow a random pattern; such a pattern would require almost 10 times as much trisaturated glycerides as have been reported by Hilditch.

TABLE III

Glyceride Composition of Cocoa Butter Calculated for Random, Even, and Kartha Distribution and Compared with Experimental Results

Glyceride	Random	Even	Kartha	Experimental results
SSS	4.14	.....	0.41	0.16
SPS	9.44	.....	1.01	.....
SSO	12.75	0.6	21.97	22.0
SPP	7.18	.....	0.82	.....
SOP	19.38	84.5	36.25	41.0
SSL	1.29	.....	1.89	.....
SOO	13.08	4.0	7.35	.....
SPL	1.97	.....	3.79	.....
PPP	1.82	.....	0.23	.....
PPO	7.37	.....	14.92	12.0
POO	9.94	.....	5.96	.....
OOO	4.47	.....	0.46	.....
SOL	2.65	10.9	1.52	.....
PPL	0.75	.....	1.82	.....
POL	2.02	.....	1.24	.....
SLL	0.13	.....	0.11	.....
OOL	1.36	.....	0.14	.....
PLL	0.10	.....	0.09	.....
OLL	0.14	.....	0.02	.....
LLL	0.005	.....	0.001	.....

The rule of even distribution is regarded by Hilditch (9) as a generalization "which covers the general trend of the observed facts more or less adequately." However, in order to make quantitative comparisons with other systems and with the experimental data, it is necessary to adhere to a "strict" even distribution even though, as indicated, conformance to a rigidly quantitative pattern was not proposed by the originator of the pattern.

Since cocoa butter contains more than 33 mole percentage of both stearic and oleic acids, each glyceride must contain both stearic and oleic acid under a strict even distribution. Then only the four constitutionally different glycerides listed in Table III are permitted. Six simultaneous equations involving fatty acid composition, iodine value, and the sum of the triglycerides can be written in a way similar to that described for soybean oil (6). The values obtained for the glycerides will differ slightly depending upon which four equations are chosen for the calculation. The values listed in Table III are based upon the four equations involving fatty acid composition.

Hilditch (8) noted signs of something more than mere even distribution. Meara (13) points out that the values found for stearopalmito-olean are much smaller than expected under an even distribution. He suggests a modified even distribution in which only two-thirds of the oleic acid forms stearopalmito-olein, and the remainder occurs as oleodistearin and oleodipalmitin.

Recently Kartha (10) has proposed a modified random distribution pattern in which an upper limit is placed upon the amount of trisaturated glycerides that may be present. He considers the remaining fatty acids to be randomly distributed.

Assuming 2.5% trisaturates, a calculation by the method of Kartha indicates 80.6% disaturated monounsaturated glycerides. This figure differs only slightly from the value of 84.5% calculated for a strict even distribution. However the method of calculation outlined by Kartha may be extended to the individual glycerides. When this is done as shown in Table III, great differences between the even and Kartha distributions become apparent in oleopalmitostearin and oleodistearin contents. The experimental compositions seem to be consistent with the Kartha pattern. Information on the amounts of the constitutionally different glycerides of cocoa butter is still too incomplete to decide definitely whether they do or do not fit such a distribution. The predominance of oleic acid in the 2 position of the glycerides (3, 11, 12, 15) implies the presence of some directing force, *e.g.*, enzymatic, in addition to random esterification. However, as Vander Wal (18) has pointed out, the presence of this directing force is not inconsistent with a "modified random" distribution since Kartha's theory makes no attempt to show how acyl groups are distributed within the triglyceride molecule.

Summary

Cocoa butter has been fractionated by countercurrent distribution between pentane-hexane and furfural-nitroethane solvent phases with the application of 1,100 transfer stages. Except for a small percentage of trisaturates and linoleic acid-containing triglycerides, oleic acid occurs at least once in each glyceride molecule. Cocoa butter is composed principally of mono-oleins: oleodistearin, 22%; oleopalmitostearin, 41%; and oleodipalmitin, 12%. Whereas the latter glyceride is not permitted under a pure even pattern, the low trisaturate content is not consistent with a random pattern. Cocoa butter follows neither a random nor an even pattern of glyceride structure.

Acknowledgment

The authors are grateful to J. C. Cowan for his interest and encouragement and are indebted to J. C. Cannon, Barbara Grimshaw, and Mary Good for their technical assistance.

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[Received March 5, 1959]

Recovery of Gossypol from Cottonseed Gums[1]

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CONSIDERABLE attention has been given to methods for the isolation of gossypol from cottonseed (1). These procedures all involve a preliminary solvent extraction of gossypol from defatted meats, or isolated pigment glands, by use of solvents such as diethyl ether or acetone. While satisfactory for small-scale preparations, the excessive costs of solvent extraction systems for the removal of a constituent present to the extent of about 1% in the cottonseed kernel mitigate against large scale use of such systems.

Cottonseed gums, obtained as a by-product, on water-washing of crude cottonseed oil processed from the seed by direct extraction, contain considerable quantities of gossypol and phosphatides (2, 3, 4) and offer a practical source of gossypol. The relatively mild conditions employed in the commercial degumming process (2) should minimize the oxidative degradation of the gossypol in the gums. It has been estimated that at one solvent-extraction plant, these gums are presently produced at an annual rate of about 2,000 tons, representing a potential source of some 200,000 lbs. of gossypol. It has also been reported that considerable amounts of gossypol can be removed from conventional screw-pressed and prepressed oils by a degumming process similar to that employed for solvent-extracted crude oils (5).

The present investigation was undertaken with the view of developing a practical process for the isolation of gossypol from this by-product of cottonseed processing and to make available sufficient amounts of gossypol to explore potential uses of this unique and reactive compound.

Basic Investigations

The proximate composition of cottonseed gums as produced by a commercial degumming process (2) is recorded in Table I. Analysis of the acetone insoluble fraction disclosed that about 70% of the total gossypol in the gums is segregated in this fraction, from which it cannot be removed by repeated acetone extractions. This suggested that most of the gossypol in gums is present in a "bound" form, presumably in chemical combination with phoshatides. Other exploratory experiments indicated that it would be necessary to cleave this gossypol combination product and to separate gossypol from the considerable amounts of surface-active phosphatides prior to its isolation.

TABLE I

Proximate Composition of Cottonseed Gums

Constituent	Range (4 samples)
	%
Moisture (6)	41.0 –44.9
Neutral oil (6)	12.4 –16.4
Total phosphorus (7)	1.16– 1.26
Phosphatides (P × 25)	29.0 –31.5
Acetone solubles (6)	17.2 –20.9
Acetone insolubles (6)	37.1 –40.3
Total gossypol (8)	4.33– 5.46

By use of mild acid hydrolysis in methyl ethyl ketone (MEK), in which both water and phosphatides have limited solubility, a fairly simple procedure was devised for cleaving the gossypol and separating it from phosphatides. In the process the gums are refluxed with MEK that contained either oxalic or phosphoric acids, using a gums-to-solvent ratio of about 1:1. Upon cooling, the mixture separates into a supernatant MEK phase which contains most of the gossypol and a lower phosphatide-water phase. The lower phase is then washed with MEK to remove practically all of the gossypol. After concentration of the combined MEK decantate and washings by distillation, addition of glacial acetic acid to the concentrate allows isolation of gossypol as the crystalline acetic-acid addition compound. The crude gossypol acetic acid is purified by two recrystallizations. Relatively pure crystalline gossypol is obtained from the pure gossypol-acetic acid complex by dispersing the complex in dilute aqueous sodium carbonate, then acidifying with a mineral acid.

Processing Variables. Optimum conditions of time and acid concentration for hydrolysis and for precipitation and purification of gossypol were established. Cottonseed gums, 500 g., were refluxed with 500 ml. of MEK containing the desired acid concentration and cooled to 50°F. After removal of the MEK supernatant the lower phase was washed by vigorous mixing with four successive 100-ml. portions of solvent. Combined decantates and washings, usually 800 ml., were concentrated by distillation to about 300 ml., and gossypol-acetic acid was isolated

[1] Presented at the 50th Annual Meeting of the American Oil Chemists' Society, New Orleans, La., April 20–22, 1959.
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