duction but also for future expansion and future innovations.

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[Received January 11, 1954]

Glyceride Composition of Fats and Oils Determined by Oxidation and Crystallization Methods[1]

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THE first application of oxidation methods to the study of glyceride composition was made by Hilditch and Lea (3). They described a method for determining trisaturated (GS_3) glycerides by oxidation of the fat in acetone solution with powdered permanganate, followed by aqueous potassium carbonate washes, which remove the acidic products from the unchanged GS_3. Attempts by several investigators (1, 4, 7) to extend the oxidation method for the determination of disaturated (GS_2U), monounsaturated (GSU_2), and triunsaturated (GU_3) glycerides by means of fractionation of the azelaoglycerides produced were unsuccessful, owing partly to hydrolysis of these products during the oxidation.

Kartha (7, 8, 9) however showed that hydrolysis can be prevented by maintaining an excess of acetic acid during the oxidation of the fat in acetone solution with permanganate. He described a procedure for separating the azelaoglycerides into two fractions, analyses of which provided a basis for calculation of the amounts of GS_2U, GSU_2, and GU_3 in the fat. He proposed an interesting hypothesis concerning the pattern of distribution of glycerides elaborated by plants and animals. The pattern is a restricted random distribution. The synthesis or elaboration of the fat by the plant or animal is thought to be of random character, but according to the nature of the plant or animal some cannot tolerate the amount of GS_3 produced. The amount of GS_3 not desired is transformed by interchange of saturated acids with unsaturated acids available from GSU_2 and GU_3. The extent to which any GS_3 enters into this rearrangement depends on the difference between the amount of this glyceride found experimentally and the amount calculated according to random theory. The effect of this rearranged "excess GS_3" superimposed on an otherwise random distribution can be calculated. Kartha found that the glyceride distribution by his oxidation method was in good agreement with that calculated according to his hypothesis for 27 fats and oils investigated. He also reported that the glyceride distribution of fats determined by either oxidation or crystallization methods does not agree in general with that calculated according to Hilditch's rule of even distribution (5).

Systematic fractional crystallization of fats from acetone at several low temperatures and analyses of fractions produced have been employed by some (6, 12, 13) as a basis for estimating the distribution of the principal classes of glycerides. Crystallizations in a much simpler way have been used to determine only the trisaturated glycerides (10).

Kartha (7) did not make any direct comparisons between results by his experimental method and those by crystallization methods on identical samples of different fats. Therefore it was considered of value to make such direct comparison on a number of dissimilar fats and oils. The results form the basis of the present report; calculations of composition according to random pattern and according to Kartha's hypothesis are included.

Experimental

Lard, palm oil, chicken fat, and cottonseed oil comprised the dissimilar fats and oils selected for investigation of glyceride composition. The lard was of high quality and composed of 25% "killing" and 75% "cutting" fat. The palm oil was from Sumatra and was alkali-refined in the laboratory. The chicken fat was rendered in the laboratory from fresh adipose tissue removed from around the gizzard and from body cavity fat depots. The cottonseed oil was of good quality and was alkali-refined.

In the oxidation method for determining glyceride composition, the procedures used for oxidizing the fat, separating the azelaoglycerides, and calculating the glyceride composition were the same as those originally described by Kartha (7, 8, 9). Briefly, the azelaoglycerides formed in the oxidation were separated as their Mg salts into soluble and insoluble fractions from an aqueous medium. The weight of the azelaoglycerides in the insoluble fraction was obtained, and the saturated acid content of each fraction was then found by the Bertram procedure. From these data the amounts of GS_2U and GSU_2 were calculated. The GS_3 was determined independently by the crystallization method described in this paper, and the GU_3 was obtained by difference.

In the crystallization method a series of fractional crystallizations from acetone at different temperatures was employed to separate the fat into relatively simple fractions, each of which contained substantially not more than 2 of the 4 classes of glycerides. The sequence of crystallization steps as outlined in Chart 1 was somewhat different from that reported in previous work (13), but, in general, the treatment and method of calculation of glyceride composition

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[1] Presented before the American Oil Chemists' Society, New Orleans, La., May 4-6, 1953.

were the same. Only dry, redistilled acetone was used, and the acetone solutions of fats were held at the crystallization temperatures overnight before being filtered. The crystallizations and filtrations at 0° and 15°C. were conducted in constant temperature rooms, those at —25° and —45° in a low-temperature cabinet equipped for vacuum filtering and for maintaining the funnel and filter flask within the refrigerated space. In every case the precipitate obtained was pressed firmly on the Büchner funnel and washed with acetone (2-3 ml./g.) precooled to the temperature of the crystallization.

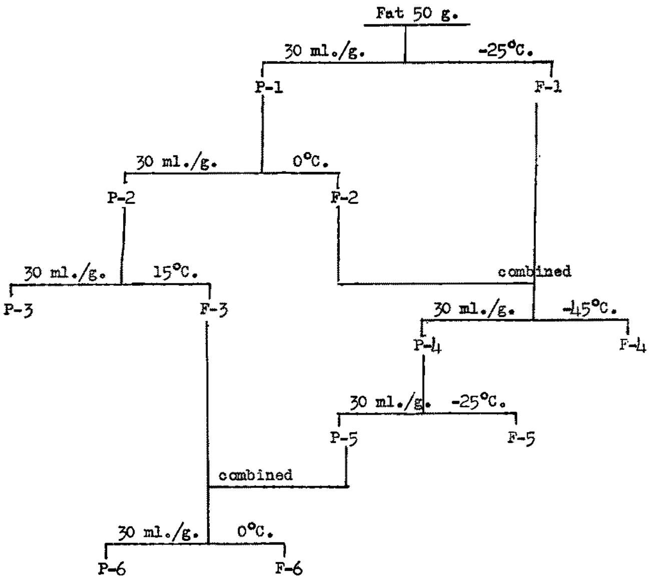

Chart 1. Steps in the fractional crystallization of fats from acetone.

Results and Discussion

Table I shows the fatty acid composition and related analyses of the four fats and oils. The cottonseed oil had an unusually high iodine value and linoleic acid content although it is within the range reported (11) for 48 different lots of cottonseed oil.

TABLE I

Analyses of Lard, Chicken Fat, Palm Oil, and Cottonseed Oil

Analyses [a]	Lard	Chicken fat	Palm oil	Cottonseed oil
Iodine number	66.2	78.5	50.1	115.2
Sapon. equivalent	285.5	284.7	279.8	286.9
Oleic acid, %	47.07	52.10	36.61	13.70
Linoleic acid, %	11.65	15.60	8.78	59.65
Linolenic acid, %	0.84	1.03	0.35	....
Arachidonic acid, %	0.30	0.27	......	....
Pentaenoic acid, % [b]	0.08	0.10	......	....
Saturated acid, %	40.06	30.90	54.26	26.65

[a] The polyunsaturated acids were determined spectrophotometrically (2), and oleic from the iodine value corrected for polyunsaturated acids; the acids are expressed as glycerides in percentage mol.

[b] Calculated as equal mixture of C_{20}-C_{22} pentaenoic acids.

Table II shows the glyceride composition of the series of fats and oils, determined by the oxidation and the crystallization methods. Considering the complexity of the fats and the many steps and analyses involved in both methods, the agreement of results by the two methods is reasonably good for lard, chicken fat, and cottonseed oil but relatively poor for palm oil.

There are a number of possible sources of error inherent in each method. The oxidation method is subject to losses of material in extraction of oxidation products at several stages, in extraction of fatty acids, and in the determination of saturated acids in the precipitate and filtrate fractions. The last-named source of error is probably the most serious because many fats contain 1 or 2% of myristic acid, which would be lost in the Bertram magnesium sulfate precipitation. If a loss of 1% of saturated acids occurred in the filtrate fraction, it would amount to 3% when calculated as GSU_2.

TABLE II

Glyceride Composition of Lard, Chicken Fat, Palm Oil, and Cottonseed Oil Determined by Oxidation and Crystallization Methods

Type of glyceride	Lard		Chicken fat		Palm oil		Cottonseed oil	
	Oxid.	Cryst.	Oxid.	Cryst.	Oxid.	Cryst.	Oxid.	Cryst.
	% mol.	*% mol.*	*% mol.*	*% mol.*	*% mol.*	*% mol.*	*% mol.*	*% mol.*
GS_3 [a]	(2.8)	2.8	(2.3)	2.3	(9.4)	9.4	(0.0)	0.0
GS_2U	24.6	27.4	18.3	17.9	47.4	48.1	13.0	14.5
GSU_2	58.9	54.8	44.2	49.2	30.5	39.3	47.7	51.0
GU_3	13.7	15.0	35.2	30.6	12.7	3.2	39.3	34.5
Sm [b]	38.8	39.4	29.2	30.4	51.2	54.6	24.6	26.7

[a] The GS_3 value was determined by the crystallization method, and was applied also to calculation of composition by the oxidation method.

[b] Sm = % mol. of saturated acids (as glyceride).

In the crystallization method slight material losses may occur in the handling of the various fractions. There are also other possible sources of error. The saturated acids in each fraction are determined by difference after analyses for unsaturated components by the spectrophotometric method and the iodine value. The percentages of saturated acids determined in this manner are usually from 0.5 to 2% higher than those determined by the Bertram method. The fractions obtained by crystallization are assumed to contain no more than 2 of the 4 classes of glycerides; slight contamination by a third class would be expected in some fractions.

Recently Quimby *et al.* (12), who used a somewhat different crystallization procedure, reported glyceride distribution for lard differing appreciably from that found in the present work and also from that reported in a previous publication by Riemenschneider *et al.* (13). It will be noted however that the former authors found 39.6% of saturated acids (calculated as acid in total acids from Table II) in lard whereas they account for only 34.4% of saturated acids in their reported glyceride composition. This apparent discrepancy of 5% of saturated acids, when calculated as GSU_2 and correspondingly less GU_3, would bring their glyceride composition in good agreement with the values reported in the present work and also with those reported by Riemenschneider *et al.* (13).

Table III shows comparisons of glyceride compositions determined by the two experimental methods, with that calculated by random or restricted random (Kartha's hypothesis) distribution. The method for calculating glyceride distribution on the basis of the restricted random hypothesis has been published recently (9); that based on random theory has also been described (13). The values by either method for lard, palm oil, and cottonseed oil are not in close agreement with those calculated by random or restricted random distribution patterns. There is good agreement however between the values by the oxidation method for chicken fat and those by both random and restricted random distribution hypotheses.

The sources of error in either experimental method would affect the determination of GSU_2 and GU_3 to the greatest extent because any error in saturated acid determination is magnified three-fold when cal-

TABLE III

Comparison of Glyceride Compositions (% mol.) Determined by Oxidation and Crystallization Methods with Values Calculated by Random and Restricted Random Distribution Hypotheses

	Oxidation					Crystallization				
	Sm	GS_3	GS_2U	GSU_2	GU_3	Sm	GS_3	GS_2U	GSU_2	GU_3
Lard										
Experimental	38.8	2.8	24.6	58.9	13.7	39.4	2.8	27.4	54.8	15.0
Restricted Random	......	2.8	32.4	43.1	21.7	......	2.8	33.4	43.0	20.8
Random	......	5.8	27.7	43.6	22.9	......	6.1	28.3	43.4	22.2
Chicken Fat										
Experimental	29.2	2.3	18.3	44.2	35.2	30.4	2.3	17.9	49.2	30.6
Restricted Random	......	2.3	18.4	43.9	35.4	......	2.3	20.0	44.2	33.5
Random	......	2.5	18.1	43.9	35.5	......	2.8	19.3	44.2	33.7
Palm Oil										
Experimental	51.2	9.4	47.4	30.5	12.7	54.6	9.4	48.1	39.3	3.2
Restricted Random	......	9.4	45.1	35.2	10.3	......	9.4	52.2	31.0	7.4
Random	......	13.4	38.4	36.6	11.6	......	16.2	40.6	33.8	9.4
Cottonseed Oil										
Experimental	24.6	0.0	13.0	47.7	39.3	26.7	0.0	14.5	51.0	34.5
Restricted Random	......	0.0	15.8	42.3	41.9	......	0.0	18.4	43.3	38.3
Random	......	1.5	13.7	42.0	42.8	......	1.9	15.7	43.0	39.4

culated as GSU_2, or conversely, in obtaining GU_3 by difference.

Summary

The glyceride composition of four dissimilar fats and oils was determined by two independent methods a) systematic fractional crystallization from acetone, and b) Kartha's modification of Hilditch's acetone permanganate oxidation method. Results by the two methods were in fair agreement for lard, chicken fat, and cottonseed oil but not for palm oil.

Calculations were also made of glyceride distribution according to the patterns of random and restricted (Kartha's hypothesis) distribution. The values calculated for either pattern however did not agree well with those obtained experimentally by either method, except for one of the four fats, chicken fat.

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[Received November 24, 1953]

Phase Relations Pertaining to the Solvent Winterization of Crude Peanut Oil in 85-15 Acetone-Hexane Mixture

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THE winterization of refined peanut oil requires the use of a solvent (1). Unless solvent refining is used, the production of a winterized oil from solvent-extracted peanut oil would involve complete removal of solvent twice, once before refining and again after winterization. Solvent winterization of the crude miscella would be advantageous in that one of these solvent-removal steps would be eliminated. The purpose of the present investigation was to determine, on a laboratory scale, the conditions under which solvent winterization of crude peanut oil is feasible.

Preliminary experiments with crude peanut oil showed that, as had been found (2, 4) for the refined oil, a mixture of 85 parts of acetone and 15 parts of hexane by weight is a preferred winterization solvent. When hexane is used as the solvent, the crystals which form present serious filtration difficulties. Satisfactory crystals are formed in acetone, but two liquid layers form at the required temperatures and oil-solvent ratios involved, interfering with the efficient removal of the solid from the liquid. By using the 85-15 acetone-hexane mixture as solvent, satisfactory crystals are formed and the separation into two liquid layers is avoided. Voogt and Seeles (5) used a mixture of acetone and benzene in the winterization of neat's-foot oil.

Materials. The commercial crude peanut oil used had the following characteristics: iodine value (Wijs), 93.9; free fatty acids as oleic, 1.14%; peroxide value, 2.7 millimoles per kg.; unsaponifiable matter, 0.41%; and moisture and volatiles, 0.07%. A commercial C. P. acetone was used. The commercial hexane was Skellysolve B.[2]

Procedure. The winterization procedure was the same as previously described in detail (2, 4). In brief, duplicate weighed samples of the desired concentrations were chilled in a constant temperature bath for a definite holding-time. The samples were then centrifuged in a refrigerated centrifuge for 30 minutes

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[2] The mention of the names of firms or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.