CHARACTERISTICS OF FREE FAT OF DRY WHOLE MILK

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ABSTRACT

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This study was undertaken to determine the compositional characteristics of the free fat fraction of dry whole milk. Adsorption chromatography on silicic acid and gas-liquid chromatography were employed to ascertain the major lipid components and the glyceride fatty acid composition, respectively, of the total lipid and free fat.

Lipid analyses by silicic acid chromatography indicated that free fat contained lower concentrations of phospholipid and free fatty acids than the total fat. Fatty acid studies by gas chromatography revealed that the free fat contained slightly higher proportions of C_{10}-C_{18} saturated acids and lower amounts of C_{18} unsaturated acids than the total lipid. These differences in fatty acid distribution were enhanced during storage. Data obtained from stored powders were inconclusive in regard to changes in the relative proportions of the major lipid components.

The results of this study indicate that the free fat extracted from dry whole milk with a mixture of petroleum and ethyl ethers is not substantially different from the total lipid.

CHARACTERISTICS OF FREE FAT OF DRY WHOLE MILK

By

Karin L. Lindquist

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INTRODUCTION

Reconstituted dry whole milk has never gained wholehearted acceptance as a beverage. Both flavor and appearance are not characteristic of normal fresh pasteurized milk. Because these defects are found to a much lesser extent in non-fat dry milk, the fat of whole milk powder has been blamed for these adverse characteristics. In particular, the guilt has been placed on that portion of the fat, usually referred to as "free" fat, extracted from whole milk powder with non-polar organic solvents.

Although researchers have hypothesized that the deleterious effects of free fat are on the flavor, dispersion and wettability of dry whole milk, the chemical composition of free fat has not been elucidated. This study was undertaken to determine if the composition of the free fat from a given whole milk powder is representative of the total fat or is somehow unique. Because off-flavors due to fat oxidation are important in dry whole milk, changes in the total lipid and free fat characteristics during storage of the powder under adverse conditions were studied.

REVIEW OF LITERATURE

Definition of "Free" Fat

In a normal extraction of the lipid of milk, alcohol and/or ammonia are used to degrade the fat globule membrane and to liberate protein-bound lipid thus enabling the extraction of the fat with common fat solvents. However, in dry whole milk, a portion of the total fat can be extracted with non-polar organic solvents alone. Holm and Greenbank (1925), believing that this fat fraction was not protected by a protein film, used the term "free" fat to describe the lipid which they obtained from dry whole milk by extraction with carbon tetrachloride. Following this work, all fat fractions extracted from whole milk powder with benzene (Favstova and Boika, 1958), petroleum ether (Litman, 1955), ethyl ether (Lampitt and Bushill, 1931), carbon tetrachloride (Greenbank and Hufnagel, 1953), carbon disulfide (Lampitt and Bushill, 1931), or hexane (Janzen, McGugan and Swanson, 1953) have been referred to as free fat or surface fat. The extraction technique has also varied. Shaking the powder and solvent (Thomas, Holgren, Jokay and Bloch, 1957), allowing the powder and solvent to stand (Lampitt and Bushill, 1931) and extracting the fat by the Soxhlet method (Nickerson, Coulter and Jenness, 1952) represent techniques.

Occurence of Free Fat

To comprehend the existing theories concerning free fat formation, some knowledge of the physical structure of spray-dried milk is desirable. The particles are small spheres containing interior air cells with protein

and small fat globules evenly dispersed in lactose, which is amorphous in fresh spray-dried milk (Coulter, Jenness and Geddes, 1951; Lampitt and Bushill, 1931; King, 1948; Choi, Tatter and O'Malley, 1951.)

At the present time, the reasons for the occurence of free fat are not completely understood. It has been hypothesized that the solids-not-fat portion of the powder particle and/or the fat globule membrane serve to protect the majority of the fat of dry whole milk from solvation (Lampitt and Bushill, 1931).

The contribution of lactose to the occurence of free fat was first proposed by Lampitt and Bushill (1931). They reported a liberation of free fat and an exhibition of optical activity when optically inactive fresh, spray-dried milk attained specific moisture levels by absorbing water from humid atmosphere. From this evidence they deduced that some of the lactose changed from an amorphous to a crystalline state at a "critical moisture content" of 8.6% to 9.2%. The significant increase in free fat at this point was attributed to lactose crystals which mechanically reptured the solids-not-fat mass rendering the fat accessible by solvents. In further support of this hypothesis, they observed that fresh, untreated roller-dried milk, which notably has much higher amounts of free fat than spray-dried milk, is optically active, indicating the presence of lactose crystals.

In agreement with Lampitt and Bushill (1931), King (1948) concluded, from optical activity studies, that fresh spray-dried milk contained only amorphous lactose, while both fresh roller-dried and stored spray-dried milk contained crystalline lactose. Also, reconstituted portions of these three powders exhibited surface fat which he called "demulsified" fat, comparable to the free fat of Lampitt and Bushill (1931). King (1948)

observed increasing amounts of surface fat in fresh spray-dried milk, stored spray-dried milk and fresh roller-dried milk in that order. To account for the quantitative differences in free fat among the three samples, he considered both the physical state of lactose and the fat globule membrane. He hypothesized that the characteristically low amount of free fat obtainable from fresh spray-dried milk was due to the impermeability of the amorphous lactose by the solvent, while in roller-dried milk, a mosaic of tiny lactose crystals formed capillary spaces admitting solvent to the interior fat. However, even in this powder he would not expect to extract fat if the fat membrane was undamaged. Probably the shearing effect of lactose crystals present in roller-dried milk and the increased denaturing effect of the higher temperatures used in roller drying collaborate to "demulsify" the fat thereby allowing its extraction with non-polar organic solvents. These two factors, although operative, occur to a lesser extent in spray-dried milk than in roller-dried milk thus accounting for the lower amounts of free fat.

By combining microscopy with special staining techniques to distinguish particle constituents, King (1955) was able to observe the mode of fat distribution in dry milk. In spray process powder which was fresh or stored under favorable conditions, he reported that tiny fat globules were distributed uniformly throughout the particle and that there was no apparent fusing of fat globules or fat patches on the surface of the particles. But under unfavorable, high-moisture storage conditions some of the fat was liberated as indicated by the appearance of coalesced fat around the interior air cells and on the surface of the powder particle. He also observed that particles of spray-dried milk which contained free

fat exhibited birefringeance in polarized light due to the crystallization of lactose, whereas powder possessing well dispersed fat showed no birefringeance. These observations support the reputed importance of the physical state of lactose in the occurence of free fat.

Choi, Tatter and O'Malley (1951) also studied the effects of moisture and 95% ethanol on the crystallization of lactose in dry whole milk by determining the quantity of α-lactose hydrate. In accord with Lampitt and Bushill (1931), they reported extensive lactose crystallization in milk powders subjected to a humid atmosphere. For spray-dried whole milk, they observed little lactose crystallization until a 6.5% moisture content was attained in the powder, which was comparable to the 8.6% to 9.2% "critical moisture content" reported by Lampitt and Bushill (1931). Choi et al. (1951) found very little α-lactose hydrate in whole powder treated with 95% ethanol. However, the alcohol treatment caused a hundred-fold increase in powder solubility and increased the free fat value from 8.0% to 25.5%. These investigators considered then that fat liberation by alcohol treatment resulted from protein coagulation rather than lactose crystallization. Contrary to the observations of Lampitt and Bushill (1931) and King (1948, 1955), Choi et al. (1951) found only amorphous lactose in some samples of roller-dried milk. From this evidence they suggested that the sizeable difference in the amount of free fat found in spray- and roller-dried milk was due to the extent of protein coagulation rather than the degree of lactose crystallization.

Significance of Free Fat

Several researchers have called attention to the deleterious effects of free fat on the reconstitutability of dry whole milk. Janzen, McGugan

and Swanson (1953) observed that fresh powder samples were easily wet and dispersed when the free fat was removed. However, after two months of storage at 70° and 110°F. the treated samples no longer displayed the improvement in wettability and dispersibility. Favstova and Boiko (1958) also reported the adverse effects of free fat relative to the keeping quality of the milk powder and the stability of the reconstituted product. Stone, Conley and McIntire (1954) stated that the percentage of total fat present in a milk powder was inversely proportional to the ease of self-dispersion. They did not cite free fat values, but Litman (1955) reported that the percentage of total fat extractable as free fat increased with the fat content of the powder.

Litman (1955) reported the appearance of an insoluble "skin" on the surface of reconstituted whole milk and flaky "scum" specks on the sides of the container, which they believed were complexed free fat and protein. In powder stored at 85°F. they observed a definite increase in scum formation concurrent with a decrease in free fat content. He postulated that free fat in the scum would be stable to non-polar solvent extraction. King (1960) applied his staining techniques to this skin material and observed: 1) clusters, which were conglomeration of casein micelles, individual fat globules and coalesced fat and 2) clumps, which were intermingled free fat and protein with imbedded air bubbles and fat globules.

Tamsma, Edmondson and Vettel (1958) reported that free fat, accounting for up to 40% of the total lipid, had no effect on the dispersibility of stored milk powders, but that higher free fat values, of 40% to 95% decreased the dispersibility of the powder. Reinke (1959) found no statistical correlation between free fat content and ease of self-

dispersion in spray-dried milk containing 10% to 69% free fat.

In addition to the problem of imperfect reconstitution, flavor defects resulting in part from fat oxidation are sometimes encountered in dry whole milk. Some researchers have indicated that the rate of flavor deterioration by fat oxidation increased with an increase in free fat content. Holm and Greenbank (1925) found that the time required for a whole milk powder to absorb oxygen decreased as the free fat content increased. Shipstead and Tarassuk (1953) obtained free fat from milk powder by extraction with petroleum ether and found it to be oxidezed to the point of tallowy flavor and complete loss of color. The fat extracted from the interior of the particle had good flavor and normal color. Greenbank and Hufnagel (1953) reported that the keeping quality of milk powders was inversely proportional to the quantity of carbon tetrachloride extractable free fat. Greenbank and Palansch (1961) studied the progress of fat oxidation in puff-dried whole milk stored at 40°F. Four fat fractions: 1) free glycerides, 2) free lipids (glycerides and phosphatides), 3) total glycerides and 4) total lipids were obtained from the milk powders and were evaluated for oxidation by the peroxide value, the thiobarbituric acid value and taste panel score. They observed that the free glycerides and free lipids began to oxidize two to four weeks before the total glycerides and lipids. Since quantitative data on these fractions were not reported, it is not known if the powders contained relatively high or low amounts of free fat. Reinke (1960) found no correlation between the quantity of free fat and the flavor scores of fresh or stored spray-dried milk containing 10% to 69% free fat.

Two related theories have been presented to explain why free fat

is more easily oxidized than the remaining fat. Washburn (1922) supported Storch's hypothesis that free fat was more easily oxidized since it was not surrounded by a gelatinous envelope which protected the fat from oxidation as long as it remained unbroken. Shipstead and Tarassuk (1953) suggested that in addition to the fat globule membrane all the solids-not-fat aid in the protection of the fat on the interior of the particle from oxidation.

Composition of Free Fat

The chemical composition of this fat fraction extractable from dry whole milk with non-polar organic solvents has not been studied. Litman (1955) reported that the fat portion of the skin material, which he believed was complexed free fat, found on the surface of reconstituted milk, had a lower iodine number and a higher melting point than the fat of the dispersed milk. King (1960) believed that the fat of this complex was a high melting fraction for it exhibited low flourescence, due to a high content of crystalline fat which does not take up dye.

EXPERIMENTAL PROCEDURE

Plan of Experiment

Seven lots of spray-dried whole milk powder were manufactured under commercial processing conditions from mixed herd milk obtained from the Michigan State University Dairy. Raw milk was preheated in a tubular heater at approximately 185°F. for five minutes. The milk was then condensed to approximately 40% total solids in a Rogers single-effect evaporator and dried in a Rogers spray drier at conditions listed in Table I. Manufacturing procedure varied slightly for powders No. 1, No. 5 and No. 7 in that the milk was pasteurized and homogenized prior to condensing.

The fresh milk powders were assayed for total lipid and free fat content and aliquots of each fraction were subjected to glyceride fatty acid analysis by gas-liquid chromatography. The fatty acid analyses were repeated during the storage of powder lots 1, 2, 3, 4 and 6. Total lipid and free fat samples extracted from fresh powders 1, 2, 3 and 7 and the total lipid of the condensed milk from which powder No. 2 was made were analyzed quantitatively for the major lipid components by adsorption chromatography on a silicic acid column. This analysis was repeated on the total lipid and free fat fractions extracted from powders No. 2 and No. 3 following one month of storage. The phosphorous content of the total lipid and free fat obtained from all seven whole milk powders was also determined. The powders were stored at atmospheric conditions in either green sample bottles (lots 1, 2 and 3) or polyethylene bags (lots 4, 5, 6 and 7).

Analytical Methods

Total fat

The solvent extraction method of Mojonnier and Troy (1925) was used to determine total fat in the dry milk powders.

Free fat

The lipid fraction representative of the free fat was extracted with a 50-50 (V/V) mixture of petroleum and ethyl ethers as described by Thomas et al. (1957). A 20 g sample of milk powder was weighed into a glass-stoppered 250 ml Erlenmeyer flask and shaken for one minute with 40 ml of solvent. The powder particles were allowed to settle for one minute prior to filtering the solvent supernatant into a suction flask fitted with a sintered-glass funnel of medium porosity. Following a second extraction, a third 40 ml portion of solvent was shaken with the milk powder for one minute and the entire flask contents were emptied into the funnel. TheErlenmeyer flask was rinsed twice with 20 and 10 ml portions of solvent and the suction filtering was continued until all the solvent was pulled into the flask. The filtrate was transferred to a weighed aluminum dish. The solvent was removed and the weight of the free fat residue was determined. Free fat values were expressed as the percentage of the total lipid of the powder.

Silicic acid chromatography

The components of the total lipid and free fat fractions were resolved by adsorption column chromatography on silicic acid according to the method of Hirsch and Ahrens (1958). The column was charged with approximately 300 mg of lipid. Petroleum ether (b.p. 60°-70°C.) and ethyl ether were used for gradient elution of the neutral fat followed by methanol to remove the phospholipids. The column eluate was collected

in weighed test tubes in 10 ml portions by a Rinco fraction collector. The solvent was evaporated in an oven at temperatures near the boiling point of the solvent phase. The tubes were reweighed under standard conditions in an air conditioned room maintained at 72° to 74°F. and 40% to 50% relative humidity. The weight of lipid residue was plotted on the ordinate against test tube number. The peaks of the resulting chromatograms were tentatively identified by reference to elution volumes of authentic lipids fractionated under the same conditions. When enough residue was available the identification was confirmed by infrared analysis with reference to spectra of standard lipids. The samples, carried in spectrophotometric grade carbon tetrachloride, were analyzed in the 2 to 14 μ range with a Beckman IR-5 infrared spectrophotometer.

Gas-liquid chromatography

Preparation of samples. Methyl esters of the neutral glyceride fatty acids were prepared by base-catalyzed interesterification of the glycerides with methanol as described by Smith and Jack (1954). Approximately one gram of fat in 4 ml of pentane was treated with 3.5 ml of absolute methanol and 0.005 ml of 1 N alcoholic KOH. The reaction mixture was allowed to stand at room temperature for two days after which the reaction was stopped by washing with three 3 ml portions of dilute hydrochloric acid. The methyl esters were extracted with pentane most of which was removed from the esters prior to gas chromatography by passing a gentle stream of nitrogen over the solvent-ester solution.

Column selection and operating conditions. Five and ten foot one-quarter inch (O.D.) copper gas chromatographic columns packed with

butanediol succinate, polyoxyalkalene adipate (Reoplex 400), diethylene glycol adipate cross-linked with pentaerythritol (LAC-446) or diethylene glycol succinate (DEGS) were prepared to find the liquid phase most suitable for the resolution of C_4 to C_{18} fatty acid methyl esters. Column performance was estimated by the 1) separation factor (stearate-oleate) and 2) the height equivalent per theoretical plate (H.E.T.P.) as suggested by Keulemans (1957). A ten foot column of 20% diethylene glycol succinate polyester (DEGS) was found to give the best resolution. The separation factor for the unresolved stearate-oleate pair was 1.15 and the H.E.T.P. was .10 inch. During the course of this study several DEGS columns were employed having approximately these same column parameters.

An Aerograph Model A-90-C Gas Chromatograph equipped with tungsten hot wire thermal conductivity detectors was employed in conjunction with a Leeds and Northrup Type G Speedomax Recorder having a 5 mv scale span and chart speed of 2 min/inch. The following operating conditions were found to be optimum for the resolution of fatty acid methyl esters (C_4-C_{18}) on the DEGS column:

Oven temperature	200°C.
Injector temperature	240°C.
Helium flow rate	50 cc/min
Detector current	265 ma

Qualitative analysis. The method of carbon numbers developed by Woodford (1960) was adopted for qualitative identification of fatty acid esters. A mixture of authentic, even-numbered, saturated acids from four to eighteen carbons and the unsaturated eighteen-carbon-acid esters was chromatographed under standard conditions. The adjusted retention volume, V_R', where

$V_R' = V_R - V_m$

V_R = the volume of gas required to elute the compound under study

V_m = the volume of gas required to elute a non-absorbed gas,

was determined for each ester and a semi-log plot of adjusted retention volumes of the saturated esters versus the length of the carbon chain was made. From the straight line, the carbon number, plotted on the abscissa, for any ester could be determined if its adjusted retention volume was known, see Figure 1. Saturated, straight-chain esters have carbon numbers identical to their chain length while unsaturated esters have non-integral carbon numbers, which are characteristic for a particular ester in a particular stationary phase.

Quantitative analysis. Approximately 0.3 g of each standard ester was weighed accurately into a 5 ml volumetric flask; a known weight of ethyl ether was added to volume. The weight, thus the number of micromoles, of each ester was therefore known for any aliquote of the mixture. One, two, three, five and ten microliter portions of the standard mixture were chromatographed and the areas of the resulting peaks were measured with a planimeter. From these measurements, the quantity-area relationship for each fatty acid ester was established by a geometric plot of the number of micromoles against the corresponding area. The identifiable peaks on chromatograms of samples of unknown fatty acid composition were also measured with a planimeter. Unresolved peaks were separated for measurement by drawing the shortest possible perpendicular line from the recorder tracing to the baseline of the chromatogram. The area percentage for each known fatty acid, based on the total identifiable area, was computed. The unassigned area was not included in this calculation.

Phosphorus

Approximately 0.5 g samples of total lipid and free fat were accurately weighed into ceramic crucibles and were treated with saturated alcoholic magnesium nitrate and ashed overnight in a muffle furnace at 600°C. according to the method of Horrall (1935). The ash was taken up in dilute hydrochloric acid and analyzed for phosphorus by the colorimetric procedure of Fiske and Subbarow (1925). The concentration of phosphorus, estimated from a standard curve, was converted to lecithin by the factor 25.94 (Horrall, 1935).

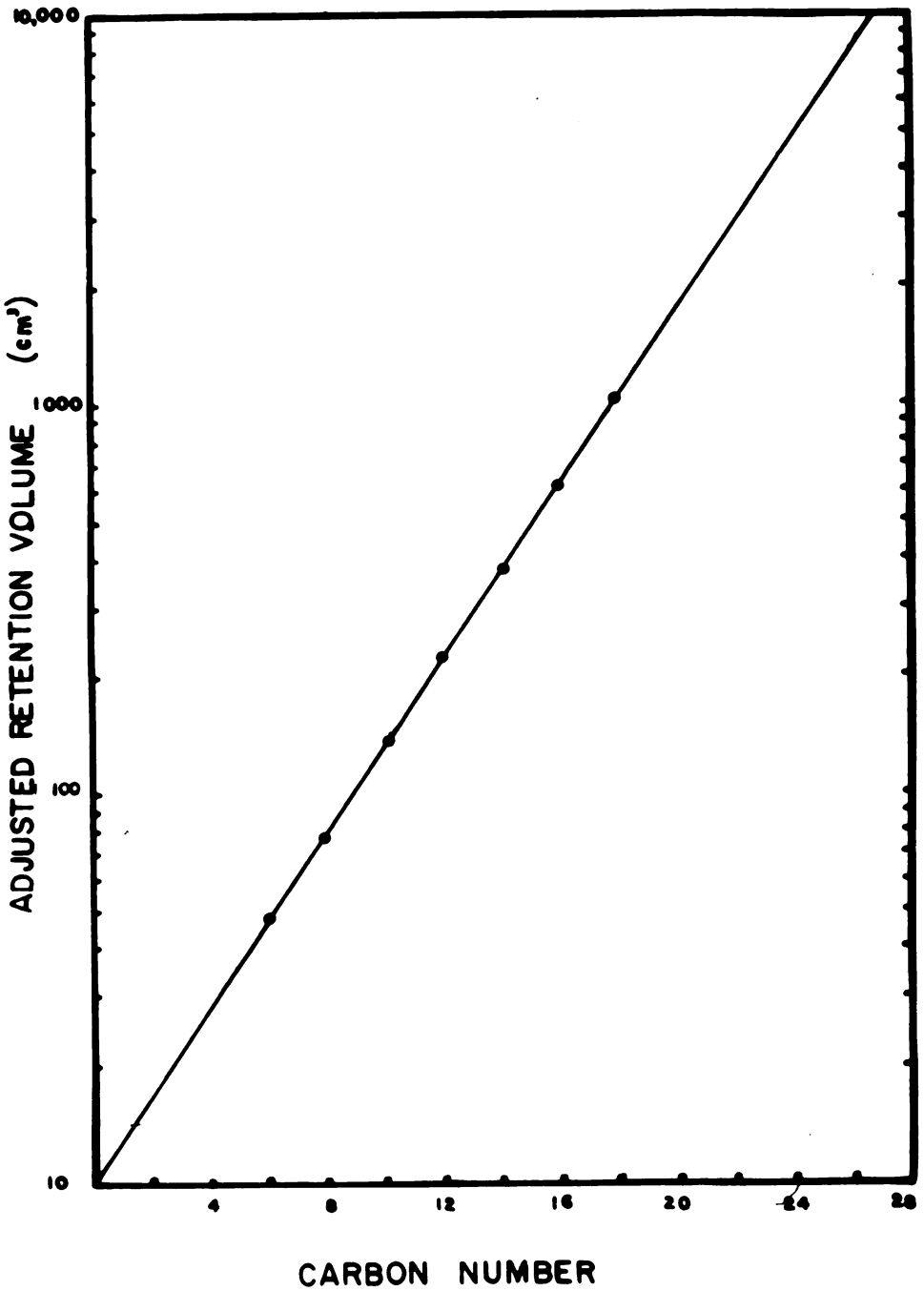

Figure 1. A plot of the adjusted retention volumes of authentic straight chain saturated fatty acids versus chain length for the identification of unknown fatty acids by the method of carbon number.

TABLE I

Manufacturing conditions for spray-dried whole milk powders

Powder lot No.	Manuf. date	Processing conditions[1]							
		Pasteurization		Homogenization prior to condensing	Preheating		Spray drying[2]		
		time	temp		Preheating time	temp	Pump pres.	Nozzle size	Exit air
		(min)	(°F.)	(psi)	(min)	(°F.)	(psi)		(°F.)
1	Sept.	HTST[3]	190	2000	15	185	2500	69/20	195
2	Nov.	--		--	5	180	2000	62/17	220
3	Jan.	--		--	5	190	2000	69/20	200
4	Mar.	--		--	*		2000	69/20	195
5	Apr.	2	195	2000	15	180	2500	69/20	195
6	May	--		--	15	180	2000	69/20	195
7	Aug.	HTST	165	2000	15	185	2500	69/20	195

[1] Vacuum-pan was operated at approximately 95°F.

[2] The incoming air temperature was maintained at approximately 300°F.

[3] HTST: equivalent to time above 165°F approximately 40-50 seconds

* Unknown

EXPERIMENTAL RESULTS

Free Fat Content

The free fat values, which represent the amount of total fat extractable as free fat, of the fresh whole milk powders ranged from 0.3% to 7.7%, see Table II. Although the observation of quantitative changes in free fat during storage was not a primary objective of this study, it was observed that the free fat value of powder No. 2 decreased slightly during six and one-half months of storage from 6.0% to 4.5% while the free fat of powder No. 3 increased from an initial value of 5.9% to 6.9% and 90.3% at five and eight and one-half months of storage, respectively. Similarly the free fat value of powder No. 4 increased from 7.6% when fresh to 86.3% in nine months. Free fat values were not determined for powders No. 5 and No. 6 when fresh, but after five months of storage they were 82.7% and 95.8%, respectively.

Neutral Glyceride Fatty Acids

The fatty acids of the total lipid and free fat samples were expressed quantitatively as percentages of the total identifiable area of the gas chromatograms. These results for all the milk powders are listed in Tables III-VIII inclusive. Quantitative comparisons of individual fatty acids gave no readily discernible information concerning the similarity or dissimilarity of the total lipid and free fat, but by grouping the fatty acids into C_4-C_8 saturated acids, C_{10}-C_{18} saturated acids and C_{18} unsaturated acids, some minor differences between the total lipid and free fat were observed. These groups of fatty acids for fresh powder, powder stored one to four months and powder stored five to nine months were averaged and recorded in Table IX. In all

three age groups the free fat contained slightly more C_{10}-C_{18} saturated acids and less C_{18} unsaturated acids than did the total fat. No differences between the total lipid and free fat were noted for the C_4-C_8 group. No discernible changes in fatty acid composition were observed in either total lipid or free fat during storage.

Gas chromatograms of total lipid and free fat extracted from powder No. 3 after four months of storage, Figures 2 and 3, exhibit peaks typical of the majority of the samples analyzed. Of the estimated twenty-five fatty acid peaks, eleven major acids were positively identified. In addition, Smith (1961) identified six of the minor peaks positively and three tentatively. The carbon numbers of the twenty-five peaks as numbered in Figure 3 are listed and identified in Table X.

Major Lipid Components

Quantitative analysis by silicic acid chromatography of total lipid and free fat extracts are listed in Table XI. The results of the three total lipid and four free fat analyses of fresh powder were averaged separately and also tabulated in Table XI. In these milk powder the free fat contained an average of 5% more triglycerides and slightly lower proportions of free fatty acids, monoglycerides and phospholipids than did the total fat. The phospholipid content determined by a chemical phosphorus analysis of total lipid and free fat from all powders are listed in Table XII. In all seven cases there was less phospholipid in the free fat than in the total fat. No differences in diglycerides or cholesteryl esters were observed.

Unusual results were obtained by silicic acid chromatographic analyses of total lipid and free fat extracted from powders No. 2 and

No. 3 when fresh and after one month of storage. In the free fat extracted from both powders, the triglyceride percentage decreased and the phospholipid proportion increased during storage. The relative amount of triglyceride in the total lipid from powder No. 3 also decreased, but the phospholipid content remained approximately the same. The diglyceride concentration in both the total lipid and free fat increased during the first month of storage.

Chromatograms of total lipid, Figure 4, and free fat, Figure 5, obtained from powder No. 7 when fresh are representative of the column chromatographic analyses. The peaks numbered in Figure 5 are identified by the numerals above the major lipid components in Table XI. Peak 5 has not been positively identified as diglyceride, for no hydroxyl absorption was observed when this fraction was subjected to infrared analysis. However, this material was definitely a glyceride for methylation and gas chromatographic analysis indicated the following fatty acid composition:

Fatty acid	Area percentage
Capric	0.5
Lauric	2.4
Myristic	12.0
Palmitic	33.5
Stearic	11.0
Oleic	35.4

Also, the elution volume of this component is nearly the same as the peak identified by Thompson (1961) as diglyceride in a similar chromatographic analysis. The elution volume for authentic diglyceride has not been determined in this laboratory.

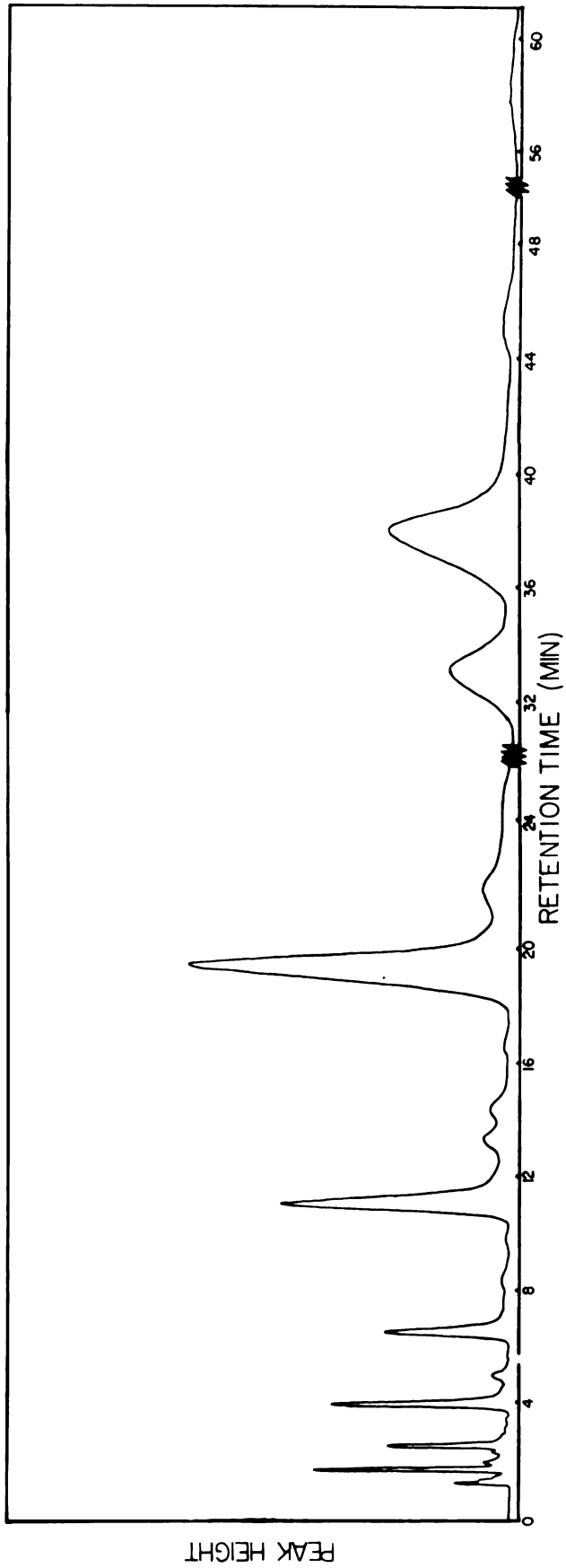

Figure 2. Gas chromatogram of fatty acid methyl esters obtained from the total lipid extracted from powder No. 3 after one month of storage.

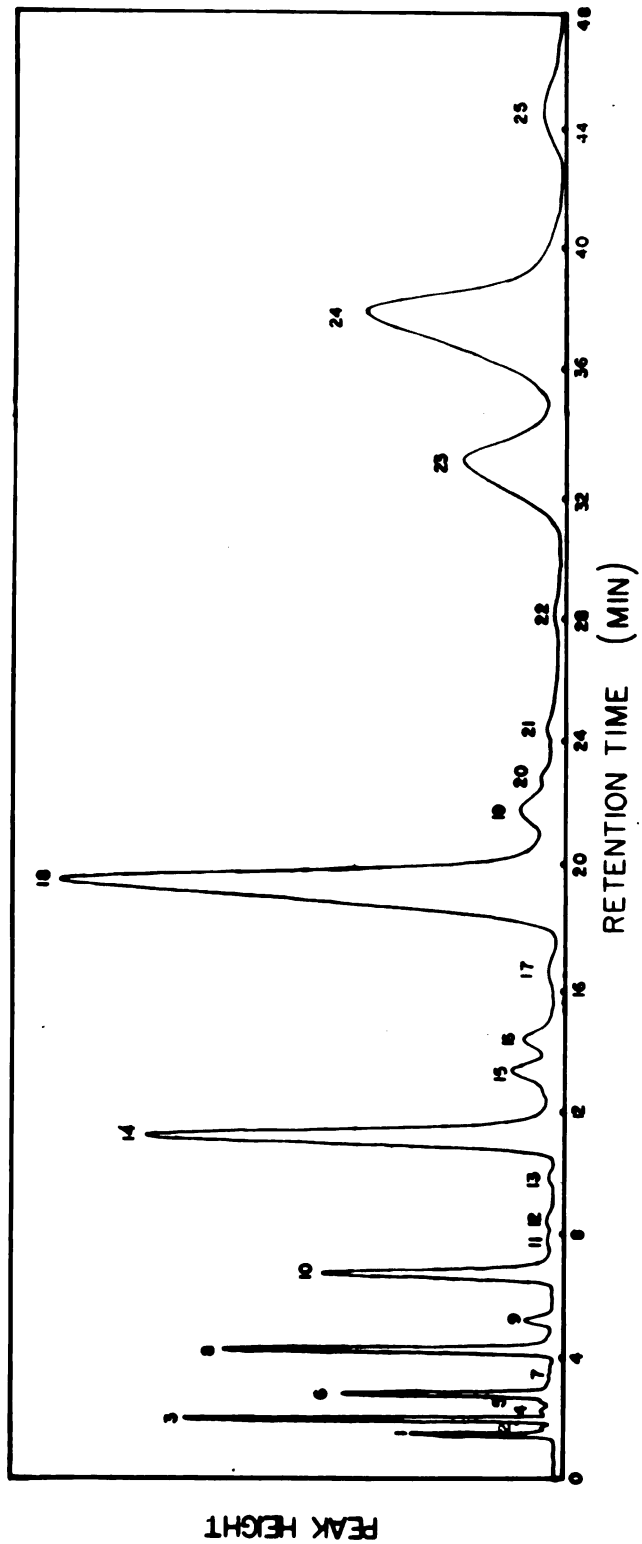

Figure 3. Gas chromatogram of fatty acid methyl esters obtained from the free fat extracted from powder No. 3 after one month of storage.

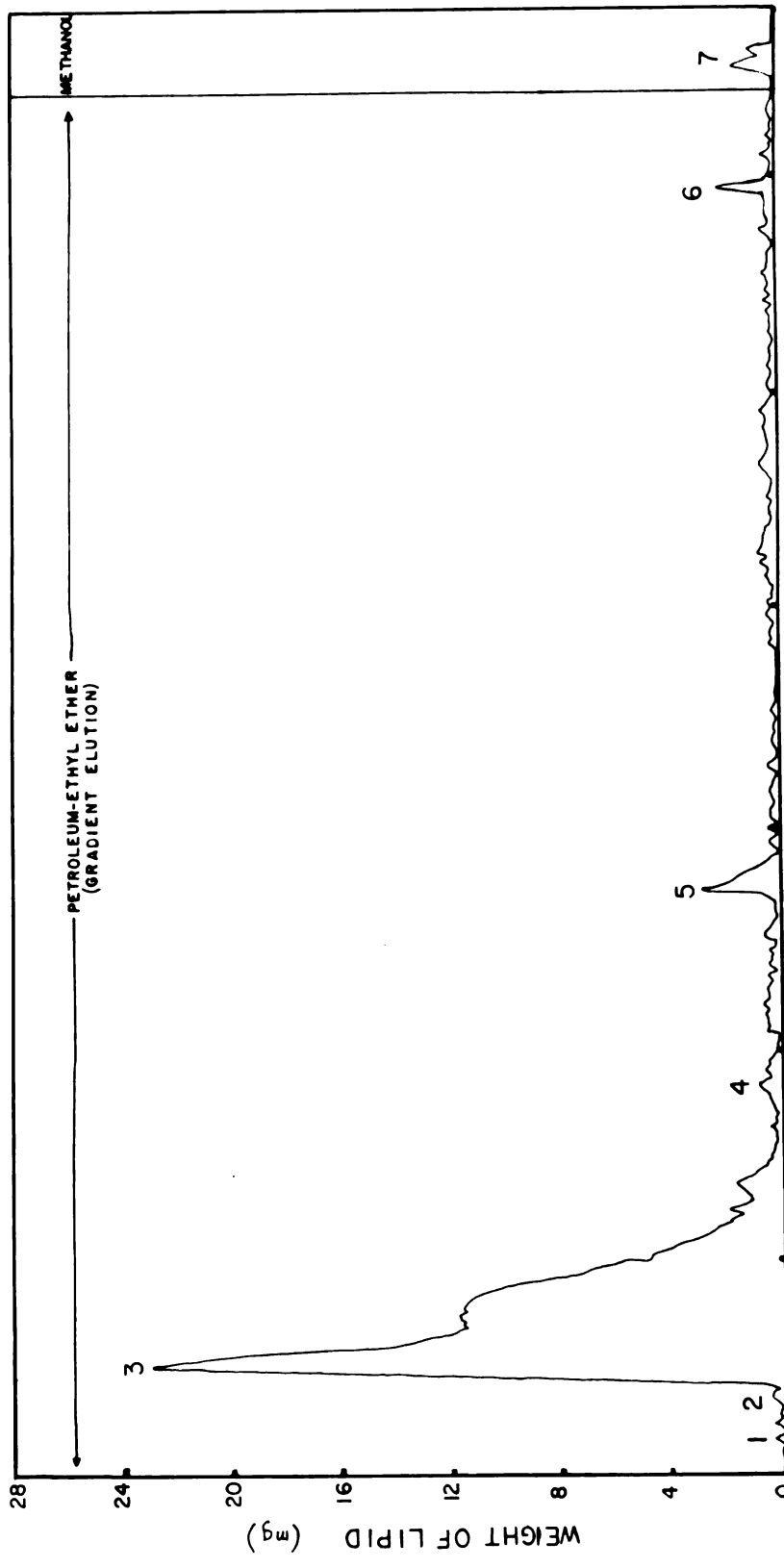

Figure 4. Silicic acid chromatogram of the total lipid extracted from powder No. 7 when fresh.

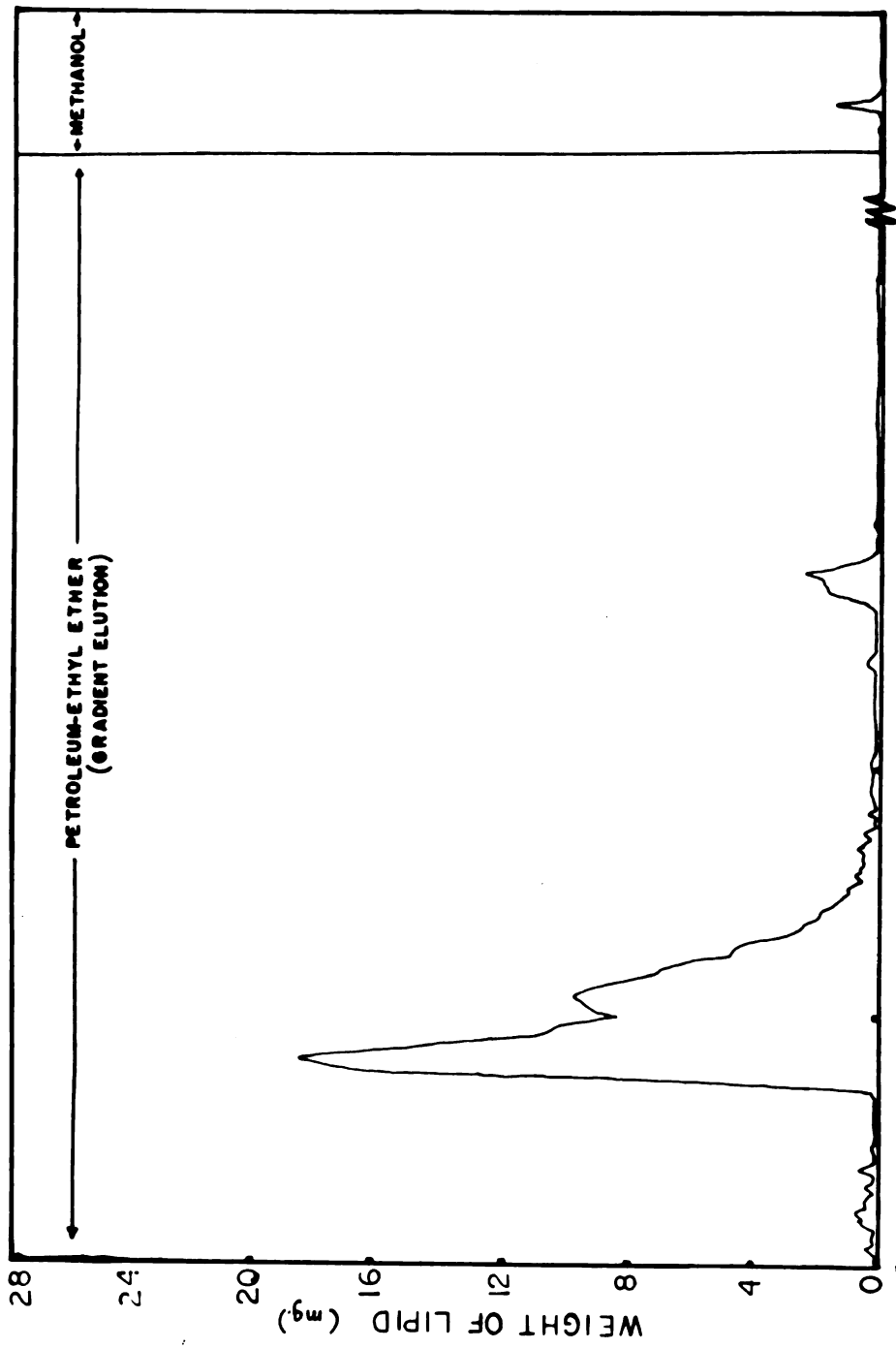

Figure 5. Silicic acid chromatogram of the free fat extracted from powder No. 7 when fresh.

TABLE II

Total lipid and free fat contents of fresh whole milk powders

Powder lot	Total lipid[1]	Free fat	Percentage of total fat extractable as free fat[2]
	(%)	(%)	
1	25.06	0.73	2.9
2	28.30	1.68	6.0
3	25.30	1.50	5.9
4	28.06	2.15	7.7
7	25.60	0.08	0.3

[1] All percentages are based on the total weight of powder

[2] Free fat values

TABLE III

Glyceride fatty acid composition of total lipid and free fat extracted from powder No. 1

Fatty acid	Storage period (months)			
	Fresh		Eight	
	Total	Free	Total	Free
	Area %			
Butyric	4.9	2.9	0.0	0.0
Caproic	4.9	2.2	3.8	1.8
Caprylic	2.4	1.5	2.3	1.4
Capric	5.7	4.4	3.4	3.0
Lauric	4.1	4.4	3.3	2.9
Myristic	9.8	10.3	10.5	11.9
Palmitic	25.4	29.4	28.4	27.6
Stearic	12.3	14.0	12.0	14.0
Oleic	25.4	27.9	31.4	32.0
Linoleic	4.1	2.9	4.1	4.5
Linolenic	0.8	0.0	0.7	1.0
Saturated acids				
C_4-C_8	12.2	6.6	6.1	3.2
C_{10}-C_{18}	57.3	62.5	57.6	59.3
Unsaturated C_{18} acids	30.3	30.8	36.2	37.5

TABLE IV

Glyceride fatty acid composition of total lipid and free fat extracted from powder No. 2

Fatty acid	Storage period (months)						
	Fresh	One		Six		Nine	
	Total	Total	Free	Total	Free	Total	Free
	Area %						
Butyric	2.3	4.2	0.7	1.9	1.5	0.0	0.0
Caproic	1.3	3.1	2.1	3.9	1.7	1.8	1.9
Caprylic	1.9	3.7	1.8	3.9	2.5	2.6	2.3
Capric	3.6	6.3	4.8	4.3	4.5	4.7	4.6
Lauric	3.6	6.3	4.6	5.2	4.7	5.3	4.1
Myristic	14.3	15.7	14.0	12.7	13.1	14.1	14.9
Palmitic	34.3	31.4	35.0	29.8	33.0	30.7	33.4
Stearic	12.3	7.9	12.6	10.3	10.5	11.2	11.6
Oleic	25.0	19.4	22.6	24.9	23.9	25.9	24.9
Linoleic	1.5	2.1	1.7	3.2	3.4	3.3	2.3
Linolenic	0.0	0.0	0.0	0.0	0.2	0.5	0.0
Saturated acids							
C_4-C_8	5.4	11.0	4.6	9.7	6.7	4.4	4.2
C_{10}-C_{18}	68.1	67.6	71.0	62.3	65.8	66.0	68.6
Unsaturated C_{18} acids	26.5	21.5	24.3	28.1	27.5	29.7	27.2

TABLE V

Glyceride fatty acid composition of total lipid and free fat extracted from powder No. 3

Fatty acid	Storage period (months)							
	Fresh		One		Four		Seven	
	Total	Free	Total	Free	Total	Free	Total	Free
	Area %							
Butyric	6.5	10.9	0.7	0.7	0.5	0.0	0.2	3.4
Caproic	2.5	3.3	2.3	1.8	1.5	4.6	0.8	3.6
Caprylic	2.2	2.4	1.8	1.6	2.8	2.9	1.0	2.6
Capric	4.1	4.3	3.8	4.3	4.5	5.0	4.4	4.7
Lauric	5.0	5.8	4.5	5.1	5.3	4.6	5.5	4.9
Myristic	13.4	12.8	12.8	13.0	13.4	13.0	14.9	13.4
Palmitic	30.3	28.6	32.6	33.6	23.0	30.9	34.3	30.6
Stearic	10.3	9.7	10.9	11.5	14.1	10.1	12.1	9.8
Oleic	24.1	20.1	25.8	25.8	27.7	25.8	26.5	22.4
Linoleic	1.6	2.1	3.5	2.5	7.3	3.1	0.4	3.1
Linolenic	0.0	0.0	1.3	0.0	0.0	0.0	0.0	1.3
Saturated acids								
C_4-C_8	11.2	16.6	4.8	4.1	4.8	7.5	2.0	9.6
C_{10}-C_{18}	63.1	61.2	64.6	67.5	60.3	63.6	71.2	63.4
Unsaturated C_{18} acids	25.7	22.2	30.6	28.3	35.0	28.9	26.9	26.8

TABLE VI

Glyceride fatty acid composition of total lipid and free fat extracted from powder No. 4

Fatty acid	Storage period (months)					
	Fresh		One		Five	
	Total	Free	Total	Free	Total	Free
	Area %					
Butyric	0.0	0.0	2.4	0.0	0.0	0.0
Caproic	3.2	1.5	3.7	3.5	0.4	0.0
Caprylic	2.0	2.1	2.4	2.7	2.1	0.8
Capric	4.2	4.2	4.0	4.6	4.4	2.9
Lauric	3.8	5.1	4.4	4.9	4.7	5.0
Myristic	13.1	14.0	12.5	14.0	14.6	17.4
Palmitic	33.8	34.8	28.8	32.5	33.0	37.3
Stearic	11.8	9.9	10.2	10.6	12.7	13.5
Oleic	26.3	22.4	26.0	24.4	26.2	23.0
Linoleic	1.7	4.3	5.6	1.8	1.9	0.0
Linolenic	0.0	1.0	0.0	1.0	0.0	0.0
Saturated acids						
C_4-C_8	5.2	3.6	8.5	6.2	2.5	0.8
C_{10}-C_{18}	66.7	68.8	59.9	66.6	69.4	76.1
Unsaturated C_{18} acids	28.0	27.7	31.6	27.2	28.1	23.0

TABLE VII

Glyceride fatty acid composition of total lipid and free fat extracted from powder No. 6

Fatty acid	Storage period (months)			
	Fresh		Four	
	Total	Free	Total	Free
	Area %			
Butyric	2.0	0.0	0.0	0.0
Caproic	2.3	4.8	0.8	4.7
Caprylic	3.3	3.3	2.0	2.4
Capric	6.1	4.8	3.7	4.9
Lauric	4.7	4.6	4.1	4.7
Myristic	11.6	13.6	13.0	12.2
Palmitic	27.6	29.5	34.2	31.3
Stearic	9.6	9.6	12.1	11.4
Oleic	28.2	26.0	28.0	25.8
Linoleic	4.6	3.8	2.1	2.6
Linolenic	0.0	0.0	0.0	0.0
Saturated acids				
C_4-C_8	7.6	8.1	2.8	7.1
C_{10}-C_{18}	59.6	62.1	67.1	64.5
Unsaturated C_{18} acids	32.8	29.8	30.1	28.4

TABLE VIII

Glyceride fatty acid composition of total lipid and free fat extracted from powders No. 5 and No. 7

Fatty acid	Powder No. 5		Powder No. 7	
	Total	Free	Total	Free
	Area %			
Butyric	0.0	0.0	0.0	0.0
Caproic	0.8	0.0	0.0	0.0
Caprylic	1.8	2.0	1.2	2.1
Capric	3.9	3.7	5.7	3.4
Lauric	4.1	4.6	4.3	4.7
Myristic	13.1	15.1	11.7	12.3
Palmitic	34.4	35.6	29.5	32.6
Stearic	12.0	12.2	14.3	14.4
Oleic	26.5	25.4	30.7	28.5
Linoleic	3.3	1.5	2.5	2.1
Linolenic	0.0	0.0	0.0	0.0
Saturated acids				
C_4-C_8	2.6	2.0	1.2	2.1
C_{10}-C_{18}	67.5	71.2	65.5	67.4
Unsaturated C_{18} acids	29.8	26.9	33.2	30.6

TABLE IX

Comparison of the total lipid and free fat average fatty acid compositions of fresh and stored whole milk powders

Fatty acid group	Total	Free	Percentage difference (free-total)
	----------Area %----------		
		Fresh powder	
Saturated acids C_4-C_8	6.5	6.5	0.0
C_{10}-C_{18}	64.0	65.5	+1.5
Unsaturated C_{18} acids	29.5	28.0	-1.5
		Stored 1 to 4 months	
Saturated acids C_4-C_8	6.4	5.9	-0.5
C_{10}-C_{18}	63.8	66.6	+2.8
Unsaturated C_{18} acids	29.8	27.4	-2.4
		Stored 5 to 9 months	
Saturated acids C_4-C_8	4.9	4.9	0.0
C_{10}-C_{18}	65.3	66.6	+1.3
Unsaturated C_{18} acids	29.8	28.4	-1.4

TABLE X

Carbon numbers and identification of fatty acid peaks from the gas chromatograms of the total lipid extracted from powder No. 3 after storage for one month, see Figure 3

Peak number	Carbon number	Fatty acid[1]
1	4.00	4:0[2]
2	4.86	5:0[5]
3	6.00	6:0[2]
4	6.77	7:0[5]
5	7.59	7:1[5]
6	8.00	8:0[2]
7	8.70	9:0[5]
8	10.00	10:0[2]
9	10.87	10:1[3]
10	12.00	12:0[2]
11	12.12	12:1[3]
12	12.83	13:0[3]
13	13.42	14:0[4] *
14	14.00	14:0[2]
15	14.82	14:1[3]
16	14.89	15:0[3]
17	15.45	15:0[4] *
18	16.00	16:0[2]
19	16.41	16:1[4]
20	16.60	17:0[4] *
21	16.88	17:0[3]
22	17.37	18:0[4] *
23	18.00	18:0[2]
24	18.45	18.1[2]
25	19.00	18:2[2]

[1] The first figure designates the number of carbons and the second the number of double bonds

[2] Identified in this laboratory

[3] Identified by Smith (1961)

[4] Proposed by Smith (1961)

[5] Proposed by this researcher

* Branched

TABLE XI

Major lipid components of total lipid and free fat samples as determined by silicic acid column chromatography

Powder lot	Peak number and identification		
	1 Hydrocarbons	2 Cholesteryl esters	3 Triglycerides
	weight %		
Fresh powder			
No. 1			
Total lipid	0.1	0.2	95.8
Free fat	0.5	0.0	96.7
No. 2			
Total lipid of condensed milk	0.4	0.1	91.4
Free fat	0.1	0.0	99.0
No. 3			
Total lipid	0.6	0.0	88.1
Free fat	1.0	0.0	84.2
No. 7			
Total lipid	0.1	0.1	82.0
Free fat	0.1	0.9	93.9
Powder stored 1 month			
Two			
Total lipid	0.1	0.0	83.5
Free fat	0.1	1.1	91.1
Three			
Total lipid	0.4	0.2	80.3
Free fat	0.4	0.0	77.4
Averages of fresh powders			
Total lipid	0.3	0.1	88.6
Free fat	0.4	0.2	93.5

TABLE XI (CONT.)

4	5	6	7	
Free fat ty acids	Digly- cerides	Monogly- cerides	Phospho- lipids	Unassigned
0.0	2.5	0.6	0,9	0.0
0.0	1.6	0.0	0.2	1.0
2.4	2.8	0.0	2.2	0.7
0.0	0.0	0.1	0.8	0.0
5.4	2.8	1.5	1.7	0.0
2.9	1.2	0.4	0.3	9.9
3.6	2.8	3.2	1.3	7.0
0.0	3.7	0.0	0.8	0.6
3.4	2.6	0.1	0.9	0.6
6.1	0.6	0.0	2.0	7.8
7.2	5.7	1.8	1.3	3.1
0.0	11.6	0.0	2.0	8.6
3.0	2.0	1.7	1.3	2.3
0.7	1.4	0.1	0.5	2.9

TABLE XII

The phospholipid[1] content of the total lipid and free fat samples

Powder lot	Total lipid	Free fat
	(%)	(%)
1	0.78	0.06
2	0.70	0.22
3	1.20	0.22
4	0.55	0.14
5	0.60	0.21
6	0.68	0.06
7	1.06	0.12

[1] Calculated as lecithin

DISCUSSION

Analytical Methods

Methyl ester preparation and gas-liquid chromatography

Base-catalyzed methanolysis was used in this study because the mild conditions of this reaction are least destructive to the unsaturated fatty acids of milk fat. Compared to acid-catalyzed methanolysis, the base catalyzed reaction is faster, more complete and less likely to alter fatty acid structures for it is capable of being used at lower temperatures (Markley, 1947). Methylation employing diazomethane was avoided because reactions may occur at ethylene bonds to form pyrazolines (Gehrke, Goerlitz, Johnson and Richardson, 1960).

Because the fatty acids of only the neutral glycerides were of interest in this study, simple methods to remove phospholipids and free fatty acids were attempted. According to Choudhury and Arnold (1961), phospholipids can be separated from neutral fat by shaking the lipid sample with silicic acid and chloroform. However, this technique was abandoned after gravimetric examination of the two fractions indicated that materials other than phospholipids were adsorbed by the silicic acid. Removal of free fatty acids and their conversion to methyl esters was attempted by the anion exchange technique described by Hornstein, Alford, Elliott and Crowe (1959). Using this method no methyl esters were recovered from the resin as indicated by gas chromatography. Either the free fatty acids were not adsorbed onto the resin or the esterification was not successful.

Theoretical considerations of the reaction described by Smith and Jack (1954) for methyl ester preparation indicated that it was unnecessary

to remove free fatty acids and phospholipids for only the neutral glycerides were active in the methylation. Under the basic conditions of this reaction no free fatty acids are methylated because carboxylic acids will convert to carboxylate anions which, because of their negative charge, are not subject to nucleophilic attack by methanol (Gould, 1960). Phospholipids will not undergo interesterification under the conditions of this reaction for the small amounts of KOH (5×10^{-5} moles) will be neutralized by the phospholipids (1.4×10^{-4} moles, based on phospholipid comprising 1% of 1 g of total lipid). The KOH catalyst would not be available for the formation of the methoxy ion. Cholesteryl esters are methylated with more difficulty than the other esters because of steric retardation by the bulky cholesteryl group in forming the reaction intermediate. These hypotheses were found to be valid by experimentation. Even if free fatty acids, phospholipids and cholesteryl esters were esterified to a slight extent, esters contributed by them could be considered to be very negligible.

The inherent error in this technique occurs in the solvent removal step prior to gas chromatography. The more volatile fatty acids, especially methyl butyrate, are lost to some extent. However, even poorer recoveries of fatty acid esters are obtained when either heat or vacuum are employed to remove solvent.

Ideally, fatty acid compositional data should be expressed as mole percentage rather than area percentage for the thermal conductivity detector responds on a weight rather than a molar basis. For comparative purposes both methods were used to indicate the fatty acid composition of free fat obtained from powder No. 6 after four months of storage, see Table XIII. The results obtained by expressing the raw data as mole

percentage and area percentage agree especially well for the fatty acids having ten or more carbons. For the shorter fatty acids, the results obtained by the area percentage method are about 1.5% lower than those arrived at by the mole percentage method of expression.

Because comparisons of the relative fatty acid compositions of the fat samples was an objective of this study, the expression of fatty acid data as area percentage was adequate. Although it is erroneous to exclude the minor fatty acids in the calculation of either area percentage or mole percentage, only the chromatographic peak areas of the eleven major fatty acids were used to compute area percentages. The unassigned areas of the chromatograms, which included the minor fatty acids and a portion of the compounds resulting from oxidative deterioration, varied considerably among the samples. The inclusion of the unassigned area would confuse the comparison of individual fatty acid concentrations among samples.

Silicic acid chromatography

Accurate quantitative analysis of a complex lipid mixture by silicic acid chromatography is complicated by at least three factors. One disadvantage is incomplete resolution of components having similar elution characteristics such as 1) triglycerides and free fatty acids and 2) cholesterol and diglycerides. Also, peak identification by reference to elution volumes of authentic lipids is dependent on exact reproduction of the conditions of the analysis. The greatest limitation in achieving reproducible results is column preparation. Because identification using elution volumes cannot be trusted completely, confirmation by infrared analysis should be employed. However, these analyses are often either

not possible or inconclusive because of insufficient amounts of sample.

Free Fat Content

The free fat values of the fresh milk powders which ranged from 0.3% to 7.7% agree well with those reported in the literature for spray-dried milk containing approximately 26% total fat. Litman (1955) reported initial free fat values of 1% to 18% and Reinke (1959) observed free fat accounting for 14.4% to 20% of the total fat in milk powders manufactured under conditions similar to those in this study. The high percentages, 70% to 80%, of the total fat extractable as free fat from powders stored five or more months is surprising and unprecedented. Reinke (1959) observed only small increases in free fat during storage in the order of two to three percent. Litman (1955) reported no changes in free fat content in powders stored at 45°F., and at higher temperatures, 85° and 110°F., a decrease in free fat value was observed.

The general increase in free fat values during storage observed in this study may be attributed to the fat freeing action of lactose crystals which form, much as described by Lampitt and Bushill (1931), when milk powders absorb moisture. Although the extent of lactose crystallization was not determined in any of the powder samples, the stored powders developed a dry, hard, powdery texture and browned considerably. Similar observations were made by Lampitt and Bushill (1931) during water absorption. The milk powders under study in this research did appear to have absorbed water for the total lipid percentage, based on the total weight of the powder, decreased during storage from 26% to 23% or 24% indicating an increase in moisture. In addition to physical destruction of the fat globule membrane by lactose crystals, protein degradation and

oxidative deterioration was probably a more important contributor to the freeing of the fat. The atmospheric storage conditions including exposure to light are unfavorable and are mainly responsible for the destruction of the fat globule membrane.

Neutral Glyceride Fatty Acids

By averaging the results of all the fatty acid analyses by gas chromatography, the free fat was noted slightly richer in C_{10}-C_{18} saturated acids and poorer in C_{18} unsaturated acids and C_4-C_8 saturated acids than the total lipid. These differences were enhanced by selecting and averaging only the data which followed this trend, see Table XIV. In these cases the free fat of fresh milk powder contained an average of 4.4% more C_{10}-C_{18} saturated acids, 2.5% fewer C_{18} unsaturated acids and 2.5% fewer C_4-C_8 saturated fatty acids than the total lipid. The minor distinction found in the C_4-C_8 group is meaningless because of the variable losses of volatile fatty acids, but the differences in concentrations of the longer fatty acids is probably significant. These findings are in agreement with those of Litman (1955) who concluded that complexed free fat found in insoluble particles of reconstituted dry whole milk has a lower iodine number and a higher melting point than the fat of the dispersed milk. Bullock and Winder (1960), without direct experimental evidence, have postulated that pressures exerted on the fat globules during drying cause a redistribution of glycerides resulting in a thin film of high-melting glycerides on the surface of the spray-dried milk particle. If the free fat extraction technique removes mostly fat on the surface of the powder particle, this hypothesis of Bullock and Winder (1960) partially accounts for the

slightly higher proportion of high melting glycerides found in the free fat.

Averages of the fatty acid data from all milk powders, Table XII, indicate that storage has no effect on the fatty acid composition of total lipid or free fat. However, the averaged selected data discussed above and listed in Table XIV indicates that the proportion of C_{10}-C_{18} saturated acids increased after storage of five or more months at the expense of C_{18} unsaturated acids in both total lipid and free fat fractions. Oxidative deterioration is probably responsible for the loss of unsaturated acids. The averaged fatty acid compositional results listed in Table XIV indicates that the free fat is more representative of the total lipid in powder stored five or more months than was initially the case. This observation can be explained by the finding that at this age the free fat accounts for 80% to 90% of the total fat.

The butyric acid concentrations of the fat fractions studied were much lower than values found by other researchers. Jenness and Patton (1959) reported that butyric acid has been found in proportions of 8.5 to 10.5 mole percentage in butterfat. The very low amounts or absence of butyric acid found in this study must be due to evaporation losses prior to gas chromatography. Linolenic acid was noted in four of seventeen total fat samples and five of sixteen free fat samples. The highest concentration of linolenic acid was found in powders manufactured in September and January. No linolenic acid was observed in powders manufactured in April, May or August. Possibly linolenic acid, although present, is not detected in all cases. This acid occurs in low concentrations in milk and because of its large retention volume it diffuses considerably during passage through the column.

Major Lipid Components

Some of the differences between total lipid and free fat elucidated by silicic acid chromatography can be attributed to the non-polar nature of the solvent used to extract free fat. The observation that lesser concentrations of free fatty acids and phospholipids are found in the free fat can be explained on this basis. Gurd (1960) reported binding of fatty acid anions to human serum albumin. A similar fatty acid association with milk albumin would certainly be stable to extraction with a 50-50 (V/V) mixture of petroleum and ethyl ethers. Even if the fatty acid anions were not associated with proteins they would be only slightly soluble in this solvent. The difference in free fatty acid concentrations between total lipid and free fat, which averaged 3.9% and 1.5% respectively, would be more outstanding if 75% of the fatty acids were not discarded as ammonium salts in the Mojonnier total fat extraction (Starr and Herrington, 1941).

Assuming that the presence of free fat indicates fat globule membrane damage for one thing, phospholipids, which are a component of the membrane, might be expected to occur at the same, if not higher, concentrations in free fat than in total fat. The finding, by both chemical and physical assay, that phospholipids occur at lower levels in free fat than total fat can also be explained by protein-lipid association. Even though the membrane might be disrupted, the majority of the phospholipids would still be complexed to membrane protein. A more polar solvent than petroleum or ethyl ether is required to break the protein-phospholipid complex. Also free uncomplexed phospholipids, due to their polar nature, may have limited solubility in the petroleum-ethyl ether mixture. These reasons help to explain the observation that free fat contains less than

half as much phospholipid as total fat.

Cholesterol, which Jenness and Patton (1959) report occurring in concentrations of 0.25 to 0.4% in butterfat, was not identified in any total lipid or free fat fractions. Cholesterol and diglycerides have similar elution characteristics on silicic acid (Hirsch and Ahrens, 1958) and may emerge from the column together as Peak 5, see Figure 4.

The decrease in triglyceride concentrations in both total lipid and free fat at the expense of an increase in diglyceride during powder storage indicates that either lipolysis is occurring or that Peak 5, tentatively identified as diglyceride, is not diglyceride at all. This fraction may be triglyceride which has been chemically altered by oxidation. These changes would effect the elution characteristics of the lipid causing it to emerge from the column after the unoxidized triglyceride. The increase in the phospholipid concentration in free fat during storage indicated extensive damage to the membrane protein resulting in a higher proportion of uncomplexed free phospholipids.

TABLE XIII

Conversion of gas chromatographic peak area to micromoles, mole percentage and comparison to area percentage[1]

Fatty acid	Area	Micro-moles	Mole %	Area %
	(cm^2)			
Caproic	2.3	0.34	6.4	4.7
Caprylic	1.2	0.16	3.1	2.4
Capric	2.4	0.32	6.1	4.9
Lauric	2.3	0.24	4.7	4.7
Myristic	6.0	0.63	11.9	12.2
Palmitic	15.4	1.63	30.8	31.3
Stearic	5.6	0.59	11.1	11.4
Oleic	12.7	1.26	23.8	25.8
Linoleic	1.3	0.14	2.6	2.6

[1] Gas chromatographic peak area data of free fat obtained from powder No. 6 after four months of storage.

TABLE XIV

Comparison of selected total lipid and free fat average fatty acid compositions of fresh and stored milk powders

Fatty acid group	No. of samples selected	Total[1] lipid	Free[1] fat	Percentage difference (free-total)
		-----Area %-----		
		Fresh powder		
C_4-C_8 saturated	3 of 6	6.7	4.2	-2.5
C_{10}-C_{18} saturated	5 of 6	63.3	67.7	+4.4
C_{18} unsaturated	5 of 6	29.9	28.0	-2.5
		Stored 1 to 4 months		
C_4-C_8 saturated	3 of 5	7.9	5.0	-3.2
C_{10}-C_{18} saturated	5 of 6	61.2	66.9	+5.7
C_{18} unsaturated	5 of 6	30.8	28.1	-2.7
		Stored 5 to 9 months		
C_4-C_8 saturated	4 of 5	5.8	3.8	-2.0
C_{10}-C_{18} saturated	4 of 5	65.3	69.3	+4.0
C_{18} unsaturated	4 of 5	28.8	26.8	-2.0

[1] Corrected to total 100

SUMMARY AND CONCLUSIONS

The free fat fraction of whole milk powder, extractable with non-polar organic solvents, has been reported to have contributed adversely to the reconstitution and flavor of that product. The purpose of this study was to determine the compositional characteristics of free fat obtained from both fresh and stored whole milk powder. The total lipid and free fat of seven spray-dried whole milk powders manufactured under commercial conditions were analyzed. Adsorption chromatography on silicic acid columns and gas-liquid chromatography were employed to ascertain semi-quantitatively the major lipid components and the glyceride fatty acid composition, respectively, of the total lipid and free fat fractions.

Lipid fractionation by adsorption chromatography indicated that free fat contained lower concentrations of phospholipid and free fatty acids than the total fat. Data obtained from stored powders were inconclusive in regard to changes in the lipid during storage. No major differences in the distribution of individual fatty acids between total lipid and free fat fractions were noted by gas chromatographic fatty acid analyses. However, by grouping the fatty acids according to chain length, subtle dissimilarities were observed. Free fat was found to be slightly richer in C_{10}-C_{18} saturated acids and poorer in C_{18} unsaturated acids than the total lipid. These differences were enhanced during storage.

The results of this study lead to the conclusion that free fat extracted from dry whole milk with a 50-50 (V/V) mixture of petroleum and ethyl ethers is not substantially different from the total lipid. Minor

dissimilarities were as follows:

1. Free fat contained lower concentrations of phospholipid and free fatty acids than the total lipid.

2. Free fat contained slightly lower concentrations of C_{18} unsaturated acids and higher concentrations of C_{10}-C_{18} saturated acids than the total lipid.

LITERATURE CITED

(1) Choudhury, B.R., and Arnold, L.K.
1960. The determination of the neutral oil content of crude vegetable oils. J. Amer. Oil Chem. Soc., 37:86.

(2) Choi, R.P., Tatter, C.W. and O'Malley, C.M.
1951. Lactose crystallization in dry products of milk. II. The effect of moisture and alcohol. J. Dairy Sci., 34: 850.

(3) Coulter, S.T., Jenness, R. and Geddes, W.F.
1951. Advances in Food Research. pp 45-118. New York: Academic Press Inc.

(4) Favstova, V. and Boiko, N.
1958. Changes in butterfat during the concentration and drying of milk. Mol. Prom., 19; 31. (Abst.) Dairy Sci. Absts., 20: 526. (Original not seen).

(5) Fiske, C.H. and Subbarow, Y.
1925. The colorimetric determination of phosphorus, J. Biol. Chem., 66: 375.

(6) Gehrke, C.W., Goerlitz, D.F., Johnson, H.D. and Richardson, C.W.
1960. Quantitative determination of the methyl esters of fatty acids by gas chromatography. Abst. material presented at Amer. Dairy Assos. 56th Ann. Meet., Logan, Utah (Mimeo).

(7) Gould, E.S.
1960. Mechanism and Structure in Organic Chemistry. p. 315. New York: Henry Holt and Company.

(8) Greenbank, G.R. and Hufnagel, C.F.
1953. The effect of the fat content of milk powder on the keeping quality of the dried powder. J. Dairy Sci., 36: 566.

(9) Greenbank, G.P. and Pallansch, M.J.
1961. The progress of oxidation in the milk powder granule. (Abst.) J. Dairy Sci. 44: 1165.

(10) Gurd, F.R.N.
1960. in Hananan, D.J. Ed., Lipid Chemistry. pp. 248. New York: John Wiley and Sons, Inc.

(11) Hirsch, J. and Ahrens, E.H.
1958. The separation of complex lipid mixtures by the use of silicic acid chromatography. J. Biol. Chem., 223: 311.

(12) Holm, G.E., Greenbank, G.R. and Deysher, E.F.
1925. The effect of homogenization, condensation and variations in the fat content of a milk upon the keeping quality of its milk powder. J. Dairy Sci., 9: 512.

(13) Hornstein, I., Alford, J.A., Elliott, L.E., and Crowe, P.F.
1960 Determination of free fatty acids in fat. Anal. Chem. 32: 540.

(14) Horrall, B.E.
1935. A study of the lecithin content of milk and its products. Purdue Univ. Agr. Expt. Sta. Bul. 401.

(15) Janzen, J.J., McGugan, W.A. and Swanson, A.M.
1953. Some factors involved in the wettability and dispersibility of dried whole milk. J. Dairy Sci., 36: 566.

(16) Jenness, R. and Patton, S.
1959. Principles of Dairy Chemistry. pp. 30-71. New York: John Wiley and Sons, Inc.

(17) Keulemans, A.I.M.
1957. Gas Chromatography. p. 113. New York: Reinhold Publishing Corp.

(18) King, N.
1948. The microscopic examination of milk powders. Netherlands Milk and Dairy J., 2: 137.

(19) King, N.
1954. Physical structure of milk powder. Dairy Ind., 19: 39.

(20) King, N.
1955. Flourescence microscopy of fat in milk and milk powder. J. Dairy Research, 22: 205.

(21) King, N.
1961. Microstructure of some non-dispersible particles in milk powder. Aust. J. Dairy Tech., 15: 77.

(22) Lampitt, L.H. and Bushill, J.H.
1931. The physico-chemical constitution of spray-dried milk powder. Fat in spray-dried milk powder. J. Soc. Chem. Ind., 50: 45.

(23) Litman, I.I.
1955. A study of a fat-protein complex in powdered milk. Ph.D thesis. State College of Washington, Pullman.

(24) Markley, K.S.
1947. Fatty Acids. p. 299. New York: Interscience Publishers, Inc.

(25) Mojonnier, T. and Troy, H.C.
1925 Technical Control of Dairy Products. 2nd Ed. p. 936. Chicago: Mojonnier Bros. Co.

(26) Nickerson, T.A., Coulter, S.T. and Jenness, R.
1952. Some properties of freeze-dried milk. J. Dairy Sci., 35: 77.

(27) Reinke, E.
1959. The effect of processing variations on the free-fat self-dispersion, and flavor of whole milk powder. M.S. thesis. Michigan State University, East Lansing.

(28) Shipstead, H. and Tarassuk, N.P.
1953. Chemical changes in dehydrated milk during storage. Agr. Food Chem., 1: 613.

(29) Smith, L.M. and Jack, E.L.
1954. The unsaturated fatty acids of milk fat. 1. Methyl ester fractionation and isolation of monoethenoid constituents. J. Dairy Sci., 37: 380.

(30) Smith, L.M.
1961. Quantitative fatty acid analysis by gas-liquid chromatography. J. Dairy Sci., 44: 607.

(31) Starr, M.P., and Herrington, B.L.
1941. The determination of fat in the presence of free fatty acids. I. The Mojonnier test of mixtures of free fatty acids and butterfat. J. Dairy Sci., 24; 165.

(32) Stone, W.K., Conley, T.F., and McIntire, J.M.
1954. The influence of lipids on self-dispersion and on ease of dispersion of milk powder. Food Tech., 8: 367.

(33) Tamsma, A., Edmondson, L.F. and Vettel, H.E.
1958. Free fat in foam-dried whole milk. J. Dairy Sci., 41: 710.

(34) Thomas, R.J., Holgren, C.J., Jr., Jokay, L. and Bloch, I.
1957. A rapid method for determining free fat in dry milks. (Abst.) J. Dairy Sci., 40: 605.

(35) Thompson, M.P.
1960. Structure of the milk fat globule membrane. Ph.D. thesis. Michigan State University, E. Lansing.

(36) Washburn, R.M.
1922. The physical analysis of dry milk. J. Dairy Sci., 5: 388.

(37) Woodford, F.P. and van Gent, C.M.
1960. Gas-liquid chromatography of fatty acid methyl esters, the carbon number as a parameter for comparisons of columns. J. of Lipid Research, 1: 188.

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