Figure 5 shows the distribution of these points along the regression line. The regression line is plotted from the regression equation:

$$y = 14.77 + .09409x$$

where y = oil content (percentage)
x = meter readings

A conversion table to translate meter readings into oil content was drawn up from the regression equation. The standard error of estimate in determining oil content by the dielectric method was found to be 0.27 in terms of percentage of oil. The coefficient of correlation was found to be + 0.98.

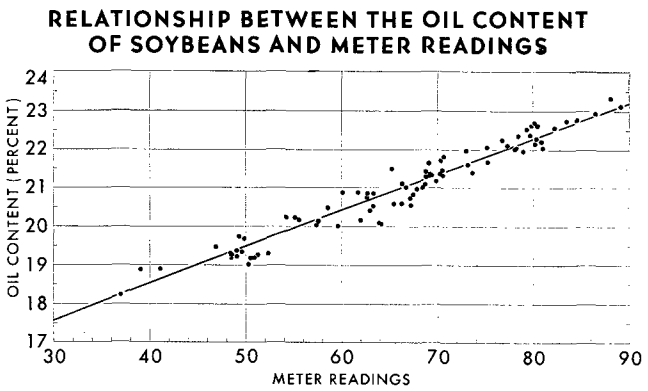

FIG. 5. Relationship between oil content of soybeans and meter readings.

Discussion. The method of analysis described above appears to be quite suitable for practical application to the rapid determination of the oil content of soybeans. Results on a single sample can be obtained in about 15 minutes, the variable being the rate of filtration. If a series of samples is analyzed, then the time per sample is further reduced because the grinding-extraction of one or more samples can take place while previous extracts are filtering. It is estimated that two analysts working with two grinder-extractors and one electronic tester could analyze from 20 to 30 samples per hour.

The technique of analysis is simple enough so that nonchemists can, with brief instructions, perform the analysis with the speed and accuracy previously indicated.

Acknowledgment

The mill and the electronic tester were built for this work by the Fred Stein Laboratories, Atchison, Kans. They have worked closely with this laboratory in developing the method, and we are deeply grateful to them for their aid in designing and redesigning the equipment, when necessary, to meet the needs of the tests. These instruments are covered by patents or patents pending (14).

REFERENCES

1. Anderson, K., Bettis, E. S., and Revinson, D., Anal. Chem. *22*, 743 (1950).
2. Arditti, R., and Heitzmann, P., Compt. rend. *229*, 446 (1949).
3. Blaedel, W. J., and Malmstadt, H. V., Anal. Chem. *22*, 734 (1950).
4. Blaedel, W. J., *et al.*, *ibid.* *24*, 198 (1952).
5. Blake, G. G., Australian J. Sci. *10*, 80 (1947).
6. Blake, G. G., *ibid.* *11*, 59 (1948).
7. Fischer, R. B., Anal. Chem. *19*, 835 (1947).
8. Gent, W. L. G., Trans. Faraday Soc. *45*, 758 (1949).
9. Hall, J. L., and Gibson, J. A. Jr., Anal. Chem. *23*, 966 (1951).
10. Jensen, F. W., and Parrack, A. L., Ind. Eng. Chem., Anal. Ed. *18*, 595 (1946).
11. Jensen, F. W., and Parrack, A. L., Texas A & M College, Eng. Expt. Sta. Bull., 92 (1948).
12. West, P. W., Burkhalter, T. S., and Broussard, L., Anal. Chem. *22*, 469 (1950).
13. West, P. W., Robichaux, T., and Burkhalter, T. S., *ibid.* *23*, 1625 (1951).
14. Stein, F. W., U. S. Patents No. 2251641 and 2440386.

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The Isolation of Monoglycerides from Lard and from Bread[1]

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SECTION A

General Procedures, Results, and Discussion

MANY experts in the field of fats have concluded that natural edible oils (at least before extensive refining) contain up to 1% of monoglycerides (1, 6-7, 9, 11-16). These conclusions were based upon the expected chemistry of fats and upon such properties of the fat as acetyl values, and periodic acid oxidation studies. Furthermore, because the main components of fats are triesters of glycerol, it seems likely that some hydrolysis will take place during cooking of a food that contains fat, and that cooked products will contain an appreciable amount of monoglycerides. Monoglycerides have been reported as being formed from a triglyceride shortening during the baking of bread (5).

This report describes a study of the monoglyceride content of lard and of baked bread by actually isolating the monoglycerides, using techniques similar to those of Jones and co-workers (10), who isolated monopalmitin from pancreas.

General Procedure

Extraction and Purification of Monoglycerides. To isolate the monoglycerides, methods were chosen which involved only solvent extraction and crystallization. The following procedure combines the best features of the several modifications presented in the experimental section. The lipids are removed by stirring with large volumes of warm petroleum ether (Skellysolve F). The Skellysolve F is preferred as a solvent to an ethyl ether-ethanol mixture since, in the extraction of bread, ether-alcohol removes large quantities of sugar, flour, and starch products. The fat recovered from the Skellysolve F extract, after removal of the solvent, is further worked up by the following procedure which is suitable for most fats. The extracted fat is first treated with 10-20 volumes of acetone. The phospholipids which are present in the fat precipitate and are removed. After removal of the acetone the fat is dissolved in 20 parts of ethyl ether and washed with distilled water. Vigorous shaking

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produces an emulsion in the water layer which is often slow to break. The washing procedure can be facilitated by withdrawing of the emulsion, centrifuging, and adding the ether layer back to the main bulk of the ether solution. The water washing is continued until no further water emulsions are formed which sometimes requires as many as 8 to 10 washes.

The washed ethyl ether solution is dried with sodium sulphate and the solvent removed. The treatment up to this point eliminates compounds more polar than monoglycerides. The washed fat is stirred with 8 to 10 volumes of warm methanol and the soluble portion decanted. Two more extractions with methanol are made and the combined extracts clarified by centrifuging. The solvent is removed under vacuum. This procedure concentrates practically all of the monoglycerides in the methanol soluble fraction.

The monoglycerides of saturated fatty acids are readily removed and purified at this stage by dissolving the methanol soluble fraction in 20 volumes of Skellysolve F and cooling the solution to +5°C. The precipitated saturated monoglycerides are purified by recrystallization from Skellysolve F at room temperature.

Substances other than monoglycerides which are soluble in methanol are the diglycerides, fatty acids, and tocopherols. The monoglycerides can be separated from these constituents by an additional extraction with methanol containing 30% water. A procedure generally easier to carry out is to redissolve the methanol soluble fraction in 20 parts of methanol and to dilute with water to make a 70% methanol solution. The 70% methanol soluble portion will contain the monoglycerides in a fairly pure form. A portion of the monoglycerides will remain in the methanol-insoluble lipid fraction and can be removed by repeated extractions. If there is an appreciable amount of free fatty acids in the original fat, some portion may remain in the 70% methanol. Fatty acids were not a factor in these experiments. If they are present, they can be removed by dissolving the 70% methanol-soluble fraction in 20 volumes of Skellysolve F and extracting with an equal volume of 80% methanol. The monoesters are extracted by the methanol and the fatty acids remain in the Skellysolve F.

Identification. The periodic acid oxidation method (8) for monoglyceride analysis was used to follow the progress of all the purification procedures. Free glycerol was determined by the method of Bradford and associates (2). The naturally occurring monoglycerides were established as essentially pure by periodic acid analysis and further identified as monoglycerides by saponification, by infrared spectroscopy, and by countercurrent distribution (Table I).

TABLE I

Properties[a] of the Fractions of Crystalline Monoglycerides of Saturated Fatty Acids and Concentrates of Monoglycerides of Unsaturated Fatty Acids

Monoglyceride extract from	Sample No.	Periodic acid assay for monoglycerides,	Free fatty acids	Melting point
		% theory	*%*	*°C.*
Saturated Monoglycerides				
Chemically reacted lard	NK-8A	98	0	71-73.5
Lard	NK-191A	96	0	71.5-73.5
6% Fat bread	NK-151A	99	0	72-73.5
2.5% Fat bread and added monoglycerides	NK-177A	99	0	71-72.5
2.5% Fat bread	NK-179A	97.5	0	71-72.5
2.5% Monoglyceride-free fat bread	NK-180A	99	0	71-72.5
2.5% Fat bread, second batch	NK-42A	96	0	72-73.5
Unsaturated Monoglycerides				
Chemically reacted lard	NK-9A	99	2.1	.....
Lard	NK-191B	96	1.6	.....
6% Fat bread	NK-153	97	....	.....
2.5% Fat bread and added monoglycerides	NK-177B	99	2.3	.....
2.5% Fat bread	NK-179B	98	3.1	.....
2.5 Monoglyceride-free fat bread	NK-180B	96	2.5	.....
2.5% Fat bread, second batch	NK-45B	84	2.1	.....

[a] "Theory" for the saturated monoglycerides is monopalmitin and for the unsaturated monoglycerides is monoolein. There was no free glycerol in any sample. Infrared analyses showed all samples to be "practically pure" monoglycerides. For other analytical data on samples NK-177A, NK-191B, and NK-179B see Table XI. By countercurrent extraction 94.1% and 85.2% of monoglycerides were recovered from samples NK-179A and NK-45B, respectively.

Results and Discussion

Lard. Prime steam-rendered lard was chosen for investigation for two reasons. It is an important constituent of most of the bread consumed by the public, and it represents one of the few unrefined fats. The 1,000 grams of fresh commercial prime steam-rendered lard with a peroxide value less than 1 and less than 0.05% of free fatty acids analyzed for 1.1% monoglycerides by periodic acid oxidation. The methanol extracts (Section B) contained 9.4 grams of monoglycerides. The methanol extract was purified by the method outlined, and 5.1 grams of essentially pure monoglycerides were obtained. 1.7 grams of monoglycerides were rejected as low potency fractions in the final purification steps. The kilo of prime steam-rendered lard thus contained from 5.1 to 6.8 grams of naturally occurring monoglycerides.

This finding confirmed some earlier work of our laboratory. Several gallons of prime steam-rendered lard purchased from Oscar Mayer and Company were distilled on the centrifugal molecular still; a 10% strip cut was taken and found to contain 6% monoglycerides in the strip cut, corresponding to an original content of 0.6% monoglyceride. While developing extraction methods, a small sample of another batch of commercial prime steam-rendered lard furnished a concentrate of 39% monoglycerides, which corresponded to an original content of 0.7% monoglyceride.

6% Fat Bread. A batch of bread was made in the laboratory, using an amount of prime steam-rendered lard equal to 6% of the flour weight (Section C). Five of the six loaves of bread from this dough were used to determine the amount of monoglycerides present in the bread. The bread was sliced and air-dried for three days and ground to a fine crumb to facilitate the extraction. Even with thorough drying and grinding only 85% of the lipid was recovered. From the recovered fat 16.5 grams of essentially pure monoglycerides were obtained and identified. Since 0.5 gram of monoglycerides was present in the prime steam-rendered lard, this represents a gain of 16.0 grams of monoglycerides in the baked bread, or 3.20 grams per loaf.

2.5% Fat Bread. Several batches of bread were made with the lard content reduced to 2.5%, based on the flour weight. The baking procedure is reported in Section D and the extraction in Section E. Fat recoveries from these breads varied between 63 and 67%.

When the lard content of the bread was reduced to 2.5%, a much smaller quantity of monoglyceride was recovered from the baked bread than from the 6% fat bread. In bread made with 2.5% of prime steam-rendered lard, there was 0.75 gram of monoglycerides per pound loaf. In the bread to which had been added

the distilled monoglycerides of cottonseed oil (MYVEROL, Type 18-85) in an amount equivalent to 0.83 gram of monoglycerides per pound loaf, 0.86 gram of monoglycerides was recovered. The bread made with lard from which all naturally occurring monoglycerides had been removed contained 0.45 gram of monoglycerides per pound loaf. It is therefore evident that the amount of monoglycerides added to a bread dough has little bearing on the quantity of monoglycerides present in the baked bread. It is also evident that it is impossible to prevent the occurrence of monoglycerides in bread by carefully removing the monoglycerides from the ingredients since the bread baked with extracted monoglyceride-free lard contained 0.45 gram of monoglycerides per pound loaf.

These experimental breads were examined for softness over a period of a week and an additional three days of air-drying elapsed before the fat was extracted. To eliminate the possibility that the monoglycerides were produced by long periods of aging, another batch of bread was made with 2.5% of prime steam-rendered lard. The bread was sliced directly after being taken from the oven and air-dried for 15 hours before grinding and subsequent fat removal. This experiment is discussed in Section E. The bread was found to contain 0.53 gram of monoglycerides per pound loaf. This is 30% less than reported for the delayed extraction bread. This loss might be attributed to the fact that the bread was not thoroughly dry, but the difference is not sufficient to indicate that monoglycerides are formed on aging. Another explanation would be that variable quantities are formed during the uncontrolled hydrolysis occurring at the time of the baking of the bread.

The presence of monoglycerides in the baked bread does not appear to contribute to bread softness. The softness of the bread appears to be due to a reaction taking place in the dough stage. Monoglycerides from any source, present at the time of the mixing of the dough, accomplish this function. The untreated prime steam-rendered lard makes the bread considerably softer than the bread made with extracted monoglyceride-free lard. A discussion of these measurements is contained in Section D.

Identification of Monoglycerides from a Reaction Product of Lard and Glycerol. The effectiveness of the extraction procedures for monoglycerides produced from lard by the customary chemical means was also studied. Prime steam-rendered lard and C. P. glycerol were reacted as discussed in Section F. The monoglycerides in this reaction mixture were purified according to the solvent procedures used to determine the naturally occurring monoglycerides. The extracted and purified fractions of the monoglyceride of saturated acid and the monoglycerides of the unsaturated acids confirmed the extraction method and proved to be identical to the naturally occurring monoglycerides found in lard and bread.

Summary

The isolation of crystalline saturated monoglycerides and the preparation of 85% or higher concentrates of the unsaturated monoglycerides from bread and lard were described. The monoesters were identified by infrared spectrophotometry, by countercurrent extraction, by monoglyceride analysis (periodic acid oxidation), by fatty acid analysis, and by glycerol analysis.

A sample of lard containing no added monoglycerides yielded 0.19% of crystalline saturated monoglyceride and 0.32% of quite pure unsaturated monoglycerides, or a total of 0.51% monoglycerides. Fractions of intermediate purity account for up to 0.2% more monoglycerides.

Bread was made with 6% (based on the flour) of lard containing no added monoglycerides; the "pound loaves" yielded a fat extract containing about 25% monoglycerides, which upon concentration yielded 3.20 grams of pure monoglycerides per loaf.

Two batches of bread made with 2.5% lard containing no added monoglycerides yielded 0.75 gram and 0.53 gram of pure monoglycerides per loaf. Bread made with 2.5% of lard from which the naturally occurring monoglycerides had been removed yielded 0.45 gram of pure monoglycerides per loaf. The bread made with this monoglyceride-free lard became hard appreciably faster than the bread made with the lard containing the natural monoglycerides.

Bread made with 2.5% lard plus 0.25% of added distilled monoglyceride concentrate (0.83 gram monoglycerides per loaf) yielded 0.86 gram of pure monoglycerides per loaf.

These experiments show that: a) lard contains an appreciable amount of naturally occurring monoglycerides; b) bread made with lard as the sole shortening will contain monoglycerides; c) the amount of monoglycerides formed in the bread depends upon the amount of fat used as shortening.

SECTION B

Extraction of Prime Steam Rendered Lard

One kilo of prime steam-rendered lard obtained from Tobin Packing Company, Rochester, New York, was melted and extracted with three portions of warm methanol.

The first and second methanol extracts were combined. Phospholipids were removed from the 21.9 grams of extracted fat remaining after assay by adding 400 ml. of acetone and filtering off the precipitated phospholipids. The acetone was removed under vacuum, and the fat dissolved in 400 ml. of ethyl ether. The ether solution was washed repeatedly with water, dried over sodium sulphate, and evaporated under nitrogen.

The acetone soluble fraction of 20.6 grams was extracted twice with 200 ml. of methanol. The methanol extracts were combined and the solvent removed under vacuum.

The methanol soluble fraction of 10.8 grams was dissolved in 200 ml. of warm Skellysolve F and cooled to +5°C. The Skellysolve F insoluble fraction was removed by filtration and recrystallized from Skellysolve F. The Skellysolve F solutions were combined and the solvent removed under nitrogen. The Skellysolve F insoluble fraction, NK-191A, has been further identified as a saturated monoglyceride by its melting point and by infrared analysis (Table I).

The Skellysolve F soluble fraction of 8.5 grams was extracted twice with 100 ml. of 95% methanol. The methanol extracts were combined and the solvent removed under vacuum.

The 95% methanol soluble fraction of 7.1 grams was extracted with 100 ml. of 85% methanol. The methanol was removed under vacuum and the fat dissolved in 100 ml. of ethyl ether. The ether solution

was water-washed and dried over sodium sulphate and the ether removed under nitrogen.

The 85% methanol soluble fraction of 5.4 grams was extracted with 100 ml. of 70% methanol. The methanol was removed under vacuum and the fat dissolved in 100 ml. of ethyl ether. The ether solution was dried over sodium sulphate and the ether removed under nitrogen. The 70% methanol soluble fraction, NK-191B, has been further identified as the monoglycerides of unsaturated acids by its fatty acid and glycerol composition (Table XI) and by its infrared analysis (Table I).

The extraction and purification data are shown in Table II.

TABLE II

Extraction and Purification of Monoglycerides From One Kilo of Prime Steam Rendered Lard

Solvent	Monoglyceride Distribution			
	Soluble fraction		Insoluble fraction	
	Wt.	Purity	Wt.	Purity
	g.	%	g.	%
First methanol	15	48	....	....
Second methanol	7	28	....	....
Third methanol	5.5	3.6	....	....
Acetone	20.6	45.8	1.3	0
Methanol	10.8	62	8.7	1.5
Skellysolve F (+5°C.)	8.5	55	2.0	96
95% Methanol	7.1	65	1.2	10.6
85% Methanol	5.4	73	1.5	30.4
70% Methanol	3.3	96	1.8	51.5

The total weight of purified monoglycerides obtained was 5.1 grams corresponding to 0.5% of the weight of the prime steam-rendered lard. A total of 1.7 grams of impure monoglycerides was rejected in the purification procedure. Inclusion of the impure monoglycerides would bring the total to 6.8 g., or 0.7% of the prime steam-rendered lard.

SECTION C

Preparation and Extraction of Bread Made with 6% Lard

Preparation of Experimental Bread. Several people connected with the bread industry have advocated the use of a bread formula containing a high percentage of fat, sugar, and milk solids. One such formula known as 6-8-6, designating 6% shortening, 8% sugar, and 6% milk solids, has been proposed by Dr. Glabau, director of the Laboratory and Experimental Baking Department of the journal, Bakers' Weekly. A batch of bread was made in the laboratory, using this formula and, as shortening, prime steam-rendered lard. Five one-pound loaves were used for the extraction and identification of the lipid constituents.

Procedure. All of the ingredients listed in Table III were mixed for two minutes on low speed in a Hobart Mixer. The bread hook was scraped down and the dough mixed for four minutes on medium speed, scraping at the end of two minutes.

TABLE III

Bread Formula

Ingredients	Quantity	Parts by weight
	grams	
Bread flour	2,000	100
Water	1,250	62.5
Milk powder	120	6.0
Sugar	160	8.0
Yeast	50	2.5
Salt	45	2.2
Malt	10	0.5
Shortening	120	6.0

The dough was allowed to rise for 2¼ hours at 84°F. and 83% humidity. The dough was punched and allowed to rise for one hour and 20 minutes at 87°F. and 89% humidity.

After molding, the 500-gram loaves were allowed to rise for 40 minutes and then baked at 425°F. for 26 minutes.

Extraction of Bread Made With 6% Lard. The bread made with 6% lard was sliced and air-dried for three days. The dried bread was ground to pass a 20-mesh screen. The 1,615 grams of crumbs were divided into three parts, and each portion was extracted three times at 37°C. with 2,000 ml. of Skellysolve F brand of light petroleum ether. The solvent was reduced to 500 ml. in the combined extracts by evaporation under nitrogen, 2,000 ml. of acetone were added to this solution and the precipitated phospholipids removed by filtration. The acetone and Skellysolve F were removed under vacuum and the fat dissolved in 400 ml. of ethyl ether. The ether solution was washed repeatedly with water, dried over sodium sulphate, and evaporated under nitrogen. The lipid fraction recovered amounted to 85% of the initial prime steam-rendered lard.

The acetone soluble fraction of 70 grams was dissolved in 750 ml. of warm Skellysolve F and cooled to +5°C. The Skellysolve insoluble fraction was removed by filtration. The Skellysolve F solutions were combined and the solvent removed under nitrogen.

TABLE IV

Extraction and Purification of Monoglycerides From Bread Made With 6% Lard

Solvent	Monoglyceride Distribution			
	Soluble fraction		Insoluble fraction	
	Wt.	Purity	Wt.	Purity
	g.	%	g.	%
Warm Skellysolve F	....	....	....	....
Acetone	70	25	3.2	0
Skellysolve F (+5°C.)	57.3	20.2	10.0	46
Acetone (+5°C.)	8.2	56	1.78	0
Skellysolve F	....	....	2.5	99
Methanol	14.4	97	47.5	0

The Skellysolve F insoluble fraction was dissolved in 100 ml. of acetone and cooled to +5°C. The precipitated fat was removed by filtration and the acetone removed under vacuum.

The Skellysolve F insoluble, acetone-soluble fraction of 8.2 grams was dissolved in 100 ml. of hot Skellysolve F and the precipitate removed by filtration at room temperature. The Skellysolve F solution was evaporated under nitrogen and added to the 57.3 grams of the first Skellysolve F soluble fraction. The Skellysolve F, recrystallized insoluble fraction, NK-151A, was further identified as a saturated monoglyceride by its melting point and by infrared analysis (Table I).

The combined Skellysolve F soluble fractions totaling 62.5 grams was extracted three times with 400 ml. of methanol. The methanol extracts were combined and the solvent removed under vacuum. The methanol soluble fraction, NK-153, has been further identified as the monoglycerides of unsaturated fatty acids by infrared analysis (Table I).

The extraction and purification data are shown in Table IV. The total weight of purified monoglycerides

obtained from the five loaves of bread was 16.5 grams. Approximately 0.50 gram of naturally occurring monoglycerides was present in the prime steam-rendered lard used as shortening. There was consequently a net gain of monoglycerides in the finished bread of 16.00 grams formed during the baking.

SECTION D

Preparation of Experimental Breads with 2.5% Lard

Since much of the bread consumed contains approximately 2.5% fat (based on the flour weight), it seemed desirable to isolate the monoglycerides which occur naturally in bread containing this level of fat. Therefore a batch of experimental bread was baked with 2.5% prime steam-rendered lard as the sole shortening. It is also desirable to know if the quantity of naturally occurring monoglycerides would be increased or decreased by the addition of monoglycerides to the dough. A batch of bread was therefore made with 2.5% prime steam-rendered lard and 0.25% of distilled monoglycerides made from cottonseed oil (MYVEROL Type 18-85). The naturally occurring lard monoglycerides reported in Section B may also have an effect upon the monoglycerides formed in bread. Therefore a third experimental bread was made containing 2.5% of prime steam-rendered lard which had previously been extracted with methyl alcohol to remove the monoglycerides.

Procedure. The formula for these breads was the same as that given in Table III except for the reduction of shortening to 50 grams. For the bread made with added monoglycerides, 5 grams of distilled monoglycerides were incorporated with the 50 grams of shortening.

The straight dough method reported in Section C was used to prepare the breads with no change except to increase the size of the molded bread from 500 grams to 570 grams.

Bread Softness Test. The bread was cooled and then wrapped. The wrapped bread was stored at room temperature and a loaf selected each day for softness tests. Softness was measured by the Baker Compressimeter method (3) with the results reported in Table V.

TABLE V

Staling of Experimental Bread

Shortening	Baker Compressimeter Data: Grams of stress resulting in 3-mm. strain (depression) for bread aged			
	19 hrs.	44 hrs.	67 hrs.	92 hrs.
2.5% Lard plus 0.25% distilled monoglycerides of cottonseed oil	11	15	19	23.6
2.5% Lard	15	28	....[a]	....
2.5% Monoglyceride-free lard	23	....[a]	....	....

[a] Off-scale readings indicating stress greater than 32 grams.

The softness of the experimental bread containing added monoglycerides was extended for 24 to 48 hours beyond the bread made with prime steam-rendered lard. The bread made with extracted monoglyceride-free lard firmed almost 24 hours before the bread made with lard containing its natural monoglycerides.

Six loaves from each experimental batch of bread were selected for the lipid extractions described in Section E.

SECTION E

Extraction of Monoglycerides from Bread

Extraction of Bread Made Without Monoglycerides. The following preferred extraction and purification scheme was used to examine the breads made with 2.5% shortening.

The 6 loaves of bread made with 2.5% prime steam-rendered lard were sliced and air-dried for three days. The dried bread was ground to pass a 20-mesh screen. The 2,140 grams of dry crumbs were divided into three parts, and each portion was extracted three times with 2,000 ml. of Skellysolve F at 37°C. The solvent was removed from the combined extracts under nitrogen. The lipid fraction recovered amounted to 67% of the initial prime steam rendered lard.

The Skellysolve F soluble fraction of 33.5 grams was dissolved in 400 ml. of acetone and the precipitated phospholipids removed by filtration. The acetone was removed under vacuum and the fat dissolved in 400 ml. of ethyl ether. The ether solution was washed repeatedly with water, dried over sodium sulphate, and evaporated under nitrogen.

The acetone soluble fraction of 31 grams was extracted twice with 300 ml. of methanol. The methanol extracts were combined and the solvent removed under vacuum.

The methanol soluble fraction of 8.06 grams was dissolved in 200 ml. of warm Skellysolve F and cooled to +5°C. The Skellysolve F insoluble fraction was removed by filtration and recrystallized from Skellysolve F. The Skellysolve F solutions were combined and the solvent removed under nitrogen. The Skellysolve F insoluble fraction, NK-179A, has been further identified as a saturated monoglyceride by its melting point and by infrared analysis (Table I).

The Skellysolve F soluble fraction of 6.6 grams was extracted twice with 100 ml. of 70% methanol. The methanol extracts were combined and the alcohol re-

TABLES VI, VII, VIII, IX

Extraction and Purification of Monoglycerides from Bread

Solvent	Monoglyceride Distribution: Soluble fraction Wt.	Soluble fraction Purity	Insoluble fraction Wt.	Insoluble fraction Purity
VI. Made with 2.5% Lard				
	g.	%	g.	%
Warm Skellysolve F	33.5	17	....	....
Acetone	31	17.4	2.5	0
Methanol	8.06	64	23	0
Skellysolve F (+5°C.)	6.6	48.5	1.7	97.5
70% Methanol	2.9	98	1.76	0
VII. Made with 2.5% Lard and Added Monoglycerides				
Warm Skellysolve F	35	31.8	....	....
Acetone	33.4	19	1.6	0
Methanol	10.5	57	22.9	0
Skellysolve F (+5°C.)	7.3	46	2.2	99
70% Methanol	3.0	99	2.0	12
VIII. Made with Monoglyceride-Free Lard				
Warm Skellysolve F	31.5	12.7	....	....
Acetone	28.6	11.9	2.8	0
Methanol	5.47	63	23	0
Skellysolve F (+5°C.)	4.0	51	0.9	99
70% Methanol	1.70	96.2	1.33	13
IX. Made with 2.5% Lard, Second Batch				
Warm Skellysolve F	26.5	13.5	....	....
Acetone	22.9	14.7	2.4	0
Skellysolve F (+5°C.)	22	11.8	0.75	96
Methanol	3.8	65.5	18	42
70% Methanol	2.2	84	1.42	0

moved under vacuum and the fat dissolved in 100 ml. of ethyl ether. The ether solution was water washed and dried over sodium sulphate and the ether removed under nitrogen. The 70% methanol soluble fraction, NK-179B, has been further identified as the monoglycerides of unsaturated fatty acids by its fatty acid and glycerol composition (Table XI) and by infrared analysis (Table I).

The extraction and purification data are shown in Table VI.

The total weight of purified monoglycerides obtained from the 6 loaves of bread was 4.5 grams. Present in the prime steam-rendered lard was 0.25 gram of naturally occurring monoglycerides. There was a net gain of monoglycerides in the finished bread of 4.25 grams formed during baking and corresponding to 8.5% of the initial prime steam rendered lard.

Extraction of Bread Made with Monoglycerides. The 6 loaves of bread made with 2.5% prime steam-rendered lard and 0.25% monoglyceride yielded 2,049 grams of dry crumbs. The lipid fraction recovered amounted to 63.5% of the initial prime steam rendered lard and monoglyceride.

The extraction and purification data are reported in Table VII.

The Skellysolve F insoluble fraction, NK-177A, has been further identified as a saturated monoglyceride by its melting point and by analysis of the fatty acid and glycerol (Table XI) and by infrared analysis (Table I).

The 70% methanol soluble fraction, NK-177B, has been further identified as the monoglycerides of unsaturated fatty acids by infrared analysis (Table I).

The total weight of purified monoglycerides obtained from the 6 loaves of bread was 5.2 grams. A total of 0.24 gram of impure monoglycerides was rejected in the purification procedure. Monoglycerides added in this bread formula amounted to 4.5 grams. There was a net gain of monoglycerides in the finished bread of 0.7 to 0.9 gram.

Extraction of Bread Made with Monoglyceride-Free Lard. The 6 loaves of bread made with 2.5% extracted prime steam-rendered lard, free of monoglycerides, yielded 2,155 grams of dry crumbs. The lipid fraction recovered amounted to 63% of the initial extracted, monoglyceride-free, steam-rendered lard.

The extraction and purification data are reported in Table VIII.

The Skellysolve F insoluble fraction, NK-180A, has been further identified as a saturated monoglyceride by its melting point, by infrared analysis, and by countercurrent extraction (Table I).

The 70% methanol soluble fraction, NK-180B, has been further identified as the monoglycerides of unsaturated fatty acids by infrared analysis (Table I).

The total weight of purified monoglycerides obtained from the 6 loaves of bread was 2.5 grams. Fractions containing impure monoglycerides amounted to 0.17 gram of monoglycerides. There was produced 2.7 grams of monoglycerides in the finished bread corresponding to 5.4% of the initial extracted, monoglyceride-free, prime steam-rendered lard.

Extraction of Second Batch of Bread Made with Lard as Sole Shortening. A second batch of bread was made to determine whether the age of the previous breads had influenced the monoglyceride content. The 6 loaves of bread made with 2.5% prime steam-rendered lard were sliced immediately after removal from the oven and the slices strung from a suspended wire to air-dry for 15 hours. The lipid fraction recovered from the 2,235 grams of dry crumbs amounted to 50% of the initial prime steam-rendered lard.

A slight modification was made in the extraction and purification procedure. The saturated acid monoglycerides were removed prior to a methanol concentration. The data are found in Table IX.

The Skellysolve F insoluble fraction, NK-42A, has been further identified as a saturated monoglyceride by melting point and by infrared analysis (Table I).

The 70% methanol soluble fraction, NK-45B, has been further identified as the monoglyceride of unsaturated fatty acids by infrared analysis and by countercurrent extraction (Table I).

The total weight of purified monoglycerides obtained from the 6 loaves of bread was 3.2 grams. A total amount of 0.25 gram of naturally occurring monoglycerides was present in the prime steam-rendered lard. There was a net gain of monoglycerides in the finished bread of 2.95 grams formed during baking and corresponding to 5.9% of the initial prime steam-rendered lard.

SECTION F

Extraction of Monoglycerides from a Reaction Product of Lard and Glycerol

A monoglyceride reaction product was made by mixing 85 grams of prime steam-rendered lard, 30 grams of C. P. glycerol, and 0.1 gram of sodium hydroxide at 180°C. for two hours and the catalyst inactivated by the addition of 0.1% phosphoric acid. The reaction mixture was dissolved in 1,000 ml. of ethyl ether and the ether solution washed four times with water, dried over sodium sulphate, and the ether solution evaporated under nitrogen. Sixty-six grams of the ether-soluble fraction were dissolved in 800 ml. of acetone and the insoluble material removed by filtration. The acetone was removed under vacuum and the fat dissolved in 600 ml. of ethyl ether. The ether solution was washed repeatedly with water, dried over sodium sulphate, and the ether solution evaporated under nitrogen.

The acetone-soluble, water-washed fraction of 63 grams was dissolved in 600 ml. of warm Skellysolve F and cooled to +5°C. The Skellysolve F insoluble fraction was removed by filtration and recrystallized from Skellysolve F. The Skellysolve F solutions were combined and the solvent removed under nitrogen. The Skellysolve F insoluble fraction, NK-8A, has been further identified as a saturated monoglyceride by its melting point (Table I) and was used as a standard for a monoglyceride of saturated fatty acid for infrared analysis (Figure 1).

The Skellysolve F soluble fraction of 50 grams was extracted twice with 100 ml. of 70% methanol. The methanol extracts were combined and the alcohol removed under vacuum and the fat dissolved in 100 ml. of ethyl ether. The ether solution was water-washed, dried over sodium sulphate, and the ether removed under nitrogen. The 70% methyl alcohol soluble fraction, NK-9A, was used as the standard for monoglycerides of unsaturated fatty acids for infrared spectrophotometry (Figure 1).

The extraction and purification data are given in Table X.

TABLE X

Extraction and Purification of Monoglycerides from a Reaction Product of Lard and Glycerol

Solvent	Monoglyceride Distribution			
	Soluble fraction		Insoluble fraction	
	Wt.	Purity	Wt.	Purity
	g.	%	g.	%
Acetone	63	46	3	0
Skellysolve F (+5°C.)	50	32.6	12.85	98
70% Methanol	6.65	99	43	22

SECTION G

Identification of Monoglycerides by Chemical Methods

Identification by Saponification. The monoglyceride of the saturated fatty acid was expected to be monopalmitin since palmitic acid is the predominant saturated acid in lard. The monoglycerides of the unsaturated fatty acids were expected to be those of oleic and linoleic acids since these acids predominate in lard. To identify further the extracted and purified samples as monoglycerides of these particular acids, several samples were split into fatty acids and glycerol. The identification of fraction NK-177A serves as an example of the analytical methods.

Isolation of Fatty Acid: NK-177A. A 0.31-gram sample of the purified ester was saponified, using 0.3 gram of potassium hydroxide dissolved in 1 ml. of water and 1 ml. of ethanol. The mixture was heated under reflux for one hour. Three ml. of water were added and the aqueous residue was acidified with dilute sulfuric acid. The precipitated acid was taken up in ether and washed repeatedly until there was no trace of mineral acid. The ether solution was dried and the ether removed under nitrogen. The dried weight was 0.24 gram, or 77.4% (theory for palmitic acid 77.5%). The neutralization value of the acid was 219 (theory for palmitic acid 219).

Isolation of Glycerol: NK-177A. As an added check an attempt was made to recover most of the small quantity of glycerol from the aqueous phase.

The water-soluble materials and all the washings from the palmitic acid were dried under vacuum. The salt residue was extracted with ethanol. The ethanol extracts were evaporated, and the resulting syrupy residue accounted for 24.8% of the original material (theory for glycerol in monopalmitin 27.9%). By periodic acid oxidation (2) this material was found to be 90.5% glycerol. It is assumed that some of the glycerol was lost in the various collection and evaporation steps.

The unsaturated monoglyceride fraction extracted from bread made with 2.5% lard and the unsaturated monoglyceride fraction extracted from the prime steam-rendered lard were also examined by saponification with the results reported in Table XI.

Melting Points. The melting points of the various saturated monoglyceride fractions obtained by the capillary method and reported in Table I are all similar. A mixture of the saturated monoglyceride fractions from the lard reaction product, NK-8A, and from the 2.5% lard bread, NK-179A, melted over the same range of 71 to 72.5°C. as did the individual fractions.

Other Chemical Properties. The purity of the monoglyceride fractions was further established by determination of the percentage of the free fatty acids and free glycerol as reported in Table I.

TABLE XI

Identification of Monoglycerides by Saponification

Sample	Fatty acid recovery		Neutralization value		Glycerol recovery	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
	%	%			%	%
Saturated monoglycerides from bread made with 2.5% lard and 0.25% monoglycerides—NK-177A	77.4	77.5[a]	219	219[a]	22.5	27.9[a]
Unsaturated monoglycerides from bread made with 2.5% lard—NK-179B	78.1	78.5[b]	199	199[b]	21.1	25.8[b]
Unsaturated monoglycerides from lard—NK-191B	78.8	78.5[b]	200	199[b]	20.7	25.8[b]

[a] Calculated as the monoglyceride of palmitic acid.
[b] Calculated as the monoglyceride of oleic acid.

SECTION H

Examination of Monoglyceride Fractions by Infrared Spectrophotometry

The infrared spectrum is now generally accepted as the finger print of an organic compound since no two organic compounds have exactly the same spectrum; in fact, different isomers of the same compound have different spectra. Because infrared absorptions are caused by intramolecular vibrations, any change or rearrangement within the molecule will affect its spectrum. As a result, a change in crystalline form will affect the infrared spectrum just as the spectra of the liquid and crystalline states of the same compound will be different. When comparing infrared spectra, all compounds should be run under similar states and conditions.

A Perkin-Elmer Model 12A infrared spectrometer was used for this work. A NaCl prism was used for all spectra over the infrared region from 2μ to 15μ A 0.002" spacer was used for the monoglycerides of saturated fatty acids and monoglycerides of unsaturated fatty acids. In addition, for better definition of the spectrum between 6.7μ and 10.75μ, a 0.001" spacer was used for the unsaturates. Since all the unsaturates were liquids, they could be run as straight oils but the crystalline saturated monoglycerides had to be melted on the cell and then solidified at room temperature. All melted saturated monoglyceride samples were allowed to stand for several days. In the crystalline state the peaks are very sharp and there are more absorption bands than in the liquid state due to the removal, through molecular interaction, of degeneracies which had existed in the free molecule. The broad nature of some of the bands in liquids may arise from the influence of intermolecular collisions, two or more close bands thus being broadened and caused to overlap by the degenerates.

Tables XII and XIII present the wavelengths and probable vibration assignments for the crystalline saturated monoglycerides and for the liquid unsaturated monoglycerides. These are based upon the examination of samples NK-8A and NK-9A but are fully compatible with earlier work done with 1-monoglycerides made from a number of fatty acids. The infrared transmission curves of samples NK-8A and NK-9A are shown on Figure 1.

Because the saturated and unsaturated portions of monoglycerides from lard are mixtures of several monoglycerides, the composition of the various samples may be slightly different and the infrared spectra may vary slightly in the intensity of some of the minor peaks. However all the major monoglyceride

TABLE XII

Infrared Absorption Bands of Crystalline Monoglycerides of Saturated Fatty Acids

Wavelengths in microns (μ)	Strength	Assignments
2.95-3.2	Strong	—O—H stretching (alcohols)
3.35-3.5	Strong	C—H stretching
5.76	Strong	C=O stretching (ester)
6.78	Strong	C—H bending (CH_2 and CH_3)
7.04	Medium	O—H bending
7.16	Strong	C—H bending (C—CH_3)
8.02	Weak	C=O bending
8.19	Weak	.....
doublet { 8.35 / 8.45	Medium / Strong	—C—O stretching (ester)
doublet { 8.91 / 9.06	Medium / Strong	C—O stretching (secondary alcohol)
doublet { 9.41 / 9.55	Strong / Strong	C—O stretching (primary alcohol)
10.08	Strong	C—C stretching
10.59	Strong	C—C stretching
10.94	Medium	C—C stretching
11.33	Weak, broad	C—C stretching
11.75	Medium	C—H rocking
12.03	Medium	C—H rocking
12.34	Weak	C—H rocking
12.84	Weak	C—H rocking
13.91	Medium	C—H rocking

characteristic peaks listed in Tables XII and XIII should be present.

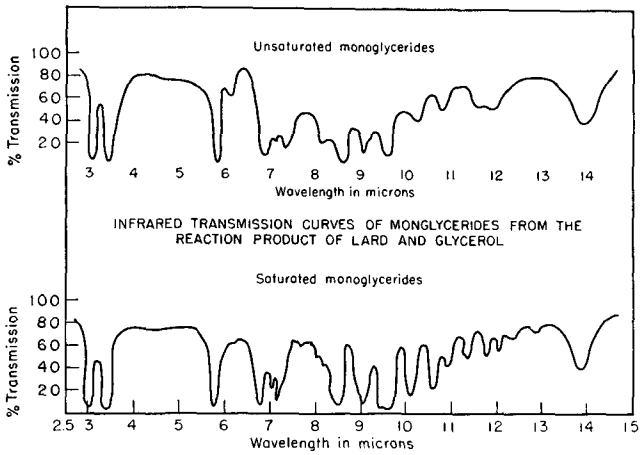

FIG. 1

Saturated Monoglycerides. All the preparations of saturated monoglycerides listed in Table I compared very well with the standard NK-8A. All the peaks were present in all the samples, and none of the absorption peaks shifted in wavelengths. A few minor variations occurred in absorption intensities, but no peaks disappeared and no new peaks appeared. A weak shoulder peak at 10.35μ and a weak peak at 11.33μ varied slightly, as did the sharpness of the weak peaks between 7.60μ and 8.36μ. It was noted that samples NK-177A and NK-179A were exactly alike in every respect by infrared.

Unsaturated Monoglycerides. The unsaturated monoglycerides listed in Table I, when compared with the

TABLE XIII

Infrared Absorption Bands of Liquid Unsaturated Monoglycerides

Wavelengths in microns (μ)	Strength	Assignments
2.8-3.1	Strong	O—H stretching alcohols
3.35-3.5	Strong	C—H stretching
5.76	Strong	C=O stretching (ester)
6.05	Weak	C=C stretching
6.8	Strong	C—H bending (CH_2 and CH_3)
7.05	Weak	O—H bending
7.25	Strong	C—H bending (C—CH_3)
8.08	Weak	C=O bending
8.52	Strong	—C—O— stretching ester
8.97	Strong	C—O— stretching (secondary alcohol)
9.52	Strong	C—O— stretching (primary alcohol)
10.14	Medium	C—C stretching
10.74	Medium	C—C stretching
about 11.46	Broad, weak	C—H rocking
about 11.78	Broad, weak	C—H rocking
13.86	Medium	C—H rocking

standard NK-9A, check very well in all respects. The slight variations observed were merely slight differences in intensities of absorption peaks. Weak shoulder peaks at 7.23μ and 9.14μ varied slightly. The intensities of the peaks at 10.14μ and 10.74μ changed in some of the samples. However all of the absorption peaks were present at the same wavelengths in all the samples. Samples NK-177B and NK-179B were exactly alike.

There is no doubt that all these samples tested by infrared are monoglycerides. Every sample has all the absorption peaks found in the standard monoglyceride sample at the exact same wavelengths. If any of these samples were a different compound, not only would the absorption peaks vary greatly in intensities but the wavelengths would shift. Slight variations are to be expected since these are mixtures of several monoglycerides, but the controlling fact is that no peaks have disappeared and no new peaks have appeared.

SECTION I

Countercurrent Extraction Experiments

Countercurrent distributions were carried out in a 50-tube apparatus described by Craig (4) and manufactured by the Otto Post Scientific Company (Otto Post Scientific Company, Massapeth, New York). The solvent systems used in all distributions were Skellysolve B and an aqueous methanol solution containing 15% water by volume. Both solvents were saturated with one another so that volume changes during the distribution would be reduced to a minimum. The procedure consisted of charging each tube of the apparatus with 10 ml. of the aqueous-methanol solvent delivered by means of an automatic pipette. Into tube O was added 10 ml. of Skellysolve B, where it was equilibrated with the aqueous methanol, allowed to separate, and then decanted into tube 1. The use of this starting charge of Skellysolve B insured complete saturation of the aqueous methanol phase with Skellysolve as it led the sample through the apparatus. To tube O was then added the sample to be fractionated;

the sample was added as a 10 ml. aliquot of a solution in Skellysolve B of the material to be tested. Equilibration consisted of 20 inversions of the apparatus. Settling was permitted for a fixed period of time and controlled for each transfer. Drainage time for decantation was also controlled. After each transfer a 10 ml. charge of the Skellysolve B was added to tube O.

The operation was continued until 50 transfers were completed. Before the 49th transfer the Skellysolve B "leader" had to be removed from the 49th tube so that this last transfer could be made. At the completion of the distribution both layers in each tube were syphoned from the tubes into weighed aluminum cups. Solvents were removed by passing warm air over the cups which were contained in a suitable chamber so that the exhaust air could be vented properly. When free of solvents, the cups were reweighed. A plot of the net weight of the cups against the tube number produced the characteristic distribution curve.

The samples of monoglycerides used to establish the positions of the peaks were prepared in the Research Laboratories of Distillation Products Industries. The monooleate was synthesized directly from 98% pure oleic acid and glycerol while the lard monoglyceride was made from prime steam-rendered lard and glycerol. Purification was accomplished by molecular distillation.

Results and Discussion

The results of the countercurrent distribution experiments are given as weight distribution curves in the accompanying figures. In Figure 2 is shown the

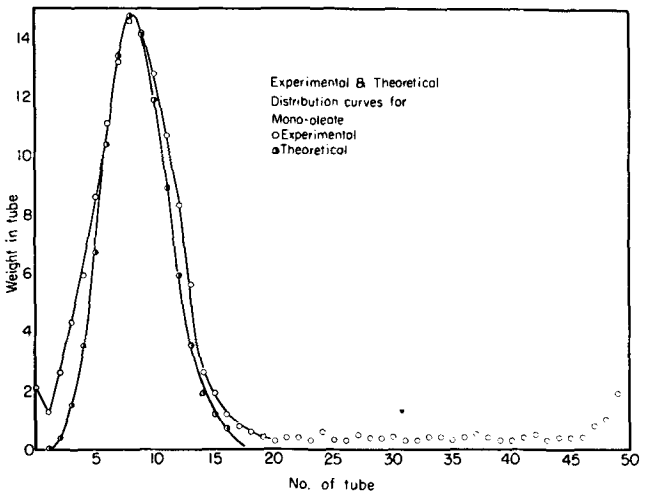

FIG. 2. Countercurrent extraction curves for monoglyceride of oleic acid.

weight-distribution curve for 0.1277 g. distilled monooleate. The position of the peak in the curve occurs between the 8th and 9th tube. Monoglycerides prepared from lard give a peak at the same position as can be seen from Fgure 3 which shows the distribution of a 0.1318 g. charge.

The theoretical curve for the distribution of pure monooleate has been calculated and is shown on Figure 2. The partition coefficient K was computed from the experimental curve by using the weights of pairs

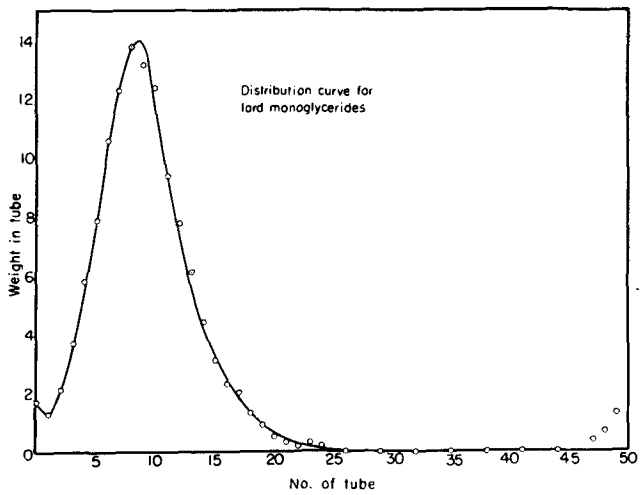

FIG. 3. Countercurrent extraction curve for distilled monoglycerides prepared from lard.

of tubes (T_r and T_{r-1}) which were chosen near the maximum and by use of the following equation (17):

$$K = T_r/T_{r-1}F \quad (1)$$

$$K = T_{r-1}F'/T_r \quad (2)$$

where K = partition coefficient
T = fraction of original substance present in tube r
n = number of transfers
r = tube number

$$F = (n + 1 - r)/r; \quad F' = (r + 1)/(n - r)$$

The value of an average partition coefficient thus computed was .210. It is the average from four pairs of tubes as follows:

r = 7;	.192
r = 8;	.2105
r = 9;	.2135
r = 10;	.2255
Avg.	.210

The theoretical value for $T_{n,r}$ (specifically $T_{49,8}$) for the maximum of the distribution was computed by the expansion of the relation:

$$T_{n,r} = n\,!\,K^r/r\,!\,(n - r)\,!\,(K + 1)^n$$

Using the values

$$n = 49$$
$$r = 8$$
$$K = .210$$

Once $T_{49,8}$ was computed, its value was inserted in equations (1) and (2) and $T_{n,r}$ values were calculated for various values of r, thus giving the theoretical curve on Figure 2.

Although the fit of the two curves is not perfect, the agreement shown in Figure 2 is sufficient to indicate that the experimental curve is that for an almost homogeneous sample of monooleate. The total amount of monoglycerides contributing to the peak of the curve can be obtained by summation of the weights of each fraction in the peak. The values for the lower ends of the peak should be obtained by extrapolation of the peak to zero ordinate. The peak in Figure 2 contains 94.6% of the monooleate sample used for this

experiment. In Figure 3 the sum of the fractions in the peak amounts to 92.0% of the lard monoglyceride sample charged to the distribution apparatus.

Since this work was initiated, a publication appeared by Zilch and Dutton (18) in which the weight distribution curve for the monoglycerides of cottonseed oil was given. The solvent system used was 80% aqueous ethanol and pentanehexane petroleum solvent. The distribution was carried out in a 25-tube apparatus. The distribution coefficient was 0.28, and the position of the monoglyceride peak occurred at the fourth tube.

While the details of procedure are different, the results of Zilch and Dutton are in good agreement with the results of the present work. The differences between the two are due to the choice of solvents and to the number of transfers applied in developing the curve. A change in the polarity of either solvent causes a variation in the distribution coefficient while increasing the number of transfers in a given distribution produces a shift in the position of the peak. This, then, is independent substantiation that the "peak" obtained by countercurrent extraction can be used to identify monoglycerides.

Because in the final use of this method the monoglycerides to be examined may be associated with triglycerides and some fatty acids, it seemed advisable to determine the effects of fats and fatty acids on the position of the monoglyceride maximum. A mixture of 21.3% distilled lard monoglycerides, 19.8% stearic acid (Hystrene 97), and 58.9% shortening (Kremit) was prepared from which a homogeneous sample amounting to .2028 g. was distributed. The results are shown in Figure 4. The monoglyceride peak occurred at the usual position, the eighth tube; but neither the fatty acid nor the triglyceride showed individual or combined peaks. The distribution curve merely approached the 49th tube asymptotically. It is of interest to point out however that the distribution of stearic acid (Hystrene 97) alone, but under identical conditions, gave a well-formed peak at the 44th tube. It appears therefore from the above experiments that the presence of either fatty acid or triglyceride has no effect on the distribution of the monoglycerides, but the distribution characteristics of the fatty acid are changed by the presence of the triglyceride shortening. The monoglyceride peak occurs at essentially the same positions either in the presence or absence of the fatty acid or triglycerides.

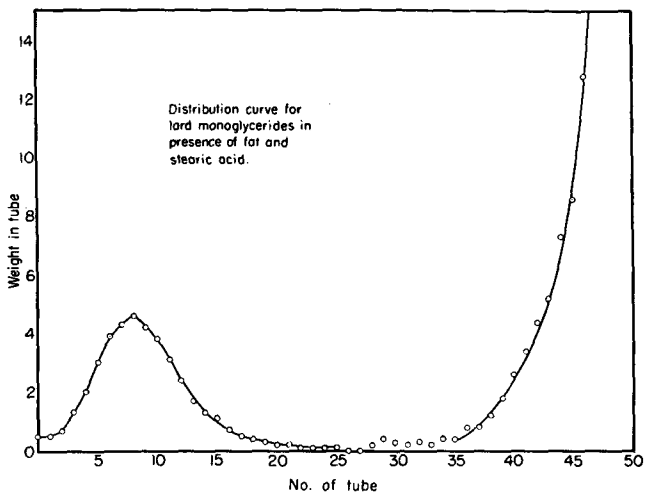

FIG. 4. Countercurrent extraction curve for lard monoglycerides in presence of fat and stearic acid.

These results then were the basis for the monoglyceride determination of bread fat extracts. The weight distribution curves for two bread fat extracts described in the earlier sections are given in Figure 5.

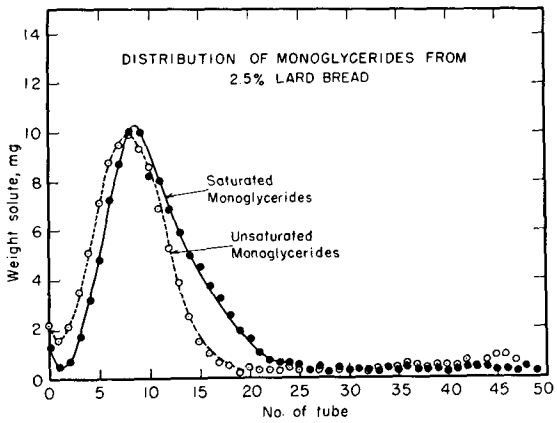

FIG. 5. Countercurrent extraction curves for monoglycerides from 2.5% lard bread.

The monoglyceride fraction, NK-45B, was liquid at room temperature and is assumed to contain mostly monoglycerides of the unsaturated fatty acids. The curve was developed from 0.1025 g. of the fraction; the peak in the curve, including the extrapolation of the sides to zero ordinate, contains 0.0873 g. or 85.2% of the original amount. The product, NK-179A, was solid and assumed to be monoglycerides of saturated fatty acids. The curve was developed from 0.1002 g., and the sum of the material represented by the peak when extrapolated to zero is 0.0943 or 94.1% of the original sample. The small peak at the right-hand side of Figure 5 may be due to free fatty acids. The results show that both samples were of high purity, the saturated monoglyceride sample being the purest.

REFERENCES

1. Bailey, A. E., "Industrial Oil and Fat Products," Interscience Publishers, New York, 2nd ed., pp. 3 and 4 (1951).
2. Bradford, P., Pohle, W. D., Gunther, J. K., and Mehlenbacher, V. C., Oil & Soap, *19*, 189-193 (1942).
3. Cereal Laboratory Methods, 5th ed., p. 164 (1947).
4. Craig, L. C., and Post, O., Anal. Chem., *21*, 500-504 (1949).
5. Dalby, G., Testimony in hearings on definition and standard for bread. FDC Docket No. 31B, pp. 12,736-12,741, 12,745. Also, Bakers Letter, Am. Dry Milk Inst. Inc., No. 117, p. 6.
6. Damle, N. R., J. Indian Inst. Sci., *9A*, 26-42 (1926).
7. Fryer, P. J., and Weston, F. E., Technical Handbook of Oils, Fats, and Waxes, Vol. II, p. 120 (1920).
8. Handschumaker, E., and Linteris, L., J. Am. Oil Chem. Soc., *24*, 143-145 (1947).
9. Hilditch, T. P., and Jones, E. E., J. Chem. Soc. Ind., *50*, 171-176T (1931).
10. Jones, M. E., Koch, F. C., Heath, A. E., and Munson, P. L., J. Biol. Chem., *181*, 755-760 (1949).
11. Lewkowitsch, J., J. Soc. Chem. Ind., *17*, 1107 (1898).
12. Lewkowitsch, J., Chem. Technol. and Analysis of Oils, Fats, and Waxes, 6th ed., Vol. 2, p. 815 (1922).
13. Mueller, H. H., and Holt, E. K., J. Am. Oil Chem. Soc., *25*, 305-307 (1948).
14. Pohle, W. D., Mehlenbacher, V. C., and Cook, J. H., Oil and Soap, *22*, 115-119 (1945).
15. Schlenker, E., and Gnaedinger, J., J. Am. Oil Chem. Soc., *24*, 239-240 (1947).
16. Trauth, J. L., Oil & Soap, *23*, 137-140 (1946).
17. Williamson, B., and Craig, L. C., J. Biol. Chem., *168*, 687-697 (1947).
18. Zilch, K. T., and Dutton, H. J., Anal. Chem., *23*, 775-778 (1951).

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