THE DISTRIBUTION OF ADDED FATTY ACIDS BETWEEN THE FAT PHASE AND THE AQUEOUS PHASE IN CREAM AND IN A BUTTERFAT-IN-WATER EMULSION

THE DISTRIBUTION OF ADDED FATTY ACIDS BETWEEN THE FAT PHASE AND THE AQUEOUS PHASE IN CREAM AND IN BUTTERFAT-IN-WATER EMULSION

By

Harry E. Ferg
Bachelor of Science
University of Wisconsin
1938

Submitted to the Department of Dairying
Oklahoma Agricultural and Mechanical College
In partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
1939

Approved:

E. L. Fouts
In Charge of Thesis

A. H. Buhlman
Acting Head of Department of Dairying

D. G. M. Dutcher
Dean of Graduate School

TABLE OF CONTENTS

	page
Introduction	1
Statement of Problem	4
Review of Literature	5
Part I	
Distribution of Added Fatty Acids Between the Fat and the Serum in Cream	7
Plan of Procedure	7
Results	11
Discussion of Results	17
Part II	
Distribution of Added Fatty Acids Between the Fat and the Water in a Butterfat-in-water Emulsion	18
Plan of Procedure	18
Results	20
Discussion of Results	22
Part III	
Comparative Rate of Volatilization of Various Volatile Fatty Acids from Fat and from Water	24
Plan of Procedure	24
Results	26
Discussion of Results	31
General Discussion of Results	32
Conclusions	35
Acknowledgments	36
Literature Cited	37

INTRODUCTION

The presence of free fatty acids in dairy products has long been recognized as the cause of certain objectionable off flavors appearing in them. Many research workers in dairy industry have defined rancid flavor in dairy products as the specific flavor which resembles that of free butyric acid and certain other low molecular weight fatty acids. This flavor development is thought to be the result of the hydrolysis of butterfat in which fatty acids and glycerol are liberated. The lower fatty acids of milk fat, especially butyric and caproic have very undesirable and readily detectable odors, even in low concentrations.

Evidence is plentiful to show that the hydrolysis of fat may be brought about by lipase, an enzyme which has often been demonstrated to have the power of splitting butterfat into its component parts and liberating fatty acids. This hydrolysis may be caused by excessive lipase being secreted into the milk by the cow or by certain microorganisms growing in dairy products containing butterfat.

The presence of appreciable amounts of free fatty acids in cream is of economic importance mainly from the standpoint of the fact that the flavors caused by them are very objectionable, the cream is difficult to churn, and the fat losses in the buttermilk are possibly greater. Also, there is some evidence that the acidity of the fat is important in hastening oxidation changes in the fat. Certain lots of raw milk or cream, usually from individual cows, develop a rancid flavor very quickly, even at relatively low temperatures. In many cases the development of rancidity is so rapid that the milk is unusable within a few hours after being drawn.

Balls and Lineweaver (1) observed that the difference in the behavior of the higher fats and the lower fats with lipase at different temperatures is very marked. They concluded the "specificity" of lipase varies with the temperature. In view of these observations it may be desirable to know how the individual fatty acids behave in cream; whether the acids dissolve in the serum or whether they remain in the butterfat. Also, such a study would be expected to throw some light upon the possibility of the removal of some of these acids by certain operations in the manufacture of butter, such as, the washing of butter, and the heat treatment which the cream receives.

Since little comprehensive material has been published upon this particular phase of the problem of rancidity in cream, the author undertook a study of the distribution of free fatty acids between fat and serum in cream, in order to obtain a fuller understanding of what becomes of the fatty acids liberated when rancid flavor develops and how the fatty acids are distributed between fat and serum.

This thesis presents experimental data relative to the behavior of certain fatty acids when added separately to cream and to prepared butterfat-in-water emulsions, and also the comparative rate of volatilization of some fatty acids from butterfat, serum, and water.

In view of the limited time allotted for this study, the effect of temperature of holding the samples, the effect of the physical condition of the butterfat (whether liquid or solid), the effect of concentration of butterfat, of the effect of time for equilibrium to be reached in the cream was not determined. The determinations were standardized for all of the above factors. Cream samples containing 30 per cent fat were held for 18 hours at a temperature of 45° C. after the fatty acids were added;

at this temperature the butterfat was in a liquid condition.

By comparing the distribution of various fatty acids in cream with the distribution of these same fatty acids in a prepared butterfat-in-water emulsion, the author intended to show the effect of serum solids of cream upon the distribution of added fatty acids between the fat and the serum of cream.

STATEMENT OF PROBLEM

The work herein reported was undertaken to obtain information regarding the behavior of certain fatty acids when added separately to cream and to prepared butterfat-in-water emulsions. Briefly, the following points were considered:

I. The distribution of added fatty acids between the fat and serum in cream.

II. The distribution of added fatty acids between the fat and water in a prepared butterfat-in-water emulsion.

III. The comparative rate of volatilization of various volatile fatty acids from fat and from water by steam distillation.

REVIEW OF LITERATURE

Distribution of Added Fatty Acids in Cream. As far as the author is aware, there has been no literature published which deals directly with the distribution of added fatty acids between fat and serum in cream. However, Hileman and Courtney (6) and Ramsey and Tracy (10) observed increases in titratable acidity during the development of rancidity in milk. The rancidity they were studying was that brought about by the lipase secreted into the milk by the cow. Increases as high as 0.17 per cent (calculated as lactic acid) were noted. It would seem from these observations that at least part of the fatty acids liberated by lipase action, was dissolved in the serum of the milk. However, from a theoretical standpoint, it is possible that some of the increased acidity was due to the projection of the polar group (-COOH) of the fatty acid molecule into the aqueous phase and subsequently titrated at the fat-water interface.

Bird (2) reported a direct but less definite relationship between the acidity of the fat of butter and that of the cream, and also, that as the cream acidity was reduced the fat acidity was likewise reduced. He stated, however, that the initial fat acidity seemed more a function of the age of the cream than its acidity, since if two creams had the same acidity and one was held longer than the other, the one held the longer gave the butter with the higher fat acidity. He concluded that since the fat-splitting enzymes have more opportunity to work in cream held the longer time and since many of the fatty acids of butterfat are more soluble in the fat than in water they remain in the fat.

Krukovsky and Sharp (8) showed that the difficulty in churning and

the abnormal foam formation observed in cream obtained from milk of cows in advanced lactation was probably due largely to the action of lipase and the concentration of the resultant soaps and fatty acids in the air-plasma interface.

Rate of Volatilization of Various Fatty Acids by Steam Distillation. A comprehensive study of the rate of volatilization of several fatty acids has been made by a number of investigators.

One of the early workers to make such a study and whose values are still often referred to in the literature was Dyer (4). His investigation in this field was conducted upon fatty acids added to water only. The values obtained by him are included later in this paper in comparison with those of the author.

Hiscox and Harrison (7) in studying the rate of volatilization of butyric, caproic, caprylic and lauric acid from water and from a fat and water mixture found that there was a retention of some of these acids by the butterfat, very marked for lauric and high for caprylic, but decreasing through caproic to butyric and the lower members of the series, in which no retention was demonstrated.

Olmsted, Whitaker, and Duden (9) in their study of the steam distillation of fatty acids were able to increase the rate of volatilization of these fatty acids by distilling them from a saturated salt solution.

It is also interesting to call attention to the work of Jensen cited by Gortner (5) in which it was observed that the distillation process in the determination of the Reichert-Meissl number of butterfat accounts for 85-88 per cent of the total butyric acid, 85 to 100 per cent of the caproic acid, and 24 to 25 per cent of the caprylic acid present in glycerides of butterfat. He reports 10 to 13.6 per cent of fatty acids of butterfat are volatile.

PART I

DISTRIBUTION OF ADDED FATTY ACIDS BETWEEN THE FAT AND THE SERUM IN CREAM

Plan of Procedure

Experimental.

Fresh cream of good quality was standardized to 30 per cent fat according to the Babcock test. The cream was pasteurized at 145° F. for 30 minutes, cooled, and formaldehyde was added at the rate of 1 part formaldehyde gas in 2,000 parts of cream. It was intended that the heat treatment should inhibit lipase action in the cream and the formaldehyde prevent growth of microorganisms during the course of the experiment.

The above cream was divided into 100 gm. portions and the following series of samples were prepared:

I. Fatty acids added only.

1. Cream - nothing added (control).
2. Cream + 0.5 ml. n-butyric acid.
3. Cream + 0.5 ml. n-caproic acid.
4. Cream + 0.5 ml. caprylic acid.
5. Cream + 0.5 ml. capric acid.
6. Cream + 0.5 gm. lauric acid.
7. Cream + 0.5 gm. myristic acid.
8. Cream + 0.5 gm. palmitic acid.
9. Cream + 0.5 gm. stearic acid.
10. Cream + 0.5 ml. oleic acid.

II. Samples same as series I except that 0.6 gm. of neutral potassium

oxalate ($K_2C_2O_4.H_2O$) was added to each sample.

Runs were also made in which lesser amounts of fatty acids were added than in the above series. Variations from the above additions of fatty acids were 0.25 and 0.1 ml. or gm. of C. P. fatty acid. In all cases the acids which were liquid at ordinary temperatures were measured and those which were solids were weighed.

All of the above samples in series I and II were placed in 100 ml. bottles and held in a 45° C. incubator for 18 hours. The samples were then centrifuged at 1500 R. P. M. for 10 minutes in separatory funnels in a size 2 International Centrifuge to obtain enough serum to test for acidity and fat. The serum obtained from the cream in series II was centrifuged again to remove the calcium oxalate precipitate. Determinations of total acidity and volatile acidity were then made upon the serum.

The remainder of the cream was then taken from the separatory funnel, cooled, and churned to obtain the fat. The butter samples thus obtained were melted, centrifuged, and the supernatant fat layer was pipetted out. Determinations of total acidity and volatile acidity were made upon aliquot portions of the fat thus obtained.

From the data obtained in this way the percentage distribution of the added fatty acids between the fat phase and the serum phase was calculated. The calculations involved are described in the section immediately following this one. Triplicate determinations were made on each of the series.

Methods.

Total Acidity of Aqueous Phase. Ten gm. of the aqueous layer weighed on a Torsion balance were titrated to the phenolphthalein end

point with 0.1 N. aqueous sodium hydroxide. The amount of fat contained in the serum was determined by the Babcock test. The total acidity of this phase was calculated as follows:

$$\frac{[\text{ml. 0.1 N. NaOH used to neutralize acidity in 10 gm.}]}{[10] - \left[(10).\left(\frac{\text{per cent fat}}{100}\right)\right]} \cdot [\text{gm. of aqueous phase}] =$$

Total acidity expressed as ml. 0.1 N. NaOH.

Total Acidity of Fat Phase. The acidity of the fat was determined by the method of Breazeale and Bird (3). In this method, 10 gm. of the fat layer were weighed into dry 125 ml. Erlenmeyer flasks on a Torsion balance. Ten ml. of absolute alcohol, 25 ml. of petroleum ether, and 10 drops of 1 per cent phenolphthalein in alcohol were added to the flask, and the solution was titrated to the phenolphthalein end point with 0.1 N. alcoholic potassium hydroxide. The total acidity of the fat phase was calculated as follows:

$$\frac{\text{ml. 0.1 N. KOH used}}{10} \cdot \text{gm. of fat in sample} = \text{Total acidity expressed as}$$

ml. 0.1 N. KOH.

The alcoholic potassium hydroxide was prepared from aldehyde free alcohol according to the method of Stout and Schuette (11).

Distribution of Added Fatty Acids between the Fat and Aqueous Phases. The percentage distribution of the added fatty acid between the fat phase and the serum phase of cream was calculated according to the following formulas:

$$\frac{F-f}{(A-a) - (F-f)} \times 100 = \text{Per cent of added fatty acid found in the fat phase.}$$

$$\frac{A-a}{(A-a) - (F-f)} \times 100 = \text{Per cent of added fatty acid found in the aqueous phase.}$$

when,

F = Total acidity of the fat phase of sample.

f = Total acidity of the fat phase of control.

A = Total acidity of the aqueous phase of sample.

a = Total acidity of the aqueous phase of control.

Volatile Acidity. Ten gm. samples were weighed into 500 ml. round bottom flasks. The samples were then steam distilled at a uniform rate for 30 minutes so that 200 ml. of distillate were collected in that time. The distillate was titrated with 0.02 N. aqueous sodium hydroxide to the phenolphthalein end point.

Reagents Used. The fatty acids used were C. P. fatty acids sold by Eimer & Amend, New York.

The alkali solutions were prepared from Merck's reagent potassium hydroxide pellets and C. P. Baker's Analyzed sodium hydroxide sticks.

The potassium oxalate used was Merck's neutral potassium oxalate ($K_2C_2O_4.H_2O$).

RESULTS

Data pertinent to the distribution of added fatty acids between butterfat and serum in cream containing 30 per cent butterfat have been summarized in tables I, II, III, and IV.

In table I is shown the percentage distribution of the added acidity in cream samples to which no neutral potassium oxalate was added. In these samples increases in titratable acidity of the serum were observed, even with the higher molecular weight fatty acids.

Tables II, III, and IV present data relating to the effect of neutral potassium oxalate upon the distribution of the increased acidity brought about by the addition of various fatty acids in varying concentrations. No increases in the titratable acidity of the serum were observed in these samples when lauric, myristic, palmitic, stearic, and oleic acids were added. The data also show that as the concentration of the added fatty acids increased a larger percentage of the acid was contained in the fat phase.

The results of the study of volatilization of various added fatty acids from fat and from the serum in samples of cream in trials 1 to 8 have been recorded in table V. The data presented tends to show that there is a partial retention of the low molecular weight fatty acids by the buffers in the serum; since, the percentage volatilization of these acids decreased markedly as the concentrations of the acids in the serum decreased. It must be pointed out that with the fatty acids only slightly soluble in the serum the experimental error was probably rather large; since only a very small amount of the acid was present in the serum and small errors in titration of the acid probably caused considerable variation in the results.

TABLE I

Percentage Distribution of Added Fatty Acids Between Fat and Serum in 30 Per Cent Cream (No Potassium Oxalate Added)

To trials 1, 2, and 3, 0.5 ml. or gm. of fatty acids were added.
To trials 4, 5, and 6, 0.25 ml. or gm. of fatty acids were added.
To trials 7 and 8, 0.1 ml. or gm. of fatty acids were added.

Fatty acid added to 100 gm. of cream	1		2		3		4		5		6		7		8	
	Per cent of increased acidity in															
	fat	serum	fat	serum	fat	serum	fat	serum	fat	serum	fat	serum	fat	serum	fat	serum
Butyric							6.0	94.0	6.0	94.0			5.4	94.6	4.0	96.0
Caproic	48.8	52.1	43.4	56.6	48.3	51.7	31.0	69.0	31.3	68.7	32.4	67.6	20.2	79.8	23.6	76.4
Caprylic	78.6	21.4	80.8	19.2	82.0	18.0	75.9	24.1	74.8	25.2	72.7	27.3	59.4	40.6	71.7	28.3
Capric	88.8	11.2	84.3	15.7	93.7	6.3	89.6	10.4	83.0	17.0	85.2	14.8	72.0	28.0	86.3	13.7
Lauric	80.5	19.5			84.4	15.6	62.0	38.0	71.1	28.8	76.8	23.2	77.1	22.9	85.7	14.3
Myristic	80.5	19.5			62.5	37.5	26.4	73.6	43.6	56.4	47.5	52.5	40.0	60.0	50.0	50.0
Palmitic	48.8	51.2	40.5	59.5	61.9	38.1										
Stearic	40.9	59.1	35.8	64.2	39.8	60.2										
Oleic	59.2	40.8	66.4	33.6	67.0	33.0	54.8	45.2	58.6	41.4	61.2	38.8	57.9	42.1	100.0	0.0

TABLE II

Percentage Distribution of Added Fatty Acids Between Fat and Serum in 30 Per Cent Cream to Which Neutral Potassium Oxalate Was Added

0.6 gm. of neutral potassium oxalate ($K_2C_2O_4.H_2O$) was added to each sample of cream. 0.5 ml. or gm. of fatty acid was used.

Trial No.	9A		10		11		12		Average	
Fatty acid added to 100 gm. cream	Per cent of increased acidity in									
	fat	serum	fat	serum	fat	serum	fat	serum	fat	serum
Butyric	9.7	90.3	8.7	91.3	9.7	90.3	9.5	90.5	9.4	90.6
Caproic			34.8	65.2	34.6	65.4	34.2	65.8	34.5	65.5
Caprylic	68.1	31.9	66.7	33.2	69.5	30.5	69.4	30.6	68.4	31.6
Capric	89.7	10.3	87.4	12.6	88.7	11.3	88.3	11.7	88.5	11.5
Lauric	100.0	0.0			96.6	3.4				
Myristic	100.0	0.0	100.0	0.0	100.0	0.0				
Palmitic	100.0	0.0	82.9	17.1	100.0	0.0				
Stearic	100.0	0.0	84.5	15.5	95.0	5.0				
Oleic	100.0	0.0			95.5	4.5				

TABLE III

Percentage Distribution of Added Fatty Acids Between Fat and Serum in 30 Per Cent Cream to Which Neutral Potassium Oxalate Was Added

0.6 gm. of neutral potassium oxalate ($K_2C_2O_4.H_2O$) was added to each sample of cream. 0.25 ml. or gm. of fatty acid was used.

Trial No.	9B		13		14		15		Average	
Fatty acid added to 100 gm. cream	Per cent of increased acidity in									
	fat	serum	fat	serum	fat	serum	fat	serum	fat	serum
Butyric	6.0	94.0	4.0	96.0	3.7	96.3	3.7	96.3	4.4	95.6
Caproic	21.8	78.2	19.3	80.7	16.0	84.0	18.5	81.5	18.9	81.1
Caprylic	63.7	36.3	50.9	49.1	48.4	51.6	49.1	50.9	53.0	47.0
Capric	93.7	6.3	67.6	32.4	83.3	16.7	87.7	12.3	83.1	16.9
Lauric	100.0	0.0								
Myristic	100.0	0.0								
Palmitic	100.0	0.0								
Stearic	100.0	0.0								
Oleic	100.0	0.0								

TABLE IV

Percentage Distribution of Added Fatty Acids Between Fat and Serum in 30 Per Cent Cream to Which Neutral Potassium Oxalate Was Added

0.6 gm. neutral potassium oxalate ($K_2C_2O_4.H_2O$) was added to each sample of cream. 0.1 ml. or gm. of fatty acid was used.

Trial No.	9C		16		17		18		Average	
Fatty acid added to 100 gm. cream	Per cent of increased acidity in									
	fat	serum	fat	serum	fat	serum	fat	serum	fat	serum
Butyric	5.9	94.1	2.4	97.6	2.4	97.6	2.5	97.5	3.3	96.7
Caproic	12.1	87.9	8.1	91.9	7.0	93.0	6.3	93.7	8.4	91.6
Caprylic	56.0	44.0	46.2	53.8	46.1	53.9	45.5	54.5	48.5	51.5
Capric	91.3	8.7	82.2	17.8	71.2	28.8	73.2	26.8	79.5	20.5
Lauric	100.0	0.0								
Myristic	100.0	0.0								
Palmitic	100.0	0.0								
Stearic	100.0	0.0								
Oleic	100.0	0.0								

TABLE V

Percentage Recovery of Added Fatty Acids by Steam Distillation from Fat and from Serum in 30 Per Cent Cream

These determinations were made upon aliquot portions of the samples described in table I.

Trial No.	1		2		3		4		5		6		7		8	
Fatty acid added to 100 gm. of cream	Per cent recovery of acid in															
	fat	serum	fat	serum	fat	serum	fat	serum	fat	serum	fat	serum	fat	serum	fat	serum
Butyric			73.0	63.6			87.1	66.2	77.2	74.8			92.0		45.0	47.9
Caproic			95.1	79.3	105.7	80.4	86.3	115.5	91.8	74.4	97.5	68.0	43.0		85.9	54.3
Caprylic			93.6	89.4	85.4	94.6	83.6	87.6	78.0	92.9	87.7	77.4	76.1		72.6	67.1
Capric			22.4	77.5	19.2	73.3	20.5	32.7	21.1	63.3	22.4	23.8			35.5	36.7
Lauric							6.3	14.3	6.4	18.6	5.1	23.3	6.5	0.0		
Myristic							0.0	17.9	4.7	18.3	0.0	9.4				
Oleic			0.0	33.0	14.3	22.2	0.0	25.0	1.2	6.1			0.0	12.7	8.0	0.0

DISCUSSION OF RESULTS

It is interesting to point out that free butyric and caproic acids, the acids generally considered to be responsible for rancid flavor in dairy products, were contained largely in the serum of the cream. Caprylic and capric acids were contained largely in the butterfat, while the higher molecular weight fatty acids were contained entirely in the butterfat.

When no neutral potassium oxalate was added to the samples of cream, increases in titratable acidity of the serum were observed when the fatty acids were added, even with the higher molecular weight fatty acids. In samples to which neutral potassium oxalate was added, the higher molecular weight fatty acids caused no increase in the titratable acidity of the serum. These results were interpreted to show that the soluble calcium and magnesium was precipitated with the higher molecular weight fatty acids as insoluble calcium soaps, which resulted in an increase in titratable acidity of the serum. The theory is that the calcium ions are replaced by hydrogen ions from the fatty acid molecule. According to Gortner (5) calcium soaps are water insoluble.

PART II

DISTRIBUTION OF ADDED FATTY ACIDS BETWEEN THE FAT AND THE WATER IN A BUTTERFAT-IN-WATER EMULSION

Plan of Procedure

Experimental.

In this experiment an attempt was made to prepare a fat-in-water emulsion which contained the same concentration of fat as the cream used in the previous experiment. This was done by weighing 70 gm. portions of distilled water containing 0.25 per cent lecithin into 150 ml. beakers. Into each 70 gm. portion of distilled water containing lecithin, 30 gm. of melted butterfat were weighed and emulsified with a hand homogenizer. The 100 gm. portions of the emulsions were then transferred to 100 ml. bottles and the following series of samples were prepared:

1. Emulsion - nothing added (control).
2. Emulsion + 0.5 ml. n-butyric acid.
3. Emulsion + 0.5 ml. n-caproic acid.
4. Emulsion + 0.5 ml. caprylic acid.
5. Emulsion + 0.5 ml. capric acid.
6. Emulsion + 0.5 gm. lauric acid.
7. Emulsion + 0.5 gm. myristic acid.
8. Emulsion + 0.5 gm. palmitic acid.
9. Emulsion + 0.5 gm. stearic acid.
10. Emulsion + 0.5 ml. oleic acid.

Runs were also made in which lesser amounts of fatty acids were

added than in the above series. Variations from the above additions of fatty acids were 0.25 and 0.1 ml. or gm. of C. P. fatty acid. In all cases the acids which were liquid at ordinary temperatures were measured and those which were solids were weighed.

The above samples were held in a 45° C. incubator for 18 hours, then cooled and churned. The butter obtained in this manner was melted and centrifuged. The supernatant fat layer was then pipetted out and an aliquot was titrated to determine the total acidity of the fat phase.

The aqueous layers decanted from the butter samples were centrifuged at 1500 R. P. M. for 10 minutes in separatory funnels in a No. 2 International Centrifuge to remove more of the fat dispersed in this layer. A lower portion of this aqueous solution was then drawn off and an aliquot was used for a fat test and a total acidity test.

From the data thus obtained the distribution of the added fatty acids between the fat phase and the aqueous phase was calculated. The calculations involved are described in part I. Triplicate determinations were made on each of the series.

Methods.

Laboratory methods and calculations have been described in part I. In this experiment an effort was made to make the determinations parallel to the experiment with natural cream in part I, so that direct comparison of results obtained in parts I and II could be made.

The reagents used were the same as those used in part I. The lecithin used to stabilize the butterfat-in-water emulsion was lecithin from eggs sold by Central Scientific Company.

RESULTS

Data pertinent to the distribution of added fatty acids between the fat phase and the aqueous phase in a butterfat-in-water emulsion containing 30 per cent fat have been summarized in table VI.

It was observed that butyric acid was contained largely in the aqueous phase indicating that butyric acid has a greater affinity for the water than for the fat. Caproic, caprylic, and capric acids were contained largely in the fat phase, while lauric, myristic, palmitic, stearic, and oleic acids were contained entirely in the fat phase.

Table VI has shown that approximately 80 per cent of the added butyric acid was contained in the aqueous phase of the butterfat-in-water emulsion regardless of the amount of butyric acid added. However, caproic, caprylic, and capric acids showed an increase in the proportional amount of those acids contained in the aqueous phase when the total addition of these acids was lessened.

TABLE VI

Percentage Distribution of Added Fatty Acids Between Fat and Water in a Fat-In-Water Emulsion Containing 30 Per Cent Fat

To trials 1, 2, 3, and 4, 0.5 ml. or gm. of fatty acids were added; to trials 5, 6, and 7, 0.25 ml. of fatty acids were added; to trials 8, 9, and 10, 0.1 ml. of fatty acids was added.

Trial No.	1		2		3		4		5		6		7		8		9		10	
Fatty acid added to 100 gm. cream	Per cent of increased acidity in																			
	fat	water	fat	water	fat	water	fat	water	fat	water	fat	water	fat	water	fat	water	fat	water	fat	water
Butyric	20.7	79.3			19.6	80.4	20.6	79.4	19.0	81.0	19.1	80.9	18.5	81.5	25.6	74.4	16.7	83.3	23.6	76.4
Caproic	78.5	21.5	80.1	19.9	77.4	22.6	77.7	22.3	72.9	17.1	72.9	27.1	74.5	25.5	67.8	32.2	70.7	29.3	67.3	32.7
Caprylic	93.6	6.4			95.6	4.4	95.5	4.5	89.6	10.4	91.5	8.5	91.3	8.7	91.8	8.2	90.4	9.6	85.4	14.6
Capric	96.5	3.5	97.7	2.3	96.2	3.8	96.1	3.9	93.6	6.8	91.9	8.1	91.9	8.1	93.6	6.4	95.1	4.9	93.6	6.4
Lauric	99.0	1.0	100.0	0.0																
Myristic	100.0	0.0	100.0	0.0																
Palmitic	98.9	1.1	99.6	0.4																
Stearic	100.0	0.0	100.0	0.0																
Oleic	98.8	1.2	100.0	0.0																

DISCUSSION OF RESULTS

This experiment was performed with the intention of finding an explanation for some of the results obtained in part I. By this comparison using an artificial cream containing no buffer substance, the effect of the buffers of cream upon the distribution of added fatty acids between the fat phase and the aqueous phase can be more easily brought out.

By comparing the distribution of various added fatty acids in cream (tables III, IV and V) with the distribution of these same added fatty acids in a prepared butterfat-in-water emulsion (table VI), the effect of serum solids of cream upon the distribution of added fatty acids between the fat and serum of cream may be observed. These data showed that a greater percentage of the added fatty acids were contained in the fat phase of the butterfat-in-water emulsion than in the fat phase of the cream samples used. This difference was less noticeable as the molecular weight of the fatty acid increased.

These results have been interpreted to show that the buffers in cream combine with a part of the added fatty acid and thereby decrease the concentration of the free fatty acid in the serum. This in turn increases the titratable acidity of the serum but will not allow this portion of the fatty acid to diffuse into the fat as in the case of a fat-in-water emulsion without buffers.

Much smaller variations were encountered in the distribution of the added fatty acids between the two phases of the butterfat-in-water emulsion, with variations in concentrations of the added acids than were experienced in the samples of cream to which varying concentrations of fatty acids were added. This observation may also be explained, in

part, by the effect of buffers upon these acids. Since the buffer capacity of all the samples of cream were the same before the acids were added, it appeared that an approximately constant amount of the acid was neutralized by these buffers. Therefore, if the concentrations of the added acids were decreased the titratable acidity of the fat was decreased to a greater extent than was the titratable acidity of the serum. This view is strengthened by the observations upon the corresponding samples of the butterfat-in-water emulsion, in which much less marked changes in the percentage distribution values with changes in concentration of the acid in question was observed. Actually, the percentage distribution of butyric acid between the fat phase and the aqueous phase in the butterfat-in-water emulsion was the same regardless of whether 0.5 ml., 0.25 ml., or 0.1 ml. of butyric acid was added. With caproic, caprylic and capric acid, a decrease in the per cent of acid dissolving in the fat was observed, but to a lesser extent than in the cream. However, it must be pointed out that caproic acid is only slightly soluble in water, caprylic and capric acids are only very slightly soluble in water. Concentrations of 0.5 ml. and 0.25 ml. of these acids in 100 gm. of the emulsion exceed their solubilities in the water contained in the emulsion.

PART III

COMPARATIVE RATE OF VOLATILIZATION OF VARIOUS VOLATILE FATTY ACIDS FROM BUTTERFAT AND FROM WATER

Plan of Procedure

Forty gm. portions of butterfat were weighed and the following series of samples were prepared:

1. Butterfat - nothing added (control).
2. Butterfat + 0.5 ml. n-butyric acid.
3. Butterfat + 0.5 ml. n-caproic acid.
4. Butterfat + 0.5 ml. caprylic acid.
5. Butterfat + 0.5 ml. capric acid.
6. Butterfat + 0.5 gm. lauric acid.
7. Butterfat + 0.5 gm. myristic acid.
8. Butterfat + 0.5 ml. oleic acid.

Other series of samples to which 0.25 and 0.1 ml. or gm. of fatty acids were added were also prepared.

The above samples of butterfat were held for 18 hours in a 45° C. incubator to allow sufficient time for the acids to dissolve in the butterfat. The total acidity of the butterfat was then determined and 10 gm. aliquots were steam distilled. The distillates were collected in 10 ml. fractions and titrated with 0.02 N. sodium hydroxide to the phenolphthalein end point. When 20 fractions were collected the distillation process was discontinued.

The steam distillation apparatus consisted essentially of: 1 2-liter roud bottom flask; 2 500-ml. round bottom flasks; 2 nitrogen connection bulbs; 2 spiral, glass, water-cooled condensers; and 7 mm. glass tubing

and rubber stoppers for connections. The steam was generated in the 2-liter flask heated with a bunsen burner. The 2-liter flask was connected with glass tubing to the 2 500-ml. flasks containing the samples. The nitrogen connection bulbs served as a connection between the 500-ml. flasks and the condensers. The distillates were collected in test tubes calibrated at 10 ml. This apparatus distilled two samples simultaneously.

A series of samples containing 40 gm. portions of distilled water instead of butterfat were also prepared as follows:

1. Water - nothing added (control).
2. Water + 0.5 ml. n-butyric acid.
3. Water + 0.25 ml. n-butyric acid.
4. Water + 0.1 ml. n-butyric acid.
5. Water + enough n-caproic acid to produce a saturated solution.
6. Water + 0.25 ml. n-caproic acid.
7. Water + 0.1 ml. n-caproic acid.
8. Water + enough caprylic acid to produce a saturated solution.
9. Water + enough capric acid to produce a saturated solution.

The experiment with the aqueous samples was conducted the same as with the butterfat and all determinations were made parallel to the trials with butterfat. These aqueous samples were steam distilled simultaneously with the corresponding fat samples in a distillation apparatus so constructed that two samples were steam distilled at the same time under identical conditions. The steam inlets to the distillation flasks were regulated with screw cocks so that the distillate could be collected at the same rate from both flasks.

RESULTS

Average results of the study of the rate of volatilization of various volatile fatty acids have been graphically illustrated in figures 1, 2, 3, and 4.

A comparison of the rate of volatilization of butyric, caproic, caprylic, and capric acids, as shown in curve I in figures 1, 2, 3, and 4, indicates that caproic and caprylic acid volatilized by steam at a somewhat faster rate than did butyric acid. Curve I in figure 4 seems to indicate that capric acid distilled from water at a slower rate than did the lower members of the fatty acid series. However since only a very small amount of capric acid was soluble in water, it is possible that the experimental error in the determination of the rate of volatilization of this acid was great enough to render values obtained for this acid insignificant. This comparison tends to show, but not conclusively, that there is a relationship between the rate of volatilization of a fatty acid from water and its molecular weight; the acid with the lowest molecular weight steam distilled at the slowest rate from water.

In contrast to the above comparison a relationship in the opposite direction was demonstrated when these acids were distilled from pure butterfat instead of water. That is, the fatty acid with the lowest molecular weight steam distilled at the fastest rate from butterfat.

By studying the completeness of volatilization of various fatty acids from water it was observed that the percentages of the acids recovered with butyric, caproic, caprylic, and capric, were 99.0, 99.0, 99.5 and 84.0 respectively; from butterfat the percentages for the same acids were 98.0, 97.0, 81.0, and 25.0, and for lauric, myristic, and oleic acids, 6.0, 1.0 and 2.0 respectively.

FIG. 1

Rate of Volatilization of Butyric Acid from Fat and from Water

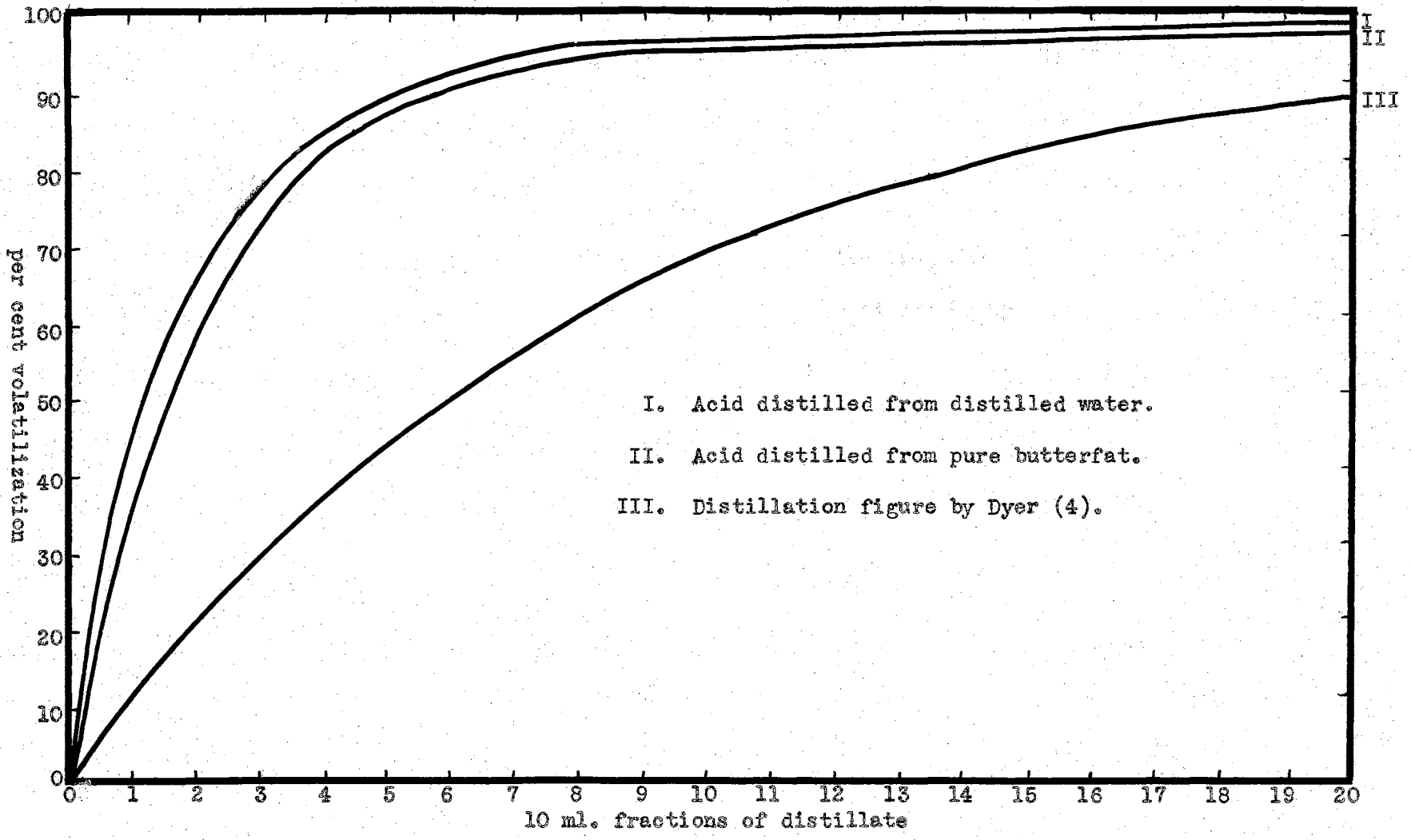


FIG. 2

Rate of Volatilization of Caproic Acid from Fat and from Water

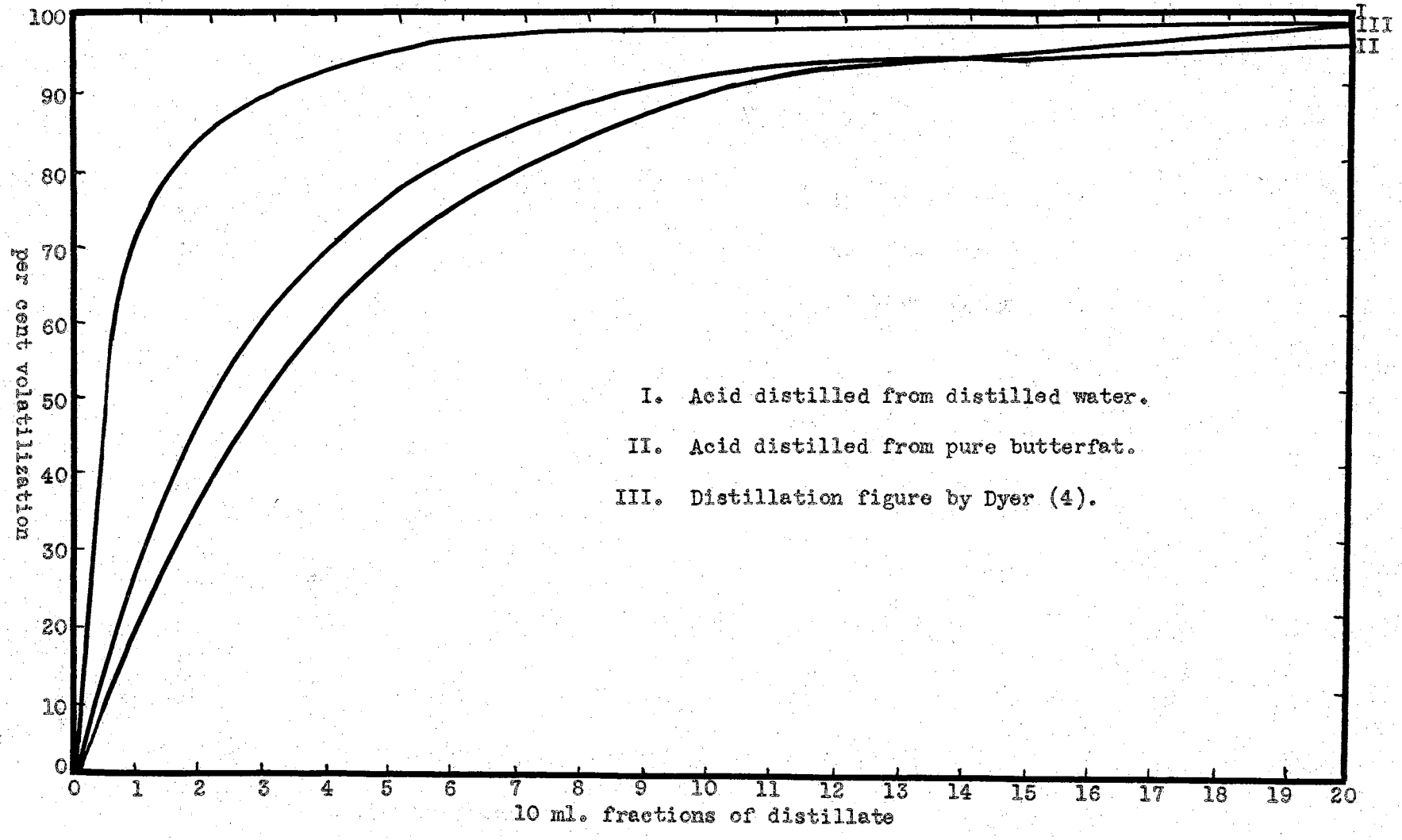


FIG. 3

Rate of Volatilization of Caprylic Acid from Fat and from Water

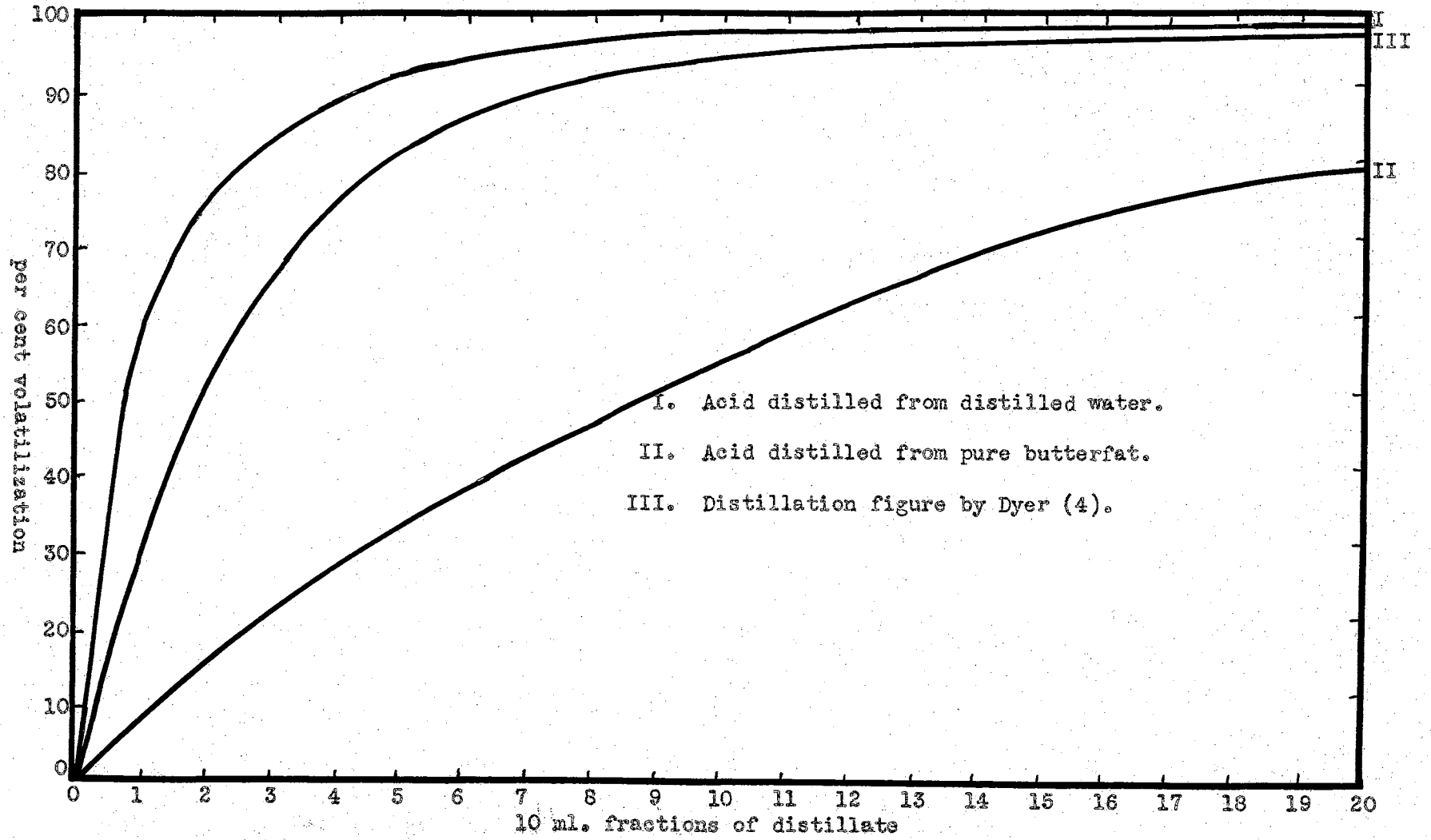
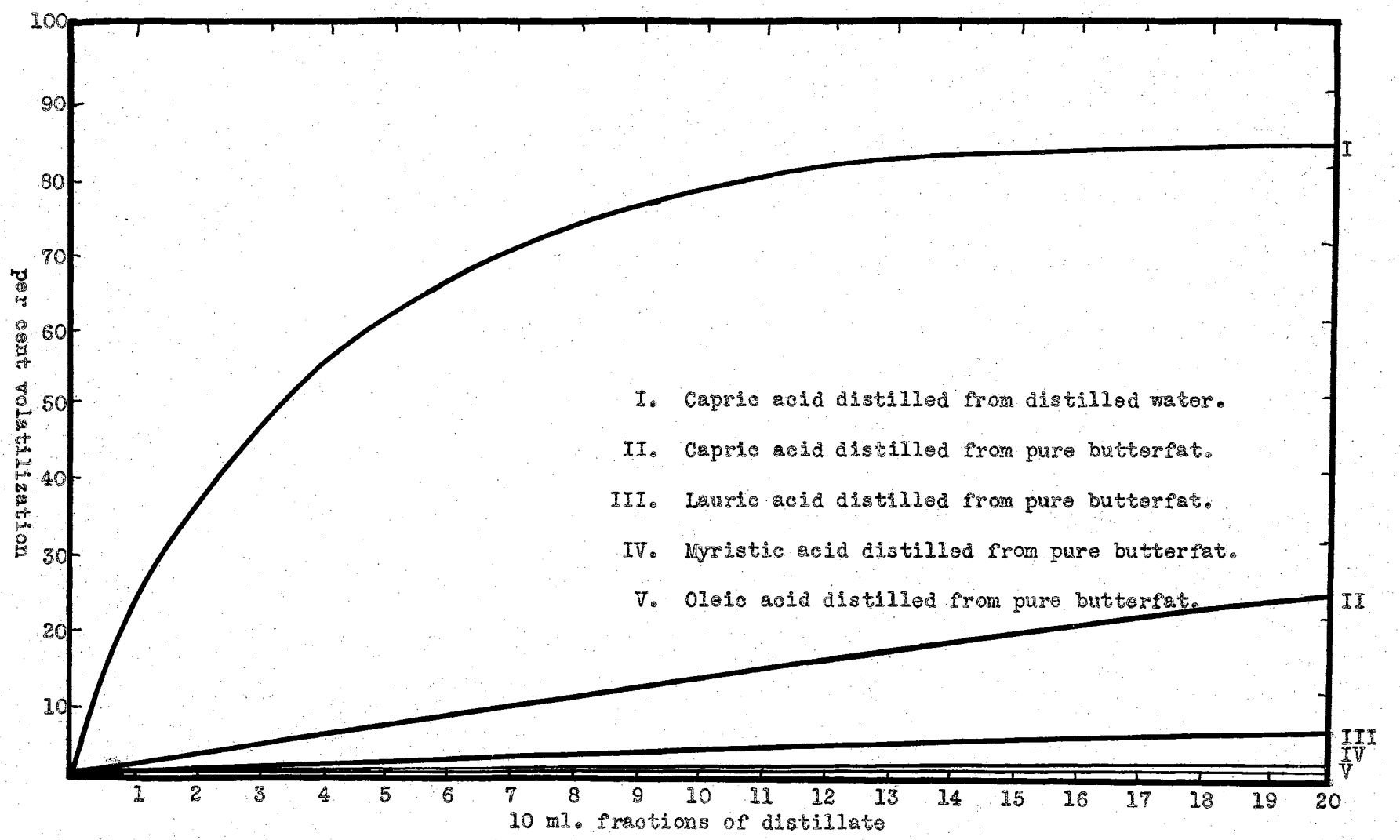


FIG. 4

Rate of Volatilization of Higher Fatty Acids



DISCUSSION OF RESULTS

In this experiment an attempt was made to make all determinations of the rate of volatilization of various volatile fatty acids from water parallel to the trials with butterfat.

It is evident from the results shown in the curves in figures 1, 2, 3, and 4, that there was a retention of some of the fatty acids by the butterfat, very marked for capric and high for caprylic, but decreasing through caproic to butyric acid, the last of which practically no retention was demonstrated.

It will be noticed that distillation rates of various lower molecular weight fatty acids given by Dyer (4) have been included in figures 1, 2, and 3. The values, however, may not be strictly comparable with those of the author, since the methods of determination differ slightly. Dyer used considerably larger amounts of the acid, steam distilled from 150 ml. of water kept at a constant volume; while the author steam distilled 10 gm. samples containing smaller amounts of the acid and did not control the volume in the distilling flask during the distillation process.

GENERAL DISCUSSION OF RESULTS

The aim of this thesis was to present data which showed where free fatty acids will distribute themselves in cream and also the rate of volatilization of some fatty acids from water, butterfat, and serum of cream.

By comparing the distribution of various fatty acids in cream with the distribution of these same fatty acids in a prepared butterfat-in-water emulsion, the author aimed to show the effect of serum solids of cream upon the distribution of added free fatty acids between the fat and the serum of cream.

It is apparent from the results of this investigation that the free fatty acids generally considered to be responsible for rancid flavor in dairy products are contained largely in the serum of the cream. Added butyric and caproic acids caused a greater increase in the titratable acidity of the serum than in the titratable acidity of the fat. Caprylic and capric acids were contained largely in the butterfat, while the higher molecular weight fatty acids were contained entirely in the butterfat.

When no neutral potassium oxalate was added to the samples of cream, increases in titratable acidity of the serum were observed when the fatty acids were added, even with the higher molecular weight fatty acids. In samples to which neutral potassium oxalate was added, the higher molecular weight fatty acids caused no increase in the titratable acidity of the serum. Those results were interpreted to show that the soluble calcium and magnesium was precipitated with the higher molecular weight fatty acids as insoluble calcium soaps, which resulted in an increase in titratable acidity of the serum. The theory is that the calcium ions are

replaced by hydrogen ions from the fatty acid molecule. According to Gortner (5) calcium soaps are water insoluble.

By comparing the distribution of the lower molecular weight fatty acids between the fat and the serum in cream with the distribution of the same fatty acids between the fat and the water in a prepared butterfat-in-water emulsion it was observed that a higher percentage of the acid was contained in the fat of the butterfat-in-water emulsion than in the fat of cream. This observation led the author to believe that the buffers in cream neutralized a part of the added lower molecular weight fatty acids, and consequently lowered the concentration of the acid and allowed less of the acid to dissolve in the butterfat. This reaction would increase the titratable acidity of the serum and lower the fat acidity.

Differences in the distribution of various low molecular weight fatty acids in cream with changes in concentration of these acids may also be explained, in part, by effect of buffers upon these acids. Since the buffer capacity of all samples of cream was the same before the acids were added, it appeared that an approximately constant amount of the acids would be neutralized by these buffers. Therefore, if the concentrations of the added acids were decreased the titratable acidity of the fat was decreased to a greater extent than was the titratable acidity of the serum. This view is strengthened by the observations upon corresponding samples of the butterfat-in-water emulsion, in which much less marked changes in the percentage distribution values with changes in concentration of the acid in question was observed. Actually, the percentage distribution of butyric acid between the fat phase and the aqueous phase in the butterfat-in-water emulsion was the same regardless of whether 0.5 ml., 0.25 ml., or 0.1 ml. of butyric acid was added. With

caproic, caprylic, and capric acid, a decrease in the per cent of acid dissolving in the fat was observed, but to a lesser extent than in the cream. However, it must be pointed out that caproic acid is only slightly soluble in water, caprylic and capric is very slightly soluble in water. Concentrations of 0.5 ml. and 0.25 ml. of these acids in 100 gm. of the emulsion exceeds the solubility of these acids in the water contained in this emulsion.

It is evident from the results shown in the curves in figures 1, 2, 3, and 4, in part III, that there was a retention of some of the fatty acids by the butterfat, very marked for capric and high for caprylic, but decreasing through caproic to butyric acid, the last of which practically no retention was demonstrated. This observation is in agreement with the findings of Hiscox and Harrison (7).

It was also observed from these results that the rate of distillation of the added fatty acids from butterfat was related to the molecular weight of the fatty acid; the acids with the lowest molecular weight steam distilled with greatest rapidity. However, when butyric, caproic and caprylic acids were steam distilled from water, the fatty acid with the lowest molecular weight steam distilled at the slowest rate.

CONCLUSIONS

Added butyric and caproic acids were contained largely in the serum of cream. Caprylic and capric acids were contained largely in the butterfat, while the higher molecular weight fatty acids were contained entirely in the fat phase.

As the concentration of the added fatty acid in cream was increased a larger percentage of the acid was contained in the fat phase.

A higher percentage of the added fatty acid was contained in the fat of the prepared butterfat-in-water emulsion than in the fat of cream. This observation led the author to believe that the buffers in cream neutralized a part of the low molecular weight fatty acids, and consequently decreased the concentration of the free acid and allowed less of the acid to dissolve in the butterfat.

Calcium of cream precipitated a part of the high molecular weight fatty acids. This precipitation caused a slight rise in serum acidity, even though the fatty acid was insoluble in the serum.

When the serum of cream was steam distilled a partial retention of the fatty acids by the buffers of the serum of cream was observed.

It was evident that when butterfat was subjected to steam distillation there was a retention of some of the fatty acids by the butterfat, very marked for capric and high for caprylic, but decreasing through caproic to butyric, the last of which practically no retention was demonstrated.

The rate of distillation of the fatty acids from fat decreased with increase in molecular weight of the fatty acid. However, when butyric, caproic and caprylic acid was distilled from water, the fatty acid with the lowest molecular weight steam distilled at the slowest rate.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. E. L. Fouts for the many helpful suggestions in this study and in the preparation of this thesis.

To Dr. J. E. Webster the author is indebted for valuable information regarding some of the chemical aspects of this problem.

LITERATURE CITED

1. Balls, A. K., and Lineweaver, Hans. Action of enzymes at low temperatures. U. S. D. A., Washington, D. C. Food Research 3: 57-67, (1938).

2. Bird, E. W. Effect of variable cream acidities. Amer. Prod. Rev., 87: 510, (1939).

3. Breazeale, D. F., and Bird, E. W. A study of methods for the determination of acidity in butterfat. J. Dairy Sci. 21: 335-344, (1938).

4. Dyer, D. C. A new method of steam distillation for the determination of the volatile fatty acids, including a series of colorimetric qualitative reactions for their identification. Jour. Biol. Chem. 28: 445-473, (1916).

5. Gortner, R. A. Outlines of biochemistry. John Wiley & Sons. Second Edition, 34, 774, (1938).

6. Hileman, J. L., and Courtney, E. Seasonal variations in the lipase content of milk. J. Dairy Sci. 18: 247-257, (1935).

7. Hiscox, E. R., and Harrison, J. Estimation of fatty acids in organic mixtures. Nature 138: 72-73, (1936).

8. Krukovsky, V. N., and Sharp, P. F. Effect of lipolysis on the churnability of cream obtained from milk of cows in advanced lactation. J. Dairy Sci. 19: 279-284, (1936).

9. Olmsted, W. H., Whitaker, W. M., Duden, C. W. Steam distillation of the lower volatile fatty acids from a saturated salt solution. Jour. Biol. Chem. 85, 190-14, (1929).

10. Ramsey, R. J., and Tracy, P. H. Rancid flavors in raw milk. Milk

Plant Monthly 22: 44, (1933).

11. Stout, A. W., and Schuette, H. A. Preparation of aldehyde-free ethyl alcohol. Ind. Eng. Chem., Anal. edition, 5: 100-101, (1933).

Typed by

Edna Shepard