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A CONTRIBUTION TO THE CHEMISTRY OF BUTTER-FAT.¹

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II. THE CHEMICAL COMPOSITION OF BUTTER-FAT.

WE have already spoken, in a somewhat general way, of those proximate components into which butter-fat is resolved by the process of saponification. The percentage of glycerol formed by the breaking up of the glycerides and the methods for its estimation were considered in a previous article.² We will confine ourselves, therefore, in the present paper to a study of the various fatty acids, both soluble and insoluble, as we obtain them from butter-fat, regarding also, to some extent, the combinations of these different acids with the glycerol base to form what we know as the glycerides, the mixture of which in varying proportions constitutes butter-fat itself.

There is no problem in analytical chemistry more difficult than that of making a quantitative separation of a mixture of different fatty acids. A mixture of two fatty acids is very hard to analyze, much more so a mixture such as we find in butter-fat where at least ten different acids are present. In a case of this kind the most that we can hope for is an approximation, since an exact quantitative separation is impossible in the present condition of analytical chemistry.

¹ Read by title before the New York meeting of the American Chemical Society, December 28, 1898.

² This Journal, 21, 612.

Bearing this in mind it need not be thought strange that the analysis of the fatty acids from butter should be a subject upon which scarcely any two writers agree. Chemists are generally quite concordant in determinations of the saponification number and other common constants pertaining to butter-fat analysis; it seems only reasonable, therefore, to attribute such discrepancies as we find in the percentages of the various acids to defective methods of analysis.

In the following table are given the percentages of the different acids in butter-fat according to several authorities:

TABLE V.

Acid.	1. Molt. ¹	2. Koefoed ²	3. Bell. ³	4 Blyth. ⁴
Oleic.....	26.52	31.11	36.1	40.40
Stearic.....	40.93	1.83	} 49.46	} 47.50
Palmitic.....	20.00	25.62		
Myristic.....		20.13	} 2.09	} 0.80
Lauric.....		7.32		
Capric.....	} 7.40	1.83	} 6.13	} 3.49
Caprylic.....		0.46		
Caproic.....		1.83		
Butyric.....		1.37		
Total.....	94.85	91.50	93.78	94.59

A difference in the composition of butter-fat itself no doubt explains to some extent the variations in the foregoing table; a difference in composition, however, would hardly warrant such wide discrepancies as we observe between columns 1 and 2 in the case of stearic acid, or between columns 2 and 3 in the case of butyric acid.

A partial separation of the acids of butter into two fractions, one soluble in water and the other insoluble, is very easily effected, as has been shown;⁵ the real difficulties of the work may be said to begin only with the final analysis of these two portions.

¹ König: *Nahrungs- u. Genussmittel*, second edition, p. 281. Original results expressed as glycerides were calculated to fatty acids.

² Koefoed: *Bulletin de l'Academie Royale Danoise*, 1891; Abstract in *Analyst*, No. 195, p. 130.

³ Bell: *Analysis and Adulteration of Foods*, Vol. II, 48. The fatty acids soluble in water were regarded as butyric acid; the 2.09 per cent. of residual acids were soluble in hot water and had a combining weight of 136.

⁴ Blyth: *Foods: Their Composition and Analysis*, fourth edition, p. 340.

⁵ This Journal, 21, 626.

To remove as far as possible the difficulties attending the presence of oleic acid, a butter-fat was selected which gave a rather low iodine absorption; the fat was examined first according to the ordinary methods of analysis and gave the following constants:

Saponification number.....	232.7
Ether number.....	232.5
Iodine number	29.28
Insoluble acids, per cent	86.40
Soluble " "	8.35
Saponification number of insoluble acids.	215.5
Molecular weight " " "	260.3
Saponification number " soluble "	572.5
Molecular weight " " "	98.0

One hundred grams of the butter-fat were taken and the soluble and insoluble acids obtained by methods already described. It is an interesting and well-known fact that in the natural fats only acids exist that have an even number of carbon atoms.¹ Besides the acids mentioned in Table V there have been found in butter-fat oxy-acids,² and also acetic and arachidic acids,³ the two latter, however, occur in such minute quantities that their presence is not worth considering.

ANALYSIS OF THE INSOLUBLE ACIDS.

The two methods most generally followed by chemists for effecting a separation of a mixture of insoluble acids are that of fractional distillation and that of fractional precipitation. In our work the latter method was selected, since it seemed to offer fewer difficulties and fewer sources of error; we have never given the distillation method a fair trial, but a few experiments seemed to indicate that there was an insufficient range in the character of the different fractions and that the whole process was attended with danger of decomposition.⁴

In making the fractional precipitations the method of Heintz⁵ was for the most part followed.

The insoluble acids (86.4 grams), obtained from 100 grams of

¹ Richter's Organic Chemistry, Smith's translation of fourth German edition, p. 186.

² Bondzynski and Rufi: *Ztschr. anal. Chem.* (1891), 1.

³ Benedikt: *Analyse der Fette*, third edition, p. 544.

⁴ Koefoed (*Vid. op. cit.* this article, p. 808), however, seems to have used the distillation method with some degree of success.

⁵ Heintz: *J. prakt. Chem.*, 66, 1.

butter-fat, were dissolved in ninety-five per cent. alcohol, using such a quantity that nothing crystallized out at 25° C. The solution was then cooled to zero, and after several hours, when crystallization was complete; filtered through a funnel kept cold by means of an ice-jacket. The precipitate of fatty acids, consisting of a white crystalline mass, we will designate as Portion I, and the acids contained in the filtrate as Portion II.

Portion I contained, besides other acids, a small quantity of oleic acid held back mechanically; to remove as much as possible of this, the precipitate of acids was dissolved in a small quantity of hot alcohol, recrystallized in the cold, and filtered as before, the filtrate being added to Portion II.

Portion I was again dissolved in about 500 cc. of hot alcohol, cooled in ice-water, and after recrystallization filtered through the ice-funnel; the precipitate constituted Part 1. The filtrate from Part 1 was evaporated to about 300 cc., cooled in ice-water, and the fat acid crystals filtered off as before; the precipitate constituted Part 2. The filtrate from Part 2 was evaporated to about 150 cc., and the process continued for one more crystallization, Part 3 being obtained; the filtrate from Part 3 was added to Portion II.

The different Parts, 1, 2, and 3, of Portion I were then washed with ether into weighed beakers; after evaporating the ether the acids were dried for a short time at 100°, after which they were cooled, weighed, and preserved for further examination.

Portion II was treated with 100 cc. of a five per cent. solution of magnesium acetate in alcohol, cooled to 0° C., and after crystallization of the magnesium salts was complete, filtered. The precipitate, Part *a*, was washed with 100 cc. of the alcoholic magnesium acetate and the washings added to the original filtrate. The filtrate from Part *a* was then rendered alkaline with ammonia and subjected to a second crystallization at 0° C., when a second precipitate, Part *b*, was obtained; this was filtered off and washed similarly to Part *a*. Nothing more separating from the filtrate at zero, the solution was treated again with ammonia until strongly alkaline; 100 cc. more of the magnesium solution were added and the liquid allowed to stand in the cold for twenty-four hours. The precipitate thus obtained was collected on a

filter and washed with small amounts of the alcoholic magnesium solution ; it constituted Part *c*.

The three fractions of Portion II, Parts *a*, *b*, and *c*, consisting of the magnesium soaps, were then decomposed with dilute hydrochloric acid, and the liberated acids thoroughly washed with hot water to remove all traces of magnesium. The fatty acids from each fraction were then washed with ether into weighed beakers, and, after evaporation of the ether, dried for a short time at 100°, when they were cooled, weighed, and preserved for further examination.

In the analysis of the different fractions from Portions I and II, the saponification number and iodine absorption were determined ; from the former constant the mean molecular weight of the mixture of fatty acids is calculated¹ and from the latter the amount of oleic acid in each fraction is determined :² the oleic acid, though sometimes present in but minute quantities, must, nevertheless, be considered in the analysis of the several fractions.

Some uncertainty prevails as to the accuracy of calculating the percentage of oleic acid in a mixture of glycerides or fatty acids from the iodine number alone. There is not only the possibility of other unsaturated acids existing besides oleic acid, but also the danger that iodine may be substituted as well as added in the course of the reaction. These objections are no doubt valid in the case of many vegetable and fish oils, but apply with less force to the common fats of animal origin, which contain, so far as known, only one unsaturated acid ; *viz.*, oleic.

The possibility of iodine being substituted, with the glycerides or acids of butter-fat at least, appears to us to be very slight ; after removing the olein or oleic acid by repeated crystallizations of the fat or fatty acids, the percentage of iodine absorbed can be reduced practically to nothing, thus showing that with the higher saturated glycerides and acids but very little, if any, substitution occurs.

We have selected as the first step in the calculations the de-

¹ By the formula $M = \frac{56100}{S}$. Benedikt: *Analyse der Fette*, third edition, p. 163.

² By the formula $O = \frac{100}{90.07} I = 1.1102 I$, 90.07 being the theoretical iodine absorption of pure oleic acid. Benedikt: *Ibid.*, p. 173.

termination of the mean molecular weight of the fatty acids in each fraction after deducting the oleic. This result is obtained by the following formula :

$$\frac{100}{m} - \frac{O}{282} = \frac{100-O}{x};$$

or
$$x = \frac{282 m (100-O)}{28200-mO}.$$

O = percentage of oleic acid as calculated from iodine number,

m = mean molecular weight of original acids.

x = " " " after deducting oleic.

If now we assume that there are not more than two acids present in each fraction besides the oleic and that these acids are adjacent homologues of even carbon atoms, then the relative percentage of each acid can be determined from the following formulas :

$$x + y = 100;$$

$$\frac{x}{m_1} + \frac{y}{m_2} = \frac{100}{m};$$

from which

$$x = \frac{100 m_1 (m - m_2)}{m (m_1 - m_2)};$$

$$y = \frac{100 m_2 (m_1 - m)}{m (m_1 - m_2)}.$$

m = mean molecular weight of the two acids.

m_1 and x = molecular weight and percentage, respectively, of the higher acid.

m_2 and y = molecular weight and percentage, respectively, of the lower acid.

The expression $m_1 - m_2$ for the saturated fatty acids of even carbon atoms equals 28; substituting this value in the previous equations :

$$x = \frac{100 m_1 (m - m_2)}{28 m};$$

$$y = \frac{100 m_2 (m_1 - m)^1}{28 m}$$

To ascertain what acids are present in any particular fraction, it is only necessary to observe between what two homologues the mean molecular weight of the fraction falls, after deducting the oleic.

The formulas, as derived from the two preceding general equations, for calculating the percentage of stearic acid (S) and palmitic acid (P) in a mixture of the two, knowing the mean molecular weight (m), are as follows :

$$S = \frac{28400 (m - 256)}{28 m};$$

$$P = \frac{25600 (284 - m)}{28 m}.$$

In the same way for palmitic acid (P) and myristic acid (M) :

$$P = \frac{25600 (m - 228)}{28 m};$$

$$M = \frac{22800 (256 - m)}{28 m}.$$

The analytical data of the various fractions belonging to Portions I and II, together with the calculated amounts of the different acids, are given in the following table :

¹ There is some difference among chemists as to the proper formula to use for calculating the percentage of two acids from the mean molecular weight. Benedikt, Hehner, and the majority of German chemists take the formula $\frac{x}{m_1} + \frac{y}{m_2} = \frac{100}{m}$, while the calculations of Allen, Prescott, and many others are based upon the formula $xm_1 + ym_2 = 100m$. The former formula seems to us the only correct one. To take for example a simple case :

	Per cent.
1 molecule stearic acid weighs 284	= 52.59
1 " palmitic " " 256	= 47.41
2 molecules of mixture weighs 540	= 100.00
Mean molecular weight of mixture = $\frac{540}{2}$	or 270.

If we determine now from the two formulas, what percentages of stearic and palmitic acids will give a mean molecular weight of 270, we obtain 52.59 per cent. stearic and 47.41 per cent. palmitic, the theoretical results, by the formula $\frac{x}{m_1} + \frac{y}{m_2} = \frac{100}{m}$; whereas the formula $xm_1 + ym_2 = 100m$ gives exactly fifty per cent. of each.

TABLE VI.

	Portion I.			Portion II.			Total per cent.
	Part 1.	Part 2.	Part 3.	Part a.	Part b.	Part c.	
Weight of fraction....	5.20 grams	6.01 grams	21.13 grams	9.22 grams	7.82 grams	5.05 grams
Mean molecular weight of fraction.....	272.2	260.0	260.7	254.6	254.5	251.2
Iodine number of fraction.....	3.44	10.94	15.03	8.41	19.85	25.12
Per cent. oleic acid in fraction.....	3.82	12.14	16.68	9.34	22.03	27.88
Mean molec'lar weight after deducting oleic	271.8	257.2	256.8	252.1	247.7	241.0
Weight of stearic acid	2.95 ¹ grams	0.25 gram	0.56 gram	3.76
" " palmitic "	2.05 grams	5.03 grams	17.05 grams	7.32 grams	4.44 grams	1.79 grams	37.68
" " myristic "	1.05 grams	1.66 grams	1.85 grams	4.56
Undetermined.....							7.90 ²

¹ Contains also the oxy-acids, a correction for which will be introduced later.

² Found by subtracting the sum of the acids determined and the oleic acid 32.50 per cent (calculated from iodine absorption) from 86.40, the total per cent. of insoluble acids.

The 7.90 per cent. of undetermined acids, which escaped precipitation, includes all of the lauric acid and no doubt a considerable part of the myristic; the percentage of each of these acids can be arrived at in another way. Having five members present in a mixture of fatty acids and knowing the molecular weight and percentage of three of them, the mean molecular weight of the other two can be determined by the following formula:

$$\frac{a}{m_1} + \frac{b}{m_2} + \frac{c}{m_3} + \frac{100 - (a + b + c)}{x} = \frac{100}{m}$$

a , b , and c are the percentages of the three known acids and m_1 , m_2 , and m_3 their respective molecular weights. m is the mean molecular weight of the whole mixture and x the mean molecular weight of the two acids sought.

Applying this formula to the case in hand we have for the three known acids, oleic, stearic, and palmitic, the following percentages:

TABLE VII.

	In 100 parts of butter-fat.	In 100 parts of acids.
Oleic acid (from iodine number)	32.50	37.61
Stearic acid	3.76	4.35
Palmitic acid	37.68	43.61
Myristic and lauric acids	12.46	14.43
Total	86.40	100.00

The mean molecular weight of the insoluble fatty acids from the butter was 260.3; substituting these values in the formula we obtain the following equation:

$$\frac{37.61}{282} + \frac{4.35}{284} + \frac{43.61}{256} + \frac{14.43}{x} = \frac{100}{260.3};^1$$

$$\text{or } 0.13339 + 0.01532 + 0.17035 + \frac{14.43}{x} = 0.38418;$$

$$\text{or } \frac{14.43}{x} = 0.06512;$$

whence $x = 221.6$.

This figure for the mean molecular weight falling between 200

¹ Strictly the formula should also take into account the oxy-acids; this, however, would make no difference with the final results, inasmuch as the mean molecular weight of the determined acids (and hence of the undetermined) would remain the same in either case.

and 228 would indicate a mixture of lauric and myristic acids. Knowing the mean molecular weight (m) of a mixture of myristic (M) and lauric (L) acids, the percentages of each may be calculated from the formulas

$$M = \frac{22800(m-200)}{28m},$$

$$L = \frac{20000(228-m)}{28m}.$$

Substituting the value 221.6 for m in the preceding equations we obtain 79.37 per cent. myristic and 20.63 per cent. lauric acid; this would give in the 12.46 parts of undetermined acids 9.89 parts of myristic and 2.57 parts of lauric acid for 100 parts of butter-fat.

Such a method for analyzing a mixture of fatty acids as the one described manifestly presents many crudities; the method of Heintz, however, "supplies the only means of obtaining an insight into a mixture of non-volatile acids."¹ Unfortunately, "there is no practical method of proving that two and only two acids are present"¹ in any particular fraction, and therein lies the principal fault of the method. Allen observes that "as a rule if the mixed fatty acids be divided into a sufficient number of fractions, each fraction will contain only two homologues,"² and this assumption seems to be a fairly reasonable one.

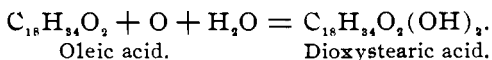
The oleic acid is a disturbing factor throughout the whole process and its presence must be taken into account in the analysis of the different fractions. We believe too little attention has been paid to this point. The method could be improved greatly if there were some good means of removing the oleic acid at the beginning of the process. The plan of converting the mixture of acids into the lead soaps and removing the lead oleate with ether would not work well on a large quantity of acids, and besides this the process does not effect a perfect separation.

We have taken no account thus far of the oxy-fat acids: that such acids are present in butter-fat seems certain from the acetyl figure which the insoluble acids always give. The oxy-acids probably do not exist in the fat as it is secreted from the lacteal

¹ Hehner and Mitchell: *Analyst*, No. 249, p. 317.

² Commercial Organic Analysis, third edition, Vol. II, Part 1, p. 242.

glands, but are later oxidation products of the oleic acid—dioxystearic¹ acid being no doubt principally formed.



Owing to the low solubility of the higher oxy-acids in cold alcohol, any oxy-acids present would come down with the first crystallization (Part I, Table VI), and this fact would alter somewhat the percentages of the acids in this fraction.

Benedikt has devised a formula by means of which the percentage of an oxy-acid in a mixture of fatty acids can be calculated from the acetyl number, provided that the constitution and molecular weight of the oxy-acid is known. The equation is

$$x = \frac{100 c m}{a(56100 - 42c)},^2$$

in which c = the acetyl number.

a = the number of hydrogen atoms in the oxy-acid replaceable by acetyl.

x = percentage sought of the oxy-acid having a molecular weight m .

Applying this formula to the insoluble acids from the average butter-fat having an acetyl number of 4.1 we would obtain for dioxystearic³ acid 1.16 per cent., or calculated to the original butter-fat 1.00 per cent. of oxy-acid.

There are no doubt other oxy-acids present besides the dioxystearic in the mixture of insoluble acids, so that the results from this method of calculation can only be considered approximate. The percentage of oxy-acids in butter-fat increases with time and is therefore never a fixed quantity. The gradual increase in the acetyl number, together with the decrease in the saponification number of the insoluble acids, with the age of a butter-fat, goes to show that oxy-acids of a high molecular weight are continually being formed. This fact will be explained more fully under the subject of "Rancidity in Butter-fat."

If we introduce a correction for the percentage of oxy-acid, as found, in the first fraction of the insoluble acids and recalculate

¹ A Scala (Staz. sperim. agr. ital. (1897), p. 613, shows that this acid is formed among the oxidation products of oleic acid.

² Benedikt: *Monatsh. Chem.* (1890), 95, and *Analyse der Fette*, third edition, p. 178.

³ For dioxystearic acid in the formula $a = 2$ and $m = 316$.

the stearic and palmitic acids, the analysis of Part I would then be :

	Grams.
Dioxystearic acid	1.00
Stearic acid	1.02
Palmitic acid	2.98

The complete analysis of the insoluble acids from 100 parts of butter-fat accordingly would be :

	Grams.
Dioxystearic acid	1.00
Oleic acid	32.50
Stearic acid	1.83
Palmitic acid	38.61
Myristic acid	9.89
Lauric acid	2.57
Total	86.40

A noteworthy fact in connection with the above results is the low percentage of stearic acid in butter-fat. This is somewhat contradictory to the statements which we find given by many authorities on the subject who regard stearic as one of the principal acids in butter-fat, it being placed, by some writers, as high even as forty per cent. We believe that these statements should be modified somewhat; when we consider that oleic acid of molecular weight 282 constitutes over one-third of the insoluble acids from butter-fat, it is self-evident that the percentage of stearic acid of molecular weight 284 must be small in comparison with that of its lower homologues, to give a mean molecular weight of 261 to the whole mixture.

Koefoed,¹ in his analysis of butter-fat, obtained only 1.83 per cent. stearic acid which, by a strange coincidence, is exactly the same quantity found by us after correcting for the oxy-acids.

Hehner² and Mitchell, by removing the oleic and lower insoluble acids with dilute alcohol and analyzing the residue, obtained only from one and three-tenths to three and six-tenths per cent. stearic acid; the same authorities, with their process³ of removing all other acids by treatment of the insoluble acids at 0° C. with a saturated solution of stearic acid in alcohol,

¹ *Vid. op. cit.*, p. 808 of this article.

² *Analyst*, No. 249, p. 321.

³ *Ibid.*, p. 322.

obtained in over 100 determinations only minute quantities of stearic acid and in many cases none at all.

This low percentage of stearic acid should rank with the high percentage of soluble fatty acids, as one of the characteristic differences between butter and other animal fats.

ANALYSIS OF THE SOLUBLE ACIDS.

A great many methods have been devised for the analysis of a mixture of the lower soluble fatty acids, but very few of them seem practicable in actual work. The method of fractional distillation, which suggests itself perhaps first of all, does not give good results when working with a small quantity of acids, the principal difficulty which we have found being that of not securing a wide enough range in the character of the several fractions.

No more satisfactory were the methods based upon the difference in the chemical affinities of the various acids.¹ A neutralized solution of the mixed soluble acids was decomposed with successive portions of tenth-normal sulphuric acid, the liquid being subjected to distillation after each addition of acid. The various distillates were neutralized with tenth-normal barium hydroxide and the mean molecular weights of the acids in each fraction determined. A difference was found in the acids of the several successive distillates, but not a distinctive one, the several molecular weights varying only from two to three units. The theory of the method is borne out, but imperfectly. In one experiment the mean molecular weight of the acids in the first fraction was 102.7; the succeeding fractions gave in their order 99.4, 96.8, 94.8, 93.9, 91.2, and 88.7. It is seen that the mean molecular weights of the acids in the successive distillates decrease until we have practically pure butyric acid in the final fraction; the differences are too small, however, to form any basis for calculations.

The method of Duclaux,² based upon the difference in ratio of vaporization of the various acids from their solution, did not appear adaptable to the present instance where more than two acids were present.

The following method, worked out by the writer, was found

¹ Allen : Commercial Organic Analysis, third edition, Vol. I, p. 487.

² Duclaux : *Ann. Chem. Phys.* [5], 11, 233.

to give a considerable range in the character of the different fractions, with but little trouble of execution; it is based upon the difference in the solubility¹ of the various lower homologues in water at different temperatures.

One gram of the mixed acids is weighed out into a small fifty cc. flask and treated with twenty-five cc. of distilled water, previously cooled to nearly 0° C. The flask is placed in a beaker of ice and agitated frequently for about five minutes; the contents of the flask are then poured into a small filter placed in a funnel surrounded by ice, and the flask rinsed out with small portions (five cc.) of the cold water, the rinsings being poured upon the filter and allowed to run into the beaker holding the original filtrate. Not more than twenty-five cc. of water are used for the washing. The acids left on the filter are washed back into the flask with about twenty-five cc. of distilled water at 30° and, after being kept at this temperature for five minutes with frequent shaking, again filtered. The flask is rinsed out and the acids on the filter washed as before with small quantities of water (about twenty-five cc. in all) at 30°, the combined filtrate and washings constituting Fraction 2. The acids remaining on the filter are then treated successively in the same manner with water at 60° and 95°, Fractions 3 and 4 being thus obtained.²

The various fractions are next titrated with standard tenth-normal barium hydroxide, using phenolphthalein as indicator; the solutions of the barium salts are evaporated to dryness in weighed platinum dishes, and the residues dried to constant weight at 100°. From the weight of the residue and the weight of barium oxide contained therein, the mean molecular weight of the acids in each fraction is determined.³ The amount of barium oxide in the residue can be calculated either from the volume of solution used to neutralize the acids, or by igniting

¹ Storer (Dictionary of Chemical Solubilities) gives the following:
Butyric acid soluble in water in all proportions:

Caproic acid	soluble in	96	parts of water at	7°	C.
Caprylic	"	400	"	100°	C.
Capric	"	1000	"	20°	C.

² The physical characters of the acids are observed to change during the process of washing. After the removal of the first fraction the acids lose their mobility and acquire a thick, oily consistency. The strong rancid smell, characteristic of butyric acid, entirely disappears with the removal of the second fraction, while the residual acids possess a very peculiar odor, resembling somewhat that of perspiration.

³ This Journal, 21, 628.

the residue with sulphuric acid and weighing the barium sulphate.

In one experiment, conducted by the method just described, there were found for the different fractions the following results:

TABLE VIII.

Fraction No.	Temperature of water used for washing.	Mean molecular weight of acids.
1.....	5° C.	91.4
" "	2..... 30° C.	113.1
" "	3..... 60° C.	123.6
" "	4..... 95° C.

The quantity of acids left after treating with water at 60° amounted to only a few milligrams and so was insufficient for an accurate molecular weight determination.

The weight of acids in each fraction can be found from the formula

$$W = \frac{Mn}{10,000},$$

in which W equals the weight in grams of acids, M the mean molecular weight of the acids, and n the number of cubic centimeters of tenth-normal alkali used for their neutralization.

If we assume that only two acids are present in each fraction, an assumption¹ probably not very far from true, the percentage of each acid can be calculated from the mean molecular weight according to the general formulas already given. For a mixture of butyric (B) and caproic (C) acids,

$$B = \frac{8800(116-m)}{28m};$$

$$C = \frac{11600(m-88)}{28m}.$$

For a mixture of caproic (C) and caprylic (C_1) acids,

$$C = \frac{11600(144-m)}{28m};$$

$$C_1 = \frac{14400(m-116)}{28m}.$$

¹ The wide difference in the solubility of the lower acids in water would almost preclude the possibility of finding more than two acids in each fraction. There is, however, this possibility, which experiments would seem to indicate, that the solubility of the higher acids in water is somewhat greater if lower acids are present. This is a point well worth investigation.

Several analyses of the soluble acids from butter-fat conducted by the method described gave the following average composition :

	Per cent.
Butyric acid.....	65.28
Caproic "	25.00
Undetermined.....	9.72

The mean molecular weight of the undetermined acids can be calculated from the equation,

$$\frac{65.28}{88} + \frac{25.00}{116} + \frac{9.72}{x} = \frac{100}{98},$$

from which $x = 154$. This value, falling between 144 and 172, would indicate that the undetermined acids consisted of caprylic and capric. From the mean molecular weight (m) the percentages of caprylic (C_1) and capric (C_2) acids can be calculated as follows :

$$C_1 = \frac{14400 (172 - m)}{28 m};$$

$$C_2 = \frac{17200 (m - 144)}{28 m}.$$

In the undetermined residue of 9.72 per cent. we find from the above formulas sixty parts of caprylic and forty parts of capric acid, or 5.83 per cent. caprylic and 3.89 per cent. capric in the total mixture of soluble acids.

Calculating the analysis to the 8.35 per cent. of soluble acids in the original butter-fat we obtain :

	Per cent. ¹
Butyric acid.....	5.45
Caproic "	2.09
Caprylic "	0.49
Capric "	0.32
Total.....	8.35

¹ In connection with these percentages for the soluble acids it will be of interest to quote the results of two French chemists :

Duclaux (*Compt. rend.*, 102, 1022) found in eight samples of butter examined 3.35 to 3.60 per cent. butyric and 2.08 to 2.26 per cent. caproic acid. Violette (*Compt. rend.*, 111, 345) found for good butter 5.32 to 6.06 per cent. butyric and 3.22 to 3.66 per cent. caproic acid. Apparently no attempts were made by either of these chemists to determine the caprylic and capric acids.

For other results see Table V.

SUMMARY.

The results of the analysis of the mixed acids from the butter-fat examined, together with the equivalent percentages of triglycerides, are given in the following table :

TABLE IX.

Acid.	Percentage of acid.	Percentage of triglycerides.
Dioxystearic.....	1.00	1.04
Oleic	32.50	33.95
Stearic	1.83	1.91
Palmitic.....	38.61	40.51
Myristic	9.89	10.44
Lauric.....	2.57	2.73
Capric	0.32	0.34
Caprylic.....	0.49	0.53
Caproic.....	2.09	2.32
Butyric.....	5.45	6.23
Total.....	94.75	100.00

Other bodies, such as coloring-matter, lecithin, cholesterin, phytosterin, etc., which occur in butter-fat in only minute quantities, have not been considered in the above analysis. These substances go to make up the unsaponifiable matter of butter-fat, the total amount of which we have found to be only about one-tenth per cent.

We have made an elementary analysis of butter-fat and find the agreement between the actual percentages of carbon, hydrogen, and oxygen, and the theoretical figures as calculated from the percentages of the various glycerides in the preceding table, to be very close.

	Carbon.	Hydrogen.	Oxygen.
Actual	75.17	11.72	13.11
Theoretical	74.86	11.71	13.43

The close agreement between these two sets of results would indicate that the figures expressing the percentages of acids and glycerides in Table IX are not very far removed from the truth.

THE GLYCERIDES OF BUTTER-FAT.

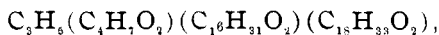
Experiments of Bell,¹ Blyth,² and other chemists indicate that there are certain glycerides in butter-fat of a complex nature ;

¹ Allen : Commercial Organic Analysis, third edition, Vol. II, Part 1, p. 180.

² Blyth : Foods; Their Composition and Analysis, fourth edition, p. 340.

that instead of simply one acid we may have two or even three different acid radicals in the same molecule.

If a mixture of tributyrin, tripalmitin, and triolein be prepared and treated with alcohol, it is possible to extract the tributyrin completely. With butter-fat, on the other hand, such an extraction cannot be made, a fact due without doubt to the butyric acid being combined with certain higher fat acids to the same glycerol radical.¹ In confirmation of this, Blyth and Robertson² have isolated from butter-fat a crystalline glyceride of the formula



a mixed ester of butyric, palmitic, and oleic acids.

While a part of the butyric, palmitic, and oleic acids in butter-fat is no doubt bound up together in one molecule, it is also certain that these acids exist in glycerides of a different nature. Experiments seem to indicate that there are present glycerides which contain at least two radicals of one acid in the same molecule and probably three. Tripalmitin certainly exists and has been isolated by us in a state of comparative purity. The separation of all the various glycerides is a problem at present beyond the power of the chemist. It is only possible to make a few rough separations and from certain analytical data draw some general conclusions.

If a melted butter-fat is allowed to stand for some time at about 25° C., a crystalline deposit will separate out. By filtering we obtain two portions,—a clear transparent oil, the so-called butter-oil, and a solid mass comprising all the glycerides of higher melting-point. If the butter-oil is cooled another deposit will form; by repeating the process of filtering and cooling, the congealing-point of the oil can be reduced from 25° C. to below 10° C. These different fractions of oil will be found to differ in chemical as well as physical properties, as can be seen from the following table :

TABLE X.

Congeaing-point.	Iodine number.	Saponification number.
25° C.	35.05	233.0
12° C.	39.24	234.0
9° C.	40.95	235.6

¹ Benedikt: *Analyse der Fette*, third edition, p. 544.

² *J. Lond. Chem. Soc., Proceedings* (1889), 5

The increase in the iodine and saponification numbers, as the congealing-point decreases, is due to an increase in the percentages of oleic and the soluble acids. The quantity of soluble acids increases much faster than the saponification number indicates, since the increase in oleic acid tends to keep this constant down.

The butter-oil of 9° congealing-point contains, according to its iodine absorption, over forty-five per cent. oleic acid, which quantity is considerably in excess of the percentage required by such a glyceride as that of Blyth and Robertson. Indications would point to the presence of glycerides containing at least two radicals of oleic acid in the molecule, and there is no reason to doubt the existence of triolein itself.

The solid mass left on the filter, after removing the butter-oil, still contains a high percentage of oleic acid; it resembles butter-fat itself in outward appearance. The body is somewhat harder, however, than butter-fat, possessing also a higher melting-point and lower content of soluble acids. For want of a better name, we have termed this residue, left after removing the oil, *butter-palmitin*. The relation between the constants of butter-oil, butter-palmitin, and the original butter-fat can be seen from the following table :

TABLE XI.

	Melting-point.	Saponification number.	Iodine number.	Reichert number.
Original butter-fat	33°	231	31.00	15.30
Butter-oil	25° ¹	233	35.05	18.18
Butter-palmitin	38°	228	27.66	11.23

By subjecting the butter-palmitin to repeated crystallizations from some suitable solvent (chloroform was found to be the best), it is possible to remove practically all of the oleins and lower glycerides and to obtain almost pure tripalmitin itself. The body possesses a beautiful, white, flaky appearance, crumbles readily between the fingers, and leaves no oily spots when spread on paper. Traces of oleic, stearic, and lower soluble acids are generally present as impurities. A specimen of the glyceride gave a saponification number of 207; the saponification number of tripalmitin, according to theory, is 208.

¹ Congealing-point.

If we treat butter-fat with hot ninety-five per cent. alcohol, then cool, filter, and evaporate the alcohol, a small quantity of a gum-like fat is left. A body so obtained melted at 22.5°C. , gave a saponification number of 257, an iodine number of 26.15, and yielded about seventy-nine per cent. insoluble and fourteen per cent. soluble acids. The small percentage of the body obtained and its high saponification number (higher than that required for an oleopalmitobutyrim), would point to glycerides containing more than one radical of the lower acids.

In conclusion, the most that we can say is that, while the evidence is strongly in favor of the existence of complex glycerides, there are equally good reasons for believing in the existence of simpler bodies. An analysis of the fatty acids from the different fractions of the butter-oil and butter-palmitin would throw much light upon the constitution of the glycerides contained therein; for such work, however, our time has been too limited. We hope, if possible, in the future to continue our work in this direction.

NOTE.

Inasmuch as the reliability of the analyses, contained in the foregoing paper, is dependent to a great extent upon the accuracy of certain chemical methods, a word should be said in regard to the extent of error which is involved in work of the latter kind.

Nearly all of the calculations concerning molecular weights and percentages were based upon the determination of two constants, the saponification number and iodine absorption. Whenever possible, duplicate, and in some cases triplicate, determinations of these constants were made. The difference between two determinations of the saponification number rarely exceeded ± 1 , and the error between duplicate determinations of the iodine absorption was seldom greater than ± 0.1 ; these differences would affect only slightly the calculation of the mean molecular weights and percentages.

In standardizing the different solutions used for the volumetric work we have employed a few methods, which for speed and accuracy we think superior to some other processes in more general use; we will therefore mention them briefly in this connection.

Tenth-normal barium hydroxide is standardized by evaporating a measured quantity (twenty to fifty cc.) in a weighed platinum dish with a slight excess of dilute sulphuric acid, igniting the residue, and weighing the barium sulphate.

Tenth-normal sulphuric acid is standardized in a similar way. Twenty cc. of the acid are measured from a burette into a weighed platinum dish and the solution carefully neutralized with tenth-normal barium hydroxide, using phenolphthalein as indicator; the liquid is evaporated to dryness, the residue ignited, and the weight of barium sulphate determined. The weight of barium sulphate found divided by 0.2335 will give the factor for the tenth-normal sulphuric acid. In this way the barium hydroxide and sulphuric acid can both be standardized by a single operation. Determinations can be made in one-fourth less time by this process than by the usual method of precipitation and with no difference as regards accuracy of results. The barium hydroxide used should be perfectly pure and free from other alkali; if not C. P. it can be easily purified by recrystallization.

The two tenth-normal solutions just mentioned can be used for standardizing the half-normal alcoholic potash and half-normal hydrochloric acid employed for determining saponification numbers. Another method, which we have been accustomed to apply as a check, has proved very successful in the case of standardizing these last-named solutions.

Ten cc. of the half-normal potash solution are carefully neutralized with the half-normal hydrochloric acid, using phenolphthalein, and the number of cubic centimeters used carefully noted; the liquid, after evaporating off the alcohol, is diluted to about fifty cc. and an excess of platinic chloride solution added. The potassium platinichloride is precipitated and determined in the usual way, a Gooch crucible being used for collecting the precipitate. The weight of potassium platinichloride divided by 1.2145 will give the factor for the half-normal alcoholic potash solution; from the number of cubic centimeters of the acid used to neutralize the potash the factor for the half-normal hydrochloric acid is found. Factors obtained for the half-normal solution by precipitation with platinic chloride and by titration against the tenth-normal solutions have always showed a very close agreement.