A Study of the Chemical Composition of Butter Fat, and Its Relation to the Composition of Butter.

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SUMMARY OF AUTHENTIC KNOWLEDGE OF THE COMPOSITION OF BUTTER FAT.

Milk fat or butter fat consists of triglycerides of fatty acids. The fatty acids of butter fat are monobasic and have the general formula $C_nH_{2n+1}CO\,OH$, except oleic acid, which is a non-saturated acid belonging to the acrylic series with the general formula $C_nH_{2n-1}\ CO\,OH$. The triglycerides of butter fat do not exist as glycerides of one fatty acid, but as a mixture of several acid radicals combined with glycerin. Glycerin is a triatomic alcohol, $C_3H_5(OH)_3$. Theoretically, therefore, the milk fat could contain triglycerides of the fatty acids present, that is, there could be tributyrin, triolein, tristearin, etc. In reality no such combination exists. Just in what order the triglycerides are present has not been definitely established. The acids present in butter fat are butyric, caproic, caprylic, capric, lauric, myristic, palmitic, oleic and stearic.

Bell[1] holds that butter fat consists of mixed glycerides, glycerides in the molecule of which the glycerol is combined with three different acid radicals forming a compound having the following composition:

$$C_3H_5\begin{cases} OC_4H_7O \\ OC_{16}H_{31}O \\ OC_{18}H_{33}O \end{cases}$$

This theory is supported by the fact that the glycerol forms triacid compounds and not compounds of one acid, which theoretically could be possible. If the glycerol formed monoacid compounds, butter fat would contain glyceryl tributyrates, caproates, stearates, etc.

SOLUBILITY OF BUTTER FATS IN ALCOHOL.

If butter fat is dissolved in alcohol, from 1.1 to 3.3 per cent of the fat goes into solution, the solubility depending on the temperature of the alcohol. If tributyrin existed in butter fat, all of the tributyrin would

[1] The Chemistry of Foods, Vol. II, page 44.

go into solution.[1] Analyses of the portion soluble in alcohol show that this is not the case. Tables I and II give the value of the constants as determined for the portion soluble in cold alcohol, the portion not soluble in cold alcohol, but soluble in hot alcohol, and the portion not soluble in either hot or cold alcohol.

TABLE I.

	Portion Soluble in Alcohol at 20 deg. C	Portion Not Soluble in Cold Alcohol, but Soluble in Alcohol at 75 deg. C.	Portion Not Soluble in Either Hot or Cold Alcohol.
Reichert-Meissl Number	48.1	29.6	20.7
Melting point	16.9°C.	31.5°C.	36.0°C.
Soluble acids (as Butyric)	9.79%	6.60%	4.26%

TABLE II.

	At 20°C.	At 75°C.
Solubility of butter fat in 95 % alcohol	1.1%	3.3%

The melting point of the portion soluble in alcohol at 20° C. is 16.9° C., while that of the portion not soluble in either hot or cold alcohol is 36° C., showing a difference of 19.1° C. The Reichert-Meissl No. in the portion soluble in alcohol is 48.1, in the portion not soluble in alcohol it is 20.7, showing a difference of 27.3. Since only 1.1 per cent of the fat is soluble in cold alcohol, this would indicate that no tributyrin exists in butter fat. This fact becomes still more evident by an examination of the molecular weight of the glycerides soluble in alcohol and those not soluble in alcohol, as calculated from the figures in Table VII.

[1] Cochran, "Action of Alcohol on Butter Fats," Analyst, Vol. 13, page 55. Lewkowitsch, "Oils, Fats and Waxes," Vol. II, page 675, 1909.

The saponification of a neutral fat yields a perfectly definite compound. This saponification takes place according to the following reaction for an ester with three molecules of acid combined with the radical of a trihydric alcohol:

$$C_3H_5\begin{cases}R_1\\R_2\\R_3\end{cases}+3KOH=C_3H_5(OH)_3+K(R_1+R_2+R_3)$$

The molecular weight of the triglyceride is calculated as follows: Determine the per cent of KOH required to saponify the fat, and divide the molecular weight of 3(KOH) by the per cent thus obtained, or multiply the saponification equivalent by three.

Thus, from the figures in Table VII the saponification equivalent of the portion soluble in alcohol was found to be 216.5. This multiplied by 3 is 649.5. This equals the molecular weight of the triglyceride.

The saponification equivalent of the portion not soluble in alcohol was found to be 260.9; this multiplied by 3 equals the molecular weight, 782.7. The molecular weight of butyrin $C_3H_5(C_4H_7O)_3$ is 302, while the molecular weight of the triglycerides of the soft portion is 649.5.

The fact that only 1 per cent of the butter fat was dissolved in cold alcohol shows clearly the absence of tributyrin, otherwise the per cent of alcohol-soluble fat would be higher. The soft portion must, therefore, be made up of mixed glycerides of the acids found in butter fat, the acids having a low melting point and a low molecular weight predominating.

FRACTIONAL SEPARATION OR CRYSTALLIZATION OF BUTTER FATS.

The same condition presents itself if butter fat is subjected to fractional separation. When butter fat is exposed to a temperature below the melting point of the harder glycerides, the softer glycerides separate from the harder glycerides. When this process is repeated by lowering the temperature after each separation, a separation can be effected whereby the constants differ widely from the original mixed glycerides.[1] Table III shows the variation of the constants of the fats thus separated. The butter used in this experiment was made in March.

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[1] Richmond Dairy Chemistry, page 37.

TABLE III.

Composition of Portions of the Butter Fat Obtained by Fractional Separation.

	Original Butter Fat.	Soft Portion.	Hard Portion.
Reichert-Meissl Number	29.06	32.65	26.74
Iodine number	34.97	42.10	30.11
Koetts. saponification number	229.5	233.87	228.8
Refractive index at 40° C.	Reading 44.1 1.4552	Reading 45.1 1.456	Reading 43.1 1.4546
Melting point	34° C.	14.5°C.	37.8°C.
Per cent insoluble acids	88.76	87.89	89.47
Melting point of insoluble acids	40°C.	36.5°C.	42.5°C.
Per cent soluble acids (as butyric)	5.89	6.67	5.46
Koetts. saponification number insoluble acids	219.5	221.35	218.8
Iodine number insoluble acids	37.36	45.05	33.48

Later in the season (in May) another sample of butter was treated similarly, separating the liquid from the solid portions of the fat, and the constants were determined as shown in Table IV.

TABLE IV.

Composition of Portions of the Butter Fat Obtained by Fractional Separation.

	Original Butter Fat.	Soft Portion.	Hard Portion.
Reichert-Meissl number	30.00	33.85	24.66
Iodine number	39.82	43.55	33.08
Koetts. saponification number	230.1	232.78	226.4
Refractive index at 40° C.	Reading 44 1.4552	Reading 44.8 1.4558	Reading 43 1.4545
Melting point	32.5°C.	13.2°C.	38.1°C.
Per cent insoluble acids	87.54	86.67	88.64
Melting point insoluble acids	39.2°C.	35.3°C.	42.4°C.
Per cent soluble acids (as Butyric)	6.09	6.90	5.17
Koetts. saponification number insoluble acids	220.53	221.6	218.7
Iodine number insoluble acids	42.14	46.2	35.66
Per cent glycerin	12.58	12.89	12.33

Tables III and IV show that the soft portions contain more volatile or soluble acids, also a greater per cent of oleic acid in combination with the glycerol base than the hard portions. The melting point of the soft portions was 22.8° C. and 24.9° C., respectively, lower than the melting point of the hard portions.

The difference in the melting points between the soft and hard portions of the insoluble fatty acids was not as great as that of the soft and hard portion of the glycerides from which the insoluble acids were derived. The reason for this must lie in the fact that the soluble fatty acids have been removed and that, therefore, the melting points of the different portions of the insoluble fatty acids depend almost entirely on the per cent of oleic acid present.

The soft portion of the glycerides is made up of a higher per cent of acids with a lower melting point, i. e., oleic and soluble acids. The soluble acids have a very low melting point. Therefore, even a slight increase in the per cent of soluble acids must cause a lowering of the melting point.

Tables V–A and V–B show a comparison of the iodine number of the soft and hard portions of the glycerides and of the insoluble acids derived from the glycerides. The iodine number of the soft and hard portions of the insoluble acids is higher than that of the corresponding portions of the glycerides of the butter fat. This is natural. The soluble acids and glycerin have been removed from the glycerides, raising the per cent. of the remaining constituents of the insoluble acids above that in the glycerides.

Table V–A.

Iodine No. of Soft and Hard Portions of Butter Fat.

	Soft Portion Iodine Number.	Hard Portion Iodine Number.	Soft Portion Per cent Olein.	Hard Portion Per cent Olein.	Gain Iodine Number.	Gain Per cent Olein.	Per cent Gain Olein of Soft Portion Over Hard Portion
From table III........	42.10	30.11	48.83	34.92	11.99	13.72	39.31
From table IV.........	43.55	33.08	50.518	38.37	10.47	12.148	31.6

Table V–B.

Iodine No. of Insoluble Acids of Soft and Hard Portions.

	Soft Portion Iodine Number.	Hard Portion Iodine Number.	Soft Portion Per cent Olein.	Hard Portion Per cent Olein.	Gain Iodine Number.	Gain Per cent Olein.	Per cent Gain Olein of Soft Portion Over Hard Portion.
From table III........	45.05	33.48	52.25	38.84	11.57	13.41	34.5
From table IV.........	46.2	35.66	53.59	41.36	10.54	12.23	29.5

CONCERNING THE SOLUBLE FATTY ACIDS.

Table VI shows the per cent of soluble fatty acids and glycerin in the soft and hard portions of butter fat. The soft portion contained 2.06 per cent more soluble acids and .56 per cent more glycerin than the hard portion, as obtained from data in Table IV.

TABLE VI.

Per Cent of Soluble Acids and Glycerin in Soft and Hard Portions.

	Soft Portion.	Hard Portion.	Gain.
Per cent soluble acids	8.23	6.17	2.06
Per cent Glycerol	12.89	12.33	.56

The soluble acids were calculated on the basis of a mean molecular weight of 104.5. This molecular weight was calculated from the amount of glycerides of the soluble acids and other data taken from Table IV.

The glycerol (C_3H_5) is calculated from the per cent of soluble acids, mean molecular weight 104.5. From this calculation the per cent of glycerin C_3H_5 $(OH)_3$ is readily determined.

The general formula for one molecule of a triglyceride is $C_3H_5(R)_3$, where R stands for mixed acid radicals R_3=104.5X3=313.5; allowing for the basic hydrogen C_3H_2=38, then the molecular weight of the triglyceride $C_3H_5(R)_3$ is 351.5.

$$351.5 : 38 = 8.23 : X$$

$$X = .888\%\ C_3H_2$$

From these results the per cent of glycerin is calculated as follows, the molecular weight of glycerin being 92:

$$38 : 92 = .888 : X$$

$$X = 2.14$$

This is the per cent of glycerin combined with the soluble acids of the soft portion.

Likewise, the per cent of the glycerin combined with the soluble acids of the hard portion is calculated:

$$351.5 : 38 = 6.17 : X$$

$$X = .888\%\ C_3H_2$$

$$38 : 92 = .666 : X$$

$$X = 1.61$$

This is the per cent of glycerin combined with the soluble acids of the hard portion.

The difference between the per cent of glycerin combined with the per cent of soluble acids of the soft portion and the per cent of glycerin combined with the per cent soluble acids of the hard portion, then, is 2.14—1.61=.53%. This agrees closely with the difference of the glycerin between hard and soft portions as shown by analyses. (See Table VI.)

The per cent of glycerin combined with the insoluble acids is nearly the same in both soft and hard portions, because the per cent of insoluble acids in the soft and hard portions differs very little. Also the variation in the composition of the insoluble acids would not materially affect the molecular weight. Therefore, it is reasonable to expect that nearly the same per cent of glycerin is combined with the insoluble acids of both the soft and the hard portions.

RELATION OF COMPOSITION OF BUTTER FAT SOLUBLE AND INSOLUBLE IN ALCOHOL TO COMPOSITION OF SOFT AND HARD PORTIONS OF FAT OBTAINED BY FRACTIONAL SEPARATION.

A comparison of the constants of the soft and hard portions with the constants of the fats soluble and insoluble in alcohol shows a close relation. The results are summarized in Table VII.

TABLE VII.

Showing the Variation of the Constants of the Soluble and Insoluble Portions in Alcohol, Also of the Soft and Hard Portions of Butter Fat Taken for the Experiment.

	A			B		
	Alcohol—Soluble Portion.	Alcohol—Insoluble Portion.	Original Butter Fat.	Soft Portion.	Hard Portion.	Original Butter Fat.
Reichert-Meissl number	48.1	20.7	27.70	33.85	24.66	30.00
Melting point	16.9°C.	36.°C.	33.5°C.	13.2°C.	38.1°C.	32.5°C.
Iodine number	34.07	39.75	37.63	43.55	33.08	39.82
Koetts. saponification number	259.14	215.06	227.4	232.78	226.4	230.1
Saponification equivalent	216.5	260.9	246.79	241.1	248.3	244.0
Refractive index at 40° C.	Reading 42.7 1.4543	Reading 45.6 1.4563	Reading 44.4 1.4555	Reading 44.8 1.4558	Reading 43 1.4545	Reading 44 1.4552
Per cent soluble acids (as Butyric)	9.792	4.26	6.60	6.90	5.17	6.09

These data give the composition of the portions of fat soluble in alcohol and of the original butter fat; also the composition of the soft and hard portions of butter fat separated by fractional crystallization and of the original butter fat. The samples A and B of butter fat used for the two experiments were not taken from the same lot of butter.

The Reichert-Meissl No. is distinctly higher in the fat soluble in alcohol and in the fat of the soft portion, than it is in the fat insoluble in alcohol and in the fat of the hard portion, as well as in the original fat.

The melting point is lowest in both the fat soluble in alcohol and in the fat of the soft portion.

On the other hand, the iodine number is lowest in the fat soluble in alcohol and highest in the fat of the soft portion.

The figures in the above table show the influence of the constants on the melting point of butter fat. The portion of fat insoluble in alcohol and the original fat from which the above portion was taken show a decidedly higher iodine number than the portion soluble in alcohol. If the melting point depended solely on the iodine number, the melting point of the fat insoluble in alcohol and of the original butter fat would be distinctly lower than the melting point of the portion soluble in alcohol. Table VII shows that this is not the case. The melting point of the portion insoluble in alcohol and of the original butter fat is a great deal higher (19.1° C. and 16.6° C., respectively, higher) than the melting point of the fat soluble in alcohol. The only factor to which this fact can be attributed is the high Reichert-Meissl No. in the case of the fat soluble in alcohol, as compared with the low Reichert-Meissl No. of the fat insoluble in alcohol and of the original butter fat. These results make it perfectly clear that the softness or hardness (melting point) of butter fat is dependent to a great degree on the per cent of soluble fatty acids present.

This table further shows, as stated in the previous chapters. that butter fat is a mixture of triglycerides of different fatty acids. The soft portion is the result of mechanical separation at different temperatures. It, therefore, contains more glycerides combined with acids of low melting points including oleic and soluble acids. Furthermore, the fat soluble in alcohol represents glycerides of acids soluble in alcohol. Since it is known that some of the glycerides of the soluble acids are soluble in alcohol. we can assume that some of the molecules in butter fat are made up of the glycerides containing a larger proportion of the soluble acids than others.

CONDITIONS AFFECTING THE COMPOSITION OF BUTTER FAT.

The composition of butter fat varies with the season of the year. A series of analyses of butter fat of butter made during each of the twelve months of the year, yielded the results summarized in Table VIII.

The results in Table VIII show that the Reichert-Meissl number was lowest in October, increasing steadily until it reached its maximum in March. After March it dropped abruptly, holding about its own till July, then taking a second drop and declining slightly toward October.

TABLE VIII.

Effect of the Season of Year on the Composition of Butter Fat.

	Reichert Meissl Number.	Iodine Number.	Melting Point.
January	30.03	31.20	33.4° C.
February	30.58	31.97	33.5° C.
March	31.30	31.94	33.5° C.
April	29.35	35.83	33.3° C.
May	29.55	36.48	32.5° C.
June	29.56	38.23	32.45° C.
July	28.90	37.10	31.9° C.
August	27.13	38.99	32.1° C.
September	27.19	35.36	33.0° C.
October	26.54	34.27	33.2° C.
November	28.36	30.65	33.4° C.
December	29.62	30.30	33.6° C.

The Iodine number was lowest in December, increasing slightly toward and including March; rising abruptly in April and continuing to rise up to and including June, then gradually declining toward October and dropping suddenly in November, followed by a slight drop in December.

The melting point followed, in general, the Iodine number reversedly. It was lowest in mid-summer when the Iodine number was highest, and it reached its maximum in December, when the Iodine number was lowest. The variations of the melting point, however, were not so abrupt as those of the Iodine number. A careful study of Table VIII suggests that, at times, the variations in the melting point may have been influenced strongly by the Reichert-Meissl number.

Experimental data produced in this country and abroad show unmistakably that the feed which the cows receive influences the per cent of olein in butter. Such feeds as cottonseed meal, bran, corn, overripe dry fodders, etc., when fed in excess, tend to decrease the per cent of olein, while linseed meal, gluten feeds, succulent pasture grasses, etc., are conducive of raising the per cent of olein.

The volatile fatty acids do not seem to be appreciably affected by the feed the cows receive. They are influenced, however, by the period of lactation as shown in Tables IX and X.[1]

TABLE IX.

Showing the Effect of the Period of Lactation on the Milk Fats.

TIME.	Reichert-Meissl Number.	Soluble Acids.	Insoluble Acids.
1st month	32.41	7.39	87.26
2d month	29.48	7.07	87.99
3d month	29.95	7.08	87.90
4th month	29.97	7.11	87.72
5th month	29.56	7.00	87.72
6th month	29.21	6.82	88.19
7th month	28.06	6.45	88.4
8th month	25.32	5.84	88.6
9th month	25.45	6.01	88.5
10th month	27.45	6.26	88.1

[1] Hunziker, Proceedings of the Indiana Academy of Science, 1908, page 144.

Table X.

Showing Effect of the Period of Lactation on the Milk Fats.

Time.	Reichert-Meissl Number.	Soluble Acids. Per Cent.	Insoluble Acids. Per Cent.
1st month	36.68	8.20	86.76
2d month	35.75	8.09	86.74
3d month	33.19	7.59	86.99
4th month	33.80	7.56	86.95
5th month	33.63	7.47	87.10
6th month	33.57	7.55	86.94
7th month	32.72	7.49	86.99
8th month	31.63	7.25	87.41
9th month	31.98	7.10	87.50
10th month	32.03	7.12	87.46
11th month	26.64	6.50	88.20
12th month	30.48	8.86	87.69

The data in Tables IX and X represent results of experiments with three cows whose period of lactation commenced in October and November respectively. They were fed on a uniform ration throughout the entire period of lactation with the exception that in July (the 9th month after calving) they were turned out on pasture.

The above tables clearly show that the soluble fatty acids are highest immediately after parturition, or at the beginning of the period of lactation. Slight irregularities excepted, they decreased as the period of lactation advanced and were lowest toward the close of the period of lactation.

It so happens that in most localities the majority of the cows drop their calves in late spring, at a time when they also change from dry feed to succulent pasture. This explains why in early summer both the per cent of volatile fatty acids and the per cent of oleic acids increase and the melting point decreases.

RELATION OF COMPOSITION OF BUTTER FAT TO COMPOSITION OF BUTTER.

During late spring and early summer, at a time when, as shown above, the Reichert-Meissl number and the Iodine number are high and the melting point is low, the butter-maker experiences usually considerable difficulty in manufacturing butter with a reasonably low moisture content. This coincidence has suggested to the writers that there may be a more or less intimate relation betwen the melting point of the butter fats and their power to absorb water during the process of butter-making. A series of experiments was, therefore, conducted bearing on this point. The results are shown in Table XI.

TABLE XI.

Per Cent of Moisture Retained by Soft and Hard Fats Churned Separately.

	Per Cent Water.		Per Cent Increase of Soft Over Hard
	Soft Fats.	Hard Fats.	
March butter	43.84	24.76	77.02
May butter	50.62	24.78	104.28
Average	47.23	24.77	90.65

Table XI covers experiments in which soft and hard portions of butter fat (butter fat with a low and a high melting point) were separated from one another by fractional crystallization of the fats and by pressure. The soft and hard portions were churned separately under identical conditions, adding the same amount of water to each churning and churning at the same temperature.

Twelve separate churnings were made each, the March butter and the May butter. In the March butter the per cent increase of the moisture of the soft fats over that of the hard fats was 77.02. In the May butter the per cent increase of the moisture of the soft fats over that of the hard fats was 104.28. These figures unmistakably show that the soft fats are capable of taking up a great deal more moisture than the hard fats. They, therefore, can leave little doubt that the material increase in the moisture content of butter made in early summer is due to the increase in the soft fats it contains.

The moisture-retaining property of the fats is largely dependent on their melting point. The lower the melting point, the greater is their power to mix with and retain water. Since the glycerides of the oleic and soluble fatty acids have a low melting point, it is reasonable that any increase in the per cent of these glycerides tends to increase the water-retaining properties of the butter.

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