

## I. THE PHYSICAL AND CHEMICAL CONSTANTS OF BUTTER-FAT.

A word should be said in regard to the preparation of samples of butter-fat for analysis. The method of procedure adopted in this laboratory is as follows: A sufficient quantity of the butter is placed in a glass jar or beaker and warmed at about 50° C., with occasional agitation, until the water and curd have separated out, leaving the layer of butter-fat quite clear. Then, without disturbing any of the water and curd, as much as possible of the melted fat is drawn off with a pipette into another vessel and allowed to stand, if necessary, for further clarification. The sample is then filtered through desiccated filter-paper into a clean, dry jar, after which it is stoppered and preserved for analysis in a cold place away from the light. The special precaution to be noted, in the preparation of the samples, is the avoidance of too high a temperature in melting. Butter-fat is an exceedingly unstable body and a temperature much above 50° C. will very soon produce chemical changes. Too long an exposure at 50° C. even, will alter the composition of butter-fat,<sup>1</sup> so that no more time than is necessary should be consumed in the processes of melting and filtering.

In Table I are given the results obtained for a few of the common constants usually determined in butter-fat analysis.

TABLE I.<sup>2</sup>

Constant.	Range.	Mean.	Number of samples represented.
Specific gravity $\frac{40^{\circ}}{15.3^{\circ}}$ .....	0.9050-0.9102	0.9073	35
Melting-point.....	31.6 <sup>2</sup> -34.6 <sup>0</sup>	33.2 <sup>0</sup>	35
Acid number.....	0.20-0.66	0.50	5
Saponification number.....	224.0-234.9	228.5	40
Ether number <sup>3</sup> .....	223.5-234.4	228	40
Iodine number.....	29.36-37.30	33.35	40
Reichert number (two and a half grams).....	15.10-17.50	16.2	40
Reichert-Meißl number (five grams) .....	22.80-32.10	28.3	10
Insoluble acids, per cent .....	86.03-88.84	87.65	10

<sup>1</sup> We have found by experiment that a butter-fat kept at 50° C. for two days suffered a decrease in its iodine number of over one unit.

<sup>2</sup> The figures from which this and succeeding tables are made up represent the mean of duplicate determinations.

<sup>3</sup> Saponification number less 0.5, the mean acid number.

The constants were all determined by the usual processes employed in the analysis of oils and fats; since these are too well known to require any detailed description, we will simply indicate the methods employed, describing only any modifications which were introduced.

The specific gravity was determined on the melted samples at 40° C. by means of a Westphal balance, which gives results accurate to the fourth decimal. Whenever greater accuracy was required a pycnometer or Sprengel tube was used.

The melting-point was determined according to the method of the official chemists.<sup>1</sup>

The acid number, or milligrams of potassium hydroxide necessary to neutralize the free acid in one gram of fat, was determined by heating about ten grams of the fat with fifty cc. of neutral alcohol in a ten-ounce flask provided with a condensing tube. After allowing the alcohol to boil for several minutes, the flask was cooled to about 50° C., when the condensing tube was removed, and the contents titrated with tenth-normal alkali (sodium hydroxide or potassium hydroxide), using phenolphthalein as indicator.

The determinations of the acid number were made upon samples of fat taken immediately after the butter was churned, so it will be observed that, even when fresh, butter-fat contains an appreciable amount of free acid. This is a fact of some importance upon which we will dwell more fully when treating of the subject of rancidity.

The saponification number, or milligrams of potassium hydroxide necessary to saponify one gram of fat, was determined according to the usual method. About two and a half grams of fat were taken and saponified with exactly twenty-five cc. of half-normal alcoholic potassium hydroxide. To secure concordant results great care must be exercised in regard to the measuring of the potassium hydroxide solution; for this reason we prefer a burette to the twenty-five cc. pipette generally recommended. The saponifications were performed in flasks provided with condensing tubes; twenty to thirty minutes were usually allowed for the reaction, after which the contents of the flask were titrated back with half-normal hydrochloric acid, using phenolphthal-

<sup>1</sup> Bulletin No. 46, Division of Chemistry, U. S. Department of Agriculture, p. 34.

eu as indicator. Blank experiments were run with every series of determinations.

The ether number, or milligrams of potassium hydroxide necessary to saponify the neutral fat in one gram of sample, is found by subtracting the acid number from the saponification number.

The iodine number, or percentage of iodine absorbed by the fat, was determined according to the method of the official chemists.<sup>1</sup> A burette was used for measuring the iodine solution as more concordant results could be secured than when a pipette was employed.

The volatile acid numbers were determined partly by the Reichert method, when two and a half grams of fat were taken, and partly by the Reichert-Meissl process, when five grams of fat were employed. With the exception of the quantity of fat weighed out the method of procedure was the same in both cases, Wollny's process<sup>2</sup> for saponification and distillation being the one adopted.

It is a well-known fact that the method of determining the volatile acids in butter-fat by the ordinary process of distillation is not a quantitative one. The number of cubic centimeters of half-normal alkali consumed is always greater by the Reichert process, as a larger percentage of the volatile acids are driven over when working with a smaller quantity of fat; the results in Table I show that the Reichert process gives a mean constant ten per cent. higher than the Reichert-Meissl method, after calculating the figures both to the same weight of fat.

Richard Meyer found that by distilling with a current of steam, the number of cubic centimeters of half-normal alkali was increased by twenty-five per cent. The experience in this laboratory has been that only from seventy-five to eighty per cent. of all the volatile acids pass over in one distillation. As a test, ten distillations were made on two and a half grams of fat, the contents of the flask being replaced with 110 cc. of water after each distillation. The first fraction took 15.5 cc. of tenth-normal alkali, the succeeding nine fractions requiring each in their order, 2.00, 0.95, 0.60, 0.55, 0.40, 0.35, 0.30, 0.30, 0.25, or

<sup>1</sup> Bulletin No. 46, Division of Chemistry, U. S. Department of Agriculture, p. 32.

<sup>2</sup> *Ztschr. anal. Chem.* (1889), 28, 721.

together 5.7 cc., giving a total of 21.2 cc., thus making the original Reichert number of 15.5 over twenty-five per cent. too small.

Instead of distilling the lower fatty acids it is possible to remove them from the higher insoluble acids by washing.<sup>1</sup> The process can be carried out in connection with the determination of the saponification number, or with the determination of the insoluble acids. If worked in connection with the former the method of procedure is as follows :

Having determined the saponification number of the fat the flask is replaced on the steam-bath until all alcohol is removed. The residue of soap is then dissolved in 100 cc. of hot distilled water, and after cooling to about 60° just enough half-normal hydrochloric acid is added to unite with all the combined alkali.<sup>2</sup> The flask is then stoppered with a condensing tube and heated on the steam-bath until the fatty acids have risen to the surface and the liquid underneath has become clear. The flask and contents are now thoroughly cooled, after which the cake of insoluble fatty acids is gently detached from the walls of the flask by shaking and the liquid filtered into a liter flask. One hundred cc. of hot water are then poured into the flask and, after the acids have melted, thoroughly shaken. The contents of the flask are poured on the filter and the flask again washed out with hot water; this is repeated until all the fatty acids are removed from the flask, when they are washed directly on the filter. As soon as the filtrate amounts to a liter it is titrated with half-normal alkali. It is needless to remark that the water used for washing should be absolutely neutral.

Although a larger percentage of the lower acids are generally removed by the above process of washing than by one distillation, the same difficulty of effecting a complete separation of the soluble from the insoluble acids is experienced. It has been found impossible to remove the last traces of soluble acids, even by continued washings, the higher and less soluble homologues, caprylic and capric acids, especially being retained by the insol-

<sup>1</sup> Allen: *Commercial Organic Analysis* (1899), Vol. II, Part 1, p. 191.

<sup>2</sup> The amount of half-normal hydrochloric acid necessary for this can be found by subtracting the number of cubic centimeters of half-normal hydrochloric acid used in titrating the excess of potassium hydroxide in the determination of the saponification number from the number of cubic centimeters of half-normal hydrochloric acid used to neutralize the potassium hydroxide in the blank experiment.

uble acids on the filter. The first filtrate (one liter) of soluble acids from a butter-fat, treated by the process of washing just described, required 15.5 cc. of tenth-normal alkali. The acids on the filter were washed nine more times, using a liter of hot distilled water at each washing. The succeeding filtrates required each in their order, 1.05 cc., 0.60, 0.50, 0.40, 0.35, 0.35, 0.30, 0.30, 0.15, making in all 19.5 cc. It is evident that, while one treatment is amply sufficient to distinguish a butter from a substitute, the method is of little value quantitatively unless the treatment of washing be repeated until the washings become neutral—a tedious operation at the best.

The process employed by us for determining the percentage of insoluble acids, or Hehner number, is as follows: Ten grams of the fat are weighed out into a wide-necked 250 cc. flask and saponified with five cc. of fifty per cent. soda and twenty-five cc. of ninety-five per cent. alcohol. The alcohol is then distilled off and the residue of soap dissolved in 150 cc. of water; 50 cc. of hydrochloric acid (1 : 1) are then slowly added with continuous shaking, after which the flask is replaced on the steam-bath and allowed to stand until the liquid beneath the layer of fatty acids has become perfectly clear. The flask and contents are now cooled; the cake of fatty acids, after hardening perfectly, is loosened from the walls of the flask by shaking gently, and the liquid underneath poured through a clean filter. The short arm of a glass siphon is then introduced, allowing the end of the tube to come in contact with the bottom of the flask; the discharging end of the siphon is closed by a piece of gum tubing fitted with a pinch-cock. The flask is now filled about three-fourths full with hot water and thoroughly agitated; after allowing the fatty acids to rise to the surface of the liquid, the siphon is filled by blowing in hot water from a wash-bottle at the lower end, the process being easily controlled by the pinch-cock. In this way also any of the fatty acids, which have risen within the siphon, can be washed back into the flask. The liquid underneath the layer of fatty acids is now siphoned off into the paper used for the first filtration, the pinch-cock serving to regulate the flow. When as much as possible of the liquid is drawn off, without taking any of the insoluble acids, the flask is again nearly filled with hot water and shaken; after washing out the siphon, the

liquid is drawn off as before. The process is continued until the washings cease to redden litmus paper; about ten washings, requiring over two liters of water, are generally necessary to effect this. When the washings are neutral the siphon is removed, any acids, which may remain adhering to it, being washed back into the flask with hot water; the flask is cooled and as soon as the acids have hardened the cake is detached and the washings poured through the filter. The flask is then placed in an inverted position over the filter and allowed to drain over night. In the morning the filter-paper, which generally contains a small quantity of insoluble acids, is washed several times with a few cubic centimeters of ether, the washings being allowed to run into the flask. The flask is then warmed at  $80^{\circ}$  until most of the ether is expelled and then dried at  $100^{\circ}$  to constant weight. We have found it expedient to dissolve out the acids, after securing a constant weight, with hot alcohol and reweigh the flask; a difference of several milligrams is generally experienced between the first and last weighings, due to the removal of soluble matter from the glass.

We believe a quicker and more thorough washing of the insoluble acids, with less danger of loss, is secured by this method than by the usual processes for determining the insoluble acids. Duplicate results can be secured which agree perfectly. The method is applicable not only to the analysis of fats and oils, but we have used it to advantage in soap analysis.

One point which we have observed in connection with the drying of fatty acids to constant weight at  $100^{\circ}$  C. is that when the acids were dried in a low evaporating dish or beaker a constant weight could never be secured; the acids continued to lose in weight even when it was evident that all moisture was removed; when, on the other hand, the drying was performed in a flask a constant weight was soon secured, after which no change was observed, even on many hours' drying. This difference we believe to be due to a partial volatilization of the insoluble acids; the escape of volatile products would be much easier from an open dish than from a flask; moreover, we have always found, after removing the flask containing the insoluble acids from the oven, that the inner surface and lower parts of the neck of the

flask were coated with a thin layer of condensed acids, showing that a volatilization must have occurred.

In Table II are given figures for a few of the constants less frequently determined in the analysis of butter-fat.

TABLE II.

Constant	Range.	Mean.	Number of samples represented.
Acetyl number.....	3.5-4.8	4.1	5
Glycerol calculated <sup>1</sup> .....	12.24-12.79	12.46	40
“ by analysis.....	12.30-12.70	12.45	10
Total fat acids calculated <sup>1</sup> ...	94.72-94.94	94.85	40
Soluble fat acids calculated ...	6.52-8.96	7.20	10
Specific gravity $\frac{2}{100}$ insoluble acids .....	0.9106-0.9242	0.9162	10
Melting-point insoluble acids	40.2 <sup>o</sup> -42.7 <sup>o</sup>	41.7 <sup>o</sup>	10
Saponification number insoluble acids.....	212.5-217.0	214.5	10
Mean molecular weight insoluble acids.....	258.1-263.5	261.0	10
Specific gravity $\frac{2}{100}$ soluble acids .....	0.9475-0.9483	0.9479	2
Saponification number soluble acids .	563.7-577.3	571.7	15
Mean molecular weight soluble acids.....	97.17-99.52	98.12	15

The acetyl number, or milligrams of potassium hydroxide necessary to combine with the acetic anhydride in one gram of acetylated fatty acids, was determined according to the method of Benedikt and Ulzer.<sup>2</sup> The process, as conducted in this laboratory, is as follows:

Fifty cc. of the melted fat are saponified, the resulting soap is decomposed with hydrochloric acid, and the insoluble acids secured according to the usual methods. Twenty-five cc. of the dried and filtered acids are boiled with twenty-five cc. of glacial acetic acid, for two hours, in a 250 cc. flask connected with a reflux condenser. The mixture is then poured into a large two-liter flask filled three-fourths full with boiling water; the flask is thoroughly agitated for some minutes and then allowed to stand until the acids have risen to the surface. After cooling perfectly under running water, the cake of acids is gently

<sup>1</sup> Calculated from figures given for ether numbers in Table I.

<sup>2</sup> *Monatsh. Chem.*, 8, 40; *Analyse der Fette*, 3rd Ed., p. 145.

detached from the walls of the flask and the liquid underneath poured out; the flask is then filled half full with hot water and the contents boiled for half an hour; a few pieces of clean pumice stone, which have been kept under water, are added to prevent bumping. The liquid underneath the acids is then removed by a siphon and the acids washed by siphonation, as under determination of insoluble acids, until the wash-water is neutral. The acids in the flask, after hardening, are allowed to drain; they are then melted, transferred to a smaller vessel, and dried at 100° until all water is removed, after which the acids are filtered through dry paper and preserved for analysis.

To determine the acetyl number, three to five grams of the acetylated acids are weighed out into a 250 cc. flask, dissolved in fifty cc. of neutral alcohol, and titrated with half-normal alcoholic potash, using phenolphthalein. The milligrams of potassium hydroxide for one gram of acid are calculated as in the determination of the acid number, and the number so obtained is designated the acetyl-acid number. An excess of the alcoholic potassium hydroxide is then added, the flask is stoppered with a condensing tube, and heated on the bath twenty to thirty minutes, after which the excess of potassium hydroxide is titrated back with half-normal hydrochloric acid. The quantity of potassium hydroxide consumed by this second process, when expressed in milligrams per gram of fat, constitutes the acetyl number. The sum of the acetyl-acid number and acetyl number is known as the acetyl-saponification number. The acetyl number then represents simply the ether number of the acetylated acid, being the difference between the acetyl-acid and acetyl-saponification numbers.

In reviewing the literature of the subject only two other values could be found for the acetyl number of butter-fat. Wachtel gives 9.6, and Bondzynski and Ruffi 18.2, numbers both considerably higher than the values given in Table II. The acetyl figure, though of little value in practical butter analysis, yet furnishes us a measure of the oxy-acids in a fat; more will be said as to the employment of this constant under the subject of rancidity.

The percentage of glycerol which a butter-fat will yield upon saponification can be readily calculated from the ether number.

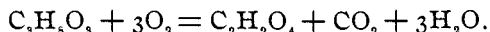


In the saponification of any triglycerid three molecules of potassium hydroxide separate one molecule of glycerol, or 168.3 parts of potassium hydroxide corresponds to ninety-two parts of glycerol; one gram, therefore, of potassium hydroxide would equal 0.5466 gram of glycerol.<sup>1</sup> If E represents the ether number of a fat, the percentage of glycerol, G, which the fat will yield, will be,

$$G = 0.05466 E.^2$$

This indirect method of estimating glycerol can be used in the analysis of any fat or oil which belongs to the class of triglycerids: with diglycerids the method is not practicable as Benedikt has shown.<sup>3</sup> With rancid fats, owing to the presence of aldehyde bodies, as will be shown later, the method gives too high results.

The percentage of glycerol was determined directly by the method of Benedikt and Zsigmondy,<sup>4</sup> which is based upon the fact that one molecule of glycerol, when oxidized in a strongly alkaline solution with permanganate, produces quantitatively exactly one molecule each of oxalic acid and carbon dioxide.



Allen's<sup>5</sup> modification of the original process of Benedikt and Zsigmondy, was followed in this laboratory with the exception of a few minor differences.

Ten grams of butter-fat are weighed out into a six-ounce saponification flask of heavy glass provided with a tightly fitting glass-stopper. Five grams of potassium hydroxide dissolved in fifty cc. of water are then added and the glass stopper wired in securely. The flask is placed in the steam-bath and shaken thoroughly every fifteen minutes, the heating being continued until the contents of the flask become thick and homogeneous, and no more liquid fat is seen swimming on the surface: eight to twelve hours is generally necessary for this. When saponification is complete the flask is cooled, unstoppered, and the contents washed out into a large beaker with 200-300 cc. of hot water. A perfectly clean solution should be obtained without any oily particles being visible.

<sup>1</sup> Zulkowski: *Ber. d. chem. Ges.*, 16, 1140.

<sup>2</sup> Benedikt: *Analyse der Fette und Wachsarten*, 3rd Ed., p. 182.

<sup>3</sup> *Ibid.*, p. 182.

<sup>4</sup> *Chem. Ztg.*, 9, 975. *Analyse der Fette*, p. 182.

<sup>5</sup> Allen: *Commercial Organic Analysis*, 3rd Ed., (1899), Vol. II, Pt. 1, p. 314.

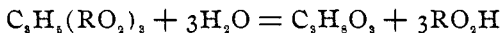
The solution of soap is decomposed with an excess of dilute sulphuric acid, and the fatty acids separated and washed in the usual way; the washings, which should be clear, are made up to one liter and thoroughly mixed. 200 cc. (two grams of fat) of this solution are measured out into a large evaporating dish or beaker, diluted to about 400 cc. and neutralized with potassium hydroxide solution using phenolphthalein. Ten grains of solid potassium hydroxide are then added, and when completely dissolved, a saturated solution of permanganate is added, at ordinary temperature, until the liquid is colored no longer green, but a very dark-blue (three to five grams of permanganate are sufficient for this). The solution is then heated to gentle boiling for about an hour, the liquid becoming finally red with precipitation of the hydrated peroxide of manganese; the volume of the liquid, during the boiling, should be kept at about 400 cc. by adding water from time to time. A strong solution of sodium sulphite is then gradually added until all color is destroyed, and the liquid above the brown precipitate is clear. The solution is next filtered through a large funnel, using heavy filter-paper, into a liter flask, and the precipitate washed with hot water until the flask is nearly full; the slight turbidity, which frequently shows in the final washings, does no harm. After cooling and making up to one liter, 500 cc. (one gram original fat) are measured into a large beaker, acidified with acetic acid, heated nearly to boiling, and precipitated with ten cc. of a ten per cent. calcium chloride solution; the liquid is heated on the steam-bath an hour or more until everything has settled out. The precipitate, consisting of calcium oxalate, with a little sulphate mixed, is filtered into a prepared Gooch crucible and thoroughly washed with hot water. The contents of the Gooch crucible, including the asbestos felt, are then washed back into the original beaker with about 200 cc. of hot water, acidified with dilute sulphuric acid, and heated on the bath for ten minutes, after which the solution is titrated with tenth-normal permanganate. One cc. of tenth-normal permanganate equals 0.0046 gram of glycerol.

The oxidation method for determining glycerol is a somewhat long and tedious one, requiring also a certain amount of practice to secure good results. The details of the method, as regards

the oxidation, must be rigidly adhered to. Benedikt states that the soluble fatty acids, butyric, etc., which are present in the same solution with the glycerol, do not yield oxalic acid on the treatment with potassium hydroxide and potassium permanganate, but that this is true only so far as regards the original method. Johnstone<sup>2</sup> and Mangold have both found that in strongly alkaline solutions butyric acid can be oxidized to oxalic acid. Experiments in this laboratory confirm this; if too great an excess of either potassium hydroxide or potassium permanganate is used for oxidation, or if during the process of boiling, the liquid is allowed to become too concentrated, abnormal results are secured. A butter-fat which gave 12.35 per cent. of glycerol, when analyzed according to the method, yielded, according to analysis, over eighteen per cent. of glycerol, by employing a large excess of potassium hydroxide and potassium permanganate, and oxidizing in a concentrated solution, thus proving beyond doubt that the soluble acids had been acted upon. By observing the necessary precautions, however, no danger need be apprehended from this source; we have found the method to yield most excellent results on many of the ordinary fats, as well as on butter-fat itself, the agreement between the percentage of glycerol as calculated from the ether number, and as determined by analysis, being very close, as the following examples will show.

Fat.	Ether number.	Glycerol calculated. Per cent.	Glycerol by analysis. Per cent.
Beef-tallow .....	195.5	10.68	10.61
Commercial stearin .....	192.3	10.51	10.56
Butter-fat.....	232.2	12.69	12.70

The total percentage of fatty acids which a butter-fat will yield can be readily calculated if we know the percentage of glycerol produced on saponification.<sup>3</sup> By inspecting the formula,



Fat + water (54) = glycerol (92) + fatty acid,

we observe that in saponifying a fat fifty-four parts of water are required for every ninety-two parts of glycerol produced. The yield of fat acids ( $F$ ) from 100 parts of fat would be represented

<sup>1</sup> *Analyse der Fette*, 3rd Ed., p. 184.

<sup>2</sup> *Chem. News*, 63, 11.

<sup>3</sup> Zulkowski: *Ber. d. chem. Ges.*, 16, 1315.

then by the formula,  $F = 100 + \frac{54}{92} G - G$ , or  $F = 100 - \frac{38}{92} G$ , in which  $G$  equals percentage of glycerol.  $G$ , as we have shown, can be obtained from the ether number ( $E$ ) of a fat by the formula,  $G = 0.05466 E$ ; by substituting this value for  $G$  in the previous equation we obtain,  $F = 100 - \frac{38}{92} \times 0.05466 E$  or  $F = 100 - 0.02258 E$ .<sup>1</sup>

The percentage of soluble acids in a butter-fat can be found by subtracting the percentage of insoluble acids (Hegner's number) from the percentage of total acids.<sup>2</sup>

The specific gravity of the insoluble acids was determined by weighing the acids in air and in water at 20°, using a sinker. For a sinker we have found a small platinum crucible cover to answer very satisfactorily, since it offers a convenient receptacle for holding the acids; a silk thread is used for suspending the cover from the beam of the balance; no air-bubbles should be present on the surface of the sinker during the operation and to prevent their appearance recently boiled distilled water is used throughout the work. The following example will illustrate the method:

Weight of sinker and fatty acids in air	=	6.5460	grams
“ “ “ alone	=	5.2110	“
“ “ insoluble fatty acids	=	1.3350	“ = $w$
“ “ sinker in water at 20°	=	4.9715	“ = $\omega$
“ “ “ and acids in water at 20°	=	4.8405	“ = $\omega'$

From the formula  $s = \frac{w}{w + \omega - \omega'}$ , we calculate the specific gravity of the insoluble acids to be 0.9106. This formula is sufficiently accurate for ordinary purposes; more strictly it should read,  $s = \frac{w(1 - s)}{w + \omega - \omega'} + s$ , in which  $s$  equals the density of air; the value of  $s$  for dry air, within the ordinary range of temperature and pressure, is 0.0012. If we desire to express the gravity in terms of water at 4° we should use the formula  $s = \frac{w(d - s)}{w + \omega - \omega'} + s$ , where  $d$  equals the density of water at the temperature of the

<sup>1</sup> Benedikt: *Analyse der Fette*, 3rd Ed., p. 162.

<sup>2</sup> *Ibid.*, p. 165.

experiment, a value easily found from tables. At  $20^{\circ}$   $d$  is 0.9982, our original specific gravity, then, corrected to  $\frac{20^{\circ}}{4^{\circ}}$  would be 0.9091 instead of 0.9106.<sup>1</sup>

The melting-point of the insoluble acids was determined by observing the temperature at which the acids became transparent when heated in a capillary tube. A small piece of capillary tubing, about one inch in length, is sealed at one end and nearly filled with the melted fatty acids; there should be no air space beneath the fatty acids and to prevent this, the filling of the tube is best accomplished with a small pipette or dropper, the end of which has been drawn out to a slender point. The tubes should be filled and cooled several hours before making the determinations.

In conducting the experiment the small tube containing the fatty acids is fastened to the bulb of a delicate thermometer and then lowered into a large test-tube, nearly full of distilled water recently boiled and cooled; the test-tube is fastened into a tall beaker nearly filled with water. Heat is now gently applied, keeping the water in the outer vessel constantly stirred by means of a blowing bulb. As soon as the temperature of the water in the beaker reaches  $38^{\circ}$ , the heat is raised more slowly, about  $1^{\circ}$  in five minutes. A difference of about  $1^{\circ}$  is generally observed between the beginning of melting and the point of absolute transparency; we have used the latter as the true melting-point, though the mean of the two readings would be perhaps a little more correct. The method of the official chemists would not be practicable in the case of determining the melting-point of the insoluble acids, owing to the solubility of the acids in alcohol.

The saponification number of the insoluble acids, which in this case would be identical with the acid number, was determined by dissolving three to five grains in hot neutral alcohol and titrating with half-normal potassium hydroxide.

From the saponification number ( $s$ ),<sup>2</sup> the mean molecular

<sup>1</sup> This method can be used as well for determining the specific gravity of butter-fat at the ordinary temperature. We have found the mean specific gravity of butter-fat corrected to  $\frac{20^{\circ}}{4^{\circ}}$  to be 0.9250.

<sup>2</sup> Analyse der Fette, p. 163.

weight ( $m$ ) of the insoluble acids can be readily calculated by means of the formula  $m = \frac{56100}{s}$ .<sup>1</sup>

For the determination of the constants of the soluble acids from butter-fat, it is necessary first to separate the acids in a state of complete purity. To effect this the following process was employed :

One hundred grams of butter-fat were saponified with fifty cc. of fifty per cent. sodium hydroxide solution and 200 cc. of alcohol in a large flask provided with a condensing tube. After distilling off the alcohol, the residue was dissolved in hot water, decomposed with excess of hydrochloric acid (1 : 1), and heated on the bath using the condensing tube, with occasional shaking until the liquid underneath the layer of acids had become clear. The contents of the flask were then cooled, the cake of acid detached, and the liquid underneath filtered through heavy paper into a large evaporating dish. The insoluble acids were then remelted, brought upon the filter with hot water and washed until the filtrate became neutral. The combined filtrates and washings were neutralized with sodium hydroxide solution using phenolphthalein and evaporated to about 500 cc.; the solution was next filtered into a large separating funnel, acidified with dilute sulphuric acid, and after cooling shaken with fifty cc. of washed ether. After standing for a few minutes, the two solutions were separated and the aqueous liquid again treated with fifty cc. of ether; the process was repeated for a third time when the combined ethereal solutions were filtered into a dry dish and the ether allowed to evaporate at the room temperature; the residue of soluble acids<sup>2</sup> (five to six grams) was dried in a vacuum over sulphuric acid and then preserved in a stoppered vial. The soluble acids thus obtained consisted of a light mobile liquid having a pale

<sup>1</sup> In the *Chem. Rev. Fett u. Harz-Ind.*, 5, [9], 169-172. R. Henriques calls attention to a difficulty in estimating the mean molecular weight of the insoluble acids from butter fat, in that the values found on drying the acids at 105°-110°C. (*viz.*, 267-270) differed from those (256.3-263) obtained when the drying was effected at the ordinary temperature in a vacuum. (Abstract in *Analyst*, No. 274, p. 7.)

The values found by Henriques on drying in a vacuum are identical with those given by us in Table II. The higher values, found on drying the acids at 105°-110°, we believe to be due to a partial volatilization of acids of low molecular weight, such as lauric and myristic, which, as will be shown in a later article, are present in the insoluble acids of butter-fat in considerable quantity. Such a volatilization may occur at even 100°, as we have already indicated (p. 618).

<sup>2</sup> A quantitative separation of the soluble acids is not effected by the treatment.

straw color,<sup>1</sup> and giving the intense odor characteristic of butyric acid.

The specific gravity of the soluble acids was determined at 20° C. by means of a small two cc. pycnometer.

The saponification number was determined by titrating one to two grams of the acids, dissolved in neutral alcohol, with half-normal potassium hydroxide. Knowing the saponification number (*s*) the mean molecular weight of the soluble acids is determined by the formula  $m = \frac{56100}{s}$ .

We are well aware that the figures given in Table II for the mean molecular weight of the soluble fatty acids are much lower than those given by certain German authorities. Benedikt<sup>2</sup> gives 139 as the mean molecular weight of the volatile fatty acids from butter-fat. This number was not found directly, however, but was calculated on the assumption that the Reichert-Meissl number is a quantitative expression for the amount of volatile acids, an assumption not warranted by experiment as has been shown.<sup>3</sup>

If the calculation be made on the volatile acid number from ten distillations instead of from one, results will be obtained which approximate very closely those expressed in Table II, as the following example will show. A butter-fat, containing 8.72 per cent. of soluble acids, required to neutralize the volatile acids (ten distillations) from 2.5 grams, 22.3 cc. of tenth-normal sodium hydroxide, or 8.92 cc. of tenth-normal sodium hydroxide per gram of fat.  $\frac{8.72}{8.92} \times 100 = 97.7$ , the mean molecular weight of the soluble acids. Practically the same figure is arrived at, if we obtain the saturation number of the soluble acids, after their complete removal by washing, according to the method already described.<sup>4</sup> Two and five-tenths grams of a butter-fat, containing seven and seven-tenths per cent. of soluble acids, required 19.5 cc. of tenth-normal sodium hydroxide to neutralize the soluble acids removed by washing. The cubic

<sup>1</sup> If distilled in a vacuum the acids can be obtained colorless.

<sup>2</sup> *Analyse der Fette*, 3rd ed., p. 546.

<sup>3</sup> This article, p. 615. Benedikt, elsewhere in his own work, calls attention to this same fact. *Analyse der Fette*, 3rd ed., p. 137.

<sup>4</sup> This article, p. 617.

centimeters per gram of fat would be seven and eight-tenths and the mean molecular weight would equal  $\frac{7.7}{7.8} \times 100$  or 98.7.

We have determined the mean molecular weight of the volatile acids in many butter-fats by still another process. The weight of the barium salts of the volatile acids, is determined and, from the amount of barium oxide contained therein, the mean molecular weight of the combined acids is calculated by means of the formula  $m = \frac{76.7(r-b)}{b} + 9$ , in which  $m$  is the mean molecular weight sought,  $r$  the weight of barium salts, and  $b$  the weight of barium oxide. The process can be carried out in connection with the determination of the Reichert or Reichert-Meissl number. After neutralizing the distillate of volatile acids with half-normal barium hydroxide, the solution is evaporated to dryness in a weighed platinum dish, and heated at  $100^\circ$  C. to constant weight. The residue is then moistened with a little strong sulphuric acid, ignited, and weighed; from the weight of barium sulphate is calculated the barium oxide. A comparison of the values obtained for the mean molecular weight of the volatile or soluble acids by the above method and by the method of direct titration is given herewith:

	Range.	Mean.
By direct titration.....	97.94-99.51	98.73
“ weighing the barium salts.....	97.17-98.97	97.92

We can verify this figure for the mean molecular weight of the volatile or soluble fatty acids in the following manner:

For the average butter-fat we may consider the following constants to be nearly correct:

Saponification number .....	228.5 (Table I)
Insoluble acids, per cent .....	87.65 “
Soluble “ “ .....	7.20 (Table II)
Saponification number of insoluble acids..	214.5 “

There are required then for the average butter-fat:

	228.5 mg. KOH to saponify 1 gram of fat
$0.8765 \times 214.5 = 188.0$	“ “ “ “ insoluble acids in 1 gram fat
Difference	$\frac{40.5}{40.5}$ “ “ “ “ soluble acids in 1 gram fat

or 40.5 mgs. of KOH are required to saponify 0.0720 gram of



soluble acids. Therefore  $\frac{40.5}{0.072} = 562.5$ , theoretical saponification number of soluble acids, and  $\frac{56100}{562.5} = 99.73$ , theoretical molecular weight of the soluble acids in the average butter-fat. While this theoretical figure is a trifle higher than any we have found in actual work, the agreement is sufficiently close to enable us to say that the mean molecular weight of the soluble acids from butter-fat lies very near to the value expressed in Table II.

We have often found it convenient in our work, where an accurate determination was impossible, either from want of time, or lack of material, to make a rough approximation of the percentage of soluble acids; for this purpose we have devised a very simple formula, the only factor necessary to know being the saponification number of the butter-fat. The equation, in which  $v$  equals per cent. of soluble acids and  $s$  the saponification number, is :

$$v = 0.0018 \left[ s - \left( 188.5 + \frac{228-s}{2} \right) \right] \times 100, \text{ or } v = 0.09(3s - 605).$$

The formula is deduced from data given in the preceding tables. The average butter-fat, having a saponification number of 228, and containing 87.65 per cent. of insoluble acids with a saponification number of 215, would require 188.5 milligrams of potassium hydroxide to neutralize the insoluble acids from one gram of fat. For any butter-fat, having a saponification number  $s$ , this value was found to be about  $188.5 + \frac{228-s}{2}$ . From the molecular weight of the volatile or soluble acids, we find that one milligram of potassium hydroxide neutralizes 0.0018 gram of soluble acids; the difference, therefore, between the saponification number of the butter-fat and the milligrams of potassium hydroxide required for the insoluble acids, multiplied by 0.0018, gives the weight of soluble acids per gram of fat; multiplying this by 100 we arrive at the percentage of soluble acids sought.

The formula is not expected to give results absolutely accurate in any case, since variations in the saponification number of a butter-fat are caused to a considerable extent by differences in the molecular weight of the insoluble acids, differences which the formula, as given, does not take into account.

THE RELATION BETWEEN THE CONSTANTS OF DIFFER-  
ENT BUTTER-FATS.

It was observed, in tabulating the results from many analyses of butter-fat, that there existed a certain ratio between some of the different constants. An inspection of the following table will illustrate this point.

TABLE III.

Butter-fat.	Saponification number.	Reichert number.	Iodine number.	Melting-point.	Specific gravity. $\frac{40^{\circ}}{15.5^{\circ}}C.$
1	226.1	15.3	54.85	33.5 <sup>o</sup>	0.9092
2	226.3	15.6	34.58	34.5 <sup>o</sup>	0.9058
3	226.9	15.7	35.45	33.8 <sup>o</sup>	0.9070
4	230.9	16.1	33.67	32.5 <sup>o</sup>	0.9058
5	232.7	16.2	30.96	33.0 <sup>o</sup>	0.9085
6	232.9	16.3	29.56	32.4 <sup>o</sup>	0.9068

We notice that with an increase of the saponification number a quite uniform increase of the Reichert number takes place, while the iodine absorption decreases; these are such variations as one would expect; a higher percentage of oleic acid of low combining power would raise the iodine number and tend to diminish the saponification value; similarly an increase in the percentage of the soluble acids of high combining power would elevate both the Reichert and the saponification numbers.

Table IV comprises a few of a large number of butter-fats analyzed at this station by Mr. W. S. Sweetser in 1894, and illustrates this relation even more pointedly than Table III.

TABLE IV.

Butter-fat.	Saponification number.	Iodine number.	Reichert number.
1	218.8	41.32	17.1
2	222.4	37.30	17.5
3	224.6	37.00	18.6
4	232.5	30.21	19.4
5	236.2	27.17	20.8
6	241.5	25.91	21.3

From these tables it would seem as if the percentages of oleic and of the soluble acids in different butter-fats bore a ratio somewhat complementary to one another. It must be remembered that the relations, which the foregoing tables represent, are only general, and by no means fixed; there are butter-fats which show constants bearing relations different from those expressed

above, and from isolated cases a table might be constructed showing exactly opposite tendencies. As regards specific gravity and melting-point, no definite relation could be discovered, though the latter showed a tendency to diminish as the saponification and Reichert numbers increased.

#### ABNORMAL BUTTER-FATS.

Different observers have reported from time to time cases in which butter-fats showed chemical and physical constants of such abnormality that, were the samples not known to be genuine, an analysis would condemn them at once as spurious.

F. W. Morse<sup>1</sup> has reported two butter-fats, from individual cows, giving Reichert–Meissl numbers of 16.5 and 11.2. “The samples were very hard, pale in color, and had an odor resembling that of tallow;” these abnormalities and the phenomenally low figures for volatile acids were attributed to an advanced period of lactation, and the presence of cottonseed products in the feed.

Samples 1 and 6 of Table IV are what might be classified as abnormal, though no note was made at the time of analysis as to any peculiarities in physical appearance. The saponification number of Sample 1 is far below the limit usually assigned to this constant, while the iodine absorption is excessively high; the irregularities of these two figures are explained by the prevalence of gluten feed in the ration.

The saponification and iodine numbers of Sample 6 vary at the opposite extreme; root crops, such as sugar-beets and mangels, predominated in the rations fed to the animals producing this butter, and no doubt account for the unusual figures.

While the butter-fat from the milk of single cows may frequently show abnormalities, such instances are less common with a full herd, since individual differences would be concealed in a general average; nevertheless, such instances do occur. Farnsteiner<sup>2</sup> and Karsch report the following constants of two butter-fats:

<sup>1</sup> New Hampshire Experiment Station Report (1893), p. 152.

<sup>2</sup> *Ztschr. Untersuch. der Nahrungs u. Genussmittel* (1898), 16–21; Abstract in *Analyst*, No. 263, p. 38.

	Saponification number.	Iodine number.	Reichert-Meissl number.
I. From 180 cows.....	218.53	49.57	22.80
II. " 25 " .....	219.74	40.00	22.11

The abnormal chemical constants of these samples were attributed to a very advanced period of lactation; no mention was made as to any anomalies of taste, odor, etc.

Sickness, peculiarities of feed, and advancement in the period of lactation are the principal causes of irregularities in the composition of butter-fat. The subject is one of special interest and illustrates the difficulty, occasionally experienced, of absolutely identifying the character of a questionable butter-fat. It will be of interest in this connection to compare the generally accepted limits for the three constants, used most universally in butter-fat analysis, with the extreme limits, where the names of the observers are bracketed.

	General limits.	Extreme limits.
Reichert-Meissl number.....	20-33	11.2[Morse]-41[Nilson]
Saponification number .....	220-236	216[Samelson]-245[Fischer]
Iodine number.....	26-38	19.5[Moore]-49.57 [Farnsteiner and Karsch]

#### INFLUENCE OF RATION UPON THE CHEMICAL COMPOSITION OF BUTTER-FAT.<sup>1</sup>

Allusion has already been made to the influence of certain feeds upon the chemical constants of butter-fat. Considerable work has been performed at the Pennsylvania Experiment Station along this line, in connection with various dairy-feeding experiments, according to plans of Dr. William Frear; the results of this work have, for the most part, not yet been published. A full discussion and tabulation of these experiments would be foreign to the nature and purposes of the present article, but certain general conclusions from the work may be briefly stated in this connection. The work of others has, in the main, been corroborated; this is true especially as regards the effect of different rations upon the volatile acid figure and iodine absorption. It is to be regretted that so few experimenters, in their work along this line, have reported determinations of the saponification number,—a constant, the value of which in butter-fat

<sup>1</sup> Bulletins 13 and 16, New Hampshire Experiment Station, contain some excellent experiments along this line. For a very full discussion of the subject see article by William Frear in *Agricultural Science*, 7, No. 3, p. 120.

analysis, as it seems of late, has been too greatly underestimated.

Briefly, the experiments conducted here indicate that, while the tendency of certain feeds, of which gluten meal is a type, is to greatly diminish the saponification number and increase the iodine absorption, other rations, such as those consisting of root products, beets, mangels, etc., have precisely an opposite effect; the extreme figures of Table IV, in butter-fats Nos. 1 and 6 were mostly, if not entirely, produced by just such a difference in ration.

It was found that other feeds, such as cottonseed, linseed, and corn-meal, all produced decided effects upon the chemical qualities of butter-fat; the three feeds named, when fed in moderate quantities, showed a tendency to elevate the saponification number, but there was a difference in other respects; while cotton and linseed meal diminished the Reichert number, corn-meal seemed to increase this constant; cottonseed and corn-meal both caused a marked decrease in the iodine absorption, whereas linseed meal produced an elevation in the iodine figure. Great care must be exercised in the interpretation of results from such experiments; other factors, such as the individuality of the cow and the stage of lactation, must not be lost sight of. The precise influence of a particular food upon the composition of the butter-fat cannot, in every instance, be foretold; we may say that the effect will always be more or less a variable quantity, since a great deal depends upon the nature of preceding rations.<sup>1</sup>

There is room for much study along this particular line, and further researches are greatly to be desired, not only as regards the effect of different kinds of food upon the chemical qualities of butter-fat, but also as regards that principle of the ration which is most active in producing a given change, as to whether it be of a carbohydrate, an albuminoid, or a fatty nature. The solution of the difficult physiological problem, dealing with the origin and formation of the fat secreted by the lacteal glands, will certainly be promoted by such investigations.

<sup>1</sup> Linseed meal, for example, if fed after a cotton meal ration, would elevate the iodine absorption, whereas, if fed after gluten meal, the iodine number would be diminished.