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PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS.

SUMMER MEETING.

A NEW departure was made this year in the arrangements of what has hitherto been known as the Country Meeting of the Society; it being decided to start from London, and to investigate the beauties of the upper reaches of the Thames. Accordingly, some thirty-five members and friends (including a fair amount of ladies), met at Paddington on the morning of the 31st ult., and travelled by rail to Maidenhead. Here they found a well-appointed steam launch in readiness, which conveyed them to Sonning. After dining here, they again embarked, and steamed to Henley, whence town was reached by train. Altogether a most enjoyable day was spent, and it was unanimously agreed by those present that the meeting had been one of the most successful of its kind which had been held in recent years. Some excellent photographs of the party were taken at Sonning by Messrs. Chattaway, Davies, and Stansell.

ON THE CONSTITUTION OF BUTTER FAT.

BY ALFRED A. ALLEN.

(Read at Meeting, June 3rd, 1891.)

IN compiling the following notes, my object has not been to offer a complete essay on the constitution of butter-fat, but rather to examine the nature of the evidence on which certain theories of its constitution have been founded, and to refute these theories so far as they have been proved inconsistent with experiment.

When butter-fat is saponified, it is well known to yield butyric acid and other acids which are generally considered as being oleic and palmitic acids, besides smaller amounts of arachidic, stearic, myristic, and lower acids of the stearic series. The result of saponifying a complex glyceride molecule containing the radicles of butyric, palmitic, and oleic acids is shown by the following equation :—

There is good reason for believing that butter-fat contains such complex glycerides as the above, for the butyrin cannot be separated by any process of fractional solution from the less soluble glycerides of the fat.

By treating butter-fat with only half the quantity of alcoholic soda necessary for its complete saponification, and precipitating the liquid with water, Dr. James Bell

obtained an oil which solidified at 4.4°C ., and on saponification yielded 88.1 per cent. of insoluble acids, but no butyric or other soluble fatty acids. This result agrees with a diglyceride of the following character, though it is to be regretted that no determination of the glycerin was made:— $\text{C}_3\text{H}_5 : \text{O.H}, \text{O.C}_{16}\text{H}_{31}\text{O}, \text{O.C}_{18}\text{H}_{35}\text{O}$.

In 1883, Messrs. J. A. Wanklyn and W. Fox enunciated a new theory of the constitution of natural fats (*Chem. News*, xlviii., p. 49; *British Association Reports*, 1883, p. 470; *ANALYST*, IX., p. 73). The theory, which was only shortly stated, was simply that the accepted view that fats are ethers of glycerin is only partially correct, and that instances of a different kind of structure occur among the natural fats and oils. According to the authors of the theory, the fats are, in fact, composed of ethers of iso-glycerin or homologues of iso-glycerin. To the wholly hypothetical compound iso-glycerin, the authors assign the formula:— $\text{CH}_3.\text{CH}_2.\text{C}(\text{OH})_3$. This theoretical compound is assumed to exist only in its ethers, for immediately on isolation it is imagined to be resolved into water and a fatty acid, thus:

Of the existence of the iso-glycerin with 3 carbon-atoms absolutely no evidence has been attempted to be given; but the authors consider that proof of the existence of ethers of the next higher homologue in butter-fat is afforded by the formation of ethyl butyrate when the fat is treated with an amount of alcoholic potash insufficient for its complete saponification. The formation of the ethyl butyrate they attribute to the decomposition of an iso-glycerin containing C_4 , this iso-glycerin splitting up into butyric acid and water, and the nascent butyric acid reacting with the alcohol to form ethyl butyrate, thus—

Wanklyn and Fox found that under favourable conditions they could obtain from butter-fat an amount of ethyl butyrate equivalent to fully 3 per cent. of butyric acid, or about one-half of the total amount which can be isolated from saponified butter. Upon this single fact, coupled with an alleged serious deficiency in the amount of glycerin yielded when fats, and especially butter-fat, are saponified, the authors erected their iso-glycerin theory. But the reaction in question was some years since demonstrated by Mr. Hehner to be true also of synthetically prepared glyceryl butyrate; besides which I believe the authors of the theory have never published a single original experiment in support of their assertion that the proportion of glycerin yielded by saponification of butter and other fats exhibits any grave departure from the amount required by the accepted view of their constitution. In view of this omission on the part of the authors to support their position by experiment, the iso-glycerin theory might well have been allowed to remain at rest, had it not recently been resuscitated by Mr. Wanklyn in a wholly unaltered form. Thus on a recent occasion Mr. Wanklyn stated that "the evidence of the existence of the isoglyceride was that reaction (*i.e.*, the formation of ethyl butyrate) and the fact that one did not get the theoretical quantity of glycerin from butter." From this concise statement of his views it would appear that Mr. Wanklyn has forgotten Mr. Hehner's destruction of his one supporting

experiment, and is ignorant of, or ignores the fact that since the original enunciation of the theory, other chemists have supplied his omission and determined the glycerin resulting from the saponification of a number of fats and by a variety of methods.

Mr. Wanklyn considers that the formation of ethyl butyrate on heating butter-fat with a limited quantity of alcoholic potash proves the presence of an isoglyceride, the iso-glycerin of which splits up into butyric acid and water, the acid reacting with the alcohol to form ethyl butyrate. He contends that if the butyric acid were a primary product of the saponification of an ordinary glyceride, instead of a secondary product of the decomposition of an iso-glycerin, it would not form ethyl butyrate, but potassium butyrate. It is certainly a curious and interesting fact that ethyl butyrate should be formed under these circumstances, seeing that it is readily converted by caustic potash into alcohol and potassium butyrate. But it must be remembered that the essential condition of its formation in notable amount, prescribed by Wanklyn and Fox themselves, is the use of the alkali in quantity insufficient to saponify the whole of the butter-fat employed. The whole argument of the reaction falls to the ground in the face of the fact, demonstrated by Mr. Hehner under the very noses of Messrs. Wanklyn and Fox, that butyric acid synthetically prepared by heating together butyric acid and glycerin, and thoroughly washing the product, develops abundance of ethyl butyrate when warmed with alcoholic potash!

The fact is that the reaction which is regarded by Mr. Wanklyn as so anomalous appears to be a perfectly normal and general one. Thus ethyl stearate may be actually prepared by boiling tristearin with solution of sodium in absolute alcohol (Duffy), or *by heating tristearin with small quantities of alcoholic potash* (Bouis, *Comp. Rend.* xlv. p., 35). Herewith is the precise condition of success in obtaining ethyl butyrate applied by Bouis to prepare the higher homologue. P. Duffy (*Jour. Chem. Soc.* v., p. 303), also obtained amyl stearate by boiling tristearin with a solution of sodium in amyl alcohol, and amyl palmitate by precisely similar means. When in the reaction of stearin and palmitin with amylate of sodium the proportion of the latter "was increased beyond one equivalent, the quantity of ether obtained diminished," and "with a quantity of sodium which was certainly not less than two equivalents, no ether was obtained."

It appears, therefore, that Mr. Wanklyn has merely re-discovered with respect to butter what was pointed out in the case of stearin and palmitin in 1853.

Duffy determined the glycerin produced by the saponification of the stearin he employed. The method of estimation consisted in evaporating the neutralised liquid resulting from the saponification nearly to dryness at a steam heat, adding dry potassium carbonate, extracting the glycerin with absolute alcohol, filtering the solution, and evaporating the filtered solution in a vacuum until the residue was constant in weight. The glycerin thus found was corrected by the deduction of the ash left on ignition. By this process, which is likely to give low results, Duffy obtained 8.94 per cent. of glycerin in two experiments, the theoretical yield from pure stearin being 10.34 per cent., a figure which does not leave Mr. Wanklyn much margin for the suggestion that the ethyl and amyl stearates were derived from an iso-glycerin, which, under the circumstances, would have to be the member with 18 carbon atoms.

The process of estimating glycerin employed by Duffy is substantially the same as

that described by Chevreul in 1823, by which that chemist isolated the following proportions of ash-free glycerin:—Human fat, 9·66 per cent.; pork, 8·82; beef, 8·60; mutton, 8·00; butter-fat, 11·85; porpoise oil, 14·00. These results, with the exception of the two last, are sensibly below the theoretical yield, as might be expected from the process employed. Further, the statement made by Mr. Wanklyn that manufacturers never obtain more than 5 per cent. of glycerin on saponifying fats, is contrary to the experience of some of the largest firms, who recover 7·5 to 8 per cent., and take no notice of the considerable loss of glycerin by evaporation during the process of concentration; besides which the process of saponification is often far from complete.

To come to more recent determinations of glycerin in fats, Mr. Hehner has published the following figures, obtained by boiling the leys from the saponification with a dilute acid solution of potassium bichromate and ascertaining the excess of oxidising agent by titration with a ferrous salt (ANALYST xii., p. 44).

Now this process is not open to the objection which has been raised against some, for it has been recently stated by Mr. Wanklyn's coadjutor (*Jour. Soc. Chem. Ind.*, x., p. 204), that dilute chromic acid mixture remained practically unchanged when boiled with butyric acid, and, further (ANALYST xvi., p. 30), that butyric acid is the only soluble non-volatile fatty acid to be detected in the glycerin mother-liquor obtained by the saponification of butter-fat.

The following are figures obtained by Mr. Hehner by the bichromate titration process:—

Fat.							Glycerin.
Olive oil	..	..	..	..	..	..	10·26 per cent.
Cod-liver oil	..	..	..	..	..	..	9·87 „ „
Linseed oil	..	..	..	..	..	..	10·24 „ „
Margarine	..	..	..	..	..	..	10·01 „ „
Butter-fat	..	..	..	..	..	..	12·40 „ „
Butter-fat	..	..	..	..	..	..	12·40 „ „

As already pointed out, it is a curious fact that the authors of the iso-glyceride theory have not, to my knowledge, published any experiments in support of their assertion that butter, tallow, and other fats yield on saponification little more than half the theoretical proportion of glycerin. This is the more curious since a valuable method of determining glycerin in fats was originally suggested by Mr. Wanklyn, and further worked out by Mr. Fox (*Oil and Colour Jour.* v., p. 1,402; *Chem. News*, liii., p. 15), and Benedict and Zsigmondy (*Chem. Zeit.* ix., p. 975, ANALYST x., p. 206). The method is based on the oxidation of the glycerin by treatment with permanganate in presence of excess of caustic alkali, whereby it is converted into oxalic acid, carbon dioxide, and water, in accordance with the equation:— $C_3H_5O_3 + 3O_2 = C_2H_2O_4 + CO_2 + 3H_2O$. The excess of permanganate is destroyed by a sulphite, the filtrate acidulated with acetic acid, and the oxalate precipitated as a calcium salt. The process has been very thoroughly investigated in my laboratory by Mr. J. C. Belcher, and proved to give very accurate results in the absence of foreign bodies yielding oxalic acid by oxidation. Alcohol is one of these, and hence to avoid all risk of its presence I have described a method of saponifying the substance with aqueous potash (ANALYST xi., p. 52; *Com. Org. Analysis* ii., p. 290). This modification, and careful proof by experiment that oxalic acid is not acted

on by permanganate in strongly alkaline solution, is the extent of my responsibility for the process in question. Benedict and Zsigmondy, however, proved by experiment that the soluble fatty acids of oils, such as acetic, butyric, caproic, etc, do not, on treatment with alkaline permanganate, yield any acids, the calcium salts of which are precipitated from acetic solutions, and hence are liable to be mistaken for calcium oxalate. The higher fatty acids of the stearic series are insoluble in water, and hence would not in any case interfere. On the other hand, certain acids of the oleic and acrylic series, and possibly oleic acid itself, yield oxalic acid by oxidation with permanganate. The higher acids of the oleic series are, however, insoluble in water, and the lower are not known to occur in fixed oils under normal conditions. Under certain circumstances, however, the method is wholly invalidated, as I found when attempting to apply it to the examination of oxidised linseed oil.

The following tabulated statement of results obtained by the alkaline permanganate process of estimating glycerin is taken from vol. ii. of my *Commercial Organic Analysis*, p. 33 :—

Kind of fatty oil.	Glycerin per cent.	
	Benedict and Zsigmondy.	A. H. Allen.
Northern whale oil	—————	11·96
Porpoise oil	—————	11·09
Menhaden oil.. .. .	—————	11·10
Lard	—————	10·83
Tallow	9·9 to 10·0	—
Butter-fat	10·2 to 11·6	11·06
Olive oil	10·1 to 11·4	—
Linseed oil	9·4 to 10·0	9·39
Castor oil	—————	·13
Cocoa-nut oil.. .. .	13·3 to 14·5	12·11
Palm-nut oil	—————	11·70
Palm oil (containing much free acid)	—————	9·71

The applicability of the alkaline permanganate process to the estimation of glycerin from butter has been recently denied (*Chem. News* xvi., p. 49, *Jour. Soc. Chem. Ind.* x., p. 204, *ANALYST* xvi., p. 49) on the ground that butyric acid itself yields oxalic acid on treatment with alkaline permanganate in the manner described by Benedict and Zsigmondy for the estimation of glycerin. The same authority states (*ANALYST* xvi., p. 30) that butyric acid is the only soluble non-volatile acid he could detect in the glycerin mother-liquor from the saponification of butter, and he also states that the oxalic acid yielded is equivalent to 11·7 to 12·23 per cent. of glycerin (*Jour. Soc. Chem. Ind.* x., p. 93). The effect of alkaline permanganate on butyric acid is alleged to be to oxidise it to oxalic acid in accordance with the following eccentric equation (*Chem. News* lxiii., p. 111, *Jour. Soc. Chem. Ind.* x., p. 204) :— $C_4H_5O_2 + O_6 = 2C_2H_2O_4 + H_4$.

The discoverer of this reaction quotes two experiments on pure butyric acid by which he obtained amounts of oxalic acid corresponding to 93·64 and 94·07 per cent. of the butyric acid taken. This result is of so startling and remarkable a nature, and so completely opposed to the experience of Benedikt and Zsigmondy and all previous observers, that it is naturally received with some reserve, especially as the observer recently published experiments (*ANALYST* xvi., p. 26) according to which tallow requires

23 per cent. of caustic potash (KHO) for its saponification against 19.3 to 19.8 per cent., which is the united experience of numerous other observers; that butter-fat requires about 27 per cent. of potash, against the general experience of 21.1 to 23.3 per cent.; and detailing other analytical results of an equally remarkable character.

As, however, the observer in question makes the distinct assertion, as the result of experiment, that butyric acid is oxidised to oxalic acid by alkaline permanganate, and hence that the estimation of glycerin in butter by the same process is invalidated, and considerable support thereby given to Mr. Wanklyn's iso-glyceride theory (*Jour. Soc. Chem. Ind.* x., p. 204), I have thought it right to ascertain by personal experiment whether the statement in question had any foundation in fact. That the assertion was baseless was already rendered extremely probable by Mr. Hehner's failure to obtain any trace of oxalic acid from butyric acid by treatment with permanganate (ANALYST xvi., p. 49).

In order to settle the point beyond the possibility of further cavil, I saponified 20 grammes of Kiel butter-fat by heating it for six or eight hours at 100° C. in a closed bottle, with a solution of 8 grammes of caustic potash in 50 c.c. of water. The soap obtained was dissolved in hot water and decomposed by dilute sulphuric acid in quantity sufficient to convert the potash into KHSO₄. The liquid was then allowed to become cold and poured off through a filter from the cake of insoluble acids. The latter was washed by boiling with water, the washings when cold being added to the main solution. This solution, containing the glycerin and soluble fatty acids resulting from the saponification, was then distilled to a small bulk, water added, and the liquid again distilled to a small volume.

The distillate was then oxidised by alkaline permanganate in the usual way. The reduced and filtered liquid gave *no trace of precipitate* when acidulated with acetic acid and treated with calcium chloride, showing that *no oxalic acid had been formed* from the butyric acid, and that *no glycerin* had distilled over.

The residual liquid remaining in the distilling flask was divided into four equal portions. Nos. 1, 2 and 3 were oxidised, respectively, by a slight excess, a moderate excess, and a large excess of alkaline permanganate, by which quantities of oxalic acid were found equivalent respectively to a production of 11.12, 11.52, and 11.78 per cent. of *glycerin* by the saponification of the butter-fat. The fourth portion of the liquid was exactly neutralised by caustic potash, and filtered from the potassium sulphate which crystallised out. The filter was washed with alcohol, and more alcohol added to the filtrate as long as a precipitate was produced. The liquid was filtered and evaporated on the water-bath till the residue was nearly constant in weight. The residue, which was syrupy and had the characters of glycerin, was ignited and the ash deducted from the weight previously observed. A direct determination of the *glycerin* by weight, in this manner, showed 11.22 per cent.

Other estimations of glycerin by the same process, but employing 20 grammes (instead of 5 grammes) of butter-fat gave 10.77, 11.53, and 12.15 per cent. of ash-free *glycerin*.

In another experiment, 20 grammes of butter-fat were saponified with aqueous

potash, the soap decomposed with acid, and the acid liquid divided into two equal portions, A and B.

A was distilled to a small bulk as before, and the residue in the flask divided into two equal portions.		B was divided into two equal parts without previous distillation to separate butyric acid.	
No. 1 showed by the oxidation with alkaline permanganate 11.52 per cent. of glycerin.	No. 2 gave Mr. Hehner by the bichromate oxidation method 11.83 per cent. of glycerin.	No. 3 showed with alkaline permanganate 10.86 per cent. of glycerin.	No. 4 was lost.

The foregoing experiments were made in my laboratory by Mr. D. Homfrey, to whom my best acknowledgments are due. The experiments appear to prove:—

1. That butyric acid does not yield oxalic acid when oxidised by alkaline permanganate in the manner prescribed by Benedikt and Zsigmondy.

2. That the amount of oxalic acid produced by the treatment of the soluble products of the oxidation of butter by alkaline permanganate is not greater when the oxidation is effected in the presence of the butyric acid than when the acid is previously removed by distillation.

3. That the oxalic acid produced by the permanganate treatment corresponds to a formation of 11 or 12 per cent. of glycerin from the butter-fat saponified.

4. That this result is fully confirmed by actual isolation and weighing of the glycerin from the saponification.

5. That there is no foundation for the iso-glyceride theory so far as butter-fat is concerned.