Mono- and Diglycerides in Industrial Fats

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IT is noteworthy that the saponification value of an industrial fat is not a satisfactory index of the amount of free fatty acid obtainable by distillation or of the nature of the glycerides present. A recent paper by Trauth and collaborators (1) mentions incidentally the "unreliability of calculating glycerine yields of low grade fats on the basis that all the neutral fat is present as triglyceride."

Discrepancies in the yield of glycerine, however, is only one aspect of the problem. Of greater importance is the fact that erroneous assumptions regarding the free fatty acid content of a hydrolized fat result in apparent losses of material. This is especially true when a batch of hydrolized fat is subjected to distillation. The apparent loss of fatty acid in this operation is due, as can be demonstrated, to the re-esterification of mono- and diglycerides by the free fatty acid. The deleterious effects of high temperature on the resulting triglycerides are the same as those attendant on the distillation of a mixture containing a high percentage of neutral fat.

The step-wise hydrolysis of fat has been demonstrated by Kellner (2) and also receives support from miscellaneous observations in practical operation. This applies as well to the slow hydrolysis of palm oil during prolonged storage as it does to an industrial splitting process such as autoclaving. Thus we have never succeeded in finding the smallest quantity of free glycerine in highly acid palm oils; in addition, these same palm oils failed to give the amount of fatty acids calculated from the saponification value when subjected to treatment with superheated steam under reduced pressure in a deacidification plant. Furthermore there is a lack of agreement between analytical data and the yield of fatty acid on distillation of autoclave-split fats.

The hypothesis is advanced that in many cases the fatty material contains various amounts of mono- and/or diglycerides and that the the latter combine with part of the free fatty acid present under the conditions employed during industrial distillation. Thus:

$$\begin{array}{l} CH_2\text{-}O\text{-}COR \\ | \\ CH\text{-}OH \\ | \\ CH_2\text{-}OH \end{array} + 2\ RCOOH \longrightarrow \begin{array}{l} CH_2\text{-}O\text{-}COR \\ | \\ CH\text{-}O\text{-}COR \\ | \\ CH_2\text{-}O\text{-}COR \end{array} + 2\ H_2O$$

$$\begin{array}{l} CH_2\text{-}O\text{-}COR \\ | \\ CH\text{-}O\text{-}COR \\ | \\ CH_2\text{-}OH \end{array} + RCOOH \longrightarrow \begin{array}{l} CH_2\text{-}O\text{-}COR \\ | \\ CH\text{-}O\text{-}COR \\ | \\ CH_2\text{-}O\text{-}COR \end{array} + H_2O$$

Verification of the above is offered by the experiments described below. A reaction flask containing the hydrolized material was equipped with two or three condensers and heated in an oil bath at 200° C. for several hours at a pressure ranging from 60 mm. to 140 mm. Hg. Stirring was accomplished by air or carbon dioxide introduced by means of a capillary tube. The use of higher vacuum was avoided in order to prevent excessive volatilization of material. It was also found that heating at 140° C. for several hours did not bring about the above conversion. Actually, heating at that temperature is used in common practice for producing mono- and di-glycerides. All material volatized during the run that was condensed was returned to the flask. Losses occurred often due to small amounts of non-condensable materials besides the weight loss accounted for by the water formed in the reaction and vaporized under the existing working conditions. No metallic catalysts were employed although it is recognized that traces of metals, inevitably present in commercial batches, may have had some effect.

The data of some pertinent experiments are summarized in the accompanying table. The results indicate that the free acid content of raw palm oil and the amount of fatty acids obtained by hydrolysis decrease appreciably if they are subjected to the described high temperature treatments. Proof is thereby furnished that mono- and/or diglycerides were present in the industrial fats. However, a quantitative estimate can be based on the presented figures only if it is assumed that the neutral part contains besides triglycerides exclusively monoglycerides or diglycerides. No attempt was made to establish the actual proportions by a determination of the glycerine content of the samples. The importance of the composition of the samples for a quantitative estimate can be illustrated by considering the decrease of fatty acid in the first experiment. The observed decrease of about 13 g. of free fatty acid would correspond to as much as 28 g. of diglycerides but only to 8 g. of monoglycerides in the original mixture.*

The experiments carried out with raw arachis oil (as exemplified by the last experiment in the table) gave less conclusive results. This is due to the relatively small amounts of free fatty acid present in arachis oil and to the high losses caused by evaporation. However, the total losses are by far less important than the difference between the amount of free acid before and after the high temperature treatment; the conclusion is therefore justified that esterification took place also in this case. Even if it is supposed, as is hardly probable, that the lost material is exclusively composed of fatty acids of low molecular weight, there remains still a difference that

TABLE

Raw material treated	Free f.a. before treating	Duration temperature and pressure of treatment	Total loss	Free f.a. after treatment
	%		g.	%
91 g. Palmoil..... 0.9 g. coal.....	30.8	4 hrs. 160° 6 hrs. 220° 60 mm.	1.2	16.3
200 g. Palmoil..... 2 g. coal.....	26.0	4 hrs. 150° 4 hrs. 200° 60 mm.	1.0	16.7
109 g. autocl. Palmoil..... 1.1 g. coal.....	93.1	6 hrs. 170° 110 mm.	0.6	82.9
120 g. autocl. Palmoil..... 1.2 g. coal.....	94.5	6 hrs. 175° 140 mm.	0.7	84.6
219 g. autocl. hydrog. Palmoil..... 2.2 g. coal.....	91.3	6 hrs. 180° 110 mm.	0.5	84.6
100 g. arachis oil..... 0.2 g. coal.....	5.0	4 hrs. 220° 110 mm.	2.4	1.4

* Assuming 358 as the molecular weight of monoglyceride, 624 that of diglyceride, and 284 that of fatty acid, then each gram decrease in fatty acid signifies the transformation of 624:284 = 2.2 g. diglyceride or 358:568 = 0.63 g. monoglyceride into triglyceride.

makes at least probable the presence of mono- and diglycerides.

Additional experiments on hydrolyzed arachis oil are being planned. Furthermore, these investigations are to be applied to other industrial fats.

Summary

1. The presence of mono- and diglycerides in raw and hydrolyzed fats and their effect on the yields of fatty acids obtained by distillation is discussed.

2. Experiments carried out under conditions suitable for the esterification of mono- and diglycerides with fatty acids to form triglycerides indicate the presence of these mono- and diglycerides.

REFERENCES

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Interrelationships of Dietary Fat and Tocopherols[1]

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DURING the first decade (1923-33) of researches establishing the existence and nature of vitamin E there occurs frequent reference to the fact that the ability of diets to induce sterility in rats varied considerably with the amount and nature of the dietary fat. The fats concerned were lard, cod liver oil, and butterfat, the latter either as whole fat or as provided by whole milk powder. In the light of present knowledge it is apparent that much of the confusion existing at that time was due to the presence of subminimal amounts of vitamin E in milk-fat and the effect of added lard and cod liver oil in (1) reducing the intake of milk fat, (2) in causing autoxidation of the vitamin in the course of storage or digestion of the diet, or (3) in increasing, in some unknown manner, the tissue requirements for the vitamin.

Evans and Bishop (1), in 1923, observed that omission of lard from their basal diet deficient in the X-substance, later designated vitamin E, resulted in increased fertility of female rats. Confirmation soon came from Mattill *et al.* (2), Sure (3), and Anderegg and Nelson (4) who noted that the reproductive adequacy of diets composed largely of whole milk powder was abolished by additions of lard or cod liver oil to the diets. On the basis of our present knowledge of the low content of tocopherol in butter fat (5), which can be determined quite accurately, it is quite obvious that diets containing about 50% of whole milk powder would provide approximately 0.055 mg. tocopherol, daily—an intake that would be definitely on the border line for reproduction and readily made inadequate by the inclusion of lard or other fats in the diet. Subsequently, Evans and Burr (6), in re-examining the capacity of various dietary fats to promote sterility, observed that the protective action of given doses of wheat germ could be nullified by incorporation of oleic acid or lard in the diet; development of rancidity greatly increased this reaction, and conferred this property upon other fats (such as butterfat and wheat germ oil) which otherwise exhibit anti-sterility activity. About this time (1927), Mattill (7) reviewed the problem of oxidative destruction of vitamins A and E, and provided experimental evidence that oxidative changes accompanying the onset of rancidity in unsaturated fats tend to destroy these vitamins. There was thus established a definite relationship between autoxidation of vitamin E and the sterility-promoting effect of cod liver oil, lard, and other fats when incorporated in diets containing border-line levels of vitamin E.

Later, in connection with the nutritional muscular dystrophy observed in the rabbit and other herbivorous animals, it was soon recognized that the muscle lesions were markedly accentuated by increasing the intake of cod liver oil or lard and, under such conditions, might occur in the face of a reasonable intake of vitamin E (8, 9). On the other hand, if cod liver oil and vitamin E supplements were fed separately and on alternate days, the lesions could be prevented or, if already present, could be repaired (10, 11). In a similar manner it was shown (12) that rancidified fat abolished the effectiveness of partially hydrogenated vegetable shortenings in preventing sterility in rats unless the two fats were fed separately, six hours apart.

It is surprising to note, in retrospect, that, in spite of this vast array of evidence of fat and vitamin E interrelationships, the response of experimental animals to fat-free diets deficient in vitamin E received little attention until 1939 when Mackenzie *et al.* (13) showed that sterility, paralysis, and growth retardation characteristic of vitamin E deficiency occurred in rats fed diets exceedingly low in lipid content (.0078%, plus 25 mg. methyl linoleate daily). They later reported (11) that rabbits exhibit severe lesions of the voluntary muscles when fed E deficient diets containing no more than 0.05% of animal fat. Although dietary fat is not a prerequisite for the production of symptoms of vitamin E deficiency, except possibly in chicks (14), there is now considerable evidence that both the quantity as well as the type of fat, especially its content of unsaturated fatty acids, may accentuate to variable degrees the onset or severity of the symptoms. This becomes a factor of major importance in the bio-assay of vitamin E, and may explain in large part the unexpectedly wide variations in bio-assay results obtained in 1939 when

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