Concerning the Chemistry of Fat Spoilage*

By PROF. K. TAUFEL, Munchen.

A Resumé of the Investigations of J. Muller, F. K. Rassow, P. Sadler and H. Thaler

ORGANIC substances in general are highly reactive. This fact makes it possible for them to undergo the necessary changes involved in metabolism. It is therefore no great error to say that the nutritive value of a food stands in close relation to this ability of its constituents.

These chemical changes which the substance undergoes under the influence of digestive and metabolistic ferments may be regarded as similar to the gradual changes which take place in most foods during their storage. In the case of carbohydrates and proteins, where our knowledge of their metabolism has led to definite, experimentally determined conceptions, there are many details which sustain these views.

Such a point of view leads to new and valuable conceptions as applied to the behavior of fats inside and outside of organisms; the fundamentals of fat metabolism are to be considered together with their changes during storage. Most of the knowledge concerning the behavior of fats in this connection is given here as merely of theoretical significance.

When I now report on some of the investigations of my co-workers as well as those of Dr. H. Thaler, it is not to be forgotten that besides the chemistry of the spoilage of fats there is in addition the view point of the nutrition and physiological food chemist.

It is characteristic of the science of chemistry that it has brought together a great amount of apparently unrelated data and has clarified it by classification. Polanzi put forward the rule that material without a formula is an untamed creature of nature. We may add that chemical reactions of unknown mechanisms cannot be depended upon as real guides or "leads." This applies especially in the case of the spoilage of fats.

A great deal of scientific work is lost in the details and secondary facts for want of a clear point of view. And it is exactly in that respect that so much confusion and contradictory material has arisen. Worthwhile contributions must therefore be made upon a broad ground work which is the result of chemical experience.

The idea of the spoilage of fats, rancidity, is as yet only a little more than a trivial definition of an, in fact unfortunate, change of this material. For now almost 150 years chemical observations have appeared in the literature on the idea of rancidity, acid formation, tallow formation, etc.; without a clear separation of the meanings one from another. The results are thereby real and apparent contradictions.

What has been usually spoken of as the so-called amount of spoilage problem, "Verdorbenheitsproben," is the attempt to correlate the subjectively obtained facts with those obtained by objective analysis. There are certain reactions which have been singled out of the complex question by certain specific facts, whose broad aspects have been closely limited and which call for a very careful, prudent and critical evaluation.

Two groups of processes are especially involved in the spoilage of fats, which are separated out from the apparent legion of reaction possibilities on the basis of the newer investigations. One can trace back the kind of spoilage to the primary idea of the unsaturated glycerides of the fat. The other group of reactions extends to a rearrangement of the originally saturated fatty acids. This appears to account for the grouping of those of average molecular weight. A third group of changes in the fat may be found in the hydrolysis of the fat by any hydrolysing influence which splits off free acids. Furthermore, one must think of every process as bound up by constant variations. This is, perhaps at best, shown as tallowy formation, and is at present the goal of investigation. And finally, the "soapiness" of water holding fats as margarine, according to the work of K. Richter and of Kiel should be mentioned. Under the influence of specific enzymatic moulds a wholly characteristic structure is formed, about which little is known. With those references I may conclude and confine myself to the two first named groups for my discourse.

I. *The Spoilage of Fats as approached from the unsaturated glycerides.*

This is also well known as "oleic rancidity," according to the work of Tschirch and Barben[2] as well as that of Powick[3] and earlier by Pritzker and Jungkunz,[4] and is primarily an antoxidation of the active double bonds of the unsaturated glycerides. So-called "moloxydes" form, about which very little of certainty is known.[5] The first step is the formation of a peroxide which finally splits into two phases from which a molecular structure follows, apparently under the direct or indirect influence of the active peroxide oxygen. The mechanism of this splitting and the kind of products formed thereby depend upon many factors such as light, temperature, moisture, catalyzers, kind of fat, etc. The glycerides may hardly be split during this process. From among the host of low molecular weight products formed (of which legion only a few are as yet known to have been separated out[5a]) various aldehydes, mono- and dicarboxylic acids as well as carbon dioxide have been found. Ketones have also been found in the present researches. An especially noteworthy end product is that of epihydrinaldehyde.

A great deal of pains has been taken to secure a reaction mechanism which will give a possible explanation for the many end products which have been found. The schemes put forward by Tschirch and Barben (loc. cit.), as well as by Powick (loc. cit.), do not go beyond merely the known products of research. I may differ with their considerations.

The expert is interested here most of all in determining the substance or substances which give rise to these spoilage characteristics which are detected by the senses. Only a partial answer can be given to this. It has been shown that undoubtedly the heptyl- and nonylaldehydes have a definite part in the odor.

Analytical examination as to the spoilage of a fat will show it to be present whereas organoleptic tests will give no worthwhile result. It happens that intense research is being given to measurements which predict the condition and nature of a fat. What the values are and their limits is a question under the present evolving points of view of the degree of spoilage reactions.

1. The determination of the variations in the charac-

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[1] Zeits. angew. Chem. 44, 597 (1931).

[2] Schweiz. Apothek., Ztg. 62, 281 (1924).

[3] Journ. agric. Res. 26, 323 (1923).

[4] Zeitschr. Unters Lebensmittel 52, 195 (1926); 54, 242 (1927); 57, 419 (1929).

[5] E. Fraenckel, Studies on the Autoxidation of Terpenes, Dissertation at Darmstadt, 1929; C. H. Lea, Proc. Roy. Soc. London B. 108, 175 (1931).

[5a] Taufel, K. and Muller, J. Biochem. Zeits. 219, 341 (1930).

teristic numbers of a fat, acid—, saponification—, iodine—, acetyl—, Reichert—, Meissl—, Numbers, etc., as measures of the degree of spoilage appear not to be sufficiently sensitive.

2. The detection and the measurement of the liquid or water-soluble and products which have been easily autoxidized give results which are somewhat ambiguous and are, therefore of little specificity; procedures according Mayshofer,[8] Issozlis,[9] Kerr,[10] Grettie and Newton,[11] etc. All may give deceptive conclusions by using the liquid oxidizable facts.

3. For the determination of the peroxides present which were formed by autoxidation, one can do it directly by weighing[11a] or by manometric measurement[12]; there is also the process by Hefter where iodine is quantitatively released by means of the peroxides present from potassium iodide added for the purpose. Lately this method has been used especially by Taffel and Revis[13] as well as by Lea who refined it.

By means of these methods the beginning of oxidation, that is, the tendency, of the fat, can be studied. A somewhat different means was followed by Holm and Greenbank,[15] as well as by Davies.[16] An evaluation of these results based upon a reaction mechanism is not possible.

4. The detection of the aldehydes which have been split off during the antoxidation present a very quick procedure to carry out. The best known method is that of Th. von Fellenberg, using acidified fuchsine; the color formed can be measured quantitatively against solutions of known aldehyde content or against solutions of potassium permanganate, using a colorimeter. The uncertainty of the composition of the presence of other reducing agents than the aldehydes detract from the value of this otherwise useful reaction.

5. The well known Kreis reaction is on the other hand, considered as a very specific reaction as compared with general reactions for aldehydes. The cause of this reaction is epihydrinaldehyde, according to Powick (loc. cit.) as well as Pritzker and Jungkunz (loc. cit.). The Kreis test has taken a strong place among all spoilage tests due to its selectivity.

A critical survey of all the above methods for spoilage reactions leaves the impression that each method more or less selects from the whole legion of possible decomposition products one single group reaction or an intermediate reaction for the identification. There are as yet no specific reactions known for those two most important aldehydes, heptyl-, and nonylaldehyde. Many more besides these are known, or their end products. A definite conclusion would be given these findings if it were possible to produce the same results organoleptically by adding these materials of decomposition to the fat. Since this is not the case, the results of the various spoilage tests are often contradictory. A real advance in this field would be first of all, a clarification of the mechanism of autoxidation. The high degree of selectivity of the Kreis test appears to us to be a very valuable help for this purpose.

Any explanation of the Kreis test[19a] must be founded upon a knowledge of the behavior of epihydrinaldehyde. We have, therefore, centered our interest upon such studies; directly with diethyl- as well as the glycolacetals of this aldehyde and indirectly, upon the oxydized fat. It has been shown that the free aldehyde is a very light liquid and partly decomposes in a neutral solution at about 100° C. It can be stored a long time in an alkaline solution; in an acid solution (1:1 hydrochloric) it is relatively stable at 0°, but decomposes quickly when the temperature is raised.

Epihydrinaldehyde is not expelled from a rancid fat by steam at 100° C. or only to a very slight amount; therefore it must be present as a heavy liquid, whose form is yet unknown. If one heats a rancid fat to 150°-160° C., it gives a negative Kreis test; the aldehydes are also destroyed.

These methods were brought together in the following modification of the Kreis test:

The oil to be investigated can be freed of the aldehydes which disturb the Kreis test by steam distillation from a suitable apparatus. After the liquid aldehydes have been driven over, a similar volume of concentrated hydrochloric acid is added under cooling. A strong current of air is then passed through the oil-acid mixture and the air laden with the free epihydrinaldehyde is caught in a suitable absorption vessel. It is here condensed in a mixture of equal parts of a 0.1 per cent alcoholic solution of phloroglucinol and conc. hydrochloric acid. The color produced is always of the same tint throughout as this is a homogeneous solution. It is thus possible to measure the color depth quantitatively against a scale and thereby obtain a measure of the amount of epihydrinaldehyde present.

This modification of the Kreis test has the following advantages[19b]:

1. It avoids the disturbing effects of the presence of peroxides which may come from either the ether or the fat iself.

2. It avoids the colors which may be formed by the acid or the reagents used and which are deceptive. For example, the allyl groups may deceive one to believe in a positive test as allylamine, allylsulfide, diallylurea, allylalcohol, etc., as well as other compounds such as euqenol, linalol, safrol, myristizin, geraniol, cinamic-vannillin, fenchone, etc.[20]

3. The sensitivity of the Kreis reaction is very great. By increasing the amount of the fat used and by purifying the phloroglucinol used, this sensitivity can be further increased. We were able to detect epihydrinaldehydeglycolacetate at a dilution of 1 :108 for certain. This is very important for physiological data.

This modification of the Kreis is meant primarily for scientific work where the usual errors are to be avoided; it is thus an analytical help. But what is the value for the commercial man? The tendency of fats to undergo autoxidation is so great that, for example, a fresh lard will show a positive reaction upon standing to the air for 12 hours, i.e., on the surface. If it has been allowed to stand in the direct sunlight the time is much shorter. This extreme sensitivity has been a personal help to me in judging a fat containing foodstuff. But in order to explain why a weakly positive test is obtained from a fat which is to the senses spoiled, one is lead into falsity. On the other hand, a strong Kreis test undoubtedly indicated that the fat has already passed through the harmless induction period of the autoxidation and is now in the process of the more rapid equilibria of the decomposition mechanism.

II. *The Spoilage of Fats as Considered from the*

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[8] Zeit. Unters. Lebens. 1, 552 (1898).

[9] Giorn. Farmac. Chim. **65**, 241, 281, 321 (1916); **66**, 245, 273 (1917); **Ann. Chim.** appl. **22**, 117 (1917).

[10] Journ. Ind. Eng. Chem. **10**, 471 (1918).

[11] Oil and Fat Ind. 1931, S. 291.

[11a] Cf. H. O. Triebold and C. H. Bailey, Cereal Chem. **9**, 50 (1932).

[12] Cf. J. Janey, Zeits, agnew. Chem. **44**, 348 (1931).

[13] Journ. Soc. chem. Ind. **50**, Transact. 87 (1931).

[14] loc. cit.

[15] Journ. Ind. Eng. Chem. **32**, 9 (1930).

[16] Journ. Soc. Chem. Ind. **47**, Transact. 185 (1928).

[19a] Vgl. auch K. Taufel, P. Sadler und F. K. Russow, Zeitschr. angew. Chemie **44**, 873 (1931).

[19b] Solid fats had to be melted or dissolved in a suitable solvent. Further work is being done to overcome this defect.

[20] Cf. Kobert, K. Zeit. anal. Chem. **46**, 711 (1907).

Viewpoint of the Saturated Glycerides.

Knoop has shown that in the metabolism of animals the saturated fatty acids decompose according to the so-called beta-oxidation process, splitting into beta-ketones:

$$\mathrm{R{\cdot}CH_2CH_2COOH \rightarrow R{\cdot}CH_2CHCOOH \rightarrow}$$
$$\mathrm{R{\cdot}CH(OH)\,CH_2COOH \rightarrow R - CO - CH_2COOH \rightarrow}$$
$$\mathrm{RCOOH + CH_3COOH}$$

If one stops the chain at the next to the last step above, decarboxylization takes place and ketones are formed, the so-called methyl ketones:

$$\mathrm{R\text{-}CO\text{-}CH_2COOH \rightarrow R\text{-}CO\text{-}CH_3}$$

A measurement of the products of this reaction may be shown in the laboratory by means of the Dakin reaction using hydrogen peroxide on ammonium salts of the fatty acids. One is indebted to Starkle[21] for the fact that the presence of moulds in saturated fatty acids of average molecular weight give rise to such methyl ketones. These have a very strong odor and they are the carriers of the aroma necessary for the ripening of high fat cheeses as Roquefort and Gorgonzola, etc. The presence of these moulds is well known in butter and in margarine. It was therefore proposed to find an analytical method which would detect these ketones that the sense has perceived objectively. The well known ketone reagents are generally not sensitive enough. Consideration of the work of Fabinyi[22] as well as Dekker and von Fellenberg[23] showed that those ketones having the grouping, —$\mathrm{CH_2COCH_2}$—, gave a condensation product with salicylaldehyde having a red raspberry color. The depth of the color produced varies from an orange-red color with acetone to a deep, violet, raspberry-red color according to the increase in the number of the carbon atoms in the ketone. The tests for ketones in fats that were rancid were carried out as follows:[24]

Procedure: A 200 cc. glass stoppered, frationating flask, the inlet tube of which is fitted with a short Liebig condenser, is used for the distillation of the fat. About 180 cc. of distilled water are placed in the flask along with a boiling stone and 25-30 cc. of water is distilled over into a suitable glass-stoppered vessel. To this 0.4 cc. pure salicylaldehyde are added and the mixture is shaken to produce a good emulsion. After the aldehyde has settled out, the liquor is decanted off to within about 4 cc. The aldehyde is again shaken up and 2 cc. of concentrated sulfuric acid are added. This addition must be made into the center of the liquid in a small stream and not allowed to run down the walls of the vessel into the liquid. The acid is well mixed by shaking vigorously and in a short time after standing the aldehyde will separate into the upper layer and the lower layer will have a milky appearance. This is the blank on the materials used and may have at most, a light yellow color. Next, 10 grams of the fat to be investigated are added to the same water in the distilling flask and a similar procedure to that of the blank followed exactly. If only a trace of ketone is present a deep red color will develop in the acid mixture in comparison to the light yellow of the blank. The color increases upon warming which is important to note in that the blank will not change color even upon heating on the steam bath for 15 minutes.

Aldehydes do not respond to this reaction. This gives an increased selectivity to this test. By using 10 grams of the substance to be investigated, it is possible to detect for a certainty, methyl-nonyl ketone at a dilution of 1:5 x 106. It is important that pure salicylaldehyde is used; technical grade will give a color in the blank test. Hence a blank is necessary.

The use of this reaction extends our boundary of knowledge. One can now test a great variety of materials in this way. As yet this reaction has not given a a deceptive result when a ketone was shown to have been present by some other means. Other odors may appear but as yet there has been only one exception to a ketone that gives a raspberry-red color and that is urea, but it is not a liquid and will settle out upon standing. This is, therefore, a typical ketone reaction. And further, Richter's "soapy" spoilage does not give rise to ketones as was earlier expected.

The former idea concerning the formation of methyl ketones as the cause of odor rancidity, especially by Starkle, can now be connected to biological factors. It is also well to pay attention to this kind of rancidity in margarine and other products using cocoa—and palmnut oils. There are other possibilities of a purely chemical nature to be considered in this connection which are being investigated now and will be reported upon later.

As pure scientists we are primarily interested first in the theory of these decompositions, and the difficulties involved in the knowledge of the reaction mechanism connected with ketone formation. The biological destruction of ketones is a subject about which little is known. In this direction the above salicylaldehyde reaction should be a very valuable help.

What practical application is there for this reaction? The next polemical fact to be added is the positive results obtained by the above prescribed procedure upon normal fats in which ketones as thus shown to be present. A light rose tint cannot be used as a harsh judgment, but a red color indicates that in spite of the senses saying there is no spoilage, the beginning is not far off. In the case of a deep red color a definite odor usually accompanies it which makes the situation clear. My opinion is that this salicylaldehyde test is an analytical help and will be found valuable in the cases of doubtful objective tests.

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[21] Biochem. Zeit. **151**, 370 (1924).
[22] Chem. Centr. **1900**, II, p. 301.
[23] Ann. **364**, 22 (1909).
[24] Cf. Taufel, K and Thaler, H. Chem. Ztg. **56**, 265 (1932).

National Oil Products Co. Expands Production Facilities

Harrison, N. J.—In order to take care of its constantly increasing business, the National Oil Products Company is erecting a new building to house part of its administrative offices and production departments—the second extension since 1929.

The new building will be a brick and steel frame structure, four stories high and basement. Each floor will contain 10,000 square feet of floor space. The contract has been let and the announced cost is $125,000.

The products to be manufactured in the new building are "Admiracion," a soapless hair shampoo, and "Vitex," a vitamin D concentrate derived from cod-liver oil.

Clemson Textile Students Awarded Fellowship

The Clemson Textile Department, South Carolina, has been awarded a fellowship by the Glyco Products Company, Inc., of Brooklyn, N. Y., makers of many new and interesting products, among which are thickening and emulsifying agents, synthetic resins, glycol and glyceryl products, and foam producers.

For this school year the fellowship has been awarded to Messrs. M. L. Huckbee and C. D. Wyatt, students at the Clemson Textile Department, who will conduct studies with a view to finding new and extended uses for the above Glyco products.