The Action of Microorganisms on Fats

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UNDER certain conditions, the preservation of edible fat is complicated by microbiological activity. These complications are, namely: (1) enzymic action in fats from presence of molds, bacteria, and yeasts, and (2) chromogenic materials elaborated by microorganisms diffusing into the substrate. The corrective measures for elimination of microbiological contamination of fats are not difficult of application, and conditions provocative of microbic growth need never occur in any refinery or plant. Fortunately, the problem of preservation of edible fat is not complicated by the presence of bacteria, molds, and yeasts that produce food poisoning or food infection.

Literature

In 1886, when the problem of microbic cleavage of fats in the intestinal tract first came into prominence, Necki[1] tested a few bacteria for lipoclastic activity. He observed some lipolytic activity in the cultures of bacteria examined. Later, VonSommaruga,[2] Schreiber,[3] and Rubner[4] found that a large number of bacteria decompose fats in the presence of atmospheric oxygen and nutrient matter, the action being a hydrolytic cleavage followed by decomposition of glycerol and oxidation of the fatty acids. Orla-Jensen[5] also observed that molds, yeasts, and bacteria produced rancidity in natural fats. He grew actinomyces in sterile butterfat; growth was accompanied by a definite increase in acidity. Eijkman,[6] whose work was confirmed by Söhngen,[7] showed that numerous species of bacteria elaborate lipoclastic enzymes. Söhngen observed that many species of bacteria grew well with fat as a source of carbon and ammonium chloride as a source of nitrogen. Wells and Corper[8] showed the existence of lipolytic enzymes in many species of bacteria killed with toluene. They found that cultures of Staph. pyogenes aureus, Ps. pyocyaneus, Bact. coli, Bact. dysenteriae, M. tuberculosis (grouped in order of lessening activity) were able to hydrolyze olive oil, triacetin, and ethyl butyrate. Van de Wall[9] demonstrated lipase activity in cultures of Ps. pyocyaneus, Staph. aureus, Bact. coli, and Bact. dysentery, Shiga-Kruse, Flexner. Kendall, Day and Walker[10] have shown lipase present in various species of bacteria (B. proteus, and B. mesentericus), and Michaelis and Nakahara[11] extended the list to include B. prodigiosus, top yeast, M. tuberculosis, S. aureus, S. albus, C. diphtheriae, dysentery, Bact. coli, Bact. typhosum, and Bact. paratyposum B. Waksman[12] writes that fats are decomposed chiefly by fungi, with the possible formation of ketones, and by a number of aerobic bacteria including S. aureus, B. prodigiosum, Bact. pyocyaneum, Bact. flourescens, and Bact. lipolyticum[13, 14, 15, 16, 17]. That microbic lipases show thermolability and thermostability was shown by Söhngen.[18] The lipases of Bact. punctatum, Pseudomona pyocyaneus, and B. liquefaciens albus were found to be more resistant to heat than those of Bact. lypolyticum, B. stutzeri, Ps. flourescens, Oidium lactis, Aspergillus niger, Penicillium glaucum, and Cladosporium butyri. Söhngen believes that he has evidence to show two bacterial lipases, alpha and beta, differing in their activity in the presence of acid.

It has been found that organisms deprived of their usual available food substances will readily utilize fats.[19] In soils, Rubner[20] found butterfat decomposed to the extent of 22.9% in one year (4.5 gms. fat in 200 gms. soil), and 38.1% in twelve years. Other fats decomposed at different rates. The fats hydrolyzed according to the general reaction:

$$C_3H_5O_3.\ R_3 + 3\ H_2O = C_3H_8O_3 + 3\ R.\ OH$$

Glycerol is, in turn, decomposed and the acid (as oleic) decomposed as follows:

$$CH_3\ (CH_2)_7\ CH = CH\ (CH_2)_7\ COOH + H_2O + \tfrac{1}{2}\ (O_2) = CH_3\ (CH_2)_7.\ CHOH.\ CHOH\ (CH_2)_7\ COOH$$

(dioxystearic acid)[21]

Glycerol fermentation depends on the species of microorganisms and environment. Usually glycerol is fermented into lactic acid, volatile fatty acids and aldehydes.[22] Glycerol fermentation may produce dihydroxyacetone, 2, 3 — butyl glycol, butyric acid, acetaldehyde, trimethylene glycol, and under anaerobic conditions, acrolein.

Lewis and Jensen encountered cultures of oxidizing bacteria imparting a tallowy flavor to meat mixtures containing fats.[23] However, Rogers, et al.,[24] write, "a purely chemical change in fat takes place in oxygen and sunlight when oxidation goes on at a slow rate. As a result, the butterfat shows tallowiness in contrast to the rancidity produced by bacterial action. It is thought that bacteria may also, at times, play a part in the oxidation of fat. It is possible that the bacteria may often be important in the first stages of oxidation and that their protoplasm may act as an acceptor in the process." Rahn[22] believes that oxidation prepares the fat so that the bacteria yeast and molds will grow well on it.

We have observed that most of the fat splitting and rancidity producing microorganisms encountered in the industry need aerobic conditions for the furtherance of their activities.

Hilditch[25] states that ". . . fatty matter is an excellent medium for the growth of many forms of molds and bacterial life and is also, when unsaturated glycerides are present, very prone to incipient oxidation at the ethylenic linkage . . . Mold spores may infect the fat and the resulting molds contain a variety of enzymes . . . Lipoclastic enzymes . . . will tend to the production of free fatty acid. There is some evidence that enzymes of the peroxidase type also induce oxidation effects, not only of the same nature as atmospheric oxidation, but also in the case of saturated fatty acids which may be converted by oxidation at the beta carbon atom from the carboxyl group into ketonic acids and ultimately into methyl ketones. $—CH_2.\ CH_2COOH —CO.\ CH_2.\ COOH —CO.\ CH_3$."

Fats decomposed under anaerobic conditions show, according to Bach and Sierp,[26] a different chemical process. During anaerobiosis, carbon dioxide is split off and the fatty acids are changed into hydrocarbons resulting

in formation of products of a lower saponification and higher iodine number than the original fat.

When heavy odors like those of aliphatic esters occur they are probably due to methyl-nonyl-ketone and allied compounds. Hilditch ascribes these decompositions to enzymic actions of anaerobic molds. Davies[27] has encountered development of ketonic rancidity due to mold growth. The liberated fatty acids are fermented to methyl carbinols and ketones which are volatilized. He distinguishes two classes of molds growing on fats, dry and oily. The dry molds are Penicillia, Aspergillia, Monila, and possess no apparent lipase. These fungi discolor fats and produce ketonic rancidity. The oily molds liberate oleic acid giving fat an oily appearance, liquefaction occurring with advanced growth. These forms are chiefly Oidium and Oospora and produce no great discolorations in fat. The dry mold ketonic rancidity is a reaction with fatty acids mainly up to capric acid (C_{10}), fatty acids from lauric upwards being toxic to them; consequently they show ketonic rancidity only in those fats containing much of the lower fatty acids up to C_{10} (butterfat, cocoanut oil). Davies believes that the competitive demand for oxygen for microbic respiration in fatty foods keeps oxygen out of the fat and that oxidative rancidity is started by the acidity formed by microbic agencies previous to the further processing of the fat.

A detailed review of the formation and decomposition of fats by microorganisms is given by Seliber.[28]

Culture Methods to Detect Fat Cleavage

Eijkman[6] devised a plate method for detecting lipase producing bacteria. The inside of a petri dish bottom is coated with molten beef tallow and permitted to solidify. A layer of inoculated agar cooled enough to prevent liquefaction of the tallow is poured into the plate. After several days' incubation, the fat becomes opaque and fragile in the vicinity of a lipase forming colony. The fat does not adhere to the glass where this action takes place. In some instances, the opaque region is surrounded by a translucent ring. B. prodigiosus and Ps. pyocyaneus demonstrate lipase activity very clearly growing in Eijkman medium.

By the use of a modification of Eijkman's method, Lieske[29] demonstrated that a large number of actinomyces are able to produce lipoclastic enzymes. This was accomplished by adding 1 to 3% of the fat to molten agar, emulsifying and then pouring plates with inoculated agar and by streaking the solidified agar-fat emulsion. Brom-phenol-blue or litmus was added as an indicator.

Buchanan[30] suggests using the fat emulsion (Lieske) medium. The fat splitting bacteria produced a transparent zone in the agar plates containing an emulsion of oil or fat.

Waksman and Daviston[31] describe the same medium containing litmus as an indicator.

Turner[32] tested a number of plating mediums for their differential usefulness in detecting lipase producing bacteria. He found that a nile blue sulphate-cottonseed oil agar medium possessed a high degree of sensitivity but inhibited the growth of certain organisms. His best results were obtained by 48 hour anaerobic growth followed by a period of aerobic growth. After the period of anaerobic growth, the colonies of fat splitting bacteria possessed a halo, deep blue in color, which, according to Lorrain Smith[33] (who introduced this dye as a specific stain for fatty acids in histological work), is due no doubt to the specific staining of free fatty acids.

Rahn[34] used an ammonium phosphate-fat medium to study fat decomposition by Penicillium glaucum. The lipoclastic activity of bacteria has been estimated quantitatively by Michaelis and Nakahara[11] who used tributyrin as a substrate. Lipase activity was measured by the stalagmometric method of Rona and Michaelis.[35] Avery and Cullen[36] estimated fat cleavage of cultures on tributyrin while Stevens and West[37] employed emulsions of ethyl butyrate or triacetin as substrate.

Eyre[38] detects lipase elaboration in broth cultures by employing the following technique:

Inosite-free isotonic broth is inoculated with the culture to be tested. After incubation the broth is filtered through a Seitz filter. Add 2 cc. of cottonseed oil to a sterile test tube and 10 cc. of the culture filtrate. Incubate 37° C. for 6 hours. Heat the mixture to 100° C. Filter through the Seitz filter. Add filtrate to 5% suspension of red blood cells. Hemolysis indicates the presence of lipase in the original culture.

In our laboratory we have employed these several methods but have found none of them entirely satisfactory. The cottonseed oil emulsion agar-nile blue medium of Turner more nearly approaches the ideal medium for the detection and enumeration of lipase forming microorganisms. However, cottonseed oil inhibits the growth of many species of microorganisms because of its high active oxygen content. It is true that freshly deodorized and otherwise refined cottonseed oil has no active oxygen content but there is no induction period and the oil immediately takes up oxygen. Most of the samples of cottonseed oil examined in this laboratory showed an active oxygen number of five to ten milliequivalents per kilogram. Such samples were about two weeks removed from the refinery. Old oil exposed to the air showed an active oxygen number ranging from 20 to 30. Most vegetable oils, refined and deodorized, tend, upon exposure to air, to possess high oxidizing capacities.

It is now an established fact that active oxygen is extremely deleterious to growth of certain species of microbes—the reducing bacteria and fungi being more sensitive to oxidation than the oxidizing flora. Accordingly, we have used a cocoanut oil medium and a palm oil (refined) medium because of their low iodine number and no active oxygen content. Lard is an exceptionally good substrate to show lipoclastic activity if one is dealing with a flora growing best at 37° C.

Our modification of emulsion medium which may be incubated either at 20° C. or at 37° C. is made as follows:

Agar stock:
- Nutrient agar 100 cc.
- Na_2HPO_4 0.5 gm.
- pH 7.4

Oil emulsion: 100 cc. refined cocoanut oil (or palm oil) 2 gms. gum tragacanth added to hot distilled water 200 cc. Add 4 gms. sodium taurocholate and place the oil and solution in shaking machine to emulsify. Shake until the globules are approximately 10 μ in diameter. Autoclave the emulsion at 15 pounds for 15 minutes.

Then as Turner suggests, we add 0.75 cc. of the oil emulsion and 0.75 cc. of 0.1% nile blue sulphate aqueous solution to each 5½ cc. of melted agar.

Portions of the well mixed medium are then cooled to 42° C., the inocula in dilutions added, and plates poured in thin layers.

Duplicate plates are poured and incubated at 20° C. and 37° C. for four days. The lipase forming colonies are at first deep blue in color, some colonies taking on a red-copper hue.

The count is made in the manner of the standard water

or milk count; i.e., numbers of lipoclastic bacteria per gram of fat from plates showing 30 to 300 colonies.

We then determine the number of microorganisms that incite and aid in oxidative rancidity, together with lipase activity. This is done by using our medium as described above without the addition of nile blue sulphate solution. Incubate as described and then test the colonies for their oxidizing activities (oxidase)[39, 40, 41, 42]. This is accomplished by pouring a 0.5% aqueous solution of dimethylparaphenylenediamine-hydrochloride over the surface of the incubated plate and observing the number of colonies that take on a rose-red color. A 0.4% aqueous solution of tetramethylparaphenylenediamine-hydrochloride is more sensitive and colors the oxidase colonies a deep purple.

Microorganisms producing lipase are characterized by a transparent or translucent zone and if upon addition of oxidase detecting dyes the colonies show both lipase and oxidase, one is probably dealing with an oxidative rancidity producing microorganism.

The Eijkman medium mentioned above may be used with good results in detecting microorganisms possessing the dual powers of lipase production and oxidase-peroxidase-peroxide formation. The number of tallow splitting colonies are counted and then the dye solutions are poured over the agar and the number of colored colonies that were shown to be lipoclastic are counted.

A typical experiment with samples of fats showing the number of lipase, oxidase, and both lipase and oxidase formers, together with other microorganisms, is shown in Table I.

for active oxygen. The results of the bacterial action on these fats are indicated in Table II.

TABLE II.

++ = Marked
\+ = Present or Positive
± = Not Marked
— = Negative

Incubated 2 weeks at 37° C.

Bacillary Culture	Test	Leaf Lard	Hardened Cottonseed Oil Shortening
Lipase former No. 3	Organoleptic	—	—
	Kreis	±	±
	Perkins-Vibrans	±	+
	Free fatty acid	++	++
	Aldehyde-Schiffs }	—	—
	Aldehyde-permanganate }		
	Active oxygen	—	++
Oxidizing No. 48	Organoleptic	± (tallowy)	± (tallowy)
	Kreis	+	+
	Perkins-Vibrans	++	++
	Free fatty acid	—	—
	Aldehyde	+	+
	Active oxygen	++	++
Combined Lipase-Oxidase No. 14	Organoleptic	++	++
	Kreis	+	+
	Perkins-Vibrans	++	++
	Free fatty acid	++	++
	Aldehyde	++	++
	Active oxygen	++	++
No Culture (control after two weeks)	Organoleptic	—	—
	Kreis	—	—
	Perkins-Vibrans	±	±
	Free fatty acid	—	—
	Aldehyde	—	—
	Active oxygen	—	+

TABLE I.

Microorganisms in Samples Carried in Laboratory Until Old and Rancid

(Lip. = Lipase; Ox. = Oxidase)

MEDIUMS	LEAF LARD (per gram)						HYDROGENATED VEGETABLE OIL SHORTENING (per gram)						BUTTER FAT (per gram)					
	Bacteria		Mold		Both enzymes		Bacteria		Mold		Both enzymes		Bacteria		Mold		Both enzymes	
	Lipase	Ox.	Lip.	Ox.	Bact.	Mold	Lip.	Ox.	Lip.	Ox.	Bact.	Mold	Lip.	Ox.	Lip.	Ox.	Bact.	Mold
Eijkman's original.	34,000	.....	150	..	.....	..	90,000	.....	1,200	..	.....	..	360	.....	1,200	..	.....	...
Lieske's	40,000	.....	180	..	.....	..	112,000	.....	900	..	.....	..	600	.....	1,400	..	.....	...
Turner's cottonseed oil-nile blue.....	15,000	.....	80	..	.....	..	44,000	.....	300	..	.....	..	25	.....	75	..	.....	...
Cocoanut oil emulsion agar	42,000	20,000	300	40	15,000	35	85,000	30,000	400	60	20,000	43	4,000	1,200	1,600	80	1,200	980
Palm oil emul. agar	44,000	15,000	260	50	10,000	40	114,000	28,000	360	54	15,000	45	6,000	1,100	1,400	92	1,420	1,200
Eijkman's tallow plate and oxidizing colony detection	34,000	26,000	120	35	20,000	55	150,000	34,000	120	100	66,000	60	420	300	980	50	53	22
Nutrient agar count			40,000						160,000						3,000			
Reducing bacteria on agar			30,000						120,000						200			
Anaerobes in glucose brain broth.			10						4,000						200			
Proteolyzers			30,000						8,200						2,800			

It is our opinion that the lipase-oxidase forming microorganisms are responsible for the more rapid lipolysis and oxidative rancidity encountered in fat. Microorganisms elaborating only lipase alone do not complicate the problem of rancidity as do the dual acting microbes.

Samples of sterile leaf lard and sterile hydrogenated cottonseed oil shortening were inoculated with (a) lipase formers, (b) oxidase and peroxide formers, (c) lipase-oxidase cultures.

After holding two weeks at 37° C. the two fats, animal and vegetable, were tested by the following methods: Kreis test, organoleptic, Perkins-Vibrans oxygen absorption,[43] free fatty acid titration, measure of aldehyde with Schiff's reagent, measure of aldehyde with permanganate, and the acetic acid-chloroform-KI-sodium thiosulfate

It is very difficult to prepare the fat or oil to be tested for microorganisms. One gram of the fat to be tested is liquefied in the 41° C. water bath and added to 99 cc. of warm (41° C.) diluent. The diluent employed is a 0.1% sodium taurocholate or 0.1% sodium oleate solution. The first dilution is well shaken so that the globules in the emulsion are under 10 μ. Obviously, the smaller the globule, the higher the count of microbes obtained.

To be sure, our technique and data are not sufficiently refined to enter into any discussion concerning the chemistry of microbically induced rancidity, but the results carry sufficient probability to shape further studies on this aspect of the rancidity problem. It must be borne in mind, however, that the shortenings and fats of animal and vegetable origin found in commerce are usually free

of serious contamination. A number of counts were made of shortenings purchased in the open market with the result that very few of the fats showed more than 10 bacteria and molds per gram.

For the majority of microorganisms, oils and fats exert a weak antimicrobic action and it is a common opinion amongst bacteriologists that certain oils are good preservatives. Hall and vanMeter[44] observed that the preservation of peanut butter, for example, is due to the germicidal action of the peanut oil present. They did state, however, that they believed the organisms died out because of the lack of available food.

The presence of strict lipophilic microorganisms in refineries is the exception, not the rule.

Discoloration of Vegetable and Animal Fats

The commonest colors of fat discoloration due to microorganisms are various shades of pink and yellow-brown. All three types of microorganisms, bacteria, yeast-torula and molds, have been shown to be causative agents in pigmentation of fats. Very little is known about the chemical configuration of microbic pigments because only in a few instances have these substances been obtained in pure or crystalline condition. It appears that the majority of these pigments are not soluble in water but are soluble in fat solvents such as ether, alcohol, acetone, chloroform and pyridine. The pigments of microbic growth are classed with the group of hydrocarbons or oxyhydrocarbons described as luteins, lipochromes, lipoxanthines, chromolipoids, or carotinoids.[45] The absorption bands of a bacterial pigment separates it into 2, 3, or 4 distinct pigments.[46, 47]

Zopf[48] described pigments in eight species of microorganisms. The intensely yellow pigment produced by B. egregium when treated with sulphuric acid and nitric acid changes into a blue compound (lipocyan reaction). The yollow pigment is fat soluble. An alcoholic solution shows absorption bands, one covering the "F" line, the other between "F" and "G." Zopf found the red pigment of Spaerotilus roseus to be fat soluble.

The yellow lipochrome of the staphylococci are soluble in fat; the mineral acids when added to the pigment produces a bright yellow color, whereas potassium hydroxide solution when added to the pigment changes it first to orange-red, then to red. A drop of sulphuric acid placed on a boiled residue of the lipochrome, which is a reddish-yellow, will give a play of colors = blue-violet to blue-green. The addition of alkali will produce a purple-red or red color.

While studying pigments of aerobic lipophilic yellow micrococci and bacilli diffused and dissolved into fats, Dr. W. Lee Lewis and the writer observed the curious phenomenon of an oxidation-reduction indicator of bacterial origin. The pigment produced by these races of micrococci and bacilli were "canary" yellow in color and very fat-soluble. Petri plates were poured with lard and beef tallow. The yellow producing bacteria were seeded into the fats and the plates incubated at room temperature 22° C. for 3 weeks. The yellow pigment diffused into the fat as the microorganisms grew. When the fat became rancid and began to show peroxide formation, the pigment changed to a greenish shade and within a few days became blue and purple. The microbes had been isolated from a purple discolored (fat side) "back-pack" ham.

Treatment of the yellow pigment (dissolved in fat) with hydrogen peroxide produced the characteristic blue color. The blue oxidation compound was then reduced to the original yellow color with sodium hyposulphite.

These purple discolorations of fats had been observed many times before the science of adequate refrigeration was perfected. The purple ("stamping ink") discolorations of the fat side of back-pack hams years ago was thought to be due to "labor troubles" because no purple colonies of bacteria could be isolated upon culturing the discolored fat.

It is said that Rembrandt, the great painter, showed on one of his canvases the interior of a meat shop of his country and faithfully depicted streaks of purple on the fatty tissue of dressed beef carcasses.

The spontaneous microbiological process involved in producing the purple pigment is as follows:

(1) Certain yellow-producing non-pathogenic cocci and some races of yellow-forming bacilli grow well on solid fats at temperatures ranging from 45° F. to 99° F.

(2) The yellow pigment formed by these bacteria diffuses slowly into the substrate.

(3) When the fat becomes rancid and peroxides appear, or if the peroxides or oxidases are elaborated by lipophilic bacteria discussed before in this paper are present, the pigment assumes a greenish color which in several weeks deepens into blue.

Bacterium luteum produces an orange-yellow fat soluble pigment, the color of which is destroyed by acids. Bacillus flavus and B. aurantiacus (Frankland) form orange-yellow pigments. The latter organism, when isolated from fats, is differentiated from B. flavus by its ability to reduce nitrates to nitrite. Sarcina flava is identified by its yellow fat-soluble pigment turning green upon addition of sulphuric acid and restoration of the yellow color by sodium hydroxide. Micrococcus rosea elaborates a red pigment soluble in fat. This pigment, when treated with sulphuric acid, becomes blue-green and sodium hydroxide restores the red color. Acetic acid destroys the pigment completely.

We have not succeeded in growing B. prodigiosus (several varieties) on pure fats. The pigment is thermolabile and gives off the odor of trimethylamine in a nitrogenous-fat medium.

The Chromobacteria studied by us are not lipophilic but in agar-oil emulsion media produce the many pigments characteristic of this group.

Pink discolorations are fairly common and operators finding a pink discoloration in their fat products should look to their processing rather than to a suspected contamination with red labels or artificial sources of dyes. Pink discolorations are usually very sensitive to heat but cannot be effaced by filtration or other physical means. These pigments are not poisonous and have no bearing on public health. Some of these pigments are carotinoids and thus are related to vitamin A.

We have encountered yeast, torula, bacilli, micrococci and molds producing pink pigments in fats both of vegetable and animal origin. Torula (the variety called "pink yeast" by bacteriologists) are with molds the commonest cause of pink fats. The torula are probably related to Cryptococcus glutinis, classified by E. C. Hansen[49] as "Saccharomyces." There are many species of red yeasts related to C. glutinis and most of them grow well in glycerol.

Piedallu[50] found a yeast which lives in oil and decomposes it. Van Tieghem[51] found a yeast (Saccharomyces olei) growing well in olive oil. Rogers[52] in an

extended study isolated torula decomposing fats with formation of fatty acids.

Occasionally some of the normally grey yeasts, like Saccharomyces ellipsoideus become pink if magnesium or phosphates are present in the substrate.[53, 54] The coloring matter of these yeasts is probably a carotin. We encountered a curious type of pink discoloration of fats due to a lipophilic torula described in 1918, as Saccharomyces pulcherrimus by Beijerinck.[55] This yeast, or torula, secretes a colorless chromogen which becomes deep red in the presence of iron salts. This yeast, when grown on emulsion-agar produces a cream-colored colony. When a trace of soluble iron is added to the medium, a pink color is produced. The iron content of some commercial fats and oils is great enough to activate the chromogen. Beijerinck suggested that the culture might be used to test for soluble iron salts.

The molds isolated from pink fat have proven to be of many varieties, both septate and non-septate. We have not classified these fungi, but it is hoped that these lipophilic forms may be the subject of a future communication.

The lipoclastic activity of fungi have received a good deal of attention. Thorough investigations have been made of yeast by Bau[56] and of molds by Camus,[57] Laxa,[58] Garnier,[59] Dox,[60] Hanzawa,[61] and Crabil and Reed.[62] Ruehle[63] studied the fat splitting power of bacterial spores.

Indol and skatol producing bacteria growing on a nitrogenous-fat substrate will produce a pink pigment upon the addition of nitrites. This type of discoloration is the result of the interaction between nitrite (containing nitrate as impurity) and indol and skatol produced by such bacteria as the colon bacilli, clostridia, hemoglobinophilic varieties, torula, etc., etc. The pigment is known as "nitro indol" and "nitroso-indol nitrate."

Traces of heated blood (hematin) in "rusty" fats together with the presence of microbic lipase cause a rapid absorption of oxygen. Lepper and Martin[64] have shown that oxygen absorption is accelerated in many fatty substances by autooxidation of unsaturated fatty acids catalysed by hematin. Lipase obviously speeds the rate of this reaction.

Summary

A review of the literature on microbic cleavage of fats brings out the fact that not a few species of bacteria, yeasts, and molds may complicate the preservation of edible fat. The microorganisms encountered in fats and oils are not pathogenic; i.e., do not cause food poisoning or food infection. Microbic activity may cause fat spoilage in two ways, lipoclastic action and discolorations due to chromogenic substances, diffusing into the substrate. Culture mediums and methods to detect fat splitting and oxidizing microorganisms are described. It is shown that cottonseed oil emulsion-agar mediums may inhibit certain types of microorganisms (reducing bacteria) because of the high active oxygen content of many samples of old cottonseed oil. It is possible that microorganisms elaborating lipase which are also oxidase-peroxidase forming may be responsible for certain types of oxidative rancidity. The microbic content of old and rancid samples of leaf lard, hydrogenated vegetable oil shortening and butter fat is shown by the use of six mediums: Eijkman's original, Lieske's emulsion agar, Turner's cottonseed oil-nile blue agar, cocoanut oil emulsion agar, palm oil emulsion agar, and Eijkman's tallow plate combined with oxidase detection. The counts show the total numbers of lipoclastic bacteria and molds together with lipase-forming and oxidase-producing bacteria and molds as well as bacteria and molds possessing both the oxidizing property and the fat splitting action.

The presence of strict lipophilic microorganisms in refineries or plants is the exception and not the rule.

Discolorations of animal and vegetable fats due to the fat-soluble pigments of bacteria, yeast, torula, and molds are described. Certain bacterial pigments were found to be oxidation-reduction indicators; blue when oxidized by bacterial oxidizing enzymes, peroxide of rancid fat or by hydrogen peroxide and when reduced by hyposulphite assumed their original yellow color.

References

1. Necki, M., Arch. exp. Path. Pharmak. **20,** 367, 1886.
2. Sommaruga, E. von, Z. Hyg. InfektKr., **18,** 441, 1894.
3. Schreiber, K., Arch. Hyg., Berlin, **41,** 328, 1902.
4. Rubner, M., Arch. Hyg., Berlin, **38,** 67, 1900.
5. Orla-Jensen, Zbl. Bakt. Abt. 11, **8,** 11, 1902.
6. Eijkman, C., Zbl. Bakt. Abt. 1, **29,** 841, 1901.
7. Söhngen, N. L., Jber. Fortschr. Chem., **41,** 788, 789, 1911.
8. Wells, H. G., and Corper, H. J., Jour. Infect. Dis., **11,** 388, 1912.
9. Van de Wall, N., Zbl. Bakt., 11, **70,** 369–372, 1927.
10. Kendall, Day and Walker, Jour. Amer. Chem. Soc., **36,** 1962–66, 1914.
11. Michaelis and Nakahara, Z. Immun Forsch. **36,** 449, 1923.
12. Waksman, S. A., Principles of Soil Microbiology, 1929, p. 464.
13. Schenker, R., Biochem. Zeitschr. **120,** 164–196, 1921.
14. Derx, H. G., "Der Oxydative Abbau der Fette durch Schimmel," Konigl. Acad. wiss, Amsterdam, **33,** 545–558, 1924.
15. Söhngen, N. L., "Lipase Production of Microbes," Konink. Acad. Wetenschap. Amsterdam, **19,** 698, 1910; **20,** 126, 1911.
16. deKruyff, E., Bull. Dept. Agr. Ind., Ne'erland, IX, Buitzenborg, 1907. (Centralbl. Bakt. 11, **20,** 610, 1908.
17. Huss, H., Zbl. Bakt. 11, **20,** 474, 484, 1908.
18. Söhngen, N. L., Thermo-tolerante lipase, Konink Acad. Wetenschap, Amsterdam **20,** 126–130, 1911.
19. Dubaquie', "Recherches sur les materieres Grasses des Vegetaux Inferieurs," Mem. Soc. Sci. Phys. Nat., Bordeaux, 1910.
20. Rubner, N., Arch. Hyg., **91,** 290, 1922.
21. Schreiner, O., and Shorey, E., Bur. Soils, U. S. Dept. Agric., Bull. **53,** 1909.
22. Rahn, O., Zbl. Bakt. 11, **15,** 54, 1905.
23. Lewis, W. Lee, and Jensen, L. B., The Cause and Prevention of Green Rings in Sausage, Bulletin 1, Non-technical discussion, Inst. Amer. Meat Packers, Chicago, p. 18.
24. Rogers, L. A., et al., "Fundamentals of Dairy Science," p. 285, 1928.
25. Hilditch, T. P., "The Industrial Chemistry of Fats and Waxes," London, 1927, pp. 276–281.
26. Bach and Sierp, Zbl. Bakt. 11, **62,** 2476, 1924.
27. Davies, W. L., Food Technology, Jan. 1932, 181–183.
28. Seliber, G., "The Formation and Decomposition of Fats by Microorganisms," Glavnauka, Leningrad, 1926 (cited by Waksman).
29. Lieske, R., "Morphologie und Biologie der Strahlenpilge," Leipzig, 1921.
30. Buchanan, R., "Agricultural and Industrial Bacteriology," 1924, 166.
31. Waksman, S. A., and Daviston, "Enzymes," 1926, p. 107.
32. Turner, R. H., Jour. Infect. Dis., **44,** 126, 141, 1929.
33. Smith, L., Jour. Path. Bact., **12,** 1, 1907.
34. Rahn, O., Zbl. Bakt. 11, **15,** 422–429, 1905.
35. Rona and Michaelis, Biochem. Z., **31,** 345, 1911.
36. Avery and Cullen, Jour. Exp. Med., **32,** 571, 1920.
37. Stevens and West, Jour. Exp. Med. **35,** 823, 1922.
38. Eyre, J. W. H., "Bacteriological Technique," 353, 1930.
39. Schultze, Zbl. Bakt. Abt. 1, **56,** 544, 1910.
40. Gordon and McLeod, Jour. Path. Bact., **31,** 185, 1928.
41. Ellingworth, McLeod and Gordon, Jour. Path. Bact., **32,** 173, 1929.
42. Jensen, L. B. and Morton, H. B., Jour. Infect. Dis., **49,** 425, 1931.
43. Lewis, W. Lee, and Perkins, G. A., "Studies on Lard," Inst. of Amer. Meat Packers, Chicago, 1929. Lewis, W. Lee and Vibrans, F. C., "Qualities of Lard," Inst. Amer. Meat Pkrs., Chicago, 1930.
44. Hall, Ivan, and Van Meter, Amer. Food Journal, **13,** 463, 1918.
45. Palmer, L. S., Carotinoids, etc., N. Y., 1922.
46. Lasseur, A. P., and Girardet, F., "Contribution a l'etude des pigments microbiens," J. Coube et fils, 1925.
47. Reader, V., Biochem. J., **19,** 1039, 1925.
48. Zopf, W., Botan. Ztg., **49,** 53–61; 69–18; 85–91, 1889. Z. wiss. Micros., **6,** 172–177, 1889. Ber. botan. Ges., **9,** 22–28, 1891. Beitr. Pysiol. Morphol nieder Organismen, **2,** 3–48, 1892.
49. Hansen, E. C., "Colores en rouge et cellules rouge resemblant a de Saccharonyces," Comp. Rend. des trav. lab. de Carlsberg (Copenhagen), Vol. 1, tome 24, 1879.
50. Piedallu, A., Comp. Rend. Soc. de Biol., **65,** 1908.
51. Tieghem Van, Bull. Soc. Bot. France, **28,** 1881.
52. Rogers, A., Zbl. Bakt., **10,** 1903.
53. Kossowicz, A., Versuchsw. in Österreich, **6,** 27, 1903.
54. Will, A., Zbl. Bakt., 11, **35,** 81, 1912.
55. Beijerinck, M. W., Arch. neerland. physiol., **2,** 609, 1918 (Chem. Abst. 13, 1082, 1919).
56. Bau, A., Wchnschr. Brau., **32,** 141–143, 151–154, 159–162, 189–191, 1915.
57. Camus, M. L., Comp. Rend. Soc. Biol., Paris, **49,** 192–193, 230, 1897.
58. Laxa, O., Arch. Hyg., **41,** 119–151, 1900.
59. Garnier, C., Comp. Rend. Soc. Biol., Paris, **55,** 1490–1492; 1583–1584, 1903.
60. Dox, Jour. Biol. Chem., **6,** 461, 467, 1909.
61. Hanzawa, Mycol. Centbl. **1,** 79–91, 1912; **5,** 230–246, 1914.
62. Crabill, C. H., and Reed, H. S., Biochem. Bul., **4,** 30–44, 1915.
63. Ruehle, C. G. A., Jour. Bact., **8,** 847–891, 1923.
64. Lepper, E., and Martin, C. J., Brit. Jour. Exp. Path., **10,** 327–334, 1929.