XIV. THE RANCIDITY OF COCONUT OIL PRODUCED BY MOULD ACTION¹.

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It is now generally agreed that the deterioration of oils and fats, whether due to the action of air, light and moisture, or to the action of micro-organisms, is an oxidative process subsequent to partial hydrolysis. In these two forms of rancidity—that of pure fats (where the absence of nidus prevents bacterial or mould growth) and that caused by micro-organisms—the course of the oxidation is not the same. In the latter form the combustion is probably more vigorous and supplies energy to the organism. Much has been written on the deterioration of pure fats, a summary and bibliography being given by Hepburn [1909]; but on biologically produced rancidity comparatively little work has been done, that of Jensen [1902] being probably the most important.

As it contains only a small proportion of unsaturated glycerides, coconut oil does not quickly deteriorate when exposed to atmospheric conditions, but eventually acquires the characteristic odour of all fats under these conditions. When subjected to mould action, however, it gives rise to a pungent “perfume” or ester-like odour quite different from that already noted. This has been observed, but not identified, by several investigators [Biffen, 1899; Walker, 1906; Jacobsen, 1918]. Biffen thought, erroneously, that it was amyl butyrate, but the author [1921] has shown it to be due to the formation of methyl ketones; Stärkle [1924] arrives at similar results and suggests that ketones are formed in a way analogous to Dakin’s synthesis of methyl ketones from fatty acids [Dakin, 1910]. Derx [1925] confirms the author’s results and his contention that *Oidium lactis* does not produce ketonic rancidity, and finds further that some *Penicillium* species produce ketonic or non-ketonic rancidity according to the composition of the medium.

The formation of methyl ketones in the decomposition of fats suggests β -oxidation, especially by analogy with the oxidation of straight chain compounds *in vivo*. β -Oxidation would explain, also, the production of methyl ketones in essential oils (e.g. methylnonyl ketone in oil of rue), and the occurrence of ketones in the “échappées” of coconut oil. Haller and Lassieur [1910] found

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that the "échappées," or oily substance which collects on the surface of the condensed steam in the deodorising of coconut oil, consists of methylheptyl, methylnonyl and methylundecyl ketones with about 12 % methylheptyl and methylnonyl carbinols. These ketones, to which the disagreeable odour and flavour of the crude, acid-free oil is due, must be formed during the drying of the copra, since coconut oil expressed from the fresh fruit has an agreeable almond-like odour and taste. Their formation may be ascribed either, as Haller and Lassieur suggest, to the decomposition under enzymic action of some hitherto unknown constituent of the coconut or, in the light of the present work, to the action of moulds, which during the sun-drying of the copra develop on it, splitting and oxidising the fat to methyl ketones. Much of the dry copra arriving from abroad is certainly infected with mould spores.

It will be noted that Haller and Lassieur found methyl ketones corresponding to three only of the known fatty acids of coconut oil.

It is possible that moulds cannot assimilate the higher fatty acids; moreover, certain fatty acids act as poisons even in weak concentration. *Penicillium glaucum* grows in a concentration of 24 mg. of caproic acid per 100 cc., but not in the same concentration of caprylic acid [Laxa, 1902]. Spieckermann [1912] states that it can grow in comparatively strong acetic acid but not in weak propionic acid, and concludes that the poisoning capacity of the acids increases with molecular weight up to caprylic acid, and then diminishes. Stärkle [1924] suggests that the poisonous acids are neutralised, as they are formed, by ammonia produced by the growing organism, and this is supported by the contention of Jensen [1902] that ammonium salts of fatty acids are produced by mould action on butter. Accordingly Stärkle gives the course of the oxidation as follows:

Dakin, however, obtained ketones even when caustic soda was used as neutralising agent. Stärkle does not explain the anomalous action of *Oidium lactis*, which, though rich in hydrolysing and oxidising enzymes, is unable to produce methyl ketones.

The secondary alcohols detected by Haller and Lassieur correspond to two of the ketones, and might therefore appear to be intermediate products in the oxidation. On the other hand they may be formed by reduction of the ketones. Thus, dextrorotatory methyl carbinols are produced by the action of yeasts on the corresponding ketones [Neuberg and Nord, 1919], but Dakin [1910] did not detect β -hydroxy-acids in his fatty acid oxidations. Again, in actions *in vitro* β -hydroxy-acids do not split off CO_2 as keto-acids readily do; while in oxidations *in vivo* the fatty acids are oxidised directly to the keto-acids, the β -hydroxyacetic acid of diabetes being probably a secondary reduction product of acetoacetic acid.

While agreeing with Stärkle that mould oxidation is analogous to Dakin's synthesis, the author believes that owing to the poisonous or narcotic effects of

volatile fatty acids on moulds, the oxidation is really abnormal. Under such unfavourable conditions the normal life processes are restrained and respiration is impeded. When the mould grows on a medium of low nutritive value, ketones are more readily formed, as is confirmed by Derx [1925].

Oidium lactis, on the other hand, offers strong resistance to external influences [Lang and Freudenreich, 1893] and can even destroy living yeast cells. Probably, therefore, it can effect a more complete or normal decomposition of fatty acids than can *Penicillium* or *Aspergillus*.

The course of the oxidations may be thus represented:

The normal form is supported by the fact that in butter *O. lactis* produces volatile acids [Jensen, 1902]. The oxidation of the non-poisonous acids, even by *Penicillium*, probably follows scheme (2), but oxidation of poisonous acids stops short at the keto-acid, which then splits up to methyl ketone. *O. lactis*, on the other hand, effects acid hydrolysis of the keto-acid to a lower acid, which in turn may be further oxidised.

The theory outlined above has formed the basis of the experimental work. This includes (I) an investigation of the products of the action of a *Penicillium* organism on coconut oil, (II) physiological experiments with *Penicillium* and *O. lactis*, (III) a repetition of Dakin's oxidation of fatty acids to determine, if possible, whether secondary alcohols are formed as intermediate products, and (IV) a more complete investigation of the "échappées" of Haller and Lassieur.

EXPERIMENTAL.

I. THE ACTION OF *PENICILLIUM* ON COCONUT OIL.

A pure culture was prepared of a mould organism isolated from rancid coconut oil margarine. The organism was identified as *Penicillium palitans* Westling [Westling, 1912], this being kindly checked by Dr Derx of Delft, Holland, to whom the author wishes to acknowledge his thanks.

Preparation of rancid coconut oil and separation of the rancidity products.

The oil was the refined deodorised oil used in margarine manufacture and was taken from a consignment from Oliefabriek Calvé-Delft, Delft. 20 cc. of just melted fat was emulsified with 20 cc. Raulin's solution containing 10 % gelatin, previously sown with spores from the pure culture, and the resulting emulsion poured into a 6-inch Petri dish placed on a cold slab. When the

emulsion had set the plates (80 in each weekly batch) were placed in an incubator at 20°. At the end of each week, when the plates showed a luxuriant growth and gave the characteristic odour, they were warmed, the melted contents poured into a 5-litre distillation flask and distilled from an oil-bath in a current of nitrogen. The delivery tube of the flask was connected by two double surface condensers to a conical receiver with a side outlet attached to a U-tube containing chloroform. This tube stood in powdered ice, its purpose being to trap any volatile matters carried away by the gas. The temperature of the bath was raised gradually to 150° and maintained at this until the water in the flask had wholly passed over. At this point the odoriferous substances had apparently completely distilled, and an oil was observed floating on the surface of the aqueous distillate. The latter was collected and preserved in tightly stoppered bottles in the dark; the fatty residue in the flask was also kept. At the end of this part of the work, 43 kg. of fat had been treated, 40 litres of aqueous distillate collected, and 4.7 kg. of mould grown. The distillate was treated five times with pure chloroform to extract the rancidity products, yielding eventually 100.5 g. crude aromatic oil.

Examination of odoriferous rancidity products.

These might be a mixture of ketones, aldehydes, esters, secondary alcohols, free fatty acid and neutral fat. Separation was accomplished as follows.

(A test for aldehydes with Fehling's solution showed only the merest trace.)

(a) *Free acid.* The liquid was washed with 10 % Na_2CO_3 solution, then with water. Only a small amount of acid was obtained.

(b) *Neutral fat.* After drying over anhydrous sodium sulphate the liquid was distilled, first at ordinary pressure, then *in vacuo*. The residue proved to be neutral fat, Wt. 5.48 g.

(c) *Alcohols.* These were separated by Haller's method [Haller and Lassieur, 1910] of conversion to the phthalic acid esters, followed by extraction with 10 % Na_2CO_3 , acidification and saponification. The alcohols had an odour resembling that of the ketonic portion, Wt. 3.3 g.

(d) *Esters.* The remaining liquid was saponified with methyl alcoholic potash. The alcohols were obtained from the unsaponifiable matter by Haller's method, as above, and the acids from the soap solution,

Weight of ester alcohols 0.30 g.

Weight of ester acids 0.93 g.

(e) *Ketones.* These constituted the remainder of the unsaponifiable matter of (d). Wt. (dried) 71.0 g.

As the ketones constituted the main part of the rancidity products they were examined first.

1. *The ketones.* The liquid was dark coloured and had a strong odour of rancid coconut oil: $D_4^{15.5}$ 0.826; it reacted with saturated sodium bisulphite solution but did not reduce Fehling's solution. After six distillations, in which a Young

"8 pear" fractionating column was used, the liquid was resolved into the following fractions:

Table I. *Wt. of substance distilled 71.0 g.*

Fraction	Temperature °	Weight g.	
1	150	1.40	
2	150-152	24.44	{ refractionated 150-2 2.45 152-7 1.65
3	152-157	4.10	
4	157-168	1.17	
5	168-173	1.40	
6	173-188	1.62	
7	188-196	8.49	
8	196-205	2.47	
9	205-215	2.64	
10	215-223	2.71	
11	223-228	9.64	
12	residue	5.54	
Total		65.62	Loss 5.38 g.

The residue was dark coloured and viscous, with a slight odour of acrolein, due probably to some decomposition during the distillation. All fractions had the strong, pungent odour of the original rancid oil, which, however, decreased in intensity with rise of the boiling-point. All reacted with an alcoholic solution of semicarbazide hydrochloride to form semicarbazones, and also with *p*-nitrophenylhydrazine.

Fraction (2) B.P. 150-152°. On refractionation the bulk of the liquid distilled at 151-152°. This fraction contained 12.20 % H and 73.63 % C; calculated for $C_7H_{14}O$, 12.28 % H, 73.68 % C.

The semicarbazone was obtained in colourless crystals, soluble in alcohol; M.P. 122.5-123°; M.P. of the semicarbazone of synthetic methyl-*n*-amyl ketone 122°; mixed M.P. 122°.

Fraction (2) was therefore methyl-*n*-amyl ketone.

Fraction (7) B.P. 188-196°. On refractionation the bulk of the liquid distilled at 193-195°. This fraction contained 12.63 % H and 75.89 % C; calculated for $C_9H_{18}O$, 12.67 % H and 76.06 % C.

M.P. of the semicarbazone 118°; M.P. of the semicarbazone of synthetic methylheptyl ketone 118°; mixed M.P. 118°.

Fraction (7) was therefore methylheptyl ketone.

Fraction (11) B.P. 223-228°. On refractionation the portion distilling at 224-226°, the B.P. of methylnonyl ketone, was collected. This fraction contained 12.93 % H and 77.74 % C; calculated for $C_{11}H_{22}O$, 12.94 % H, 77.64 % C.

M.P. of the semicarbazone 122-122.5°; M.P. of the semicarbazone of synthetic methylnonyl ketone 123°; mixed M.P. 122°.

Fraction (11) was therefore methylnonyl ketone.

Fractions (3)-(6); (8)-(10). Semicarbazones were prepared from each fraction and, in most cases, pure substances were obtained after from 5 to 13 crystallisations from alcohol. No new substance was found, this indicating that the fractions were mixtures of the ketones already identified.

Fraction (1) B.P. up to 150°. The semicarbazone was found to be that of methylamyl ketone. Methylpropyl ketone could not be identified.

Fraction (12). The liquid was not wholly soluble in cold alcohol. The insoluble oil being separated, the clear alcoholic solution was used to prepare the semicarbazone, which proved to be that of methylnonyl ketone. The semicarbazone of methylundecyl ketone could not be found.

2. *Neutral fat.* Saponification value 246.8, iodine value 13.7; the fat was completely saponifiable. It therefore closely resembled coconut oil.

3. *Alcohols.* The yield of alcohols being only about 4 cc., it was decided to examine the products formed on oxidation. On distillation from a very small flask, the liquid was resolved into two fractions, (1) 160–200°, (2) 200–240°, and a drop of dark residue. The fractions were oxidised with chromic acid mixture as follows. 2 g. finely powdered potassium dichromate were added to each liquid, contained in a small flask surrounded by crushed ice. A mixture of 3 g. H_2SO_4 and 4 g. water was allowed to drop slowly from a tap funnel into each flask, so that the whole of the acid was added in 12 hours. After dilution with water, the reaction products were extracted with ether, washed free from acid and dried. On evaporation of the ether, 2 cc. of a pungent smelling liquid was in each case obtained. With Fehling's solution there was no reaction, but with *p*-nitrophenylhydrazine each liquid gave a positive reaction for ketones.

The alcohols, therefore, consisted of secondary alcohols.

The ketones were now converted to their semicarbazones.

Semicarbazones (crystallised from alcohol):

(i) Alcohol fraction B.P. 160–200°

	1st crop	2nd crop
	M.P.	M.P.
5th crystallisation	117–118°	117–118°
6th " "	118–118.5°	120–121°
7th " "	—	121–122°

(ii) Alcohol fraction B.P. 200–240°.

	1st crop	2nd crop
	M.P.	M.P.
6th crystallisation	119–120°	not suf-
7th " "	121°	ficient for
8th " "	122–123°	determination

(i) *Semicarbazone from 1st crop (6th crystallisation)* M.P. 118–118.5°.

Analysis: 10.65 % H, 60.71 % C; calculated for $\text{C}_{10}\text{H}_{21}\text{ON}_3$, 10.55 % H, 60.30 % C; mixed M.P. with semicarbazone of synthetic methylheptyl ketone 117–118°.

Hence alcohol fraction (i) contained methylheptyl carbinol.

(i) *Semicarbazone from 2nd crop (7th crystallisation)* M.P. 121–122°.

Mixed M.P. with semicarbazone of methylamyl ketone 120–121°.

Hence alcohol fraction (i) contained methylamyl carbinol. (Analysis on 0.023 g. gave an unsatisfactory result.)

(ii) *Semicarbazone from 1st crop (8th crystallisation)* M.P. 122–123°.

Analysis: 11.64 % H, 63.10 % C; calculated for $\text{C}_{12}\text{H}_{25}\text{ON}_3$, 11.01 % H, 63.43 % C; mixed M.P. with semicarbazone of methylnonyl ketone 121–122°.

Hence alcohol fraction (ii) contained methylnonyl carbinol.

4. *Ester acids and ester alcohols.*

(i) *Acids.* Neutralisation value, 382.7; mean molecular weight, 146.6. Calculated for caprylic acid *m.wt.* 144.

The barium soap was prepared and crystallised from hot water. 1st crop of crystals, Ba 32.30 %; 2nd crop of crystals, Ba 32.65 %; required for barium caprylate, Ba (C₈H₁₅O₂)₂, Ba 32.45 %.

The esters were, therefore, principally those of caprylic acid.

(ii) *Alcohols.* Owing to the possibility of ethyl esters being present in the rancidity products, the acid phthalic esters were saponified with methyl alcoholic potash (ester alcohol separation). This precaution was justified, for the quantity of alcohols insoluble in water (0.3 g.) was too small to account for the ester acids found. The aqueous liquid containing methyl alcohol and any soluble alcohols was neutralised and submitted to several successive distillations until a concentrated alcoholic distillate was obtained. Ethyl alcohol was identified in this distillate by the iodoform reaction and by oxidation to acetaldehyde.

The alcohols insoluble in water yielded, on oxidation with chromic acid mixture, a small quantity of ketones. The semicarbazone was prepared, but the quantity was insufficient for purification. The secondary alcohols were probably identical with those present in the free state.

The discovery of ethyl esters suggested the possible presence of free ethyl alcohol, but the method of investigation had precluded its detection. Accordingly, a further 1600 cc. of rancid coconut oil was distilled, and, the ketonic oil being separated, the aqueous distillate at length yielded a concentrated alcoholic fraction. Ethyl alcohol was here definitely identified. It was probably produced by fermentation of the sugar in the medium.

5. *Free acid.* This was added to the fatty acids obtained from the aqueous part of the distillate.

Examination of aqueous part of distillate.

The liquid was made alkaline with sodium hydroxide and evaporated to small bulk. Ammonia was liberated in considerable quantity, showing the presence of ammonium salts. The barium soaps of the fatty acids were prepared and fractionally crystallised from hot water, and by their analysis lauric, capric and caprylic acids were identified. Caproic acid was probably also present, as a final crystallisation product gave a mean molecular weight of 140.8 (caprylic acid *m.wt.* 144). The lower fatty acids, including acetic acid could not be detected.

Examination of the coconut oil from which the volatile rancidity products had been removed.

After the determination of the analytical characteristics of the fat, the free fatty acids were separated and converted to their methyl esters, which were

then fractionally distilled *in vacuo*. The original coconut oil was analysed in the same way.

Table II.

	Fresh oil	Rancid oil	Rancid oil after distillation	The fatty acids separated from rancid oil
Sapon. value	258.9	259.7	258.0	264.5
Iodine value	8.0	8.55	8.4	15.6
Reichert Meissl	7.54	7.81	7.7	5.39
Polenske	16.35	16.70	14.95	11.35
Acetyl No.	1.2	7.7	6.2	—

Composition of the original coconut oil and of the free acids separated from the rancid fat (calculated from fractional distillation of methyl esters):

Table III.

	Original oil %	Separated free acids %
Caproic acid	0.2	—
Caprylic acid	7.2	0.2
Capric acid	10.7	10.4
Lauric acid	48.7	42.5
Myristic acid	17.5	23.9
Palmitic acid	5.4	4.5
Stearic acid	0.8	1.4
Oleic acid	9.0	16.6
	99.5	99.5

The analyses were carried out under the same conditions, but, as the calculations were based on the assumption that each fraction consisted of not more than two esters and oleic ester, the results are useful only for comparison.

The most striking feature of the results is the decreased percentage of caprylic acid, with the corresponding increase of the higher acids. Caprylic acid, therefore, is apparently almost completely consumed by the mould, while the higher acids are attacked either to a less extent or not at all. This is confirmed by the analysis of the ketones from the rancid fat, where methylamyl ketone was found to predominate.

If the glycerides of the various fatty acids of coconut oil are split equally by mould lipase (and this is the view of several observers [Jensen, 1902]) it follows that acids higher than lauric acid are not oxidised to ketones. This is confirmed by the analysis of the ketones. It is possible that the higher fatty acids, if absorbed, are partially decomposed according to the complete oxidation scheme suggested for *Oidium lactis*.

II. PHYSIOLOGICAL EXPERIMENTS WITH *PENICILLIUM PALITANS* AND *OIDIUM LACTIS*.

To obtain further information of the selective action of moulds on fatty acids and light on the anomalous behaviour of *Oidium lactis*, various series of experiments were carried out with both organisms under the same conditions. Pure cultures were grown on gelatin—Raulin's solution, containing graduated

proportions of pure fatty acids, secondary alcohols, methyl ketones and keto-esters (prepared by the acetoacetic ester synthesis). The products formed after seven days' incubation at 20° were obtained by steam-distilling the cultures and extracting the aqueous distillate with ether and were examined. Ketones were tested for by means of the *p*-nitrophenylhydrazine or the semicarbazide reaction.

(a) *Action of the moulds on pure fatty acids.*

Table IV.

Acid	<i>P. palitans</i>		<i>O. lactis</i>	
	Quantity inhibiting growth %	Ketone formation	Quantity inhibiting growth %	Ketone formation
Caproic	>0.10	+	>0.04	—
Caprylic	>0.04	+	no growth at 0.025	—
Capric	>0.8	+	>0.20	—
Lauric	>25	+	>25	—
Myristic	>25	?	>25	—
Palmitic	>25	—	>25	—
Stearic	>25	—	>25	—
Butyric	>0.04	—	>0.5	—

In the experiments with *Penicillium*, in which growth took place, the mould remained white, with little conidia formation, the mycelium and hyphae tending to grow into the air. The results support the earlier conclusion, that only acids up to lauric acid are oxidised to ketones. The poisonous character of the acids was clearly demonstrated. Caprylic acid exerted the greatest effect; the higher acids affected the organism very little, if at all, this being due probably to their non-absorption on the mycelium.

The results for *O. lactis* confirm the author's previous work. It would seem that the lower fatty acids are more poisonous to *O. lactis* than to *Penicillium*. The case of butyric acid, where the order is reversed, is noteworthy, and it may be that, its normal habitat being milk and butter, *O. lactis* has acquired tolerance towards butyric acid.

(b) *Action of the moulds on secondary alcohols.*

Table V.

Secondary alcohol	<i>P. palitans</i>		<i>O. lactis</i>	
	Quantity inhibiting growth %	Ketone formation	Quantity inhibiting growth %	Ketone formation
Methylamyl	>0.8	+ semicarbazone M.P. 122°	0.1	—
Methylheptyl	>2.0	+ semicarbazone M.P. 117°	0.2	—
Methylnonyl	>4.0	+ semicarbazone M.P. 121°	2.0	—

The results support the idea that secondary alcohols may be intermediate products in the oxidation of fatty acids by moulds of the *Penicillium* genus.

P. palitans exerts a powerful oxidising action on the alcohols, which exhibit little poisonous action. *O. lactis*, on the other hand, suffers a marked poisonous effect, and oxidation, where it occurs, is presumably more complete.

(c) *Action of the moulds on methyl ketones.*

Table VI.

Ketone	<i>P. palitans</i>	<i>O. lactis</i>
	Quantity inhibiting growth %	Quantity inhibiting growth %
Methylamyl	>0.2	>0.1
Methylheptyl	>0.8	>0.1
Methylnonyl	>2.0	>0.2

The two organisms show a great difference in their behaviour. *Penicillium* can withstand considerable quantities of methyl ketones, which it partially destroys; *Oidium* very little.

(d) *Action of the moulds on keto-esters.*

The cultures after incubation were examined for ketones and acidity. Although the acidity figures were checked against blank experiments, they cannot be taken as reliable, as more or less acid may be consumed by the mould, or neutralised by formation of ammonium compounds.

Table VII.

Keto-ester	<i>P. palitans</i>			<i>O. lactis</i>		
	Quantity inhibiting growth %	Ketone formation	Acidity N/10 NaOH cc.	Quantity inhibiting growth %	Ketone formation	Acidity N/10 NaOH cc.
Acetoacetic ester	>0.2	—	3.0	>0.4	—	0.4
Butylacetoacetic ester	>0.4	—	2.0	>0.4	—	0.6
Amylacetoacetic ester	>1	—	1.9	>2.0	—	4.1

Since in none of the cultures were ketones detected, while the production of acid was evident, it would seem that both organisms decompose the keto-esters by acid hydrolysis. The lower acidity figures and the negative result for *O. lactis* must be explained by its greater capacity for producing ammonia. Keto-esters (or perhaps the fatty acids produced by their decomposition) are poisonous to the organisms. This was confirmed by the appearance of the cultures; growth, at first vigorous, became more and more feeble, both organisms tending to throw out aerial hyphae. In the *Penicillium* experiments conidia formation was observed only in the cultures with the smallest ester concentration; in the other cultures the mycelium remained white.

A similar set of experiments, with sterilised milk as medium, also showed acid formation by both organisms; especially by *O. lactis*, which in some cases produced an acidity equivalent to 20 cc. N/10 NaOH in the culture.

The normal process of decomposition of keto-esters (keto-acids) is apparently the same for both organisms; ketonic fission must be abnormal.

Further experiments were conducted with 25 % beef-fat emulsified with the medium. Beef-fat, consisting essentially of the glycerides of palmitic, stearic and oleic acids, does not yield ketones under mould action; this was confirmed by the blank experiments, where no keto-esters were used.

Action of the moulds on keto-esters in presence of 25 % beef-fat.

Table VIII.

Keto-ester	<i>P. palitans</i>		<i>O. lactis</i>	
	Quantity inhibiting growth %	Ketone formation	Quantity inhibiting growth %	Ketone formation
Blank	—	—	—	—
Acetoacetic ester	0.4	+	0.4	—
Butylacetoacetic ester	2.0	+	2.0	—
Amylacetoacetic ester	2.0	+	2.0	—

Identical results were obtained, using palmitic acid in place of beef-fat.

The positive reactions for ketones were quite distinct but the quantities produced were evidently small. The reason is probably that the beef fatty acids, formed by the original hydrolysis, have a lesser clogging effect on the mycelium than the volatile acids.

The experiments show that with *Penicillium*, but not with *Oidium*, a medium containing fat or fatty acid, and therefore hindering respiration, causes ketonic decomposition of the keto-ester. This explains the difference in the behaviour of the organisms towards fats. The fatty acids (up to lauric acid) formed by hydrolysis are absorbed or adsorbed on the mycelium of the organism and, being poisonous, are oxidised by excited oxidase secretion to keto-acids, which tend to split up to methyl ketones. The ketones are still deleterious to the *Penicillium*, though to a lesser extent than the fatty acids. To *Oidium*, on the other hand, they are specially harmful; but, possessing more powerful enzymic properties, *Oidium* is able to restrain the tendency of the keto-acids to split off CO₂ and to hydrolyse them to lower fatty acids and acetic acid, which are less harmful than ketones. Acetic acid is utilised by the organism; thus the fat is gradually decomposed and consumed.

This theory, though not without fault, seems to be in best accord with the experimental data. It is in agreement moreover, with the properties of *O. lactis* in its natural habitat, milk. The organism prefers an acid medium, the acid being of low carbon content (lactic acid), and in butter-fat finds a fat admirably suited to its peculiarities; the highly poisonous acids, caproic and caprylic, are present in small quantity, while butyric acid is, as experiment showed, readily utilised as a source of energy. *Penicillium*, as it would be expected, produces only traces of ketones in butter-fat.

III. THE DAKIN SYNTHESIS WITH SPECIAL REFERENCE TO SECONDARY ALCOHOLS AS INTERMEDIATE PRODUCTS.

100 g. of the fatty acids of coconut oil were neutralised with ammonia and a slight excess of ammonia added. After boiling with a large excess of hydrogen peroxide for 12 hours under a reflux condenser, the mixture was distilled. The ketonic products were extracted from the distillate with ether and, after treatment by Haller's method for the separation of alcohols, yielded 5-6 g. ketones and 0.5 g. of a dark-coloured oil. Oxidation of this oil in the cold by chromic acid mixture produced a few drops of liquid which reacted with semicarbazide hydrochloride.

Further experiments were conducted with (a) 30 g. caprylic acid, (b) 41 g. capric acid and (c) 220 g. lauric acid. In (a) and (b) only a few drops of alcohols were obtained; in (c) however 0.33 g. resulted, giving 0.108 g. on oxidation. In all experiments the presence of ketone was detected in the products of oxidation of the alcohols, and in (c) the semicarbazone was prepared and crystallised from alcohol: 2nd crystallisation, M.P. 121-122°; semicarbazone of methylonyl ketone, M.P. 122-123°; mixed M.P. 120°.

The ketone in (c) was therefore methylonyl ketone corresponding to methylonyl carbinol.

The ketones, from which the alcohols had been separated, were identified as methylamyl, methylheptyl and methylonyl respectively, thus confirming Dakin's work.

The results appear to indicate that secondary alcohols are intermediate products in the oxidation of fatty acids, but the evidence is not convincing. The identification of secondary alcohols by oxidation to the same ketones from which they were originally separated is certainly a weak point; the yield, however, was too small for such a test as the pseudo-nitrol reaction. Again, the fact that *Penicillium* oxidises secondary alcohols to ketones cannot be taken as proof that they are intermediate products. But if not intermediate products, they must be produced by reduction of ketones; that is, the mould must, at a later stage, probably when narcotised by the poison, reduce, not oxidise. Possibly it seeks for a fresh source of oxygen, by freeing a reducing substance (hydrogen?) which reacts with the ketone. In this case it would be expected that a pinacone would also be formed, but this, though specially looked for, could not be found. In the absence of further evidence, therefore, and on the data of the foregoing work, the author explains the course of the oxidation of fatty acids by moulds thus:

THE "ÉCHAPPÉES" OF HALLER AND LASSIEUR.

The work of Haller and Lassieur was repeated and extended, substances being found additional to those recorded by Haller and Lassieur, and it is proposed, therefore, to make this work the subject of a separate paper.

SUMMARY.

1. The rancidity of coconut oil, caused by a typical *Penicillium* organism, is due essentially to the presence of methylamyl, methylheptyl and methyl-nonyl ketones. Methylamyl ketone occurs in the greatest quantity and is responsible for the characteristic "perfume" odour of the rancid oil. There are also present secondary alcohols corresponding to the ketones, ethyl alcohol (probably by fermentation of sugar), esters of the secondary alcohols and ethyl alcohol with caprylic acid (and probably other fatty acids), and free fatty acids.

2. The production of methyl ketones by moulds shows that here, as in the higher animals and man, oxidation of a chain compound takes place primarily at the β -carbon atom, with formation of a keto-acid.

3. The normal course of the decomposition of the keto-acid by moulds is the formation of a fatty acid containing two carbon atoms less, and acetic acid; but in the case of *Penicillium* the absorption of poisonous fatty acids on the mycelium impedes respiration, and, an abnormal condition being induced, the keto-acid is decomposed to methyl ketone and carbon dioxide.

4. The poisoning capacity of the fatty acids towards *Penicillium* increases with the molecular weight up to caprylic acid, then decreases. Only acids up to lauric acid are absorbed; consequently, ketones of higher molecular weight than methyl-nonyl ketone are not formed.

5. The fatty acids with the exception of butyric acid and perhaps the lower acids, are more poisonous to *O. lactis* than to *Penicillium*. *Oidium*, nevertheless, does not induce ketonic fission of the keto-acid, its greater enzymic activity making possible the normal decomposition to a lower acid and acetic acid.

6. Secondary alcohols are probably intermediate products of the mould oxidation, but the experimental evidence is not yet conclusive.

7. Dakin's oxidation of saturated fatty acids has been repeated, but the formation of secondary alcohols as intermediate products could not be satisfactorily demonstrated.

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