Action of Microorganisms on Fats and Oils: Oleic Acid

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Abstract

Fungi were cultivated on media containing oleic acid and the amount of residual determined. Ketone formation was demonstrated. None of the cultures exhibited marked destruction of the oleic acid or greatly changed the iodine and saponification numbers.

Mixed cultures of bacteria under anaerobic and aerobic conditions and pure cultures of *Aerobacter aerogenes* attacked oleic acid slightly, but the recovered acid showed little change. *Clostridium acetobutylicum* reduced oleic acid to a slight extent at the double bond and at the carboxyl group.

IN surveying the literature on the activities of microorganisms papers dealing with fats and higher fatty acids as substrates seem almost rare in comparison with the mass of published information on carbohydrate decomposition. Many papers discuss bacterial or fungal hydrolysis of fats or growth on fats or fatty acids as a sole source of carbon, but rarely has the extent of decomposition or identification of products been determined. Primary obstacles to such work are the relatively few microorganisms capable of producing extensive changes in higher fatty acids and the slow rate of these changes.

In determining if fats are attacked by microorganisms, Rahn[1] suggested slanting a layer of solid fat along the side of a flask and growing microorganisms in a layer of liquid medium in the bottom of the flask. Pitted and eroded areas appear on the surface of the fat.

Quantitative determinations of fat and fatty acid utilization by microorganisms have been reported by a few workers. Rahn[1] found that Penicillium and other molds as well as two unidentified bacilli decomposed palm fat to an extent varying from 8 to 95 per cent. The same organisms oxidized 19 to 57 per cent of the stearic acid and 9 to 42 per cent of the palmitic acid supplied in media.

Of a number of organisms, von Fürth and Schwartz[2] found *B. fluorescens* to be the most active in decomposing the sodium salts of oleic, ricinoleic, stearic and oxystearic acids. Forty-eight per cent of the added oleic acid and a larger percentage of the other fatty acids was oxidized.

Söhngen[3] noted that a number of microorganisms decomposed sodium and calcium salts of fatty acids, benzine, petroleum, and paraffin. Carbon dioxide, water, and traces of volatile acids were the only products found.

Rubner[4] followed the decomposition of butter fat, cod liver oil, and oleic acid in soil over a period of 35 years. At the end of the first year 22.9 per cent of the butter fat, 9.9 per cent of the cod liver oil and 9.7 per cent of the oleic acid had been decomposed. In 12 years the figures had risen to 38.1 per cent and 70 per cent respectively for butter fat and cod liver oil. No figure was given for oleic acid. These last figures remained unchanged after a total time of 35 years.

According to Zikes[5] the growth of bacteria and fungi on fatty materials is possible only when impurities are present. Washed fats and waxes were found to give much slower growth of microorganisms. Using impure waxes Citromyces formed 174 to 214 mgm. of CO_2 in 6 weeks from white wax, yellow wax and paraffin. Jensen and Grettie[6] showed the importance of the presence of small amounts of moisture for the development of bacteria and molds in fats.

Mycobacterium tuberculosis was found by Sédych and Seliber[7] to give decomposition of olive oil and triolein to the extent of 4 to 47 per cent. Pigulewski and Charik[8] also used olive oil as a substrate for the growth of bacteria. Purple bacteria attacked the oil to a slight extent, but a bacillus destroyed 26 per cent more than was lost in the control. The iodine, acid, and ester numbers of the oil showed light decreases.

Haag[9] demonstrated oxidation by a number of organisms of oleic, palmitic, and stearic acids, triolein, tripalmitin, tristearin and tributyrin. Oleic acid in general seemed to be most susceptible to attack.

Penicillium glaucum growing on triolein produced an increase in the acid and hydroxy numbers, but little change in the other constants (Oeffner[10]).

The action of microorganisms on fats may be classified according to Jensen and Grettie[11] and later by Horowitz-Wlassowa and Livschitz[12] as either lipolytic or oxidative. Jensen and Grettie[6] have extended this classification to include: (1) oxidative rancidity caused by organisms producing lipase and peroxidase, (2) lipolysis by those producing lipase alone, (3) oxidative changes (tallowiness), and (4) production of small amounts of flavor materials. von Fürth and Schwartz[2] concluded in the absence of residual acids, ketones, aldehydes or alcohols after bacterial oxidation of fatty acid salts that the process was one of intracellular oxidation.

Products of the decomposition of fats by microorganisms may be very simple. Anaerobic decomposition of fatty acids to and including C_{18} acids gives carbon dioxide, methane, and small quantities of lower volatile fatty acids according to Neave and Buswell[13] and Tarwin and Buswell[14]. Volatile acids have also been recovered by Rahn[1], fatty acids and aldehydes by Seduikh[15], and ketostearic acid by Pigulewski and Charik[8]. Ketones have been reported by a number of workers. Derx[16] states that methyl ketones are produced from fatty acids by the action of fungi, but are formed only under unfavorable conditions. Stärkle[17] identified the methyl ketones from *Penicillium glaucum* cultures on different fatty acids as follows: methyl n-propyl ketone from caproic acid, methyl n-amyl from caprylic, methyl n-heptyl from capric, and methyl n-nonyl from lauric acid. No ketone was obtained from myristic acid. Täufel, Thaler, and Löweneck[18] report that methyl ketone formation took place when lauric acid was acted upon by *Penicillium glaucum* and *Aspergillus nigar*, but no ketones were produced from oleic, palmitic or stearic acids. That the result with oleic acid is due to peculiarities of the long carbon chain rather than the double bond was demonstrated, they believe, by the fact that formation of methylketone from undecenylic (1) (11) acid occurred. Acklin[19], investigating the mechanism of ketone formation obtained no ketones from butyric or

B-hydroxybutyric acids, but found that B-hydroxycaproic acid was a source of methyl ketone. A number of ketones were isolated by Stokoe[20] from coconut oil medium on which *Penicillium palitans* had grown. Methyl n-amyl, methyl n-heptyl, and methyl n-nonyl ketones as well as methyl n-heptyl and methyl n-nonyl carbinols were identified. Small quantities of unidentified alcohols and ketones were also present.

EXPERIMENTAL

In a number of experiments fatty acids, usually oleic, were exposed to the action of molds and bacteria under conditions favorable for the growth of these organisms. The fatty acid was then recovered and the extent to which it had been changed was determined.

Molds capable of attacking long-chain fatty acids were isolated from soil, river mud, and manure by inoculating suspensions of these materials onto agar medium in which oleic acid had been emulsified. The medium used in these experiments contained 0.2 per cent $NaNO_3$, 0.1 per cent KH_2PO_4, 0.05 per cent $MgSO_4$, 0.05 per cent KCl, and a trace of $FeSO_4$. When solid medium was required as in this case 1.5 per cent agar was also added. Oleic acid was added to the extent of 1.0 per cent to the melted agar, emulsified by shaking, and plates poured at once. The oleic acid emulsion plates inoculated with material from various sources yielded eight different molds. Incubation of mold cultures was at about 30° C.

Each of the eight molds was tested for action on oleic and stearic acids by inoculating spores into flasks in which a shallow layer of asbestos fibre had been placed together with 25 cc. of mineral salt solution, 0.3 gm. of tricalcium phosphate and 25 gm. of oleic or stearic acid. In 6 days, a slight surface growth was apparent, and after 20 days, when the analyses were made, heavy mats of mold mycelium were present in the flasks. Recovery of the residual fatty acids was effected by acidifying the contents of the flasks with sulfuric acid, evaporating to dryness and extracting with petroleum ether. The residues after evaporation of the solvents were weighed, and the percentage recoveries given in Table 1. The fungi utilized very little oleic or stearic acid.

Mold cultures 2, 6, and 8 were used for further experiments. In

TABLE 1
UTILIZATION OF OLEIC AND STEARIC ACID BY FUNGI

Mold culture number	Oleic acid per cent recovered	Stearic acid per-cent recovered
1	94.0	94.0
2	89.5	92.1
3	94.0	96.0
4	84.0	89.3
5	80.0	84.8
6	78.3	93.7
7	98.5	100.0
8	84.0	78.0

TABLE 2
UTILIZATION OF OLEIC ACID IN INORGANIC AND ORGANIC MEDIA BY FUNGI

Mold culture number	Nitrate medium on sand per cent oleic acid-recovered	Peptone medium on glass wool per cent oleic acid recovered
2	85	85
		+$NaHSO_3$ 90
6	95	86
8	88	82

TABLE 3
UTILIZATION OF EMULSIFIED OLEIC ACID BY FUNGI

Mold culture	Incubation period	Oleic acid recovered	Constants of recovered oleic acid: Iodine number	Constants of recovered oleic acid: Saponification number
number		per cent		
Uninoculated	—	95	89.3	190.4
2	2 weeks	91	91.1	189.3
	4 "	83	90.6	186.1
	6 "	80	94.3	185.9
	8 "	79	85.0	185.0
6	2 weeks	86	94.5	189.0
	4 "	81	97.3	185.0
	6 "	73	97.4	180.2
	8 "	69	95.2	179.4
8	2 weeks	91	90.2	188.3
	4 "	89	90.5	188.0
	6 "	89	90.3	187.4
	8 "	88	91.8	186.9

TABLE 4
UTILIZATION OF EMULSIFIED OLEIC ACID BY MIXED CULTURES OF BACTERIA

Inoculum and air relations	Incubation period	Oleic acid recovered	Constants of recovered oleic acid: Iodine number	Constants of recovered oleic acid: Saponification number
		per cent		
Uninoculated	—	98	89.5	191.3
Soil				
Aerobic	4 weeks	75	95.1	189.2
"	8 "	59	94.3	189.0
Anaerobic	4 "	95	89.3	190.4
"	8 "	91	88.9	191.5
River water				
Aerobic	4 weeks	87	93.3	192.3
"	8 "	68	94.9	195.0
Anaerobic	4 "	90	91.5	190.3
"	8 "	89	91.0	185.7

TABLE 5
ACTION OF BACTERIA ON OLEIC ACID

Treatment and constants		Clostridiun acetobutylicum	Aerobacter aerogenes
Oleic acid emulsified with sodium hydroxide			
Iodine number		79.7	86.8
Saponification number		177	189.6
Oleic acid emulsified with gum arabic			
Iodine number		83.4	84.3
Saponification number		173.3	188.0
Original Oleic acid			
Iodine number	89.3		
Saponification number	193.9		

one test 900 gm. of sand and 100 gm. of oleic acid mixed thoroughly in Fernbach flasks and 112 cc. of mineral nutrient solution then added were inoculated with spore suspensions. In three days surface growth was evident and a ketone odor was noted. Water extracts of the sand substrate gave traces of precipitate with 2, 4-dinitrophenylhydrazine. After an incubation period of 13 days, the contents of the flasks were mixed by grinding and an aliquot used for recovery of oleic acid. The results are given in Table 2. Iodine and saponification numbers of the recovered oleic acid were insignificantly different from those values for the original acid.

In a second experiment with molds 2, 6, and 8 organic nitrogen was made available to encourage more vigorous growth. The molds were grown in Fernbach flasks on one inch pads of glass wool. As nutrient 100 gm. of oleic acid and 100 cc. of the mineral salt solution with 0.5 per cent peptone added were supplied. To one of the flasks inoculated with mold number 2, 20 gm. of sodium bisulfite were added to fix methyl ketones as they were produced in an attempt to prevent further oxidation. Incubation of the flasks was at 37° C. Good mycelial pads had developed after eight days. At this time a definite ketone odor was apparent in the flask containing the sodium bisulfite. After 21 days incubation, the contents of the flasks were treated for recovery of the oleic acid. The water solution in the flask containing sodium bisulfite gave a purple color with sodium nitroprusside, no precipitate with dimedon (5, 5-dimethylcyclohexanedion-1, 3), and a precipitate with 2, 4-dinitrophenylhydrazine. These tests indicated the presence of ketones. The dinitrophenylhydrazone was derived from a mixture of ketones as indicated by the range of its melting point (75-81° C.). Insufficient amount of the hydrazone was obtained for purification and identification. Thus, small amounts of ketones are formed from oleic acid though other workers[17] failed to detect them. The saponification and iodine numbers of the recovered oleic acid were very close to those of the original acid. The percentages of oleic acid recovered are given in Table 2.

It was hoped that emulsification of the oleic acid would facilitate attack by fungi. 2.78 per cent gum arabic was dissolved in the mineral nutrient solution and to 90 cc. of solution, 10 gm. of oleic acid added with shaking. This suspension was run through a hand homogenizer and sterilized by autoclaving. The emulsion was very stable, and even after autoclaving only a few drops of oleic acid had risen to the surface. Inoculation of the flasks were made with spore suspensions of molds 2, 6, and 8. After incubating for 2, 4, 6, and 8 weeks flasks were taken for analysis, the residual oleic acid determined as also the extent to which the iodine and saponification numbers had changed. Table 3 gives the complete results. After 4 weeks there was no appreciable utilization of oleic acid by cultures 2 and 8, but culture 6 did oxidize more oleic acid from the 4th to the 8th week. However, the amount of fatty acids utilized in this or the other experiments reported here was very unpromising. The iodine and saponification numbers of the recovered oleic acid indicated very little conversion to other compounds.

The action of bacteria on oleic acid was tested by inoculating emulsions prepared with sodium hydroxide or gum arabic. In the first experiment mixed cultures of bacteria were employed, and in others pure cultures of bacteria possessing strong reducing activity. The incubation temperature was 37° C.

As inoculums for crude or mixed cultures, soil and river water were added to flasks of the gum arabic-oleic acid emulsion. Two flasks receiving each inoculum were incubated anaerobically and two flasks aerobically to compare the change produced in the oleic acid under these two conditions. Mold developed on the surface of the aerobic cultures. 4 and 8 weeks after inoculating, oleic acid was recovered for determination of the iodine and saponification numbers and the amount of acid remaining. Table 4 gives the results. While the aerobic cultures showed appreciable utilization of the oleic acid, the anaerobic cultures were much less active. The constants of the recovered oleic acid were not greatly different from those of the original acid.

The ability of Clostridia of the acetone-butyl alcohol forming group to reduce acetic, propionic, and butyric acids suggested a trial of their activity in reducing higher fatty acids. In the first experiment 40 gm. of oleic acid emulsified in water containing 5 gm. of sodium hydroxide was added to an actively fermenting corn mash culture of *Clostridium acetobutylicum*. Analyses were made on the culture after 4 days. As considerable separation of oleic acid had taken place, a second experiment was set up in which a gum arabic emulsion was added to medium containing 2 per cent glucose, 0.3 per cent K_2HPO_4 and 0.5 per cent peptone. This culture was analyzed after 5 days.

Similar experiments were performed with a culture of *Aerobacter aerogenes* which was able to convert acetic acid to 2, 3-butylene glycol. The oleic acid was added to a glucose-peptone medium after fermentation had begun, in one case emulsified in a sodium oleate-oleic acid mixture and in the other emulsified in gum arabic solution. Analyses were made after 8 days. The results are given in Table 5. *Clostridium acetobutylicum* reduced oleic acid to some extent at the double bond and the carboxyl group while *Aerobacter aerogenes* produced little or no change.

SUMMARY

Cultures of fungi and bacteria were grown on media containing oleic acid. From the fungi cultures 69 and 98 per cent and from the bacterial cultures 59 to 95 per cent of the original amount of oleic acid was recovered with little or no change in the iodine and saponification numbers. Formation of small amounts of ketones from oleic acid by the action of fungi was demonstrated. *Clostridium acetobutylicum* reduced oleic acid to a slight extent.

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