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LIPOLYTIC BACTERIA IN DRY SAUSAGES

I

THE ACTIVITY OF MICROCOCCACEAE ON SWINE FAT

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INTRODUCTION

Good ripening and formation of flavours in dry sausages are the consequence of complex phenomena depending on a number of factors, extrinsic (temperature, humidity, etc.) and intrinsic, correlated with their bacterial flora (nitrate reduction, hydrolysis of proteins, fats, carbohydrates etc.).

Recent investigations performed by us (Giolitti, 1960, Giolitti & Massacra, 1963) and by others (Ten Cate, 1960, Terplan, 1962, Maillet & Henry, 1962, Niinivaara, 1964, Poja & Niinivaara, 1964) have pointed out that microorganisms, and among them the lipolytic ones play an important rôle in the ripening of sausages and in the formation of flavours.

In our former investigations we pointed out that among the microorganisms responsible for the breakdown of neutral fats the Micrococci could be considered most important. With the aim of checking this hypothesis, in a previous paper (Cantoni, Massacra, Renon & Giolitti, 1964) the lipolytic activity on swine fat of Micrococcaceae was investigated (some of the strains tested were isolated from sausages), the most important fatty acids released were identified and a correlation between the amount of free fatty acids and bacterial growth was demonstrated.

Therefore it seemed to us of theoretical and practical interest to investigate more carefully the activity of lipolytic microorganisms on swine fat by identifying not only the fatty acids released by the bacterial lipases, but the products of their breakdown too, taking into consideration the fact that this investigation could contribute to a better knowledge of the substandes involved in the production of flavours and in controlling the bacterial growth during ripening.

METHODS

To this aim three bacterial strains belonging to Micrococcaceae were used: Micrococcus strain D10, Micrococcus strain C13, both isolated from dry sausages and identified as belonging to the Lactis-saprophyticus group according to Baird-Parker, 1961, and Staphylococcus aureus strain 78 ATCC, coagulase positive.

The strains were kept in the following medium:

Peptone g. 5, yeast extract (Difco) g. 5, NaCl g. 20, Agar (Oxoid n. 3) g. 12, distilled water ml 1,000. pH 6,5 to 6,7.

The medium used to detect the action of the strains under examination on swine fat was the following:

Peptone g. 10, yeast extract (Difco) g. 5, NaCl g. 5, distilled water ml 1,000. pH 6,5 to 6,7.

To 1,000 ml of this medium still hot, 100 gr of tragacanth gum were added in order to make the emulsion of fat easier, and 500 ml of melted lard.

The mixture was homogenized in a mixer until a stable emulsion was obtained.

INVESTIGATIONS ON THE C_4 - C_{20} FATTY ACIDS

METHODS: Flasks containing 3,000 ml of the culture medium were inoculated with the growth on agar slants of the different strains incubated for 48 hours at 30°C (Micrococci) and at 37°C (Staphylococcus) suspended in saline to such an amount to have a concentration of 10^7 cells per ml of the liquid medium. The flasks were incubated at 30°C (Micrococci) and 37°C (Staphylococcus) for 28 days.
Samples of the cultures were taken to be analyzed after 4, 8, 12, 16, 20, 24, 28 days of incubation.
The lard used in all experiments was analyzed too, after being saponified, in order to find out its fatty acid composition. For this purpose two methods were used: interesterification (Stoffel, 1959) and metilation in sealed vials with sulphuric acid and methanol (Benassi,1964) using the soaps obtained by treating the fat with methylalcoholic potassium hydroxyde. The gas-chromatographic analysis gave the same results with both methods.

Detection of free fatty acids in the culture medium before being inoculated and during the bacterial growth.

20 ml of the culture medium were extracted with ethyl ether and petrol ether (b.p. 30° - 50°C) 4 : 6 (v/v) in a separatory funnel before being inoculated and at the times already shown after inoculum.
The watery phase was discarded and to the ether phase ethanol with some drops of phenolphtalein were added to 1/1 ratio (v/v). NaOH 4n. was added dropwise until a pink colour appeared and then distilled water until two distinct layers were obtained.
The soap solution was concentrated in a water bath after having washed it with ether in order to eliminate the residual fat, the residue transferred in a capsule, dryed in an oven at 105°C. and weighed.
The esterification was performed with the Benassi's method treating the sodium salts with methanol and sulphuric acid 40 : 60 (w/v) in a sealed vial.
The gas-chromatographic analysis were performed with a flame ionization chromatograph firm "Erba", model D. The operating conditions were: WEAS column (Chromosorb + succinic acid esters) 2 m lenght, column temperature 185°C., injection block temperature 320°C, detector temperature 270°C, pressure in the column 0,65 Kg/cm^2, hydrogen (chromatographic grade) pressure 0,35 Kg/cm^2, oxygen pressure (air, chromatographic grade) 1,0 Kg/cm^2, carrier gas: nitrogen (chromatographic grade), paper speed 100mm per minute. In each analysis the inoculum was of 1 - 2 microliters.
RESULTS The results are shown in table 1; in column A the total fatty acids detected in lard are listed. In column B the free fatty acids present in the culture medium

before being inoculated, and in the others the fatty acids released by the tested strains at the various periods of incubation. All the figures are referred to 100 g. of neutral fat.

DISCUSSION: At it has been previously pointed out (Cantoni et Al., 1964) the Micrococcaceae tested are strongly lipolytic, the most active strain being Micrococcus C13, the lesser one Staphylococcus strain 78 ATCC.

Qualitatively all the strains have given the same fatty acids, differences however are observed in their amounts.

28 fatty acids released by the bacterial lipolytic enzymes have been detected: the lard used contained 22 of them, in the sterilized culture broth only 21 could be shown. As a consequence of the bacterial metabolism, we have observed the appearance of amounts progressively increasing of free fatty acids, among them those with a chain of a number of carbon atoms lower than 8 did not exist in the lard and must be considered as a product of the breakdown of fatty acids with a longer chain. While the total amount of free fatty acids increases with the age of the culture, the percent values of the individual fatty acids shows differences which may depend on the neutral fats structure and from the subsequent utilization of the free fatty acids liberated by the microorganisms.

The fatty acids more easily released are oleic, and to a lesser extent myristic, palmitoleic and linoleic acids; palmitic and stearic acids are released with a greater difficulty.

Among the 28 fatty acids which have been detected, 20 were certainly identified, 8 have not been and are indicated with the symbols X-0,.....X-7. Among them X-2, X-4, X-5, X-7 are present in the lard.

As an hypothesis we suppose that X-4 and X-5 could be the cis and trans isomers of linoleic acid, X-7 could be perhaps identified as arachidonic acid, X-0, X-1, X-3 and X-6 could not be identified.

INVESTIGATIONS ON THE $C_1 - C_5$ FATTY ACIDS

The fatty acids with short carbon atom chain play probably a very important rôle in the ripening of dry sausages. Their antibacterial and flavouring properties may influence the composition of the microflora during ripening and contribute to the flavours formation.

METHODS: 300 ml of the fat containing medium inoculated as previously described were taken after 4, 8, 12, 16, 20, 24, 28 days of incubation; they were made acid with concentrated phosphoric acid to pH 3 and then distilled. The distillate was collected in 10 ml of NaOH 1n.

The sodium salts of the fatty acids were concentrated to a small volume in water bath, transferred in a capsule and dryed in an oven at 105°C.

The gas chromatography was performed with the above mentioned apparatus. Two methods were used for the fatty acids evaluation: the Ralls's method (1960) and another set up by one of us (Molnar, unpublished). With the Ralls's method, which analizes the ethyl esters obtained by pyrolyzing the alcaline salts in the presence of potassium ethylsulphate, we have estimated quantitatively the formic, propionic, butyric, iso=butyric, valeric and isovaleric acids, but not the acetic acid because in the chro=matogramme the ethanol peak, resulting from the decomposition of the ethylsulphate, is superimposed on that of the ethylacetate.

To overcome this difficulty we have used Molnar's method, which is based on the py=rolysis of sodium or potassium salts of fatty acids in the presence of potassium hydrogen sulphate:

$$RCOOK + KHSO_4 = RCOOH + K_2SO_4$$

Using this method we succeeded in obtaining a chromatogramme which allowed us to estimate the acetic acid togeter with the other acids except formic acid which cannot be detected by the flame ionization detector. The amounts of fatty acids which could be detected with both methods were the same and this fact was considered confirmatory for their reciprocal effectiveness.

The operating conditions were:

1) Ethyl esters analysis: Column WEAS, 2m length, column temperature 60°C, injection block temperature 190°C, detector temperature 120°C, hydrogen pressure 0,2 Kg/cm^2, nitrogen pressure 0,36 Kg/cm^2, oxygen pressure (air) 1,0 Kg/cm^2, carrier gas : ni=trogen, paper speed: 100 mm/ minute.

2) Free fatty acids analysis: WEAS column, 2m length, column temperature 120°C, injection block temperature 230°C., detector temperature 170°C, hydrogen pressure 0,30 Kg/cm^2; nitrogen pressure 0,67 Kg/cm^2, oxygen pressure (air) 1,0 Kg/cm^2, car=rier gas: nitrogen, paper speed 100 cm/ minute.

The pyrolysis was performed in an apparatus devised by one of us (Renon) at a tempe=rature of 350°C for 30 seconds.

RESULTS: the results are shown in table 2, where the percentages of free fatty acids (volatile) produced by the strains under examination are tabulated as referred to 100 g of neutral fat.

Micrococcus D10 was the higher producer of volatile fatty acids (from 0,002 to 0,096 mg per cent), Staphylococcus 78 the lower (from 0,002 to 0,022 mg per cent) and Mi=crococcus C13 yielded intermediate values (from 0,002 to 0,033 mg per cent).

The total amount of volatile fatty acids increases with the age of the culture and the highest quantities are produced between the 24 and the 28th day of growth.

DISCUSSION: All the examined strains are able to yield variable amounts of the same volatile fatty acids, among these propionic and butyric acids are produced to a greater extent.

By examining the quantitative behaviour of the C_1 - C_5 fatty acids during the bac= terial growth, it may be observed that their amount increases in connection with the amount of fat which is metabolized, whilst - as it has been already observed for the long chain fatty acids - during the growth of the culture quantitative changes were observed in the individual fatty acids, which are probably due to their utilization by the microorganisms.

The production of volatile fatty acids seems to be of interest for two reasons: in the first place because it shows that the Micrococcaceae are able to carry out the breakdown of long chain fatty acids, in the second place because the amount of these fatty acids increases with the age of the culture.

It may be assumed that in natural conditions they could influence the composition of the bacterial flora of dry sausages during ripening and especially the gram negati= ve by inhibiting it, and the lactobacilli by stimulating their growth because seve= ral fatty acids may act as growth factors for them.

INVESTIGATIONS ON THE CARBONYL COMPOUNDS

As a complement to the investigations on the fatty acids metabolism during the growth of Micrococcaceae, we have considered very important the investigation of the behaviour of carbonyl compounds (aldehydes and ketones) which in preliminary tests appeared to be present in the culture media containing fat, with the aim to complete the knowledge on fat metabolism of these microorganisms.

METHODS: as a preliminary step all the solvents were made free from carbonyls by boiling them under reflux with dinitrophenilhydrazine and hydrochloric acid and then distilled.

For the detection of carbonyl compounds the Ralls's,1960 and Niinivaara and Eser, 1963 methods were used with the following modifications: 100 ml of the fat contai= ning culture medium (the same used in all these investigations) were steam distil= led in an all-glass apparatus and the distillate (300 ml) was collected in a Frese= lius flask containing 75 ml of 2:4-dinitrophenylidrazine 4% in hydrochloric acid 6n (p/v). The distillate was brought to a known volume, one fraction (A) was used for the quantitative analysis of carbonyls; the other (B) was extracted several times with benzene, which after having been washed with a small amount of distilled water was dried in order to obtain the phenylhydrazones which were examined by gas chroma= tography.

For the quantitative analysis of carbonyls the solution (A), containing the dinitro= phenylhydrazones was made alcaline by adding methylalcoholic potassium hydrate, brought to a known volume and examined spectrophotometrically (Beckman DU spectro= photometer) at a wavelength of 480 millimicrons. The results were compared with a standard solution of 2:4-dinitrophenylhydrazone of the butyric aldehyde.

The gas chromatographic analysis of the carbonyl compounds was performed with the apparatus used in the former investigations. Operating conditions: column packed with carbowax 1150 (polyethyleneglicol) 2m length, column temperature 190°C, detector temperature 240°C, column pressure 0,18 Kg/cm^2, hydrogen pressure 0,30 Kg/cm^2, oxygen pressure (air) 1,0 Kg/cm^2, carrier gas, nitrogen., paper speed 100 mm/minute. Pyrolysis : 240°C - 260°C per 6 seconds.

RESULTS: the results are shown in table 3, Micrococcus strain C13 yielded the greatest amount of carbonyl compounds (660 - 1490 micromoles per litre of culture medium), Micrococcus strain D10 (42 - 815 micromoles/litre) and Staphylococcus 78 (70 - 825 micromoles/litre) a lower one. All the strains yielded the same compounds even if in different amounts.

DISCUSSION: All the examined strains of Micrococcaceae yielded a relevant quantity of carbonyl compounds: they vary quantitatively and qualitatively during the growth (28 days) in connection with both production and utilization. A similar phenomenon was observed by us with regard to diacetyl and by other Authors with regard to other metabolic products (Beerens,1952, Eddy,1961).

We were able to recognize 15 compounds, among them 11 have been certainly identified, 4 were not: X-1, X-2, X-3, X-4 (X-2 probably corresponds to the crotonic aldehyde). The major components were isovaleric and propionic aldehydes.

The carbonyl compounds, as a complex medium containing fat and peptone was used, could have originated from different sources: from fatty acids, from aminoacids and from the oxydation of glycerol. In fact the formic, acetic, propionic, butyric, valeric, isovaleric aldehydes etc. may result from the breakdown of the corresponding aminoacids, acrolein from glycerol and the short chain aldehydes from the breakdown of fatty acids.

The origin of acetone, methylketone, isopropylketone is difficult to explain and for the present we do not think to formulate any hypothesis.

INVESTIGATIONS ON DIACETYL AND FORMALDEHYDE

Among the carbonyl compounds detected by gas chromatography we could detect also the presence of diacetyl and formaldehyde, but the flame ionization detector could not allow us to evaluate their amount, therefore we were compelled to resort to other methods.

METHODS: The same strains previously indicated were used, they were grown on the same medium containing fat, but in greater quantities (5,000 ml instead of 3,000). The incubation temperatures were those already indicated.

Analysis were performed after 48 hours and 3, 4, 5, 6, 7, 8, days of growth.

Detection of diacetyl: 500 ml of the culture broth were distilled in an all-glass apparatus, 20 ml of the distillate were collected in 5 ml of hydroxylamine (these

conditions were checked in preliminary experiments), the diacetyl was analyzed with the Lemoigne's et Al.,1952, method, the readings were performed with a BeckmanDU spectrophotometer at a wavelength of 502 millimicrons.

Detection of formaldehyde: 500 ml of the culture broth were distilled in an all-glass apparatus and 50 ml of the distillate were collected in 5 ml of iced water in an ice-bath. The formaldehyde was determined by the chromotropic acid method (Schryver, 1909) Readings were performed at a wavelength of 570 millimicrons.

RESULTS: Results are shown in table 4. All the tested strains produced diacetyl in amounts varying from 0.30 to 1.8 mg /litre, all the strains produced formaldehyde in amounts varying from 1.0 to 6.0 mg/litre.

DISCUSSION: The presence of diacetyl and formaldehyde in the culture media where Micrococcaceae had grown seems to us rather interesting: generally it is believed that diacetyl originates mainly from carbohydrates, to have detected it in a medium devoid of them confirms the hypothesis that it may originates also from fat metabo= lism. Its appearance follows the formation of fatty acids with different chain lengths and saturation bonds. It may be assumed that also in sausages diacetyl may be produced.

As regard to the formaldehyde, we are lacking informations about its biogenesis, although it seems that it may originate from glycine: in support of this hypothesis however we were not able to find any experimental data.

It is of interest to notice that the Micrococcus strain C13, which is the stronger lipolytic yields the most considerable amount of diacetyl and formaldehyde, this fact leads to a possible correlation between the breakdown of fats and the produc= tion of volatile compounds.

If the production of formaldehyde is demonstrated also in sausages, its presence could contribute to flavours production and to the composition of the microbial flo= ra togeter with some other products of the metabolism of Micrococcaceae among those which have been examined in this paper.

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Table 1.

Percent of free fatty acids (C-5- C -20) released by Micrococcaceae from swine fat.

Fatty acids	MICROCOCCUS C 13							MICROCOCCUS D 10							STAPHYLOCOCCUS SP 28							A	B
	4	8	12	16	20	24	28	4	8	12	16	20	24	28	4	8	12	16	20	24	28		
C-5	0,06	0,12	0,07	0,07	0,05	0,09	0,05	0,03	0,07	0,05	0,10	0,10	0,10	0,09	0,02	0,03	0,04	0,84	0,05	0,03	0,04	0,000	0,000
C-6	0,02	0,13	0,01	0,03	0,04	0,03	0,04	0,02	0,02	0,01	0,01	0,04	0,07	0,02	0,01	0,01	0,01	0,24	0,01	0,01	0,01	0,000	0,000
C-7	0,01	0,27	0,05	0,04	0,19	0,11	0,12	0,04	0,05	0,02	0,02	0,05	0,13	0,09	0,06	0,05	0,04	0,43	0,04	0,05	0,05	0,000	0,000
C-8	0,02	0,08	0,02	0,02	0,06	0,07	0,05	0,02	0,02	0,01	0,02	0,03	0,03	0,02	0,02	0,02	0,02	0,23	0,01	0,01	0,02	0,000	Tr.
C-9	0,10	0,19	0,06	0,06	0,16	0,16	0,10	0,02	0,06	0,03	0,04	0,05	0,16	0,09	0,06	0,06	0,05	0,80	0,04	0,05	0,04	Tr.	0,000
C-10	0,05	0,06	0,02	0,01	0,08	0,04	0,11	0,02	0,03	0,02	0,02	0,03	0,06	0,04	0,03	0,03	0,02	0,22	0,02	0,03	0,01	0,000	Tr.
C-11	0,26	0,29	0,13	0,13	0,13	0,26	0,15	0,07	0,09	0,08	0,10	0,11	0,14	0,12	0,09	0,08	0,12	0,25	0,07	0,05	0,10	0,04	0,06
C-12	0,02	0,02	0,01	0,01	0,02	0,01	0,05	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,47	0,05	0,01	0,01	Tr.	0,000
C-13	0,20	0,25	0,13	0,11	0,10	0,21	0,13	0,09	0,09	0,08	0,11	0,12	0,12	0,12	0,10	0,10	0,13	0,27	0,09	0,06	0,09	0,05	0,07
C-14	0,01	0,05	0,03	0,01	0,01	0,02	0,01	0,01	0,01	0,01	0,01	Tr.	0,01	0,01	0,01	0,01	0,01	0,20	0,01	0,01	0,01	Tr.	Tr.
C-15	Tr.	0,03	0,02	0,01	Tr.	0,01	Tr.	Tr.	0,01	Tr.	Tr.	0,01	0,01	Tr.	Tr.	0,02	0,35	0,01	Tr.	Tr.	Tr.	0,000	0,000
C-16	1,92	2,02	1,67	1,25	1,33	1,90	1,60	1,32	1,55	1,20	1,36	1,41	1,47	1,48	1,11	1,10	1,73	1,19	1,36	1,13	1,42	0,99	0,34
C-17	0,03	0,06	0,03	0,01	0,05	0,06	0,07	0,05	0,06	0,04	0,09	0,05	0,03	0,03	0,06	0,06	0,05	0,39	0,06	0,04	0,04	0,03	Tr.
C-18	0,04	0,06	0,05	0,02	0,03	0,07	0,12	0,04	0,05	0,02	0,03	0,03	0,04	0,03	0,03	0,03	0,04	0,10	0,01	0,01	0,02	0,02	0,03
C-19	Tr.	0,01	0,02	0,01	Tr.	0,01	0,03	0,02	0,01	0,01	0,01	0,01	0,01	Tr.	Tr.	0,01	0,01	0,05	0,02	0,01	0,01	0,000	Tr.
C-20	21,76	16,69	23,12	13,95	18,55	18,83	21,25	23,81	24,28	17,57	14,07	15,40	14,97	12,58	14,85	13,37	26,02	17,13	20,11	19,10	17,61	23,79	22,65
C-21	2,51	2,96	2,45	2,34	2,27	3,00	2,74	2,62	2,57	2,18	2,74	2,00	2,54	3,29	2,06	1,83	3,01	3,66	2,35	1,72	2,34	1,78	3,05
C-22	0,09	0,06	0,13	0,08	Tr.	0,07	0,17	0,25	0,29	0,27	0,15	0,03	0,11	0,03	0,10	0,06	0,24	0,30	0,09	0,10	0,12	0,14	Tr.
C-23	Tr.	0,06	0,01	Tr.	Tr.	0,05	0,03	0,00	Tr.	Tr.	Tr.	Tr.	0,03	0,08	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	0,000	0,000
C-24	0,19	0,19	0,24	0,21	0,24	0,09	0,17	0,19	0,26	0,15	0,24	0,10	0,20	0,10	0,25	0,26	0,27	0,63	0,22	0,24	0,21	0,14	0,28
C-25	8,12	7,13	11,12	8,48	6,85	7,68	9,97	10,43	11,28	7,54	11,86	12,15	9,55	5,87	9,52	10,42	12,22	7,89	10,10	16,23	9,16	14,69	12,11
C-26	51,55	56,01	48,75	58,67	55,80	55,80	51,68	47,82	46,65	62,25	55,40	56,18	58,77	62,69	59,89	59,19	45,91	51,74	52,93	51,37	56,36	46,74	50,60
C-27	10,44	11,71	9,50	13,37	8,71	8,66	9,11	10,61	10,14	7,43	11,30	10,02	7,59	10,07	9,38	10,60	7,72	7,90	10,14	6,90	10,48	9,89	8,90
C-28	0,05	0,03	0,09	0,02	0,45	0,09	0,17	0,10	0,11	Tr.	0,05	0,04	0,12	0,03	0,21	0,23	0,14	Tr.	0,10	0,54	0,09	0,14	0,02
C-29	0,44	0,45	0,37	0,20	0,99	0,68	0,50	0,31	0,26	0,22	0,29	0,23	0,26	0,45	0,46	0,53	0,35	0,29	0,41	1,90	0,30	0,21	0,16
C-30	0,98	0,58	1,31	0,51	1,90	0,87	1,16	1,20	1,10	0,53	1,35	1,25	1,94	1,49	1,03	1,12	0,90	2,37	1,04	1,00	1,11	1,01	0,83
C-31	0,75	0,33	0,30	0,14	1,13	0,73	0,36	0,40	0,57	0,06	0,33	0,25	0,99	0,78	0,35	0,36	0,39	1,09	0,25	0,75	0,18	0,000	0,90
C-32	0,36	0,18	0,30	0,21	0,85	0,31	0,17	0,50	0,37	0,10	0,46	0,31	0,56	0,38	0,42	0,39	0,53	0,96	0,41	0,52	0,24	0,34	0,000
TOTAL	12,6	2092	21,9	86,5	26,6	27,4	45,2	2,1	3,1	4,6	10,4	18,8	20,2	23,1	2,0	4,2	4,5	9,1	9,7	10,5	17,5	100	0,34

A) Fatty acids calculated for 100gr.of fat

B) Fatty acids released from swuine fat after saponification

Free fatty acids in the culture medium

TABLE 2. Percent of free Fatty Acids (C_1 - C_5) prpduced by Micrococcaceae from swine fat breakdown.

	Micrococcus C 13							Micrococcus D 10							Staphylococcus St 78							
Fatty acids:	4	8	12	16	20	24	28	4	8	12	16	20	24	28	4	8	12	16	20	24	28	Blanc
formic	4,1	3,7	2,5	5	2,5	5	1,9	1,5	1,4	3,1	1,5	1,3	1,0	1,0	2,5	5,9	4,5	2,2	1,0	3,0	5,0	absent
acetic	16,5	18,5	25,9	7,9	15,3	12,2	10,7	22,1	40,6	16,7	11,8	9,0	8,7	6,9	19,0	29,5	24,1	35,3	27,1	30,0	21,9	"
propionic	72,5	68,5	62,9	75,4	74,2	73,7	82,4	69,2	40,8	73,2	85,9	89,0	89,7	91,8	71,9	61,4	58,3	53,8	58,0	50,0	42,4	"
Tecbutyric	0,1	0,5	0,1	0,1	0,3	0,1	0,2	0,3	0,1	tr.	tr.	tr.	tr.	tr.	0,2	0,1	0,1	0,3	0,1	0,6	1,0	"
butyryc	5,9	5;9	6,1	10,6	7,2	8,2	4,4	5,1	12,7	5,2	0,6	0,5	0,5	0,3	3,9	1,3	9,6	7,3	9,2	15,9	28,3	"
isovaleric	0,1	3,0	1,7	0,5	0,3	0,3	0,3	1,5	3,1	1,3	0,1	0,1	0,1	tr.	1,8	1,2	2,0	1,0	1,1	1,3	1,3	"
valeric	0,2	Tr.	0,5	0,2	0,2	0,2	0,1	0,3	1,0	0,5	0,1	0,1	tr.	tr.	0,5	0,7	1,1	0,1	0,2	0,2	0,2	"
P.p.m.	6	9	9	9	30	65	150	6	6	6	15	120	75	260	6	35	6	6	6	60	35	
% weigth (+)	0,02	0,003	0,002	0,003	0,01	0,023	0,050	0,002	0,002	0,002	0,015	0,06	0,025	0,087	0,002	0,012	0,002	0,002	0,002	0,020	0,012	

(+) per cent Weigth from gr.100 of fat

TABLE N.3. Carbonyl compounds produced during swine fat breakdown by Micrococcaceae.

	Micrococcus C 13						Micrococcus D 10						Staphylococcccus St 78					
	Days of growth						Days of growth						Days of growth					
	4	8	12	16	20	24	4	8	12	16	20	24	4	8	12n	16	20	24
Acetaldehyde	4,8	6,6	10,8	11,8	14,3	3,5	1,5	1,6	7,9	1,3	18,6	5,4	1,5	1,0	1,7	11,5	1,8	17,8
X -1	1,3	2,1	3,4	2,6	1,4	0,8	0,4	Tr	4,2	0,4	3,8	0,1	0,7	0,6	0,8	0,7	2,5	3,2
Propionald.	12,2	14,5	21,2	9,3	8,3	10,0	2,5	64,2	33,1	4,9	8,6	58,6	3,7	24,8	31,7	1,1	4,1	10,1
Aceton+ I-but.	12,6	10,5	4,6	3,0	7,3	0,8	9,1	1,1	7,6	3,2	6,0	1,7	14,8	5,3	3,1	0,9	2,4	1,5
Acrolein	2,1	2,3	2,1	1,0	3,3	0,8	0,3	Tr.	2,3	Tr.	2,4	0,4	1,5	1,2	0,6	0,3	1,6	2,8
Butiryc ald.	0,9	0,6	Tr.	Tr.	0,6	Tr.	0,4	Tr.	Tr.	Tr.	0,4	Tr.	1,4	1,0	Tr.	Tr.	Tr.	Tr.
Met-et-ket.	4,0	3,5	4,8	1,7	1,9	1,2	1,8	11,1	5,9	2,0	1,2	15,8	2,4	5,6	6,3	1,1	1,5	6,7
I-valerald.	43,2	48,9	42,5	64,1	57,8	79,4	70,8	16,2	37,3	74,9	50,6	11,3	67,6	49,5	49,0	88:0	80,3	10,9
I-prop-m-ket.	1,0	0,8	0,4	0,2	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
Valeraldheyde	2,3	0,8	0,7	0,3	0,8	0,2	1,3	Tr.	1,7	2,3	0,8	0,3	1,2	0,7	0,6	0,5	0,2	2,2
Diethyl-ket.	5,3	0,9	0,4	0,7	0,6	0,3	0,7	0,5	Tr.	1,3	Tr.	Tr.	0,1	0,3	0,8	Tr.	0,1	Tr.
Diacetyl	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	0,4	Tr.	0,1	Tr.	0,9	Tr.	0,5
X -2	11,1	6,6	4,1	4,1	3,1	2,3	11,2	3,2	Tr.	7,0	3,5	2,9	5,0	7,0	3,9	2,9	1,6	5,6
X-3	1,2	1,9	1,2	1,2	0,4	0,7	Tr.	Tr.	Tr.	2,7	1,9	0,4	Tr.	Tr.	0,6	Tr.	0,4	1,9
X-4	Tr.	Tr.	TR.	Tr?	tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	3,1	Tr.	Tr.	Tr.	Tr.	Tr.	3,7
Micromoles/l	891	1450	930	980	1060	667	815	177	81	42	342	660	825	318	72	81	224	630

Legenda :Tr = traces
Propionald.= propionaldehyde. Aceton+ I-But.= Acetone+ Iso-butyraldehyde. Butyric ald.= butyraldheyde. Met.et.ket.=Methyl-ethyl-Ketone. I-Valerald.=Iso-valeraldehyde.I-Prop-m.ch.= Iso-propyl-methil-Ketone. Diethylket.= Dietilketone

TABLE 4. Production of Diacetyl and Formaldehyde from swine fat by MICROCOCCACEAE

DIACETYL (p.p.m.)

Strains	Days of growth						
	2	3	4	5	6	7	8
C 13	1,05	1,10	1,49	1,45	1,30	1,8	1,46
D 10	0,92	1,20	0,95	0,75	0,60	0,3	0,15
St 78	0,18	0,65	0,47	0,06	0,12	0,75	0,45
Blanc	0	0	0	0	0	0	0

FORMALDEHYDE (p.p.m.)

C 13		5,6	2,3	6	2,8
D 10		3,6	1	2,1	1,8
St 78		1,2	1	3,4	2,2
Blanc		0	0	0	0

Bibliography

1) Baird - Parker A. C., (1961) J. Appl. Bact. 25, 352
2) Beerens H., Guillaume J., (1952) Ann. Inst. Pasteur, 82, 93
3) Benassi R., (1964) Il Latte, 38, 335
4) Cantoni C., Massacra G., Renon P., Giolitti G., '1964), Atti Soc. It. Scienøe Vet. 18, 455
5) Cate,Ten. (1960) Tijdschr. Diergeneesk. 85, 999
6) Eddy B. P., (1961) J. Appl Bact. 27, 41
7) Eser H., Niinivaara F.P., (1963) Zbl. Leb. Unters. u. Forschung. 124, 245
8) GioDitti G., (1960) Arch. Vet. Ital. 12, 23
9) Giolitti G., Massacra G., (1963) Atti Soc. Ital. Sc. Veter. 17, 541
10) Lemoigne M. Giraud M., Giacobelli G.,(1952) Ann. Inst. Pasteur, 83, 824
11) Maillet J., Henry M.,(1960) VI° Symposium sur les substances étrangères dans les aliments, Madrid, 10th - 15th Oct. 1960, p. ; Ministerio de Agricultura, Madrid, 1961
12) Molnar M. R.., (1965) unpublished data
13) Niinivaara F.P. , Nurmi E., (1964) X° European Meeting of Meat Res. Workers Roskilde,
14) Poja ., Niinivaara F.P., (1964) Fleischwirt. 44, 435
15) Ralls J. W., (1960) Anal. Chem. 32, 332
16) Renon, P., (1965) Unpublished data
17) Stoffel W., Chu F., Aherens E.H. Jr (1959) Anal. Chem. 31, 307
18) Schreyver R., (1909) Analyst, 34, 470
19) Terplan G., (1962) Biologische, chemische und physikalische Vorgaenge beim der Herstellung von gepoekelten und gereiften Fleischwaren. Institut fuer Nahrungsmittelkunde, University, Munchen.

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SUMMARY

The metabolism of swine fat by Micrococcaceae has been investigated in a culture medium containing fat.

28 free fatty acids have been shown and among them 6 with a chain shorter than 8 carbon atoms were not present in the original fat. In addition to free fatty acids a number of carbonyl compounds were detected and the most part of them identified. The results of these findings were discussed and correlated with the production of flavours and with their possible influence on the microflora of sausages.