LIPOLYTIC ACTIVITY OF MEAT STARTER CULTURES.

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INTRODUCTION

The lipolytic activity of bacteria is influenced by a number of factors, both in model systems and in food. The physiological state of the bacteria, the incubation temperature, the pH, the composition of the test medium and the substrate concentration (the lipid content and type) all have a considerable effect on the amount of fatty acids liberated from fats. A number of bacteria have been shown to possess the ability to hydrolyse fatty acids from triglycerides. Thus, lipolytic activity was shown in meat spoilage bacteria like staphylococci micrococci (4,6,7) lactobacilli and streptococci (5,11) and Gram negative bacteria like pseudomonades (2). Studies were done on pure triglycerides in model systems, however, also on natural fat, acids were extracted and titrated with alkali, without any attempt to evaluate the nature of the acids liberated.

Although lipolytic activity may be regarded as a spoilage characteristic of food, especially the hydrolysis of butyric acid from tributyrate in milk, the activity in fermented meat products may be regarded advantageously. There is a lack of knowledge concerning the lipolytic activity of starter cultures used in the meat industry, both regarding the total activity if present, and the activity towards specific fatty acids. The present study was done in order to evaluate some starter cultures used in the industry for possible activity towards natural fats from pork, lamb and beef.

MATERIALS AND METHODS

Fat tissue: pork, lamb and beef fat consisted all of the fatty capsule from the kidney. The fatty tissue was trimmed free from any lean tissue and melted on boiling water, and the fat was collected for immediate use in the model system.

An emulsion consisting of 10 % gum arabic and 5 % lipid in water was homogenized by passage through a milk homogenizer (Rannie/APV) at high pressure (900bar). A reaction mixture was produced by mixing :0.2 ml fat emulsion0.1 ml 1.0 M Tris-HCl pH 7.2, 0.1 ml 1.0 M NaCl and 0.6 ml of a suspension of bacteria. This volume (1ml) was used for titration, while a larger volume was used for gas chromatographical analyses (GC).

Bacteria used were :Staphylococcus xylosus, Staphylococcus carnosus, Pediococcus acidilactici, Pediococcus pentosaceus and Lactobacillus plantarum, kindly supplied by Christian Hansen A/S.

The staphylococci were grown in Caso broth (Merck), the other bacteria were grown in MRS (Oxoid) broth. All strains were grown as stationary cultures at 25oC, and harvested during logaritmic growth phase. Centrifugation was performed at 5000 r.p.m. for 15 min. and cells were washed in 5 mM Tris-HCl pH 7.2. The suspension was adjusted to an OD of 1.0 at 660 nm. The bacteria were tested for activity towards tributyrin by spreading bacteria on the surface of tributyrin agar plates with 5 and 10 % tributyrin. After incubation at 20°C and 37°C plates were examined for clearing zones. Tests were also made using round holes made in the agar, and stainless steel cylinders placed on the agar, both filled with bacterial suspension and with filtrate after centrifugation of the cultures. Tests were also made on the staphylococci after disintegrating cells in the homogenizer, both on the cell fraction and on the cell filtrate.

The reaction mixtures were incubated at 20°C and 37°C along with blanks without fat in capped glass tubes. Reaction mixtures for GC analyses were incubated at 37°C only.

The reaction was stopped by adding 0.2 ml water and 0.2 ml 0.2 M H_2SO_4 in 2 M NaCl (for 1 ml volumes). Extraction was done directly in the glass tubes, using a mixture of diethylether:pentan (2.75:1) (3). Extraction was done for 15 min; the tubes were kept on ice and shaken heavily every 5 min for a period of

1 min. After separation of the two layers 3 ml of the upper phase were removed and titrated with 2 mM alcoholic NaOH, with a freshly prepared solution of 0.002 % thymol blue as indicator (3). Duplicate smaples were made for all bacteria.
The bottles with the larger amount for GC analyses were treated similarly the upper phase was collected and evaporated to dryness under vacuum.
Thin layer chromatography plates were used for separating the free fatty acids from the rest of the extracted lipids;especially the triglycerides, were made with silica gell (Merck 60 G),which was spread on glass plates in a layer of 0.5 mm. The lipid extract was solubilized in a small amount of diethyl ether and applicated onto the silica. Plates were developed in a solvent of hexan:diethyl ether:glacial acetic acid (80:20:1) for app. 35 min using a wett chamber. The different lipid fractions were detected using UV light after spraying with 2,3-dichlofluorescein. The fatty acids were scraped off the plates and methylated.
Methylation was done by boiling the fatty acid containing silica directly with 2-3 ml 20% BF_3 on a 80°C water bath with reflux for 5 min. 0.75 ml 0.1% hydroquinon was added as an oxidant.A hexan extract of the methylated fatty acids was evaporated and the fatty acid distribution was determined by GC.
Gaschromatography: gas chromatograph (Microlab GE 82). Column (J & W Sci.) DB-WAX 30 omega (0.25µ, Carbowax 20M) 30 m x 0.32 mm ID. Injection temperature 250°C. Detector:FID 250°C. Carrier gas : N_2 2.3 ml/min.Split 1: 30. Temperature program : delay 0.5 min, 150-220°C with 4°C/ min.

RESULTS AND DISCUSSION

Results on tributyrin agar showed that S.xylosus possessed a strong lipolytic activity and S.carnosus a very small activity towards tributyrin. The other bacteria did not show any activity.Results were similar using the different methods, and no lipolysis was observed using culture filtrate either before or after cell disintegration.
The study with natural lipids showed, however, that all bacteria had in fact lipolytic activity at both 20°C (Tab. 2) and 37°C (Tab.1). Lipolytic activity (liberated fatty acids in µmol/ hr ml bacterial suspension) was higher at 37°C than at 20°C for a specific bacteria. The activity at 37°C was 3.7-15.4 times the activity at 20°C. Generally the activity for a specific bacteria was higher on lamb and beef fat than on pork,both at 20°C and at 37°C. The activity on the former lipids was up to c. 6 times higher than on pork. The lipolytic activity varied considerably among the bacteria,. no bacteria had a higher activity than other bacteria on all three fats, neither at 20°C nor at 37°C. The activity towards pork fat showed a pronounced influence on which type of fat was used i.e. the fatty capsule from the kidney or back fat. The effect was,however, only found at 37°C. At this temperature the activity on back fat was 2-3 times higher than on the fat from the kidney region. One would expect to find an effect at 20°C too, because any difference in fat acid composition should be more pronounced at lower temperature, due to solidification of triglycerides and acids. The influence of temperature on the lipolytic activity of bacteria has been examined in several studies. Often activity has been shown to have a maximum between 35°C and 40°C (1,4,8),although results may vary for different bacteria and substrates (7,10). Activity may be reduced at high temperature due to enzyme denaturation (8), and activity may be altered due to fatty acid specificity i.e. triglycerides with preferentially hydrolyzed fatty acids may be more or less solid at a given temperature.

Preliminary investigation on the composition of the mixture of free fatty acids liberated due to lipolytic activity from the five bacteria were performed by GC. Peaks were identified by comparison to standard fatty acid methyl esters. In this study no attempt to a quantitative determination was made.
The peak pattern for all investigations, was independent of fat type or bacteria strain , and showed that four

to five fatty acids were dominating in the liberated fatty acid mixture. These fatty acids were: myristic acid, palmitic acid, stearic acid, oleic and linoleic acid. Many minor components, some probably including isomers of unsaturated fatty acids, could be detected, but has not been identified.

Results from the GC analysis of free fatty acid methyl esters are shown in table 3 to 5. GC analysis was carried out with four bacteria on the fatty capsule from the kidney from lamb, beef and pork. The P.pentosaceus was only tested on pork fat; S.xylosus on both types of pork fat. Tables are given on the relative composition of the above mentioned five fatty acids.

Lamb fat: results shows (Tab.3) no great variation between strains in the relative constitution of the fatty acids liberated. Palmitic acid (C 16:0) is the major component - about 37%, while stearic acid (C 18:0) and oleic acid (C 18:1) contributes nearly as much i.e. 32% and 27% respectively. Myristic acid (C 14:0) is a minor component with about 3 % and linoleic acid (C 18:2) nearly negligible with only 1 %.

Beef fat: results shows, that also for beef fat no great variation between strains can be observed (Tab.4) Again palmitic acid is a major component (41%), and the amount of the unsaturated oleic acid is considerably higher (33%) than the corresponding saturated stearic acid (24%).

Pork fat: results shows a greater variability between strains than was seen with lamb and beef fat (Tab.5). For S.carnosus, P.acidilactici and P. pentosaceus, palmetic acid is the major component (40%), but while the staphylococcus shows considerable more stearic acid (34%) than oleic acid (25%), the two others have nearly equal amounts of this fatty acid (26%) For all the bacteria myristic acid amount to less than 1% but P.pentosaceus liberates more than the dobble amount of linoleic acid (7%) than S.carnoseus (2%) and P.acidilactici (3%).

Staphylococcus xylosus and L.plantarum shows a different pattern. The major component here is oleic acid (35-40%), and the amount of palmetic acid is higher (26-34%) than stearic acid (21-22%). Again the amount of unsaturated linoleic acid is considerably higher (9-13%) than observed with lamb and beef fat.

The S.xylosus was tested on two different type of pork fat. As seen (Tab. 5) the back fat and the fatty capsule of the kidney gives quite different relative amount of the fatty acids. However, the fatty acid pattern is similar i.e. oleic acid as the major component (back fat:48%, kidney fat: 34%) and a higher amount of palmitic acid (back fat:27%, kidney :34%) than stearic acid (back fat:19%, kidney: 22%).

Umemoto et al. (11) have shown a variation between strains in free fatty acids liberated from butterfat. Fatty acids with carbon chain length from 10 to 18 were detected by GC analysis on a packed column. The relative amounts of the individual fatty acids were not determined.

Studies with isolated lipases have shown that substrate specificity differs among bacterial lipases i.e. lipase B from Chromobacterium viscosum had specificity to long chain and saturated fatty acids but stearic esters were poor substrates (9). Such differences in substrate specificity could be an explanation for the different fatty acid pattern observed with the five bacteria in the present study. On the other hand the experiments were carried out with bacterial suspensions and not isolated enzymes. It may well be that more than one enzyme is involved in the degradation of fat triglycerides each with a different substrate specificity. The different fatty acid pattern might reflect a difference in activity of involved lipolytic enzymes. This activity differing among bacteria is consistent with results from titration on analysis of liberated free fatty acids as a whole.

The different fatty acid pattern for each bacteria depending on fat type might be due to the difference in amounts and type of the fatty acids bound in triglycerides. If enzyme activity is dependent on fat type used, then the amount of individual

fatty acids could be correlated to the composition of fat triglycerides. Probably a mixture of mechanisms are involved and a more quantitative investigation might elucidate some of these aspects.

CONCLUSION

The investigation showed that only the staphylococci were active towards tributyrin, and that the activity was cell bound. Further, the activity towards tributyrin is not a good indication of lipolytic acivity as all starter cultures in fact were lipolytic towards natural fats.

There was a large variation in lipolytic activity among the culutres with no special preference between the pork, lamb and beef fat. However, all strains are active at a temperature relevant to production of meat products with starter cultures, and therefore will influence the flavor development.

This preliminary investigation showed a considerable influence of fat type used in the test system, not only between fat from different species but also between fat from different regions in the same species. Although variations between the bacteria could be detected - especially on pork fat- there seen to be less influence from species type than from fat type. The difference between strains was not significant for any of the fatty acids selected.

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Lipolytic activity of starter cultures on lamb, beef and pork fat. Activity in µmol x 10^3/hr ml bacterial suspension.

Table 1. 37^oC.

	Lamb	Beef	Pork	
S.x	48.6^c	63.9^b	$^x97.1^a$	33.7^b
S.s	28.7^b	83.5^a	84.8^a	35.0^b
P.p	33.6^c	25.0^{bc}	49.0^a	14.7^b
P.a	31.7^b	95.5^c	47.0^a	19.4^b
L.p	41.0^a	51.5^a	46.7^a	21.1^b

Table 2. 20^oC.

	Lamb	Beef	Pork	
S.x	30.7^b	17.1^c	$^x 6.3^a$	6.1^a
S.s	22.0^b	20.4^b	10.8^a	6.5^a
P.p	37.9^b	17.1^c	7.9^a	4.0^a
P.a	20.7^c	26.6^c	10.4^a	3.9^b
L.p	22.8^b	26.0^b	8.2^a	6.2^a

S.x = S.xylosus; S.s = S.carnosus; P.p = P.pentosaceus; P.a = P.acidilactici; L.p = L.plantarum.

^{a} means in the same row with different superscript are significantly different ($P < 0.05$).

x first column is back fat, other results are for fat from the kidney region.

% Fatty acid produced by different bacteria from lamb, beef and pork fat.

Table 3. Lamb fat.

	S.x	S.s	P.a	L.p
C 14:0	3.3	3.6	3.0	2.3
C 16:0	36.3	37.8	36.9	36.6
C 18:0	30.0	31.3	31.5	37.0
C 18:1	29.0	26.3	27.6	24.0
C 18:2	1.4	1.0	1.0	0.1

Table 4. Beef fat.

	S.x	S.s	P.a	L.p
C 14:0	2.0	1.1	1.2	1.4
C 16:0	40.5	42.3	39.0	40.3
C 18:0	22.8	24.0	24.6	23.2
C 18:1	33.3	32.0	34.3	34.1
C 18:2	1.4	0.6	0.9	1.0

Table 5. Pork fat.

	S.x		S.s	P.a	P.p	L.p
C 14:0	^{x} 0.3	0.7	0.8	0.6	1.0	0.7
C 16:0	27.1	33.8	38.0	42.2	40.2	26.0
C 18:0	19.1	21.8	33.9	26.3	25.3	21.2
C 18:1	47.5	34.4	25.0	27.7	26.3	39.5
C 18:2	6.0	9.3	2.3	3.2	7.2	12.6

x first column is back fat, other results are for fat from the kidney region.