SCREENING OF BACTERIAL STARTER CULTURES FOR FERMENTED MEAT PRODUCTS.

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INTRODUCTION

The use of bacterial starter cultures for the production of fermented meat products, in particular dry sausage, has become common during the last decades, and several reviews on the use of this technology have been published (e.g. Leistner 1986; Lücke and Hechelmann 1986; Lücke 1986; Goekalp and Ockerman 1986; Incze 1987). It is pointed out by several of these authors that it is possible to produce fermented sausages without the use of bacterial starter cultures, but if used, this ensures a much more reliable production method, with formation of lactic acid for preservation purpose, which in addition results in a fast drying rate.

In some cases micrococci are added, traditionally to ensure nitrate reduction, but also to prevent accumulation of peroxide, which causes discoloration. Most authors also mention that the starter cultures will promote aroma formation, but this is often taken as an additional advantage, the preservation function is always stressed as the primary cause for their use.

The purpose of the experiments reported below has been to attempt to screen for suitable bacterial starter cultures, where aroma formation was considered as an important property, although their ability to preserve the product, primarily through lactic acid formation, was retained.

MATERIALS AND METHODS

Starter cultures

Bacterial starter cultures to be tested were partly micro-organisms isolated from commercially available fermented dry sausages, partly commercially available bacterial-starter cultures. Three preparations were included in the further procedures: preparation A: a mixture of *S.carnosus* and *L.pentosus*, preparation B: a mixture of *S.carnosus* and *P.pentosaceus*, and preparation C: *P.acidilactici*.

Starter cultures from the sausages were obtained through isolation on APT-agar and MRS-agar. Representative colonies were selected from primary plates and restreaked on APT and MRS-agar. The selected strains were characterized by microscopy, fermentation mode and examined for growth at 5, 11, 20, 25, 30 and 370 C. Strains were also examined for catalase activity, gelatinolytic activity and lipolysis by testing them for hydrolysis of Tween 80. All tests were done using fresh cultures, i.e. cultures grown overnight.

For the proper screening tests were used inocula equivalent to approximately 10^6 organisms per ml. This is equivalent to the concentrations often used for fermentation of sausages.

SCREENING TESTS

Besides testing for temperature limits for growth, the ability for a starter culture to lower pH is important. The former tests have been described above, but for pH measurements were partly used a medium, M3, earlier described by Nordal and Slinde (1980), partly a meat slurry to examine the possibilities in a meat model. The method has also been described previously (Zeuthen and Gotfredsen 1982).

The initial isolation procedures from sausages obtained commercially showed that practically all isolates were capable of growing both at 10-11°C and at least up to 37°C, but none grew at 5°C. However, none of the isolates showed proteolysis on gelatine or lipolysis by break-down of Tween 80, not even isolates which were described as typical micrococci. Based on the preliminary tests, the isolates could be classified as: *Micrococcus* (4 samples), *Lactobacillus* (7 samples), and *Streptococcus* or *Pediococcus* (21 samples).

PRODUCTION OF A LABORATORY MODEL SAUSAGE

A sausage mix was made according to the following recipe: lean pork meat: c. 58 per cent, pork fat tissue: c. 38 per cent, glucose: c. 1 per cent, sodium chloride: 2.5 per cent, and sodium nitrite 100 ppm. After mixing and inoculation with the appropriate starter culture, the blend is stuffed in a 30 mm artificial casing, especially suited for dry sausages.

Drying: The sausages are hung on a rack in a large glass cylinder furnished with a magnetic stirrer. A layer of saline in the bottom of the cylinder is added increasing sodium chloride during the drying period to simulate drying conditions in a drying chamber. The cylinder is placed in a room at 20°C. Considering the small diameter of the casings, drying was accomplished, i.e. showed a drying loss of c. 20 per cent already after 7 days, and was thus considered "finished".

BACTERIAL FORMATION OF DL-LACTIC ACID

This property is also considered important to measure and was therefore determined using the enzymatic method described by Boehringer and Mannheim.

DETERMINATION OF CARBONYLS

Since carbonyls constitute an important class of aroma compounds it was decided to include an assessment of these in the screening procedure. Carbonyls were therefore determined as 2,4-dinitrophenylhydrazones (2,4 DNPH) by thinlayer chromatography. The method is based on procedures described by Esterbauer et al 1982), Lawrence (1985) and Thomas et al. (1971). Sixteen 2,4 DNPH derivatives were made as standards, but carbonyl standards above $C_{10:2}$ and $C_{11:1}$ were not available commercially, primarily because they are very unstable.

RESULTS AND DISCUSSIONS

Figures 1 and 2 show the results of the pH changes in M3 broth and meat slurry caused by typical representatives from each of the selected bacterial groups and from the starter cultures A, B and C. It is interesting to notice that although the commercial starter cultures could cause a fast pH decrease and the lowest ultimate pH in the M3 broth, they showed a different behaviour in the meat slurry. pH measurements of the model sausages

Table 1. Analytical results of changes in sausage models during fermentaion (1a Micrococcus, 2a Streptococcus, A, B and C commercial starter cultures).

Culture	1a	2a	A	B	C
pH-start	5.4	5.4	5.4	5.4	5.4
pH-ult.	5.2	4.8	5.1	5.5	4.6
Brineconc. start	5.7	5.7	5.7	5.7	5.7
Brineconc. ult.	15.1	16.3	11.6	18.7	17.8
Weight loss	16.6	17.0	16.9	18.4	18.3
% lactic acid	0.56	1.29	0.59	0.41	1.28

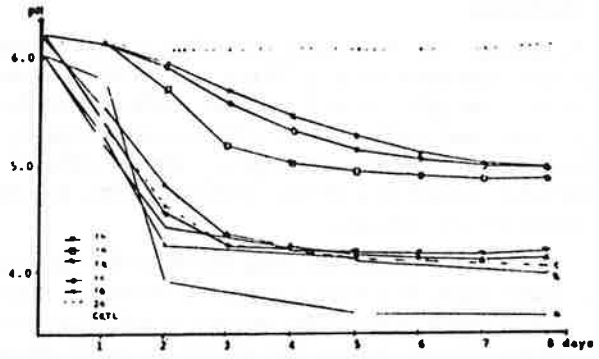

Fig. 1. pH changes in M_3 broth at 20° C as function of time for bacterial isolates from fermented sausages (1a-d Micrococci, 1h, q, 2a Streptococci and commercial starter cultures A, B and C).

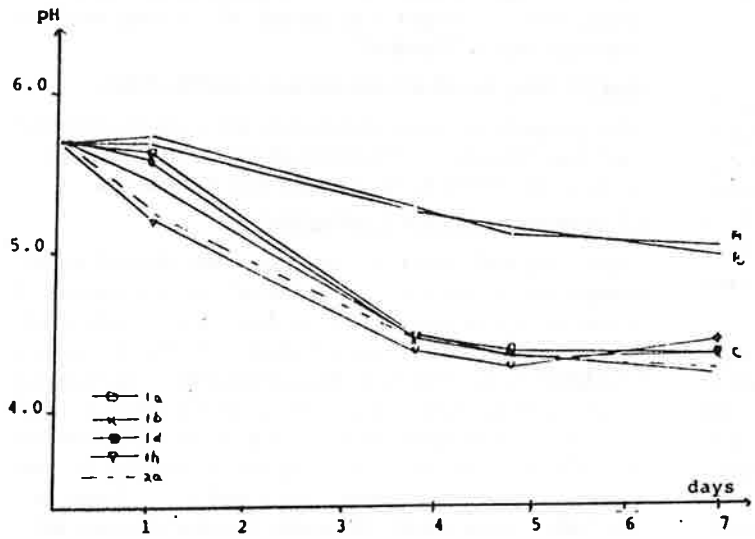

Fig. 2. pH changes in meat slurries at 20° C as function of time for bacterial isolates (1a, b, d Micrococci, 1h, 2a Streptococci and commercial starter cultures A, B and C).

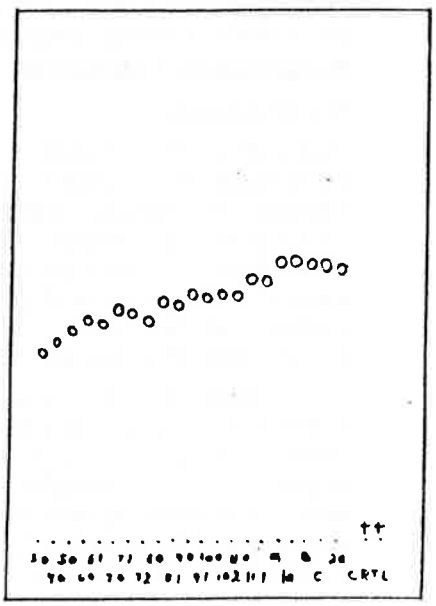

Figure 3. Migration of DNPH derivatives on TLC with CH_2Cl_2/Toluene (1:1) after 45 minutes.

confirmed this result, only the commercial starter culture C, the pure Pediococcus, could lower pH more efficiently than any of the cultures isolated at random from the sausages.

The results of analyses of the model sausages during fermentation are shown in Table 1. As will be seen, the commercial starter culture C, was capable of decreasing pH the most, whereas the end-pH was the same or lower using the two isolates as starter cultures, than using preparation A or B.

The results also show that the end-pH is very closely correlated with the measurable concentration of lactic acid.

Figure 3 shows the results of attempts to determine the carbonyls from the fat phase as an expression of the aroma components. As mentioned, carbonyls with a chain length of 16 to 18 carbon atoms are unavailable, so they could not be included as 2,4 DNPH derivatives in this investigation. The experiment was made in the hope that also short and middle chained carbonyls were formed and could be proved, and that they would comprise part of the aroma components. As will be seen, though, this is not the case. All migrations were above the standards available. Overall, the above experiments seem to show that the same ultimate pH may be reached using an isolate picked at random, as with a commercially available starter culture, but, on the other hand, commercially available starter cultures do differ in the final pH of a fermented sausage, according to the manufacturer's specification.

Regarding the aroma components in fermented sausages, it appears that at least as far as carbonyl compounds are concerned, these seem to be long chained, and since the starter cultures at least in this experiment hardly seem implicated, they are not showing lipolytic activity. Therefore, any probable carbonyls formed are due to spontaneous oxidation during the fermentation and drying period.

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