USE OF CERTAIN GRAM-NEGATIVE BACTERIA IN DRY SAUSAGE

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Gram-negative bacteria are known to be useful in curing brines and improve the aroma and flavour of the cured meat products. Only some investigations into the effects of gram-negative bacteria have been made. KOHNLE (1953) stated that some Achromobacter and Escherichia strains produce the characteristic aroma of dry sausage and that micrococci and lactobacilli do not produce any kind of aroma. Alcaligenes, Pseudomonas and Aerobacter species are also able to create characteristic aromas. KELLER (1954) investigated the effect of bacterial strains isolated by Kohnle and belonging to the genera Alcaligenes, Pseudomonas, Achromobacter, Aerobacter and Escherichia on the aroma of dry sausage and found one Escherichia strain and two Achromobacter strains to have the best effect. KELLER and MEYER (1954) inoculated dry sausages with 5 Escherichia, 2 Alcaligenes, 1 Pseudomonas and 2 Achromobacter strains and found one Escherichia strain to have an especially favourable effect on the flavour of dry sausage, this being caused by fat breakdown products. MEYER (1954) also investigated the effect of Kohnle's strains on the aroma of dry sausage. One Escherichia strain proved best. LOSEM (1956) also stated that this strain improves the flavour of dry sausage. He also isolated it from dry sausage after a ripening period of 9 weeks.

LÜCKERT (1958) inoculated dry sausage with Micrococcus M_{53} (NIINIVAARA 1955) together with an Escherichia strain and obtained sausages whose colour and aroma were especially good. Both strains disappeared from the sausage during the ripening period of 40 weeks. The ripening time of the sausages recorded by the school of Keller was long compared with processing times nowadays. Also, the pH value of the sausages was high - over 6,0 - making it possible for gram-negative bacteria to grow and thrive in dry sausage. NURMI (1966) inoculated dry sausage with two nitrate-reducing strains, one being Escherichia coli and the other Aerobacter cloaceae according to BERGEY's MANUAL (1957). The strains were used together with Lactobacillus plantarum. Aerobacter + Lactobacillus sausages had a peculiar mild flavour characteristic of sausages inoculated with lactobacilli. When Escherichia coli was used with lactobacilli the peculiar flavour was not found. The colour of the sausages was essentially a lighter red than of sausages inoculated with lactobacilli and micrococci.

Because most of these previous studies date from the 1950's and are not quite applicable to modern processing techniques it was established whether or not it is possible to use gram negative bacteria as a starter culture in dry sausage.

This is done by inoculating dry sausage with the following bacteria both alone and with lactobacilli: Aeromonas x (isolated from dry sausage of good quality), Aeromonas 19 (isolated from dry sausage of good quality), Vibrio costicolus (isolated from curing brine), Achromobacter 22 (isolated from curing brine), Achromobacter guttatus (received from Zwitzerland), Achromobacter x (isolated from dry sausage), Escherichia coli (Institute of Microbiology, University of Helsinki) and Proteus vulgaris (Institute of Microbiology, University of Helsinki). Sausages prepared with addition of Staphylococcus + Lactobacillus were used as control sausages.

MATERIAL AND METHODS

Preparation of sausages

Twenty-two series of experiments were used in the investigation. Each series contained 6 different sausage groups and a group of 6-7 identical sausages. Sausages were prepared using the following formula: Beef 33,4 %, Pork 33,4 %, Pork fat 30,0 % and seasoning salt

mixture 3,2 % (sodium chloride 89,7 %, glucose 9,0 %, potassium nitrate 0,625 % and white pepper 0,7 %). Sausage mass was inoculated with 5 x 10^6 cells/g of each strain used. The sausage mass was stuffed into a casing 60 mm in diameter (Visko PKX, manufactured by Oy Visko Ab, Hanko, Finland), the weight of one sausage being about 0,5 kg. The sausages were ripened as follows: 0-4 days temperature 22°C and humidity 95 %, 4-7 days temperature 21°C and humidity 90 %, 7-21 days temperature 15-16°C and humidity 80 %.

Organoleptic evaluation

The panel carrying out the evaluation consisted of five persons familiar with the organoleptic evaluation of dry sausage. The experimental sausages were evaluated when they were 1, 3, 7, 10, 14 and 21 days old. The following properties were evaluated: colour of sliced surface, consistency, aroma and flavour. The flavour of 1 and 3 day old sausages was not evaluated. A scoring system and a descriptive method were used side by side as the evaluation method.

Physical and chemical examinations

The following examinations were made after 0, 1, 3, 7, 10, 14 and 21 days of ripening: pH value, consistency measured with Instron instrument, weigt loss, nitrite and nitrate.

Microbiological examinations

Experimental sausages were studied microbiologically after 0, 1, 3, 7, 10, 14 and 21 days of ripening. The following examinations were made:
Total number of bacteria on plate count agar, staphylococci on mannitol-salt agar, lipolytic bacteria on tributyrine agar, lactobacilli on Rogosa agar, faecal streptococci on Slanez agar and coliforms on VRB agar.

RESULTS and DISCUSSION

Use of Aeromonas x and 19 as a starter culture

Aeromonas x and 19 strains had a favourable effect on the colour of dry sausage but otherwise the quality of the samples was very similar to that of the sausages with no inoculations. Aeromonas strains did not form observable amounts of acid in dry sausage and so did not improve the consistency. The aroma and flavour were better than in the control sausages.

Aeromonas x and 19 strains had a very favourable effect on the quality of dry sausage when inoculated together with lactobacilli. Both strains reduced nitrate, the colour forming during the first 3 days. The pH value of Aeromonas + Lactobacillus sausages decreased even more quickly and to a lower value than in Staphylococcus + Lactobacillus sausages, being about 5,20 after 3 days of ripening (Fig.1). The consistency of Aeromonas + Lactobacillus samples developed within one week and was at least as good as and often firmer than in Staphylococcus + Lactobacillus sausages (Fig.2). The consistency of Aeromonas 19 + Lactobacillus sausages was better at the 0,05 significance level than that of Staphylococcus + Lactobacillus samples. The aroma and flavour of Aeromonas + Lactobacillus sausages were as good as or better than those of Staphylococcus + Lactobacillus sausages (Fig.3). The aroma and flavour of Aeromonas 19 + Lactobacillus sausages were significantly better (significance level 0,001) than Staphylococcus + Lactobacillus sausages. The flavour of Aeromonas x + Lactobacillus sausages was better at the 0,05 level than Staphylococcus + Lactobacillus sausages.
The number of bacteria, roughly the number of lactobacilli, was often significantly higher in Aeromonas + Lactobacillus sausages, (rance 2-4 x 10^8/g between 3 and 21 days of ripening) than in Staphylococcus + Lactobacillus sausages (range 8 x 10^7-1,5 x 10^8/g between 3 and 21 days of ripening) (Fig.4). For this reason the former sausages ripened more quickly than the latter.

Use of Vibrio costicolus as a starter culture

V. costicolus did not thrive in dry sausage. The amount of V. costicolus fell noticeably

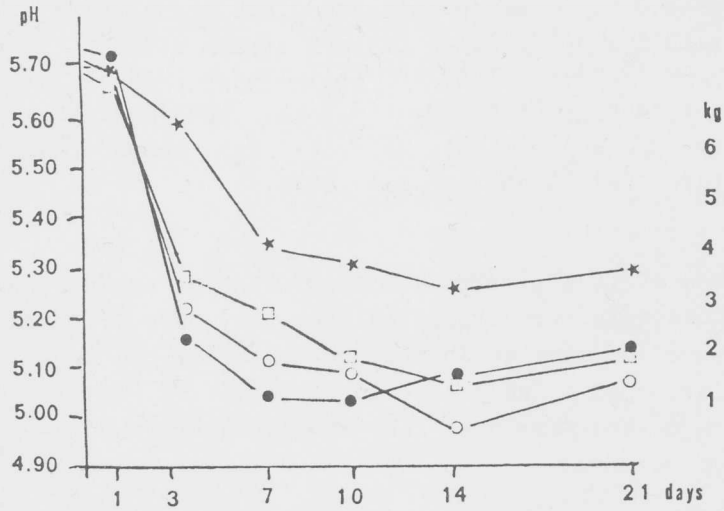


Figure 1. Effect of different bacterial inoculations on pH value of sausage.

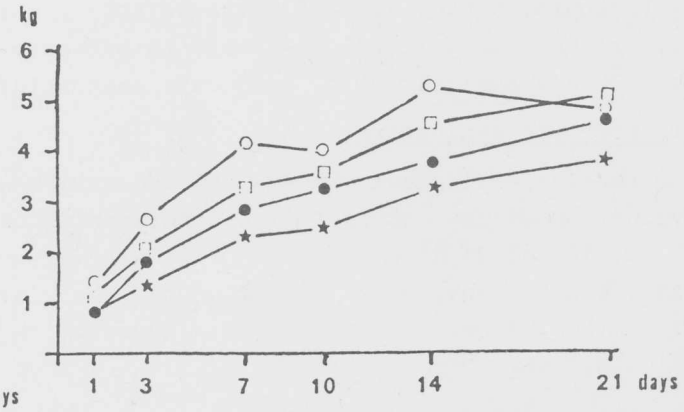
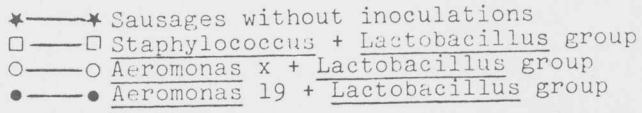


Figure 2. Effect of different bacterial inoculations on consistency (determined by Instron apparatus) of sausage.



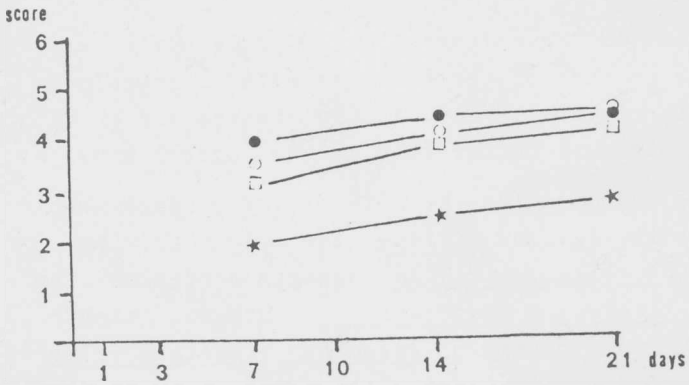


Figure 3. Effect of different bacterial inoculations on flavour of sausage.

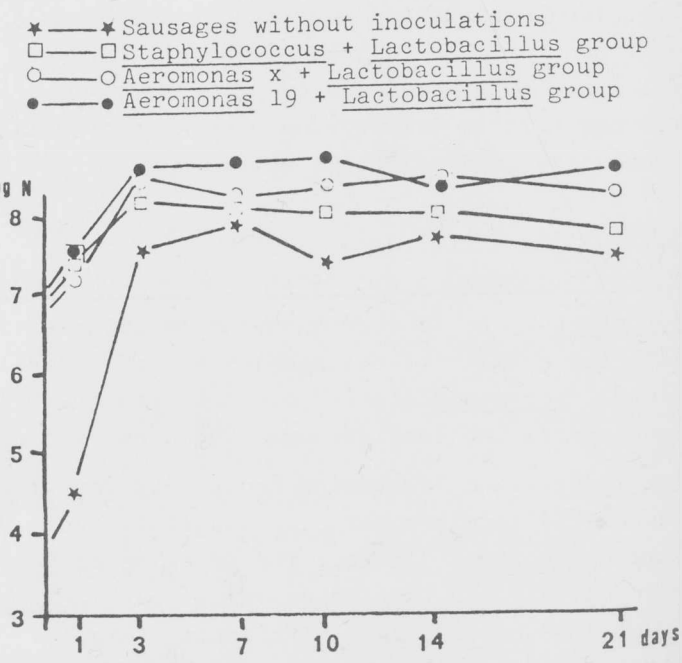
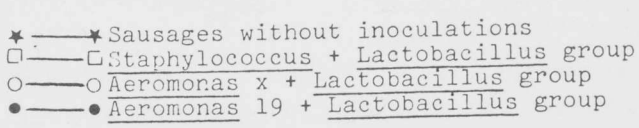
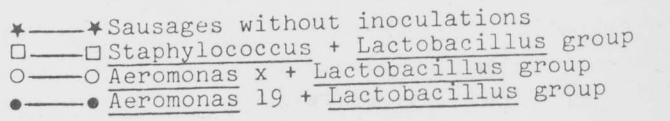


Figure 4. Number of lactobacilli/g on Rogosa agar in different sausage groups.





during the first 3 days of ripening from 10^7/g to about 10^3/g. In 7 day old sausages no V. costicolus was found. As ripening began V. costicolus reduced nitrate, producing 20-30 ppm nitrite in the first 3 days of ripening thereby securing colour formation. This nitrate reduction did not happen regularly in every experimental series. When lactobacilli were inoculated along with V. costicolus the colour formation was missing because vibrios disappeared and the lactobacilli therefore produced discolourations. The consistency of these sausages was firm. On the basis of these observations, especially that of the disappearance of V. costicolus from sausage, it can be concluded that V. costicolus cannot be used as a starter culture for dry sausage.

Use of Achromobacter strains as a starter culture

Achromobacter 22 failed to thrive in dry sausage. It disappeared completely after one week. Clearly then, it did not have any marked effect on the quality of dry sausage. The sausages inoculated with it alone were in the same class as control sausages and those inoculated with Achromobacter 22 and lactobacilli had better consistency than the control. Two experimental series were carriet out.

The Achromobacter guttatus strain also failed to grow in dry sausage and disappeared even more quickly than Achromobacter 22. It had no effect on the quality of dry sausage. Only one series of experiments was carried out.

Dry sausages were inoculated with Achromobacter X alone and with lactobacilli in three experimental series. In one series the inoculum was succesful. Achromobacter X thrived in dry sausage, the number of cells being about 2×10^7/g after 7 days of ripening after which it fell. The effect of the Achromobacter strain used when inoculated with lactobacilli was the same as that of staphylococci: the colour was red, consistency firm and aroma and flavour good. When inoculated with just Achromobacter X the colour of the sausages was good but the consistency, aroma and flavour were in the same class as sausages with no inoculation.

In two other experimental series Achromobacter X not only failed to thrive but disappeared during the first 3 days of ripening. Neither did this strain survive very well after lyophilization. It can be concluded then, that none of the Achromobacter strains used is suitable for use as a starter culture in dry sausage.

Use of Escherichia coli as a starter culture

Escherichia coli was selected as a standard enterobacter for an experimental starter culture. Three experimental series contained sausages inoculated with E. coli alone and with E. coli and lactobacilli. The sausages inoculated with E. coli alone were in the low quality class. However the quality of E. coli + Lactobacillus sausages was clearly better though not as good as that of either Aeromonas x or 19 + Lactobacillus or Staphylococcus + Lactobacillus sausages. The colour was not as clear, consistency not as firm and aroma and flavour not as palatable as in these latter samples.

The pH value of sausages inoculated with E. coli alone was quite high (5,40). The pH values of E. coli + Lactobacillus sausages were between 5,0 and 5,10. E. coli reduced nitrate quite strongly, producing a meam nitrite content after 3 days of ripening of 48 ppm in E. coli + Lactobacillus sausages and 44 ppm in E. coli + Lactobacillus sausages. The number of E. coli in sausages inoculated with E. coli alone and E. coli + Lactobacillus decreased during ripening from 10^6-10^7/g to 10^5-10^6/g.

Use of Proteus vulgaris as a starter culture

Proteus vulgaris was inoculated alone and with lactobacilli into dry sausage in three experimental series to investigate how this kind of strongly proteolytic and spoiling bacteria grows in dry sausage and affects its properties. When dry sausages were inoculated with P. vulgaris alone, the bacteria had an adverse effect on the quality of the sausages. The consistency did not develop. Instead, colour was produced, which is natural since P. vulgaris is a strong nitrate reducer. As a rule, however, the colour faded in the later stages of ripening. The aroma and flavour of P. vulgaris sausages were unpalatable.

When P. vulgaris was inoculated together with lactobacilli into dry sausage the consistency of the sausage was only slightly better than without lactobacilli. The colour did not develop in P. vulgaris + Lactobacillus sausages and the aroma and flavour were unsatisfactory.

The pH value of P. vulgaris sausages decreased, as in the sausages not inoculated.The pH value of P. vulgaris + Lactobacillus sausages decreased, as in the sausages inoculated with staphylococci and lactobacilli as a rule. However, in an experimental series of sausages containing more than 10^7 Proteus cells/g the pH value remained above 5,35 throughout ripe-

ning.

The number of Proteus cells changed considerably. In one experimental series there were more than 10^7 cells/g throughout ripening while in two other series the number decreased from a level of 3×10^6/g and P. vulgaris bacteria practically disappeared after 3-7 days of ripening.

Pathogenicity of Aeromonas x and 19

The examination was carried out by inoculating intraperitoneally different numbers of bacterial cells into white mice. As a result of the apathogenicity examination of Aeromonas x and 19, the LD_{50} value lies between 5×10^7 and 10^8. It is known that gram-negative bacteria produce endotoxins and are so able to kill mice when inoculated in sufficient quantities.

According to MÄKELÄ (1975) gram-negative bacteria having an LD_{50} value of 10^8 or more are apathogenic. VALTONEN (1970) considered SALMONELLA TYPHIMURIUM strains having a value of 10^8 avirulent.

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