

THE EFFECT OF SOME GRAM-NEGATIVE BACTERIA ON THE RIPENING AND QUALITY OF DRY SAUSAGE

Selostus: Eräiden gram-negatiivisten bakteerien vaikutus kestromakkaran kypsymiseen ja laatuun

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Abstract. The possible use of gram-negative bacteria as starter cultures in dry sausage was studied by inoculating the following gram-negative bacteria into dry sausage both alone and with *Lactobacillus plantarum*: *Aeromonas* x, *Aeromonas* 19, *Vibrio costicolus*, *Achromobacter* 22, *Achromobacter guttatus*, *Achromobacter* X, *Escherichia coli*, or *Proteus vulgaris*. Sausage without any inoculation and sausage containing *Micrococcus* sp. + *Lactobacillus plantarum* were used as references.

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, and the colour formed during the first 3 days. The pH value of *Aeromonas* + *Lactobacillus* sausages decreased so quickly that the consistency of these sausages developed within one week and was at least as good as, and often firmer than in *Micrococcus* + *Lactobacillus* sausages. The consistency of *Aeromonas* 19 + *Lactobacillus* sausages was better at the 0.05 significance level than that of *Micrococcus* + *Lactobacillus* samples. The aroma and flavour of *Aeromonas* + *Lactobacillus* sausages were as good as or better than those of *Micrococcus* + *Lactobacillus* sausages. The aroma and flavour of *Aeromonas* 19 + *Lactobacillus* sausages were significantly better (significance level 0.001) than *Micrococcus* + *Lactobacillus* sausages. The flavour of *Aeromonas* x + *Lactobacillus* sausages was better at the 0.05 level than *Micrococcus* + *Lactobacillus* sausages.

The number of bacteria, roughly the number of lactobacilli, was often significantly higher in *Aeromonas* + *Lactobacillus* sausages (range $2-4 \times 10^8$ /g between 3 and 21 days of ripening) than in *Micrococcus* + *Lactobacillus* sausages (range $8 \times 10^7 - 1.5 \times 10^8$ /g between 3 and 21 days of ripening). For this reason the former sausages ripened more quickly than the latter.

Vibrio 21 and three *Achromobacter* strains did not thrive in dry sausage and disappeared during the first 7 days of ripening. When inoculated together with lactobacilli, *Escherichia coli* had almost as favourable an effect as micrococci on the quality of dry sausage. Inoculations of *E. coli* alone and *Proteus vulgaris*, both alone and with lactobacilli, did not improve the quality of dry sausage. *P. vulgaris* actually had a detrimental effect.

Conclusion

The investigation has proved that gram-negative bacterial strains *Aeromonas* x and 19 inoculated with lactobacilli improve the quality of dry sausage and that, especially with the *Aeromonas* 19 + *Lactobacillus* inoculum, better dry sausage was obtained than with micrococci and lactobacilli.

1. Introduction

The manufacture of fermented food products, e.g. various dairy products, is based on the action of micro-organisms. The use of microbes ensures ripening and avoids the development of faulty products. The use of bacterial cultures

in dairy products or different starting portions of ripe material in vegetable and bread products has been known for quite a long time. On the other hand, starter cultures in meat products have only been used during the last three decades.

Bacteria used as starter culture for dry sausage have been solely gram-positive, generally lactic acid bacteria and micrococci. Mixing lactic acid bacteria with micrococci has proved beneficial for dry sausage. In cured meat products gram-negative bacteria have also been important. The traditional method has been to add old brine to new brine — to transfer bacterial inoculum to the new brine. Gram-negative bacteria often prevail in curing brines. They have also been inoculated artificially into the brines because they improve the colour formation and flavour of cured products. Some investigations into the effects of gram-negative bacteria on the quality of dry sausage have been made, but in practice gram-negative bacteria have not been used as starter culture for dry sausage. Most previous studies date from the 1950's and are not quite applicable to modern processing techniques, because shorter ripening times are used nowadays.

Because gram-negative bacteria can have a beneficial influence on meat curing and because their use in the manufacture of dry sausage has been little studied, the effect of some gram-negative bacteria on the ripening and quality of dry sausage will be clarified in this investigation. Attention will primarily be directed to the question of whether or not gram-negative bacteria can be used as starter culture for dry sausage.

2. Review of the literature

2.1. Natural bacterial flora of dry sausage

According to CORETTI (1956 a, 1956 b) dry sausage contains mixed bacterial flora; the number of bacteria as ripening begins is about $4 \times 10^6/g$. MÖLLER (1955) investigated the bacterial flora of the dry sausage masses from different factories and observed gram-negative bacteria dominating the flora. The number of bacteria increased according to CORETTI (1956 a, 1956 b) from $4 \times 10^6/g$ to $3 \times 10^8/g$ over 2 weeks. The number of enterococci varied from $1.6 \times 10^4/g$ to $5 \times 10^5/g$, the number of yeasts being $4 \times 10^3-3.1 \times 10^4/g$. According to the same investigation the number of lactobacilli was very small when ripening began while in the ripened product lactobacilli predominated. According to SZCZEPANIAK and PEZACKI (1974) cocci formed the dominant microbial flora in dry sausage and smoking resulted in the destruction of gram-negative rods and yeasts. The total microbial counts increased from one million/g to 6–33 million/g during ripening.

REUTER et al. (1968) observed that the number of bacteria increased from $10^4-10^5/g$ to $10^8/g$ during the first 2–3 days of ripening. The increase in lactobacillus counts during that time was from $10^2-10^4/g$ to $10^8/g$. The number of bacteria belonging to the genera *Leuconostoc* and *Pediococcus* was 0– $10^2/g$

during the first 4 days, increasing later to a level of 10^3 – 10^4 /g. The sausage mass before stuffing contained 10^4 – 10^5 enterobacter cells per gram, the count after 2–3 days being 10^2 or less per gram. The prevailing level of streptococcus and bacillus cells was 10^4 – 10^5 /g throughout ripening.

LERCHE (1956 a, 1956 b) found that the lactobacillus count at the beginning of ripening was low but increased considerably over 4–5 days. During drying almost all gram-negative and a great part of the gram-positive bacteria disappeared and the ready product contained almost exclusively lactobacilli, micrococci and yeasts. FISZER (1970) stated that during the first 10 days of ripening the heterofermentative lactobacillus strains prevailed in dry sausage and thereafter the homofermentative strains dominated. However URBANIAK and PEZACKI (1975) proposed that the situation is just the opposite — that the homofermentative strains predominate at the beginning with heterofermentative strains being found increasingly later on. They also stated that the number of gram-negative bacteria decreased during ripening.

NIINIVAARA and POHJA (1956, 1957 a, 1957 b) found that during the beginning of the ripening of dry sausage the number of bacteria increased abundantly, micrococci forming the majority. The number of lactobacilli was also considerable but the count of gram-negative bacteria decreased, especially after smoking. The final flora were chiefly composed of lactobacilli.

TEN CATE (1960) observed that gram-negative bacteria disappeared from dry sausage as early as the first 2 or 3 days of ripening. Lactobacilli and Lancefield D group streptococci remained in the sausage. Also, according to ERIKSON (1961) gram-negative bacteria generally disappears completely and the micrococcus count decreases while *Leuconostoc* strains form the majority of bacterial flora. CORETTI (1958) found *Leuconostoc* species in faulty dry sausages.

KASBOHM (1954) studied salami and cervelat sausages and found that the good products contained the most bacteria, especially lactobacilli. There was also an abundance of lactostreptococci, micrococci and yeasts. The sausages contained small but constant amounts of bacteria belonging to the mesentericus-subtilis group, and gram-negative bacteria.

2.2. Use of gram-positive bacteria and moulds in dry sausage

2.2.1. MICROCOCCI

It has been shown in many investigations that micrococci play an essential part in the ripening of dry sausage. When studying the bacterial flora of dry sausage, LERCHE (1956 b, 1957) found about 50 bacterial species, with micrococci forming an essential part of the microbial flora. He also stated that smoking reduced the number of micrococci in dry sausage and that micrococci improved the colour of dry sausage depending on their ability to reduce nitrate.

NIINIVAARA (1955) carried out extensive investigations into the influence of *Micrococcus* strains on the ripening process of dry sausage. A great number of *Micrococcus* strains were isolated in his work. A strain denoted M₅₃ proved to be the best as to its starter culture properties. It accelerated the drop in the

pH value so that in sausages inoculated with micrococci, a pH value of 5.4 was achieved in 2 days while in control sausages 5 days were needed for this. According to Niinivaara the development of colour depends on the reduction of nitrate and nitrite and on the pH value. The development of normal colour took 5 days in the control sausages but only 2 days in the sausages inoculated with micrococci. Further, Niinivaara observed that the control sausages were sometimes spoiled because of the influence of other bacteria. The *Micrococcus* strain M₅₃ was found to have an antagonistic effect on the growth of other bacteria. Inoculation ensured the successful ripening of dry sausage and it was also possible to reduce the ripening time from 14 days for control sausages to 9 days for sausages containing micrococci. The acceleration and assurance of ripening are based on the following properties of *Micrococcus* M₅₃: its ability to reduce nitrate and to reduce the pH value of sausage, and its antagonism towards other microbes.

NIINIVAARA and POHJA (1956) further clarified the role of micrococci in the preparation of dry sausage. According to them the micrococcus count increased more than that of other bacteria during the predrying of dry sausage. They also found, like LERCHE (1956 b), that smoking reduced the number of bacteria in dry sausage. In their later investigations (NIINIVAARA 1958, NIINIVAARA and POHJA 1957 a, 1957 b, 1957 c, POHJA and NIINIVAARA 1957) they stated that the *Micrococcus* strain M₅₃ had an antagonistic effect upon most of the bacteria isolated from dry sausage, but not, however, upon the *Lactobacillus* strains identified as *Lactobacillus plantarum* and *Lactobacillus leichmannii*.

In 1957 the *Micrococcus* strain M₅₃ began to be used in the meat industry in the form of a preparation called »Baktoferment» (manufactured by Rudolf Müller & Co., Giessen, FRG). This strain, isolated originally by Niinivaara, lost its activity fairly quickly and the strains used at present were isolated later. It is important, therefore, that new active strains be continually sought. POHJA (1960) has presented a method for the selection of new strains suitable for manufacturing dry sausage.

KUCHLING (1963) found that nitrate-reducing micrococci promoted the development of a red colour but only during the first day after preparation. He further showed that micrococci have no effect on the aroma of the sausage but that they may disturb acid formation and so bring about faulty products. KUCHARKOVA et al. (1963) found a *Micrococcus aquatis* strain which improves the quality of dry sausage. In Bulgaria a *Micrococcus* strain called P₄ has been used in the manufacture of dry sausage (DJEVIZOV 1973, DJEDJEVA 1973). It improves the quality of sausage and shortens the processing time.

2. 2. 2. LACTIC ACID BACTERIA

JENSEN and PADDOCK (1940) were the first to clarify the possible use of lactobacilli in the preparation of semi-dry sausage. The strains used were *Lactobacillus plantarum*, *Lactobacillus brevis* and *Lactobacillus leichmannii*. They used both pure and mixed cultures and stated that lactobacilli reduced the ripening time, prevented the development of faulty products and improved the aroma of sausages.

NIVEN et al. (1955, 1958) and DEIBEL et al. (1961 a, 1961 b) extensively studied the possible use of lactic acid bacteria in semi-dry sausage — called summer sausage — in the USA. They found a *Pediococcus cerevisiae* strain which accelerated the ripening of semi-dry sausage and produced a firmer product with a strong, clean aroma. This strain does not reduce nitrate but forms lactic acid from glucose. Its optimum temperature is +37° C and it is therefore applied in the manufacture of summer sausage, the internal temperature of which rises to 35–40° C. The preparation takes some days. This strain has not been adopted for use in the manufacture of dry sausage in Europe.

The *Pediococcus cerevisiae* strain isolated by Niven and Deibel has been used commercially as a lyophilized preparation called »Accel» (manufactured by Merck & Co., Rahway, N. J., USA). Merck & Co. later developed a frozen pediococcus preparation (Lactacel) which is more active than the lyophilized one (EWEYERSON et al. 1970). More recently frozen cultures of *Lactobacillus plantarum* (Lactacel DS) and a mixture of *L. plantarum* and *P. cerevisiae* (Lactacel MC) have been made available (DEIBEL 1974).

The starter culture preparations Saga and Saga II (Manufactured by Micro-life Technics, Sarasota, Fda, USA) contain lactic acid bacteria as a frozen suspension. Saga is for use in sausages fermented at smokehouse temperatures of 32–37° C and Saga II in sausages fermented at greenroom temperatures of 18–32° C (SHAH 1977). The cultures of lactic acid bacteria are also manufactured in France (LIEPE 1975). According to CORETTI (1977) the French pre-prepare called »Fermentes lactiques» contains lyophilized streptococci (*Streptococcus lactis*) in dried fat-free milk powder.

2. 2. 3. THE MIXTURE OF MICROCOCCI AND LACTIC ACID BACTERIA

NIVEN (1952 a, 1952 b) found that both bacteria producing lactic acid and nitrate-reducing bacteria are required in the ripening of dry sausage. In spite of this, mixed starter cultures containing only homofermentative and heterofermentative lactobacilli were used in many plants in the USA (LEISTNER 1963).

NURMI (1965, 1966) developed a method in which both micrococci and lactobacilli were used in the preparation of dry sausage. According to Nurmi, lactobacilli used alone to inoculate sausage produced an essentially better consistency than micrococci. The consistency of control sausages was also much poorer. However, when the usual nitrate quantity was used, lactobacilli brought about discolourations and an off-flavour. This was caused by hydrogen peroxide formed by lactobacilli and oxidation of fat caused by hydrogen peroxide. The inoculation of just micrococci did not markedly accelerate the decrease in pH value and so the consistency developed no better than in the control sausages. The micrococci alone improved the colour of the cut surface of dry sausage a little.

When lactobacilli were inoculated along with micrococci into dry sausage, the bacteria were observed to considerably improve the quality of dry sausage. The lactobacillus inoculation markedly accelerated the ripening process while,

on the other hand, micrococci prevented the discolourations caused by lactobacilli and reduced nitrate, thus promoting the formation of colour. According to Nurmi it is probable that the catalase formed by micrococci destroys hydrogen peroxide before it causes any harm. ROZIER et al. (1970) also observed the catalase activity of micrococci necessary to assure the colour formation and stabilization by decomposing peroxides formed by lactic acid bacteria. Nurmi's experimental sausages inoculated with lactobacilli and micrococci ripened on average within 6–7 days with a weight loss of about 10 %. The ripening time of control sausages was 19 days and respective weight loss 20 %. The lactobacillus inoculation required was given as 1–10 million cells per gram. Nurmi used a micrococcus inoculation of 5–10 million cells per gram.

Nurmi concluded from his investigations that it is better to use lactobacilli and micrococci together as starter culture than either separately. He stated that lactobacilli considerably accelerate the decrease in pH and so promote the formation of a firm consistency. On the other hand micrococci promote colour formation and prevent discolouration and bad flavour which might be caused by inoculating only lactobacilli into sausages. From Nurmi's investigations a starter culture called »Duploferment 66» has been developed containing frozen or lyophilized micrococci and lactobacilli (manufactured by Rudolf Müller & Co., Giessen, FRG).

The starter culture Saga III (manufactured by Microlife Technics, Sarasota, Fda, USA) also contains frozen micrococci and lactic acid bacteria and is used for dry sausages fermented at 10–32° C (SHAH 1977). Some mixed culture preparations are also on the market in Spain. According to CORETTI (1977), these preparations called »Lamirlac» are mixtures of lyophilized lactobacilli, pediococci and micrococci and are prepared for different dry sausages, and dried and cooked hams.

INAL (1969) investigated the effect of *Micrococcus aurantiacus* and *Pediococcus cerevisiae* inoculated together on the ripening and quality of dry sausage. This starter culture combination ensured ripening and improved the colour, consistency and aroma of the sausages.

SUTIC and JOKSIMOVIC (1973) inoculated dry sausage separately with *Micrococcus* strain M-104 and *Streptococcus* strain Ak-60 and with both strains together. The best quality, especially taste and aroma, was achieved with a culture containing both streptococci and micrococci. There were only small differences between other experimental sausage types. The pH value of the sausages did not decrease during ripening.

REUTER (1970, 1972) has studied the lactic acid bacteria of dry sausage. In addition to finding normal lactobacilli he found lactic acid bacteria which he called atypical streptobacilli. These bacteria were coccoid, grew at a lower temperature than normal lactobacilli and needed less oxygen than typical lactobacilli. REUTER (1972) also inoculated some atypical streptobacilli together with micrococci into dry sausage and observed that some strains had an unfavourable effect and some improved the quality, especially the flavour. Some strains even predominated in dry sausage over typical lactobacilli.

2. 2. 4. BACILLI

In the 1950's the role of bacilli was also studied. SCHÖNBERG (1953), SCHÖNBERG and WALZ (1954), BALDENIUS (1954) and WALZ (1958) found that bacteria belonging to the genus *Bacillus* caused colour and flavour defects in dry sausage. CORETTI (1958) inoculated *Bacillus subtilis* pure culture into dry sausage without any adverse effect on the products. In his opinion lactobacilli were the main reason for faulty products. POHJA and NIINIVAARA (1960) came to the conclusion that bacilli do not bring about any kind of change in dry sausage.

2. 2. 5. MOULDS

Many countries manufacture dry sausage with mould flora on the surface. Most natural moulds are toxic and so not suitable for foodstuffs. MINTZLAFF and LEISTNER (1972), however, have isolated a mould, *Penicillium nalgiovensis*, which is not toxic and has the right properties for use as a starter culture for dry sausage. The name of the commercial product developed from it is Edelschimmel Kulmbach 72 (manufactured by Rudolf Müller & Co. Giessen, FRG).

2. 3. Gram-negative bacteria and their use in fermented meat products

2. 3. 1. TAXONOMY

BERGEY'S MANUAL (1974) classifies gram-negative bacteria according to cell shape, oxygen demands and metabolic character in the following way:

- aerobic rods and cocci
- facultatively anaerobic rods
- anaerobic bacteria
- cocci and coccobacilli
- anaerobic cocci
- chemolithotrophic bacteria.

Gram-negative aerobic rods and cocci includes the following families:

- *Pseudomonadaceae*
- *Azotobacteraceae*
- *Rhizobiaceae*
- *Methylomonadaceae*
- *Halobacteriaceae*.

This group also contains genera of uncertain affiliation: *Alcaligenes*, *Acetobacter*, *Brucella*, *Bordetella*, *Francisella* and *Thermus*.

Gram-negative facultatively anaerobic rods includes two families: *Enterobacteriaceae* and *Vibrionaceae*. *Enterobacteriaceae* contains 12 genera, among them being *Escherichia*, *Salmonella*, *Shigella*, *Serratia* and *Proteus*. *Vibrionaceae* contains five genera: *Vibrio*, *Aeromonas*, *Plesiomonas*, *Photobacterium* and

Lucibacterium. There are also genera of uncertain affiliation like *Chromobacterium*, *Flavobacterium*, *Haemophilus*, *Pasteurella* etc.

Gram-negative anaerobic bacteria includes the family *Bacteroidaceae* and genera of uncertain affiliation.

The *Neisseriaceae* family and two associated genera belong to the gram-negative cocci and coccobacilli group. Gram-negative anaerobic cocci consists of the family *Veillonellaceae*. Gram-negative chemolithotrophic bacteria contains the *Nitrobacteriaceae* family of organisms which oxidize ammonia or nitrite. This group also contains organisms that metabolize sulphur and organisms that deposit iron or manganese oxides.

2.3.2. METABOLIC PROPERTIES

Among gram-negative bacteria there are metabolically versatile organisms forming acid from carbohydrates, alcohols etc., and which are both lipolytic and proteolytic. Nearly all gram-negative bacterial genera contain the strains that form acids from sugars and other compounds (BERGEY'S MANUAL 1974). According to CASELITZ (1965, p. 31, 125) lipolytic strains are to be found among bacteria belonging to the genera *Pseudomonas* and *Aeromonas*. Of the family *Enterobacteriaceae* the genus *Proteus* contains the most lipolytic strains (EDWARDS and EWING 1972, p. 324—328). The genera *Pseudomonas* and *Aeromonas* contain many proteolytic strains (CASELITZ 1965, p. 22, 119, BERGEY'S MANUAL 1974). There are also proteolytic bacteria which belong to the *Enterobacteriaceae* family. According to EDWARDS and EWING (1972, p. 324—328) the genus *Proteus* in particular contains proteolytic strains.

The lipolytic, proteolytic and saccharolytic activities of gram-negative bacteria are factors important in the aroma and flavour when these bacteria are used as starter cultures in dry sausage (KELLER 1954, KELLER and MEYER 1954). According to BUTTIAUX (1959) aeromonads play an important part in the biological processes on which the manufacture of raw sausage is based. Many gram-negative bacterial strains such as pseudomonads, aeromonads and enterobacteria reduce nitrate to nitrite (BERGEY'S MANUAL 1974). As is known the nitrate reduction is an important factor in the manufacture of dry sausage.

2.3.3. TOXINS AND PATHOGENICITY

Most gram-negative bacteria possess endotoxins. Endotoxins are complex lipopolysaccharides derived from the walls of bacterial cells and often liberated when bacteria lyse. The physiopathogenic effects of all endotoxins are similar, although they appear to be distinct antigenically. The sequence of monosaccharide units imparts antigenic specificity to the molecule. The specific antibody combines with the polysaccharide portion of the molecule (JAWETZ et al. 1968, p. 294).

The different types of toxins of gram-negative bacteria are classified according to their antigenic nature as follows: somatic (O), flagellar (H) and capsular (K) antigens. The common antigen (CA) and a thermostable antigen of salmo-

nellae are also known (EDWARDS and EWING 1972, p. 48, 54). MÄKELÄ and MEYER (1976) suggest that all bacteria belonging to the family *Enterobacteriaceae* have the common antigen (ECA) and that other gram-negative bacteria do not. The presence of this antigen can be a virulence factor.

It is known that there are many pathogenic organisms among gram-negative bacteria, especially bacteria belonging to facultatively anaerobic rods such as enterobacteria. Bacteria belonging to the genera *Salmonella* and *Shigella* of the *Enterobacteriaceae* family are the most pathogenic. However, some *Escherichia coli* and *Proteus* strains are also pathogenic. Some *E. coli* strains may even be very pathogenic and cause diarrhoeal inflammations and other infections (BERGEY'S MANUAL 1974).

Among pseudomonads *Pseudomonas aeruginosa*, and among vibrios *Vibrio comma*, are generally known to be pathogenic. Most *Pseudomonas* and *Vibrio* bacteria are, however, apathogenic and saprophytic. The genus *Aeromonas* includes bacteria which are pathogenic for fish, frogs and other water animals (CASELITZ 1965, p. 57—59). According to CASELITZ (1965, p. 59—60) *A. hydrophila* is also pathogenic for mice, guinea pigs, rats, pigeons, and rabbits. Caselitz also stated that pathogenic aeromonads are haemolytic. DAHLE and NORDSTOGA (1968) also found aeromonas bacteria pathogenic for mice and possibly for blue fox but not for mink or seals. CASELITZ (1965, p. 74—77) presents five cases in which *Aeromonas hydrophila* has been pathogenic for man. The diagnosis was septicæmia twice, diarrhoea twice and osteomyelitis once.

2.3.4. GRAM-NEGATIVE BACTERIA IN MEAT CURING

Bacteria with a beneficial effect on meat curing may be both gram-positive and negative. In many countries a curing method in which old brine is mixed with new is used. The purpose of this method is to transfer bacteria to the brine to be made before the curing process. LEISTNER (1960) examined the effect of bacteria in brines made in the above way on the brine itself, and on the products cured in those brines. In his opinion micro-organisms may improve the colour or flavour of the ready products, and stabilize brines.

Leistner observed that micrococci and lactobacilli improve the stability of curing brines and prevent their spoilage. He is also of the opinion that bacteria belonging to the genera *Vibrio*, *Spirillum*, *Achromobacter*, *Alcaligenes* and *Micrococcus* improve colour formation by reducing nitrate or affecting the pH and redoxpotential values favourably.

Leistner observed the influence of bacteria on the flavour of cured products. At least micrococci improve the flavour. Also, the optimal mixed cultures have a beneficial effect on the flavour of cured meat products. One mixed culture which Leistner found beneficial contained bacteria belonging to the following genera: *Micrococcus*, *Vibrio*, *Spirillum*, *Achromobacter*, *Alcaligenes*, *Lactobacillus*, *Microbacterium* and *Corynebacterium*. The effect is stronger if the culture is inoculated both into the cover brine and the pumping brine.

BUTTIAUX (1957) isolated a nitrate-reducing *Vibrio costicolus* strain which was used in meat curing in France. It was of great importance because the use

of nitrite was not allowed in France. In Germany *V. costicolus* was also found to have a beneficial effect on the flavour of cured products. In Great Britain *Achromobacter* strains have been used in brines to improve the quality of meat products (HAWTHORN and LEITCH 1962, p. 281). These strains have been found to have the same biochemical properties as the *Vibrio costicolus* of Buttiaux.

PETÄJÄ (1972, 1973) found *Vibrio costicolus* and *Achromobacter* strains which proved successful in meat curing, used both in cover brine and especially in the brine to be injected into meat. The strains were used as starter culture in dried hams. They had a favourable effect on the colour, consistency and flavour of hams. The respective control hams were raw after 7 days of ripening.

GORBATOV et al. (1974) used *Achromobacter guttatus* with *Streptococcus lactis*, *Lactobacillus plantarum*, *Micrococcus caseolyticus* and *Micrococcus varians* in the curing of canned «Vetchina hams» and found that this kind of mixed culture improves the flavour of hams.

2. 3. 5. GRAM-NEGATIVE BACTERIA IN THE PREPARATION OF DRY SAUSAGE

The influence of gram-negative bacteria on the quality of dry sausage, especially on the aroma and flavour, was investigated in the 1950's. 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.

ECKERT (1958) inoculated dry sausage with *Micrococcus* M₅₃ (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 cloacae* according to BERGEY'S MANUAL (1957). The strains were used together with *Lactobacillus plan-*

tarum. *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 that of sausages inoculated with lactobacilli and micrococci.

3. Experimental

3.1. Purpose of the study

Gram-negative bacteria are known to be useful in curing brines and improve the aroma and flavour of the cured meat products. The purpose of this investigation is to establish whether or not it is possible to use gram-negative bacteria as a starter culture in dry sausage and to find new, improved strains to be used 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, *Aeromonas* 19, *Vibrio costicolus*, three *Achromobacter* strains, *Escherichia coli* and *Proteus vulgaris*. The effect of inoculated bacteria on the ripening, quality and bacterial flora of dry sausage is examined.

3.2. Material

3.2.1. 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 %
Seasoning salt mixture	3.2 %
	100.0 %

Seasoning salt mixture:

Sodium chloride	89.7 %
Glucose	9.0 %
Potassium nitrate	0.625 % (200 ppm in sausage)
White pepper	0.7 %
	100.0 %

One batch contained 34.2 kg of sausage mass; the raw materials were mixed and ground in a cutter of 200 l capacity (Seydelmann, manufactured by Maschinenfabrik Seydelmann, Stuttgart, FRG). The mixture was left quite rough and divided into 6 equal parts each containing 5.7 kg. Each portion was inoculated and grinding was completed in a 40 l cutter (Seydelmann, Rasant

K-40, manufactured by Maschinenfabrik Seydelmann, Stuttgart, FRG). 100 ml of cell suspension was used for each 5.7 kg. The control portion was prepared first and the cutter was cleaned after the preparation of each portion. The sausage mass was stuffed into a casing 60 mm in diameter (Visko PK X, manufactured by Oy Visko Ab, Hanko, Finland), the weight of one sausage being about 0.5 kg. The sausages were ripened as follows:

ripening time	temperature	humidity
0— 4 days	22° C	95 %
4— 7 days	21° C	90 %
7—21 days	15—16° C	80 %

The ripening of the sausages was followed for 21 days. The samples inoculated with lactobacilli ripened in 10 days while sausages with other inoculations or without any inoculation sometimes did not ripen even in 21 days. After 14 days the sausages had ripened so that they could best be compared with each other.

3. 2. 2. BACTERIAL CULTURES USED

3. 2. 2. 1. *Micrococcus* sp.

The strain employed was isolated from the commercial product »Bactoferment 61» (manufactured by Rudolf Müller & Co, Giessen, FRG). The cells were cultivated in the nutrient broth (see 3. 3. 3. 1. h) by aeration, the incubation temperature being 30° C. It took 1—2 days to produce enough cells. The cells were separated centrifugally. The cell mass was covered with glucose, frozen and lyophilized (Lyophilizer Christ Delta 1/1 a, manufactured by Martin Christ, Osterode am Harz, FRG). The lyophilized cells were stored for 4 days before use. Lyophilized cells were suspended in 2 % sodium chloride solution just before inoculation into the sausage mass. The inoculations varied between 10^6 and 10^7 cells/g of sausage mass.

3. 2. 2. 2. *Lactobacillus plantarum*

The *Lactobacillus* strain used was *L. plantarum* (NURMI 1966). It was isolated from the commercial product »Duploferment 66» (manufactured by Rudolf Müller & Co, Giessen, FRG). The cells were cultivated in MRS broth (see 3. 3. 3. 1. i) by aeration, the incubation temperature being 30° C and the time one day. The cells were separated, covered with glucose, frozen, lyophilized and stored for 4 days before use, as with micrococci. Lyophilized cells were suspended in 2 % sodium chloride solution before inoculation. The inoculations varied between 10^6 — 10^7 cells/g of sausage mass.

3. 2. 2. 3. *Aeromonas* x

Aeromonas x was isolated from dry sausage (control sausage of experimental series IV in this study). The cell preparates used as an inoculum were made in

the same way as micrococci preparates. However the cultivation of one day was enough. The inoculations varied between 10^6 and 10^7 cells/g of sausage mass.

Some properties of *Aeromonas x* were investigated. According to the results, *Aeromonas x* is a gram-negative rod with polar flagella. Its properties are as follows:

- fermentative
- oxidase positive
- catalase positive
- lipolytic
- grows well in salt concentrations under 5 % and at temperatures of 20–37° C
- reduces nitrate
- liquefies gelatin
- forms acids from the following substrates: glucose, fructose, mannose, galactose, sucrose, maltose, xylose, raffinose, mannitol, sorbitol, glycerol and salicin.
- does not form acid from the following substrates: lactose, arabinose, rhamnose and esculin.
- not haemolytic

Aeromonas x has been classified as belonging to *Aeromonas* genus on the basis of BERGEY'S MANUAL (1974). In Bergey's Manual three species (*A. hydrophila*, *A. punctata*, *A. salmonicida*) and some subspecies are presented. The properties of *Aeromonas x* do not correspond to the properties of the three species and subspecies described.

Pathogenicity

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*, the LD₅₀ 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 LD₅₀ value of 10^8 or more are apathogenic. VALTONEN (1970) considered *Salmonella typhimurium* strains having a value of 10^8 avirulent.

3. 2. 2. 4. *Aeromonas 19*

Aeromonas 19 was isolated from dry sausage (control sausage of experimental series XVII in this research). The cell preparation was made and inoculated in the same way as that of *Aeromonas x*. The inoculations varied between 10^6 and 10^7 cells/g of sausage mass.

Aeromonas 19 is a gram-negative rod with polar flagella. Its properties are as follows:

- fermentative
- oxidase positive
- catalase positive

- lipolytic
- grows well in salt concentrations under 5 % and at the temperatures of 20–37° C
- reduces nitrate
- liquefies gelatin
- forms acids from the following substrates: glucose, fructose, mannose, galactose, sucrose, maltose, xylose, mannitol, sorbitol, glycerol and salicin.
- does not form acid from the following substrates: lactose, raffinose, rhamnose and esculin. Acid was formed only weakly from arabinose.
- not haemolytic

Aeromonas 19 has also been identified according to BERGEY'S MANUAL (1974) and does not correspond exactly with three species or subspecies presented, either.

Pathogenicity

The LD₅₀ value of *Aeromonas* 19 was observed to be about 10⁸. So according to what has just been said (chapter 3. 2. 2. 3.) it can be regarded as apathogenic.

3. 2. 2. 5. *Vibrio costicolus*

The *Vibrio costicolus* strain used had been isolated from curing brine (PETÄJÄ et al. 1973). A lyophilized *V. costicolus* preparation was made and inoculated in the same way as that of *Aeromonas* strains, the inoculum being 10⁶–10⁷ cells/g of sausage mass.

3. 2. 2. 6. *Achromobacter* strains

Achromobacter 22 (PETÄJÄ et al. 1973), *Achromobacter guttatus* (received from Nora Winterhalter of Eidgenössische Technische Hochschule, Zürich) and *Achromobacter X* (isolated from control dry sausage, experimental series XIV) were used as starter cultures. The strains were classified according to BERGEY'S MANUAL (1957). The lyophilized preparations were made and inoculated in the same way as those of *Aeromonas* x and 19, the inoculum being 10⁶–10⁷ cells/g of sausage mass.

3. 2. 2. 7. *Escherichia coli*

The strain of *Escherichia coli* used was obtained from the Institute of Microbiology of the University of Helsinki. *E. coli* cells were inoculated as a lyophilized preparation made in the same way as *Aeromonas* preparations. The cells were suspended in 2 % sodium chloride solution just before inoculation, the inoculum being 10⁶–10⁷ cells/g of sausage mass.

3. 2. 2. 8. *Proteus vulgaris*

The strain of *Proteus vulgaris* used originated from the Institute of Microbiology of the University of Helsinki. *P. vulgaris* was inoculated as a lyo-

philized preparation made in the same way as *Aeromonas* preparations. The cells were suspended in 2 % sodium chloride solution just before inoculation, the inoculum being 10^6 – 10^7 cells/g of sausage mass.

3. 3. Methods

3. 3. 1. ORGANOLEPTIC EVALUATION

The panel carrying out the evaluation consisted of five persons familiar with the organoleptic evaluation of dry sausage. Before evaluation, the quality desirable in experimental sausages was established. This was achieved by training evaluations. 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. The choices offered to the panel members were as follows:

Colour of sliced surface

- 3 bright red
- 2 red
- 1 brown, yellowish red
- 0 other bad discolourations

Consistency

- 3 firm
- 2 quite firm
- 1 slightly soft
- 0 soft

Aroma

- 3 excellent
- 2 good
- 1 odourless
- 0 unpalatable

Flavour

- 3 excellent
- 2 good
- 1 moderate
- 0 bad

Because the panel members could also award half points the points were multiplied by 2 before statistical processing.

3.3.2. PHYSICAL AND CHEMICAL EXAMINATIONS

3.3.2.1. *pH value*

pH values were measured after 0, 1, 3, 7, 10, 14 and 21 days of ripening using a Findip digital pH & mV meter 555A (manufactured by Oy Findip Ab, Kauniainen, Finland).

3.3.2.2. *Consistency*

Consistency was measured using an Instron instrument MO821 TM-M (manufactured by Instron Limited, High Wycombe, Bucks, England). A 7 cm long piece of sausage was used in the measurements. The sausage was compressed 1 cm with the measuring device which was 5.6 cm in diameter and the instrument registered the force needed. Measurements were made in three positions and their mean value was calculated. The results were expressed in kilograms. Consistency was measured after 1, 3, 7, 10, 14 and 21 days of ripening at room temperature.

3.3.2.3. *Weight losses*

Weight losses were determined by weighing the sample sausages to be studied before other determinations. This means that figures for weight losses were obtained after ripening times of 1, 3, 7, 10, 14 and 21 days.

3.3.2.4. *Nitrite*

The quantity of nitrite was determined according to the method of STOYA (1969). The protein was precipitated using di-sodium tetraborate-10-hydrate and zinc sulphate-7-hydrate. In this study the precipitate was separated centrifugally instead of by filtration. Nitrite was determined from the liquid phase using sulfanilic acid and α -naphthole. The determination was carried out after 0, 1, 3, 7, 10, 14 and 21 days of ripening.

3.3.2.5. *Nitrate*

The nitrate quantity was also determined by the method of STOYA (1969). The protein precipitation took place in the same way as in the nitrite determination. In the liquid phase the nitrate was reduced to nitrite by cadmium released by reducing cadmium sulphate with zinc powder. The total nitrite content was determined using the sulfanilic acid and α -naphthole reagents. The nitrate content was calculated using nitrite and nitrate standards determined in conjunction with the analyses. The nitrate was determined after 1, 3, 7, 10, 14 and 21 days of ripening.

3. 3. 3. MICROBIOLOGICAL EXAMINATIONS

3. 3. 3. 1. *Substrates used*

- a) Plate count agar for counting total number of bacteria, Merck No. 5463. Manufactured by Merck, Darmstadt, FRG.
- b) Mannitol salt agar for counting staphylococci and micrococci. Manufactured by Orion Oy, Helsinki, Finland.
- c) Tributyrine agar for counting lipolytic bacteria. Manufactured by Orion Oy, Helsinki, Finland.
- d) Rogosa agar for counting lactobacilli (ROGOSA et al. 1951). Manufactured by Orion Oy, Helsinki, Finland.
- e) Slanetz agar for counting enterococci and streptococci of the Viridans group (SLANETZ and BARTLEY 1957). Manufactured by Orion Oy, Helsinki, Finland.
- f) VRB (violet red bile) agar for counting coliform bacteria. Manufactured by Orion Oy, Helsinki, Finland.
- g) Hugh-Leifson semi-solid agar for fermentation and oxidation examinations in identification tests (HUGH and LEIFSON 1953).
- h) Nutrient broth according to PETÄJÄ (1977) for mass cultivation of different bacteria (developed for *Vibrio costicolus* at the Institute of Meat Technology of the University of Helsinki).
- i) MRS broth for mass cultivation of lactobacilli (de MAN et al. 1960).

3. 3. 3. 2. *Methods employed*

Experimental sausages were studied microbiologically after 0, 1, 3, 7, 10, 14 and 21 days of ripening. Dilution 1 was made in the following way: 10 grams of sausage was weighed into 90 ml of 2 % sodium chloride solution with a sterile spoon. The mixture was homogenized using an Ultra Turrax homogenizer TP 18/2 (manufactured by Janke & Kunkel KG, Staufen i. Br., FRG). The other necessary dilutions were also made in 2 % sodium chloride solution. If possible the count of bacteria was made by using plates on which the number of colonies varied between 30 and 300.

The following examinations were made:

- a) Cultivation on plate count agar, plating method.
Incubation for 4 days at 30° C.
The total number of bacteria was determined on plate count agar. All the colonies were recorded.
- b) Cultivation on mannitol salt agar, spreader method.
Incubation for 2 days at 37° C.
Round and entire colonies were recorded, the count including both micrococci and staphylococci.
- c) Cultivation on tributyrine agar, spreader method.
Incubation for 4 days at 30° C.

The lipolytic bacteria, of which inoculated *Aeromonas* strains separately, were determined on tributyrine agar by counting the colonies having a distinct lipolysis halo. The total number of bacteria was also counted.

- d) Cultivation on Rogosa agar, plating method.

Incubation for 4 days at 30° C.

The number of lactobacilli was determined on Rogosa agar. All the colonies were recorded.

- e) Cultivation on Slanetz agar, spreader method.

Incubation for two days at 37° C.

On Slanetz agar all red and reddish entire colonies were counted, the results thus including enterococci and streptococci of the Viridans group.

- f) Cultivation on VRB agar, plating method.

Incubation one day at 37° C.

Coliform bacteria form deep red colonies on VRB agar 1–2 mm in diameter. Only these kinds of colonies were recorded.

- g) Identification of *Aeromonas* strains.

Some strains that grew well in good quality dry sausage were identified. The first studies consisted of the following examinations: gram staining and microscopic examination, growing experiments at temperatures of 20–37° C and in NaCl concentrations of 0–5 %, reduction of nitrate, catalase and oxidase tests and further proteolytic, lipolytic and starch hydrolytic properties. Later the oxidation and fermentation tests and acid formation from the following substrates were investigated: glucose, fructose, mannose, galactose, sucrose, maltose, lactose, xylose, arabinose, raffinose, rhamnose, mannitol, sorbitol, glycerol, salicin and esculin.

- h) Determination of pathogenicity of *Aeromonas* strains.

The apathogenicity of *Aeromonas* strains was examined by inoculating intraperitoneally different numbers of bacterial cells into white mice. These studies were carried out in the State Veterinary Medical Institute, Helsinki.

3.3.4. STATISTICAL METHODS

The results of the organoleptic evaluation were tested by analysis of variance both without separating the samples of different ages and also by handling sausages of different ages separately. The comparison of different sausage group pairs was carried out using the t-test. The different properties were treated separately. The following notations were used to express the degree of significance:

+++	samples different, significance level	<0.001
++	» » »	<0.01
+	» » »	<0.05
–	» » »	>0.05

The presence of any significant differences between evaluations of panel members was tested in order to criticize the organoleptic evaluation. The standard deviation of the mean was used in the calculation of all the results.

3.3.5. GROUPING OF EXPERIMENTAL SAUSAGES

There were altogether twenty-two experimental sausage series. The composition of these series varied so that in different sequences the effect of different bacteria was studied. The sequences of investigation can be recorded in the following way according to the experimental series:

Experimental series	Inoculated gram-negative bacteria
1-6	<i>Vibrio</i> 21 and different <i>Achromobacter</i> strains
4-13	<i>Aeromonas</i> x, <i>Escherichia coli</i> and <i>Proteus vulgaris</i>
14-16	<i>Achromobacter</i> X
17-22	<i>Aeromonas</i> 19

This work deals mainly with the effect of *Aeromonas* x and *Aeromonas* 19 on the quality of dry sausage. This is done by comparing their properties with those of control and *Micrococcus* + *Lactobacillus* sausages. The following schema will be used in describing the results:

1. Control group
2. *Micrococcus* + *Lactobacillus* group
3. *Aeromonas* x group
4. *Aeromonas* x + *Lactobacillus* group
5. *Aeromonas* 19 group
6. *Aeromonas* 19 + *Lactobacillus* group

A short account will be given of the effect of other inoculated gram-negative bacteria on the ripening of dry sausage.

3.4. Results

3.4.1. USE OF *AEROMONAS* STRAINS AS A STARTER CULTURE

3.4.1.1. Organoleptic evaluation

Colour of sliced sausage

Fig. 1 and Table 1 show the differences in the mean scores of the sausages after different ripening periods. The sausage groups containing *Lactobacillus* inoculations are the best, the differences between them being very small. The colour of *Micrococcus* + *Lactobacillus* sausages is slightly better than that of *Aeromonas* x + *Lactobacillus* and *Aeromonas* 19 + *Lactobacillus* samples after 14 days of ripening. The colour develops at about the same rate in all three groups.

Table 1. Effect of bacterial inoculations on colour of sliced surface of experimental sausages (scores 0-6).

Sausage group	3 days	7 days	14 days	21 days
1. Control				
number of samples	21	21	20	19
mean	1.2	2.9	3.2	3.2
s.d.m.	0.9	1.4	1.5	1.7
2. <i>Micrococcus</i> + <i>Lactobacillus</i>				
number of samples	15	15	15	13
mean	2.9	4.1	4.9	4.6
s.d.m.	1.1	1.0	0.9	0.9
3. <i>Aeromonas</i> x				
number of samples	5	5	5	5
mean	1.3	2.6	3.1	4.0
s.d.m.	1.3	1.8	1.3	1.4
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>				
number of samples	6	6	6	6
mean	2.6	4.3	4.2	4.4
s.d.m.	1.2	1.1	0.9	0.9
5. <i>Aeromonas</i> 19				
number of samples	3	4	4	3
mean	2.7	3.0	3.9	3.6
s.d.m.	0.1	1.7	1.6	1.6
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>				
number of samples	6	6	6	5
mean	2.9	4.0	4.3	4.4
s.d.m.	1.0	1.0	1.2	1.0

s.d.m. = standard deviation of mean

In the control and *Aeromonas* x sausages the colour was significantly worse than in other samples (Table 2), being brownish and developing slowly. The

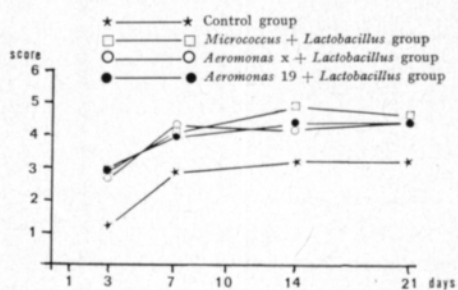


Figure 1. Effect of different bacterial inoculations on colour of sliced surface of sausage.

colour of *Micrococcus* + *Lactobacillus*, *Aeromonas* x + *Lactobacillus* and *Aeromonas* 19 + *Lactobacillus* sausages proved significantly better (significance level 0.05) than that of other samples. There was, however, no significant difference between them. *Aeromonas* 19 sausages were better than the control and *Aeromonas* x samples (Table 2).

Table 2. Comparison of colour of sausage pairs using the t-test. Scores for sausages of different ages are not separated.

Samples	Control	<i>Micrococcus</i> + <i>Lactobacillus</i>	<i>Aeromonas</i> x	<i>Aeromonas</i> x + <i>Lactobacillus</i>	<i>Aeromonas</i> 19
<i>Micrococcus</i> + <i>Lactobacillus</i>	+++	←			
<i>Aeromonas</i> x	-	+++			
<i>Aeromonas</i> x + <i>Lactobacillus</i>	+++	-	+++	←	
<i>Aeromonas</i> 19	+++	+++	+	+	
<i>Aeromonas</i> 19 + <i>Lactobacillus</i>	+++	-	+++	-	+

Consistency

The mean consistencies of sausages of different ages are shown in Fig. 2 and Table 3. *Aeromonas* 19 + *Lactobacillus* sausages are generally the best with *Micrococcus* + *Lactobacillus* samples the next best followed by *Aeromonas* x + *Lactobacillus* sausages. This was also the order after 14 days of ripening. Taken throughout ripening, *Aeromonas* 19 sausages were better than the control and *Aeromonas* x sausages.

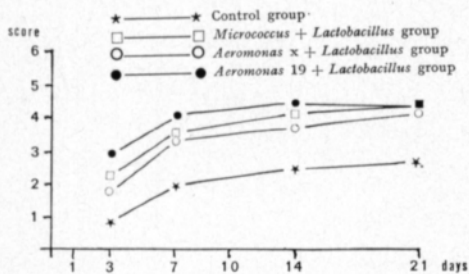


Figure 2. Effect of different bacterial inoculations on consistency of sausage.

The consistency of the control and *Aeromonas* x sausages was significantly worse than that of other samples (Table 4). The samples made using *Lactobacillus* inoculation were best. Sausages inoculated with micrococci and lactobacilli were not significantly better than *Aeromonas* x + *Lactobacillus* samples. Instead *Aeromonas* 19 + *Lactobacillus* and *Micrococcus* + *Lactobacillus* sausages differed at the 0.05 level, the former being the better. *Aeromonas* 19 + *Lactobacillus* samples were better than *Aeromonas* x + *Lactobacillus* ones at the 0.001 level. *Aeromonas* 19 sausages were better than samples with *Aeromonas* x.

Table 3. Effect of bacterial inoculations on consistency of experimental sausages (scores 0-6).

Sausage group	3 days	7 days	14 days	21 days
1. Control				
number of samples	21	21	20	19
mean	0.9	1.9	2.5	2.7
s.d.m.	1.0	1.2	1.5	1.2
2. <i>Micrococcus</i> + <i>Lactobacillus</i>				
number of samples	15	15	15	13
mean	2.2	3.5	4.2	4.5
s.d.m.	1.3	0.9	1.0	1.1
3. <i>Aeromonas</i> x				
number of samples	5	5	5	5
mean	0.6	1.5	2.5	3.0
s.d.m.	0.8	1.4	0.9	1.3
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>				
number of samples	6	6	6	6
mean	1.8	3.4	3.8	4.2
s.d.m.	1.2	0.7	0.8	1.2
5. <i>Aeromonas</i> 19				
number of samples	3	4	4	3
mean	1.3	2.5	3.2	3.5
s.d.m.	1.2	1.3	1.1	1.5
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>				
number of samples	6	6	6	5
mean	2.9	4.1	4.5	4.4
s.d.m.	1.4	0.9	1.1	0.9

s.d.m. = standard deviation of mean

Table 4. Comparison of consistency of sausage pairs using the t-test. Scores for sausages of different ages are not separated.

Samples	Control	<i>Micrococcus</i> + <i>Lactobacillus</i>	<i>Aeromonas</i> x	<i>Aeromonas</i> x + <i>Lactobacillus</i>	<i>Aeromonas</i> 19
<i>Micrococcus</i> + <i>Lactobacillus</i>	+++ ←	↑			
<i>Aeromonas</i> x	-	+++			
<i>Aeromonas</i> x + <i>Lactobacillus</i>	+++ ←	-	+++ ←		
<i>Aeromonas</i> 19	+++ ←	+++ ↑	++ ←	↑ ++	
<i>Aeromonas</i> 19 + <i>Lactobacillus</i>	+++ ←	+ ←	+++ ←	+++ ←	+++ ←

Aroma

The *Aeromonas* 19 + *Lactobacillus* sausages were the best for most of the ripening time (Fig. 3 and Table 5). The order of preference of the *Micrococcus* + *Lactobacillus*, *Aeromonas* x + *Lactobacillus* and *Aeromonas* 19 sausages varied during ripening. The order of preference of sausages with *Lactobacillus* inoculation after 14 days of ripening was as follows: *Aeromonas* 19 + *Lactobacillus* sausages were the best, second came *Aeromonas* x + *Lactobacillus* samples and third *Micrococcus* + *Lactobacillus* samples.

The aroma of the control and *Aeromonas* x sausages was significantly worse than that of other samples (Table 6). The *Aeromonas* 19 samples were clearly better than these, being statistically as good as *Micrococcus* + *Lactobacillus* and *Aeromonas* x + *Lactobacillus* samples, but worse than *Aeromonas* 19 +

Table 5. Effect of bacterial inoculations on aroma of experimental sausages (scores 0-6).

Sausage group	3 days	7 days	14 days	21 days
1. Control				
number of samples	21	21	20	19
mean	1.4	2.4	3.0	2.7
s.d.m.	1.0	1.3	1.2	1.3
2. <i>Micrococcus</i> + <i>Lactobacillus</i>				
number of samples	15	15	15	13
mean	1.9	3.0	3.6	3.6
s.d.m.	0.9	1.0	1.0	0.8
3. <i>Aeromonas</i> x				
number of samples	5	5	5	5
mean	1.1	2.2	2.0	3.4
s.d.m.	1.0	1.4	0.9	0.7
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>				
number of samples	6	6	6	6
mean	1.6	3.2	4.0	4.3
s.d.m.	1.2	1.0	1.0	0.7
5. <i>Aeromonas</i> 19				
number of samples	3	4	4	3
mean	2.2	3.1	3.5	2.9
s.d.m.	1.4	1.4	1.1	1.3
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>				
number of samples	6	6	6	5
mean	2.5	3.9	4.6	4.2
s.d.m.	0.8	0.9	0.8	1.0

s.d.m. = standard deviation of mean

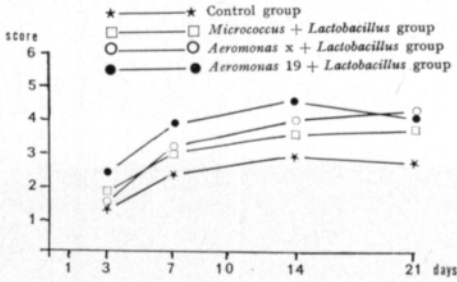


Figure 3. Effect of different bacterial inoculations on aroma of sausage.

Lactobacillus samples. There was no statistical difference between *Micrococcus* + *Lactobacillus* and *Aeromonas* x + *Lactobacillus* sausages, while *Aeromonas* 19 + *Lactobacillus* samples were significantly better (significance level 0.001) than the other ones.

Table 6. Comparison of aroma of sausage pairs using the t-test. Scores for sausages of different ages are not separated.

Samples →	Control	<i>Micrococcus</i> + <i>Lactobacillus</i>	<i>Aeromonas</i> x	<i>Aeromonas</i> x + <i>Lactobacillus</i>	<i>Aeromonas</i> 19
<i>Micrococcus</i> + <i>Lactobacillus</i>	+++ ←				
<i>Aeromonas</i> x	-	↑ +++			
<i>Aeromonas</i> x + <i>Lactobacillus</i>	+++ ←	-	+++ ←		
<i>Aeromonas</i> 19	+++ ←	-	++ ←	-	
<i>Aeromonas</i> 19 + <i>Lactobacillus</i>	+++ ←	+++ ←	+++ ←	+++ ←	+++ ←

Flavour

The flavour of *Aeromonas* 19 + *Lactobacillus* sausages was best after ripening times of 7 and 14 days (Fig. 4 and Table 7). Sausages with *Aeromonas* x + *Lactobacillus* and *Micrococcus* + *Lactobacillus* were next in this order throughout ripening. *Aeromonas* 19 sausages were better than both the control and *Aeromonas* x sausages throughout the ripening period.

The flavour of control and *Aeromonas* x sausages was the worst (Table 8). *Aeromonas* 19 samples were significantly better than these. However, the sausages inoculated with lactobacilli were best. *Aeromonas* x + *Lactobacillus*

Table 7. Effect of bacterial inoculations on flavour of experimental sausages (scores 0–6).

Sausage group	7 days	14 days	21 days
1. Control			
number of samples	17	18	18
mean	1.9	2.5	2.9
s.d.m.	1.0	1.2	1.2
2. <i>Micrococcus</i> + <i>Lactobacillus</i>			
number of samples	14	14	13
mean	3.1	3.9	4.2
s.d.m.	1.0	0.8	0.7
3. <i>Aeromonas</i> x			
number of samples	4	5	5
mean	1.9	2.6	2.7
s.d.m.	1.6	1.1	0.9
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>			
number of samples	6	6	6
mean	3.6	4.0	4.5
s.d.m.	0.7	0.9	1.0
5. <i>Aeromonas</i> 19			
number of samples	4	4	3
mean	2.5	3.7	3.3
s.d.m.	1.1	1.1	1.0
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>			
number of samples	6	6	5
mean	4.0	4.5	4.4
s.d.m.	0.8	1.1	0.9

s.d.m. = standard deviation of mean

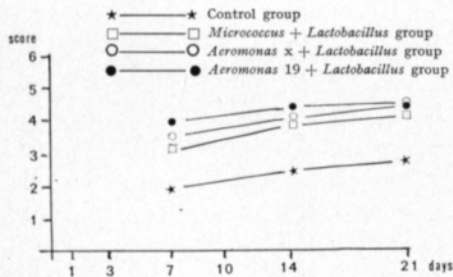


Figure 4. Effect of different bacterial inoculations on flavour of sausage.

sausages were better at the 0.05 significance level and *Aeromonas* 19 + *Lactobacillus* sausages better at the 0.001 significance level than *Micrococcus* + *Lactobacillus* sausages. However, *Aeromonas* 19 + *Lactobacillus* samples were not significantly better than *Aeromonas* x + *Lactobacillus* samples.

Table 8. Comparison of flavour of sausage pairs using the t-test. Scores for sausages of different ages are not separated.

Samples	Control	<i>Micrococcus</i> + <i>Lactobacillus</i>	<i>Aeromonas</i> x	<i>Aeromonas</i> x + <i>Lactobacillus</i>	<i>Aeromonas</i> 19
<i>Micrococcus</i> + <i>Lactobacillus</i>	+++	←	↑		
<i>Aeromonas</i> x	-	+++			
<i>Aeromonas</i> x + <i>Lactobacillus</i>	+++	←	+++	←	
<i>Aeromonas</i> 19	+++	↑	++	↑	+++
<i>Aeromonas</i> 19 + <i>Lactobacillus</i>	+++	←	+++	-	+++

3.4.1.2. Physical and chemical examinations

3.4.1.2.1. pH value

a) Control group

The pH value of control sausages showed the slowest drop. However, in these experiments the mean pH value of 7 day old sausages was 5.33 (Fig. 5 and Table 9). The pH value 5.30 or lower was attained in 54.4 % of 7 day old samples. During the next 2 weeks the pH decreased only slightly, the mean being 5.29 after 21 days of ripening. Fifty percent of the samples had a pH of 5.30 or lower.

b) *Micrococcus* + *Lactobacillus* group

The pH of *Micrococcus* + *Lactobacillus* sausages decreased so that its mean in 7 day old sausages was 5.20 (Fig. 5 and Table 9). 67.7 % of samples that were only 3 days old attained a pH of 5.30 or lower. The pH changes during the next 2 weeks were small. The mean pH of 3 week old sausages was 5.11 and the pH of 86.7 % of samples was 5.30 or lower.

c) *Aeromonas* x group

The pH of sausages inoculated with *Aeromonas* x strain was high throughout the ripening time, the mean always being over 5.30 (Table 9). *Aeromonas* x did not reduce the pH of dry sausage.

d) *Aeromonas x + Lactobacillus* group

The pH of the *Aeromonas x + Lactobacillus* sausage group decreased more quickly than that of the *Micrococcus + Lactobacillus* group, the mean being 5.22 when the sausages were 3 days old (Fig. 5 and Table 9). 67.7% of the samples had a pH of 5.30 or lower. After 2 weeks the mean pH value was 4.97 and all samples had a pH value of 5.30 or lower.

e) *Aeromonas 19* group

The pH of *Aeromonas 19* sausages was lower than that of sausages inoculated with *Aeromonas x*. After 10 days of ripening the mean pH value was less than 5.30, being 5.24 in 3 week old sausages (Table 9).

Table 9. Effect of bacterial inoculation on pH value of experimental sausages.

Sausage group	0 days	1 day	3 days	7 days	10 days	14 days	21 days
1. Control group							
number of samples	22	20	22	22	22	22	20
mean	5.70	5.70	5.58	5.33	5.30	5.25	5.29
s.d.m.	0.11	0.12	0.18	0.17	0.15	0.16	0.14
% samples, pH \leq 5.30	0	0	9.1	54.5	46.5	72.2	50.0
2. <i>Micrococcus + Lactobacillus</i>							
number of samples	15	14	15	15	15	15	14
mean	5.69	5.66	5.28	5.20	5.10	5.06	5.11
s.d.m.	0.69	0.33	0.23	0.27	0.16	0.15	0.14
% samples, pH \leq 5.30	0	0	67.7	73.3	86.7	86.7	86.7
3. <i>Aeromonas x</i>							
number of samples	4	4	4	4	4	4	4
mean	5.74	5.72	5.67	5.40	5.37	5.38	5.42
s.d.m.	0.10	0.10	0.16	0.21	0.17	0.21	0.18
% samples, pH \leq 5.30	0	0	0	50.0	50.0	50.0	25.0
4. <i>Aeromonas x + Lactobacillus</i>							
number of samples	6	6	6	6	6	6	6
mean	5.72	5.70	5.22	5.09	5.09	4.97	5.07
s.d.m.	0.10	0.10	0.09	0.19	0.19	0.20	0.15
% samples, pH \leq 5.30	0	0	67.7	83.3	83.3	100	100
5. <i>Aeromonas 19</i>							
number of samples	4	4	4	4	4	4	3
mean	5.75	5.76	5.57	5.32	5.28	5.25	5.24
s.d.m.	0.05	0.11	0.21	0.27	0.20	0.25	0.26
% samples, pH \leq 5.30	0	0	0	50.0	50.0	75.0	75.0
6. <i>Aeromonas 19 + Lactobacillus</i>							
number of samples	6	6	6	6	6	6	5
mean	5.73	5.71	5.16	5.04	5.04	5.07	5.12
s.d.m.	0.08	0.09	0.21	0.14	0.19	0.13	0.19
% samples, pH \leq 5.30	0	0	83.3	100	100	100	100

s.d.m. = standard deviation of mean

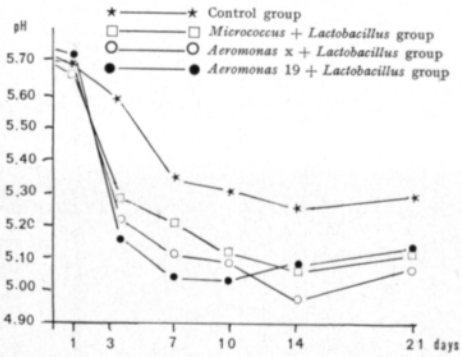


Figure 5. Effect of different bacterial inoculations on pH value of sausage.

f) *Aeromonas* 19 + *Lactobacillus* group

The decrease in pH value of *Aeromonas* 19 + *Lactobacillus* sausages was quickest (Fig. 5 and Table 9). The mean pH value of 3 day old sausages was 5.16 and all the sausage samples attained pH 5.30 or lower after 7 days of ripening. The pH differences were small during the next 2 weeks.

3. 4. 1. 2. 2. Consistency

a) Control group

The consistency of control sausages developed slowly and was not firm enough. Some sausages reached a consistency value of 3 kg after 14 days and these comprised 53.8 % of the total sample (Fig. 6 and Table 10). The mean consistency value of 21 day old sausages was 3.83 kg.

b) *Micrococcus* + *Lactobacillus* group

The consistency of *Micrococcus* + *Lactobacillus* sausages was clearly firmer than that of control sausages. After 7 days 41.7 % of samples attained a consistency value of 3 kg or over (Fig. 6 and Table 10). After 3 weeks of ripening the mean was 5.08 kg.

c) *Aeromonas* x group

The consistency values of the control and *Aeromonas* x sausages were of the same order. However, after 7 days of ripening the consistency values of 33.3 % of *Aeromonas* x sausages were 3 kg or over (Table 10). Later the respective percentages were higher than those of the control group. The mean of 21 day old sausages was 3.86 kg.

d) *Aeromonas* x + *Lactobacillus* group

After 3 days of ripening 50.0 % of samples had a consistency value of 3 kg or over (Fig. 6 and Table 10). So the consistency can be said to develop more quickly in the *Aeromonas* x + *Lactobacillus* group than in the *Micrococcus* + *Lactobacillus* group. The mean consistency values were also higher throughout ripening with the exception of 3 week old sausages, where the mean for *Micrococcus* + *Lactobacillus* sausages was 0.24 kg higher, the mean for *Aeromonas* x + *Lactobacillus* sausages being 4.84.

Table 10. Effect of bacterial inoculations on consistency (kg) of experimental sausages.

Sausage group	1 day	3 days	7 days	10 days	14 days	21 days
1. Control						
number of samples	10	13	12	13	13	11
mean	0.87	1.40	2.37	2.53	3.32	3.83
s.d.m.	0.14	0.65	0.88	0.65	3.21	0.93
% samples, ≥ 3 kg	0	0	0	0	53.8	90.9
2. <i>Micrococcus</i> + <i>Lactobacillus</i>						
number of samples	11	12	12	13	13	12
mean	1.07	2.09	3.33	3.58	4.51	5.08
s.d.m.	0.39	1.11	1.36	1.16	1.34	1.41
% samples, ≥ 3 kg	0	0	41.7	61.7	92.3	100
3. <i>Aeromonas</i> x						
number of samples	3	3	3	3	3	3
mean	1.45	1.68	2.57	2.55	3.82	3.86
s.d.m.	0.45	0.72	0.42	0.73	0.43	1.36
% samples, ≥ 3 kg	0	0	33.3	66.7	66.7	100
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>						
number of samples	3	4	4	4	4	4
mean	1.35	2.61	4.17	3.98	5.27	4.84
s.d.m.	0.45	1.86	2.68	1.12	1.76	1.40
% samples, ≥ 3 kg	0	50.0	75.0	75.0	100	100
5. <i>Aeromonas</i> 19						
number of samples	4	4	4	4	4	3
mean	0.88	1.06	1.62	2.18	2.93	3.83
s.d.m.	0.11	0.11	0.51	0.76	0.60	0.74
% samples, ≥ 3 kg	0	0	0	0	75.0	100
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>						
number of samples	6	6	6	6	6	5
mean	0.86	1.86	2.91	3.27	3.78	4.74
s.d.m.	0.13	0.33	0.73	1.05	1.00	1.38
% samples, ≥ 3 kg	0	0	16.7	66.7	83.3	100

s.d.m. = standard deviation of mean

e) *Aeromonas* 19 group

The consistency values stayed under 3 kg during the first 10 days of ripening, until after 2 weeks 75.0 % of samples had values over 3 kg. The sausages of this group were softest throughout ripening up to 3 weeks when the mean consistency value was the same as that of the sausages of the control group — 3.83 kg (Table 10).

f) *Aeromonas* 19 + *Lactobacillus* group

In *Aeromonas* 19 + *Lactobacillus* sausages the consistency developed surprisingly slowly especially at the beginning of ripening. After 7 days only 16.7 %

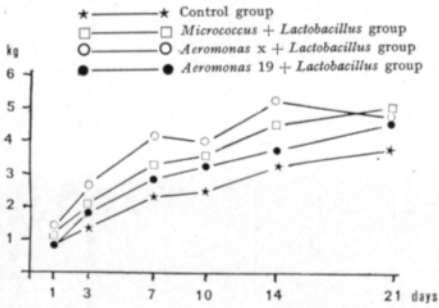


Figure 6. Effect of different bacterial inoculations on consistency (determined by Instron apparatus) of sausage.

of sausages had attained the consistency value of 3 kg or over (Fig. 6 and Table 10). The corresponding percentage in *Micrococcus + Lactobacillus* sausages was 41.7 and in *Aeromonas x + Lactobacillus* sausages 75.0. After 21 days the mean consistency value of *Aeromonas 19 + Lactobacillus* sausages was 4.74 being 0.34 kg lower than that of *Micrococcus + Lactobacillus* sausages.

3. 4. 1. 2. 3. Weight losses

The mean weight losses for each sausage group are presented in table 11. The table shows that the weight losses for each group developed similarly. The ranges of the means for all sausage groups are as follows:

Table 11. Percentile weight losses of experimental sausages.

Sausage group	1 day	3 days	7 days	10 days	14 days	21 days
1. Control						
number of samples	12	15	13	16	17	14
mean	2.6	7.1	13.9	17.1	19.0	24.6
s.d.m.	1.0	1.8	2.3	3.1	4.4	3.6
2. <i>Micrococcus + Lactobacillus</i>						
number of samples	10	16	13	16	15	12
mean	2.7	8.4	14.1	17.8	20.6	25.4
s.d.m.	1.0	2.1	1.4	2.1	2.7	2.8
3. <i>Aeromonas x</i>						
number of samples	4	5	5	5	4	5
mean	2.5	7.8	15.3	18.0	20.6	26.0
s.d.m.	1.0	2.1	2.3	3.2	4.9	2.8
4. <i>Aeromonas x + Lactobacillus</i>						
number of samples	5	6	6	6	5	5
mean	3.3	8.3	15.2	18.9	20.5	25.3
s.d.m.	1.4	2.3	2.5	2.4	1.7	6.1
5. <i>Aeromonas 19</i>						
number of samples	3	4	4	3	3	3
mean	3.0	7.8	13.5	17.2	20.5	24.9
s.d.m.	0.6	1.9	1.5	1.0	2.6	1.0
6. <i>Aeromonas 19 + Lactobacillus</i>						
number of samples	5	6	6	6	5	5
mean	3.3	8.8	14.5	18.1	22.4	26.7
s.d.m.	1.5	2.2	1.6	2.0	1.6	0.8

s.d.m. = standard deviation of mean

1 day	3 days	7 days	10 days	14 days	21 days
2.5–3.3 %	7.1–8.8 %	13.5–15.3 %	17.1–18.9 %	19.0–22.4 %	24.6–26.7 %

When the control sausages ripen for 3 weeks and *Micrococcus* + *Lactobacillus*, *Aeromonas* x + *Lactobacillus* and *Aeromonas* 19 + *Lactobacillus* sausages ripen for 10 days, the weight losses during ripening can, in the last 3 cases, be considerably reduced. The following mean weight losses for ripened sausages can be taken from table 11:

— control group	24.6 %
— <i>Micrococcus</i> + <i>Lactobacillus</i> group	17.8 %
— <i>Aeromonas</i> x group	26.0 %
— <i>Aeromonas</i> x + <i>Lactobacillus</i> group	18.9 %
— <i>Aeromonas</i> 19 group	24.9 %
— <i>Aeromonas</i> 19 + <i>Lactobacillus</i> group	18.1 %

It was sometimes found that in the control, *Aeromonas* x and *Aeromonas* 19 groups the sausages did not always ripen within 3 weeks.

3. 4. 1. 2. 4. Nitrite

The nitrite content of experimental sausages rose during the first 3 days of ripening so that the percentages of samples containing 30 ppm or more of sodium nitrite for the different sausage groups were as follows:

— control group	29.6 %
— <i>Micrococcus</i> + <i>Lactobacillus</i> group	66.7 %
— <i>Aeromonas</i> x group	80.0 %
— <i>Aeromonas</i> x + <i>Lactobacillus</i> group	66.7 %
— <i>Aeromonas</i> 19	25.0 %
— <i>Aeromonas</i> 19 + <i>Lactobacillus</i>	0 %

(Table 12).

The highest mean nitrite values after 3 days of ripening were found in sausages inoculated with *Aeromonas* x (56.5 ppm), *Aeromonas* x + *Lactobacillus* (37.2 ppm) and *Micrococcus* + *Lactobacillus* (34.1 ppm) (Table 12). Even the control sausages contained 25.6 ppm sodium nitrite while *Aeromonas* 19 sausages contained only 12.0 ppm and *Aeromonas* 19 + *Lactobacillus* sausages only 9.4 ppm. After 3 days of ripening nitrite concentrations decreased

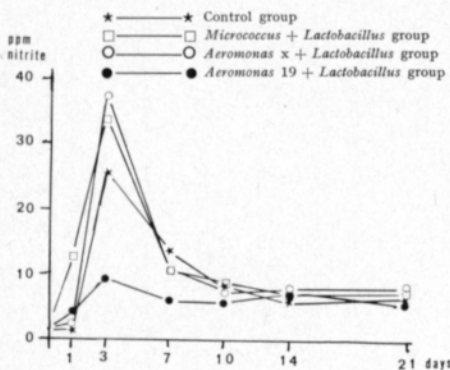


Figure 7. Effect of different bacterial inoculations on sodium nitrite content of sausage.

so that 7 day old sausages contained about 10 ppm sodium nitrite. During the next 2 weeks there was a small decrease in nitrite concentration in all sausage groups (Fig. 7 and Table 12). The results can be interpreted as showing that *Aeromonas* x and *Micrococcus* are effective nitrate reducers. On the other hand the content of nitrate is also lower in *Aeromonas* 19 + *Lactobacillus* sausages (p. 145). Consequently *Aeromonas* 19 could be regarded as a stronger nitrate reducer.

Table 12. Effect of bacterial inoculations on sodium nitrite content (ppm) of experimental sausages.

Sausage group	0 days	1 day	3 days	7 days	10 days	14 days	21 days
1. Control							
number of samples	20	19	21	21	22	22	20
mean	1.0	1.4	25.6	13.3	8.0	6.4	6.4
s.d.m.	0.7	1.3	25.0	9.4	3.5	3.7	3.0
% samples, ≤ 30 ppm	0	0	29.6	0	0	0	0
2. <i>Micrococcus</i> + <i>Lactobacillus</i>							
number of samples	15	14	15	15	15	15	14
mean	1.4	12.5	34.1	10.4	8.6	7.0	6.9
s.d.m.	1.2	20.8	18.0	5.4	3.7	2.9	3.6
% samples, ≤ 30 ppm	0	14.4	66.7	0	0	0	0
3. <i>Aeromonas</i> x							
number of samples	5	5	5	5	5	5	5
mean	0.8	2.3	56.5	13.3	12.8	9.6	8.7
s.d.m.	0.6	2.8	28.3	9.3	7.5	4.7	3.3
% samples, ≤ 30 ppm	0	0	80.0	0	0	0	0
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>							
number of samples	6	6	6	6	6	6	6
mean	0.8	1.8	37.2	10.1	7.5	8.2	8.1
s.d.m.	0.8	1.7	24.6	3.6	4.0	4.3	2.4
% samples, ≤ 30 ppm	0	0	66.7	0	0	0	0
5. <i>Aeromonas</i> 19							
number of samples	4	4	4	4	4	4	4
mean	1.2	8.0	12.0	11.1	5.5	7.6	6.5
s.d.m.	0.5	7.0	12.6	11.3	1.4	3.9	5.1
% samples, ≤ 30 ppm	0	0	25.0	25.0	0	0	0
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>							
number of samples	6	5	6	6	6	6	5
mean	1.3	3.6	9.4	6.8	6.5	7.1	6.4
s.d.m.	0.5	4.6	7.5	1.2	1.7	2.4	2.7
% samples, ≤ 30 ppm	0	20.0	0	0	0	0	0

s.d.m. = standard deviation of mean

3. 4. 1. 2. 5. Nitrate

The potassium nitrate content decreased most markedly in sausages inoculated with lactobacilli. After one day the means were as follows:

– <i>Micrococcus</i> + <i>Lactobacillus</i> group	119.0 ppm
– <i>Aeromonas</i> x + <i>Lactobacillus</i> group	100.3 ppm
– <i>Aeromonas</i> 19 + <i>Lactobacillus</i> group	122.4 ppm

(Table 13).

The mean potassium nitrate contents of other samples after one day of ripening were as follows:

– control group	176.9 ppm
– <i>Aeromonas</i> x group	176.6 ppm
– <i>Aeromonas</i> 19 group	159.0 ppm

(Table 13).

Table 13. Effect of bacterial inoculations on potassium nitrate content (ppm) of experimental sausages.

Sausage group	1 day	3 days	7 days	10 days	14 days	21 days
1. <i>Control</i>						
number of samples	13	11	13	11	14	12
mean	176.9	179.7	82.8	97.8	64.7	53.5
s.d.m.	55.3	62.0	43.8	54.8	41.7	23.6
2. <i>Micrococcus + Lactobacillus</i>						
number of samples	11	12	12	13	12	9
mean	119.0	106.0	86.6	54.9	42.8	39.1
s.d.m.	39.0	32.4	43.3	37.7	22.9	21.5
3. <i>Aeromonas x</i>						
number of samples	5	5	5	6	4	6
mean	176.6	107.8	94.0	90.8	87.0	81.6
s.d.m.	65.0	65.5	73.0	54.4	29.1	51.9
4. <i>Aeromonas x + Lactobacillus</i>						
number of samples	6	7	7	7	6	6
mean	100.3	115.1	73.7	71.0	36.7	46.5
s.d.m.	33.1	35.8	18.9	26.5	13.5	30.0
5. <i>Aeromonas 19</i>						
number of samples	3	3	3	3	3	3
mean	159.0	99.7	55.3	57.7	44.7	55.3
s.d.m.	18.7	70.5	38.3	33.7	5.5	45.2
6. <i>Aeromonas 19 + Lactobacillus</i>						
number of samples	5	5	6	6	5	5
mean	122.4	72.4	68.2	49.0	50.6	33.2
s.d.m.	27.1	26.8	50.5	37.0	33.0	24.9

s.d.m. = standard deviation of mean

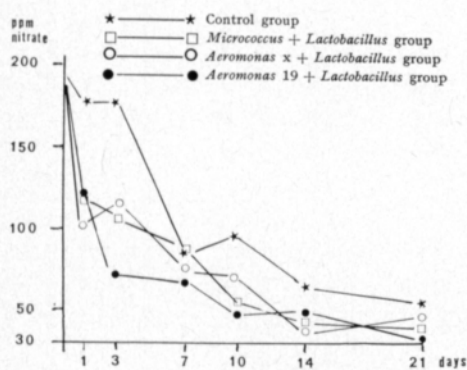


Figure 8. Effect of different bacterial inoculations on potassium nitrate content of sausage.

The potassium nitrate concentration decreased throughout the time of ripening in sausages inoculated with lactobacilli and some other bacteria (Fig. 8). After 3 weeks of ripening the lowest mean values were in these sausages the values being as follows:

- *Micrococcus + Lactobacillus* group 39.1 ppm
 - *Aeromonas x + Lactobacillus* group 46.5 ppm
 - *Aeromonas 19 + Lactobacillus* group 33.2 ppm
- (Table 13).

The mean potassium nitrate contents in other sausages after 3 weeks of ripening were as follows:

— control group	53.5 ppm
— <i>Aeromonas</i> x group	81.6 ppm
— <i>Aeromonas</i> 19 group	55.3 ppm

(Table 13).

3.4.1.3. Microbiological examinations

3.4.1.3.1. Total bacteria

The results of total bacterial counts are presented in Fig. 9 and Table 14. On the first day of ripening there was little or no increase in numbers of bacteria in any of the sausage groups. During the next 2 days the essential growth of bacterial counts took place in all groups. After that the total bacterial counts stayed at the same level or changed only slightly. The highest numbers of bacteria were in the sausages of *Aeromonas* x + *Lactobacillus* and *Aeromonas* 19

Table 14. Number of bacteria $\times 10^6/g$ on plate count agar in different sausage groups.

Sausage group	0 days	1 day	3 days	7 days	10 days	14 days	21 days
1. <i>Control</i>							
number of samples	15	14	15	14	15	15	14
mean	3.2	2.7	82.6	86.4	70.5	59.9	66.9
s.d.m.	4.6	3.8	84.0	69.9	41.2	46.2	40.3
2. <i>Micrococcus</i> + <i>Lactobacillus</i>							
number of samples	10	13	13	14	14	13	12
mean	16.4	23.1	135.1	143.6	92.5	77.4	77.7
s.d.m.	8.3	15.4	107.0	145.2	71.6	52.3	70.0
3. <i>Aeromonas</i> x							
number of samples	4	4	4	4	4	4	4
mean	17.3	17.3	38.8	92.5	65.3	71.4	65.7
s.d.m.	10.9	10.2	54.3	80.2	52.4	68.1	25.5
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>							
number of samples	6	6	6	6	6	6	6
mean	16.7	12.7	356.7	208.0	195.3	171.5	211.8
s.d.m.	8.9	8.7	215.1	81.3	99.0	77.6	86.6
5. <i>Aeromonas</i> 19							
number of samples	4	4	3	4	3	4	3
mean	12.0	12.5	49.0	42.8	39.7	69.8	213.3
s.d.m.	4.6	3.4	5.6	12.7	15.6	65.2	161.7
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>							
number of samples	6	6	6	6	6	6	5
mean	15.0	36.0	350.0	360.0	391.0	264.8	698.0
s.d.m.	7.6	39.8	284.7	152.2	352.6	170.8	436.0

s.d.m. = standard deviation of mean

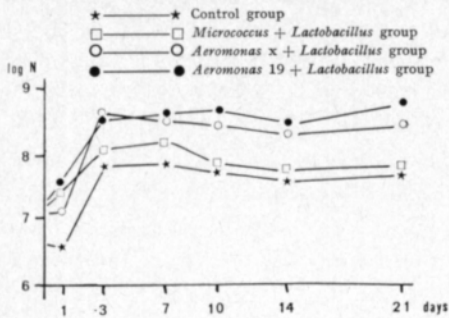


Figure 9. Number of bacteria/g on plate count agar in different sausage groups.

of *Aeromonas* x and *Aeromonas* 19 sausages remained between $10^7/g$ and $2 \times 10^8/g$ throughout ripening. The reason is that they did not contain inoculated lactobacilli.

3. 4. 1. 3. 2. Micrococci

Mannitol salt agar was used to determine, in particular, the number of inoculated micrococci. Staphylococci also grow on this medium, and this must be borne in mind when checking the results.

+ *Lactobacillus* groups, with mean values well over $10^8/g$. In the *Micrococcus* + *Lactobacillus* group the mean bacterial count increased to a little over $10^8/g$ during the first 3 days of ripening and decreased to less than $10^8/g$ after 7 days of ripening. The control sausages contained slightly fewer bacteria than the sausages inoculated with micrococci and lactobacilli. The bacterial counts

Table 15. Number of micrococci $\times 10^4/g$ on mannitol salt agar in different sausage groups.

Sausage group	0 days	1 day	3 days	7 days	10 days	14 days	21 days
1. Control							
number of samples	20	19	17	14	19	15	17
mean	7.5	6.0	14.9	33.2	10.0	5.1	8.7
s.d.m.	11.9	6.6	13.7	33.6	11.5	6.9	11.3
2. <i>Micrococcus</i> + <i>Lactobacillus</i>							
number of samples	15	13	14	15	15	14	14
mean	870	1267	1607	1240	1547	1593	1293
s.d.m.	566	849	1440	771	1164	1408	975
3. <i>Aeromonas</i> x							
number of samples	5	5	5	5	5	5	5
mean	1.5	2.9	1.9	3.4	4.3	4.2	4.5
s.d.m.	1.2	2.1	1.1	3.3	4.7	2.3	4.5
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>							
number of samples	6	6	6	6	6	6	6
mean	3.1	3.3	3.3	2.1	1.4	0.9	0.3
s.d.m.	3.0	3.2	3.0	2.4	1.7	0.9	0.2
5. <i>Aeromonas</i> 19							
number of samples	4	4	4	4	4	4	4
mean	7.5	23.3	10.6	23.3	1.9	3.6	4.8
s.d.m.	10.4	24.5	8.8	24.4	1.4	2.6	4.7
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>							
number of samples	6	6	6	6	6	6	4
mean	7.3	4.4	4.1	0.8	2.1	2.7	0.6
s.d.m.	9.0	3.4	4.9	0.7	2.2	2.5	0.8

s.d.m. = standard deviation of mean

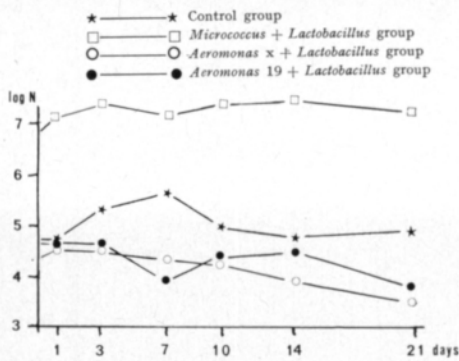


Figure 10. Number of micrococci/g on mannitol salt agar in different sausage groups.

The number of micrococci in control sausages was about $10^5/g$ throughout ripening (Fig. 10). The sausages inoculated with micrococci and lactobacilli contained the most micrococci. Throughout ripening the mean count was a little over $10^7/g$. The number of micrococci in *Aeromonas x + Lactobacillus* and *Aeromonas 19 + Lactobacillus* sausages decreased from about $5 \times 10^4/g$ to about $5 \times 10^3/g$ during ripening (Fig. 10 and Table 15). The mean

micrococcus count of *Aeromonas x* sausages stayed between $10^4/g$ and $5 \times 10^4/g$ while that of *Aeromonas 19* sausages exceeded $10^5/g$ after one day and stayed there about one week before falling back to between $10^4/g$ and $10^6/g$.

3. 4. 1. 3. 3. Total bacteria on tributyrine agar

The total number of bacteria in control sausages remained lower on tributyrine agar than on plate count agar, the mean lying between $10^6/g$ and $10^7/g$ throughout ripening (Figs. 9, 11 and Tables 14, 16). The total bacterial count

Table 16. Number of bacteria $\times 10^6/g$ on tributyrine agar in different sausage groups.

Sausage group	0 days	1 day	3 days	7 days	10 days	14 days	21 days
1. Control							
number of samples	19	16	21	15	22	21	21
mean	1.4	1.3	7.1	10.0	7.1	4.9	6.2
s.d.m.	1.2	1.5	8.9	9.1	4.7	6.8	7.2
2. <i>Micrococcus + Lactobacillus</i>							
number of samples	15	14	15	13	14	13	14
mean	8.1	11.2	14.5	11.1	6.6	7.0	6.0
s.d.m.	5.2	9.1	13.3	9.0	3.5	4.3	4.7
3. <i>Aeromonas x</i>							
number of samples	5	5	5	5	5	5	5
mean	12.5	43.8	45.4	38.2	59.2	39.8	35.6
s.d.m.	8.7	36.3	27.9	27.9	55.2	27.7	24.3
4. <i>Aeromonas x + Lactobacillus</i>							
number of samples	6	6	6	6	6	5	4
mean	20.6	33.8	57.0	10.6	7.1	4.0	2.1
s.d.m.	17.1	13.0	50.0	3.7	4.8	3.0	3.3
5. <i>Aeromonas 19</i>							
number of samples	4	4	3	4	4	4	3
mean	22.1	28.0	10.8	35.5	45.8	49.1	25.0
s.d.m.	13.8	6.5	8.0	28.8	46.0	52.2	15.0
6. <i>Aeromonas 19 + Lactobacillus</i>							
number of samples	6	5	6	6	6	6	5
mean	32.0	23.0	49.2	13.3	132.1	78.6	20.2
s.d.m.	32.0	11.8	36.9	12.7	151.7	87.2	19.3

s.d.m. = standard deviation of mean

of *Micrococcus* + *Lactobacillus* sausages on tributyrine agar reflects most closely the number of micrococci. The mean count first rose to a little over $10^7/g$ and then fell to less than $10^7/g$ after one week. On mannitol salt agar the mean count remained in excess of $10^7/g$.

The mean bacterial count of *Aeromonas* x + *Lactobacillus* sausages increased

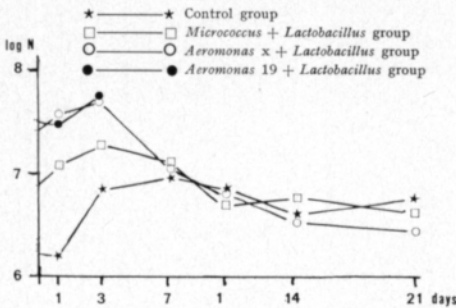


Figure 11. Number of bacteria/g on tributyrine agar in different sausage groups.

(Fig. 11 and Table 16). The mean quantity of bacteria in the *Aeromonas* x and *Aeromonas* 19 sausage groups varied between $10^7/g$ and $6 \times 10^7/g$ during ripening.

(Fig. 11 and Table 16). Thereafter the count fell to less than $10^7/g$ and after 3 weeks of ripening was around $10^6/g$. The total number of bacteria in *Aeromonas* 19 + *Lactobacillus* sausages ranged between $10^7/g$ and $10^8/g$ during ripening. The numbers of bacteria shown greatly resemble the lipolytic bacterial count (= inoculated *Aeromonas* bacteria) because lactobacilli do not grow on tributyrine agar

3. 4. 1. 3. 4. Lipolytic bacteria

The sausage was cultivated on tributyrine agar to determine the number of inoculated *Aeromonas* strains. In the sausages inoculated with *Aeromonas* x + *Lactobacillus* and *Aeromonas* 19 + *Lactobacillus* the mean lipolytic bacterial counts decreased from about $2 \times 10^7/g$ to about $2.5 \times 10^6/g$ during ripening (Fig. 12 and Table 17). The numbers of strain x were a little smaller than those of strain 19. The mean quantity of lipolytic bacteria in sausages inoculated

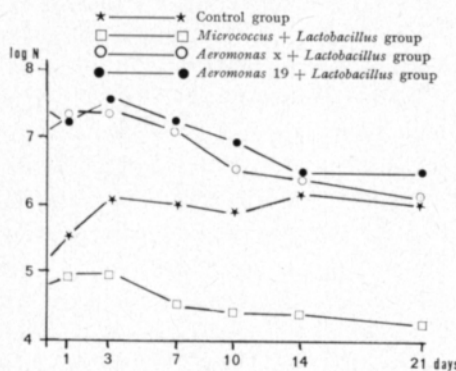


Figure 12. Number of lipolytic bacteria/g on tributyrine agar in different sausage groups.

with *Aeromonas* x and *Aeromonas* 19 varied between $10^7/g$ and $5 \times 10^7/g$ (Table 17).

The mean lipolytic bacterial counts in the control sausages rose from about $10^5/g$ to about $10^6/g$ during the first 3 days of ripening and then remained at a level of $10^6/g$ (Fig. 12 and Table 17). The number of lipolytic bacteria varied between $10^5/g$ and $10^4/g$ in *Micrococcus* + *Lactobacillus* sausages during ripening.

Table 17. Number of lipolytic bacteria $\times 10^6/g$ on tributyrine agar in different sausage groups.

Sausage group	0 days	1 day	3 days	7 days	10 days	14 days	21 days
1. Control							
number of samples	19	17	19	19	19	19	19
mean	0.2	0.4	1.3	1.0	0.8	1.5	1.0
s.d.m.	0.3	1.2	2.0	2.4	1.4	2.5	2.4
2. <i>Micrococcus</i> + <i>Lactobacillus</i>							
number of samples	12	13	14	11	13	11	14
mean	0.07	0.09	0.1	0.04	0.03	0.03	0.02
s.d.m.	0.1	0.2	0.2	0.06	0.07	0.04	0.03
3. <i>Aeromonas</i> x							
number of samples	5	5	5	5	5	5	4
mean	15.0	26.2	42.2	32.6	17.6	16.2	4.7
s.d.m.	14.3	19.9	27.0	26.7	11.4	14.7	7.0
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>							
number of samples	6	6	6	6	6	6	6
mean	14.8	25.8	30.2	11.2	3.8	2.4	1.2
s.d.m.	11.2	17.8	27.9	5.0	2.5	2.2	2.2
5. <i>Aeromonas</i> 19							
number of samples	4	4	3	4	4	4	3
mean	22.0	28.0	41.0	35.5	35.3	9.1	25.5
s.d.m.	13.9	6.5	15.0	28.8	35.5	6.8	30.4
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>							
number of samples	6	5	6	6	6	6	5
mean	32.0	23.0	49.2	18.5	9.4	3.0	3.3
s.d.m.	31.4	11.8	36.9	16.8	7.1	3.7	4.0

s.d.m. = standard deviation of mean

3. 4. 1. 3. 5. Lactobacilli

The mean lactobacillus counts of the control sausages rose steeply during the first 3 days of ripening from $5 \times 10^3/g$ to $4 \times 10^7/g$ and ranged between

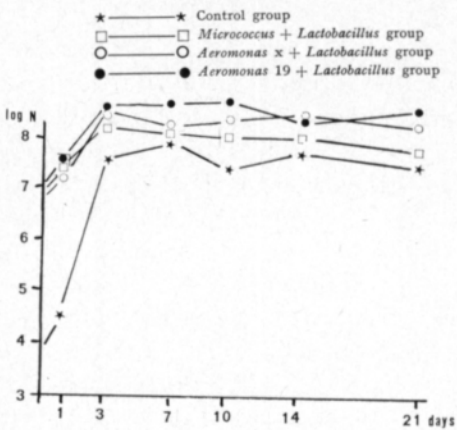


Figure 13. Number of lactobacilli/g on Rogosa agar in different sausage groups.

$10^7/g$ and $10^8/g$ for the rest of the ripening time (Fig. 13 and Table 18). The mean quantity of lactobacilli in sausages inoculated with lactobacilli rose from $8-10 \times 10^6/g$ to over $10^8/g$ in 3 days. Thereafter the mean lactobacillus counts remained at more than $8 \times 10^7/g$ for the rest of the ripening time so that the mean lactobacillus level in *Micrococcus* + *Lactobacillus* sausages was less than that in *Aeromonas* x + *Lactobacillus* and *Aeromonas* 19 + *Lactobacillus* sausages, the mean lactobacillus counts after 14 days of ripening being as follows:

Table 18. Number of lactobacilli $\times 10^6/g$ on Rogosa agar in different sausage groups.

Sausage group	0 days	1 day	3 days	7 days	10 days	14 days	21 days
1. Control							
number of samples	18	18	21	20	20	17	20
mean	0.005	0.02	43.8	94.6	34.1	56.1	40.1
s.d.m.	0.006	0.02	52.5	90.5	24.1	55.6	44.8
2. <i>Micrococcus</i> + <i>Lactobacillus</i>							
number of samples	13	12	15	15	15	12	12
mean	9.2	17.8	147.3	112.2	103.9	102.0	77.2
s.d.m.	7.2	14.4	143.2	116.8	109.4	101.1	78.3
3. <i>Aeromonas</i> x							
number of samples	5	5	5	5	5	5	5
mean	0.0031	0.014	10.0	44.6	41.4	41.8	41.6
s.d.m.	0.0053	0.022	8.1	30.8	22.5	34.3	24.5
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>							
number of samples	6	6	6	6	6	6	6
mean	8.0	14.7	300.0	162.7	247.3	320.0	187.5
s.d.m.	8.2	9.7	248.9	75.7	162.5	234.8	95.0
5. <i>Aeromonas</i> 19							
number of samples	4	4	3	4	4	4	4
mean	0.0073	0.037	9.7	41.0	78.0	65.5	102.7
s.d.m.	0.0083	0.043	7.2	36.4	50.9	69.9	64.7
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>							
number of samples	6	6	6	6	6	6	5
mean	10.0	31.2	311.7	345.0	423.0	240.7	456.0
s.d.m.	3.9	27.6	190.8	190.0	300.0	144.9	218.0

s.d.m. = standard deviation of mean

- *Micrococcus* + *Lactobacillus* group $1.0 \times 10^8/g$
- *Aeromonas* x + *Lactobacillus* group $3.2 \times 10^8/g$
- *Aeromonas* 19 + *Lactobacillus* group $2.4 \times 10^8/g$

The number of lactobacilli in *Aeromonas* x and *Aeromonas* 19 sausages rose from $10^3-10^4/g$ to a mean value of $10^7/g$ during the first 3 days (Table 18). During the rest of the ripening the mean lactobacillus counts varied between $4 \times 10^7/g$ and $10^8/g$.

3. 4. 1. 3. 6. Streptococci

The mean streptococcus counts of control sausages rose from about $10^3/g$ to $5 \times 10^5/g$ during the first 3 days of ripening (Fig. 14 and Table 19). Thereafter the number of streptococci rose further attaining a mean value of $2 \times 10^6/g$ after 10 days of ripening. After that, the value remained in excess of $10^6/g$.

The mean quantity of streptococci in sausages inoculated with lactobacilli and some other bacteria rose from $3 \times 10^2-2 \times 10^3/g$ to values between $10^4/g$

Table 19. Number of streptococci $\times 10^4/g$ on Slanetz agar in different sausage groups.

Sausage group	0 days	1 day	3 days	7 days	10 days	14 days	21 days
1. Control							
number of samples	21	18	19	19	20	21	20
mean	0.091	0.21	45.0	84.7	201.5	166.0	107.0
s.d.m.	0.100	0.18	35.0	93.0	311.0	235.0	140.0
2. <i>Micrococcus</i> + <i>Lactobacillus</i>							
number of samples	12	13	13	13	13	15	14
mean	0.05	0.13	2.3	3.1	1.9	3.4	2.9
s.d.m.	0.04	0.15	3.1	5.8	3.0	5.7	6.7
3. <i>Aeromonas</i> x							
number of samples	5	5	5	5	5	4	5
mean	0.30	0.21	20.2	133.0	84.6	53.5	103.3
s.d.m.	0.46	0.23	14.7	161.8	79.9	71.3	90.6
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>							
number of samples	6	6	6	6	6	6	4
mean	0.20	0.11	4.6	4.4	5.7	6.1	2.6
s.d.m.	0.35	0.052	5.8	4.7	7.7	8.6	3.4
5. <i>Aeromonas</i> 19							
number of samples	3	4	3	4	3	4	3
mean	0.038	0.70	58.8	90.0	333.3	132.7	217.3
s.d.m.	0.025	0.89	37.1	132.1	305.5	171.3	300.3
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>							
number of samples	6	6	5	6	6	6	5
mean	0.027	0.42	8.2	14.4	13.4	7.4	23.4
s.d.m.	0.023	0.37	7.4	20.9	21.6	8.0	33.7

s.d.m. = standard deviation of mean

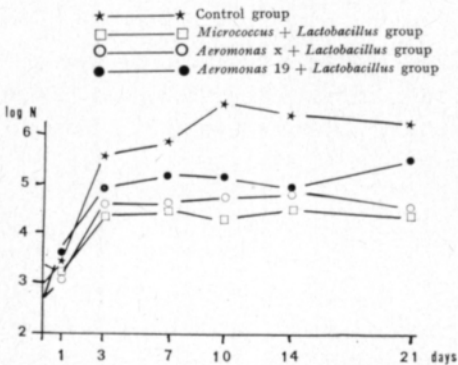


Figure 14. Number of streptococci/g on Slanetz agar in different sausage groups.

and $10^5/g$ over 3 days (Fig. 14 and Table 19). Thereafter the streptococcus counts remained at a level of about $10^4/g$ to $10^5/g$ so that *Aeromonas* 19 + *Lactobacillus* samples contained most and *Micrococcus* + *Lactobacillus* sausages least streptococci.

The mean streptococcus counts in *Aeromonas* x and *Aeromonas* 19 sausages resembled the values for control sausages, exceeding $10^6/g$ after 7 and 10 days of ripening and the latter remaining in excess of this

figure for the rest of the ripening time (Table 19).

Table 20. Number of coliform bacteria $\times 10^2/g$ on VRB agar in different sausage groups.

Sausage group	0 days	1 day	3 days	7 days	10 days	14 days	21 days
1. Control							
number of samples	17	18	15	13	16	13	18
mean	7.1	12.6	14.7	15.7	14.4	15.5	21.7
s.d.m.	10.8	12.9	25.2	18.5	23.5	20.3	29.3
2. <i>Micrococcus</i> + <i>Lactobacillus</i>							
number of samples	12	13	12	12	13	13	12
mean	16.3	21.5	12.9	11.3	4.6	4.0	3.2
s.d.m.	15.8	24.9	15.2	21.7	5.9	8.8	6.8
3. <i>Aeromonas</i> x							
number of samples	6	6	6	6	6	6	6
mean	2.1	3.6	1.0	1.3	0.1	0.8	1.8
s.d.m.	3.1	4.3	1.4	1.1	0.2	0.8	1.8
4. <i>Aeromonas</i> x + <i>Lactobacillus</i>							
number of samples	6	6	6	6	6	6	6
mean	1.6	1.2	2.0	0.9	1.0	0.6	0.7
s.d.m.	1.3	1.5	2.6	1.2	0.6	0.7	0.6
5. <i>Aeromonas</i> 19							
number of samples	4	4	4	4	4	4	4
mean	0.6	9.3	15.6	5.3	3.8	4.4	4.7
s.d.m.	0.5	15.3	18.7	5.1	5.5	4.0	6.4
6. <i>Aeromonas</i> 19 + <i>Lactobacillus</i>							
number of samples	6	6	6	6	6	5	4
mean	1.0	3.6	6.3	5.3	3.8	4.1	4.2
s.d.m.	1.2	3.4	6.8	4.9	5.1	3.9	6.2

s.d.m. = standard deviation of mean

3. 4. 1. 3. 7. Coliform bacteria

The mean quantity of coliform bacteria in control sausages rose from $7 \times 10^2/g$ to a little over $10^3/g$ and stayed at this level for the rest of the ripening time (Fig. 15 and Table 20). The mean level of coliform bacteria in *Micrococcus* + *Lactobacillus* sausages, on the other hand, decreased from $2.0 \times 10^3/g$ to less than $10^3/g$ after 10 days of ripening and remained at less than $10^3/g$ for the rest of the ripening time.

The mean quantity of coliform bacteria in *Aeromonas* x + *Lactobacillus* sausages decreased from just over $10^2/g$ to less than $10^2/g$ after 10 days of ripening (Fig. 15 and Table 20). The corresponding number of bacteria in *Aeromonas* 19 + *Lactobacillus* sausages ranged between $10^2/g$ and $10^3/g$. The mean quantity of coliform bacteria in *Aeromonas* x and *Aeromonas* 19 sausages ranged between $10^2/g$ and $10^3/g$ throughout ripening (Table 20). The highest values appeared after one day or 3 days of ripening.

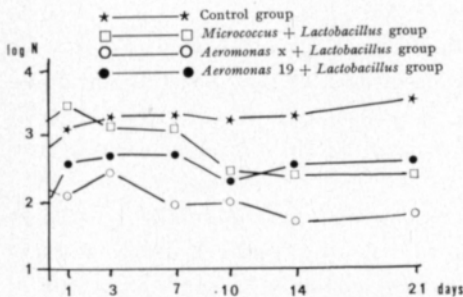


Figure 15. Number of coliform bacteria/g on VRB agar in different sausage groups.

3. 4. 2. USE OF *VIBRIO COSTICOLUS* AS A STARTER CULTURE

The effect of *Vibrio costicolus* on the quality of dry sausage was studied. The strain was isolated from curing brine and improves the quality of dried hams (PETÄJÄ 1972, PETÄJÄ et al. 1973).

V. costicolus did not thrive in dry sausage. The amount of *V. costicolus* fell noticeably 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 dry sausage, it can be concluded that *V. costicolus* cannot be used as a starter culture for dry sausage.

3. 4. 3. USE OF *ACHROMOBACTER* STRAINS AS A STARTER CULTURE

The effect of *Achromobacter* 22, *Achromobacter guttatus* and *Achromobacter X* strains on the quality of dry sausage was studied. The first strain was isolated from curing brine (PETÄJÄ et al. 1973), strain X from a control dry sausage in this research and *A. guttatus* had been received from Nora Winterhalter of Eidgenössische Technische Hochschule, Zürich, Switzerland.

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 carried 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 successful. *Achromobacter X* thrived in dry sausage, the number of cells being about $2 \times 10^7/g$ 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 micrococci: 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 control sausages.

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.

3. 4. 4. 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 same quality class as control sausages. 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 *Micrococcus* + *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 mean nitrite content after 3 days of ripening of 48 ppm in *E. coli* 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.

3. 4. 5. 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 control sausages. The pH value of *P. vulgaris* + *Lactobacillus* sausages decreased, as in the sausages inoculated with micrococci 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 ripening.

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 3×10^8 /g and *P. vulgaris* bacteria practically disappeared after 3–7 days of ripening.

4. Discussion

4.1. Effect of bacterial inoculations on the ripening of dry sausage

It is accepted that the use of bacterial cultures improves the quality of dry sausage. Since the *Pediococcus cerevisiae* starter culture developed by NIVEN et al. (1955) and the *Micrococcus* starter culture developed by NIINIVAARA (1955), many new starter cultures have been developed: different Lactacel and Saga products in the USA, French and Spanish starter cultures, and starters in Bulgaria, the Soviet Union, Yugoslavia, the GDR etc. Perhaps the most important of these is the mixed culture of micrococci and lactobacilli developed by NURMI (1966). Also, according to LIEPE (1971, 1975, 1976) the use of lactobacilli and micrococci is necessary to assure the controlled ripening of dry sausage. In Nurmi's method lactobacilli produce the lactic acid necessary for the development of the consistency and aroma of dry sausage. Micrococci reduce nitrate to nitrite and avoid the discolourations which lactobacilli would produce. This method also considerably reduces the processing time. This mixed starter culture of micrococci and lactobacilli is used in Europe in particular, but in other countries, too. In addition to »Duploferment 66» developed on the basis of the studies of Nurmi, there are some other commercial products which contain micrococci and lactobacilli (Saga III).

The *Micrococcus* + *Lactobacillus* starter culture is also the reference with which other experimental sausages are compared in this investigation. Like the bacteria of this starter culture, all cultures used in dry sausage have in practice been gram-positive. The effect of gram-negative bacteria on the aroma and flavour of dry sausage has been studied most notably by KOHNLE (1953), KELLER (1954), KELLER and MEYER (1954), MEYER (1954), LOSEM (1956) and ECKERT (1958). The purpose of this examination was to investigate the possible use of gram-negative bacteria in dry sausage.

The effect of the following gram-negative bacteria on the quality of dry sausage was studied by inoculating the bacteria alone and with *Lactobacillus plantarum* (NURMI 1965, 1966): two *Aeromonas* strains, *Vibrio costicolus*, three *Achromobacter* strains, *Escherichia coli* and *Proteus vulgaris*.

Aeromonas strains x and 19

According to BUTTIAUX (1959) *Aeromonas* bacteria play an important part in the biological process on which the manufacture of raw sausage is based. When *Aeromonas* strains x and 19 were inoculated alone their favourable effect on the quality of dry sausage was restricted to improving colour due to the nitrate-reducing capacity of the *Aeromonas* strains. When inoculated along with lactobacilli, both *Aeromonas* x and *Aeromonas* 19 improved the ripening of dry sausage. The colour developed over 3 days and was at least as good as that of *Micrococcus* + *Lactobacillus* sausages throughout the ripening period.

The pH value of sausages with *Aeromonas* x or 19 + *Lactobacillus* inoculations decreased as quickly as in *Micrococcus* + *Lactobacillus* sausages and often

even more quickly. The pH values, too, are as low or a little lower than in *Micrococcus* + *Lactobacillus* sausages. According to NURMI (1965) the inoculation of lactobacilli caused a rapid decrease of the pH value of dry sausage. Concurrently the desired consistency also developed rapidly. Also, the consistency scores of sausages inoculated with *Micrococcus* + *Lactobacillus*, *Aeromonas* x + *Lactobacillus* or *Aeromonas* 19 + *Lactobacillus* were best. The last of these, however, was better (significance level 0.05) than that of *Micrococcus* + *Lactobacillus* samples and better (significance level 0.001) than that of *Aeromonas* x + *Lactobacillus* samples. Results from samples of different ages were taken into account for this comparison. The consistency, measured mechanically, was more often firmer in *Aeromonas* + *Lactobacillus* sausages than in *Micrococcus* + *Lactobacillus* sausages. However, the values obtained did not differ very much from each other.

The aroma of sausages inoculated with the *Aeromonas* 19 strain and lactobacilli was better (significance level 0.001) than the aroma of sausages inoculated with micrococci and lactobacilli. There were no statistical differences between *Micrococcus* + *Lactobacillus* and *Aeromonas* x + *Lactobacillus* samples. The flavour of *Aeromonas* x + *Lactobacillus* (significance level 0.05) and *Aeromonas* 19 + *Lactobacillus* (significance level 0.001) sausages was significantly better than the flavour of *Micrococcus* + *Lactobacillus* sausages. The flavour of all the sausages mentioned was certainly better than in the other sausage groups. NURMI (1965) observed that micrococci and lactobacilli improved the flavour of dry sausage. The *Aeromonas* + *Lactobacillus* inoculations used have proved to have an even more beneficial effect on the flavour. The scores of the evaluations of the sausages of different ages were processed together using the t-test when the aroma and flavour of different sausage groups were compared.

There were no significant differences between the evaluations of different panel members with respect to each property or with respect to the products. Products of different ages were tested separately.

Nitrate was strongly reduced by *Micrococcus* and *Aeromonas* strains, the nitrite level ranging from 10 to 100 ppm after 3 days of ripening. After 3 days the nitrite content was lower in sausages inoculated with lactobacilli because of the low pH value. Many countries have discussed banning the use of nitrate. PUOLANNE (1977) concluded that it is safe from the point of view of technology to manufacture dry sausage using 150 ppm KNO_3 or 100 ppm $NaNO_2$ or 50 ppm $NaNO_2$ and 75 ppm KNO_3 when starter culture is used. LEISTNER et al. (1973) state that gram-negative bacteria do not resist sodium nitrite. The count of enterobacteria in 12 day old brine (11 % NaCl) is 10^5 /ml but only 10^2 /ml when 0.06 % nitrite is used. In some experimental series of this investigation the effect of nitrite on *Aeromonas* strains in dry sausage has also been examined. Sausages were prepared using nitrite and *Aeromonas* x + *Lactobacillus* and *Aeromonas* 19 + *Lactobacillus* inoculations. According to the results nitrite does not disturb the growth or prevent the *Aeromonas* strains used from thriving.

Based on the weight losses, the following calculations can be made. If control sausages ripen in 3 weeks with a mean weight loss of 24.6 % and the *Micro-*

coccus + *Lactobacillus* and *Aeromonas* x or 19 + *Lactobacillus* sausages ripen in 10 days with a mean weight loss of about 18.3% this represents a saving in weight loss of 6% units. NURMI (1966) found that sausages with *Micrococcus* + *Lactobacillus* inoculation ripened in just 6–7 days with a weight loss of 10%. In this study too, the sausages often ripened in one week but were not fit for sale before they had ripened for 10 days.

Vibrio costicolus and *Achromobacter* strains

Neither the *Vibrio costicolus* strain nor the *Achromobacter* strains thrived in dry sausage and both disappeared during the first few days of ripening. This means that their effect on the ripening of dry sausage is very small, both alone and when inoculated together with lactobacilli.

Escherichia coli

Escherichia coli did not have a very beneficial effect on the ripening of dry sausage when inoculated alone. When inoculated with lactobacilli its effect on the quality of the sausage very much resembled that of micrococci inoculated with lactobacilli. However, the colour, consistency and flavour were not as good.

Proteus vulgaris

Inoculation with *Proteus vulgaris* was used to establish how this kind of strongly proteolytic organism affects dry sausage. The results show that it is harmful even when inoculated with lactobacilli. The consistency, colour, aroma and flavour were not acceptable.

4.2. Effect of bacterial inoculations on bacterial flora of dry sausage

According to NURMI (1966) *Lactobacillus* inoculation is necessary to reduce the pH value and so to develop the consistency of dry sausage. The lactobacilli alone produced discolourations because they form hydrogen peroxide and little or no catalase to decompose hydrogen peroxide. Therefore bacteria which are catalase positive and which reduce nitrate are needed besides lactobacilli to prevent discolourations and to produce a good colour.

NIINIVAARA (1955) had developed a method in which micrococci were used as starter culture in dry sausage. So Nurmi used micrococci to prevent discolourations. In this research two gram-negative *Aeromonas* strains were used instead of micrococci and good quality sausages resulted. NURMI (1966) stated that micrococci have a strongly depressing effect on the number of lactobacilli in dry sausage — the number of lactobacilli is smaller when inoculated together with micrococci. In this investigation the number of lactobacilli was often significantly higher when inoculated with *Aeromonas* x or 19 than when inoculated with micrococci (Table 21). This property of *Aeromonas* strains proved favourable. It was stated earlier that most *Aeromonas* + *Lactobacillus* sausages were better than *Micrococcus* + *Lactobacillus* samples in colour, con-

19, *Vibrio costicolus*, *Achromobacter* 22, *Achromobacter guttatus*, *Achromobacter* X, *Escherichia coli* or *Proteus vulgaris*. Sausages without any inoculation and sausages inoculated with micrococci and lactobacilli were used as references in the examination. Twenty-two series of dry sausage experiments were carried out and examined over a ripening period of 21 days. The following conclusions can be drawn concerning the effect of bacterial inoculations on the ripening process and the quality of dry sausage:

1. The colour, consistency, aroma and flavour of the samples without inoculum were worst as a rule, *Micrococcus* + *Lactobacillus* sausages being much better and competing in quality with other sausages. The good colour of sausages inoculated with micrococci and lactobacilli developed in 3 days and the firm consistency in 7 days. The aroma and flavour were also acceptable. Micrococci reduced nitrate in 3 days so that the mean nitrite content reached 34.1 ppm and thereafter decreased. The number of micrococci was a little over $10^7/g$ throughout ripening while the number of lactobacilli rose from about $10^7/g$ to $10^8/g$ in 3 days. The pH value decreased in 3 days from 5.70 to 5.28.
2. *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 control sausages. *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.
3. *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 *Micrococcus* + *Lactobacillus* sausages, being about 5.20 after 3 days of ripening. The consistency of *Aeromonas* + *Lactobacillus* samples developed within one week and was at least as good as and often firmer than in *Micrococcus* + *Lactobacillus* sausages. The consistency of *Aeromonas* 19 + *Lactobacillus* sausages was better at the 0.05 significance level than that of *Micrococcus* + *Lactobacillus* samples. The aroma and flavour of *Aeromonas* + *Lactobacillus* sausages were as good as or better than those of *Micrococcus* + *Lactobacillus* sausages. The aroma and flavour of *Aeromonas* 19 + *Lactobacillus* sausages were significantly better (significance level 0.001) than *Micrococcus* + *Lactobacillus* sausages. The flavour of *Aeromonas* x + *Lactobacillus* sausages was better at the 0.05 level than *Micrococcus* + *Lactobacillus* sausages.

The number of bacteria, roughly the number of lactobacilli, was often significantly higher in *Aeromonas* + *Lactobacillus* sausages, (range $2-4 \times 10^8/g$ between 3 and 21 days of ripening) than in *Micrococcus* + *Lactobacillus* sausages (range $8 \times 10^7-1.5 \times 10^8/g$ between 3 and 21 days of ripening). For this reason the former sausages ripened more quickly than the latter.

4. *Vibrio* 21 and three *Achromobacter* strains not only failed to thrive in dry sausage, but disappeared completely during the first 7 days of ripening. The sausages inoculated with *Escherichia coli* alone were in the same quality class as the control sausages. When inoculated with lactobacilli, *E. coli* had very much the same effect as micrococci. However, *Micrococcus* + *Lactobacillus* sausages were slightly better in every respect than *E. coli* + *Lactobacillus* sausages. *Proteus vulgaris* had adverse effects when inoculated into dry sausage both alone and even with lactobacilli.

Conclusion

The investigation has proved that gram-negative bacterial strains *Aeromonas* x and 19 inoculated with lactobacilli improve the quality of dry sausage and that, especially with the *Aeromonas* 19 + *Lactobacillus* inoculum, better dry sausage was obtained than with micrococci and lactobacilli.

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SELOSTUS

Eräiden gram-negatiivisten bakteerien vaikutus kestopakkarin kypsymiseen ja laatuun.

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Gram-negatiivisten bakteerien mahdollista käyttöä starterkulttuureina kestopakkarissa tutkittiin siirrostamalla seuraavia gram-negatiivisia bakteereita kestopakkarin yksinään ja *Lactobacillus plantarum* kanssa: *Aeromonas* x, *Aeromonas* 19, *Vibrio costicolus*, *Achromobacter guttatus*, *Achromobacter* 22, *Achromobacter* X, *Escherichia coli* tai *Proteus vulgaris*. Ilman bakteerilisyistä valmistettuja makkaroina sekä mikrokokki + laktobasillilisyksellä valmistettuja makkaroina käytettiin vertailunäytteinä tutkimuksessa. Kaksikymmentäkaksi koesarjaa kestopakkarin valmistettiin ja tutkittiin kypsymisajan ollessa 21 vuorokautta. Seuraavia johtopäätöksiä voidaan tehdä bakteeri-inokulaatioiden vaikutuksista kestopakkarin kypsymisprosessiin ja laatuun:

1. Ilman bakteerilisäystä valmistettujen näytteiden väri, konsistenssi, aromi ja maku olivat yleensä huonoimmat mikrokokki + laktobasillimakkaroiden ollessa paljon parempia ja jopa kilpailen laadussa muiden koemakkaroiden kanssa. Mikrokokeilla ja laktobasilleilla siirrostettujen makkaroiden väri kehittyi kolmessa ja konsistenssi seitsemässä vuorokaudessa. Aromi ja väri olivat myös hyväksyttävää viikon vanhoissa näytteissä. Mikrokokki pelkisti nitraattia kolmessa päivässä siten, että keskimääräinen nitriittimäärä nousi 34.1 ppm:ään ja laski sen jälkeen. Mikrokokkimäärä oli hieman yli $10^7/g$ koko kypsymsajan, kun taas laktobasillimäärä kohosi $10^7/g$:sta $10^8/g$:aan kolmessa päivässä. pH-arvo laski kolmessa vuorokaudessa 5.70:sta 5.28:aan.
2. Kannat *Aeromonas x* ja *Aeromonas 19* vaikuttivat suotuisasti kestromakkaran väriin, mutta muuten näytteiden laatu oli kontrollimakkaroiden tasoa. *Aeromonas*-kannat eivät muodostaneet makkaran laatuun vaikuttavia määriä happoa kestromakkarassa eivätkä siten parantaneet konsistenssia. Aromi ja maku olivat hieman parempia kuin kontrollimakkaroissa.
3. *Aeromonas x* ja *19* kannoilla oli varsin suotuisa vaikutus kestromakkaran laatuun laktobasillien kanssa inokuloituna. Molemmat kannat pelkistivät nitraattia värin muodostuessa kolmen ensimmäisen päivän aikana. *Aeromonas* + laktobasillimakkaroiden pH-arvo laski jopa nopeammin ja alemmaksi kuin mikrokokki + laktobasillimakkaroissa ollen noin 5.20 kolmen päivän kypsytyksen jälkeen. *Aeromonas* + laktobasillinäytteiden konsistenssi kehittyi yhdessä viikossa ja oli ainakin yhtä hyvä ja usein parempikin kuin mikrokokki + laktobasillimakkaroissa. *Aeromonas 19* + *Lactobacillus*-makkaroiden konsistenssi oli parempi kuin *Micrococcus* + *Lactobacillus*-näytteiden merkitsevyydellä 0.05. *Aeromonas* + laktobasillimakkaroiden aromi ja maku olivat yhtä hyviä tai parempia kuin mikrokokki + laktobasillinäytteiden. *Aeromonas 19* + *Lactobacillus*-makkaroiden aromi ja maku olivat merkitsevästi (merkitsevyydellä 0.001) parempia kuin *Micrococcus* + *Lactobacillus*-makkaroiden. *Aeromonas x* + *Lactobacillus*-näytteiden maku oli merkitsevyydellä 0.05 parempi kuin *Micrococcus* + *Lactobacillus*-makkaroiden.

Kokonaisbakteerimäärä, vastaten suurinpiirtein laktobasillimäärää, oli usein merkitsevästi suurempi *aeromonas* + laktobasillimakkaroissa (vaihteluväli $2-4 \times 10^8/g$ 3-21 kypsytyksivuorokauden aikana) kuin mikrokokki + laktobasillimakkaroissa (vaihteluväli $8 \times 10^7 - 1.5 \times 10^8/g$ 3-21 kypsytyksivuorokauden aikana). Tästä syystä edelliset makkarat kypsyivät nopeammin kuin jälkimmäiset.

4. *Vibrio costicolus* ja kolme *Achromobacter*-kantaa eivät vain viihtyneet huonosti kestromakkarassa, vaan hävisivät täydellisesti seitsemän ensimmäisen kypsytyksivuorokauden aikana. Pelkästään *Escherichia coli* bakteerilla siirrostetut makkarat olivat laadultaan samaa luokkaa kuin kontrollimakkarat. Laktobasillien kanssa inokuloituna *E. coli* vaikutti paljolti mikrokokkien tavoin. Kuitenkin *Micrococcus* + *Lactobacillus*-makkarat olivat joka suhteessa hieman parempia kuin *E. coli* + *Lactobacillus*-makkarat. *Proteus vulgaris* vaikutti haitallisesti kestromakkaran laatuun sekä yksin että laktobasillien kanssa inokuloituna.

L o p p u p ä ä t e l m ä

Tutkimus osoitti, että gram-negatiiviset bakteerikannat *Aeromonas x* ja *19* laktobasillien kanssa inokuloituna vaikuttavat edullisesti kestromakkaran laatuun. Etenkin *Aeromonas 19* + *Lactobacillus*-inokulaation avulla saatiin parempaa kestromakkaraa kuin lisättäessä makkaraan mikrokokkeja ja laktobasilleja.