Utrecht 1960

paper no 4 67

Studies on the Microbial Flora in Swedish Dry Sausages.

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Salted and cold-smoked meat products normally support a variegated microbial flora which gives the particular product its characteristics and quality and which contributes towards its keepability. The microbiology of such products and especially these aspects already involved in manufacture have been studied by several investigators in recent years. One approach has been the inoculation of large amounts of bacteria isolated from sausages of desirable type in order to ensure products of high and uniform quality. The most important contributions in this field have been made by N i i n i v a a r a (1,2), K o h n l e (3) and K e l l e r (4), working towards somewhat different ends. Niinivaara has isolated a Micrococcus strain, M 53, and has demonstrated that generous inoculation of the raw sausages with this strain improves the colour and the durability of the colour in the finished product. Kohnle on the other hand, has worked with "aromatic" bacteria. His strain, 25 c, a Gram-negative rod, when added in large amounts to raw sausages gives better aroma and colour in the saleable product. Both of these bacteria have been used with good results as a combined inoculum by E c k e r t (5). The improved colour depended at least in part upon ability of these strains to reduce nitrate in the salt to nitrite. Nitrite is then bound to myoglobin to give the

desirable red colour. In France, Lactobacillus strains and in the U. S., Pedicoccus strains have been utilized to attain the same result.

These bacteria are now available commercially in pure culture for addition to raw sausages. Experience with these preparations has been good. In Sweden, however, these cultures are not generally used in the manufacture of sausages, partly because food hygiene regulations are quite restrictive in regard to the addition of foreign materials and partly because manufactures strive to maintain a low bacteria content in these products.

Cold-smoked Swedish sausage, "medvurst", resembles the German "Dauerwurst" although the method of manufacture is somewhat different. Efforts to simplify the manufacturing process and to reduce the effects of high labour costs have led to shortening of the various phases. Nowadays, sausages are generally held for ripening for two days at 16 to 20°C and then smoked for two days at 24 to 30°C. It can be mentioned that according to regulations, smoked "medvurst" can contain only meat salt and spices, and that the water content cannot exceed 50 per cent.

The short ripening and smoking periods and the relatively high water content compared with genuine "Dauerwurst" can be expected to produce a microbial flora of quite different composition to that in "Dauerwurst". The significance of this flora for the quality of the finished product is probably not as great as is claimed for the German sausage.

To study the microflora of cold-smoked medvurst, the manufacture of this particular type of sausage was followed

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at three of the larger factories in Stockholm. As well as the bacteriological surveys, the water and salt contents were followed to ascertain that the variations were not of sufficient magnitude to affect the growth or composition of the microflora. In conjunction with the preparation of sausages in the usual size, four " test sausages " about 15 cm long, were filled and allowed to follow the rest of the batch trough the various procedures. One of these sausages was then remowed for study at the following stages

1. immediately after filling
2. at the end of the ripening period, when the rest of the batch went on to the smoke room
3. immediately after the conclusion of smoking
4. the finished product after storage for a week in a refrigerater at approx. 5°C.

Ten g of sausages contents were taken for pH reading after homogenisation in 90 ml distilled water with an "Ultra-Turrax", filtration, and allowing the filtrate to stand for 30 minutes. The pH of raw sausage was about 6 and then sank to about pH 5,8 during the ripening period and to about 5 during smoking and remained at this level during and after smoking.

Both quantitative and qualitive studies were carried out on the microflora, especially the bacterial flora.

For these studies representative 5 g samples were taken from the test sausages described above. These samples were carefully homogenised in a sterile mortar with 45 ml dilutent made up of Ringerś solution supplemented with 0,5% per cent pepton. In our experience peptone appears to be

able to reduce the bacteriecidal effect of physiological saline solutions upon bacteria. The diluted sample was further diluted in ten-fold series until a suitable end-point was reached, generally after nine such dilutions.

After unsuccessful efforts to follow the development of the bacterial flora during the various phases of manufacture using selective media, a changeover was made to meat-extract-peptone agar(pH 7,2) with 5 per cent difibrinated bovine blood. Total bacterial counts were estimated on meat-extract-peptone agar with 5 per cent steril horse serum. The presence of coli-aerogenes was established through the use of violet-red-bile agar. To some extent, Packers crystal-violet and sodium azide agar (7) was used to ascertain the presence of some types of streptococci.

Total counts were made after incubation for 3 days at 30°C and included all colonies visible to the naked eye. The results obtained are given in table 1.

Table 1.

Test sausages	1	2	3	4
Total bacteria/g (log)	6,31	7,68	8,00	8,40
Coli-aerogenes/g (")	2.12	2.34	0.35	0.55

The bacteria content of sausage filling is high, probably because the meat used is salted and generally mature. A large portion of this flora is comprised of micrococci, some strains of which contribute towards a desirable colour in the finished product through their ability to reduce nitrate to nitrite which in turn reacts with myoglobin to form the red compound nitrosomyoglobin. For stability of this compound it is likely that reducing substanses are required

and there are probably furnished by bacterial enzymes.

Ripening at room temperature establishes a suitable environment for the development of all the bacterial species likely to be encountered. Increase in the coliforms is relatively much less than the increase in the total bacteria content. Since it was impossible to obtain an accurate evaluation of the proportional relationships between the different species with the exception of the coliforms, an estimation has been based upon growth properties and morphological characteristics.

During the ripening phase, the flora was qualitatively uniform and consisted largely of micrococci and several types of Gram-negative rods. The Gram-negative bacteria could be classified as Enterobacteriaceae, Achromobacteriaceae and Pseudomonadaceae. Only a few Bacillaceae were encountered in the samples. Of the Gram-positive anaerobic spore-formers, Clostridium perfringens was the only species specifically looked for with negative results on all occasions. In addition to the bacteria listed above, some Gram-positive coccoid rods belonging to the Lactobacillaceae were seen. During smoking, there are extensive changes in the character of the flora. Most or practically all the Gram-negative bacteria and some of the micrococci disappear leaving the flora dominated by short, Gram-positive coccid rods, an observation which agrees in part with L e r c h e's (6) results. A large number of strains were isolated and studied both morphologically and biochemically. Special attention was paid to the Gram-positive coccoid rods; 92 such strains were investigated.

These appeared on the primary blood plates after

2 to 3 days incubation as small, partially transparent, slighty convexed colonies surrounded by a distinct haemolytic zone. This zone can be described as α-haemolysis but on occasion the zone has a distinct greenish colour when examined against transmitted light. In smears from the primary colonies, the organisms appear as very short rods and since they often occur in chains, they are difficult for the inexperienced to distinguish from streptococci. For tabular presentation, the strains isolated have been grouped according to their fermentation properties.

A. form large, often mucoid colonies on 5 per cent saccharose agar

    a. ferment lactose with acid formation

    b. do not ferment lactose

B. form small, clear, greyish-white colonies on 5 per cent saccharose agar

    a. ferment glucose with acid formation but not lactose

    b. ferment glucose and lactose with acid formation.

The fermentation reactions listed here were carried out in a standard manner, i.e. as single series in fermentation - tubes containing nutrient broth (pH 7.2) with 1 per cent of the particular sugar or alcohol added. The other biochemical reactions were also carried out in a customary manner. Incubation was at 30°C and readings were made on alternate days during 14 days. The biochemical properties of the Gram-positive coccoid rods are summrized in table 2.

Table 2

Group	Aa	Ab	Ba	Bb
No of strains	6	34	25	27
Gelatin	0	0	0	0
Nitrate	0	0	0	0
Indol	0	0	0	0
Arabinose	6	0	3	14
Glucose	6	34	25	27
Dulcitol	0	0	0	0
Inulin	0	0	0	0
Lactose	6	0	0	27
Maltose	5	34	25	27
Mannitol	4	1	1	17
Salicin	4	5	9	20
Saccharose	6	28	18	21
Sorbitol	3	2	1	16
Aesculin	4	13	7	20
Levulose	6	34	25	27
Mannose	6	31	24	26
Trehalose	5	8	6	24
Galactose	6	25	21	27
Raffinose	2	0	0	8
Litmus milk:				
Acid and Red.	2	5	3	18
Acid	3	18	15	9
No reaction	1	10	5	0

According to these results, the Gram-positive coccoid rods on the whole fit in the genus Leuconostoc as described in Bergeyś Manual, 7th ed.. Since biochemical variations have often been noted among the different Leuconostoc species, a definite typing of the strains isolated is not possible. The biochemical and morphological properties in table 2 permit a rough equivalation between Leuconostoc mesenteroides and type Aa, Leuconostoc dextranicum and type Ab and Leuconostoc citrovorum and types Ba and Bb.

Of the strains of micrococci isolated, 45 could not be grouped according to the reactions described in Bergeyś Manual. It can be pointed out that the sausages contained a large number of micrococci strains. As for the Gram-negative rods, with the exception of the coliforms, the 35 strains isolated and studied biochemically were evenly distributed between the genus Achromobacter and the genus Alcaligenes. A few samples also contained strains belonging to the genus Flavobacterium.

Streptococci were encountered in these sausages although to a lesser content than the **short** Gram-positive rods. They were extremely difficult to identify solely by colonial morphology since the colonies resembled those of Gram-positive coccoid rods. Some samples were examined for the presence of faecal streptococci using Packerś crystal violet and sodium azide agar. From 100 to 1000 bacteria per g of this type were counted and there were no remarkable alterations during the manufacturing process. At an incubation temperature of 39,5°C, Packerś medium is considered to be selective for faecal streptococci. Two hundred streptococcal colonies were transferred from this medium to nutrient broth at pH 9.6. Most strains were capable of growth at this high pH level, an indication that they probably were in fact faecal streptococci.

Available reports indicate that Gram-positive rods are present in large numbers in cold-smoked meat products (1, 2, 3). This observation was also made on the corresponding Swedish products. The significance of these bacteria for quality of the finished products is not clear. Judging from studies which have been carried out, the enzymatic ac-

tivity of these bacteria upon protein and to a certain extent fat seems to be weak. Activity upon carbohydrates and alcohols on the other hand, is much more evident. In a few trials dealing with the realtionships between these bacteria and other species it appeared that in some concentrations they are capable of inhibiting to a degree at least the growth of Escherichia coli but have no inhibitory effect upon Salmonella typhi murium.

To summarize these investigations, then, the normal bacterial flora in cold-smoked Swedish medvurst is largely composed of Leuconostoc species and that these may be present in numbers as great as several hundred milions per g. The major growth of these bacteria takes place during smoking, a process which offers a practically optimal temperature för growth. Growth is also facilitated by the concomitant decrease in the pH-level since according to Bergey's Manuel, these species thrive at pH walues about or below 6.

The number of other bacterial species present in the raw sausages stuffing are reduced to varying degrees during manufacture to give count of less than 100 000 per g. Micrococci are reduced, especially during smoking, and at the same time the Gram-negative rods are to a great extent inhibited or killed. The coli-aerogenes group, often present in raw sausages stuffing, are also greatly reduced or killed during smoking. If the coliform count in raw sausages was less than 1000 per g these species could not be detected in the final sausages with the method used here.

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