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THE ROLE OF HIGH COUNT OF LACTIC ACID BACTERIA AND LOW COUNT OF STAPHYLOCOCCI IN THE FORMATION OF DISCOLORATIONS IN DRY SAUSAGE

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INTRODUCTION

Incidents of discolourations of dry sausage are due to the oxidation of NO-myoglobin by hydrogen peroxide, which is produced by lactic acid bacteria when enough oxygen is available (Lücke, 1985). Generally, hydrogen peroxide is decomposed by catalase produced by the catalase positive bacteria, especially *staphylococci* (Lücke and Hechelmann, 1986). Although *staphylococci* are used as a starter culture in dry sausage, discolorations are not always prevented (Petäjä, 1992). Decreased counts of *staphylococci* and high counts of lactic acid bacteria have regularly been found in discoloured sausages. In such circumstances, the catalase capacity of *staphylococci* has apparently not been sufficient to decompose all the hydrogen peroxide. Lactic acid bacteria differ in their capacity to produce hydrogen peroxide (Lücke and Hechelmann, 1986). Lactic acid bacteria used as a starter culture are weak hydrogen peroxide producers. On the other hand, the natural bacterial flora of raw meat may contain lactic acid bacteria which produce large amounts of hydrogen peroxide. The discoloured sausages also contain, along with the lactic acid bacteria of the starter culture, wild-type peroxide-producing lactic acid bacteria. The count of *staphylococci* at that time being too low to rapidly decompose all the peroxide that is formed. So, the low count of staphylococci and high count of wild-type peroxide-producing, lactic acid bacteria are risk factors for discolouration of dry sausage. Because discolourations of dry sausage appears in Finland from time to time, especially in summer, the bacterial flora of dry sausage batches having discolourations were studied during sausage ripening at a meat plant. Special attention was paid to the count of *staphylococci* and the count of peroxide forming lactic acid bacteria, as the aim was to find out how low the count of *staphylococci* and how high the count of peroxide-forming wild-type lactic acid bacteria has to be to produce discolouration of dry sausage.

MATERIAL AND METHODS

Manufacture of the sausages

Sausages were manufactured using the following formula: Beef 25.6%, pork 46.5%, pork fat 4.3%, NaCl 2.9%, glucose 0.3%, $NaNO_3$ 0.022%, Na-ascorbate 0.2% and seasoning (white pepper, black pepper) 0.2%. The beef and pork (temperature -1 to +2.5°C) were ground first with salt in a 200l cutter (Laska, Laska Gmbh, Wien, Austria).

Thereafter other additives, seasons and starter culture were mixed into the sausage mass. Finally, pork fat (temperature -1 to +0.5°C) was added and the sausage mass was cut until the particle size was 3mm in diameter. The final temperature of the sausage mass was -4°C. The sausage mass was stuffed into a 70mm fibre casing (Visko light, Visko Oy, Hanko, Finland). The ripening program of the sausages was as follows:

Ripening time (days)	Temperature (°C)	Humidity (%)	Smoking (h/day)
1	23	92	
2-8	29-21	93-84	3
9-23	17	82	3
23-37	17-13.5	78-72	

Microbiological studies

Each batch of sausage was examinedmicrobiologically immediately after manufacture (0 day) and after ripening for 1, 2 and 4 weeks. A different sample was taken at each time. Samples were dispatched from the plant to the laboratory by express mail (12 hours). The following determinations were made: Aerobic total plate count (Plate count agar, Merck 5463, three days at 30°C); *staphylococci* (Baird-Parker agar, Labm 85 and X085, two days at 37°C); lactic acid bacteria (Rogosa agar, Merck 5413, four days at 30°C; blood-o-dianisidin-agar [BOD-agar], Chr. Hansens Lab., two days at 30°C) and peroxide forming lactic acid bacteria (BOD-agar, Chr. Hansens Lab., two days at 30°C).

Measurement of pH-values

pH-values were measured after manufacture (0 day) and after 1, 2 and four weeks of ripening using a Knick portames 651 pH-meter (Knick Elektronische Allesgäräte, Berlin, Germany). Each sample was measured at three sites and the mean of the three values was used.

RESULTS AND DISCUSSION

Microbiological studies

At the beginning of ripening the mean of the aerobic total count was 6.5log cfu/g, the mean count of *staphylococci* was 6.4log cfu/g and the mean count of lactic acid bacteria was 6.1log cfu/g (Table 1). The counts of lactic acid bacteria were relatively low, as in five samples out of 11 the counts were below 6.0log cfu/g (Table 2). At the beginning of ripening, the counts of *Brochothrix thermosphacta* ranged from 2.0 to 5.0log cfu/g and the count of pseudomonads from 2.0 to 3.6log cfu/g. Both counts decreased to <2.0 log cfu/g during the first 10 days of ripening. After one week of ripening, the mean count of lactic acid bacteria had risen to 8.0log cfu/g, but the mean count of staphylococci had decreased to 5.8log cfu/g (Table 1). The counts of peroxide forming lactic acid bacteria were over 6.0log cfu/g in only four of the 11 experimental series at the beginning of ripening (Table 2). However, the mean of their counts increased during the first week of ripening, to 8.0log cfu/g, then decreased a little thereafter (Table 1). The fractions of peroxide formers in the total counts of lactic acid bacteria were about 1/3. Those numbers of peroxide formers pose a high discolouration risk. The lactic acid bacteria of starter cultures also produce small amounts of hydrogen peroxide. After one week of ripening the counts of hydrogen peroxide formers were over 8log cfu/g in six of 11 experimental series (Table 2). If at the same time the number of *staphylococci* is 5.0log cfu/g or lower, discoloration is likely. However, during this test in only one experimental series did the count of *staphylococci* decrease below 5.0log cfu/g.

pH-value

pH-values ranged from 5.63 to 6.12 at the beginning of ripening and from 5.0 to 5.5 after one week of ripening (Table 2). The minimum pH-values were attained after two weeks of ripening. The pH values then ranged from 4.93 to 5.44, the value mean being 5.13. In two experimental series, unusually high pH-values of over 5.4 were measured during ripening.

CONCLUSION

It appears that when the count of *staphylococci* is not less than 5.0log cfu/g and the count of hydrogen peroxide formers not more than 8.0log cfu/g, discolouration of dry sausage does not occur. However, because these counts of *staphylococci* and peroxide-forming, lactic acid bacteria have been found also in discoloured dry sausages, more investigation on the topic is necessary.

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Table 1. The total count of bacteria and the count of staphylocci and micrococco of experimental sausages during ripening. The number of experimental series=11. The bacterial counts are expressed as log CFU/g.

	0 week	1 week	2 weeks	4 weeks
# Samples	11	10	9	11
Total plate count:				
Mean	6.5	7.8	7.6	7.4
Std Dev.	0.6	0.8	0.2	0.4
Range	6.1-7.8	7.0-8.6	7.3-7.9	6.7-8.2
Staphylococci + micrococci				
Baird-Parker-agar				
Mean	6.4	5.8	5.8	5.6
Std. Dev.	0.2	0.6	0.5	0.4
Range	6.1-6.8	4.5-6.4	4.5-6.2	4.5-6.0
Chapman-agar				
Mean	6.2	5.7	5.6	5.4
Std. Dev.	0.3	0.6	0.4	0.4
Range	5.5-6.5	4.3-6.0	4.3-6.1	4.4-5.7

Table 2. The count of lactic acid bacteria and the count of peroxide forming lactic acid bacteria and pH-value of experimental sausages during ripening. Number of experimental series=11. The bacterial counts are expressed as log CFU/g.

	0 week	1 week	2 weeks	4 weeks
# Samples	11	10	10	11
Lactic acid bacteria				
Rogosa-agar				
Mean	6.1	8.0	7.6	7.4
Std. Dev.	1.0	0.3	0.4	0.4
Range	5.1-8.1	7.6-8.4	7.1-8.1	6.5-8.2
BOD-agar				
Mean	6.7	8.3	7.9	7.9
Std. Dev.	0.9	0.4	0.3	0.5
Range	6.0-8.2	7.6-8.8	7.4-8.3	7.1-8.5
Peroxide forming lactic acid bacteria (BOD-agar)				
Mean	4[1]	8.1	7.8	7.9
Std. Dev		0.4	0.4	0.4
Range		7.7-8.7	7.2-8.4	7.0-8.4
pH-value				
Mean	5.92	5.23	5.13	5.16
Std. Dev.	0.18	0.16	0.16	0.14
Range	5.63-6.12	5.00-5.50	4.93-5.44	4.98-5.42

[1] Number of samples containing over 6,0logCFU/g.

Table 3. Grouping of samples according to selected bacterial counts after 0, 1, 2 and 4 weeks of ripening.

	0 week	1 week	2 weeks	4 weeks
# Samples	11	10	9	11
Staphylococci + micrococci				
Baird-Parker-agar				
$<10^6$CFU/g	0	6	8	9
$<5x10^5$CFU/g	0	3	1	2
Chapman-agar				
$<10^6$CFU/g	2	7	8	11
$<5x10^5$CFU/g	1	3	3	6
Lactic acid bacteria				
Rogosa-agar				
$<10^6$CFU/g	5	10	9	11
BOD-agar				
$<10^6$CFU/g	10	10	9	11
Peroxide forming lactic bacteria				
BOD-agar				
$<10^6$CFU/g	4	10	9	11
$<10^7$CFU/g	2	10	9	10
$<10^8$CFU/g	0	6	2	2