

## HEAT RESISTANCE OF TWO STREPTOCOCCI ISOLATED FROM THE CORE OF COOKED MEAT PRODUCTS MADE FROM COARSELY GROUND PORK

E. PETÄJÄ

Department of Food Technology, Meat Section, University of Helsinki, Viikki, E-building, 00710 HELSINKI, Finland

**SUMMARY:** At 72°C the number of colony forming units (cfu) of the streptococcus strains (3b1 and 7b1) decreased in APT-broth more steeply than in coarsely ground cured pork in which the decrease was about 1 log unit over 15 min. When heated in APT-broth according to the heating process used for cooking sausages thermal death began steeply at 60°C (strain 3b1) and at 65°C (strain 7b1) diminishing when the maximum temperature of 72°C had been attained. Strain 3b1 did not survive after heating process in APT-broth. When heated in coarsely ground cured pork according to the heating process for cooking sausages the thermal death process happened more slowly than in APT-broth, the cfu number decreasing by 3 (strain 3b1) and 2 (strain 7b1) log units during the heating process. When the heat treated and cooled coarsely ground porks were stored for 4 weeks at 4°C the cfu number rose by a maximum of 1 log unit.

In cooked meat products lactic acid bacteria appear which survive the cooking process. However, these bacteria probably do not constitute a spoilage problem because their cfu number decreases some log units during cooking and rises only a little during storage.

**INTRODUCTION:** The bacterial flora of cooked meat products made of coarsely ground meat and the heat resistance of the dominating strains of the flora have been investigated by PETÄJÄ (1991). The bacterial flora contained mostly lactic acid bacteria but pseudomonads were also found in large numbers in the core of the products. Most of the core strains survived after heating for 30 min at 72°C in APT-broth at least in three tests out of six. The pseudomonads survived after heating for 15 min at 72°C in coarsely ground cured pork. This article deals with the survival of two core streptococci in APT-broth and in coarsely ground pork after heating at 72°C and after a heating process corresponding to that of cooking sausages.

### MATERIAL AND METHODS:

**Bacterial strains:** The two bacterial strains used were isolated on APT-agar from the core of cooked meat products made of coarsely ground pork. They are thought to belong to the genus *Streptococcus* on the basis that they are gram positive cocci growing in chains. Because they grow at 10°C or less but not at 45°C and not in broth containing 6.5% NaCl they should belong to the lactic acid streptococci group according to BERGEY'S MANUAL (1986). However their fermentation patterns do not correspond to any species of this group. The fermentation tests were made by API 50CH-strips (API System, Mautalieu Vercieau, France). The strain fermented ribose, D-glucose, D-fructose, saccharose, trehalose and D-turanose and the strain 7b1 D-glucose, D-fructose, D-mannose, a methyl-D-glucoside, N acetyl glucosamine, cellobiose, maltose, melibiose, saccharose, trehalose, 6 gentiobiose and D-turanose.

**Thermal death of the strains during heating at 72°C in APT-broth and coarsely ground cured pork:** APT-broth: Five millilitres of APT-broth (Merck 10454) heated in a test tube to 72°C were inoculated with 0.05 ml of 18 h old APT-broth culture to be tested. Different tubes were prepared for each heating period. The tubes were heated for 0.5, 1, 1.5, 2, 5, 15, 30, 60 or 120 min and cooled for 10 min at 10°C in water. The number of colony forming units (cfu) of inoculated strain in each test tubes after inoculation and after heating was determined on APT-agar (Merck 10453, 3 d at 30°C). Six experiments were conducted for both strains.

Coarsely ground cured pork: One experimental batch contained 150 g pork, 0.67 g glucose and 15 g water. The pork originated from pig slaughtered as aseptically as possible. The pork obtained from the ham was also handled and trimmed aseptically. The following additives were used: NaCl (2%), NaNO<sub>2</sub> (0.012%), phosphates (0.15% P<sub>2</sub>O<sub>5</sub>) and Na-ascorbate (0.04%). The bacterial inoculum (the aim 10<sup>7</sup>/g meat) was added as APT-broth culture (15 ml). The coarse grinding of the meat and mixing of additives and bacteria were done in a Moulinex mixer (Moulinex France). The mixed batches were packed in the 10 ml glass tubes, different tubes being used for each heating period. The tubes inoculated with the strain 3b1 were heated in a water bath for 0.5, 2, 5 and 15 min and the tubes inoculated with the strain 7b1 for 0.5, 2, 5, 15, 30 and 60 min after the temperature had been raised to 72°C (3 min). After heating the tubes were cooled for 10 min at 10°C in water. Four experiments were conducted for both strains.

The total count of bacteria (=the number of inoculated bacteria) of the inoculums and the pork tubes after inoculation and after heating was determined on APT-agar (Merck 10453, 3 d at 30°C). *Staphylococcus* + *micrococcus* (Baird-Parker-agar, Labm 85 and

X085, 2 d at 37°C), pseudomonas (GSP-agar, Merck 10230, 3 d at 25°C) and Brochothrix thermosphacta (STAA-agar, GARDNER 1965, 2 d at 22°C) determinations were made on the inoculated but not heated meat tubes.

**Thermal death of the strains during heat treatment corresponding to the heat treatment used in cooking sausages: APT-broth:** The 5 ml APT-broth in test tube at 22°C was inoculated with 0.05 ml 18 old APT-broth culture of the strain to be tested. Different tubes were made for each heating period. The broth tubes were heated in a water bath by raising the temperature from 22°C to 72°C the mean of raising time being 45 min, then by keeping the broth tubes for 5 min at 72°C and thereafter decreasing the temperature to 55°C over 47 min (mean). Then the last broth tubes were removed from the water bath and cooled for 10 min to 30°C. The temperature profile is presented in the Table 2 and in the Figure 2. During heating one broth tube was removed from the water bath when the following temperatures (°C) had been reached: 22 (0 min), 55 (27 min), 65 (36 min), 72 (45 min), 72 (46 min), 72 (47 min), 72 (50 min), 65 (62 min), 55 (97 min) and 30 (103). The removed tubes were cooled for 10 min at 10°C in water and the number of surviving colony forming units was determined on APT-agar (Merck 10453, 3 d at 30°C). Also the cfu number of inoculums were determined on APT-agar. Six experiments were conducted for both strains.

**Coarsely ground cured pork:** The same kind of cured pork batches inoculated with the strain to be tested were made as in studying the heat resistance of the strains at 72°C (as explained earlier). Instead of bacterial inoculum 15 ml water was added to control batches. The mixed meat batches were packed in 10 ml test tubes, different tubes for each heating period. The tubes were heated in water bath by raising the temperature from 22°C to 72°C the mean of raise time being 48 min, then by keeping the tubes for 5 min at 72°C and thereafter by decreasing the temperature to 55°C during 44 min (mean). Then the last pork tubes were removed from the water bath and cooled over 10 min to 30°C. The temperature profile is presented in Figures 2 and 3. During heating one pork tube was removed from water bath when the following temperatures (°C) had been reached: 22 (0 min), 55 (29 min), 65 (40 min), 72 (48 min), 72 (53 min), 30 (103 min). The removed tubes were cooled for 10 min at 10°C in water and studied microbiologically. One tube in each experimental series was reserved to be stored after cooling for 4 weeks at 6°C. Before microbiological study. Four experiments were conducted for both strains.

The following microbiological determinations were made on inoculated and heated meat tubes: Total count of bacteria in respect to the count of surviving colony forming units of inoculated strain (APT-agar, Merck 10453, 3d at 30°C), staphylococci + micrococci (Baird & Parker-agar, Labm 85 and X085, 2 d at 37°C), pseudomonads (GSP-agar, Merck 10230, 3 d at 25°C) and Brochothrix thermosphacta (GARDNER 1965, 2 d at 22°C). The cfu number of inoculums were determined on APT-agar.

## RESULTS AND DISCUSSION:

**The thermal death of the strains during heating at 72°C in APT-broth and coarsely ground cured pork: APT-broth:** The number of cfu/ml of the strain 3b1 decreased during 0.5 min by 2 log units from 6.3 to 4.2 log cfu/ml (Table 1, Fig. 1). The respective decrease of the strain 7b1 was 1 log unit from 6.5 to 5.2 log cfu/ml. The thermal death of both strains diminished after heating for 0.5 min being 2 log units for 3b1 and 1.5 log units for 7b1 over the next 14.5 min.

**Coarsely ground cured pork:** The number of colony forming units of both strains decreased during heating in coarsely ground cured pork more slowly and less than in APT-broth. The decrease for both strains was about 1 log unit during heating for 2 min (Table 1, Fig. 1). The cfu numbers of the porks before heating were 6.8 log cfu/ml (strain 3b1) and 7.6 log cfu/ml (strain 7b1). The thermal death of both strains diminished also in coarsely ground cured pork when the heating was continued being about 2 log units for 3b1 and 0.5 log unit for 7b1 over the next 13 min. Coarsely ground experimental pork contained staphylococci and micrococci only accidentally over 2 log cfu/g but pseudomonads or Brochothrix thermosphacta never.

**D-values:** The thermal death curves for both strains are not straight lines as they should be theoretically. So different D-values could be determined for different heating periods. However, if safe D-values are required they should be determined according to cfu numbers after heating for 5 and 15 min after the steep decrease of colony forming units at the beginning of heating. The D-values determined according to this heating period for the strains are the following: The strain 3b1 20 (APT-broth), 8.3 (coarsely ground cured pork) and the strain 7b1 16.7 (APT-broth), 20 (coarsely ground cured pork).

**Thermal death of the strains during heat treatment corresponding to the heat treatment used in cooking sausages: APT-broth:** The number of cfu/ml of the strain 3b1 decreased 2.5 log units from 6.0 to 3.4 log cfu/ml when heated in APT-broth from 22°C to 65°C over 36 min (Table 2, Fig. 2). When the temperature was raised thereafter to 72°C over 9 min the number of cfu/ml decreased 1 log unit. Thereafter heating for 5 min at 72°C and cooling for 53 min to 30°C decreased the cfu number so that there were no



samples containing colony forming units of the strain. When heated in APT-broth from 22°C to 65°C over 36 min the cfu number of the strain 7b1 did not decrease (Table 2, Fig 2). When the temperature was raised to 72°C over 9 min the number of cfu/ml decreased by 3 log units from 6.9 to 3.8 log cfu/ml. Thereafter heating for 5 min at 72°C and cooling to 30°C during 53 min did not decrease the cfu number of the strain.

**Coarsely ground pork:** The number of cfu/g of the strain 3b1 decreased 2 log units from 7.2 to 5.2 log cfu/g when heated in coarsely ground cured pork from 22°C to 72°C over 36 min (Table 2, Fig. 3). Thereafter heating for 5 min at 72°C decreased the cfu number by 1 log unit and cooling to 30°C over 53 min by 0.7 log unit. When heated in coarsely ground cured pork from 22°C to 72°C over 36 min the cfu number of the strain 7b1 decreased 0.7 log unit from 7.7 to 7.0 log cfu/g (Table 2, Fig. 3). Thereafter heating for 5 min at 72°C decreased the cfu number about 1 log unit and cooling to 30°C over 53 min by 0.5 log unit.

When the heat treated and cooled experimental coarsely ground cured porks were stored at 4°C for 4 weeks the cfu number of the strain 3b1 rose by 1 log unit to 4.5 log cfu/g and the cfu number of the strain 7b1 by 0.5 log unit to 6.0 log cfu/g.

Coarsely ground experimental porks contained staphylococci and micrococci only accidentally over 2 log cfu/g but pseudomonads or *Brochothrix thermosphacta* never.

**CONCLUSION:** In cooked meat products lactic acid bacteria appear which survive after the cooking process. However, these bacteria probably do not constitute a spoilage problem because their cfu number decreases some log units during cooking and rises only a little during storage.

**REFERENCES**

BERGEY'S MANUAL of Systematic Bacteriology, vol 2 1986. Ed. by P. H. A. Sneath, N. S. Mair, M. E. Sharpe and J. G. Holt. The Williams & Wilkins, Baltimore Md.

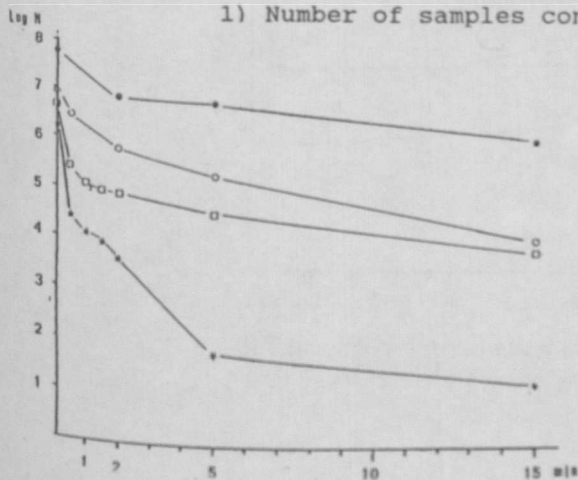
GARDNER, G. 1966. A selective medium for the enumeration of *Microbacterium thermosphactum* in meat and meat products. *J. Appl. Bact.* 29: 455 - 460.

PETÄJÄ, E. 1991. Meat products made of coarsely ground meat: Survival of lactic acid bacteria and pseudomonads after heat treatment. *Proc. 37th International Congress of Meat Science and Technology*: 605 - 608. Kulmbach.

**Table 1.** Thermal death of bacterial strains 3b1 and 7b1 at 72°C in APT-broth (6 test series) and in coarsely ground cured pork (CCP) (4 test series).

Heating time, min	Cfu/ml in APT-broth				Cfu/g in CCP			
	3b1		7b1		3b1		7b1	
	X	s	X	s	X	s	X	s
0	6.3	0.9	6.5	0.2	6.8	0.5	7.6	0.5
0.5	4.2	0.8	5.2	0.5	6.3	1.0		
1	4.1	0.8	5.0	0.4				
1.5	4.0	1.7	5.0	0.3				
2	3.5	1.3	4.8	0.5	5.7	0.7	6.8	0.9
5	1.7	1.9	4.3	1.0	5.2	0.5	6.5	0.6
15	1.2	1.4	3.7	1.1	4.0	1.2	6.0	1.3
30	1) 4		2.1	0.9			5.5	1.1
60	1) 1		1) 2				5.3	1.4
120	1) 0		1) 1					

Cfu = colony forming unit  
 X = mean of colony forming units/ml  
 s = standard deviation of mean  
 1) Number of samples containing colony forming units



**Figure 1.** Thermal death of lactic acid bacteria strains 3b1 and 7b1 during heating at 72°C in APT-broth and in coarsely ground pork.

★ 3b1 in APT-broth  
 ○ 3b1 in coarsely ground pork  
 □ 7b1 in APT-broth  
 ● 7b1 in coarsely ground pork

**Table 2.** Thermal death of the bacterial strains 3b1 and 7b1 in APT-broth (6 test series) and in coarsely ground cured pork (CCP) (4 test series) during heating corresponding to the heat treatment used in cooking sausages.

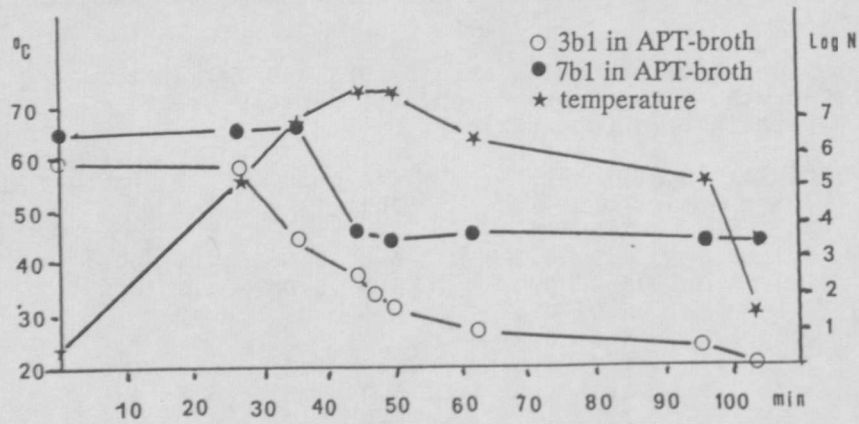
Temperature, °C	Time, min	Cfu/ml in APT-broth				Time, min	Cfu/inCCP			
		3b1		7b1			3b1		7b1	
		X	s	X	s		X	s	X	s
22	0	6.0	0.1	6.5	0.3	0	7.2	0.3	7.7	0.5
55	27	5.7	1.1	6.7	0.5	29	6.5	1.2	7.6	0.4
65	36	3.4	0.6	6.9	0.3	40	5.6	1.0	7.1	0.4
72	45	2.4	0.9	3.8	0.8	48	5.2	1.3	7.0	0.5
72	46	2.6	0.2	3.5	1.2					
72	47	2.0	0.9	4.1	0.6					
72	50	1.6	1.2	3.6	1.3	53	4.2	1.7	6.1	0.7
65	62	1.0	1.1	3.9	0.4					
55	97	0.5	1.0	3.7	0.4					
30	103 1)	0		3.5	0.7	107	3.5	0.5	5.5	0.9
						4 weeks at 6°C	4.5	1.7	6.0	1.9

Cfu = colony forming unit

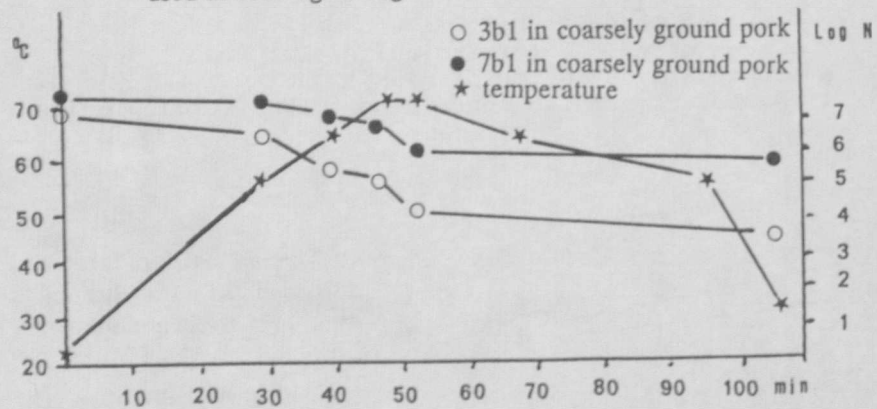
X = mean of the cfu counts

s = standard deviation of mean

1) number of samples containing colony forming units



**Figure 2.** Thermal death of lactic acid bacteria strains 3b1 and 7b1 in APT-broth during heating corresponding to the heat treatment used in cooking sausages.



**Figure 3.** Thermal death of lactic acid bacteria strains 3b1 and 7b1 in coarsely ground pork during heating corresponding to the heat treatment used in cooking sausages.