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THE EFFECT OF COLD INCUBATION ON THE HEAT RESISTANCE 0F A STREPTOCOCCUS STRAIN AND OF A PSEUDOMONAS STRAIN ISOLATED FROM THE CORE OF COOKED MEAT PRODUCTS MADE FROM COARSELY GROUND PORK

E. PETÄJÄ and E. PUOLANNE
Department of Food Technology, Meat Section, Viikki, E-Building, P. O. Box 27, SF-00140 University of Helsinki, Helsinki, Finland

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BACKGROUND AND OBJECTIVES

The heat resistance of streptococci and pseudomonads isolated from the core of cooked meat products made from coarsely ground meat has earlier been described by Petäjä (1991, 1992) and Petäjä and Puolanne (1993). Most of the strains were remarkably heat resistant, surviving after heating for 30 min at 72°C in APT-broth in 50 % or more of the tests. When inoculated in coarsely ground cured pork, the most heat resistant pseudomonads survived after heating for 15 min at 72°C. The most heat resistant streptococci survived after a heat treatment corresponding to that experienced in cooking sausages and products made from coarsely ground meat. When the most heat resistant Streptococcus and Pseudomonas strains were inoculated into such products to study the effect of glucose addition or the pH of the meat raw-material on the keepability of the products, they did not survive heating as well as in earlier studies (Petäjä, Puolanne and Korteniemi 1994, Petäjä and Puolanne 1994). These experiments differed from the former heat resistance studies in that the inoculated products were incubated at 6°C for 20 h before heating. The effect of such cold incubation on the subsequent heat resistance of the same Streptococcus and Pseudomonas strains was studied in this investigation.

MATERIAL AND METHODS

Bacterial strains: Streptococcus strain 7b1 and Pseudomonas strain 7b2 were isolated on APT-agar (Merck 10453) from the core of cooked meat products made from coarsely ground pork (Petäjä 1991, Petäjä and Puolanne 1993). In the earlier studies, both strains proved to be heat resistant to the extent that when heated in coarsely ground pork, the Pseudomonas strain survived after heating for 15 min at 72°C and the Streptococcus strain was resistant to a heating treatment corresponding to the cooking of cooked sausage (Petäjä 1991, Petäjä 1992).

Thermal death of the strains during heat treatments simulating the cooking of sausage

1. In APT-broth: 5 ml of APT-broth (BBL 10918) preheated in a test tube to 22°C was inoculated with 0.05 ml of 18 h APT-broth culture of the strain to be tested (the target 10^7 cfu/ml in inoculated APT-broth). Different culture tubes were inoculated for each heating period. Two series of inoculated tubes were made one to be heated at once and another after incubation at 6°C for 20 hours.The culture tubes were placed in a water bath and the temperature was increased from 22°C to 72°C over 45 min (mean), maintained at 72°C for 5 min and then decreased to 55°C over 47 min (mean). Then the final tube was removed from the water bath and cooled to 30°C during 10 min. The temperature profile is presented in Tables 1 and 2. During heat treatment cycle, one broth tube was removed from the water bath at the following temperatures: 22°C (0 min), 65°C (39 min), 72°C (46 min), 72°C (51 min), 65°C (56 min) and 30°C (82 min). After removal, each tube was cooled for 10 min in water at 10°C, the number of surviving colony forming units (cfu) being determined on APT-agar (BBL 10918, 3 d at 30°C, spreader method). The cfu number of inoculums were also determined on APT-agar. Five experiments were conducted for each of the strains.

2. In coarsely ground cured pork: One exprimental batch contained 150 g pork, 0.67 g glucose and 15 g water. The pork was obtained from outer fillets aseptically cut aseptically from carcasses. Handling, trimming and cutting of the fillets into pieces were done aseptically. The following additives were used: NaCl (2 %), $NaNO_2$ (0.012 %), phosphates (0.15 % as P_2O_5) and Na-ascorbate (0.04 %). The bacterial inoculum (the target 10^7 cfu/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 into the 10 ml glass test tubes, different tubes being used for each heating period. Two series of inoculated tubes were made one to be heated at once and another after incubation at 6°C for 20 hours. The tubes were subjected to the following heating cycle in water bath: the temperature was increased from 22 to 72°C over 48 min (mean), maintained at 72°C for 5 min and then decreased to 55°C over 44 min (mean). The final test tubes were removed from the water bath and cooled over 10 min to 30°C. The temperature profile is presented in Tables 1 and 2. During the heating cycle tubes were removed from the water bath at the following temperatures: 22°C (0 min), 55°C (28 min), 65°C (35 min), 72°C (45 min), 72°C (50 min), 30°C (83 min). The removed tubes were cooled for 10 min in water at 10°C. Two tubes in each experimental series were stored after cooling, one for 1 day at 6°C and another for 2 weeks at 6°C. Four experimental series were conducted for each strain.

Total cfu counts of bacteria were determined on APT-agar (BBL 10918, 3d at 30°C, spreader method). The following microbiological determinations were made on those tubes heated to 65°C and on the test tubes which received the whole heat treatment and were cooled to 30°C: staphylococci + micrococci (Baird-Parker-agar, Labm 85 and X085, 2d at 37°C), pseudomonads (GSP-agar, Merck 10230, 3 d at 25°C) and Brochothrix thermosphacta (Gardner 1965, 2 d at 22°C). Inocula levels were determined by plating on APT-agar.

RESULTS AND DISCUSSION

1. The effect of cold incubation before heat treatment on the heat resistance of the strains: The incubation for 20 h at 6°C did not decrease the subsequent heat resistance of Streptococcus 7b1 and Pseudomonas 7b2 in APT-broth or in coarsely ground cured pork (Tables 1 and 2). The numbers of cfu detected in samples heated just after inoculation and in samples heated after preincubation for 20 h at 6°C were not significantly diffferent over the heating cycle. Hence, the decrease in heat resistance (Petäjä et al. 1994; Petäjä & Puolanne 1995) cannot be explained by cold incubation before heating.

2. Survival of the strains after heating:

- **Streptococcus 7b1:** The heat resistance of Streptococcus 7b1 was lower in this investigation than in the original studies (Petäjä 1991, Petäjä 1992). The number of cfu/ml of strain 7b1 decreased about 4 log units from a level of about 7 log cfu/ml to about 3 log cfu/ml when heated in APT-broth from 22°C to 65°C over 39 min (Table 1). When the temperature was raised to 72°C over a further

7 min and maintained for 5 min at 72°C, the number of cfu decreased to approximately 1.5 log cfu/ml. During 2 weeks of storage at 6°C, the tubes heated just after inoculation retained viable streptococci but in the tubes incubated for 20 h at 6°C before heating, the streptococci were below the level of detection.

When heated in coarsely ground cured pork, the number of cfu/g decreased more steeply than in APT-broth the mean counts being about 1 log cfu/g of pork after heating to 72°C over 45 min and heating at 72°C for 5 min (Table 2). After cooling to 30°C and 2 weeks of storage at 6°C, only 3 samples out of 24 contained inoculated streptococci at 2 log cfu/g or abowe. Heated test tubes did not contain staphylococci and micrococci, pseudomonads or Brochothrix thermosphacta on the level of detection.

- **Pseudomonas 7b2:** The heat resistance of Pseudomonas 7b2 was also lower in this study compared to those in which the heat resistance of the strain was investigated for the first time (Petäjä 1991 and 1992). The number of cfu/ml of Pseudomonas strain 7b2 decreased steeply during heating, especially from 55°C to 72°C, the survivors being at the level of 1 log cfu/ml in APT-broth and under 2 log cfu/g in coarsely ground cured pork (Table 2). After decreasing the temperature to 30°C and storing the samples for 2 weeks at 6°C the broth samples contained survivors of the pseudomonads inoculated, but the counts were below 2 log cfu/ml. Only 4 coarsely ground cured pork samples out of 24 contained pseudomonads at levels above 2 log cfu/g. Heated test tubes did not contain staphylococci and micrococci or Brochothrix thermosphacta on the level of detection.
- **Disappearance of heat resistance:** In the studies in which the effects of glucose content and the pH of the meat raw-material were studied (Petäjä and Puolanne 1995, Petäjä et.al. 1993), Streptococcus 7b1 and Pseudomonas 7b2 survived more poorly than in the original heat resistance studies (Petäjä 1991, 1992). The present study indicates that the decreased survival cannot be explained by the cold incubation before heating: Streptococcus 7b1 and Pseudomonas 7b2 survived almost as poorly as in the glucose and pH studies. The poor survival was seen in all experimental series and in both the APT-broth and the pork series. On the basis of the results of these three studies it can be concluded, that Streptococcus 7b1 and Pseudomonas 7b2 have lost their heat resistance during 2-3 years storage as stock cultures at 6°C. The stock cultures have been subcultured every two months. Attempts were made to recover heat resistance by successive heatings but without any success. The loss of heat resistance appears permanent. From the practical point of view, this result means that bacteria surviving in the cores of meat products have an even less harmful potential than it has been considered because in addition that they do not generally grow in the core and spoil the product, they may lose their heat resistance.

CONCLUSIONS

1. A cold incubation before heating does not account for the decreased heat resistance of the Streptococcus and Pseudomonas strains used in the study.
2. The heat resistant bacteria studied may lose a large portion of their heat resistance. This must be realized when research work is done with heat resistant bacteria.
3. Due to the loss of heat resistance the heat resistant bacteria may be less harmful than previously believed.

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Table 1. Thermal death of Streptococcus strain 7b1 in APT-broth (5 test series) and in coarsely ground cured pork (CCP) (4 test series) when heated just after inoculation (A) or after incubation for 20 h at 6°C after inoculation (B). Heating simulated that used in cooking sausages.

Temperature, °C	Time, min	Cfu/ml in APT-broth A x	A s	B x	B s	Time, min	Cfu/g in CCP A x	A s	B x	B s
22										
55	0	6.8	0.2	7.1	0.7	0	7.6	0.4	7.7	0.4
65	30	4.9	1.1	4.4	2.1	28	3,5	0.4	3.5	0.4
72	39	3.2	1.9	3.0	1.8	35	2.5	0.9	2.0	0.8
72	46	2.3	1.9	2.1	1.3	45	2.1	0.2	1.4	0.4
65	51	1.7	1.8	1.4	2.1	50	1.2	0.3	1.0	0.4
55	56					55				
30	66					62				
	82	1.7	1.7	1.0	1.1	83	1)1		1)0	
6°C										
6°C	1 d	1.5	1.6	0.2	9.5	1 d	1)2		1)0	
	14 d	2.5	3.3	0.0	0.0	14 d	1)0		1)0	

Cfu = colony forming unit in log cfu/ml (APT-broth) /g (CCP)
x = mean
s = standard deviation of mean
1) = number of samples out of four containing colony forming units 2 log cfu/g or more
2) = number of samples containing colony forming units

Table 2. Thermal death of Pseudomonas strain 7b2 in APT-broth (5 test series) and in coarsely ground cured pork (CCP) (4 test series) when heated just after inoculation (A) or after incubation for 20 h at 6°C after inoculation (B). Heating simulated that used in cooking sausages.

Temperature, °C	Time, min	Cfu/ml in APT-broth A x	A s	B x	B s	Time, min	Cfu/g in CCP A x	A s	B x	B s
22	0	5.4	0.2	6.3	0.3	0	7.1	0.2	7.2	0.1
55	30	5.0	0.1	5.6	0.5	28	4.8	0.2	4.7	0.2
65	39	2.9	0.7	2.1	0.6	35	2.2	1.9	1.3	1.0
72	46	1.3	0.2	0.9	1.0	45	1)1		1)1	
72	51	1.4	1.6	2.4	0.9	50	1)1		1)1	
65	56					55				
55	66					62				
30	82	0.8	1.0	1.8	1.5	83	1)0		1)0	
6°C	1 d	0.3	0.5	1.6	1.8	1 d	1)1		1)0	
6°C	14 d	2)1		2)1		14 d	1)2		1)1	

Cfu = colony forming unit in log cfo/ml (APT-broth) /g (CCP)
x = mean
s = standard deviation of mean
1) = number of samples out of four containing colony forming units 2 log cfu/g or more
2) = number of samples containing colony forming units