

Meat products made of coarsely ground meat: Survival of lactic acid bacteria and pseudomonads after heat treatment

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SUMMARY: The main strains in the surface layer of the cooked meat products made of coarsely ground pork were coccoid lactic acid bacteria surviving only accidentally after heating for 15 minutes at 72°C in APT-broth. The counts ranged from 3.5 to 7.8 log cfu (colony forming units)/g in the surface layer. The core of the products contained coccoid lactic acid bacteria or coccoid lactic acid bacteria and pseudomonads or only pseudomonads as the main bacterial strains the counts ranging from 2.6 to 6.0 log cfu/g. Most of these strains survived after heating for 30 minutes at 72°C in APT-broth at least in three tests out of six. It must be pointed out that all pseudomonads were able to survive after heating for 60 minutes at 72°C in APT-broth and often after heating for 15 minutes at 72°C in coarsely ground, cured pork. The numbers of inoculated pseudomonads decreased when the heated experimental porks were kept at 4°C. This indicates that they probably do not constitute a serious spoilage factor in cooked meat products.

INTRODUCTION: The manufacture and consumption of the cooked meat products made of coarsely ground meat has grown over the last ten years in Finland. These products are heated to internal temperatures of 68-72°C. In spite of that they sometimes spoil too quickly so that not only the surface layer but also the core of the product spoils. There is not much available information on the keeping times or the microbial flora of this kind of products. These products can be assumed to resemble bologna type cooked sausages and luncheon meat in these respects. In these products high bacterial counts may be found although the products would not allways have spoiled. PARADIS and STILES (1978) have found 60 % of commercial bologna type sausages contain over 10⁶ bacteria/g. According HILL et al.(1976) 4-week-old bolognas contain over 10⁷ bacteria/g. According to DUITSCHAEVER (1977) 50 % of commercial luncheon meats contain over 5 x 10⁶ bacteria/g. HILL et al. (1976) have found that 71 % of luncheon meats stored for 3 weeks at 4 - 7°C contain over 10⁶ bacteria/g. In this study the bacterial flora of the surface layer and the core of cooked meat products made of coarsely ground pork was investigated at the moment of spoilage. The dominating strains were isolated and their heat resistance was studied to clarify the nature of microbial spoilage in cooked coarsely ground meat products.

MATERIAL AND METHODS:

Product samples and their microbiological examination: Seven 2-4-week-old samples of cooked and vacuum-packed coarsely ground pork products were received directly from their manufacturers and kept at 4°C and 7°C. At the moment of the first signs of spoilage on the surface (smell and/or slime after 3 weeks at 4°C and 1 week at 7°C) the following examinations were made: The total count of bacteria on plate count agar (Difco 0479, 3 d at 30°C), on APT-agar (Merck 10453, 3 d at 30°C, 9 d at 15°C) and on blood agar (Orion, Espoo, Finland, 2 d at 37°C), the total count of anaerobic bacteria on SPS-agar (Merck 10235, 2 d at 37°C anaerobically), the number of lactic acid bacteria on Rogosa-agar (Merck 5463, 4 d at 30°C), the number of Brochothrix thermosphacta on STAA-agar (GARDNER 1965, 2 d at 22°C), the number of staphylococci and micrococci on Baird-Parker-agar (Labm 85 and X085, 2 d at 37°C), the number of coliforms on VRB-agar (Labm 31, 1 d at 37°C). The former determinations were made on the surface layer 1 - 2 mm in thickness and on the core of the product samples. The results were tested with variance analysis (Epistat program) in relation to surface layer and core and storage temperature. The main types of bacteria were isolated from the surface layer and core of all product samples.

Survival of the isolated strains after heating on solid and in liquid medium: Solid medium. The survival of the strains after heating on solid medium was studied with a Gradiplate W10-temperature-gradient incubator (Biodata OY, Helsinki, Finland). In the incubator a stepless temperature gradient can be achieved in a 10°C range. The survival ability of the strains was investigated by keeping them on APT-agar (Merck 10453) in cuvettes for 30 min at the temperature range of 50 - 70°C and incubating cuvettes for 2 d at 30°C. The inoculation was carried out by rolling a drop (0.05 ml) of the 18 h old APT-broth culture onto the surface of the

agar. Three determinations for each strain were made. Liquid medium. The survival of the strains after heating in APT-broth at 72°C was investigated side by side with the experiments on solid medium. The 5 ml APT broth in test tube heated to 72°C was inoculated with 0.05 ml 18 h old APT-broth culture. Different tubes were prepared for each heating period. The tubes were heated for 5, 15, 30, 60, 90 or 120 min and incubated at 30°C for 2 d and the growth (turbid broth) was recorded. Six experiments were made for each strain.

Survival of four isolated *Pseudomonas* strains after heating at 72°C in coarsely ground cured pork: One experimental batch contained 150 g pork, 0.67 g glucose and 15 g water. The following additives were used: NaCl (2 %), NaNO₂ (0.012 %), phosphoric acid (0.15 % P₂O₅) and Na-ascorbate (0.04 %). The *pseudomonas* inoculum (the aim 10⁷ cfu/g meat) was added as APT-broth culture (15 ml). Instead of the inoculum 15 ml water was added to control batch. The coarsely grinding of the meat and mixing additives and bacteria were done in a Moulinex mixer (Moulinex, France). The mixed meat batches were packed in the 10 ml glass test tubes for each heating period. The inoculated tubes were heated in water bath for 5, 15 and 30 min after the temperature had been raised to 72°C (3 min) and cooled for 10 min at 10°C in water and for 1 h at 22°C. The following microbiological determinations were made for the non-heated and heated coarsely ground meats and for the meats heated for 5 min and stored for 2-3 weeks at 6°C: Total plate count on APT-agar, *pseudomonads*, staphylococci and micrococci, lactic acid bacteria and *Brochothrix thermosphacta*. The same media and incubations were used as in microbiological studies of meat product samples. The cfu number of inoculums were determined on APT-agar by incubating for 2 d at 30°C. The surviving of inoculums after heating in APT-broth at 72°C for 5, 15, 30, 60 and 90 min was also tested side by side with the coarsely ground pork experiments. The thermal resistance experiments with coarsely ground pork were made three times for each strain.

RESULTS AND DISCUSSION:

Bacterial flora of the product samples: The total number of aerobically growing bacteria in the surface layer of the products ranged from 4.9 to 7.8 log cfu/g at the moment of spoilage when the products were stored at 4°C. The respective range was 3.5 - 7.8 log cfu/g after storing at 7°C. The cores contained at the moment of spoilage 4.6 - 6.0 log cfu/g when the products were stored at 4°C and 2.5 - 5.6 log cfu/g when they were stored at 7°C the mean of bacterial counts at 7°C being lower ($p < 0.05$) than at 4°C. The means of the bacterial counts in the core were both at 7°C and 4°C ($p < 0.05$) lower than in the surface layer. The incubation temperatures 30°C and 15°C resulted the total numbers of bacteria on the same level. The number of lactic acid bacteria growing on Rogosa-agar ranged from 1.0 to 7.0 log cfu/g the surface layer containing higher ($p < 0.05$) counts than the core at 4°C. The samples contained *Brochothrix thermosphacta* and staphylococci/micrococci the counts being higher in the surface layer than in the core. No samples contained coliforms over 1.0 log cfu/g. The counts for anaerobically growing bacteria ranged from 1.0 to 6.0 log cfu/g the counts being higher ($p < 0.05$) in the surface layers than in the cores and after storing at 4°C higher ($p < 0.05$) than after storing at 7°C.

Lactic acid bacteria growing on Rogosa-agar, *Brochothrix thermosphacta*, staphylococci/micrococci or anaerobically growing bacteria did not form the main bacterial types of the bacterial flora of the products. However, the bacterial flora of each product sample contained 1 - 3 main bacterial types which were isolated.

Isolated bacteria: The strains isolated from the surface layer of the products were gram-positive cocci or coccibacilli (Table 1). The core of two products also contained gram-positive cocci as the main bacterial types (5 pcs.). The core of two products contained gram-positive coccus and gram-negative rod as the main bacterial strains whereas the core of three products contained only gram-negative rods as a main strain. Gram-negative rods proved *pseudomonads*. The gram-positive coccus strains isolated were lactic acid bacteria. It was surprising that the core of the products contained *pseudomonads* because it is supposed that gram-negative bacteria are destroyed by heat treatment (HALLENBACH and POTTER, 1981, PALUMBO et al. (1974). Lactic acid bacteria have been reported to be the main bacterial group in cooked meat products (MANTEL and BECK, 1977, DAELMAN and HOOFF, 1977), which

proved also by the results of this study. However, also GARDNER (1966) has found that cooked sausage may contain gram-negative bacteria, pseudomonads and achromobacteria.

Survival of isolated bacterial strains after heat treatment in APT-broth and on APT-agar: Seven surface layer strains survived after heating for 15 min at 72°C in one or two of the six tests (Table 1). Five surface layer strains did not survive at all. The highest surviving temperatures of most surface layer strains on APT-agar were on both sides of 50°C, at most 58°C. Because the surface layer strains are not very heat resistant, they are probably due to contamination after cooking. Most lactic acid bacteria and pseudomonas strains isolated from the cores survived after heating for 30 min at 72°C in APT-broth at least in three of the six tests (Table 1). Two lactic acid bacteria and one pseudomonad survived after heating for 30 min at 72°C in all six tests. All pseudomonads survived after heating for 60 min in two of the six tests. The highest surviving temperature of the core strains was distinctly lower on APT-agar than in APT-broth. Only three strains on APT-agar had the highest surviving temperature over 60°C in some experiments.

Survival of four isolated Pseudomonas strains after heating at 72°C in coarsely ground cured pork: Strain 7b₂ survived best after heating in coarsely ground cured pork (Table 2). The number of cfu decreased from log 7.2/g to log 3.6/g during heating for 15 min. After heating for 30 min two tests of the three contained inoculated pseudomonads over log 2.0 cfu/g. After heating strains 1b₂, 4b and 6b in coarsely ground cured pork for 15 min each strain appeared over log 2.0 cfu/g in 1 - 2 tests of the three the numbers ranging between log 2.0 and 4.3 cfu/g.

The total plate counts of bacteria in heat treated coarsely ground experimental pork ranged between log 3.1 - 5.6 cfu/g. The control pork (not inoculated) contained pseudomonads and staphylococci/micrococci only accidentally over 2.0 cfu/g. They did not contain lactic acid bacteria over log 1.0 cfu/g and *Brochothrix thermosphacta* over log 2.0 cfu/g. The non-heated coarsely ground cured pork also did not exceed these values. When the inoculated pork samples, which had been heated for five min at 72°C, were stored for 2 - 3 weeks at 6°C, the numbers of all inoculated strains decreased. This indicates that although pseudomonads would survive after heat treatment they probably are not a serious spoilage factor in cooked meat products made of coarsely ground meat or in cooked meat products generally.

REFERENCES:

- DAELMAN, W. & VAN HOOFF, J. 1975. Einfluss des pH-wertes, der Verwendung von Polyphosphat und der Lagerung auf die bakteriologische Beschaffenheit von Brühwurst und Brühwurstaufschnitt. *Archiv für Lebensmittelhygienia* 26: 213 - 217.
- DUITSCHAEVER, C. L. 1977. Bacteriological evaluation of some luncheon meats in Canadian retail market. *J. Food Prot.* 40: 382 - 384.
- GARDNER, G. 1966. A selective medium for the enumeration of *Microbacterium thermosphactum* in meat and meat products. *J. Appl. Bact.* 29: 455 - 460.
- HALLENBACH, C. H. & POTTER, N. N. 1981. Effects of nitrite and sorbate on bacterial populations in frankfurters and thuringer Cervelat. *J. Food Prot.* 44: 341 - 346.
- HILL, W. M., REAUME, J. & WILCOX, J. C. 1976. Total plate count and sensory evaluation as measures of luncheon meat shelf life. *J. Milk Food Techn.* 39: 759 - 762.
- MANTEL, T. & BECK, G. 1977. Zur microbiologischen Situation vorverpackter Brühwurst. *Fleischwirtsch.* 5: 245 - 247.
- PALUMBO, S. A., HUHTANEN, C. N. & SMITH, J-L. 1974. Microbiology of frankfurter's process: *Salmonella* and natural aerobic flora. *Appl. Microbiol.* 27: 724 - 732.
- PARADIS, D. C. & STILES, M. E. 1978. A study of microbial quality of vacuum packaged sliced bologna. *J. Food Prot.* 10: 811 - 815.

Table 1. The main bacterial strains of the surface layer (a series) and the core (b series) of the cooked meat products made of coarsely ground pork, their survival after heat treatment at 72°C in APT-broth and their highest surviving temperature on APT-agar (Gradiplate method).

Product/ main strains	gram + or -	cell shape	Range of bacte- rial counts (log cfu) inocula- ted in 5 ml APT-broth	Survival at 72°C in APT-broth (po- sitive tests of 6)					The highest survival tem- perature (°C) on APT-agar (range of 3 tests)
				Minutes					
				15	30	60	90	120	
a series									
1/1	+	coccob.	6.1-6.8	2	0	0	0	0	<45.5-49.1
1/2	+	coccob.	7.0-7.5	2	0	1	1	0	<46.4-52.9
2/1	+	coccob.	7.3-7.4	1	0	0	0	0	<46.2-51.1
2/2	+	coccob.	7.5-8.2	1	0	0	0	0	48.8 (1 test)
3/1	+	coccob.	7.4-7.7	0	0	0	0	0	47.4-49.0
3/2	+	coccob.	7.5-8.3	1	0	1	0	0	<45.5 (1 test)
3/3	+	cocci	7.2-8.2	0	0	0	0	0	47.3-49.1
4/1	+	cocci	6.6-8.2	2	1	0	0	0	<46.2-52.6
4/2	+	coccob.	4.8-7.3	1	0	0	0	0	55.3-58.2
5/1	+	cocci	3.7-6.2	0	0	0	0	0	47.3-47.3
7/1	+	cocci	5.7-7.2	0	0	0	0	0	48.1-50.2
7/2	+	coccob.	4.2-6.8	0	0	0	0	0	47.8-55.0
b series									
1/1	+	cocci	6.5-7.1	3	2	0	0	1	49.0-50.8
1/2 (1b2	-	rods	6.8-6.9	6	6	5	5	4	48.5-52.7
2/1	+	cocci	6.0-7.5	3	2	0	0	0	<45.5 (1 test)
2/2	+	cocci	6.0-7.9	5	4	0	0	0	46.8-51.3
3/1	+	cocci	5.7-7.4	6	6	6	5	2	50.3-56.6
3/2	+	cocci	6.9-8.2	5	4	0	0	0	<46.4 (1 test)
3/3	+	cocci	5.9-7.1	1	1	2	2	2	48.5-50.3
4/1 (4b)	-	rods	7.2-7.8	6	4	3	2	0	50.5-58.9
5/1	-	rods	6.1-6.9	2	2	2	0	0	51.0-60.0
6/1 (6b)	-	rods	6.9-8.2	4	3	2	2	0	50.1-56.7
7/1	+	cocci	7.7-7.9	6	6	6	6	0	54.8-60.6
7/2 (7b2)	-	rods	6.5-7.1	3	4	3	3	3	58.8-62.2

Table 2. Survival of some *Pseudomonas* strains after heat treatment at 72°C in APT-broth and in coarsely ground cured and cooked pork. The strains were isolated from the core of cooked meat products made of coarsely ground pork.

Strain	Range of bacterial counts (log cfu) inoculated in 5 ml APT-broth	Survival at 72°C in APT-broth (positive tests of 6)					Range of bacterial counts (log cfu/g) inoculated in pork	Survival at 72°C in coarsely ground pork (log cfu/g)			
		Minutes						Minutes			
		5	15	30	60	90		0	5	15	30
1b ₂	6.8-8.7	-	4	4	4	4	7.1-8.0	7.0	- 1)	1	2
4b	6.7-7.8	5	4	3	4	2	7.0-7.2	6.0	1	1	2
6b	6.4-7.1	6	4	4	4	2	7.4-7.7	5.8	2	2	0
7b ₂	7.3-8.2	6	6	4	5	2	7.6-8.5	7.2	3.4	3.6	2

1) Number of samples containing over 100 cfu/g. Number of samples = 3.