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Listeria monocytogenes: heat resistance and behaviour during storage of milk and whey and making of Dutch types of cheese*

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Summary

The heat resistance of *Listeria monocytogenes* suspended in milk was determined with a plate pasteurizer and glass capillary tubes. The reduction of the number of *Listeria* obtained by the plate pasteurizer was ≤5 log 10 units at 64.0 °C for 10 s and 6-7 log 10 units at 67.0 °C for 10 s. The heat resistance of freely suspended and phagocyted *Listeria* was investigated with glass capillary tubes. The surviving *Listeria* were determined with and without resuscitation at 4 °C. Using the resuscitation method it appeared that phagocytosis did increase the heat resistance of one of the two tested *Listeria* strains by factor 2. With the resuscitation method up to 30 times more *Listeria* could be recovered than without resuscitation. The estimated decimal reduction times for the most heat-resistant strain was about 17 s at 64.0 °C and 8 s at 66.0 °C. Incubation tests of *L. monocytogenes* in raw and HTST-pasteurized milk (15 s 72 °C) at 4 and 7 °C showed some injury of *Listeria* during the first 1-2 days. Thereafter growth commenced. In intensively pasteurized milk (30 min 98 °C) *Listeria* was not injured.

Gouda and Maasdam types of cheese were made from *Listeria*-contaminated milk. During the manufacture *Listeria* was concentrated in the curd by factor 10, and a limited growth occurred. During 6 weeks of ripening at 13 °C the number of *Listeria* was almost constant.

1 Introduction

Outbreaks of listeriosis in the United States have been associated with the consumption of pasteurized milk (1) and non-acidified fresh cheese (2). *Listeria monocytogenes* was detected in low numbers in raw milk (3). The sources of *L. monocytogenes* on the dairy farm could be silage of inferior quality and subsequently faeces of clinically healthy carriers among cattle (4). *Listeria*

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may also be excreted in the milk after a *Listeria* abortion, but these kinds of abortions have become rare in the Netherlands (4). The occurrence of clinically healthy excretors in milk are even more rare. When *Listeria* is excreted in the milk the bacteria can be encapsulated by phagocytes. Survival and recontamination after pasteurization might be possible contamination routes to liquid milk consumed as such. With regard to cheese making from raw milk, it is known that certain pathogenic bacteria such as *Staphylococcus aureus* can grow to high numbers if the acidification rate is slow (5).

Data published on the heat resistance of *L. monocytogenes* are not always consistent. Donnelly & Briggs (6) using freely suspended cells found at 62.7 °C decimal reduction (D) values from 24 to 60 s depending on the strain. From the results of Bradshaw et al. (7) a similar D-value of 25 s at 62.7 °C and a D-value of 0.9 s at 71.7 °C can be estimated. A higher D-value of 3 s at 71.7 °C was determined by Doyle et al. (8) using phagocytosed bacteria of strain Scott A and a plate pasteurizer instead of tubes. However, Bunning et al. (9) found no significant difference in heat resistance between freely suspended and phagocytosed bacteria of strain Scott A. The controversion on heat resistance was increased according to a report of Fernandez Garayzabal et al. (10) who detected *L. monocytogenes* in samples of pasteurized milk produced by a commercial plant using a plate pasteurizer at 78 °C for 15 s; however, leakage in the plate pasteurizer and recontamination may explain these findings.

Survival of *L. monocytogenes* during pasteurization is affected both by its heat resistance and by the number of bacteria in the milk before the heat treatment. Hence the storage period and temperature before pasteurization are important. In the Netherlands the milk on the farm is usually stored in a cooled tank at 4 °C for 2-3 days. In dairy plants raw milk may be stored at 4 °C for another 24 h or first thermisized (65 °C for 10 s) and subsequently stored at 4 °C for 24 h. Then the milk is pasteurized at 76 °C for 15 s and packaged. In cheese factories raw milk is thermisized (65 °C for 10 s) within a few hours after arrival. Then the milk is stored at a maximum of 6 °C for 1-3 days. The milk is pasteurized (72 °C for 15 s) immediately before the cheesemaking process. Because no data on the growth of *L. monocytogenes* in raw and thermisized milk are available, an estimation of the numbers of *L. monocytogenes* in the milk before pasteurization cannot be made.

During the manufacture of Camembert and Cheddar cheese no growth of *L. monocytogenes* was observed (11, 12), due to a long lag time (13) and the inhibition by lactic acid produced by the starter culture. During ripening *L. monocytogenes* showed rapid growth to high numbers on Camembert (11) and only little growth in Cheddar (12). No such data are available for Gouda

and Maasdam cheese which have a slower acidification rate and a higher pH than Cheddar.

In this study the behaviour of *L. monocytogenes* in milk during cold storage and the effects of thermization and pasteurization on freely suspended and phagocytosed bacteria were tested. Moreover growth of *L. monocytogenes* was tested during Gouda and Maasdam cheese manufacture and ripening. Furthermore, the influence of a slow acidification rate and high moisture content during ripening on the number of *Listeria* was investigated.

2 Materials and methods

2.1 Cultures

Listeria monocytogenes strains 1, 13 and 669 and LM4B were isolated from soft cheeses, whereas strain LM4B originated from the collection of National Institute of Public Health and Environmental Hygiene. The strains were cultured separately in tryptose broth (Difco) at 37 °C for 2 days, diluted in skim milk or peptone-saline solution (peptone 1 g, sodium chloride 8.5 g, water 1000 ml, pH 7.0) to the appropriate concentration. Equal numbers of bacteria of the different strains were used for inoculation.

2.2 Thermization in a plate pasteurizer

800 litres pasteurized cheese milk (3.5 % fat) were inoculated with equal numbers of strains 1, 13, 669 and LM4B to 9.10^4 bacteria per ml. The milk was heated in an APV plate heat exchanger pasteurization unit to 67.0 and 70.0 °C in the first experiment and to 64.0, 67.0 and 70.0 °C in the second experiment. Temperature deviations were less than 0.1 °C. The pasteuriser consisted of a heating section, a holding tube and a cooling section. To obtain an average residence time in the holding tube of 10 s the flow rate was 820 l/h.

The residence time distribution was calculated using a computer program developed by Van Boxtel (14, 15). It was shown that 10 % of the milk had a residence time of 8.0 s or less, and that 1 % of the milk had a residence time of 6.5 s or less. The residence time distribution was twice that of modern types of plate pasteurization units in order to simulate less ideal processing circumstances.

After thermization the milk was sampled. 0.1 ml was streaked on a trypaflavin-nalidixic acid-serum-agar (TNSA) consisting of 25 g/l nutrient broth No. 2 (Oxoid), 1.25 g/l $Na_2HPO_4 \cdot 2H_2O$, 19.0 g/l Bacto agar, pH 7.7. To one litre of the basal medium, 50 ml horse serum, 4 ml 1 % nalidixic acid and 4 ml 0.2 % trypaflavin were added (16, 17). For enrichment a non-selective medium was used. Therefore 1, 10 and 100 ml of thermisized milk was mixed with

a 9-fold volume of tryptose broth (20 g/l tryptose, 1 g/l glucose 1 g, 5 g/l sodium chloride, pH 7.2) and incubated at 30 °C. After 24 h a loopful of the culture was streaked on a TNSA plate and the rest of the culture was kept at 4 °C. After 2 weeks a loopful was again streaked on a TNSA plate. The plates were incubated at 37 °C. After 48 h they were examined for pale yellow translucent, slightly raised colonies. Such colonies were confirmed according the method described by Beckers et al. (3).

2.3 Thermization in capillary tubes

The technique for the heat resistance test was carried out according to Franklin et al. (18). Glass capillary tubes (75 long and 1.0 mm internal diameter) were filled with a bacterial suspension in skim milk using a micro-syringe. For each test 10 capillaries were used. After sealing, the capillaries were immersed for 15 s in a waterbath of the appropriate temperature, thereafter cooled in chlorinated ice water, dried and opened. The contents were collected by a micro-syringe, diluted in a peptone-saline solution and plated on two series of brain heart infusion agar (Difco). One series was incubated at 37 °C for 48 h, whereas the other was incubated first at 4 °C for 5 days and further at 37 °C for 2 days to obtain a cold resuscitation of the heated bacteria (L. Dominguez Rodriguez, personal communication).

2.4 In vitro phagocytosis

The phagocytosis of *L. monocytogenes* strains 1 and LM4B was carried out according to the method of Van Furth et al. (19), as adapted by Vecht et al. (20). A 48 h culture in tryptose broth incubated at 37 °C was washed twice in Hanks' balanced salt solution (300 mM, pH 7.2) (HBSS). For opsonization the bacterial suspension was incubated in 6 % pooled bovine serum at 37 °C under slow rotation during 25 min. The phagocytosis reaction was initiated by incubation of the opsonized bacteria with bovine polymorphonuclear leucocytes (PMN) at 37 °C under slow rotation during 15 min. The concentration of bacteria and PMN were both 10^7 per ml. The incubation time of 15 min proved to be an optimum time during which a maximal number of viable bacteria were internalized.

The intracellular bacteria were separated from the extracellular bacteria by centrifugation (4 min, 110 g). The pellet of PMN was resuspended in skim milk, resulting in 2.4×10^5 CFU of strain 1 per ml and 1.9×10^5 CFU of strain LM4B per ml. The PMN suspensions were used for the thermization test in capillaries. After thermization the PMN suspensions were removed from the capillaries and 1 ml suspension was mixed with 3 ml HBSS, diluted in a peptone-saline solution and plated out on BHI agar plates.

2.5 Storage of milk and whey

Cultures of strains 1, 13, 669 and LM4B were grown in tryptose broth and stored at 0 °C for two days. One ml of an appropriate dilution of each culture was added to pre-incubated screw-capped bottles with 250 ml of freshly drawn raw milk, HTST-pasteurized milk (15 s 72 °C), milk pasteurized more intensively (30 min 98 °C) and thermisized (68 °C for 10 s) whey of pH 6.5. The bottles were incubated at different temperatures in a waterbath. After different periods of time samples were taken and tenfold dilutions were made in a peptone-saline solution. From each dilution 0.1 ml was streaked on 2 to 5 TNSA plates which were incubated at 37 °C for 48 h. To investigate the decreased number found at low temperaure, dilutions of HTST-pasteurized milk were also streaked on nutrient agar medium. This agar medium includes the base of TNSA without the selective agents. The colonies were counted after 2 days incubation at 37 °C. The Gram-negative bacteria were enumerated on plate count agar supplemented with 2 mg of crystal violet per 1 after incubation at 30 °C for 3 days. Colonies were examined by Gram-stain.

2.6 Cheesemaking and ripening

Equal numbers of strains 1, 669 and LM4B grown in tryptose broth were added to three cheese vats of 200 litres. The first vat was used to make 4 Gouda cheeses of 4.5 kg and 1 cheese of 1 kg according to the normal procedure with a renneting temperature of 30.0 °C and a cooking temperature of 35.5 °C. 0.6 % of the mesophilic mixed-strain starter Bos was added. The second vat was also used to make Gouda cheese, however the dose of starter Bos was only 0.3 % to obtain a slower acidification and an extraordinarily high moisture content (approx. 45 %) of the cheese. The third vat was used to make Maasdam cheeses of the same size as that mentioned for the Gouda cheeses. Besides starter Bos, a culture of propionic acid bacteria was added. After the pH had decreased to 5.5, the cheeses were laid in brine at a temperature of 13 °C. After brining, the Gouda cheeses of vat 1 were ripened at 13 °C and 88 % R.H. The cheeses of vat 2 were ripened at 13 °C and 92 % R.H. The Maasdam cheeses were ripened for 2 weeks at 13 °C, then for 2 weeks at 18 °C and thereafter at 4 °C, continuously at 88 % R.H. Samples of whey from vat 1 were examined with TNSA plates to estimate the distribution of *Listeria* over the curd and the whey.

Six and 24 h after inoculation of the cheese milk, and after 2 and 6 weeks samples of 25 g were taken from the interior of the cheese. After 2 and 6 weeks surface samples of 25 cm^2 and 2 mm thickness were also taken. The samples were homogenized in a Stomacher in 225 ml tryptose broth prewarmed to 45 °C. Tenfold dilutions were made in a peptone-saline solution

and 0.1 ml of the dilutions were streaked on TNSA plates. Colonies were counted after 2 days incubation at 37 °C. Moreover, the number of *Listeria* in the Gouda cheese with a normal moisture content was also counted on nutrient agar plates.

3 Results

3.1 Thermization in a plate pasteurizer

The results of the experiments with the plate pasteurizer are shown in Table 1. In the first experiment *Listeria* could not be detected in volumes of 100 ml of milk thermized at 67.0 and 70.0 °C for 10 s. In the second experiment *L. monocytogenes* could be detected after thermization at 67.0 °C for 10 s in a volume of 100 ml of milk. After thermization at 64.0 °C for 10 s *L. monocytogenes* could be detected using a non-selective medium in a volume of 1 ml of milk, but not in a volume of 0.1 ml using a selective agar medium.

This means that the number of *Listeria* was reduced by ≤ 5 log 10 units at 64.0 °C and by 6-7 log 10 units at 67.0 °C. This equals decimal reduction times of ≥ 2 s at 64.0 °C and about 1.5 s at 67.0 °C.

3.2 Effect of phagocytosis

The effect of phagocytosis on the thermal resistance of *L. monocytogenes* in capillary tubes is demonstrated in Table 2. Using the determination method without cold resuscitation the heat resistance increased by phagocytosis by factor 1.4 for strain 1 and by factor 3.2 and 3.8 for strain LM4B at 64.0 and

Table 1. Survival of *Listeria monocytogenes* in cheese milk[a] after pasteurization in a plate heat exchanger.

Heating time (s)	Heating temperature (°C)	*L. monocytogenes* detected in volumes (ml) of			
		0.1[b]	1[c]	10[c]	100[c]
		1st experiment			
10	70.0	–	n.t.[d]	n.t.	–
10	67.0	–	n.t.	n.t.	–
		2nd experiment			
10	70.0	–	–	–	–
10	67.0	–	–	–	+
10	64.0	–	+	+	+

[a] Inoculated with equal numbers of strains 1, 13, 669 and LM4B, in total 9×10^4 per ml.
[b] Detection on trypaflavin-nalidixic acid-serum agar.
[c] Detection by enrichment in tryptose broth.
[d] Not tested.

Table 2. Thermization of free and intracellular *Listeria monocytogenes* in skim milk.

Bacterial strain	Heating time (s)	Temperature (°C)	Reduction factor[a]	
			free bacteria	intracellular bacteria
1	15	63.0	150 (210)[b]	75 (150)
LM4B	15	64.0	9 (32)	6 (10)
LM4B	15	66.0	94 (3200)	110 (830)

[a] Quotient of colony-forming units before and after thermization.
[b] Without brackets: with cold resuscitation; within brackets: without cold resuscitation.

66.0 °C respectively. Using the cold resuscitation method the heat resistance of strain LM4B was either not or only a little increased by phagocytosis, while that of strain 1 increased by factor 2.

3.3 Effect of resuscitation

If *Listeria* is allowed to resuscitate during 5 days at 4 °C before incubation at 37 °C approx. 2 to 34 times more *Listeria* could survive (Table 2). The estimated decimal reduction times for the most heat resistant strain LM4B after resuscitation at 4 °C is about 17 s at 64.0 °C and 8 s at 66.0 °C. Besides brain heart infusion agar plates for counting *Listeria* after heating, TNSA plates were also used. However, the recovery on TNSA plates was 100 times less than on brain heart infusion agar plates (results not shown in Table 2).

3.4 Storage of milk and whey

Fig. 1 demonstrates the behaviour of *L. monocytogenes* in freshly drawn raw milk, HTST-pasteurized milk and intensively pasteurized milk. In all cases except intensively pasteurized milk part of the *Listeria* could not be recovered with TNSA plates during the first 1-3 days. The largest effect was determined at 4 °C after 2 days, showing that 86 % of *L. monocytogenes* could not grow on TNSA. Counting *Listeria* on nutrient agar medium showed that the bacteria were still present, but appeared to be in a lag phase. No such lag phase occurred in intensively pasteurized milk. Using a inoculum of young bacterial cells of a culture incubated for 18 h in intensively pasteurized milk, the lag phase was similar to that shown in Fig. 1.

After the onset of growth, doubling times of 0.6 to 3.5 days at 4 °C and 0.4 to 1.0 day at 7 °C were measured depending on the medium and the stage of growth. After 7 days in raw milk at 4 °C the growth rate increased to a doubling time of 0.8 day (not shown in the Figure). The number of Gram-negative bacteria was lower than that of *Listeria* during the first three days of incubation.

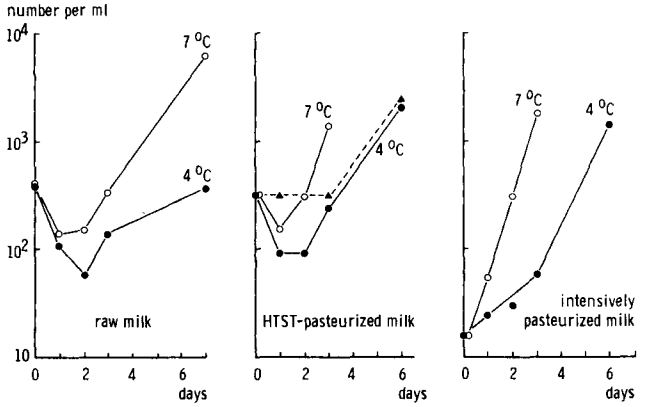

Fig. 1. Growth of *Listeria monocytogenes* strains 1, 13, 669 and LM4B in raw, HTST-pasteurized and intensively pasteurized milk incubated at 4 and 7 °C (— enumerated on trypaflavin-nalidixic acid serum agar medium, – – – enumerated on nutrient agar medium).

Fig. 2 shows the behaviour of *L. monocytogenes* in thermisized whey. Again the number of *Listeria* counted on TNSA plates decreased during the first period of incubation. The decrease was at the highest at the lowest temperature. The decrease in number must be contributed to a lag phase during which part of the the *Listeria* are not able to grow on TNSA plates. After the lag phase, *L. monocytogenes* multiplied with doubling times of 12 h, 6 h, 3 h, and 40 min at 7, 12, 20 and 30 °C, respectively.

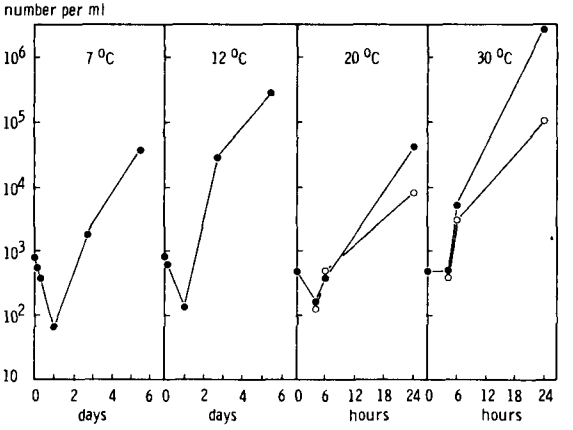

Fig. 2. Growth of *Listeria monocytogenes* strains 1, 13, 669 and LM4B in thermized whey incubated at 7, 12, 20 and 30 °C and enumerated on trypaflavin-nalidixic acid-serum agar medium (●—● aerobic culture, ○—○ anaerobic culture).

Table 3. Number of *Listeria monocytogenes* during making and ripening of Gouda cheese with normal and extraordinarily high moisture content, and of Maasdam cheese[a]).

Cheese type	Moisture content	R.H. during ripening (%)	Number inoculated in cheese milk[b])	Number in cheese after					
				6 h	24 h	2 weeks		6 weeks	
						surface	interior	surface	interior
Gouda	normal	88	5.0×10^2 [c]	1.9×10^4	1.3×10^4	$<1.0 \times 10^2$	1.5×10^4	1.3×10^3	1.6×10^3 [d]
Gouda	high	92	5.0×10^2	2.9×10^4	2.8×10^4	$<1.0 \times 10^2$	2.0×10^4	2.2×10^4	7.8×10^3
Maasdam	normal	88	5.0×10^2	2.1×10^4	1.5×10^4	$<1.0 \times 10^2$	9.3×10^3	1.0×10^2	3.6×10^3

[a] Enumerated on trypaflavin-nalidixic acid-serum agar.
[b] Equal numbers of *L. monocytogenes* strains 1, 669 and LM4B were used for inoculation.
[c] A similar number of *L. monocytogenes* was counted on nutrient agar medium.
[d] On nutrient agar medium the number counted was 10 times higher.

3.5 Cheese making and ripening

The acidification rates of the cheeses were determined by measuring the pH 6 h after renneting. The pH of the Gouda cheese of vat 1 and the Maasdam cheese of vat 3 were 5.48 and 5.44, respectively. These pH values are considered as normal. The pH of the Gouda cheese of vat 2 was 6.02, which reflects a slow acidification. In Table 3 the fate of *L. monocytogenes* during cheese making and ripening is shown. Based on the number of *Listeria* in the whey of vat 1, 5 per ml, it was estimated that the Listerias were concentrated by factor 10 due to the entrapment of the bacteria in the curd. After deduction of factor 10, the factor of increase by growth was 3.8, 5.8, and 4.2 respectively in the three vats. During two weeks of ripening the concentration of *Listeria* remained fairly constant, although no Listerias could be detected in the surface layer. After six weeks of ripening, *Listeria* in the surface layer could be detected again. The number on the surface of the wet-stored cheese was almost similar to the original number, but the number on the surface of the cheeses from vat 1 and 3 were low. The numbers in the interiors of all cheeses had decreased after six weeks.

However, this decrease could be attributed to a sub-lethal injury, since the number counted on nutrient agar was tenfold higher than that counted on TNSA.

4 Discussion

4.1 Thermization

The heat resistance of the phagocytosed bacteria differed either a little or not at all from the freely suspended bacteria, depending on the strain tested. This is in agreement with the results of Bunning (9) who found no difference. There is an indication that the method for counting *Listeria* after heat treatment did influence the recovery. When the bacteria were incubated at 4 °C on a non-selective agar medium before further incubation at 37 °C, up to 30 times more bacteria could be detected. Counting heat-injured *Listeria* on the selective TNSA plates produced even much lower recoveries. Therefore, in counting heat-injured *Listeria* a non-selective pre-enrichment medium or a non-selective agar medium incubated at 4 °C may be advantageous. Further investigations are necessary to optimize the composition of the pre-enrichment medium and the period of cold resuscitation.

4.2 Storage of milk and whey

The incubation test with freely suspended *Listeria* in milk simulated the situation on the farm when milk may be contaminated by faeces of cows. The num-

ber of *L. monocytogenes* decreased during the first two days of storage of raw milk and HTST-pasteurized milk. This may be attributed to bactericidal factors in milk such as lactoperoxidase, lysozyme and agglutinines. The effect of agglutinines was possibly counteracted by shaking the incubated milk before sampling. The Gram-negative bacteria had probably no effect on *Listeria* because of their low number as we confirmed. After three days at 7 °C growth of *Listeria* commenced in raw milk, indicating that during the cold storage of the milk at the farm no increase of Listeria occurs.

From the results the concentration of *Listeria* in pasteurized milk can be estimated as follows. Based on the data of Beckers et al. (3) it is assumed that 5 % of the milk from different farms could contain 10 *L. monocytogenes* per ml. This means that mixed milk collected from many farms contains 5 *Listeria* per 10 ml. After thermization at 65 °C for 10 s with a D-value of 12 s the *Listeria* concentration becomes 1 per 16 ml. Assuming no growth in thermisized milk during 1 day at 4 °C, pasteurization at 76 °C for 15 s with a D-value of 0.3 s (7) reduces the *Listeria* concentration to 1 per 10^{49} ml. So, the risk of surviving *Listeria* in pasteurized milk seems to be extremely small. Examination of samples of recontaminated pasteurized milk for *L. monocytogenes* did not reveal the bacterium (results not published).

4.3 Cheese-making and ripening

In cheese factories thermisized milk may be stored up to 3 days during which *L. monocytogenes* may multiply by factor 5 to a concentration of 1 per 3 ml, assuming an initial concentration as mentioned above. Pasteurization at 72 °C for 15 s with a D-value of 1 s (7) reduces the *Listeria* concentration to 1 per 3×10^{12} l, which makes the risk for survival by *Listeria* low.

During cheese making the concentration of *L. monocytogenes* increased by factor 10 due to entrapment in the curd and furthermore by approximately factor 4 by growth in normal Gouda and Maasdam cheese. During an extraordinarily slow acidification there was slightly more growth. So, it appeared that the acidification rate is not such an important factor for the growth of *L. monocytogenes* as has been noticed for the growth of *Escherichia coli* (21) and *Staphylococcus aureus* (22). Moreover, no growth was determined during the first 2 weeks of ripening as was found in Cheddar cheese (12). There were no significant changes in the number of *Listeria* in the normal Gouda, Maasdam and the high moisture Gouda cheese. This result indicates that moisture and ripening temperature are not critical with regard to growth of *Listeria*. The increase of the number of Listeria in the surface layer of wet-stored Gouda cheese may reflect a repair from sub-lethal injury due to brining.

From these data the occurrence of *L. monocytogenes* in raw-milk cheese

can be estimated. Starting with 10 *Listeria* per ml of cheese milk, the concentration of *Listerias* could amount 400 per g of cheese. However, the number of *Listeria* found in raw-milk Gouda cheese was lower than the calculated concentration indicating that the *Listeria* concentration in raw milk is lower than assumed (Beckers, unpublished results).

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Samenvatting

M. D. Northolt, H. J. Beckers, U. Vecht, L. Toepoel, P. S. S. Soentoro en H. J. Wisselink, *Listeria monocytogenes: hitteresistentie en gedrag tijdens de bereiding van Goudse kaas en bewaring van melk en wei*[1]

De hitteresistentie van *L. monocytogenes* werd bepaald met een platenpasteur en met glazen capillairen. Met de platenpasteur kon bij 64,0 °C en bij 67,0 °C een reductie van het aantal *Listeria* bereikt worden van respectievelijk ≤5 log-10-eenheden en 6-7 log-10-eenheden. Met de glazen capillairen werd de hitteresistentie van vrij gesuspendeerde en gephagocyteerde bacteriën bepaald. De overlevende *Listeria* werden bepaald met en zonder resuscitatie bij 4 °C. Uit de bepaling met resuscitatie bleek dat phagocytose slechts bij één van de twee geteste stammen een toename van de hitteresistentie met factor 2 tot gevolg had. Met resuscitatie kon tot 30× meer *Listeria* werden aangetoond na verhitting dan zonder resuscitatie. De geschatte decimale reductietijden van de meest hitteresistente stam is ca. 17 s bij 64,0 °C en 8 s bij 66,0 °C. In bewaarproeven van *L. monocytogenes* in rauwe en HTST-gepasteuriseerde melk bij 4, 7 en 10 °C werd tijdens de eerste 1-2 dagen een deel van de *Listeria* subletaal beschadigd. Daarna volgde groei. In intensief gepasteuriseerde melk werd geen beschadiging waargenomen en vond direct groei plaats. Tijdens bereiding van Goudse en Maasdammer kaas nam het aantal *L. monocytogenes* toe met een factor 10 door ophoping en een factor 4 door groei. Het aantal *Listeria* bleef tijdens 6 weken rijpen bij 13 °C nagenoeg constant.

References

1. D. W. Fleming, S. L. Cochi, K. L. MacDonald, J. Broudum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Andurier, C. V. Broome & A. L. Reingold, *N. Engl. J. Med.* 312 (1985) 404-407.
2. S. M. James, S. L. Fannin, B. A. Agee, B. Hall, E. Parker, J. Vogt, G. Run, J. Williams, L. Lieb, C. Salminen, T. Pendergast, S. B. Werner & J. Chin, *Morbid. Mortal. Week. Rep.* 34 (1985) 357-359.

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3. H. J. Beckers, P. S. S. Soentoro & E. H. M. Delfgou-van Asch, *Int. J. Food Microbiol.* 4 (1987) 249-256.
4. R. G. Dijkstra, in: A. L. Countieu, E. P. Espaze & A. E. Reynaud (Eds), Proceed. 9th Int. Symp. on the problems of Listerioses, Nantes, Sept. 17-20, 1985. pp. 291-293.
5. J. Stadhouders, M. M. Cordes & A. W. J. van Schouwenburg-van Foeken, *Neth. Milk Dairy J.* 32 (1978) 193-203.
6. C. W. Donnelly & E. H. Briggs, *J. Food Prot.* 49 (1986) 994-998.
7. J. C. Bradshaw, J. T. Peeler, J. J. Corwin, J. M. Hunt, J. T. Tierney, E. P. Larkin & R. M. Twedt, *J. Food Prot.* 48 (1985) 743-745.
8. M. P. Doyle, K. A. Glass, J. T. Beery, G. A. Garcia, D. Jeffrey Pollard & R. D. Schultz, *Appl. Environ. Microbiol.* 53 (1987) 1433-1438.
9. V. K. Bunning, R. G. Grawford, J. G. Bradshaw, J. T. Peeler, J. T. Tierney & R. M. Twedt, *Appl. Environ. Microbiol.* 52 (1986) 1398-1402.
10. J. F. Fernandez Garayzabal, L. Dominguez Rodriguez, J. A. Vazquez Boland, J. L. Blanco Cancelo & R. Suarez Fernandez, *Can. J. Microbiol.* 32 (1986) 149-150.
11. E. T. Ryser & E. H. Marth, *J. Food Prot.* 50 (1987) 372-378.
12. E. T. Ryser & E. H. Marth, *J. Food Prot.* 50 (1987) 7-13.
13. E. M. Rosenow & E. H. Marth, *J. Food Prot.* 49 (1986) 847-848.
14. A. J. B. van Boxtel, *Zuivelzicht* 78 (1986) 502-504.
15. A. J. B. van Boxtel, *Zuivelzicht* 78 (1986) 559-561.
16. B. Ralovich, A. Forray, E. Mérö, H. Málovics & I. Százados, *Zbl. Bakteriol. Hyg. I. Abt. Orig. A.* 216 (1971) 88-91.
17. E. H. Kampelmacher & L. M. van Noorle Jansen. *Zbl. Bakt. Hyg., I. Abt. Orig. A.* 246 (1980) 211-227.
18. J. G. Franklin, D. J. Williams & L. F. L. Clegg, *J. appl. Bact.* 21 (1958) 51-57.
19. R. v. Furth, Theda L. van Zwet & P. C. J. Leijh, in: P. M. Weir (Ed.), Handbook of experimental immunology, 3rd edn., Ch. 32, Blackwell Scientific Publications, Oxford.
20. U. Vecht, H. J. Wisselink & A. T. J. Bianchi, in: M. Sandholm (Ed.), Proceed. Symp. on mastitis control and hygienic production of milk, Espoo, Finland June 10-12, 1986.
21. M. D. Northolt, *Neth. Milk Dairy J.* 38 (1984) 135-150.
22. J. Stadhouders, M. M. Cordes & A. W. J. van Schouwenburg-van Foeken, *Neth. Milk Dairy J.* 32 (1978) 193-203.