Survival of *Listeria monocytogenes* During the Manufacture and Ripening of Trappist Cheese

IVANA KOVINCIC1, IVICA F. VUJICIC1, MELINA SVABIC-VLAHOVIC3, MIRJANA VULIC1, MAJA GAGIC1, and IRENE V. WESLEY2*

1Faculty of Agriculture, 21000 Novi Sad, Yugoslavia; Faculty of Medicine, 1100 Belgrade, Yugoslavia; 2National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010 U.S.A.

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ABSTRACT

Trappist cheese constitutes more than one-third of the semi-soft cheese production in Yugoslavia. The ability of *Listeria monocytogenes* to survive the Trappist cheese-making process and persist during 90 d of ripening and storage was examined. Trappist cheese was manufactured from pasteurized milk (trials A, B, C) and from whey (trials D and E) inoculated with *L. monocytogenes* (2.46-5.38 log10 CFU/ml). An increase in *L. monocytogenes* counts was detected after 30 d of ripening in all of the five trials. After ripening and storage for 90 d, the *L. monocytogenes* counts ranged from 2.72-5.64 log10 CFU/g cheese. A decline in the population of *L. monocytogenes* was correlated with a decrease in cheese moisture and pH and with an increase in NaCl and titratable acidity.

*L. monocytogenes* has caused foodborne epidemi­ics (9,14,20-22) and sporadic cases of listeriosis (7,8) in which cheese was the contaminated vehicle. *L. monocytogenes* has been detected in cheeses (2,5,9,13,14,19-22), in raw milk (12,15,23), and in the dairy plant environment (3,4,10), which poses a major source of postpasteurization contamination.

The growth characteristics of *L. monocytogenes*, a high risk cheese-associated pathogen (20), during the manufacture and ripening process has been documented in an assortment of cheeses (11,14,20,21). These studies suggest that the composition of cheese, ripening and storage conditions, lactic acid starters, pH, salt and moisture concentration, virulence of the strain evaluated, and the concentration of the inoculum used, influence the survival and growth of *L. monocytogenes*.

Originally formulated in 1885 in Bosnia, Trappist cheese constitutes more than one-third of the semi-soft cheese production in Yugoslavia and may have export potential. It is produced in France as Port du Salut cheese, as well as in Hungary, Austria, Germany, and Czechoslovakia. The Canadian Oka Trappist cheese and the American Gethsamani Trappist cheese are the New World counterparts (1).

Herein, we report on the survival of *L. monocytogenes* during ripening and storage of Trappist cheese manufactured from pasteurized milk and from whey in order to simulate environmental contamination.

MATERIALS AND METHODS

The strain of *L. monocytogenes* (serotype 4b) used in this study was isolated from milk obtained from a clinically healthy, naturally infected cow, which had aborted.

The lactic starter culture of *Streptococcus lactis* subsp. *lactis* and *Str. lactis* subsp. *cremoris* was incubated (20-24 h at 20°C) in UHT sterilized low-fat (1.8%) milk before use.

Trappist cheese was manufactured from pasteurized (72°C, 20 s) milk standardized for 3.2% fat content to provide at least 45% fat in dry cheese matter. After warming the milk to 32°C, the 1% (w/w) lactic starter culture and the *L. monocytogenes* inoculum (trials A, B, and C) were added and incubated for 30 min. The milk was coagulated for 30-40 min after addition of a commercial fluid rennet (Vojvodjanka, J. Kostic Co., Indjija, Yugoslavia). In trials D and E, *L. monocytogenes* was added during the early coagulation phase. The coagulum was cubed (1 cm3 each), the curd was allowed to separate (15 min), and scalded (39°C) with stirring for 45 min. Settled curd was transferred to perforated hoops, drained, and pressed (10-12 h). The cheese was salted in brine (18% NaCl, 4 h), dried (5 d at 16-18°C), waxed and aged (10°C). Samples for chemical analyses and for *L. monocytogenes* enumeration were taken at 1, 15, 30, 60, and 90 d of ripening.

Five batches (trials A-E) of cheese were made in the standard wheel (16-cm diameter x 6-cm height, 1.3 kg). *L. monocytogenes* was added to pasteurized milk (trials A, B, C) just prior to cheese-making (trials A, B, C) and to the curd-whey mixture (trials D and E). *L. monocytogenes* was added to the pasteurized milk to achieve the following starting concentrations: trial A (2.46 log10/ml milk), trial B (3.72 log10/ml milk), and trial C (5.38 log10/ml milk). In trials D and E, *L. monocytogenes* was added after the addition of rennet, when the pasteurized milk contained approxi­mately 20% curd. The initial concentrations (CFU log10/ml whey) were 2.49 (trial D) and 4.28 (trial E).

Sample plugs of Trappist cheese were taken radially from the outer vertical face to the center of the wheel with a 10-mm cheese trier (18). The cone-shaped plugs were ground in a sterile cheese grinder and suspended in physiological saline.

Milk, whey, and cheese dilutions (10-1 to 10-6) were prepared in physiological saline and were plated in triplicate on tryptose blood agar.
agar with 50 mg/L nalidixic acid added. Representative presumptive colonies of *Listeria* were identified by biochemical assays.

The percentage of moisture and NaCl were determined according to International Dairy Federation Standard Methods (16-18). The pH of samples was determined by pH meter (Iskra MA 5703) using a 50% cheese emulsion prepared in physiological saline. Acidity was determined by the titration of a 10-g cheese sample suspended in water with 0.25 M NaOH and phenolphthalein indicator and reported as % lactic acid. Data are reported as the mean of two replicates. The mean (± standard error), moisture (%), NaCl (%), and titratable acidity for the five trials was determined on day 90.

**RESULTS AND DISCUSSION**

In this study, *L. monocytogenes* colonies were detected throughout the 90 d of ripening and storage of Trappist cheese manufactured from pasteurized milk inoculated with three concentrations of *L. monocytogenes* (Fig. 1, milk A, B, C). Counts (log, _CFU/g_ recovered during manufacturing and storage were directly related to the concentration of the initial inoculum. In the first trial (milk A; starting concentration of 2.46 log, _CFU/ml pasteurized milk_), *L. monocytogenes* populations reached a maximum (3.64 log, _CFU/g cheese_) by day 60. In the second trial (milk B; starting concentration of 3.72 log, _CFU/ml milk_), the maximum recovery occurred on day 30 (4.59 log, _CFU/g_). With the highest inoculum (milk C; starting concentration of 5.38 log, _CFU/ml milk_), the maximum recovery of *L. monocytogenes* occurred on day 30 (6.83 log, _CFU/g cheese_). For all trials, the viable cell counts recovered on day 90 of storage (10°C) varied with the initial inoculation and were less than the maximum titers achieved.

Similar data were obtained for cheese made from pasteurized milk which was contaminated with *L. monocytogenes* shortly after the addition of rennet (Fig. 2, whey D and E). In trial whey D (starting concentration of 2.49 log, _CFU/ml whey_), the maximum recovery of *Listeria* (3.75 log, _CFU/g cheese_) occurred by day 30. In trial whey E (starting concentration of 4.18 log, _CFU/ml whey_), an increase of 1.9 log, _CFU/g cheese_ occurred by the first 15 d of ripening. By day 90 of aging *L. monocytogenes*, populations were 5.36 CFU log, _CFU/g cheese_. As noted with the cheese prepared from contaminated milk (milk A, B, C), the numbers of *L. monocytogenes* recovered on day 90 exceeded those initially present in the whey. In contrast to trials A, B, and C, viability counts on day 90 of trials D and E did not decline as precipitously.

Results of moisture, salt, pH, and lactic acid determinations for fresh cheese 1 d after salting, and for ripened cheese after 90 d of storage at 10°C are shown in Table 1. The decline of *L. monocytogenes* counts on day 90 from peak values was associated with the changes in the mean value (± standard error) of these physical parameters. *L. monocytogenes* was recovered from the cheese on day 90 when final determinations for the five trials of pH (4.88 ± 0.099), moisture content (30.06% ± 1.942), lactic acid (0.838%), and salt (1.435 ± 0.051) were made. Published values for comparison are as follows: salt (1.3-2.5%) and moisture (40-46%) (1). Although growth of *L. monocytogenes* is optimal in neutral to slightly alkaline medium,

replication occurs in a pH milieu of 4.5 to 5.0 (24). The average final pH of 4.88 (s.e. ± 0.099) achieved for these five trials, while not optimal for growth, did not totally inhibit *L. monocytogenes*. The influence of NaCl (%) on the growth of *L. monocytogenes* was minimal in these trials since the NaCl (%) achieved on day 90 has not been shown to inhibit its replication (20). *L. monocytogenes* can tolerate 4.5% NaCl milieu for prolonged intervals at 5°C, but not at 30°C, and can survive for up to 8 weeks in 20% NaCl at 4°C (20). The decline in the *L. monocytogenes* population at day 90 may also reflect competition with *Streptococcus cremoris* and *S. lactis* starter cultures and the resultant secretion of compounds (e.g., nisin) inhibitory to *L. monocytogenes* (20).

The infectious dose of *L. monocytogenes* is unknown and has been estimated to be as low as 10^2 CFU/g, although infection of immunocompetent individuals has followed consumption of soft cheese contaminated with 30-50 million *Listeria* per gram (7). Therefore, Trappist cheese, like a variety of cheeses examined to date, if contaminated with *L. monocytogenes* during manufacturing, would support its replication during storage and thus pose a potential public health concern.
TABLE 1. Moisture, salt, pH and lactic acid content in Trappist cheese contaminated with Listeria monocytogenes. Data are the mean of two samplings.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Age of cheese (days)</th>
<th>Moisture (%)</th>
<th>Salt (%)</th>
<th>pH (%)</th>
<th>Lactic acid (%)</th>
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<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>43.9</td>
<td>1.14</td>
<td>5.42</td>
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<td></td>
<td>90</td>
<td>28.3</td>
<td>1.47</td>
<td>5.13</td>
<td>0.92</td>
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<tr>
<td>B</td>
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<td>45.00</td>
<td>1.19</td>
<td>5.40</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>27.50</td>
<td>1.55</td>
<td>5.12</td>
<td>0.88</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>42.87</td>
<td>0.71</td>
<td>5.02</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>29.00</td>
<td>1.54</td>
<td>4.73</td>
<td>0.76</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
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<td>1.10</td>
<td>5.00</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>90</td>
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<td>1.43</td>
<td>4.70</td>
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</tr>
<tr>
<td>E</td>
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<td>0.78</td>
<td>5.03</td>
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<tr>
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<td>90</td>
<td>27.75</td>
<td>1.27</td>
<td>4.73</td>
<td>0.76</td>
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</table>

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REFERENCES