Chemical Composition of and *Listeria monocytogenes* Survival in White Pickled Cheese

O. M. ABDALLA¹, G. L. CHRISTEN,* and P. M. DAVIDSON²

Department of Food Science and Technology, Agricultural Experiment Station, University of Tennessee, Knoxville, Tennessee 37901-1071

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**ABSTRACT**

White pickled cheese was made from pasteurized milk with 8% salt and preserved in the whey at 4°C. About 5.0 log₁₀ CFU/ml cells of *Listeria monocytogenes* were inoculated into milk, and the survival of the pathogen was studied during the storage period. The chemical composition of the cheese was also determined. *L. monocytogenes* were not inhibited by 8% salt, but lactic acid bacteria were unable to grow and produce acid to inhibit *L. monocytogenes*. During ripening, the pH never decreased below 6.0. While fat, protein, total solids, and ash contents decreased in curd during ripening, the acidity and salt did not change. In cheese whey, fat, protein, acidity, and salt showed a slight increase, while total solids and ash contents were unchanged.

White pickled cheese of the Sudan is a product traditionally made from raw milk to which salt (6-20%) has been added (5,16). Although this high salt level is meant to control foodborne pathogens, it has a major influence on acid development by limiting the growth of naturally occurring lactic acid bacteria (4,8,15,16). Recent foodborne listeriosis outbreaks due to consumption of milk (13) and cheese (18) have generated an interest in the survival of *Listeria monocytogenes* in cheeses (7,21,22, 26-28,33), milk and milk products (11,25,30-32), yogurt (30), and cream (25).

Although different food products were tested for the survival of *L. monocytogenes*, high salt foods were not studied except for cabbage juice which was found to inhibit growth of the pathogen at salt levels of up to 5% (9). White pickled cheese is traditionally made from raw milk with more than 5% salt added. This study was designed to determine the effect of salt and/or lactic acid bacteria on the growth of *L. monocytogenes*, in this cheese made by traditional procedures. Additionally, composition of the cheese was determined since this information is not available in the literature.

**MATERIALS AND METHODS**

**Bacterial cultures**

Frozen (-70°C) starter culture (Chr. Hansen’s Laboratory Inc., Milwaukee, WI) was thawed. One milliliter was transferred to 10 ml sterile rehydrated nonfat dry milk (10% wt/vol) and incubated at 30°C for 24 h. One milliliter was transferred to 10 ml M17 broth and incubated at 30°C for 24 h. The culture was serially diluted and spread plated on prepoured M17 agar plates and incubated at 30°C for 48 h. Colonies were picked and streaked on M17 agar plates and incubated at 30°C for 48 h. The streaking was repeated three times to isolate a pure culture. A single colony presumptively identified as *Lactococcus* sp. by Gram staining, catalase and oxidase tests was picked and grown in M17 broth and on an agar slant. The organism was further verified by Gram staining, catalase and oxidas tests and growth at 10, 40, and 45°C, and in 2, 4, and 6.5% NaCl. Biochemical tests were completed as described by Garvie and Farrow (14). Cultures were maintained frozen (-70°C) in 20% glycerol. When needed, they were thawed and incubated in M17 broth for 24 h.

**Cheese manufacture**

Nine kilograms of pasteurized milk (62.7°C for 30 min) was cooled to 37.8-40.0°C in a 5-L bucket. NaCl (8% wt/wt) and CaCl₂ (0.2% wt/wt) were added to milk prior to addition of rennet (11 ml rennet per 45.5 kg milk). The rennet (Chr. Hansen’s Laboratory Inc., Milwaukee, WI) was diluted to 1:40 with water prior to addition to the milk. Two treatments were used, the control to which only salt, CaCl₂, and rennet were added and the other to which starter culture (log₁₀ 8 CFU/ml) was added as well. After addition of rennet (at 38°C), milk was stirred and left to develop a curd. The curd was cut with a knife into small cubes. Whey was drained and curd laddled into small cylindrical metal molds (14.5 cm inside diameter x 13 cm height) lined with cheesecloth and pressed lightly (627.3 kg weight) overnight. The following day, the curd was cut into small cubes (ca. 5.5 x 3.5 x 2.0 cm) and stored in the collected whey at 4°C. The first experiment was designed to study the chemical composition of cheese and whey, while the second experiment was designed to evaluate the pH and to study the survival of *L. monocytogenes* in cheese curd and whey.

**Enumeration of L. monocytogenes**

*L. monocytogenes* Scott A (1 ml of a 24-h culture) was added to milk before rennet addition. Populations in the milk were ca. log₁₀ 5 CFU/ml. Cheese and whey were analyzed for *L. monocytogenes* and pH (Fisher Accumet pH meter Model 825 MP with
Orion System 8163 Ross combination pH probe) in milk at day 0 (milk before addition of rennet), and in curd and whey at 1, 3, 10, 20, 30, 40, 50, and 60 d. Cheese curd (11 g) and whey (11 ml) were diluted 1:10 with sterile 0.1% peptone water, diluted serially, and spread plated on Listeria Selective Agar Base (Oxford Formulation, Oxoid, Columbia, MD, supplemented with 0.1% colistin and 0.2% moxalactam, Sigma). Cheese curd was blended in a stomacher (Stomacher 400, Tekmar Cincinnati, OH) for 2 min prior to plating. Plates were incubated at 35°C for 48 h. Typical colonies (creamy white surrounded with black zone) were counted. Representative colonies (four colonies from a plate) were picked for confirmatory tests which included Gram staining, tumbling motility, catalase, oxidase, umbrella motility, xylene, manninol, and rhamnose utilization.

Evaluation of chemical composition of cheese

Milk (at day 0) and cheese and whey (at days 1, 10, 30, and 60) were analyzed for chemical composition.

Fat (Babcock method) and salt (Mohr method) were determined according to standard methods (24). The Kjeldahl method (method #920.105) was used for protein determination as described in the Association of Official Analytical Chemists (AOAC) (6) with the following modifications. One gram of sample was used and 15 ml H2SO4 were added followed by two tablets (Kjeltabs - 175 mg HgO and 3.5 g K2SO4 in each tablet). The mixture was digested for 45 min using a Kjeltec digester (Tecator 1015 digester, Fisher Scientific, Pittsburgh, PA). After the sample was cooled, 10 ml of sodium thiosulfate (80 g in 1 L distilled water) were added. Boric acid (25 ml) was used to receive the distillate which was then titrated with 0.1 N HCl. Protein (%) was calculated using a conversion factor of 6.38.

The method number 920.124 described in AOAC (6) was used for the determination of titratable acidity in cheese and brine. Total solids (method #926.08) and ash (method #935.42) were determined as in AOAC (6) except that a sample size of 2 g was used in each case. In addition, milk and cheese for total solids analyses were heated overnight in a vacuum oven at 70°C. Proteolysis in cheese was measured according to the method of Polychroniadou (23).

Statistical analyses

Statistical analyses were performed using the Statistical Analysis System (29). Each experiment was repeated twice. General linear models were used to determine the effects of days of storage, treatment (control, starter culture), replication, and the interaction between day and treatment. These variables were analyzed using general linear models to test the effect on fat, protein, salt, titratable acidity, catalase, oxidase, umbrella motility, xylene, manninol, and rhamnose utilization.

RESULTS AND DISCUSSION

Chemical composition

Raw milk had 3.0% fat, 3.31% protein, 12.19% total solids, 0.78% ash, 0.24% NaCl, and 0.18% titratable acidity (Table 1). The average composition of cheese made with or without starter was similar (Table 1), except the cheese made without starter had significantly higher protein than that made with starter. Composition of white pickled cheese varies among countries. Kosikowski (17) listed average composition of ripened white pickled cheeses made from sheep milk from various countries. Fat ranged from 20.3 to 32.2%, protein from 13.4 to 25.0%, total solids from 40.3 to 62.0%, ash from 2.3 to 5.3%, and salt from 2.3 to 5.3%. Fahmi and Sharara (12) provided information on the average composition of Domiati cheese made from cows' or buffaloes' milk. Fresh cheese made from cows' milk had average total solids of 41%, average fat of 18%, and average salt of 4.5%. Ripened (4-6 months) cheese was higher total solids (45%), fat (20%), and salt (4.9%).

Fat content. Fat content in cheese curd showed a decrease over time in both control and starter culture treatments (Fig. 1). Fat was not detectable in whey in either treatments on day 1 and increased slightly by day 60 (Fig. 2). Addition of starter culture did not affect (P > 0.05) fat content (Table 1).

The decrease in fat content of curd over time is in agreement with reports of Alla Gabo (5) and Nofal et al. (20) but does not agree with reports of Dariani et al. (70) and Zaki et al. (34). Fat content of whey started at 0% and increased with time. Some fat must have leaked from curd into the whey/brine solution, which could partially explain the decrease in fat content in curd during storage.

<table>
<thead>
<tr>
<th>Composition (%)</th>
<th>Raw milk</th>
<th>Control</th>
<th>Starter culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Curd</td>
<td>Whey</td>
<td>Curd</td>
</tr>
<tr>
<td>Fat</td>
<td>3.00</td>
<td>16.00</td>
<td>14.88</td>
</tr>
<tr>
<td>Protein</td>
<td>3.31</td>
<td>12.45</td>
<td>11.61</td>
</tr>
<tr>
<td>Total solids</td>
<td>12.19</td>
<td>37.56</td>
<td>36.03</td>
</tr>
<tr>
<td>Ash</td>
<td>0.78</td>
<td>8.59</td>
<td>8.80</td>
</tr>
<tr>
<td>Salt (8% added)</td>
<td>0.24</td>
<td>5.15</td>
<td>5.55</td>
</tr>
<tr>
<td>Titratable acidity</td>
<td>0.18</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>A420</td>
<td>-</td>
<td>0.06</td>
<td>-</td>
</tr>
</tbody>
</table>

**Values within the same row between treatments for curd or whey followed by different superscripts are significantly different (P < 0.05).**

A420 = Absorbance at 420 nm, a measurement of proteolysis (breakdown to amino acids) in cheese.

Figure 1. Change in fat, protein, and salt in cheese curd during storage at 4°C for 60 d. Cheese was made from pasteurized milk with 8% salt. Starter culture (1%; L. lactis ssp. cremoris) was added to the starter treatment.

TABLE 1. Average chemical composition of milk, curd, and salted whey of white pickled cheese over all sampling times.
Protein content. Protein content in cheese curd decreased during storage (Fig. 1). In whey, protein content showed an increase at the end of the storage period (Fig. 2). Differences (P < 0.05) between control and starter treatments in curd but not in whey were observed (Table 1).

Decreased protein content during pickling was a direct result of protein degradation leading to the formation of water soluble compounds, some of which were lost in the pickling solution leading to increased nitrogen content of whey (5,10,20,34).

Salt content. The percent salt in curd increased slightly in the control but stayed the same in the starter treatment (Fig. 1). In whey, salt concentration increased slightly toward the end of the storage period for both treatments (Fig. 2). Mean value of salt content was higher in curd made with starter than in the control (P < 0.05), while there was no significant difference (P > 0.05) in whey (Table 1).

The general trend did not show a pronounced decrease or increase of salt content in either curd or whey. Previous reports (4,10) showed a gradual decrease in salt content of curd with advanced ripening, which was attributed to dissolution of cheese salt into the pickling whey to obtain equilibrium (34).

Total solids. While total solids in cheese curd decreased gradually over time in both treatments (Fig. 3), in whey it decreased slightly at day 10 after which it showed a slight increase (Fig. 4). No statistically significant differences between total solids of control and starter in curd or whey (P > 0.05) were detected (Table 1).

Total solids content is the portion of the cheese other than water. The decrease in total solids of cheese curd during pickling could be explained by degradation of total protein, dissolution of salt and fat into the pickling solution, or absorption of pickling whey by curd (10). The increase in total solids of whey was due to the same factors. However, others have reported that moisture content of cheese decreased during pickling, a phenomenon attributed to curd contraction and expulsion of whey as a result of acid development (20,34).

Ash content. Ash content constitutes the inorganic part of the solid matter. The ash content of curd decreased at day 10 and remained constant throughout the storage period (Fig. 3). In whey, ash content remained around 8% throughout the storage period (Fig. 4). Addition of starter did not affect (P > 0.05) ash content of curd or whey (Table 1).

During pickling, ash content followed a trend somewhat similar to total solids, indicating that ash content in curd and whey was very much affected by diffusion of salt from curd into whey.

Protein degradation. Protein breakdown into amino acids was measured to evaluate the effect of proteases. During the 60-d ripening period, absorbance changed slightly (Fig. 5). All values were small (<0.1 absorbance units), indicating only small quantities of free amino groups maintained in the curd. The concentration of free amino acids was the same (P > 0.05) in both treatments (Table 1). Nitrogen content of curd decreased during ripening while that of whey increased (Fig. 1 and 2). Perhaps free amino groups were diffusing into whey, and we were unable to measure them in the curd.

Heat treatment inactivated the natural microflora of milk, while salt added at high levels inhibited starter bacteria added.
to milk (34) resulting in reduced proteolysis. Similar results were found in the present study where milk was pasteurized and high salt levels inhibited starter bacteria.

**Acidity.** The acidity in curd did not change during the storage period in either treatment and remained at 0.01% from day 1 to day 60 (Fig. 6). In whey, acidity slightly increased from day 1 to a maximum at day 30, then decreased toward the end of the storage period (Fig. 6). Whey from cheese made with starter culture had higher mean acidity value (P < 0.05) than the control (Table 1).

In cheese made in the same way but inoculated with *L. monocytogenes*, pH of cheese curd increased irregularly until day 30, then decreased at day 50 after which it increased to a maximum value at the end of the storage period (Fig. 7). The pH of the cheese made with starter was always below that of the control. The pH of whey increased steadily over time with a slight increase and a slight decrease observed at days 30 and 50, respectively (Fig. 8). The mean pH value for the starter culture treatment was lower (P < 0.05) than control in both curd and whey (Table 2).

Lactic acid bacteria are unable to grow in high salt levels, thus low acidity resulted (16). The strain used in the present study was found to grow in salt content up to 4% (data not shown). Pasteurization inactivated the naturally occurring microflora in milk. There is an inverse relationship between salt content and acid production (10,16,34). In cheese made from 4% salt and preserved in 4% brine solution, lactic acid bacteria were able to grow and produce acid such that they inhibited growth of some foodborne pathogens (2).

*Survival of L. monocytogenes in cheese curd and whey*  
*L. monocytogenes* in cheese curd showed a similar trend in control and starter treatments (Fig. 7). Numbers (log10 CFU/g) increased steadily until day 40, after which a slight decrease occurred at day 50, and an increase was seen at the end of the storage period.

During cheese manufacture, cells diffused into the whey so that by day 1, approximately log10 3.2 and 3.4 CFU/ml
TABLE 2. Mean log₁₀ CFU/g (ml) of
L. monocytogenes Scott A and pH for curd and whey of white pickled cheese made from pasteurized
milk with 8% salt.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial population in milk (Log₁₀ CFU/ml)</th>
<th>Curd*</th>
<th>Whey*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.0</td>
<td>7.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Starter culture</td>
<td>5.1</td>
<td>7.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Log₁₀ CFU/g (ml) and pH values represent the mean of all sampling days.
<sup>b</sup> Values within each column followed by different superscripts are significantly different (P < 0.05).

were detected in control and starter treatments, respectively (Fig. 8). Numbers increased sharply until day 10, after which numbers in the control remained constant throughout the storage period, while numbers in starter treatment increased steadily until the end of storage (Fig. 8). Significantly higher numbers of cells were detected in curd and whey of the starter treatment (P < 0.05) than the control (Table 2).

Salt did not inhibit L. monocytogenes in this study. The pathogen has been shown to survive for 10 d or 1 year in salt concentrations of 30.5 or 10%, respectively, in other foods depending on temperature and pH (19). In cabbage juice, L. monocytogenes was able to grow at 5°C in the presence of 5% NaCl (9). Two recent outbreaks of listeriosis were associated with the consumption of pasteurized milk and Mexican-style cheese made from pasteurized milk contaminated with unpasteurized milk (13,18). Soft cheeses are considered to be effective vehicles for L. monocytogenes because they are treated with brine solution during production or storage which leads to high concentration of NaCl that may inhibit competing microorganisms, particularly, lactic acid bacteria (18). In addition, soft cheeses never acquire strong acidic environment to discourage bacterial growth since they are not aged (18). Traditionally, white pickled cheese is made with raw milk and high salt level (6-20%). If contaminated with L. monocytogenes, white pickled cheese could be unsafe.

Salt suppressed starter culture growth in this study, thereby preventing acid production. High NaCl content inhibited growth of lactic acid bacteria (4,8,15). Additionally, the presence of starter culture in 8% NaCl appeared to enhance L. monocytogenes growth (Fig. 7 and 8). However, the use of 4% NaCl in cheese with added starter and preserved in 4% brine solution inhibited L. monocytogenes (1,3). In order to produce a safe cheese of this type, pasteurization, starter addition, and use of 4% salt are recommended. An alternative to use of low salt levels is to develop mutant starter organisms that are able to grow at high salt levels. Even without pasteurization, cheese made with 4% salt and starter culture may be safe if stored for at least 60 d (1).

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REFERENCES


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