Fate of *Listeria monocytogenes*, *Salmonella Typhimurium* DT104, and *Escherichia coli* O157:H7 in Labneh as a Pre- and Postfermentation Contaminant

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ABSTRACT

Commercially pasteurized milk (~2% milkfat) was heated at 85 to 87°C/30 min, inoculated to contain 2,000 to 6,000 CFU/ml of *Listeria monocytogenes*, *Salmonella Typhimurium* DT104, or *Escherichia coli* O157:H7, cultured at 43°C for 4 h with a 2.0% (wt/wt) commercial yogurt starter culture, stored 12 to 14 h at 6°C, and centrifuged to obtain a Labneh-like product. Alternatively, traditionally salted and unsalted Labneh was prepared using a 3.0% (wt/wt) starter culture inoculum, similarly inoculated after manufacture with the aforementioned pathogens, and stored at 6°C and 20°C. Throughout fermentation, *Listeria* populations remained unchanged, whereas numbers of *Salmonella* increased 0.33 to 0.47 logs during the first 2 h of fermentation and decreased thereafter. *E. coli* populations increased 0.46 to 1.19 logs during fermentation and remained that these levels during overnight cold storage. When unsalted and salted Labneh were inoculated after manufacture, *Salmonella* populations decreased >2 logs in all samples after 2 days, regardless of storage temperature, with the pathogen no longer detected in 4-day-old samples. Numbers of *L. monocytogenes* decreased from 2.48 to 3.70 to <1.00 to 1.95 logs after 2 days with the pathogen persisting up to 15 days in one lot of salted/unsalted Labneh stored at 6°C. *E. coli* O157:H7 populations decreased from 3.39 to 3.7 to <1.00 to 2.08 logs during the first 2 days, with the pathogen no longer detected in any 4-day-old samples. Inactivation rates for all three pathogens in Labneh were unrelated to storage temperature or salt content. Unlike *L. monocytogenes* that persisted up to 15 days in Labneh, rapid inactivation of *Salmonella Typhimurium* DT104 and *E. coli* O157:H7 suggests that these emerging foodborne pathogens are of less public health concern in traditional Labneh.

Labneh, a type of soft cheese, is particularly popular in Syria, Lebanon, and other Middle Eastern countries where it plays an important dietary role (5, 7, 11, 28). The traditional method for Labneh production affords many opportunities for microbial contamination from cloth bags used for whey drainage during concentration, which is also a time-consuming process (6, 12, 27). This product is sometimes sold from large uncovered containers where the risk of microbial contamination is high. At homes and in restaurants, manual mixing of Labneh with spices, fresh herbs, and other uncooked materials can lead to pathogen introduction and an increased microbial load (9). The fact that final product is consumed without further heat treatment raises additional public health concerns. However as a fermented dairy product, Labneh is a highly nutritious food that is also beneficial for the relief of human intestinal disorders (6, 8, 12).

Milk used for commercial production of yogurt and Labneh (concentrated yogurt) is heated at 185 to 203°F (85 to 94°C) for 2 to 30 min (5, 10). While this treatment assures complete destruction of all milkborne pathogens, postprocessing contamination can occur (22). Production of Labneh in Middle Eastern homes begins with boiling of the milk for several minutes, which in addition to nearly complete sterilization, serves to concentrate the total solids. Contamination of milk after heating is expected when yogurt and Labneh are produced at home, a practice still common in many countries (4, 7, 12, 28).

When carelessly handled or produced, milk and milk products can easily become a vehicle for many foodborne pathogens. *Listeria monocytogenes*, *Salmonella Typhimurium* DT 104 (an emerging multiantibiotic-resistant strain), and *Escherichia coli* O157:H7 are of special importance due to their proven or likely acid tolerance and their ability to cause major dairy-related outbreaks in the United States and elsewhere (20). Milkborne infections involving these pathogens should occur more frequently in developing countries such as Syria than in major industrialized nations, because many dairy products, like Labneh, are produced at home from boiled milk (13). However, listeriosis, salmonellosis, and hemorrhagic colitis have been only rarely attributed to yogurt or Labneh. One such outbreak occurred in England in 1991 and was reportedly linked to consumption of farm-produced yogurt containing *E. coli* O157:H7 (15). These observations prompted us to assess the behavior of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella Typhimurium* DT104 in Labneh as a pre- and postfermentation contaminant.

MATERIALS AND METHODS

Experimental design. In the fermentation study, pasteurized cow’s milk (150 ml) was inoculated to contain a cocktail of the

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TABLE 1. Chemical composition of Labneh

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<td>21.19</td>
<td>3.95</td>
<td>1.53</td>
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<sup>a</sup> Inoculated with Salmonella Typhimurium DT104 and L. monocytogenes.
<sup>b</sup> Inoculated with E. coli O157:H7.

Pathogenic strains. L. monocytogenes (strains CWD17, CWD95, and CWD246), E. coli O157:H7 (strains AD317, AR, and AD305) (provided by C. W. Donnelly, University of Vermont, Burlington, Vt.), and Salmonella Typhimurium DT104 (strains: G10601, G10127, and G01074) (provided by B. Swaminathan, Centers for Disease Control and Prevention, Atlanta, Ga.) were subcultured twice in trypticase soy broth (Difco Laboratories, Detroit, Mich.) (24 h/35°C) from stock cultures stored at −80°C. Cocktails of each pathogen were prepared by combining equal volumes (3 ml) of the three strains.

Preparation of traditional Labneh. Commercially pasteurized cow’s milk (2.5 kg) containing ~2.0% (wt/wt) milkfat was heated at 85 to 87°C for 30 min. After cooling to 46°C, the milk was inoculated with a commercial yogurt culture (3.0% wt/wt) containing Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, and L. delbrueckii subsp. lactis (Chr. Hansen Laboratories, Milwaukee, Wis.) previously grown in heat-treated (85 to 87°C) skim milk. Milk was fermented in 9.5-liter plastic containers at 43°C for 3.5 h. The end product was stored at 2.7 to 3.3°C overnight (12 to 14 h) and then poured into cloth bags (5 kg/bag). After hanging 48 h at 7.2 to 7.7°C for whey drainage, Labneh was removed from the bags, gently mixed, and divided into two equal portions, one of which was salted at a level of 1.0% (wt/wt). Salted and unsalted Labneh were inoculated to contain up to 6,000 CFU/g of each pathogen cocktail, manually mixed for 2 min, packaged in 237-ml plastic yogurt containers and stored at 6 or 20°C. Ten-gram samples were then taken initially and after 2, 4, 7, 10, and 15 days of storage, diluted in 90 ml of 0.1% peptone solution, and shaken for 2 min. Thereafter, serial dilutions prepared in sterile 0.1% peptone were surface-plated on modified Oxford agar (Difco), MacConkey sorbitol agar (Difco), and MacConkey agar (Difco) for enumeration of Listeria, E. coli, and Salmonella, respectively, following 24 to 48 h of incubation at 35°C.

Inoculation and preparation of centrifuged Labneh. Commercially pasteurized milk containing ~2.0% milkfat was heated at 85 to 87°C for 30 min, cooled, poured into centrifuge bottles (150 g each), and cultured with 2% (wt/wt) of the aforementioned commercial yogurt starter culture. Each bottle was then inoculated with one of the pathogen cocktails at a level of 2,000 to 6,000 CFU/g, incubated at 43°C for 4 h, stored at 6 to 7°C for 12 h, and centrifuged (Sorvall Super T 21, Newtown, Conn.) for 6 min at 5,500 × g to obtain a Labneh-like product containing ~22% total solids. Numbers of pathogens in 10-g samples were determined after 0, 2, and 4 h of fermentation, after overnight storage, and after centrifugation as previously described.

Chemical evaluations. Titratable acidity, % milkfat, and % total solids of the traditional Labneh were determined by titration, the Babcock method, and vacuum oven drying, respectively, as described in “Standard methods for the examination of dairy products” (14). The pH was measured using a digital pH meter (model 8417, Hanna Instrument, Woonsocket, R.I.) equipped with a standard combination electrode.

RESULTS AND DISCUSSION

Chemical composition of traditional Labneh. All five batches of traditional Labneh (Table 1) were compositionally similar to commercially available Labneh that reportedly contains 8 to 11% milkfat and 22 to 26% total solids with typical pH and titratable acidity values ranging from 3.86 to 4.17 and 1.4 to 2.5, respectively (6, 12).

Behavior of L. monocytogenes. Growth of L. monocytogenes during fermentation was suppressed by the thermophilic starter culture as previously reported (23) with populations of this psychrotrophic pathogen increasing only 0.07 to 0.18 logs following overnight cold storage (Table 2).

Depending on inoculum size, pH, and storage temperature, L. monocytogenes can reportedly survive 3 to 25 days in yogurt (3, 24, 25). The type of acid and fermentation temperature also markedly influence Listeria survival with the growth rate for L. monocytogenes decreasing at pH ≤ 5.2 (21, 22) and generally ceasing at pH ≤ 4.5 (16, 22). Modest Listeria growth has been reported during thermophilic fermentation with S. thermophilus and Lactobacillus bulgaricus (23) suppressing L. monocytogenes, particularly when used at inoculum levels of ≥2.0%. However, multiplication of Listeria during fermentation is not a simple process, being further complicated by the rate of acid de-
TABLE 3. Numbers of L. monocytogenes (log CFU/g) surviving in three batches of salted and unsalted Labneh during storage at 6 and 20°C

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<th>Type of Labneh</th>
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<td>2.00</td>
<td>&lt;1.00</td>
<td>—</td>
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</table>


* — not tested.

development, type of acid produced, type of starter culture, bacteriocin production, and incubation temperature (1, 9, 23).

In our study, addition of Listeria at levels ≥2,600 CFU/g decreased the rate of acid production and increased the curdling time, thus suggesting some inhibition of the starter culture. As expected, higher Listeria populations were obtained in the first three batches of Labneh-like product (~22% total solids) that were obtained as a result of centrifugation. However, separation of the whey by centrifugation (after overnight cold storage) to convert the last three batches into a Labneh-like product was only possible after an additional 1 h of incubation at 43°C. The lower numbers of Listeria present in these sediments compared to those obtained without additional incubation further suggest partial injury of the pathogen during incubation at a low pH.

When L. monocytogenes was inoculated into Labneh after fermentation, Listeria populations decreased rapidly in all samples within the first 2 days of storage (Table 3). These findings were expected because the pH and titratable acidity (primarily lactic acid) of Labneh ranged between 3.99 to 4.21 and 1.36 to 1.5%, respectively. Inhibition of Listeria was most likely due to the starter culture (9, 23) coupled with high levels of undissociated lactic acid.

After 4 days of storage, Listeria was no longer detected in 9 of 12 samples examined by direct plating, 5 of which contained 1.0% salt. However, the pathogen was recovered from two salted and two unsalted samples examined at day 7 with one sample each stored at 6°C still harboring Listeria at day 15. Because L. monocytogenes can grow in media containing up to 12% salt and survive in the presence of 26% NaCl (22), addition of 1.0% salt to Labneh had little adverse impact on Listeria survival. However, the pathogen was detected at day 7 in salted and unsalted Labneh stored

at 20°C. These findings differ somewhat from those of Gohil et al. (9) who reported that Listeria could not survive more than 2 days in Labneh (27% total solids and pH 3.8 or 4.5) stored at 20°C or 5 days at 10°C. These discrepancies are at least partially explained by differences in Labneh composition and pH. Although the shelf life for Labneh is considerably shorter at ambient storage temperatures, Listeria is more readily inactivated under such conditions, thereby decreasing the risk of listeriosis.

Behavior of Salmonella Typhimurium DT104. Salmonella populations increased 0.33 to 0.47 logs 2 h after the start of fermentation as expected because the initial milk acidity and numbers of competing starter microorganisms were low (Table 4). After the fermentation, populations in three of four batches decreased to levels near the original inoculum. Although numbers of salmonellae decreased an additional 0.44 to 0.82 logs during storage, the end product (pH ≤ 4.4) still contained 2.3 to 3.1 log Salmonella CFU/g. As expected, centrifugation to produce a Labneh-like product further concentrated the pathogen. These findings are in contrast to an early study by Park and Marth (17, 18) in which a non-DT104 strain of Salmonella Typhimurium was inactivated within 18 h when autoclaved skim milk was fermented at 42°C with a 1% starter culture containing equal numbers of Salmonella Thermophilus and L. bulgaricus.

When Salmonella Typhimurium DT104 was introduced into Labneh as a postfermentation contaminant, populations decreased ≤2.42 logs 2 days after inoculation with the pathogen no longer detected by direct plating in 4-day-old samples, regardless of storage temperature. The pH and titratable acidity values for this Labneh ranged between 3.99 to 4.21 and 1.36 to 1.5%, respectively. Salmonella inactivation rates in all samples were similar, irrespective of salt content or storage temperature, indicating that the low pH and high lactic acid content of Labneh were sufficient to inactivate the pathogen (19).

Under circumstances prevailing in Middle Eastern countries, contamination of Labneh with Salmonella would not be expected to reach the inoculation levels used in this study. Hence, rapid inactivation of chance contaminants from high levels of acid (26), especially at ambient temperatures that encourage lactic acid production, suggests that Labneh should not be a serious source of salmonellosis.
particularly when the product is stored several days before consumption.

**Behavior of E. coli O157:H7.** Populations of E. coli O157:H7 increased 0.46 to 1.19 logs during the 4-h fermentation period and then generally remained stable after overnight cold storage (Table 6). As was true for *Listeria*, the time needed for milk coagulation compared to the control also increased for samples containing >4,750 CFU/g with an additional hour of incubation at 43°C required to obtain sediment by centrifugation. E. coli O157:H7 populations in sediment from the three batches requiring an additional 1 h of incubation at 43°C for whey separation were lower than those observed following overnight cold storage, which indicates additional inhibition of the pathogen as was also observed for *Listeria*.

In Labneh inoculated after fermentation, numbers of E. coli O157:H7 decreased 1.4 to >2.7 logs following 2 days of storage, regardless of storage temperature or salt content, with the pathogen no longer detected in 4-day-old samples by direct plating. While commercial yogurt starter cultures are reportedly inhibitory to E. coli O157:H7 with the pathogen inactivated in yogurt within 48 h of manufacture, E. coli persisted 60 days when commercially produced yogurt was inoculated to contain ≥10 E. coli O157:H7 CFU/g and stored at 4°C (2). Compositional differences between yogurt and Labneh (concentrated yogurt) are most likely responsible for the rapid demise of E. coli O157:H7 in our study.

These results demonstrate that traditional Labneh has an environment that will prevent growth and long-term survival of the aforementioned pathogens, particularly *Salmonella* Typhimurium DT 104 and E. coli O157:H7. The risk of contracting salmonellosis or hemorrhagic colitis from traditional Labneh appears to be very low because the causative agents are rapidly inactivated in the product shortly after manufacture. Consumption of contaminated Labneh could, however, induce listeriosis, because *L. monocytogenes* has a relatively low oral infective dose for susceptible individuals and can persist in the product during storage. Hence, contamination of Labneh must be minimized through hygienic preparation and appropriate packaging or handling to minimize the risk of infection through its utilization.

**ACKNOWLEDGMENT**

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**REFERENCES**


**TABLE 5. Numbers of Salmonella Typhimurium DT104 (log CFU/g) surviving in three batches of salted and unsalted Labneh during storage at 6 and 20°C**

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<tr>
<th>Type of Labneh</th>
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**TABLE 6. Fate of E. coli O157:H7 during Labneh manufacture**

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<tr>
<th>E. coli O157:H7 population (log CFU/g or ml)</th>
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<th>Sediment after centrifugation</th>
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*a* Centrifugation following 1 h of additional incubation at 43°C for whey separation.

**TABLE 7. Numbers of E. coli O157 (log CFU/g) surviving in three batches of salted and unsalted Labneh during storage at 6 and 20°C**

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