Development of a Probiotic Soft White Jordanian Cheese

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Abstract: Probiotic soft white cheese was produced from cow’s milk using *Lactobacillus acidophilus* and *Bifidobacterium lactis* and a combination of the two. The probiotic bacteria were either added to the cow’s milk used in the production before renneting or to the curd before pressing. We concluded that it is better to add the probiotic bacteria to the milk than to the curd. Most cheese treatments had probiotic counts >10^6 CFU/g after 1 week. All soft white cheeses produced in this study were rated acceptable by a sensory panel at the end of the study.

Key words: Probiotic - Soft white cheese - Probiotic cheese

INTRODUCTION

With growth in the functional food research area, interest has been focused on the incorporation of probiotic bacteria such as bifidobacteria and lactic acid bacteria into cultured dairy products to enhance the health and nutritional value of these products [1].

Although marketing and research attention has focused on fermented milks and yogurt as food carriers for bifidobacteria, these products are not optimal for the maintenance of high concentrations of some strains, as evidenced by poor viability (particularly some strains of *Bifidobacterium*) in commercial yogurts [2]. An alternative means of maintaining the viability of the bifidobacteria would be to incorporate the organisms into a product like cheese where the pH, lipid content, oxygen level and storage conditions are more conducive to the long-term survival of bifidobacteria during processing and digestion [1]. The biopreservative effect can be enhanced by the combination of probiotic strains with other lactic acid bacteria [3].

The first attempts to produce probiotic cheese may have been the use of dressing fermented by *B. infantis* in the production of cottage cheese [4, 5]. Recently the potential production of probiotic cheese has been investigated. Cheddar cheese [6, 7, 8, 9], Gouda cheese [10], Crescenza cheese [11], mozzarella cheese [12], panela cheese [13], Iranian feta cheese [2], Fior Di Latte cheese [14], Scamorza ewe milk cheese [15], fresh cheeses [16, 17, 18], Turkish white brined cheese [19], feta-type cheese [20], edam cheese [21], Emmental cheese [22], Domiati cheese [23], soft cheese [24] and cheese-based dips compare favorably with yogurt as delivery systems for viable probiotic micro-organisms [25].

Consumption of probiotic cheese has been found to attenuate exercise-induced immune suppression [26], improve symptoms of constipation [27] and improve body mass index and blood pressure indices [28, 29]. Some strains of bifidobacteria produce antimicrobial compounds that reduce levels of *Pseudomonas* in cottage cheese [30]. There are commercial probiotic cultures of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* subsp. *Shermanii* with anti-clostridial effects and activity against cheese-contaminating yeasts and molds [31].

The major cheese type produced in Jordan is soft white cheese (jubin balady) [32]. According to the Jordanian standards for soft cheese [33], soft cheeses are defined as unripened soft cheeses ready to eat directly after processing. Their acidity should not exceed 1.9% as lactic acid and they should be kept under refrigeration.
In Jordan, soft white cheese is usually produced seasonally from ewe or goat milk, or a combination of both, without addition of a starter culture or salt, under unmechanized or artisanal conditions and is manipulated by hands at various stages of manufacture [34]. The method of production may be summarized as coagulation of the milk for 40–60 min with rennet (a natural complex of enzymes produced in the mammalian stomach to digest mother's milk and often used in the production of cheese) after heating to about 35°C and then pressing in cheesecloth [34]. The cheese is usually directly consumed after production or used in the production of Arabian confectioneries such as kunafeh. This soft white cheese has a very limited keeping ability even under refrigeration; various types of microorganisms may enter the cheese during manufacture and subsequent handling [35, 36, 34]. A large portion of the soft white cheese produced seasonally in Jordan is used in the production of boiled white cheese.

The objective of this study was the development of a tasty probiotic soft white cheese using a traditional Jordanian manufacturing procedure.

**MATERIALS AND METHODS**

**Bacterial Cultures and Propagation:** Six probiotic cultures, including *Bifidobacterium lactis* FD BB-12° (Chr. Hansen-Denmark), *Lactobacillus acidophilus* HOWARU NCFM° (Danisco), *L. acidophilus* (ATCC 4356) (Microbiologics, UK), *B. lactis* HOWARU HN019 (Danisco), *B. breve* Yakult (Yakult, Japan) and *B. lactis* from a supplement (Theralac probiotic master) were evaluated through cultivation on reconstituted skim milk (RSM) to identify cultures yielding the highest numbers of probiotic bacteria. Two cultures were selected for application in cheese production, *L. acidophilus* and *B. lactis*.

All strains were tested for purity according to the method of Cowan and Steel (1993). Cultures of *L. acidophilus* and *B. lactis* (beginning with 1% inoculum) were propagated weekly according to the method of Tharmaraj and Shah [37].

**Culture Cultivation:** DeMan rogosa sharpe (MRS) agar (Oxoid, UK) supplemented with 0.5 g L⁻¹ L-cysteine-HCl monohydrate (Euro OTC pharma GMPH) was prepared and autoclaved at 121°C for 15 min. This medium was used for cultivation of the above cultures. Cultures were incubated anaerobically at 37 ± 1°C for 2 d. (Anaerobic Gen Gas-Pack 2.5L, Oxoid, UK) [37].

**Intermediate Medium for Probiotic Addition to Cheese:**

UHT skim milk (Nadeec, Saudi Arabia) with 0.1 g fat/100 ml and pH 6.6 was used for cultivation and propagation of probiotic bacteria. Two presterilized 250-ml bottles were opened aseptically for the addition of 0.05% L-cysteine-HCl monohydrate. The bottles were filled with UHT skim milk and mixed thoroughly; 2.5 ml of refrigerated culture preserved on RSM (final concentration 1%) was added and mixed thoroughly.

The inoculated bottles were incubated anaerobically at 37 ± 1°C for 2 days in jars using anaerobic Gen 2.5 L gas packs (Oxoid, UK). After 2 d, the skim milk was coagulated. Cultures were transferred to fresh UHT skim milk three times in succession for activation. The probiotic counts were determined for bottles using MRS agar fortified with 0.05% L-cysteine-HCl monohydrate. These cultures were then available for cheese fortification.

**Conventional and Probiotic Cheese Production:** Fig. 1 shows the main steps of conventional and probiotic cheese production under hygienic conditions and utensil sterilization. Conventional soft white cheese (control) was produced from pasteurized cow’s milk as described by [35]. As can be seen in Fig. 1, the processing steps were as follows: 16 kg of pasteurized milk (72 °C for 15 s) was warmed to 35°C. Calcium chloride (0.02%) was then added and the warmed milk was coagulated by addition of rennet (Reniplus, Proquiga, Spain, 750 IMCUL/ml) according to the manufacturer's recommendation. After 40 minutes, the coagulated curd was cut using sharp stainless steel knife into 1 cm³ cubes and they were allowed to stand for 10 min. The curd was then strained through cheesecloth and the resulting solid residue was pressed (using a pressure equivalent to 1.91 × 10¹ Pascal) for up to 2 h. The pressed cheese was then cut into small rectangular blocks of dimension 8 × 4 × 2 cm. Probiotic cheese was produced by adding the probiotic culture (*L. acidophilus* (A), *B. lactis*(B), or both (AB)) either to the milk (1 h before rennet addition) or to the strained curd before pressing. In both cases, probiotic culture was added to give a count of at least 10⁶ CFU/g. Seven types of cheese were produced. Each type was placed in twelve glass jars (200 g/jar), brine was added and salt concentration in the brine was determined based on the results of the preliminary experiments described below. Jars were labeled and kept refrigerated at 4°C. Two jars were assigned randomly to each of six different storage periods: 0, 2, 4, 7, 14 and 21 days. The experiment was repeated three times.
Determining the Most Preferred Salt Concentration in the Cheese Brine: A preliminary experiment was conducted to determine the salt concentration of the brine based on sensory preference. Different brine solutions (0, 2, 4, 6, 8, 10, 12, 14 and 16% w/w) were prepared using table salt (Sasi, Jordan). A clean 500-ml jar was prepared for each of the nine concentrations. Each jar received ca. 200 g cheese (prepared by the conventional method described below) before filling with each brine concentration. The jars were left at 4°C for 2 days. Twelve panelists were recruited to identify the sample with the most preferred saltiness. The salt concentration in the sample preferred by the highest number of panelists was chosen for the production of probiotic and conventional cheese after the cheese was placed in glass jars.

Chemical Analysis of Milk and Cheese: Milk was analyzed for pH, fat and specific gravity. Fat and specific gravity were measured with a milk analyzer (Milkostron Ltd., Bulgaria). A pH meter (Hanna instruments, USA) was used to measure the pH of milk, brine and cheese. The percentage of NaCl in brine was determined by the method of Hooi et al. [38].
Microbiological Analysis of Cheese: Samples of probiotic cheese from each manufactured batch were analyzed directly after packing (zero time) and at specified time intervals (2, 4, 7, 14 and 21 days) during the shelf life of the refrigerated (at 4°C) product. Testing included the following steps: first, probiotic bacteria counts for treatments were determined as described previously and then methylene blue staining was performed by the method of Cowan and Steel [39]. For control (conventional) cheese, lactic acid bacteria count (LABC) was measured at zero time and after 21 days.

Sensory Evaluation of Cheese: The testing was conducted in the sensory evaluation lab. at the University of Jordan, where the research had been conducted in the dairy pilot plant. Room temperature and relative humidity were controlled at 25°C and 75%, respectively. Thirty panelists (15 males and 15 females) aged between 22 and 40 years from the staff and students of the Faculty of Agriculture at the University of Jordan were recruited to evaluate samples of cheese collected after pressing and stored for the assigned time intervals. Testing included the control cheese. A hedonic scale of nine points, with five sensory attributes (appearance, smell, taste, consistency and overall acceptability) was used for evaluation [40]. Cheese samples were prepared by cutting the cheese into cubes of 1 cm³ and refrigerating them at 4°C. Samples were served in disposable odor-free plastic dishes and allowed to reach room temperature before serving. Sample size was 30 g. The identities of samples were blinded to panelists. Each sample was coded with a randomly selected 3-digit number and a balanced order of sample presentation to panelists for tasting was used.

Statistical Analysis: Statistical analysis for microbiological levels and physical and chemical tests was performed using F tests, t tests (LSD), Tukey’s studentized range and correlation with SAS version 9; and table curve program version 5.1 for correlation and regression analysis [41].

RESULTS

Culture Selection: B. lactis FD BB-12 and L. acidophilus HOWARU NCFM were chosen for the production of soft white cheese, for the following reasons: successful use in preliminary studies of the production of probiotic dairy products [42, 43, 37], successful experiments in cheese [44, 45], isolation from human intestine [43], growth improvement in the presence of calcium [43], resistance to bile acids [42,43] and conferral of multiple confirmed health effects [42, 43, 46, 47, 48, 49].

Intermediate Medium for Probiotic Addition to Cheese: The probiotic counts of B. lactis and L. acidophilus in Nadec skim milk after activation three or more successive times ranged between 10⁷ and 10¹¹ CFU/ml. The pH of the previously cultured skim milk (intermediate medium for probiotic addition to cheese) was 4.1–4.7 and 6.1–6.6 for L. acidophilus and B. lactis, respectively. The morphology of cultures stained with methylene blue after 8, 24 and 48 h showed densely packed, well-oriented rods with rounded ends for L. acidophilus, but short, curved, club-shaped, bifurcated Y-shaped, bifid-formed, or pleomorphic rods occurring singly or in many-celled chains or clumps for B. lactis. This cultured milk was then used for cheese fortification.

Chemical Analysis of Milk and Cheese: Fat, specific gravity and pH of the fresh whole cow’s milk used in the production of the cheese were 3%, 1.031 and 6.8, respectively. pH of the brine and cheese remained almost constant throughout the experiment, with averages of 6.7 and 6.5, respectively. A rise in salt concentration (NaCl%) was noticed in the cheese at the second day of storage and remained almost constant throughout the experiment in all cheeses. A drop in salt concentration in the brine was noted on the second day, followed by a slight rise near the end of the experiment.

Monitoring Probiotic Culture Counts in Probiotic Cheese During Storage: Figure 2 shows changes in probiotic bacteria count in soft white cheese during storage at 4°C for 21 days. With the exception of the B. lactis count, counts were >10⁶ CFU/g directly after production of the cheese and remained at these levels after a week of storage. A drop in the counts was noted during refrigerated storage. After 2 weeks, the counts remained at =10⁶ CFU/g in all cheeses. Generally, B. lactis counts were lower than L. acidophilus counts.

There were significant (p < 0.0001) differences between the counts of the probiotic cheese types directly after production (Figure 2). At 2 days there were significant (p < 0.0001) differences among the counts. The differences were between addition of L. acidophilus to milk (A/m) and addition of L. acidophilus to curd (A/c) and addition of B. lactis to milk (B/m) and addition of B. lactis to curd (B/c), but not between addition of L. acidophilus and B. lactis to milk (AB/m) and addition
Fig. 2: Development of the counts of probiotic bacteria used in the production of soft white cheese when added to the milk or the curd, during refrigerated storage (4 C). (A= L. acidophilus, B= Bifidobacterium lactis, m=addition to the milk, c=addition to curd)

Table 1: Results of taste preference testing of soft white cheese kept in brine of different salt concentrations at 4°C for 48 h

<table>
<thead>
<tr>
<th>Salt (NaCl%) in brine</th>
<th>0%</th>
<th>2%</th>
<th>4%</th>
<th>6%</th>
<th>8%</th>
<th>10%</th>
<th>12%</th>
<th>14%</th>
<th>16%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of panelists</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: Sensory evaluation of probiotic soft white cheese samples produced using Lactobacillus acidophilus, Bifidobacterium lactis, or a combination of the two bacteria during storage for 21 days at 4°C

<table>
<thead>
<tr>
<th>Sensory attribute</th>
<th>Control</th>
<th>A/m*</th>
<th>A/c</th>
<th>B/m</th>
<th>B/c</th>
<th>AB/m</th>
<th>AB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>7.8**</td>
<td>7.5</td>
<td>6.9</td>
<td>7.8</td>
<td>7.4</td>
<td>6.9</td>
<td>6.9</td>
</tr>
<tr>
<td>Smell</td>
<td>7.4^a</td>
<td>7.5</td>
<td>7.2</td>
<td>7.4</td>
<td>7.2</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td>Taste</td>
<td>7.4^b</td>
<td>7.4</td>
<td>7.0</td>
<td>7.7</td>
<td>7.2</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Consistency</td>
<td>7.6^c</td>
<td>7.4</td>
<td>7.0</td>
<td>7.7</td>
<td>7.6</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>7.4^d</td>
<td>7.4</td>
<td>7.1</td>
<td>7.8</td>
<td>7.4</td>
<td>6.9</td>
<td>6.9</td>
</tr>
<tr>
<td>Average</td>
<td>7.5</td>
<td>7.4</td>
<td>7.0</td>
<td>7.7</td>
<td>7.4</td>
<td>7.6</td>
<td>6.9</td>
</tr>
</tbody>
</table>

* A/m = L. acidophilus addition to the milk, A/c = L. acidophilus addition to the curd, B/m = B. lactis addition to the milk, B/c = B. lactis addition to the curd, AB/m = L. acidophilus and B. lactis addition to the milk, AB/c = L. acidophilus and B. lactis addition to the curd.
** Only scores followed by different letters are significantly different.

of L. acidophilus and B. lactis to curd (AB/c). At 4 days, there were significant (p < 0.0001) differences among the counts. The differences were between Am and A/c, B/m and B/c and AB/m and AB/c. At 7 days, there was a significant difference (p < 0.001) among the counts. The significant differences were between A/m and A/c, but there were no significant differences between B/m and B/c or AB/m and AB/c. At 14 days, there were no significant differences among the counts except between A/c and all other types. At 21 days, there were significant (p < 0.01) differences among the counts. The differences were between A/m and A/c, B/m and B/c and AB/m and AB/c.

Monitoring LABC for the Control Cheese: LABC in the control cheese ranged between log 2.8 and 5.1 for 0 and 21 days, respectively.
Determining the Most Preferred Salt Concentration in the Cheese Brine: Six of the panelists preferred cheese kept in 6% NaCl brine and four preferred 8% (Table 1). Accordingly, the cheese for the experiments was kept in 7% NaCl brine.

Sensory Evaluation of Cheese: A summary of the results of sensory evaluation of probiotic soft white cheese produced using \textit{L. acidophilus} and \textit{B. lactis} during storage for 21 days at 4°C is shown in Table 2. With the exception of the cheeses produced using a combination of \textit{L. acidophilus} and \textit{B. lactis} in the curd and \textit{L. acidophilus} in the curd, no significant differences in the appearance of the cheese were noted. The only significant difference in smell was noted between the cheeses produced using a combination of \textit{L. acidophilus} and \textit{B. lactis} in the milk and using a combination of \textit{L. acidophilus} and \textit{B. lactis} in the curd. A significant difference in taste was noticed between cheese produced using \textit{B. lactis} in the milk and cheese produced using a combination of \textit{L. acidophilus} and \textit{B. lactis} in the curd, with the first of these showing the highest acceptability. Results of the other treatments were intermediate. With respect to consistency control, cheese produced using \textit{B. lactis} in the milk and curd and cheese produced using a combination of \textit{L. acidophilus} and \textit{B. lactis} in the curd had the highest scores, but with no significant difference. Cheese produced using \textit{L. acidophilus} and \textit{B. lactis} in the curd was significantly the least preferred, with respect to consistency. Accordingly and as can be seen from the overall testing and average scores, cheese produced using \textit{B. lactis} in the milk was the most preferred, followed by cheese produced using a combination of \textit{L. acidophilus} and \textit{B. lactis} in the milk and control. Cheese produced using a combination of \textit{L. acidophilus} and \textit{B. lactis} in the curd was the least preferred but was not rejected by the panelists.

**DISCUSSION**

The production of probiotic dairy products based on fermented milk has an established tradition, which is reflected in the global availability of commercial probiotic cultured milk. To the best of our knowledge, this is the first time in Jordan to produce a probiotic product based on traditional dairy food. Production of commercial probiotic cheese is still in its early stages compared with probiotic cultured milk. Cheese has certain advantages as a carrier of probiotics compared with more acidic fermented dairy products such as yogurt. These advantages are represented by the high buffering capacity and by the dense matrix and high fat content, which are important factors for protecting probiotic bacteria in the stomach [2].

In our study, besides the selection of suitable probiotic bacteria, the production of probiotic cheese faces three major difficulties: determination of the right step of cheese production at which to add the probiotic bacteria (at the right concentration), the survival of probiotic bacteria during storage and the effect of probiotic bacteria addition on sensory properties of the finished cheese.

In this study, it was possible to produce a probiotic cheese using \textit{L. acidophilus} and \textit{B. lactis}. Both of these bacteria have been established as probiotic cultures. Given that the addition of probiotic bacteria to the curd did not result in higher numbers, we conclude that adding them to milk is much preferable. Furthermore, direct addition to milk is easier (saving time and effort) and more hygienic and ensures better distribution of the bacteria in the cheese. Songisepp et al. [50] found the addition of lactobacilli into milk during the production of Pikantne cheese (a semisoft cheese based on an Estonian open-texture, smear-ripened cheese) to be the preferred method of incorporation when compared with inoculation into drained curds in which the probiotic additive would be partially drained off in whey.

For realization of the health benefits of probiotic organisms, recommendations for the minimum viable counts of each probiotic strain, in counts per gram or milliliter of probiotic product, are highly variable. In general, the food industry has applied the recommended level of \(10^6\) CFU/g at the time of consumption for probiotic bacteria [2]. In our experiment, the bacteria were still viable at the end of the experiment. Most of the cheese treatments showed probiotic counts \(>10^6\) CFU/g after 1 week. Numbers at 2 weeks were \(<10^6\) CFU/g. Counts of probiotic bacteria did not increase in the cheese, for two reasons: the time between the introduction of the bacteria and transfer of the cheese pieces into brine was short and no growth of the probiotic bacteria in the cheese in the brine under refrigeration occurred, because these bacteria are not psychrotrophic in nature and salt inhibits their growth. To increase the viable count in the developed cheese to the recommended level, we recommend increasing the inoculum of each bacterium, for example by doubling the amount of cultured skim milk containing the probiotic bacteria. However, the effect of increasing the level of probiotic culture in probiotic cheese on sensory properties should be investigated before this measure is
applied. Gomes et al. [51] reported that some negative sensory effects occurred when a high level of supplementation with *L. acidophilus* was used in probiotic cheese processing.

For a probiotic bacterium to provide a benefit to human health, it must survive passage through the upper gastrointestinal tract and arrive alive at its site of action and must be able to function in the gut environment [12]. Further studies are needed to evaluate the tolerance of the culture used in the developed probiotic cheese to gastric conditions.

Aside from the viability of probiotics in cheese, it is important that incorporation of probiotic bacteria should not affect the sensory characteristics (flavor, texture and appearance) expected of conventional (non-probiotic) cheeses [52]. All soft white cheeses produced in this study showed high sensory preference compared with conventional cheese. Probiotic bacteria used in this study did not affect sensory quality because these bacteria have limited proteolytic and lipolytic activity [53]. Keeping the traditional soft white cheese in brine with 7% salt (NaCl) proved to be most suitable, because at this concentration the taste of the cheese was not negatively affected. The use of probiotics in trials of Pecorino ovine cheeses did not adversely affect preference or acceptability; in fact, panelists scored probiotic cheeses higher in preference over traditional cheese, although not significantly [54]. De Souza et al. [55] stated that addition of *L. acidophilus*, either solely or in co-culture with a thermophilic lactic starter culture, resulted in good acceptance of Minas fresh cheese (a typical Brazilian fresh cheese), improving sensory performance of the product during storage for up to 14 days. Overall, probiotics may have no significant effect on sensory characteristics (flavor, texture and appearance) of cheese, may adversely affect these attributes, or may improve them [52]. Further studies are needed for sensory profiling of the probiotic cheese we have described. Albenzio et al. [15] defined sensory properties of Scamorza ewe-milk cheese and established a specific quantitative vocabulary for sensory analysis and a reference frame for assessor training for systematic monitoring the quality of this new type of ewe milk cheese. Further studies of the quality of cheese and of the levels of salt that are acceptable in the production of cheeses from a health perspective are also needed. The heightened intake of sodium from processed foods is of great public health concern worldwide. Felicio et al. [56] found that more than 70% of cheeses examined in their study could be classified as high-sodium cheeses, with sodium contents exceeding 400 mg Na/100 g of product and accordingly they suggested that cheese manufacturers need to reformulate their products and that public health authorities need to take additional measures to curb sodium intake from cheese consumption, including demand-specific labeling and implementation of educational campaigns to inform the public about the dangers associated with high sodium intake. Cruz et al. [57] reported that various types of cheeses have been developed with reduced sodium content by reduction of NaCl or partial/total substitution of this salt with KCl, MgCl, and CaCl. The results were mostly positive; most cheeses are acceptable, but at times there was a sour residual taste resulting from the substitution of NaCl.

**CONCLUSIONS**

All probiotic soft white cheeses produced in this study received high sensory preference. Cheese produced using *B. lactis* in the milk was the most preferred, followed by cheese produced using *L. acidophilus* and *B. lactis* in the milk and the control. Cheese produced using *L. acidophilus* and *B. lactis* in the curd were the least preferred. Probiotic bacteria were still viable in the cheese at the end of the experiment. Most of the cheese treatments used resulted in probiotic numbers >10⁶ CFU/g after 1 week. Additional work is needed to increase storage stability of the cheese while maintaining the viability of probiotic bacteria and sensory properties. Further studies are also needed to confirm the ability of the probiotic culture in the cheese to survive gastric conditions.

**REFERENCES**


