Microbial Properties of Traditional Cheese (Jar Cheese) during Ripening

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ABSTRACT

This study was performed aimed at providing health products of milk and indigenous knowledge in the preparation of a traditional cheese (jar). Jar Cheese is one of the best known and most famous cheeses in Iran without adding any starter and is made from raw milk. Important issue in the production of this product is the risk of pathogenic microbes. In local conditions for the safe storage, cheese is maintained in earthenware jar for one month and it is used without knowledge of pollution. In this study, microbiological characteristic of a fresh jar cheese and effect of ripening process was studied within 90 days. In local conditions for safe storage of jar cheese, it must maintain at least a month into the jar, well as some salt is added to cheese, during of producing. In fact, salt is used as a protective material due to reduction of water activity and inhibitory effect of presence and activity of bacteria. Jar cheese has a mild acidic salty taste, and a pleasant flavor and smell. In its manufacture milk warm up to around 30-35°C and then lamb rennet is added. The coagulum is cut into pieces that are then transferred to drain the whey. The resulting curd mass is cut into small cubes and the cubes are packed in 10–12% salt brine in a clay jars. Ripening takes place in deep natural or man-made caves for 3–4 months at an average temperature of 10–12°C. We investigated microbiological characteristic such as existence or nonexistence of some bacteria like Mesophilic, Coliform, Escherichia coli, lactobacillus, Staphylococcos aureus, mold and yeast. Microbial investigations showed that during the period of ripening, coliform and Staphylococcus aureus were inhibited and the amounts of them reduced So that at the end of the third month, any Coliform weren’t isolated. Also the amounts of mold and yeast did not increase during the period of ripening significantly. An increase was observed in Lactobacillus bacteriaduring the first month and then they showed a reduction. A jar cheese is consumed after one month, while this survey showed that in the first month, None of the biological properties of cheese samples was not in accordance with national standard of ripen cheese, and favorable conditions didn’t have for consumption.

Key words: Jar cheese; microbiological; Coliform; Escherichia coli; Staphylococcus aureus; Mesophilic; lactobacillus.

Introduction

After meat, milk is the most important animal product. According to wide variation in the production of dairy nomadic group products in Iran; unfortunately, experts of dairy industry have low attention in order to introduce these products in Iran while in many countries such as France and Denmark diversity of traditional dairy products is one of the national and cultural prides. In Iran, Researches on milk traditional products, is mostly about geography, which in the following can be noted:

Dairy products of TuranShahrud region [1], dairy products of Bakhteyariha[2], familiarity with nomadic population [3], evaluation of dairy products in the migrant tribes of North Khorasan[4], dairy supply chain in the tribes of Semnan province[5].

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traditional cheese of Kazeroun by Marhamati Zadeh and colleagues [6].

*Escherichia coli* are considered as an indicator in the researches. The results of Hesami Rudd and colleagues’ research [7] have confirmed the existence of *Escherichia coli* in a jar fresh cheese, in West Azarbaijan. Also, Aghazadeh Meshgi [8] indicated the existence of *E. coli* in the jar cheeses with less than 6 months of ripening period.

Changes that occur in cheese during ripening have important role in microbial changes and the inhibitory effect on the growth of unwanted bacteria in the cheese. These changes are reduction in the amount of moisture and pH and also, increasing in the acidity which prepare conditions for survival of harmful bacteria [8, 9]. Numerous studies have been done in order to achieve the effect of ripening conditions on the microbiological characteristics of traditional cheeses produced from raw milk such as jar cheese [8, 7, 10 and 11].

**Materials and Methods**

**Microbiological tests:**

At first, in this study we integrated 10 g of each sample with 90 ml of peptone water 1% sterile. Subsequent dilutions were made in peptone water 1%. All cultures were performed in triplicate. Total count of *Mesophilic* (Nutrient Agar, Merck) was done at 30 °C for 48 h and total count of *coliform* (Violet Red Bile Agar, Merck) was done at 37° C for 48 hours. In order to check *Escherichia coli*, part of the purple colonies with a diameter of 0.5 mm were transferred to tubes containing lactose broth along with Durham tube and were placed in incubator at 37 °C for 24 h.

Microorganisms were investigated in terms of concentration of gas (carbon dioxide) in the Durham tube. Also biochemical tests IMVIC were investigated after the incubation at 37 °C for 48-24 hours.

Among microorganisms *E. coli* produced gas and biochemical test results of them showed indole positive, methyl red positive, Voges Proskauer negative, and citrate negative. *Lactobacillus* count (MRS agar, Merck) was done after incubation for 5 days at 37 °C and count of *Staphylococcus aureus* (Baird-Parker Agar, Merck) was done after incubation at 30 °C for 48 hours. The count of mold and yeast (SDA, Merck) were performed at 25-22 °C for 3-7 days.

**Results and Discussions**

**Microbial investigation:**

Important issue in the production of jar cheese is probability of high microbial load and contamination to pathogenic microbes of staphylococcal and enterococci groups because of use of raw milk and secondary contamination of milk after milking and in the production process.

The results of microbial studies are expressed in the table 1.

<table>
<thead>
<tr>
<th></th>
<th>First day of ripening</th>
<th>Thirty day of ripening</th>
<th>Sixtieth day of ripening</th>
<th>Ninetieth day of ripening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesophilic (CFU/g)</td>
<td>1.89×10⁶</td>
<td>6.93×10⁴</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coliform (CFU/g)</td>
<td>0</td>
<td>0</td>
<td>1.99×10⁵</td>
<td>0</td>
</tr>
<tr>
<td>E. coli</td>
<td>2.47×10⁴</td>
<td>1.3×10⁵</td>
<td>2.1×10⁵</td>
<td>1.4×10⁵</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>2.4×10⁵</td>
<td>2.4×10⁵</td>
<td>2.4×10⁵</td>
<td>2.4×10⁵</td>
</tr>
<tr>
<td>Mold and yeast</td>
<td>3.7×10⁵</td>
<td>4.0×10⁵</td>
<td>6.3×10⁵</td>
<td>6.3×10⁵</td>
</tr>
</tbody>
</table>

**The Mesophilic bacteria:**

Maximum amounts of *Mesophilic* bacteria (0.05 ≥ P) were observed in fresh jar cheese (1.89×10⁶ CFU/g) and their amounts decreased sharply during the first month so, we did not observe any Mesophilic after 60 days of ripening. These results are similar to the obtained results by Kurtand colleague [12] and Hayaloglu and colleague [13] on Tulum cheese. Inhibitory factors of *Mesophilic* bacteria growth were reduction in the amount of moisture and increasing in the salt.

**Lactic acid bacteria:**

We did not add any starter and used raw milk in preparation of jar cheese, the isolated *lactic acid* bacteria were native lactic in milk flora so, *lactic acid* bacteria (NSLAB) were non-starter. As, is seen in the table 1, the amounts of lactic acid bacteria in jar cheese increased in the first month of ripening that were not significant statistically (0.05 ≥ P) but then they had a slightly reduction. Perhaps reason of this reduction is ripening temperature (refrigerated conditions) because based on the results of Sheehan and colleagues [14], temperature of ripening has certain effect on the growth of these bacteria. So, similar to these results are presented in Kashar traditional cheese by Cetinkaya and Soyutemiz [9].

Since jar cheese is prepared of raw milk without adding starter, the presence of native lactic in flora
milk are important factors in the development of flavor and aroma in this kind of cheese. Higher amounts of non-starter lactic acid bacteria show increase enzymic content of cheese texture which is according to research of Hannon and colleagues [15] in development of cheese flavor [13].

Coliform bacteria:

Coliform were not observed in the cultured fresh cheese that it means low amounts of Coliform in the milk used (raw milk). The amounts of Coliform increased after placing the cheese inside the jar during first month, and reached to $10^5 \times 99.1$ CFU/g on the thirtieth day, which were much higher than permissible limit by national standards (Institute of Standards and Industrial Research of Iran, 2008). Contamination of jar or workplace can lead to cheese contamination and its increase during first month.

According to the results of confirmatory tests, isolated coliform were a kind of citrobacter. Coliform levels decreased significantly after 30 days $(0.05 \geq P)$ and reached the peak on ninetieth day. According to the researches of Akgunand colleagues [16] and Atamer and colleagues [17], development of non-starter lactici acid bacteria in cheese is an inhibitor of the coliform growth. Also, pH of cheese, low amount of moisture and high amount of salt are as inhibitor factors [10, 11].

Effect of ripening process in coliform bacteria reduction was reported in jar cheese of western Azerbaijan [7]. Atamar and colleagues [17] reported that coliform weren’t identifiable from Kashar traditional cheese after 30 and 60 days of ripening.

Staphylococcus aureus bacteria:

Survey of fresh cheese showed lack of Staphylococcus aureus. However, after transferring cheese to a jar, their amounts increased and reached to $4.2 \times 10^5$ CFU/g in thirtieth day, that contamination was because of secondary contamination from jar or our hands and generally lack of sanitation. In the subsequent periods, Staphylococcus aureus was negative in the samples and it is because of microbial competition of bacteria for producing lactic acid, reduction of pH, production of hydrogen peroxide by lactoperoxidase system, competition to obtain food and production metabolites such as nisin during the ripening [8].

Mold and yeast:

Count results of yeast and mold showed that fresh cheese was without mold and yeast but they were found after transferring to the jar. During the process of ripening an increase was observed in the number of yeast and mold that $(0.05 \geq P)$ wasn’t significant statistically.

References