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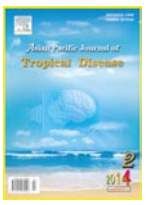

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Monitoring microbial quality of commercial dairy products in West Azerbaijan province, northwest of Iran

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ABSTRACT

Objective: To evaluate the extent of microbial contamination such as coliform, *Escherichia coli*, positive coagulase *Staphylococcus aureus*, molds and yeast in cheese, buttermilk, yogurt, and milk in West Azerbaijan province.

Methods: Between March and November 2012, 93 samples of cheese, buttermilk, milk, and yogurt were collected from factories of West Azerbaijan province, northwest of Iran. The samples were tested by standard numbers 5486, 5234, 6806, and 10154 for monitoring their microbial quality.

Results: The results of this study revealed that 33% of cheese samples were unauthorized. Also, 22% of buttermilk, 23% samples of yogurt, and 15% of milk samples were unauthorized. Other examples of microbial aspects were normal.

Conclusions: It is necessary to determine the critical control points inorganizing factories and automated control systems in order to eliminate or minimize the threat of pollution. Microbial quality of the present products was excellent. Meanwhile, training and familiarizing manufacturers of dairy products are very important in terms of health standards.

1. Introduction

Despite the advances of modern production methods, food safety and consumer safety are increasingly in public health importance. In industrialized countries, 30% of people have been estimated to suffer from eating disorders once a year. So, it is required to reduce or eliminate pathogenic microorganisms in food sources using different methods[1,2]. Dairy foods refer to all the milk products. The first material for producing dairy products is cow milk. However, the milk of other mammals such as goats, sheep, *etc.* is sometimes used. Milk is a substance that is secreted from the breast tissue of cattle and mammals[3].

Cheese or other fresh products that are obtained from milk cheese juice after coagulation and exit of whole milk, cream, buttermilk, butter, milk fat, or a mixture of them is obtained[3]. Iranian yogurt drink is made by mixing water and enough salt[3]. Yoghurt is the fermented product of milk, which is highly consumed in the Mediterranean, Asian, and Central European countries. Bulgaria has been identified as the original country in terms of yoghurt production[3].

Dairy foods are always exposed to pathogenic microorganisms and infections. Controlling microorganisms in food is one of the most important maintenance aspects[4], because in a country like the United States, 6.5 to 33 million people are annually infected by the disease-causing germs in food, which entails damage of 2.9 to 6.7 billion dollars[5].

Coliform is found in human and animal fecal and is usually plentiful in nature. It is extremely risky and toxic for food and water and could cause an intestinal disease. Coliforms are divided into two categories of non-fecal and fecal, which only live in the intestines, however, some coliforms are not only in the intestine, but also can be seen in the soil and plants. *Escherichia coli* (*E. coli*) is one of the

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coliforms that exists in large numbers in the human intestine, it also can be present in water, food, and the environment after fecal contamination[6-8].

Staphylococcus aureus (*S. aureus*) is one of the most common infectious pathogens due to having various enzymes such as coagulase, hyaluronidase, uclease, lipase, hemolysin, and leukocidin, which is able to cause an infection anywhere in body[9].

In West Azerbaijan province, there are many traditional farms for produce milk. So milk is produced the form of non industrial. This study was designed to access microbial quality of diry products.

2. Materials and methods

2.1. Sampling

Between March and November 2012, 93 samples of cheese, buttermilk, milk, and yogurt were collected from factories of West Azerbaijan province, northwest of Iran.

2.2. Microbial tests

Technique and reference methods under International Standardization Organization standards were used for monitoring the samples' microbial quality.

2.3. Coliform (standard no. 5486)

About 1 mL of the samples was taken and dilution 10^{-3} was prepared thus added to sterile plates. The medium (lauryl sulfate tryptose broth) was then added to tube. The plates were incubated at 30 °C for 1-3 d[10].

2.4. E. coli (Iranian national standard no. 5234)

About 1 mL of sterile sample was poured and added to lauryl sulfate tryptose medium. Then, it was incubated at 37 °C. If gas was formed, the sample was reported as negative; and after culturing the samples, if gas was observed, it was positive. From positive (gas+) samples were taken and was added to the second tube. One was added to peptone water tube and incubated at 44 °C and for another tube and other tube to the EC broth. On Day 3, if the EC broth was positive, pepton water medium was added. Ultimately of the tube shall be counted[10].

2.5. S. aureus (positive coagulase) (Iranian national standard no. 6806)

The samples were prepared and incubated. If black colonies were observed, the sample test was positive[10].

2.6. Mold and yeast (Iranian national standard no. 10154)

First, standard dilution was prepared and into 15 mL of medium containing tetracycline and chloramphenicol oxytocin was inoculation into the plates and incubated at 45 °C. The colonies were then counted by the following formula:

$N=\Sigma c/(n_1+0.1N_2)\ d$

Where Σc is total number of colonies on selective plates from two successive dilutions; n_1 is number of plates with minimum 10 and maximum 150 colonies in the countable dilution; n_2 is number of plates with minimum 10 and maximum 150 colonies to be counted in the second dilution; d is coefficient dilution in the first choice.

If more than 2 dilutions were counted between 10 and 150 colonies, the results are going to change formula so that subsequent dilution are taken into account[10].

3. Results

After examination of microbial and fungal contamination, the samples were observed low. Survey results showed that milk production was processed with the mechanization of good quality and was sanitary. The results of this study revealed that 33% samples of cheese, 22% samples of buttermilk, 23% samples of yogurt, and 15% samples of milk were unauthorized. Other examples of microbial aspects were normal.

Detailed results of the microbial quality of the tested samples for cheese, buttermilk, yoghurt, and milk are given in Tables 1-5.

According to Table 1, 8 out of 30 samples of cheese were above coliform. Six of the *E. coli* were positive. *Staphylococcus* was observed in 2 and 4 of them were from standard molds and yeast. In total, 18 out of 27 samples were acceptable. Microbial quality of the present products was excellent. About 67% of the cheese samples were acceptable in terms of microbial.

According to Table 2, for buttermilk samples were determined. For the 4 cases were positive to coliform. All samples of buttermilk were negative *E. coli* and no cases of *Staphylococcus* were reported. In total, 25 out of 32 samples of buttermilk were acceptable. Microbial quality of the products was excellent. About 78% of the buttermilk samples were acceptable in terms of microbial.

Based on Table 3, 18 out of 23 samples were acceptable. Four and three samples were contaminated with coliform and *E. coli* and were positive, respectively. In the present samples, one *S. aureus* was positive and three samples were also contaminated with mold and yeast. Microbial quality of

Table 1

Results of microbial tests for cheese.

Sample of tested cheese	Coli form	*E. coli*	Positive coagulase *S. aureus*	Mold and yeast	Result
1	2.4×10^2	+	−	<10	unacceptable
2	<10	−	−	<10	acceptable
3	<10	−	−	<10	acceptable
4	1.5×10^2	+	4.75×10^2	1.2×10^3	unacceptable
5	<10	−	−	<10	unacceptable
6	<10	−	−	<10	acceptable
7	<10	−	−	<10	acceptable
8	1.1×10^2	+	−	6.1×10^2	unacceptable
9	<10	−	−	<10	acceptable
10	1.1×10^2	+	−	<10	unacceptable
11	<10	−	−	<10	acceptable
12	<10	−	−	<10	acceptable
13	<10	−	−	6.4×10	acceptable
14	<10	−	−	6.4×10	acceptable
15	<10	−	−	<10	acceptable
16	7.3×10^3	−	−	<10	unacceptable
17	2.4×10^2	−	−	<10	unacceptable
18	<10	−	−	<10	acceptable
19	2.1×10	+	−	<10	unacceptable
20	<10	−	−	<10	acceptable
21	6.1×10^2	−	−	<10	unacceptable
22	<10	−	−	<10	acceptable
23	<10	−	−	<10	acceptable
24	<10	−	−	<10	acceptable
25	<10	−	−	<10	acceptable
26	<10	−	−	<10	acceptable
27	<10	−	−	<10	acceptable
28	2.4×10^2	+	2.8×10^2	−	unacceptable
29	22	<10	−	−	acceptable
30	22	<10	−	−	acceptable

Table 2

Microbial tests for buttermilk.

Sample of tested yoghurt	Coli form	*E. coli*	Positive coagulase *S. aureus*	Mold and yeast	Result
1	20.7×10^2	−	−	<1	unacceptable
2	<1	−	−	<1	acceptable
3	<1	−	−	<1	acceptable
4	<1	−	−	<1	acceptable
5	<1	−	−	<1	acceptable
6	<1	−	−	<1	acceptable
7	<1	−	−	<1	acceptable
8	<1	−	−	<1	acceptable
9	<1	−	−	<1	acceptable
10	<10	−	−	<10	acceptable
11	<1	−	−	<1	unacceptable
12	3.6×10^3	−	−	<1	unacceptable
13	<1	−	−	<1	acceptable
14	<1	−	−	<1	acceptable
15	<1	−	−	<1	acceptable
16	6.6×10^2	−	−	<1	unacceptable
17	<1	−	−	<1	acceptable
18	<1	−	−	<1	acceptable
19	<1	−	−	<1	acceptable
20	<1	−	−	<1	acceptable
21	<1	−	−	<1	acceptable
22	<1	−	−	<1	acceptable
23	2.8×10^3	−	−	<1	unacceptable
24	<1	−	−	<1	acceptable
25	<1	−	−	<1	acceptable
26	<1	−	−	<1	acceptable
27	<1	−	−	<1	acceptable
28	<1	−	−	<1	acceptable
29	<1	−	−	<1	acceptable
30	<1	−	−	<10	unacceptable
31	<1	−	−	<1	unacceptable
32	<10	−	−	<1	acceptable

Table 3

Results of microbial tests for yogurt.

Sample of tested yoghurt	Coli form	*E. coli*	Positive coagulase *S. aureus*	Mold and yeast	Result
1	<10	−	−	<10	acceptable
2	1.5×10^2	+	1.45×10^3	<10	unacceptable
3	<10	−	−	<10	acceptable
4	<10	−	−	<10	acceptable
5	<10	−	−	2.5×10^2	unacceptable
6	<10	−	−	2.5×10^2	acceptable
7	<10	−	−	<10	acceptable
8	4.02×10^2	−	−	2.5×10^2	unacceptable
9	2.4×10^2	+	−	<10	unacceptable
10	2.4×10^2	+	−	<10	unacceptable
11	<10	+	−	<10	acceptable
12	<10	−	−	<10	acceptable
13	<10	−	−	<10	acceptable
14	<10	−	−	<10	acceptable
15	<10	−	−	<10	acceptable
16	<10	−	−	<10	acceptable
17	<10	−	−	<10	acceptable
18	<10	−	−	<10	acceptable
19	<10	−	−	<10	acceptable
20	<10	−	−	<10	acceptable
21	<10	−	−	<10	acceptable
22	<10	−	−	<10	acceptable
23	<10	−	−	<10	acceptable

Table 4

Results of microbial tests for fresh and pasteurized milk.

Sample of tested fresh milk	Total count of microorganisms	Coli form	*E. coli*	Microbial deterrent test	Result
1	7.15×10^2	<1	−	−	acceptable
2	4×10^3	2.4×10	+	−	unacceptable
3	2×10^4	2.4×10	+	−	unacceptable
4	3.71×10^2	<10	−	−	acceptable
5	2.76×10^2	<10	−	−	acceptable
6	7.15×10^2	<1	−	−	acceptable
7	2.92×10^2	<1	−	−	acceptable
8	1.01×10^3	<1	−	−	acceptable
9	2.92×10^2	<10	+	−	unacceptable
10	×	×	<1	<1	acceptable

×: Experiments were no performed. None of the milk samples had a bump and protuberance and all the samples were acceptable. Fresh milk was positive in 3 cases (for *E. coli*) and 3 cases were unacceptable. Microbial quality of the products was excellent.

Table 5

Results of microbial tests for pasteurized milk.

Sample of tested pasteurized milk	Incubated at 30 °C for 10 d	Incubated at 55 °C for 7 d	Total count at 30 °C for 10 d	Total count at 55 °C for 10 d	Result
1	−	−	<1	<1	acceptable
2	−	−	<1	<1	acceptable
3	−	−	<1	<1	acceptable
4	−	−	<1	<1	acceptable
5	−	−	<1	<1	acceptable
6	−	−	<1	<1	acceptable
7	−	−	<1	<1	acceptable
8	−	−	<1	<1	acceptable
9	−	−	<1	<1	acceptable
10	−	−	<1	<1	acceptable

−: No bagginess and leakage. A total of 85% of the pasteurized milk samples were acceptable in terms of microbial.

the products was excellent. About 77% of the yogurt samples were acceptable in terms of microbial.

None of the milk samples had a bump and protuberance and all the samples were acceptable. Fresh milk was positive in 3 cases (for *E. coli*) and 3 cases were unacceptable. Microbial quality of the products was excellent. 85% of the pasteurized milk samples were acceptable in terms of microbial.

It can be seen in Figure 1 that in all kinds of dairy products (cheese, yogurt, buttermilk, and milk), suspected microbial

quality was good in most cases of and apparently healthy controls were good. In most studies have been reported worldwide contamination (microbial contamination in food always happen), the microbial quality of the samples was at an acceptable level. The sampling was performed in the case of suspected and in total, microbial quality of the products were excellent.

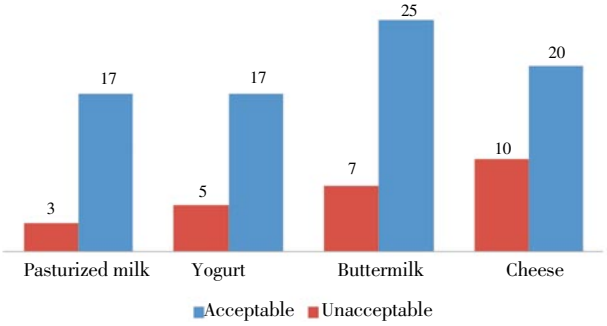

Figure 1. Comparing the acceptable and unacceptable number of cheese, buttermilk, yogurt, and milk in terms of microbial quality.

4. Discussion

Murinda *et al.* carried out a study in America in which *E. coli* O157:H8 was isolated from 30 dairy product factories[11]. Results of this study showed that cheese was a carrier of *E. coli*[12]. Bonyadian *et al.* demonstrated that the contamination of traditional cheese in Chaharmahal and Bakhtiari province, Iran with O157:H7 was zero; but, other *E. coli* serotypes were isolated[13]. The study by Marhamatizadeh *et al.* on Kazerroun traditional cheese diagnosed *Listeria monocytogenes* and positive-coagulase *Staphylococcus* as the cause[14]. Ogier and Serror[15] showed that among Mediterranean cheeses, a number of enterococcus was reached cheese 10^5–10^7 CFU/g[16].

In the present study, 2 cases of *Staphylococcus* infection was observed in the cheese and buttermilk samples. *S. aureus*-caused disease has been the third-ranking factor of food poisoning in the world[17].

Aragon-Alegro *et al.* in Brazil reported that 15.1 dairy and take away food samples were contaminated with *S. aureus* (positive coagulase)[18]. Akineden *et al.* in Germany declared that 17.7% of goat cheese had positive coagulase *S. aureus*[19].

The results of a study in Yazd, Iran demonstrated that 7.1% of samples were unacceptable (microbial quality) and 40.5% of yogurt samples were contaminated with *Staphylococcus*[20].

Jayarao *et al.* concluded that coliform in a herd cattle US which health management and good governance to be effective in improving the quality of raw milk[21].

A study by Desai *et al.* was conducted in India and microorganisms has been reported to be 10^4 to 1.8×10^6[22].

The results of studies in Lorestan province, Iran showed coli form and *E. coli* contamination of raw milk in the region[23].

Van Schaik *et al.* believed that there were gathers milk from letdown stage until delivery to factory raw milk has a significant effect on bacterial load[24], which were consistent with the results obtained in this study.

The findings of Sangatashetal *et al.* demonstrated that the yogurt drink not respecting the standards of plant design and production of natural resources there are several pollution of the factories. Also, a variety of bacterial and fungal contamination was reported in the mentioned study[25].

Microbiological and chemical qualities of ayran manufactured by two methods such as of handy and industrial to cities of in Turkey (Ankara and Karz) emphasized that number of mold and yeast was more from standard level[26].

The role of milk and milk products in human nutrition, especially in children and child nutrition and restriction for food's human in some countries and the risk of existence aflatoxin in human foods and animal that is more important[27]. The results showed that a large percentage of the cheese produced from milk of cattle farms was contaminated with aflatoxins or *Aspergillus* spores[28]. In other studies, contamination of dairy products and cheese with mold and aflatoxinm has been reported[29-32].

Kaniou-Grigoiadou *et al.* suggested that the contamination of starter of buttermilk, washing with contaminated water, contaminated packaging materials, and lounge air with yeast are causes of yeast infection for buttermilk[33]. Microbial quality of the products was excellent.

However, processed and raw foods are frequently contaminated with a variety of pathogens. Therefore, it is necessary to determine the critical control points in organizing factories and automated control systems in order to eliminate or minimize the threat of pollution.

The use of herbs spice that have the anti-microbial effects in dairy products is a good strategy to reduce the microbial load of the product[34-58].

Conflict of interest statement

We declare that we have no conflict of interest.

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