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ORIGINAL ARTICLE

# Microbiological and Physicochemical Analysis of Traditional Dairy Products in Shiraz, Southern Iran

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ABSTRACT

**Background:** The production of milk and dairy products is an important part of Iran's economy. Traditional products such as dairy products due to poor hygienic conditions during storage, improper heat treatment, and secondary pollution can be a suitable medium for the growth, reproduction as well as transmission of a wide range of microbial pathogens. This study aimed to investigate the physical, chemical, and microbial properties of traditional dairy products of Shiraz in comparison with the Iran National Standards Organization (INSO).

**Methods:** In an experimental study, 130 samples of traditional dairy products were randomly purchased from retail markets in Shiraz, southern Iran. The traditional dairy products included 25 samples of cheeses, 25 pieces of kashk, 25 dishes of yogurt, 25 bottle of dough, 15 package of butter, and 15 vessel of sarshir (cream). The test methods were based on Iranian Standard Organization.

**Results:** High microbial contamination, presence of heavy metals, adulterations, and out-of-standard physical properties were observed in some traditional dairy products.

**Conclusion:** There were two categories of food safety concerns in dairy products including microbiological and chemical hazards such as primarily foodborne pathogens and chemical-related concerns such as antibiotics, pesticides, and herbicides. Therefore, to prevent microbial and chemical contaminations, several management programs are required to improve the quality of milk and dairy products. Continuous monitoring of dairy products and their distribution units should be one of the essential programs for policy makers and health officials.

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## Introduction

Milk and dairy products play an important role in the human diet. The traditional milk and dairy market in Iran has expanded over the years. Consumption of traditional dairy products has been considered for several reasons, including local production, cultural factors, good taste, and belief in the health benefits of natural and unprocessed products. However, the consumption of these products is associated with risks and challenges

due to insufficient supervision (1, 2). Milk and dairy products are one of the important sources, which could be infected by biological agents. It is estimated that 70% of infectious diseases could be transmitted to human from unhealthy food and over 450 types of viral, fungal, parasitic and microbial diseases could be transmitted to human as zoonotic food sources regarding milk and dairy products as the major parts of these foods. If the production and distribution are not in line with hygienic methods and are not aseptic, it could cause transmission of zoonotic diseases and food poisoning (3, 4).

Dairy products are contaminated by microorganisms during the stages of production, supply, and storage until the expiration date due to non-observance of hygiene principles (5, 6). Fungi are a very large, diverse, and heterogeneous group of microorganisms that can be found in almost every climate (7, 8). Mold growth in dairy products causes economic damage through texture weakening, color change, aroma, and flavor. However, a more serious concern about molds is that some of them are able to produce a variety of mycotoxins such as aflatoxin, ochratoxin, patulin and citrinin, which will have harmful effects such as carcinogenicity on the health of consumers (9-11). Another possible contamination of milk and dairy products was contamination with heavy metals such as lead and cadmium (12, 13). Cadmium, for example, can accumulate in tissues such as the liver and kidneys and causes cancer, anemia, and high blood pressure. High concentrations of lead were demonstrated to have harmful effects on the nervous system, kidneys, lungs, bone marrow, and blood (14, 15). Heavy metals represent a special group of food contaminants and the entry of heavy metals into the environment has recently increased dramatically due to rapid industrialization and the use of new technologies that require heavy metals (14, 16).

Other problems with dairy products include adulterations such as addition of palm oil as a substitute for natural fats, baking soda to contaminated and spoiled milk by ranchers, formalin to prevent microbial growth, starch to thicken the crop, etc. (17). Traditional dairy products have received a lot of attention from consumers in recent years, so the review of these products is very important for both government agencies and consumers. Therefore, the analysis of these products in order to identify risk factors is very important in increasing consumer awareness. The aim of this study was to find the most important health risk factors of traditional dairy products including traditional cheeses, traditional kashk, traditional yogurt, traditional dough (kind of drink), traditional butter, and traditional sarshir (cream) that were distributed in Shiraz, southern Iran and to evaluate their physical, chemical, and microbial properties based on Iranian National Standard.

## Materials and Methods

A total of 130 samples of traditional dairy products were randomly purchased from retail markets in Shiraz, Iran (Figure 1). The traditional dairy products included 25 samples of cheeses, 25 pieces of kashk, 25 dishes of yogurt, 25 bottle of dough, 15 package of butter, and 15 vessel of sarshir. The samples were collected in 4-6°C and transported to the laboratory inside a cold box. The pH and acidity of the dairy products were measured using a pH meter (Mi 180 Bench Meter, Szeged, Hungary) based on titration method and INSO No. 2852 (18). The protein content of cheese and kashk was measured applying the Kjeldahl method according to INSO No. 1811 and 639, respectively. Kashk samples were evaluated for heavy metals of lead and cadmium using the atomic absorption spectroscopy (Shimadzu, Japan), and the results were compared with the maximum allowable levels set by the Codex Alimentarius Commission.

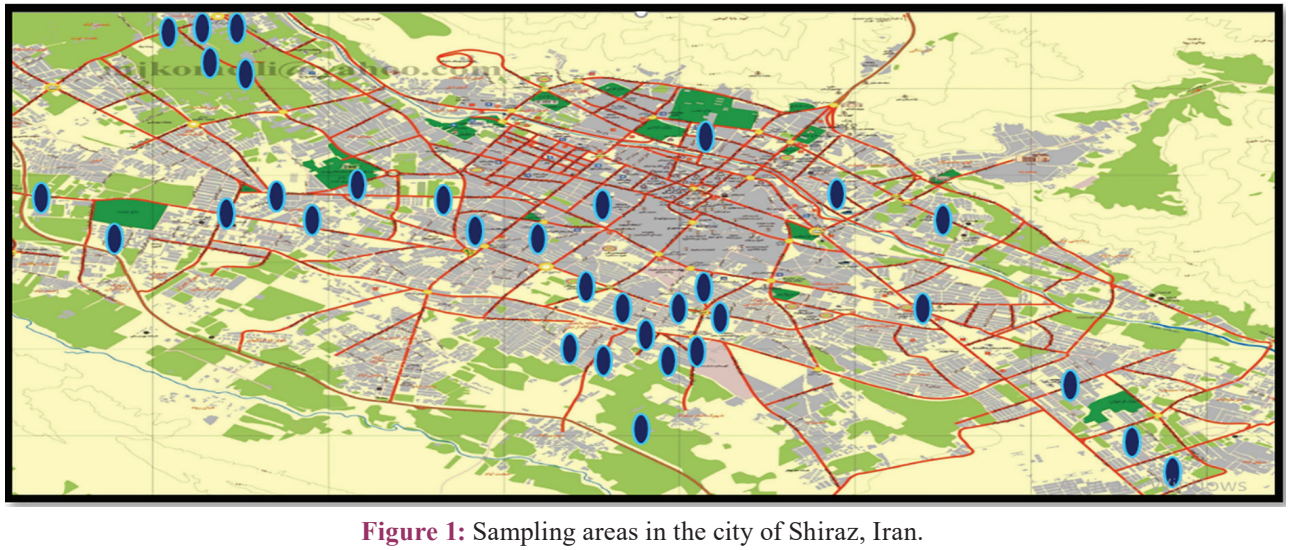

**Figure 1:** Sampling areas in the city of Shiraz, Iran.

The gas chromatography (GC) method was used to measure the plant sterol levels. The first and most important step in the analysis of phytosterols was sample preparation that was applied on a thin layer of chromatographic plate (TLC). Standard cholesterol solution with a concentration of 1 mg/mL for plant samples and botulin solution with the same concentration for animal samples were spotted at a distance of 1 cm on both sides of the TLC plates. The plates were placed in a TLC tank containing 65 mL hexane and 35 mL of ethyl ether. The solution was then injected into the GC-MS with a syringe (19).

Butter and sarshir samples were evaluated for peroxide value (PV). PV was determined according to the AOCS method of Cd8-53. Molds and yeasts, coliforms, and psychotropics bacterial count were determined in the samples by the INSO methods of 10154, 11166 and 3451 or 2406, respectively (20). Yeast glucose chloramphenicol agar was used to count mold and yeast. Violet red bile agar was used to assess coliform count by the pour plate method. Also, a nutrient medium such as plate count agar was employed to count psychrophilic bacteria. All tests were performed in three replications. Data analysis was performed using Microsoft Office Excel (version 2016, USA) and results were expressed as mean±standard error. Data were compared with the INSO.

## Results

The pH and acidity of dairy products including yogurt, cheese, kashk, dough, and sarshir were shown in Table 1 and Figure 1-5. Based on the standard values, only 4% of cheese samples showed a pH higher than the standard range. Moreover, the pH value of 53% of the samples was out of the standard range of INSO 2006. As shown in Table 1,

**Table 1:** The pH and acidity values of dairy product samples.

| Product | Property | Standard | Standard Error | Out of range (%) |
|---|---|---|---|---|
| Yogurt | pH | Max 4.6 | 3.97±0.030 | - |
| | Acidity | 1.3-2 | 1.28±0.047 | 8 |
| Cheeses | pH | Max 6.6 | 6.00±0.096 | 4 |
| | Acidity | Max 0.4 | 6.00±3.212 | 8 |
| Kashk | pH | Max 4.5 | 3.47±0.034 | - |
| | Acidity | 1.3-2 | 1.95±0.315 | 58.4 |
| Dough | pH | Max 4 | 3.43±0.041 | - |
| | Acidity | 0.7-1 | 1.21±0.044 | 84 |
| Sarshir | pH | 6.5-6.8 | 6.43±0.051 | 53 |
| | Acidity | 0.09-0.15 | 0.26±0.016 | 93 |
| Butter | pH | - | - | - |
| | Acidity | Max 0.4 | 0.58±0.073 | 67 |

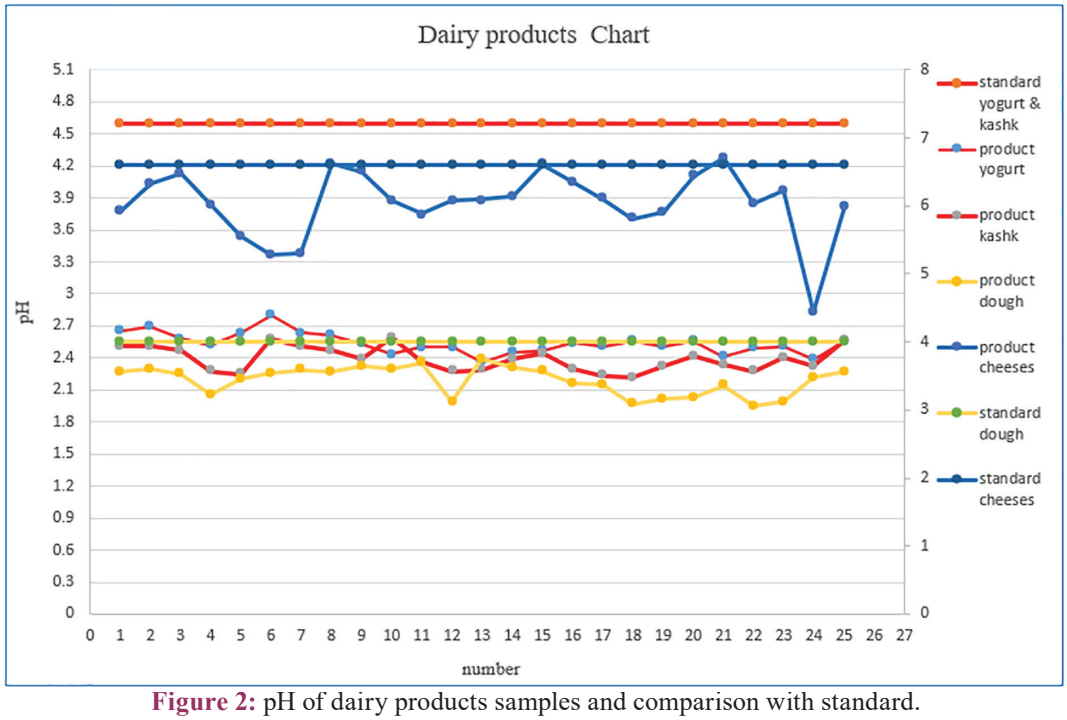


**Figure 2:** pH of dairy products samples and comparison with standard.

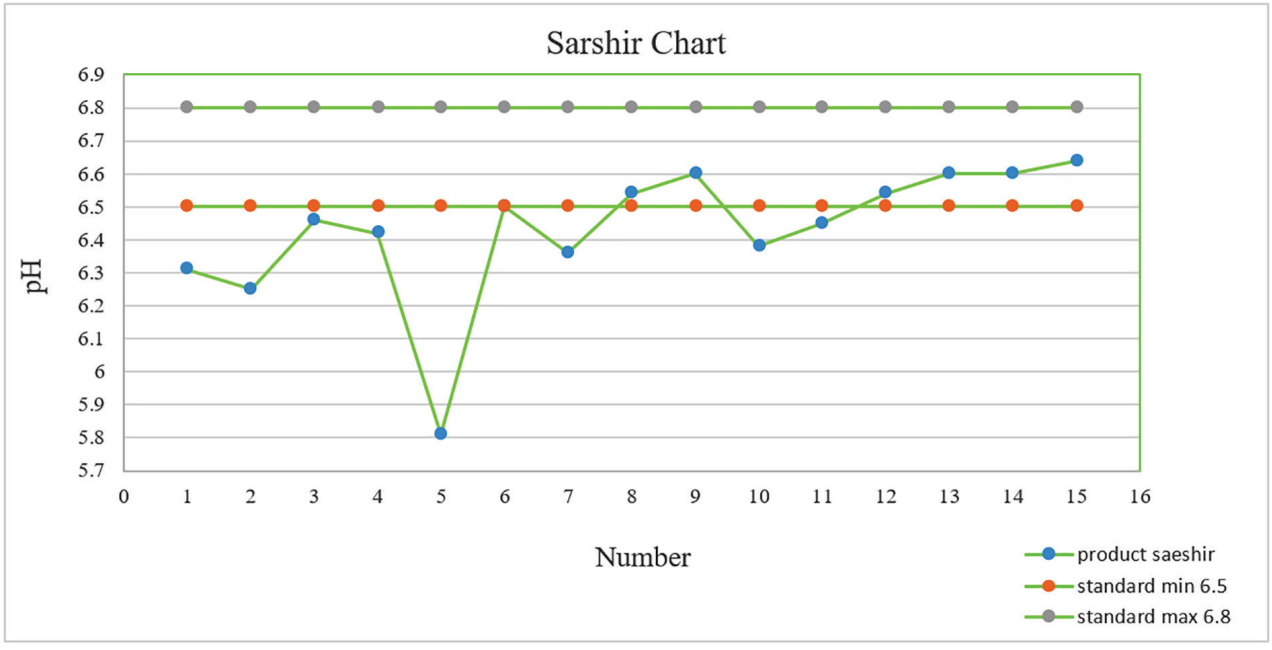


**Figure 3:** pH of sarshir samples in comparison with standard values.

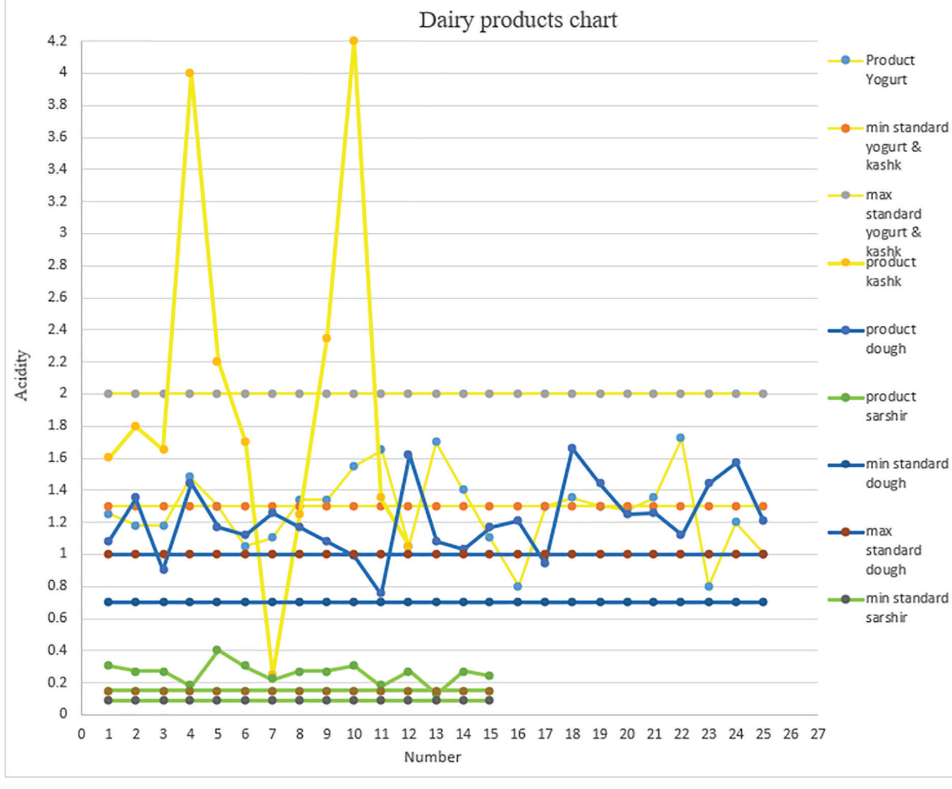


**Figure 4:** Acidity of dairy products samples in comparison with standard values.

the acidity values of 8% of yogurt, 8% of cheese, 58.4% of kashk, 84% of dough, 67% of butter, and 93% of sarshir samples were out of the standard value. Based on standards values of INSO No.2452 and 6629, 4% of cheese and 4% of whey samples were out of the standard range. The protein content of cheese and kashk samples was a minimum of 12 and 13, respectively that was within the standard range. The maximum concentration of lead and cadmium recommended by the Codex 2000 was 0.01 ppm and 0.01 ppm, respectively. The amounts of lead in the kashk samples were much less than the 0.01 ppm. On the contrary, the amounts of cadmium were much more than the limit set by the Codex 2000.

In the investigations carried out on phytosterols in yogurt, only one sample contained phytosterols with a concentration exceeding the standard. The amount of phytosterols in this sample was approximately 3%.

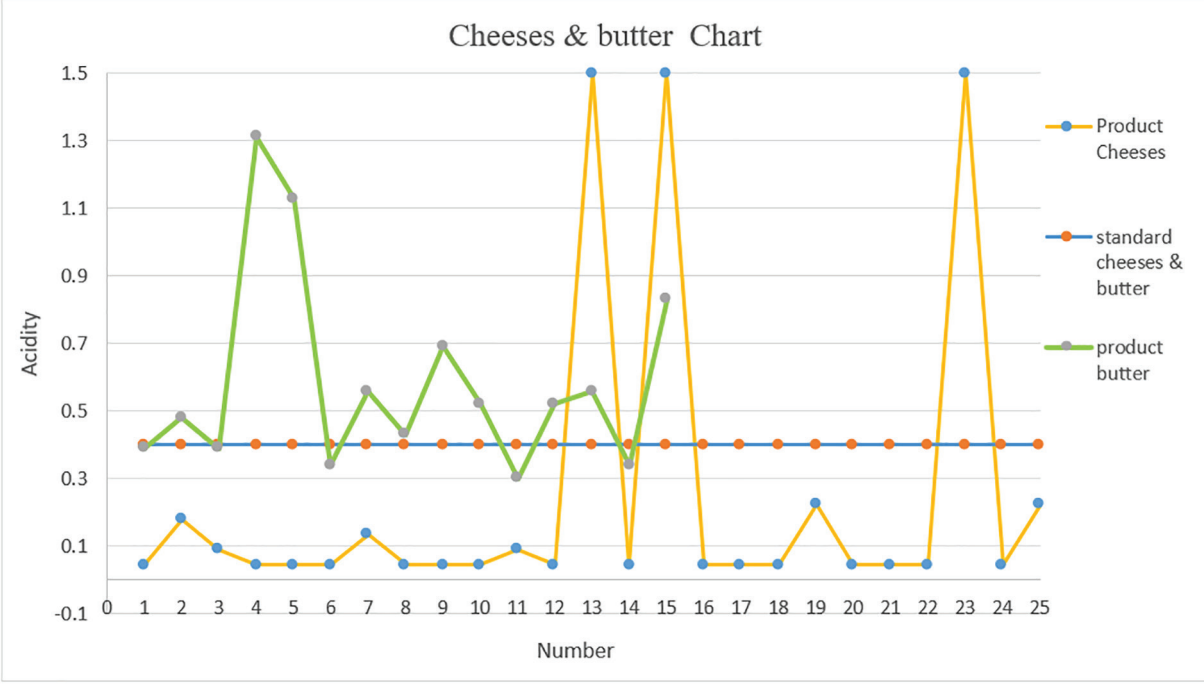


**Figure 5:** Acidity of cheese and butter samples in comparison with standard values.

**Table 2:** Microbial contamination (CFU/g) of dairy product samples.

| Product | Mold and yeast | | Coliform | | Psychrophilic bacteria | | Total count | |
|---|---|---|---|---|---|---|---|---|
| | Standard | Out of range% | Standard | Out of range% | Standard | Out of range% | Standard | Out of range% |
| Yogurt | Max 100 | 72 | Max 10 | 32 | - | - | - | - |
| Cheese | Max 100 | 100 | Max 10 | 100 | - | - | - | - |
| Kashk | Max 100 | 100 | Max 10 | 72 | - | - | - | - |
| Dough | Max 100 | 40 | Max 10 | 80 | - | - | - | - |
| Sarshir | 0 | 100 | Max 10 | 60 | Max $10^4$ | 53 | Max $2\times10^4$ | 33 |
| Butter | Max 100 | 93 | Max 10 | 93 | Max $10^4$ | 87 | Max $2\times10^4$ | 87 |

In this study, peroxide valves of 33% of the butter samples were higher than the standard limit; while peroxide values of sarshir samples were within the standard range. Dairy products were evaluated for microbial contamination including molds and yeasts, coliforms, psychrophilic bacteria, and total count. Table 2 shows the microbial contamination of traditional dairy products that in comparison to the standard values, most samples were highly contaminated.

## Discussion

The pH value of 53% of the samples was out of the standard range of INSO 2006. As shown, the acidity value of 8% of yogurt, 8% of cheese, 58.4% of kashk, 84% of dough, 67% of butter, and 93% of sarshir samples were out of the standard value (18). The pH was shown to be affected by the type of milk and especially by the fermentation time. The pH value can be changed due to the fermentation activity of microorganisms such as lactic acid bacteria and the production of fatty acids and amino acids (21). Total acidity is an inherent factor that can affect the shelf life, as well as the taste and aroma of dairy products (22). Prolonged and uncontrolled fermentation process can affect the acidity level. In traditional dairy products, due to the lack of appropriate measurement system and absence of skilled qualified personnel, the fermentation process is not performed properly, which can have adverse effects on the characteristics of the final product. Overgrowth of lactic acid bacteria can lead to high production of lactic acid and an increased acidity. On the other hand, increasing the concentration of SNF causes a significant increase in the acidity of products (23).

Among different dairy products, the protein contents of cheese and kashk samples were evaluated due to the importance of protein contents in these products. Based on standards values INSO No.2452 and 6629, 4% of cheese and 4% of kashk samples were out of the standard range. The importance of proteins and their role in the ripening period of cheese is such that the percentage of soluble proteins

in the total cheese protein is considered the ripening index of cheese. During the production stage and ripening period, under the influence of renin and other protease enzymes, long-chain peptides are broken down into short-chain peptides. Proteolysis was demonstrated to affect the flavor of cheese too. Lactobacilli are part of the microbial flora of cheese that plays an important role during ripening. This group of microorganisms increases the levels of short-chain peptides, free amino acids, and free fatty acids in cheese samples (24). In the case of kashk, the low content of protein may be related to the type of yogurt used for the production of kashk (25). On the other hand, lipolytic and proteolytic activities of molds and yeasts can affect the amounts of protein (26).

Heavy metals including lead and cadmium were measured in kashk due to the distribution of this product in bulk (unpackaged) and its exposure to polluted air and car smoke. The amounts of lead in the kashk samples were much less than 0.01 ppm. On the contrary, the amount of cadmium was much more than the limit set by the Codex 2000. Rapid and uncontrolled growth of the industry in developing countries can be the main reason for the increased level of heavy metals in food products (27). The application of industrial wastewater for agriculture has led to soil pollution, which represents a threat to the environment and food security. Moreover, drinking contaminated sewage by animals has caused the accumulation of heavy metals in their bodies (28). Lead and cadmium levels were measured in milk samples collected from Zabol City and were shown to be within the standard permitted level (28). Abo El-Makarem *et al.* illustrated that the amount of lead and cadmium in the milk and dairy products in Egypt were higher than the standard permitted level (29).

Cholesterol is the predominant sterol in animal fats, while vegetable oils contain a mixture of phytosterols and mainly beta-sitosterol (30). The presence of beta-sitosterol in dairy products is an indicator of adulteration. Cholesterol, along with polar lipids, proteins, glycoproteins, gangliosides, and enzymes, is a key component of milk fat cell membranes and is responsible for maintaining membrane fluidity and integrity (19). The beneficial effects of phytosterols in reducing the harmful effects of cholesterol in the body as well as preventing bowel cancer are well-known, but it should be noted that one of the main rights of consumers is full knowledge of the composition of foods consumed (30).

In this study, peroxide valve of 33% of the butter samples was higher than the standard limit; while peroxide value of sarsir samples was within the standard range (31). The high moisture content of traditional butter may affect its microbiological, physical, and chemical quality. The presence of water in butter can activate lipases, stimulate the growth of microorganisms, and cause the hydrolysis of triglycerides (32). Butter contains saturated fatty acids and cholesterol and their oxidation process occurs during the storage period and can be accelerated by factors such as exposure to air, light, and temperature (33). Moreover, the high peroxide value of traditional butter samples can be attributed to the presence of some peroxidants such as copper or some enzymes.

Milk and dairy products were evaluated for microbial contamination including molds and yeasts, coliforms, psychrophilic bacteria, and total count (34). It was shown when our samples were compared with the standard value; most of them were highly contaminated (20). The microbial population of dairy products may be affected by the chemical composition of milk, the microbial population of raw milk, differences in traditional processing methods, packaging materials, storage conditions, and post-processing contamination (34). The spoilage microorganisms in dairy products include Gram-negative psychrophiles, coliforms, lactic acid bacteria, yeasts, and molds. Extracellular lipases are one of the most important enzymes secreted by organisms involved in the breakdown of lipids in dairy products (such as unsalted white butter) (35). Based on the optimum growth temperature of molds and yeasts and its comparison with the growth temperature of lactic acid and thermophilic bacteria, the high population of molds and yeasts in yogurt can be due to its storage at room temperature; which is optimum (about 25°C) for the growth of molds and yeasts (36). Other factors affecting the growth of molds and yeasts in dairy products include their ability to grow at low temperatures, to ferment sucrose and lactose, to produce a variety of extracellular enzymes for hydrolysis of different substrates such as fat and protein molecules, and to produce lactic acid and citric acid in products and resistance towards preservatives (37).

Moreover, contamination of dairy products can be divided into two categories of direct and indirect contaminations. In the direct type, contamination is accidental, such as contamination caused by poor hygiene in the production and supply process. Indirect contamination is caused by moldy livestock feeding or feeding of foods containing fungal spores (38). In general, the quality of raw milk, pasteurization process, hygiene of packaging containers, air condition of the production hall, filling equipments, nozzles, and the water used for

washing can be a possible source of contamination with coliform bacteria, molds, and yeasts that can have a great influence on the microbial quality of the final product (39, 40).

## Conclusion

Our findings revealed a high microbial contamination, presence of heavy metals, adulterations, and out-of-standard physical properties in some traditional dairy products. Therefore, to prevent the microbial and chemical contaminations, several management programs are required to improve the milk quality and the quality of the dairy products. Continuous monitoring of dairy production and distribution units can be one of the essential programs. Dairy farms health is also of special importance to reduce the milk contamination. The production environment, by hygiene practices in herd management and good milk storage is also of great importance. Traceability of milk and dairy products, from the production-distribution chain as well as consumption can be a good policy for quality assurance and reducing the public health risks.

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## Authors' Contribution

RNS: Software, methodology, data curation, and writing the original draft. MM: Writing the review and editing, validation, supervision, resources, project administration, methodology, investigation, funding acquisition, and conceptualization. MG: Writing the review andediting, validation, supervision, resources, project administration, methodology, investigation, funding acquisition, and conceptualization. ME: Software, methodology, data curation, and writing the original draft. MHE: Validation and methodology.

## Conflict of Interest

The authors declare no conflict of interest.

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