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Evaluation of the microbial quality of the traditional yogurt and kashk in Rasht City

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ABSTRACT

Milk and dairy products such as yogurt and kashk have long been used as part of the human diet and are among the most important suppliers of a large part of the human need for calcium. This study aimed to investigate the microbial quality of traditional yogurt and kashk samples in Rasht City. 25 samples of traditional yogurt and 25 samples of traditional liquid kashk were randomly collected from the supply centers of Rasht from December to March 2021. The microbial quality of the samples was investigated in terms of contamination with coliform, *Escherichia coli*, coagulase-positive *staphylococci*, sulfite-reducing *clostridia*, mold, and yeast. The contamination with coliform in traditional yogurt and kashk was 0.35 ± 0.12 and 0.11 ± 0.08 log CFU/g, and the contamination with mold and yeast in traditional yogurt and kashk was 2.07 ± 0.52 and 0.60 ± 0.18 log CFU/g, respectively. No significant difference was found between the samples of traditional yogurt and kashk in terms of contamination with *E.coli*, but a significant difference was between traditional yogurt and kashk samples in terms of contamination with mold and yeast and their contamination level was much higher. According to the results, the microbial quality of traditional yogurt was low. Therefore, health control of production units and monitoring of traditional yogurt production in Rasht City should be done continuously until the contamination in traditional yogurt is reduced.

1-Introduction

Since milk and its derivatives are high in nutrients and promote rapid body growth, they are an essential component of the human diet. Some microorganisms found in milk and its derivatives are advantageous, while others are harmful. Humans can get infections from milk and its byproducts [1].

One of the most well-known fermented foods in the world, particularly in the Middle East, is yogurt. The process of making yogurt involves heating milk to a boiling temperature and then adding yogurt starter to the milk once it has cooled. However, due to unhygienic manufacturing circumstances, traditional goods like yogurt made in the traditional method may provide an environment conducive to the development and reproduction of harmful microbial organisms [2].

One fermented milk product that may be made by boiling and condensing yogurt, buttermilk, or buttermilk is kashk. It is a fermented milk beverage that is typically enjoyed outside in the sun. It is known as Kurut in Eastern Anatolia, Turkey; the word comes from the Turkish language and signifies drying. This substance can be made either dry or liquid. The milk used to make kashk might be from sheep, goats, cows, or a combination of them. Kashk is produced in the food business by industrial methods and is made straight from milk as liquid kashk. Dry kashks, which are typically manufactured in an unpasteurized state, are ground and diluted to create traditional liquid kashks [3 and 4].

Some of the studies that have been done on the microbiological quality of kashk and yogurt in Iran are discussed. Haji Mohamadi Farimani et al. investigated the microbiological and chemical aspects of traditional nomadic yogurt. The province of Khorasan Razavi nomadic regions provided the samples. Together with chemical properties, the total amount of form, coagulase-positive Staphylococcus aureus, yeast, mold, and bacteria that produce lactic acid were examined. Only one yogurt sample, made from milk that had only been heated to 40 ^{o}C, had positive Escherichia coli and 2.38 log CFU/g of coliform. Every sample tested negative for Staphylococcus aureus. Certain samples included significant concentrations of mold and yeast, which suggested unhygienic conditions both before and after fermentation [5].

Rahbar et.al examined the microbiological state and quality of the traditional yogurt made in Rasht City, focusing on the identification of mold, yeast, Staphylococcus aureus, and Escherichia coli. Regarding coliform, Escherichia coli, and Staphylococcus aureus contamination, every sample fell within the accepted range. Five samples of traditional yogurt were found to be contaminated with mold [6]. The microbiological quality of twenty-four yogurt samples was examined in Turkey. Unhygienic production conditions are indicated by the presence of coliform in five cow yogurts and *Escherichia coli* in one [7]. The current study aims to investigate the microbiological quality of yogurt and traditional kashk supplied in Rasht City, given that these products are not evaluated for microbial quality at production centers and regulatory agencies only collect samples and assess microbial quality in response to customer complaints.

2- Materials and methods

2.1. Sampling

A cross-sectional, descriptive research design was used in the current study. 25 samples of traditional liquid kashk and 25 samples of traditional yogurt were randomly selected from supply centers in Rasht City from December to March 2021. The samples were delivered in sterile containers with refrigeration to the microbiology laboratory. According to the Iranian national standard, microbial tests were conducted on the samples, including counting coliforms, detecting *Escherichia coli*, detecting positive *Staphylococcus aureus* coagulase, counting mold and yeast for yogurt and kashk, and counting regenerating *clostridium* for kashk.

2.2. Culture method

The national standard number 5486 was followed for the overall counting of the form. First, 90 ml of buffered peptone water was used to dilute 10 grams of the sample. Then, 10-fold serial dilutions were made, and 1 ml of each dilution was put on an empty plate. Finally,

Violet-Red Bile Agar molten media (HiMedia, India) was added, and the plate was moved in the shape of eight Latins on a level surface. The cultivation process used a two-layer pour plate. A 48-hour incubation period at 30 °C was conducted. After counting the plates with 10 to 150 colonies, the quantity of coliforms was ascertained by applying the formula (8).

$$N = \frac{\Sigma c}{(n1 + 0.1\, n2) \times f}$$

N: the quantity of coliform colonies in one gram of the sample
Σc: the total number of colonies detected in two dilutions with between 10 and 150 colonies
f: The amount of sample in the first selected dilution;
n1: The number of plates in the first selected dilution;
n2: The number of plates in the second selected dilution
Ten red-colored coliform colonies were transferred to ten tubes containing Brilliant Green Bile Broth (HiMedia, India) and Durham tube, and the tubes were then placed in an incubator at 37 °C for 24 h to confirm the formation of gas. It was thought that the discovery of gas bubbles would verify that the samples contained general form [8].
The existence of thermotropic coliform in the samples was first verified before examining the Escherichia coli. The samples were stored in an incubator for 24 h at 44.5 °C, while the verified cultivars were cultivated in a bright green environment at the coliform stage. The IMViC test was repeated on each positive tube if turbidity was detected, which verified the presence of thermotropic coliform in the tubes. The sample was cultivated in SIM, MRVP, and Simmons' Citrate Agar (HiMedia, India) medium and maintained at 37 °C for 24 h in an incubator to perform the IMViC test. When three drops of Kovac's reagent are added to a tube containing SIM media for the indole test, if a red color develops in the upper layer, the test is deemed positive. The methyl red test was performed by adding methyl red reagent to a tube containing MRVP medium. The test was deemed positive if a consistent red color was seen.
Alpha-naphthol reagent and potassium solution were added to the tube with MRVP medium for the Voges-Proskauer test. A color complex is deemed to indicate a positive test result. When placing the citrate test sample in the incubator, the test was deemed positive if a blue color was noticed. *Escherichia coli* was thought to be present based on the IMViC pattern, which read - - + + [9].

Ten grams of the sample were combined with ninety ml of buffered peptone water, and the mixture was then cultivated for twenty-four hours at 37 °C in Giolitti-Cantoni Broth (HiMedia, India). Subsequently, the sample was grown linearly in Mannitol Salt Agar (HiMedia, India) and Baird-Parker Agar (HiMedia, India), which included egg yolk and potassium tellurite, and was maintained in an incubator for twenty-eight hours at 37 degrees Celsius. The tube coagulase test and rabbit plasma were used to examine and validate the coagulase characteristics of *Staphylococcus aureus* strains [9].
To count the mold and yeast, 10 grams of the sample were added to 90 ml of peptone water, and 0.1, 0.01, and 0.001 dilutions were made using the national standard number 10154. Subsequently, 0.1 ml of every dilution was added to the DRBC Agar (Dichloran-Rose Bengal Chloramphenicol Agar) growth medium (HiMedia, India) and allowed to grow superficially. The plates containing 10 to 150 colonies were then counted after being maintained at 25 °C for 5 days [10].
The kashk sample was subjected to sulfite-reducing clostridium counts using National Standard No. 2197. One milliliter of the diluted sample was put onto two empty plates after 10 grams of kashk sample and 90 milliliters of peptone water were well mixed. The plates were filled with 10 ml of molten Sulfite Polymyxin Sulfadiazine Agar medium (HiMedia, India) and mixed using a figure-eight motion on a level surface.
Following the medium's hardening, 5 ml of molten media were added, and the plates were then left in an anaerobic jar with a 37 °C temperature for 20 h. 150 black colonies on a plate were chosen and counted. Verification tests were run on the five chosen black pages. Gelatin melting, nitrate reduction, and culture in mannitol and lactose sugar medium were among the confirmatory assays. The quantity of Clostridium in the culture sample was measured upon confirmation of the positive colonies [11].

2.3. Statistical analysis

The traditional yogurt and traditional liquid kashk sample groups were compared using the t-test for the studied variables. The percentage of samples with microbial contamination over the allowable limit was compared using the chi-square test. SPSS software (version 24) was used for all statistical computations. Furthermore, this study's significance level ($p < 0.05$) was deemed to be less than 0.05. The Ethics Committee of Tabriz Islamic Azad University of Medical Sciences provided the study's ethics ID code (IR.IAU.TABRIZ.REC.1401.068).

3- Results and Discussion

Table 1 displays the results of the microbiological quality analysis of samples of traditional kashk and yogurt from Rasht City. No significant difference was found regarding the levels of coliform contamination in the two types of products. *Escherichia coli* contamination was only found in the conventional yogurt sample. In both kinds of samples, *Staphylococcus aureus* contamination was observed. The traditional yogurt sample had the greatest level of mold and yeast contamination, and it differed from the traditional kashk sample considerably ($p < 0.05$). Kashk was found to be contaminated with sulfite-reducing *Clostridium*. The Iranian national standard, No. 2406, is the basis for determining the sample standard limit [12].

Table 1. Comparison of microbial quality of yogurt and kashk samples in Rasht City

Test	Traditional yogurt (Mean ± SEM)	Traditional kashk (Mean ± SEM)	Acceptable level [12]	*P* value
Coliform log CFU/g	0.35 ± 0.12	0.11 ± 0.08	1	0.111
Escherichia coli	Positive	Negative	Negative	-
Staphylococcus aureus- Coagulase positive	Positive	Positive	Negative	-
Mold and Yeast log CFU/g	2.07 ± 0.52	0.60 ± 0.18	2	0.002
Sulfite-reducing *clostridia* log CFU/g	-	0.08 ± 0.06	2	-

The significance level is calculated based on the t-test.

The comparison of the number of samples contaminated over Iran's national standard's allowable level is displayed in Table 2. The results demonstrated that traditional yogurt had the maximum number of non-conforming samples ($p < 0.05$) considering the number of samples that contained mold and yeast above the standard limit. The level of contamination in the contaminated samples was less than the allowable limit considering the sulfite-reducing *Clostridium* in kashk.

Table 2. Comparison of the number of samples with microbial contamination exceeding the acceptable level in Rasht City

Test	Traditional yogurt		Traditional kashk		*P* value
	No (%) of samples exceeded the acceptable level	No (%) of samples at an acceptable level	No (%) of samples exceeded the acceptable level	No (%) of samples at an acceptable level	
Coliform	3 (12%)	22 (88%)	2 (8)	23 (92%)	0.637
Escherichia coli	1 (4%)	24 (96%)	0	25 (100%)	0/312
Staphylococcus aureus- Coagulase positive	3 (12%)	22 (88%)	3 (12%)	22 (88%)	1
Mold and Yeast	8 (32%)	17 (68%)	0	25 (100%)	0.002
Sulfite-reducing *clostridia*	-	-	0	25 (100%)	-

The significance level is calculated based on the chi-square test.

Numerous manufacturers are engaged in the manufacturing of various fermented milk products in Iran. Given that these products are mostly acidic, there are instances when there is a lack of attention to health regulations throughout the production process. It is crucial to ensure hygienic quality because these items do not undergo heat treatment before ingestion [6]. Except for the amount of mold and yeast contamination in traditional yogurt, the condition of the samples that were collected, according to the present study conducted in Rasht City, revealed satisfactory conditions in other situations.

The typical yogurt sample had higher levels of mold and yeast contamination than the traditional kashk sample. Food molds have the potential to produce fungus-induced toxins that can have a long-term negative impact on consumer health. Controlling mold contamination of food is crucial since mycotoxins are known to cause cancer. Both developed and developing countries have seen an increase in the prevalence of food-borne illnesses in recent years [13].

Rahbar et al.'s investigation on the microbiological quality of 23 samples of traditional yogurt in Rasht City found that there was no evidence of *Staphylococcus aureus* or *Escherichia coli* contamination, and the coliform contamination was within the expected range [6]. However, in the current research, *Escherichia coli* contamination was found in 12% of the conventional yogurt samples and coliform levels surpassed the acceptable limit.

Saprophytic mold-contaminated milk products were isolated and identified in a study conducted in the city of Isfahan. From various parts of Isfahan City, 200 distinct samples of pasteurized milk products—70 samples of cheese, 60 samples of doogh, 40 samples of yogurt, 20 samples of kashk, and 10 samples of cream—were collected and tested for the presence of saprophytic molds. 33 (16.5%) of the samples were unsatisfactory, 50 (25%) were acceptable, and 117 (58.5%) were favorable overall. The maximum levels of mold contamination (beyond the allowable limit) were found in cheese, cream, kashk, doogh, and yogurt, respectively [14]. The present study's findings indicated that samples of yogurt and kashk were contaminated with both mold and yeast. The rate of contaminated samples was greater compared to the study carried out in Isfahan City. This difference may have resulted from the samples pasteurized in the Isfahan study.

Keshavarzpour et al. (2014) examined the microbiological quality of milk and its traditional and industrial products in Isfahan City. A total of 155 samples were collected, comprising 30 samples of milk, 26 samples of yogurt, 50 samples of doogh, and 49 samples of cheese. Overall, the rate of samples that exceeded the allowed limits for coliform was 9.7% (15 samples), *Escherichia coli* was found in 11 samples (77.1%), and yeast and mold in 20 samples (13%) [15].

Karajibani et al. investigated the microbiological contamination of pasteurized milk and its products in Zahedan City. Over ten months, 551 samples—244 pasteurized milk samples, 165 yogurt samples, and 142 pasteurized doogh samples—from Zahedan City's milk and its product manufacturing units were chosen at random, and the microbiological quality was assessed. The data indicated that 13.1% of the pasteurized milk samples did not meet the norm. Additionally, the pasteurized yogurt and doogh samples had deviations from the norm of 18.28% and 4.9%, respectively. *Staphylococcus aureus* accounted for the majority of the microbiological contamination in the samples (45.5%) [16]. The fact that the current study's sampling season was colder than the prior study's may have contributed to the lower rate of infected samples in the current research.

Azimi et al. analyzed the chemical, microbiological, and sensory properties of kashk that were sun- and hot air-dried. The kashk samples were dried at 50, 60, and 70 ^{o}C using two methods: hot air and the sun. Chemical characteristics, general form, total microbial population, mold, and yeast were evaluated upon drying.
The findings indicated that kashk dried at 50 degrees Celsius had the maximum microbiological burden, whereas kashk dried at 60 and 70 ^{o}C had the minimum microbial load. Every sample of kashk was clear of coliform. [3]. In the current study, 8% of the kashk samples had coliform counts higher than expected. Coliform contamination in food often suggests that the handling and processing of food was not done with proper cleanliness. Three products—kashk, Qaraqorut, and butter oil—produced in the nomadic regions of the provinces of Chaharmahal and Bakhtiari were evaluated by Bagheri and Shaviklo for their microbiological quality. Kashk samples did not include *Staphylococcus aureus* or *Escherichia coli*. Mold and yeast counts in 25% of the kashk samples were higher than the allowable limits, whereas coliform counts in every sample were below the allowable limits [17].
Amin Ekhlas et.al (2022) examined the microbiological quality of traditional liquid kashk samples in Hamedan City. Sampling from six traditional kashk makers in Hamadan City was done for this investigation. The samples' mean concentrations of coliform, mold, and yeast were 1.64, 7.39, and 1.64 log CFU/g, respectively. The samples had higher than mean levels of mold and yeast contamination [18]. Rasht City had less coliform, mold, and yeast contamination than Hamedan City.
Çelik et.al examined the chemical, physical, and microbiological qualities of traditional yogurts collected from Ordu and Giresun cities in Tukey. A total of 24 conventional yogurt samples were collected. Unhygienic manufacturing conditions were indicated by the coli form found in five cow yogurts and the Escherichia coli infection in one case. Homemade yogurts were satisfactory in terms of compositional value but not microbially suited. Five yogurt samples (20%) showed signs of coliform contamination, and one sample (4.1%) showed signs of *Escherichia coli* contamination [7]. The results of the current study supported the presence of *Escherichia coli*, but there was less coliform contamination in the yogurt samples from Rasht City than there was in the previous study.
Ifeanyi et al. reported that four commercial brands of yogurt were sampled in one of the cities of Anambra state, which was conducted in the southeast of Nigeria. The amount of yeast, coliforms, and heterotrophic bacteria in the samples was counted to evaluate the microbiological quality. Three species of fungus from the genera *Aspergillu*s, *Rhizopus*, and *Saccharomyces*, as well as *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* species, *Lactobacillus* species, and *Bacillus* species, were isolated from the samples. Every sample had *Escherichia coli* in it. The findings demonstrated that the yogurt samples from Anambra state had inadequate microbiological quality [19].
The microbiological quality of 152 yogurt samples was examined in a study conducted in the Canadian province of Ontario. There was

evidence of *Staphylococcus* contamination in 27.6% of the samples. It was shown that 13.8%, 17.8%, and 26.3% of yogurt samples were contaminated with coliform, mold, and yeast, respectively [20].
The microbiological quality of forty Sa'eedi Kishk samples was examined in a study conducted in Egypt. Eighty percent of the samples had coliform contamination, with the maximum mean contamination being 1.01 ± 0.18 log CFU/g. There was no evidence of *Staphylococcus aureus* contamination in the samples. Mold and yeast contamination affected 50% of the samples [21]. The results did not align with the current research. According to the current study, Rasht City's conventional kashk distribution had no microbiological contamination.

4- Conclusion

The results show that while there is little microbiological contamination in the conventional yogurt and kashk samples provided by Rasht City, there is not enough quantity of mold and yeast contamination (32%), which is evident in the yogurt samples. The high degree of mold and yeast contamination in food is a sign of incorrect product storage, environmental pollution, and poor storage conditions. It appears that producers should abide by health regulations by taking part in health education courses in the manufacturing and supply chain, and regulatory authorities should routinely monitor the microbiological purity of traditional products. The microbiological quality of the finished product will not be optimal and might endanger the health of customers if personal and environmental health suggestions are not followed during the preparation of traditional products. In addition, it is important to stop the distribution of items of poor microbiological quality in municipal supply centers. It appears that requiring traditional product manufacturers to adhere to national standards can significantly enhance the healthfulness of traditional items.

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ارزیابی کیفیت میکروبی ماست و کشک سنتی در شهر رشت

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چکیده

شیر و فرآورده‌های آن نظیر ماست و کشک از دیرباز به‌عنوان بخشی از رژیم غذایی مورداستفاده قرار می‌گیرد و یکی از مهم‌ترین تامین کنندگان بخش بزرگی از نیاز انسان به کلسیم است. هدف از این مطالعه بررسی کیفیت میکروبی ماست سنتی و کشک سنتی عرضه‌شده در شهر رشت بود. در این مطالعه ۲۵ نمونه ماست سنتی و ۲۵ نمونه کشک مایع سنتی از مراکز عرضه شهر رشت از دی تا اسفند ۱۴۰۰ به‌صورت تصادفی ساده جمع‌آوری شد. کیفیت میکروبی نمونه‌ها ازلحاظ آلودگی کلی‌فرمی، *اشرشیاکلی*، *استافیلوکوکوس اورئوس* کوآگولاز مثبت، *کلستریدیوم‌های* احیاکننده سولفیت و کپک و مخمر بررسی شدند. میزان آلودگی کلی فرمی در ماست سنتی و کشک سنتی به ترتیب۰/۱۲ ±۰/۳۵ log CFU/g و ۰/۰۸ ±۰/۱۱ log CFU/g و میزان آلودگی با کپک و مخمر در ماست سنتی و کشک سنتی به ترتیب ۰/۵۲ ±۲/۰۷ log CFU/g و۰/۱۸ ±۰/۶۰ log CFU/g بود. در بین نمونه‌های ماست سنتی و کشک سنتی تفاوت معنی‌داری ازنظر میزان آلودگی به *اشرشیاکلی* وجود نداشت، اما ماست‌های سنتی ازنظر آلودگی به کپک و مخمر تفاوت معنی‌داری با کشک‌های سنتی داشته و میزان آلودگی در آن‌ها بسیار بالاتر بود. با توجه به نتایج حاصله کیفیت میکروبی ماست سنتی پایین بود. بنابراین کنترل بهداشتی واحدهای تولیدکننده و نظارت بر تولید ماست سنتی در شهر رشت باید به‌طور مستمر انجام گیرد تا میزان آلودگی در ماست‌های سنتی کاهش یابد.