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Research Article

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Investigating the Amount of Microbial Contamination of Pasteurized Milk in Kermanshah City with Coliform and the Total Number of Bacteria

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Abstract

Background and aim: One of the most widely consumed dairy products is milk, which has a high nutritional value and is favorable for the growth of microorganisms due to its environmental nutrients and due to the sensitivity of this product, ensuring the health and bacteriological quality after Pasteurization is of particular importance. The purpose of the present study was to investigate the amount of coliform contamination and the total number of bacteria in pasteurized milk in kermanshah city.
Materials and Methods: To measure the microbial load of pasteurized milk in Kermanshah city, coliforms were performed using the most probable number of MPN on lactose culture medium and to count the total number of bacteria from the standard number 5272. Data were analyzed using one-way ANOVA and SPSS software version 22.
Results: The results of testing 144 samples of pasteurized milk performed in twelve months, showed that the contamination rate was 46% (29 out of 144 cases) and the highest contamination with coliforms was in July with 4 cases (33.3%) and the lowest contamination was related to August and November with 1 case (8.33%). Also, the highest amounts of bacteria were related to July, August and September, respectively, and the lowest was related to January.
Conclusion: According to the obtained analyzes, it was found that the amount of coliform contamination and the total number of bacteria was less than standard and indicates the standardization of monitoring systems, HACCP and health in pasteurization chain.

Key words: *Coliform, Totalcount, Pasteurized milk, Microbial contamination*

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Introduction

Today, the consumption of milk and its products in every society is one of the most important indicators of cultural development. Milk is a complete food with a lot of nutritional value, which is a part of the diet in every age group and has high nutritional importance (Ciliberti *et al.,* 2022; Fontecha et al., 2020). Raw milk is rich in easy-to-digest proteins, vitamins A, B1, B2, B6 and pantothenic acid, saturated and unsaturated fatty acids, and many salts. Milk is poor in terms of abundance of vitamins K and B12. The temperature of pasteurization does not have much effect on the reduction of vitamins A and K, but it can cause a significant decrease in the amount of vitamin D, C and folic acid (Dror & Allen, 2018; Sharabi *et al.,* 2018). This food is a favorable growth environment for the activity of various pathogenic microorganisms. The growth and proliferation of pathogenic microorganisms on the taste and other organoleptic characteristics of milk, including its smell, color and taste, have adverse effects, which in addition to spoilage in milk, cause even fatal diseases (Claeys *et al.,* 2013).

Milk production in the world was 843.4 million tons in 2018, and India is the largest producer of milk in the world with 21% of the world production. Milk production in the world was 843.4 million tons in 2018, and India is the largest producer of milk in the world with 21% of the world production. The 10 major milk producing countries in 2018 include India, America, China, Pakistan, Germany, Russia, Brazil, New Zealand, France and Turkey, which produce about 62% of the world's milk; Iran's share in milk production this year is about 1% of the total milk production in the world. The per capita consumption of milk for each Iranian person in the household in 2016 is about 30.2 kg, and in 2017 this figure has reached 28.2 kg; While the per capita consumption of milk in the world is 113 kg, different statistics of global milk consumption are presented; But it is clear that the per capita consumption of milk in Iran is far from the global per capita and developing countries (Khosravi *et al.,* 2013; Muaz *et al.,* 2022; Soltanali *et al.,* 2015).

If the lactating animal is suffering from a certain disease, the milk is a completely sterile liquid during secretion. But when the milk leaves the animal's body and approaches the end parts of the nipple, the contaminations present in this part enter the milk. When milk leaves the animal's body, it has a temperature between 36-38 degrees Celsius, which is the best condition for the growth of microorganisms; As a result, the milk should be cooled to 4 degrees Celsius immediately after milking (Kabelitz *et al.,* 2021). This is while in the developing countries, cold chain preservation is not observed by the producers in the case of many of the milks presented to the milk collection centers (Chambers, 2005).

Contamination of raw milk takes place from several areas, which include: contamination inside the breast, outside the breast and teats, the surfaces of storage equipment and carrying milk (Wanjala *et al.,* 2018). The number and type of microorganisms in freshly milked milk depends on factors such as animal health, milking tools and equipment, season, feeding, and most importantly, the conditions of keeping the animal in barns and stables (Wanjala *et al.,* 2018; Perin *et al.,* 2019). The abundance of bacteria in milk is not only important in relation to its proper evaluation for human consumption, but it is also one of the basic factors; because there are pathogenic bacteria in that field. Some of these pathogenic bacteria include: Salmonella, including: Salmonella, Escherichia coli, coliforms, Staphylococcus aureus, Streptococcus agalactiae, Enterococci, Listeria monocytogenes, Enterococci and They are corynebacteria.

The presence of coliforms indicates the contamination of milk with pathogenic microorganisms, which indicates a low level of hygiene in the stables and barns of livestock farms, milking equipment, and if present in pasteurized milk, It indicates thermal insufficiency and the possible presence of bio-film in the heat transfer systems of factories, which prevents the transfer of pasteurization temperature to milk (Joubrane *et al.,* 2022).

Coliforms include two categories of microorganisms: a) fecal coliforms and b) non-fecal coliforms. They are gram-negative and bacilli-shaped, without endospore and both stimulatory and non-stimulatory, which are able to ferment lactose sugar by producing acid and gas when exposed to 37-35 degrees (Heidarzadi *et al.,* 2021).

The coliforms include: Citrobacter, Enterobacter, Hafnia, Klebsiella and Escherichia coli, etc., and

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some sources consider Escherichia coli to be the most important (Yates, 2007). Escherichia coli is one of the most important contaminants of food. This microorganism is an important member of Enterobacteriaceae family, one of the important causes of gastroenteritis in humans. The main place of this microorganism is the digestive system of humans and warm-blooded animals. Shigatoxin-producing serotypes, the most important of which is serotype O157H7, can cause diarrhea and hemorrhagic colitis and eventually cause death in humans (Heidarzadi *et al.,* 2021).

One of the traditional and common methods of increasing the shelf life of raw milk and making it healthy in order to prevent spoilage and remove pathogens is pasteurization; during this process, all pathogenic factors are destroyed.

Slow pasteurization and fast pasteurization are among the most common methods of making milk healthy, which are being done in most countries of the world.

Slow pasteurization and fast pasteurization are among the most common methods of making milk healthy, which are being done in most countries of the world. LTLT (Low Temperature Long Time) slow pasteurization includes a temperature of 63 degrees Celsius for 30 minutes and HTST (High Temperature Short Time) fast pasteurization at a temperature of 72 to 75 for 15 to 20 seconds(Bousbia *et al.,* 2021). However, some sporulating bacteria are not destroyed during this process. On the other hand, psychrotrophic bacteria such as Pseudomonas, which cause the production of extracellular enzymes, are not destroyed during pasteurization, which subsequently reduces the shelf life of pasteurized, sterilized milk and dairy products. It endangers the health of consumers (MA *et al.,* 2019).

Estimating the microbial load is one of the most important factors in milk quality evaluation. Total microbial count of pasteurized milks according to the instructions of Iran Industrial Research and Standard Institute and the results match the values of the existing standards. Among the usual laboratory instructions in all milk factories, it is performed on the raw milk and also on the produced product (pasteurized milk). The purpose of this article is to investigate the amount of contamination in pasteurized milk in Kermanshah province with coli form and the total number of bacteria.

Materials and Methods

In order to investigate the prevalence of coliform contamination and the total number of bacteria in pasteurized milk in Kermanshah city, 144 samples of pasteurized milk from factories in Kermanshah city with a volume of 200 milliliters were collected in all months of 2018 and then It was taken to the laboratory for the tests of the total number of bacteria and coliform count.

Total count of microorganisms

Total counting of microorganisms in milk using different dilutions and the number of colonies formed in terms of CFU (Colony-Forming Unit) per milliliter of milk using Plate Count Skim Milk Agar culture medium according to the standard 5272, the standard department was carried out.

Total number of forms

MPN (Most Probable Number) method was used to count coliforms. Five dilutions of 10-5-10-1 were used for the experiment. In this way, 1 milliliter of pasteurized milk was added to a test tube containing 9 milliliters of sterile physiological serum and mixed, then 15 milliliters of Violet Red Bile Agar medium was added to it, and mixed in the form of 8 until well mixed. And after about 5 minutes when the environment was closed, it was incubated at 30 degrees Celsius for 24 hours and colony growth was checked (da Silva Malheiros et al., 2010). For statistical analysis, SPSS software version 22 and one-way ANOVA statistical analysis were used; Also, the significance level of $P<0.05$ was considered.

Results

The results of the analysis of 144 samples of pasteurized milk that were carried out during twelve months, It showed that the highest coliform contamination was in July with 33.3% and the lowest contamination was in August and November with 8.33%.

Out of a total of 144 tested pasteurized milk samples, 29 samples were positive and contaminated. The results of this research showed that there was no significant relationship between different seasons of the year and the amount of pollution ($P<0.05$).

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Discussion

Among the most important factors that cause spoilage in stored pasteurized milk include: A) Intrinsic factors in milk, including free fatty acids and its natural metal content. b) External and processing factors include manipulation, stirring, storage, unfavorable temperature, exposure to light and secondary contamination by metals or microorganisms. The type of packaging used in pasteurized milk may have a general effect on the quality characteristics of milk by directly controlling the amount of oxygen and light available for reaction with the product and also providing a suitable barrier to prevent contamination by microorganisms after pasteurization.

Sampling months	Number of samples of pasteurized milk	Total contamination in pasteurized milk		Positive percentage (contamination)
		Negative	Positive	
April	12	9	3	25%
May	12	10	2	16.67%
June	12	10	2	16.67%
July	12	8	4	33.3%
August	12	11	1	8.33%
September	12	9	3	25%
October	12	9	3	25%
November	12	11	1	8.33%
December	12	9	3	25%
January	12	10	2	16.67%
February	12	9	3	25%
March	12	10	2	16.67%

Table 1. The frequency of contamination with coliforms in pasteurized milk of Kermanshah city.

Sampling months	Coliforms	Total count	pH	Acidity degree
April	3 ± 0.03^{a}	$2*10^{-3}\pm0.01^{bc}$	6.7 ± 0.02^{a}	0.15 ± 0.02^{a}
May	3 ± 0.03^{a}	$2*10^{-3}\pm0.01^{ab}$	6.73 ± 0.01^{a}	0.15 ± 0.02^{a}
June	3 ± 0.03^{a}	$2*10^{-3}\pm0.01^{ab}$	6.79 ± 0.03^{a}	0.15 ± 0.02^{a}
July	5 ± 0.03^{a}	$3*10^{-3}\pm0.00^{a}$	6.63 ± 0.01^{a}	0.15 ± 0.02^{a}
August	1 ± 0.03^{a}	$3*10^{-3}\pm0.00^{a}$	6.67 ± 0.03^{a}	0.15 ± 0.02^{a}
September	4 ± 0.03^{a}	$3*10^{-3}\pm0.02^{a}$	6.65 ± 0.02^{a}	0.15 ± 0.02^{a}
October	3 ± 0.03^{a}	$0.002*10^{-3}\pm0.02^{d}$	6.63 ± 0.01^{a}	0.15 ± 0.02^{a}
November	1 ± 0.03^{a}	$0.002*10^{-3}\pm0.03^{d}$	6.69 ± 0.00^{a}	0.15 ± 0.02^{a}
December	3 ± 0.03^{a}	$0.002*10^{-3}\pm0.03^{d}$	6.65 ± 0.00^{a}	0.15 ± 0.02^{a}
January	2 ± 0.03^{a}	$1*10^{-3}\pm0.01^{acd}$	6.61 ± 0.00^{a}	0.15 ± 0.02^{a}
February	2 ± 0.03^{a}	$2*10^{-3}\pm0.01^{bc}$	6.63 ± 0.00^{a}	0.15 ± 0.02^{a}
March	2 ± 0.03^{a}	$1*10^{-3}\pm0.03^{bc}$	6.71 ± 0.00^{a}	0.15 ± 0.02^{a}

Table 2. Microbial and chemical analyzes based on the sampled month.

In the study of Ombui et al., on 246 pasteurized milk samples, coliforms were found in 26% of all samples; The results of this research showed that 29 of the 144 samples were infected, which is somewhat proportionate (Ombui *et al.,* 1994). In the investigation of the microbial quality of 348 samples of pasteurized milk in Brazil by Silva et al., in 2008, 38.5% of the samples were in accordance with the standard, which is in line with the results of this research.In another study conducted in Brazil by da Silva Malheiros et al., in 2010 on the microbial characteristics of pasteurized milk. It was found that the number of coliforms in 50% of pasteurized milk samples washigher than the standard, which is not consistent with the present study

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(da Silva Malheiros *et al.,* 2010). Examining the results of microbial contamination of pasteurized milk in 2013 by Zulfiqari et al., in Qom province showed that the amount of coliforms was reported as 4.7%, and in this research, the amount of coliforms was 46% (Zolfaghari *et al.,* 2012). In a study carried out in 2009 by Dobradran et al., on the investigation of the microbial quality of pasteurized and sterilized milk sold in Bushehr city. It was shown that 15.2% of 98 pasteurized milk samples were contaminated with coliforms, and the results of this research showed that 46% were contaminated with coliforms (Dobaradaran *et al.,* 2014). During the study conducted on the bacterial contamination of pasteurized milk in Tehran province in 2009 by Faramarzi et al., it was shown that out of 256 tested samples, 9.84% had coliform contamination, which is lower than the present study (Faramarzi *et al.,* 2012). The results of Fadaei et al.'s studies in 2015 in Shahrekord showed that 80.5% of pasteurized milk was contaminated with coliforms out of a total of 242 cases, which is much higher than the results of the present study with 46% (Fadaei *et al.,* 2008). In a study in Brazil by Abdul-Raouf et al., out of a total of 75 pasteurized milk samples, it was shown that 25% of the samples had coliform contamination, which is consistent with the results of this research (Abdul-Raouf *et al.,* 1996). Guaranteeing the safety of pasteurized and even raw milk is obvious to guarantee the health of consumers; However, for raw and pasteurized milk, some standards have been set by Iran's national standard. But due to reasons such as lack of proper organization and necessary investment in this field, the amount of microbial load and the physical and chemical properties of milk can be affected. Before the milk is pasteurized, a series of quality control tests are performed on it when it enters the factory. Among these tests, we can refer to checking freshness and oldness, the presence or absence of antibiotics, the amount of milk fat and protein, aflatoxin and the presence of infection in milk. One of the contaminations of pasteurized milk and the failure of pasteurization in milk is the presence of coliforms. These microbes are destroyed during the pasteurization process; If the pasteurized milk contains a very high amount of these bacteria, it indicates the insufficiency of the thermal process during the pasteurization chain. The results of the present study indicate that the contamination of pasteurized milk in Kermanshah city is significantly lower than many other regions and is higher than some studies conducted in other regions. However, this study shows that the pollution is according to the standards of the country, which can be caused by greater accuracy and precision in observing the health standards during the production and disinfection of the equipment and also to be the precise and continuous supervision of the experts of the units under his supervision.

The reasons for the increase in bacterial contamination of pasteurized milk can be attributed to defective pasteurization equipment, pasteurization process effluent, pollution after pasteurization due to poor production process, transportation, or the poor health status of factory workers (Fadaei et al., 2008). Also, training of livestock farmers in the field of animal hygiene and breast washing before milking, control of mastitis, training of operators of milk collection and storage centers in order to keep the milk at a temperature of 1-4 degrees Celsius before reaching the factory. It can minimize the high contamination load of raw milk and subsequently reduce the possibility of secondary contamination.

Conclusion

Foodborne diseases are one of the major concerns around the world. About 250 different diseases are caused by food, and bacteria are responsible for two-thirds of food-borne diseases. The fight against bacterial food borne diseases is facing new challenges due to rapid change. Recent data from developing or developed countries show that at least 10% of the population may experience a foodborne illness. The situation in developing countries is equally serious. In 2002, the Centers for Disease Control in the United States reported 76,000 cases of foodborne illness, most of which were of bacterial origin. The microbiological safety of food during storage is related to many factors. Ready-made food products are consumed without any treatment between the final production stage and consumption. The highly nutritious nature of dairy products makes them a good environment for the growth of microorganisms. Spoilage occurs when microorganisms break down carbohydrates, proteins, and fats in milk and produce harmful end products. Dairy products can contain various organisms, including many common bacteria

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between humans and animals such as Brucella abortus, B. melitensis, Campylobacter jejuni, Escherchia coli, Listeria monocytogense, Mycobacterium bovis, M. tuberculosis. Salmonella, Staphylococcus aureus, Enterocoliticas. It is impossible to prevent the contamination of milk with microbes. Therefore, the microbial content of milk is a major feature in determining its quality in terms of safety. It is important to mention that pasteurized milk can contain pathogenic bacteria such as E. coli, L. monocytogenes, Salmonella spp., S. aureus and Yersinia enterocolitica. Therefore, with all the mentioned issues, it is necessary to pay utmost attention to the consumables.

Conflict of interest

The authors have no conflicts of interest to declare.

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بررسی میزان آلودگی میکروبی شیرهای پاستوریزه شهرستان کرمانشاه به کلی فرم و تعداد کل باکتری ها

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چکیده

زمینه و هدف: یکی از پرمصرف ترین محصولات لبنی، شیر می باشد که از ارزش غذایی بالایی برخوردار است و به دلیل دارا بودن موادمغذی، محیط مطلوبی برای رشد میکروارگانیسم ها می باشد که به دلیل حساس بودن این فرآورده، اطمینان از کیفیت بهداشتی و باکتریولوژیکی بعد از پاستوریزاسیون، از اهمیت ویژه ای برخوردار است. هدف از مطالعه حاضر بررسی میزان آلودگی کلی فرمی و سنجش تعداد کلی باکتری ها در شیرهای پاستوریزه استان کرمانشاه می باشد.

مواد و روش‌ها: برای سنجش میزان آلودگی بار میکروبی شیرهای پاستوریزه شهرستان کرمانشاه، کلی فرم ها را به روش محتمل ترین تعدا MPN بر روی محیط کشت لاکتوز برات و جهت شمارش کلی باکتری ها از استاندارد با شماره ۵۲۷۲ انجام گرفت. داده ها با استفاده از آزمون آماری آنوا یک طرفه و نرم افزار SPSS نسخه ۲۲ انجام گرفت.

یافته‌ها: نتایج حاصل از آزمایش ۱۴۴ نمونه شیر پاستوریزه که در طی دوازده ماه انجام گرفت، نشان داد که میزان آلودگی ۴۶ درصد (۲۹ مورد از ۱۴۴ مورد) بود و بیشترین میزان آلودگی به کلی فرم در تیرماه با ۴ مورد (۳۳/۳ درصد) و کمترین میزان آلودگی مربوط به ماه های مرداد و آبان با ۱ مورد (۸/۳۳ درصد) بود. همچنین بیشترین مقدار آلودگی تعداد کلی باکتری ها به ترتیب مربوط به ماه های تیر، مرداد و شهریور و کمترین مربوط به دی ماه بود.

نتیجه‌گیری: با توجه به آنالیزهای به دست آمده، مشخص شد که میزان آلودگی کلی فرمی و تعداد کلی باکتری ها، کمتر از میزان استاندارد بوده و نشان دهنده استاندارد بودن سیستم های نظارتی، HACCP و بهداشتی در زنجیره پاستوریزاسیون است.

واژه‌های کلیدی: *کلی‌فرم، توتال کانت، شیر پاستوریزه، آلودگی میکروبی*

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