Food safety assessment of milk-based Indian sweets

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Abstract

Milk-based sweets are a staple of Indian cuisine, particularly in regions like Bikaner, Rajasthan, known for their rich dairy culture. However, these sweets are highly perishable and susceptible to contamination by pathogens such as *Bacillus cereus*, which can cause foodborne illnesses. The present study focuses on the isolation and biochemical characterization of *Bacillus cereus* from milk-based sweets sold in Bikaner. A total of 300 mawa based and chhana based sweets samples were collected from various local sweet shops in Bikaner. Several biochemical tests (like malonate, Voges Proskauer, catalase, carbohydrate utilization tests etc.) were performed to confirm the *B. cereus* isolates using Hi*Bacillus*™ kits. Out of which 39 (13%) tested positive for *Bacillus cereus*. Microbiological analyses revealed the highest contamination in mawa based sweets (16.66%; 25/150) as compared to chhana based sweets (9.33%; 14/150). The findings highlight the urgent need for stringent hygiene practices and quality control measures to ensure food safety in milk-based sweet production.

Keywords: *Bacillus cereus*, milk-based sweets, food safety, biochemical characterization.

Introduction

Milk is regarded as an ideal diet for humans, meeting their basic nutritional needs at every stage from birth to old age. Nonetheless, milk is a nutrient-rich medium that can sustain the development of many microbes along the lengthy chain of milk processing, employee handling, distribution, and storage (Hefny *et al.*, 2020). The dairy farmers are dependent on dairy food industry for remunerative prices and global dairy food industry is always striving for maintaining the food safety standards in their products (Kokaeva etal., 2020; Yuldashbaev et al., 2020).

With an estimated production of 239.3 million tons and a daily availability of 471 grams per person in 2023–2024, India is the world's largest producer of milk, accounting for 24% of worldwide production (NDDB, 2024). Meanwhile, sweets have been consumed in India for centuries due to their nutritional and health benefits, as well as their delicious flavour. India's cultural legacy includes the preparation of many sweets dating back to centuries. Many sweets are regarded auspicious and sacred, and their production is crucial in celebrating festivals (Balkrishna *et al.*, 2024). Khoa and chhana are the two basic ingredients used to make almost all milk sweets in India (Aggarwal *et al.*, 2018). Bikaner, a city in Rajasthan, India, is famous for its milk-based sweets which are widely consumed in India due to their rich flavour and cultural significance. These products are often prepared in bulk under conditions that may not adhere to stringent hygiene standards, increasing the risk of microbial contamination. However, these products are vulnerable to contamination by microbial pathogens during preparation, storage, and distribution. Milk besides a rich source of nutrients also serves as an excellent culture and protective medium for certain microorganisms, mainly bacterial pathogens. Out of numerous numbers of pathogens, particularly food borne pathogens, *Bacillus cereus* is one of the 22 food-borne pathogens that the World Health Organization (WHO) has selected for use in assessing the burden of food-borne illnesses (Kirk *et al.* 2015). *Bacillus cereus* is a spore-forming bacterium is a significant concern as it can survive high temperatures and cause emetic or diarrheal syndromes upon ingestion (Kramer & Gilbert, 1989). *Bacillus cereus* has been isolated from a wide range of food products, including dairy, eggs, and meat (Kramer and Gilbert, 1989; Ombui *et al.*, 2008). This bacterium can thrive in most foods with a pH above 4.5 and at temperatures exceeding 4°C (Reyes *et al.*, 2001; Svensson *et al.*, 2007). The global incidence of foodborne illnesses has been on the rise, particularly in developing countries where food products are often exposed to contaminated environments during processing and subjected to temperature abuse during transportation and storage at retail outlets (WHO, 2007). Foodborne illness caused by *B. cereus* manifests in two distinct forms i.e., emetic and diarrheal syndromes (Haddaji *et al.*, 2022). Growing awareness and improved recognition of *B. cereus*-related illnesses have led to a significant increase in reported cases of this type of food poisoning. Beyond gastrointestinal infections, *B. cereus* is also implicated in various non-gastrointestinal conditions such as meningitis, endophthalmitis, endocarditis, periodontitis, osteomyelitis, wound infections, and septicaemia in humans (Schoeni and Wong, 2005). Due to the widespread presence of *Bacillus cereus* in the environment, completely preventing food contamination by this bacterium or its spores is nearly impossible. Its presence in milk and milk-based sweets is of public health concern due to its heat resistance and pathogenic potential. The swift detection of *B. cereus* in food is crucial for implementing quality control measures to eliminate the bacterium from food products and enhance the diagnosis of food poisoning outbreaks (Ombui *et al.*, 2008).

Because the milk-based sweets are widely consumed in the Bikaner city of Rajasthan (India), this study was conducted to isolate and biochemically characterise *Bacillus cereus*, in locally sold sweets of Bikaner.

Materials and Methods

Sample Collection

A total of 300 samples of milk-based Indian sweets were collected from different local vendors in Bikaner, Rajasthan. Samples included commonly consumed 150 mawa based sweets and 150 chhana based sweets samples which were collected aseptically in sterile containers and all the aseptic precautions were taken during the sample collection and avoid external contamination. Each sample was procured in its original retail condition and swiftly delivered to the laboratory under refrigerated conditions with minimal delay. The selection of sweet shops was randomized to cover a wide geographic area within the city, ensuring representative sampling (Acharya *et al.*, 2013).

Isolation of *Bacillus cereus*

The samples were thoroughly processed for isolation of *Bacillus cereus*. After the collection of samples, each sample (1g) was homogenized in 9 mL of Brain Heart Infusion (BHI) broth and incubated at 37 °C for 24 hours to promote the growth of *B. cereus*. Following enrichment, the samples were plated on Polymyxin Egg Yolk Mannitol Bromothymol Blue Agar (PEMBA) supplemented with PEMBA supplement and egg yolk emulsion using a primary, secondary, and tertiary streaking pattern. This method facilitated the isolation of bacterial colonies, and the plates were incubated at 37 °C for 24 hours (Tewari *et al.*, 2015).

Biochemical Characterization

The confirmed presumptive colonies characterized by peacock-blue coloured colonies on PEMBA agar with a zone of precipitation were selected and their further confirmation was performed using Gram's staining and other biochemical tests using Hi*Bacillus*™ identification kit (KB013) provided by Himedia. KB013 is a test kit consisting of 12 assays designed for the identification of *Bacillus* species. The kit included sterile media for various tests, such as malonate, Voges-Proskauer, citrate, ONPG, nitrate reduction, catalase, and arginine. Additionally, it provides media for the utilization of five different carbohydrates: sucrose, mannitol, glucose, arabinose, and trehalose.

Results

Microbiological isolation of *B. cereus*

Following enrichment by Brain Heart Infusion (BHI) broth, the samples were plated on Polymyxin Egg Yolk Mannitol Bromothymol Blue Agar (PEMBA) and the suspected *B. cereus* colonies were identified based on their characteristic peacock blue coloration on PEMBA agar in mawa based sweets samples (Fig 1) and chhana based sweets samples (Fig 2).

Identification of *B. cereus*

To confirm the identity of the isolates, Gram's staining and a series of biochemical tests were performed. The Gram's staining results revealed that all isolates appeared purple due to the retention of crystal violet dye in their thick peptidoglycan layer showing Gram-positive, rod-shaped bacilli, either arranged singly or in chains, which is typical of *B. cereus* (Fig 3). Biochemical tests further supported the identification, as the isolates tested positive for the Voges-Proskauer test, citrate utilization, catalase activity, and the ability to ferment glucose and trehalose, while being negative for malonate, ONPG, mannitol, and arabinose fermentation. Additionally, delayed reactions were observed in nitrate reduction, arginine utilization, and sucrose fermentation, all of which further affirmed their confirmation as *B. cereus*.

The detailed results of different biochemical tests for *B. cereus* isolates from mawa based sweets samples and chhana based sweets samples have been presented in Table 2 and depicted in Fig 4 and Fig 5. For mawa based sweets samples, *Bacillus cereus* exhibited 100% positive reaction for citrate test, catalase test and glucose sugar tests, 96% positivity for trehalose sugar test, 92% positivity for Voges Proskauer test, 84% positivity for nitrate reduction, 80% positive reaction for arginine and sucrose test, and 12% positive reaction for arabinose sugar test. While 100% negative reaction for malonate test, ONPG test and mannitol sugar test were observed.

For chhana based sweets samples, *Bacillus cereus* exhibited 100% positive reaction for citrate test, catalase test and glucose sugar tests, 92.85% positivity for Voges Proskauer test and trehalose sugar test, 85.71% positive reaction for sucrose test, 78.57% positivity for nitrate reduction and arginine test, and 7.14% positive reaction for arabinose sugar test. While 100% negative reaction for malonate test, ONPG test and mannitol sugar test were recorded.

The findings of the current investigation showed that among the 300 samples analysed, 39 (13%) isolates of *B. cereus* were obtained. The number of isolates of *B. cereus* were higher in mawa based sweets (16.66%; 25/150) as compared to chhana based sweets (9.33%; 14/150) (Table 1).

Table 1. Number of *Bacillus cereus* isolates obtained from different types of milk-based sweets

S. No.	Sample type	No. of samples	No. of *B. cereus* isolates obtained
1.	Mawa based sweets	150	25 (16.66%)
2.	Chhana based sweets	150	14 (9.33 %)
	Total	300	39 (13 %)

Table 2. Biochemical tests for *B. cereus* isolated from mawa based & chhana based sweets samples using Hi*Bacillus*™ commercial kits

S. No.	Test	Mawa based Sweets No. (%)	chhana based sweets N0. (%)
1.	Malonate	0/25	0/14
2.	Voges Proskauer	23/25 (92%)	13/14 (92.85%)
3.	Citrate	25/25 (100 %)	14/14 (100%)
4.	ONPG	0/25	0/14
5.	Nitrate reduction	21/25 (84 %)	11/14 (78.5 %)
6.	Catalase	25/25 (100)	14/14 (100 %)
7.	Arginine	20/25 (80%)	11/14 (78.5 %)
8.	Sucrose	20/25 (80%)	12/14 (85.71%)
9.	Mannitol	0/25	0/14
10.	Glucose	25/25 (100 %)	14/14
11.	Arabinose	3/25 (12%)	1/14 (7.14%)
12.	Trehalose	24/25 (96%)	13/14 (92.85%)

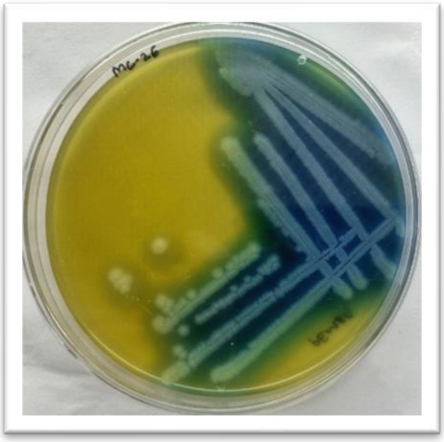

Fig 1. Isolation of *B. cereus* on PEMBA agar (Mawa based sweet sample) Peacock-blue colored colonies

Fig 2. Isolation of *B. cereus* on PEMBA agar (Chhana based sweet sample) Peacock-blue colored colonies

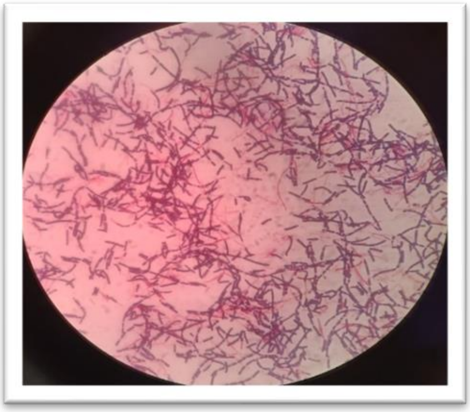

Fig 3. Gram's staining of *B. cereus* Gram positive large rods

Discussions

Milk-based sweets hold significant cultural and economic value, particularly in countries like India, where they are integral to celebrations, rituals, and dietary customs. Despite their widespread popularity, these sweets are often produced in small-scale or unregulated settings, which increases the risk of contamination. Due to their rich nutritional profile and high moisture content, milk-based sweets such as rasgulla, peda, and burfi create favourable conditions for the growth of spoilage and pathogenic microorganisms, including *Bacillus cereus*. The bacterium's potential to produce both emetic and diarrheal toxins poses a direct threat to consumer safety, and contamination events can result in costly product recalls and significant reputational damage. Therefore, the present study was undertaken to isolate and identify *B. cereus* strains isolated from milk-based sweets, with the aim of evaluating food safety and assessing the public health risks associated with its presence.

The present study revealed that milk-based sweet samples obtained from various retail sweet shops in Bikaner, Rajasthan, were contaminated with *Bacillus cereus*. This contamination of *B. cereus* is contributed by multiple factors including inadequate hygiene standards, unsanitary processing methods, the absence of proper

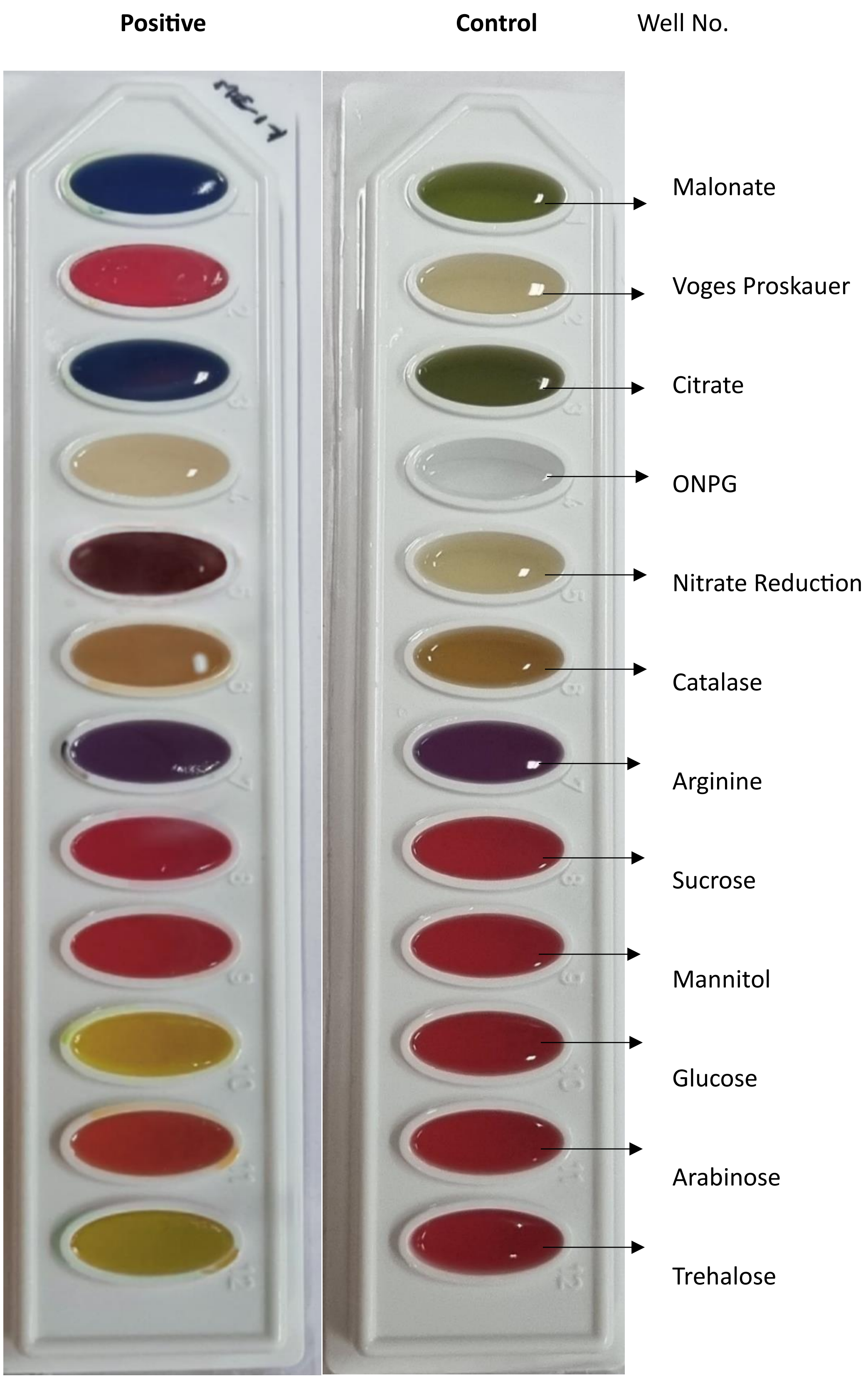

Fig 4. Biochemical tests for *B. cereus* of mawa based sweet sample

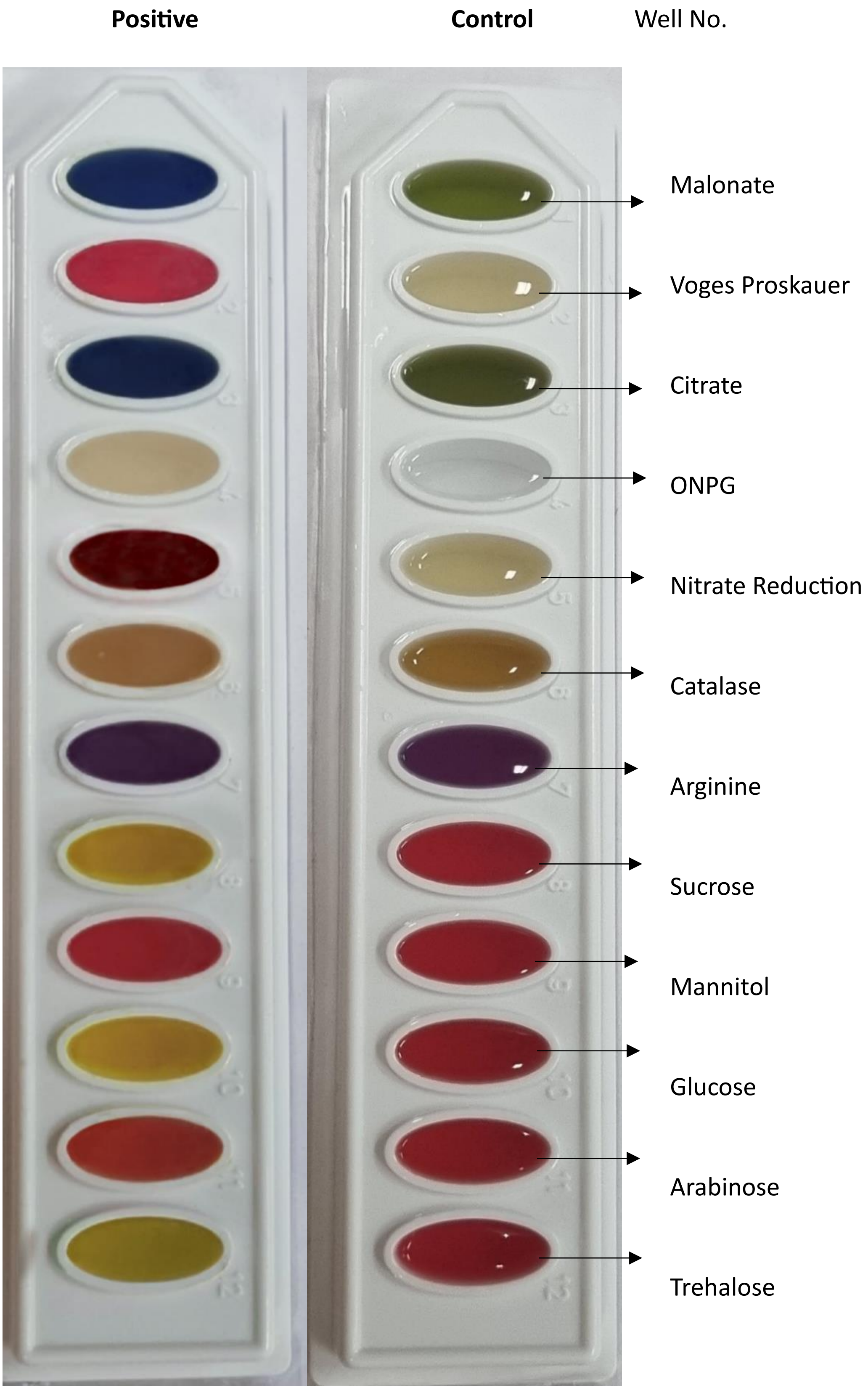

Fig 5. Biochemical tests for *B. cereus* of chhana based sweet sample

sanitation facilities, environmental contamination such as polluted water sources, and improper handling during transportation and storage. These findings emphasize on implementing Good Manufacturing Practices (GMP) in local sweet production, educate vendors about food safety standards, and conduct regular microbiological testing to ensure compliance with safety norms to ensure the safety of milk-based sweets.

Musvi (2011) conducted a study on 125 dairy product samples from the Kashmir Valley, including cheese, barfi, rasgulla (white), rasmalai, and ice cream, with 25 samples of each type. *Bacillus cereus* emetic strains were detected in 20 samples, resulting in an overall prevalence of 16%, which aligns with the findings of the present study. The highest contamination rate was recorded in rasgulla (6/25; 24%), followed by barfi (5/25; 20%), ice cream (4/25; 16%), rasmalai (3/25; 12%), and cheese (2/25; 8%). These findings highlight the necessity of stringent hygiene measures across the production and supply chain to mitigate the prevalence of *Bacillus cereus*.

Mahadeva (2024) examined 120 dairy product samples and reported a *Bacillus cereus* prevalence rate of 14.1% (17/120), which is comparable to the results of the present study. Similarly, Ewida *et al.* (2024) analysed 250 samples, including marketable milk, Ras cheese, Domiati cheese, and ice cream, sourced from retail outlets in Assiut City, Egypt. They reported a prevalence of 5.6%, which is lower than the findings of this study.

Yusuf *et al.* (2018) investigated 215 samples, consisting of 30 raw milk samples and 185 milk product samples (29 barfi, 28 rasgulla, 28 rasmalai, 25 ice cream, 25 paneer, 25 pastry, and 25 kalaari). *Bacillus cereus* was detected in 61 samples, yielding an overall prevalence of 28.37%. The individual prevalence rates were 26.66% in raw milk, 34.48% in barfi, 39.28% in rasgulla, 25% in rasmalai, 44% in ice cream, 16% in paneer, 32% in pastry, and 8% in kalaari—values notably higher than those reported in the current study. The contamination was attributed to factors such as milk adulteration with water or rice flour, unclean utensils, and prolonged storage under unhygienic conditions at retail outlets, leading to a higher bacterial load in raw milk.

Sekar (2018) analysed 150 samples, including 25 khoa samples, and found a 24% prevalence rate of *Bacillus cereus* in khoa, which exceeds the rate observed in this study. This highlights the importance of maintaining proper hygiene throughout the production and distribution of dairy products.

Dai *et al.* (2024) examined 41 milk samples and reported a *Bacillus cereus* prevalence rate of 19.27%, which is higher than the rate found in this study. Their findings underscore the bacterium's potential risk to the dairy industry and its broader public health implications.

Conclusion

The high contamination rate of *B. cereus* in the milk products in the present study raises a significant concern for both food handlers and consumers. Future research should explore seasonal variations and intervention strategies to mitigate contamination.

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