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Microbiological Quality Assessment of Ice Cream Produced in Yemen

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Abstract

The microbiological quality of ice cream produced in Yemen was assessed to evaluate compliance with food safety standards and identify potential health risks. A total of 100 samples were collected from three Yemeni governorates (Sana'a, Ibb, and Hodeidah) and analyzed for aerobic plate count, Enterobacteriaceae, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, mold, and yeast counts. The results showed that 61% of the samples were contaminated, failing to meet acceptable microbial quality standards. Significant disparities were observed between large and small factories, with 52% of samples from large factories meeting the standards compared to only 26% from small factories (P = 0.013). *Escherichia coli* contamination was detected in 28% of small factory samples, while yeast and mold contamination was significantly higher in small factories (72%) compared to large factories (36%) (P = 0.013). Packaging type also had a significant impact on microbial quality (P = 0.003), with unpackaged samples showing substantially higher contamination rates than packaged samples. No *Salmonella* spp. or *Staphylococcus aureus* was detected in any samples, reflecting effective control of these pathogens. The findings emphasize the importance of implementing stringent hygiene practices and improving microbiological monitoring, especially in small-scale production facilities, to ensure the safety and quality of ice cream in Yemen.

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1. INTRODUCTION

Ice cream, a widely consumed frozen dessert, holds a special position due to its popularity across all age groups, particularly children, who represent a vulnerable demographic. Its year-round consumption, combined with its nutritional value, makes ice cream both a dietary indulgence and a potential vector for foodborne pathogens [1, 2]. The history of ice cream in Yemen dates back to the early 20th century, likely introduced during the British colonization of Aden under the administration of the British East India Company. Initially, ice cream was produced using traditional manuals, with small factories emerging in areas such as Tawahi and Al-Mualla. The introduction of electricity enabled the establishment of Yemen's first modern ice cream factory, Nana, in Al-Hodeida in the early 1980s. Since then, the industry has expanded, with factories and small-scale operations established in several governorates, including Sana'a, Aden, Ibb, and Hadhramaut [3]. Despite this growth, many small-scale producers still rely on manual methods and struggle to meet consistent quality and sanitary standards. Ice cream production can be categorized into industrial methods, which are characterized by stringent hygiene standards, and traditional methods, which are often associated with small-scale operations that may not consistently adhere to established safety protocols [4]. These traditional methods are prevalent in many countries, including Yemen, where sanitary conditions in small-scale operations often fall below acceptable levels owing to inadequate hygiene practices and a lack of regular microbiological control. Yemen, a country enduring

the devastations of war and economic hardship, is facing significant public health challenges. With widespread poverty, low individual incomes, and limited access to healthcare services, the population is highly vulnerable to the spread of infectious diseases. Foodborne pathogens, in particular, pose a considerable threat as early detection and surveillance systems are often absent or underdeveloped. These conditions emphasize the importance of providing baseline data on bacterial and viral pathogens transmitted through food. Such investigations play a vital role in disease surveillance and risk management, as demonstrated by previous studies on infectious agents in Yemen [5–7]. The microbiological quality of ice creams is influenced by both intrinsic and extrinsic factors. Ingredients such as milk, cream, fruits, and stabilizers may introduce microorganisms into the final product, whereas external factors such as manufacturing practices, storage conditions, and handling further contribute to contamination risks [8, 9]. Primary sources of contamination include raw milk and water, whereas secondary sources include utensils, handling, and flavoring agents [10]. Several studies have highlighted the microbial risks associated with ice cream, including contamination by pathogenic bacteria such as *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, and Listeria *monocytogenes* [9]. Fungal contaminants, such as yeasts and molds, have also been reported and are often linked to the use of inadequately processed raw sugar cane in the production process [11]. These contaminants pose significant health risks, particularly in children, and have been associated with outbreaks of gastrointestinal diseases worldwide [12]. In Yemen, while larger manufacturers often implement advanced measures such as pasteurization and freezing to mitigate microbial risks, small-scale producers frequently lack the necessary resources and infrastructure to ensure product safety. This disparity underscores the importance of comprehensive studies to evaluate and improve the microbiological quality of ice creams in Yemen, where data on microbial contamination are limited. This study aimed to investigate microbial contaminants, including bacteria, yeasts, and molds, and to analyze variations in contamination levels based on factory type, packaging, and production methods.

2. MATERIALS AND METHODS

2.1. Materials

- **Chemical Reagents:**
  All chemicals used in this study were of analytical grade. Media preparation and sterilization were performed using standard reagents, including buffered peptone water, Rappaport-Vassiliadis (RV) broth, and Xylose Lysine Deoxycholate (XLD) agar for *Salmonella* spp., Plate Count Agar for Total Aerobic Count (TAC), Violet Red Bile Glucose Agar for Enterobacteriaceae, and Baird Parker Agar for *Staphylococcus aureus*. All media were sourced from HiMedia, India.
- **Laboratory Equipment:**
  Key laboratory equipment included autoclaves (for media and sample sterilization), incubators (for microbial growth at controlled temperatures), colony counters (for enumeration of microbial colonies), and iceboxes for sample transportation.

2.2. Methods

- **Sample Collection:**
  A total of 100 random samples were collected from food-processing factories across three Yemeni governorates: Sana'a, Ibb, and Hodeidah. These governorates were chosen because of their diverse climatic, geographic, and socioeconomic conditions, that may influence the microbiological quality of food products. Sana'a, located in the northern highlands, is the largest city in Yemen with a mild semi-arid climate [13], while Ibb, known as the "Green Province," has a temperate climate that supports agricultural activities. In contrast, Hodeidah, situated along the Red Sea coast, experiences hot and humid tropical conditions [14] and poses unique challenges to food storage and safety. This regional diversity provides a representative sample for assessing the impact of environmental and production factors on ice cream quality.
- **Sample Transportation and Storage:**
  Samples were transported in sterilized iceboxes with temperature maintained at 4-5 ℃ and stored under the same conditions until microbiological testing.
- **Microbiological Testing:**
  This study primarily relied on the Yemeni Standard Specifications for Ice Cream Testing Methods (YSMO 817:2012) and the Microbiological Criteria for Foodstuffs (YSMO 1016:2019). These standards provide a comprehensive framework for evaluating the microbiological quality and safety of ice cream products, including detailed methodologies and acceptable limits for key parameters such as aerobic plate count, Enterobacteriaceae, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, yeast, and mold counts [15, 16].

1. **Total Aerobic Count (TAC):**
   Enumeration of total aerobic bacteria was performed using Plate Count Agar. Plates were incubated at 37 ℃ for 48 h, and colony counts were expressed as colony-forming units per gram (CFU/g) of sample [17].
2. **Detection of Enterobacteriaceae:**
   Violet Red Bile Glucose Agar was used to detect Enterobacteriaceae. The plates were then incubated at 37 ℃ for 24 h. Colonies were counted, and char-

acteristic red colonies with a precipitation zone were recorded as positive [18].

3. **Detection of *Salmonella* spp.:**
   Salmonella spp. detection involved enrichment in Buffered Peptone Water (pre-enrichment at 37℃ for 18 h), followed by selective enrichment in RV broth at 42℃ for 24 h. Samples were streaked on XLD agar, and characteristic black-centered colonies were confirmed biochemically [19].
4. **Detection of *Escherichia coli*:**
   The Most Probable Number (MPN) technique was used for *Escherichia coli* detection. Tubes showing gas formation in Lauryl Tryptose Broth were streaked onto Eosin Methylene Blue (EMB) agar. Biochemical confirmation was performed using the IMViC test [20].
5. **Detection of *Staphylococcus aureus*:**
   Staphylococcus aureus was detected using Baird Parker Agar. The plates were then incubated at 37℃ for 24–48 h. Characteristic black colonies with halos were confirmed using coagulase tests [21].
6. **Yeasts and Molds Count:**
   Potato Dextrose Agar supplemented with chloramphenicol was used for enumeration of yeast and mold . The plates were incubated at 25℃ for 5–7 days. The colonies were counted and expressed as CFU/g [22].

2.3. Statistical Analysis

Statistical analysis was performed using **Epi Info** software (version 7) to evaluate the relationships between microbial quality and various factors, including factory type, ice cream type, and packaging type. Descriptive statistics were used to calculate the frequencies and percentages of acceptable and unacceptable samples. Chi-square tests were used to assess the significance of the associations between categorical variables, with a significance level set at $P < 0.05$. These methods were employed to ensure robust analysis of the data and highlight the critical factors affecting the microbial quality of ice cream samples.

3. RESULTS

Microbiological analysis of the 100 ice cream samples. The results indicated that only 39% of the samples were deemed acceptable, whereas 61% failed to meet the required microbial quality standards, as shown in Figure 1. This finding underscores the widespread challenges in ensuring the safety of ice cream products, particularly in the context of small-scale production facilities with limited resources and infrastructure.

A comparison between large and small factories demonstrated notable differences in microbial quality ,Table 1. Among the samples collected from large factories, 52% met the acceptable standards, whereas 48%

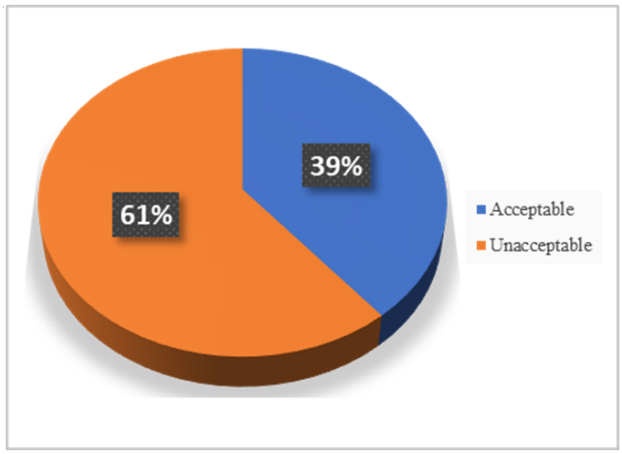

Figure 1. Illustrates the distribution of the 100 ice cream samples into two categories: acceptable and unacceptable.

were classified as unacceptable. In contrast, only 26% of the samples from small factories were acceptable, with 74% failing to meet standards. Statistical analysis revealed a significant association between factory size and microbial quality ($P = 0.013$), highlighting the superior hygiene practices typically observed in large-scale operations. Additionally, the analysis revealed disparities in the quality of the ice cream types. Water ice samples exhibited better microbial compliance, with 58.62% meeting acceptable standards, compared to only 30.99% of semi-ice milk samples.

Semi-ice milk samples showed a higher proportion of unacceptable results (69.01%) than water ice (41.38%), and this difference was statistically significant ($P = 0.013$). Packaging also plays a critical role in influencing the microbial quality, with funnel packaging showing the highest rate of acceptable samples (75%), followed by biscuit packaging (62.5%). In contrast, the cup- and stick-packaged samples had acceptance rates of 27.27% and 31.25%, respectively. Table 2 highlights significant differences in the microbiological quality of ice cream samples from large and small factories, focusing on aerobic plate count (APC), Enterobacteriaceae, and yeast and mold counts. For APC, large factories showed a lower proportion of unacceptable samples (90.0%) compared to small factories (96.0%), with average counts of 1.07×10^5 CFU/g and 6.72×10^4 CFU/g, respectively. Enterobacteriaceae contamination was more prevalent in small factories, where 54.0% of samples were unacceptable, compared with 26.0% in large factories, with average counts of 4.9×10^3 CFU/g and 2.2×10^3 CFU/g, respectively. Similarly, yeast and mold contamination was significantly higher in small factories, with 72.0% of samples classified as unacceptable compared to 36.0% in large factories, and average counts of 6.0×10^4 CFU/g and 9.0×10^3 CFU/g, respectively. These findings indicate that small factories are more susceptible to microbial contamination across all parameters, likely because of inadequate sanitation and post-production handling, em-

Table 1. Microbial Quality Analysis of Ice Cream Based on Factory Type, Ice Cream Type, and Packaging Type

Category	Subject No. (%)	Acceptable No. (%)	Unacceptable No. (%)	P. Value
Factory Type				
Large Factories	50 (50.0%)	26 (52.0%)	24 (48.0%)	**0.013**
Small Factories	50 (50.0%)	13 (26.0%)	37 (74.0%)	
Ice Cream Type				
Semi Ice Milk	71 (71.0%)	22 (30.99%)	49 (69.01%)	**0.013**
Water Ice	29 (29.0%)	17 (58.62%)	12 (41.38%)	
Packaging Type				
Biscuit	8 (8.0%)	5 (62.50%)	3 (37.50%)	**0.003**
Cup	44 (44.0%)	12 (27.27%)	32 (72.73%)	
Funnel	16 (16.0%)	12 (75.00%)	4 (25.00%)	
Stick	32 (32.0%)	10 (31.25%)	22 (68.75%)	
Total	**100%**	**39 (39.0%)**	**61 (61.0%)**	

Table 2. Microbiological Examination of Ice Cream Samples: Proportion of Unacceptable Samples and Average Counts by Factory type

Microbiological examination	YSMO 1016/2019 limit	Large factories (No. =50)		Small factories (No. =50)	
		Unacceptable sample No. (%)	Average count	Unacceptable sample No. (%)	Average count
Aerobic plate count	$5x10^4$-10^5 CFU/ g	45 (90.0%)	10^5×1.07	48 (96.0%)	10^4×6.72
Enterobacteriaceae	10-10^2 CFU/ g	13 (26.0%)	10^3×2.2	27 (54.0%)	10^3×4.9
Yeast and Mold count	10^2-10^4 CFU/ g	18 (36.0%)	10^3×9.0	36 (72.0%)	10^4×6.0

phasizing the need for improved hygiene practices in small-scale production facilities.

The detailed microbial quality assessment, outlined in Table 3, identified specific contaminants that contributed to the overall results. The aerobic plate count (APC) exceeded the acceptable limits in 17% of the total samples, with small factories contributing to the majority of these cases. Enterobacteriaceae contamination was observed in 40% of the samples, with a higher prevalence in small factory products (54%) than in large factory products (26%). Similarly, *Escherichia coli* was detected in 25% of the samples, with a higher occurrence in small factory samples (28%) than in large factory samples (22%). Notably, no *Salmonella* spp. or *Staphylococcus aureus* were detected in any of the samples, reflecting the effective control of these specific pathogens. However, yeast and mold contamination posed significant challenges, particularly in small factories, where 72% of the samples exceeded acceptable yeast counts, compared to 36% in large factories. The mold contamination exceeded the permissible limits in 12% of the samples, with the majority originating from small factories.

4. DISCUSSION

The microbiological quality of food, particularly ice cream, is significantly influenced by factors such as the raw material quality, production environment, processing hygiene, and storage conditions. APC is a crucial indicator of microbial contamination and reflects overall food quality. In this study, the APC levels for large factories ranged from 2x10 to $3.6x10^6$ CFU/ml, with an average of $1.07x10^5$ CFU/ml. These findings align with the results of Yusuf et al. in Nigeria [23], although 10% of large factory samples exceeded the limits established by Yemeni standards (YSMO 1016/2019) [15]. Meanwhile, small factories showed higher APC levels, ranging from 2.0x10 to $5.04x10^5$ CFU/ml, with an average of $6.72x10^4$ CFU/ml. This is higher than the results reported by Vica et al. in Romania [24] but lower than those reported by Mokbul et al. in Palestine [9]. The significant difference ($P<0.05$) in microbiological quality between large and small factories emphasizes the need for improved hygiene and processing practices in small-scale facilities. The high microbial load in small factories can be attributed to inadequate heat treatment, poor equipment

Table 3. Microbial Quality Assessment of Ice Cream Samples

Microbial Examination	Frequency	Percentage (%)
Aerobic plate count ($5x10^4$-10^5 CFU/ g)		
Acceptable	83	83.00%
Unacceptable	17	17.00%
Enterobacteriaceae (10-10^2 CFU/ g)		
Acceptable	60	60.00%
Unacceptable	40	40.00%
***Escherichia coli* (absent /g)**		
Acceptable	75	75.00%
Unacceptable	25	25.00%
***Salmonella* spp. (absent/25g)**		
Acceptable	100	100.00%
Unacceptable	0	0
***Staphylococcus aureus* (10-10^2 CFU/ g)**		
Acceptable	100	100.00%
Unacceptable	0	0
Mold count (10^2-10^4 CFU/ g)		
Acceptable	88	88.00%
Unacceptable	12	12.00%
Yeast count (10^2-10^4 CFU/ g)		
Acceptable	47	47.00%
Unacceptable	53	53.00%
TOTAL	**100**	**100.00%**

sanitation, and unhygienic handling practices. The analysis of Enterobacteriaceae contamination revealed that 26% of large factory samples were contaminated, with an average count of $2.2x10^3$ CFU/ml, while 54% of small factory samples showed contamination, with an average of $4.9x10^3$ CFU/ml. These values were higher than those reported by Kahraman and Kolanciyan in Istanbul, Turkey [25] but lower than the contamination levels reported by Yaman et al. in Kars, Turkey [26]. The presence of Enterobacteriaceae indicates poor microbiological quality and potential post-pasteurization contamination, which could be due to inadequate heat treatment or unhygienic practices during handling and storage [1]. However, the absence of *Salmonella* spp. and *Staphylococcus aureus* in all samples is consistent with previous studies by Kahraman and Kolanciyan in Istanbul, Turkey [25], Nayak et al. in India [27], and Alsagher et al in Libya [28]. *Escherichia coli* was observed in 22% of large factory samples and 28% of small factory samples, indicating potential issues with the water quality used during production [2]. These results were higher than those reported by El-Malt et al. in 2013 [8] but lower than those reprted by Nayak et al. in 2020 [27]. Additionally, yeast and mold counts were higher in small factory samples (72%) compared to large factories (36%), with averages of $6.0x10^4$ and $9.0x10^3$ CFU/ml, respectively. These results suggest contamination from ingredients, poor sanitation, and inadequate thermal processing. High yeast and mold counts may lead to undesirable changes in the product, rendering it unmarketable or unsuitable for consumption [8, 29]. The differences between packaged and unpackaged ice creams from small factories were notable. Packaged samples showed no microbial growth, while 48% of unpackaged samples exceeded the Yemeni standard limit of 10^5 CFU/ml [15]. Enterobacteriaceae contamination was detected in 8% of the packaged samples and 100% of the unpackaged samples, further highlighting the risks associated with unpackaged products. The absence of *Escherichia coli* in packaged samples and its presence in 56% of unpackaged samples reinforces the need for strict packaging and handling practices to minimize contamination risks [4]. Yeast and mold contamination was also significantly higher in unpackaged samples (96%) than in packaged samples (48%), which is consistent with the findings of El-Malt et al.(2013) [8]. Although this study provides valuable insights into the microbiological quality of ice cream produced in Yemen, several limitations should be considered when interpreting the findings. First, the study focused solely on bacterial and fungal contamination without assessing the presence of viral pathogens, which could pose significant public health risks. Second, the sampling was limited to three governorates, which, although diverse in climate and socio-economic conditions, may not fully represent the entire country. Third, variations in production practices within large and small factories were not explored in de-

tail, which could provide further context for the observed disparities in microbial contamination. Additionally, the cross-sectional design of the study limits its ability to capture seasonal variations or longitudinal trends in the contamination levels. Future research should address these limitations by including a broader range of pathogens, expanding the geographical scope, and exploring the production practices in greater detail. Longitudinal studies could also provide a deeper understanding of the factors that influence microbial quality over time. In addition, it is essential to explore novel approaches to enhance microbial safety during ice cream production. One approach involves the incorporation of natural antimicrobial agents. For instance, *Boswellia sacra* (frankincense) may serve as both a preservative and a flavor-enhancing agent. *Boswellia sacra* has been reported to exhibit significant antimicrobial activity against a range of bacterial and fungal pathogens [30], which could contribute to extending shelf life and improving the microbiological safety of ice cream. Future studies should investigate the potential of such natural ingredients to reduce microbial contamination while maintaining product quality and consumer acceptability.

5. CONCLUSION

This study revealed significant differences in the microbiological quality of ice cream from large and small factories in Yemen. Large factories showed better adherence to Yemeni standards, with lower microbial contamination, whereas small factories had higher contamination rates. These differences underscore the importance of enforcing strict hygiene practices and improving microbiological monitoring, particularly in small facilities, to ensure the safety and public health of ice cream.

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