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Physico-Chemical and Microbial Properties of Ice Used for Various Purposes in Kangra, Himachal Pradesh

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ABSTRACT:
This study aimed to assess the physicochemical and microbiological qualities of ice blocks sold at various locations in Kangra, Himachal Pradesh. Samples were collected from four different sites and microbiological quality was assessed using the presence or absence of coliforms as an indicator organism. The physicochemical parameters analyzed included pH, temperature, dissolved oxygen (DO), total dissolved solids (TDS), total suspended solids (TSS), electrical conductivity (EC), and chloride content. The results were compared with the WHO Drinking Water Standards. The findings revealed that All samples tested positive for coliforms, indicating potential health risks. The TDS values ranged from 68.00- 271.67 mg/L, the pH from 7.03-7.50, the TSS from26.00-37.67 mg/L, the ice melting temperatures from of 3.23-4.07^{0}C, the DO from 15.2- 21.5 mg/L, the EC from 97.79-375.00 µs/cm, and the chlorides concentrations from 55.50- 60.09 mg/L. These findings underscore the necessity for ice manufacturers to comply with FDA-recommended good manufacturing practices (GMP).

Keywords:
Ice quality, Physico-chemical analysis, Microbiological analysis, Coliforms, Water quality

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1. INTRODUCTION

The formation of ice is a consequence of the cooling and subsequent solidification of water vapor molecules. A remarkable phenomenon occurs within a temperature range of 0–4°C, wherein water experiences contraction and an increase in density (Mako et al., 2014). This initial density increase was attributed to the adoption of open-structure arrangements by water molecules at 0 °C, resembling those found in ice crystals. These configurations facilitate a more compact molecular structure, consequently leading to an observed increase in the density across this temperature range. As the temperature continues to decrease, this process becomes a crucial stage in the transformation of liquid water into solid ice.

Ice has served a variety of functions, owing to which the manufacturing of ice, particularly ice cubes, is growing, and generates large quantities of this product with the help of cube-maker machines (Gaglio et al., 2017). According to the U.S. In the Public Health Service Food Service Sanitation Manual, ice is classified as food and is subject to the Good Manufacturing Practices (GMP) Regulations found in CRF21, Chapter 1, parts 20 and 110 (Moyer et al., 1993). These regulations cover ice manufacturing facilities, the quality of source water, and employee sanitary practices. While ice is commonly used in food premises for cold drinks and is generally perceived as safe owing to its frozen state, studies have revealed the presence of harmful pathogens in ice cubes used for beverage preparation (Thongkaow et al., 2021). Despite its seemingly innocuous nature, the potential health risks associated with contaminated ice underscore the importance of proper handling and hygiene practices (Lateef et al., 2006), especially in food establishments to ensure the safety of consumable ice used in beverages (Hampikyan et al., 2017). Most of the ice was supplied by commercial plants, which produce blocks of ice by mechanical freezing. While freezing is detrimental to a large number of organisms in the environment, microbial contaminants in ice can approach unsafe levels for human consumption (Ladanyi & Morrison, 1968). Ice used with or as food must have the same microbiological quality as that of drinking water. Owing to the growing trend of production in this industry, a thorough assessment of the hygienic quality of the items on the market is urgently needed (Caggiano et al., 2020).

Numerous investigations on the microbiological quality of food ice have revealed that it may serve as a conduit for foodborne illnesses and various types of pathogens, such as *Escherichia coli* (Gaglio et al., 2017; Brar et al., 2023), *Pseudomonas aeruginosa*, *Clostridium perfringens* spores, *C. perfringens*, *Salmonella spp., Shigella spp., Campylobacter spp., Vibrio cholera* (Waturangi et al., 2013), *Aeromonas spp*., and *Yersinia spp*. (Gerokomou et al., 2011; Wilson et al., 1997). If these bacteria remain viable during storage, ice consumption may directly or indirectly cause human infections including diarrheal illnesses (Falcão et al., 2004), hepatitis, and typhoid fever. Contaminated ice can be a source of large disease outbreaks, including cholera, dysentery, and cryptosporidiosis.

According to the World Health Organization, ice intended for consumption or that comes into direct contact with food should be as safe and of the same quality as drinking water. Thus, the analysis of its physicochemical quality has also become an important aspect for ensuring its best benefits for consumer health, as does the need to evaluate the overall stability of the water quality (Salazar et al., 2018).

Therefore, the objective of this study was to investigate the microbiological and physicochemical qualities of ice used in the Kangra District. Kangra encompasses multiple ice production factories across different locations, specializing in commercial ice manufacturing and offering a diverse range of products, including block ice, cube ice, tube ice, flaked ice, plate ice, and shaved ice. This comprehensive array caters to the requirements of numerous establishments such as hotels, food service outlets, restaurants, convenience stores, supermarkets, bars, cafes, hospitals, and industries. These ice products fulfil a wide spectrum of functions within these sectors, underscoring the significance of Kangra’s ice manufacturing industry.

2. MATERIAL AND METHODS

Study area

The present study was conducted in District Kangra, 32^{0}5'59.2944'' N and 76^{0}16'8.7744'' E of the state Himachal Pradesh, India. Four sampling sites were selected for this study: (i) The Ice manufacturing factory Nagrota Bagwan, (ii) The Ice manufacturing factory Tanda, (iii) The Local juice vendor Nagrota Bagwan, and (iv) The Local juice vendor Rait. Table 1 presents the sample numbers and corresponding sampling locations.

Sample collection and analysis

Sampling was conducted between February and April 2022. Samples were aseptically collected from each location in sterile glass bottles and transported to the laboratory for subsequent analysis. The sample preparation was completed within 24 h of sample collection. The samples were maintained at temperatures below 5°C until further analysis. The interval between collection and analysis was approximately 1 h to allow ice to melt. Each sample was subsequently separated into two sterilized plastic bottles, one for physicochemical parameter analysis (Table 2) and the other for microbiological analysis at room temperature to ensure complete ice melting.

Physicochemical parameters analysis:

The physicochemical parameters of the ice samples were analyzed using the procedure recommended by the American Public Health Association (APHA, 2012). The ice samples were assessed for temperature, dissolved oxygen, pH, TDS, TSS, EC, and chloride content**.** Table 2 lists the different methods employed to analyze the physicochemical parameters.

Microbiological analysis

The bacteriological quality of the ice samples was determined by assessing total coliform bacteria and faecal coliform bacteria using the most probable number (MPN) method (Kumar, 2013). The coliform group of bacteria includes aerobic, facultative anaerobic, gram-negative, and non-sporulating bacilli. A multiple-tube fermentation technique was employed to enumerate the positive presumptive and confirmed coliform tests. Serial dilutions of the samples (1:10) were prepared for the determination of aerobic microorganisms.

An initial presumptive test was conducted, and fermentation tubes were used for each sample and filled with 9 ml of MacConkey broth. One Durham´s tube was placed in each tube in an inverted condition. The diluted samples were then added to the fermentation tubes. After shaking, tube samples were incubated at 35-37°C for 48 h. Tubes showing gas production in Durham´s tubes were recorded as positive (+ve). The negative control tubes were incubated for an additional 24 hours to confirm the results.

Following the acquisition of positive results, the culture was purified using a confirmatory test. Brilliant Green Lactose bile (BGLB) broth medium was used to inhibit the growth of all fermenters, except coliforms. Consequently, gas formation in the BGLB constitutes a confirmed test, indicating the presence of coliforms. The results of the test are expressed as the most probable number (MPN), as the count is based on statistical analysis of sets of tubes in a series of serial dilutions. By definition, MPN is related to a sample volume of 100 ml.

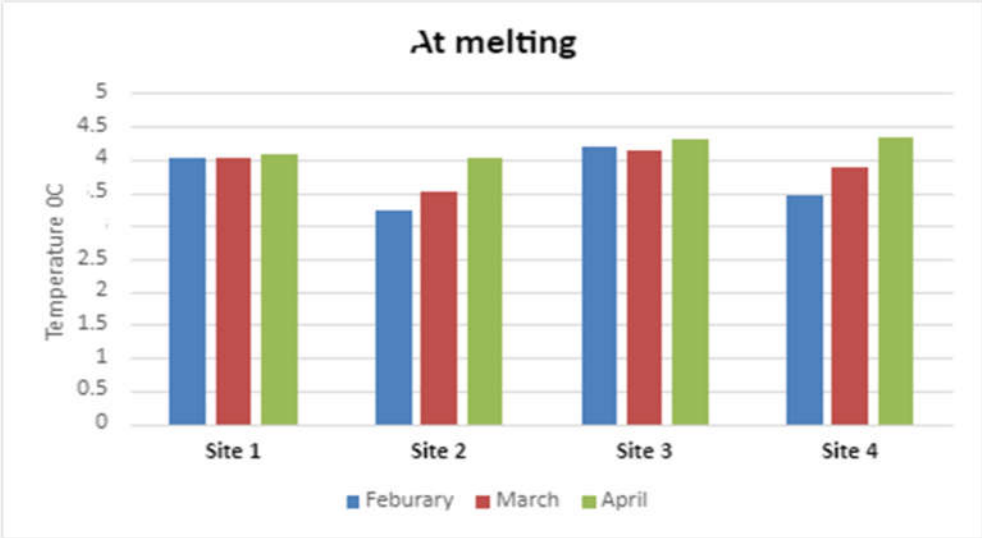
Graphic presentation: Different types of graphs were applied to present the data based on the investigation.

3. RESULTS AND DISCUSSION

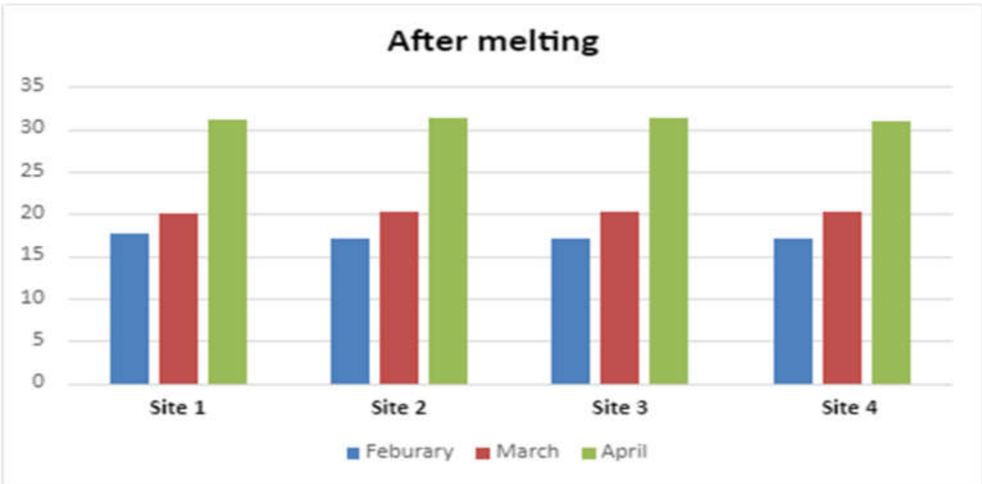
This study examined the total and fecal coliform bacteria as well as the physicochemical characteristics of packaged ice from many Kangra localities. The presence of these microorganisms and physicochemical parameters are used to evaluate the hygiene and sanitation conditions of production areas, as well as the potential presence of pathogens.

Physicochemical Parameters

Physicochemical parameter studies are essential for obtaining precise information regarding water quality, enabling the comparison of various physicochemical parameter values with established standards. Physicochemical parameters are presented in Tables 3, 4, and 5. The present study revealed the monthly temperature variations among the four sites. At each site, the maximum temperature during ice melting was observed in April, ranging from 4.07 to 4.33 ^{0}C, while the minimum temperature was recorded in February, ranging from 3.23 to 4.03 ^{0}C (Graph 1). After the melting of ice, the maximum temperature ranged from 30.40 to 31.17^0 °C in April, whereas the minimum temperature ranged from 17.18 to 17.60^0 °C in February (Graph 2).



Graph 1: Monthly variation in temperature (°C) of collected ice samples during melting time



Graph 2: Monthly variation in temperature (°C) of collected ice samples after melting time

Table 1: Samples and Sampling locations

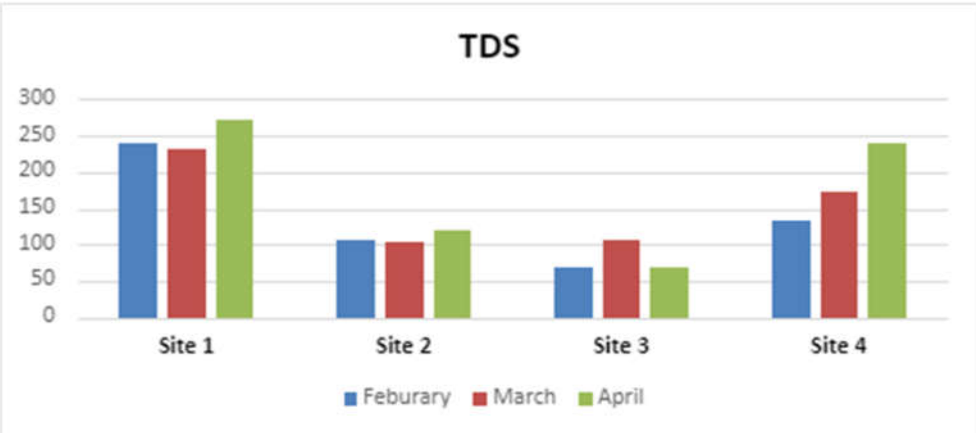
Sample	Sampling sites	Coordinates of sites
A	Ice manufacturing factory Nagrota Bagwan	32°6'34.1352"N, 76°22'33.7908"E
B	Ice manufacturing factory Tanda	32°5'59.892"N, 76°17'17.447"E
C	Local juice vendor, Nagrota Bagwan	32°6'24.912"N, 76°22'39.791"E
D	Local juice vendor, Rait, Kangra	32°11'8.988"N, 76°12'42.803"E

Table 2: The physico-chemical parameters

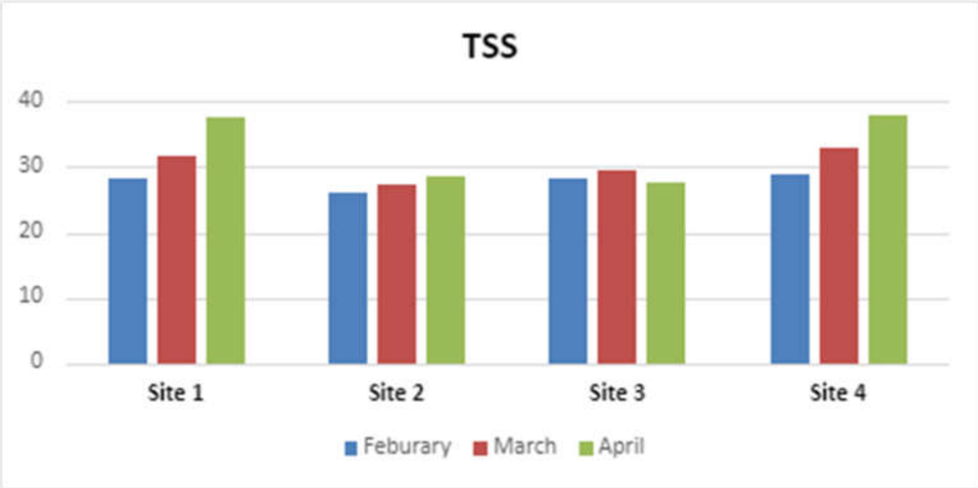
Parameter	Methods	Units
Temperature	Digital Thermometer (Range -50°C -+300°C)	°C
Dissolved oxygen	Winkler titration method	mg/L
pH (Hydrogen ion concentration)	pH meter (eco Testr pH 1 Range 0.0-14.0pH)	
Total Dissolved Solids	Filtration and evaporation method	ppm
Total Suspended Solids	Filtration method	mg/L
Electrical conductivity	Electrical conductivity meter (Cyber Scan 600)	µs/cm
Chlorides	Iodometric Methods I	mg/L

Particles in water exist in either suspended or dissolved states. The TDS level recommended by the WHO ranges from 50-300 mg/L; however, our findings ranged from 68.00- 271.67 mg/L. The maximum value was observed in sample A (271.67±7.51) in April, whereas the minimum value was recorded in sample C (68.00±4.58) in April and February (Graph 3). The values from both sites were within the acceptable range according to WHO guidelines.

The TSS concentration increased from February to April, with values ranging from 26.00-37.67 mg/L (Graph 4). The minimum TSS value was observed in sample A (26.00±2.65) in February, while the maximum was recorded in sample D (38.33±3.21) in April. Notably, in sample D, the TSS concentrations in February and March exceeded the WHO permissible limit of 30 mg/L for drinking water.



Graph 3: Monthly variation in TDS (ppm) of collected ice samples



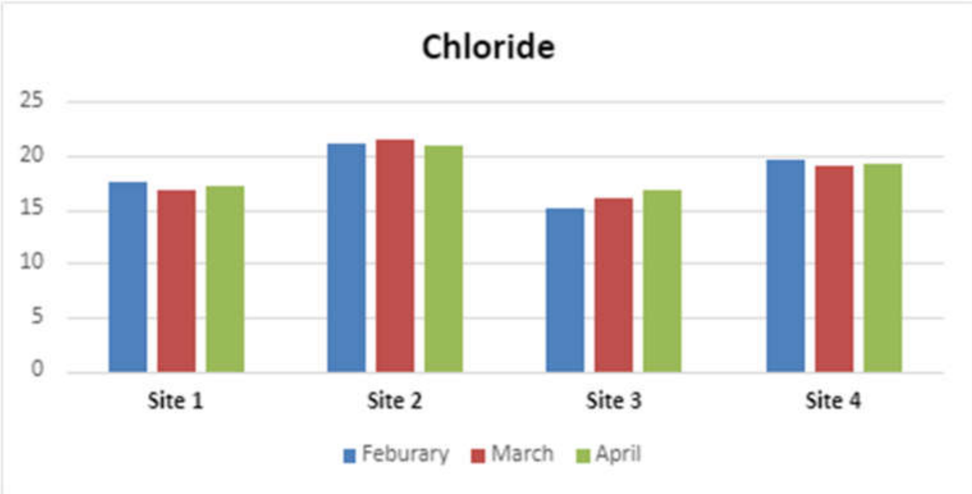
Graph 4: Monthly variation in TSS (mg/L) of collected ice samples

DO quantifies the level of pollution caused by organic matter and degradation of organic substances, as well as the capacity of the water body for self-purification (Chapman, 2021). DO values ranged from 15.2- 21.5 mg/L in February, 16-21.5 in March, and 16.9-21 in April (Graph 5). For drinking water, the dissolved oxygen concentration must be 6.5-8 mg/L. The highest DO value was observed in Sample B (21.5 mg/L) in March, whereas the minimum value was recorded in Sample C (15.2 mg/L) in February.

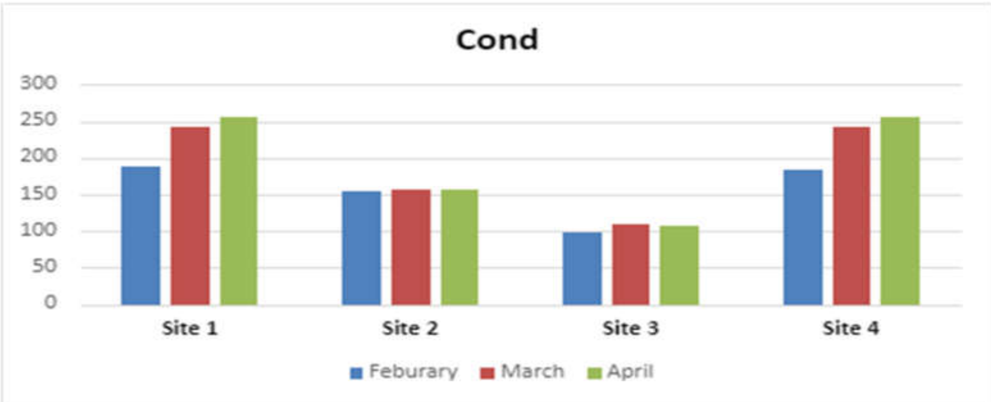
Graph 5: Monthly variation in DO (mg/L) of collected ice samples

Chlorides generally have minimal adverse effects on human health; however, their excessive consumption may lead to significant issues. In our study, chloride concentrations (mg/L Cl) varied between 55.50- 60.09 mg/L (Graph 6). According to the World Health Organization (WHO), chloride levels in public drinking water should not exceed 250 mg/L, as adverse health impacts are unlikely to occur above this threshold. Sample A exhibited the maximum concentration (60.07±0.65) in April, while Sample C exhibited the lowest concentration (55.50±0.62) in February. A previous study conducted on ice samples revealed that chloride concentrations varied between 5 mg/L and 110 mg/L, with the majority of samples (93%) being below 10 mg/L (Gerokomou et al., 2011).

Water conductivity is a valuable measure of salinity and the total salt content. It is directly proportional to the dissolved mineral matter content (Acharya et al., 2008). In this study, the value of electrical conductivity (as µs/cm) was highest in April and lowest in February, ranging from 97.79-375.00 µs/cm (Graph 7), which was under the expected range of WHO regulation recommended for drinking water (400 µs/cm). Sample A exhibited the highest electrical conductivity (375.00±0.72 µs/cm) in April; the lowest electrical conductivity was exhibited by Sample C (97.79µs/cm) in February.

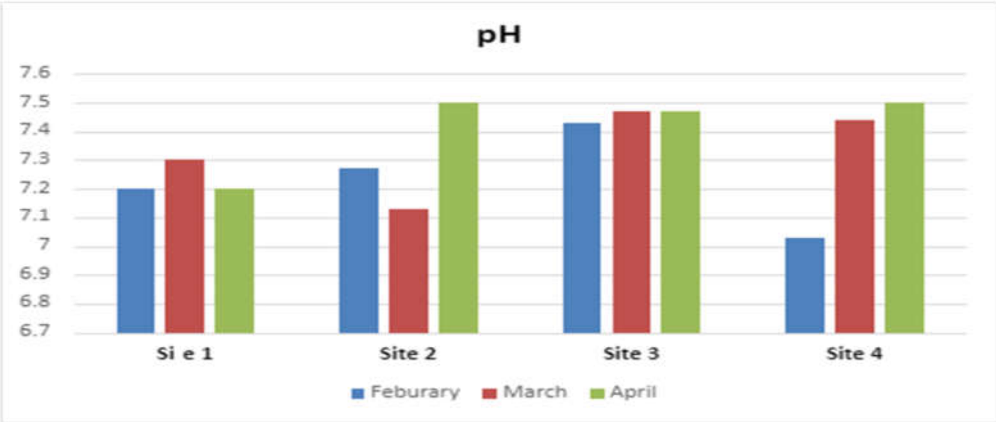


Graph 6: Monthly variation in Chloride (mg/L) of collected ice samples



Graph 7: Monthly variation in Electrical Conductivity (µs/cm) of collected ice samples

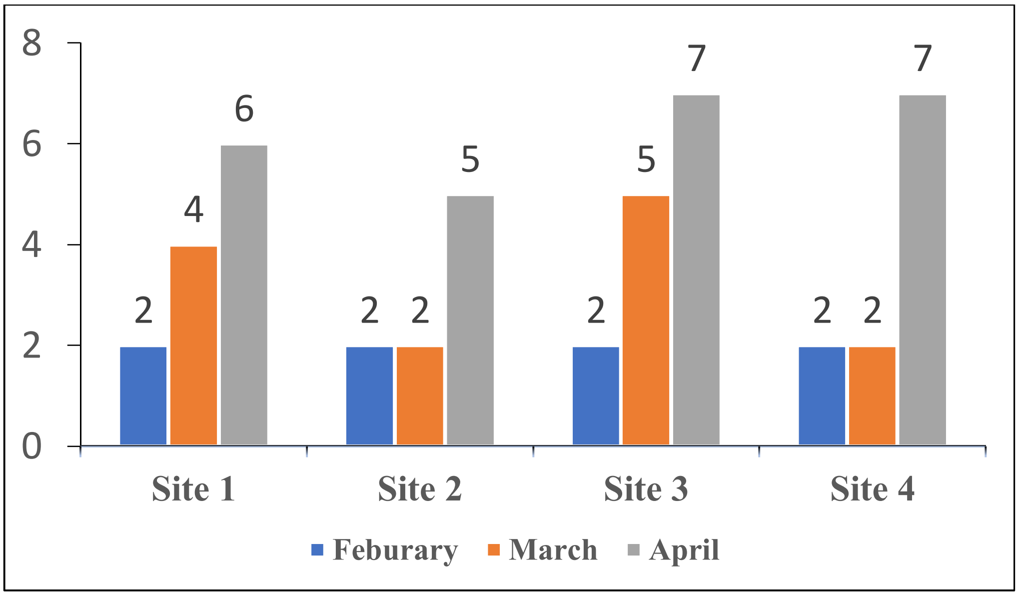
The pH of the water can be used to determine its acidity and alkalinity. In this study, the pH values ranged from 7.03-7.50 in each Sample A (Graph 8). The maximum pH value was observed in April in Samples B (pH 7.50±0.10) and D (7.50±0.15), while the minimum was detected in Sample A (7.20±0.20). The observed pH range falls within the prescribed range of the WHO, which is 6.5 to 8.5 for drinking water. In February, the lowest pH value was recorded in Sample D (7.03±0.15) and the highest in Sample C (7.43±0.45). pH values fluctuate due to changes in the levels of CO2, carbonate, and bicarbonate in water (Asaolu&Olaofe, 2004; Thakur et al., 2025). A previous study conducted on ice samples revealed that pH values ranged from 7.2 to 8.3 with a mean value of 7.7 (Gerokomou et al., 2011). Normally, drinking water has a pH of 7 (Brar et al., 2023).



Graph 8: Monthly variation in pH of collected ice samples

Microbiological analysis

The results are shown in Tables 3, 4, and 5, and total coliform bacteria (MPN/100 ml) and fecal coliform bacteria (MPN/100 ml) were isolated from four samples. The International Packaged Ice Association (IPIA) establishes limits for specific indicator tests to ensure consumer safety while maintaining quality control. According to these guidelines, the heterotrophic plate count of water should not exceed 500 MPN/ml and the acceptable range for coliforms is <1 MPN /100 ml. Coliforms serve as indicators of hygienic conditions (Hazen 1988).In this study, at each site, the maximum total coliform bacteria was observed in April, ranging from 5-7 MPN /100 ml, while the minimum range was observed in February, which was <2 MPN /100 ml (Graph 9).



Graph 9: Monthly variation in Total Coliform Bacteria (MPN/100ml) of collected ice samples

Table 3: Average value of physico-chemical and microbiological parameters of ice sample in the month of February

Parameters	Sample A	Sample B	Sample C	Sample D
Temperature (^{0}C)				
At melting time	4.03±0.15	3.23±0.25	4.20±0.20	3.47±0.50
After melting	17.60±0.40	17.73±0.47	17.17±0.31	17.13±0.31
Conductivity (µs/cm)	328.33±3.51	155.33±7.51	97.79±3.68	241.33±4.16
pH	7.20±0.20	7.27±0.25	7.43±0.45	7.03±0.15
Chlorides (mg/l)	56.20±0.36	59.17±0.78	55.50±0.62	59.40±0.89
Total Dissolved solids (ppm)	238.39±1.96	105.00±6.00	68.33±5.51	241.33±6.11
Total Suspended Solids (mg/l)	28.33±3.51	26.00±2.65	28.33±5.51	29.00±2.00
Dissolved oxygen (mg/l)	17.5	21.2	15.2	19.6
Total Coliform Bacteria (MPN/100ml)	<2	<2	<2	2
Faecal Coliform Bacteria (MPN/100ml)	Absent	Absent	Absent	Absent

Table 4: Average value of physico-chemical and microbiological parameters of ice sample in the month of March

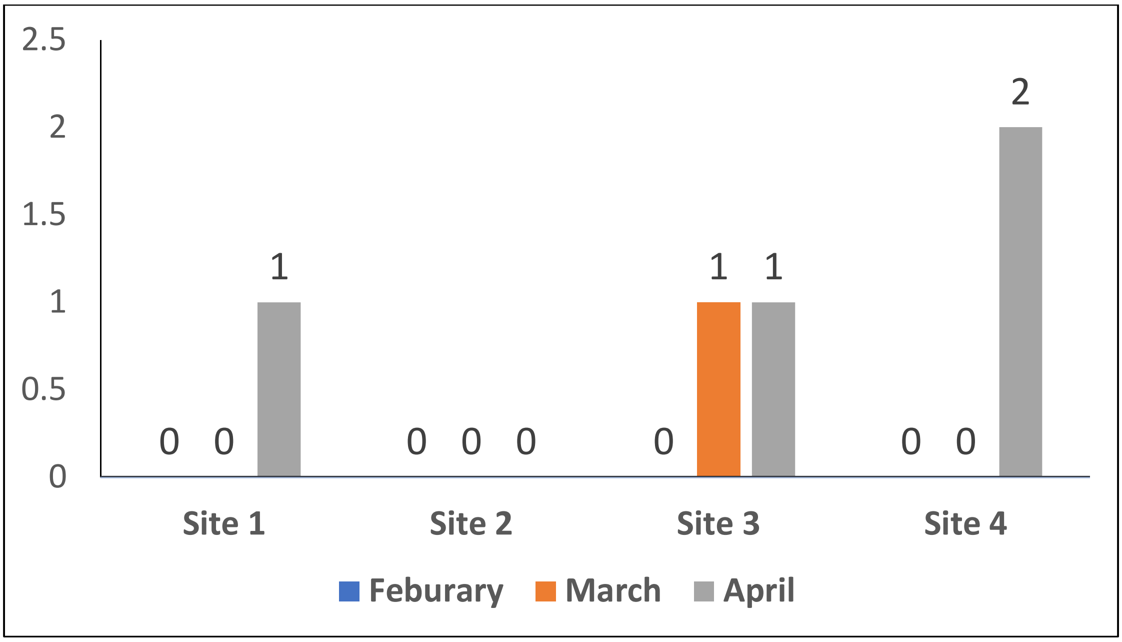
Parameters	Sample A	Sample B	Sample C	Sample D
Temperature (^{0}C)				
At melting time	4.03±0.15	3.53±0.51	4.13±0.15	3.87±0.42
After melting	19.80±0.36	21.10±1.14	20.17±0.87	20.57±1.26
Conductivity (µs/cm)	328.00±6.00	157.67±5.51	110.33±6.51	241.33±4.16
pH	7.30±0.10	7.13±0.15	7.47±0.40	7.44±0.14
Chlorides (mg/l)	58.87±0.42	59.40±0.70	56.10±0.30	60.09±0.39
Total Dissolved solids (ppm)	231.67±4.51	103.33±4.04	106.33±7.51	172.00±9.00
Total Suspended Solids (mg/l)	31.67±2.52	27.33±3.51	29.67±4.04	33.00±4.58
Dissolved oxygen (mg/l)	16.8	21.5	16	19
Total Coliform Bacteria (MPN/100ml)	4	<2	5	2
Faecal Coliform Bacteria (MPN/100ml)	Absent	Absent	1	Absent

Table 5: Average value of physico-chemical and microbiological parameters of ice sample in the month of April

Parameters	Sample A	Sample B	Sample C	Sample D
Temperature (°C)				
At melting time	4.07±0.15	4.03±0.15	4.30±0.26	4.33±0.42
After melting	30.40±0.72	31.17±0.25	31.40±0.62	31.00±0.70
Conductivity (µs/cm)	375.00±13.53	157.33±4.51	108.00±4.58	183.33±4.04
pH	7.20±0.20	7.50±0.10	7.47±0.35	7.50±0.40
Total Dissolved solids	271.67±7.51	118.33±6.03	68.00±4.58	132.67±7.09

(ppm)				
Total Suspended Solids (mg/l)	37.67±3.06	28.67±2.52	27.67±3.51	38.33±3.21
Dissolved oxygen (mg/l)	17.2	21	16.9	19.2
Total Coliform Bacteria (MPN/100ml)	6	5	7	7
Feacal Coliform Bacteria (MPN/100ml)	>1	Absent	1	>2

The highest total coliform bacteria concentrations were observed in samples C and D, that is, 7 MPN /100 ml, which is seven times higher than the acceptable range of IPIA limits, substantiating the assertion of a sanitation issue. Both samples were obtained from local juice vendors. While fecal coliform bacteria were detected only in April, their concentrations ranged between 1-2 MPN /100 ml at each site (Graph 10), with the exception of Sample B.



Graph 10: Monthly variation in Fecal Coliform Bacteria (MPN/100ml) of collected ice samples

Izani et al. (2012) assessed fecal coliform contamination in ice cubes from food outlets in Kelantan, revealing that fecal coliforms ranged from 1CFU/100 ml to >50CFU/100 ml (Noor Izani et al., 2012). Gerokomou et al., (2011) study isolated Total coliforms bacteria from ice samples, which ranged from 1 to 97 cfu/100 ml, fecal coliforms bacteria, which ranged from 1 to 100 cfu/100 ml (Gerokomou et al., 2011). Nichols et al. (2000) in the United Kingdom emphasized the high contamination levels in ice used to cool food, particularly coliforms and enterococci (10^2CFU/100 ml) (Nichols et al., 2000). Schmidt and Rodrick (1999) investigated the quality of packaged ice in Florida, highlighting discrepancies in label information and instances where one sample exceeded the state regulatory limits for aerobic plate and coliform counts (500 CFU/ml) (Schmidt & Rodrick, 1999). Moyer et al. (1993) noted elevated heterotrophic plate counts in packaged ice, particularly in samples from convenience stores, exceeding the levels approved by the Packaged Ice Association (Moyer et al., 1993).

A comprehensive review of various studies on the physicochemical and microbiological characteristics of ice highlights the critical importance of water quality management, particularly in the context of ice production and consumption. The findings from these studies reveal significant concerns regarding microbial contamination of ice, potentially leading to health risks for consumers (Chavasit et al., 2011). Consequently, the management and protection of ice, as well as the assurance of the proper quality of ice consumption, are imperative.

4. CONCLUSION

An evaluation of ice blocks sold in Kangra, Himachal Pradesh, examining their physicochemical and microbiological properties has uncovered notable safety issues for consumption. The detection of coliforms in each sample suggests a possible health hazard, emphasizing the importance of rigorous monitoring and enhanced production methods. Although certain physicochemical parameters such as pH, temperature, dissolved oxygen, and total dissolved solids, electrical conductivity, and chloride concentrations are within acceptable ranges, other aspects such as total suspended solids, require additional scrutiny to ensure that the ice meets the optimal standards for human use. These findings emphasize the crucial need for ice producers to follow the FDA's Good Manufacturing Practices (GMP). To reduce the risk of foodborne illnesses, it is essential to prioritize educational initiatives focusing on hygiene, sanitation, and the dangers associated with contaminated water and ice. Furthermore, implementing regular microbiological testing is necessary to ensure adherence to safety regulations and to safeguard public health. In conclusion, a comprehensive strategy incorporating strict regulatory supervision, upgraded manufacturing procedures, and increased awareness among ice manufacturers is vital to tackle the potential health risks identified in this study and to guarantee the safety of ice products marketed in the area.

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