

# Physicochemical and Microbiological Studies of Various Brands of Vanilla Ice Cream Manufactured in Bangladesh

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## ABSTRACT

Ice cream enjoys widespread popularity in Bangladesh, particularly during the hot summer months. Despite its reputation for high fat and sugar content, ice cream also boasts essential nutrients like calcium, protein, and vitamins. The Bangladesh Standards and Testing Institution (BSTI) has established standards to ensure the quality of ice cream manufactured by various industries operating under different brand names. This study aims to scrutinize four prominent vanilla ice cream brands in Bangladesh, assessing their quality variations and adherence to BSTI standards. The quality of ice cream relies on its physical, chemical, and sensory attributes, influenced by factors such as ingredient types, processing conditions, and storage. The study utilized comprehensive physicochemical analysis, encompassing total solids, fat, solid non-fat, protein, lactose, acidity, pH, and viscosity, coupled with microbial analysis for safety. The findings uncovered notable variations in both physicochemical (especially total solid, fat, protein and lactose content) and microbial characteristics among the four ice cream brands, despite all selected brands complying with the standards set by BSTI.

**Keywords:** Ice cream, physicochemical analysis, microbial safety, quality evaluation

## INTRODUCTION

Ice cream is made by freezing a mixture of cream, milk, sugar, and flavorings with agitation to incorporate air to assure uniform consistence (Arbuckle, 2013). During the warmer months in Bangladesh, people of all ages savor the delight of a refreshing serving of ice cream. Despite the high amounts of sugar and fat included in many ice cream recipes, milk is a common ingredient, and fruit is occasionally added during production, therefore ice cream is generally seen as a healthy dish (Cruz et al., 2009).

The quality of ice cream is dependent on its physical, chemical, and sensory attributes (Clarke, 2015). Several factors influence the quality attributes of ice cream, such as the type of ingredients used, processing conditions, and storage. Milk and cream are the key sources of fat and solids in ice cream. These milk and cream ingredients give ice cream its smoothness, richness, and creamy consistency. (Tharp & Young 2012). Milk provides a source of protein, lactose, and minerals such as calcium, magnesium, and potassium, while cream contributes to the fat content and texture of the ice cream (Sinha, 2007). The amount of fat included in the milk and cream affects the sensory qualities of the ice cream, such as its taste, texture, and melting

rate (Goff, 1997). Sugar is not only added to sweeten and enhance the flavor of ice cream but also contributes to its texture, body, and freezing point.

Besides, ice cream's physicochemical and microbial characteristics are crucial factors as they determine its safety for consumption. During production, storage, and handling, ice cream can be contaminated with various microorganisms, including bacteria, yeasts, and molds, some of which, such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp., are potential foodborne pathogens (Marshall et al., 2003). Using contaminated raw materials, inadequate pasteurization, and improper storage can increase the microbial load in ice cream and lead to spoilage and foodborne illnesses (Pal, 2016).

The market offers various local and international ice cream brands. With the increasing demand for ice cream, it is crucial to assess physical, chemical, and microbial parameters to evaluate these products' quality differences and microbial safety. The primary objective of this study was to evaluate four well-known ice cream brands in Bangladesh in terms of physicochemical and microbial quality parameters, assessing their quality variance and adherence to the standards set by the Bangladesh Standards and Testing Institution (BSTI).

## MATERIALS AND METHODS

### Sample collection

From the retail market in Dhaka, Bangladesh, a total of 12 samples of vanilla flavor plain cup ice cream (100 ml) were obtained. These samples were taken from four famous brands, designated as S1, S2, S3 and S4. The samples within the same brand with the same manufacturing dates were purchased and transported in an ice box and kept in the laboratory's freezer until it was time to analyze them. The experimental analysis was performed between January to July, 2022.

### Physicochemical analysis

#### Total solid

The gravimetric technique recommended by AOAC was used to determine total solids (AOAC, 1990). The method involves weighing a 5.0 gm sample and placing it into a moisture can, evaporating it to dry using a steam bath, and drying it in an oven at 105°C for 3 hours. The sample was weighed again after cooling and returned to the oven for final drying until a constant weight was achieved. The moisture content was computed as a proportion of the examined sample's weight over moisture content. The mathematical equation below.

$$\%TS = [(W3 - W2) / W1] \times 100$$

Where,

W3 = Weight of the sample and moisture can after the final drying

W2 = Weight of the empty moisture can before adding the sample

W1 = Weight of the sample used for analysis.

#### Fat

The Gerber technique was used to ascertain the amount of fat in the ice cream samples (Devide, 1977). 10 ml of 98% concentrated sulfuric acid was added to 5 ml of the sample's contents in a butyrometer, followed

by 1 ml of amyl alcohol. After agitating the mixture for six minutes, it was centrifuged to separate the fat components. The butyrometer was conditioned in a water bath, and the fat content was measured from the scale.

### **Solid not fat**

The solid not fat (SNF) was estimated by subtracting the fat percentage from the percentage of the total solids (Holsinger, 1997). The formula below was used to calculate the SNF.

$$\% \text{ SNF} = \% \text{ Ts} - \% \text{ fat},$$

Where,

Ts = the total solids percentage.

### **Protein**

The Kjeldahl technique was used to determine the protein content in the ice cream samples (Bradstreet, 1954). The method involved digesting 2g of each sample with 15 ml of 98% concentrated sulphuric acid and a 0.8g Kjeldahl mixture as a catalyst, then diluting the mixture with distilled water after cooling. The resulting mixture was placed into a Kjeldahl analyzer, where ammonia was liberated by heating the digestion. The ammonia was distilled for about four minutes in a conical flask that contained 50 milliliters of 40% sodium hydroxide and 25 milliliters of 2% boric acid.

$$\% \text{ Nitrogen} = (0.28 \times A) / \text{Weight of sample in gram}.$$

Where,

A = amount of 0.1 M H<sub>2</sub>SO<sub>4</sub> in volume (ml)

### **Lactose**

A sample of ice cream was subjected to Fehling's solution procedure to determine the amount of sugar content (lactose) in ice cream (Woldu&Tsigie, 2015). The sample was dissolved in water, mixed with Fehling's solutions A and B, and boiled for a few minutes to produce a reddish-brown precipitate of cuprous oxide. The solution is filtered, treated with sodium hydroxide and sulfuric acid, and diluted. An instrument called a spectrophotometer is used to determine the absorbance of the solution at 540 nm. The amount of lactose in the solution is then determined using a standard curve.

### **Acidity**

The acidity of ice cream was determined as described by Lees (1971). A 10 gm sample was combined with distilled water to produce a 20 ml solution. After adding a few drops of an indicator composed of phenolphthalein, the liquid was titrated with 0.1 M NaOH until it achieved a bright pink endpoint. The following is the calculation that was used to get the percent acidity.

$$\% \text{ Acidity (as lactic acid)} = [(0.009 \times \text{Vol. of M}/10) / \text{Weight of the sample}] \times 100$$

### **pH**

The pH of the supplied samples was tested by a pH meter (CPH-102). The pH of the supplied samples was tested by a pH meter (CPH-102). After being turned on, the pH meter immediately stabilized. The electrode was immersed in a clean buffer solution beaker, and the temperature was adjusted. After removing the

buffer solution, the electrode was washed with distilled water. Finally, the electrode was placed into the sample, and the pH measurement once it stabilized.

### Viscosity

The viscosity of the mixture was assessed at  $5 \pm 1$  °C through a viscometer following 1 minute of shear (spindle 7, 100 r.p.m.). The viscosity values are expressed in centipoise.

### Microbial test

#### Enumeration of total viable count

The total viable count (TVC) was computed using the methodology described in Faruque et al., 2019. A new pipette was employed to transfer 0.1 milliliters of each successive dilution solution onto nutrient agar plates. A sterile glass spreader was used to distribute the diluted samples throughout the Petri dish's surface; however, a unique pure spreader was used for each petri dish. After 24 hours of incubation at 37 °C, the plates were counted for the number of colonies they contained. The total viable count, expressed as the number of organisms or colony-forming units per gram (CFU/gm) of the ice cream sample, was determined by multiplying the average colony count in a particular dilution by the dilution factor. This allowed for the calculation of the total viable count.

#### Coliform test

To assess the overall coliform count in the ice cream, 1 milliliter of each dilution that had been decreased by a factor of ten was put into MacConkey agar and incubated. Each dilution was performed on five test plates comprised of MacConkey agar. After that, the agar plates were incubated at 37 °C for 24 hours. Total coliform count was calculated using the same methods used by Faruque et al., (2019). The findings of the total coliform organism count of the ice cream specimen were given as the number of organisms or colonies formed per gram (CFU/gm).

#### Data analysis

The statistical analyses were conducted using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, N.Y., USA). The obtained results represent the mean of triplicates with standard deviation, and ANOVA was employed for comparing means.

## RESULTS AND DISCUSSION

### Physicochemical quality

The table 1 shows the test results of several physicochemical parameters conducted on four different ice cream samples.

Table 1. Physicochemical characteristics of ice cream samples

Test parameters	S1	S2	S3	S4
Total solid (%)	38.56±0.15 <sup>a</sup>	40.5±0.1 <sup>b</sup>	37.5±0.2 <sup>c</sup>	43.4±0.2 <sup>d</sup>
Fat (%)	10.42±0.03 <sup>a</sup>	11.01±0.08 <sup>b</sup>	10.73±0.07 <sup>c</sup>	12.23±0.15 <sup>d</sup>
Solid not fat (%)	28.24± 0.14 <sup>a</sup>	29.58± 0.07 <sup>b</sup>	26.7± 0.1 <sup>c</sup>	31.5± 0.10 <sup>d</sup>
Protein (%)	5.27± 0.06 <sup>a</sup>	5.33± 0.09 <sup>a</sup>	5.37± 0.07 <sup>a</sup>	5.15± 0.05 <sup>b</sup>

Lactose (%)	7.49± 0.05 <sup>a</sup>	7.35± 0.11 <sup>ac</sup>	7.60± 0.05 <sup>b</sup>	7.26± 0.08 <sup>c</sup>
Acidity (%)	0.13± 0.00 <sup>a</sup>	0.14± 00 <sup>a</sup>	0.14± 00 <sup>a</sup>	0.14± 00 <sup>a</sup>
pH	6.65 ± 0.01 <sup>a</sup>	6.64 ± 0.01 <sup>a</sup>	6.66 ± 0.01 <sup>a</sup>	6.68 ± 0.01 <sup>a</sup>
Viscosity (cP)	160.33±1.52 <sup>a</sup>	179.66±1.52 <sup>b</sup>	180.66±0.57 <sup>b</sup>	181.33±1.53 <sup>b</sup>

Values are presented as mean ± SD of triplicates. Values in the same column sharing the same letter are not significantly different where  $p < 0.05$ .

The total solid content of the ice cream samples ranges from 37.5% to 43.4%. In general, a higher total solid percentage indicates a denser and richer ice cream (Marshall et al., 2003). Among the four samples, S4 has the highest total solid percentage (43.4±0.16 %), indicating it has the most dense and creamy texture. Ice cream containing greater total solids content exhibited a tendency to melt faster compared to ice cream with lower total solids content, as observed in the study by El-Nagar et al. in 2002. The phenomenon is characterized by a freezing point depression of 1.86 °C for each mole of solute added to 1 kilogram (kg) of water, meaning that if one mole of sugar is dissolved in 1 kg of water, the freezing point shifts from 0 °C to -1.86 °C (Rohrig, 2014).

The fat level in the ice cream specimens ranges from 10.42% to 12.23%. Fat is essential to ice cream as it contributes to its texture and flavor (Marshall et al., 2003). Among the four samples, S4 has the highest fat percentage (12.23±0.15 %), which suggests it will have a more indulgent and creamy taste than the other samples.

The protein content in ice cream samples ranged from 5.15 % to 5.37%, similar to the study carried out by Martinou-Voulasiki (1990). S1 and S2 have slightly higher protein content in the given samples than S3 and S4. Protein is an essential component of ice cream, as it helps to stabilize the mixture and prevent the formation of ice crystals (Tharp & Young, 2012).

Lactose is a type of sugar found in milk, and is one of the main components in ice cream. Among the four samples, S1 and S2 have slightly higher lactose content than S3 and S4. Lactose, one of the less sweet sugars among the main sweeteners, has a sweetness range from 0.2 to 0.4 when compared to sucrose, the reference sugar, wherein a solution of 30 g/L of sucrose at 20 °C is regarded as having a sweetening power of 1 (Dominici et al., 2022) However lactose hydrolysis by enzyme treatment can increase the sweetness in ice-cream (Abbasi and Saeedabadian, 2015). The lactose content of the ice cream specimens ranges from 7.26% to 7.60%. The highest lactose content is observed in S3, while the lowest is in S4. These differences in lactose content may contribute to variations in the sweetness and texture of the ice cream samples (Holsinger, 1997). In ice cream and frozen dairy products, the presence of lactose leads to the crystallization of  $\alpha$ -lactose during the freezing process, resulting in the development of a sandy texture (Matak, 1999).

The acidity of the ice cream is an essential element since it influences both the taste and the stability of the combination (Marshall et al., 2003). In the provided samples, the acidity is relatively low, ranging from 0.13% to 0.14%, consistent with the findings of Ahmed et al. (2023), who reported an acidity range of 0.18% to 0.19% in ice cream made with various percentages of stevia. The pH parameter represents the ice cream sample's acidity or basicity level. The results show that the pH level of the four samples is consistent, ranging from 6.64 to 6.68. A pH level of around seven is considered neutral.

Viscosity is crucial in determining ice cream's texture and consistency; the higher the viscosity, the thicker and creamier the ice cream will be (Tharp & Young, 2012). The mean viscosity of all four ice cream samples is relatively consistent, with the values ranging from 160.33 to 181.33 cP (centipoise), which means they may have a similar texture and consistency. The viscosity measurement is affected by the presence of

fat, protein, stabilizers, bulking agents, and premium ingredients (Ahmed et al., 2023).

### Microbial quality

Table 2 shows the microbial test result (total viable count and coliform test) of ice cream samples.

Table 2. Microbial parameters of ice cream samples

Taste parameter	S 1	S 2	S3	S4	BSTI Standard
Total viable count (CFU/gm)	$4.5 \times 10^3$	$5 \times 10^3$	$8.4 \times 10^3$	$9 \times 10^3$	Max 100000
Coliform (CFU/gm)	0	0	0	0	Below 10 CFU/gm

BSTI= Bangladesh Standards and Testing Institution

The total viable count in four samples ranges from  $4.5 \times 10^3$  to  $9 \times 10^3$  CFU/gm, which is relatively low compared to the maximum limit of 100,000 CFU/gm set by BSTI standards (Table 2). Another study by Hossain et al. (2012) reported a microbial count of 3267-20916 CFU/gm in ice cream samples in Bangladesh. This suggests that the ice cream samples will likely be safe for consumption. However, the total viable count should be kept as low as possible to ensure the quality and shelf-life of the product. The absence of coliform bacteria in all four samples is a good sign, as coliform bacteria can indicate fecal contamination and potentially cause illness if present in high numbers.

### CONCLUSION

This study concluded on different parameters of ice cream and found variations in total solid, fat, solid not fat, protein, and lactose content. S1 and S2 brands had a higher fat content than S3 and S4, while S3 had the highest protein content. All samples were within the acceptable limits for microbial safety according to BSTI standards, with no evidence of coliform bacteria. These results will provide more insights to the consumer on ice cream for a more educated choice. It would be useful to perform additional tests, such as sensory evaluation, to assess the samples' taste, texture, and overall quality.

### CONFLICT OF INTEREST

The authors state unequivocally that they do not have any known conflicting financial interests or personal connections with third parties that would have given the appearance of influencing the work disclosed in this study.

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