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Evaluation of microbiological quality of Ice-cream available in Kolkata and its Suburbs

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Abstract
A study was undertaken to enumerate the Microbiological quality of ice-cream available in Kolkata and its Suburbs. 29 samples of ice-cream were collected from sweetmeat shops and street vendors in this locality. The standard plate count of ice-cream samples ranged between 2×10^4 and 5.25×10^7 c.f.u./gm and the coliform count of ice-cream ranged between 0 and 1.325×10^4 c.f.u./gm. Samples of ice-cream showed large number of yeast and mould contaminants ranging from 1.75×10^2 to 1.95×10^4 c.f.u./gm. In the experiment, psychrotrophic count and staphylococcal count of ice-cream samples ranged from 2.10×10^3 to 4.75×10^4 c.f.u./gm and from 0.35×10^4 to 3.55×10^4 c.f.u./gm respectively. The titratable acidity of ice-cream samples ranged from 0.235% to 0.275% lactic acid. Only 2 out of 6 samples fulfilled the PFA (1955) standard and other crossed the limit. Only 21 samples, out of 29 samples met the required fat percentage prescribed by Prevention of Food Adulteration Rules (1976) and only 2 samples from street vendors did not maintain the prescribed standard for total solids percentage given by Prevention of Food Adulteration Rules (1976). We have got mixed results from the investigation, 4 nos. of the samples fulfilled the BIS requirement and twenty-one nos. of samples met the specification for Prevention of Food Adulteration (PFA).

Keywords: Ice cream, standard plate count, coliform count, psychrotrophic count, staphylococcal count, titratable acidity, fat percentage, total solids

Introduction

Ice cream as an industry in India is of comparatively recent origin and may be said to have started in the nineteen-sixties. Today ice cream may be considered a luxury item, although its popularity is increasing rapidly. Ice cream is an excellent source of food energy. The fat content of the ice cream is twice than that of milk and fully 50% of total solids content is sugar, including lactose, a very desirable food item for growing children and persons who need to put on weight.

Among milk products, ice cream is also a rich source of calcium, phosphorous and other minerals of vital importance in building good bones and teeth. The milk protein contained in ice cream are of excellent biological value, because they contain all the essential amino acids.

Ice cream is also rich in essential vitamins like vitamin A, Thiamine (vitamin B_1), Riboflavin (vitamin B_2), Vitamin B_6, Vitamin B_{12}. The digestibility and palatability of ice cream is also very high. Ice cream probably came to the United States with the early English colonists. In 1851, the first wholesale ice cream industry in the United States was established in Baltimore, Maryland, by Jacob Fussell. Ice cream, a rich source of nutrients, is subject to microbial contamination at various stages of manufacture and during addition of ingredients. The handling by the operators who happen to be carries of certain diseases may also contaminate the product with potential pathogenic organisms. Hence, to ensure the safety of the product, microbiological quality of ice cream should be as high as possible and it must be free from any of the pathogens as the product is largely consumed by the children.

In order to safeguard the consumer health, which is of primary concern, there is a need to impose firm control on the quality of ice cream. In the present investigation, the microbiological examinations will help in determining the quality of the product, the sanitary and hygienic conditions maintained and the effect of public health for the marketed product available in Kolkata region.

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Materials and Methods
Methods of Analysis
For the estimation of various microbial populations in the sample of ice cream the microbiological examinations mentioned below along with testing of titratable acidity have been carried out.
1. Standard plate count (IS : 1479 ; BIS : 1962)
2. Coliform count (Manual in Dairy Bacteriology by ICAR, 1972)
3. Yeast and mould count (IS : 5403 ; BIS : 1969)
4. Psychrotrophic count (APHA, 1992)
5. Staphylococcal count (APHA, 1978)
6. Titratable acidity (IS : 1479 ; 1960)
7. Determination of fat percentage [SP : 18 (Part XI) – 1981]
8. Determination of total solids [SP : 18 (Part XI) – 1981]

Results and Discussion
(a) Standard plate count
Standard plate count reflects the viable microbial population. Thus it reveals the microbiological quality of product examined and also reflects on the raw materials quality, and the degree of hygiene and cleanliness maintained during manufacture, handling and storage of the product.

The Standard plate count of ice cream samples ranged from 2×10^4 to 5.25×10^7 c.f.u./gm (Table-1). Guha *et al.* (1979) [4] also reported a higher standard plate count (mean 0.41×10^6 c.f.u./gm) in samples of ice cream collected from 10 manufacturers in Kolkata. The results of the present investigation also laid down significant difference in standard plate count from sample to sample of different brands within the same locality. It may be due to the different sanitary practices adopted in different ice cream plants and outlets during manufacture, packaging, storage and distribution of the product and also due to ingredients added after pasteurization of the mix (colour, essence, fruit, nut, sauce etc).

In the present investigation 13 out of 29 samples met the standard prescribed by ISI/BIS and the standard plate counts of ice cream samples collected from street vendors (4.4×10^7 c.f.u./gm and 5.25×10^7 c.f.u./gm) were far exceeding the limit.

(b) Coliform count
The coliform group bacteria comprise all aerobic and facultative anaerobic, gram-negative, non-sporing rods, which are able to ferment lactose and produce acid and gas at $32\ ^0C$ within 48 h. These are also indicative organisms of faecal contamination. In portion to the numbers present, existence of these organisms in ice cream is suggestive of unsanitary conditions and practices during processing, packaging and storage.

The coliform count of ice cream ranged between 0 and 1.325×10^4 c.f.u./gm (Table-1). According to BIS standard, coliform count of ice cream should not be more than 90 coliform/gm. But in the present investigation, only 7 samples (24.14%) fulfilled this requirement. In case of street vendor's ice cream coliform counts (1.25×10^4 c.f.u./gm and 1.325×10^4 c.f.u./gm) were abnormally very high as per BIS standard. Bathla and Rao (1972) [1] also reported higher coliform count (0-174 million/ml, mean 3.68 million/ml) in ice cream sample in Agra city. Coliform count of 425.8 c.f.u./gm was founded by Guha *et al.* (1979) [4] in the ice cream samples available in Kolkata. Coliforms (*Klebsiella* spp., *Enterobacter colacae*, *E. aerogenes, E. hafniae, Escherichia coli*) were detected by Rajalakshmi (1983) [5] in the samples of ice cream sold in Hyderabad city. Coliform counts in ice cream were affected by time and temperature of holding of ice cream mix prior to freezing and it was recommended that the mix should not be stored for more than 48h at below $40\ ^0F$ (Bigalke and Chappel, 1984) [2]. *Escherichia coli* contamination proved to be a good indicator of unsatisfactory heat treatment (Stenzel, 1978) [7]. Other sources of coliform contamination are ingredients added after pasteurization and environmental factors such as compressed air, faults in storage tanks, cracks in the plant and packaging material. Basic ingredients should have low total counts and coliform-free.

(c) Yeast and mold count
Yeasts have been found to be most contaminants in dairy products causing gassiness and flavour defects. Yeast and moulds get entry into the product from the atmosphere, utensils and human hands under natural conditions. They can even tolerate high acidic condition.

Samples of ice cream showed large number of yeast and mould contaminants ranging from 1.75×10^2 to 1.95×10^4 c.f.u./gm (Table-1). Though there is no prescribed standard for yeast and mould in ice cream. But again the two samples of street vendors showed exceptionally high yeast and mould count comparing with other samples of ice cream of reputed brands. Excluding the two samples of street vendor, other samples were more or less homogenous (Code B) in yeast and mould count. But among the other samples of reputed brands only one sample (B_6 Kessar pista) showed abnormally high yeast and mould count. Bathla and Rao (1972) [1] also reported higher yeast and mould count (0-216 million/ml and 0-1.1 million/ml respectively) in the ice cream samples in Agra city. Higher yeast and mould count (3.77×10^3 c.f.u./gm) was found by Guha *et al.* (1979) [4] during the study on microbiological quality of ice cream available in Kolkata. The presence of moulds such as *Aspergillus, Penicillium* and *Mucor* spp. was detected by Vibha *et al.* (1996) [8] in the samples of ice cream available in Mumbai region. These yeast and moulds may get entry in ice cream from equipments, utensils, human hands and atmosphere. To control contamination yeast and mould in ice cream, it is recommended that high sanitary conditions, health education and proper storage facilities can minimize the higher prevalence of this micro-flora in the product.

(d) Psychrotrophic count
The presence of psychrotrophic bacteria in milk and milk products could be of great significance as these organisms may grow and proliferate during the storage even at low temperatures such as 5^0C and bring about the spoilage of these products.

The psychrotrophic count of ice cream ranged from 2.10×10^3 to 4.75×10^4 c.f.u./gm (Table-1). The sample (B_9) of street vendor showed high psychrotrophic count (4.75×10^4 c.f.u./gm). Although there is no specific standard for psychrotrophic count in ice cream. *Yersinia enterocolitica* was detected by Centorbi *et al.* (1989) [3] in ice cream samples. *Listeria monocytogenes* was identified by Pednekar *et al.* (1997) [6] in the product. Presence of psychrotrophs in high count in ice cream samples indicates post processing contamination during freezing, packaging, storage and distribution of the product. In this respect ageing period is very important. There is ample chance of proliferation of psychrotrophs at ageing temperature (at 0-5 ^0C). So mix

should not be stored for long period. The possible sources of contamination of the product by psychrotrophs may be uncleaned hands of workers, poor quality of water used for cleaning of equipments and containers, poor sanitary condition of the packaging and storage room and some untreated frozen ingredients like fruit, nut, sauce, colour, flavour etc. which are added as such to the product.

(e) Staphylococcal count

The presence of staphylococcus organisms in milk products is great significance in relation to consumer health because of the fact that some strains are highly pathogenic producing enterotoxins, which lead to food poisoning.

Staphylococcal counts of ice cream ranged from 0.35×10^4 to 3.55×10^4 c.f.u./gm (Table-1). In the present investigation further identification and confirmation tests were not done due to lack of facilities. So it will be too early to draw specific conclusion about the nature of pathogenicity only depending upon the isolation and enumeration experiments done in this research work. But it is definitely alarming about the probable hazardness due to the consumption of the product. Most of the samples along with two samples of street vendors showed high Staphylococcal count. *Staphylococcus aureus* was isolated from ice cream sample (Rajalakshmi, 1983) [5]. This organism was found more frequently in the cheaper varieties of ice cream. *Staphylococcus aureus* was also present in 14% of ice cream samples tested by Centorbi *et al*. (1989) [3]; 12, 0, 4 and 4% of the samples contained <10, 10-100, 101-1000 and 1001-10000 c.f.u./gm respectively. The 15 *Staphylococcus aureus* strains examined (26.6% biotype A, human ecovar and other biotype C, bovine ecovar) were all positive in the following biochemical tests; coagulase tube; fixed coagulase; thermo-nuclease; acidity from glucose and manitol in anaerobiosis; lysostaphin sensitivity. The possible source of staphylococcal entry into the product may be unclean equipments, dirty water, unhygienic storage conditions, nose or septic lesions of persons involved. Presence of *Staphylococcus* is highly undesirable in the product.

(f) Titratable acidity

In the present investigation, only the plain (Vanilla) ice creams were tested for titratable acidity. The titratable acidity of ice cream ranged from 0.235% lactic acid to 0.275% lactic acid (Table-1). Only 2 out of 6 samples met the required standard presented by Bureau of Indian Standard (BIS). Higher acidity definitely indicates the inferior quality and more metabolic activity of the microorganisms in the product. Proper heat treatment, clean and sanitized equipments and utensils, proper sanitary condition in manufacturing process and storage and personnel hygiene may check the development of the acidity.

(g) Determination of fat percentage

In the present investigation, twenty-nine samples were tested for percentage of fat. The fat percentage of ice cream ranged from 6.4 to 11.2 (Table-2). Only 21 samples, out of 29 samples met the required standard prescribed by Prevention of Food Adulteration Rules (1976). Science the milk fat has higher value; the manufacturers are interested in economic use of it, which is not permitted by regulatory standards. Sometimes milk fat slightly hinders, rather than improves the whipping ability.

(h) Determination of total solids

The results pertaining to the percentage of total solids of different ice cream samples were depicted in Table-2. The total solids percentage of ice cream samples varies from 35.30 to 39.00. Only two samples from street vendors did not maintained the prescribed standard given by Prevention of Food Adulteration Rules (1976). This may due to cheapest value of sugar and the milk-solids-not-fat should be added in ice cream as large a quantity as possible without risking the danger of sandiness.

Table 1: Different microbiological counts including acidity of market samples of ice cream of reputed brands and street vendors along with DMRT* results.

Characters under study		Standard plate count × 10⁵ c.f.u./gm	Coliform count × 10² c.f.u./gm	Yeast & mould count × 10² c.f.u./gm	Psychrotrophic count × 10³ c.f.u./gm	Staphylococcal count × 10⁵ c.f.u./gm	Titratable Acidity % L.A.
Brand	Variety						
B₁	Vanilla	2.50 B**	1.75 B	4.25 B	5.50 CD	3.050 AB	0.255 BC
	Kessar pista	3.75 B	2.75 B	3.50 B	8.00 BCD	3.5000 A	-
	Butter scotch	4.50 B	3.50 B	4.75 B	9.75 BCD	2.400 ABCDEF	-
	Chocobar	4.00 B	3.50 B	4.50 B	9.00 BCD	2.700 ABCD	-
B₂	Two-in-one	0.20 B	0.20 B	3.25 B	2.75 CD	0.350 G	-
	Kessar pista	0.525 B	2.00 B	4.75 B	3.75 CD	0.525 FG	-
	Butter scotch	0.60 B	2.75 B	4.50 B	4.25 CD	0.600 FG	-
	Vanilla	0.30 B	0.15 B	2.50 B	2.50 D	0.375 G	0.235 D
B₃	Chocobar	7.50 B	0.30 B	2.00 B	12.50 BCD	3.100 AB	-
	Malai- pista	2.20 B	0.00 B	1.75 B	9.00 BCD	1.950 ABCDEFG	-
	Butter scotch	7.25 B	1.40 B	2.25 B	15.00 BCD	2.800 ABC	-
B₄	Vanilla	0.70 B	4.25 B	5.75 B	2.10 D	3.400 A	0.245 CD
	Chocobar	2.10 B	5.00 B	10.00 B	5.25 CD	3.550 A	-
	Butter scotch	1.75 B	5.50 B	9.25 B	4.50 CD	3.250 A	-
	Kessar pista	2.50 B	4.75 B	9.75 B	5.25 CD	2.750 ABCD	-
B₅	Rainbow	9.50 B	0.20 B	7.75 B	4.75 CD	0.750 EFG	-
	Butter scotch	12.50 B	4.25 B	5.00 B	11.00 BCD	0.925 CDEFG	-
	Vanilla	7.50 B	2.50 B	6.75 B	5.75 CD	0.600 FG	0.275 A
	Chocobar	10.50 B	1.60 B	8.00 B	13.00 BCD	0.725 EFG	-
B₆	Butter scotch	2.00 B	1.75 B	6.50 B	25.00 B	1.075 CDEFG	-
	Chocobar	2.60 B	0.20 B	4.75 B	17.50 BCD	1.250 BCDEFG	-
	Vanilla	1.50 B	0.25 B	4.50 B	15.00 BCD	0.850 DEFG	0.255 BC

	Kessar pista	2.10 B	2.50 B	52.50 B	20.00 BC	1.200 BCDEFG	-
B7	Kessar pista	6.50 B	1.75 B	12.00 B	15.00 BCD	1.050 CDEFG	-
	Vanilla	5.25 B	1.65 B	7.50 B	9.75 BCD	1.125 CDEFG	0.265 AB
	Butter scotch	7.25 B	1.65 B	13.50 B	16.00 BCD	2.050 ABCDEFG	-
	Twin-zee	8.00 B	2.25 B	11.00 B	17.50 BCD	1.925 ABCDEFG	-
B8	Street vendor (Stick)	525.00 A	125.00 A	180.00 A	17.00 BCD	3.250 A	-
B9	Street vendor (Cup)	440.00 A	132.50 A	195.00 A	47.50 A	2.600 ABCDE	-
	LSD (5%)	185.9	15.14	51.79	14.46	1.614	0.0173
	SE m	64.26	5.235	17.91	4.999	0.5581	0.005
	Range	0.20-525	0-132.50	1.75-195	2.10-47.50	0.35-3.55	0.235-0.275

* : DMRT : Duncan's Multiple Range Test.

** : Similar alphabetical codes signify statistical homogeneity of respective treatment means.

: Not done.

Table 2: Comparison of percentage of Fat and Total solids of different varieties of Ice cream samples (Including street vendors)

Characters under study		Fat (%)	Total solids (%)
Brand	**Variety**		
B1	Vanilla	10.50	38.50
	Kessar pista **	8.40	36.50
	Butter scotch	10.40	37.20
	Chocobar	9.60	36.70
B2	Two-in-one	9.80	37.10
	Kessar pista **	8.33	36.90
	Butter scotch	10.40	37.90
	Vanilla	9.60	37.80
B3	Chocobar	9.40	38.00
	Malai- pista **	8.70	37.90
	Butter scotch	9.70	37.80
B4	Vanilla	10.80	37.90
	Chocobar	10.50	38.20
	Butter scotch	9.70	38.50
	Kessar pista **	7.80	36.90
B5	Rainbow	10.30	38.70
	Butter scotch	10.90	38.90
	Vanilla	11.00	39.00
	Chocobar	9.50	37.60
B6	Butter scotch	9.20	36.80
	Chocobar	10.20	37.30
	Vanilla	9.80	37.00
	Kessar pista **	8.40	36.60
B7	Kessar pista **	8.60	36.60
	Vanilla	11.20	38.40
	Butter scotch	9.70	36.90
	Twin-zee	9.50	36.70
B8	Street vendor (Stick)	6.40	35.30
B9	Street vendor (Cup)	7.10	35.90

*Average of three replicates.

** Ice cream sample containing fruits and nuts.

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