Research Article

Isolation and identification of bacteria from Ice cream samples and their Proteolytic and Lipolytic activity

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Abstract

20 ice cream samples from areas in and around Vandavasi, Thellar, Tindivanam and Pondy area were collected and processed for the isolation and identification of bacteria and molds using routine methods. First the samples were processed for isolation and identification of mesophilic bacteria using standard plate count method. Using MPN (most probable number) technique the ice cream samples were processed for the identification of coliforms. *Escherichia coli* was identified in six samples and confirmed by morphological, cultural and biochemical tests. *Salmonella typhi* was isolated in three samples and confirmed by morphological, cultural and biochemical tests. Presence of both *E.coli* and *Salmonella typhi* indicates faecal pollution water. Hence water act as main source of contamination. Almost 55% of the samples were positive for coagulase positive *Staphylococcus aureus* and confirmed by their growth in the Baired parker potassium Tellurite agar. Four samples were positive for *Micrococcus*. Both *Staphylococcus aureus* as well as *Micrococcus* are normal flora of human skin. Presence of these organisms indicates contamination due to improper handling of food by food handlers.*Bacillus spp* was isolated from four samples and *Pseudomonas* was isolated from three samples. This indicates contaminated air, incomplete pasteurization of milk and improper handling of food. *Aspergillus, Mucor, Penicillium* was isolated. These molds indicate contamination from air in plant, during transportation and improper handling. Four samples were absolutely negative for bacteria and eight samples for molds because of complete care taken for each and every step in the ice cream production, storage and handling. As ice cream consumption is increasing year by year as re-creative food by children as well as adults, it should be prepared with proper care for prevention of contamination.

Keywords: ice cream samples, identification of coliforms, morphological, cultural and biochemical tests.

Introduction

Milk is a nutritious basic food. It is rich in proteins, carbohydrates, fats, calcium, phosphorus, riboflavin and other B vitamins. Milk and milk products are most popular and are widely consumed by people all over the world. There are several kinds of milk products available and among them the most important is ice cream.

Ice cream is frozen dairy Product. It is a rich source of milk nutrients and a luxury food product. Ice cream is

the result of an evolution covering a period of about five centuries. It may be said to have its beginning in the practice of a kind of beverages with snow from snow capped mountains. With the discovery of this phenomenon **Salt Peter** made freezing mixtures with ice which not only chilled but actually frozen beverages resulting in a product like ice cream.

The commercial ice cream production began in 1851 with the invention of the hand cranked freezer in 1846.

Water ices were definitely known in the year 1950. Eventually milk and cream were added into these mixtures making a product rich in taste, flavour and texture. This product resembled our present day ice cream. The popularity of ice cream is attributed to its refreshingly cool and sweet characteristics. Though the origin of ice cream goes back to a few centuries, the future of ice cream seems endless.

The percentage of the milk utilization for ice cream is 0.7% of total milk produced in the country. India produces annually 313 million liters of ice cream and related products. The growth rate for ice cream industry was estimated to be 25% - 35%.

The richness in nutritive constituents of ice cream although has been realized by all but the problem lies in the production and handling of this food is very complex and is associated with many problems. Since ice cream is a milk-based product, it is rich in all milk nutrients and act as a growth medium for various microorganisms. The growth and multiplication of containing microorganisms are possible during production, storage, handling, transportation and marketing of these products. The contaminating microorganisms in the food are traced in ingredients added and environmental factors such air, faults in storage tank, and cracks in the plant and packaging materials (Bigalke and Chappell, 1984).

The micro flora of ice cream depends on the following factors like

- Health condition of milking animals
- Initial flora of ingredients
- Nature of cream
- Type of processing treatments.
- Storage conditions and duration of storage.
- Post processing contamination.
- Personal hygiene of handlers.

The initial content of micro flora of the raw water has a considerable bearing on the the ultimate quality of the product. Heat treatment kills most of the micro flora but does not affect the stable toxins. If the mixture is held for sufficient time at room temperature, the organism gets opportunity to grow and produce enterotoxin.The frozen state of ice cream gives protection to the disease causing microbes to survive for long period by utilizing the ingredients as

food. And also freezing temperature extends the life time of the organisms by reducing metabolic activities. The contaminating microorganisms include not only the saprophytes but also pathogenic forms that cause diseases to humanity.

PATHOGENS IN ICECREAM

Ice cream is one of the most popular nutritious milk products. Hence specific group of organisms bring about a characteristic, changes in ice cream. Some of them are *Coliform bacteria* and *Psychrophiles*. Aerobic and anaerobic spore formers also cause characteristic change in ice cream. *Bacillus, Salmonella species, Staphylococcus species* and *Listeria monocytogens* are involved in causing food borne illness.

A wide variety of bacterial species including *Micrococcus*, *Streptococcus, Escherichia coli, Aerobacter, Pseudomonas spp* etc, have also been isolated in the ice cream.

Ice cream stored in the deep freezers, cold rooms have been analyzed and the information reveals that *Salmonella typhimurium* survived at -25ºC for 3 years. Coliforms at -25º C for 150 days. *Enterococci* at 18 - 25ºC for 2 years and *Brucella abortus* remained viable for longer than 30 minutes.

Certain fungus like *Penicillium, Mucor, Rhizopus, Aspergillus* etc and Yeasts has also been isolated in ice cream.

Ice cream has been implicated in certain out breaks of food borne infection and food poisoning, various outbreaks of *Staphylococcus* (Hobbs and Gilbert, 1978),*Typhoid fever* (Evans, 1978), *Listeriosis* (Dirksen and Flagg, 1988) were reported due to the consumption of ice cream.

In this study, 15 ice cream samples from various areas in and around Vandhavasi, Thellar, Tindivanam and Pondy area were collected processed to identify the microbiological quality. The total count, MPN (Most probable number) count, Proteolytic and Lipolytic count, Yeast and mold count were followed. In particular this study concentrated more on the isolation and identification of bacterial contaminants.

Ice creams samples were processed with following objectives

To isolate and identify bacteria from Ice cream samples.
To compare the samples with bacteriological standards of Ice cream as lay down by BIS (Bureau of Indian standards).
To isolate and identify the fungi from ice cream samples.
To identify the proteolytic and lipolytic activity of isolated bacteria.
To study the prevalence of microbial load on ice cream.
To study the effect of pH on the growth of isolated organisms.
To study the Nutritional value of ice cream..

Materials and Methods

To entire study of ice cream was carried out under following procedures.

CLEANING OF GLASSWARES

The glass wares of Borosil grade were used in all the experiments. The glass wares were cleaned by soaking in chromic acid solution (100 mg Potassium dichromate dissolved in 1 liter water with 500 ml concentrated sulphuric acid) for two hours and washed in water.

STERILIZATION

All the glass wares were sterilized in hot air oven at 180ºC for 2 hrs. All the prepared media were sterilized in autoclave at 121ºC for 15 minutes at 15 lbs pressure.

SELECTION OF ICE CREAM BRANDS

A total of 20 different brands of ice cream representing the microbiological quality, sold in and around Vandhavasi, Thellar, Tindivanam and Pondy area were randomly selected. The brand names of these samples were arranged alphabetically. All the 20 samples were collected from various ice cream parlours, private ice cream producing units and from saluted fast food centers.

COLLECTION OF ICE CREAM SAMPLES

Samples were collected in their original containers from the shop in an ice box and kept in a frozen state at all times until ready for analysis.

PREPARATION OF SAMPLE FOR THE TEST

About 2.5 gm of frozen product was weighed under aseptic condition and added directly into a flask containing 22.5 ml of buffered peptone water. The contents were shaken vigorously. This suspension was kept as master dilution. 1 ml of sample from this suspension was aseptically transferred in to 9 ml of saline in test tubes using sterile 1 ml pipette. This suspension was shaked and labeled as 10^{-1} dilution. Using sterile 1 ml pipette, 1 ml of sample from 10^{-1} dilution tubes was transferred into another tube and shaked vigorously. This was labeled as 10^{-2} dilution. The same process was repeated up to 10^{-6} dilution, control tube was also included.

ENUMERATION OF MESOPHILIC AEROBIC BACTERIA

STANDARD PLATE COUNT

About 0.1 ml of the diluted homogenate were plated on previously solidified plate count agar medium and spread with a sterile L – rod. Duplicate plates were also prepared from each dilution. Then the plates were incubated for 24 – 48 hours at 37ºC. After the incubation period, the colonies were counted.

ENUMERATION OF COLIFORMS

The method most probable number (MPN) is used for the enumeration of coliforms. The coliform group includes all the aerobic and facultative anaerobic gram negative, non-sporing, rod shaped bacteria which ferment lactose with gas production within 48 hours at 37ºC. The standard test for the estimation of number of coliform group may be carried out by multiple tube dilution by fermentation technique. There are three steps to estimate the coliform group by MPN technique. Viz.,
 a) **Presumptive test**
M b) **Confirmed test**
 c) **Completed test**

Presumptive test (coli form test)
Media used: Mac conkey broth

Measured amount of single and double strength modified Mac Conkey broth was sterilized in test tubes containing Durham's tubes to show the gas production. Totally three sets of tubes were used each of which having five tubes. The first sets of tubes were provided 10ml double strength medium and the other two set with 5 ml single strength medium.10ml of sample was inoculated in double strength first set and 1.0ml and 0.1ml of sample was inoculated in single strength second and third set tubes. All these tubes were incubated at 37° c for 24hrs and analyzed for gas production in Durham's tubes and colour change in the medium indicates the acid production, which are the positive results.

Confirmed test
Media used: Brilliant green lactose bile broth

From the positive test tubes, one (or) two loop of culture was transferred to Brilliant green lactose bile broth tubes with Durham's tubes. The tubes were incubated at 37° c for 48 hrs. After incubation the formation of gas in any amount constitute the positive confirmed test.

Completed test
Media used: Eosin methylene blue agar

The positive confirmed test tubes were re-incubated to 40 c for 24 hrs. After incubation, the tubes were examined for gas production, which indicated positive result. From such tubes one loopful of inoculum was transferred to Eosin Methylene blue Agar (EMB) plate and streaked. These plates are incubated at 37° c for 24 hrs. After 24 hrs of incubation, the colonies were observed for their characteristic morphology called sheen indicating the growth of *E.coli.*

DETECTION OF *SALMONELLA*

Pre-enrichment

Media used: Buffered peptone water

About 2.5gms of ice-cream samples was transferred into 22.5ml of the buffered peptone water and it was incubated at 37 c for 12 hrs (over night).

Enrichment
Media used: Tetra thionate broth
Bismuth sulfite agar

About 5 ml of each pre-enrichment Broth was transferred to 10ml of tetrathionate broth and incubated at 42° c for 24 hrs. Following incubation, a loopful of enrichment culture was streaked on Bismuth sulfite agar and plates were incubated at 37° c for 24 hrs. The plates were examined after 24 hrs to 48 hrs for typical colonies of *Salmonella spp* and colonies were brown, black (or) in colour.

IDENTIFICATION OF BACTERIAL PATHOGENS
MORPHOLOGICAL STUDY
MICROSCOPIC EXAMINATION

Gram staining

A smear of the test culture was made on a clean glass slide, air –dried, heat fixed and gram stained. The smear was flooded with crystals violet solution and allowed to stand for one minute. Then it was washed with water, flooded with iodine solution and left for one minutes. It was then drained and decolorizes with 95% ethanol and washed gently in running water. It was then counter stained for 30 seconds to one minute with saffranine and washed. Then the stained smear was observed under microscope.

Spore Staining

A smear of suspected colony was made on a clean glass slide, air dried and heat fixed. This was covered with malachite green and allowed to act for 2 minutes and then heated till it steams. Then the slide was allowed to act for 2 minutes. Then the smear was washed with water and counter stained with safranine for 1 minute. Then washed with water and observed under microscope.

Motility test

The semisolid agar medium was stabbed with loopful of culture, incubated at 37ºC for 24 hours. The motility was confirmed by diffused growth of the organism around the stabbed area.

Nutrient Agar

A small portion of suspected colony from Standard plate count was streaked on nutrient agar plate and incubated at 37ºC for 24 hours.

Blood Agar

A small portion of suspected colony was streaked on blood agar and incubated at 37ºC for 24 hours.

Mac Conkey Agar

A small portion of suspected colony was streaked on Mac Conkey agar and incubated at 37ºC for 24 hours.

Mannitol Salt Agar

A small portion of suspected colony was streaked on Mannitol salt agar and incubated at 37ºC for 24 hours.

Baired Parker agar

A small portion of suspected colony was streaked on Baired Parker agar and incubated at 37ºC for 24 hours.

Eosin Methylene Blue Agar

A small portion of suspected colony was streaked on EMB agar and incubated at 37ºC for 24 hours.

BIOCHEMICAL TESTS

Catalase Test

A drop of 3% of hydrogen peroxide was placed on a glass slide and a loopful of culture was added and observed for the occurrence of brisk effervescence, which indicated a positive result.

Oxidase Test

A small amount of culture was streaked smoothly on an oxidase disc (Tetra methyl Para Phenylene Diamino Dihydrochloride) with a wire loop. A positive reaction was indicated by an intense deep purple colour appearing with in five to ten seconds.

IMViC Tests

IMViC test was performed by using the test culture.

Indole Test
Medium used: Indole broth

7 ml of sterile peptone broth was inoculated with the test culture and incubated at 37ºC for 24 hours cultures producing a red ring following the addition of Kovac`s reagent indicates indole positive. Absence of red ring indicates negative result.

Methyl Red test
Medium used: MR-VP medium

7 ml of sterile glucose broth was inoculated with the test culture and incubated at 37ºC for 24 hours. Methyl red was added after incubation. Change in colour indicates negative result.

Voges Proskauer test

6 ml of sterile glucose broth was inoculated with the test culture and incubated at 37ºC for 24 hours. Barrit`s reagent was added after incubation, change in colour of the broth from yellow to pink indicates positive result. No colour change indicates negative result.

Citrate utilization test
Medium used: Simmons citrate agar

The test organism was inoculated in Simmons citrate agar slants. The tubes were incubated at 37ºC for 24 to 48 hours. Following incubation, citrate positive cultures were identified by the growth of the organism and also blue coloration of the medium. Citrate negative cultures will show no growth and the medium will remain green.

Triple sugar iron agar test

The culture was streaked on TSI agar slope surface and stabbed into the butt and incubated at 37ºC for 24 hours. Alkaline slant and acid butt with H_2S gas indicates the presence of Salmonella spp.

Sugar fermentation test
Medium used: Carbohydrate broth

One drop of culture was inoculated into individual carbohydrate tubes and incubated at 37ºC for 18-24 hours and observed for color change from blue to yellow due to acidity from fermentation.

Coagulase test
Slide coagulase test

To a drop of overnight broth culture on a slide, a drop of rabbit plasma was added and observed. Evenly coarse clumping visible to the naked eye within 5- 10 seconds shows positive reaction.

Tube coagulase test

To 6.3 ml of culture 0.5 ml of rabbit plasma was added in a small tube and incubated at 37ºC. Then the tubes were examined for clotting after 6 hours. The formation of a distinct clot was evidence of coagulase activity.

IDENTIFICATION OF LIPOLYTIC ORGANISMS

Medium used: Tributyrin agar

About 0.1ml of the prepared samples was flooded on the surface of previously dried Tributyrin agar medium and spread with a sterile L – rod. Then the plates were incubated at 37ºC for 24 hours. After incubation, the plates were observed for clear zone around the colonies, which indicated lipolytic activity of the organisms.

IDENTIFICATION OF PROTEOLYTIC ORGANISMS

Medium used: Skim milk agar

About 0.1 ml of the prepared sample was flooded on the surface of the pre- dried agar plates and spread with a sterile L- rod. The plates were incubated at 37ºC for 24 hours. After incubation, the plates were observed for clear zone around the colonies, which indicated the proteolytic activity of the organisms.

IDENTIFICATION OF MOLDS FROM ICE CREAM SAMPLES

Dilutions of the sample homogenate were prepared as in standard plated count method. About 0.1 ml of the diluted sample were plated on the previously solidified Sabouraud`s Dextrose Agar medium and spread with a sterile L – rod. Then the plates were incubated at 25ºC for 3 days and colonies were observed.

EFFECT OF pH ON THE GROWTH OF ISOLATED ORGANISMS

Saline suspensions of nutrient broth culture, adjusted to an OD of 0.05 at a wavelength of 600nm**.**Four sets of tubes containing nutrient broth with pH values 3, 5, 7 and 9 were taken. The first set of tubes were inoculated with 0.1ml saline culture of *E.coli* and second set with *Staphylococcus aureus*, third set with *Bacillus spp* and fourth set with *Micrococcus spp* were also inoculated. All the tubes were incubated at 37ºc for 24 to 48 hours. After incubation, the optical densities of all cultures were recorded.

ANALYSIS OF NUTRITIONAL VALUE OF ICE CREAM ESTIMATION OF PROTEIN

About 0.2 to 1ml of working stock of BSA was pipette out in 5 different test tubes. Similarly and 0.5, 1ml of the food sample extract was taken in two other tubes marked as F1 and F2. The final volumes of the tubes were made up to 1ml with distilled water. Then to all these tubes, 4.5ml of alkaline copper reagent and 0.5ml of Folins phenol reagent were added and observed for the color development and it was read at 640nm after 20minutes.

ESTIMATION OF CARBOHYDRATE

About 0.2 to 1ml of working stock of glucose solution was pipetted out in 5 different test tubes. Similarly 0.2 and 0.4ml of food sample extract was taken into another two tubes marked F1 and F2.The final volume of the tubes was made up to1ml with distilled water. Then to all these tubes, 4ml of Anthron's reagent was added and mixed well. The tubes were kept in boiling water bath for 10minutes. The tubes were then cooled to room temperature and color development. The tubes were then read at 620nm.

ESTIMATION OF FAT

About 0.5 to 2.5ml of working standard solution (cholesterol) was pipetted out in 5 different test tubes and 0.5; 1ml of food sample extract was taken in two other test tubes marked as F1 and F2. The final volume was made up to 5ml with ferric chloride and added and mixed well. The tubes were allowed to stand for 10-20minutes. A pinkish red color was developed which was read at 560 nm using a green filter..

Microbiological quality of the ice cream samples was discussed based on standard plate count for bacteria, most probable number method, lipolytic activity and proteolytic activity of bacterial isolates and mold identification.

The names of the ice cream companies used for the microbiological analysis were tabulated in Table 1.

STANDARD PLATE COUNT FOR BACTERIA:

A total of 20 ice cream samples were processed for standard plate count.   The results for total number of mesophilic aerobic bacterial count from ice cream samples were tabulated in Table 2. The bacterial colonies isolated from ice cream samples on plate count agar were shown in Plate 1.

MOST PROBABLE NUMBER (MPN) METHOD

The MPN technique was performed for 16 samples to identify the coliforms present in ice cream samples. The results for MPN technique were tabulated in Table 3 and were shown in plate 2. Out of the 16 ice cream samples processed, sample 6, 10, 13, 18 and 20 showed positive results.

CHARACTERIZATION OF BACTERIAL ISOLATES

The morphological and cultural characteristics of the bacteria isolated from ice cream samples are tabulated in Table 4 and shown in Plate 3, 4 and 5.

The biochemical characteristics of the isolated bacteria were tabulated in Table 5 and shown in Plate 6 and 7.

The results for sugar fermentation analysis of the isolated bacteria are tabulated in Table 6.

The isolated and identified bacteria from ice cream were tabulated in Table 7.

Out of the 6 different bacterial isolates, 3 isolates were identified up to their generic level as *Bacillus, Pseudomonas* and *Micrococcus.*

The other bacterial isolates were identified as *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*.

The identified bacteria are shown in figure 1 and their percentage was shown in Figure 3a.

LIPOLYTIC ACTIVITY

Lipolytic activity of the bacterial isolates from ice cream samples were tabulated in Table 8

PROTEOLYTIC ACTIVITY

The Proteolytic activities of the bacterial isolates were shown in Table 9

MOLD IDENTIFICATION

The morphological and cultural characteristics of isolated molds are tabulated in Table 10

The isolated and identified molds from ice cream samples were tabulated in Table 11 .

EFFECT OF $_p$H ON THE GROWTH OF ISOLATED ORGANISMS

The optical density of the isolated cultures various pH were tabulated in Table 12

ANALYSIS OF NUTRITIONAL VALUE OF ICE CREAM

The nutritional value of ice cream was studied by estimating the protein, carbohydrate and fat present in the ice cream. The values were tabulated in Table 13

Discussion

Out of 20 Ice cream samples processed for the isolation and identification of microorganisms. Only 4 samples showed negative results for bacteria and only 9 samples showed negative results for molds. These results showed that the most of the ice creams are in poor standard. As ice cream is a rich source of milk nutrients i.e., Casein (Milk protein), Lactose (Milk sugar), added sugars and cream rich in lipid, it act as good medium for growth and multiplication of microorganisms mainly bacteria and fungi. The organisms isolated in this study are mostly mesophilic, but they can also lead a latent life in psychrophilic

Table 1 names of the ice cream companies used for micrbiological analysis

S. NO	NAME OF THE COMPANY
1	Aavin
2	Amirtha
3	Amul
4	Arun
5	Bharat
6	Cadbury`s
7	Cornetto
8	Enzo
9	Joy
10	Kashmir
11	Kovai
12	Kwality wall`s
13	Lazza
14	Maharaja
15	Maharani
16	Modern
17	Ramya
18	Simla
19	Vadilal
20	Venkateswara

TABLE 2 TOTAL NUMBER OF MESOPHILIC AEROBIC BACTERIAL COUNT FROM ICE CREAM SAMPLES

S.NO	NAME OF THE COMPANY	TOTAL PLATE CONUT / GM
1	Aavin	Nil
2	Amirtha	20×10^4
3	Amul	Nil
4	Arun	2×10^4
5	Bharat	50×10^4
6	Cadbury`s	19×10^4
7	Cornetto	Nil
8	Enzo	47×10^4
9	Joy	70×10^4
10	Kashmir	300×10^4
11	Kovai	90×10^4
12	Kwality wall`s	4×10^4
13	Lazza	270×10^4
14	Maharaja	80×10^4
15	Maharani	210×10^4
16	Modern	70×10^4
17	Ramya	450×10^4
18	Simla	346×10^4
19	Vadilal	Nil
20	Venkateswara	TNTC

TNTC   - Too numbers to count.

TABLE 3

MOST PROBABLE NUMBER METHOD (MPN)

The MPN values / 100 ml of the samples are given below

S.No	DS (10ml)	SS (1 ml)	SS (o.1 ml)	MPN/100 ml
1	Nil	Nil	Nil	Nil
2	0	0	0	0
3	Nil	Nil	Nil	Nil
4	0	0	0	0
5	5	0	5	95
6	0	0	0	0
7	Nil	Nil	Nil	Nil
8	0	0	0	0
9	0	0	0	0
10	5	3	1	110
11	0	0	0	0
12	0	0	0	0
13	5	3	2	140
14	0	0	0	0
15	0	0	0	0
16	5	3	2	120
17	0	0	0	0
18	5	4	2	220
19	Nil	Nil	Nil	Nil
20	5	5	5	1600

TABLE 4

MORPHOLOGICAL AND CULTURAL CHARACTERISTICS   OF THE ORGANISMS ISOLATED FROM ICE CREAM SAMPLES

S.No	Gram`s Staining	Motility	Nutrient Agar	Blood Agar	MacConkey Agar
1	Nil	Nil	Nil	Nil	Nil
2	Gram positive cocci	Non motile	Golden yellow	Beta haemolytic	Small pink
3	Nil	Nil	Nil	Nil	Nil
4	Gram positive cocci	Non motile	Golden yellow	Beta haemolytic	Small pink

5	Gram positive cocci Gram negative rod	Non motile Motile	Golden yellow Mucoid	Beta haemolytic Haemolytic	Small pink Large pink
6	Gram positive cocci Gram negative rod	Non motile Motile	Yellow Mucoid	Alpha haemolytic Non-haemolytic	Small pink Pink
7	Nil	Nil	Nil	Nil	Nil
8	Gram positive	Non motile	Golden yellow	Beta haemolytic	Small pink
9	Gram positive rod Gram negative rod	Non motile Motile	White rhizoidal Large transparent	Non haemolytic Non haemolytic	Pink Colourless
10	Gram negative rod Gram positive cocci	Motile Non motile	Mucoid Yellow	Haemolytic Alpha	Large pink Small pink
11	Gram positive Gram negative	Non motile Motile	Golden yellow Mucoid	Beta haemolytic Non haemolytic	Small pink Pink
12	Gram positive rod	Non motile	White rhizoidal	Non haemolytic	Pink
13	Gram positive cocci Gram negative rod	Non motile Motile	Golden yellow Mucoid	Beta haemolytic Haemolytic	Small pink Large pink
14	Gram positive cocci	Non motile	Yellow	Alpha	Small pink

15	Gram positive cocci	Non motile	Golden yellow	Beta haemolytic	Small pink
	Gram negative rod	Non motile	Mucoid	Non haemolytic	Pink
16	Gram positive rod	Non motile	White rhizoidal	Non haemolytic	Pink
17	Gram positive cocci	Non motile	Golden yellow	Beta haemolytic	Small pink
18	Gram positive cocci	Non motile	Golden yellow	Beta haemolyic	Small pink
19	Nil	Nil	Nil	Nil	Nil
20	Gram negative rod	Motile	Mucoid	Haemolytic	Large pink
	Gram positive cocci	Non motile	Golden yellow	Beta haemolytic	Small pink

TABLE 5
BIOCHEMICAL CHARACTERISTICS OF THE ISOLATED ORGANISMS

S. No	Indole	Methyl Red	Voges proskauer	Citrate utilization	Coagulase	Catalase	Oxidase
1	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	-	+	+	ND	+	+	-
3	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4	-	+	+	ND	+	+	-
5	-	+	+	ND	+	+	-
	-	+	-	+	-	-	-
6	ND	ND	ND	ND	-	+	+
	-	-	+	+	ND	+	+
7	Nil	Nil	Nil	Nil	Nil	Nil	NIl
8	-	+	+	ND	+	+	-
9	ND	ND	ND	ND	-	-	ND
	-	+	-	+	-	-	-
10	+	+	-	-	-	-	+
	ND	ND	ND	ND	-	-	-
11	-	+	+	ND	+	+	-

	-	-	+	+	ND	+	+
12	ND	ND	ND	ND		-	ND
13	-	+	+	ND	+	+	-
	+	+	-	-	-	-	-
14	ND	ND	ND	ND	-	-	+
15	-	+	+	ND	+	+	-
	-	-	+	-	ND	+	+
16	ND	ND	ND	ND	-	-	ND
17	ND	ND	ND	ND	-	-	-
18	-	+	+	ND	+	+	-
19	Nil	Nil	Nil	Nil	Nil	Nil	Nil
20	+	+	-	-	-	-	-
	ND	ND	ND	ND	-	-	+

ND ---- Not Detected.

TABLE 6

SUGAR FERMENTATION ANALYSIS OF THE ISOLATED ORGANISMS

S.No	GLUCOSE	LACTOSE	SUCROSE	MANNITOL
1	Nil	Nil	Nil	Nil
2	Acid	Acid	Acid	Acid
3	Nil	Nil	Nil	Nil
4	Acid	Acid	Acid	Acid
5	Acid	Acid	Acid	Acid
	Acid, Gas	Acid, Gas	Acid, Gas	Acid, Gas
6	-	-	-	-
	Acid, Gas	Acid, Gas	Acid, Gas	Acid, Gas
7	Nil	Nil	Nil	Nil
8	Acid	Acid	Acid	Acid
9	Acid	Acid	Acid	Acid
	Acid	-	-	Acid
10	-	-	-	-
	Acid, Gas	Acid, Gas	Acid, Gas	Acid, Gas
11	Acid	Acid	Acid	Acid
	-	-	-	-
12	Acid	Acid	Acid	Acid
13	Acid	Acid	Acid	Acid
	Acid, Gas	Acid, Gas	Acid, Gas	Acid, Gas
14	Acid	Acid	Acid	Acid
15	-	-	-	-
	Acid	Acid	Acid	Acid
16	Acid	Acid	Acid	Acid
17	Acid	Acid	Acid	Acid
18	Acid, Gas	Acid, Gas	Acid, Gas	Acid, Gas
19	Nil	Nil	Nil	Nil
20	Acid	Acid	Acid	Acid
	-	-	-	-

TABLE 7
ORGANISMS ISOLATED AND IDENTIFIED FROM ICE CREAM SAMPLES

S.No	Total number of samples	Number of positive samples	Name of the organisms	Number of organisms
1	20	16	*Staphylococcus aureus*	11
			E. coli	6
			Salmonella typhi	3
			Micrcoccus spp	4
			Bacillus spp	4
			Pseudomonas spp	3

TABLE 8

LIPOLYTIC ORGANISMS ISOLATED AND IDENTIFIED FROM ICE CREAM SAMPLES

S.No	Sample Name	Activity	Organism
1	Aavin	No growth	No growth
2	Amirtha	Clear zone around the colony	*Staphylococcus aureus*
3	Amul	No growth	No growth
4	Arun	Clear zone around the colony	*Staphylococcus aureus*
5	Bharat	Clear zone around the colony	*Staphylococcus aureus*
6	Cadbury`s	Clear zone around the colony	*Micrococcus spp*
7	Cornetto	No growth	No growth
8	Enzo	Clear zone around the colony	*Staphylococcus aureus*
9	Joy	Clear zone around the colony	*Bacillus spp*
10	Kashmir	Clear zone around the colony	*Micrococcus spp*
11	Kovai	Clear zone around the colony	*Staphylococcus aureus*
12	Kwality wall`s	Clear zone around the colony	*Bacillus spp*

| 13 | Lazza | Clear zone around the colony | *Staphylococcus aureus* |

S.No	Sample Name	Activity	Organism
14	Maharaja	Clear zone around the colony	*Staphylococcus aureus* *Micrococcus spp*
15	Maharani	Clear zone around the colony	*Staphylococcus aureus*
16	Modern	Clear zone around the colony	*Bacillus spp*
17	Ramya	Clear zone around the colony	*Staphylococcus aureus* *Bacillus spp*
18	Simla	Clear zone around the colony	*Staphylococcus aureus*
19	Vadilal	No growth	No growth
20	Venkateswara	Clear zone around the colony	*Staphylococcus aureus* *Micrococcus spp*

TABLE 9

PROTEOLYTIC ORGANISMS ISOLATED AND IDENTIFIED FROM ICE CREAM SAMPLE

S.No	Sample Name	Activity	Organism
1	Aavin	No growth	No growth
2	Amirtha	No growth	No growth
3	Amul	No growth	No growth
4	Arun	No growth	No growth
5	Bharat	No growth	No growth
6	Cadbury`s	Clear zone around the colony	*Micrococcus spp*
7	Cornetto	No growth	No growth
8	Enzo	No growth	No growth
9	Joy	Clear zone around the colony	*Bacillus spp*
10	Kashmir	Clear zone around the colony	*Micrococcus spp*
11	Kovai	No growth	No growth
12	Kwality wall`s	Clear zone around the colony	*Bacillus spp*
13	Lazza	No growth	No growth
14	Maharaja	Clear zone around the colony	*Micrococcus spp*
15	Maharani	No growth	No growth
16	Modern	Clear zone around the colony	*Bacillus spp*
17	Ramya	Clear zone around the colony	*Bacillus spp*
18	Simla	No growth	No growth
19	Vadilal	No growth	No growth
20	Venkateswara	Clear zone around the colony	*Micrococcus spp*

TABLE 10

MORPHOLOGICAL, CULTURAL & SPECIAL CHARACTERS STUDY OF ISOLATES FROM ICE CREAMS

S.No	Microscopic appearence	Morphology	Colour	Special characteristics
1	Nil	Nil	Nil	Nil
2	Brush like conidia	Folded colony	Greenish	Finger like appearance
3	Nil	Nil	Nil	Nil
4	Nil	Nil	Nil	Nil
5	Conidiospores	Woolly	Black	Sterigmata double
6	Nil	Nil	Nil	Nil
7	Nil	Nil	Nil	Nil
8	Sporangiospores	Cottony	white	Rhizoids absent
9	Conidiospores	Velvetty	Green	Sterigmata single
10	Conidiospores	Woolly	Black	Sterigmata double
11	Nil	Nil	Nil	Nil
12	Nil	Nil	Nil	Nil
13	Brush like conidia	Folded colony	Greenish	Finger like appearence
14	Nil	Nil	Nil	Nil
15	Conidiospores	Velvetty	Green	Sterigmata single
16	Conidiospres	Woolly	Black	Sterigmata double
17	Brush like conidia	Folded colony	Greenish	Finger like appearence
18	Sporangiospores and columella	Cottony	White	Rhizoids absent
19	Nil	Nil	Nil	Nil
20	Conidiospores	Woolly	Black	Sterigmata double

TABLE 11
ORGANISMS ISOLATED AND IDENTIFIED FROM ICE CREAM SAMPLES

S.No	Total number of samples	Number of positive samples	Name of the Organisms	Number of the organism
1	20	11	*Aspergillus niger*	4
			Aspergillus fumigatus	2
			Mucor spp	2
			Penicillium spp	3

TABLE 12
EFFECT OF pH ON THE GROWTH OF ISOLATED ORGANISMS
(Optical Density Readings)

S.No	Microbial species	pH 3	pH 5	pH 7	pH 9
1	*Staphylococcus aureus*	0.05	0.20	0.36	0.10
2	*Escherichia coli*	0.05	0.19	0.43	0.25
3	*Bacillus spp*	0.07	0.33	0.45	0.43
4	*Micrococcus spp*	0.06	0.23	0.41	0.35
5	*Salmonella typhi*	0.05	0.27	0.39	0.25
6	*Pseudomonas spp*	0.09	0.32	0.43	0.30

TABLE 13
ESTIMATION OF PROTEIN, CARBOHYDRATE AND FAT IN ICE CREAM

S.No	Food Sample	Protein (mg/100mg)	Carbohydrate (mg/ 100mg)	Fat (mg/ 100mg)
1	Ice cream	3.0	12.2	8.8

environment and thus can grow and multiply even at a low storage temperatures of ice cream. Good ice creams are nutritive and are not responsible for any ill effects and diseases of the consumers. Whereas the contaminated ice creams are mostly responsible for various diseases like sore throat, tonsillitis, typhoid and food borne illness. The nutritional value of ice cream is also reduced due to the growth of proteolytic and lipolytic organisms which utilizes the protein, fat and other nutrients present in ice cream.

The source of contamination of ice cream is many but the main sources are water and milk. Water used for the preparation of ice cream may act as a main source. The water may contain faecal coliforms and thus may contaminate ice creams. The milk used for production of ice cream may not be properly pasteurized which can also be a reason for the persistence of contamination.

The other major sources of contamination are utensils and equipments used for the ice cream preparation, air in the plant and food handlers either suffering from diseases or being at a state of healthy carriers harboring the organisms in nose or throat. The risk for rheumatic fever, a serious immunological and physiological problem which is mostly affecting the heart of children is because of *Streptococcus pyogenes*. One among the main reasons for this disease is the contaminated ice creams. Tonsillitis leads to tonsillectomy (Surgical removal of tonsils). This is commonly seen in children consuming ice creams frequently.

Staphylococcus aureus can cause *Staphylococcus* food poisoning, tonsillitis, Pharyngitis and septicemia. *Escherichia coli* can cause diarrhea in infants and adults. *Salmonella typhi* can cause typhoid.

Aspergillus spp can cause *Aspergilloma* or fungus ball. *Mucor spp* can cause sinus problems. Ice cream though some times not directly involved in above diseases, they are undoubtedly the main factors responsible for the deterioration of the pre-existing of the diseases. The people particularly children should not be allowed to take ice creams which are locally made by people who are not aware of microbiological standards of the ice creams.

Care must be taken in all steps involved in ice cream production right from milk collection till reaching the hand of the consumers. The quality should be improved by applying **Hazard Analysis Critical Control Point (HACCP)** system to ice cream production.

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