COURSE TITLE: MICROBIOLOGY OF MILK PRODUCT

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Microbiology of Ice- Cream

RAKESH KUMAR
ASSOCIATE PROFESSOR (DAIRY MICROBIOLOGY)
FACULTY OF DAIRY TECHNOLOGY
S.G.I.D.T., BVC CAMPUS,
P.O.- BVC, DIST.-PATNA-800014

History

No one knows exactly when ice cream was first produced. Ancient manuscripts tell us that the Chinese liked a frozen product made by mixing fruit juices with snow – what we now call water ice. This technique later spread to ancient Greece and Rome, where the wealthy in particular were partial to frozen desserts. After disappearing for several centuries, ice cream in various forms reappeared in Italy in the Middle Ages, most probably as a result of Marco Polo returning to Italy in 1295 after some 17 years in China, where he had acquired a liking for a frozen dessert based on milk. From Italy, ice cream spread through Europe during the 17th century, long remaining a luxury product for the royal courts. Industrial ice cream production began at the end of the 19th century when the first mechanical refrigerators were pioneered.

THE HISTORY HANDOUT

Ice Cream

- China is believed to be the origin of ice cream as they were the ones who invented the device that produces sorbets and ice cream at around **200 B.C.**
- In **37-68 AD**, it was recorded that Emperor Nero had ice brought from the alps to create chilled delicacies mixed with fruit toppings.
- In **16th century**, Mughal emperors used horsemen to obtain ice from the Hindu Kush to produce fruit sorbets served on their table.
- In **1674**, the first recipe of flavoured ice appeared followed by various recipes which was later began to be published in **1694**.
- From the mid **18th century**, ice cream had become popular and became less expensive making it readily available to ordinary people.
- In **1888**, ice cream cone was first mentioned in **Mrs. A. B. Marshall's** book and eventually the edible cone became popular in the US during the **1904** World's Fair in Missouri.

www.imin.com

Typical ice cream formulas

Type of ice cream	Fat % wt	MSNF % wt	Sugar % wt	E/S % wt	Water % wt	Overrun % vol
Dessert ice	15	10	15	0.3	59.7	110
Ice cream	10	11	15	0.5	63.5	100
Milk ice	4	12	13	0.6	70.4	85
Sherbet	2	4	22	0.4	71.6	50
Water ice	0	0	22	0.2	77.8	0
Sorbet	0	0	22	0.5	77.5	30-50

Fat — Milk, cream, butter or vegetable fat
MSNF — Milk solids-non-fat (protein, lactose, salts)
Sugar — Sucrose, glucose/dextrose or syrups
E/S — Emulsifier and stabilizer, e.g. monoglycerides, locust bean gum (LBG), guar gum or carrageenan

Overrun — Amount of air added to the product
Other ingredients — Flavours, colours, fruit, nuts and chocolate pieces may be added during processing

Microbiology of Ice cream

Through ingredients

Dairy products, Gelatin, Sugar, Flavouring materials, Colouring materials, Eggs, Air

Contamination at manufacture and handling

SPC, Coliform, Yeast & Moulds, Thermoduric counts.

The various mix ingredients that may act as the source microorganisms to the ice cream fall under the above mentioned heads. Ingredients added to ice cream mix before pasteurization normally constitute little microbial contamination except spores. However, microbial analysis is very important in establishing the quality of ingredients such as milk, cream, other dairy products, chocolate, cocoa, eggs, emulsifier, stabilisers and other food additives.

Microbiological Quality of Ingredients

The ultimate quality of the finished ice cream depends not only on the numbers but also the types of bacteria present in the mix ingredients. In considering the mix ingredients we should, therefore, discuss not only numbers but also types of organisms, as far as possible.

Contamination at manufacture and handling

Presence of higher bacteria in frozen dairy products and ingredients are indications of improper sanitation and of processing and handling conditions.

Bacterial contamination varies with raw milk used, separation process, Inadequate refrigerated storage, Long distant transport

Usually cream contains high bacterial counts than the milk from which it is made and such cream may be a single chief source of bacteria in ice cream.

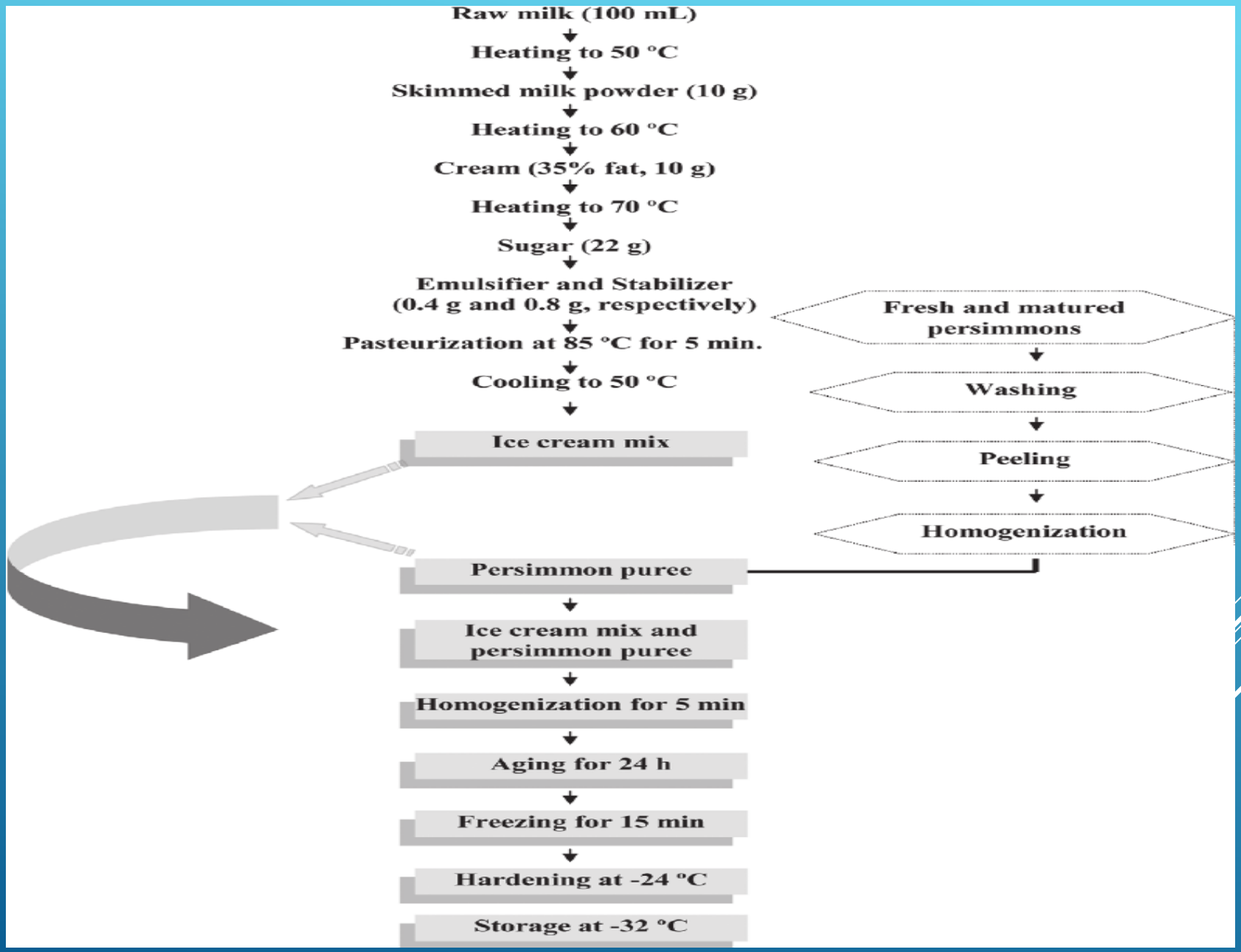
Overall Quality -Micro environment of Ice - Cream

Micro environment refers to the immediate conditions that surround the ice cream. Micro environment may include the temperature, storage container, packaging material that surrounds the ice cream etc… During storage the temperature should be maintained at around -20°C without any major fluctuations. If there is variation in the temperature with time it may lead to quality deterioration in the ice cream and microbial spoilage. The surface of the container in which the ice cream is stored also plays a major role in the quality. The contact of ice cream with the container serves as a medium of heat transfer to maintain the storage temperature. High heat conducting, odourless, non-corrosive stainless steel material may be used to contain the ice cream to maintain the ice cream in a fixed frozen temperature. If packed in packaging materials prior to storage, they should not impart any colour, odour etc. There should not be any air pockets in the ice cream storage, as they serve as the sources of spoilage during storage.

Safety aspect of Ice-cream

The potential enteropathogens encountered in ice cream are mainly originating from either the gradients or as environmental contaminants. Amongst the ingredients, raw milk and cream are the likely source of *Campylobacter jejuni*, *Salmonella dublin*, *E. coli*, *Listeria monocytogenes* and *Yersinia enterocolotica*. Sweeteners, especially systems have been known to contribute *E. coli* and salmonella. The pathogenes of importance from egg and eggs products are are Proteus, Salmonella, Enterococcus spp. Similarly, Colouring material can also be a source of *E. coli* and Salmonella from the environmental sources the major pathogens of importance are *Listeria monocytogenes*, *Yersinia enterocolitica* and Salmonella spp. They may be found more in wet than in dry areas of dairy plants.

Manufacturing process of Ice- Cream



Raw Milk

The dairy products that is used in the mix vary widely in their bacterial counts. Some of the dairy products such as liquid milk, cream and skim milk concentrate should have been subjected to adequate heat treatment, cooling and storage. The original raw milk may have a count of less than 10,000 per ml. if produced under sanitary conditions and properly cooled, or it may have a count as high as 5×10^6 to 1×10^8 cfu/ml, if carelessly produced and improperly cooled.

The numbers and types of micro-organisms in milk immediately after productions directly reflect microbial contamination during production, collection and handling. A use full indicator for monitoring the sanitary conditions present during the production, collection, and handling raw milk is the 'total' bacterial count or standard plate count (SPC). SPC values for raw milk can range from < 1000 cfu/ml.

The most commonly occurring psychrotrophs in raw milk are the Gram negative Roads. Pseudomonas spp. accounts for at least 50% of the GNRs with Pseudomonas fluorescens predominating. Other species include pseudomonas putida, P. fragi, P. aeruginosa, Flavobacterium, Acinetobacter-Moraxella, Achromobacter, Alcaligenes, Chromobacterium, Aeromonas, Klebsiella, and Coliform group comprise most of the remaining psychrotrophsic Gram negative Roads. The majority of these coliforms are the Aerobacter spp. Microflora of pasteurized milk.

The microflora of pasteurized milk is primarily bacterial in nature, originate from

- Thermoduric organisms present in raw milk supply
- Raw milk contact with contaminated handling and processing equipment
- Entry after the pasteurization process.
- Typical total bacterial numbers in freshly pasteurized milk are less than 1000 cfu/ml.

Cream

Best ice cream is made from sweet and unneutralized cream which has not been subjected to microbial action. Cream with off flavour if used, will be reflected in the finished product.

•Tests should be done for Yeasts & Moulds, Mesophilic bacteria. Coliforms and Lipolytic bacteria.

•The important spoilage organism is Pseudomonas fragi which causes un pleasant taste and taints. The spoilage is usually the result of chemical changes producing rancid and other off flavours.

•If butter is used, it should preferably be stored at–18° to –20°C. Whole milk/Skim milk: High quality is desirable and it should be freefrom toxin producing microorganisms.

Raw milk should have SPC less than 2,00,000/ml

Pasteurized milk SPC should be less than 30,000/ml. In fresh cream, the predominating organisms

at 50°C -- Pseudomonas, Alcaligenes, Acinetobacter Aeromonas, Achromobacter and

At 30°C--Corynebacterium, Bacillus, Micrococcus, Lactobacillus and Staphylococcus.

Butter

Butter has not been regarded as high risk products. However, the presence of a potentially pathogenic genus given an indication of a post process contamination problem. Butter and anhydrous milk fat (butter oil) are products made under careful control from cream that has been heat treated at a relatively high temperature. Therefore, a very high microbiological quality is to be expected, and spoilage is usually the result of chemical changes producing rancid and other off flavours. Tests for yeasts, moulds, mesophilic bacteria, coliforms and the presence of lipophilic organisms should be carried out. However, in particular the presence or absence of Pseudomonas fragi should be noted, as these organisms can cause unpleasant taints in butter. Butter should preferably be stored at a temperature below -20°C and as for all the ingredients, careful stock control should be ensured.

Dried and Concentrated Milk

Skim milk powder may contain number of Bacillus cereus, though not a major health hazard but the number should be as low as possible. Bacilli reduce methylene blue rapidly and canproduce clotting in milk; they grow at relatively low temperature and in extreme cases causes spoilage of the mix. Sweetened condensed milk (SCM) and Dried milk (DM): The bacterial standards intended for ice cream manufacturing should SCM – SPC 500/ml DM - SPC 30,000/ml and Coliform 90/ml

•Condensed milk – Should be stored in manner that prevent entrance of insects, bacteria and mold spores.

Dry milk – should be stored in reduced humidity and in places of free from insect & dust contamination.

Listeria monocytogenes may survive the typical spray drying process and thus dried milk may serve as a source of Listeria.




Sugar

High temperature used in processes like refining and deodorizing processes. They contain almost no moisture and therefore should contain very few organisms indeed. Granulated sugar and dextrose, should be almost sterile. Total count in any sugar should not exceed 200/g. The most common organism present in small number is Yeasts. Sugar contains only small number of bacteria and is of little importance as a source of bacteria in ice cream. Sugar syrups contain osmophilic yeasts and moulds may grow on the surface if contaminated.

Thermophilic spores –not more than 125/10g - 150/10g

Yeasts – not more than 10/10g

Molds – not more than 10/10g

Flat sour spores - not more than 50/10g

Sugar

The number of organisms contributed to the ice cream mix by the sugar is usually insignificant. However it was found to be necessary to heat the sugar solution separately in the form of a solution to 112.7° to 115.5°C, in order to meet the rigid standards which classify ice cream with a count of less than 10,000/g as good and with a count of less than 50,000/g as fair. Granulated sugar, as well as other dry sugars such as dextrose, should be almost sterile, and the only organisms that may normally be present are small numbers of yeasts. Sugar syrups, sucrose, corn syrups, or mixtures of these, or lactose and whey syrups, again may contain some yeasts. It is suggested that tests for yeasts should be made on bulk deliveries of sugar and sugar syrups.

Coliforms, Yeasts and moulds, Anaerobic thermophilic, H2S and non-H2Sproducers, Aerobic spore formers, Thermophiles Mesophilic acid producers------Tests to be performed.

Stabilizers

Production of ice cream with high overrun is an interesting tool for cost saving. However, the perceived quality by the consumer has to be kept in mind. The sensory attributes such as creaminess and smoothness as well as resistance to shrinkage and melting cannot be compromised as these properties are very closely linked to consumer preferences.

Creaminess as well as melting resistance is related to the distribution of air cells in the product. A more uniform air cell distribution in the ice cream results in a creamier and slower melting ice cream. Emulsifiers like mono- and diglycerides are well known for their positive influence in this respect. The options when choosing stabilisers are far greater than in the case of emulsifiers. Most countries allow the use of a wide range of stabilisers. The most commonly used stabilisers in ice cream are:

Guar gum
Locust bean gum
Cellulose gum
Alginate
Carrageenan

Stabilizers used to maintain smooth texture and body by preventing formation of large ice crystals.

•Stabilizers should not present any problem, but gelatin as an animal products, may be a hazard. The common organism in gelatin is aerobic spore former.

•Tests for stabilizers: SPC, Coliform, Yeast &Mould, Aerobic spore formers which indicate degree of unsanitary handling.

•Gelatin should be obtained from reputable supplies and store as cool & dry.

•Gelatin - SPC- not more than 10,000/g and Yeast & Mould: not more than 100/g Stabilizers are kept dry and properly protected from dust and dirt, the number of bacteria present tend to reduce as a result of death of organisms.

Emulsifier

Very essential when butter, butter oil or vegetable fats are used to provide surface active material in place of natural fat globule membrane. Homogenization reduces fat globule size and increases surface area which has to be protected. An emulsifier reduces the energy required to maintain the integrity of fat globules. Assists in obtaining more uniform and large number of smaller, air cells.

Tests: SPC, Coliform, Yeasts & Moulds, ß. haemolytic, streptococci, Salmonella

Egg products – SPC should not exceed 10,000/g

_Important source of contamination in ice cream as they are added after mix is pasteurized.

Fruits – canned, fresh and frozen and should be of satisfactory in microbiological standards particularly canned fruits.

Fresh & frozen fruits – may contain Yeasts. Nuts may be infected with moulds. Coconuts may be contaminated with Salmonellae. Walnuts-Mould and parasitic infection

Mono- and diglycerides of fatty acids (E471) are the most commonly used emulsifier in ice cream. Mono- and diglycerides of fatty acids are produced by interesterification of glycerol and fat. The selection of fat determines the functional properties of the emulsifier. The mono- and diglycerides can be further esterified with organic acids. For instance lactic acid is used for formation of E472b lactic acid esters of mono- and diglycerides of fatty acids, so called lactic acid esters.

In short terms the functionality of the emulsifier in ice cream is seen as:

- Improved fat emulsification in the mix
- Controlled fat agglomeration and coalescence
- Facilitated air incorporation
- Improved dryness on extrusion
- Improved melting resistance
- Improved heat-shock stability
- Improved smoothness and creaminess

Egg yolk

It is highly valuable in foods and helps in other flavors blending for desirable properties. Ice cream cost is increased by egg yolk addition. It provides desirable flavor to ice cream but if product of egg has any off flavor that can be easily detectable in ice cream with egg. Protein and lecithin complex in egg yolks is highly preferred in mixes where total solids are lowered and prepared with butter oil or butter.

Functions

i. Delicate characteristic flavor

ii. Texture and body improvement

iii. Viscosity increase

iv. Improved whip ability

Flavoring and Coloring Agents

Other food stuffs added to ice cream include flavoring and coloring materials. They include fruits [canned, fresh or frozen (in concentrated sugar syrup)], nuts, chocolate, broken biscuit, colors and flavors. Most of these should be of a satisfactory microbiological standard, particularly canned fruits, but fresh and frozen fruits may contain yeasts; nuts may be infected with molds (with the risk of aflatoxin); and desiccated coconut may be a hazard because it can be contaminated by Salmonella and should be heat – treated. The examination of these materials should include a visual inspection and the enumeration of mesophilic bacteria, coliform, yeasts and molds. Colors may be infected by careless handling and this must be avoided by maintaining good management control. Flavors are normally added to the mix after it has been heat treated, and so they must also be handled with great care to avoid contamination. Flavouring ingredients, mainly vanilla extract, is mostly an insignificant source of bacteria as the concentration of alcohol in the extract effectively prevents any growth. Fresh strawberries have been reported to be a source of *E. coli*.

- Fresh & frozen fruits Tests: Visual inspection, SPC, Coliform, ß-haemolytic streptococci, Yeasts& Moulds.
- Canned / fresh nuts are sterilized in ethylene oxide and CO_2 in1:9 or boiling in sugar solution(50%) followed by drying at 250°C for few minutes. Coconuts should be heat treated when desiccated
- Alcoholic colors add only a fewer bacteria. Flavours have low counts due to presence of alcohol. Tests: Similar to fruits & nuts.
- Careless handling – defects avoided by maintaining good management control.

Air--Air is an important component in ice cream affecting the physical and sensory properties as well as the storage stability. Ice cream normally has around 100 % overrun meaning that the air makes up 50 % of the ice cream volume. The amount of air incorporated into the mix influences the sensory attributes of the ice cream. If a lower amount of air is applied, the resulting ice cream is dense, heavy and more cold eating. If a higher amount is used, the texture is lighter, creamier and more warm eating. Production of ice cream with high overrun is an interesting tool for cost saving. However, the perceived quality by the consumer has to be kept in mind. The sensory attributes such as creaminess and smoothness as well as resistance to shrinkage and melting cannot be compromised as these properties are very closely linked to consumer preferences. Creaminess as well as melting resistance is related to the distribution of air cells in the product. A more uniform air cell distribution in the ice cream results in a creamier and slower melting ice cream. Emulsifiers like mono- and diglycerides are well known for their positive influence in this respect. Therefore, it is of crucial importance that the air be filtered so as to ensure that no contamination can be attributed due to the introduction of air.

High shear benefits

In order to obtain an ice cream with high overrun and a homogeneous air cell distribution, it was necessary to apply higher shear in the freezer i.e. the dasher speed in the continuous freezer was increased. Higher shear also means higher degree of churning out in the freezer and hence stronger air cell walls and higher stability of the created ice cream structure. This was also confirmed in the melt-down and heat-shock tests. It results for ice cream stored at constant temperatures as well as heat-shocked ice cream are shown. For comparison also melting properties of a 6 % fat ice cream with 100 % overrun are included.

Suggests tests for raw materials:

1. Milk - SPC, Coliform
2. Milk powder - SPC, Spore forming org.
3. Butter - SPC, Coliform, Ps. fragi, lipolytic, Y&M.
4. Cream - SPC, Coliform
5. Anhydrous milk fat - Coliform, lipolytic, Y&M.
6. Vegetable fats - Coliform, lipolytic, Y&M.
7. Sugar - SPC, Coliform, Y&M.
8. Stabilizer/emulsifier - SPC, Coliform
9. Fruits - SPC, Coliform, Y&M
10. nuts - SPC, coliforms, Y&M

Advice to trade Ice-Cream

Key steps of manufacturing steps which should be observed by the persons involved in trade. These include –

(a) Obtain ingredients, such as milk, cream and ice-cream mix, from licensed and reputable sources;

(b) Store ingredients at proper temperature (frozen items: -18°C or below; chilled items: 0-4°C);

(c) Maintain the ice-cream mix (< 7°C) and ice-cream products (< -18°C) at adequate temperatures from manufacturing factory to retail outlets;

(d) Discard the defrosted products and do not re-freeze any melted ice-cream for sale;

(e) Drain off and discard the leftover of soft ice-cream daily;

(f) Maintain all equipment and utensils in clean and good condition; and

(g) Observe hygienic practices during all preparation and handling processes.

POTENTIAL MICROBIOLOGICAL HAZARDS

Ice-cream, a milk-based product, is a good media for microbial growth due to high nutrient value, almost neutral pH value (pH ~6-7) and long storage duration of ice-cream . However, pasteurization, freezing and hardening steps in the production can eliminate most of the microbiological hazards. Ice-cream must be heat-treated during the production process. Pasteurization is most commonly applied heat treatment in the dairy industry. This can destroy almost all pathogenic bacteria in milk. The subsequent process that subjects the mixtures to freezing temperature can also inhibit the growth of any remaining flora. Hardening is also the important control point that further reduces the hazards. Furthermore, as automatic machines are commonly used for ice-cream making in dairy industry, the chance of contamination through direct hand manipulation can be reduced.

Heat treatment by pasteurization can destroy most of the specific pathogens that pose risk to public health. However, the potential microbiological hazards found in the final products can still be introduced after pasteurization through adding contaminated ingredients and improper handling procedures. This is especially important in the preparation of soft ice-cream as its final stage of the production is carried out at point of sale. Some pathogens that can survive in food even at low temperature include *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp. and *Yersinia* spp. For ice-cream products, *L. monocytogenes* is of significant food safety concern worldwide because the microbiological limits for *Listeria monocytogenes* in the guidelines of FSSAI for ready-to-eat food are considered for the strict monitoring.

Potential sources of contamination of various microbial groups to ice cream

Source	Microbial groups	Remarks
Honey	Yeasts (e.g., *Zygosaccharomyces* spp.)	Yeasts are natural habitats of flowers.
Flavorings	Vegetative microbial cells Bacterial spores (<100 per g)	Extracts contain alcohol that sufficiently dehydrate and destroy vegetative cells. Cocoa and chocolate liquor consist bacterial spores when contaminated during or after pressing and grinding.
Fruits	Yeasts (e.g., *Saccharomyces* and *Cryptococcus*) Molds (e.g., *Alternia*, *Aspergillus*, *Botrytis*, *Fusarium*, *Geotrichum*, *Mucor*, *Penicillium*, *Rhizopus*) Bacteria (e.g., *Bacillus*, *Pseudomonas*, *Achromobacter*)	Relatively low pH of fruits favors the growth yeasts and molds. Freezing destroys some microorganisms but does not guarantee complete inhibition. Adding hypochlorite to wash water is an effective way of reducing microbial counts of fruits before freezing. Candied or glaciéd fruits have a_w low enough to prevent microbial growth.
Nuts	Various microorganisms	Nuts consist microorganisms originating from mainly soil. Treatments applied to separate nut meats and shell may reduce the microbial count. a_w of most nuts (i.e., 0.70) prevent microbial growth. Nuts (both tree nuts and ground nuts) are considered to be free of pathogens. However, mycotoxins carry a potential risk.
Egg yolks	*Salmonella* spp. and *Micrococci* in fresh eggs Gram-negative rods (e.g., *Pseudomonas*, *Proteus*)	Egg is usually contaminated during laying. Iron is essential for the growth of most bacteria. Glycoproteins present in shell membrane bind iron. Also, ovotransferrin chelates iron, making them unavailable for bacteria. Binding biotin and riboflavin by avidin and ovoflavoprotein, respectively, inhibits bacteria that need these two vitamins for their growth. Transovarian infection of *Salmonella* is a major concern. Pasteurization and freezing reduce the counts of bacteria to a great extent. *Bacillus*, *Micrococcus* and *Enterococcus* may withstand pasteurization and freezing.

Predominant pathogenic microorganisms of ice cream in retail sale

Predominant pathogen	Country	References
Salmonella enteritidis	Poland (survey period of 1961–1991)	Głośnicka and Kurnikoska (1994)
Listeria spp.	England and Wales	Greenwood et al. (1991)
Listeria monocytogenes	Finland (survey period of 1990–1997)	Miettinen et al. (1999)
Listeria monocytogenes *Listeria innocua*	California/USA (an incidence of 12% among 922 samples)	Walker et al. (1991)
Listeria monocytogenes	USA (survey period of 1997–1999 with six re-calls)	FDA Enforcement Report (www.fda.gov/Safety/Recalls/EnformenetReports/defaut.html)
Listeria monocytogenes	India (53% of packed and 100% of open ice creams were contaminated)	Warke et al. (2000)
Fecal coliforms	Turkey (15 of 46 samples contained coliforms above the legal limits)	Kocak et al. (1998)
Fecal coliforms *Staphylococcus aureus* *Salmonella* spp.	Italy (survey period of 1987–1988)	Massa et al. (1989)
Fecal coliforms	Pakistan (66% of the samples contained coliforms above the legal limits)	Masud (1989)
Enterobacteriaceae	Venezuella (77% of the samples failed to comply with legal requirements)	de Tamsut and Garcia (1989)
Escherichia coli O157:H7 (detected by RTi PCR)	India	Singh et al. (2009)
Verocytotoxin-producing *E. coli* O145 and O26	Belgium	Eurosurveilance (European Communicable Disease Bulletin 2008)

In India, the Bureau of Indian standards (BIS) has prescribed a maximum limits for total count of 2,50,000/ g and coliform count of 10/g. Also the ice cream should be phosphatase negative. However the BIS standards are not mandatory.

THANK YOU