

COMPARATIVE STUDY OF QUALITY OF SELECTED DAIRY ICE CREAM AVAILABLE IN BANGLADESH

**A THESIS
BY
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Examination Roll No.: MS 070719
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Khulna-9208, Bangladesh**

September, 2008

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A Thesis

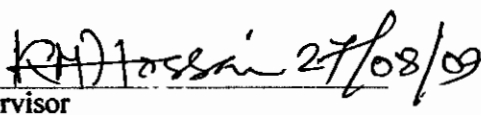
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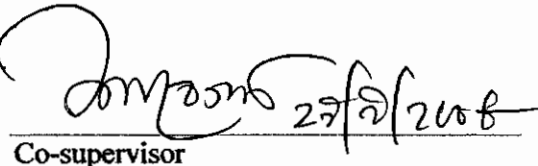
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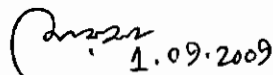
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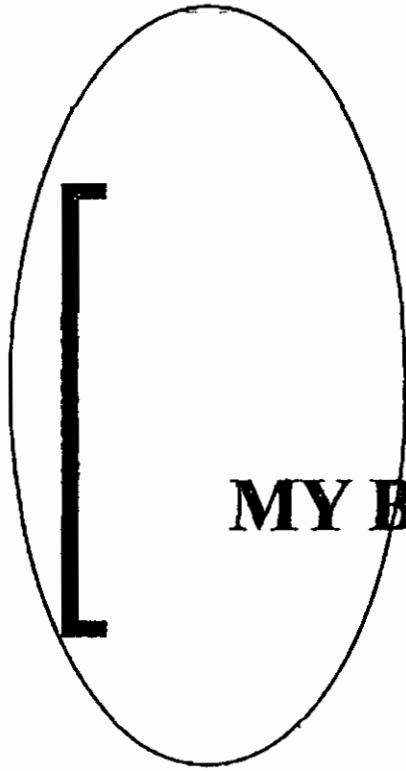
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September, 2008



**DEDICATED
TO
MY BELOVED PARENTS**



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**IN THE NAME OF ALLAH, THE ALMIGHTY, MOST GRACIOUS AND MOST
MERCIFUL, HE WHO OWNS ALL GRATITUDE AND PRAISES**

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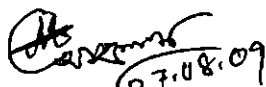
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COMPARATIVE STUDY OF QUALITY OF SELECTED DAIRY ICE CREAM AVAILABLE IN BANGLADESH

DECLARATION

This thesis reports the original research work of the author, except as otherwise stated. The material presented has not been submitted either in whole or in part for a degree from this or any other university. The findings of the research has not been/will not be published anywhere without the concurrence of the concerned supervisor/co-supervisor.



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ABSTRACT

Ice cream is popular and nutritionally enriched congealed dairy product. Since it harbor misappropriate cream mix and many potent pathogen, its microbial quality has always been crucial important to public health. The experiments were subjected to measure the physical, chemical and microbiological qualities of Milk Vita, Igloo and Kwaliti brands ice cream in the market of Bangladesh. The experiment results show that there was regarding physical parameters (specific gravity, flavor, body texture, melting quality and color) no significant differences between Milk Vita and Igloo. However, significant differences regarding specific gravity, body texture between Milk Vita and Kwaliti was observed. Regarding chemical tests, the total solid were 344.3 ± 0.3 , 297.5 ± 2.29 , 270.4 ± 0.3 g/kg respectively, fat were 101.1 ± 0.36 , 92.16 ± 0.76 , 81.0 ± 0.5 g/kg respectively, protein were 35.6 ± 0.47 , 34.9 ± 0.51 , 31.5 ± 0.5 g/kg respectively, ash were 10.22 ± 0.035 , 4.49 ± 0.18 and 7.95 ± 0.37 g/kg respectively and acidity were 2.23 ± 0.057 , 1.93 ± 0.57 and 1.1 ± 0.1 g/kg respectively in Milk Vita, Igloo and Kwaliti ice cream respectively. The experimental results show that selected ice cream samples regarding chemical parameters are within acceptable range. However, significant differences were observed regarding percentage of total solid, fat and ash among Milk Vita vs Igloo, Milk Vita vs Kwaliti and Igloo vs Kwaliti ice cream samples. In protein and acidity have no significant differences between Milk Vita and Igloo, however significant differences Milk Vita vs Kwaliti and Igloo vs Kwaliti were observed. Regarding total bacterial count in ice cream of Milk Vita, Igloo and Kwaliti varied from 1500 to 2500 with an average of 2000 cfu/ml, 2000 to 3000 with an average 2566.66 cfu/ml, and 4500 to 6000 with an average 5166.66 cfu/ml respectively. The coliform count varied from 2 to 3 with an average 2.33 cfu/ml and 4 to 6 with an average 5 cfu/ml in Igloo and Kwaliti respectively but in case of Milk Vita, it was found negative. It was observed that fecal coliform was negative in all selected brands of ice cream. The experimental results showed that the ice cream samples from Milk Vita, Igloo and Kwaliti maintained recommended standard obliged by Bangladesh Standard Testing Institute. However to reduce contamination with microorganism to a minimum and obtain good keeping quality of the product, the raw material are to be examined; the equipment containing the food is to be adequately cleaned, sanitized and tested; the preserving processes is to be checked; packaging and storage are to be supervised.

ABBREVIATIONS

°C	Degree centigrade
-	Negative reaction
+	Positive reaction
%	Percentage
>	more than
<	less than
Approx.	Approximate
BSTI	Bangladesh Standard Testing Institute
cfu	Colony forming unit
df	Degree of freedom
dw	Distilled water
Dr.	Doctor
e.g.	Example gratita (For example)
<i>et al.</i>	et alibi (and others)
FAO	Food and Agriculture Organization
g or gm	Gram
GMP	Good Manufacturing Practices
GHP	Good Handling Practices
h or hr	Hour(s)
H ₂ SO ₄	Sulphuric Acid
i.e.	id est (that is)
kGy	Kilogray
L	Litter
mg	Milligram
MR	Methyl Red
ml	Millilitre
mFC	Faecal Membrane Coliform
ND	Not detection
No.	Number
NPU	Net protein utilization
NaOH	Sodium Hydroxide
NS	Non significant
pH	Negative logarithm of hydrogen ion
S	significant
SNF	Solid-non-fat
sp. or spp.	Species
TCC	Total coliform count
TS	Total Solid
TVBC	Total viable bacterial count
vs	Versus
viz	Videli (namely)
VP	Voges proskauer
WHO	World Health Organisation
Wt	Weight
µg	Microgram

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Chapter One

INTRODUCTION

INTRODUCTION

Ice cream has a long history as a popular dairy food item. Two types of ice-cream, soft and hard, are available on the market. Ice cream is a nutritionally enriched congealed dairy product produced by freezing pasteurized mixture of milk solid other than fat, sugar, emulsifier and stabilizer. Flavor enrichment of ice cream is because of optional addition of fruit nuts, candies, syrups and other flavoring ingredients (*Food and environmental hygiene department*, 2001). Evolution of ice cream in its present form is because of gradual timely changes brought about according to human taste by changes in its preparation.

The characteristics of aged ice cream mixes (110 gkg^{-1} milk fat, 110 gkg^{-1} non-fat milk solids, 140 gkg^{-1} sugar, 2 gkg^{-1} stabilizers and 2 gkg^{-1} emulsifier) and the final ice cream prepared with various milk solids non-fat were examined (Udabage, *et al.* 2005). Dairy ice cream is a complex matrix consisting of ice, air, fat and the concentrated unfrozen serum. The approximate phase volumes are $\sim 250 \text{ mL}^{-1}$ for ice, $\sim 500 \text{ mL}^{-1}$ for air, $\sim 50 \text{ mL}^{-1}$ for fat and $\sim 200 \text{ mL}^{-1}$ for serum (Campbell and Pelan, 1998.). The ice cream properties are determined by its formulation and the processing conditions used in its manufacture (Goff, 1997). A typical regular ice cream formulation consist of $350\text{--}370 \text{ gkg}^{-1}$ total solids (TS) with $100\text{--}120 \text{ gkg}^{-1}$ fat, $100\text{--}110 \text{ gkg}^{-1}$ milk solids non-fat (MSNF), $130\text{--}160 \text{ gkg}^{-1}$ sweetener and $2\text{--}4 \text{ gkg}^{-1}$ stabilisers and emulsifiers (Marshall and Arbuckle, 1996).

The source of milk solids used in an ice cream formulation, as well as the conditions used in the preparation of the milk ingredient, both influence the ice creams' properties (Udabage and Augustin, 2003). Improved melt resistance of ice cream was obtained when lower temperatures for longer times were used for pasteurization (Loewenstein and Haddad, 1972). Pre-processing of whey proteins to form microparticulated whey proteins is reported to stabilize ice cream foam and increase its resistance to meltdown (Koxholt, *et al.*, 2001). The major steps in the manufacture of ice cream include preparation of the ice cream mix, homogenization, pasteurization, cooling, ageing, freezing and churning. The formulation and stability of the ice cream emulsion is an integral part of ice cream manufacture and has a significant effect on the properties of the final ice cream. The stability of emulsions is influenced by the surface coverage of

the fat globule, the dimensions of the adsorbed layer and the surface viscosity (Euston and Hirst, 2000). The ability of a protein to form and stabilize an emulsion is dependent on its ability to adsorb, rapidly unfold and spread at the droplet interface. During emulsification of the ingredients (i.e. before heating), caseins are preferentially transferred to the oil-water interface in comparison to whey proteins, dominating the adsorbed layer thickness and producing a stabilizing layer (Dickinson and Parkinson, 2004). Upon heating, the whey proteins competitively displace the caseins from the interface except κ -casein which resists displacement due to complex formation with β -lactoglobulin (Dickinson and Parkinson, 2004). Churning of the ice cream mixes prior to hardening sets up the ice cream structure as it results in fat destabilization and the partial coalescence of globules that leads to the formation of a fat network (Kokubo, *et al.*, 1996). The agglomerated fat network is capable of stabilizing the foam lamellae and reducing drainage which leads to increased melt resistance in ice cream (Koxholt, *et al.*, 2001).

Ice cream is undoubtedly one of most popular and favorite food product in Bangladesh among children and adults especially during summer season. Several international imported and Bangladeshi national brands of ice cream in variety of flavors have been marketed here. Quality of ice cream depends on both extrinsic factors that include manufacture procedure, and intrinsic factors that include proportion of ingredients used. Ice cream, a milk based product is good media for microbial growth due to high nutrient value, almost neutral pH value and long storage duration. Primary sources of microbial contamination to ice cream include water and raw milk whereas secondary sources include flavoring agents, utensils and handling. Although pasteurization, freezing and hardening steps in production can estimate most of the microbial hazards, but still numerous health hazards are persistent due to various conditions.

The biological agents contaminated with in food are traced to ingredients added post pasteurization and environmental factors such as air, faults in storage tank, cracks in the plant and packaging materials (Bigalke and Chappel, 1984). In developed countries ice cream receives quality control measures to increase its shelf life as well as prevent potential threat of public health. Bangladesh is still backward in this respect. Due to no enforcement of inspection act and lack of maintenance of standard relation to hygienic quality of ice cream, the consumers of this country are deprived of getting quality ice

cream. In our country, some commercial company are manufacturing and marketing ice cream in the local market. The microbiological status of ice cream for public health significance in Germany is known (Furh, 1986 and M-E-Elahi, *et al.*, 2002). However not much research works have not been conducted regarding quality investigation of ice cream in Bangladesh. Therefore, the present research program has been undertaken the following objectives:

- I. To evaluate the physical and chemical quality of commercially produced ice cream in Bangladesh.
- II. To ascertain the sanitary status of commercially produced ice cream in Bangladesh and to made awareness among consumer regarding quality of ice cream.

Chapter Two
LITERATURE REVIEW

REVIEW OF LITERATURE

Numerous scientific works have been carried out in different countries of the world for determination the quality of ice cream. Some of the important findings are reviewed and presented below.

2.1 Physio-chemical properties

Mann (1971) reported that ice cream mix containing 10% fat, 12% serum solids, 15% sugar and 0.4% gelatin yielded ice cream of the best body, texture and flavor.

Parijez (1975) studied on analysis of 150 ice-cream samples, which averaged 4.23% fat, 32.15% TS. Consideration is given to the possibility of introducing into the Yugoslav market a low-calorie ice-cream with non-milk fat, although current Yugoslav regulations prohibit the use of vegetable fats in ice cream.

Adam (1978) proposed an evaluation scheme for organoleptic testing of ice cream, which involved five point scales for scoring color, shape, odor, flavor and consistency respectively. The scoring system for each characteristic was complemented by a series of standard descriptive phrases, which have the meaning for all panel members and have been evolved as a result of thousands of organoleptic evaluations of ice cream at the German Federal Research Institute for nutrition karlsruhe. Samples were classed in three grades according to their score: Grade I (score 9-7), desirable properties: Grade II (score 6-4), satisfactory and suitable for market: Grade III (score 3-1) unsatisfactory and unsuitable for sale. The following weighing factors were recommended for converting the individual scores to an overall score, 1 for shape and odor, 2 for color, 3 for flavor and consistency.

Berline (1979) worked on requirements relating to ingredients manufacturing procedures, physical, chemical and sensory characteristics, testing, labeling, packaging, transport. The author laid down for 6 types of ice cream (Eiskream), with 3, 6, 10 or 15% milk fat or with 3 or 6% vegetable fat. The minimum TS content of the 6 types were 27, 30, 34 38 37 and 30% respectively. The sucrose content was 10%.

flavor was obtained using 14.1% fat and 15.4% sugar and optimum texture or mouthful was attained with 13.2% fat and 14% sugar.

Rothwell (1995) studied that high quality strawberry ice cream can be made from an ice cream mix containing >10% milk fat, sucrose plus glucose, a good stabilizer or emulsifier system and high quality fruit mix plus flavoring. He also studied that luxury or premium ice cream contains 12-16% milk fat, 14-16% sugar and dried egg yolk as emulsifier.

Udabage, *et al.*, (2005) studied on physical behavior of dairy ingredients during ice cream processing that the characteristics of aged ice cream mixes (110 gkg⁻¹ milk fat, 110 gkg⁻¹ non-fat milk solids, 140 gkg⁻¹ sugar, 2 gkg⁻¹ stabilizers and 2 gkg⁻¹ emulsifier) and the final ice cream prepared with various milk solids non-fat were examined. The milk solids non-fat powders used were low heat skim milk powder (LHSMP), whey protein concentrate with 350 gkg⁻¹ crude protein (WPC 35), and unheated and heated blends (1:1 ratio) of LHSMP and WPC 35. The source of the milk solids non-fat and the heat treatment applied during powder manufacture influenced the characteristics of the ice cream mix and the properties of the final ice cream. The results suggest that alteration of the emulsion characteristics of ice cream mixes with the use of milk solids non-fat with different proportions of caseins to whey proteins and increased whey protein denaturation altered the ice cream properties. The use of a high heat-treated blend of LHSMP and WPC 35 resulted in ice creams with the best melt resistance.

2.2 Microbiological parameters

Marino (1952) worked on the maximum limits of bacterial count in ice cream. After examination the problems from the public health point of view, the author concluded that the maximum permitted bacterial count should vary according to the type of ice cream. For fresh ice cream bacterial count showed not allowed more than 10,000 per ml.

Boese (1953) carried out bacteriological investigation of ice cream. The bacteriological examination of 350 samples of ice cream obtained from towns and villages in the Moers (Rhineland) district showed that only, 42-44% of samples

contained <300000 microorganisms per ml. and 15-34% or < 10 coliform per ml. Marked improvements in the bacteriological quality of ice cream were, however supervised, particularly in the town. Pathogenic bacteria were isolated from two of the samples of ice cream.

Widell (1957) studied on the bacteriological ice cream control. A total of 694 commercial samples of ice cream were examined by methylene blue test and by two modifications of and results compared with the bacteriological quality of ice cream as determined by total bacterial count and coliform count. Although the results showed up certain limitations of methylene blue test, another considered that suitable as a preliminary test for estimating the hygienic quality of ice cream and gave details of modification of original English method.

Citerni (1958) carried out bacteriological examination of ice cream in Acquila province, Italy was reported that both total bacterial counts and coliform counts were found to be markedly higher in samples taken in the province than those taken in the town. No relationship was found between total bacterial count and the presence or absence of coliform bacteria.

Crowhurst (1959) worked on the bacteriological grading of ice cream lollies. They concluded from the examination of 25 samples of ice cream lollies that the existed little relationship between total bacterial count and methylene blue reduction time. However, the presence of coliform bacteria, even in small numbers, invariably resulted in a low grading by methylene blue test.

Nutting, *et al.*, (1959) estimated the coliform bacteria in ice cream by the use of membrane filter. A membrane filter procedure based on the method of Fifield *et al.*, used in conjunction with a dilute wetting agent (Triton X 100) was used successfully as a direct 1-day method for the method was comparable to standard deoxycholate lactose agar plate method for both vanilla and flavored ice creams.

Overby (1960) reported about the factors influencing determination of the bacterial count of ice cream. Duplicate plantings were performed of 420 samples of different types of ice-cream from 42 factories and plates counted after 2-3 days at 30°C. The

extra-days incubation gave counts increasing from 3 to 8.73 with an average 82%. There were wide variations between duplicates after both 2 and 3 days, which were apparently related to the factory rather than the type of ice cream manufactured. The weight of 1 ml of ice cream taken out with a pipette after melting at 30°C varied from 0.31 to 1.00 gm. It would therefore be more accurate to state results as bacterial counts per gm than per ml melted product.

Irujo and Liono (1960) studied on the hygienic conditions of the manufacture and scale of ice cream in Bibao and results of bacteriological analysis of 125 samples of ice cream from Biabo were presented which showed an improvement between 1961 and 1962. In 1961, 60 of 61 samples contained coliform only 59 of 93 did so in 1962. *Citrobacter freundii* was the most common organism. Pathogenic *staphylococci*, mainly *staphylococcus aureus*, were detected in 44 and 58 samples respectively and the average total bacterial counts were 785,000 and 151,000 per ml in the two years.

Masek and Maja (1964) carried out study on the methods for the estimation of coliform bacteria. In connection with the introduction of a new official medium (meat peptone agar + 0.22% TTC) for coliform estimation in Czechoslovakia, the authors compared several selective media using both the plate and paper strip techniques. The result showed that the new medium gave values closely correlated with those obtained on deoxycholate and Levine media. The incorporation of agar into paper strips had beneficial effects, facilitating the counting of colonies and reducing the variations in counts.

Archambault *et al.*, (1965) reported the bacteriological quality of ice cream of England. Ice cream sold in the district of Montreal was analyzed by the fermentation tube calorimetric method. A total of 305 samples were examined, 210 were from unopened container (i) and 95 from containers whose contents had been partly consumed (ii) for (i), 98% had total counts <100,000 per gm and 81.2% <5000 per gm; for (ii) the corresponding figures were 51.6% and 34.7% the probable coliform counts were: for (ii) 85.1% <1000 per gm and 53.9% <50 per gm.

Boey (1966) reported the microbiological studies of ice cream in Bulgaria. The total colony counts of 27 samples from batches of ice cream from a dairy factory in Warn

taken between March 1963 and October 1964 ranged (with one exception of 210,000 per ml) from 700 to 42000 per ml. 18 samples showed no coliform growth and 9 counts of 10-2710 per ml. *Salmonellae*, haemolytic *streptococci* and *staphylococci*; were not detected. In 25 samples (19 ice cream and 6 fruit ice) taken from resultants, shops or portable retail freezers, the total counts ranged from 2200 to 7500000 per ml with overgrown plates for 2 samples. Nine samples showed no coliform growth and 16 counts of 10-312000 per ml ice cream samples were failed on total count and 13 on high coli-titre.

Tamieri and Roche (1967) studied on the microbiological control of ice cream produced on small scale. In 1964, 582 samples of ice cream were taken from 361 small scale producers in 9.3 districts in Cremona province of Italy. Eighty nine of the samples had total bacteria counts of $>2 \times 10^5$ per gm and 233 contained >10 coliform per ml in all, 20 samples (41.2%) considered unsatisfactory by these two criteria. The highest incidence of unsatisfactory samples was in July and August, in districts with lower populations, and from procedures whose premises had been inspected only once or twice, in subsequent years, the bacterial quality improved 1965, 33.6% unsatisfactory; 1966, 29.2%; 1967, 27.1%. In 1964, 2 cases of food poisoning were traced to *Salmonella typhimurium* in ice cream.

Seidel (1969) determined coliform bacteria with particular reference to use of TTC agar. Analysis of ice cream showed modified leifsonagar to be superior to TTC agar (1:450) for determination of presence of coliform bacteria. A TTC pour plate culture (48hr. and 0.1ml sample) does not permit accurate determination of the count, but a TTC surface culture (24hr.) permits better determination between cocci and bacilli.

Bester (1977) determined coliform bacteria in dairy products (test procedure). He gave details of 4 stages in testing for coliform, (i) presumptive test, using a solid or liquid medium; (ii) confirmatory test, to eliminate false-positive results caused by organism which fermented sugars other than lactose e.g. in ice-cream, fruit yogurt or sweetened condensed milk; (iii) final confirmatory tests on colonies which had grown on eosin methylene blue or endo agar plate (part of the colonies being transferred to lactose broth and part to agar slants) or in brilliant green bile broth in which gas had formed

(transfer to agar slants) and (iv) the modified Eijkman test for fecal coliform, which at 44°C produced gas in brilliant blue green bile broth and also indole (tryptone water).

Humann and Weber (1978) detected *Enterobacteriaceae* in ice cream qualitatively and quantitatively. He also compared different test systems. From 104 samples of ice cream (45 from industrial and 59 from small scale procedures), two hundred ninety four strains of *Enterobacteria* were isolated and examined for biochemical characteristics. The majorities were *Enterobacter spp.* and *Klebsiella spp.*; *Citrobacter* and occasionally *Escherchia coli* were also identified. One hundred thirty nine strains were subjected to comparative tests by (i) the API 20E system, (ii) Enterotube or Fermentube system, and (iii) a conventional series of biochemical tests. Both (i) and (ii) could be used instead of (iii); (ii) offered advantage in time and labor saving. Results confirmed that small scale ice cream producers did not always meet the hygiene requirements while all industrially produced samples were unobjectionable.

Moiseeva and Bukanova (1978) worked on the bacteriological assessment of ice cream according to local standard ice creams, manufactured in 93 dairies and refrigerating plants. In 94.5% of samples the bacterial contamination was $<10^5$ per ml; 97.6% of samples had <0.3 titres for coliform bacteria. The quality of frozen creams compiled bacteriologically with OSI'4973-74 standards.

Berlin (1979) worked on microbiologically quality of ice cream. He suggested that bacterial count should not exceed 100000 per ml with not more than 10 coliform and 10 coagulase-positive *Staphylococci*/ml.

Marcy and Adam (1979) observed that a commercial brand of vanilla ice cream was contaminated with *S. typhimurium* (8×10^3 /ml), *E. coli* (2×10^3 /ml), *Ent. cloacae* (6×10^3 /ml) and stored for 3 years at -28°C. A steady fall in viable count was observed for all organisms to 0.35×10^3 and 0.3×10^3 and 2.2×10^3 respectively at the end of 3 yr. Results suggested that coliform especially *E. coli* may be suitable indicator organism during long-term storage at deep freezing temperature.

Aleksieva and Mirkou (1983) reported that the coating base consisting of cocoa; milk mixture with 0.8-1.4% moisture found to be fully satisfactory initially and during

storage for 6 months in paper/nylon packaging; coliform, pathogenic staphylococci and salmonellae were absent, and total bacteria counts ranged from 200-4600/gm initially to 100-2100/gm after storage. However, during mixing of comminuted base with butter for production of coating to be applied to ice cream in the enrolling machine, total bacteria count increased to 13200-79000/gm, and *E. coli* titre reached 1000/gm.

Bigalike and Chappel (1984) reported that coliform counts are affected by time and temperature of holding ice cream mix prior to freezing and it is recommended that he mix should not be stored for more than 48 hours at below 40°C. 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 be coliform free.

Kumar *et al.*, (1989) studied on microbiological quality of ice cream marketed in Madras City, India, by (i) organized dairy companies (ii) private manufactures and (iii) small vendors/push carts. Mean plate coliform and psychrotrophic bacterial counts respectively for (i) were 4.1×10^4 , 5.9×10^2 and 3.9×10^3 ; for (ii); 3.6×10^5 , 2.82×10^2 and 1.6×10^4 ; and for (iii) were 3.6×10^6 ; 4.2×10^3 and 1.3×10^5 . Quality was in the order (i) > (ii) > (iii).

Maifreni *et al.*, (1993) collected and analyzed 396 samples of artisanal ice cream had been from different ice-cream shops in Udine and province in the course of the years 1990 and 1991. All tested flavors contained aerobic germs, coliform, *Enterococci* and yeasts in different quantities.

Kivanc (1994) reported the microbiological quality of ice cream marketed in Esakisehir, Turkey, during May-August 1992. twenty three ice cream samples were collected over this period; total aerobic bacteria, psychrophilic bacteria, coliform, *Escherichia coli*, *Staphylococcus aureus*, faecal Streptococcus, yeast and fungi counts were evaluated and tests were carried out to detect *Salmonella spp.* or *Shigella spp.* It was concluded that the microbiological quality of ice cream samples was unacceptable.

Feldsine *et al.*, (1997) analyzed five foods representative of a variety of food products using Visual Immunoprecipitate Assay (VIP) and the Bacteriological Analytical Manual (BAM) culture method for the presence of *E. coli* 0157:H7. During this study, a total of 1377 samples and controls were analyzed and confirmed, of which 508 were positive and 867 were negative by both methods. Two samples were positive by BAM and negative by VIP.

Curiale *et al.*, (1997) inoculated a dry-film coliform count plate that with 5 ml sample was compared with the Violet Red Bile Agar plate method in a collaborative study by 18 laboratories. Significantly higher ($P < 0.05$) numbers of coliform were recovered by the dryfilm method from 2% milk samples at the 3 inoculum levels, the chocolate milk at the low and high-inoculum levels, and the cream at the high-inoculum level. Significantly higher counts were obtained by the agar method for cottage cheese samples at the low-inoculum level.

Erol (1998) reported that 100 ice cream samples, including 30 vanillas, 26 chocolate and 44 fruit-flavored were analyzed microbiologically and observed that 63.2-73.1% of vanilla, 57.4-73.0% of chocolate and 52.1-61.3% of fruit-flavored ice cream samples did not meet statutory requirements for total bacterial count and coliform count, 20.0-30.8 of the samples contained 10^2 - 10^4 cfu/g coagulase positive *staphylococci*, while *F. coli* and *Salmonella* were isolated from 2% of the samples.

Graceleah *et al.*, (1999) conducted microbial analysis of ice cream produced by big-scale and small-scale manufacturers in metro Manila that ice cream produced by three large-scale and three small-scale manufacturers were studied to determine the microbial load or colony forming units per gram (cfu/g) using the standard aerobic plate count. Analyses for the most probable number/gram (MPN/g) of total coliform and fecal coliform, presence of coagulase positive *Staphylococcus* and *Salmonella* were also done in order to determine if the samples conformed to the standards set by the Bureau of Food and Drugs (BEAD). All ice cream samples produced by the big-scale manufacturers tested were within the limits set by BFAD. On the other hand, although samples from the small-scale manufacturers had cfu/g which were acceptable

in terms of BFAD standard, all the samples tested were positive for coagulase positive *Staphylococcus*, coliform, and fecal coliform.

Little (1999) reported a study on a total of 1983 soft ice cream and liquid and dried mix samples obtained from 1370 outlets (78% retail premises and 22% mobile vendors) in the UK. Aerobic plate counts (APC) of $>10^5$ cfu/g, was reported 15% contained *Enterobacteriaceae* ($>10^4$ cfu/g), 0.5% contained *Staphylococcus aureus* ($>10^2$ c.f.u./g), 5% contained *Bacillus spp.* ($<10^4$ cfu/g), and 0.8% contained *Listeria monocytogenes* ($>10^2$ cfu/g). On the basis of published microbiological guidelines, 26% of samples were unsatisfactory, and one sample was unacceptable due to the presence of *S. aureus* ($>10^4$ cfu/g). The APC was $>10^5$ /g (or ml) in 21% of 597 mix samples and 7% contained $>10^4$ *Enterobacteriaceae*/g; *S. aureus* were isolated from 14, *L. monocytogenes* from 2 and *Listeria spp.* From 3 mix samples, all at $<10^2$ /g. *Bacillus spp.* Were present, all at $<10^4$ /g, in 36 liquid and 3 dried mixes. Liquid mixes tended to be of poorer microbiological quality than dried mixes. The microbiological quality of the ice cream was dependent upon type of mix, dispensing machine used and frequency of cleaning and results indicated that the microbiological quality of soft ice cream from some outlets, in the remains a cause for concern.

Food and Environmental Hygiene Department HKSAR, (2001) reported that ice-cream is a frozen dairy product. It is sold in soft or hard status. The production of ice-cream includes totally eight steps. Pasteurization, freezing and hardening are the main steps to eliminate the microbiological hazards. In Hong Kong, sale of ice-cream is governed by the Public Health and Municipal Services Ordinance and its subsidiary legislations, especially the Food and Drugs (Composition and Labelling) Regulations and the Frozen Confections Regulation. Reviewing the microbiological surveillance findings on 16,379 ice-cream samples collected from 1998 through 2000, 543 samples (3.3%) were found unsatisfactory in either total bacterial count or coliform count. Trend analysis showed that the unsatisfactory rates of all ice-cream decreased from 4.7% in 1998 to 3.2% in 1999, and then, to 2.2% in 2000. Comparing the unsatisfactory rates of both types of ice-cream, the relatively high unsatisfactory rates of soft ice-cream were observed. In addition, 198 additional samples were taken for *Listeria monocytogenes* examination in the past three years. No sample was found to contain *L. monocytogenes*. A gradual improvement in the hygienic quality of ice-cream was

observed in the period of 1998-2000. To ensure food safety, the trade and the public are advised to pay attention on some key areas from the preparation to consumption of ice-cream.

Baylis *et al.*, (2000) compared the ability of four rapid methods and a standard cultural method to detect low levels of heat-injured cells of *Salmonella typhimurium* in ice cream and skimmed milk powder. The detection of *Salmonella* in samples contaminated with low levels (< 10 cfu/g) was significantly greater with the novel broth method than with the other methods ($P \leq 0.01$).

Windrantz and Arias (2000) evaluated the presence of total and fecal coliform, *E coli*, *Listeria spp.* and *Salmonella spp* in 65 samples of both commercial and homemade ice cream. 37.1 % of homemade ice cream and 20% of commercial ice cream did not fulfill the international standard for total coliform. At the same time 82.9% of home made samples and 56.7% of commercial ones presented fecal coliform. *E coli* were found in 51.4% of home made samples and 26.7% of commercial ones. Sixteen *Listeria spp.* isolates were obtained, 50% corresponded to *Listeria monocytogenes* and 50% to *L. innocua*. The overall presence of *L. monocytogenes* in ice cream samples was of 12.3% and it was isolated in all cases, from homemade ice cream samples. *Salmonella* was not isolated from the samples analyzed.

Kruy *et al.*, (2001) studied the microbiological quality of ice creams sorbets sold on the streets of Phnom Penh City. Microbiological analysis performed in the laboratory of Pasteur Institute of Cambodia indicated the poor bacteriological quality of the samples. The proportions of samples classified unsafe according to microbiological criteria were 83.3% for total bacterial count at 30°C, 70% for total coliform, 30% for faecal coliform, 12.2% for *Staphylococcus aureus* and 1.9% for presence of *Salmonella spp.* These bacterial results suggest that many other food products sold in the streets may threats. Regulation of the street food sector should be part of a larger strategy for enhanced food safety and environmental quality in the city.

M-E-Elahi *et al.*, (2002) did conduct study on the sanitary quality of commercially produced ice cream of Milk Vita, Igloo, Polar and Savoy regarding total bacterial count (TBC), coliform count and Staphylococcal count. Each brand of ice cream was

collected from retail stores of Dhaka City. The TBC reported in ice cream of Milk Vita, Igloo, Pollar and Savoy varied from 2800 to 3800 with an average of 3280 cfu/ml, 2800 to 4000 with an average of 3450 cfu/ml, 10,000 to 19,000 with an average of 15,000 cfu/ml and 26,800 to 56,000 with an average of 42,460 cfu/ml respectively. The coliform count varied from 4 to 18 with an average 11.60 cfu/ml and 18 to 42 with an average of 28 cfu/ml in Polar and Savoy but in case of Milk Vita and Igloo, it was found negative. The Staphylococcal count in the samples of Igloo and Savoy varied from 2 to 7 with an average of 3.80 cfu/ml and 7 to 17 with an average of 11.20 cfu/ml respectively, whereas it was found negative in the samples of Milk Vita and Polar.

Joshi *et al.*, (2004) examined the microbial quality of ice cream sold in Kathmandu and observed that out of total 44 samples (61.1%), 49 samples (68.1%) and 16 samples (22.2%) exceeded standard value of mesophilic aerobic count, total coliform count and Staphylococcal count respectively. However, only one sample (1.4%) was found to be contaminated with *Salmonella spp.* The microbial quality of 'with additive' ice cream samples was poorer in comparison to plain samples. Comparison of the quality of Nepalese and foreign brands was not significant.

Conclusion

High quality ingredients like whole milk, cream powder milk sugar, emulsifier, flavoring agent etc must be used during ice cream manufacture. To reduce contamination with microorganism to a minimum and obtain good keeping quality of the product, the raw material are to be examined; the equipment containing the food is to be adequately cleaned, sanitized and tested; the preserving processes is to be checked; packaging and storage are to be supervised. To ascertain the sanitary status of commercially produced ice cream in Bangladesh and to made awareness among consumer regarding quality of ice cream.

Chapter Three
MATERIALS & METHODS

MATERIALS AND METHODS

The study was carried out at the Quality Control Laboratory, Milk Vita, Dhaka Dairy Plant and Microbiological Laboratory of Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh during the period of December 2007 to August 2008.

3.1 Sample Collection

To perform the study selected ice cream brands being produced by different companies of Bangladesh like Milk Vita, Igloo and Kwaliti was chosen. The source and collection places of selected ice cream samples together with names are shown in Table 1.

Table 1: Source and collection places of selected ice cream samples

Trade name of samples	Sources of samples	Place of collection
Milk Vita	Bangladesh Milk Producer Co-operative Union Ltd., Dhaka, Bangladesh.	Dhaka Dairy Plant, Mirpur, Dhaka.
Igloo	Abdul Momen Ltd. Dhaka, Bangladesh.	Mirpur-1, Dhaka.
Kwaliti	Sanowara Drinks and Beverage Industry Ltd., Dhaka.	Mirpur-1, Dhaka.

3.2 Analysis of the sample

Total samples for each test was analyzed in this study. The collected samples was transported in well insulated flask and put in deep freeze at -20°C until subject to physical, chemical and microbiological analysis. The following tests were performed with collected samples.

➤ Physical Analysis

- i. Specific gravity
- ii. Test for flavor
- iii. Test for color and package
- iv. Test for body and texture
- v. Test for melting quality

➤ Chemical Analysis

- i. Determination of total solid percentage
- ii. Determination of fat percentage
- iii. Determination of protein percentage

- iv. Determination of ash percentage
- v. Determination of acidity

➤ **Microbial Examination**

- i. Total viable bacterial count
- ii. Coliform count
- iii. Detection of total coliform bacteria
- iv. Detection of fecal coliform bacteria

3.2.1 Physical Analysis

Immediately after collection of selected samples from different retail shops, samples were subjected to determine specific gravity and judged to organoleptic evaluation of flavor, color and package, body and texture and melting quality and thereby overall of physical score of samples by a panel of expert judges. Specific gravity was determined by lactometer method.

3.2.2 Chemical Analysis

3.2.2.1 Determination of total solid percentage

The percentage of total solid of ice cream samples were carried out according to the procedure suggested by Aggarwala and Sarma (1960) using the following formula:

$$\% \text{ TS} = \frac{\text{Weight of the sample after drying}}{\text{Weight of the sample before drying}} \times 100$$

3.2.2.2 Determination of fat percentage

The percentage of fat in ice cream samples were determined by Gerber fat test method.

3.2.2.3 Determination of protein percentage

The percentage of protein in ice cream samples were determined by Kjeldahl method according to American of Official Agricultural Chemist (AOAC, 2003) using following equation

$$\% \text{ of Nitrogen} = \frac{0.014 \times 0.1N \times \text{ml acid}}{\text{Wt of sample}} \times 100$$

Where, 0.014= milli equivalent weight of N₂

Thus, nitrogen percent obtained was multiplied by 6.38 in order to obtain protein percent.

3.2.2.4 Determination of ash percentage

The percentage of ash in ice cream samples were determined by adopting AOAC method (2003) using following equation.

$$\% \text{ of Ash} = \frac{\text{Wt of ash}}{\text{Wt of sample}} \times 100$$

3.2.2.4 Determination of acidity

The percentage of acidity of ice cream samples were determined by titration methods described in the Technique of Dairy Plant Testing by Goss (1953). The amount of acidity is expressed in term of percent lactic acid as follows:

$$\% \text{ of acidity} = \frac{\text{ml. of 0.1N NaOH used}}{\text{Wt of sample}} \times 100$$

3.2.3 Microbial Examination

3.2.3.1 Sample preparation

The intact ice cream packages were purchased and immediately transported to the Quality control Laboratory of Dhaka Dairy Plant, Milk Vita, Dhaka. Samples were stored at deep freeze; standard procedure was followed for the microbial quality control of dairy products recommended by World Health Organization (WHO).

3.2.3.2 Microbiological analysis of the samples

Microbiological analysis was done according to Bergey's Manual of Determinative Bacteriology, 8th edition, (Buchanan and Gibson 1984). Decimal dilution techniques followed by standard spread plate count were used for microbial enumeration.

Media used for microbial study were as follows:

Nutrient agar (NA) media (Difco) for total viable bacterial count.

MacConkey agar media (Difco) for total coliform count.

Biochemical test for coliform detection.

3.2.3.3 Sterilization of glassware

The glassware's used for microbiological analysis were sterilized in a hot air oven (Memmart, Model TV 10-8042407, Germany) at 180° C for two hours.

3.2.3.4 Preparation of media

All the above mentioned media used for culture preparation and some other media used for biochemical test (e.g. Carbohydrate fermentation broth, Nitrate broth, Simmons citrate agar, SIM medium, MR-VP broth, etc.) were prepared by adding ingredients in cleaned conical flasks as per description by Difco manuals (Difco Manual of dehydrated culture media and reagents for Microbiological and Clinical Laboratory Procedures, 1977). An electronic balance (Model no. 604569, Switzerland) was used for accurate weight measurement. Then the conical flasks were sealed with cotton plug and aluminum foil for sterilization. pH of the liquid media, solution, etc. was measured by using a TDA p^H meter (Model- HM-10K, Japan). The preparations were then autoclaved, cooled and dispensed into sterilized plates or tubes inside the laminar airflow cabinet (Environment Air Control inc. USA.).

3.2.3.5 Sterilization of media and solution

In an autoclave (Gallenkamp, Model- APP No.-713-105 3F England) under 15 lbs/in² pressure for 15 minutes at 121°C temperature, media and solution used for the study were sterilized. Starch ampicillin agar media was cooled to about 50°C and ampicillin was added at the concentration of 10µg/ml.

3.2.3.6 Processing of samples

The samples were transferred into sterile conical flasks aseptically and mixed by Vortex mixer (Model VM2000, D.S. Instruments "Ins. Taiwan). For dilution, 10 ml of ice cream sample was taken in 90 ml sterile normal saline (0.9% NaCl), by micropipette and was homogenized to give a homogeneous suspension of 10⁻¹ dilution. Then 1.0 ml suspension was transferred from 10⁻¹ dilution to 9 ml of sterile normal saline in a test tube to give a 10⁻² dilution. In this way 10-fold serial dilution of the samples were made from 10⁻¹ to 10⁻⁴ in sterile normal saline. For each dilution, sample was thoroughly mixed using vortex mixer. Before starting any experiment, hands were rinsed with 70 % alcohol. All of the experiments were performed at the laminar airflow cabinet and the results were also recorded in just time.

3.2.3.7 Inoculation of the samples

Standard Plate Count (SPC) method recommended for dairy products (American Public Health Association, 1953) was followed for quantitative analysis of microorganisms. From each dilution, 0.1 ml of serially diluted sample was inoculated with sterile pipette and spread into different agar plates in triplicate with the help of

sterile bent glass rod. The whole operation was done aseptically on a laminar flow bench.

3.2.3.8 Incubation of media

Nutrient agar media plates, MacConkey agar media plates, staphylococcal agar media plates and starch ampicillin agar plates were incubated at 37°C for 24 hours. Membrane fecal coliform (mFC) agar media plates were incubated at 44.5°C for 24 hours. PDA plates were incubated at 28°C to 30°C for 72 hours.

3.2.3.9 Enumeration of total viable bacteria

Nutrient agar medium was used for total viable bacterial count (TBC); pH of the medium was adjusted at 6.8 prior to sterilization. Inoculated plates were incubated at 37°C for 24 hours to facilitate viable bacterial growth. After incubation, the inoculated plates having 30 to 300 colonies were considered for counting using colony counter (Gallenkamp, England) and following back calculation total count was expressed as colony forming unit per milliliter (cfu/ml).

3.2.3.10 Enumeration of total coliform bacteria

Like total viable bacteria, same method was used for total coliform count (TCC). The medium used for coliform was MacConkey Agar. Inoculated plates in triplicate were incubated at 37°C for 24 hours. After incubation using colony counter and following back calculation, the total count was expressed as cfu/ml.

3.2.3.11 Enumeration of total fecal coliform bacteria

For total fecal coliform count (TFCC), selective membrane fecal coliform (mFC) agar medium was used. The medium was not sterilized by autoclave according to formulation; the medium was heated to boil. The inoculated plates were incubated at 44.5°C for 24 hours. Selected bluish colonies were considered for counting. Back calculation method was used to express the total count by colony forming unit per ml cfu/ml.

3.2.3.2 Identification of bacterial isolates

To identify contaminating bacteria of different selected ice cream samples, the following procedures were followed:

3.2.3.2.1 Isolation of the bacteria

Agar plates with different discrete colonies were selected for isolation. Morphologically dissimilar well-spaced colonies were picked up with the help of a sterile loop from the plates. Each colony was streaked on freshly prepared plates of the same media and incubated at 37°C for 24 hours. After incubation, typical pure colonies were taken as isolates.

3.2.3.2.2 Purification and preservation of the selected bacterial isolates

The selected isolates were then purified through repeated streak plating on nutrient agar plates until a pure culture was obtained. When plating produced only one type of colony in a particular plate, it was considered to be pure. The purified isolates from single colony were then transferred to nutrient agar slant in one drum screw capped culture vial and preserved as stock culture. For long time preservation, the vials were filled with paraffin oil, tightly capped and wrapped with paraffin and properly marked. The vials were then kept at 4°C in refrigerator.

3.2.3.3 Morphological studies for identification of the selected bacterial isolates

With a view to identify the selected bacterial isolates, the following morphological characteristics were studied carefully.

3.2.3.3.1 Colony morphology

The morphological characteristics of the pure isolates on agar plates were studied after 24 hours incubation carefully. The shape, size, textures, opacity, edge elevation and pigmentation of the isolated colonies were observed using hand lens.

3.2.3.3.2 Microscopic examination

For all microscopic examinations, the slides were scrupulously cleaned and dried for having a good staining. The following techniques were employed for microscopic examination of bacterial strains.

3.2.3.3.3 Preparation of smear

Bacteria were grown on nutrient agar slants at 37°C for 24 hours or more according to necessity. A loopful of normal saline solution (0.9% NaCl) was placed in a clean slide and a portion of bacterial culture was transferred on it using a sterile loop. A very thin film was made which was allowed to dry in air.

3.2.3.3.4 Fixation of smear

The smear was fixed by slightly heating the slide over the gas flame. The slide was allowed to cool before staining.

3.2.3.3.5 Application of staining

The fixed smear was used for the following staining

3.2.3.3.6 Gram staining

This is one of the most important and widely used differential staining techniques. In this process the fixed bacterial smear was subjected to a number of solutions. The fixed smear was treated with ammonium oxalate crystal violet solution for 30 seconds. This was washed in tap water and Lugol's iodine solution was applied for 30 seconds. This in turn was tipped off and acetone was then applied for 2-3 seconds to decolorize the stain followed by washing thoroughly in tap water. Finally 0.5% safranin solution was used as a counter stain for 30 seconds. This slide was washed thoroughly in water and dried by heat and examined under microscope. Gram positive bacterial were blue or purple and gram negative organisms were red in color.

3.2.3.3.7 Acid-fast staining

During acid-fast staining, a dried and fixed smear was flooded with solution of strong carbol fuchsin and heated the smear over a hot copper plate for 5 minutes. The slide was washed thoroughly with tap water. After washing off the excess dye, the smear was treated with acid alcohol to decolorize. When decolorization was complete, again the slide was washed in water. Loeffler's methylene blue was applied for one minute as a counter stain. The slides were washed and kept on standing for dehydration and then were examined under microscope. If there was any acid fast bacterium, it was appeared red in color.

3.2.3.4 Physiological and biochemical studies of the isolated strains

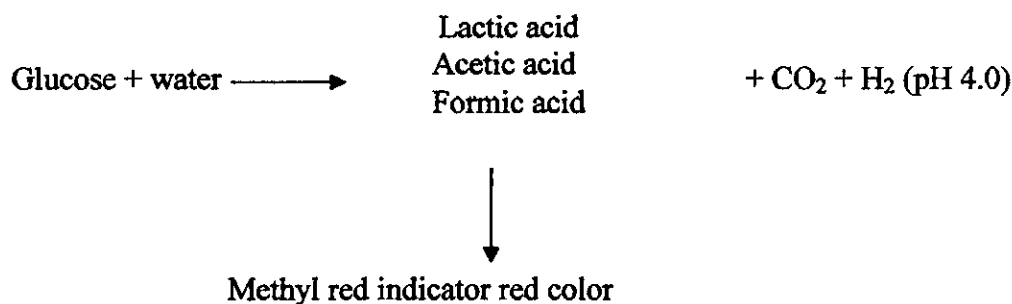
The following experiments were done based on the physiological and biochemical activities of the organisms.

3.2.3.4.1 Indole test

Indole is a nitrogen containing compound that can be produced from degradation of the amino acid, tryptophan, by the action of bacteria which contain tryptophanase enzyme. The indole test is important because certain bacteria form indole and it can be readily detected chemically. For Indole test, SIM agar medium is used which contains the substrate tryptophan. SIM agar deep tube was inoculated with 24 hours old culture and incubated at 37°C for 24 hours. After incubation, 10 drops or 0.5 ml of Kovac's reagent was added into tubes and shaken vigorously for one minute. A red color in the reagent layer indicated indole production.

3.2.3.4.2 Methyl-Red (MR) test

To differentiate between a glucose oxidizing enteric organisms, particularly *Escherichia coli* and *Enterobacter aerogenes*, methyl red test is performed. The glucose is the major substrate oxidized by all enteric organisms for energy production. The end product of this process varies depending on the specific enzymatic pathways present in the bacteria. In this test, the pH indicator methyl red detects the presence of large concentration of acidic end products. Although all enteric microorganisms ferment glucose with the production of organic acid, this test is of value in the separation of *E. coli* and *Enterobacter aerogenes*. Both of these organisms initially produce organic acid end products during the early incubation period. The low acidic pH (4.0) is stabilized and maintained by *E. coli* at the end of incubation. During the later incubation period, *Enterobacter aerogenes* enzymatically converts this acid to non-acidic end products such as 2, 3-butanediol and acetone (acetylmethylcarbinol), resulting in an elevated pH of approximately 6, the glucose fermentation reaction generated by *E. coli* is given below.



As shown, the methyl red indicator in pH range of 4 will turn red, which is indicative of a positive test. At a pH of 6, still indicating the presence of acid but with a low hydrogen ion concentration, when the indicator turns yellow, it is a negative test.

For this test, tubes of MR-VP broth were inoculated with 24 hours old culture of test organism and incubated at 37°C for 48 hours. After growth, two drops of methyl red solution was added. The tubes were then well shaken and examined. A distinct bright red color indicated MR positive and yellow color indicated MR negative.

3.2.3.4.3 Voges-proskauer test (VP) test

This test was used to differentiate the species within the group of *Enterobacter*. For this test, tubes of MR-VP broth were inoculated with 24 hours old culture of test organism and incubated at 37°C for 48 hours. After incubation, 0.6 ml 5% α -naphthol solution was added to each tube followed by 0.2 ml of 40% KOH aqueous solutions. The tubes were then well shaken for 1-2 minutes and examined. A distinct bright orange red or pink-red color indicated VP positive and yellow color indicated VP negative.

3.2.3.4.4 Citrate utilization test

This test was used to determine the ability of certain bacteria to utilize citrate as the sole source of carbon for metabolism with resultant alkalinity. The test organism was inoculated in Simmon's citrate agar by stabbing in the butt and streaking on the slant. The inoculated tube was then incubated at 37°C for 24 hours. The change in color of the medium from green to blue indicated a positive activity.

3.3 Statistical Analysis

Data generated from the experiment was statistically analyzed according to the Steel and Torrie (1980) using completed Randomized Design (CRD).

Chapter Four
RESULTS & DISCUSSION

RESULTS AND DISCUSSION

The results of various physical properties, chemical composition and microbiological sanitation of selected ice cream brands of Bangladesh like Milk Vita, Igloo and Kwality and a summary of those analyses are shown in Table 2, Table 3, Table 4, Table 5 and Table 6.

4.1 Physical parameters

Ice cream samples were served to a panel of judges for physical parameters (organoleptic evaluation) using a score card (Appendix A). The panelists were randomly selected from Milk Vita officials. The panelists were asked to assign appropriate score to each product tested on the characteristics scale of flavor, color, body and texture and melting quality of ice cream. Specific gravity was determined by butyrometer.

4.1.1 Specific gravity

Mean specific gravity of Milk Vita, Igloo and Kwality ice cream were 1.091 ± 0.0036 , 1.082 ± 0.0023 and 1.0083 ± 0.0015 respectively (Table 2) which recommended by BSTI (Draft B.D.S. for ice cream 1983). Statistical analysis showed that there was no significant difference in Milk Vita vs Igloo. However, significant difference in Milk Vita and Kwality and Igloo vs Kwality were observed (Appendix Table 1).

Table 2: Specific gravity of selected ice cream samples

Parameters	Sample source			Level of significance	
	Milk Vita	Igloo	Kwality		
Specific gravity	1.091	1.082	1.0083	Milk Vita vs Igloo	NS
	± 0.0036	± 0.0023	± 0.0015	Milk Vita vs Kwality	S
				Igloo vs Kwality	S

NS= not significant; S= significant

4.1.2 Flavor

Flavor scores of selected ice cream samples of Milk Vita, Igloo and Kwality were 3.8 ± 0.836 , 4.4 ± 0.892 , 2.6 ± 0.891 , respectively (Table 3). Statistical analysis showed that there was no significant difference among the flavor scores of different ice cream samples (Appendix Table 2). However the flavor score was highest in Milk Vita and lowest in Kwality ice cream. It may be mentioned that most of the ice

cream factories of Bangladesh use strong vanilla flavor and the flavors are highly acceptable. Lucas (1929) found that a vanilla flavor shows no tendency to disappear from ice cream during its storage period. However, the flavors of Igloo seemed to be better than other two ice cream brands.

4.1.3 Body and texture

The body and texture score of Milk Vita, Igloo and Kwality were 4.6 ± 0.547 , 4.0 ± 1.0 , 2.8 ± 0.836 , respectively (Table 3). Statistical analysis showed that there were no significant differences among the scores of different ice cream brands (Appendix Table 3). The highest score observed in Milk Vita and lowest in Kwality. Josephson (1963) reported that a high quality ice cream for its body and texture depends very much on good stabilizer. Rothwell and Palmer (1965) showed that satisfactory body and texture obtained when the stabilizers were used in proper concentration and combination. From this experiment, it is clear that the body and texture of different ice cream differed slightly although the difference was non-significant.

4.1.4 Package color

The package color score of selected ice cream samples of Milk Vita, Igloo and Kwality were 3.8 ± 0.447 , 4.0 ± 0.707 , 3.8 ± 0.836 , respectively (Table 3). Statistical analysis showed that there was no significant difference among the scores of selected ice cream samples (Appendix Table 4). Consumer acceptability depends on color. But Milk Vita had the least attractive package. Therefore, it is recommended that if attractive package is used, then it will be more popular to the consumer.

4.1.5 Melting quality

The melting quality score of Milk Vita, Igloo and Kwality were 4.6 ± 0.542 , 3.8 ± 0.831 , 2.6 ± 0.547 respectively (Table 3). Statistical analysis showed that there was no significant difference among the scores of different brands (Appendix Table 5). In this experiment, melting quality score was highest in Milk Vita and lowest in Kwality brands. Melting resistance depends upon amount and quality of stabilizer used and degree of over run. If proper quality of stabilizer is used, then melting quality will be increased.

4.1.6 Total score of physical parameters

The total mean score of selected available ice cream samples of Milk Vita, Igloo and Kwaliti were 16.6 ± 0.547 , 16.2 ± 1.09 , 12.0 ± 1.00 respectively (Table 3). Significance differences ($P < 0.01$) were found (Appendix Table 6). It was observed that overall score was the highest in Milk Vita (16.6 ± 0.547) and lowest in Kwaliti (12.0 ± 1.00) ice cream.

Table 3: Organoleptic score of selected dairy ice cream samples

Parameters	Sample source			Level of significance	
	Milk Vita	Igloo	Kwaliti		
Flavour	3.8	4.4	2.6	Milk Vita vs Igloo	NS
	± 0.836	± 0.892	± 0.891	Milk Vita vs Kwaliti	NS
				Igloo vs Kwaliti	NS
Melting quality	4.6	3.8	2.6	Milk Vita vs Igloo	NS
	± 0.542	± 0.831	± 0.547	Milk Vita vs Kwaliti	NS
				Igloo vs Kwaliti	NS
Body and texture	4.6	4.0	2.8	Milk Vita vs Igloo	NS
	± 0.547	± 1.0	± 0.836	Milk Vita vs Kwaliti	S
				Igloo vs Kwaliti	NS
Package color	3.8	4.0	3.8	Milk Vita vs Igloo	NS
	± 0.447	± 0.707	± 0.836	Milk Vita vs Kwaliti	NS
				Igloo vs Kwaliti	NS
Overall score	16.6	16.2	12.0	Milk Vita vs Igloo	NS
	± 0.547	± 1.09	± 1.00	Milk Vita vs Kwaliti	S
				Igloo vs Kwaliti	S

NS= not significant; S= significant

4.2 Chemical Parameters

The chemical experiments were studied to know the percentage of total solid, fat, protein, ash and acidity. The chemical parameters of selected ice cream samples are presented in Table 4.

4.2.1 Total solids (TS)

The mean TS content of Milk Vita, Igloo and Kwaliti ice cream were 344.3 ± 0.3 , 297.5 ± 0.29 , 270.4 ± 0.3 g/kg respectively (Table 4). Statistical analysis showed that there were significant differences ($P < 0.01$) within the TS content of different samples (Appendix Table 7). TS content was highest in Milk Vita and lowest in Kwaliti. Parjez (1975) reported that total solid content of ice cream was 321.5 g/kg.

Similar type of result was reported by Ortin *et al.* (1990). In their work they observed that TS content of ice cream varied from 31.03 to 40.17%. Therefore, the present experimental result shows that Milk Vita has the desired level of TS compared to other brands.

4.2.2 Fat

The mean fat content of Milk Vita, Igloo and Kwaliti ice cream were 101.1 ± 0.36 , 92.16 ± 0.76 , 81.0 ± 0.5 g/kg respectively (Table 4). There was significant difference ($P < 0.01$) within the fat content of different samples (Appendix Table 8). The fat content was higher in Milk Vita and lower in Kwaliti ice cream. Kitu (2004) found that the fat content Milk Vita was 101.3 g/kg. The results of fat content of present study are similar to the previous findings. Therefore, Milk Vita and Igloo ice cream had a desired level of fat content compared to Kwaliti ice cream.

4.2.3 Protein

The mean protein content of selected ice cream brands of Milk Vita, Igloo and Kwaliti were 35.6 ± 0.47 , 34.9 ± 0.51 , 31.5 ± 0.5 g/kg respectively (Table 4). The protein content of Milk Vita ice cream and other dairy ice cream had a significant difference ($P < 0.01$) (Appendix Table 9). Milk Vita had highest protein content and Kwaliti ice cream had the lowest. Sebastian (1985) reported that the minimum protein content of ice cream was 3.5%. Therefore, only the Milk Vita and Igloo ice cream had a desired level of protein content compared to Kwaliti ice cream.

4.2.4 Ash

The mean ash content of Milk Vita, Igloo and Kwaliti ice cream were 10.22 ± 0.035 , 4.49 ± 0.18 and 7.95 ± 0.37 g/kg respectively (Table 4). There was a significant difference ($P < 0.01$) among the ash contents (Appendix Table 10). The ash content was highest in Milk Vita and lowest in Igloo. Heinz (1958) observed that the ash content of ice cream was 0.81% all of brands had an acceptable ash content.

4.2.5 Acidity

The mean acidity of Milk Vita, Igloo and Kwaliti ice cream were 2.23 ± 0.057 , 1.93 ± 0.57 and 1.1 ± 0.1 g/kg respectively (Table 4). Statistically the acidity scores did differ significantly ($P < 0.01$), (Appendix Table 11)). Sebastin (1985) found that the ice cream acidity was 0.25. A similar result was also reported by Khan (1983). The present experimental result shows that the acidity percent of Milk Vita and Igloo ice cream was within the standard range compared to Kwaliti ice cream whose acidity percent did exceed the standard range. Body texture and melting quality of ice cream will be lower due to exceed acidity of ice cream.

Table 4: Chemical test of selected dairy ice cream samples

Parameters (g/kg)	Sample source			Level of significance	
	Milk Vita	Igloo	Kwaliti		
Total solid	344.3	297.5	270.4	Milk Vita vs Igloo	S
	± 0.3	± 0.29	± 0.3	Milk Vita vs Kwaliti	S
				Igloo vs Kwaliti	S
Fat	101.1	92.16	81.0	Milk Vita vs Igloo	S
	± 0.36	± 0.76	± 0.5	Milk Vita vs Kwaliti	S
				Igloo vs Kwaliti	S
Protein	35.6	34.9	31.5	Milk Vita vs Igloo	NS
	± 0.47	± 0.51	± 0.5	Milk Vita vs Kwaliti	S
				Igloo vs Kwaliti	S
Ash	10.22	04.49	07.95	Milk Vita vs Igloo	S
	± 0.035	± 0.18	± 0.37	Milk Vita vs Kwaliti	S
				Igloo vs Kwaliti	S
Acidity	2.23	1.93	7.95	Milk Vita vs Igloo	NS
	± 0.057	± 0.57	± 0.37	Milk Vita vs Kwaliti	S
				Igloo vs Kwaliti	S

NS=not significant; S= significant

4.3 Microbiological parameter

The microbiological parameters of Milk Vita, Igloo and Kwaliti ice cream of Bangladesh are given in Table 5, 6 and 7.

4.3.1 Total viable bacteria

The bacteriological status of four brands of ice cream is presented in Table 5. The average bacterial count of Milk Vita, Igloo and Kwaliti ice cream were 2000 cfu/ml, 2566.66 cfu/ml and 5166.66 cfu/ml respectively (Table 5). The mean TBC in use samples of ice cream of Milk Vita 2000 cfu/ml was found lower in comparison to use samples of Igloo 2566.66 cfu/ml and Kwaliti 5166.66 cfu/ml which recommended by BSTI (Draft B.D.S. for ice cream 1983) should have a maximum count of 10,000 cfu/ml. These findings support the results of Marino (1954), Keller *et al.*, (1987) and M-E-Elahi *et al.*, (2002) who suggested that fresh ice cream contained not more than 1,00,000 cfu/ml of total bacterial count (TBC) per ml. From the above findings, it was revealed that all brands of ice cream samples were within acceptable limit of public health safety because the samples did not exceed the total viable count 1,000,00 cfu/ml which were in agreement with that of Marino (1954) and Hankin *et al.*, (1984). It is clear from the overall results that ice cream samples of Milk Vita was comparatively better because the counts of total bacteria were less than recommended microbiological standard of Food and Drug Administration and USPHS (1965) and compared to Igloo and Kwaliti brands of ice cream Rossi (1990) reported that the ice cream might be contaminated due to improperly cleaned servers and debris falling into uncovered tubes at the at the point of sale.

4.3.2 Coliform count

The average bacterial count of Milk Vita, Igloo and Kwaliti ice cream were Nil, 2.33 and 5 respectively (Table 5). The highest mean coliform count was recorded with the ice cream of Kwaliti 5 cfu/ml followed by Igloo 2.33 cfu/ml and nil in the samples of Milk vita (Table 5). As per BSTI standard (Draft B.D.S. for ice cream 1983) coliform count should not exceed of 10/ml. The Coliform standards for ice cream should not over 10 cfu/ml (Frazier, 1958 and James, 1978). Therefore, this study demonstrates that the samples of Milk vita and Igloo did meet the recommended criteria of USPHS (1965). M-E-Elahi *et al.*, (2002) found coliform varied from 4 to 18 with an average 11.60 cfu/ml who recommended the findings of this experiment.

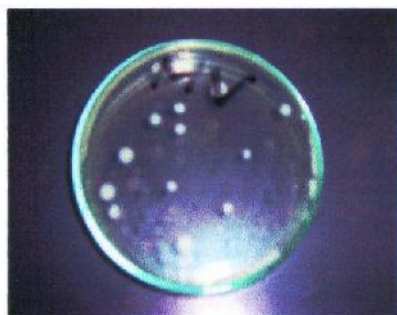


Plate1: Milk Vita ice cream



Plate2: Igloo ice cream

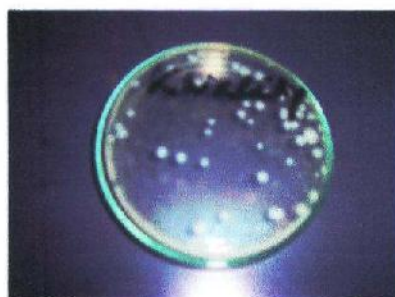


Plate3: Kwaliti ice cream

Fig 1: Total bacterial count of selected dairy ice cream



Plate1: Milk Vita ice cream



Plate1: Igloo ice cream



Plate1: Kwaliti ice cream

Fig 2: Coliform count of selected dairy ice cream

Table 5: Bacteriological status of selected ice cream samples

Ice cream brands	Average bacterial count cfu/ml	Average coliform count cfu/ml	Faecal coliform count cfu/ml
Milk Vita	2000	Nil	Nil
Igloo	2566.66	2.33	Nil
Kwality	5166.66	5	Nil

4.3.3 Identification of bacterial isolates

Standard tests were done for identification of coliform bacteria, such as, morphological (Appendix Table 11) and biochemical tests (Table 6). Coliform bacteria were then identified on the basis of the above mentioned tests according to Bergey's Manual of Determinative Bacteriology (Buchann and Gibbons, 1984) and Manual for the identification of Medical Bacteria (Cowan and Steel 1975). These findings support the results of Boey (1966), Humann and Weber (1978), Windrantz and Arias (2000) and Kruey *et al.*, (2001).

Table 6: Biochemical characteristics of isolated coliform (non-fecal) from selected ice cream samples

Ice cream brands	Indole	M.R	V.P	Citrate
Milk Vita	ND	ND	ND	ND
Igloo	—	—	+	+
Kwality	—	—	+	+

ND = not detection

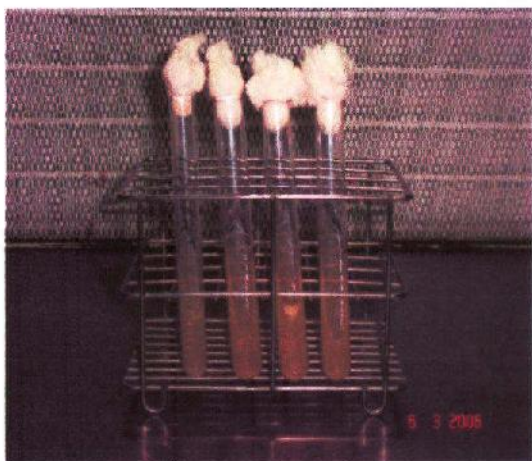


Fig 3: Preserve bacteria on Agar slant

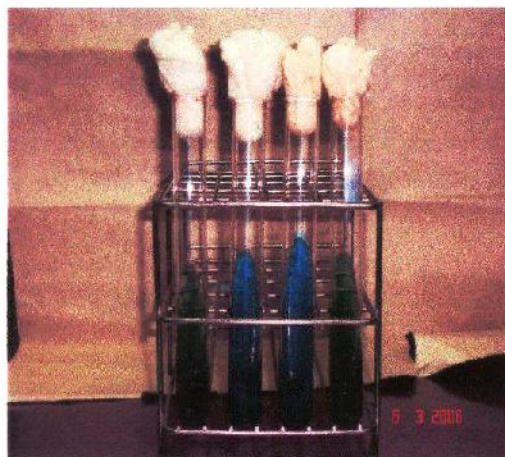


Fig 4: Citrate utilization test

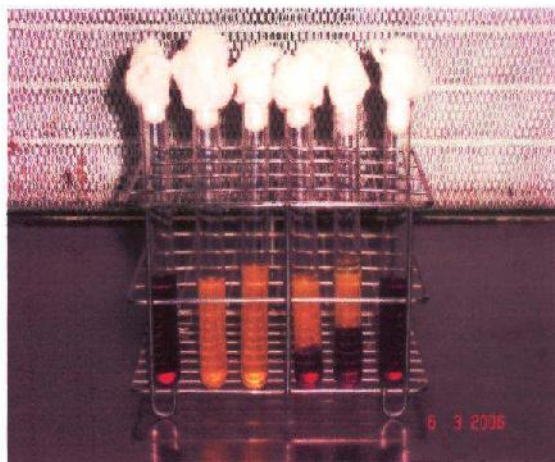


Fig 5: Methyl-Red test



Fig 6: Voges-proskaure (VP) test

Chapter Five
CONCLUSION

Conclusion

Ice cream is a very popular dairy product and due to increasing its popularity and increased demand the ice cream factory is increasing day by day. Organoleptic, chemical and microbial quality of ice cream is very important before the product being marketed to ensure the consumer that ice cream is desirable containing ingredient mix and free from contamination. The experiment results show that there was regarding physical parameters (specific gravity, flavor, body texture, melting quality and color) no significant differences between Milk Vita and Igloo. However, significant differences regarding specific gravity, body texture between Milk Vita and Kwality was observed. Regarding chemical tests, the total solid were 344.3 ± 0.3 , 297.5 ± 2.29 and 270.4 ± 0.3 g/kg respectively, fat were 101.1 ± 0.36 , 92.16 ± 0.76 and 81.0 ± 0.5 g/kg respectively, protein were 35.6 ± 0.47 , 34.9 ± 0.51 and 31.5 ± 0.5 g/kg respectively, ash were 10.22 ± 0.035 , 4.49 ± 0.18 and 7.95 ± 0.37 g/kg respectively and percent of acidity were 2.23 ± 0.057 , 1.93 ± 0.57 and 1.1 ± 0.1 g/kg respectively in Milk Vita, Igloo and Kwality ice cream respectively. Regarding total bacterial count in ice cream of Milk Vita, Igloo and Kwality varied from 1500 to 2500 with an average of 2000 cfu/ml, 2000 to 3000 with an average 2566.66 cfu/ml, and 4500 to 6000 with an average 5166.66 cfu/ml respectively. The coliform count varied from 2 to 3 with an average 2.33 cfu/ml and 4 to 6 with an average 5 cfu/ml in Igloo and Kwality respectively but in case of Milk Vita, it was found negative. It was observed that fecal coliform was negative in all selected brands of ice cream. The significant differences ($P < 0.01$) prevail pair selected ice cream dairies have significance difference but the parameter of organoleptic, chemical and microbial quality did not exceed the standard suggested by American Public Health Association (1967) and Bangladesh Standard Testing Institute (BSTI, 1983). Statistical analysis shows for both organoleptic and chemical cases Milk vita is highest and lowest Kwality. No coliform found in Milk Vita, Igloo but Kwality maintain coliform in standard level.

Source of inappropriate ingredients could be mixed and contamination may be due to adulterating, mishandling and lacking of proper care during processing, preparation, packaging or storage. Good Manufacturing Practices (GMP) and Good Handling Practices (GHP) contain basic requirements in any food safety program and it is the prerequisites of Hazard Analysis Critical Control Points (HACCP). The following steps should be taken to prevent or minimize.

- High quality ingredients like whole milk, cream powder milk sugar, emulsifier, flavoring agent etc must be used during ice cream manufacture.
- Use of hazard analysis and monitoring critical control points so as to suggest precaution in food handling, processing and preservation.
- Government should adopt effective rules and regulation to assure the consumers hygienic aspects served by the industry.
- To reduce contamination with microorganism to a minimum and obtain good keeping quality of the product, the raw material are to be examined; the equipment containing the food is to be adequately cleaned, sanitized and tested; the preserving processes is to be checked; packaging and storage are to be supervised.
- Gamma radiation source can be used for preservation of food after packaging because a major portion of microorganisms are killed in 10 kGy dose level except spore-forming bacteria without any hamper of nutrition value.

Chapter Six
REFERENCES

REFERENCES

- Abou-Donia, S., Ismail, A. A. and Abd-E-Rehim, N. Z. 1980. Chemical composition of ice cream in Alexandria city. J. of Dairy Sci., 63: 1, 37.
- Adam, R. 1978. Proposal for an evaluation scheme for organoleptic testing of ice cream. Lebensmittelwissenschaft und technologisch, 11 (3): 164-168.
- Aggarwala, A. C. and Sarma, P. C. (1960). A laboratory Manual of Milk Inspection. Bombay Calcutta, New Delhi, India. pp:87.
- Akyuz, N. and Andic, S. 1992. A study for determining the sensor, chemical and microbiological quality of ice cream of produced in Van. Yuzuncu-Yil-Universitesi-Ziraat-Fakultesi-Dergisi (Turkey). 2 (2): 13-28.
- Aleksieva, V. and Mirkov, M. 1983. Microbiological study of "Eskimo" ice cream. Veterinarnomeditsinski Nauki (Bulgaria), 20 (3-4): 80-85.
- American Public Health Association. 1953. Standard Methods for Examination of Dairy Products. 10th ed. APHA Inc. New York, pp.144-148.
- AOAC, 2003. Official method of analysis. Association of Official Agricultural Chemists. Washington, D.C., USA.
- Archambault, J. Caprioli, T. and McNad, A. G. 1965. Bacteriological quality of ice cream. Queb. lait, 24 (11): 8-10.
- B.D.S. 1983. Ice cream BSTI. (Draft B.D.S. for ice cream)
- Baylis, C. L., MacPhee, S. and Betts, R. P. 2000. Comparison of methods for the recovery and detection of low levels of injured *Salmonella* in ice cream and milk powder. Lett. Appl. Microbiol., 30(4): 320-324.
- Berlin, 1979. Ice cream quality requirements. German-Demonstratic Republic-Standard. TGL: 29126-01, pp: 8.
- Bester, B. H. 1977. Determination of coliform bacteria in dairy products. (Test procedure) South African Journal of Dairy Technology (1977) 9(4) 139-142 Cited from Dairy Sci, Abst. 40 (10): 1978.
- Bigalke, D. and chappel, R. 1984. Controlling coliform and other microbial contamination in ice cream. Dairy and Food Sanitation. 4: 318-319.
- Boese, W. Z. 1953. Bacteriological investigation of ice cream and its results. Hyg. Infektkr. 137 (3): 268-283.

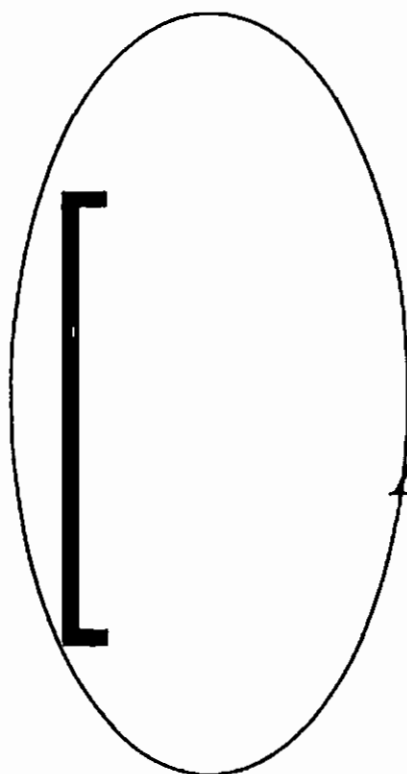
- Boey, B. 1966. Microbial Studies of Ice cream. *Vet. Med. Nauki, Sof*, 3(10): 1065-1072.
- Buchanan, R. E., and N. E. Gibson. 1984. *Bergey's Manual of determinative bacteriology*. (8th Ed.), The Williams and Wilkins Company, Baltimore.
- Campbell, I. J. and Pelan, B. M. C. 1998. The influence of emulsion stability on the properties of ice cream, in: Buchheim W. (Ed.), *Proceedings of the International Symposium on Ice Cream*, Special issue 9803, Int. Dairy Fed., Brussels, Belgium. pp: 25-36).
- Citerni, M. 1958. The bacteriological examination of ice cream in Acquila province. *Riv. Ital. Igiene*. 18 (1/2): 35-39.
- Cowan and Steel, 1975. *Manual for the Identification of Medical Bacteria* (6th Ed.) AVI publishing company, Inc., Westport, conn.
- Crowhurst, B. 1959. The bacteriological grading of ice cream lollics. *Ice Cr. Frog. Confect.* 11(5): 250-253. 1959 (Cited from Dairy Sci. Abst. 21:1959).
- Curiale, M. S., Gangar, V., Donorio, A., Gambrel-Lenarz. S. and McAllister, J. S. 1997. High-sensitivity dry rehydratable film method for enumeration of coliform in dairy products: collaborative study. *J. AOAC*. 80(3): 505-516.
- Dickinson, E., and Parkinson, E. L. 2004. Heat induced aggregation of milk protein stabilized emulsions: sensitivity to processing and composition, *Int. Dairy J.* 14: 635-645.
- Erol, L., Kuplulu, O., Siriken, B. and Celic, T. H. 1998. Determination of microbiological quality of ice cream from various patisseries in Ankara. *Turk Veterinerlik-ve-Hayvancilik Dergisi*. 22(4): 345-352.
- Euston S. R. and Hirst R. L. (2000). The emulsifying properties of commercial milk protein products in simple oil in water emulsions and in a model food system, *J. Food Sci.* 65: 934-940.
- Feldsine, P. T., Falbo-Nelson, M. T., Brunelle, S. L. and Forgey, R. L. 1997. Visual immunoprecipitate assay (VIP) for detection of enterohemorrhagic *Escheerichia coli* (EHEC) 0157:H7 in selected foods: collaborative study. *J. AOAC. Int.*, 80(3): 517-529.
- Food and environmental hygiene department, microbiological risk assessment of Ice-cream. 2001. Risk assessment studies report no. 7, Hong Kong.
- Frazier, W. C. and Westhoff, D. C. 1958. *Pasteurized milk in milk ordinance code*. USPHS. Food microbiology. 1st ed., Mc Grawhill Inc., New Delhi, pp: 112.

- Fuhr, R. 1986. Bacteriological quality of different types of ice cream and milk shake during 1980-1985. In; arbeitsstagung des Arbeitsgebietes " lebensmit-telhygiene" Leitthema: Forschung und praxis der Lebensmittelhygiene" 9(12): 257-261.
- Goff, H. D. 1997. Colloidal aspects of ice cream. A review, Int. Dairy J. 7: 363-373.
- Goss (1953). The quality of ice cream and it importance in human nutrition. J. Dairy Sci. 83: 31-32.
- Graceleah O. Orallo, Aileneli H. Pangan, Esperanza C. Cabrera, Ph.D. 1999. Microbial Analysis of Ice Cream Produced by Big-Scale and Small-Scale Manufacturers in Metro Manila. Phil J Microbiol Infect Dis. 28(3):99-101.
- Guinard, J. X., Zovmas-Mors, C. and Panyam, D. 1995. Effects of butter and sugar on the sensory properties and acceptability of ice cream. IFA Annual methyl, 231.
- Hamann, R. and Weber, H. 1978. Quantitative and Qualitative detection of Enterobacteriaceae in ice cream. Comparison of different test systems, Archiv, fur Lebensmittenthgiene. 29 (1): 5-16.
- Hankin, L. and Hanna, J. G. 1984. Quality of ice cream and ice milk. Bulletin Contecticuit Agricultural Experiment Station, No.818.p: 6.
- Heinz, H. and J.G.O. 1958. Nutrient content of vanilla ice cream. Nutritional data .3rd Ed.2nd revised printing.
- Irujo, J. M. and Liona, J. I. 1963. Hygienic conditions of the manufacture and sale of ice cream in Bibao. Rev. esp. Leech. 48: 87-94.
- James, M. and Jay, J. M. 1978. Indices of Food Sanitary quality and microbiological standards and criteria, modern Food Microbiology, 2nd ed., p: 311.
- Josephson, D. V. 1963. Same observation on body and texture of ice cream Int. Rev Sugar and cofect 16: 360-364.
- Joshi, D. R., Shah P. K., Manandhar, S., Sharma, S. and Banmali, P. (2004). Microbial Quality of Ice Cream Sold in Kathmandu. Journal of Nepal Health Research Council Vol. 2 No. pp: 37-40.
- Keller, J. J., Steinmann, M. A and Wentzel, B. S. 1987. The quality of South African ice cream. Suid Afrikaanse Tydskrif virSuiwelkunde, 19:145-147.
- Khan, A. K. 1983. Studies on the bacteriological quality of ice cream produced under Bangladesh condition. M. S. Thesis, department of Dairy Science, BAU, Mymensingh.

- Kitu, S. 2004. Comparative analysis of ice cream of different dairies available in Bangladesh. Thesis, Biotechnology and Genetic Engineering Discipline, Khulna University. pp: 31-35.
- Kivance, K. Yamac, M. and Kunduhoglu, B. 1994. The microbiological analysis of ice cream marketed in Eskisehir. *Gida*, 19(5): 317-322.
- Kokubo, S., Sakurai K., Hakamata, K., Tomita, M., and Yoshida, S. 1996. The effect of manufacturing conditions on the de-emulsification of fat globules in ice cream, *Milchwissenschaft* 51: 262-265
- Koxholt, M. M. R., Eisenmann, B. and Hinrichs, J. 2001. Effect of the fat globule sizes on the meltdown of ice cream. *J. Dairy Sci.* 84: 31-37.
- Kruy, S. L., Soares, J. L., Ping, S. and Sainte Marie, F. F. 2001. Microbiological quality of ice cream (sorbet) sold on the street of Phnom Penh. *Wlletin de la Societe de pathologic Exotique*. 94:5, 411-414.
- Kumar, C. N., Khan, M. M. H., Thangavel, K., Ernest, J., Ramakrishnan, M. and Domodhiran, L. 1989. Bacteriological quality of market ice cream from various sources. *Cherion*. 18(2): 67-70.
- Little, C. L., Louvois, J. and de-Louvois, J. 1999. Microbiological quality of soft ice cream from fixed premises and mobile vendors. *International journal of Environmental Hcalth I,aboratorv Services (CDSC)*, 61 Colindale Avenues, London, UK.
- Loewenstein, M. and Haddad, G. S. 1972. High temperature short time and ultra high temperature pasteurization of ice cream, *Amer. Dairy Rev.* 34: 82, 84, 90, 92, 117, 119.
- Lucas, P.S. 1929. Vanilla won't Freeze out ice cream. *Trade J.* 25(8):48.
- Maifreni, M., Civilini, M., Domenis, C., Manzano, M., Di Prima, R. and Comi, G. 1993. Microbiological quality of artisanal ice cream. *Zentralbl. Hyg. Umweltmed.* 194(5-6): 553-570.
- Mann, E.J.N.D.D. 1971. Ice cream Tech. *Dairy Ind.* 36:294-295.
- Marcy, G. and Adam, W. 1979. Decrease of *Salmonella typhimurium*, *Escherichia coli* and *Enterobacter cloacae* in ice cream during long storage at 28°C *Archiv for Lebensmittelhygiene*, 30(2): 70-72.
- Marino, V. 1954. Maximum limits of bacterial count in ice cream. *Ann. Sanita Pub.* 13(4): 1075-82.

- Marshall R. T. and Arbuckle W. S. 1996. Ice Cream, 5th edn., Chapman & Hall, New York, USA,
- Masek, J. and Maja Svata. 1964. Study of Methods for the estimation of coliform bacteria. *Prumysl potravín*. 15(9): 466-469; 1964 (Cited from Dairy Sci. Abst. 26:1964.
- M-E-Elahi, A. T. M., Habib, S., Rahman, M. M., Rahman, G. I. and Bhuiyan, M. J. U. 2002. Sanitary Quality of Commercially Produced Ice Cream Sold in the Retail Stores. *Pakistan Journal of Nutrition* 1(2): 93-94.
- Moiseeva, J. and Bukanova, H. 1978. Changes in the total count and coliform content of ice cream during storage *Maelkeritidende*. 79(7): 129-136.
- Nutting, L. A., Lomot, P. C. and Barber, F. W. 1959. Estimation of coliform bacteria in ice cream by use of the membrane filter. *Appl Microbiol*. 7(4): 196-199.
- Ortin, L. and Sanchez, A. T. 1990. Mean composition of milk production, V. Ice cream. *Alimentaria*, 27 (217):21-23.
- Overby, A. J. 1960. Factors influencing determination of the bacterial content of ice cream. *Maelkeritidene*. 73(14) 271-277: (15) 293-300): 1960).
- Parijez, S. 1975. The quality of ice cream and its importance in human nutrition. *Hrana-i-Ishrana*, 16(7): 353-357.
- Rossi, C. 1990. Bacteriological quality of soft ice cream. *Environmental Health*, 98:161-163.
- Rothwell, J. 1995. Report premium or luxury dairy ice cream. *Ice Cream*, 46(4):146.
- Rothwell, J. and Palmer, M. N. 1965. Modern trends in ice cream stabilizers, *Dairy Ind.* 30:107-108 and 118.
- Sebastian, J. Unnikrishnan, V. and Rao, M. B. 1975. Chemical quality of market ice cream. *Journal of Food Science and Technology*. India. 12: 202
- Seidel, G. (1969). Determination of coliform bacteria in ice cream with particular reference to use of TTC agar. *Nahrung* 13(5) 427-429 (Cited from Dairy sci. Abst. 32:1970).
- Steel, R. G. D. and Torrie, T. A. 1960. Principles and procedures of statistics. McGraw-Hill, New York. pp: 481.
- Tamieri, C. L. and Roche, J. G. 1967. Microbiological control of ice cream. *Revue Italy. institute Pasteur delyom*, 10(4): 363-373.

- Udabage P. and Augustin M. A. 2003. Dairy ingredients for ice cream, *Aust. J. Dairy Sci. Technol.* 58: 21–25.
- Udabage P., Augustin M. A., Cheng, L. J. and Williams, R. P. W. 2005: Physical behaviour of dairy ingredients during ice cream processing. *EDP Sciences. Lait* 85; 383–394
- USPHS, 1965. Standard method for examination of milk and dairy product.
- Widell, S. 1957. Bacteriological ice cream control. *Varfoda.* 9(8): 33-36.
- Windrantz, P. and Arias, M. L. 2000. Evaluation of the bacteriological quality of ice cream sold at San Jose, Costa Rica. *Arch. Latinoam. Nutr.* 50(3): 301-3.



APPENDICES



APPENDICES

Appendix A: Score card for judging of ice cream

Judge No. _____ Group No. _____

Grading Score	Point
Excellent	5
Very good	4
Good	3
Poor	2
Very poor	1

Please asses the products for the following characteristic (5-1) point.

Sample Name	Flavour	Body & Texture	Color & Package	Melting quality	Total Judging point	Comments
Milk Vita						
Igloo						
Kwality						

Name of the judge:

Address:

Appendix Table 1: Paired Samples test for specific gravity of selected ice cream

	Paired Differences					
	Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference		t
				Lower	Upper	
Pair 1 Milk Vita - Igloo	.008667	.005508	.003180	-.022892	.040226	2.726
Pair 2 Milk Vita - Kwaliti	.08267	.00379	.00219	.06097	.10436	37.820
Pair 3 Igloo - Kwaliti	.074000	.001732	.001000	.064075	.083925	74.000
						df
						2
						2
						2
						Sig.(2-tailed)
						.112
						.001
						.000

Appendix Table 2: Paired Samples test for flavor of selected ice cream

	Paired Differences					
	Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference		t
				Lower	Upper	
Pair 1 Milk Vita - Igloo	-.600	1.140	.510	-2.948	1.748	-1.177
Pair 2 Milk Vita - Kwaliti	1.200	.837	.374	-.523	2.923	3.207
Pair 3 Igloo - Kwaliti	1.800	1.643	.735	-1.583	5.183	2.449
						df
						4
						4
						4
						Sig. (2-tailed)
						.305
						.033
						.070

Appendix Table 3: Paired Samples test for body and texture of selected ice cream

	Paired Differences					
	Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference		t
				Lower	Upper	
Pair 1 Milk Vita - Igloo	.600	.548	.245	-.528	1.728	2.449
Pair 2 Milk Vita - Kwaliti	1.800	.447	.200	.879	2.721	9.000
Pair 3 Igloo - Kwaliti	1.200	.837	.374	-.523	2.923	3.207
						df
						4
						4
						4
						Sig. (2-tailed)
						.070
						.001
						.033

Appendix Table 4: Paired Samples test for color and package of selected ice cream

	Paired Differences					
	Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference		t
				Lower	Upper	
Pair 1 Milk Vita - Igloo	-.400	.894	.400	-2.242	1.442	-1.000
Pair 2 Milk Vita - Kwaliti	-.200	1.304	.583	-2.885	2.485	-.343
Pair 3 Igloo - Kwaliti	.200	1.304	.583	-2.485	2.885	.343
						df
						4
						4
						4
						Sig. (2-tailed)
						.374
						.749
						.749

Appendix Table 5: Paired Samples test for melting quality of selected ice cream

	Paired Differences					
	Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference		t
				Lower	Upper	
Pair 1 Milk Vita - Igloo	.800	1.304	.583	-1.885	3.485	1.372
Pair 3 Igloo - Kwaliti	1.200	1.304	.583	-1.485	3.885	2.058
						df
						4
						4
						Sig. (2-tailed)
						.242
						.109

N.B: At pair 2, the correlation and t can not be computed because the standard error of the difference is zero.

Appendix Table 6: Paired Samples test for overall score of selected ice cream

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 Milk Vita - Igloo	.200	1.095	.490	-2.056	2.456	.408	4	.704
Pair 2 Milk Vita - Kwaliti	4.600	.548	.245	3.472	5.728	18.779	4	.000
Pair 3 Igloo - Kwaliti	4.400	1.517	.678	1.277	7.523	6.487	4	.003

Appendix Table 7: Paired Samples test for total solid of selected ice cream

	Paired Differences						t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference					
				Lower	Upper				
Pair 1 Milk Vita - Igloo	4.6733	.2159	.1247	3.4359	5.9107	37.483	2	.001	
Pair 2 Milk Vita - Kwaliti	7.38000	.04583	.02646	7.11741	7.64259	278.938	2	.000	
Pair 3 Igloo - Kwaliti	2.70667	.20984	.12115	1.50425	3.90908	22.341	2	.002	

Appendix Table 8: Paired Samples test for fat of selected ice cream

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1	Milk Vita - Igloo	.89333	.10066	.05812	.31651	1.47015	2	.004
Pair 2	Milk Vita - Kwaliti	2.01000	.08544	.04933	1.52042	2.49958	2	.001
Pair 3	Igloo - Kwaliti	1.11667	.07638	.04410	.67902	1.55431	2	.002

Appendix Table 9: Paired Samples test for fat of selected ice cream

	Paired Differences					
	Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference		t
				Lower	Upper	
Pair 1 Milk Vita - Igloo	.07000	.02646	.01528	-.08160	.22160	4.583
Pair 2 Milk Vita - Kwality	.41333	.03512	.02028	.21210	.61457	20.385
Pair 3 Igloo - Kwality	.34333	.06028	.03480	-.00206	.68873	9.866
						df
						2
						2
						2
						Sig. (2-tailed)
						.044
						.002
						.010

Appendix Table 11: Paired Samples test for ash of selected ice cream

	Paired Differences					
	Mean	Std. Deviation	Std. Error Mean	99% Confidence Interval of the Difference		t
				Lower	Upper	
Pair 1 Milk Vita - Igloo	.572667	.015044	.008686	.486461	.658873	65.931
Pair 2 Milk Vita - Kwality	.226667	.035105	.020268	.025513	.427820	11.184
Pair 3 Igloo - Kwality	-.346000	.020421	.011790	-.463012	-.228988	-29.347
						df
						2
						2
						2
						Sig. (2-tailed)
						.000
						.008
						.001