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Evaluation of the Microbioical Quality of Flavoured Ice Cream in Khartoum State

تقويم الجودة الميكروبيولوجية للآيسكريم المنكه بولاية الخرطوم

By

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DEDICATION

To my mother

To my sisters

To my all teachers

To all whom I respect and appreciate.

To all people of this country

Omer Hassan

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Thanks and gratefulness firstly and lastly to “ALLAH” who gave me health and patience to carry out this study successfully.

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ABSTRACT

The microbial quality of mango and chocolate flavoured ice cream samples were investigated. The samples (factory and homemade flavoured ice cream) were collected from three regions namely Khartoum, Khartoum North and Omdurman. The samples were collected randomly. The mean (cfu/ g) value of total bacterial counts, *Staphylococcus*, moulds and yeasts, total colifrom and spore forming bacteria (SFB) of chocolate and mango flavoured ice cream were in the range of 3.0×10^3 to 8.9×10^3, 0.0 to 2.1×10^2, 0.0 to 5.2, 1.4×10 to 2.5×10 and 0.0 to 3.0 cfu/g, respectively. The highest total bacterial count observed in homemade mango flavoured ice cream produced in Khartoum North area (8.9×10^3 cfu/g) and the lowest count was observed in homemade chocolate flavoured ice cream produced in Khartoum (3.0×10^3 cfu/g). On the other hand homemade ice cream produced in Omdurman obtained the highest *Staphylococci* count (2.1×10 cfu/g), while factory chocolate flavoured ice cream produced in Khartoum North and homemade chocolate flavoured ice cream produced in Khartoum were nil. The highest count of yeasts and moulds were obtained by mango flavoured homemade ice cream produced in Khartoum (8.2×10 cfu/g), while mango and factory chocolate flavoured ice cream produced in Khartoum and Omdurman were nil. The total coliform count was highest in factory chocolate flavoured ice cream and the lowest count obtained by homemade ice cream. The tested samples showed no counts for spore forming bacteria except chocolate and factory mango flavoured ice cream produced in Khartoum and homemade chocolate favored ice cream produced in Khartoum.

E. coli were observed in all tested samples, while *salmonella* were observed in mango and chocolate flavoured ice cream in Omdurman area. *Pseudomonas* bacteria were absent in all tested samples.

From this study, it is concluded that mango flavoured ice cream had a significantly higher frequency of bacterial counts compared with chocolate flavoured ice cream products. Homemade mango flavoured ice cream had a significantly higher frequency of bacterial counts compared with factory mango flavoured ice cream products.

The current investigation has indicated a poor overall level of hygiene in the service of chocolate and mango flavoured ice cream in the Khartoum State of Sudan.

الملـخـص

لقد تمت الدراسة على الجودة الميكروبية للآيسكريم والمنكهة بالشيكولاتة والمانجو. تم جمع العينات من أحد المصانع ومن المنازل ومن ثلاث مناطق هي: **الخرطوم، الخرطوم بحري وأم درمان.**

كان متوسط القيم (cfu/g) للعدد الكلي للبكتريا، والبكتريا العنقودية، والأعفان والخمائر، وباكتريا القولون والبكتريا المكونة **للأبواغ** لأنواع الآيسكريم المنكهة في عينات بالشيكولاتة والمانجو يتراوح ما بين (10^3×3.0 -10^3×8.9 ،10^2×2.1-0.0 ،5.2-0.0 ،2.5×10-1.4×10 ،3.0-0.0 cfu/g) **على التوالي.** أعلى قيمة للعدد الكلي للبكتريا لوحظ في آيسكريم المنازل المنكه بالمانجو في منطقة **الخرطوم بحري** (10^3×8.9 cfu/g)، وأقل أعداد للبكتريا الكلية كان في منطقة الخرطوم في آيسكريم المنازل المنكه بالشيكولاتة (10^3×3.0 cfu/g).

من ناحية أخرى فإن الآيسكريم المنتج بالمنازل بأمدرمان أعطى أعلى عدد من البكتريا العنقودية في حين أن الآيسكريم المصنع المنكه بالشيكولاتة بمنطقة الخرطوم بحري، وكذلك آيسكريم المنازل المنكه بالشيكولاتة **بمنطقة الخرطوم لم تحتوي** على البكتريا العنقودية (10×2.1 cfu/g).. **أعلى رقم للخمائر** والأعفان كان في عينات المنازل المنكه بالمانجو بمنطقة **الخرطوم** (10×8.2 cfu/g). عينات المصنع المنكهة بالمانجو والشوكولاتة بمنطقتي الخرطوم وأم درمان لم يوجد بهما خمائر وأعفان. بكتريا القولون كانت عالية في عينات المصانع المنكهة بالشيكولاتة في حين أن عينات المنازل سجلت **أعداداً أقل.**

تم ملاحظة بعض الأعداد من البكتريا المكونة للأبواغ في عينات المصنع المنكهة بالشيكولاتة والمانجو بمنطقة الخرطوم وفي عينات المنازل المنكهة بالشيكولاتة بمنطقة الخرطوم كذلك. الباكتريا البرازية كانت حاضرة في كل العينات. أما باكتريا السالمونلا تمت ملاحظتها في العينات المنكهة بالشيكولاتة **والمانجو بمنطقة أم درمان.** أما الباكتريا الزائفة فلم توجد في كل العينات.

من هذه الدراسة يمكن أن نخلص إلى أن عينات الآيسكريم المنكهة بالمانجو هي الأكثر احتواءً على الباكتريا مقارنةً بعينات الشيكولاتة. كما أن عينات المنازل المنكهة بالمانجو ذات أعداد باكتيرية أعلى **مقارنةً** بمثيلتها في المصانع.

خلصت هذه الدراسة إلى مستوى صحي متدني في **إنتاج** الآيسكريم المنكه بالشيكولاتة والمانجو بولاية **الخرطوم بالسودان.**

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CHAPTER ONE

INTRODUCTION

Ice cream is a nutritionally enriched congealed dairy product consumed by all age groups particularly children, during summer (Sharif *et al*., 2005). The ingredients of ice cream may be various combinations of milk, cream, evaporated or condensed milk, dried milk, colaring material, flavours, fruits, nuts, sweetening agents, eggs, eggs products and stabilizer. Any of these may account for the various specific species of bacteria (Yaman *et al*., 2006) such as *Salmonella* spp., *Listeria* spp., *Yersinia* spp., *Staphylococcus aureus*, *Escherichia coli*, coliforms, *Bacillus* spp. In many countries ice cream is a nutritious food for human and also an excellent medium for the growth of many microorganisms some of which may cause diseases in human beings such as cholera, typhoid and bacillary dysentery.

Contaminated ice cream causes several outbreaks of gastrointestinal diseases in a number of countries in Asia, Europe and North America (Dijuretic *et al*., 1997). In England and Wales two outbreaks of *S. enteritidis* phage type 4 infections were reported in 1990 and 1995due to consumption of ice cream (Hennessy *et al*., 1972).

Quality of ice cream depends on extrinsic factors that include manufacture procedure, as well as intrinsic factors that include the proportion of ingredients used. Primary sources of microbial contamination of ice cream include water and raw milk, whereas secondary sources include flavouring agents, utensils and handling. Although freezing and hardening steps in production can estimate most of the microbial hazards, but still numerous health hazards are persistent due to various conditions (Ahmad and Hussein, 2008).

The objective of this study was to investigate the microbioical quality of mango and chocolate flavoured ice cream produced in Khartoum State.

CHAPTER TWO

LITERATURE REVIEW

2.1 Composition of ice cream

The ingredients of ice creams usually consist of cream, the source of fat, concentrated nonfat milk solids, sugar, stabilizer and emulsifier, and flavouring materials (Mehu, 1993). Most of these ingredients in ice cream are multifunctional, contributing to different aspects of ice cream manufacture, product quality and stability.

2.1.1 Nonfat milk solids

The nonfat portion of milk, called nonfat milk solids (NMS), is composed of approximately 55% lactose, 37% protein, 8% minerals and others, such as vitamins, acids and enzymes (Arbuckle, 1986). The amount of NMS in ice cream mixes ranges from 9-12% and usually varies inversely with the fat content. NMS are critical for texture and body of ice cream (Hegenbart, 1996). According to U.S. standards of ice cream, most milk and non-fermented milk products are permitted in ice cream, but there are some specific limits in some ingredients. For example, amounts of whey solids or modified whey solids are limited to 25% of the NMS content. There are several reasons for the limitations, including: the higher lactose content in whey solids increases the potential of crystallization of the sugar the high concentrations of lactose and minerals result in lowered freezing point, protein content is decreased in ice cream (Marshall and Goff 2003).

NMS can come from several sources, such as skimmed milk, condensed whole milk and buttermilk. Buttermilk solids can be a superior substitute for NMS, especially in mixes made with butter, butter oil or anhydrous milk fat as the source of fat. Buttermilk contains a higher concentration of fat globule membrane phospholipids than does skim milk (Marshall and Goff 2003).

The added amount of NMS to an ice cream mix is a primary factor for improving the quality of ice cream (Campbell and Marshall 1975). Overall, NMS can lower the freezing point, slow the rate of melting and increase the amount of unfrozen material by adding dissolved serum solids (Mangino 1984).

Stampanoni Koeferli *et al*., (1996) also indicated NMS can cause a decrease in coldness, ice crystal and melting rate perception and an increase in creaminess and mouth coating. In addition, it also can increase overrun attainable without producing poor textures and strengthen resistance to heat shock and shrinkage (Goff, 1995). Lactose in NMS causes a lower freezing point, and milk protein provides a slight flavour, water holding and also affects the formation of size of air cells in ice cream (Campbell and Marshall, 1975). Nevertheless, high concentrations of NMS could cause some side effects in the quality of ice cream, such as cooked or condensed flavours and sandiness. Sandiness is particularly produced by high amounts of lactose which crystallizes in ice cream. The crystals dissolve slowly during eating and give a gritty mouth feel (Goff, 1995).

2.1.2 Milk fat

Milk fat, a complex arrangement of triglycerides, is a primary determinant of the texture or body of ice cream, which forms the structured network. Milk fat, containing at least 10% and no more than 20%, is an ingredient of major importance in ice cream because its correct use is vital not only to balance properly the mix but also to satisfy legal standards (Marshall 1998). The effect of milk fat in ice cream is dependent on several factors including the type and level of fat, the amount of non-fat milk solids and sugar, as well as variety of flavour compounds used in ice cream (Marshall and Arbuckle, 1996).

Milk fat comes from milk and cream, the best source, and provides the desirable flavours and aids in good melting properties and in decreasing the

size of ice crystals (Hyde, 2001). Furthermore, milk fat also affects textural attributes such as viscosity, tenderness, elasticity, emulsification, ice crystallization and other desirable attributes such as richness, smoothness (Marshall and Arbuckle, 1996; Hyde, 2001). The possible mechanism of milk fat contributing necessary structure and texture of ice cream is due to trapped water, creating an oil-in-water emulsion with the interaction of stabilizers and emulsifier during whipping (Hyde, 2001).

During the freezing process, milk fat globules concentrate towards the surface of air cells to support the air cell structure as air is incorporated into the mix and are surrounded by casein subunits absorbed at the fat-water interface (Goff, 1995; Koxholt *et al*., 2001; Marshall and Goff, 2003). Milk fat coalesces partially during freezing to form and stabilize the ice cream structure and to give body to the ice cream (Goff, 1995).

Stampanoni *et al*, (1996) showed that within the 3% to 12% range, fat increased primarily buttery, creamy, and mouth coating characteristics of ice cream and lowered its melting rate, coldness and ice crystal perception, whereas sugar increased sweetness, caramel and vanillin notes and lowered milky flavour. Excessive milk fat decreases whipping ability and results in excessive richness as well as high caloric value (Goff, 1995).

2.1.3 Sweeteners

Sweetness control in ice cream is very important in order to achieve maximum consumer acceptance and minimum production cost (Wilson-Walker, 1982). Many kinds of sweeteners, such as cane and beet sugars, many types of corn sweeteners, maple syrup, honey, inverted sugar, fructose and malt syrup, have been used in ice cream mixes (Stogo, 2001; Salama, 2004). Ice cream is formulated normally with a sweetness equivalent to 13%-15% sucrose content, with 25% or more of the sweetness provided by corn-based sweeteners (Schaller-Povolny and Smith, 1999). Sucrose, corn syrup

and high fructose corn syrup are principal sweeteners used in ice cream (Bodyfelt *et al.*,1988; Salama, 2004).

The amount of sweeteners added to ice cream is very important. Besides creating desirable flavour properties, sweeteners also are the major ingredients to lower the freezing point, which is one of the influential factors for quality of the ice cream mixes (Baer and Baldwin, 1984). When sweetener content is about 16% or higher, ice cream tends to become too soft or too dense and chewy, depending on the type of sweeteners used in excess, due to lowering the freezing point. In other words, hardness of ice cream is dependent upon the sugars and other materials (Baer and Baldwin 1984; Grotta, 1994).

If a mix with a lower freezing point causes less water to be frozen as the ice cream exits the freezer, the storage life of ice cream is shortened due to being more susceptible to increases in ice crystal size during temperature fluctuations (Schaller-Povolny and Smith, 1999). Furthermore, as sweetener concentration is increased, viscosity of the mix and firmness of the ice cream increased.

Monosaccharides, such as fructose and glucose, equally lower the freezing point of the mix, although they do it to a greater degree than disaccharides. High molecular weight sugars, such as corn syrup, do not depress the freezing point as much as disaccharides on a weight to weight basis (Bodyfelt *et al.*, 1988).

Sweeteners and various flavourings are added to dairy products to improve flavour balance and to partially mask acetaldehyde flavour (Kagan, 1985). In general, approximately 45% of the sucrose can be replaced with corn syrup for economic, handling or storage reasons (Clark, 1994). However, corn syrup with low to medium dextrose equivalents (DE) may impart off-flavours and should constitute no more than about one-third of the total sweetener solids. In fact, low DE sweeteners, made by hydrolysis of starches,

do not contribute much sweetness, but they provide great water binding properties (Goff, 1995).

The percentage of the sweetening agents obtained from different sources added to the ice cream mix is influenced mainly by the desired concentration of sugar in the mix total solids content of the mix effect on the properties of the mix concentration of sweeteners other than sugar and relative inherent sweetening power of the sweeteners other than sucrose (Marshall *et a.,l* 2003).

2.1.4 Emulsifiers

Mono-, diacylglycerides and sorbitan esters, especially polysorbate 80, are usually used in ice cream as the emulsifiers (Marshall *et al*., 2003), and they are supplied to manufacturers as blends with stabilizers. The normal requirements in ice cream ingredients for emulsifiers are between 0.1%- 0.5 % (Clark, 1994; Marshall *et al*., 2003). Emulsifiers added to ice cream have several important functions, including that they lower the fat and water interfacial tension in the ice cream mix so that they cause protein displacement from the fat globule surface, which in turn reduces the stability of the fat globule causing it to partial coalesce during the whipping and freezing process promote nucleation of fat during aging of mixes, thus reducing the time needed to age mixes before freezing (Goff, 1995) decrease tendencies for shrinkage and lower the rate of melting (Bodyfelt *et al*.,1988) cause a high amount of agglomeration of the fat during freezing. Therefore, the surfaces of the fat globules must have a relatively high concentration of emulsifier (Goff, 1995).

In sum, emulsifiers contribute greatly to texture and meltdown properties. They aid in developing the appropriate fat structure and air distribution in the ice cream and provide smoothness and a stiffer body, enabling production of smaller and more evenly distributed air cells, increasing resistance to melting and shrinkage, and improving dryness (Clark, 1994).

2.1.5 Stabilizers

The stabilizer usually is a kind of polysaccharides such as gelatins, gums, egg yolk solids and seaweed (Bodyfelt *et al*., 1988). The amount used in regular ice creams may range from 0-0.5% (Clark, 1994). The stabilizer contributes several functions in ice cream: It can increase the viscosity of products decreasing water migration, maintain homogeneity and control ice crystal growth during the freezing process (Hagiwara and Hartel, 1996). Stabilizers in ice cream cause a slight increase in melting rate (Arbuckle, 1986). During storage, stabilizers play a role in resisting structural changes during "heat shock", which is the inevitable temperature-cycling during storage that creates ice crystal growth and other types of deterioration due to structural changes (Goff, 1995). During consumption, stabilizers provide uniform meltdown, mouthfeel and texture. Stabilizer can contribute to a smoother more resistant body and texture (Arbuckle, 1986). However, too much stabilizer can make the mix too viscose, making the ice cream heavy and soggy. In short, stabilizer added to ice cream is to control ice crystal growth during hardening and storage, especially during temperature fluctuations, to give body and stiffness during freezing for air Corporation and to impart smoothness in body and texture (Arbukle 1986; Marshall *et al*.,2003). The sensory quality and overall acceptability of ice cream with various stabilizers has been reported (Minhas *et al*., 1997).

2.1.6 Flavourings

Flavour is the most important positive attribute of ice cream. Vanilla, chocolate and strawberry flavour are among the most preferred ice cream products (Marshall *et al.*, 2003). The type and intensity of flavour in ice cream are the two important flavour characteristics. Flavours should only be intense enough to be recognized easily and to present a delicate pleasing taste. Too much or too little flavouring, unnatural or atypical flavouring, and

too much or too little sweetness could cause important defects in ice cream (Marshall *et al*., 2003).

Some factors, including mix composition, ingredient quality, process variables, freezing and storage conditions, age of product, temperature of consumption, pH, fat content, sweetness level, stabilizers, incorporation of air and flavourings added, affect the overall flavouring of ice cream (Marshall, 1998). In addition, temperature, hydration, surface area, enzyme saliva and binding phase inversion also affect flavour release during eating (Marshall and Arbuckle,1996).

The procedure for adding flavourings to ice cream usually depends on the type of flavouring used, such as liquid, syrup, semisolid, and solid forms, and the type of freezer.

Other than liquid forms, flavourings are added to the batch freezer prior to completion of the freezing process in most situations. Liquid flavourings are added to ice cream mixes prior to freezing. In addition, the performance and type of the freezer also is a critical factor in the use of the mix composition and flavour. For example, ice cream mixes with added chocolate flavour, whip more slowly than mixes of most other flavours (Marshall and Goff, 2003). The amount of flavouring added to ice creams needs to be high enough to ensure good impact at the low temperatures of consumption and also to be balanced with sweetness (Marshall *et al*., 2003). The typical consumption temperature of ice cream is between -20C and -5C. The lower the temperature, the lower the flavour impact perceived.

2.2 Manufacturing of ice cream

The basic steps in the manufacturing of ice cream generally consist of two distinct stages: mix manufacture and freezing operations. Mix manufacture consists of combining and blending of ingredients, batch and continuous pasteurization, homogenization and mix aging. Freezing operations usually

include a two-step process: under high shear and under quiescent conditions (Caldwell *et al*., 1992; Marshall and Goff, 2003).

2.2.1 Pasteurization

The main purpose of pasteurization, the bioical control point, in ice cream is to destroy the pathogenic bacteria. In addition, it also helps hydrate proteins and stabilizers of the ice cream mix. Both batch pasteurization and the continuous high temperature short time (HTST) method are generally used in the manufacture of ice cream (Goff, 1995). Batch pasteurization may be accomplished by heating either to 65.5 C for not less than 30 minutes or to 71.1 C for not less than 10 minutes, which is the legal minimum for ice cream in a batch pasteurization system (Hyde, 2001). The batch method is more suited to the smaller manufacturers, who will mix their ice cream and pasteurize it in the same vessel. One advantage of batch pasteurization is that is produces a better mouth feels of the ice cream, due to more of the whey proteins being denatured. HTST is more fitting for larger manufacturers who use a plate heat exchanger, which heat-treats a continuous stream of product. The pasteurization temperature for ice cream is higher than that for milk because the sugar added to ice creams coats the bacterial cells and helps to protect them (Goff, 1995; Marshall and Goff ,2003; Beattie, 2004).

2.2.2 Homogenization

Homogenization causes ice creams to have a stable, uniform, smooth texture and be whipped more easily during the freezing process, although not all ice cream manufacturers use a homogenizer in the production process line (Goff, 1995; Marshall *et al*., 2003).

Homogenization of ice cream mixes causes the formation of a fat emulsion by breaking down or reducing the size of the fat globules found in milk or cream to less than 1 μm (Berger, 1990). It provides some functions in ice cream manufacture, including size reduction of fat globules increasing surface area membrane formation to make a smoother ice cream creating

greater apparent richness and palatability better whipping ability decreasing the danger of churning the fat enabling the use of butter, frozen cream, etc., and increasing resistance to melting (Goff, 1995; Marshall *et al*., 2003). The mix should be homogenized in a two-step homogenizer with the pressure of the first stage between 2000-2500 pounds and between 500-1000 pounds for the second stage (Goff, 1995). The higher the fat and total solids in the mix, the lower should be the pressure of the homogenizer. By doing a two-step homogenization, clumping or clustering of the fat is reduced, which produces a thinner more rapidly whipped mix and melt-down is also improved (Beattie, 2004).

2.2.3 Aging

The aging process of ice cream mix is performed overnight in insulated and refrigerated storage tanks at a temperature maintained as low as possible without freezing (Hyde, 2001). In the process, the fat cools down and crystallizes, and the proteins and polysaccharides (stabilizers) fully hydrate in the ice cream mix. Aging provides some essential functions including that it improves whipping qualities of the mix improves the body and texture of the ice cream causes the reduction in stabilization of the fat globule causes fat crystallization and increases viscosity (Goff, 1995; Marshall *et al*., 2003; Beattie, 2004).

2.2.4 Freezing and hardening

The flavour and color usually is added to the aged ice cream mix before operating the freezing process because off-flavours do not occur once the ice cream mix has been aged. Freezing ice cream mix is a dynamic process that freezes a portion of the water and whips air into the frozen mix by the operation of a freezer pump, resulting in an ice cream that has a consistency close to that of soft-serve ice cream (Goff, 1995).

If a soft-serve ice cream is made, a horizontal batch freezer specifically designed to dispense the ice cream is used, because the ice cream has higher

solids content (Beattie, 2004). The longer dwell time and lower overrun results in a product with a higher proportion of unfrozen water and consequently there is a greater risk of larger ice crystals to occur (Goff ,1995; Beattie, 2004).

If the product is going to be sold in liter containers, small tubs or being destined for scooping, one of two types of freezers, batch freezers and continuous freezers may be used. Continuous freezers are more efficient with a shorter dwell time, higher level of air incorporation is possible and a lower proportion of unfrozen water in the product is achieved (Beattie, 2004)

Ice cream contains a considerable quantity of air, up to half of its volume. This air is referred to as overrun. This gives the product its characteristic lightness. Without air, ice cream would be similar to a frozen ice cube. The batch type of freezer produces ice creams with a low overrun and is better with recipes, which contain about 30-32% total 23 solids (Beattie, 2004).

After the ice cream has been partially frozen, inclusions, such as nuts, candy or fruit, are added, and the freezing process is continued by subsequent hardening. The ice cream usually is packaged and is placed into a blast freezer at -30°C to -40°C where most of the remainder of the water is frozen.

Some of the important factors affecting hardening rate are size and shape of package, air temperature, air circulation, positioning within the freezer room, drawing temperature from the freezer, composition of the mix and percentage of air incorporated.

Quickly freezing the ice cream avoids the formation of large ice crystals. Below about -25°C, ice cream is stable for indefinite periods without danger of ice crystal growth; however, above this temperature, ice crystal growth is possible, and the rate of crystal growth is dependent upon the temperature of storage. This limits the shelf life of the ice cream (Goff, 1995; Marshall *et al*., 2003; Beattie, 2004).

2.2.5 Shelf life

The shelf life of ice cream is mainly dependent on the storage conditions. Ice cream can last as long as one year, or as little as two weeks. The finished product will have a shelf life of about 12-18 months if kept at around -20°C to -25°C. In order to maintain shelf life, some factors must be taken into consideration, including proper formulation of the ice cream, such as the addition of stabilizer and sugar, freezing the ice cream quickly, hardening the ice cream rapidly and avoiding temperature fluctuations during storage and distribution (Goff 1995; Marshall *et al*., 2003; Lee *et al*., 2005).

2.2.6 Potential microbioical hazards of ice cream

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 (Bell and Kyriakides,1998). However, pasteurization, freezing and hardening steps in the production can eliminate most of the microbioical hazards.

According to the Frozen Confections Regulation under Chapter 132, 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 (Andreasen and Nielsen, 1998). 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.

Nevertheless, there are some steps in the production of ice-cream that can lead to the microbioical hazards. (ICMSF,1996).

Heat treatment by pasteurization can destroy most of the specific pathogens that pose risk to public health. However, the potential microbioical hazards found in the final products can still be introduced after pasteurization through

adding contaminated ingredients and improper handling procedures (Marshall,1998). 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. (ICMSF,1996).

For ice-cream products, *L. monocytogenes* is of significant food safety concern worldwide (Bell,1998). In Hong Kong, regular surveillance to monitor *L. monocytogenes* in ice-cream has been in place. In 1997, *L. monocytogenes* was found in 4 imported ice-cream samples. Consequently, the importer had voluntarily recalled all incriminated ice-cream products on sale. No report of any persons being affected was received in that case.

2.3 Detection of microbial contaminants

Currently, so many techniques are available for enumeration and isolation of microbial contaminants in foods. But, techniques explained by American Public Health Association (APHA, 1984) and International Commission on Microbioical Specification for Foods (ICMSF, 1978) for enumeration and isolation of bacteria are widely acceptable.

However, several novel methods such as immunoical, chemical, biochemical, biophysical, nucleic acid probe, Polymerase Chain Reaction (PCR) and more recently biosensor based techniques have been developed to monitor the incidence of pathogenic bacteria in foods (Fung, 1995). Inherent problems associated with such techniques include difficulties in the recovery of bacterial species from meat and other foods, as some co-extractive materials comes at the time of enrichment in selective broth medium or even successful, they are time consuming to carry out and can significantly extend duration of the isolation and detection procedure (Duffy *et al*., 1999). Inefficiencies in extraction of the target pathogens from the food matrix and poor separation from elements of the competitive micro flora, can lead to subsequent

problems in the accurate detection and/or differentiation of target organisms. Thus, co-extractive materials can interfere with DNA hybridization test in PCR assay and immunoassay (Beumer and Brinkman, 1989). Furthermore, these methods require approval by any Governmental Organization or other agencies such as CAC, AOAC, APHA, ISO etc. Traditional cultural and seroical methods play an utopian goal in this area of concern, though time consuming and labour intensive.

Traditional and standardized analysis of food for presence of bacteria relies on the enrichment and isolation of presumptive colonies on solid media, using approved diagnostic artificial media. The International Organization for Standardization (ISO) has elaborated several standards for the detection of important pathogenic bacteria by traditional method. For *example, Salmonella* (ISO 6579), *Listeria monocytogenes* (ISO 10560), *thermo-tolerant Campylobacter* (ISO 10272), *E. coli* O157 (ISO 16654) and *Staphylococcus* sp. (ISO 6888) were enumerated by several workers (Anonymous, 1999, 2001).

2.3.1 Total bacterial count

Total Bacterial Count (TBC), also known as Heterotrophic Plate Count (HPC), Heterotrophic Colony Count (HCC), Aerobic Plate Count (APC), Total Plate Count (TPC), or Standard Plate Count (SPC), represents the total bacterial load in a given sample. It is a test to detect all viable microorganisms that could grow aerobically on plate count agar at appropriate incubation condition (usually 37^{o}C, 48hrs). The TBC tests could reflect the general hygiene condition of a sample. Guideline values of TBC depend on nature of samples, and they may be varied in different countries. The following shows some of the guideline values of TBC.

Swimming Pool Water - not exceeds 200 cfu/mL (Hong Kong), Drinking Water - not exceed 100 cfu/mL (China), Cooling Tower Water - not exceed

105 cfu/mL (Hong Kong), Ready-to-eat Food - < 10^3 to 10^7cfu/g depends on food types (Jolt *et al*,1994).

Enviro Labs Ltd. (ELL) holds HOKLAS accreditation for testing Heterotrophic Plate Count or Aerobic Plate Count in water, wastewater, marine water, and food samples.

2.3.2 Coliform count

The coliform count is the number of colonies in a sample that grow and form distinctive countable colonies on Violet Red Bile Agar after being held at 37°C (90°F) for 24 hours. Coliforms are generally only present in food that have been faecally or environmentally contaminated.

Coliform bacteria are commonly found in the intestine of humans and warm blooded animals. A specific subgroup of coliform bacteria is faecal coliform bacteria, with the most common member in the subgroup being (*E.coli*).The presence of faecal coliform bacteria, though being relatively less health hazardous to human, indicates that samples may be faecal contaminated. As faecal coliform bacteria is abundant in faeces and is relatively easy to detect, it is usually used as an indicator organism of contamination. Positive results of faecal coliform or *E.coli* in the samples also indicate the existence of other pathogenic organisms, thus reflecting the potential health risks for individuals being exposed to samples being tested.

2.3.3 *E. coli*

Escherichia coli is a Gram negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some, such as serotypeO157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and by preventing the establishment of pathogenic bacteria within the intestine.(Podschun and Ullmann, 1998).

E. coli are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for faecal contamination. The bacteria can also be grown easily and its genetics are comparatively simple and easily manipulated or duplicated through a process of metagenics, making it one of the best-studied prokaryotic model organisms, and an important species in biotechnoy and microbioy.

2.3.4 *Salmonella*

Salmonella infection usually occurs when a person eats food contaminated with the bacteria (associated with faeces of human and animals). Symptoms of *Salmonella* infection include diarrhea, abdominal cramps, and fever. *Salmonella* outbreaks are commonly associated with eggs, meat and poultry, but these bacteria can also contaminate other foods such as fruits and vegetables. Foods that are most likely to contain *Salmonella* include raw or undercooked eggs and chicken, raw milk, raw or undercooked meats, and contaminated water. Enviro Labs Ltd. (ELL) uses international recognized method to detect *Salmonella* in food samples.

2.3.4.1 Characteristics of *Salmonella*

Salmonella are gram-negative facultative intracellular anaerobes that cause a wide spectrum of disease. This spectrum can range from a gastroenteritis, enteric fever (caused by typhoid and paratyphoid serotypes), bacteremia, focal infections, to a convalescent lifetime carrier state. The type of infection depends on the serotype of *Salmonella* and host factors .It maintains a broad although the taxonomy of *Salmonella* can be confusing; all *salmonella* serotypes are members of a single species, *S.enterica*. More than 2500 serovar shave been described of which humans are almost exclusively infected by *S. enterica* subspecies *enterica* serotypes *typhi*, *typhimurium*, and *cholera esuis* worldwide (Chambers *et al.*, 2008). In the United States, *S. Enteritidis* (20%),

S.Typhimurium (16%), *S. Newport* (10%), and *S. Javiana* (6%) account for nearly one half of the human isolates.

2.3.4.2 Salmonellosis

Caused by *S. Enteritidis* is the most common bacterial infections cause of food-borne disease .Ninety-five percent of cases of *Salmonella* infection are food-borne; however, the incidence of direct contact exposure with animal carriers is on the rise. Once infected, salmonellosis harbors a significant morbidity and mortality. One third of untreated patients experience complications and account for three fourths of deaths associated with salmonellosis. *Campylobacter* and *Salmonella* are the most common bacterial pathogens found in stool cultures recovered from patients presenting with gastroenteritis or severe diarrhea. *Salmonella* has a widespread distribution in the environment and certain host factors make humans particularly susceptible to infection. Its increasing antimicrobial resistance, prevalence, virulence, and adaptability are a challenge worldwide (Oburn, *et al*. 2007).

2.3.5 *Pseudomonas*

Pseudomonas is a genus of gamma proteobacteria, belonging to the larger family of *pseudomonas*.

Recently, 16S rRNA sequence analysis has redefined the taxonomy of many bacterial species. As a result the genus Pseudomonas includes strains formerly classified in the genera *Chryseomonas* and *Flavimonas*. Other strains previously classified in the genus Pseudomonas are now classified in the genera *Burkholderia* and *Ralstonia*.

"*Pseudomonad"* literally means 'false unit', being derived from the Greek *pseudo* (false) and *monas* (a single unit). The term "monad" was used in the early history of microbioy to denote single-celled organisms.

Because of their widespread occurrence in water and in plant seeds such as divots, the *pseudomonas* were observed early in the history of microbioy. The generic name *Pseudomonas* created for these organisms was defined in rather

vague terms as a genus of Gram-negative, rod-shaped and polar-flagella bacteria. Soon afterwards, *Pseudomonas* was isolated from many natural niches and a large number of species names were originally assigned to the genus. New methodoy and the inclusion of approaches based on the studies of conservative macromolecules have reclassified many strains (Cornelis, 2008).

Pseudomonas aeruginosa is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several different epidemioical studies indicate that antibiotic resistance is increasing in clinical isolates.

In the year 2000, the complete genome sequence of a *Pseudomonas* species was determined; more recently the sequence of other strains have been determined including *P. aeruginosa* strains PAO1 (2000), *P. putida* KT2440 (2002), *P. fluorescens* Pf-5 (2005), *P. syringae* pathovar tomato DC3000 (2003), *P. syringae* pathovarsyringae B728a (2005), *P. syringe* pathovarphaseolica 1448A (2005), *P. fluorescens* PfO-1 and *entomophila* L48 (Cornelis, 2008).

2.3.5.1 Characteristics of *Pseudomonas*

Members of the genus display the following defining characteristics:

* Rod shaped
* Gram-negative
* One or more polar flagella, providing motility
* Aerobic
* Non-spore forming
* Positive catalase test

Other characteristics which tend to be associated with Pseudomonas species (with some exceptions) include secretion of pyoverdin (fluorescein), a fluorescent yellow-green siderophore under iron-limiting conditions. Certain Pseudomonas species may also produce additional types of siderophore, such as pyocyanin by *Pseudomonas aeruginosa* and thioquinolobactin by *Pseudomonas fluorescens*,. *Pseudomonas* species also typically give a

positive result to the oxidase test, the absence of gas formation from glucose, glucose is oxidised in oxidation/fermentation test using Hugh and Leifson O/F test, beta hemolytic (on blood agar), indole negative, methyl red negative, Voges-Proskauer test negative, citrate positive (Nzai *et al.*,2000).

2.3.5.2 Pathophysioy

Pseudomonal infection, as described by Pollack, (1984), occurs in three stages: Bacterial attachment and colonization, followed by local invasion and dissemination and systemic disease.

In healthy children, disease is primarily limited to the first 2 stages (as in diseases such as otitis externa, urinary tract infections (UTIs), dermatitis, cellulitis, and osteomyelitis), although recent case reports describe bacteremia, sepsis, and GI infections in previously healthy children. In immuno-compromised hosts, including neonates, infection can progress rapidly through the 3 stages and cause pneumonia, endocarditis, peritonitis, meningitis, ecthymagangrenosum (EG), bacteremia, and overwhelming septicemia.

2.3.6 *Staphylococcus aureus*

S. aureus (pronounced, literally the "golden cluster seed" or "the seed gold" and also known as golden *Staph.* and *Oro staphira*) is a facultatively anaerobic, Gram-positive coccus and is the most common cause of *Staph.* infections. It is frequently part of the skin flora found in the nose and on skin. About 20% of the human population are long-term carriers of *S. aureus* (Kluytmans *et al.*,1997). The caroten oidpigment staphyloxanthin is responsible for *S. aureus*' characteristic golden colour, which may be seen in colonies of the organism. This pigment acts as a virulence factor with an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system. *Staph*. organisms which lack the pigment are more easily killed by host defenses.

2.3.6.1 The illnesses caused by *S. aureus*

S. aureus can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulites folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections. Abbreviated to *S. aureus* or *Staph aureus* in medical literature, *S. aureus* should not be confused with the similarly named and similarly dangerous (and also medically relevant) species of the genus *Streptococcus.*

S. aureus was discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses. Each year some 500,000 patients in American hospitals contract a staphylococcal infection (Bowersox, 1999). *S. aureus* is a facultatively anaerobic, Gram-positive coccus, which appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates**. (**Ryan and Ray, 2004). The golden appearance is the etymoical root of the bacteria's name; *aureus* means "golden" in Latin.

2.3.6.2 Role in disease

Strains are responsible for food poisoning through the production of an enterotoxin and pathogenicity is also associated with coagulase positivity.

S. aureus may occur as a commensal on skin; it also occurs in the nose frequently in about a third of the population (Whitt and Salyers, 2002), and throat less commonly *S. aureus* can infect other tissues when barriers have been breached (e.g., skin or mucosal lining). This leads to furuncles (boils) and carbuncles (a collection of furuncles). In infants *S. aureus* infection can

cause a severe disease staphylococcal scalded skin syndrome (Curran and Al-Salihi, 1980).

S. aureus infections can be spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroys tissues, and contact with objects such as towels, sheets, clothing, or athletic equipment used by an infected person. Deeply penetrating *S. aureus* infections can be severe. Prosthetic joints put a person at particular risk for septic arthritis, and staphylococcal endocarditis (infection of the heart valves) and pneumonia, which may be rapidly spread.

CHAPTER THREE

MATERIAL S AND METHODS

3.1 Materials

Ice cream samples: Twelve chocolate flavoured ice creams and mango flavoured ice cream samples were collected from three regions namely Khartoum, Khartoum North and Omdurman. From each regions 4 samples were collected, two samples with chocolate flavour and two samples with mango flavour from a certain factory, two samples with chocolate and mango flavour from traditional home made ice cream. The samples were collected during June, 2012 to October, 2012 randomly. They were placed in an ice-filled container during transport to inhibit the multiplication of any microbial flora that may be present, and then kept in freezer prior to analysis within 72 hrs.

3.2 Microbioical analysis

3.2.1 Preparation of serial dilutions

Ten grams of ice cream sample were aseptically added to 90 ml sterile 0.1% peptone water in a conical flasks and homogenized (Colworht stomacher, A.J. Sewar and Co. Ltd., London). This solution was referred to as stock solution (dilution 10^{-1}). One ml of first dilution (10^{-1}) transferred to 9 ml peptone water (10^{-2})ml and serial decimal dilution up to 10^{-6} were prepared as described by Harrigan (1998).

3.2.2 Sterilization of glassware

According to Harrigan, (1998) glassware were washed thoroughly and left to dry, then they were sterilized in a hot air oven(Karl Kolb, Screntific Technical Supplies, Frankfurt, Germany) at 160°C for three hours. Inoculating wires, loops and metal instruments such as spatulas, knives and forceps were sterilized by flaming after dipping in ethanol.

3.2.3 Preparation of culture media

3.2.3.1 Plate count agar

This medium was used to determine total bacterial count(TBC) and for sub culturing of bacteria. It was obtained in a dehydrated form. The component of the medium was peptone, yeast extract, Lab-Lemco powder, sodium chloride and agar. It was prepared according to the manufacturer's directions by using 40g in one liter of distilled water and boiled in a water bath until it was completely dissolved. The pH was adjusted to pH 7.2 and then autoclaved at 121°C for 15 minutes.

3.2.3.2 Nutrient agar

Thirty grams were dissolved in one litre distilled water heated to boiling until the ingredients of the medium dissolved, after which the conical flask was covered by cotton and aluminum foil and sterilized by an autoclave at 121°C for 20 minutes.

3.2.3.3 Baird Parker medium

Baird parker medium was prepared by suspending 63g of-Media Baird parker agar Base in 950 ml distilled water, and boiled to dissolve the medium completely. Then sterilized in an autoclave at 121°C for 15 minutes, cooled to 50°C and 50ml of Hi- Media egg yolk tellurite emulsion (FD046) were added, well mixed and poured into Petri-dishes.

3.2.3.4 Staphylococcus medium No. 110

This is a selective medium for isolation and differentiation of pathogenic staphylococci (Chapman and pratt, 1982). On a basis of salt tolerance, pigmentation, mannitol, fermentation and gelatin liquefaction. Pathogenic staphylococci (coagulase-positive) are able to grow on the high salt mannitol medium to form orange colonies which give positive reactions for acid production, and gelatin liquefaction. One hundred and fifty (150) grams were

dissolved in one litre of distilled water, heated to boil and dissolved completely, and sterilized by autoclaving at 121^0C for 15 minutes (Oxoid, 2006).

3.2.3.5 Pseudomonas medium

1- BAM Medium Mize: Pseudomonas Agar F. bacterioical Analytical Manud Mize.

- Proteose peptone No 3 (Difco): 20.0g
- Tryptone (Difco) 10.0g
- Dipotassium phosphate 1.5g
- Magnesium sulfate 0.73g
- Glycerol (Difco) 10.0g
- Distilled water 1 liter.

3.2.3.6 Malt extract agar

It was used to cultivate, count and isolate the yeasts and moulds. It was obtained in a dehydrated form. The medium was composed of malt extract, yeast extract, peptone, glucose and agar. It was prepared according to the manufacturer's directions by using 50g in one liter of distilled water. The medium was allowed to boil in water bath until it was completely dissolved. The pH was adjusted to pH 5.4 and then autoclaved at 115°C for 10 minutes.

3.2.3.7 Starch milk agar

This medium was used to determine spore-forming bacteria. It consisted of nutrient agar, 1% skim separated milk and soluble starch.

3.2.3.8 MacConkey broth

This selective and differential medium was used for detection of coliform bacteria by multiple tube technique. It was obtained in a dehydrated from. The medium was composed of peptone, lactose, bile salt, sodium chloride and bromocresol purple.

Forty grams were dissolved in one litre distilled water in a conical flask.

Using a pipette,9 ml were put into each tube (about 5 cm length) with an inverted Durham tubes inside "10 tubes were prepared to each sample, 9 for the sample and one for' the control' covered by cotton and aluminum foil, sterilized by autoclave at 121°C for 15 minutes.

3.2.3.9 Brilliant green bile lactose broth

This selective medium was used for detection of *E. coil* by the multiple tube technique. It was obtained in dehydrated from. The, medium was composed of peptone, lactose, purified bile and brilliant green. It was prepared according to the manufacturer's instructions by suspending 40g in one liter distilled water. The pH was adjusted to 7.4 distributed in 5ml amounts in 150 x 16ml test tubes with inverted Durham tubes and then sterilized in the autoclave at 121°C for 15 minutes.

3.2.4 Enumeration of total bacteria

One ml was pipetted from10^{-1}, 10^{-2}, 10^{-3} or 10^{-4} dilution into Petri dishes. Promptly, 15 ml of PC agar medium melted and allowed to cool to 45°C was poured into the Petri-dishes. Immediately, aliquots were mixed with agar medium by tilting the dish to for 5 times in one direction, rotating it clockwise 5 times, tilting it to for again 5 times in direction at right angles to that used the first time and rotating it counterclockwise 5 times. After solidification, the Petri-dishes were inverted and incubated at 37°C for 48h. Colony counter was used to count all colonies. The number of presumptive colony-forming units was computed per g of specimen by multiplying by the reciprocal of the dilution used.

3.2.5 Enumeration of yeasts and moulds

From suitable dilutions (10^{-1}) of sample 0.lml was aseptically transferred into only one dilution to a solidified malt extract agar (MEA) containing 0.1g chloramphenicol per one liter to inhibit bacterial growth. The sample was spread all over the plates using sterile bent glass rod.

Selective medium of the total count of yeasts and moulds are malt extract

agar (M.E.A). These are surface plate medium, which were poured into sterilized Petri dishes and left to solidify. A 0.1 ml of diluted sample was transferred using sterilized pipette into the media plates, then hockey stick was used to distribute sample all over the media. Dilutions used were 10^{-2}, 10^{-3}. and 10^{-4}.Inoculated plates were incubated at 25^{0}C for 72 hours according to Harrigan and MacCance (1976).

3.2.6 Enumeration of total spores

The colony count method to determine the total spores was followed as described by Harrigan, (1998). A test tube of suitable dilution is heated in water bath at 80°C for 10 minutes. Then the tube is cooled and one ml from this dilution was aseptically transferred into sterile Petri-dishes. The inoculums were mixed with the medium and allowed to solidify. The plates were then incubated at 37°C for 2 days.

3.2.7 Enumeration of *Staphylococcus aureus*

A dilution of 0.lml was pipetted over the surface plates containing Baird Parker agar medium and each portion was spread with a sterile spreader and incubated at 37°C for 48h. Colony counter was used to count black shiny colonies with narrow white margins and surrounded by clear zones. Colony forming units (cfu) were calculated by multiplying by the reciprocal of the dilution used.

The medium used was *Staphylococcus* medium No. 110. This medium is surface plate medium poured into sterilized Petri dishes, and left to solidify. A 0.1 ml of diluted sample was transferred using sterilized pipette into the medium plates, then hockey stick was used to distribute sample all over the medium. Dilutions used were 10-^{3} and 10-^{4}• Inoculated plates were incubated at 37°C for 48 hours.

3.2.8 Detection of *Salmonella*

The stage of the pre-enrichment of *Salmonella* was done by mixing 25g of the sample with 225ml of buffer peptone water in a sterile conical flask. The pre-enrichment culture was incubated for 24h at 37^{O}C. The stage of the selective enrichment of *Salmonella* was done by pipetting 1 ml of pre-enrichment culture to l0 ml of selenite cystine broth and incubating at 37°C for 24h. The stage of the plating on selective agar medium was done by transferring a loopful of the selective enrichment medium to the surface of each one of the three selective agar medium (xylose lysine decxycholate, bismuth sulphite agar and brilliant green agar) and spreading to obtain isolated colonies.

Brilliant green agar plates were incubated for 24h at 37^{o}C. Typical colonies of *Salmonella* in brilliant green agar medium were colurless, pink to fuchsia, or translucent to opaque with the surrounding medium pink to red. Bismuth sulfite agar plates were incubated at 37°C for 48h. Typical *Salmonella* colonies on bismuth sulfite agar appeared brown or gray to black, sometimes with metallic sheen. The medium surrounding the colony was usually brown at first, then turned black as the incubation time increased. XLD agar plates were incubated at 37^{o}C for 24 hours. Typical *Salmonella* colonies on XLD agar appeared with black centre.

Biochemical screening test was done by taking one loopful of the culture and transferring it into the sodium chloride solution ampule. A drop of the solution was transferred to each cupule in the Rapid ID 20 E strip. A drop of paraffin oil was added to the Urea, LDC, ODC and ADH cupules to make anaerobic conditions. The strip was incubated at 36°C for 24h.

Medium used were lysine iron agar and triple sugar iron agar. By sterile steel loop, colonies from the plating medium bismuth sulphite (B.S.) and selanite broth (B.S.) were streaked on prepared lysine iron agar and triple sugar iron agar tubes and incubated at 37°C for 48 hours. A positive result was

indicated by presence of gas in the triple sugar iron agar tubes by the slant red and butt yellow colour, in lysine iron agar purple colour.

3.2.9 Detection of coliform bacteria

The medium used was MacConkey broth. The dilutions used from10^{-1}, 10^{-2} and 10^{-3}. One milliliter of diluted sample was transferred into each of the MacConkey tubes (three tubes for each dilution). Tubes were then incubated for 24-48 hours at 37°C. Positive results were indicated by presence of acid and gas (Harrigan, 1998).

3.2.10 Detection of *E.coli*

The medium used was Brilliant Green Bile Lactose Broth (Oxoid). The dilutions used were 10^{-1}, 10^{-2} and 10^{-3}. One milliliter of diluted sample was transferred into each of the Brilliant Green Broth tubes (three tubes for each dilution). Tubes were then incubated for 24-48 hours at 44°C. Positive results were indicated by presence of acid and gas.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1: Microbial counts of chocolate and mango flavoured ice cream

Tables (1, 2) revealed the mean value of TBC, *Staph*. moulds and yeasts total coliform and spore forming bacteria (SFB) of chocolate and mango flavoured ice cream.

According to the result presented in table (1) the mean value of TBC, *Staph*. moulds and yeasts, total coliform and spore forming bacteria (SFB) of chocolate flavoured ice cream were in range of (3.0×10^3 to 8.9×10^3, 0.0 to 2.1×10^2, 0.0 to 5.2, 1.4×10 to 2.5×10 and 0.0 to 3.0 cfu/g), respectively. The highest bacteria count of total bacterial count in chocolate flavoured ice cream were observed in homemade ice cream produced in Omdurman area 8.9×10^3 and the lowest count were observed in homemade ice cream produced in Khartoum 3.0×10^3. On the other hand homemade ice cream produced in Omdurman obtained the highest *Staphylococci* count (2.1×10 cfu/g), while chocolate flavoured factory ice cream produced in North Khartoum and homemade chocolate flavoured ice cream produced in Khartoum were nil. The highest count of yeasts and moulds were obtained by chocolate flavoured factory ice cream produced in Khartoum North 5.2 while chocolate flavoured factory ice cream produced in Khartoum and Omdurman were nil. The total coliform count was highest in homemade chocolate flavoured ice cream and the lowest count obtained by factory ice cream. The tested samples showed no counts for spore forming bacteria except chocolate flavoured factory ice cream produced in Khartoum (2.0 cfu/g) and homemade chocolate flavoured ice cream produced in Khartoum (3.0 cfu/g).

Table 1: Microbial counts (cfu/g) of chocolate flavoured ice cream

Microorganism	Factory ice cream			Home ice cream		
	Location					
	Khartoum	Omdurman	K. North	Khartoum	Omdurman	K. North
Total bacterial count	4.5×10^3	6.4×10^3	3.8×10^3	3.0×10^3	8.9×10^3	7.7×10^3
Staphylococcus aureus	7.0	1.3×10	0.0	0.0	2.1 ×10	8.0
Yeast and Moulds	0.0	0.0	5.0	5.0	5.0	3.0
Coliform	1.4×10	1.4×10	4.5×10	1.4×10^2	1.1×10^2	2.5×10^2
Spore forming bacteria	2.0	0.0	0.0	3.0	0.0	0.0

Table 2: Microbial counts (cfu/g) of mango flvuerd ice cream

Microorganism	Factory ice cream			Home ice cream		
	Location					
	Khartoum	Omdurman	K. North	Khartoum	Omdurman	K. North
Total bacterial count	2.5×10^5	2.0×10^5	2.5×10^5	2.6×10^5	2.5×10^5	3.6×10^5
Staphylococcus aureus	8.0	1.3×10	1.1×10	0.0	2.1×10	4.0
Yeast and Moulds	0.0	0.0	3.6×10	8.2×10	4.7×10	0.0
Coliform	1.4×10^2	1.4×10^2	1.4×10^2	1.4×10^2	1.1×10^2	2.5×10^2
Spore forming bacteria	2.0	0.0	0.0	3.0	0.0	0.0

According to the result presented in table (2) the mean (cfu/g) value of TBC, *Staph*. moulds and yeasts, total coliform and spore forming bacteria (SFB) of mango flavoured ice cream were in range of 2.0×10^5 to 3. 6×10^5, 0.0 to 2.1×10, 0.0 to 8.2×10, 1.1×10 to 2.5×10^2 and 0.00 to 3.0 cfu/g, respectively. The highest total bacterial count in mango flavoured Ice cream were observed in homemade ice cream produced in Khartoum North area (3.6×10^5 cfu/g) and the lowest count observed in mango flavoured factory ice cream produced in Omdurman (2.0×10^5 cfu/g). On the other hand homemade ice cream produced in Omdurman were obtained the highest Staphylococci count (2.1×10 cfu/g), while mango flavoured factory ice cream produced in Khartoum North and homemade mango flavoured Ice cream produced in Khartoum were nil. The highest count of yeasts and moulds were obtained by mango flavoured homemade ice cream produced in Khartoum (8.2 cfu/g) while mango flavoured factory ice cream produced in Khartoum and Omdurman were nil. The total coliform count was highest in mango flavoured factory ice cream and the lowest count obtained by homemade ice cream. The tested samples showed no counts for spore forming bacteria except mango flavoured factory ice cream produced in Khartoum (2.0 cfu/g) and homemade chocolate flavoured ice cream produced in Khartoum (3.0 cfu/g).

The results were in agreement with the finding of Keller *et al*,(1987) who reported that the fresh ice cream contain no more than 100,000 cfu/g of total bacterial counts, but above that result obtained by Zeinab, (2005) of total bacterial counts of chocolate and mango flavoured loli ice cream and homemade ice cream.

The result of total bacterial counts of the tow flavour were in the range of the Sudanese standard specification (SSMO, 2007) of ice cream, ice cream mixes, combined milk ice cream and water based ice cream (No 308). The highest total bacterial counts of homemade chocolate flavoured ice cream may be due to unhygienic practices or improper sterilized tools. Homemade chocolate flavoured ice cream had a significantly higher frequency of staphylococci

compared with chocolate flavoured factory ice cream products. This could be attributed to poor handling during preparation and contamination from the environment during sale.

The presence of yeasts and moulds may be due to contamination of sugar.

4.2 Pathogenic bacteria in chocolate and mango flavored ice cream

The presence of *E. coli*, *Salmonella* and *Pseudomonas* bacteria in chocolate and mango flavoured ice cream were presented in table 3 and 4 which revealed the presence of *E. coli* in all tested samples. Also the results indicated the presence of *salmonella* in mango and chocolate flavoured ice cream in Omdurman area. While *Pseudomonas* bacteria were absent in all tested samples.

Coliforms and *E. coli* may contaminate ice cream during processing through contaminated water or faecal material as a result of poor sanitary practices.

The presence of *Salmonella spp* observed in one location (Omdurman), but being potent pathogen it should be taken considerably. Although pathogenic microorganisms are resistant to freezing temperatures, in certain bacterioic media, particularly selective ones, they can exhibit poor or no development.

The contamination of flavoured ice cream by *Salmonella* may be a result of contamination of non pasteurized liquid eggs.

Kanbakan *et al* (2004) reported that the water used during the production and sale of ice cream, particularly home-made one, may have served as a vehicle for *E. coli* and other coliform to contaminate the ice cream studied. It is relevant to mention that at the sale outlet, particularly when sold in open containers which require dispensing of the product for sale, scoop water may serve as vehicle of coliform contamination of ice cream.

Table 3: Pathogenic bacterial detection of chocolate ice cream

Bactria	Factory ice cream			Home ice cream		
	Location					
	Khartoum	Omdurman	K. North	Khartoum	Omdurman	K. North
E. coli	+ve	+ve	+ve	+ve	+ve	+ve
Salmonella	-ve	+ve	-ve	-ve	+ve	-ve
Pseudomonas	-ve	-ve	-ve	-ve	-ve	-ve

Table 4: Pathogenic bacterial detection of mango ice cream

Bactria	Factory ice cream			Home ice cream		
	Locations					
	Khartoum	Omdurman	K. North	Khartoum	Omdurman	K. North
E. coli	+ve	+ve	+ve	+ve	+ve	+ve
Salmonella	-ve	+ve	-ve	-ve	+ve	+ve
Pseudomonas	-ve	-ve	-ve	-ve	-ve	-ve

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions:

1. From this study it is concluded that there was a great difference between the two ice creams flavours (chocolate and mango). The type of methods and location of flavoured ice cream production had an effect on microbial counts.
2. The highest total bacterial counts were observed in mangoes flavoured ice creams particularly in homemade ice creams and this may be due to unhygienic practices or improper sterilized tools.
3. Homemade chocolate flavoured ice cream had a significantly higher frequency of staphylococci compared with chocolate flavoured factory ice cream products.
4. The presence of some groups of pathogenic bacteria such as *Salmonella* species may pose a risk for public health particularly for children and vulnerable elderly people.
5. The current investigation has indicated a poor overall level of hygiene in the service of flavoured ice cream in the Khartoum State.

5.2 Recommendations

1. In Sudan, ice cream should receive quality control measures to increase its shelf life as well as to prevent potential threat of public health.
2. Due to non-enforcement of inspection act and lack of maintenance of standard relation to hygienic quality of ice cream, the consumers of such countries are deprived of getting poor quality ice cream.

3. In the manufacturing processes, pasteurization should be effective to destroy most of pathogenic bacteria.
4. Automatic machines that are widely used for ice-cream production in dairy industry minimize direct hand manipulation and possibility of cross contamination.
5. Good manufacturing practice (GMP) would be a good way to improve hygienic quality of soft ice cream, especially in all steps after pasteurization.
6. For the consumers, they should also observe some key points to avoid the exposure to microbioical hazards, specially children.

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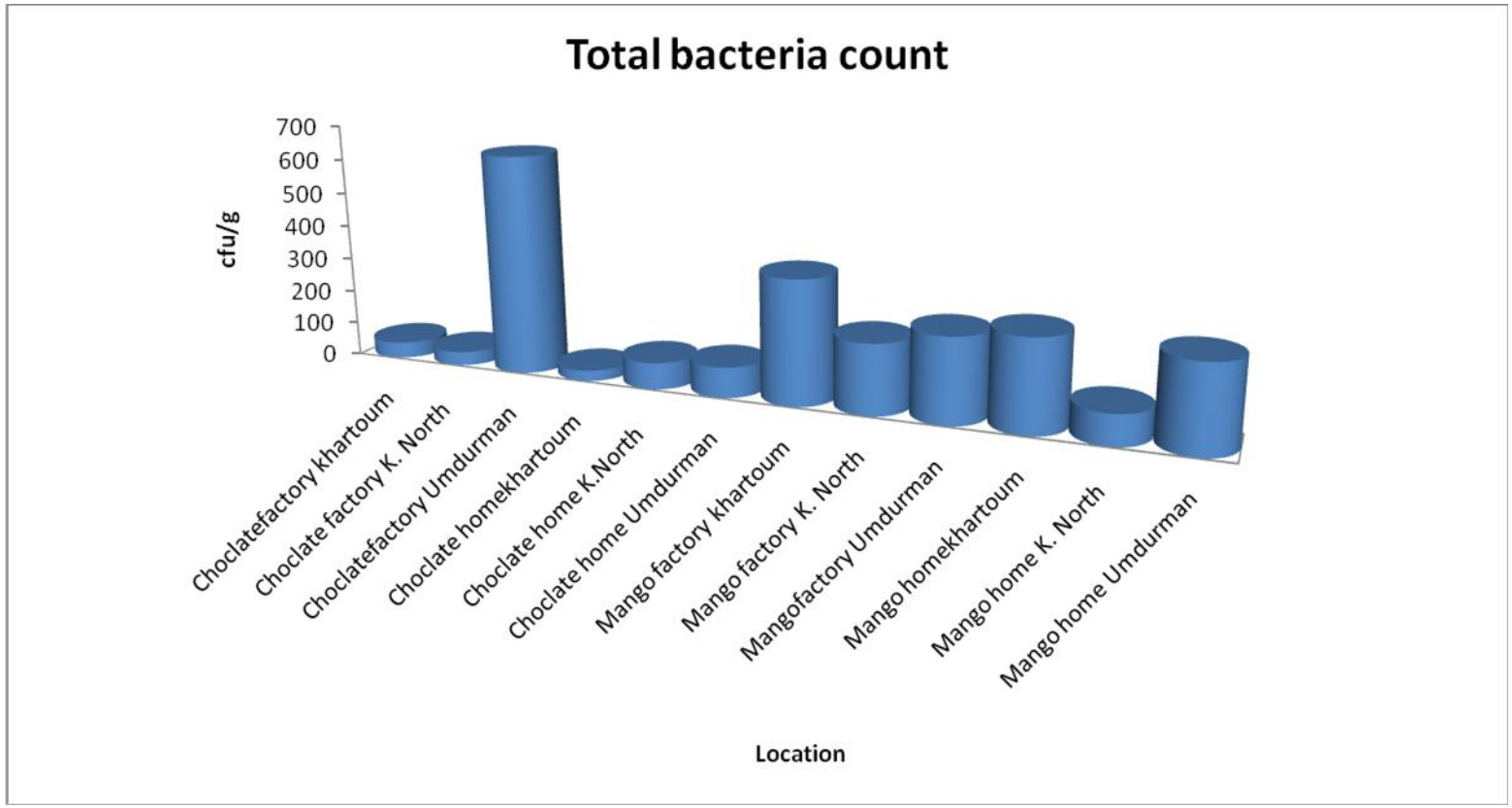
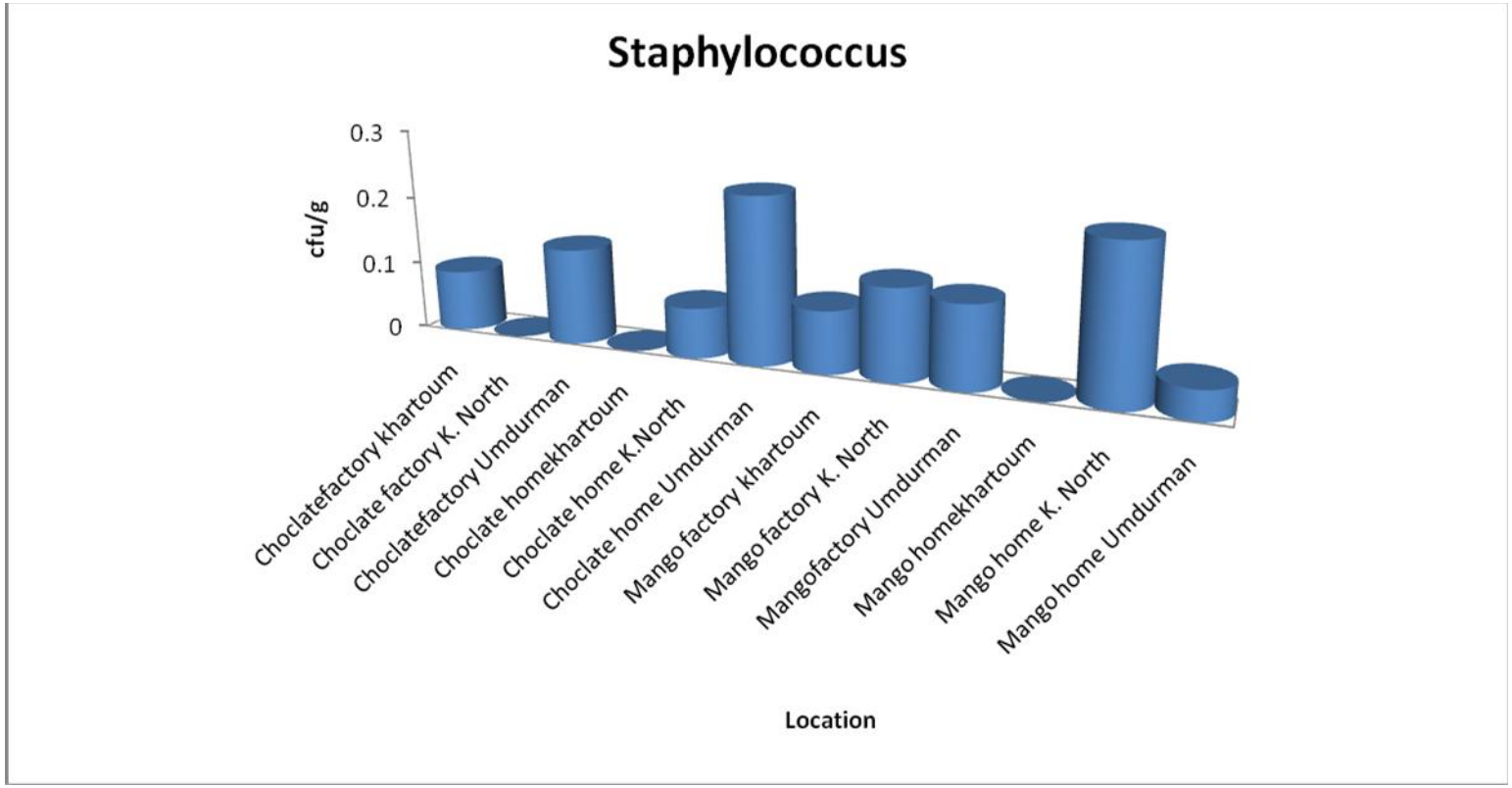
APPENDICES

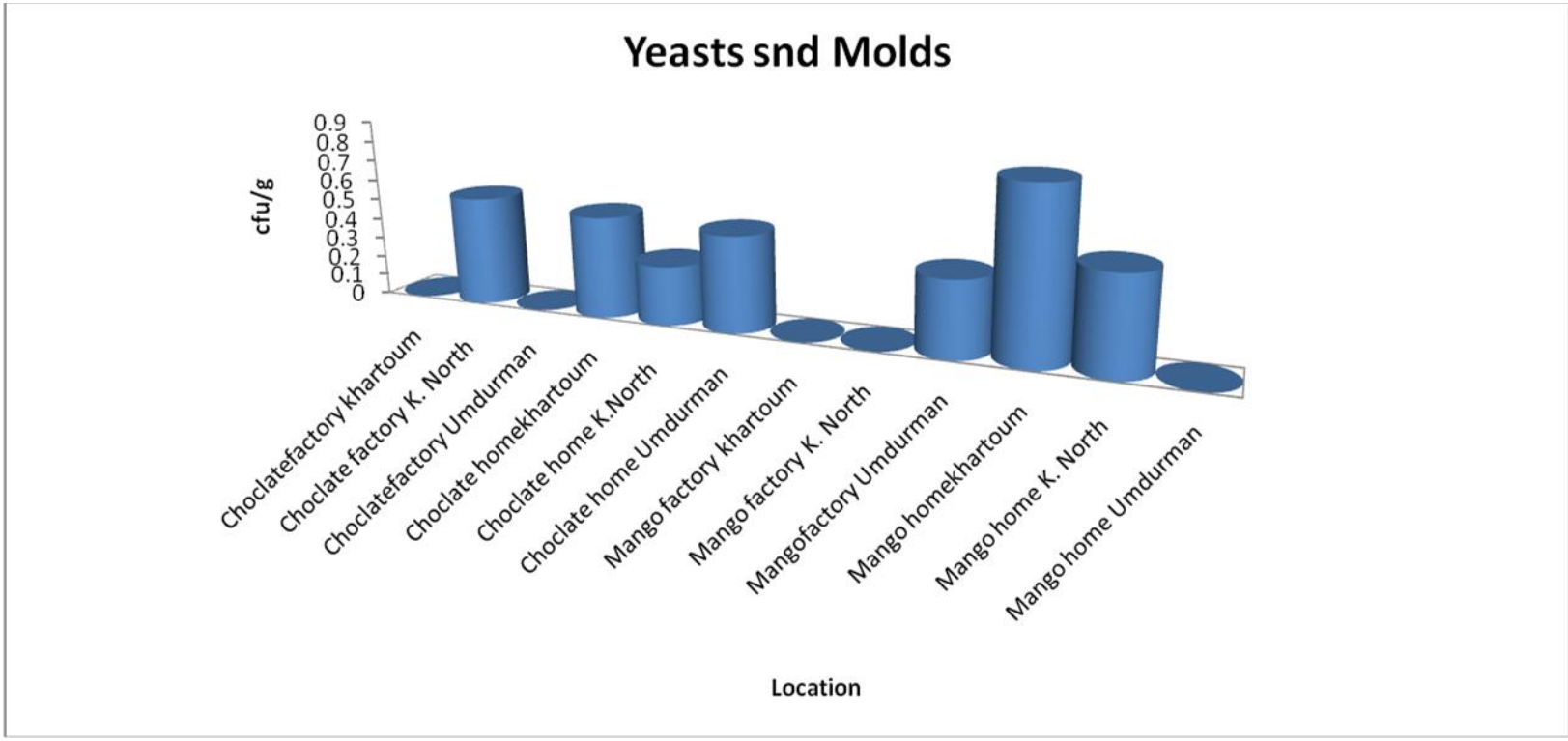
Appendix (1)



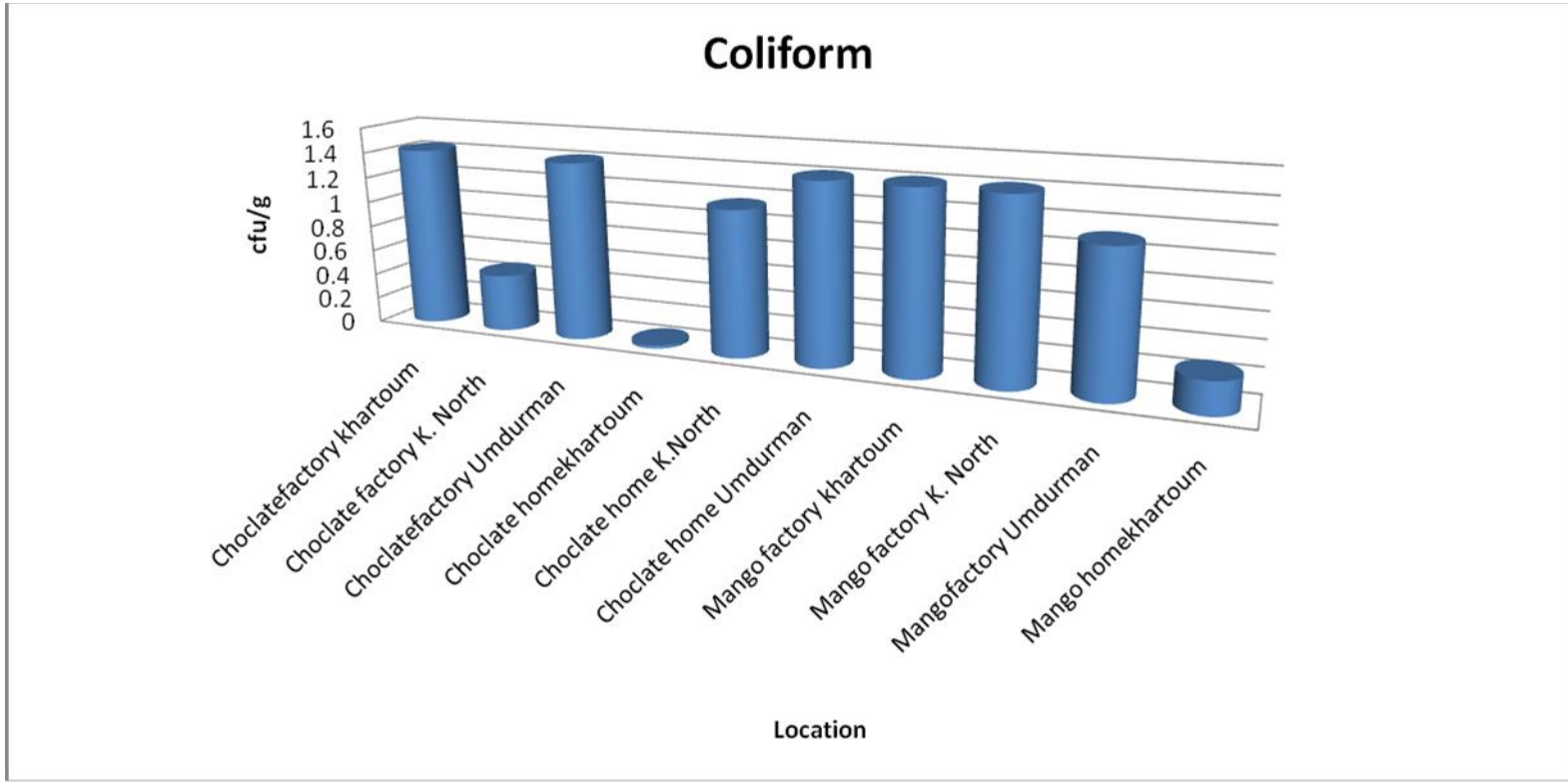
Appendix (2)


Staphylococcus
cfu/g
0.3
0.2
0.1
0
Choclatefactory khartoum
Choclate factory K. North
Choclatefactory Umdurman
Choclate homekhartoum
Choclate home K.North
Choclate home Umdurman
Mango factory khartoum
Mango factory K. North
Mangofactory Umdurman
Mango homekhartoum
Mango home K. North
Mango home Umdurman
Location

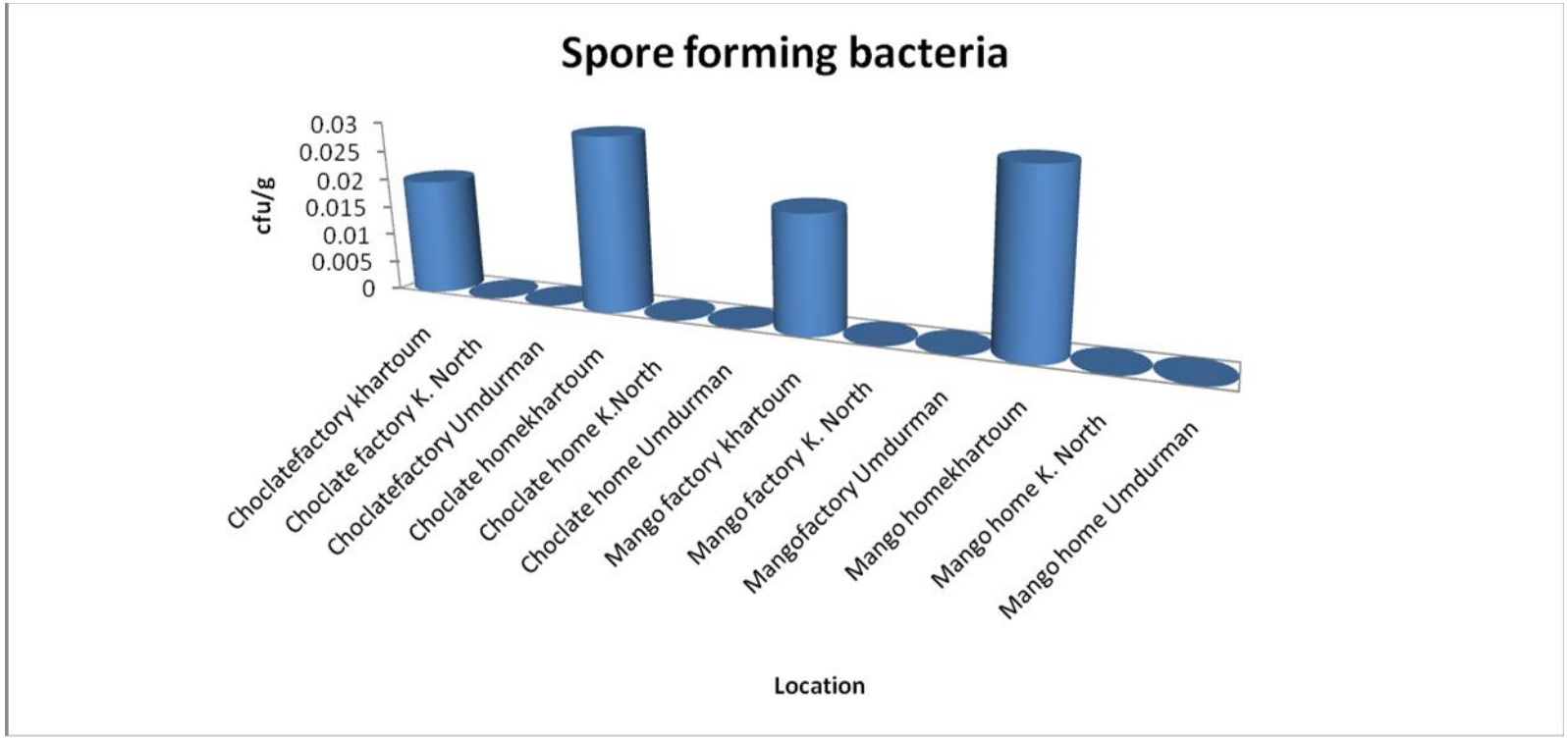
Appendix (3)


Yeasts snd Molds
cfu/g
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Choclatefactory khartoum
Choclate factory K. North
Choclatefactory Umdurman
Choclate homekhartoum
Choclate home K.North
Choclate home Umdurman
Mango factory khartoum
Mango factory K. North
Mangofactory Umdurman
Mango homekhartoum
Mango home K. North
Mango home Umdurman
Location

Appendix (4)


Coliform
cfu/g
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Choclatefactory khartoum
Choclate factory K. North
Choclatefactory Umdurman
Choclate homekhartoum
Choclate home K.North
Choclate home Umdurman
Mango factory khartoum
Mango factory K. North
Mangofactory Umdurman
Mango homekhartoum
Location

Appendix (5)


Spore forming bacteria
cfu/g
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0.02
0.015
0.01
0.005
0
Choclatefactory khartoum
Choclate factory K. North
Choclatefactory Umdurman
Choclate homekhartoum
Choclate home K.North
Choclate home Umdurman
Mango factory khartoum
Mango factory K. North
Mangofactory Umdurman
Mango homekhartoum
Mango home K. North
Mango home Umdurman
Location