MICROBIOLOGICAL QUALITY ASSESSMENT OF BRANDED RETAIL ICE CREAM PRODUCTS IN LAGOS METROPOLIS, SOUTHWEST NIGERIA

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ABSTRACT

Ice cream is a nutritionally enriched dairy product consumed by all age groups. This study evaluated the microbial quality of some brands of retail ice cream products randomly purchased from vendors in Lagos metropolis by isolation, identification and characterization of the microbial isolates. A total of six different brands of ice cream products were collected and brought into the laboratory and kept in the refrigerator at 4ºC prior to analysis. The microbiological quality of some brands of ice cream products obtained from vendors in Lagos metropolis was determined following Standard and conventional methods for the identification and characterization of microbial isolates. Five bacterial species were prominent: Staphylococcus aureus, Klebsiella species, Bacillus species, Escherichia coli and Enterobacter species. Bacterial growth were observed for all samples ranging from 1.14 x 10^3 to 6.0 x 10^4 (cfu/ml). Staphylococcus aureus recorded highest percentage occurrence of 100%, followed by Klebsiella species 66% and Bacillus species 66%, Escherichia coli 50% while the least value of 5% was obtained for Enterobacter species. Therefore, the storage and processing methods should be investigated for possible points of contamination. The presence of Escherichia coli in ice cream products showed the possibility of acquiring food-borne pathogen from this product and this is suggestive of faecal contamination. This is of public health significance and this calls for proper education of hawkers and processing hands to maintain highest standard of hygienic practices.

Keywords: *Contamination*; *Food - borne pathogen; Good Manufacturing Practice; Ice cream; Quality control.*

INTRODUCTION

Ice cream is a frozen dairy dessert obtained by freezing the ice cream mix with continuous agitation (Deosarkar *et al*., 2016). Ice cream is considered nutritive food due to its composition which includes milk proteins, fat and lactose as well as other compounds such as emulsifiers, stabilizers, candies, syrups, fruits and nuts for flavour enhancement (Osamwonyi *et al*., 2011; Balthazar *et al.*, 2017). Ice cream production technology has further developed by its supplementation with prebiotic ingredients or probiotic bacteria (Cruz *et al*., 2009). A typical ice cream contains 12% fat, 11% non-fat milk solids, 15% sugar and about 1% minor

ingredients, the rest being water (Ojokoh, 2006). The protein and vitamin contents of ice cream are fairly low, but as a source of calcium it is as valuable as milk (Osamwonyi *et al*., 2011). The richness in the nutritive constituents of ice cream has been realized by all but the problem lies in the production and handling of this food. The world is faced with the problem of food shortage, these milk products are considered a partial solution to this problem in developing countries. However, these products are vulnerable to spoilage by certain microorganisms, some of which are beneficial and others are harmful to human beings (Owni, 2011). Ice cream is commonly enjoyed by people of all ages (Karaman *et al*., 2014), the production and consumption of ice cream continue to increase year on year (Smith, 2015). Dairy products pose a major concern to the dairy industry and public health authorities (Melo *et al*., 2015) as they can be important sources of food borne pathogens (Garcell *et al*., 2016). Primary sources of these microorganisms in ice cream have been reported to include raw materials used for the composition of ice cream mix, such as milk and milk powder, cream, flavouring and colouring substances, sanitizer and air during processing (Ahmed *et al*., 2008).

Ice cream production is a relatively complex operation, with series of steps which, in both compositional and microbiological terms contributes to the overall quality of the ice cream (Owni, 2011). Although, ice cream is not a sterile product, it contains no harmful microorganisms when it is produced by an approved process and under hygienic conditions (Marshall and Arbuckle, 2003). The microbiological quality of ice cream depends on both the manufacturing procedure and the proportion of ingredients used. Primary sources of microbial contamination to ice cream include water and raw milk whereas secondary sources include flavouring agents, utensils and handling. However, pasteurization, freezing and hardening steps in production can eliminate most of the microbiological hazards, but still, numerous health hazards are persistent due to various conditions (Marshall and Arbuckle, 2003).

Many psychrophiles and psychro-tolerant microorganisms like *Listeria monocytogenes,* coliforms, *Staphylococcus aureus, Bacillus* sp., *Salmonella* sp., *Shigella sp., Streptococcus sp., Pseudomonas sp., Campylobacter sp., Brucella sp.* and other bacteria are generally found in ice cream (Hossain, 2012). The presence of microorganisms in pasteurized ice cream could be due to their ability to survive the pasteurization process as the case with spore - formers and they may persist in ice cream product thereafter (Ojokoh, 2006). Time - dependent heating during ice cream production reduces largely the vegetative forms of microorganisms. On the

other hand, spore - bearing microorganisms may constitute health hazards through consumption of this kind of milk products. Coliforms were historically used as indicator microorganisms to serve as a measure of faecal contamination and the presence of enteric pathogens in food. *Escherichia coli* is of interest since its presence is suggestive of recent faecal contamination with the possibility of accompanying presence of enteric pathogens (Osamwonyi, 2011). Contributing factors to ice cream contamination includes poor sanitary practices during processing, use of contaminated ingredients; particularly post - process contamination and improper storage temperatures (Kanbakan, 2004).

Ice cream products are usually named by the dominant additives added to the basal plain. Thus, chocolate ice cream requires the addition of considerable quantity of cocoa or chocolate, vanilla flavour to the vanilla type, so also the banana ice cream (Ojokoh, 2006).

Food safety and quality problems caused by microbial, chemical or physical contamination of foods tend to be expensive, particularly if these result in costly consumer recalls. Apart from traditional measures of food safety and quality control, potential contamination can be alleviated by "build-in" hygiene into the food processing equipment and utilities from the start. Furthermore, good hygienic design of closed equipment and components may ensure compliance with compelling national and international food safety legislation as well as food safety management systems built on the well-known concepts of Good Manufacturing Practices (GMPs), Hazard Analysis Critical Control Points (HACCP) and pre-requisite food safety and quality programmes (Moerman, 2017). The HACCP concept is a scientific approach to assess hazards associated with food production and establish control systems to ensure food safety (FAO, 1997).

Classical quality control methods which only emphasized on hygienic quality of final products are inadequate to control hazards occurring at early stages of the production process. In order to provide safe food and prevent foodborne illness outbreaks, HACCP programmes are recommended. The implementation of traceability and HACCP concepts under ISO 22000 standards in dairy food processing has proven to be a novel technology in tracking and tracing ice cream products thereby enhancing the microbiological quality of ice creams (Allata *et al*., 2017).

MATERIALS AND METHODS

Sample Collection

The samples obtained were made up of six brands and one - litre of each sample was used, namely; (Sc), (Sv),(Gsc), (Phc), (Phv) (Gsv) ice cream were obtained from retail outlets in various locations in Lagos, Nigeria.

Microbiological analysis

The ice cream samples were allowed to defrost at 28±2 ºC for 6 hours in sterile covered beakers. Total aerobic cell count was carried out using the serial dilution method. Thereafter, 10ml of each sample was measured aseptically with a sterile micropipette and transferred into 90ml of distilled water and homogenized by vortex. Subsequent serial dilutions up to 10^5 were made in duplicates. The enumeration of microorganisms in the sample was by pour plate technique. Nutrient agar was used for bacteria, MacConkey agar for Coliforms and potato dextrose agar to which streptomycin (0.14g/L) was added for fungi. The plates were allowed to set and incubated inversely aerobically at 37 ºC for 48 hours for bacteria while fungi plates were incubated at 25 ºC for 3 - 5 days. The resultant microbial colonies were counted at the end of the incubation period (Smibert and Krieg, 1981; Sospedra *et al*., 2013). The viable counts per ml was calculated by multiplying the average numbers of colonies by the reciprocal of the dilution and reported as colony forming unit per milliliter (cfu/ml). Bacterial isolates were identified by cultural, morphological characteristics as well as biochemical test and utilization of sugars in accordance with methods of Buchanan and Gibbons (1974).

Characterization and identification of bacterial isolates

Pure cultures of bacterial isolates were identified on the basis of their morphology and biochemical characteristics. The organisms were subsequently characterized according to the taxonomic scheme of Buchanan and Gibbons (1974). The following tests were performed on each isolate.

Colonial morphology

The shape, size, pigmentation, elevation, and marginal characteristics of the bacterial species were examined on Nutrient agar plates after appropriate incubation period (Smibert and Krieg, 1981).

Biochemical reactions

The biochemical reactions conducted in order to characterize the isolates were; Gram stain reaction, Motility test, Catalase production test, Oxidase test, Indole

production test, Citrate utilization test, Coagulase test, Urease test, Methyl-red / Voges-Proskauer test, Carbohydrate utilization test, Growth on MacConkey agar (Smibert and Krieg, 1981).

RESULTS

The mean total aerobic mesophilic count of the respective ice cream samples are presented in Table 1 while Table 2 showed the type of bacteria isolated from various ice cream brands. It was observed that Sc, Ph and Gs brands had *Klebsiella* species, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus* species while Sv brand had *Enterobacter* species amongst others.

The percentage frequency of occurrence of the bacterial isolates are shown in Figure 1 with *Staphylococcus aureus* having the highest percentage frequency of occurrence (100%), *Klebsiella* species and *Bacillus* species having 66% while that of *Escherichia coli* was 50% . Fungi (yeast and moulds) were not detected in all the ice-cream samples using potato dextrose agar supplemented with streptomycin at 0.14g/L.

Table 1: Total aerobic mesophilic microbial counts in ice cream

Samples	Bacteria count (cfu/ml)	Fungi count (yeast & mould)
Sc	1.14×10^3	Nil
Sv	1.38×10^4	Nil
Gsc	1.16×10^4	Nil
Gsv	1.22×10^4	Nil
Phc	7.8×10^3	Nil
Phv	6.0×10^4	Nil

Table 2: Micro-organisms isolated from different brands of ice cream

Brand	Bacteria
Sc	*Klebsiella species*
	Escherichia coli
	Staphylococcus aureus
	Bacillus species
Sv	*Staphylococcus aureus*
	Klebsiella species
	Enterobacter species
Gsc	*Staphylococcus aureus*
	Klebsiella species
	Escherichia coli
	Bacillus species
Gsv	*Staphylococcus aureus*
	Escherichia coli
	Bacillus species
Phc	*Bacillus species*
	Klebsiella species
	Staphylococcus aureus
Phv	*Staphylococcus aureus*
	Bacillus species

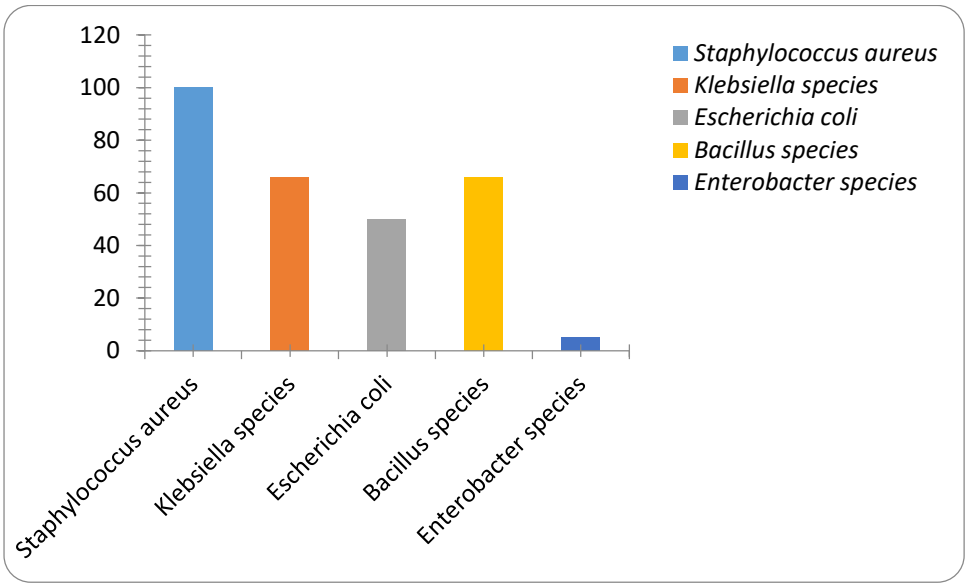

Fig. 1: Percentage (%) frequency of occurrence of bacterial isolates

Table 3: Morphological and Biochemical characterization of isolates

Isolates	Pigment	Gram Reaction	Catalase	Oxidase	Coagulase	Motility	Indole	Citrate utilization	Methyl red	Voges Proskauer	Urease	Glucose	Lactose	Sucrose	Maltose	Fructose	Mannitol	Probable Organism
I	Cream	+ Rods	+	+	-	+	-	+	-	-	-	+	+	+	-	+	+	*Bacillus cereus*
II	Pink	- Rods	+	-	-	+	+	-	+	-	-	+	+	+	+	-	+	*Escherichia coli*
III	Pink	- Rods	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	*Klebsiella pneumoniae*
IV	Pink	+ cocci	+	-	+	-	-	-	-	-	+	+	+	+	+	-	+	*Staphylococcus aureus*
V	Pink	- Rods	+	-	-	+	-	-	-	+	-	+	+	+	+	-	+	*Enterobacter aerogenes*

Key: - = Negative + = Positive

DISCUSSION

Ice cream, which is a milk product, is consumed globally and most consumptions occur outside homes and amongst children during the summer months in temperate countries and all year round in tropical countries (Kanbakan, 2004). Ice cream is also an excellent medium for the growth of many microorganisms and thus its bacteriological quality has always been crucially important to public health. This study particularly discovered food borne pathogens in randomly collected ice cream products from retail outlets which are of public health significance (Table 2). This corroborated the findings of Hesham (2012) and Garcell *et al*. (2016).

The result obtained from the microbial quality assessment showed that ice cream products are contaminated by both Gram positive and Gram negative bacterial species such as *Klebsiella* species, *Escherichia coli, Staphylococcus aureus, Bacillus* species and *Enterobacter* species. *Staphylococcus aureus* was present in all the samples analyzed having a 100% occurrence compared with other microorganisms. This is in agreement with the report of Ojokoh (2006) and Anil (2012) which comparatively had reported higher frequency of *Staphylococcus aureus* occurrence to other bacterial isolates. The possible sources of these organisms could be nose where it is commonly found, hands, skin and clothing of handlers (Kanbakan *et al.*, 2003). Coughing, talking and sneezing could also produce droplets which could settle on ice cream products during transportation, distribution and retailing (Ahmed *et al*., 2008).

Bacillus species were isolated from four out of six samples analyzed giving a percentage frequency of 66%. This is in contrast with the findings of Osamwonyi (2011) who reported a percentage frequency of 93.7%. The detection of *Bacillus* species is suggestive of a favourable environment within the ice cream favoring its growth (Ojokoh, 2006). Coliform organisms such as *Klebsiella* species, *Escherichia coli* were also isolated from the analyzed samples. The presence of coliforms most especially *Escherichia coli* has been described as index for food hygiene (Frazier, 1978). However, this may also suggest feacal contamination and the possibility of presence of other intestinal pathogens responsible for a variety of diseases. Coliforms being non - spore formers should be susceptible to pasteurization and their post - pasteurization presence in ice cream products may suggest insufficient heat treatment, unhygienic or low hygienic tools used, contaminated water or good manufacturing practice not being followed (Yaman *et al*., 2006). Microorganisms such as *Salmonella* species, and *Shigella* species were not isolated from any of the ice cream analyzed. This is in agreement with

the reports of Osamwonyi *et al*. (2011) but in contrast with reports of Ojokoh (2006) and Joshi (2004) which reported presence of *Salmonella* and *Shigella* in ice cream products. The findings from this study corroborated the fact that consumption of contaminated ice cream and other dairy products by children, elderly people and immunosuppressed patients could be source of acquisition of food - borne diseases (Joshi, 2004; Oliver *et al*., 2005; Melo, 2015). Hence, the need for "built-in" hygiene into the food processing equipment and utilities from the start. Furthermore, good hygienic design of closed equipment and components may ensure compliance with national and international food safety legislation as well as food safety management systems built on well-known concepts of Good manufacturing Practice (GMPs), Hazard analysis critical control points (HACCP) and prerequisite food safety and quality programs(Daniels *et al*.,2002; Moerman, 2017), that guarantees both traceability and effective implementation of these quality control schemes (Allata *et al*., 2017).

CONCLUSION

Ice cream is a popular and nutritionally enriched congealed dairy product, its microbial quality has always been crucially important to public health. The presence of *Escherichia coli* in ice cream products show the possibility of acquiring food borne pathogens by infants, children, elderly and immunosuppressed people. *Escherichia coli* is described as an index for fecal contamination and its presence is suggestive of the possibility of presence of other intestinal pathogens.

RECOMMENDATION

It is recommended that relevant government regulating agencies should continue to monitor local companies which produce this dairy product. The quality of raw materials, processing devices and packaging process as practiced by these organizations should be scrutinized repeatedly. Vendors should be educated on the importance of personal hygiene and the need for proper handling of ice cream. Consumers should always insist on purchasing properly stored products since the problem of bacteriological qualities lie mainly in handling and storage conditions.

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