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Full Length Research Article

BACTERIOLOGICAL QUALITY ASSESSMENT OF ICE CREAM SOLD IN SELECTED EATERIES WITHIN KADUNA METROPOLIS

*Mohammed S.S.D., Wartu J.R. and Akpami J. N.

Department of Microbiology, Faculty of Science, Kaduna State University, Kaduna, Nigeria

*Corresponding Author Email Address mosada78@gmail.com or mohammed.sambo@kasu.edu.ng Phone: +2348035861774

ABSTRACT
Ice cream is a frozen dairy product. Ice cream has an outstanding nutritional quality, but is also an excellent medium for bacteria growth. The study was conducted to evaluate the bacteriological quality of ice creams sold in selected eateries within Kaduna Metropolis. Fifteen (15) samples of ice cream were examined for proximate and bacteriological quality. The proximate analysis were determined for vanilla, strawberry and banana ice cream. Total viable count and coliform count were carried out on the ice cream samples using the pour plate technique, the samples were analysed by culturing on Nutrient, MacConkey and *Salmonella-Shigella* Agar media. Gram staining and biochemical test were carried to identify the organisms. The antibiogram of the selected antibiotics were evaluated against the organisms isolated. The proximate composition of moisture content was high in sample Y with 51.02%; Z sample had the highest ash content with 1.77%; sample Y and Z were both high in protein content with 7.41%. Sample X had a high crude fat content with 10.22%, sample Y was high in crude fibre content with 0.26% and sample Z was high in carbohydrate content with 30.28%. The total viable count ranged from $2.2x10^5$to $24.7x10^5$CFU/mL. The selected eatery from M site had the highest total viable count of bacteria while D had the lowest total viable count. The ice cream samples were contaminated with *E. coli*, *Salmonella*, *Staphylococcus aureus, Klebsiella* and *Shigella* species. *E. coli* and *Staphylococcus areus* which were observed in all the samples obtained from the sampling sites. The *S. aureus* was resistant to Spectinomycin at 10µg concentration. *E. Coli* and *Klebsiella* were more susceptible to the antibiotics used at different concentration. Pefloxacin, Gentamicin and Ciprofloxacin were observed to be the more potent antibiotics at 10µg concentration. The presence of the bacteria isolate lack proper hygienic conditions during preparation, preservation or serving of ice cream. These results suggest that consumption of these ice creams might cause GI disturbances, stomach abscess, diarrhoea and other diseases. The presence of potential pathogens in the ice cream samples revealed the significance of implementation of quality control measures in productive, storage and marketing ice creams thus reducing the public health hazards.

Keywords: Ice cream, proximate, bacteria, antibiogram, quality assessment.

INTRODUCTION

Milk is a nutritious basic food. It is rich in proteins, carbohydrates, fats, calcium, riboflavin, and other B vitamins. Milk and milk products are most popular and widely consumed by people all over the world. There are several kinds of milk products available and among them the most important is ice cream (Senthikumaran, 2014). Ice cream is a frozen dairy product made by freezing the ice cream mix with agitation. It is composed of a mixture of food ingredients like milk products, sweetening materials, stabilizers, colours, flavours, and egg products. Ice cream mix is the unfrozen mixture of the ingredients, consisting of all the ingredients of ice cream with the exception of air and flavouring materials. The composition of ice cream is usually expressed as a percentage of its constituents, for example, the percentage of milk fat, milk solids not fat, sugar, egg solids, stabilizers (which are the compounds added in very small quantities to strongly influence the formation and growth of ice crystal in the ice cream so as to render the product with desired body and texture) and the total solids. Ice cream and related products are classified as frozen dessert (Kalyankar*et al*., 2016) it is sold in packages or in open containers at retail outlets/ice cream colours, the open variety being distributed manually in scoops, cones, or sundaes across the counter. During production, transportation and storage, it may become contaminated with several microorganisms. (Matthew *et al*., 2013). The ingredients of ice cream may be various combination of milk, cream, evaporated or condensed milk, dried milk, colouring materials, flavours, fruits, nut, sweetening, agents, eggs and egg products and stabilizers. Any of these may contribute microorganisms and affect the quality of the product as judged by its bacterial load or its content of various specific species of bacteria. Time dependent heating during the ice cream making reduces largely the vegetative forms of the microorganisms. On the other hand spore bearing microorganisms may well pose risks through consumption of this kind of milk products. Furthermore, the presence of pathogen in ice cream sample is mostly by means of tools and equipment, water; workers, environment, packaging materials and contamination during the transportation and distribution of ice cream (Yaman *et al*., 2008). The presence of microorganisms in ice cream such as *Salmonella* sp*, Staphylococcus aureus, Escherichia coli* have been well documented but examination of pathogenic bacteria like *Bacillus cereus*, *Yersinia enterocolitica*, *Listeria monocytogenes, Brucella* spp*,* and *E. coli* 0157:H7 have been rarely studied. The initial content of the microflora of the raw water has a considerable bearing on the ultimate quality of the product. Heat treatment kills most of the microflora but does not affect the stable toxins. If the mixture is held for a sufficient time at room temperature, the organism gets opportunity to grow and produce enterotoxins. The frozen state of the ice cream gives protection to the disease causing microbes to survive long periods by utilizing the ingredients as food and also freezing temperature extend the life time of the microorganisms by reducing metabolic activities. The contaminating microorganisms include not only saprophytes but also pathogenic forms that cause disease to humanity (Senthikumaran *et al.,* 2014). Ice cream had its origin in Europe and was introduced later in the United States where it developed into an industry. It is widely believed that ice cream

evolved from ice beverage and water ices (Kalyankar *et al*., 2016). Ice cream probably came to the United States with the early English colonists. In 1851, the first wholesale ice cream industry in the United States was established in New York, Saint Louis, Chicago, Washington, and Cincinnati. The ice cream soda was introduced in 1879, and ice cream cone and Eskimo pie were introduced in 1904 and 1921 respectively (Kalyankar *et al.,* 2016). Microbiological quality of ice cream reflects the sanitary conditions during processing and packaging stages and is an indication of food safety. The study was conducted to evaluate the bacteriological quality of ice creams sold in selected eateries within Kaduna Metropolis.

MATERIALS AND METHODS

Study Area

Selected eateries from Barnawa, Sabon Tasha, Unguwan Rimi, Romi, Gonin Gora, Narayi, Television, Tudun Wada, Kakuri, and Kabala Doki of Kaduna Metropolis were used for the study.

Collection of Samples

A total of 50 samples of ice cream were collected from selected eateries and transported in ice to the Microbiology laboratory within an hour of collection and were preserved in the refrigerator prior to the commencement of the analysis.

Proximate Analysis of ice-cream

Determination of Moisture Content

According to the method described by Adeniran and Ajifolokun (2018), moisture content was determined using "Gallenkamp" hot air oven method. Exactly 5g of the ice cream sample was weighed into porcelain dish of known weight and was heated in a "Gallenkamp" hot air oven at 105^{o}C for 3hours. The ice cream sample was cooled in desiccators and weighed. The sample was subsequently heated, weighed and cooled until a constant weight was attained.

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$$\% \text{ Moisture content} = \frac{\text{loss in weight of sample}}{\text{Original weight}} \times 100$$

Determination of Ash Content

Ash content determination was carried out by using the method which defines ash as the inorganic residue remaining after the organic matter has been burnt. Ash content of sample was determined using AOAC According to the method described by Adeniran and Ajifolokun (2018), moisture content was determined using "Gallenkamp" hot air oven method. Exactly 2g of well mixed ice cream sample was weighed into an ignited, cooled and weighed crucible. Few drops of glycerol was added and mixed thoroughly with the sample and was heated gently over a Bunsen burner until the sample charred. The crucible was transferred into a muffle furnace at about 550^{o}C until a white grey ash was obtained. The crucible was cooled in desiccators and reweighed according to the method described by Adeniran and Ajifolokun, 2018, the percentage ash content was calculated as;

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

Determination of Protein Content

According to the method described by Adeniran and Ajifolokun, (2018), about 2g of each ice cream sample was weighed into a digestion tube. Kjedhal tablet (0.8g) was used as catalyst and the samples were digested with 15ml of concentrated sulphuric acid using an automatic dispenser. The digestion tube was placed on a preheated digester at 420℃ for about 30min. in a fume cupboard and digested until a clear homogenous mixture was obtained. After digestion, the digestion tube was removed, cooled and diluted with about 50ml of distilled water. The digestion tube was placed into a micro-kjedhal analyzer (digestion this was heated up to liberate ammonia which was distilled into a conical flask containing unit). The analyzer was dispensed; 50ml of 40% NaOH into the digested sample and 25ml of 2% boric acid for about 4min. The % nitrogen was calculated as:

$$\% \text{ Nitrogen} = \frac{0.28 \times A}{\text{Weight of sample in gram}}$$

Where A = volume (ml) of 0.1 M H_2SO_4

Determination of Crude Fat Content

According to the method described by Adeniran and Ajifolokun, (2018), exactly 2g of the ice cream sample was weighed into a free extraction thimble and plugged lightly with cotton wool: the thimble was placed in the extractor and fitted up with reflux condenser and a 250ml soxhlet flask which has been previously dried in the oven, cooled in desiccators and weighed. The soxhlet flask was then filled to two-thirds of its capacity with n-hexane and boiled on a heating mantle. The heater was put on for 6hours with constant running water from the tap for condensation of hexane vapor.

$$\% \text{ Ether extract} = \frac{\text{Final weight of flask} - \text{initial weight of flask}}{\text{Sample weight}} \times 100$$

Determination of Crude Fiber Content

The fat free extract obtained after ether extract that has been collected was placed in a beaker and 700ml boiling 1.25% sulphuric acid was added. The beaker with its content was heated for 30min. while been rotated periodically to remove adhered solids to its sides. Therefore, the solution was filtered and rinsed with 50ml boiling water. This was repeated with three 50ml boiling portions of water and subsequently sucked dry. The entire residue was removed and replaced in a beaker with boiling 1.25% NaOH was added. It was boiled again and was washed with 25ml boiling 15 sulphuric acids, three portions of 50ml water and 25ml of ethanol. The residue was transferred into an ashing dish and dried at 130℃. The dish was cooled in desiccators and weight was taken. The residue was thereafter ignited at 600℃ for 30min, cooled in desiccators again and was reweighed according to the method described by Adeniran and Ajifolokun, (2018), the percentage crude fibre is thus calculated;

$$\% \text{ Crude fibre} = \frac{\text{weight of residue} - \text{weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of Carbohydrate Content

The carbohydrate content of the ice cream sample was calculated by difference, a method as described by Adeniran and Ajifolokun, (2018) .The total of all the previously proximate parameters was subtracted from 100 represent the carbohydrate content;

% Carbohydrate = 100% - (%moisture + %crude protein + % crude fat + %crude fibre + %ash).

Media Preparation

Nutrient Agar, MacConkey Agar Plate Count Agar and *Salmonella-Shigella* Agar were prepared according to manufacturer's instructions. Nutrient agar was prepared by weighing 5.6g of powder in 200ml of distilled water and was allowed to stand for 10 minutes; it was mixed and then sterilized by autoclaving at 121^{o}C for 15 minutes to obtain a pure culture of the isolates (Gordon and Lowy, 2008). Exactly 11.1g of MacCokey Agar was dissolved in 500 ml of distilled water. The manufacturer's instruction was followed as a template in preparing the required quantity of the media (Liu, 2009). While 12.6 g of *Salmonella-Shigella* Agar was dissolved in 500ml of distilled water and mixed thoroughly. The media was heated with frequent agitation and boiled for one minute; it was then allowed to cool down and then poured into the Petri dishes (Liu, 2009).

23.5 g of PCA was dissolved in 1 L of distilled water and mix thoroughly. It was heated with frequent agitation and boiled for one minute to completely dissolve the powder. It was then autoclaved at 121^{o}C in the autoclave for 15 minutes (Difco and BBL Manuel).

Microbiological Examination of Ice Cream

Isolation of Bacteria from Ice Cream

In serial dilution 25ml of ice cream was aseptically transferred into 225 ml of distilled water and homogenized by vertex. Subsequent serial dilutions was be made up to 10^{-5}.1 ml sample was cultured on Nutrient agar (Oxoid) and incubated at 37^{0}C for 24 hours. The total number of colonies was counted after culturing for 24 hours. The bacterial morphology was observed and identified. Separated colonies were sub-culture on freshly prepared Nutrient agar, MacConkey (Oxoid) and *Salmonella Shigella* Agar (Oxoid) plates (Khater, 2005).

Enumeration of Total Bacteria from Ice Cream

The method described by Khater (2005) was used. From each dilution, 1 ml sample was aseptically transferred into sterile Petri dishes in duplicate, followed by adding 10 ml of standard plate count agar at 45-46°C. The Petri dishes were covered and mixed by gentle rotation then allowed to solidify. The plates were inverted and incubated at 37ºC for 48 hours. The developed colonies were counted using colony counter, plates 25-250 or less than 25 colonies were selected. The average number of colonies in each dilution was multiplied by the reciprocal of the dilution factor and recorded as colony forming units/ gm (Khater, 2005).

Enumeration of Coliform Bacteria from Ice Cream

The method described by Khater, (2005) was used. From each dilution, 1 ml sample was aseptically transferred into sterile Petri dishes followed by addition of 10 ml Mac Conkey Agar medium at 44-46°C. The contents were allowed to solidify (5-10 minutes) on a leveled surface, then additional 3 ml Mac Conkey Agar were added to each Petri dishes as on overlay to completely cover the surface of the solidified medium to inhibit surface colony formation. The plates were then inverted and incubated at 37°C for 48 hours. The number of dark red colonies measuring ≥0.5 mm in diameter on (15-150 CFU/ML) plates were counted and resulted were recorded as follows (Khater, 2005).

Characterization and Identification of Bacteria Isolates from Ice Cream

Gram Staining

Crystal violet was added to the surface of a glass slide having a fixed smear of the isolate and allow to stand for 30 seconds, and rinsed with distilled water, Lugol`s iodine was then then added and allowed to stand for 30 seconds. The glass slide was afterwards decolorized with acetone-alcohol for 2-3 seconds and rinsed with distilled water followed by the application of a counter stained (safranin) for 30 seconds, and rinsed with distilled water, air dried and examined under microscope using oil Immersion objective lens. Gram-positive bacteria appear purple, while Gram-negative bacteria appear red (Asghar, 2014).

Catalase Test

One drop of 3% of hydrogen peroxide was placed on a clean slide using a sterile wire loop. A reasonable colony of the isolate was then collected and placed on the top of the hydrogen peroxide. Production of gas bubbles indicates the presence of catalase, hence a positive catalase test.

Oxidase Test

A piece of filter paper will be placed on a glass slide. The filter paper will be soaked with freshly prepared oxidase reagent and the isolate was picked with a sterile glass rod and streaked across the filter paper. A development of blue-purple colour within a few seconds indicated positive result (Senthikumaran *et al.,* 2014)

Coagulase Test

Two drops of human plasma was placed on two different spot on a slide. The first drop was inoculated with the test organism. The second was inoculated with *Staphylococcus aureus* as control (positive). The slide was then rocked for one (1) minute after which the test microorganism spot was compared with the positive and negative controls. A clumping of the plasma indicates a positive coagulase test (Senthikumaran *et al.,* 2014).

Methyl-Red Test

A colony of the test organism from EMBA was inoculated in 0.5 mL of sterile glucose phosphate broth. After overnight incubation at 37°C, a drop of methyl red solution was added. Appearance of a bright red color was taken as a positive methyl red test (Senthikumaran *et al.,* 2014).

Voges-Proskauer Test

Two drops each of alpha-naphthol and potassium hydroxide were added to the glucose phosphate broth (Voges-Proskauer broth) which has been inoculated with bacteria. The test tube was observed for colour change. A cherry red colour indicates a positive result, while a yellow-brown colour indicates a negative result. A reversal in the order of the reagents being added may result in a weak-positive or false-negative reaction

(Senthikumaran *et al.,* 2014).

Triple Sugar Iron Agar (TSI)
Sterile straight wire loop was used to pick a well-isolated colony. The TSI was inoculated by first stabbing through the center of the medium to the bottom of the tube and then streaked on the surface of the agar slant. The cap of the tube was left loosely before it was incubated at 35^0C in ambient temperature for 18 to 24 hours. Result; H_2S production shows blackening. H_2S: *Salmonella typhi.* (Senthikumaran *et al.,* 2014).

Indole Test
The test organisms were inoculated into the tubes of peptone water (5ml) and one test tube was left un-inoculated as control. The tubes were then incubated at 37°c for 48 h. After incubation 1 ml of KOVAC's reagent was added to all the tubes including the control. The tubes were shaken gently and allowed to stand for 1-2 min after which they were observed for formation of cherry red ring. Indole production was detected by KOVAC's or Ehlrich's reagent, which contains p-dimethylaminobenzaldehyde that reacts with indole to produce a red colored compound.

Antimicrobial Susceptibility Testing
Mueller Hinton Agar was prepared according to the manufacturer's instructions. Susceptibility testing was conducted using disk diffusion technique on Muller Hilton Agar (MHA) plates as described by NCCLS (2009) and Ali *et al.* (2013). Inoculation was carried out by dipping a sterile swab into the inoculums suspension adjusted to turbidity of 0.5 McFarland standards (10^6cells/ml) and the agar surface was streaked across in four directions. Gram negative antibiotic disc was then placed on the streaked media after which the plate was incubated at 37°C for 24 hours.

RESULTS

Proximate Composition of Ice Cream
Table 4.1 showed the proximate composition of ice cream samples sold within Kaduna metropolis. Vanilla ice cream, Strawberry ice cream and Banana ice cream showed the proximate compositions for moisture content as 50.26%,51.02%,50.18% for sample X, Y, Z respectively, Ash content were 1.76%,1.54%,1.77% for sample X, Y, Z respectively, Protein content were 7.39%,7.41%,7.41% for sample X, Y, Z respectively, Crude fat content were 10.22%,9.99%,10.13% for sample X, Y, Z respectively, Crude fiber content were 0.24%,0.26%,0.23% for sample X, Y, Z respectively and carbohydrate content 30.13%,29.78%, 30.28% for sample X, Y, Z respectively.

Total Viable Count and Coliform Count of Ice Cream
Table 4.2 showed result of total viable count (CFU/ml) and coliform count (per 100 ml) of ice cream sold at Kaduna Metropolis within selected eateries at Barnawa, Sabon Tasha, Unguwan Rimi, Romi, Gonin Gora, Narayi, Television, Tudun Wada, Kakuri, and Kabala Doki. Each having a code from A-O with values (4.5, 10.3, 9.6, 2.2, 8.3, 13.1, 11.4, 22.9, 3.4, 5.2, 7.5, 11.2, 24.7, 4.0 and 4.9) X 10^5 CFU/mL respectively. Sample site M had the highest total viable count of $24.7x10^5$CFU/mL from Kakuri and sampling site D had the lowest total viable count of $2.2x10^5$CFU/mL from Romi.

Morphological and Biochemical Characterization of Isolates from Ice Cream Sold Within Selected Eateries in Kaduna Metropolis
Table 4.3 showed the Gram reaction and biochemical characteristics of the isolates from ice cream within Kaduna metropolis. The organism showed characterization of either cocci or bacilli shape and gram reaction of either positive or negative with biochemical characterization of catalase, `e, oxidase, methyl-red, Voges-Proskauer and triple sugar iron agar. A total of five (5) bacteria species namely *E. coli, S. aureus, Salmonella* sp*, Shigella* sp *and Klebsiella* sp were identified from vanilla, strawberry, and banana ice creams.

Antibiogram of Selected Antibacterial against the Bacterial Isolates
Table 4.4 showed the antibiogram of selected antibacterial against the bacteria isolates from ice cream sold within Kaduna Metropolis in selected eateries. The selected antibacterial drugs Augmentin (30μg) inhibited the growth of *S. aureus* with the zone of inhibition of 13mm, *E. coli* with 13mm, *Salmonella* with 06mm, *Shigella* with 06mm, and *Klebsiella* sp with 13mm zone of inhibition. Chloramphenicol (30μg) inhibited the growth of *S. aureus* with 12mm*, E. coli* with the zone of inhibition 15mm, *Salmonella* with 11mm, *Shigella* with 11mm, and *Klebsiella* with 12mm zone of inhibition. Pefloxacin (10μg) inhibited the growth of *S. aureus* with the zone of inhibition 15mm, *E. coli* with 19mm, *Salmonella* sp with 24mm, *Shigella* sp 16mm and *Klebsiella* sp with 20mm zone of inhibition. Tarivid (10μg) inhibited the growth of *S. aureus* 19mm, *E. coli* 16mm, *Salmonella* sp 20mm, *Shigella* 06mm, and *Klebsiella* sp with 14mm. Septrin (30μg) inhibited the growth of *S. aureus* with 13mm*, E.coli* with 18.0mm, *Salmonella* with 13mm, *Shigella* sp with 23mm and *Klebsiella* 26mm. Gentamycin (10μg) inhibited the growth of *S. aureus* with the zone of inhibition 19mm, *E. coli* with 18mm, *Salmonella* sp with 19mm, *Shigella* sp with 22mm and *Klebsiella* sp with 21mm. Ciprofloxacin (10μg) inhibited the growth of *S. aureus* with the zone of inhibition 17mm, *E. coli* with 23mm, *Salmonella* with 20mm, *Shigella* sp with 23mm and *Klebsiella* sp with 14mm, Amoxicillin (30μg) inhibited the growth of *S. aureus* with the zone of inhibition of 14mm, *E. coli* with 19mm, *Salmonella* sp with 11mm, *Shigella* with 06mm and *Klebsiella* sp with 14mm, Streptomycin (20μg) inhibited the growth of *S. aureus* with the zone of inhibition of 15mm, *E. coli* with 13mm, *Salmonella* sp with 19mm, *Shigella* with 06mm and *Klebsiella* sp with 19mm and Spectinomycin (20μg) inhibited the growth of *S. aureus* with the zone of inhibition 11mm, *E. coli* with 18mm, *Salmonella* sp with 15mm, *Shigella* sp with 15mm and *Klebsiella* sp with 18mm zone of inhibition. Most of the isolates were resistant to Augmentin, Chloramphenicol, and Amoxacilin.

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Table 1: Proximate Composition of Selected Ice Cream from Selected Eateries within Kaduna Metropolis

Parameters	Sample		
	X	Y	Z
Moisture content (%)	50.26	51.02	50.18
Ash contents (%)	1.76	1.54	1.77
Protein content (%)	7.39	7.41	7.41
Crude fat content (%)	10.22	9.99	10.13
Crude fiber content	0.24	0.26	0.23
Carbohydrate content (%)	30.13	29.78	30.28

X = Vanilla Ice cream
Y = Strawberry Ice cream
Z = Banana Ice Cream

Table 2: Total Viable Count and Coliform Count from Ice Cream($X10^{-4}$)

S/No	Sample site	Sample code	Total Viable Count (CFU/ml) X 10^5	Coliform count
1	Barnawa	A	4.5	3
2	Sabon Tasha	B	10.3	12
3	Tudun Wada	C	9.6	6
4	Romi	D	2.2	3
5	Gonin Gora	E	8.3	5
6	Narayi	F	13.1	11
7	Television	G	11.4	32
8	Ungwan Rimi	H	22.9	66
9	Sabon Tasha	I	3.4	15
10	Kabala Doki	J	5.2	25
11	Sabon Tasha	K	7.5	4
12	Barnawa	L	11.2	7
13	Kakuri	M	24.7	12
14	Ungwan Rimi	N	4.0	3
15	Barnawa	O	4.9	5

Table 3: Characterization of Bacteria Isolates from Ice Cream

Gram Reaction	Catalase	Indole	Oxidase	MR	VP	TSI			Probable Organisms
						H_2S	Slant	But	
+cocci	+	-	-	-	+	-	R	R	*Staphylococcus aureus*
-rod	+	V	-	+	-	-	R	R	*Shigella* sp.
-rod	+	+	-	+	-	-	R	R	*Escherichia coli*
-rod	+	-	-	+	-	+	Y	Y	*Salmonella* sp.
-rod	+	-	-	-	+	-	R	R	*Klebsiella* sp.

Keys: +: Positive, -: negative, V: Variable, VP: Vogues Proskauer, R: Red, Y: Yellow, sp : species

Table 4: Antimicrobial Activity of Selected Antibiotics against Bacteria Isolates from Ice Cream

Organisms	Zone of Inhibition(mm)									
	AU (30µg)	CH (30µg)	PEF (10µg)	OFX (10µg)	SXT (30µg)	CN (10µg)	CPX (10µg)	AM (30µg)	S (20µg)	SP (10µg)
S. aureus	13	12	15	19	13	19	17	14	15	11
E. coli	13	15	19	16	18	18	23	19	13	18
Salmonella	06	11	24	20	13	19	20	11	19	15
Shigella	06	11	16	06	23	22	23	06	06	15
Klebsiella	13	12	20	14	26	21	14	14	19	18

Keys; Augmentin (AU), Chloramphenicol (CH), Pefloxacin (PEF), Tarivid (OFX), Septrin (SXT), Gentamycin (CN), Ciprofloxacin (CPX), Amoxicillin (AM), Streptomycin (S), Spectinomycin (SP).

Sensitive: 12 mm and above
Intermediate: 7mm to 11 mm
Resistant: 0 mm to 6 mm

DISCUSSION

The result obtained in this study represent the current status of bacteria quality of ice cream being sold within Kaduna metropolis. The moisture content of the ice cream sample were found to be 50.26% for vanilla, 51.02% for strawberry and 50.18% for banana. According to Goff (2008) the moisture content of ice cream ranged between 55% -64% which comes from the milk or other ingredients. The ash content of vanilla ice cream were found to be 1.76%, 1.54% for strawberry ice cream and 1.77% for banana ice cream, this implies that banana flavor has a higher ash content. Ash content is an indication of mineral content of the ice cream samples as reported by Umelo (2014). The Ash content, Protein content, Crude fat content, Crude fiber content and Carbohydrate content did not fall within the stipulated range as reported by (Khater, 2005). According to Indian Food Safety Standards and Regulations (2011), total viable count (TVC) of ice cream should not exceed 2.5 x 10^5CFU/ml. In the present study, it was observed that the TVC of the ice cream ranged from 2.2 x 10^5 – 24.7x10^5CFU/ml, with Kakuri having the highest Total Viable Count and Romi having the lowest total viable count. Nine (9) of the samples tested were not within the BIS limits for total viable count, thereby indicating high levels of contamination and higher risk of infection. The bacteria counts in ice cream samples may have resulted from inadequate processing, such as initial improper cooling of the hot ice cream mix, which may lead to multiplication of microorganisms present in ice cream immediately after pasteurization as reported by Ojokoh (2006). In the present study the maximum coliform count was 66/ml and the minimum coliform count was 3/ml, which did not exceed 100/ml as reported by the India Food Safety Standards and Regulations (2011).The presence of coliform organisms is taken as an indication that other pathogenic organisms may also be present in the sample as reported by Jadhav and Raut (2014). The presence of coliforms in ice cream samples may indicate insufficient heat treatment, unhygienic or low hygienic materials or tools used, water being contaminated or good manufacturing practice being not followed (Yamen *et al.*, 2006). *Staphylococcus* sp was present in all the ice cream samples analyzed. The possible source(s) of this organisms in ice cream could be from nose where it is commonly

found; hands, skin, and clothing of handlers, coughing, talking and sneezing which produces droplets which could settle on ice cream during transportation, storage and retailing. Coliforms isolated from the samples, being non-spore formers should be susceptible to pasteurization. *Klebsiella, Proteus, Salmonella* and *Staphylococcus aureus* indicates fecal contamination. The presence of this high level transmission of all these bacteria is fecal-oral route and or via common house flies. The results suggest negligence such as poor sanitation during the preparation and/or storage of these products as reported by Ahmed *et al*., (2009). Their post pasteurization presence in ice cream may be due to faulty heat process or to post pasteurization contamination by handlers with poor sanitary practices. The level of presence of these organisms in food has been described as index of food hygiene (Ojokoh, 2006). The presence of other organisms could be attributed to unhygienic conditions during preparation, handling and serving of ice creams. Soft ice creams may also get contaminated if the ice cream preparation machines are exposed to dust and flies. Health education of the vendors and strict implementation of hygienic standards may help to reduce the contamination rates. Ambily and Beena (2012) reported that *Staphylococcus aureus* can survive better in frozen products like ice creams and can elaborate enterotoxins leading to food poisoning outbreaks and the presence of starch and protein are reported to favour enterotoxin production. Isolation of *E. coli* could be due to fecally contaminated water used in milk production and raw materials and storage environment. *Escherichia coli* have been reported to be linked to diarrhea diseases, urethra-cystitis, prostatitis and pyelonephritis (Umofia *et al*, 2014). The findings of Yamen *et al*., (2006) showed that *E. coli* in samples may indicate a lack of good manufacturing practice during the production and ice cream produced in domestic or catering premises may be relatively important vehicles for the causes of gastrointestinal diseases. The presence of *Salmonella* sp. in ice cream may possibly be due to the either fresh eggs or egg powder used in the ice cream production as being stated in previous works. The presence of *Salmonella* sp. may pose a great risk for public health since *Salmonella* outbreaks from ice cream have been reported previously. Most of the isolates were resistant to Augmentin, Chloramphenicol and Amoxacilin. This is because they have become multi resistant to these therapeutic agents, rendering these antibiotics ineffective as treatments of choice for infections caused by these pathogens (Bolaji *et al*., 2018) *S. aureus* was sensitive to the antibiotics except for Spectinomycin. *E. coli* was susceptible to Augmentin, Chloramphenicol, Pefloxacin, Tarivid, Septrin, Gentamycin, Ciprofloxacin, Amoxacilin, Streptomycin and Spectinomycin. *Salmonella* sp was resistant to Augmentin, Chloramphenicol and Amoxacilin. *Salmonella* sp was resistant to Chloramphenicol as reported by Nwinyi *et al*., (2017). The use, misuse and under-use of antibiotics are responsible for resistance development to bacterial antimicrobials worldwide (Bolaji *et al*, 2018).*Shigella* sp was susceptible to Augmentin, Chloramphenicol, Tarivid, Amoxacilin and Streptomycin. *Klebsiella* sp was susceptible to Augmentin, Chloramphenicol, Pefloxacin, Tarivid Septrin, Gentamycin, Ciprofloxacin, Amoxacilin, Streptomycin, and Spectinomycin. Susceptibility of the isolates to the antibiotics can be attributed to non-presence of resistant genes, high concentration of antibiotics or previous non-exposure of antibiotics to the isolates. Sensitivity of most isolates to Pefloxacin, Gentamycin, Septrin, Ciprofloxacin was in agreement with the findings of Damian (2012) who said the effectiveness of Ofloxacin might be attributed to the fact that Ofloxacin is a relatively new antibiotic and has not been extensively used to warrant resistance developing against it pathogens. Antibiotic resistance of isolated bacteria from milk products may be a reflection of the harmful effects of self medication. Many antibiotics have been reported to be persistent in the environment and have been isolated from ground water which could probably be used at times in the preparation of milk products. This could enhance the emergence and spread of bacterial resistance among people who may consume these milk products.

Conclusion

Findings from this work showed that though ice cream is nutritious, it serves as a vehicle for transmission of pathogens. The proximate composition of vanilla, strawberry and banana ice cream were in close range within themselves. Ice cream within selected eateries in Kaduna metropolis showed the presence of *E. coli*, *Salmonella*, *Staphylococcus aureus, Klebsiella* and *Shigella* species. The total viable count was high and some exceeded the Food Safety and Standards Authority guide lines. Most organisms were susceptible to antibiotics, though some of the isolates were resistant to Augumentin, Chloramphenicol and Amoxicillin. The counts of microorganisms above the recommended criteria and the presence of some groups of pathogenic bacteria may pose a risk for public health particularly for children and vulnerable elderly people.

Recommendations

1. Ingredients such as milk, cream and ice-cream mix, should be obtained only from licensed and reputable sources.
2. Ingredients used for ice cream production should be stored at proper temperature (frozen items; - 18^0C or below, chilled items; 0- 4^0C.
3. The use of good quality raw materials and automatic machines to minimize handling will be effective in assuring quality.
4. The quality of ice cream should be monitored. Improper practices in ice cream production, bad hygiene practices, faulty packaging and storage should be discouraged.
5. Adoption of good sanitation practices and application of the HACCP principles in the system along with education of workers on personal hygiene will definitely improve the quality of ice cream.
6. Consumers should purchase ice cream from reputable shops or eateries by observing the hygienic condition of the environment.

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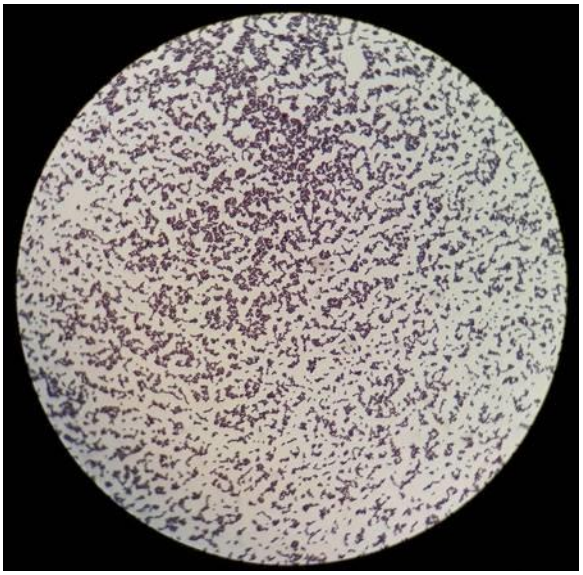
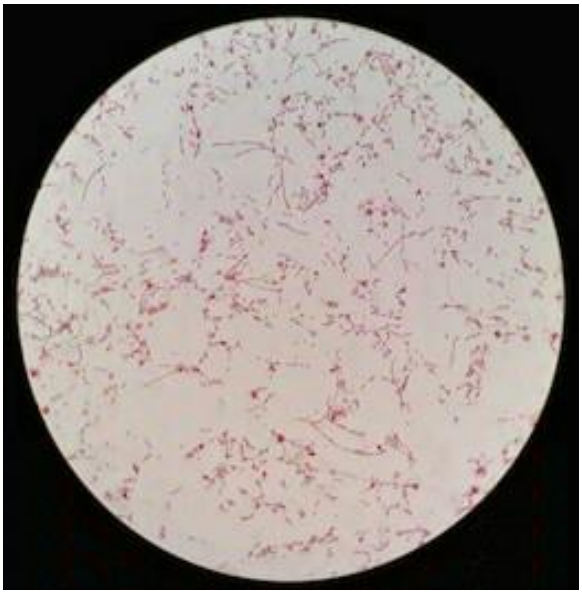
Appendix I: Standard Bacteriological Limits in Ice Cream

S/No	Characteristics	Requirement
1	Total colony count per gram (Standard plate count)	Not more than 250, 000
2	Coliform count	Not more than 100/ml
3	Acidity present (as lactic acid)	Maximum of 0.25

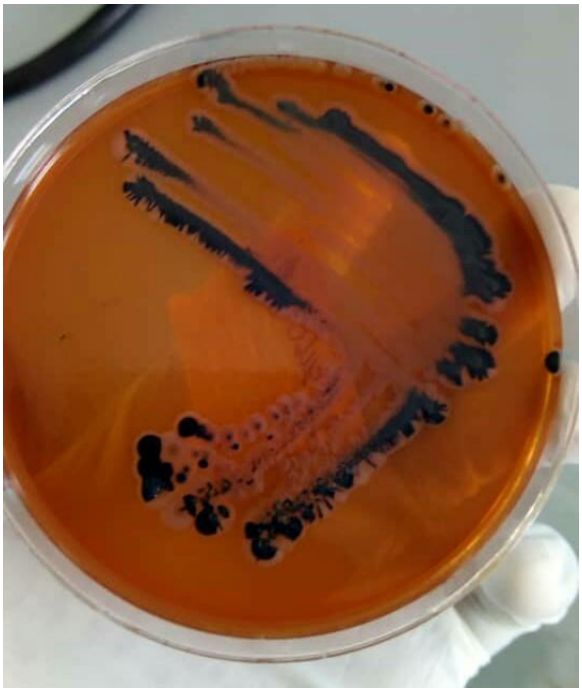
Source: Bureau of Indian Standards, BIS (1981) Cited in Arshi*et al.* (2014).



Appendix II: Microscopic view of *S.aureus* (Gram positive)



Appendix III: Microscopic view of *E.Coli* (Gram negative)



Appendix IV: Colony growth of *Salmonella* Sp (streak method)