

**COMPARATIVE STUDY OF THE MICROBIOLOGICAL QUALITY OF  
ICE CREAM SOLD IN AUCHI, ETSAKO WEST L.G.A, EDO STATE,  
NIGERIA**

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**BEING A PROJECT WORK PRESENTED TO THE DEPARTMENT OF  
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AWARD OF HIGHER NATIONAL DIPLOMA (HND) IN SCIENCE  
LABORATORY TECHNOLOGY.**

**DECEMBER, 2022**

## **CERTIFICATION**

This is to certify that this project titled “**COMPARATIVE STUDY OF THE MICROBIOLOGICAL QUALITY OF ICE CREAM SOLD IN AUCHI, ETSAKO WEST L.G.A, EDO STATE, NIGERIA**

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in the department of Biological Science Laboratory Technology (Microbiology Option), Auchi Polytechnic, Auchi. Having completed my course work, it is accepted in partial fulfillment for the Higher National Diploma (HND) in Biology/Microbiology.

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PROJECT SUPERVISOR

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**DATE**

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**MR. OHIMAI A.**  
(HEAD OF DEPARTMENT)

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**DATE**

## **DEDICATION**

With all sincerity of heart, we dedicate this project work to God Almighty, the giver of life, strength, knowledge, wisdom, understanding and ever loving kindness throughout our stay in school.

## **ACKNOWLEDGEMENT**

All thanks to God Almighty for giving us the grace, wisdom and understanding to complete this project work.

Our special thanks go to our project supervisor Mr. Okosun S.R. for making this project work a successful one, may God bless you Sir.

We sincerely acknowledge our HOD Mr. Anthony Ohimai and to all our lecturers in Biological Science Laboratory Technology for the knowledge they have impacted on us.

Our special appreciation goes to our parents for their prayer and financial support throughout our academic pursuit, may God in His infinite Mercy bless you and keep you save (Amen).

We also want to appreciate our wonderful siblings, family members, course mates and well wishers for their prayers and words of advice throughout our years in school.

## **ABSTRACT**

Ice cream is a delicious dairy product commonly consumed by all age groups especially children. Due to its composition, it can harbor many potent pathogens. Most ice creams become contaminated with microbes during production, transit and preservation. Such contaminated food product can be responsible for food borne infections in children, elderly people and immune-suppressed patients. Therefore, the study was conducted to evaluate the microbiological quality of ice creams sold in different areas of Auch. Three ice cream samples randomly collected and analyzed for total viable count, and fungi count. The result revealed that the mean value of total viable count of the Ice cream samples were  $1.5 \times 10^2$  to  $2.5 \times 10^2$  and  $1.5 \times 10^1$  to  $2.0 \times 10^1$  cfu/g for bacteria and fungi count respectively. *Lactobacillus* spp., *staphylococcus* spp., *Escherichia coli* could be isolated and identified from the examined ice cream samples. It is recommended to launch awareness programs to minimize the contamination of ice cream products.

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# CHAPTER ONE

## INTRODUCTION

### 1.1 Background of the Study

Ice cream is the major dairy product which is one of the favorite food items in large segments of the population. It is a nutritionally enriched frozen dairy product consumed by all age groups particularly children mostly during summer (Sharif *et al.*, 2018). Ice cream is a product of mixture consisting milk, sweetening and stabilizing agents together with flavoring and coloring matter (Graff - Johnson, 2014). Different ingredients like milk, cream, evaporated or condensed milk, dried milk, coloring material, flavors, fruits, nuts, sweetening agents, egg products and stabilizers are used in ice cream preparation. Also, there are many parameters which should be controlled accurately during processing to obtain a high quality ice cream with the required taste, flavor, viscosity, consistency and appearance (Yaman *et al.*, 2006).

Due to its composition, it can harbor many potent pathogens. Handling and storage conditions are some of these variables because they affect the physical, chemical and microbial quality of product (Marshall and Arbuckle, 2017, Warke *et al.*, 2017, Champagne *et al.*, 2020, Daniels *et al.* 2002). Most ice creams become contaminated with microbes during production, transit and preservation. The possibility of acquiring food borne diseases by infant, children, elderly people and immune-suppressed patients is more due to contaminated ice creams. During last few decades, it has been seen that consumption of contaminated ice creams are responsible for disease outbreaks in many countries from Asia, Europe, and North America (Chug, 2017; Djuretic *et al.*, 2018; Digrak and Ozcelik, 2010). Since the consumption of ice-cream is higher among children of vulnerable age groups, there is a need to maintain a high microbiological safety standard (Champagne *et al.*, 2020). It is a known fact that relatively low storage



temperature and pasteurization steps during processing of ice creams are considered to eliminate most of the pathogenic microorganisms. However, the addition of contaminated ingredients, improper handling and the use of improper storage temperatures are the factors that contribute to the potential hazards of the finished products especially during processing after pasteurization processes (Verma *et al.*,2017).

Bacteriological quality of ice cream reflects hygienic practice in production and is an indication of good practice in production. Also, these products are vulnerable to spoilage by certain microorganism, some of which are beneficial and others are harmful to human beings (Esmail, 2018). All types of ice cream, whether machine, canned, car ice cream are present in Auchi. But unfortunately it is not checked by public health authorities. So a hazard of being contaminated is greatest (Esmail, 2018). The richness in nutritive constituents of ice cream has been realized by all; however some hazards may lies between production and handling. So great difficulties with regard to microbial quality of ice cream (Bigalke and Chappel, 2008). Hence, the present study was taken up to determine the microbial quality of packed, open and pop ice cream sold and marketed in Auchi and to assess the potential of these frozen products to pose risk to public health.

## **1.2 Objective of the Study**

The main objective of this study is to investigate the;

1. Microbiological quality of ice cream of packed , open and pop ice cream sold and marketed in Auchi.
2. To assess the potential of these frozen products to pose risk to public health.

### **1.3 Scope of the Study**

The scope of this study is centered on investigation of microbial quality of open and pop ice cream sold and marketed in Auch.

### **1.4 Significance of the Study**

The significance of this project is that it could lead to good quality and contamination free ice cream sold and marketed in Auch. By the time the findings and recommendations are strictly adhered to, the people will be aware of the effects of consuming contaminated ice creams and therefore take necessary precautions.

### **1.5 Limitations of the Study**

Instability/non availability of power supply which was required to carry out the work.

### **1.6 Definition of Terms**

**Microbial:** Relating to or characteristic of a microorganism, especially a bacterium causing disease or fermentation.

**Quality:** The standard of something as measured against other things of a similar kind; the degree of excellence of something.

**Ice Cream:** Ice cream is a sweetened frozen food typically eaten as a snack or dessert. It is usually made from dairy products, such as milk and cream, and often combined with fruits or other ingredients and flavors.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.1 Definition of ice cream:**

Ice cream may be defined as a food prepared with one or more milk products, sweetened with one or more optional saccharine ingredients and contains one or more other ingredients such as egg or egg products, flavoring, fruit juices, confectionery, stabilizer, color or water and the resulting mixture is frozen, while being stirred and is hardened by low temperature (Tressler and Evers, 2017).

#### **2.2 History of ice cream:**

The history of ice cream, in various forms, goes back at least as far the ancient Greeks and Romans, who cooled their wine with mountain snow and ice (Mariani, 2006). Marco Polo brought back from the orient a recipe for a frozen dessert based on milk and there is evidence that some forms of ice cream were brought by Catherine de Medicis from Italy to France. Most of the ice cream during that period was made through a method of beating cream in a pewter pot that was shaken in a larger pot of salt and ice (Mariani, 2006). The first improvement in the manufacture of ice cream was given to us by a New Jersey Woman, Nancy Johnson, who in 1846 invented the hand cranked freezer. This device is still familiar to many who make home made ice cream today (Davidson, 2010).

Ice cream became a popular Luxury food, but almost all of it was made at home until 1851, in where, Jacob Fussel, a Baltimore milk dealer, established the first ice cream plant (Mariani, 2006). Ice cream became a national favorite during the early 1900 after Soda fountains introduced Sodas, Sundaes and other new ways of serving it.

During world war one ice cream was declared an “essential foodstuff” so that its ingredients were not rationed, and by 1919 Americans were eating 230 million gallons of ice cream per year and it became known as an “American typical food”, like hamburgers and hotdogs and in 1940 the figure was up to 318 million gallons (Mariani, 2006). About 1926 the first successful commercially continuous process freezer was perfected. Today multicylinder continuous freezers can turn out over 3800 liters of uniformly frozen ice cream per hour (Potter, 2017).

The ice cream industry as we know it today has been mainly developed in the United States. The development of the ice cream industry can be most quickly told by listing the approximate dates of some important methods of processing and merchandising (Arbuckle, 2010).

The term, “ice cream”, is found in an advertisement which appeared in the New York Gazette of May 19, 1777, by Philip lenzi, who termed himself a “confectioner from London”, which reads as follows: “May be had almost every day, ice cream; like wise, ice for refreshing wines, etc”.

### **2.3 Nutritive value of ice cream:**

The nutritive value of ice cream varies with its composition; however, all the constituents of milk are present in a concentrated form (Eckles and Macy, 2008). To ice cream may be added such materials as eggs, gelatin, fruits, nuts, chocolate, and bakery products, all of which add to its nutritive value. The milk products which go into the mix contain the same constituents as does whole milk, but in different amounts. Milk and its products, such as ice cream, are among the richest sources of calcium, phosphorus, and other minerals of vital importance in building good bones and teeth (Arbuckle, 2010). Small amounts of iron, copper, zinc, aluminum, cobalt,

iodine, traces of silicon and boron are present in milk. The distribution of calcium in foods other than milk and dairy products is not extensive (Eckies *et al.*, 2008).

## **2.4 Ice cream ingredients:**

The fact that ice cream comes in many flavors and types leads a person to believe that ice cream is a complex and confusing product (Varnam and Sutherland, 2017). All ice cream has a general formula, which can be added to or slightly modified to create the desired product. The major constituents (ingredients) in the ice cream formula backbone are milk fat, milk solids not fat, sweetener, stabilizer and/or emulsifiers, water and air (Varnam, 2017).

## **2.5 Microbiology of ice cream ingredients:**

A commercial prepared ice cream must be made under sanitary conditions, as the bacteria can grow easily in milk mixtures (Foster *et al.*, 2017). Many cities and states have no bacterial standards for raw or pasteurized cream. Some ice cream is still made from cream with high total bacterial counts and such cream may be the chief dairy product source of the bacteria in ice cream (Foster *et al.*, 2017). Skim milk powder may, on occasions, contain numbers of *Bacillus cereus* and although this is not often a health hazard, it is preferable for the numbers to be kept as low as possible (Robinson, 2008). Sugar syrup, whether sucrose or mixtures of sucrose and corn syrups again should contain only a few yeasts, but it should be remembered that osmophilic yeasts may be able to grow in these syrups (Robinson, 2008). Stabilizers and emulsifiers rarely present problems, but gelatin as an animal product, may be a hazard and should be obtained from reputable supplies and kept quite cool and dry, as indeed should all the dry materials which are used in ice cream manufacture (Robinson, 2008). The numbers of bacteria which are present in ice cream will depend very largely upon the numbers and types in the raw materials, especially milk, cream, condensed or dried milk (Eckles and Macy, 2008). Ice cream should be made from

high grade raw materials and be handled as carefully as any dairy product, even though low temperatures protect it against deterioration in storage (Eckles and Macy, 2008).

The various Gram negative and Gram positive psychrotrophic species are listed and with respect to pathogenic psychrotrophs, emphasis is given on *Listeria monocytogenes*, *Yersinia enterocolitica* and *Bacillus cereus*. The influence of psychrotropic bacteria on the quality of raw milk, pasteurized milk, UHT milk, butter, ice cream, cheese and milk powders is examined. Methods that can be used to eliminate or control the development of psychrotropic bacteria include low or high temperature, chemicals, gases, the lactoperoxidase system, lactic acid bacteria, micro filtration, bactofugation, lactoferrin related protein, sanitation, flavors and naturally occurring spore germinants.

#### **2.5.1 Potential microbiological hazards associated with ice cream:**

Vasavada (2003) Padhy and Doyle (2002) demonstrated that many bacterial agents are capable of causing diseases or intoxication in a susceptible host through consumption of raw milk or milk products. Ice cream, a milk based products are good media for microbial growth due to high nutritive value, almost neutral pH value (pH 6-7) and long storage duration of ice cream. However, pasteurization, Freezing and hardening steps in the production can eliminate most of the microbiological hazards (Bell and Kyriakides, 2010). Pasteurization is the most commonly applied heat treatment in the dairy industry (Andreasen and Nielsen, 2020). 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 an important control point that further reduces the hazards (Andreasen and Nielsen, 2020). Furthermore they reported that 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 microbiological hazards (ICMSF, 2017). Heat treatment by pasteurization can destroy most of the specific pathogens that pose risk to public health. However, the potential microbiological hazards found in the final products can still be introduced after pasteurization through adding contaminated ingredients and improper handling procedures (Marshall, 2020). This is especially important in the preparation of soft ice cream as its final stage of 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, 2017).

In order to produce ice cream which will not only be a pleasant and nutritious food, but also one which does not present a health hazard, it is necessary to pay attention to a wide range of details (Robinson, 2008). These include careful selection and testing of the raw materials, the use of correct processing conditions in equipment properly cleaned and adequately sanitized and finally satisfactory handling of the product at the sales point. Only a small quantity of ice cream has to be contaminated to produce a hazard for a large number of people and to avoid this, it is essential that all operators are properly trained in every way, that bacteriological control should be carried out carefully and the results acted upon (Robinson, 2008).

### **2.5.2 Major diseases transmitted through ice cream:**

The same dangers of illness caused by drinking raw milk are inherent in ice cream either made from raw milk and cream or handled under unsanitary conditions (Silliker *et al.*, 2007). With few exceptions, outbreaks occurred in recent years have been caused by ice cream made not in commercial establishments but rather at homes where a combination of faulty practices occurred such as use of raw milk, cream and eggs, inadequate heat treatment and contamination (Silliker *et al.*, 2007).

Table (2-1): Hazards and typical control in the production of ice cream:

Process	Hazard	Control measure
Raw materials	Presence of pathogens	<ul style="list-style-type: none"> <li>• Purchase materials from reputable suppliers</li> <li>• Intake testing</li> </ul>
Pasteurization	Survival of the pathogens	<ul style="list-style-type: none"> <li>• Corrected time/ temp. control</li> <li>• Maintenance of equipment</li> <li>• Effective disinfection programme of equipment</li> </ul>
	Recontamination Growth of microorganisms	<ul style="list-style-type: none"> <li>• Hygienic design/ cleaning/ disinfection of equipment and utensils</li> <li>• Temp.: &lt;5°C</li> </ul>
Filling in packaging step	Recontamination	<ul style="list-style-type: none"> <li>• Hygiene design/ environmental hygiene of equipment of utensils</li> </ul>
Addition of ready to eat ingredients (e.g.: fruits, nuts or syrup)	Recontamination	<ul style="list-style-type: none"> <li>• Purchase materials from reputable suppliers</li> <li>• Environmental hygiene of storage area, equipment and utensils</li> <li>• Hygiene of addition practice</li> </ul>
Hardening	Recontamination	<ul style="list-style-type: none"> <li>• Cleaning and disinfection of equipment and utensils</li> </ul>
Storage and transportation	Growth of microorganism	<ul style="list-style-type: none"> <li>• Tem. &lt; - 18°C</li> <li>• Discard the defrosted products</li> </ul>

Source: ICMSF. Microorganisms in foods.

Ice cream has been incriminated as a transmitter of pathogenic bacteria, but out breaks of disease due to commercially manufactured ice cream are rare (Foster *et al.*, 2017). At various times ice cream has been found to harbor typhoid fever, paratyphoid fever, diphtheria and scarlet fever organisms (Foster *et al.*, 2017). Many major food poisoning outbreaks have been caused by human contamination. One, in 1945, was due to staphylococci carried by a worker in the cook house of an army hospital, which were introduced into batches of ice cream mix after the ingredients had been cooked (Robinson, 2008). Moreover around 700 people were affected by a staphylococcal toxin which developed during that period. There are still cases of food poisoning



caused by ice cream reported from overseas, although most countries, in which there is any appreciable production and sale of ice cream, have relatively strict standards and heat treatment requirements (Robinson, 2008). Gastroenteritis caused by Salmonella in ice cream is characterized by abdominal cramps and diarrhea, Vomiting, fever and headache. Antimicrobial therapy is not indicated in uncomplicated gastroenteritis, which typically resolves within one week (Noakes *et al.*, 2019).

### **2.5.3 Storage and distribution:**

The ice cream must stay frozen solid for quality assurance. When the ice cream leaves the factory, it must be stored at a constant, uninterrupted, freezing cycle at low temperatures to avoid problems (Goff, 2018). Problems at retail level can arise from overfilling of the display cabinet, heat from the display lamps or hot air from incorrectly positioned circulation fans or displaying ice cream together with the semi frozen goods (Goff, 2008). Eckies and Macy (2008) mentioned that refrigerated, insulated trucks are commonly used to transport ice cream from the manufacture to the retail distributor. The shelf life of any food commodity should combine the two considerations of safety and organoleptic property of the product. Moreover they reported that it is more economical to ship the products of which ice cream is made to a point from which the finished ice cream can be easily distributed.

## **2.6 Defects of ice cream:**

### **2.6.1 Body and texture defects:**

Body and texture defects include coarse icy texture, which is due to the presence of ice crystals of such a size that is noticeable when the ice cream is eaten. The term body, used in relation to ice cream, refers to the consistency or richness of the product (Peckham, 2021). Desrosier (2018) mentioned that body defects are commonly described as crumbly, soggy and weak, while the common texture defects are coarse, icy fluffy, sandy and buttery. He also

reported that a crumbly body or a flaky, snowy characteristic in ice cream is caused by low stabilizer or emulsifier, low total solids or coarse air cells.

### **2.6.2 Flavor defects:**

Flavor defects can be classified in five different ways. This includes the flavoring system, which is that it lacks flavor or the flavor is too high or that the flavor is the unnatural (Goff, 2018). The dairy ingredient flavor defects include acid, salty, old ingredient, oxidized/metallic, rancid or whey flavor (Smith *et al.*, 2016). The most commonly used system in flavor assessment for ice cream is the dairy ingredient flavor defect system claimed off flavors in butterfat can be carried to second products, such as ice cream and affect consumer acceptance. However, milk fat with a high mono unsaturated fatty acid content compared with a high polyunsaturated fatty acid content did not exhibit oxidation problems.

### **2.6.3 Shrinkage defects:**

A very troublesome defect in ice cream is shrinkage because there appears to be no single cause or remedy (Goff, 2018). This defect shows up in hardened ice cream and manifests itself in reduced volumes of ice cream, usually by pulling a way from the top and/or sides of the container. They also added that structurally, it is caused by a loss of spherical air bubbles and formation of continuous air channels. Goff (2018) indicated that some factors believed to be associated with the defect include that Some emulsifier seem to enhance shrinkage, freezing and hardening, both low and high storage temperatures appear to contribute, ultra smooth ice cream as can be produced in continuous freezer, type of container, partial destabilized protein, season of the year as more shrinkage occurs in winter months and methods of handling in grocery store cabinets.

#### **2.6.4 Color defects:**

Ice cream should possess a pleasing color, if its color is too high or if it lacks color, it is objectionable (Eckles and Macy, 2008). The ideal color is the considered of the flavor, true in shade and neither too pale nor too intense (Arbuckle, 2010). Uniform, natural color is desirable ice cream. An uneven color results if the color is not properly added and also it care is not exercised when changing flavor. Excessive color is the result of adding too much artificial color to the mix. An unnatural color describes defects due to insufficient (pale) color, excess (intense) color and colors that are not characteristics (true in shade) of the flavor (Arbuckle, 2010).

## **CHAPTER THREE**

### **MATERIALS AND METHOD**

#### **3.1 Materials**

The materials use for these study include; incubator, autoclave, sterile test tubes, Sabrose Dextrose Agar (SDA), Beakers, sterile pipette, distilled water, weighing balance, conical flask, measuring cylinder, microscope, slide, Nutrient Agar (NA), cotton wool, petri dishes, Bunsen burner, cool box, wire loop, crystal violet, lugol's iodine, safranin, aluminum foil, acid alcohol, methylated spirit, masking tape, hand gloves, face mask, oil immersion.

#### **3.2 Sterilization of Apparatus**

All apparatus were sterilized to ensure they are free from contamination. The incubator, autoclave and disinfectant were used for this purpose.

#### **3.3 Samples Source and Methods of Collection**

The ice cream samples were obtained from Auchi, Etsako West Local Government Area, Edo State, Nigeria. All the samples were commercially made. they were purchased from Valchi fast food, GT fastfood and Matice. A total of 3 ice cream sample were collected and examined. Samples were transported to the laboratory in a cool box and stored at 20°C prior to examination.

#### **3.4 Preparation of Sample**

The preparation of the samples was carried out in accordance with the method of (Warke et al., 2017). Ice cream samples was kept in water bath at 45 after thawing, complete liquefaction was observed and the sterile pipette could be introduced for collection of samples. A quantity of about 10mls of liquid ice cream was used for serial dilution.

### **3.5 Preparation of Media**

Media used for work are nutrient agar (NA) and PDA for isolation of bacteria and fungi respectively. The NA and PDA were prepared according to manufacturer instruction and sterilized using autoclave for 15 minutes at 121°C. It was then removed and allowed to cool before it was poured into petri-dishes. The petri-dishes were then allowed to set, after which 1ml of ice cream sample from the serial diluted tube was inoculated on the surface of the Nutrient agar and was incubated at 37°C for the period of 18-24 hours while the inoculated PDA was incubated at room temperature for 72 hours (3 days). Each bacteria isolate obtained was counted and estimated according to the method of (Miles and Mistril, 2011) while the fungi colonies were also counted and estimated.

### **3.6 MICROBIAL ANALYSIS**

Samples were analysed for the presence of microorganisms. (Bacteria and fungi). The total viable plate count was done; colonies that developed after the period of incubation was counted as colony forming unit per gram (cfu/g) for sample. Isolated bacteria were identified using cultural, morphological and biochemical tests.

Result obtained was determined and was in accordance with the Beygey's manual of determination bacteriology (1989). The fungi isolated were identified by its cultural and microscopic characteristics. This was done by staining with lactopenol blue and subsequent examination under the microscope using x10 and x40 objective lenses.

### **3.7 Gram Staining**

Discrete colony was taken from nutrient agar plates with a sterile wire loop and emulsified in a drop of normal saline on a grease free slide and smear made.

The smear so made was heat fixed by passing over a flame and allowed to cool, the slide was flooded with crystal violet solution and allowed to cool. The slide was flooded with crystal violet solution and allowed to stay for 30 seconds after which it was washed off with water immediately after 30 seconds. It was flooded with safranin and left for about 30 seconds after which it was washed with water and allowed to air dry.

The stained smear was then examined under the microscope using the oil immersion objective (x100). The morphology and the gram reaction of each isolated was recorded.

A gram positive organism retain the purple colour of the primary stain (crystal violet) while the gram negative organism retain the red colour of safranin.

### **3.7 Bio-Chemical Tests**

#### **3.8.1 Oxidase Test**

Some drop of 1% solution of *tetramethyl-phenylene* thiamin was poured on a whatman filter paper. Pour colony of the test organism was picked using the edge of a clean slide and smear on the filter paper organisms which produce the enzymes *cytochrome oxidase* turn the colour to the filter paper to intense purple when held for 5 to 20 seconds which indicates a positive test.

#### **3.7.2 Indose Test**

This test is used to know the ability of bacteria to release indole from the splitting of the amino acid tryptophan. The test organism was inoculated into 6ml sterile peptone water and incubated for 3hours at 37°C Kovac's reagent about 0.2-0.3ml *dimethylamino benzaldehyde* was added to the culture after incubation the bottles were shaken and allow to stand when later absence in reddish brown ning colouration at the top of the culture indicates positive results.

### **3.7.3 Catalase Test**

This test is used to identify organism that can produces the enzymes catalase. This was done by placing a drop of distilled water clean slide inoculums from the pure culture was emulsified into it, a few drops of hydrogen peroxide was added to the suspension of the organisms production of bubbles indicated a positive test.

## CHAPTER FOUR

### 4.0 Results and Discussion

#### 4.1 Results

Bacteria isolates were identified with reference to Cowan and Steel's manual for the identification of Medical Bacteria (Cowan, 1985) and Bergey's manual of determinative Bacteriology (Holt et al., 2020).

Fungal isolates were identified base on their morphological and cultural characteristics as recommended by Sampson et al (2020).

The result obtained from the total viable plates count is as presented in the tables below:

**Table 1A: Total Viable Bacterial Count of Packed Ice Cream (cfu/g)**

Sample	Count
A	$2.5 \times 10^2$
B	$2.2 \times 10^2$
C	$1.5 \times 10^2$

Where;

A = Ice cream from Valchi

B = Ice cream from GT

C= Ice cream from Matice



**Table 1B: Total Viable Bacterial Count of Open Ice Cream (cfu/g)**

Sample	Count
A	$2.0 \times 10^1$
B	$1.5 \times 10^1$
C	$1.5 \times 10^1$

Where;

A = Valchi Plaza

B = GT ice cream

C= Maticce

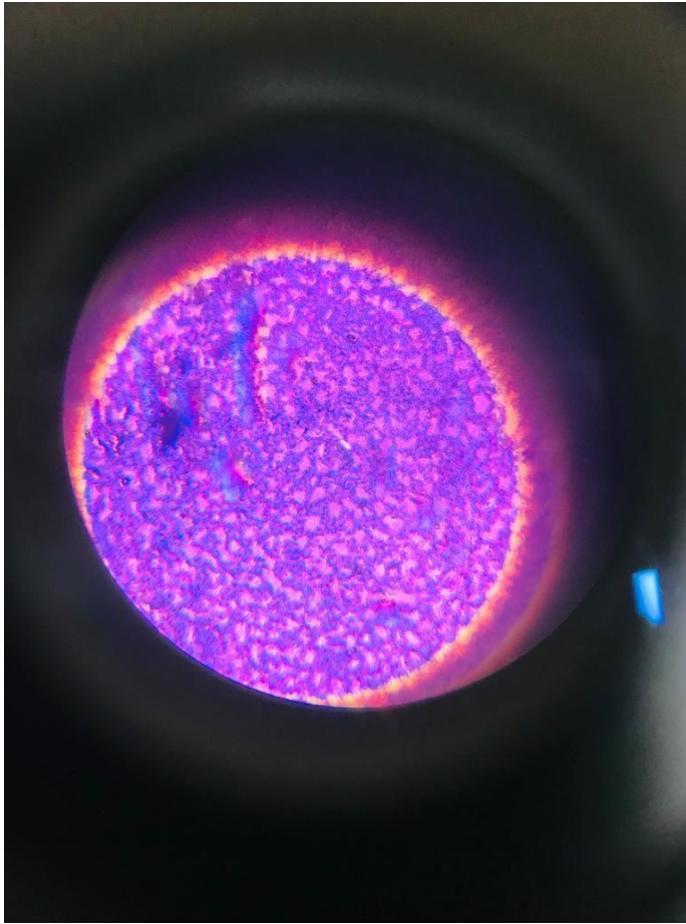
**Table 3: Cultural, Morphological and Biochemical Characterization of Isolates.**

Isolates	Cultural Characteristics	Morphological Characteristics	Biochemical Test			
			Gram stain	Indole	Catalase	Oxidase
Lacto-bacillus spp	Spreading colonies that were creamy white	They appear as rod shape in branches	+	—	+	+
Staphylococcus Spp	Pin point colonies that were creamy in colour, round and smooth with entire margins and convex elevation	Organism appear as cocci, some occurring singly while majority are in grape-like clusters	+	—	+	+
Escherichia coli	Pink colony on MacConkey agar 3-4mm colonies are entire in shape	Straight rod shape that are motile	—	+	+	+

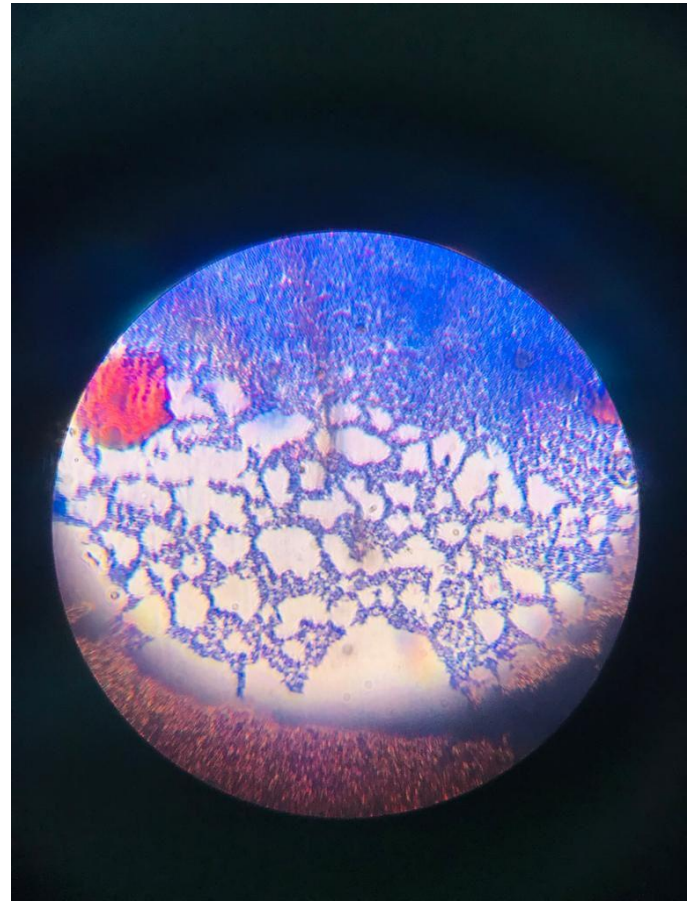
— = Negative

+ = positive

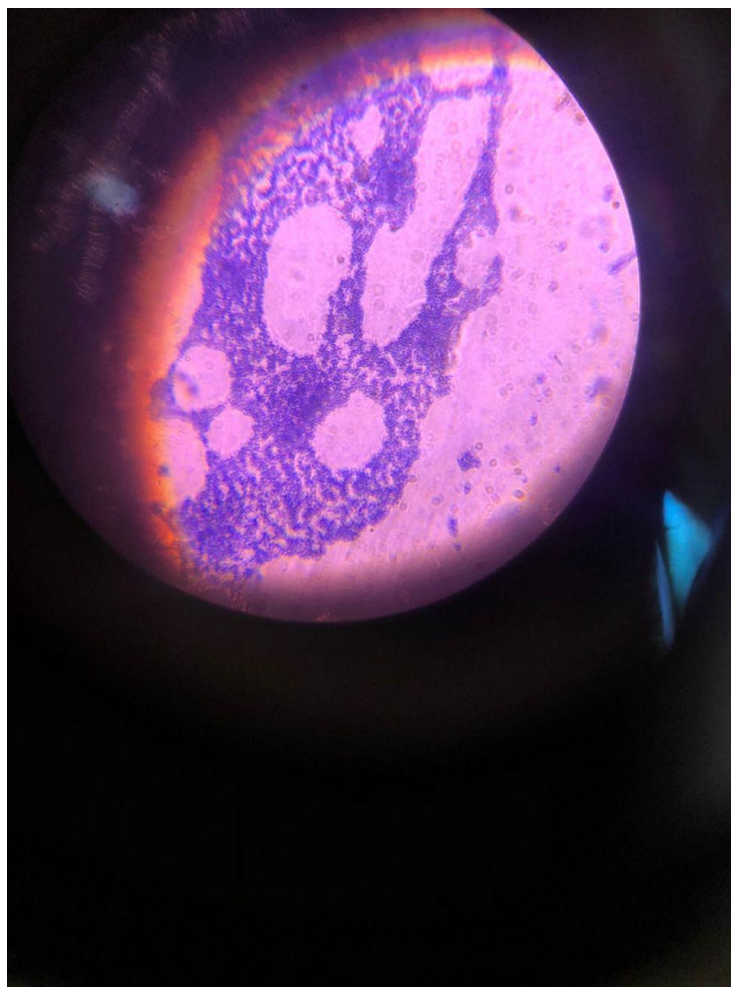
**Fig. 4 Shapes and arrangement observed during microscopy from the samples used**



**Fig 4.1. Sample A**



**Fig. 4.2 Sample B.**



**Fig. 4.3 Sample C**

## **4.2 DISCUSSION**

It has been found that the total viable bacteria count of ice cream used in this research, range from  $1.5 \times 10^2$  to  $2.5 \times 10^2$  (cfu/g). These counts may return result from the high load of initial microflora of raw milk and other ingredients and their quality, the environment, insufficient heat treatment and poor personal hygiene. It has been previously stated that the production of ice cream on a small scale rather than industrially is an important factor associated with the contamination of ice cream (Bostan and Akin, 2002; Kanbakan et al., 2017).

The incidence of *Enterobacteriaceae* count in the ice cream samples was similar to those given by Erol et al (1998) who reported that the incidence of *Enterobacteriaceae* in ice cream sample was (53%). The presence of *Enterobacteriaceae* in food products resulted from

fecal contamination. Therefore, in this study the microbial quality of ice cream samples seemed to be high due to insufficient heat treatment, unhygienic treatment, unhygienic handling of the ice cream before *and* during storage (Jay, 2017).

The existence of *Enterobacteriaceae*, *coliforms* and *E.coli* in examined ice cream samples might indicate the lack of a good manufacturing practice during the production which has an important role in gastrointestinal diseases therefore, implementing regulatory measures like good manufacturing practices, hygienic distribution and retail storage practices important for microbiological safety of ice cream sold are necessarily, Kanbakan and Con (1999) reported that coliform contamination on the hands of persons in sales department was higher than on the hands of factor workers.

The presence of coliforms in ice cream samples might result from insufficient heat treatment, unhygienic materials or tools used and contaminated water. Much attention has been paid towards *E.coli* because of its importance as an organism of true fecal origin associated with enteric pathogens (Tsen et al., 1998). Inadequate cleaning of the hands, same person selling ice cream and collecting money, open cones, and unclean cloth for cleaning the scoops can contribute to high coliform count (Kanbakan et al., 2017).

Pasteurization kill coliform organism so their post pasteurization presence in ice creams refer to faulty heat process during preparation. Meanwhile, contamination may arise from water, bad personal hygiene of the ice cream manufacturer and utensils used for ice cream (Jadhay and Raut, 2014).

Currently, the incidence of coliforms was 46% and 22% in open and packed ice cream samples. Those results agreed with El-Ansary (2019) who reported that the incidence of

coliforms in examined ice cream sample was 21% of with a mean value of  $4.58 \times 10^3 \pm 1.50 \times 10^3$ , on the contrary, a higher incidence of coliforms in ice cream samples (56%) was given by Abou-El Khair et al. (2014).

According to Egyptian standards (2018) for ice cream which stipulated that the coliform counts must not exceed 10 cells/g, 78% of packed ice cream samples and 54% of unpacked ice cream samples comply with Egyptian standard, it is evident from the results that coliform contaminate high percent street-vendor pop ice cream samples this may attributed to poor hygienic measure carelessness during handling and distribution, poor quality ingredients, ineffective sanitizing method and prolonged storage of the mixture.

From table 2 of a total viable fungi count (cfu/g), the count range from  $1.5 \times 10^1$  to  $2.0 \times 10^1$  cfu/g.

Table 3 shows the cultural, morphological and biochemical characteristics of isolates. Irrespective of the count recorded, , the present of microorganisms in the different samples is an indicator that the samples were contaminated.

## CHAPTER FIVE

### 5.0 CONCLUSION AND RECOMMENDATION

#### 5.1 Conclusion

This study is an analyses of the microbiological quality of ice cream sold in Auchi, Etsako West Local Government Area.

The result obtained from this study has indicated a poor level of hygiene in the production of ice cream in Auchi. It is clear from the previous and current studies that there is necessity for improving the hygiene status of locally produce ice cream in domestic or catering premises especially in all steps, post-pasteurization and at retail level in Auchi. The fungi contamination of diary product can occur from environment, equipment, handlers and packaging materials. (Ahmed et al., 2020).

Many types of fungi which include mould and yeast have been isolated from milk product or mycological media by employing standard techniques among this fungi are *Aspergillus spp.*, *Pennicillium* and *Fusarium* are important as they produce *microtoxins* which can cause serious health hazard among the susceptible individuals. Hence, it is advised that fungi contaminated milk products should not be consumed as it can be potential source of *mycotoxicosis* in humans.

#### 5.2 Recommendation

From the result and conclusive statement of this study, it is therefore recommended that general hygiene conditions of premises, the quality of the raw material prior to process, storage of the products under appropriate conditions to prevent the infections resulting from pathogenic microorganisms, workers and sales people in the point of sell should be regularly checked for

sustaining the favorable hygienic status. Also, to avoid fungi contamination, adequate environmental sanitation, proper sterilization of equipment and packaging materials and hygiene of handlers should be observed consistently.

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