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Microbiological Quality of Banana from different markets in Bahry Town

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الآية

قال تعالى:

(وَفَاكِهَةٍ مِمَّا يَتَخَيَّرُونَ (20) وَلَحْمِ طَيْرٍ مِمَّا يَشْتَهُونَ (21)

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Dedication

We dedicate this study to:

Our families, Teachers and friends

And all people who helped us to complete this study

With love.

Maab, Fatima &Hosna

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First of all we thank Allah for giving us such wonderful opportunity and made this study see light.

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Abstract

This study was conducted to evaluate the microbiological quality for Banana samples which has been taken from different location in Bahry town from central market of Bahry, Bahry market and street.

Two samples of each location has been taken ripe and over ripe sample and then subjected to the microbial tests total count, staphylococcus, total coliform, *E.coli*, *Salmonella* and yeast and mould also chemical test moisture content, protein, fat, ash, carbohydrate and acidity test.

The different microbial load was higher in over ripe sample compared to ripe sample and this may be due to improper supply and surrounding environment. In addition to that the absence of *E.coli* and *Salmonella* in ripe sample,also there were no significant difference in the results of the chemical test in the targeted markets.

ملخص الدراسة

أجريت هذه الدراسة لمعرفة الجودة الميكروبيولوجية لعينات الموز المأخوذة من مناطق مختلفة في مدينة بحري تمثلت في السوق المركزي ببحري ، سوق بحري والشارع.تم أخذ عينتين من كل منطقة ناضجة وزائدة النضج وأجريت عليها الإختبارات الميكروبية التالية العدد الكلي للبكتريا،البكتريا العنقودية، بكتريا القولون، *E.coli*، السالمونيلا والأعفان والخمائر. وإختبارات كيميائية تتمثل في الرطوبة، البروتين، الدهن، الرماد، الكربوهيدريت، واختبار درجة الحموضة.

وكانت مستويات الميكروبات المختلفة أعلي في العينات الزائدة النضج مقارنة بالعينات الناضجة في كل الأسواق وقد يكون هذا بسبب العرض غير السليم والبيئة المحيطة. بالإضافة إلي عدم وجود ال*E.coli* والسالمونيلا في العينات الناضجة.

أيضا لم يوجد اختلاف معنوي في نتائج التحاليل الكيميائية ودرجة الحموضة في كل من الأسواق المعنية.

CHAPTER ONE

INTRODUCTION

The Banana (genus Musa) is one of the top three fruits in the world, coming after citrus and grapes **(Hui, 2006)**. Also, it considered as one of the most import five fruits crops in Sudan. In African, the banana total production was estimated at 7.141.000 metric tons **(FAO, 2010).**

Banana plants are monocotyledonous perennial and important crop in the tropical and Sub tropical world regions **(Valmayor*et al.*, 2000)**. Within the bunch are clusters of double rows of fruit called "hands" and individual fruit called "fingers". **(Ogazi, 1996).**

Bananas are harvested unripe and green, because they can ripen and spoil very rapidly **(Daniel's *et al.*, 2001).** FAO (2004) data sources put the world production of plantains at about 60 million tons **(FAO, 2010).** In West Africa, plantain production increased at an average annual rate of between 2.3% to 2.6% **(FAO, 2010).**

Banana fit well with recommendations on nutrition and human needs for increased consumption of foods low in fat, Cholesterol, and salt. Their low lipid and high energy contents make them very useful in low –fat diets. They also have a special place in the feeding of obese patients .They are usually the only raw fruit permitted to people suffering from peptic ulcers **(FAO,1989).**

On other hand Food safety is an increasingly important public health issue. Governments all over the world are intensifying their efforts to improve food safety. These efforts are in response to an increasing number of food safety problems and rising consumer concerns. Food safety begins in the field, and should be of special concern, since a number of outbreaks of foodborne illnesses have been traced to contamination of produces in the field**(Kumar *et al*., 2015).**

The number of documented outbreaks of human infections associated with the consumption of raw fruits, vegetables, and unpasteurized fruit juices has increased. Outbreaks with identified etiology were predominantly of bacterial origin, primarily *Salmonella.* More recently, Salmonellosis has been linked to tomatoes, seed sprouts, cantaloupe, apple juice, and orange juice contamination **(Beuchat, 2002).**

Among the greatest concerns with human pathogens on fresh fruits and vegetables are enteric pathogens e.g., E. coli O157:H7 besideSalmonellathat have the potential for growth prior to consumption or have alow infectious dose. Farther Bacterial pathogens have been isolated from a wide variety of fresh produce. However **(Beuchat, 2002).**

In Sudan no many documented study on safety of fruits are available, demanding more studies on safety of different fruits displayed and sold in local market. Therefore, the objectives of this study are:

1- To determine the microbiological safety of banana collected from different local markets in Bahary town.

2- To examine the physicochemical propertiespH of the collected banana.

3- To determine the proximate composition of different banana.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Classification

Family: *Musaceae*

Latin Name: *Musa SPP*

English Name: Banana **(Simmonds, 1987).**

2.2 Description

The banana plant, *Musa paradisiaca*, is the world's largest herbaceous perennial plant. It is grown for its fleshy, curved banana fruit. The plant is tall, tropical and tree-like with a sturdy main pseudo stem (not a true stem as it is made of rolled leaf bases) with the leaves arranged spirally at the top. The leaves are large blades with a pronounced central midrib and obvious veins. They can reach up to 2.7 m (8.9 ft) in length and up to 0.6 m (2.0 ft) in width. Each pseudo stem produces a group of flowers which may also be called the 'banana heart' from which the fruits develop in an hanging cluster. The banana fruits are comprised of a protective outer layer, or skin, with numerous long, thin strings that run between the skin and the edible inner portion. The seeds are tiny black specks running through the center of the fruit. In commercial plantations, the parent banana plant dies after harvest and is replaced with a daughter plant. However, a plantation can grow for 25 years or more if managed properly **(Ogazi, 1996).**

2.3 Economic value of banana

Banana is the cheapest, most plentiful, and most nourishing of all fruits. It contains nearly all the essential nutrients, including mineralsand vitamins and has several medical properties. Banana is a rich source of energy;about 24 bananas each weighing around 100g, would provide the energy requirement (2400 Cal /day) of a man **(FAO, 1989).**

2.4 Maturity indices of musaspp

Banana require about three months from the beginning of flowering until harvest. Multiple fruits are produced on a large bunch, weighing between 50-200kg **(Ogazi, 1996).** Within the bunch are clusters of double rows of fruit called "hands" and individual fruit called "fingers"**(Ogazi, 1996).**

Maturity standards for banana are more precise several different external and internal fruit characteristics can be used to determine banana maturity. These include fruit diameter, age of the bunch, angularity of the fruit, length of the fruit, and peel color **(Johnson *et al.*, 1998).** The stage of maturity for harvest depends on the intended market destination **(Johnson *et al.*, 1998).** Locally marketed banana can be harvested at a more advanced maturity stage compared to export market fruit. Export market destined fruit should be harvested the day before or the same day of shipment **(Ogazi, 1996).**Banana maturity is related to the diameter of the fingers. This is determined by measuring the diameter of the fruit at its mid-point with a pair of calipers **(Ogazi, 1996).**

Another method for estimating banana maturity is to record the age of the bunch. The time from which the fruit bunch first becomes visible (Shooting) is recorded. Bunches can be tagged

with different colored ribbons at the time of shooting, and subsequently harvested after the appropriate time for the particular cultivar, based on the season of the year and experience **(Johnson *et al.*, 1998).** The color of the ribbons is changed weekly to coincide with the time of shooting and subsequently the age of the bunch **(Johnson *et al.*, 1998).**

A third method used to determine harvest maturity of banana is to observe the shape (fullness) and angularity of the fruit. Immature fruit is angular in cross sectional shape and has distinct ridges **(Ogazi, 1996).** As the fruit matures, it becomes less angular and more rounded or full. The degree of roundness differs between cultivars and location of the hand on the bunch. Typically, the fullness of the fruit on the middle hand is measured. The appropriate shape to harvest the fruit depends on the market destination. Fruit intended for the domestic market should be harvested when the fruit shape is nearly round **(Johnson *et al.*, 1998).**

2.5 Chemical composition of banana

2.5.1 Moisture

The moisture content of banana pulp normally increase during ripening from about 69% to about 74%, the water derived from the breakdown of carbohydrate, presumably during respiration, contributes to thisincrease, probably, a more significant factor is the osmatic withdrawal of moisture from the peel, this osmatic transfer of moisture is reflected by the changes in the weight ratio of pulp to peel which is about 1.2 - 1.6 in the

green fruit and about 2.0-2.7 when the fruit is fully ripe **(FAO, 2010).**

2.5.2 Carbohydrates

About 20-25 % of the banana pulp the fresh green fruit is starch, from initiation completion of ripening, the starch is almost completely hydrolyzed, only1-2% remaining is the fully ripe fruit, sugar is normally 1-2% in the pulp of green fruit and increases to about 15-20% in the ripe pulp total carbohydrates decreases 2-5% during ripening presumably as sugar are utilized in respiration, the green peel contains about 3% starchlocalizedduring ripening with concomitant accumulation of sugars. **(FAO, 2010)**

2.5.3 Proteins

Banana Protein content varies from 1% to 25%, depending on type variety,altitude and climate. The banana protein was found to increase over ripening process 3.8-8.2%**(FAO, 2010).**

2.5.4 Fat

Fat content in banana pulp remains almost constant (1%) during ripening process.Banana peel was found to contain 2.2-10.9% lipids which was very rich in poly unsaturated fatty acids , particularly linoleic, linolenic and palmatic as the major fatty acids in both peel and pulp **(FAO,2010).**

2.5.5 Pectin

Ripe banana pulp was reported to contain 0.7%to 1.2% pectin.During ripening, insoluble protopectin is usually converted

into soluble pectin that causes lessening of cell wall and texture degradation leading to softening of banana fruit.Pectin as a jell-forming material is commonly used in jam, jellies and marmalades, as thickener, texturizer, emulsifiers,fat or sugar replaced **(FAO, 2010).**

2.5.6 Pigments

Banana peel is green when fully mature, gradually turns yellow and in some case brown spots is found. Similar changes are also observed in banana pulp **(FAO, 2010).**

2.5.7 Fibers

In general, the banana peel has much more fiber than the pulp .However, banana pulpat harvest was found to contain 2-3% celloulose**(FAO, 2010).**

2.5.8 Minerals and vitamins

Banana pulp is rich in vitamin A, B vitamins (thiamine 40µg, riboflavin70µg, niacin 610µg, pantothenic acid 280µg, and pyridoxine 470 µg and folic acid23µg) and ascorbic acid. Potassium is found to the most abundant mineral present in banana inedible protein, followed by magnesium, calcium,phosphorous and iron,whereas copper is found in very small quantity. Due to its nutritive value, process banana when accompanied with some legume based product, can be served as excellent baby by food and snack food (**FAO, 2010).**

2.5.9 Volatile compounds

There are 350 volatile compounds separated from ripe banana pulp. The volatiles are mainly a complex mixture of esters, but alcoholics, aldehydes, ketones, and aromatic compounds are also present. The odor component during ripening arising mainly from metabolism of the ester component, alcoholic, carbonic compound and amino acids like valine, leucine and isoleucine which are the main sources of alcoholic isoamyl, isobutyle and their esters. Also, the fatty acids like palmatic, linolenic and linoleic increased during ripe respiration**. (FAO, 2010).**

2.6 Medical uses

All parts of the banana plant have medicinal applications: the flowers is used in bronchitis and dysentery and on ulcers; cooked flowers are given to diabetics; the astringent plant sap in cases of hysteria, epilepsy, leprosy, fevers, hemorrhages, acute dysentery and diarrhea, and it is applied on hemorrhoids, insect and other stings and bites, young leaves are placed as poultices on burns and other skin afflictions, the astringent ashes of the unripe peel and of the leaves are taken in dysentery and diarrhea and used for treating malignant ulcers, the roots are administered in digestive disorders, dysentery and other ailments; banana seed mucilage is given in cases of catarrh and diarrhea in India **(Naryana, 2015).**

Antifungal and antibiotic principles are found in the peel and pulp of fully ripe bananas. The antibiotic acts against Mycobacteria. A fungicide in the peel and pulp of green fruits is active against a fungus disease of tomato plants. Norepinephrine, dopamine and serotonin are also present in the ripe peel and pulp.

The first two elevate blood pressure; serotonin inhibits gastric secretion and stimulates the smooth muscle of the intestines **(Naryana, 2015).**

Table (1): The Chemical composition and nutritional of bananas per 100g of edible fresh portion (Chandler, 1995).

Nutrients	**Amount**	Daily recommended values
Water	74%	240ml
Carbohydrates	23 %	300 grams
Protein	1%	50 grams
Fats	0.5%	65 grams
Fiber	2.5%	25 grams

2.7 Processing quality

The bulk of the banana, cooking banana are eaten as raw, in the ripe state, or as a cooked vegetable, and only a very small proportion are processed in order to obtain a storable product. Generally, preserved products do not contribute significantly to the diet of the millions of people who eat banana, cooking banana, however in some countries or areas, the processed or preserved products are important in periods when food is scarce. Processing is recognized as a way of preserving the fruit. Yet the proportion of fruits processed and the suitability of the various *Musa* groups to processing is relatively unknown. New *Musa* hybrids should therefore be screened for their processing quality or suitability for processing **(Thompson, 1995).**

The ripe banana is utilized in a multitude of ways in the human diet, from simply being peeled and eaten out of hand to being sliced and served in fruit cups and salads, sandwiches, custards and gelatins, being mashed and incorporated into ice cream, bread, muffins and cream pies **(Adeniji *et al.*, 2006).** Ripe plantains are often sliced lengthwise, baked or boiled, and served (perhaps with a garnish of brown sugar or chopped peanuts) as an accompaniment for ham or other meats. Ripe plantain may be thinly sliced and cooked with lemon juice and sugar to make jam or sauce, stirring frequently during 20 or 30 minutes until the mixture jells. Whole, peeled plantain can be spiced by adding them to a mixture of vinegar, sugar, cloves and cinnamon which has boiled long enough to become thick and then letting them cook for 2 minutes **(Chandler, 1995).**

Banana puree is important as infant food and can be successfully canned by the addition of ascorbic acid to prevent discoloration. The puree is produces on a commercial scale in factories close to banana fields and packed in plastic-lined 10 cans and 55-gallon metal drums for use in baby foods, cake, pie, ice cream, cheesecake, doughnuts, milk shakes and many other products **(Ogazi, 1996).**

Through experimental work with a view to freezing peeled, blanched, sliced green banana, it has been found that, with a pulp-to-peel ratio of less than 1:3 the fruits turn gray on exposure to air after processing and this discoloration is believed to be caused by the high iron content (4.28p/m) of the surface layer of the flesh. Its reaction to the tannin normally present in green bananas. At pulp to peel ratio of 1.0, the tannin level in green bananas is 241.4mg; at 1.3, 151.0mg, and at 1.5, 112.6mg, per 100g **(Ogazi,**

1996). Therefore, it is recommended that for freezing, green bananas should be harvested at a stage of maturity evidenced by 1.5 pulp-to-peel ratio. Such fruits have a slightly yellowish flesh, higher carotene content, and are free of off-flavors. The slices are cooked by the consumer without thawing **(Ogazi, 1996).**

Completely green banana are 50% flesh and 50% peel **(Ogazi, 1996).**Banana for freezing should have a pulp content of at least 60% for maximum quality in the ultimate food product, but a range of 55 to 65% is considered commercially acceptable **(Ogazi, 1996).**

Banana flour, or powder, is made domestically by sun drying slices of unripe fruits and pulverizing**.** Commercially, it is produced by spray-drying, or drum-drying, the mashed fruits**.** The flour can be mixed 50-50 with wheat flour for making cupcakes. Two popular Puerto Rican foods are “paste less” and “alcapurais” both are pastry stuffed with meat, the first is wrapped in plantain leaves and boiled the latter is fried. The pastry is made of banana flour or a mixture of plantain with cassava or cocoyam **(Ogazi, 1996).**

Commercial production and marketing of fried green banana chips has been increasing in various parts of the world over the past 25 years and these products are commonly found in retail groceries alongside potato chips and other snack foods**(Ogazi, 1996).**

In Africa, ripe bananas and plantains are also processed into beer and wine. The Tropical Products Institute in London has established a simple procedure for preparing acceptable vinegar from fermented banana rejects **(Ogazi, 1996).**

2.8 Food Safety

According to the Centers for Disease Control andPrevention, in the U.S. the number of reported produce-related outbreaks per year doubled between the period 1973-1987 and 1988-1992 (2,24). During both time periods, the etiologic agent was unknown in more than 50% of outbreaks.**(Beuchat, 2002).**

Escherichiacoli O157:H7 infection has been associated with lettuce, sprouts, and apple juice, and enterotoxigenic *E. coli* has been linked to carrots. Documented associations of shigellosis with lettuce, scallions, and parsley; cholera with strawberries; parasitic diseases with raspberries, basil, and apple cider; hepatitis A virus with lettuce, raspberries, and frozen strawberries; and Norwalk/Norwalk-like virus with melon, salad, and celery have been made. Among the greatest concerns with human pathogens on fresh fruits and vegetables are enteric pathogens (e.g., *E. coli* O157:H7 and *Salmonella*) that have the potential for growth prior to consumption or have a low infectious dose. Bacterial pathogens have been isolated from a wide variety of fresh produce. **(Beuchat, 2002).**

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Materials

Three samples of ripe banana fruit were collected from differentmarkets in Bahrytown in Khartoum state.

3.1.1 Microbial Media

- Plate Count Agar
- Nutrient Agar
- Potato – Dextrose Agar
- Maconkey Broth
- Brilliant Green 2% Bile Broth
- EC Broth
- Eosin Methylene Blue Agar
- Selenite Cysteine Broth
- Bismuth Sulphite Agar
- Baird-Parker Agar
- Used Diluent 0.1% Peptone Solution

3.2. Methods

3.2.1Microbial methods

3.2.1.1 Sterilization of Glassware

Petri dishes, test tubes, flasks, pipettes…etc., were sterilized in hot air oven at 160 – 180º C for 2 to 3 hours before they were put in the oven they were washed dried and packed in stainless steel cans or sometimes in aluminum foil.

3.2.1.2 Sterilization of Media

Culture media were prepared following manufacturing instructions then sterilized Sterilization was achieved by autoclaving at 121ºC for 15 minutes.

3.2.1.3Preparation of serial dilutions

Aseptically 10 grams of the sample were homogenized in 90 ml of sterile diluent (0.1% Peptone water). It was mixed well to give dilution (10^{-1}) by using sterile pipette 1 ml was transferred aseptically from dilution (10^{-1}) to a test tube containing 1 ml of sterile diluent (10^{-2}). In the same way the preparation of serial dilution was continued until the dilution (10^{-6}). One ml of each dilution was transferred into sterile petri dish, and then 15 ml of sterile melted Plate Count Agar medium were added to each plate. The inoculum was mixed with medium and allowed to solidify.

The plates were incubated at 37º C for 48 hours. A colony counter was used to count the viable bacterial colonies after incubation and the results were reported as colony-forming units (CFU) per gram.

3.2.1.4 Total Viable Count of Bacteria

It was carried out by using the spread plate count method as described by **Harrigan (1998).**

3.2.1.5 Determination of Coliform Bacteria

It was carried out by using the Most Probable Number (MPN) technique as following:

3.2.1.5.1 Presumptive Coliform test

10, 1.0 and 0.1 ml prepared samples was inoculated in triplicates of MacConkey Broth test tubes containing Durham tubes. The tubes were incubated at 37º C for 48 hours. The production of acid together with sufficient gas to fill the concave of the Durham tube is recorded as positive presumptive test.

3.2.1.5.2 Confirmed test for Total Coliforms

From every tube showing positive result a tube of Brilliant Green 2% Bile Broth was inoculated by using a sterile loop. The tubes were inoculated at 37ºC for 48 hours, and then the tubes showing positive and negative result were recorded. The Most Probable Number (MPN) of total coliform was found out by using the Most Probable Number (MPN) tables.

3.2.1.5.3 Confirmed E. coli test

Medium used was EC Broth. From every tube showing positive result in the presumptive test used to inoculate a tube of EC Broth containing Durham tube were inoculated at 44.5º C for 24 hours. Tubes showing any amount of gas were considered positive. For further confirmation of E. coli tubes of EC Broth showing positive results at 44.5º for 24 hours were streaked on Eosin Methylene Blue Agar (EMB) plates. The plates were incubated at 37º C for 48 hours. Colonies of E.coli are usually small with metallic green sheen on EMB Agar.

3.2.1.6*Staphylococcus aureus*

Medium used was Baird-Parker Agar; 0.1 ml from every dilution was transferred onto the surface of each well dried Baird-

Parker Agar medium plates. The inoculum was spreaded all over the plate using sterile bent glass rod. The plates were incubated at 37º C for 24 hours, after that period of incubation the plates were examined and counted *Staphylococcusaureus*appear black shiny convex and surrounded by a zone clearing 2-5 mm in width of colony.

3.2.1.7 Yeasts and Moulds

From suitable dilutions of sample 0.1 ml was aseptically transferred onto solidified Potato-Dextrose Agar containing 0.1 gram chloramphenicol per one liter of medium to inhibit bacterial growth. The sample was spread all over the plates using sterile bent glass rod. Plates were incubated at 28º C for 72 hours. Colonies were counted using a colony counter and the result were presented as CFU/gram.

3.2.1.8 Detection of *Salmonella*

Ten gram of the sample were added to a conical flask containing 90 ml of sterile Nutrient Broth and incubated at 37º C for 24 hours. A loopfull of 24 hours incubated Nutrient Broth was transferred aseptically to sterilized Selenite cysteine Broth and incubated at 37º C for 24 hours. A loopfull of 24 hours inoculum of Selenite Cysteine Broth was streak on Bismuth Sulphite Agar surface and incubated at 37º C for 24 – 72 hours. Black metallic sheen discrete colonies indicated the presence of *Salmonella*.

3.2.2 Chemical methods

3.2.2.1 Moisture content

The moisture content was determined according to the

standard method of the Association of Official Analytical Chemists **(AOAC, 2003).**

Principle: The moisture content in a weighed sample is removed by heating the sample in an oven (under atmospheric pressure) at 105 °C. Then, the difference in weight before and after drying is calculated as a percentage from the initial weight.+66.

Procedure: A sample of 2 g ±1 mg was weighed into a pre-dried and tarred dish. Then, the sample was placed into an oven (No.03-822, FN 400, Turkey) at 105 ± 1 °C until a constant weight was obtained. After drying, the covered sample was transferred to desiccators and cooled to room temperature before reweighing. Triplicate independent results were obtained for each sample and the mean value was reported to two decimal points according to the following formula:

$$\text{Moisture content (\%)} = \frac{(Ws - Wd)}{\text{Sample weight (g)}} \times 100\%$$

Where:

Ws = weight of sample before drying.

Wd = weight of sample after drying.

3.2.2.2 Crude protein content

The protein content was determined in all samples by micro-Kjeldahlmethod using a copper sulphate-sodium sulphate catalyst according to the official method of the **AOAC (2003).**

Principle: The method consists of sample oxidation and conversion of its nitrogen to ammonia, which reacts with the

excess amount of sulphuric acid forming ammonium sulphate. After that, the solution was made alkaline and the ammonia was distilled into a standard solution of boric acid (2%) to form the ammonia-boric acid complex which is titrated against a standard solution of HC1 (0.1N). The protein content is calculated by multiplying the total N % by 6.25 as a conversion factor for protein.

Procedure: A sample of two grams (2 gm.) was accurately weighed and transferred together with, 4g NaSO4 of Kjeldahl catalysts (No. 0665, Scharlauchemie, Spain) and 25 m1 of concentrated sulphuric acid (No.0548111, HDWIC, India) into a Kjeldahl digestion flask. After that, the flask was placed into a Kjeldahl digestion unit (No.4071477, type KI 26, Gerhardt, Germany) for about 2 hours until a colorless digest was obtained and the flask was left to cool to room temperature.

The distillation of ammonia was carried out into 25m1 boric acid (2%) by using 20 ml sodium hydroxide solution (45%). Finally, the distillate was titrated with standard solution of HC1 (0.1N) in the presence of 2-3 drops of bromocreasol green and methyl red as an indicator until a brown reddish color was observed.

$$\text{Crude Protein (\%)} = \frac{(\text{ml Hcl sample} - \text{ml Hcl blank}) \times N \times 14.00 \times (F)}{\text{Sample weight (g)} \times 1000} \times 100\%$$

Where:

N: normality of HCl.

F: protein conversion factor = 6.25

3.2.2.3 Fat content

Fat content was determined according to the official method of the **AOAC (2003).**

Principle: The method determines the substances which-are soluble in petroleum ether (65-70 °C) and extractable under the specific conditions of Soxhlet extraction method. Then, the dried ether extract (fat content) is weighed and reported as a percentage based on the initial weight of the sample.

Procedure: A sample of 5g ± l mg was weighed into an extraction thimble and covered with cotton that previously extracted with hexane (No.9-16-24/25-29-51, LOBA Cheme, and India). Then, the sample and a pre-dried and weighed extraction flask containing about 100 ml hexanes were attached to the extraction unit(Electro thermal, England) and the extraction process was conducted for 6 hrs. At the end of the extraction period, the flask was disconnected from the unit and the solvent was redistilled. Later, the flask with the remaining crude ether extract was put in an oven at 105 °C for 3 hrs. , cooled to room temperature in a desiccators, reweighed and the dried extract was registered as fat content according to the following formula:

$$\text{Fat content (\%)} = \frac{(W2 - W1)}{W3} \times 100\ \%$$

Where;

W_2 =Weight of the flask and ether extract

W_1 =Weight of the empty flask

W_3=initial weight of the sample

3.2.2.4 Ash content

The ash content was determined according to the method described by the **AOAC (2003).**

Principle: The inorganic materials which are varying in concentration and composition are customary determined as a residue after being ignited at a specified heat degree.

Procedure: A sample of 5g ±1 mg was weighed into a pre-heated, cooled, weighed and tarred porcelain crucible and placed into a Muffle furnace (No.20. 301870, Carbolite, England) at 550 to 600 °C until a white gray ash was obtained. The crucible was transferred to a desiccator, allowed to cool to room temperature and weighed. After that, the ash content was calculated as a percentage based on the initial weight of the sample.

$$\text{Ash (\%)} = \frac{[(\text{Wt of crucible} + \text{Ash}) - (\text{Wt of empty crucible})]}{\text{Initial weight (Wt)}} \times 100\%$$

3.2.2.5Total carbohydrates

Total carbohydrates were calculated by difference according to the following equation:

Total carbohydrates = 100% - (Moisture + Protein + Fat + Ash).

3.2.3 Physiochemical methods

3.2.3.1 Hydrogen ions concentration

The Hydrogen ions concentration (pH) of the different samples was determined as described by **Ranganna (2001).**

Principle: The pH value of the different samples was measured with a pH-meter. After standardization of the pH-meter electrodes with buffer

solution, the reading of the sample is recorded as pH value.

Procedure: After standardization of the pH-meter (N0.478530, Hanna, India) with buffer solutions (pH 4.01 and 7.01), the electrode of the pH-meter was rinsed with distilled water, immersed in the sample and left to stand until a staple reading was achieved. All the readings were expressed as pH to the nearest 0.01-pH units.

3.3 Statistical analysis method

The results were subjected to Statistical Analysis (Minitabsoftware) by using two sample t test to compare significant different between means.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Microbiological count of raw samples from Central market

The result presented in table (2) indicated significant increase in levels of total count,*Staphylococcusaureus,* total coliform and*E.coli* in over ripped banana samples as compared with sound ripped banana sample. *Staphylococcusaureus*, total coliform and *E.coli* was not found in ripe banana sample. And also *Salmonella* was not detective in the same samples however over ripe sample was contaminated by *Salmonella.*

From the table (2) the total viable of bacteria in ripe and over ripe banana was4.59and 7.87log cfu/g,respectively.Aljedah and Robinson (2002) reported that the total count bacteria in banana juice was 6.34log cfu/g.these result was higher than ripe banana and lower than over ripe banana. In the same table the total coliformin ripe and over ripe banana wereNF and 35.5MPN/g, respectively.Aljedah and Robinson (2002) reported that the total coliformin banana juice was 3.51MPN/g .This result was lower than over ripe banana. These different may be due to the source of the banana or to the experimental conditions.Also in table 2 yeast/mouldin ripe and over ripe banana were 1.24 and 2.74 log cfu/g, respectively.Aljedah and Robinson (2002) reported that the total count bacteria in banana juice was 4.53MPN/g these result was higher than result in table 2.These different may be due to the source of the banana juice or to the experimental conditions.

Table (2): Microbiological quality of banana samples collected from Central Market.

Type of banana	Total viable count 0f bacteria log cfu	*Staphylococcus aureus* Log Cfu	Total coliform (MPN)	E. coli (MPN)	*Salmonella*	Yeast\Mould Log cfu
Ripened	4.59 ± 0.15[a]	NF	NF	NF	NDT	1.24± 1.75[a]
Over-ripened	7.87 ± 0.02[b]	3.78 ±0.04	35.50± 0.71	12.50±0.71	DT	2.74±0.057[a]

*Values are mean ± ST of replicate in depended sample.

*Values that carry that same superscript latter in same column are not significant different.

*NF = not found.

*NDT = not detected.

*DT = detected.

4.2 Microbiological quality of raw samples from Bahry market

The result presented in table (3) in detected significant increase in levels of total count,*Staphylococcusaureus*, total coliform, *E.coli*and yeast/mould in over ripped banana samples as compared with sound ripped banana sample. Total coliform, *E.coli* and yeast/mouldwere not found in ripe banana sample. And also *salmonella* was not detective in the same sample however over ripe sample was contaminated by *Salmonella.*

From the table 3 the total count of bacteria in ripe and over ripe banana were 4.64and 7.93 logcfu/g, respectively. Aljedah and Robinson (2002) reported that the total count of bacteria in banana juice was6.34log cfu/g.these result was higher than ripe banana and lower over ripe banana. In the same table the total coliform in ripe and over ripe banana were NF and 43.50 MPN/g, respectively.Aljedah and Robinson (2002) reported that the total coliform in banana juice was3.51MPN. This result was lower than over ripe banana. Also in table 3 yeast/mould in ripe and over ripe banana were NF and 2.88 log cfu/g, respectively.Aljedah and Robinson (2002) reported that the yeast/mouldin banana juice was4.53cfu/g,these result was higher than result in table 3.These different may be due to the source of the banana juice or to the experimental conditions.

Table (3): Microbiological quality of banana samples collected from Bahary market.

Type of banana	Total viable count 0f bacteria log cfu	*Staphylococcus aureus* Log Cfu	Total coliform (MPN)	*E. coli* (MPN)	*Salmonella*	Yeast\Mould Log cfu
Ripened	4.64± 0.06[a]	2.54± 0.08[a]	NF	NF	NDT	NF
Over-ripened	7.93± 0.01[b]	3.82± 0.0[b]	43.50± 0.71	17.50± 2.12	DT	2.88± 0.04

*Values are mean ± ST of replicate in depended sample.

*Values that carry that same superscript latter in same column are not significant different.

*NF = not found.

*NDT = not detected.

*DT = detected.

4.3 Microbiological quality of raw samples from Street

The result presented in table (4) in detected significant increase in levels of total count,*Staphylococcusaureus*, total coliform,*E.coli* and yeast/mould in over ripped banana samples as compared with sound ripped banana sample. *Staphylococcusaureus*, E.coli and yeast/mould was not found in ripe banana sample .and also*Salmonella* was not detective in the same sample however over ripe sample was contaminated by *Salmonella.*

From the table 4 the total count of bacteria in ripe and over ripe banana were 5.69 and 8.87 log cfu/g respectively. Aljedah and Robinson (2002) Reported that the total count bacteria in banana juice was 6.34.these result was higher than ripe banana and lower than over ripe banana. In the same table the total coliform in ripe and over ripe banana were3.50 and 48.50 MPN/g, respectively. Aljedah and Robinson (2002) Reported that the total coliform in banana juice was 3.51 MPN/g.This result was similar to ripe banana and lower than over ripe banana. Also in table 4 yeast/mould in ripe and over ripe banana were NF and 3.87 log cfu/g, respectively.Aljedah and Robinson (2002) Reported that the yeast/mould in banana juice were 4.53 log cfu/gthese result was higher than resultin table 4.These differences may be due to the source of the banana juice or to the experimental conditions.

Table (4): Microbiological quality of banana samples collected from the street.

Type of banana	**Total viable count 0f bacteria log cfu**	***Staphylococcus aureus* log Cfu**	**Total coliform (MPN)**	***E. coli* (MPN)**	***Salmonella***	**Yeast\Mouldlogcfu**
Ripened	5.69± 0.21[a]	NF	3.50± 0.71[a]	NF	NDT	NF
Over-ripened	8.87± 0.03[b]	3.89± 0.02	48.50±6.36[b]	20.50± 0.71	DT	3.87±0.05

*Values are mean ± ST of replicate in depended sample.

*Values that carry that same superscript latter in same column are not significant different.

*NF = not found.

*NDT = not detected.

*DT = detected.

4.4 Chemical composition raw samples from different markets

4.4.1Moisture content

The results presented in table (5) show the moisture content of moisture contentof banana samples collected from the three different markets. Results of moisturecontentwere not significantly different between sound ripened samples and over ripened one in each market.

These results were in disagreement with those stated by **Salunkhe*and Kadam*. (2005).**Who stated that the moisture content of banana was 75.7%.This variation may be due to Climatic conditions, genotypesvariation, and agricultural practices.

Table (5): Moisture Content of banana samples collected from different markets.

Type of sample	Central market	Bahry market	Street
Ripened	83.05± 0.78[a]	82.95±0.92[a]	83.30±0.40[a]
Over-ripened	82.40±1.27[a]	82.65±0.50[a]	82.10±2.12[a]

4.4.2 Crude protein

The results presented in table (6) show the crud proteinof banana samples collected from the three different markets. Results ofCrude protein were not significantly different between sound ripened samples and over ripened one in each market.

These results were in disagreement with those stated by **Salunkheand Kadam. (2005).**Who stated that thecrud proteinof banana was 1.1%. This variation may be due to Climatic conditions, genotypesvariation, and agricultural practices.

Table 6: crud protein of banana samples collected from different market

Type of sample	Central market	Bahary market	Street
Ripened	1.95± 0.01[a]	2.35±0.06[a]	2.41±1.01[a]
Over-ripened	1.90±0.00[a]	2.43±0,04[a]	2.54±0.06[a]

4.4.3Fat content

The results presented in table (7) showthe Fat content ofbanana samples collected from the three different markets. Results ofFat contentwere not significantly different between sound ripened samples and over ripened one in each market.

These results were in disagreement with those stated by**Salunkhe*and Kadam*. (2005).**Who stated that theFat contentof banana was 0.2%.

This variation may be due to Climatic conditions, genotypesvariation, and agricultural practices.

Table (7): Fat content of banana samples collected from different markets

Type of sample	Central market	Bahary market	Street
Ripened	1.03± 0.04[a]	0.98± 0.04[a]	1 ± 0.04[a]
Over-ripened	1.08±0.21[a]	0.98± 0.04[a]	1.03± 0.08[a]

4.4.4 Ash content

The results presented in table (8) showtheAsh content of banana samples collected from the three different markets. Results were not significantly different between sound ripened samples and over ripened one in each market.

These results were in disagreement with those mentioned by **Salunkheand Kadam. (2005).**Who stated that theAsh content of banana was 0.6%.

This variation may be due to Climatic conditions,

genotypesvariation, and agricultural practices.

Table 8: Ash content of banana samples collected from different markets

Type of sample	Central market	Bahary market	Street
Ripened	0.18± 0.172[a]	0.30± 0.16[a]	0.61± 0.007[a]
Over-ripened	0.25± 0.17[a]	0.26± 0.22[a]	0.65± 0.08[a]

4.4.5 Total Carbohydrate

The results presented in table (9) showtheCarbohydrate content of banana samples collected from the three different markets. Results were not significantly different between sound ripened samples and over ripened one in each market.

These results were in disagreement with those mentioned by **Salunkhe and Kadam. (2005).**Who stated that theCarbohydrate content of banana was 12.6%.

This variation may be due to Climatic conditions, genotypesvariation, and agricultural practices.

Table 9: Total Carbohydrate of banana samples collected from different markets.

Type of sample	Central market	Bahary market	Street
Ripened	13.90± 0.79[a]	12.42± 0.67[a]	13.68± 0.007[a]
Over-ripened	14.13± 0.79[a]	12.89± 0.43[a]	13.69± 2.34[a]

4.5 Physiochemical characteristic raw samples from different markets.

4.5.1 pH

The results presented in table (10) showthe pH of banana samples collected from the three different markets. Results were not significantly different between sound ripened and over ripenedsamples in each market.

Table (10): pH of banana samples collected from different markets

Type of sample	Central market	Bahary market	Street
Ripened	6.82± 0.07[a]	6.85± 0.01	6.88 ± 0.01
Over-ripened	6.60± 0.04[b]	6.65± 0.04	6.70± 0.04

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the results obtained in this study wecan conclude that, the over ripe banana was in high microbial contamination.statetotal viable count, yeasts and mould, *staphylococcusaureus* , colon bacteria, *E.coli*, *Salmonella* were detected and counted.

For other analysis there were no significantdifference($p < 0.05$) in(Moisturecontent, protein, Fat, Ash, carbohydrateand PH). There for ripe banana sample was beter as compare to over ripe one from microbial safety base.

5.2 Recommendations

1) Provide clean prepared places for supply and selling of banana fruit.
2) Consume should avoid consumption of over ripe banana which may be contamination with pathogenic According to the obtained results we recommend not to eat the opened over riped banana because of the presence of pathogenic microorganisms.
3) Further researches are needed.

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