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RESEARCH ARTICLE

Bacteriological Assessment of Ready-to-Eat Bakery Products Sold in Zuru Metropolis, Kebbi State, Nigeria

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ABSTRACT

Ready-to-eat foods made from wheat and flour are rich in essential nutrients such as proteins, fats, minerals, and carbohydrates which may increase exponential microbial proliferation in these products when kept under improper storage conditions or through unhygienic handling and as a result cause mild-to-severe illnesses when consumed. This study was conducted to determine the bacteriological value of bakery products sold in Zuru metropolis, in Kebbi state, Nigeria. A total of 20 samples were collected from different bakeries and hawkers and examined using the conventional bacterial isolation, identification, biochemical tests, and enumeration. The total viable bacterial number of the samples demonstrated the highest (12.638×10^7) count in sample (doughnut), while the lowest (1.519×10^7) count was observed in bread factory. The biochemical identification of the isolates revealed the presence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus,* and *Pseudomonas aeruginosa*. The frequency and percentage occurrence of the isolates indicated the highest frequency of 8 and a percentage occurrence of 40% for *E. coli*, while the lowest frequency of 2 and a percentage occurrence of 10% for *S. aureus*. This level of bacterial contamination may be attributed to poor handling, preparation, and/or marketing of the baked foods. Moreover, as such safe hygienic practices are recommended during the production and service of these foods.

Keywords: Bakery, Microbial, *Staphylococcus aureus*, Zuru

INTRODUCTION

Bakery products are one of the most important foods eaten outside homes globally.[1] These Bakery products supply different essential nutrients and calories required every day, which include lipids, vitamins, minerals, proteins, and carbohydrates.[2] Food safety is, however, a basic necessity for human well-being, and regardless of this, many foods are habitually adulterated with naturally occurring bacteria pathogens. These bacteria (such as *Escherichia coli*, *Salmonella* spp., *Bacillus* spp., and *Staphylococcus aureus*) cannot be detected the organoleptic way (smelled, tasted, or seen), but can inflict diseases of varying gravity, and even death, particularly when preserved in conditions that increase the exponential proliferation of those microorganisms that could reach considerable levels for contamination. This brings about a substantial threat to food safety and as such is of major significance to public health agencies globally.[3]

The measurement of worldwide occurrence of ailments from these food sources is of immense challenge. In the year 2016, over 200 people in Zuru town suffered from diarrhea that was

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linked to intake of baked food. Ailments resulting from the ingestion of unsafe food have turned out to be among the common dilemmas faced by human health agencies in the present time.[4] In several developing nations, a primary source of baked foods (bread, doughnut, snacks, and cake) is processed or vended at open locations such as markets, institutions, and roadsides. Microbiologists believe that such foods are mostly kept at temperatures that are inappropriate and through unhygienic handling by retailers who sell at unhygienic environment.[5] Besides, most of the food handlers are uneducated while some had only a limited number of years in school and as a result lacks sufficient understanding of safe handling of food and how they contribute in the introduction of deleterious pathogens in these bakery products.[6]

Concurrently, a huge amount of individuals who patronize the meals are mostly concerned about how easy it is to access more than the subject of microbial value and safety. Food-borne illness epidemics related to baked foods have been linked with countless food borne etiologic agents such as *E. coli*, *S. aureus, Salmonella* spp., and *Bacillus* spp.[7] The preliminary bacteriological loads on baked foods are inhibited by baking temperatures while conditions in which the food must have been stored, handled, or dispensed may warrant the proliferation of the bacteria before the stage of consumption.[8] These bakery products are routinely made without proper hand sanitary measures and this may precede an amplified occurrence of bacteria growth by possible pathogenic bacteria, such as *Staphylococcus* species.[9]

Statement of problem

Baked foods are the most known form of food that contains pathogenic and non-pathogenic microorganisms which may cause mild-to-severe illnesses in humans and sometimes animals. It is known that *S. aureus* and *E. coli* are some of the most common microorganisms found in most baked foods and they have higher ecological role on or in the human body, this matter has necessitated advanced investigations.[10] Therefore, if baked foods are not properly processed, before or during productions may contain undesirable microorganisms.

Justification

Baked foods are made and sold in various forms within the community in which a lot of contaminations are encountered, it is, however, important to further investigate these microbial interactions to contribute to public health and the medicine of prevention. The rampant consumption of baked foods has resulted in many food-borne disease outbreaks associated with a higher morbidity in Nigeria, especially in Kebbi State. This has necessitated the need to conduct research in the bakery product industries and markets in Zuru metropolis. So as to enable public health agencies to create awareness on health hazards with respect to the consumption of bakery products sold in Zuru metropolis.

Aims and objectives

This study aims to assess the bacteriological value of bread, doughnut, cake, and snacks sold in Zuru town.

The specific objectives are:

i. To enumerate bacteria from some selected baked food such as bread, doughnut, cake, and snacks
ii. To isolate and identify bacteria from such baked foods
iii. To promote quality of baked food.

MATERIALS AND METHODS

Study area

This study was focused in Zuru town, Kebbi State, Nigeria. Zuru lies at the extreme northwest of Nigeria on latitude 11° 25 49'' North 5° 14' 15'' East and it original name (with diacritics) is Zuru, the town covers a total of 653 square kilometers of land mass[11] with a population of about 165,547 inhabitants according to 2006 census.[12] It has a mixed rural and urban population.

Sample collection

An aggregate of 20 samples was collected from different centers as follows; five bread samples from Ujabil Bread Factory, Sidi Bread Factory, Rahama Bread Factory, Saha Bread Factory, and *Salafiya* Bread Factory. Each of the five samples of doughnut, snacks, and cakes was collected from hawkers. All samples were retrieved using sterile polythene bags. The polythene bags were placed in the packed containers and transported to the Microbiology Laboratory, Department of Microbiology, Faculty of Life Science, Kebbi State University of Science and Technology, Aliero, Kebbi State, for microbial assessment.

Media preparation

Nutrient agar

Weighing balance was used to obtain 28 g of nutrient agar powder and dissolved in a liter of distilled water on a hot plate. This was followed by autoclaving for 121°C in 15 min, the media was poured in Petri dishes and allowed to cool.[13]

Methyl red-Voges-Proskauer (MR-VP) medium

The medium was prepared by dissolving 17 g into 1000 ml of sterile distilled water in a conical flask and allowed to fully dissolve on a hot plate. The medium was subsequently autoclaved for 15 min at 121°C; this was followed by filling Petri dishes with the media. The media were allowed to cool and incubated overnight to ensure sterility.[13]

Simons citrate agar

34.5 g of the agar was taken using a weighing balance and dissolved in a liter of distilled water. After complete dissolution, it was sealed and autoclaved at 121°C for 15 min, the media was poured into culture plates and allowed to cool. This was also incubated overnight to ensure sterility.[13]

Methodology

Serial dilution

Six test tubes containing 9 ml of distilled water. One milliliter of the sample was added to a single test tube and subsequently taken and transferred to a corresponding test tube until all the six test tubes are serially diluted.[13]

Inoculation of samples

Serially diluted samples were inoculated by taking 0.1 ml from tubes 3 and 5. The 0.1 ml was placed in a sterile Petri dishes and 20 ml of molten nutrient agar was poured into the plate. It was swirled through mixing and allowed to solidify before incubating at 37°C for 24 h.[13]

Enumeration of bacteria

After 24 h of incubation, plates that showed evidence of bacterial growth were enumerated: A plate was divided into four quadrants, and one quadrant was counted and recorded. To obtain the total number of colonies, the counted quadrant was multiplied by four. To get the total number of bacteria/ml, the total number of colonies on the plates was multiple by the reciprocal of the dilution factors. The final number is reported in colony-forming units per ml (cfu/ml).[14]

Isolation and purification of bacteria

All test organisms were grown separately on nutrient agar plate. A Wire loop sterilized by flame was used to transfer a loopful of the specimen into nutrient agar plate and emulsified in a small area of the plate. The wire loop was resterilized and used to streak the inoculums in parallel lines followed by streaking for isolation. Incubation of the Petri dishes took about 24 h at 37°C. Colonial morphologies were observed and recorded. Desired

Table 1: The total viable bacterial number of the samples demonstrated the highest (12.638×10^7) count in sample (doughnut), while the lowest (1.519×10^7) count was observed in bread factory

Total viable count in cfu/g/different samples Bacterial count of sample × 10^x in cfu/g			
Sample	**Total count**	**Mean count (SD)**	**Range**
B	6.63×10^7	1.519×10^7	3.598×10^7
C	1.036×10^7	2.895×10^3	2.440×10^6
S	7.359×10^6	1.671×10^6	3.799×10^6
D	3.362×10^7	12.638×10^7	3.166×10^7

B: Bread sample, C: Cakes, SK: Snacks, DN: Doughnut

Table 2: The biochemical identification of the isolates revealing the presence of *Bacillus cereus*, *E. coli*, *Salmonella* spp., *S. aureus*, and *Pseudomonas aeruginosa*

Isolates	Ind	Cat	Ur	Coa	Citr	Mot	MR	VP	TSI	GR	Organisms
1	–	+	–	–	+	+	–	+	+	–	*B. cereus*
2	+	+	–	–	–	–	+	–	–	–	*E. coli*
3	–	+	–	–	–	+	+	–	–	–	*Salmonella* spp.
4	–	+	+	+	+	–	+	+	–	+	*S. aureus*
5	–	+	–	–	+	–	–	–	–	–	*Pseudomonas aeruginosa*

+: Positive, –: Negative, *E. coli*: *Escherichia coli*, *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, Ind: Indole, Cat: Catalase, Ur: Urease, Co: Coagulase, Ci: Citrate, Mt: Motility, MR: VPTSI: Triple sugar iron[13]

Table 3: The frequency and percentage occurrence of the isolates indicating the highest frequency of 8 and a percentage occurrence of 40% for *E. coli*, while the lowest frequency of 2 and a percentage occurrence of 10% for *S. aureus*

Identified organisms	Frequency of occurrence	Percentage of occurrence (%)
B. cereus	4	20
E. coli	8	40
Salmonella spp.	3	15
S. aureus	2	10
Pseudomonas aeruginosa	3	15
Total	20	100

E. coli: *Escherichia coli*, *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*

colonies were further inoculated on a plated for confluent growth. From this growth, a little growth was transferred to slants for further analysis.[13]

Identification of bacteria

Smear preparation

Small amount of the colony was placed at a center of clean glass slide. With the aid of an inoculating loop, it was dispersed to an even thin film. It was allowed to air dry, fix over a gentle flame.[13]

Grams staining

Crystal violet was poured on the smear and stood for 60 s and rinsed with distilled water. Iodine solution was also poured on the smear and allowed for 60 s this was followed by rinsing with water. Ethanol was added to serve as a decolorizing agent rinsed immediately and counterstained using basic fuchsin. After rinsing and drying, the slide was examined under microscope oil immersion × 100 objective lens.[13]

Indole test

Kovacs indole reagent was added to a 9 ml of peptone water containing the test organism culture. Changes were observed and recorded.[13]

Catalase test

A smear of a suspected colony was made and few drops of hydrogen peroxide were added on the smears, reactions were observed and recorded.[13]

Coagulase test

A suspected colony was picked with a wire loop and used to make a smear on a clean glass slide, and heat fixed. Few drops of human plasma were added to the slide. The slide was observed for possible clumping and recorded.[13]

Urease test

Urease agar was prepared in a test tube slants and inoculated with the test organism. The slants were incubated for 48 h, observations were recorded.[13]

Citrate utilization test

Test tubes containing prepared Simon citrate agar were inoculated with the test organism. This was followed by incubation for 48 h.[13]

Motility test

A drop of normal physiological saline was placed on a clean glass slide. Fresh growths were added to the glass slide and viewed under the microscope.[13]

MR-VP test

An MR-VP broth was prepared and with the aid of a straight sterile wire the test organism was inoculated

into the broth. The broth was incubated for 24 h at 37°C and followed by the addition of a small quantity of methyl red (MR). Changes in color were noticed and recorded. This was also followed by the addition of few drops of hydrogen peroxide and 5% alpha-naphthol in another tube containing the broth. Changes in color were observed after an hour.[13]

Triple sugar iron agar test (TSI)

A sterile medium was inoculated with the test organism using a straight wire and by surface streaking. The inoculated medium was incubated for 24 h at 37°C. Changes and reactions were observed and recorded.[13]

RESULTS

The bacteriological assessment of ready to eat bakery products obtained within Zuru Metropolis as shown in Table 1 where the total viable bacterial number of the samples demonstrated the highest (12.638×10^7) count in sample (doughnut), while the lowest (1.519×10^7) count was observed in (bread factory). The biochemical identification of the isolates revealing the presence of *bacillus cereus, Escherichia coli, salmonella* spp, *Staphylococcus aureus,* and *pseudomonas aeruginosa* are shown in Table 2 while Table 3 depicts the frequency and percentage occurrence of the isolates indicating the highest frequency of 8 and a percentage occurrence of 40% for *Escherichia coli,* while the lowest frequency of 2 and a percentage occurrence of 10% for *Staphylococcus aurous.*

DISCUSSION

The total viable bacterial number shows the highest count of 12.638×10^7 cfu/g in doughnut samples. This exceeds the maximum threshold set by notable Nigerian food quality agencies which asserts that the count of aerobic bacteria must not be more than 100 cfu/g and this can be linked to the fact that doughnut factories could be prone to bacterial contamination due to poor personal hygiene from the workers, vendors, and the machines used in making doughnuts. Most times, doughnut makers do not put on gloves or other protective clothing during preparation, baking and packaging of the doughnut.

The lowest count of 1.519 x 107 was observed in Bread samples; perhaps due to the nature of packaging adopted by most bread bakers and as a result, the main implication of highest bacterial number associated with baked foods is food-borne illnesses and food poisoning, and *S. aureus* accounts for the most prevalent pathogen associated with baked food; due to its ubiquitous nature.[15]

Another microbial assessment of bread carried out by Ijah *et al*.[16] depicts a 3×10^5 cfu/g which is higher than what was obtained from bread samples in this study. This contradicts the findings reported in an analogous study carry out by the New South Wales Food Authority[17] which discovered that 97.8% of samples had satisfactory bacteriological thresholds. Only a single sample, a custard-filled product, had a possible dangerous level of *B. cereus*. The biochemical identification of isolates revealed the presence of *E. coli*, *Salmonella* spp., *Bacillus* species, *S. aureus,* and *Pseudomonas aeruginosa.* The bacterial isolates found in this study were different from those identified by Adebayo *et al.*,[18] but the isolates were almost similar with those that identified by Begum.[19] The high bacteriological values obtained in this study are also in conformity with the data reported by Olunlade *et al*.[20] in a stored biscuit, in which the bacterial isolates identified in their studies include *B. cereus*, *S. aureus*, *Klebsiella aerogenes,* and *Proteus mirabilis.*

CONCLUSION

The total viable bacterial number and biochemically identified organisms found in this study, confirms the bacteriological contamination of the samples, which may be due to poor handling, preparation, and/or marketing of the bakery products. And as such, it could be deduced that the baked foods could have a detrimental (mainly food-borne illnesses and food poisoning) effects to the consumers, therefore warrant a call for outmost public attention.

RECOMMENDATIONS

Maintaining proper hygiene by food handlers and vendors and by developing adequate cleaning

system of the production floor, frequently fumigating production and storage area, proper cleaning and sterilization of manufacturing equipment and machineries, controlling insect and rodent access into the production area, maintaining appropriate storage temperature, and maintaining proper temperature during transportation, in retail shops according to recommended temperature (–18°C) will reduce the bacterial load and as result prevent pathogens from invading the bakery products which could invariably minimize the health risks to the consumers.

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