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Research Article

ENUMERATION OF MICROORGANISMS ASSOCIATED WITH THE DIFFERENT STAGES OF BREAD PRODUCTION IN FUTMIN BAKERY, NIGERIA

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ABSTRACT
Microbiological analysis of different stages of bread which includes dry mixture, non-dry mixture, mixture of ingredient with flour, dough formation, milling, cutting, proofing, baking and cooling stage were obtained from FUTMIN bakery and analyzed using standard microbiological and biochemical methods. Various microbial species identified at different stages of bread production include bacterial species such as *Staphylococcus, Bacillus, Pseudomonas, Streptococcus, Escherichia coli*. Fungal species include *Aspergillus, Mucor, Cephalosporium, Saccharomyces cerevisiae and Torulopsis glabrata* were among the yeast isolates. The total aerobic Bacteria count ranged from 2.85 x 10^4 cfu/g to 6.21 x 10^6 cfu/g, Coliform count ranged from 1.19 x 10^4 cfu/g to 2.05 x 10^6 cfu/g, species of *Staphylococcus* count ranged from 2.00 x 10^4 cfu/g to 5.52 x 10^5 cfu/g, while Fungi count ranged from 4.00 x 10^3 to 1.40 x 10^6 cfu/g. The bacterial with the highest frequency of occurrence were *Staphylococcus* and *Escherichia coli* (24.24%), followed by *Bacillus* sp (21.21%), *Staphylococcus epidermidis* (12.12%), *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Streptococcus* sp had the least frequency of occurrence (6.06%). Fungal isolates with the highest frequency of occurrence was *Aspergillus* sp (36.84%), followed by *Cephalosporium* sp (21.21%), and *Aspergillus fumigatus* (15.79%). *Mucor* sp and *Saccharomyces cerevisiae* had same frequency of occurrence (10.53%) while *Torulopsis glabrata* had the least frequency of occurrence (5.26%). Bacterial counts at stage one, two, and five of the production process which had to do with mixing and milling were high while the baking stage had a reduced microbial load. The results revealed that three stages (stage one, two and five) were the critical control points. It therefore means that application of adequate control measures to these points will greatly reduce microbial hazard of bread.
KEYWORDS: Enumeration, Microorganisms, Bread production, Futmin bakery.

INTRODUCTION

Food is any substance that can be taken through ingestion into the system by an organism to supply nutrient and energy for growth and development. The food eaten has direct influence on health of people. It is therefore pertinent to keep food free from contamination by pathogenic organism, particularly processed food like bread[1]Bread is one of the most ancient of human foods, and is produced with the help of microorganisms[2].

Food safety and hygiene are related to sanitary control of food aimed towards good health. This has led to the enactment of laws to protect the public from buying decomposed, adulterated, unpreserved or misbranded food products. It also provides for safe handling of potentially dangerous foods and products such as milk[3]. While food is necessary for life it also acts so as a potent vehicle for transmission of diseases and even death when its safety and hygiene is unguaranteed. Majority of food poisoning cases occurs at the end of the food handling chain. This is due to ignorance or indifference of such handlers to food processing and storage. In about 80% of the cases, food poisoning was caused by foods of pathogens. In 20% of cases, unsanitary handling and sloppy conditions of the handler were implicated[4].

Food poisoning may be due to heavy metals (zinc, tin, lead or copper), allergic condition or bacterial contamination due to processing, unhygienic conditions, poor quality of raw materials, food handlers, cooking utensils or poor storage. In Nigeria food poisoning and contamination arise from poverty, dirty and indiscriminate sewage disposal. More so, cooked foods are hawked unprotected from dust and flies while most source of drinking water is surface water with high faecal load[5]. Considering this background, the aim of this study is to enumerate, isolate and identify microorganisms associated with different stages of bread making in our locality.

MATERIALS AND METHODS

Sample Collection

A total of sixty three samples were aseptically collected during the various stages involved in bread making from FUTMIN bakery using a sterilized aluminium spoon and knife, and immediately transferred into sterile specimen bottles with screw caps. These samples include; dry

mixture (sugar, milk, salt, nutmeg, preservative, yeast), non-dry mixture (butter, oil flavor, vegetable oil), mixture of ingredient with the addition of flour, dough stage, milling stage, molding stage, proofing stage, baking stage and cooling stage.

Preparation of Samples

One gram of bread sample to be analyzed was weighed using electronic weighing machine balance and was homogenized with the aid of a little quantity of distilled water and then shaken to obtain homogenized solution. One milliliter (1ml) of the solution was serially diluted in a set of 5 test tubes containing 9ml of sterile, distilled water each as diluents and aseptically 0.1ml of appropriate dilution was plated on Nutrient Agar (NA), Mannitol Salt Agar (MSA), MacConkey Agar (MCA), and Sabouraud Dextrose Agar (SDA) using pour plate technique. The NA, MSA, and MCA plates were incubated at 37^{o}C for 24 hours while the SDA plates were incubated at room temperature 28± 2^{o}C for 3-5days.

Isolation of Pure Cultures

After incubation, colonies which developed from pour plate culture were counted using a colony counter and expressed as colony forming units per milligram (cfu/g) of samples. Colonies differing in size, shape and colour were selected from the different plates and further subcultured on Nutrient Agar by the streak plate technique and incubated at 37^{o}C for 24 hours after which were maintained on agar slants for further characterization and identification.

Identification of Bacterial Isolates

The biochemical tests carried out on the bacterial isolates are listed below: Gram staining, Catalase test, Coagulase test, Methyl red-voges proskauer (MR-VP) test, Starch hydrolysis**,** Indole test**,** Citrate utilization test**,** Mannitol Salt, Carbohydrate fermentation test.

RESULTS

Table 1 shows the total aerobic bacterial counts at different stages of bread production. The highest bacterial count was obtained in stage 1 with the value of 6.21 x 10^6 cfu/g and the least in stage 8 with the value of 2.85 x 10^4 cfu/g. Table 2 shows the total coliform counts at different stages of bread production. The highest coliform count was obtained in stage 5 with the value of 2.05 x 10^6 cfu/g and the least in stage 1 with the value of 1.19 x 10^4 cfu/g. Table 3 shows the total species of *Staphylococcus* counts at different stages of bread production. The highest species of *Staphylococcus* count was obtained in stage 2 with the value of 5.52 x 10^5 cfu/g and the least in stage 9 with the value 2.00 x 10^4cfu/g. Table 4 shows the total fungal counts at different stages of bread production. The highest fungal count was obtained in stage 1 with the value of 1.40 x 10^6cfu/g and the least in stage 7 with the value of 4.00 x 10^3 cfu/g. Table 5 and 6 represent the frequency of occurrence of bacterial and fungal isolates respectively in the different stages of bread production. *Staphylococcus aureus* and *Escherichia coli* has the highest frequency of occurrence (24.24%) in bread production followed by *Bacillus* spp. (21.21%) and *Staphylococcus epidermidis* (12.12%). Similarly, *Bacillus subtilis, Streptococcus* sp and *Pseudomonas aeruginosa* had the least frequency of occurence (6.06%) in bread production. It was observed that *Aspergillus niger* had the highest frequency of occurrence (36.84%) in bread production followed by *Cephalosporium* (21.05%) and *Aspergillus fumigatus* (15.79%). *Mucor* sp. and *Saccharomyces cerevisiae* had same frequency of occurrence (10.53%) while T*orulopsis glabrata* had the least frequency of occurrence (5.26%).

DISCUSSION

The study has revealed that microorganisms occurred at different stages of bread production. International microbiological standards recommended units of bacterial contaminations for foods in the range of 10^3-10^2 cfu/g of food for coliform organisms and less than 10^3cuf/g of food for total bacterial plate counts. This work revealed that Bacterial counts ranges from 2.85 x 10^4 – 6.21 x 10^6 cfu/g, Coliform counts ranged from 1.19 x 10^4 – 2.05 x 10^6cfu/g, species of *Staphylococcus* counts ranged from 2.00 x 10^4 – 5.52 x 10^5cfu/g and Fungal counts ranged from 4.00 x 10^3 - 1.40 x 10^6cfu/g. It was observed that almost all the stages of bread samples examined had microbial load above the acceptable limit and are therefore microbiologically unacceptable. Various microbial species were identified at different stages of bread production. The organisms were species of *Staphylococcus, Bacillus, Pseudomonas, Streptococcus, Escherichia coli, Mucor, Aspergillus, Saccharomyces cerevisiae* and *Torulopsis glabrata*. Frazier[6] *et al* reported that heat kills some micro-organisms in food. While heat plays a key role in the production process, the baking was unable to eliminate the *bacillus* species. This is because these organisms form spores which enable then to survive unfavourable conditions and later germinate[7]. Obuekwe[8] *et al* and Talaro[9] *et al* 1993 reported that *Staphylococcus* species are widely distributed in the environment and they occur on the skin and nostrils of human. *S. epidermidis* might have been introduced from handlers being a normal flora on the skin of humans[10]. Majority of food contamination involving Coliforms are usually due to improper handling arising from contact with handlers, their feaces or feacally contaminated objects[11] which is a very

common occurrence amongst local baked food handlers and retailers. The prevalence of *Pseudomonas* species in the stages 5 and 6 of bread production agrees with the reports of Cruickshank[12] *et al.* who stated that the bacterium could be extensively found in warm and moist situations. The fact that some organisms are absent from some stages of the production process suggests that the activities at those stages do not favor their growth; for example, the baking processing (stage 8) eliminated yeast isolates identified earlier in other stages. It is also important to mention that *Aspergillus* species survived the baking process (stage 8). This organism is widely distributed in the environment and produce spores which allow for their proliferation. According to Gilbert[13] the presence of these organisms brought about by improper hygiene during production and storage could cause food poisoning, potential systemic and non-gastroenteric pathogenic effects. Poor sanitation, temperature control and time of baking employed by local bakers are possible sources of reasons behind microbial contamination. Ubiquity of microorganisms in soil, air and water environment and their characteristics as potential spoilers should serve as a major worry for regulatory agencies on food as food is rapidly spoiled when microbial loads exceeds threshold levels.

CONCLUSION

This study revealed that there is contaminant in bread which may pose a biohazard to consumers. The high prevalence of *Staphylococcus* sp in this study agrees with Ijah and Antai[5] that the organism should not be taken lightly because is associated with food poisoning or intoxication. Since, bread is a highly nutritional food consumed by all groups of people ranging from infants to the elderly in Nigeria irrespective of sex, religion, status. Its microbiological quality should therefore be enhanced which agrees with Hobbs and Robert[11], that microbiological standard of food provides safe, sound and wholesome quality and also protects the health of consumers.

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Table 1: Total Aerobic Bacterial Counts (cfu/g) at Different Stages of Bread Production

Stages of Production	Event	Total Aerobic Bacterial Counts (cfu/g)
1	Dry ingredient mixture	6.21 X 10^6
2	Non-dry ingredient mixture	7.95 X 10^5
3	Mixture of ingredient with flour	6. 07 X 10^5
4	Dough formation	4.17 X10^5
5	Milling	4.85 X 10^5
6	Molding	1.87 X 10^5
7	Proofing	2.65 X10^6
8	Baking	2.85 X 19^4
9	Cooling	4. 37 X 10^5

Table 2: Total Coliform Counts (cfu/g) at Different Stages of Bread Production

Stages of production	Event	Total Aerobic Bacterial Counts (cfu/g)
1	Dry ingredient mixture	1.19 X 10^4
2	Non-dry ingredient mixture	2.98 X 10^4
3	Mixture of ingredient with flour	2. 30 X 10^4
4	Dough formation	1.67 X10^6
5	Milling	2.05 X 10^6
6	Molding	5.43 X 10^5
7	Proofing	3.09 X10^4
8	Baking	Nil
9	Cooling	8. 66 X 10^4

Table 3: Total *Staphylococcus* Counts (cfu/g) at Different Stages of Bread Production

Stages of production	Event	Total Aerobic Bacterial Counts (cfu/g)
1	Dry ingredient mixture	9.67 X 10^4
2	Non-dry ingredient mixture	5.52 X 10^5
3	Mixture of ingredient with flour	2. 27 X 10^4
4	Dough formation	9.12 X10^4
5	Milling	2.25 X 10^4
6	Molding	1.55 X 10^5
7	Proofing	1.57 X10^5
8	Baking	Nil
9	Cooling	2. 00 X 10^4

Table 4: Total Fungal Count (cfu/g) at Different Stages of Bread Production

Stages of production	Event	Total Aerobic Bacterial Counts (cfu/g)
1	Dry ingredient mixture	1.40 X 10^6
2	Non-dry ingredient mixture	1.36 X 10^5
3	Mixture of ingredient with flour	1. 21 X 10^5
4	Dough formation	1.01 X10^5
5	Milling	1.90 X 10^5
6	Molding	1.42 X 10^5
7	Proofing	4.00 X10^3
8	Baking	9.42 X10^4
9	Cooling	8. 00 X 10^3

Table 5: Frequency of Occurrence (%) of Bacterial Isolates

Isolates	No of isolates	Frequency of occurrence (%)
Bacillus sp	7	21.21
Bacillus subtilis	2	6.06
Staphylococcus aureus	8	24.24
Staphylococcus epidermidis	4	12.12
Streptococcus sp	2	6.06
Pseudomonas aeruginosa	2	6.06
Escherichia coli	8	24.24
Total	**33**	**99.99**

Table 6: Frequency of Occurrence (%) of Fungal Isolates

Isolates	No of isolates	Frequency of occurrence (%)
Aspergillus niger	7	36.84
Aspergillus fumigatus	3	15.79
Cephalosporium sp	4	21.05
Mucor sp	2	10.53
Saccharomyces cerevisiae	2	10.53
Torulopsis glabrata	1	5.26
Total	**19**	**100**

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