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Evaluation of Microbial Load of Some Foods Rehandled and Repackaged in Open Markets in Diobu, Port Harcourt, Rivers State, Nigeria

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Abstract

Evaluation of some packaged food sold in open markets in Port Harcourt was carried out to determine microbial load on the food due to re-handling and re-packaging practices embarked by retailers. Samples of re-packaged sugar, bread, and groundnuts were purchased from retailers and taken to the laboratory for microbial analysis. Analysis involved standard microbiological procedures. Results obtained showed that re-packaged bread had amicrobial load of 1.1 x 10 5 cfu/g, while sugar and groundnuts had 2.3x10 4 cfu/g, and 4.4 x10 4 cfu/g respectively.The bacterial counts of Escherichia coli (9.0 x 10 3 cfu/g) and Staphylococcus aureus (4.8 x 10 3 cfu/g) obtained were significantly different (P<0.05). While the heterotrophic plate count (HPC) bacteria in the food samples for bread, sugar, and groundnuts were 6.9 x 10 4 cfu/g, 5 x 10 3 cfu/g, and 2.8 x10 4 cfu/g respectively, showing a significant difference at P<0.05. However, heterotrophic fungi showed count of 2 x 10 3 cfu/g for bread, 3x10 3 cfu/g counts for cube of sugar samples and, 8 x 10 3 cfu/g counts for roasted groundnut (P>0.05). Notable microbes isolated and identified were Escherichia, Staphylococcus, Salmonella, Proteus and Aspergillus. The microbial load of Escherichia coli, Salmonella spp. and Staphylococcus aureus counts in the food samples were at acceptable levels. Bread was the most contaminated food sample re-handled and re-packaged in the market space with higher load of microbes. Hence, re-handling and re-packaging of bread should be discouraged and if practiced, should be done hygienically.

Keywords: bread, foods, groundnuts, handle, microbial load, open markets, package, retailers, sugar

1. INTRODUCTION

Packaging of food is a process of enclosing food to protect it from damage, contamination, spoilage, pest attack and tampering during and after storage and sale. The package is often labeled with information such as amount of ingredients, nutritional content and cooking instructions (Ahenainen, 2003). Food packaging retards product deterioration and maintains or increases the quality and safety of food (Ajo, 2003). Packaging of food protects the food from external influence such as chemical, biological and physical entities that could deteriorate the food (Cassey, 2015). Hence, distinctive or innovative packaging could boost sales in a competitive market (Ajo, 2003). Food packaging may be designed for convenience such as ease of access, handling and many more (Ahenainen, 2003). Retailers re-package food to boost sales in an open-competitive market for which re-handling might influence or affect the manufacturers' packaged innovation or idea (Cassey, 2015). Consequently, the right selected re-packaging material such as leaves, plastic, aluminum foil, paper, polythene, among others should be able to maintain product freshness and quality (Marsh & Bugusu, 2007). The leaves of plants can be used to package foods. However, not all leaves are useful in food packaging as some may possesses poisonous bioactive substances (Casey, 2015). Packaging food with the use of paper is not always reliable. Papers do not protect food for long period of time because paper bags have poor barrier property due to their light and weak nature (Ahenainen, 2003; Marsh & Bugusu, 2007). Sugar manufacturers in Nigeria, package sugar cubes with paper bags. The outcome of re-handling and re-packaging of table sugar cubes in Nigeria have increase with the use of polyethene or nylon bags. Economically, this practice is encouraged as a way to meet the market demand (Marsh & Bugusu,

2007).The paper board used by manufacturers to package sugar cubes are severally, disadvantaged as a result of moisture penetration, where the sugar loses its strength with increasing humidity. And also, the possibility of the paper tearing apart exposes the cubes to biological, physical or chemical influence. The un-professional practice of handling and re-packing of roasted groundnut and bread foods with the use of polyethene bag have increased sales exponentially; this practice gives the product good clarity and prevents the product from oxygen interference (Casey, 2015). Although, re-handling of these aforementioned foods are major factors in packaging practice which are not in-line with standard practices of National Agency for Food and Drug Control (NAFDAC).The retailers practice of re-handling and re-packaging a finished product into small units for the purpose of convenience, subsidized price and availability for consumers may introduce microbes into the finished product and thus lead to food borne illness (Marsh & Bugusu, 2007). Therefore, the aim of this study was to evaluate the microbial quality of table (cube) sugar, roasted groundnut and sliced bread that are often re-handled and re-packaged and retailed in the market space of Port Harcourt metropolis, Nigeria.

2. MATERIALS AND METHODS

2.1 Study area

The study was conducted at Rumuokoro axis of Port Harcourt metropolis, Rivers State, Nigeria. The area is densely populated with huge commercial activities. The huge commercial activities thus, necessitated competition amongst food vendors, geared towards meeting customers' satisfaction. With the up-spring of micro-industries such as the bakery, food vendors in their attempt to meet customers demand, embark on re-handling/re-packaging of these food (groundnuts, bread and sugar) items for customers or consumers satisfaction via food accessibility and availability.

2.3 Sample collections

Thirty (30) samples of re-handled / re-packaged cubes of sugar, bread and roasted groundnuts, comprise of 10 samples each were randomly purchased from retail-vendors. The purchased samples were transferred into sterile bags and transported to the Demonstration and Diagnostic Laboratory, Rivers State College of Health Science and Management Technology, Port Harcourt for standard microbiological analyses.

2.4 Microbial load determination

The bioload on the food samples were determined with the spread plate technique as employed by Muhammed *et al*. (2011). The technique involved taken a gram each of the food samples aseptically into a 9ml of normal saline. The composition was then serially diluted to 10^{-1}. An aliquot (0.1ml) of 10^{-1} dilution was then transferred onto freshly prepared Nutrient agar media, Mannitol Salt agar media, Salmonella/Shigella agar media, Eosin Methylene Blue agar media and Potato Dextrose agar media as carried out by Bryan *et al.* (2020) for enumeration of heterotrophic bacteria, faecal, *Salmonella*, Staphylococcal and heterotrophic fungi. The result in which the colonies were defined was determined by multiplying the number of colonies, the dilution factor and volume of inoculums, and then later expressed as colony forming unit per gram (cfu/g).

2.5 Microbial morphology and identification

Pure cultures of the isolates were characterized morphologically and identified based on their appearance on the media plates, and their biochemical and Gram stain reactions (Benson, 2002).

2.6 Statistical analysis

Data collected were analyzed and presented in tabular forms as employed by Okolie (2007). The data were analyzed using descriptive statistical methods namely; measure of central tendency, measure of variability and simple percentages. Inferential statistical method, t-test, was used to determine the level of significance at *P* value less than 0.05 between different variables studied.

3. RESULTS

3.1 Microbial load on sliced bread, cube of sugar and roasted groundnut samples

Table 1 shows the mean faecal counts of 6 x 10^3cfu/g, 2 x 10^3cfu/g, and 1 x 10^3cfu/g derived from sliced bread, cube of sugar, and roasted groundnuts samples respectively. This shows an insignificant difference at a probability level of greater than 0.05. *Staphylococcus aureus* load on sliced bread was 2.8 x 10^4cfu/g, that of sugar were 1.3 x 10^4cfu/g, and that of groundnut was 7 x 10^3cfu/g. The differences in these counts were not statistically significant (P>0.05). *Salmonella/Shigella* had no count of bacteria derived from the food samples. Heterotrophic bacteria had counts of 6.9 x 10^4cfu/g, 5 x 10^3cfu/g, and 2.8 x10^4 cfu/g for bread, sugar and groundnut food samples respectively, showing a significant difference at P<0.05. However, heterotrophic fungi showed counts of 2 x 10^3cfu/g for bread, 3x10^3cfu/g counts for cube of sugar samples and for roasted groundnut, 8 x 10^3cfu/g counts were derived, showing an insignificant difference (P>0.05). Total microbial load on bread samples was 1.05 x 10^5cfu/g, while sugar samples had 2.3 x 10^4cfu/g, and groundnut samples had 4.4 x 10^4 cfu/g counts.

Table 1**: Mean microbial load on sliced bread, cube of sugar and roasted groundnut samples**

Microbial Isolates	Bread (cfu/g)	Sugar (cfu/g)	Roasted Groundnut (cfu/g)	Total Microbial Load (cfu/g)	NAFDAC Permissible Limit (cfu)
Faecal	6 x 10^{3a}	2x10^{3a}	1.x10^{3a}	9 x 10^3	<10
Staphylococcal	2.8x10^{4a}	1.3x10^{4a}	7 x 10^{3a}	4.8 x 10^4	<100
Salmonella/Shigella	0.0^a	0.0^a	0.0^a	0.0	0
H-bacteria	6.9x10^{4b}	5 x10^{3a}	2.8x10^{4b}	1.02 x 10^5	<100
H-fungi	2 x 10^{3a}	3x10^{3a}	8 x 10^{3a}	1.3 x 10^4	<100
Microbial Load	1.05x 10^5	2.3 x 10^4	4.4 x 10^4		

Keys: cfu/g = Colony-forming unit/gram, [a] =, [b] =, NAFDAC=National Agency for Food and Drug Control
H-bacteria=Heterotrophic bacteria; H-fungi=Heterotrophic fungi

3.2 Microbial phenotypic features and identification

Table 2 shows the phenotypic features of bacteria isolated and their differential biochemical reactions. *Escherichia coli* isolates presented a gray, round, and small phenotypic features. Biochemical reaction of the isolate revealed a lactose positive reaction. Yellow, round and large colonies were prime phenotypic features for *Staphylococcus aureus*, while biochemical reaction using coagulase test identified them positive.
For *Proteus* sp., they presented a colourless colony and showed a positive reaction to urease reagent.

Table 2**: Phenotypic features and biochemical reactions of bacteria isolates**

Colour	Shape	Size	Coagulase	Sucrose	Lactose	Urease	Catalase	Bacteria
Green Metallic Sheen	Round	Small	N	N	P	N	P	*E. coli*
Yellow	Round	Large	P	P	P	N	P	*S. aureus*
Colourless	Round	Large	N	P	N	P	P	*Proteus* sp.
Gray	Round	Tiny	N	N	P	N	N	*Salmonella* sp.

P=Positive reaction; N=Negative reaction

Table 3, shows the fungi phenotypic features for *Aspergillus candidus*, *Aspergillus niger* and *Candida* sp. with phenotypic features such as white grey, black growths, and creamy white and smooth respectively on the plates.

Table 3**: Phenotypic features of heterotrophic fungal isolates**

Macroscopic Feature	**Probable Fungi**
Smooth white grey colony growth	*Aspergillus candidus*
Black powdery growth	*Aspergillus niger*
Creamy white and smooth	*Candida* sp.

3.3 Frequency distribution of bacterial and fungal isolates

Tables 4a and 4b show the frequency distribution of bacterial and fungal isolates derived from sliced bread, cubes of sugar and roasted groundnut food samples respectively. For Table 4a, *Escherichia coli*, *Staphylococcus aureus*, and *Proteus* sp. accounted for 13%, 27%, and 60% of bacterial occurrence in the food samples respectively. In Table 4b, *Candida* sp. accounted for 38% of the fungal distribution in the food sample. Additional distribution of fungal in the food samples also reported *Aspergillus candidus* and *Aspergillus niger* with 29% and 33% occurrence respectively.

Table 4a: **Frequency distribution of bacterial isolates**

Bacteria	**Sliced Bread (n=10)**	**Cube of Sugar (n=10)**	**Roasted Groundnut (n=10)**	**Frequency**	**Percentage (%)**
Escherichia coli	2	0	0	2	13
Staph. aureus	2	0	2	4	27
Salmonella sp.	0	0	0	0	0
Proteus sp.	5	1	3	9	60

Table 4b: **Frequency distribution of fungal isolates**

Fungal	**Sliced Bread (n=10)**	**Cube of Sugar (n=10)**	**Roasted Groundnut (n=10)**	**Frequency**	**Percentage (%)**
Candida sp.	1	3	5	9	38
A. candidus	2	3	2	7	29
A. niger	2	1	5	8	33

A= *Aspergillus*; n = Number of samples investigated

5. DISCUSSION

The loads of *Escherichia coli* in the food samples (cube of sugar, sliced bread and roasted groundnut) were significantly not different. The loads of *Escherichia coli* satisfied the permissible level for *Escherichia coli* in ready-to-eat food according to National Agency for Food and Drug Control of Nigeria (NAFDAC) and thus may not present health risk although. Staphylococcal counts in the food samples showed roasted groundnut had heavy load, while sugar cubes had the least load. Basically, the loads reported in this study satisfied NAFDAC permissible level of Staphylococcal bacteria in ready to eat food. The presence and high levels of Staphylococcal bacteria in roasted groundnut may indicate frequent handling during preparation, processing and sorting (Mosupeye & Holy, 2000). Staphylococcal bacteria are part of normal flora of the human body, particularly the hand. The human body harbors large amount of *Staphylococcus aureus*, an opportunistic pathogen which transit to a new host, and becomes pathogenic (in its new environment). Staphylococcal organisms may have freely being dispersed and thus contaminated the food items.

Salmonella/Shigella loads were completely absent in the food samples, these satisfies NAFDAC's permissible level of *Salmonella* sp. and *Shigella* sp. in ready to eat food. NAFDAC recommends that there should not be any *Salmonella* sp. and *Shigella* sp. present in ready to eat food. Heterotrophic bacteria load in bread were significantly different from that obtained in roasted groundnut and cube of sugar. The loads of heterotrophic bacteria in bread were higher than groundnut and sugar, suggesting a likelihood of danger emanating from elevated level of bacteria which may pose issues in the host body when the foods are consumed (Amanidaz, 2015). Heterotrophic fungi

load in the food samples, specifically, roasted groundnut was higher than sliced bread and sugar cube, however, the loads were significantly not different probably because the fungal; *Candida* and *Aspergillus niger* were most predominant in groundnut than the other food samples as reported by Sangoyomi *et al*.(2016). Groundnut is frequently predisposed to invasion by fungi and subsequent contamination by mycotoxin-producing fungi especially in areas with humid climate which favours their growth (Bhatnagar & Ehrlich, 2002). High occurrence of *Aspergillus niger* in roasted groundnut in this study could be associated with airborne fungal spore, which may have entered the repackaged food and survived during groundnut exposure to the atmosphere (Wada *et al*., 2018). The least of fungal load on the food samples were noticed on bread thus shows a divergent view of Muhammed *et al*. (2011) report. Muhammed *et al*. (2011) reported a high microbial load on bread sold in Zaria. Roasted groundnut is consumed in high quantities in Nigeria and by implication could lead to foodborne diseases associated with *Aspergillus niger* as documented in this study. The presence of microbes in sugar cubes are questionable, according to Solanki and Sheth (2015) proper use of salt, spices, nitrates and sugar are important means of preventing food spoilage via the control of microbes.

5.1 CONCLUSION AND RECOMMENDATION

The evaluation of microbial load of some foods packaged and sold in open-markets in Rumuokoro, Port Harcourt implicated bread as the most contaminated food sample re-handled and re-packaged in the market space. Microbial contamination may have originated from packaging material or un-hygienic processes, hence, materials for repackaging must be thoroughly screened, to ward off possible hazard pose by contaminants. Picking of food with utensil should be encouraged for re-packaging exercise rather than bare handling. All foods should be handled properly and hygienically to promote food safety.

6. References

Ahenainen, R. (2003). *Novel food packaging techniques*. England: Wood Head Publishers.

Ajo, J.A. (2003). *Food packaging, origin, trend, principles and applications*. Nigeria: Amana Publishers.

Amanidaz, N. (2015). The intervention between heterotrophic bacteria, Coliform, faecal Coliform in the water supply. *Applied and Environmental Microbiology, 12*, 53-60.

Benson, H.J. (2002). *Microbiological applications: Laboratory manual in General Microbiology* (8th ed.). New York:Mc Graw Hill.

Bhatnagar, D., Yu, J., & Ehrich, S. F. (2002). Toxins of filamentous fungi. *Chemical Immunology and Allergy,* 81, 167-206.

Bryan, F.L., Michanie, S.C., Alvarez, P., & Panigua, A. (1988). Critical control points of street-vended foods in the Dominican Republic. *Journal of Food Production 51*, 373-383.

Cassey, N.G. (2015). Plant leaves in food preparation and packaging. *Agricultural Science Journal, 1* (4), 35-39.

Marsh, K., & Bugusu, B. (2007). Food packaging: Roles, materials and environmental issues. *Journal of Food Science, 72* (3). 39-55.

Mosupye, F.M., & Holy, A. V. (2000). Microbiological hazard identification and exposure assessment of street food vending in Johannesburg, South Africa. *International Journal of Food Microbiology, 6*, 137 – 145.

Muhammed, M.A., Idris, R., Abubakar, S.C., & Lawal, S.W. (2011). Bacteriological assessment of local bread sold within Ahmadu Bello University Campus Zaria, Nigeria.

Okolie, J.C.N.(2007). *Fundamental of Research Methodology for Nigerian Tertiary Institutions* (2nd ed.). Owerri, Imo State: Okson Printing Service.

Sangoyomi, T.E. (2016). Fungal infection and aflatoxin contamination of market-sold groundnut seeds in Ibadan and Iwo, South West, Nigeria. *Nigerian Journal of Mycology, 8*, 113-121.

Solanki, S., & Sheth, J.H. (2015). Healthy food selection: The role of nutritional information of packaged foods on consumer purchase intentions. *Indian Journal of Marketing*, 45. Doi:10.17010/ijm/2015/i9/79807

Wada, N., Shehu, K., Abubakar, A.L., & Sanic, F.K. (2018). *A review on isolation and identification of fungi Associated with Fruit Rot of Sweet Pepper in North Western, Nigeria*. Mycological Society of Nigeria, Book of Abstract, Nigeria.