JOURNAL OF SCIENTIFIC RESEARCH IN ALLIED SCIENCES
ISSN NO. 2455-5800
Contents available at: www.jusres.com

NUTRITIONAL AND MICROBIAL EVALUATION OF COMMERCIAL BREADS SOLD AT BUS TERMINALS IN OSUN STATE, EDE, NIGERIA.

A.A.T Taleat and *Bolaji A.S
Science Laboratory Technology Department,
Federal Polytechnic, Ede

ARTICLE INFO

Article History
Received: Oct' 2018
Accepted: Dec' 2018

Keywords:
Bread, Nutritive values, contamination, Heavy metals, micro-organisms

Corresponding author
A. A. Taleat*

ABSTRACT

ORIGINAL RESEARCH ARTICLE

Major diseases and illness of men were caused by consumption of contaminated food substances. Bread, a major staple food item eaten by people was analyzed for its nutritional value, the heavy metal and microbial contaminations using standard methods of analysis. Samples were found to contain about 40 % moisture content, 9 % protein, the crude fibre ranged between 0.35 – 0.57 % while the crude fat was about 3 %. Selected heavy metals analysed were Zn (181.10 mg/100g), Fe (85.72 mg/100g), Cd (0.29 mg/100g), and Pb (1.05 mg/100g) found in the exposed bread samples sold at bus terminal in Osogbo while exposed sample from Ikirun was found to contain Zn (105.36 mg/100g), and Fe (46.84 mg/100g). Cadmium (0.17 mg/100g) was detected in fresh bread sample from Ede. All other heavy metals analysed were not detected in other fresh bread samples. A total of 30 bacterial isolates were found in the bread samples and identified by biochemical tests. Enterobacter aerogenes (23 %), Salmonella typhi (20 %), Pseudomonas aeruginosa (17 %), Staphylococcus aureus (17 %) and Klebsiella pneumonia (13 %) were found to be present in the bread samples analysed. The samples were found to be nutritive and the exposed samples were found to contain worrisome amount of heavy metals. Pathogenic micro-organisms were found in the bread samples though in limited amounts. Bread should be well packed in a protective manner for handling and marketing to prevent cross contamination and safeguard the health of the consumers.

INTRODUCTION

Bread is an important universally accepted and convenient food of all populations. Its origin dates back to the Neolithic era and is still one of the most consumed and acceptable staple food products in all parts of the world (Oluwajoba *et al*, 2012). It is a good source of nutrients (macronutrients and micronutrients (Ijah *et al*., 2014). Bread is an important staple food that requires no further processing before consumption. The consumption of bread cut across socio-cultural and religious barriers and is a food of choice for both rich and poor in Nigeria (NAFDAC, 2010). Bread is the second most

widely consumed non-indigenous food product after rice. It is consumed extensively in most homes, restaurants, and hotels. Bread is an important diet that provides as 50-90% of total calorie and protein intakes.

Food contamination is defined as the presence of harmful chemicals and microorganisms in food which can cause consumers illness. These chemicals and organisms may be introduced into the food at various stages such as during production, processing, packaging, transport and/or marketing. Food contamination has become a matter of serious concern all over the world especially in the developing countries like Nigeria.

Heavy metals such as cadmium, lead, zinc, iron and mercury are natural chemical elements. They can be present at various levels in the environment: soil, water and atmosphere. Metals can also occur as residues in food as a result of their presence in the environment. People can be exposed to these metals through the environment or by ingesting contaminated food or water. The accumulation of these metals in the body can lead to harmful effects over time. Consumption of contaminated food is one of the major paths for heavy metals to enter into human body. The effect of environmental pollution on food contamination and on their safety for human consumption is a serious global public issue that needs to be closely monitored. There is a concern in relation to the toxicity of lead in the general population and it effect on brain is intellectual development in young children, while long-term exposure in both children and adults can cause damage to the kidneys, reproductive and immune systems along with effects on the nervous (Feyzi *et al.*, 2017).

Heavy metals have a wide spectrum of effects on health and the fact that these toxic metals accumulate in the body, it is essential to monitor contents of foodstuffs in order to protect human health. Several cases of human disease, disorders, malfunction and malformation of organs have been attributed to the toxicity of heavy metals (Jurup, 2003). Contamination of bread by heavy metals could be due to flour, which may have been produced from contaminated wheat, water used for backing bread or the contaminated environment could be the source of heavy metal contamination (Magomya et al., 2013) Microbial contamination of food is another source of concern. Microbial contamination of food can results from food processing, packaging, transportation and handling. The aim of this study is to determine the nutritional compositions, microbial and heavy metal contamination of commercial bread sold at bus terminals in Osun state , Nigeria.

Methodology

Samples Collection

Bread samples were collected from three towns in the three Senatorial districts of Osun State. Fresh bread samples from each district was collected independently and equally from different Bakery. Another set of samples of the same brands of bread was collected from bread-sellers in each of the bus terminals. Six brands of commercial bread were sampled for the study in March 2017 (n = 36). The samples were kept in separate clean polythene bags and were taken to the Laboratory for analysis.

Sample Preparation and Heavy Metal Determination

The glassware containers used for analysis were washed with detergent solution, and thoroughly rinsed with tap water to remove absorbance due to detergent. The containers were thereafter soaked overnight in 6 mol/dm^3 HNO_3 analytical grade (*Merck*) solutions and finally rinsed with distilled water. All aqueous solutions and dilutions were

prepared with distilled water. 10 g each of bread sample was weighed by a digital analytical balance with ± 0.001g precision and transferred into a beaker containing 10 mL HNO_3 (65%) and 4 mL $HClO_4$. The mixture was covered with a watch glass and heated to decreasing the volume to 3 – 5 mL through evaporation. 10 – 15 mL distilled water was added to the solution and then filtered through an acid washed paper filter. The filtrate was finally diluted to 50 mL with distilled water in an acid washed volumetric flask. The toxic and essential metals were determined from the sample solutions by ICP-OES. The blank solution was prepared in similar way without bread. Recovery determination was performed by spiking blank samples at concentration levels of 15, 25, 75, 150, 250, 500 and 750 µg/mL in triplicates for each concentration levels treated according to the procedure described in sample preparation. The recoveries were calculated using the spiked calibration curves.

Nutrient Analysis

Nutrient parameters such as moisture content, crude protein, crude fat, crude fiber and ash of the bread samples were determined by standard methods (A.O.A.C Int'l, 2000). Moisture content was determined by gravimetric method (oven drying the samples) at 105 ^{0}C for 3 hours. Protein content (% N x 6.25) was determined by the *Kjedahl* method. Crude fat test was carried out based on *Soxhlet* extraction method using hexane as solvent. The ash content was determined by dry ashing in muffle furnace at 550 ^{0}C. Dietary fiber was determined by digesting defatted samples with diluted sulphuric acid (1.25%) solution for 30 minutes at boiling point followed by digestion with sodium hydroxide (1.25%) solution for the same duration. All the analysis were carried out in triplicates and on dry weight basis. Carbohydrate was determined using estimation by difference (AOAC Int'l., 2000).

Microbiological Analysis

Total mesophilic (total viable bacterial counts) and fungi counts (yeast and mould counts) were carried out on the bread samples to determine the microbial load of the samples (APHA, 2001).

Bread samples were prepared by mashing and mixing in peptone water. The sub-samples were serially diluted and 0.1mL aliquots were spread plated on nutrient agar (NA), MacConkey agar (MCA),and potato dextrose agar (PDA) for the enumeration of aerobic viable bacteria, coliforms, and fungi, respectively. The NA and MCA plates were incubated at 37 ^{0}C for 48 hours while PDA plates were incubated at room temperature (28 ± 2 ^{0}C) for 5 days The colonies were then counted and expressed as colony forming units per gram (cfu/g) of samples. All counts were done in duplicate using the scientific colony counter. Observed colonies were sub-cultured repeatedly on media used for primary isolation to obtain pure cultures.

Characterization and Identification of Isolates.

The bacterial isolates were identified using Gram reaction and biochemical tests and were identified by comparing their characteristics with those of known *taxa* as outlined in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1994). The fungal isolates were identified based on macroscopic and microscopic examination.

Statistical Analysis

Statistical analysis were performed using the SPSS (version 20). Difference in proximate composition and heavy metals were detected using one-way analysis of variance (ANOVA). A significance level of ($P < 0.05$) was used for the study.

Results

Table 1: Proximate Compositions of the Bread Samples

Sample	Moisture content (%)	Crude protein (%)	Crude fibre (%)	Crude fat (%)	Total ash (%)	Carbohydrate (%)
OEB	37.94±0.06	7.29±0.01	0.35±0.01	2.39±0.02	1.32±0.01	50.96±0.14
OFB	37.80±0.01	7.68±0.01	0.42±0.01	2.57±0.02	1.39±0.02	50.11±0.02
EEB	38.54±0.06	8.30±0.01	0.48±0.02	2.66±0.02	1.38±0.02	48.82±0.19
EFB	37.94±0.02	7.96±0.01	0.48±0.02	2.46±0.02	1.52±0.01	49.14±0.45
IEB	37.78±0.07	8.50±0.02	0.57±0.02	2.68±0.01	1.48±0.02	49.17±0.04
IFB	37.71±0.02	8.36±0.02	0.53±0.01	2.66±0.03	1.54±0.01	49.11±0.13

Values are mean triplicate determinations ± Standard deviation

Table 2: Heavy Metals Analysis of Bread Samples

Sample	Zn(mg/100g)	Fe (mg/100g)	Cd (mg/100g)	Pb (mg/100g)
OFB	N.D	N.D	N.D	N.D
EFB	N.D	N.D	0.17± 0.01	N.D
IFB	N.D	N.D	N.D	N.D
OEB	181.10 ±2.10	85.72±2.12	0.29 ±0.01	1.05 ± 0.01
EEB	N.D	N.D	N.D	N.D
IEB	105.35± 1.41	46.84±0.56	N.D	N.D

Values are mean triplicate determinations ± Standard deviation

Table 3: Total viable count (Cfu/g) of bacteria and fungi isolated from the bread samples.

Location	Code	Bacteria MCA (Cfu/g)	Fungi PDA(Cfu/g)
OSOGBO	OFB	$2.67 \pm 0.23 \times 10^4$	$2.20 \pm 0.25 \times 10^4$
	OEB	$3.72 \pm 0.35 \times 10^5$	$5.15 \pm 1.23 \times 10^4$
EDE	EFB	$3.67 \pm 0.43 \times 10^4$	$4.33 \pm 0.85 \times 10^4$
	EEB	$3.53 \pm 0.35 \times 10^5$	$6.73 \pm 1.13 \times 10^5$
IKIRUN	IFB	$4.66 \pm 0.73 \times 10^4$	$3.33 \pm 0.43 \times 10^4$
	IEB	$9.21 \pm 1.33 \times 10^5$	$7.36 \pm 1.45 \times 10^4$

MCA –MacConkey Agar, PDA – Potato Dextrose Agar, OFB= Osogbo Fresh Bread, OEB= Osogbo Exposed Bread, EFB = Ede Fresh Bread, EEB= Ede Exposed Bread, IFB= Ikirun Fresh Bread, IEB= Ikirun Exposed Bread

Table 4: Frequency of Occurrence of Bacterial Isolates

Isolate	OFB	EFB	IFB	OEB	EEB	IEB	Total	Percentage (%)
Enterobacter aerogenes	0	1	0	3	1	2	7	23
Salmonella typhi	0	1	1	0	1	2	6	20
Pseudomonas aeruginosa	1	1	0	2	1	1	5	17
Staphylococus aureus	0	1	0	2	2	1	5	17
Klebsiella pneumoniae	2	0	1	2	2	0	4	13
Enterococus faecalis	0	1	1	0	1	0	3	10
Total							30	100

Table 5: Biochemical characterization of the bacteria isolates and their identification

Isolate code	Colonial morphology	Citrate	Catalase	SUGAR FERMENTATION								Motility	Indole	Urease	Methylred	Voge's	Gram - reaction	Organism
				Glucos	Surcos	Maltos	Fructo	Lactos	Galact	Oxidas	Congu							
OFB1	Cream, round, raised	+	+	-	-	+	+	-	-	-	-	+	-	-	+	-	-ve rod	*Salmonella typhi*
OFB2	Green, round	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-ve rod	*Pseudomonas aeruginosa*
OFB3	White, transparent	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+ve cocci	*Staphylococcus aureus*
OFB4	Pink, raised	-	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-ve rod	*Enterobacter aerogenes*
OFB5	White, transparent	-	+	+	+	+	+	+	+	-	+	-	-	+	-	+	+ve cocci	*Staphylococcus aureus*
OEB1	Pink, round raised	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-ve rod	*Enterobacter aerogenes*
OEB2	Green, round	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-ve rod	*Pseudomonas aeruginosa*
OEB3	Pink, raised	-	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-ve rod	*Enterobacter aerogenes*
EFB1	Green, round	+	+	-	-	-	-	-	-	+	-	+	-	-	-	+	-ve rod	*Pseudomonas aeruginosa*
EFB2	Cream, round	+	+	-	-	+	+	-	+	-	+	+	-	-	+	-	-ve rod	*Salmonella typhi*
EFB3	Pink, raised	-	-	+	+	+	+	+	+	-	+	+	-	+	-	-	-ve rod	*Enterobacter aerogenes*
EFB4	Cream, flat	-	-	+	+	+	+	-	+	+	+	+	-	-	+	+	+ve cocci	*Enterococcus faecalis*
EFB5	Pink, round	+	+	+	+	+	+	+	+	-	+	-	-	+	-	+	-ve rod	*Klebsiella pneumoniae*
EFB6	Pink, raised	-	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-ve rod	*Enterobacters aerogenes*
EEB1	Cream, round	+	+	-	-	+	+	-	+	-	+	+	-	-	+	-	-ve rod	*Salmonella typhi*
EEB2	Pink, round	+	+	+	+	+	+	+	-	+	-	+	-	-	-	+	-ve rod	*Klebsiella pnermoniae*
EEB3	White, transparent	-	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+ve cocci	*Staphylococcus aureus*
EEB4	Cream, flat	-	-	+	+	+	+	+	+	-	+	-	+	-	-	+	+ve cocci	*Enterococcus faecalis*
EEB5	Pink round, raised	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+	-ve rod	*Klebsiella pnermoniae*
EEB6	White, transparent	+	+	+		+	+	+	+	-	+	+	-	+	-	-	+ve cocci	*Staphylococcus aureus*
EEB7	Green, round	+	+	-	+	+	-	-	-	+	-	+	-	-	-	+	-ve rod	*Pseudomonas aeruginosa*
IFB1	Pink, round	+	+	+	+	+	+	+	+	-	+	-	+	-	-	+	-ve rod	*Klebsiella pnermoniae*
IFB2	Cream, round	+	+	-	-	+	+	-	+	-	+	+	-	-	+	-	-ve rod	*Salmonella typhi*
IFB3	Cream, flat	-	+	-	+	+	+	-	+	+	+	+	-	-	+	+	+ve cocci	*Enterococcus faecalis*
IFB4	Green, raised	-	+	-	+	+	-	-	+	+	-	+	-	-	-	+	-ve rod	*Pseudomonas aeruginosa*
IFB5	Cream, raised	+	+	-	-	+	+	-	+	-	+	+	-	-	+	-	-ve rod	*Salmonella typhi*
IFB6	Pink, raised	-	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-ve rod	*Enterobacter aerogenes*
IEB1	Cream, round	-	+	-	-	-	+	+	-	+	-	+	+	+	-	-	-ve rod	*Salmonella typhi*
IEB2	Pink round	-	+	+	+	+	+	+	+	-	+	-	-	+	-	+	-ve rod	*Enterobacter aerogenes*
IEB3	White, transparent	-	+	+	+	+	+	+	+	-	+	-	-	+	-	+	+ve cocci	*Staphylococcus aureus*

Discussion

The proximate compositions of the bread samples were shown in table 1. The moisture content of all the samples was almost 40 %. Moisture content is a very important factor in the keeping quality of bread and high moisture can have an adverse effect on storage stability The bread samples analysed may therefore have reduced shelf life. The crude protein ranged from7.29 % (OEB) to 8.36 % (IFB). From this result, bread can be regarded as lean protein food source. It should therefore be eaten with a compliment food items by bread consumers to have a balanced diet. Higher concentration of protein was reported by Ijah *et al*., (2014). The protein content of bread generally depends on the protein richness of the raw materials used in barking the bread. The bread samples been investigated are commercial samples and this may be the reason while it has low protein content.

Crude fibre content of the bread samples ranged from 0.35 to 0.53 %. The values were lower than the recommended the maximum allowable value (2.0 %) by Nigerian Raw Materials. Similar results were reported for cookies made from potato flour (Raji, 2010). The carbohydrate content of all the samples was nearly 50%. There is no significant difference ($P < 0.05$) in the nutritive compositions of all the commercial bread samples investigated.

Result of total microbial count in Cfu/g on Osogbo bread samples, Ede bread samples and Ikirun bread samples are presented in Table 3 microorganisms. The total viable count of microbes isolated from the bread samples analyzed. Fresh bread samples bacteria count ranged from 2.67 x 10^4 to 4.66 x 10^4 Cfu/g. While the exposed bread samples bacteria count ranged from 3.53 x 10^5 to 9.20 x 10^5 Cfu/g. Similarly, fungi count for fresh bread samples ranged from 2.00 x 10^4 to 4.00 x 10^4 Cfu/g and that of exposed bread samples ranged from 5.00 x 10^4 to 6.70 x 10^5 Cfu/g. The exposed bread samples were found to contain higher loads of microorganisms compared to the fresh samples. The presence of microorganisms in food can generally be attributed to the low level of hygiene and sanitation (Isong *et al.*, 2013). Bread and other food items should be well protected from the being exposed to environmental contaminations.

A total of 30 bacterial isolates were isolated and identified by biochemical tests (Table 5). The frequency of occurrence of bacterial isolates are presented in Table 4. E*nterobacter aerogenes* had the highest proportion of (7; 23%), followed by *Salmonella typhi* (6; 20%), *Pseudomanas aeruginosa* and *Staphylococcus aureus* having (5; 17%) respectively, *Klebsiellapneumoniae* (4; 13%) and *Enterococcus faecelis* (3; 10%).

Selected nutritive (Iron and Zinc) and toxic (Cadmium and Lead) heavy metals were determined from the samples (Table 2). The highest concentration of Iron (Fe) (85.72±0.03) mg/100g was found in OEB and the least concentration of Fe (46.84±0.26) mg/100g was found in IEB samples. Iron is an essential trace element required by all forms of life. It plays a key role in the immune system of human. Iron is the most important constituent of different enzyme systems and other important constituents like myoglobin, the cytochromes and catalase. Various groups (male, female, children, pregnant, lactating) differ regarding their requirement for iron. Iron may also exhibit its health benefits in curing anemia (Feyzi *et al*., 2017). Consumption of iron rich food could help in the treatment of iron deficiency anemia.

Zinc is an essential trace element for human. It is vital for many biological functions and plays a crucial role in enzymes activities in human body. It is especially important in pregnancy, skin care, wound healing and immune resistance. A high level of zinc in human body has been

associated with acute effects such as vomiting and gastrointestinal irritation (Feyzi *et al*., 2017). Highest concentration of Zinc (Zn) (181.10±0.20) mg/100g was found in Osogbo exposed bread samples (OEB) while the least concentration (105 .36±0.41) mg/100g was present in Ikirun exposed bread samples (IEB). These values are lower than the permissible level of Zn in foods (5000mg/100g) (USDA (2003). The of level of zinc in the present study was in agreement with the previous studies (Demironzi *et al*., 2003; Dawood, 2011).

The level of Cadmium in the bread samples ranged from 0.016- 0.029 mg/100g. The values were above the permissible limit of Cadmium in food is 0.005 mg/100g. All the samples had higher levels of cadmium than permissible. The principal toxic effect of cadmium is its toxicity to the kidney, although it is has been found to be associated with lung diseases (including induction of lung tumors) and skeletal changes in occupationally exposed populations (Alimentrarius, 1994: Dawood, 2013). Generally, it was observed that higher concentrations of heavy metals were present in the exposed bread samples being hawked at bus terminals than the fresh samples. However, the statistical analysis of the data revealed there is no significant difference between heavy metals present in bread samples from different bus terminals across the sampling areas ($p < 0.05$).

Conclusions

The results of this study have shown that bacterial strain isolated from both fresh and exposed bread samples have the capability of exposing the consumers to serious diseases. The isolates obtained from the bread samples reveal that there is high population of microbial load in exposed bread than the fresh bread samples. This may be as a result of careless exposure of the bread to environmental contaminants and improper hygiene being practiced by the food (bread) handlers. The bacteria isolated from the bread samples were identified as *Enterobacteraerogenes* (7), *Salmonella typhi* (6), *Pseudomonas aeruginosa* (5), *Staphylococcus aureus* (5), *Klebsiellapneumoniae* (4), and *Enterococcus faecalis* (3) respectively.Similarly, the fungi species isolated from the bread samples include the species of *Aspergillus*, *Mucor* and*Penicillium* respectively. Results of heavy metals analysis showed that the bread samples contained Fe, Zn, Cd and Pb at level that were at wide variance with those specified by such bodies as NAFDAC or WHO. There is need to place a close surveillance on bakers and retailers of bread in order to ensure better compliance with health regulations and safe guarding the health of bread consumers..

References

- AOAC International (2000). Official Methods of Food Analysis, *Association of Official Analytical Chemists.* Washington, DC,USA, 16th edition.
- APHA (2001). Compendium of Methods for the Microbiological Examination of Foods, *American Public Health Association*. Washington, DC, USA, 4th edition.
- Demirozu, B., Saldamh, T., Gursel, B., Ucak, A., Cetinyokus, F., Yuzbazi, N (2005). Determination of Some Metals Which are Important for Food Quality Control in Bread. *Journal of Cereal Science.* 37: 171- 177.
- Feyzi,Y., Malekirad, A., Fazilati, M., Salavati, H., Habibollahi, S., Rezaei1, M.(2017). Metals that are Important for Food Safety control of Bread Product. *Advances in Bioresearch*. 8(1): 111-116.
- Ijah, U. J. J., Auta, H. S., Aduloju, M.O., Aransiola, S.A (2014). Microbiological, Nutritional, and Sensory Quality of Bread Produced from Wheat and Potato Flour Blends. *International*

Journal of Food Science and Technology. 1-6.

- Isong, N. B., Akpan, M.M., Udota, H.I. and Barber, L (2013). Antibacterial and Microbial Assessment of Breads in Aba Local Government Area, Nigeria, West Africa. *Journal of Microbiology and Biotechnology Research*, 3 (3):155-159.
- Jarup, L (2003). Hazards of Heavy Metal Contamination. *British Medical Bulletin*, 68, 167-182.
- Krieg, N.R.K., Holt, J.G (1994). Bergey's Manual of Systematic Bacteriology.
- Magomya, A. M., Yebpella, G. G., Udiba, U. U., Amos, H. S., Latayo, M. S. (2013). Potassium Bromate and Heavy Metal Content of Selected Bread Samples Produced in Zaria,Nigeria. *International Journal of Science and Technology*, 2, 232-237.
- Oluwajoba, S. O., Malomo, O., Ogunmoyela, O. A. B.,Dudu, O. E. O., Odeyemi, A (2012), Microbiological and Nutritional Quality of *Warankashi* Enriched Bread. *Journal of Microbiology, Biotechnology and Food Sciences*, 2(1): 42–68.
- Raji, R. A (2010). Effect of Pre-treatment on Sweet Potato Flour for Cookies Production. [M.S. Thesis]. University of Agriculture, Abeokuta, Nigeria.
- USDA. (2003). Zinc is Food: Draft for Comment. Foreign Agricultural Service (GAN Report) CH3043.