

## Original Research Article

### Effect of Storage Condition on the Microbial and Proximate Composition of Bread Made From Wheat Flour and White Flour

#### ABSTRACT

Bread is one of the most consumed staple foods in the world. It is a good source of nutrients, such as carbohydrates, protein, and fat and micronutrients (minerals and vitamins) which are essential for human health. The aim of ~~the this~~ research is to determine the effect of storage conditions on the microbial and physico-chemical composition of bread from wheat flour and white flour. The fresh samples (white and wheat ~~bought from the super market~~) were cultured according to the standard microbiological procedures. The Total Heterotrophic Bacterial Count (THBC) of the fresh white flour bread sample (FB) obtained was  $1.3 \times 10^4$  CFU/g (4.11 logCFU/g) while that of Wheat bread (WB) sample was  $1.2 \times 10^4$  CFU/g (4.00 logCFU/g). The total Staphylococcal Count (TSC) of the samples was recorded as  $4.9 \times 10^3$  CFU/g (3.69 logCFU/g) which was higher in comparison to that of wheat bread with the count of  $1.4 \times 10^3$  CFU/g (3.14 logCFU/g). Decrease in THBC of wheat bread and white bread sample stored at refrigeration temperature as compared to the bread samples stored at ambient temperature in relation to the days of storage was observed. Refrigeration had little effect on the growth of fungi as observed in this study as the Total Heterotrophic Fungal count (THFC) of refrigerated white bread sample increased from  $2.0 \times 10^3$  CFU/g (3.30 logCFU/g) on the day 6 to  $8.9 \times 10^3$  CFU/g (3.94 logCFU/g) on the day 8. The microorganisms isolated were *Micrococcus* sp, *Staphylococcus* sp, *Bacillus* p, *Aspergillus niger*, *A. flavus*, *Rhizopus* sp, *Mucor* sp, *Penicillium* sp, *Candida* sp, and *Saccharomyces* of which *Bacillus* and *Aspergillus* were most occurring among bacteria and fungi respectively. The moisture content, crude lipid, crude protein of the white bread sample were higher than those of wheat bread while the ash content, crude fiber and carbohydrate were higher in the wheat bread sample as compared to those of White bread sample. From the result obtained in ~~the this~~ study, it was observed that refrigeration temperature was effective in the increase in shelf life of bread by the reduction in bacteria growth in bread thus, it can be recommended for the storage of bread.

Comment [E1]: this information may be cited in materials section

KEYWORDS BREAD, PATHOGEN AMBIENT TEMPERATURE , REFRIGERATION TEMPERATURE

Comment [E2]: not adequate, see comments of reviewer

#### 1. Introduction

Bread is one of the most consumed staple food in the world. It's origin dates back to the Neolithic era. It is a good source of nutrients, such as carbohydrates, protein, and fat and micronutrients (minerals and vitamins) which are essential for human health (1, 2). After rice, bread has become the second most widely consumed non indigenous food product ~~after rice~~ in Nigeria. It is consumed extensively in most homes, restaurants, and hotels. Flour from wheat has been the major raw material in the production of bread (2). The increase in bread consumption has been the result of a number of factors including urbanization and a growing population, a shift in food preferences towards snacks (biscuits, bread, etc.) and increased wealth in tropical countries (3).

Comment [E3]: repeated in abstract

According to Sibanda *et al.* (4), it is expected that demand for foods based on this cereal, such as bread, will increase by 2050. Unfortunately, wheat is a temperate crop that is not conducive to tropical areas because of soil and adverse weather conditions. As a result, the countries in these areas, most of which are in the process of development, are spending huge sums (25,000 billion CFA francs in 2011) on importing wheat (3).

Starch known as polysaccharides is the main component of wheat flour in higher amount. There are different kinds of flour used in cooking, they are; all-purpose flour, self-rising flour, and cake flour including bleached flour. Constituents such as protein and ash generally influence the end-use of flour also. The higher the protein content the harder and stronger the flour, and the more it will produce crusty or chewy breads. The important quality parameters for wheat flour performance are moisture, wet gluten, gluten index, ash, weevils count and flour microbiology (5). White flour is a type of flour that made mainly from the endosperm only. A total of 57% of processed wheat flour is used in the baking and confectionery industry, 16% is used for domestic consumption, 17% for dough, 12% for cookies and 2% for the production of drugs, glue and animal feeding (14, 5).

All microorganisms have a defined temperature range in which they grow, with a minimum, maximum, and optimum. An understanding of the interplay between time, temperature, and other intrinsic and extrinsic factors is crucial to selecting the proper storage conditions for a food product. Temperature has dramatic impact on both the generation time of an organism and its lag period. However, fresh bread has short shelf life, and numerous chemical and physical alterations, known as staling, occur during its storage period. The texture and flavor of fresh bread deteriorate rapidly, and as a result, it loses its freshness and crispiness, while crumb firmness and its rigidity increase. Overtime as a result of this deterioration, the pleasant bread flavour disappears, and a stale taste can be observed. These preservation problems have required advanced technology for longer stable dough storage. Freezing is advantageous processing system to preserve the quality of dough and bread (6).

Storage temperatures significantly influence on the moisture contents and other physico-chemical parameters of wheat flour during storage which might result in the changes in the quality of bread (7). Temperature is an important factor that influences the growth of microorganisms and as such, determines the shelf-life of food including staple food such as bread. Low temperature results in the reduction in water activity needed for growth of deteriorating microorganisms (6).

The aim of ~~the~~ this research was to determine the effect of storage conditions on the microbial and physico-chemical composition of bread from wheat flour and white flour. Shelf-life of food (especially staple like bread) is an important condition to be considered in the quality of food as it helps to ascertain the length of time after production and packaging during which food retains a required level of quality at certain condition, this in turn determine the quantity of food to be bought or prepared.

**Comment [E4]:** this paragraph should be placed before the objective

## 2. Materials and Methods

### 2.1 Sample Site/ Sample Collection

Freshly prepared loaves of white and wheat bread were purchased from Market square supermarket at Choba junction, Obio/Akpor Local Government Area (4°53'54''54N, 6°54''E). The samples were transported aseptically and immediately to the laboratory for analysis. The samples were aseptically shared into different zip-lock bags and labelled according as RWB, RFB, RmFB, RmWB. RWB and RFB samples were stored in the refrigerator at 4°C while RmFB and RmWB were stored at ambient temperature.

Comment [E5]: add the contry

Comment [E6]: not necessary

### 2.2 Sample Preparation

The samples (white and wheat bread) were stored at two different temperature, ambient temperature (28-30°C) and refrigeration temperature (4°C) and monitored for microbial load for the period of eight days (day 0, day 2, day 4, day 6 and day 8) to determine the effect of storage conditions on the microbial quality of the samples in relation to days of storage.

### 2.3 Microbial Analysis of the Samples

The total mesophilic bacteria and fungi of the sample were analysed by weighing 10g of the samples (bread) into ~~90ml~~ 90mL of 1% sterile peptone water (diluent). Serial dilution was carried out aseptically using sterile pipette. After dilution, aliquot (0. ~~1ml~~ 1mL) of the diluted samples were cultured on different media (MacConkey, NA, PDA and MSA) using sterile hockey stick. The cultured plates were incubated aerobically at 35°C (for MacConkey, NA and MSA) for 24 hours and at 28-30°C (for PDA) for 48 hours. This analysis was repeated for the samples (white and wheat bread) stored at the ambient temperature and refrigerated temperature for five days (day 0, day 2, day 4, day 6 and day 8).

After culture incubation, the Total Heterotrophic count of the bacteria and fungi were determined by counting the colonial growth on the cultured plates and the CFU/MI (colony forming unit) were calculated. The different isolates of the cultures were purified by streaking the bacterial isolates on the freshly prepared nutrient agar plates based on their different cultural morphological characteristics and incubated at 37°C for 24 hours to obtain pure cultures of the isolates. Based on the cultural morphology (macroscopy), the fungal isolates were subcultured on freshly prepared PDA plates and incubated for 30°C for 48-72 hours. Different biochemical tests carried out in the research to determine identify the isolated bacteria were; methylred-voges proskauers (MRVP) test, sugar fermentation tests, catalase, indole, production test, test for hydrogen sulphide and gas production, citrate utilization test.

The fungal microscopy of the fungal isolates were determine. To appreciate the microscopic feature of the fungi isolated, lactophenol cotton blue was dropped on a clean glass slide, little growth of the fungus was removed with a sterile inoculating needle, and the

preparation was covered with a clean coverslip and examined under the microscope with  $\times 10$  magnification.

#### 2.4 Proximate Analysis

Proximate composition of the whole wheat and the white bread samples were determined using Mongi *et al.*, (16) methods. Moisture content (% MC) was determined by drying samples in an oven at 105°C for 24 hours. Crude protein percentage (% CP) was determined by Kjeldahl method and the percentage nitrogen obtained was used to calculate the % CP using the relationship:  $\% \text{ CP} = \% \text{ N} \times 6.25$ . Percentage ash (%) was determined by the method of AOAC, (15) it was determined by incinerating the samples in a muffle furnace at 550°C for four hours. The ash was cooled in a desiccator and weighed. Crude fibre percentage (% CF) was determined by dilute acid and alkali hydrolysis AOAC (15) method and the ~~Carbohydrate~~ was calculated by ~~estimation by difference~~ subtraction (15).

#### Statistical analysis

All measurements was done in duplicates for each ~~of the~~ samples, and data were reported for duplicate analyses of the same extract. All statistical analyses were carried out using analysis of variance (ANOVA)-. Significance of the differences was ascribed at the 0.05 level for ANOVA

Table 3.1: Microbial counts of the fresh bread samples on the first day (Day 0)

	Samples	Dilution	Counts	CFU/g	Log CFU/g
<b>THBC</b>	FB	10 <sup>-2</sup>	13	1.3×10 <sup>4</sup>	4.11
	WB	10 <sup>-2</sup>	12	1.2×10 <sup>4</sup>	4.00
<b>Total Coliform</b>	FB	10 <sup>-1</sup>	Nil	Nil	Nil
	WB	10 <sup>-1</sup>	Nil	Nil	Nil
<b>Total Staphylococcal Count</b>	FB	10 <sup>-1</sup>	49	4.9×10 <sup>3</sup>	3.69
	WB	10 <sup>-1</sup>	14	1.4×10 <sup>3</sup>	3.14
<b>THFC</b>	FB	10 <sup>-1</sup>	Nil	Nil	Nil
	WB	10 <sup>-1</sup>	Nil	Nil	Nil

### 3 Results

**Comment [E7]:** authors must describe each results with corresponding table or figures and interpretation of each result statistically

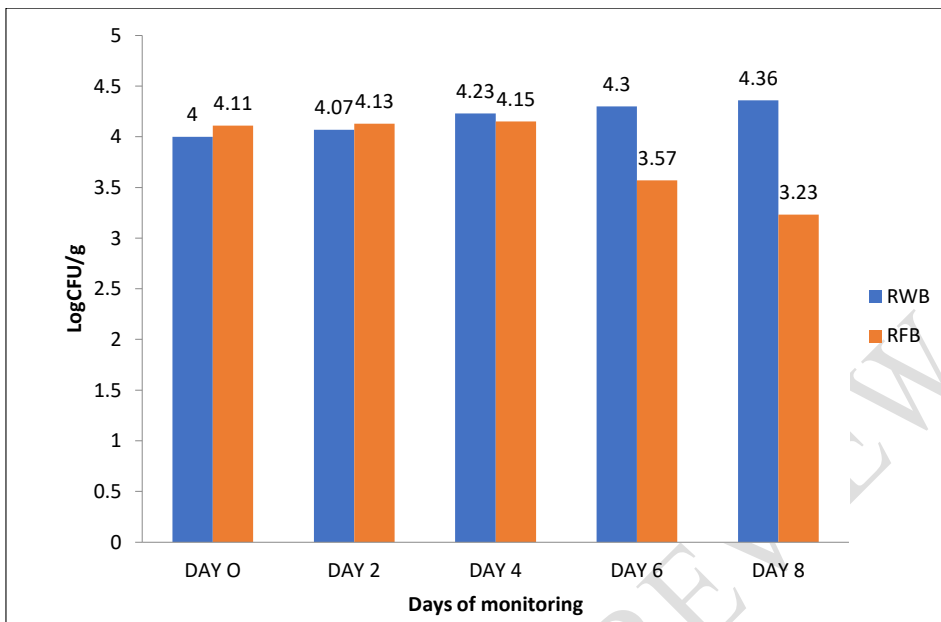


Fig 3.1: Total Viable Count of the samples, White bread (RFB) and Wheat bread (RWB) at refrigeration temperature in relation to the days of monitoring

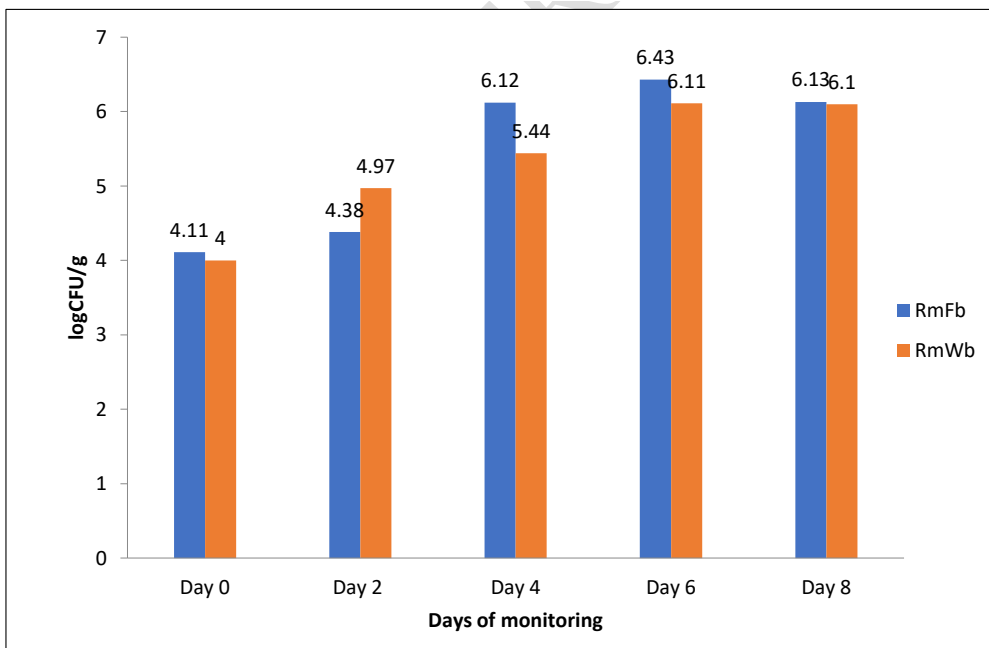


Fig 3.2: Total Viable Count of the samples, White bread (RmFB) and Wheat bread (RmFW) at ambient temperature in relation to the days of monitoring

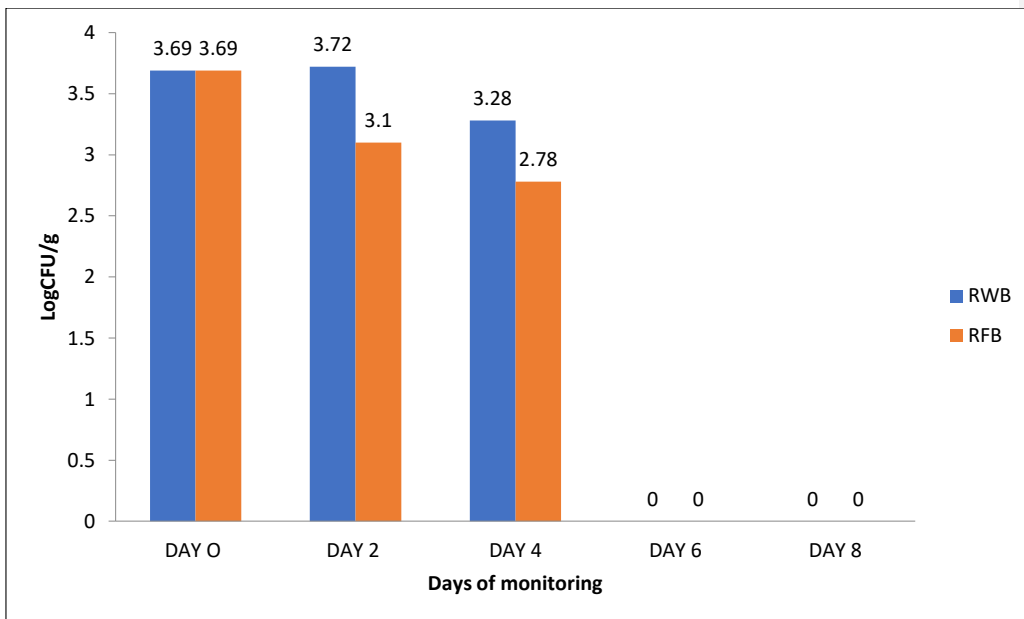


Fig 3.3: Staphylococcal Counts of the White bread (RWB) and Wheat bread (RFB) samples at refrigeration temperature in relation to the days of monitoring

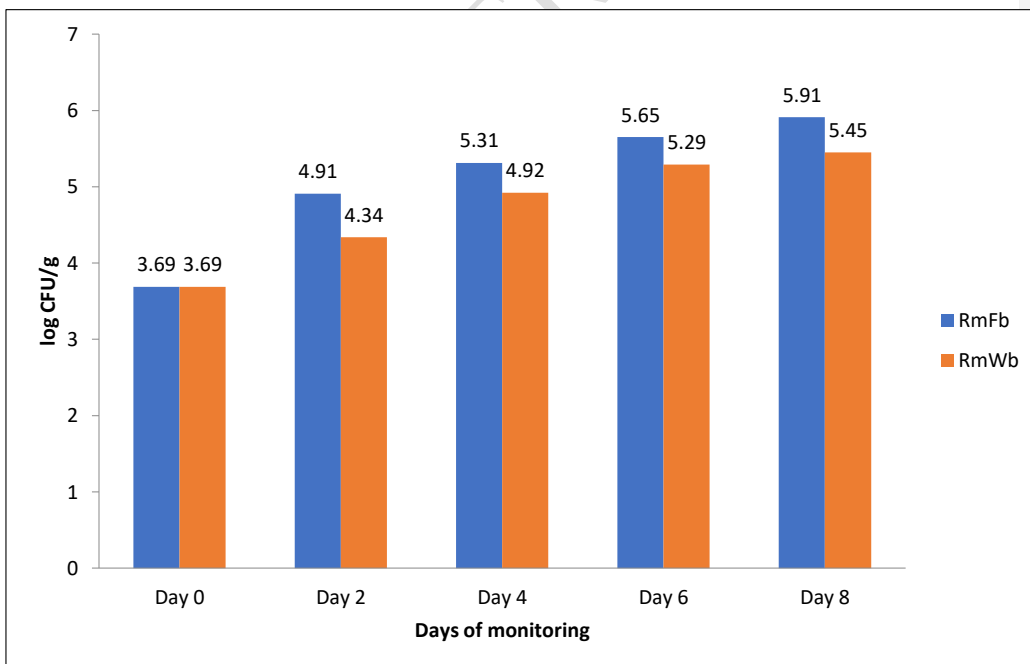


Fig 3.4: Staphylococcal Counts of the White bread (RmFB) and Wheat bread (RmFB) samples at ambient temperature in relation to the days of monitoring

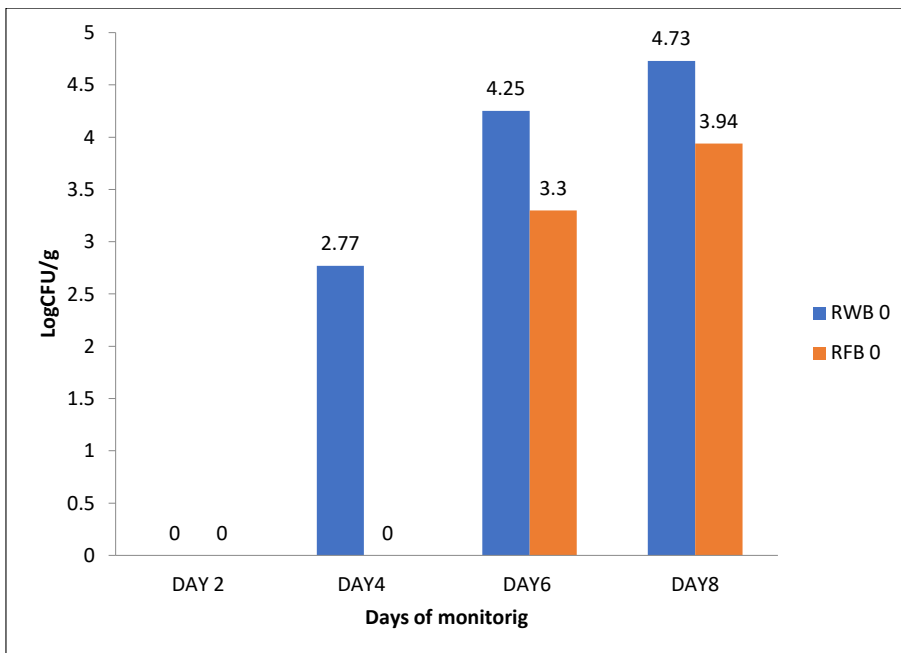


Fig 3.5: Total Heterotrophic Fungal Counts of the White bread (RFB) and Wheat bread (RWB) samples at refrigeration temperature in relation to the days of monitoring

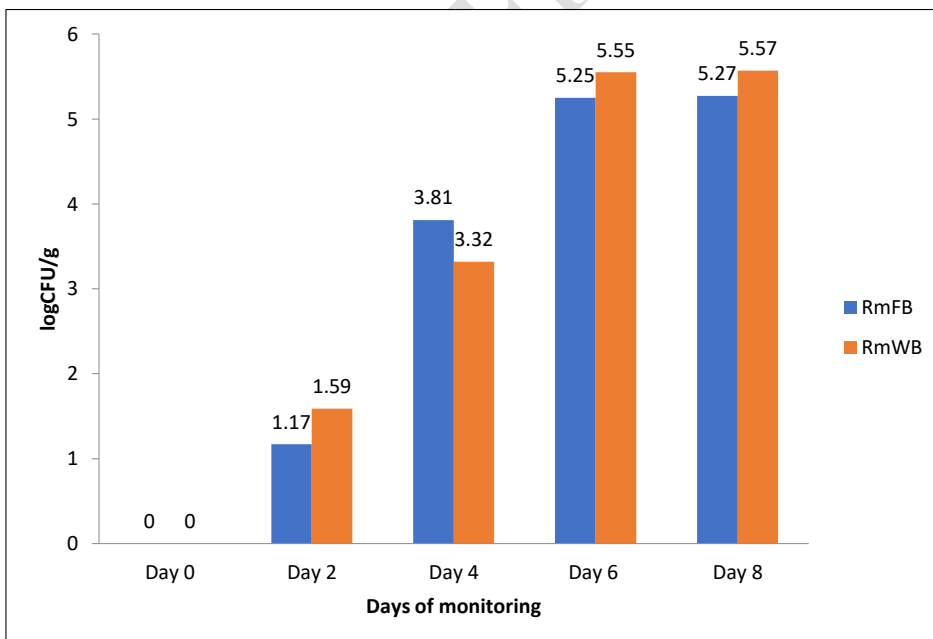


Fig 3.6: Total Heterotrophic Fungal Counts of the White bread (RmFB) and Wheat bread (RmWB) samples at ambient temperature in relation to the days of monitoring

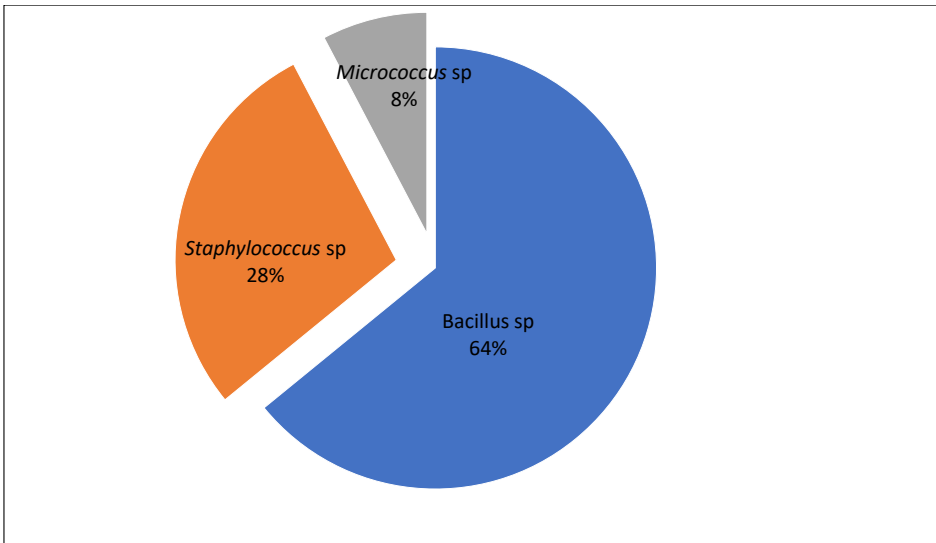


Fig 3.7: Percentage occurrence of the bacteria identified from the various samples

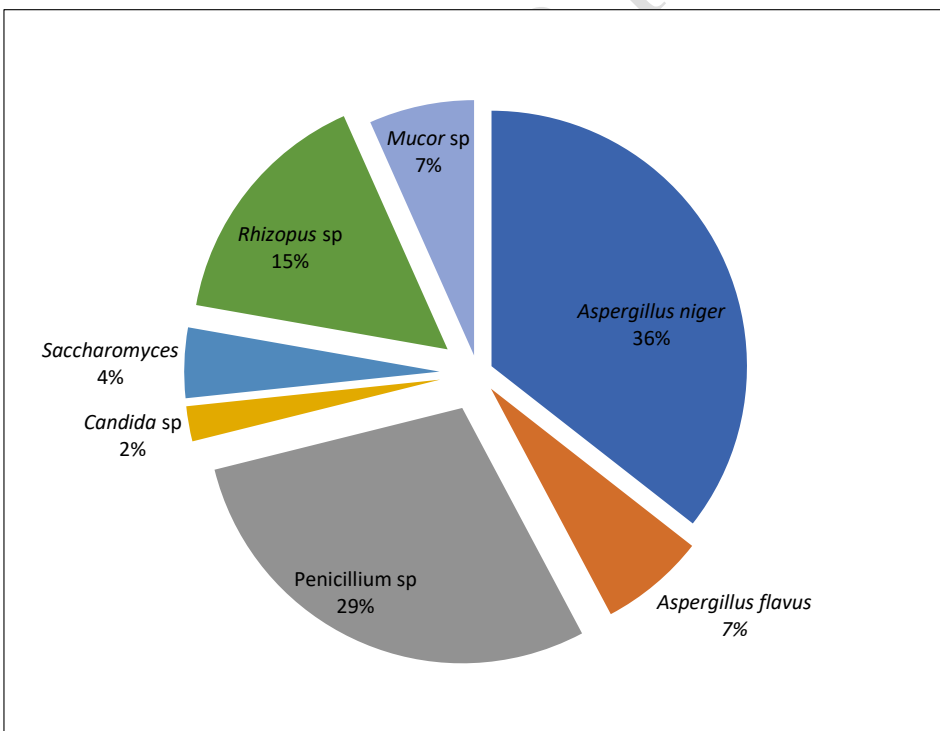


Fig 3.8: Percentage occurrence of the fungi identified from the various samples

Sample	Moisture content %	Ash (%)	Crude Lipid (%)	Crude fibre (%)	Crude Protein (%)	Carbohydrate (%)
White bread	8.26	0.89	1.34	1.05	12.96	75.5
Wheat bread	6.83	1.18	1.02	3.94	9.4	77.61

Table 3.2: Proximate analysis result of the samples

#### 4. Discussion

The microbial analysis of the fresh sample (as shown in Table 3.1) revealed the total heterotrophic bacterial count ranged from  $1.2 \times 10^4$  CFU/g ( $4.00 \log$  CFU/g) to  $1.3 \times 10^4$  CFU/g ( $4.11 \log$  CFU/g) with white bread sample significantly higher in heterotrophic bacterial count than the wheat sample ( $p < 0.05$ ). The total Staphylococcal counts of  $4.9 \times 10^3$  CFU/g ( $3.69 \log$  CFU/g) for white bread and  $1.4 \times 10^3$  CFU/g ( $3.14 \log$  CFU/g) of wheat sample were also obtained from the fresh samples. The heterotrophic bacteria count of the samples is in contrast to the findings of Ijah *et al.*, (2) in which there was higher count of heterotrophic bacteria. No count of coliform and fungi was observed in the fresh samples. The microbial count of heterotrophic bacteria and *staphylococcus* obtained in the fresh sample of bread is above the limit set by the standard organisation of Nigeria which states that the count of aerobic bacteria must not exceed 100 CFU/g but was within the expectation of the governing body in terms of coliforms presence which was absent from the samples, thus the fresh white and wheat bread sample was safe for consumption as a result of the absent of faecal

**Comment [E8]:** this must be placed in results section

contamination. The higher bacterial count in white bread sample might be as a result of higher moisture content compared to the wheat sample (8).

To assess the microbial stability of the bread samples under two storage condition (ambient temperature and refrigeration temperature), microbial count and isolation technique was employed on day 2, day 4, day 6 and day 8. The Total Heterotrophic Bacterial Count (THBC) (as presented in fig 3.1) shows reduced bacterial growth in refrigerated white bread sample from day 2 to day 8 in which there was very slow or little increase in bacterial count in relation to the storage time in the cold storage condition. For the sample of wheat bread at refrigerated temperature, there was declined in the bacterial growth after the day 4 compared to the bacterial growth in white bread which increased but at a reduced rate. As shown in fig 3.2, increased in heterotrophic bacteria was observed in both samples stored at ambient temperature although white bread sample showed a significant higher count ( $p < 0.05$ ) which might have been as a result of their higher moisture content and protein content. The reduced count of heterotrophic bacterial in refrigerated samples may be as a result of reduction in the amount of water activities thus, increase in their shelf-life of bread can be attained at refrigeration temperature. Moisture is a very important factor in the keeping the quality of bread and higher moisture content may therefore reduce the shelf-life in bread (9). No growth count of coliform was observed during the monitored storage period. This is similar to the report by Alpers *et al.*, (9) in which there was no coliform present in the microbial analysis of bread.

Figure 3.3 and Figure 3.4 shows the staphylococcal count at both refrigeration and ambient temperature respectively. The staphylococcal count in relation to storage time showed a reduced growth count in refrigerated bread samples of wheat and white bread that is to infer that the staphylococcal count reduced with longer time in cold storage temperature. When considering growth rate of microbial pathogens, time and temperature are integral and must be considered together. Increases in storage and/or display temperature will decrease the shelf life of refrigerated foods since the higher the temperature, the more permissive conditions are for growth (10).

The total heterotrophic fungi count of the samples stored at ambient temperature increased exponentially from day 2 to day 6 and day 8 where reduced growth was observed (as shown in Figure 3.5) and the fungi count were higher in wheat bread stored at ambient temperature compared to the white bread (as shown in Figure 3.6). No total heterotrophic fungi was observed from the day 2 and 4 for refrigerated wheat bread and refrigerated white sample respectively and an increased was observed on day 6 before gradual increased growth count was observed on the day 8 for both refrigerated samples. The exponential growth of fungi in the refrigerated samples could be because moulds are much more tolerant to cold than heat and may also be as a result of the ability of mould to occur in reduced water activities (10).

The bacterial isolated from the samples are *Micrococcus* sp, *Staphylococcus* sp and *Bacillus* sp of which *Bacillus* sp was the most occurring bacteria isolated (as shown in Figure 3.7). These bacteria as similar to those isolated from Ijah *et al.*, (2) from the microbial analysis of bread. *Bacillus* occurred predominantly up to the day 8 when other bacteria were reduced or

absent in both refrigerated sample and sample at ambient temperature. *Bacillus* sp form spores which enable the bacteria to survive unfavourable conditions like hot and cold temperature. Spoilage organisms are heat-resistant spores of bacteria belonging to *Bacillus* genera, which survive the baking process. Members of the *Bacillus* genus that bring about bacterial spoilage of bread are known as rope. This is of major economic concern to the baking industry. Ropiness, which is the most important spoilage of bread after moldiness, occurs particularly in summer when the climatic conditions favor the growth of bacteria. It is mainly caused by *Bacillus subtilis* but *Bacillus licheniformis*, *Bacillus megaterium* and *Bacillus cereus* have also been associated with ropy bread. Most important rope formers are *B. subtilis*, *B. licheniformis* and *B. mesentericus* (11). *Staphylococcus* species are widely distributed in the environment and occur on the skin and nostrils of human from where the organisms can contaminate food through cross contamination.

The fungi isolated in the course of the study were *Aspergillus niger*, *A. flavus*, *Rhizopus* sp, *Mucor* sp, *Penicillium* sp, *Candida* sp, *Saccharomyces*. *Aspergillus* had the highest percentage of occurrence than other fungi isolated (as presented in Figure 3.8). This is in line with the report of Dantyan and Nwokwu (12). Mold growth even occur in refrigerators, because molds are much more tolerant to cold than heat. Molds can grow at reduced water activities and can be a problem in improperly processed dry and semi-dry fermented products. Molds, such as *Aspergillus*, *Rhizopus*, and *Penicillium*, are responsible for the spoilage of bread (10).

The chemical composition of white and wheat flour bread samples was determined as represented in Table 3.2 showed that white bread had 8.26% moisture content, 0.89% ash content, 1.43% crude lipid, 1.05% crude fibre, 12.96% crude protein and 75.5% carbohydrate while the wheat bread had 6.83% moisture content, 1.18% ash content, 1.02% crude lipid, 3.94% crude protein and 77.61% carbohydrate. The white bread sample contained higher amount of moisture content, crude lipid than wheat bread as analysed. Also, the wheat-bread sample was high in ash content, crude fiber and carbohydrate. The wheat bread sample was high in dietary fiber (3.94%) compared to those of white bread (1.05%). This is similar to the report by Bae *et al.*, (13) and Ijah *et al.*, (2). The high value of fiber in wheat bread sample might arise from the fact that whole grain wheat used in wheat flour includes fractions of germs and brans that are removed during the milling process of white wheat flour (13). Moisture content and crude protein of white bread sample was higher than that recorded in wheat bread sample contrary to the report by Bae *et al.*, (13) where wheat flour produce higher moisture content. Wheat bread sample produced higher carbohydrate content compared to white bread sample thus wheat bread should produce more calories of energy compared to white bread which is in line with the report of Ijah *et al.*, (2) where wheat flour produced more carbohydrate content.

## 5 Conclusion

From the results it is apparent that humidity and temperature are important factors to be considered in microbial shelf life of bread. Reducing the temperature to refrigerated temperatures was shown to be able to decelerate the growth rate of spoilage microorganisms

in bread. Consequently, both factors should be considered when designing suitable storage methods for bread. From the result of the study, it will be advised that bread not completely consumed after opening should be stored at refrigeration temperature to inhibit or reduce the growth of spoilage and possible pathogenic microorganisms as cold temperature was seen to be effective in the increase in shelf life of bread by the reduction in bacteria growth in bread although not completely effective in the reduction of fungal growth thus, storage of bread at ambient temperature for a long time should be discouraged to reduce microbial contamination.

**Comment [E9]:** unclear, please consider rewriting with English editing

### REFERENCES

1. Oluwajoba, S. O., Malomo, O., Ogunmoyela, O. A. B., Dudu, O. E. O. and Odeyemi, A. (2012). Microbiological and nutritional quality of warankashi enriched bread. *Journal of Microbiology, Biotechnology and Food Sciences*, 2:42–68
2. Ijah, U. J., Auta, H. S., Aduloju M. O. and Aransiolo S. A. (2014). Microbiological Nutritional and Sensory Quality of Bread Produced from Wheat and Potato Flour Blends. *International Journal of Food Science*, 10:24-30
3. Badje D. S., Soro D., Yeo M. A. and Koffi E. K. (2018). Physico-chemical, Functional and Sensory Properties of Composite Bread Prepaid from Wheat and Defaulted Cashew (*Anacardium occidentale* L.) Kernel Flour. *International Journal of Environmental and Agriculture Research*, 4(4):88-92
4. Sibanda, T., Ncube, T., and Ngoromani, N., (2015). Rheological Properties and Bread Making Quality of White Grain Sorghum-Wheat Flour Composites. *International Journal of Food Science and Nutrition Engineering*, 5(4): 176-182.
5. Sabaragamuwa K., Fernando, K. M. C. and Sparke, D. L. (2018). Nitrogen uptake utilization and use efficiency of different wheat species. *University Journal*, 18(1):55-65
6. Ribotta P. D., Perez, G. T., Leon, A. E, and Anon, M. C. (2004). Effect of emulsifier and guar gum on microstructural, rheological and baking performance of frozen bread dough. *Food Hydrocolloids*, 18(2):305-313
7. Saranji, P. and Geetha, M. (2012). Microbial Spoilage of Bakery products and its Control by Preservatives. *International Journal of Pharmaceutical and Biological Archives*, 3(1):38-48
8. Willey, J. M., Sherwood, L., Woolverton, C. J., Prescott, L. M., and Willey, J. M. (2011). *Prescott's Microbiology*. New York: McGraw-Hill

9. Alpers, I., Kerpes R., Friolli M., Nobis A., Hoi K. I., Bach A., Jekle M., and Beckers T. (2021). Impact of Storing Condition on Staling and Spoilage Behaviour of Bread and Their Contribution to Prevent Food Waste. *Journal of food microbiology*, 10:76-81
10. Moral, U., Nagar P., Maan, S. and Kaur, K. (2017). A Growth of Different Types of Microorganisms, Intrinsic and Extrinsic Factors of Microorganisms and Their Effect in Food: A Review. *International Journal of Current Microbiology and Applied Science*, 6(1):290-298
11. Ybar, A., Cetinkaya, F. and Soyutemiz, G.E. (2012). Detection of rope-producing Bacillus in bread and identification of isolates to species level by Vitek 2 System. *Journal of Biological and Environmental Sciences*, 6: 243–248.
12. Dantyan, S. Y. and Nwokwu, O. E. (2011). “Enumeration of microorganisms associated with the different stages of bread production in FUTMIN bakery, Nigeria,” *International Research Journal of Pharmacy*, 2: (7)88–91
13. Bae, w., Lee, B., Hou, G. G., and Lee, S. (2014). Physicochemical characterization of whole Grain wheat flour in frozen dough system for bakery technology. *Journal of Cereal Science*, 60:520-525
14. Chang, Y. K. and Ferrari, M. C. (2014). A study on the methods used for wheat flour analysis and wheat gluten quality. *Food Science and Technology*, 34(2):235-242.
15. Bae, W., Lee, B., Hou, G. G., and Lee, S. (2014). Physicochemical characterization of whole Grain wheat flour in frozen dough system for bakery technology. *Journal of Cereal Science*, 60:520-525
16. Mongi R. J., Ndabikunze B. K., Chove B. E. Mamiro P., Ruhembe C. C. and Ntwenya J. G. (2011). Proximate composition, bread characteristics and sensory evaluation of cocoyam-wheat composite breads. *African Journal of Food, Agriculture, Nutrition and Development*, 11(7):5586-5599

Formatted: Font: Italic