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Investigation on Microbiological, Organoleptic and Shelf Life Characteristics of Wheat-cassava Bread

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Authors' contributions

This work was carried out in collaboration among all authors. Author CLO design the research, handled the laboratory work and wrote the draft. Author BJOE modified and approved the design and supervised the research. Author OCE co-supervised, read and approved the manuscript. All authors approved the manuscript.

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ABSTRACT

Aim: Microbiology, sensory and shelf life evaluation of wheat-cassava bread from raw materials to finished products.
Study Design: Completely randomized design with two replications and average values calculated for mean comparison.
Place and Duration of Study: Food/Industrial Laboratory, Department of Microbiology, University of Port Harcourt, Nigeria and Department of Medical Laboratory Science, Niger Delta University, Nigeria, from June 2018 to March, 2019.
Methodology: Microbiological, proximate, physicochemical, sensory and shelf-life characteristics of wheat-cassava bread produced using different formulations (90:10; 80:20; 70:30; 60:40) and control 100% wheat-bread were investigated during storage at 32±2°C, using standard methods. Bread were baked at 180°C and 200°C. Two independent determinations were analyzed using ANOVA and significance of the mean differences determined at *P*=.05.
Results: Mean total heterotrophic counts (THC) of raw flour samples increased from 5.41 to 7.62 log_{10} cfu g^{-1}; total coliform from, 1.08 to 1.90 log_{10} cfu g^{-1}; and total fungal counts from 2.46 to 4.88 log_{10} cfu g^{-1} with increasing cassava flour substitution. Mean values for THC and total fungal counts of baked bread samples increased from 1.88 to 7.73 log_{10} cfu g^{-1} and 0.48 to 4.05 log_{10} cfu g^{-1}

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respectively from day 0 to day 6. However, coliforms were not detected in baked bread. The predominantly isolated bacteria were: *Bacillus*, *Aerococcus* and *Staphylococcus* while fungi were: *Aspergillus, Penicillium* and *Fusarium*. Proximate composition showed that carbohydrate and ash contents increased from 46.07 to 55.29% and 1.35 to 3.79% respectively while crude protein and moisture contents decreased from 14.01 to 8.08% and 29.0 to 23.8% respectively and pH from 6.59 to 6.14 with increasing cassava flour substitution.

Conclusion: The overall acceptability of the wheat/cassava bread reduced with increasing cassava flour concentration, however, there was no significant difference *(P*=.05) in the overall acceptability of the 100% wheat bread and that of 90:10% till day four.

Keywords: Bread; cassava flour; microbial load; sensory attributes; shelf-life.

1. INTRODUCTION

Bread has become the second most widely consumed non-indigenous food product in Nigeria. In recent times, bread consumption has increased dramatically in many of the developing countries such as Nigeria, due to changes in eating habits, work schedule, ease of preparation of breakfast involving bread.

Bread is relatively expensive because it is made from imported wheat that is grown sparingly in Nigeria for climatic reasons. As a result of the increased rate of bread consumption in Nigeria, the country has to depend on imported wheat for the production of bread and other products. Nigeria imports wheat of more than a million tons annually, making it among largest wheat importers globally [1]. This importation has placed a huge burden on the Nigerian foreign reserve and the manufacturing sector. As a result of the huge depletion of our foreign reserve, the Federal Government directed the use of cassava as a supplement to wheat flour for baking by adding a minimum of 10% cassava flour to wheat for a start up to a maximum of 40% [2].

Because of the high cost and demand for wheat flour, efforts have been made to promote the use of composite flours in which flour from locally farmed crops such as cassava, yam, sweet potato, protein-rich flours including soybean, peanuts and bambara nut and other cereals (including maize, rice, millet and sorghum) replace a portion of wheat flour for use in bread, thereby decreasing the demand for imported wheat. Several reports have indicated that the composite bread can be made by substituting up to 40% cassava flour for wheat flour [1]. Cassava flour is a good substitute for wheat flour in bread making because of its ease to use in recipes in place of traditional grain-based flours or even gluten-free flour blends; its neutrality in terms of taste; it does not have dry, strong or unfamiliar taste or texture that often comes with using some gluten-free flours. Its texture blends well in baking products like biscuits, cookies and denser bread. It does not have sour taste or smell that fermented, sprouted grain flours contain [3,4].

Notwithstanding efforts made by the Nigerian government to sustain high quality cassava flour (HQCF) which has a significant economic advantage, many Nigerians are hesitant to incorporate different levels of HQCF into wheat flour for bread making. Those in the bakery sector have given several reasons for the slow adoption of this technology, including ease of deterioration of cassava bread due to its high moisture and microbial load, fear of the presence of toxic components in cassava, and bulkiness [1].

This work focused on the microbiology, sensory attributes and shelf life evaluation of wheat-cassava bread from raw materials to finished products.

2. MATERIALS AND METHODS

2.1 Sample Collection

The cassava flour used in this study was obtained from Shonghai Rivers Development Initiative, Bunu, Tai, Ogoni, Rivers State while wheat flour and other ingredients used (granulated sucrose, baking yeast and baking fat) were bought from Giant Bakery Shop Port Harcourt, Nigeria.

2.2 Preparation of Composite Flours

Wheat flour was mixed with cassava flour at several ratios: 90:10, 80:20, 70:30, and 60:40. The control bread was produced with 100% wheat flour. The recipe for the formulation of bread production is shown in Table 1.

Table 1. Recipe for bread production

Ingredients (g)	A	B	C	D	E
Wheat Flour	100	90	80	70	60
Cassava Flour	0	10	20	30	40
Yeast	2.0	2.0	2.0	2.0	2.0
Sugar	8	8	8	8	8
Salt	1	1	1	1	1
Fat	4	4	4	4	4
Water (ml)	60	60	60	60	60

2.3 Bread Production by the Straight Dough Method

The straight dough method was used to produce the bread. This method involves the addition of all the ingredients (flour, salt, water, sugar, yeast, fat) at mixing stage and kneading same to obtain the dough [5]. The different dough samples were placed in baking pans smeared with vegetable oil and covered for the dough to ferment resulting in gas production and gluten development for 1½h. The dough was then baked in the oven at 180°C and 200°C respectively for 30 min. The baked loaves were carefully removed from the pans and allowed to cool and packaged in polyethylene bags for analysis. Two samples of bread were prepared per formulation.

2.4 Microbial Analysis of Cassava Flour, Wheat Flour and Bread Samples

The total viable bacterial and fungal counts of cassava and wheat flour and each bread sample were determined using the pour plate technique. Ten grams of each sample was homogenized in 90 ml of sterile peptone water to obtain 10^{-1} and further serial dilutions up to 10^{-6} were prepared and analysed at intervals of 2 days for 6 days. Aliquot (1 ml) of each dilution was plated using pour plate method on plate count agar (PCA) for total viable bacterial count; MacConkey agar for total coliform count, and potato dextrose agar (PDA), which was modified with streptomycin to inhibit bacterial growth, was used for fungal count. Plates for bacterial counts were incubated at 37°C for 18-24 h while plates for fungal enumeration were incubated at 30±2°C for 72 h. The different colonies on each plate were isolated, purified, and stored on Nutrient agar slants (for bacteria) and PDA slants (for fungi) for further characterization and identification.

2.5 Identification of Isolates

The bacterial isolates were identified on the basis of their cultural, biochemical (indole, methyl red, Voges Proskauer, catalase test, citrate utilization test, oxidase test, urease test, glucose fermentation, lactose fermentation, and sucrose fermentation test) and molecular characteristics [6,7,8], while the fungal isolates were identified on the basis of their cultural and microscopic characteristics [9].

2.6 Phylogenetic Analysis

This was carried out using Saitou and Nei, [6] while the evolutionary distance was computed using Jukes-cantor method [10].

2.7 Proximate Analysis of the Samples

Proximate analyses were carried out on the samples (raw materials and baked samples). The percentage of total carbohydrate, moisture content, crude fat, crude protein, crude fibre, ash content were determined as described by AOAC [11].

2.8 Physicochemical Analysis of Samples

2.8.1 Cyanide content

The hydrogen cyanide content of the raw cassava flour was determined as described by AOAC [11].

2.8.2 The pH

A 10 g portion of the bread samples were homogenised in 20 ml of sterile distilled water. The pH of homogenate was determined using Jenway meter (3510). The pH of the sample was measured and recorded [12].

2.9 Sensory Evaluation of COMPOSITE Bread

Coded samples of the baked breads were evaluated for sensory attributes such as colour, texture, flavour, taste, appearance and overall acceptability at intervals of 2 days for 6 days by ten members' panelists who are familiar with quality of bread. The samples were evaluated using a hedonic scale of 1-7 [13] where:

> 1=Dislike extremely; 2=Dislike moderately; 3=Dislike slightly; 4= Neither like nor dislike; 5=like slightly; 6=like moderately; and 7= like extremely.

2.10 Shelf-life Study

This was done to determine the keeping quality of the baked products of the various formulations. The bread samples were stored at ambient temperature (32±2°C) and observed for 6 days. Visual observations for mould growth were carried out on the samples stored. Analysis (microbiological and pH determination) were done at 2 days interval for 6 days.

2.11 Statistical Analysis

Statistical analysis of the data obtained from two independent determinations was carried out using ANOVA and the significance of the mean differences determined at P=.05 using SPSS (version 20).

3. RESULTS AND DISCUSSION

3.1 Microbial Counts and Identification

The mean total heterotrophic counts (THCs) of the raw flour samples increased from 5.41 to 7.62 log_{10} cfu g^{-1}; total coliform from, 1.08 to 1.90 log_{10} cfu g^{-1}; and total fungal counts from 2.46 to 4.88 log_{10} cfu g^{-1} with increasing cassava flour substitution (Fig. 1). Bacterial isolates were identified as *Enterobacter aerogenes*, *Staphylococcus epidermidis, Bacillus subtilis, Aerococcus viridans, Shewanella chilikensis, Providencia vermicola, Proteus mirabilis* and *Klebsiella* sp (Fig. 2) while fungal isolates were identified as *Candida tropicalis, Candida krusei, Fusarium solani, Penicillium citrinum, Kodamaea ohmeri, Aspergillus niger, Candida rugosa* and *Aspergillus terreus* (Fig. 3). Figs. 4 and 5 show Phylogenetic characteristics of evolutionary relationship between the fungal isolates and bacterial isolates.

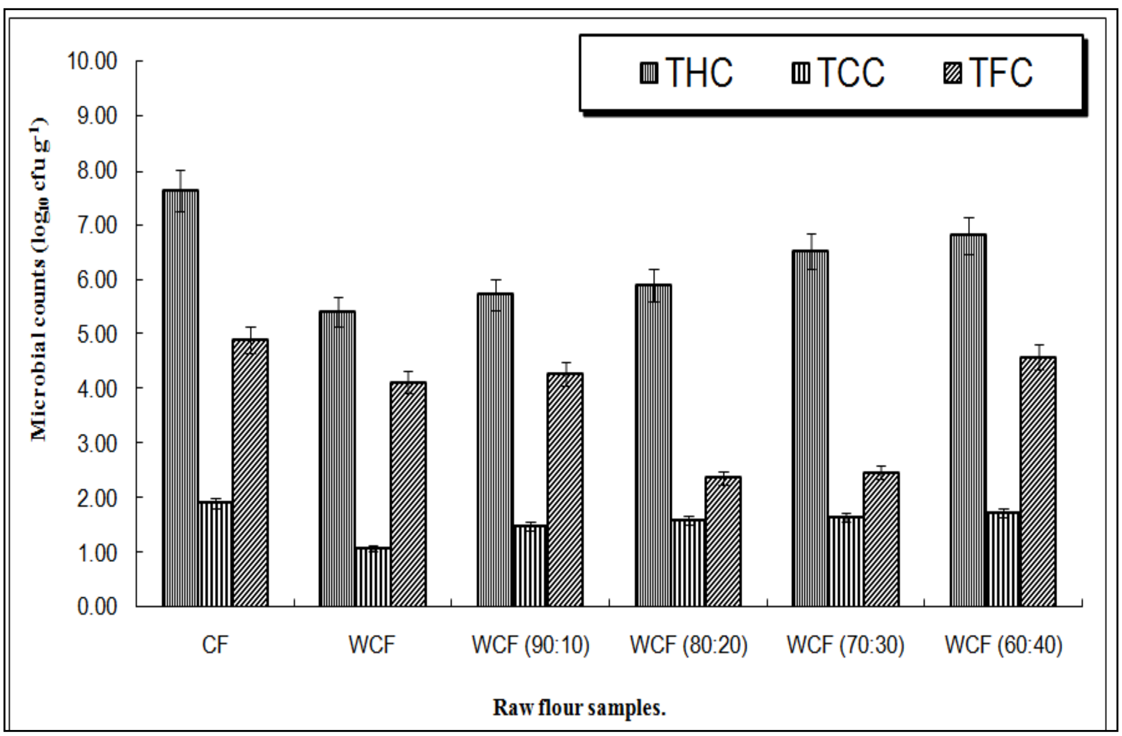

Fig. 1. Microbial counts from raw flour samples used in the study

THC = Total heterotrophic count; TCC = Total coliform count; TFC = Total fungal count; CF = Cassava flour; WF =Wheat flour; WCF (90:10) = Wheat and cassava flour (90:10); WCF (80:20) = Wheat and cassava flour (80:20); WCF (70:30) = Wheat and cassava flour (70:30); WCF (60:40) = Wheat and cassava flour (60:40). Error bars represent mean ± standard deviation of duplicate determinations

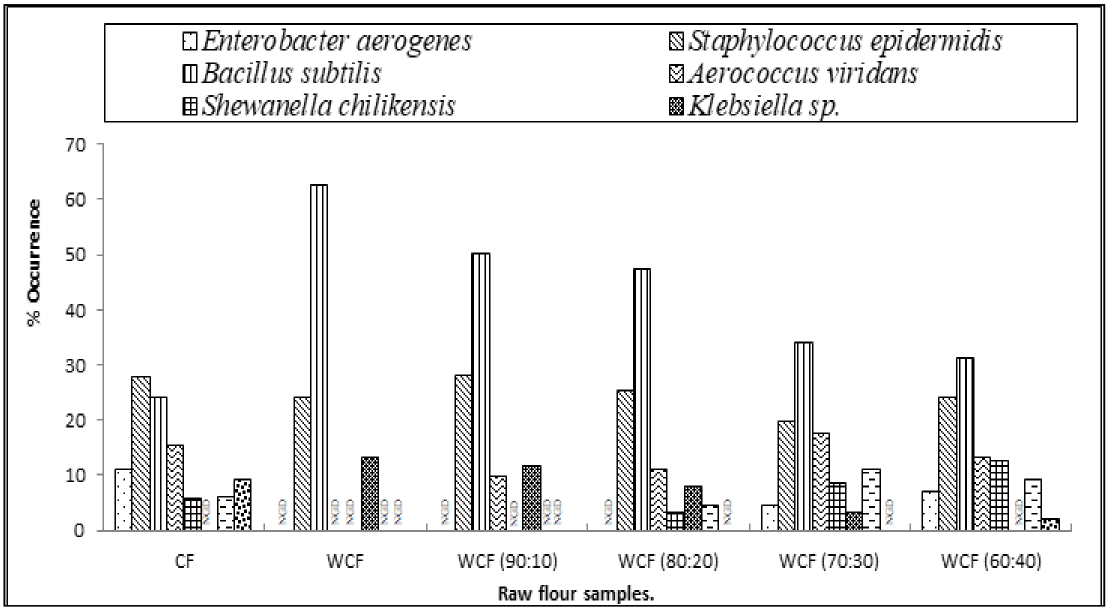

Fig. 2. Percentage occurrence of bacteria from the different raw flour samples
CF= Cassava flour; WF= Wheat flour; WCF (90:10) = Wheat and cassava flour (90:10); WCF (80:20) =Wheat and cassava flour (80:20); WCF (70:30) = Wheat and cassava flour (70:30); WCF (60:40) = Wheat and cassava flour (60:40); NGD = No growth detected

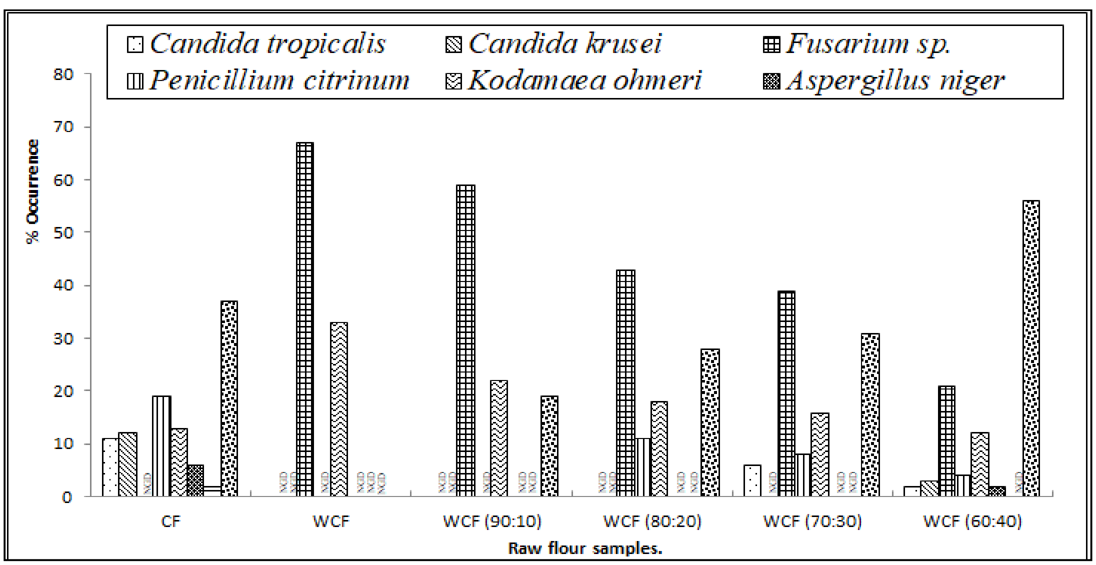

Fig. 3. Percentage occurrence of fungal species from the different raw flour samples
CF= Cassava flour; WF= Wheat flour; WCF (90:10) = Wheat and cassava flour (90:10); WCF (80:20) =Wheat and cassava flour (80:20); WCF (70:30) = Wheat and cassava flour (70:30); WCF (60:40) = Wheat and cassava flour (60:40); NGD = No growth detected

3.2 Proximate Composition of Raw Flour Samples

Cassava flour had the highest contents of carbohydrate (91.74%) and crude fibre (1.94%) while wheat flour had the highest protein content of (7.42%). However, carbohydrate and crude protein content of the wheat flour decreased with increasing cassava flour concentration while protein content of the wheat flour decreased with increase in cassava flour concentration (Table 2).

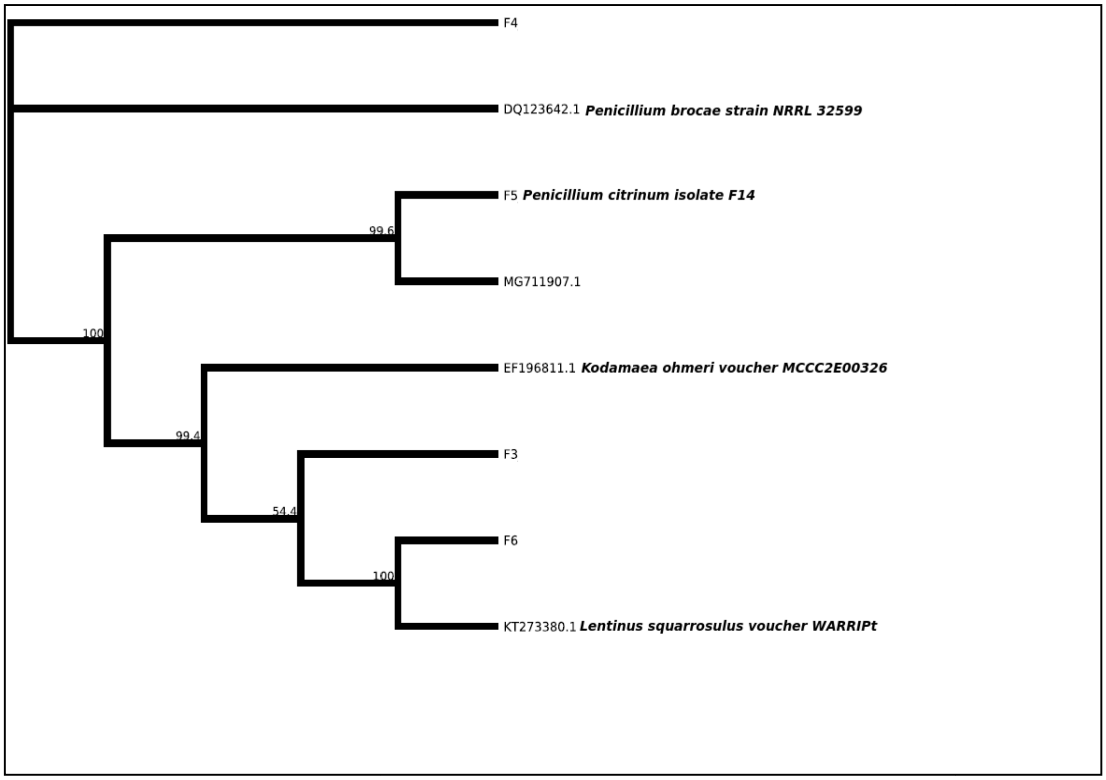

Fig. 4. Phylogenetic characteristics of evolutionary relationship between the fungal isolates

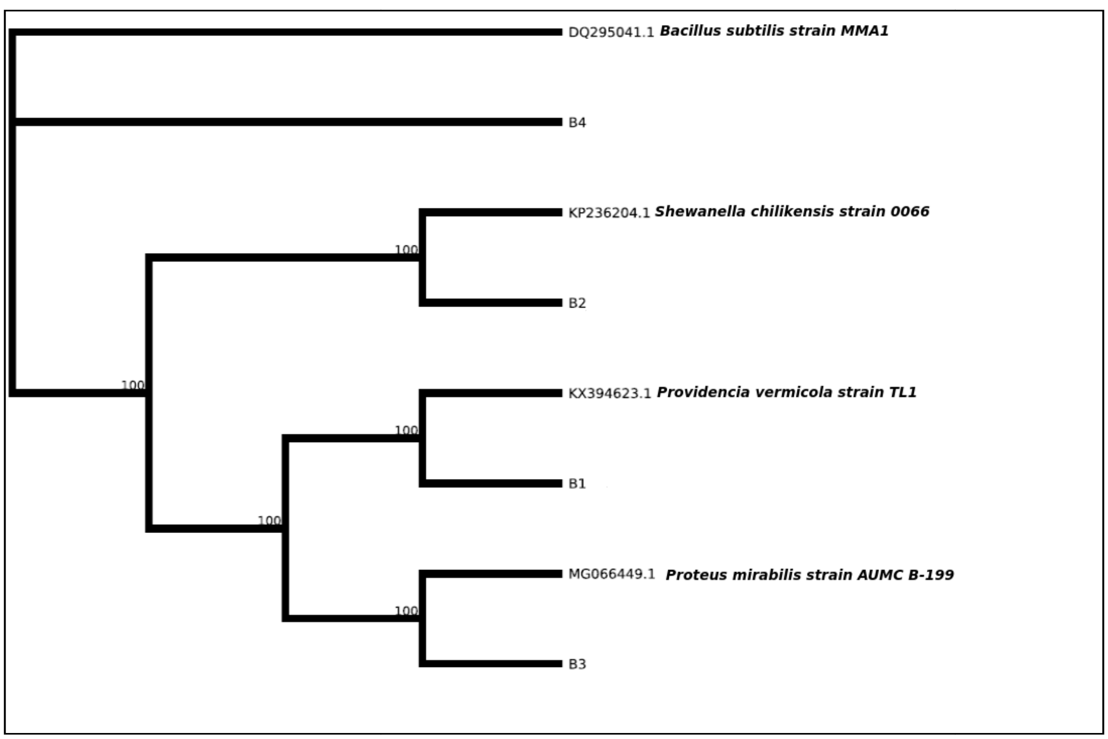

Fig. 5. Phylogenetic characteristics of evolutionary relationship between the bacterial isolates

Table 2. Proximate composition of the raw flour samples

Raw flour sample	Moisture content (%)	Ash content (%)	Crude lipid (%)	Crude protein (%)	Crude fiber (%)	Carbohydrate (%)
CF	7.39	0.94	0.94	1.28	1.94	91.74
WF	5.16	0.73	0.86	7.42	0.58	87.83
WCF (90:10)	5.29	0.75	0.87	7.03	0.66	88.21
WCF (80:20)	5.64	0.81	0.89	6.87	0.86	88.78
WCF (70:30)	5.98	0.83	0.89	6.22	0.93	89.44
WCF (60:40)	6.21	0.87	0.90	5.75	1.37	89.91

3.3 The pH of the Raw Flour Samples

The pH value decreased from 6.79 to 6.42 with increasing cassava flour concentration (Table 3).

3.4 Microbial Counts and Identification of Isolates from Baked Bread Samples at Different Temperatures

The mean total heterotrophic counts (THCs) of the bread samples baked at 180°C and 200°C increased from 3.83 to 7.73 log_{10} cfu g^{-1} and from 1.88 to 5.89 log_{10} cfu g^{-1} respectively from day 0 to day 6 (Fig. 6). However, no fungal growth was detected for bread samples baked at 180°C and 200°C on day 0, but with subsequent storage days, fungal growth increased to 4.05 and 3.32 log_{10} cfu g^{-1} respectively (Fig. 7). The bacteria isolated for both bread samples baked at 180°C and 200°C were: *Staphylococcus epidermidis, Bacillus subtilis, Aerococcus viridans, Providencia vermicola,* and *Proteus mirabilis* (Figs. 8 and 9). Fungal isolates from bread samples baked at 180°C (Fig. 10) were: *Fusarium solani, Penicillium citrinum, Kodamaea ohmeri, Aspergillus niger, Penicillium brocae, Rhizopus stolonifer* and *Aspergillus terreus*, while those from bread samples baked at 200°C (Fig. 11) were: *Aspergillus terreus, Penicillium citrinum, Fusarium solani, Penicillium brocae, Rhizopus stolonifer, Aspergillus niger* and *Lentinus squarosulus.*

3.5 Proximate Properties of Baked Bread Samples

Carbohydrate and ash contents increased from 48.11 to 55.29% and 1.35 to 3.30% respectively while the crude protein and moisture contents decreased from 14.01 to 8.08% and 29.0 to 24.6% respectively for bread samples baked at 180°C. On the other hand, carbohydrate and ash contents also increased from 46.07 to 53.55% and 1.71 to 3.79% respectively while the crude protein and moisture contents decreased from 13.66 to 8.12% and 26.3 to 23.8% respectively for bread samples baked at 200°C (Figs. 12 and 13).

3.6 Cyanide Content of the Crude Cassava Floor

The cyanide content of the cassava floor was 6.4 mg/kg.

3.7 The pH of the Baked Bread Samples

The mean pH of the bread samples baked at 180°C decreased from 6.59 to 6.14 (Table 4) while those baked at 200°C also decreased from 6.53 to 6.30 with increasing cassava flour concentration (Table 5).

3.8 Sensory Properties of Baked Bread Samples

The bread baked at 180°C and 200°C were highly acceptable on day 0 because of their freshness, but acceptability generally reduced with increasing number of storage days and cassava flour concentration (Tables 3 and 4).

3.9 Discussion

Several microorganisms are associated with both raw flour samples (wheat and cassava) and baked products of different wheat-cassava formulations. The occurrence of several microorganisms in the raw flour is a clear indication that they are suitable microbial substrates. Some of the bacteria isolated from the raw materials and baked products are shown in Figs. 2, 8 and 9. These microorganisms have been previously reported by several authors [1,14,15,16].

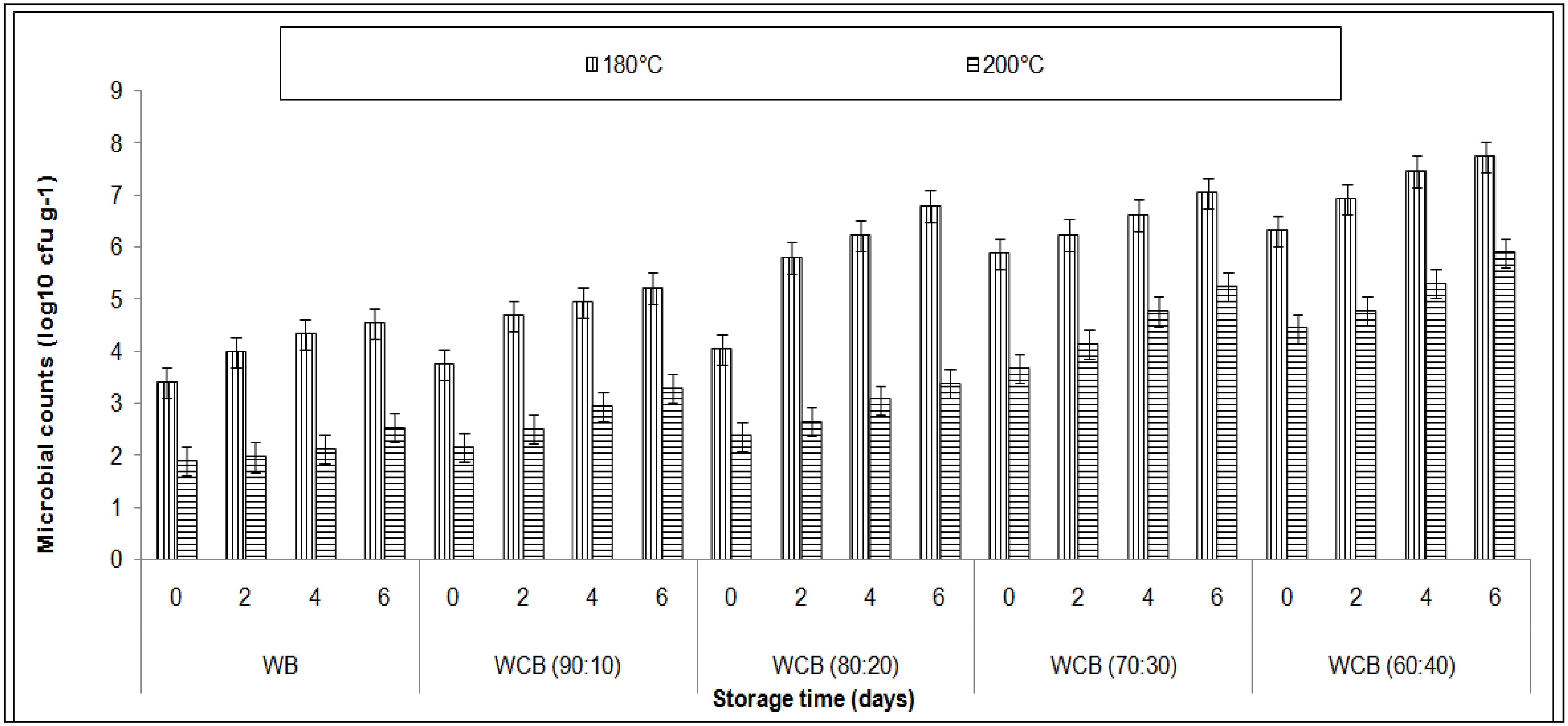

Fig. 6. Total heterotrophic counts obtained from the different bread samples baked at 180°C and 200°C on different storage days

WB = Wheat bread; WCB (90:10) = Wheat and cassava bread mixture (90:10); WCB (80:20) = Wheat and cassava bread mixture (80:20); WCB (70:30) = Wheat and cassava bread mixture (70:30); WCB (60:40) = Wheat and cassava bread mixture (60:40).
Error bars represent mean ± standard deviation of duplicate determinations

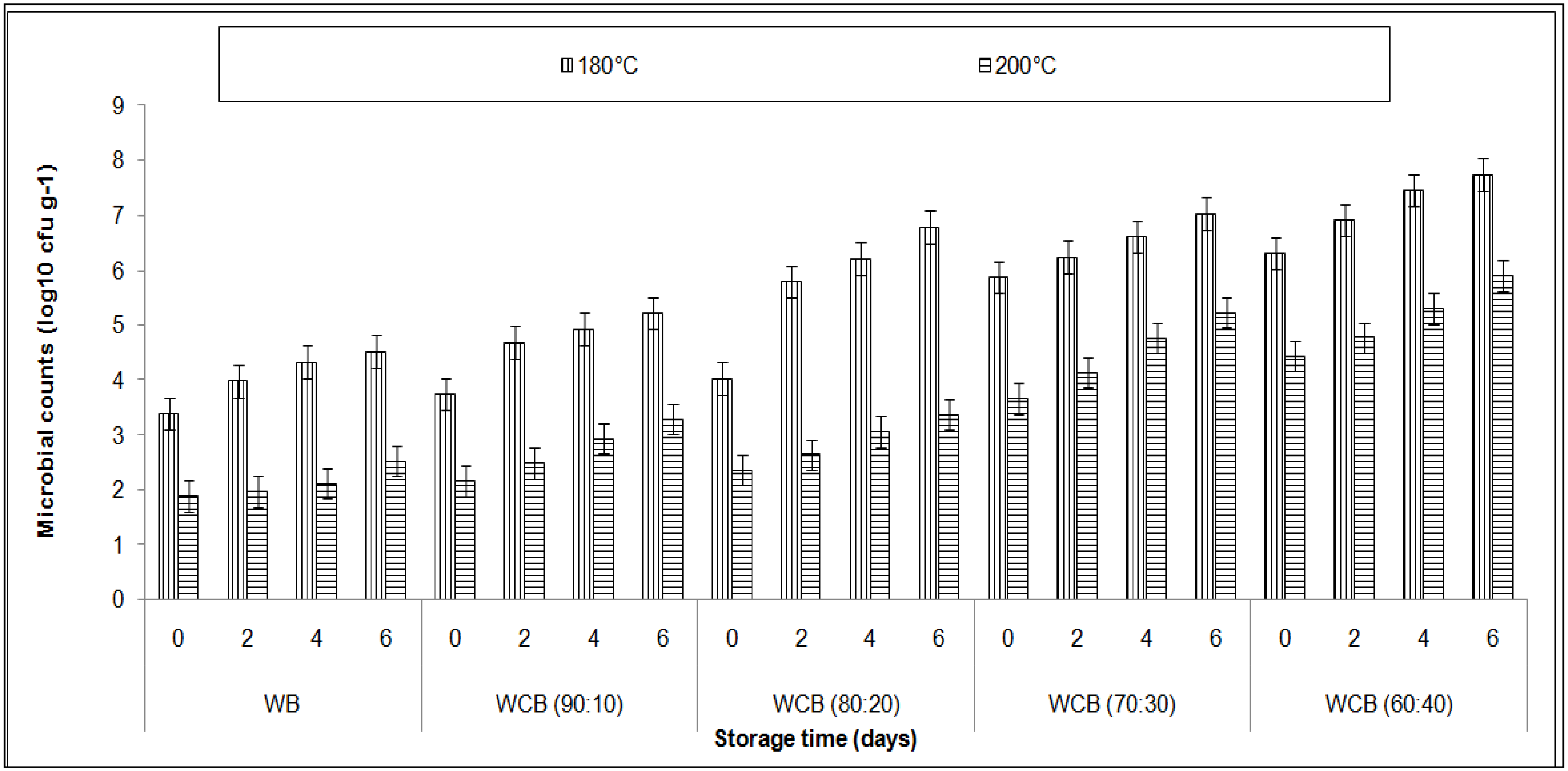

Fig. 7. Total fungal counts obtained from the different bread samples baked at 180°C and 200°C at different storage days

WB = Wheat bread; WCB (90:10) = Wheat and cassava bread mixture (90:10); WCB (80:20) = Wheat and cassava bread mixture (80:20); WCB (70:30) = Wheat and cassava bread mixture (70:30); WCB (60:40) = Wheat and cassava bread mixture (60:40).
Error bars represent mean ± standard deviation of duplicate determinations

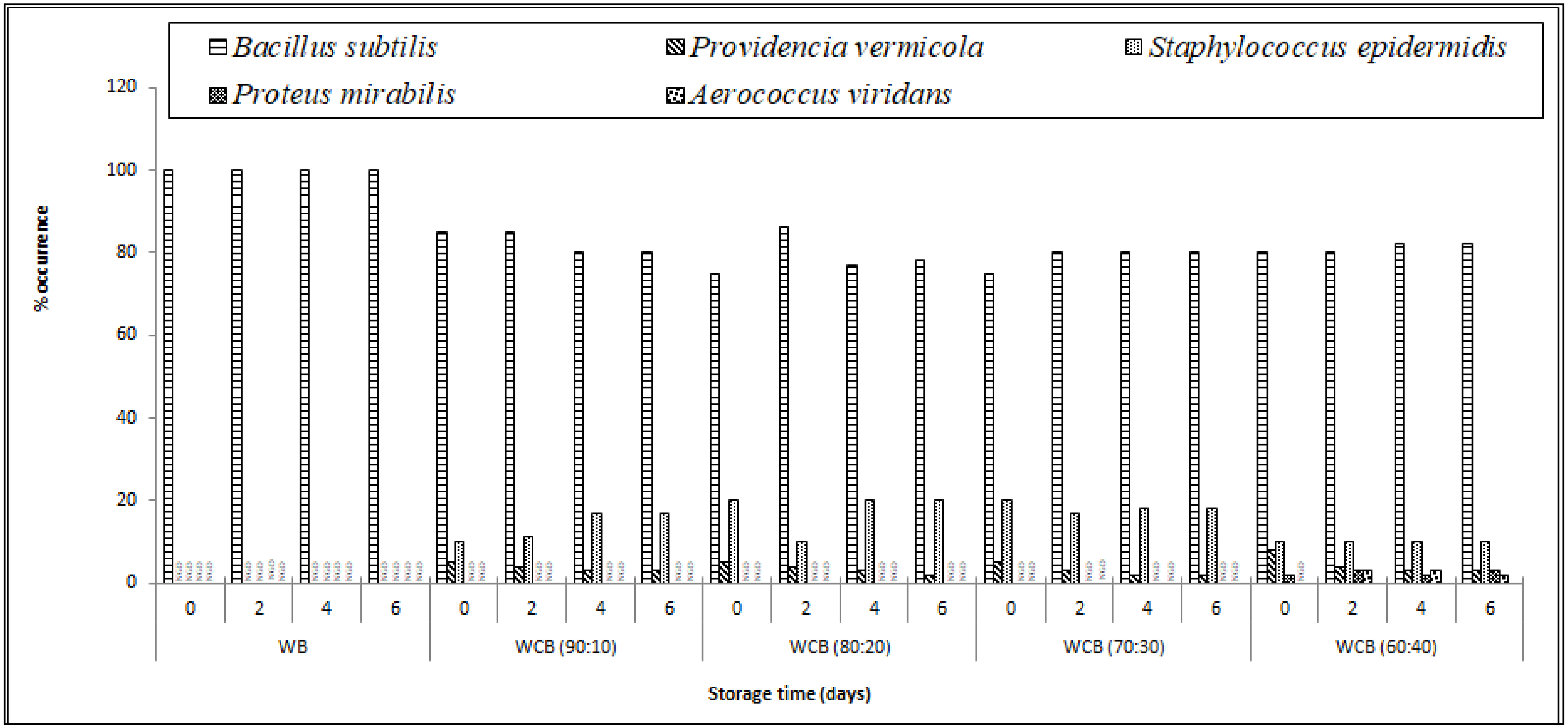

Fig. 8. Percentage occurrence of different bacteria from bread samples baked at 180°C on different storage days

WB = Wheat bread; WCB (90:10) = Wheat and cassava bread mixture (90:10); WCB (80:20) = Wheat and cassava bread mixture (80:20); WCB (70:30) = Wheat and cassava bread mixture (70:30); WCB (60:40) = Wheat and cassava bread mixture (60:40). NGD = No growth detected

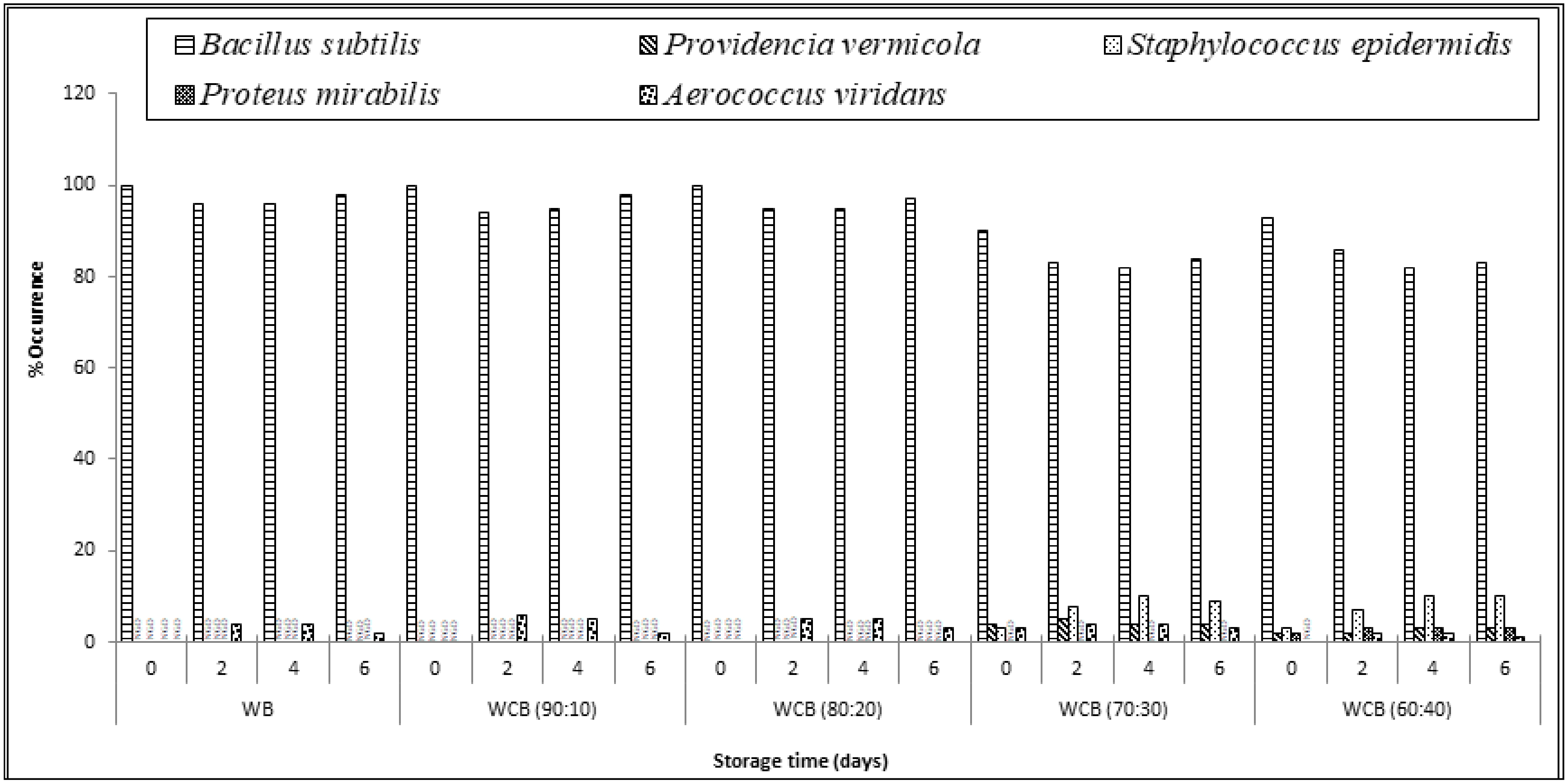

Fig. 9. Percentage occurrence of different bacteria from bread samples baked at 200°C on different storage days

WB = Wheat bread; WCB (90:10) = Wheat and cassava bread mixture (90:10); WCB (80:20) = Wheat and cassava bread mixture (80:20); WCB (70:30) = Wheat and cassava bread mixture (70:30); WCB (60:40) = Wheat and cassava bread mixture (60:40). NGD = No growth detected

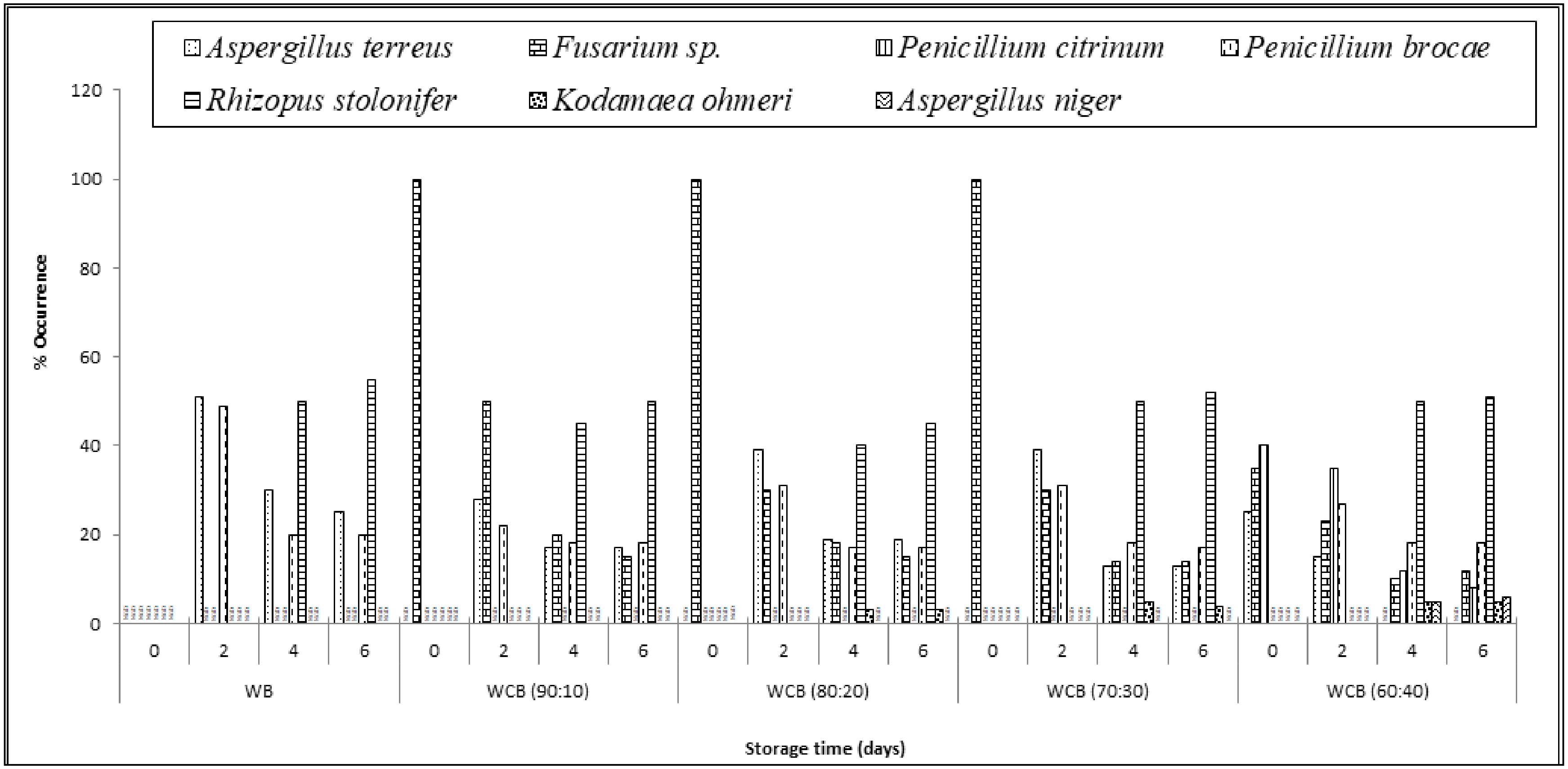

Fig. 10. Percentage occurrence of different fungi from bread samples baked at 180°C on different storage days

WB = Wheat bread; WCB (90:10) = Wheat and cassava bread (90:10); WCB (80:20) = Wheat and cassava bread (80:20); WCB (70:30) = Wheat and cassava bread (70:30); WCB (60:40) = Wheat and cassava bread (60:40). NGD = No growth detected

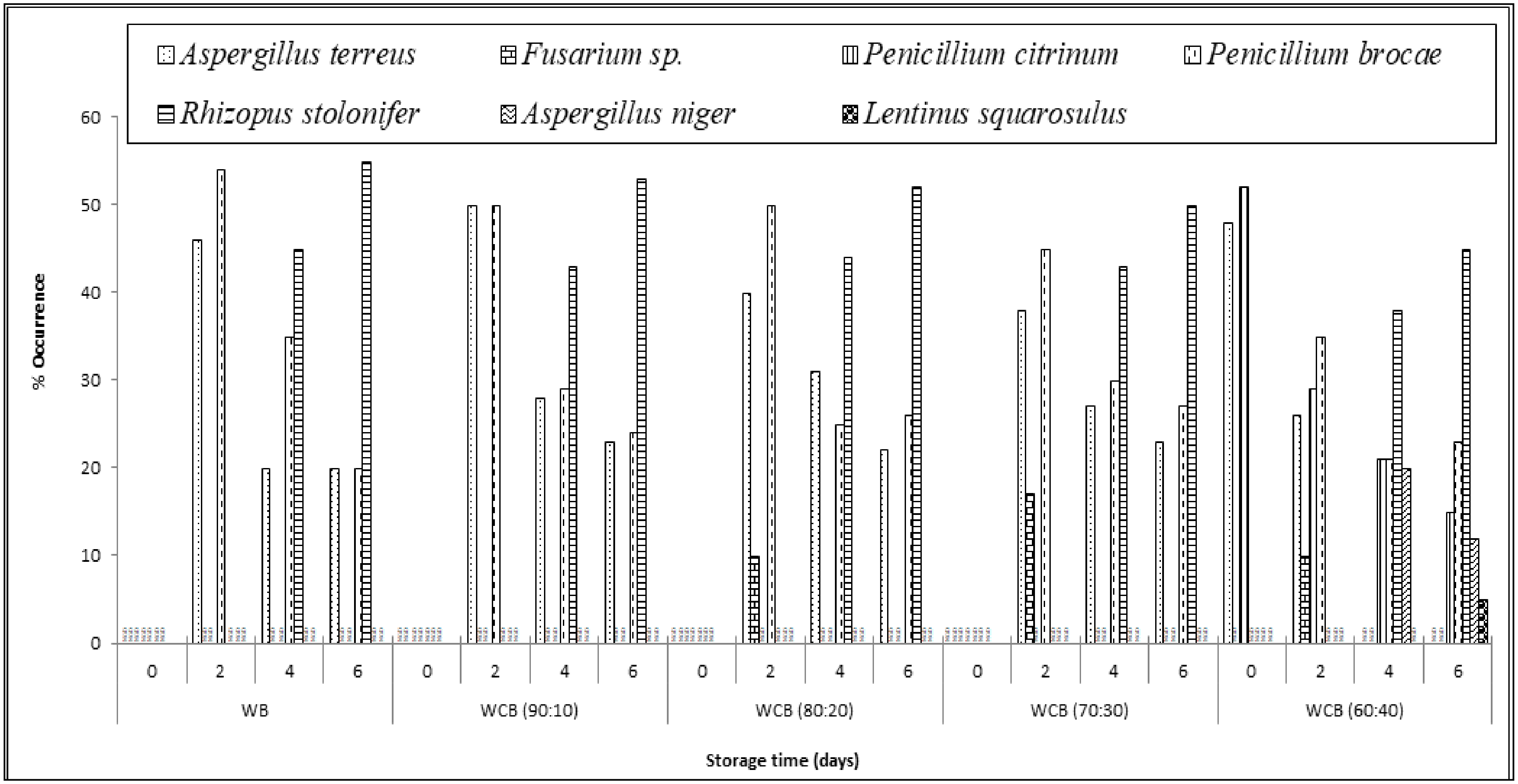

Fig. 11. Percentage occurrence of fungi from bread samples baked at 200°C on different storage days

WB = Wheat bread; WCB (90:10) = Wheat and cassava bread (90:10); WCB (80:20) = Wheat and cassava bread (80:20); WCB (70:30) = Wheat and cassava bread (70:30); WCB (60:40) = Wheat and cassava bread (60:40). NGD = No growth detected

Table 3. The pH of the raw flour samples used in the study

Flour samples	CF	WF	WCF (90:10)	WCF (80:20)	WCF (70:30)	WCF (60:40)
pH	6.42	6.79	6.59	6.56	6.55	6.49

CF=Cassava flour; WF= Wheat flour; WCF=Wheat and cassava flour

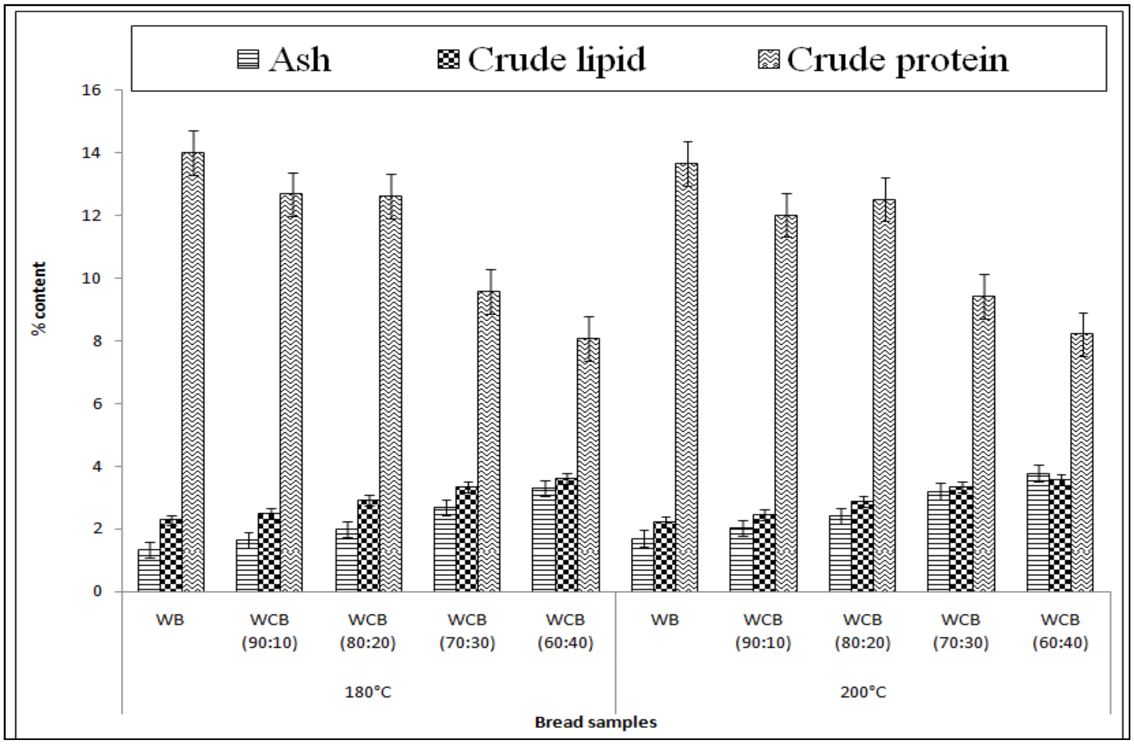

Fig. 12. Changes in ash, crude lipid and crude protein content of the bread samples baked at 180°C and 200°C on storage day 0

WB = Wheat bread; WCB (90:10) = Wheat and cassava bread (90:10); WCB (80:20) = Wheat and cassava bread (80:20) WCB (70:30) = Wheat and cassava bread (70:30); WCB (60:40) = Wheat and cassava bread (60:40). Error bars represent mean ± standard of duplicate determinations

Table 4. The pH of bread samples baked at 180°C on different storage days

Bread samples	Days			
	0	2	4	6
WB	6.59	6.42	6.47	6.40
WCB (90:10)	6.40	6.47	6.35	6.29
WCB (80:20)	6.32	6.40	6.33	6.25
WCB (70:30)	6.28	6.37	6.30	6.20
WCB (60:40)	6.24	6.32	6.18	6.14

The total heterotrophic count (THC) of the cassava flour (7.62 $\log_{10}$ cfu g^{-1}) was more than that of the wheat flour (5.41 $\log_{10}$ cfu g^{-1}), with the most isolated bacteria from the raw flour samples (Fig. 2) belonging to the genus *Bacillus*, a spore forming organism widely distributed in nature and in foods. Others are: *Aerococcus* and *Staphylococcus*. The fungal isolated from the raw flour samples in this study (Fig. 3) are: *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp., and *Fusarium* sp and these species have been frequently reported to be present in these samples [16]. However, no report of isolation and identification of *Kodamaea ohmeri* from raw wheat and cassava flours had been previously published to the knowledge of the authors. Perhaps, use of molecular method of identification of the isolates from the samples contributed to its detection and identification. *Aspergillus* sp. was the predominant fungal isolate in the raw flour samples. Their prevalence could be attributed to the fact that they are commonly found in many tropical and humid environments [17].

Bacteria isolated from control bread (WB) and WCB sample as shown in Figs. 8 and 9 belonged to *Bacillus subtilis*, *Staphylococcus epiderdermis, Proteus mirablis*, and *Providencia sp*. However, coliforms were not isolated. This could be as the result of the high temperatures used during baking. It also conforms to the limit set by the African Organization for Standardization [18], which states that the counts of aerobic bacteria must not exceed 10^4 cfu/g and coliform growth must not be detected in bread samples. This corroborates the findings by Daniyan and Nwokwu [19], Eleazu et al. [1] and Saranraj and Sivasakthivelan [20], who reported the presence of *Bacillus* and *Staphylococcus* in bread. However, in the present study, the occurrence of *Providencia sp.*, *Aerococcus viridans* and *Proteus mirablis* in WCB might be due to incorporation of the cassava flour from where these organisms were previously isolated.

Table 5. The pH of bread samples baked at 200°C on different storage days

Bread samples	Days			
	0	**2**	**4**	**6**
WB	6.53	6.47	6.45	6.43
WCB (90:10)	6.44	6.45	6.44	6.41
WCB (80:20)	6.41	6.43	6.42	6.37
WCB (70:30)	6.36	6.39	6.38	6.34
WCB (60:40)	6.33	6.34	6.32	6.30

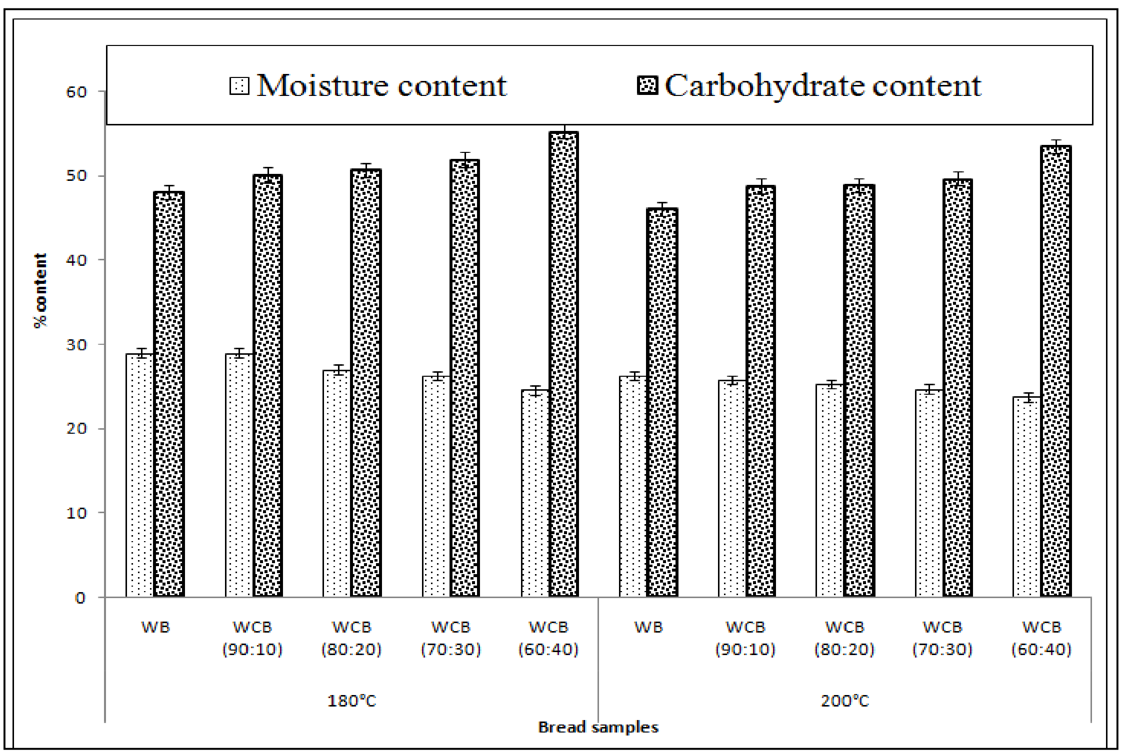

Fig. 13. Changes in moisture and carbohydrate content of the bread samples baked at 180°C and 200°C on storage day 0

WB = Wheat bread; WCB (90:10) = Wheat and cassava bread (90:10); WCB (80:20) = Wheat and cassava bread (80:20); WCB (70:30) = Wheat and cassava bread (70:30); WCB (60:40) = Wheat and cassava bread (60:40). Error bars represent mean ± standard of duplicate determinations

Table 6. Sensory properties of the bread samples baked at 180°C on different days

Days	Sensory attributes	Baked bread label				
		WB	**WCB (90:10)**	**WCB (80:20)**	**WCB (70:30)**	**WCB (60:40)**
0	Appearance	6.0±0.48^{a}	5.8±0.28^{a}	5.6±0.08^{b}	5.2±0.32^{b}	5.0±0.20^{b}
	Texture	6.4±1.12^{a}	5.6±0.32^{b}	5.6±0.32^{b}	4.8±0.52^{c}	4.0±1.52^{c}
	Taste	6.2±1.12^{a}	5.8±0.72^{b}	5.6±0.52^{b}	4.6±0.48^{c}	3.2±1.88^{d}
	Aroma	6.0±0.48^{a}	5.8±0.28^{b}	5.6±0.08^{b}	5.6±0.08^{b}	4.6±0.92^{c}
	Overall Acceptability	6.2±1.12^{a}	5.8±0.72^{b}	5.6±0.52^{b}	5.1±0.48^{c}	4.2±1.88^{d}
2	Appearance	5.8±0.64^{a}	5.6±0.44^{a}	5.4±0.24^{a}	5.0±0.16^{a}	4.8±1.16^{b}
	Texture	5.8±0.84^{a}	5.4±0.44^{a}	5.4±0.44^{a}	4.8±0.16^{b}	3.8±1.56^{c}
	Taste	6.0±1.08^{a}	5.8±0.88^{b}	5.6±0.68^{b}	4.6±0.32^{c}	3.0±2.32^{d}
	Aroma	5.6±0.92^{a}	5.0±0.32^{a}	4.6±0.08^{b}	4.4±0.28^{b}	4.0±0.88^{b}
	Overall Acceptability	6.0±1.08^{a}	5.7±0.88^{b}	5.6±0.68^{b}	4.7±0.32^{c}	3.9±2.32^{d}
4	Appearance	5.0±0.12^{a}	4.8±0.12^{a}	4.6±0.12^{a}	2.8±0.08^{b}	2.6±0.28^{b}
	Texture	4.6±0.88^{a}	4.0±0.12^{a}	3.8±0.08^{b}	2.6±0.12^{b}	1.6±0.82^{c}
	Taste	4.4±0.80^{a}	4.2±0.60^{a}	3.6±0.60^{a}	2.6±0.20^{b}	1.0±1.80^{c}
	Aroma	3.8±0.60^{a}	3.5±0.20^{a}	2.6±0.01^{b}	2.4±0.20^{b}	2.0±0.60^{b}
	Overall Acceptability	4.4±0.80^{a}	4.1±0.60^{a}	3.4±0.60^{b}	2.6±0.20^{b}	1.0±1.80^{c}
6	Appearance	1.4±0.02^{a}	1.3±0.02^{a}	1.1±0.02^{a}	1.2±0.02^{a}	1.4±0.02^{a}
	Texture	1.1±0.05^{a}	1.2±0.01^{a}	1.5±0.04^{a}	1.3±0.01^{a}	1.1±0.02^{a}
	Taste	1.0±0.04^{a}	1.4±0.04^{a}	1.3±0.01^{a}	1.1±0.03^{a}	1.2±0.01^{a}
	Aroma	1.5±0.01^{a}	1.1±0.03^{a}	1.4±0.05^{a}	1.2±0.01^{a}	1.3±0.03^{a}
	Overall Acceptability	1.1±0.02^{a}	1.0±0.02^{a}	1.2±0.02^{a}	1.2±0.02^{a}	1.0±0.02^{a}

WB = Wheat bread; WCB (90:10) = Wheat and cassava bread (90:10); WCB (80:20) = Wheat and cassava bread (80:20); WCB (70:30) = Wheat and cassava bread (70:30); WCB (60:40) = Wheat and cassava bread (60:40).

Values in rows with different superscripts for the different bread samples are significantly different at P=.05

Table 7. Sensory properties of the bread samples baked at 200°C on different days

Days	Sensory attributes	Baked bread label				
		WB	**WCB (90:10)**	**WCB (80:20)**	**WCB (70:30)**	**WCB (60:40)**
0	Appearance	5.8±0.44[a]	5.6±0.24[a]	5.4±0.04[a]	5.0±0.36[a]	5.0±0.36[a]
	Texture	5.8±0.64[a]	5.6±0.44[a]	5.6±0.44[a]	4.8±0.36[b]	4.6±1.60[b]
	Taste	6.0±0.96[a]	5.8±0.76[b]	5.6±0.56[b]	4.6±0.44[c]	3.2±1.84[d]
	Aroma	6.0±0.48[a]	5.8±0.28[b]	5.6±0.08[b]	5.6±0.08[b]	4.6±0.92[c]
	Overall Acceptability	6.0±0.96[a]	5.8±0.76[b]	5.6±0.56[b]	4.6±0.44[c]	3.2±1.84[d]
2	Appearance	5.6±0.64[a]	5.6±0.64[a]	5.2±0.24[a]	4.8±0.16[b]	3.6±1.32[c]
	Texture	5.6±0.84[a]	5.4±0.64[a]	4.8±0.04[b]	4.6±0.16[b]	3.4±1.36[c]
	Taste	6.0±0.68[a]	5.8±0.88[b]	5.4±0.48[b]	4.0±0.92[c]	2.6±2.32[d]
	Aroma	5.6±0.92[a]	5.0±0.32[a]	4.8±0.12[b]	4.4±0.28[b]	3.8±0.88[c]
	Overall Acceptability	6.0±1.24[a]	5.8±1.04[b]	5.4±0.64[b]	4.0±0.76[c]	2.6±2.16[d]
4	Appearance	4.6±0.12[a]	4.4±0.12[a]	2.8±0.12[b]	2.6±0.08[b]	2.4±0.28[b]
	Texture	4.0±0.84[a]	3.8±0.24[a]	2.6±0.04[b]	2.4±0.16[b]	1.6±0.96[c]
	Taste	4.0±0.80[a]	4.0±0.60[a]	3.4±0.6[b]	2.6±0.20[c]	1.0±1.80[d]
	Aroma	3.8±0.60[a]	3.6±0.20[a]	2.6±0.0[b]	2.4±0.20[b]	2.0±0.60[b]
	Overall Acceptability	4.1±0.80[a]	4.0±0.60[a]	3.4±0.6[b]	2.6±0.40[c]	1.0±1.80[d]
6	Appearance	1.3±0.02[a]	1.2±0.03[a]	1.6±0.03[a]	1.3±0.03[a]	1.2±0.05[a]
	Texture	1.2±0.01[a]	1.3±0.01[a]	1.3±0.04[a]	1.4±0.05[a]	1.3±0.02[a]
	Taste	1.2±0.01[a]	1.4±0.05[a]	1.3±0.03[a]	1.6±0.01[a]	1.1±0.03[a]
	Aroma	1.1±0.03[a]	1.1±0.01[a]	1.1±0.02[a]	1.7±0.02[a]	1.1±0.05[a]
	Overall Acceptability	1.6±0.02[a]	1.6±0.04[a]	1.0±0.01[a]	1.2±0.02[a]	1.5±0.06[a]

WB = Wheat bread; WCB (90:10) = Wheat and cassava bread (90:10); WCB (80:20) = Wheat and cassava bread (80:20); WCB (70:30) = Wheat and cassava bread (70:30); WCB (60:40) = Wheat and cassava bread (60:40).

Values in rows with different superscripts for the different bread samples are significantly different at P=.05

Saranraj and Geetha [21], Eleazu et al. [1], Udeme et al. [22], and Saranraj and Sivasakthivelan, [20] reported *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* in bread. *Penicillium* sp. was the predominant fungal isolate in the bread samples. Their prevalence could be due to the fact that the genus is broad and encountered almost everywhere and is very common in foods and feeds [23]. The higher total heterotrophic counts and total fungal counts observed in WCB samples compared with WB samples may be attributed to the influence of cassava flour which was incorporated into the bread. This compared favourably with the findings of Eleazu et al. [1]. The microbial counts and variations also increased with increasing storage days.

The mean scores for appearance, taste, texture, aroma and overall acceptability of the baked bread products are shown in (Tables 6 and 7). The mean sensory scores of quality attributes of the products indicated that, generally, panelist expressed preference for three bread samples out of the five presented. The bread samples were WB (100% wheat bread), wheat cassava bread WCB (90:10) and WCB (80:20). Bread made from WCB (70:30) and WCB (60:40) were least preferred by the panelist. These results are similar to findings by some authors [1,3,24,25,26], who reported that breads baked with 10 and 20 % cassava-wheat composite flour were not significantly different in sensory attributes compared with those baked with 100% wheat flour. The baked products were highly acceptable on day 0 but generally reduced with increasing number of storage days, with day 6 samples being the least acceptable because of the staling and spoilage of the bread.

The moisture content of all the bread samples had comparable moisture values (Fig. 13). The high moisture content of the bread sample may be attributed to the amount of water added during baking. Increase in the level of cassava flour resulted in decrease in the protein content from 14.01% in 100% wheat bread (WB) to 8.08% in bread made from WB (60:40). This may have been due to the low protein content of the cassava flour which must have diluted the protein content of the wheat flour, thus reducing the protein level of the mixed flour. This is similar to earlier findings where protein content of snacks reduced with supplementation with starch-based products for bread fruit flour [27], for plantain and for cassava flour [28]. The blending ratios had a significant effect on carbohydrate content of the composite bread samples (Fig. 13). The increase in the carbohydrate content with increase in cassava supplementation is not unexpected as recorded by some other authors [3,22,29], but the high level of carbohydrate is desirable in baked products because on heating starch granules in the presence of water, it swells and forms a gel which is important for the characteristic texture and structures of baked goods [30].

The cyanide content is below the maximum (≤10 mg/kg) recommended by African Organization for Standardization [18]. The pH values of wheat flour and cassava flours obtained in this study compare favourably with values obtained by previous workers [16,26,31]. The pH of the baked bread samples (Tables 4 and 5) differed from these found by Eleazu et al. [1]. This may be attributed to difference in cultivars or processing procedures.

4. CONCLUSION

From the results of this study, it can be concluded that a blend of cassava and wheat flour is acceptable in bread production. More so, cassava is readily available and nutritious. Microbial load of the bread increased with increasing number of days, thus, reducing the sensory attributes and shelf-life of the bread. There was no significant difference (*P*=.05) in microbial quality between the 100% wheat bread and that of 90:10%. The overall acceptability of the wheat/cassava bread reduced with increasing cassava flour concentration, however, there was no significant difference (*P*=.05) in the overall acceptability of the 100% wheat bread and that of 90:10%.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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