Microbial, Proximate and Sensory Evaluation of Bread Produced from a Blend of Wheat and Corn Flour

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Abstract: Wheat flour, which is the major raw material used in baking bread, is being imported in Nigeria and other countries where cultivation of wheat is hampered by unfavourable climate. This has necessitated the rising cost of bread beyond the reach of the poor in Nigeria. This study was aimed at assessing the quality of bread produced from a blend of wheat and corn flour. Composite breads were produced from wheat flour (WF) and wheat flour: corn flour (WF: CF) blends; 100% WF, 80% WF: 20% CF and 60% WF: 40% CF. The following analysis including microbial, proximate, sensory attributes and shelf life were used to assess the quality of the bread. Data was analyzed using SPSS software. The fiber, fat and protein content of the blend; 80% wheat flour bread (WFB): 20% corn flour bread (CFB) was significantly higher ($p < 0.05$) than the other flour blends. 100% WFB recorded the highest ash and moisture content ($p < 0.05$) of 2.73±0.02% and 29.56±0.25% respectively. However, the carbohydrate content was highest ($p < 0.05$) in 60% WF: 40% CF blends. There was no significant difference ($p > 0.05$) in the mean sensory scores of 100% WFB and 80% WFB: 20% CFB, with respect to taste and overall acceptability. The microbial counts were below the maximum permissible limits recommended by the Standard Organization of Nigeria, thus the bread is safe for human consumption. Bread could be produced from up to 20% CF substitution in WF without compromising the quality.
Key word: Bread, corn flour, microbial counts, taste, wheat flour

INTRODUCTION

Bread is a staple food consumed by people around the world and it is an important part of many cultures diet. It is one of the oldest foods prepared by baking dough of flour, usually wheat, and it plays essential role in both religious rituals and secular society (Bread, 2023). Bread may be leavened by naturally occurring bacteria or yeasts (sourdough), chemicals (baking soda) or industrially produced yeast or by using a high-pressure aeration which creates gas bubbles that make raised dough (Vincent *et al*., 2024). Bread has been reported to be a good source of energy and nutrients such as carbohydrates, proteins, fats, as well as minerals and vitamins essential for human health (Prieto-Vázquez *et al*., 2022).

In Nigeria, bread ranked second to rice as the most widely consumed non indigenous food product and it is produced from wheat flour as the major raw material (Henry-Unaeze and Amadi, 2022). However, wheat production is limited in Nigeria by unfavourable climate and as a result; huge amount of foreign exchange is used annually on importation of wheat flour to meet the local flour needs for bakery products (Umar and Muhammad, 2021). The Nigerian government in conjunction with the Food and Agricultural Organization (FAO) had made concerted efforts to minimize the importation of wheat in Nigeria, by encouraging the use of composite flours and blends of non-wheat flours or meals to produce bread and other aerated products (Ayoade *et al*., 2020). The incorporation of wheat flour with local or indigenous products is on the increase due to high demand for baked foods (Ayoade *et al*., 2020), and the recent changes that focused on healthy eating, enhancing the use of indigenous product such as local cereals and legumes in baking industries (Feyera, 2020). Thus, many developing countries have called for the introduction of this strategy to utilize the possibility of replacing wheat flours with other flours made from indigenous foods (Ayade *et al*., 2020).

Corn or maize (*Zea mays*) is widely cultivated and readily available in Nigeria and ranked as the second most widely produced cereal crop worldwide. Due to its high productivity, it is by far the most economical cereal to produce (Abo Raya *et al*., 2022). Corn flour is rich in nutrients and contains high levels of many important vitamins and minerals (Kumari *et al*., 2019). This study thus investigated the microbial, proximate and sensory quality of bread produced from a blend of wheat and corn flour.

MATERIALS AND METHODS

Sample collection: Wheat grains (hard red variety) and yellow sweet corn variety were purchased from Hausa Market in Oko Kingdom, Delta State, Nigeria. Baking ingredients such as sugar, salt, butter, dry yeast, vegetable oil and egg were purchased from Ogbe-Ogonogo Market in Asaba, Delta State, Nigeria.

Preparation of wheat flour: The wheat grains (10 kg) was cleaned by hand picking out all foreign materials and the cleaned wheat was washed and soaked in 15 liters of water for 60 minutes, after which it was drained, dried in an oven at 60^{o}C for 24 hours (Oluwafemi and Seidu, 2017). The dried wheat grains were ground into flour using pestle and mortar. The flour was sieved through a 0.25 mm sieve and packaged in polyethylene bags for further use.

Preparation of corn flour: The maize grains (5 kg) were sorted to remove stones and other foreign particles. It was washed with 10 liters of clean water and dried in hot air oven at 60^{o}C for 24 hours. The grains were ground to powder using pestle and mortar. The flour was screened through a 0.25 mm sieve and packaged in polyethylene bags for further use.

Blend formulations and ingredients: The blend formulations and ingredients used in baking were prepared using the modified method of Ayoade *et al*. (2020). The wheat and corn flour blends were as follows: 100% (500 g) wheat flour (WF), 80% (400 g) WF: 20% (100 g) corn flour (CF) and 60% (300 g) WF: 40% (200 g) CF. The recipes used include; sugar (2%), salt (0.5%), water (36%), lightly beaten eggs (2%), melted butter (2%), yeast (2%) and vegetable oil (1%).

Bread baking process: The straight dough method (Chuahan *et al*., 1992) was used in baking the bread. The blend formulations and all ingredients were mixed in Havells mixer (Premio-i, India) for 5 minutes to form the dough. The dough was transferred into bowls, covered with wet clean muslin cloth and allowed to ferment for 60 minutes. After fermentation, the dough was punched to release excess gas, scaled to 250 g dough pieces and allowed to proof in a proofing cabinet for 90 minutes at 30^{o}C and 85% relative humidity. Subsequently, the dough was baked at 220^{o}C for 45 minutes. The baked bread samples were de panned, allowed to cool at 25^{o}C and stored in Ziploc bags for analysis.

Microbial quality of the bread samples: Microbial analysis of the bread samples was carried out using the spread plate method as described in American Public Health Association (APHA, 2015). The bread samples were mashed and mixed with peptone water and 1.0 ml of the aliquots were serially diluted up to 10^{-2} dilutions. Subsequently, 0.1 ml of the dilutions were spread plated on the surface of solidified nutrient agar, Sabouraud dextrose agar and MacConkey agar plates for bacterial, fungal and coliform enumeration respectively (Ijah *et al*., 2014).

Proximate analysis of the bread samples: The moisture, crude protein, crude fat, crude fiber and ash content were determined using the standard method (AOAC, 2010). Carbohydrate was estimated by difference; % carbohydrate = 100 - % (protein + moisture + fat + crude fiber + ash).

Sensory quality of the bread samples: The bread samples from different blends of wheat and corn flour were subjected to sensory evaluation in terms of loaf colour, crust colour, flavour, crumb texture, taste and overall acceptability, using a 9-point Hedonic rating scale. The rating was with 1 representing the least score (dislike extremely) and 9 representing the highest score (like extremely) (Nazir and Nayik, 2016).

Shelf life of the bread samples: The bread samples were stored at room temperature (25°C) and virtual observations was carried out on a daily basis to ascertain the onset of changes especially in colour and texture, caused by the activities of spoilage microorganisms, commonly mould and bacteria. At the onset of spoilage, the bread samples were mashed, mixed with peptone water and cultured on nutrient and Sabouraud dextrose agar plates for bacterial and fungal isolation respectively. Bacteria isolates were identified by gram reaction and biochemical tests with reference to the Bergey's Manual of Systemic Bacteriology (Krieg and Holt, 1994). Fungal isolates were identified by cultural and microscopic characteristics with reference to the fungal atlas (Watanabe, 2002).

Statistical analysis of data obtained: Data obtained were subjected to one way analysis of variance (ANOVA) using SPSS software version 20. Duncan's new multiple range test and the least significant difference (LSD) was used to detect significant differences among the means at $p < 0.05$ level.

RESULTS

The microbial counts of the freshly prepared bread are presented in Table 3. Highest bacterial and fungal counts of 8.0 × 10 cfu/g and 5.0 × 10 sfu/g were observed in the 100% WFB. However, zero (0) coliform count was observed in all the bread samples.

The proximate compositions (Table 1) revealed that the ash and moisture content of the 100% WFB are significantly higher ($p < 0.05$) than the other samples substituted with 20% and 40% CF. However, the blend, 80% WFB: 20% CFB recorded significantly higher ($p < 0.05$) fiber, fat and protein content. In addition, the blend 60% WFB: 40% CFB recorded significantly higher ($p < 0.05$) carbohydrate content than the other flour blends. However, Duncan's new multiple range test and the least significance difference (LSD) revealed that there was no significant difference ($p > 0.05$) between the carbohydrate content of the100% WFB and that of 80% WFB: 20% CFB.

Table 2 depicts the sensory attributes of the bread samples. The bread produced with 100% WF recorded significantly higher ($p < 0.05$) mean sensory scores with respect to flavour and crumb texture, while the bread produced with 20% CF substitution in WF recorded significantly higher ($p < 0.05$) mean sensory scores with respect to loaf colour and crust colour. However, with respect to taste and overall acceptability, there was no significant difference ($p > 0.05$) in the mean sensory scores of the bread produced with 100% WF and 20% CF substitution in WF. Moreover, the bread produced with 40% CF substitution in WF seems not to be acceptable by the panelists, and thus recorded significantly lower mean sensory scores in all the sensory parameters except crumb texture, where it showed no significant difference with the bread produced with 20% CF substitution in WF.

The shelf life of the bread samples are presented in Table 4. The bread samples lasted for 6 to 7 days before obvious spoilage was noticed. In addition, 100% WFB and 80% WFB: 20% CFB lasted for 6 days while 60% WFB: 40% CFB lasted for 7 days. On culturing, the microorganisms obtained include bacteria such as *Bacillus* sp. and *Staphylococcus aureus* and fungi such as *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp. and *Saccharomyces cerevisiae* (Tables 5 and 6).

Table 1: Microbial counts of the bread samples

Bread samples	TBC (×10^2cfu/g)	TFC (×10^2 sfu/g)	TCC (×10^2 cfu/g)
100% WFB	0.80	0.50	0
80% WFB: 20% CFB	0.62	0.32	0
60% WFB: 40% CFB	0.58	0.35	0

Key: TBC; total bacterial counts, TFC; total fungal counts, TCC; total coliform counts

Table 2: Proximate composition (%) of the bread samples

Parameters	100% WFB	80% WFB: 20% CFB	60% WFB: 40% CFB
Fiber	3.34±0.02[a]	4.49±0.01[b]	2.63±0.04[c]
Fat	1.14±0.02[d]	3.27±0.06[e]	2.52±0.03[f]
Ash	2.73±0.02[a]	1.09±0.02[b]	0.62±0.01[c]
Moisture	29.56±0.25[a]	26.69±0.25[b]	26.85±0.20[b]
Protein	10.50±0.05[a]	11.20±0.17[b]	10.15±0.03[a]
Carbohydrate	52.73±0.50[a]	53.25±1.31[a]	57.23±0.42[b]

Key: Values are mean ±SEM of three replicates. Mean with different superscripts within the row are significantly different at $p < 0.05$ level

Table 3: Sensory quality of the bread samples

Parameters	100% WFB	80% WFB: 20% CFB	60% WFB: 40% CFB
Loaf colour	7.1±0.17[a]	8.3±0.11[b]	6.60±0.05[c]
Crust colour	7.60±0.14[d]	8.50±0.04[e]	6.8±0.23[f]
Flavour	8.2±0.14[g]	7.6±0.09[h]	6.1±0.14[i]
Crumb texture	8.5±0.14[b]	6.10±0.09[a]	5.5±0.28[a]
Taste	8.5±0.02[a]	8.40±0.05[a]	6.9±0.25[b]
Overall acceptability	8.7±0.11[c]	8.5±0.14[c]	6.4±0.20[d]

Key: Values are mean ±SEM of three replicates. Mean with different superscripts within the row are significantly different at $p < 0.05$ level

Table 4: Shelf life of the bread samples (days)

Bread samples	Shelf life
100% WFB	6
80% WFB: 20% CFB	6
60% WFB: 40% CFB	7

Table 5: Identification of bacteria isolated from the bread samples after 7 days of storage

Test	Isolate 1	Isolate 2
Colony	Large golden-yellow colonies	Creamy, white, large and mucoid
Shape	Cocci	Rods
Gram stain	+	+
Motility	-	+
Catalase	+	+
Coagulase	+	+
Indole	-	-
Glucose	+	+
Sucrose	+	+
Maltose	+	+
Mannitol	+	+
Sorbitol	-	-
Suspected bacteria	*Staphylococcus aureus*	*Bacillus* sp.

Key: +; positive reaction, -; negative reaction

Table 6: Identification of fungi isolated from the bread samples after 7 days of storage

Cultural characteristics	Microscopic characteristics	Suspected fungi
Shades of green	Chains of single celled conidia	*Penicillium* sp.
Slightly rough black. Reverse side is white to yellow	Septate hyphae. Long colourless smooth conidiophores.	*Aspergillus* sp.
White/grey fuzzy colonies	Non septate hyphae. Septa only present where gametes form	*Rhizopus* sp.
Flat, smooth, moist, glistening and creamy in colour	Oval or spherical in shape	*Saccharomyces cerevisiae*

DISCUSSION

The highest bacterial and fungal counts observed in 100% WFB could be attributed to the high moisture content observed in the bread sample, which supports microbial growth. Furthermore, the bacterial and fungal counts recorded in the bread samples could have emanated from the raw materials or from the baking environment or storage facilities. However, as stated by the Standard Organization of Nigeria, the maximum permissible level of aerobic bacteria in bread is 100 cfu/g and that of coliform must be zero (Ijah *et al*., 2014). Therefore, the counts recorded in this study conform to the regulatory standard, which suggests that the bread is safe for human consumption in terms of microbial quality.

Ash is a measure of the mineral or inorganic constituents in food. Thus, the high ash content in 100% WFB indicates that the WF is of good quality and contains high amount of minerals needed for the body. This findings was in line with the Oluwafemi and Seidu (2017), who reported significantly higher ash content in wheat flour compared to corn flour. Similar observation was also reported by Ijah *et al*. (2014). Moisture or water content is a very important parameter in the food industry due to its role in determining the products shelf life and overall stability. Thus, the higher moisture content recorded in 100% WFB suggests that the product could support microbial growth and as such could have lower keeping quality. However, the moisture content recorded in all the bread samples are above the acceptable moisture limit of 15% for dry products (Ijah *et al*., 2014). This could be attributed to the processing methods employed in the preparation of the breads.

However, the finding of the moisture content recorded in this study was similar to the findings of Begum *et al*. (2013), who reported that moisture content of composite breads decreased with maize substitution by a range of 37.39 to 35.31%. The fiber content recorded in this study suggests that bread substituted with 20% CF is richer in dietary fiber, therefore good for human health. A high fiber diet lowers the blood cholesterol level and stimulates bacterial fermentation in the colon. This contributes to the benefits of dietary fiber for health promotion and disease prevention such as heart disease, diabetes, obesity and some types of cancer (FDA, 2016). The significantly higher fat and protein content in 80% WFB: 20% CFB further suggests that substitution of 20% CF in WF improved the nutritional status of the bread. Abo Raya *et al*. (2022) reported fat content of 4.26% in corn flour and 1.6% in wheat flour, similar to the report of 3.27% in 20% CF substitution and 1.14% in 100% WFB recorded in this study. In addition, Begum *et al*. (2013) reported that fat content of composite bread increases with maize flour substitution by a range of 4.04 to 4.16%. Furthermore, the protein content of all the bread samples produced in this study exceeded the minimum value of 10%, recommended by the Food and Agricultural Organization and the World Health Organization (Ifesan *et al*., 2020). This suggests that the bread produced with 20% and 40% CF substitution in WF still retained its nutritional composition similar to the bread produced with 100% WF.

The carbohydrate content of the bread samples increased with the incorporation of CF to the WF (52.73 to 57.23%). This could be attributed to the higher content of carbohydrate in maize compared to wheat. This finding was in agreement with the Jocelyne *et al*. (2020), who reported high value of carbohydrate in maize (75.48%) compared to wheat (73.91%). Begum *et al*. (2013) also reported a value of 42.24% carbohydrate in WFB and 45.34% in WFB supplemented with 25% CF.

The low mean sensory scores recorded in 60% WFB: 40% CFB may be attributed to the decrease in gluten content (with increase in CF substitution), which is responsible for bread sensory and baking quality. Bread produced with 40% CF substitution was hard, dry and sandy as reported by the panelists. However, the sensory scores of the bread produced with 20% CF substitution was similar to the Begum *et al*. (2013), who reported that bread containing up to 10% maize flour was most acceptable in terms of sensory evaluation.

The reason for the higher shelf life of 60% WFB: 40% CFB could be attributed to the increase in corn flour substitution up to 40%, which enhance its ability to retain moisture and reduce staling that could encourage microbial growth. This finding was in line with the report of Mbachu *et al*. (2022) who observed a shelf life of 6 days in WFB and 7 days in WFB supplemented with 50% potato flour. Similar finding of a shelf life of 6 days in WFB was also observed by Ijah *et al*. (2014). Spoilage was indicated by visible black, yellow and green colouration, as well as a slimy texture of the bread, indicating microbial growth. When the bread samples were cultured after spoilage (7 days of storage), the microorganisms identified consists of bacteria such as *Bacillus* sp. and *Staphylococcus aureus*. Fungi identified include *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp. and *Saccharomyces cerevisiae*. These microorganisms could be responsible for the spoilage of the bread. Similar observation was obtained by Ayoade *et al*. (2020), who identified *Aspergillus* sp., *Rhizopus* and *S. cerevisiae* from composite bread after 7 days of storage. Dzomeku *et al*. (2012) reported that *Aspergillus flavus, A. niger*, *Penicillium*, *Rhizopus* and *S. cerevisiae*, are the fungi associated with the spoilage of bread at room temperature.

CONCLUSION

This study has demonstrated that the bread produced from 20% CF substitution in WF was comparable to that of the 100% WFB, with respect to microbial, nutritional and sensory quality. Therefore, bread could be produced from up to 20% CF substitution in WF without compromising the quality. If this is adopted by bakers, it will accrue great savings in the scarce resources of most developing countries where wheat cultivation does not thrive for climate reasons, and as well reduce the cost of bread and other baked products.

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