



CHANGES IN NUTRITIONAL, TEXTURE, RANCIDITY AND MICROBIOLOGICAL PROPERTIES OF COMPOSITE BISCUITS PRODUCED FROM BREADFRUIT AND WHEAT FLOURS ENRICHED WITH EDIBLE FISH MEAL

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Abstract

The use of indigenous crops in the preparation of nutritious snacks such as biscuits has been reported as a means of alleviating the perennial problem of malnutrition among Nigerians, especially children. However, storage has been recognised as a factor affecting the attributes of these biscuits. This study investigated the quality changes of biscuit produced from fish meal enriched-composite flour of breadfruit and wheat. Freshly harvested seedless variety of breadfruit, matured catfish, wheat flour and other ingredients were procured from local outlets in Ogun State, Nigeria. Breadfruit flour (BF) was produced by washing, manual peeling, washing, grating, bagging, dewatering, pulverizing and drying. Edible fish meal (EFM) was produced by washing, eviscerating, steaming and drying Catfish. Five blends of BF, WF and EFM were obtained from the optimised solutions of the D-optimal mixture design. Samples were stored (HDPE; 28 ± 2 °C) for 12 weeks. The proximate and mineral compositions, rancidity, texture profile and microbial counts of the biscuits were determined. Data were subjected to Analysis of Variance (ANOVA) and independent sample t-test. The means from ANOVA were separated using Duncan's Multiple Range Test at $p \leq 0.05$. At the end of the storage period, the biscuit blends were significantly ($p \leq 0.05$) different in moisture, protein, fat, fibre, ash, carbohydrate, calcium, iron and zinc. Increased levels of EFM in the blends led to increase in protein, fat, calcium, peroxide value (PV) and free fatty acid (FFA) value of the biscuits. At the end of the storage period, moisture increased, while fat decreased. The PV and FFA of most samples increased significantly ($p \leq 0.05$) with storage, and were within the maximum permissible level. Total bacterial and mould counts also increased significantly ($p \leq 0.05$) and exceeded the permissible level after 4 weeks of storage. Hence, the biscuits are suitable for consumption within 4 weeks under the investigated storage conditions.

Keywords: Storage, Biscuit, Breadfruit, Edible fish meal, Wheat

Introduction

Biscuits are one of the mostly eaten bakery products by all age groups, especially children. They are readily available, affordable and have a long shelf life (Klunklin and Savage, 2018). They are also ready-to-eat, widely consumed snack with good eating quality (Hasmadi *et al.*, 2014; Adeola and Ohizua, 2018) and can be more readily produced from composite flour than bread. Wheat flour is a common ingredient in biscuit making. However, wheat flour lacks some essential amino acids such as lysine, tryptophan and threonine which are required by school age children who are the major

consumers of biscuit (Gayas *et al.*, 2012). It is also necessary to explore the use of non-wheat adjuncts in biscuit making. Nigeria's wheat imports may grow at 5 per cent per annum and the country could be importing as much as 10mmt per annum by 2030 (AEGIC, 2015). Incorporation of non-wheat adjuncts that can play complementary roles in reducing protein energy malnutrition and having potentials to alleviate associated social problems is therefore highly desirable in biscuit production.

Breadfruit is an underutilized indigenous crop in Nigeria with less competing uses (Bakare *et al.*, 2012;

Bakare *et al.*, 2014). It has also been reported to be good source of fibre, calcium, magnesium thiamine and niacin (Ragone, 2003). It has greater yield per tree per year that can be sustained for many years; an average-sized tree reportedly produced 400 to 600 fruits per year. Its horticultural features can also be explored at household level to mitigate the effect of climate change in addition to its economic advantage. Breadfruit is enzyme active even during processing to flour. It is therefore necessary to improve the quality of its flour by modifying processing procedure and reducing processing time.

Fish is an important source of high quality protein (Ohen and Abang, 2007), providing about 16% of animal protein consumed by the world's population (FAO, 1997). It provides 40% of the dietary intake of animal protein of the average Nigerian (Federal Department of Fisheries, 1997). It also supplies essential amino acids such as lysine and methionine that are deficient in plant protein (Tibaldi *et al.*, 2015). Catfish is highly nourishing (contains lysine and vitamin A that is necessary for healthy growth). It contains some quantities of calcium, phosphorus, fat and other nutrients needed for human growth and health (Kwasek *et al.*, 2020). Catfish is a major source of protein to an average Nigerian home. Catfish farming is presently undertaken by a large number of people especially the small-scale farmers in Nigeria (Kareem *et al.*, 2008) who needed to be encouraged by expanding the value addition chain for their product. Fish has been used in different forms (concentrate, powder, fillet, etc.) in biscuit production (Ibrahim, 2009; Mohamed *et al.*, 2014; Abou-Zaid and Mohamed, 2014; Abraha *et al.*, 2018). However, its use as means of nutritional enrichment presents storage stability problems (Mohamed *et al.*, 2014) that need to be monitored along with other technological challenges associated with product development efforts in biscuit making.

Herb and spice have been incorporated into biscuit making recipes (Manley, 1983). This may have been as a result of changes in food habits of different countries, appreciation of the phytochemical functionality of its nutraceuticals, and phenolic constituents (Bakare *et al.*, 2016). African and Asian countries are phyto-active in their dietary practices. Development of biscuits with spicy characteristics for segments of such market would therefore not be unusual. Spices contain important antioxidant properties that are useful to human health, extend shelf life of food product and also reduce the level of microorganisms in food items (Klunklin and Savage, 2018).

Storage is an important aspect in the production, distribution and consumption of food products, as it causes changes in their quality attributes (Chowdhury *et al.*, 2012; Nagi *et al.*, 2012; Olunlade *et al.*, 2013; Rios *et al.*, 2014; Godase *et al.*, 2020). Development of nutritionally enriched biscuits may therefore require the profiling of some of its microbiological characteristics and chemical indices that may affect its stability during

storage. This study evaluated the effects of storage period on some quality attributes of biscuit produced from the blends of Improved Quality Breadfruit Flour (IQBF), wheat flour (WF), Edible Fish Meal (EFM) that were formulated from predicted optimised solutions of D-optimal mixture design. Specifically, it monitored the proximate, some mineral (calcium, iron, zinc), microbiological quality of the biscuit during storage, some of its index of rancidity (peroxide value, free fatty acid value) and textural properties.

Materials and Methods

Source of Materials

About 600 pieces of freshly harvested seedless variety of Breadfruit (*Artocarpus communis* Forst) and matured Catfish (*Clarias gariepinus*) were procured from farms in Idiroko and Ijebu-Ode, Ogun State, Nigeria respectively. The Honeywell brand of wheat flour (WF) and other ingredients were obtained from shops in Abeokuta.

Methods

Design of the experiment and validation of optimized Solutions

This study was part of an earlier experiment (Bakare *et al.*, 2020) where the nutritional and other technological qualities of biscuit were profiled and predictions about possible optimisation solutions were made. The predicted solutions were validated by producing five new formulated samples of biscuits on the basis of the optimized solutions and subjected to storage studies. Changes that were monitored during the 12-week storage period were proximate (moisture content, crude protein, fat, fibre, total ash, carbohydrate), some mineral (calcium, iron, zinc), microbiological (total bacterial and mould counts) qualities, textural properties and rancidity (peroxide value, free fatty acid value) profile of the biscuit. Earlier in the study, D-optimal experimental design was used to show the effects of each component {Improved Quality Breadfruit Flour (IQBF), wheat flour (WF) and Edible EFM (EFM)} of the blends of flour on biscuit quality. The design consist of sixteen (16) sets of experimental runs with five replications at the central point, which allows for estimation of pure error sum of squares. The coded and actual variables at the three levels (-1, 0 and +1) in which attributes of each of the factors {IQBF (60.00, 80.00 and 100), WF (0.00, 20.00 and 40.00) and EFM (0.00, 20.00 and 40.00)} at high, central and low levels were chosen based on preliminary experiments. Fitness of each of the models were analysed to identify the model that can best be used as a response predictor. The models were assessed for their adequacy for the experimental conditions and significant terms in each of the models were identified and numeric optimisation of the mixture blends done on the basis of set targets. The five optimised predicted solutions from the study thus served as the baseline for this storage study.

Preparation of improved quality breadfruit flour (IQBF)

A modified method of Bakare *et al.* (2012) was used to

produce IQBF. The production was done within 24hrs of harvest. The method of production involved washing, manual peeling, washing, grating, bagging, dewatering, and pulverizing. Drying of the pulverized mash was done in a flash dryer (Nobex Flash dryer, Nobex Technical Company Limited, Idimu Lagos, Nigeria) with the following conditions; loading time, tube temperature, and inlet air temperature were 10 min, 180°C and 200°C respectively. On the other hand, the feed moisture content, density and rate were 45%, 1,380 kg/m³ and 820 kg/hr respectively. The powder was allowed to settle and discharge at every 10min. Milling of the dried breadfruit was done using a locally fabricated hammer mill, sieved (W.S. Tyler, 8570 Blvd, Mentor, OH, United States) through a 250-µm mesh sieve, and sealed in polythene bags prior to analyses.

Preparation of spice mixture

Formulated Instant Spice mixture (ISM) was prepared as described by Bakare *et al.* (2016). and added to confer

flavour and functionality properties on the biscuit.

Preparation of edible fish meal (EFM)

EFM was produced as described by Bakare *et al.* (2020), with some modifications. About 500g Catfish was washed, eviscerated, steamed at 95°C for 25min, and dried in forced convectional air dryer [Nexus, NX-AF3100(2), Deekay Group (Nig.) Ltd, Nigeria] at 180°C for 45min and pressed. The dried catfish was left to cool, milled to powdery form using locally fabricated Hammer mill, sieved (W.S. Tyler, 8570 Blvd, Mentor, OH, United States) through a 250-µm mesh sieve, packed in an airtight container, and stored for subsequent use.

Preparation of flour blends

The IQBF, WF and EFM flours were blended as presented in Table 1.

Table 1: Experimental Design and Optimization Goals for Process variables

Predicted Optimised Solutions (POS)	Blends		
	IQBF	WF	EFM
1	61.33	38.6	0.08
2	60.0	39.51	0.49
3	63.22	36.71	0.07
4	66.2	33.8	0
5	79.5	0	20.5

IQBF = Improved quality breadfruit flour; WF = Wheat flour; EFM = Edible fish meal

Biscuit production

The biscuit was produced as described by Manley (1983) and modified by Bakare *et al.* (2014). The biscuit recipe (%) includes: blends of flour (63.5), sugar (12.7), fat (15.9), invert syrup (6.35), sodium bicarbonate (0.5), baking powder (0.5), and ISM (0.6).

Analyses

All analyses were performed in triplicates. Moisture (AOAC method 950.46B), ash (AOAC method 920.153), protein (AOAC method 955.04), and lipid (AOAC method 991.36) contents were determined according to Association of Official Analytical Chemists procedures (AOAC, 2012). Carbohydrate levels were calculated by the equation: % carbohydrates = 100% - (% moisture + % protein + % ash + % lipid). Crude fibre content of flour was determined by trichloroacetic acid method as described by Entwisted and Hunter (1949). Mineral content of the biscuit samples was determined using the method described by Adeniji and Tenkouano (2008). In the analysis, 1g of sample was weighed into a pyrex glass conical flask and 10ml of concentrated nitric acid was introduced into the flask with a straight pipette, then 5ml of perchloric acid was also added. The mixture was heated until a clear digest was obtained, then the digest was cooled to room temperature and diluted to 50ml with distilled water. The diluent was filtered into a plastic vial for atomic absorbance spectrophotometer analysis.

Peroxide value

Peroxide value was determined by dissolving oil sample (5g) in glacial acetic acid – chloroform (3:2v/v) solution, 0.5ml saturated potassium iodide and starch indicator were added and titrated with 0.01N Sodium thiosulphate. Similar procedure was carried out for blank sample (AOCS, 2009) and peroxide value was calculated thus:

$$\text{Peroxide value } \left(\frac{\text{meq}}{\text{kg}} \right) = \frac{V1 - V2 \times N \times 1000}{W}$$

Where:

V1- Volume of Sodium thiosulphate (Na S O) consumed for sample,

V2- Volume for blank,

W- Weight of sample in grams and

N- Normality of Sodium thiosulphate.

Free fatty acid

The free fatty acid (FFA) content of oil was determined by dissolving the test sample (1g) in 1:1v/v ethyl alcohol and titrated with potassium hydroxide (0.1N) using phenolphthalein indicator. Blank sample was subjected to similar treatment and FFA calculated thus;

$$\% \text{ FFA (as oleic)} = \frac{(V - B) \times N \times 28.21}{W}$$

Where:

V- Volume of KOH utilized by sample,

B- Volume for blank and

N- Normality of KOH

Texture profile of biscuit

The texture of the biscuits was determined as described by Bakare *et al.* (2020) using the Universal testing machine (model M500-100AT, Testometric, England). The parameter measured were hardness, chewiness, gumminess, cohesiveness, springiness, stringiness, and force at the peak, deformation at the peak, energy to peak, adhesiveness and energy to break.

Microbial analysis

Aerobic colonies in the biscuits during storage; bacterial, yeast and total mould counts were carried out using the method described by Rinku *et al.* (2017). The stock solution of the biscuit was prepared by dissolving 1g of sample into 9ml distilled water and allowed to stand for 30min and serial dilution was made for each sample. Nutrient (7g) and potato dextrose agar (10g) were weighed separately and suspended in 250ml of distilled water and then autoclaved at 121°C for 15min. It was allowed to cool to about 45- 50°C before pouring into sterile petri dishes. One milliliter of appropriate dilution was pipetted into sterile petri dishes and sterile agar was poured, rocked and allowed to gel before incubating at 37°C for 24h. The bacterial and fungal colonies were counted and recorded in colony-forming unit (cfu/g).

$$\text{cfu/g} = \frac{\text{number of count}}{\text{volume plated} \times \text{diution}}$$

Storage

Prepared biscuits were packed and kept under ambient conditions (28 ± 2 °C). The moisture content, peroxide value, free fatty acid and microbial quality were monitored at 2 weeks interval over the period of 12 weeks, while the proximate and mineral composition and texture profile were measured at zero and 12th week of storage.

Data analysis

Data on the various responses were subjected to independent sample t-test and analysis of variance. Differences between mean values were separated using a Duncan multiple range test (Duncan, 1955) using the statistical analysis package SPSS 17 for Windows (IBM, Armonk, NY, USA).

Results and Discussion

Changes in proximate and mineral compositions

Food products rich in protein content are nutritionally important for reducing the incidence of protein malnutrition (UNICEF, 2015). Protein is required for growth, development and maintenance of health. Proteins build and repair body tissues, play major roles in regulating various body functions, and provide energy when there are insufficient carbohydrate and fat in the diet (Elango and Ball, 2016; Roth, 2011; Wardlaw

and Kessel, 2002). The crude protein content of the biscuit ranged from 6.41 to 12.73% with samples containing 20.5% EFM and 0.0% EFM with significantly ($p \leq .05$) higher and lower protein values respectively (Table 2). These observations agreed with previous reports of Ibrahim (2009), Mohamed *et al.* (2014) and Abou-Zaid and Mohamed (2014). Results of independent t-test indicated that the protein content of the biscuit did not change significantly ($t = 0.026$, $df = 8$, $P = 0.98$) during the 12 weeks of storage.

Fat, apart from its nutritional value, is also a functional ingredient in achieving the desired texture, mouthfeel and the crispiness of biscuit (Davidson, 2019). The fat contents of the biscuit samples ranged from 13.67 to 20.95% and were similar to those reported by Kumar *et al.* (2016) and Omran *et al.* (2016). The fat content of the biscuits increased significantly ($p < .05$) with the inclusion of EFM, indicating appreciable contribution of the EFM to the fat content of the biscuit samples (Table 1). The fat content of the samples also decreased significantly ($t = 3.42$, $df = 8$, $P = 0.009$) during storage. This observation was in agreement with Rinku *et al.* (2017) who reported that fat content of the biscuit produced from wheat supplemented with fenugreek seed powder decreased with storage time. Reduction in the fat content during storage may be due to hydrolysis and oxidation of oil component in the biscuit (Lee and Cho, 2012; Khatun *et al.*, 2016).

Fibre is known to aid digestive system of humans (Li and Komarek, 2017). The crude fibre content of the biscuit samples increased as the inclusion of breadfruit flour increased, except the sample prepared from blend 2 (60.00:39.51:0.49; BF:WF:EFM). The range of value (0.66- 1.06%) obtained in this study (Table 2) was lower than values reported by Usman *et al.* (2015) but higher than 0.47-0.80% reported by Agu and Okoli (2014). The fibre content of the biscuit samples did not change significantly ($t = 0.71$, $df = 8$, $P = 0.49$) during storage.

The ash content of food materials could be used as an index of mineral constituents of the food. It is the inorganic residue remaining after water and organic matter have been removed by heating (Samia *et al.*, 2018). It is also an indication of the mineral composition of food materials. Except for POS 2 (60.00:39.51:0.49; BF: WF: EFM), the ash contents (1.74-4.05%) of the biscuit samples increased with increased inclusion of BF and EFM in the blends (Table 2). The increase in the ash content can be attributed to the effect of combination of fish with breadfruit, which is a good source of calcium, potassium, zinc and iron (Ragone, 2003). The reduction in the ash content (Table 2) of the biscuit during storage was not significant ($t = 1.76$, $df = 8$, $P = 0.12$) enough to alter the mineral quality of the biscuit.

The carbohydrate content of the biscuit samples were within the range (53.39-75.19%) reported by Omran *et al.* (2016). The lower the content of EFM in the blends, the higher the carbohydrate contents of the biscuit samples (Table 1). The range of values obtained from

this study was higher than the range of 42.10- 42.99% reported by Kumar *et al.* (2016). The carbohydrate contents of the blends did not change significantly ($t = 0.36$, $df = 8$, $P = 0.73$) during storage.

Minerals are essential for the maintenance of the overall mental and physical development of bones, teeth, tissues, muscles, blood and nerve cells (Wardlaw and Kessel, 2002; Ohizua *et al.*, 2017). This study analyzed calcium, iron and zinc which have been reported as being essential for the vulnerable age group. Calcium is essential for teeth formation, muscle contraction and maintenance of cell membrane (Sharif *et al.*, 2009; Muhammad *et al.*, 2018). The calcium contents of the biscuit samples increased as the level of EFM increased in the blend with POS 5 having significantly higher value than other POS. The range of values (Table 2) for calcium was in agreement with that reported by Tsikritzi *et al.* (2014). The reduction observed in the calcium contents of the samples during storage were not statistically significant ($t = 0.12$, $df = 8$, $P = 0.91$) to infer negative changes in biscuit quality during the storage period. In spite of probable utilization of some nutrients by microbes during the storage period, consumption of 100g of the biscuit can still provide up to 24.5% of recommended allowance of 1,200 mg (NRC, 1989) for children within the ages 11 to 24 years and 36.8% for older age groups.

Iron is a constituent of hemoglobin, myoglobin, and a number of enzymes and, therefore, is an essential nutrient for humans (Muhammad *et al.*, 2018; Bothwell *et al.*, 1979). It is also stored as ferritin and hemosiderin in the spleen, liver, and bone marrow, while a small amount is associated with the blood transport protein transferrin. The iron content ranged from 81.15mg/100g (POS 4) to 134.2mg/100g in POS 2 (60.00:39.51:0.49; BF: WF: EFM) and can adequately meet up with the 10 to 15mg/day (NRC, 1989). The reduction in the iron contents of the samples during storage were not statistically significant ($t = 0.48$, $df = 8$, $P = 0.65$).

Zinc is essential for good immune system, hormone secretion, mental wellbeing, foetus growth and normal development of humans (Vidyavati *et al.*, 2016). The range of values (9.25 to 20.50mg/100g) obtained in this study for zinc was similar to that reported by Tsikritzi *et al.* (2014). Although these values decreased during storage, there was no significant ($t = 0.95$, $df = 8$, $P = 0.37$) difference between samples at the end of the storage period.

Changes in rancidity indices of biscuit

Rancidity is a term generally used to denote unpleasant odours and flavours in foods resulting from deterioration in the fat and oil portion of food. Rancidity can be hydrolytic (reaction of fats with water) or oxidative (reaction of fats with air). According to Vaclavick and Christian (2005), hydrolytic rancidity (or lipolysis) is catalysed by heat and enzymes (lipases), while oxidative rancidity (or autoxidation) is catalysed

by heat, light, certain metals (iron and copper) and enzymes (lipoxygenases). Oxidative rancidity reduces the nutritional quality and safety of food (Nawar, 1985).

Peroxide value is used to detect the onset of oxidative rancidity in food (Abraha *et al.*, 2018). The peroxide value of the biscuit (Table 3) increased significantly ($p \leq 0.05$) with storage time for all the samples. The results agreed with reports on previous studies on peroxide value of biscuit during storage (Rinku *et al.*, 2017; Takeungwongtrakul and Benjakul, 2017; Omran *et al.* 2016). The mean peroxide value ranged from 1.50 to 2.46 meq/kg with POS 4 (BF: WF: EFM; 66.2:36.8:0.00) and 5 (BF: WF: EFM: 79.45:0.00:20.5) having the lowest and highest values respectively. This implied that samples without EFM (Table 1) have reduced tendencies to oxidative rancidity, while the onset of oxidative rancidity is more pronounced with increasing inclusion of EFM. The peroxide values of samples were not significantly different except for POS 5 containing 20.5% EFM. The values obtained in this study were below the values reported by Mohamed *et al.* (2014) but higher than that of Abraha *et al.* (2018) for biscuits containing fish protein concentrate. However, peroxide values of the biscuit at the end of the storage period were below FAO Codex standard value of 10 meq/kg (FAO, 1999). The lower the peroxide value the better the quality of the biscuit.

Free fatty acid value is used to determine hydrolytic rancidity. It indicates the extent of decomposition of glycerides by lipase action (Onwuka, 2018). The free fatty acid value of the biscuit increased significantly ($p \leq 0.05$) with storage time for all the tested POS (Table 3), but the rate of increase was more pronounced with increase in EFM. Similar observations were reported by Nagi *et al.* (2012) and Kumar *et al.* (2016). Free fatty acid in biscuits from POS 5 were significantly different ($p < 0.05$) from other samples (Table 3). The free fatty acid values, however, were within the FAO Codex standard value of 1% maximum (FAO, 1999), except for biscuit from POS 2 (60.0:39.51:0.49; BF: WF: EFM) and POS 5 (79.45:0.00:20.55; BF: WF: EFM). This suggested a storage period of less than 10 weeks for biscuit containing these levels of EFM inclusion.

Texture profile of the biscuits

Texture is a very important quality attribute that influences consumer acceptance of biscuit. Hardness is the peak force measured during the first bite (Hussein *et al.*, 2018). The lower the values obtained for hardness, the softer the biscuit. The hardness of the biscuit (Table 4) ranged from 343.9N to 1633.5N in POS 2 and 5 respectively. It increased significantly ($p \leq 0.05$) as the inclusion level of breadfruit increased in the samples. There was a significant reduction ($t = 3.14$, $df = 18$, $P = 0.006$) in the hardness values of the biscuit during storage. Morais *et al.* (2018) also reported a decrease in the hardness of biscuit during storage.

Chewiness is the energy needed to masticate solid food to a state of readiness for swallowing (Karaoglu and

Kotancilar, 2009; Pereira *et al.*, 2013; Chandra and Shamasundar, 2015). It is directly related to hardness as it is estimated as the product of hardness, cohesiveness, and elasticity (Rosenthal, 1999). The samples were significantly different ($p \leq 0.05$) in chewiness property. The values obtained for chewiness of the biscuits ranged from 19.4N to 434.47N in POS 3 and 5 respectively. In spite of the reduction in values observed in most of the biscuit samples, results from t-Test was unable to establish a significant difference ($t = 1.02$, $df = 18$, $P = 0.323$) in the chewiness properties of the biscuit before and after storage.

Gumminess is the product of hardness and cohesiveness which simulates the energy required to disintegrate a food product before swallowing (Hussein *et al.*, 2018). The gumminess values of the biscuit samples ranged from 242.2N to 964.49N with POS 2 and 5 with lowest and highest values respectively. The observed reduction in the gumminess of the samples during storage was not significant ($t = 1.06$, $df = 18$, $P = 0.309$) enough to establish a difference in the gumminess property of the biscuit before and after storage.

Cohesiveness reflects the strength of the internal bonds binding the food particles together and suggests how well the biscuit withstands a second deformation relative to its resistance under the first deformation. The higher the cohesion value, the greater the ability of the biscuit to break when subjected to stress (Bakare *et al.*, 2020; Hussein *et al.*, 2018). The cohesiveness values of the biscuits ranged from 0.41 to 0.67 in POS 3 and 2 respectively. These results were within the range (0.59-0.78) reported by Pereira *et al.* (2013) for Maria type cookies, but higher than that (0.01-0.02) reported by NoorAziah *et al.* (2012). There was no uniform trend in the changes observed in the cohesiveness values of the biscuits during storage. The cohesiveness values of biscuits prepared from POS 2, 3 and 5 increased, while those obtained from POS 1 and 4 decreased during storage. It could however be concluded that the biscuit samples were significantly different ($t = 2.59$, $df = 18$, $P = 0.018$) before and after storage. The lower the cohesiveness values of biscuit, the higher its fragility (Pereira *et al.*, 2013).

Changes in moisture and microbial load of biscuit

The total bacterial count of the biscuit samples is presented in Table 5. The total bacterial count of the biscuit samples ranged from 0.5×10^1 in POS 2 to 1.5×10^1 in POS 3, 4 and 5. In all the samples, the bacterial load of the biscuit samples increased significantly ($p \leq 0.05$) during storage. The value obtained at 12 weeks of storage period was more than the maximum accepted value of 10,000 cfu/g (UNICEF, 2017).

Mould growth was not detected before storage of the biscuit samples. According to Frazier and Westhoff (1986), baking temperatures are usually high enough to kill all mould spores in and on baked foods. There was a significant ($p \leq 0.05$) difference in the total mould count of the biscuits during storage. At 2 weeks of storage,

total mould count ranged from 1.50×10^1 to 3.00×10^1 cfu/g. The total mould count of the biscuit samples produced was found to increase with storage period for all the blends. The total mould count of the biscuit samples at 12 week of storage was higher than the maximum allowable level of 300 cfu/g (UNICEF, 2017). The significant increase in the total bacterial and mould counts during storage may be as a result of increase in moisture content and the permeability of the packaging material. The moisture content of the samples at the commencement of storage ranged from 4.77 to 5.73% and was significantly different from each other with POS 5 and 1 having the lowest and highest values respectively. It increased significantly ($t = -27.68$, $df = 8$, $P = 0.00$) by about 100% after 12 weeks of storage which implies possible permeability error in the packaging material used or as a result of syneresis associated with high setback viscosity property of breadfruit flour (Bakare *et al.*, 2020). The high final viscosity, setback viscosity and swelling power of 636.00 ± 47.02 RVU, 204.21 ± 13.38 RVU and 891.56 ± 1.59 observed for the IQBF used in this study was relatively higher than values for conventional breadfruit flour (Bakare *et al.*, 2014). These have implications on the re-association and retrogradation, or of starch molecules of the biscuit made from it during cooling and storage. Setback viscosity of flours had also been correlated with the texture of various products (Adeyemi and Idowu, 1990; Michiyo *et al.*, 2004). This may also explain the significant changes observed in the texture (hardness) of the biscuit at the end of the storage period.

Conclusion

The study evaluated biscuits produced from five predicted optimized solutions of an earlier study, stored in high density polyethylene at 28 ± 2 °C for 12 weeks and evaluated for changes in some of its nutritional [proximate and mineral (Ca, Fe and Zn)] quality, rancidity (peroxide value and free fatty acid) profile, texture (Hardness, chewiness, gumminess and cohesiveness) and Microbiological (total bacterial and mould counts) qualities. Moisture content of the biscuits increased ($t = -27.68$, $df = 8$, $P = 0.00$), fat content decreased ($t = 3.42$, $df = 8$, $P = 0.009$) and the texture quality [Hardness ($t = 3.14$, $df = 18$, $P = 0.006$) and cohesiveness ($t = 2.59$, $df = 18$, $P = 0.018$) were significantly altered. The peroxide value (1.50-2.46 meq/kg) and free fatty acid (0.38-0.69%) increased significantly ($P \leq 0.05$) with storage, but were within the bench mark standard of 10meq/kg and 1% respectively specified by UNICEF for high energy biscuit. The microbiological integrity of the biscuit could only be sustained within 4 weeks of storage as the total bacterial and mould counts increased significantly ($p \leq 0.05$) with storage, and exceeded the permissible level of 10,000cfu/g and 300cfu/g respectively after 12 weeks of storage. The quality attributes within the storage period were moisture (4.77-5.73%), protein (6.41 to 12.73%), fat (13.67 to 20.95 %), fibre (0.66- 1.06%), ash (1.74-4.05%), carbohydrate (57.41-71.64%), Ca (42.50-310.0 mg/100g), Fe (81.15- 134.2mg/100g) and Zn (9.25 to 20.50 mg/100g).

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Table 2: Proximate and Mineral Compositions of Biscuits during Storage

Storage period (weeks)	Composition	POS 1	POS 2	POS 3	POS 4	POS 5
Changes in Proximate Contents (%)						
0	Moisture	5.52±.02 ^d	5.73±.01 ^c	5.31±.01 ^c	5.04±.04 ^b	4.77±.01 ^a
12		11.66±.03 ^b	11.67±.02 ^b	11.84±.02 ^c	11.06±.07 ^a	11.24±.14 ^a
0	Crude protein	10.41±.00 ^c	11.26±.02 ^d	10.03±.03 ^b	6.41±.08 ^a	12.73±.14 ^e
12		10.36±.04 ^c	11.18±.07 ^d	10.03±.04 ^b	6.42±.07 ^a	12.66±.22 ^e
0	Crude fat	17.58±.02 ^c	18.55±.14 ^d	17.03±.03 ^b	13.67±.14 ^a	20.95±.00 ^e
12		13.12±.07 ^c	13.72±.03 ^d	12.70±.07 ^b	11.93±.03 ^a	14.63±.02 ^e
0	Crude fibre	0.68±.01 ^a	0.66±.01 ^a	0.96±.01 ^b	0.97±.01 ^b	1.06±.01 ^c
12		0.64±.01 ^a	0.62±.01 ^a	0.85±.00 ^c	0.82±.01 ^b	1.01±.01 ^d
0	Total ash	2.05±.00 ^b	1.74±.00 ^a	2.82±.00 ^c	3.07±.00 ^d	4.05±.00 ^e
12		1.08±.01 ^a	1.00±.00 ^a	2.06±.01 ^b	2.43±.01 ^c	2.55±.07 ^d
0	Carbohydrate	64.41±.01 ^c	62.55±.07 ^b	64.78±.01 ^d	71.64±.01 ^e	57.41±.01 ^a
12		63.68±.01 ^d	62.40±.07 ^b	63.28±.01 ^c	68.04±.01 ^e	58.45±.21 ^a
Changes in Mineral Contents (mg/100g)						
0	Calcium	50.50±.70 ^a	52.75±.35 ^a	48.50±.71 ^a	42.50±.70 ^a	310.0±14.14 ^b
12		41.68±.07 ^a	42.91±.14 ^a	41.03±.49 ^a	40.75±.56 ^a	294.00±7.07 ^b
0	Iron	95.35±.70 ^d	134.2±2.12 ^e	90.80±.70 ^c	81.15±1.4 ^a	87.25±1.41 ^b
12		88.25±1.4 ^b	125.8±7.07 ^c	85.60±4.95 ^{a,b}	76.80±1.41 ^a	81.50±.70 ^{a,b}
0	Zinc	17.00±.70 ^d	20.50±.70 ^c	16.50±.70 ^c	9.25±.35 ^a	13.85±.49 ^b
12		14.23±.70 ^c	18.05±.70 ^d	13.65±.70 ^{b,c}	6.25±.35 ^a	12.23±.21 ^b

POS 1 = 61.33: 38.6: 0.08 (IQBF:WF:EFM); POS 2 = 60.0: 39.51: 0.49 (IQBF:WF:EFM); POS 3 = 63.22: 36.71: 0.07 (IQBF:WF:EFM); POS 4 = 66.22: 33.8: 0.0 (IQBF:WF:EFM); POS 5 = 79.5: 0.0: 20.5 (IQBF:WF:EFM); IQBF = Improved quality breadfruit flour; WF = Wheat flour; EFM = Edible fish meal Mean values with different superscripts within the row are significantly different at p<0-0

Table 3: Changes in Rancidity Indices of biscuits during storage

Storage Period (weeks)	Peroxide values (meq/kg)									
	1	2	3	4	5	1	2	3	4	5
0	0.830±.00 ^a	0.84±.01 ^a	0.83±.00 ^a	0.82±.01 ^a	1.32±.01 ^a	0.18±.01 ^a	0.19±.00 ^a	0.17±.01 ^a	0.15±.01 ^a	0.24±.04 ^a
2	1.15±.01 ^b	1.15±.01 ^b	1.15±.01 ^b	1.06±.04 ^b	1.98±.01 ^b	0.24±.01 ^b	0.29±.02 ^b	0.22±.00 ^a	0.19±.01 ^b	0.38±.04 ^{ab}
4	1.61±.00 ^c	1.69±.01 ^c	1.53±.00 ^c	1.31±.00 ^c	2.33±.00 ^c	0.32±.01 ^c	0.36±.04 ^{bc}	0.31±.01 ^b	0.29±.02 ^c	0.53±.09 ^{bc}
6	1.81±.00 ^d	1.87±.01 ^d	1.81±.00 ^d	1.51±.00 ^d	2.62±.55 ^d	0.38±.03 ^d	0.42±.04 ^c	0.37±.02 ^b	0.34±.01 ^d	0.63±.11 ^c
8	2.01±.02 ^e	2.16±.04 ^e	2.02±.00 ^e	1.83±.00 ^e	2.87±.08 ^e	0.48±.02 ^e	0.55±.04 ^d	0.44±.03 ^c	0.41±.01 ^e	0.85±.04 ^d
10	2.07±.00 ^f	2.23±.01 ^f	2.06±.00 ^f	1.92±.00 ^f	3.01±.01 ^f	0.64±.01 ^f	0.77±.05 ^e	0.61±.04 ^d	0.53±.03 ^f	1.07±.06 ^e
12	2.12±.04 ^g	2.31±.01 ^g	2.09±.00 ^g	2.06±.02 ^g	3.08±.02 ^g	0.81±.03 ^g	1.11±.02 ^f	0.80±.04 ^e	0.73±.01 ^g	1.24±.04 ^f

Means values with different superscripts within the column are significantly different (p ≤ 0.05). POS 1 = 61.33: 38.6: 0.08 (IQBF:WF:EFM); POS 2 = 60.0: 39.51: 0.49 (IQBF:WF:EFM); POS 3 = 63.22: 36.71: 0.07 (IQBF:WF:EFM); POS 4 = 66.22: 33.8: 0.0 (IQBF:WF:EFM); POS 5 = 79.5: 0.0: 20.5 (IQBF:WF:EFM); IQBF = Improved quality breadfruit flour; WF = Wheat flour; EFM = Edible fish meal

Table 4: Changes in Texture profile of biscuits during storage

Storage period (weeks)	Parameters	POS 1	POS 2	POS 3	POS 4	POS 5
0	Hardness (N)	1353.0±3.5 ^d	343.9±.0 ^a	497.1±2.8 ^c	463.4±1.4 ^b	1633.5±4.9 ^e
12		101.8±1.4 ^a	217.5±2.8 ^b	452.7±2.8 ^c	291.4±1.4 ^c	384.1±1.4 ^d
0	Chewiness (N)	324.3±2.1 ^d	166.6±3.5 ^c	19.4±.0 ^a	106.3±1.4 ^b	432.5±2.8 ^e
12		31.61±.4 ^a	116.7±1.4 ^c	361.8±2.1 ^e	85.7±1.4 ^b	135.3±1.4 ^d
0	Gumminess (N)	916.7±7.1 ^d	242.2±2.1 ^a	261.9±1.4 ^b	274.6±2.1 ^c	963.5±1.4 ^e
12		41.0±1.4 ^a	190.9±.7 ^c	463.9±1.4 ^e	121.6±2.1 ^b	268.1±3.5 ^d
0	Cohesiveness	0.67±.02 ^c	0.67±.04 ^c	0.41±.01 ^a	0.61±.01 ^{bc}	0.58±.01 ^b
12		0.61±.01 ^b	0.73±.01 ^c	0.98±.01 ^d	0.31±.01 ^a	0.72±.03 ^c

POS 1 = 61.33: 38.6: 0.08 (IQBF:WF:EFM); POS 2 = 60.0: 39.51: 0.49 (IQBF:WF:EFM); POS 3 = 63.22: 36.71: 0.07 (IQBF:WF:EFM); POS 4 = 66.22: 33.8: 0.0 (IQBF:WF:EFM); POS 5 = 79.5: 0.0: 20.5 (IQBF:WF:EFM); IQBF = Improved quality breadfruit flour; WF = Wheat flour; EFM = Edible fish meal. Mean values with different superscripts within the row are significantly different at $p < 0.05$.

Table 5: Total Bacterial and Mould Count (cfu/g) of Biscuits during Storage

Storage Period (weeks)	POS 1			POS 2			POS 3			POS 4			POS 5		
	TBC	TMC	TBC	TBC	TMC	TBC	TBC	TMC	TBC	TMC	TBC	TMC	TBC	TMC	
0	$1.00^a \times 10^1$	ND	$0.50^a \times 10^1$	ND	ND	$1.50^a \times 10^1$	$1.50^a \times 10^1$	ND	$1.50^a \times 10^1$	ND	$1.50^a \times 10^1$	ND	$1.50^a \times 10^1$	ND	
2	$4.00^b \times 10^2$	$3.00^a \times 10^1$	$3.50^b \times 10^2$	$3.00^a \times 10^1$	$1.50^a \times 10^1$	$1.00^b \times 10^2$	$1.00^b \times 10^2$	$1.50^a \times 10^1$	$2.50^b \times 10^2$	$1.50^a \times 10^1$	$2.50^b \times 10^2$	$1.50^a \times 10^1$	$2.50^b \times 10^2$	$3.00^a \times 10^1$	
4	$2.25^c \times 10^4$	$1.30^b \times 10^2$	$5.10^c \times 10^4$	$2.35^b \times 10^2$	$1.05^b \times 10^2$	$5.95^c \times 10^4$	$1.05^b \times 10^2$	$1.05^b \times 10^2$	$3.10^c \times 10^4$	$1.00^b \times 10^2$	$3.65^c \times 10^4$	$1.00^b \times 10^2$	$3.65^c \times 10^4$	$1.50^b \times 10^2$	
6	$1.16^d \times 10^6$	$2.40^c \times 10^5$	$2.18^d \times 10^6$	$3.90^c \times 10^5$	$1.45^c \times 10^5$	$3.15^d \times 10^5$	$1.45^c \times 10^5$	$1.45^c \times 10^5$	$3.00^d \times 10^5$	$2.80^c \times 10^5$	$3.00^d \times 10^5$	$2.80^c \times 10^5$	$3.00^d \times 10^5$	$3.15^c \times 10^5$	
8	$2.25^e \times 10^8$	$1.08^d \times 10^8$	$3.25^e \times 10^7$	$2.93^d \times 10^8$	$3.30^e \times 10^7$	$3.30^e \times 10^7$	$3.90^d \times 10^7$	$3.90^d \times 10^7$	$3.00^e \times 10^7$	$1.30^d \times 10^7$	$3.10^e \times 10^7$	$1.30^d \times 10^7$	$3.10^e \times 10^7$	$3.55^d \times 10^8$	
10	$2.64^f \times 10^9$	$2.21^e \times 10^9$	$2.58^f \times 10^9$	$2.71^e \times 10^9$	$2.71^e \times 10^9$	$2.58^f \times 10^9$	$1.72^e \times 10^9$	$1.72^e \times 10^9$	$2.51^f \times 10^9$	$1.69^e \times 10^9$	$1.34^f \times 10^9$	$1.69^e \times 10^9$	$1.34^f \times 10^9$	$1.31^e \times 10^9$	
12	$1.15^g \times 10^{10}$	$1.58^f \times 10^{10}$	$6.70^g \times 10^9$	$5.55^f \times 10^9$	$1.06^f \times 10^{10}$	$9.05^g \times 10^9$	$1.06^f \times 10^{10}$	$1.06^f \times 10^{10}$	$7.40^g \times 10^9$	$1.68^f \times 10^{10}$	$5.35^g \times 10^9$	$1.68^f \times 10^{10}$	$5.35^g \times 10^9$	$3.45^f \times 10^9$	

POS 1 = 61.33: 38.6: 0.08 (IQBF:WF:EFM); POS 2 = 60.0: 39.51: 0.49 (IQBF:WF:EFM); POS 3 = 63.22: 36.71: 0.07 (IQBF:WF:EFM); POS 4 = 66.22: 33.8: 0.0 (IQBF:WF:EFM); POS 5 = 79.5: 0.0: 20.5 (IQBF:WF:EFM); IQBF = Improved quality breadfruit flour; WF = Wheat flour; EFM = Edible fish meal. Mean values with different superscripts within the column are significantly different ($P \leq 0.05$). TBC = total bacterial count; TMC = total mould count. ND = Not detected.

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