International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Effect of Fermented Plantain Flour Inclusion on the Glycemic Response, Nutritional Composition and Anti-Inflammatory Properties of Wheat Bread

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Abstract
Bread is a staple food worldwide, but the increasing prevalence of gluten-related disorders and the need for nutritious alternatives have led to exploring composite flours in bread production. This study investigated the effects of incorporating fermented plantain flour into wheat bread on its nutritional composition, anti-inflammatory properties, glycemic response, and sensory characteristics. Composite bread samples were analyzed for proximate composition, amino acid profile, anti-inflammatory activities (protein denaturation, membrane stabilization, and enzyme inhibition), glycemic index, and sensory attributes. Results showed that 30% plantain flour substitution (ZOM3) significantly enhanced the nutritional profile, with increased protein (23.16%), fiber (3.51%), and improved amino acid composition. This formulation demonstrated superior anti-inflammatory properties, with protein denaturation inhibition (IC50 ~26 μg/ml) comparable to diclofenac, and showed remarkable anti-lipoxygenase (IC50 16.01 μg/ml) and anti-cyclooxygenase (IC50 19.20 μg/ml) activities. The glycemic load was significantly reduced in ZOM3 (25.1) compared to the control (51.26). Sensory evaluation revealed high acceptability scores (8.12-8.67) for all composite bread formulations. These findings suggest that fermented plantain flour can be successfully incorporated into wheat bread up to 30% substitution level, creating a functional food product with enhanced nutritional value, improved health-promoting properties, and acceptable sensory characteristics.

Keywords: Composite flour, fermented plantain, anti-inflammatory, glycemic index, Bread

INTRODUCTION

Bread is a staple food consumed worldwide, traditionally made from wheat flour, water, yeast, and salt. its popularity stems from its versatility, taste, and nutritional value (Cappelli & Cini, 2021). However, the increasing prevalence of gluten-related disorders and the desire for more nutritious alternatives have led to the exploration of composite flours in bread production (Boukid et al., 2023). One such alternative is the incorporation of fermented plantain flour into wheat-based bread, which may enhance both nutritional and anti-inflammatory properties (Adebo et al., 2022). Recently, there has been growing interest in enhancing the nutritional profile of bread by incorporating alternative flours, such as pro-vitamin A cassava and plantain flour to replace a portion of wheat for use in bread, thereby decreasing the demand for imported wheat and producing protein enriched bread (Giami et al., 2015). Although there is now a substantial amount of available composite bread technology, such breads still require at least 70 % of wheat flour to be able to rise (Eggleston, 2017).

Inflammation is the body's natural response to injury or infection, characterized by redness, swelling, heat, and pain (Chen et al., 2021). While acute inflammation is essential for healing, chronic inflammation is associated with various diseases, including cardiovascular diseases, diabetes, and certain cancers (Liu et al., 2023). Diet plays a crucial role in modulating inflammation, with certain foods either exacerbating or mitigating inflammatory responses (Netto Candido et al., 2021). Whole grains, for instance, are rich in dietary fiber, antioxidants, and phytochemicals, which may exert anti-inflammatory effects and reduce chronic disease risk (Vanegas et al., 2022).

Wheat flour is the primary ingredient in traditional bread-making, valued for its gluten content, which imparts elasticity and structure to the dough (Johansson et al., 2023). It is a significant source of carbohydrates, providing energy, and contains essential nutrients such as fiber, vitamins, and minerals (Dewettinck et al., 2022). However, the gluten in wheat can cause adverse reactions in individuals with celiac disease or gluten sensitivity. Moreover, some studies suggest that refined wheat products may contribute to inflammation, whereas whole-grain wheat may offer anti-inflammatory benefits due to its higher nutrient and fiber content (Leonard et al., 2023). The predominance of wheat flour for baking of aerated (leavened) breads is due to the properties of its elastic gluten protein, which helps in producing a relatively large loaf volume with a regular, finely vesiculated crumb structure. If the wheat flour used in bread making is to be substituted with flour produced from other crops, it must be milled to acceptable baking quality (Opara et al., 2016).

Plantain (Musa paradisiaca) is a type of banana commonly found in tropical regions, are rich in fibre, vitamins, and minerals, offering potential health benefits when included in the diet (Jones et al., 2020). However, about 35 – 60 % of the plantain produced annually is lost post-harvest due to poor infrastructure (Olorunda et al., 2020). Plantain has a versatile culinary uses and nutritional benefits with a starchy texture and slightly sweeter taste when ripe, plantains serve as a staple food in many cuisines around the world. Rich in essential nutrients such as potassium, fibre, and vitamins A and C, plantains offer numerous health benefits (Onabanjo et al., 2017). From Savory dishes like fried plantains to sweet treats like plantain desserts, this versatile fruit plays a significant role in global gastronomy and nutrition. Fermentation of plantain flour involves the action of microorganisms that break down carbohydrates and proteins, enhancing the bioavailability of nutrients and introducing beneficial compounds. Fermented foods have been associated with improved digestion and potential anti-inflammatory effects. Incorporating fermented plantain flour into bread not only diversifies nutrient intake but may also improve the bread's sensory properties and health benefits (Oloyede et al., 2013).

MATERIALS AND METHODS

Source of Material

Wheat, unripe plantain, starter culture, baker’s yeast, sugar, and other ingredients were purchased at Odo-Oba Central Market, Ogbomoso, Oyo state, Nigeria. The materials were authenticated at the Department of Crop Production and Soil Science, LAUTECH, Ogbomoso. All chemicals used are of analytical grade.

Preparation of Wheat Flour

The wheat flour was produced from wheat grains according to method described by (Bolarinwa et al., 2015). The grain was cleaned and sorted to remove dirt, stone, and other extraneous materials. The cleaned grains were then washed, dried and dry milled using a Binatone kitchen blender (mode BLG 4O2, Zhongshan, Haishang) and the resultant flour sieved to obtain a uniform size of 400 µm and packaged for further analysis.

Preparation of Fermented Plantain Flour

The fermented plantain flour was produced from unripe plantain according to method described by (Oloyede et at., 2013). The unripe plantain was washed, peeled, diced, then steam blanched for 10 min. The blanched plantain was oven dried, milled, sieved, and then mixed with water and starter culture, it was allowed to ferment for 24hrs. After fermentation, the flour was oven dried again, milled using a Binatone kitchen blender (mode BLG 4O2, Zhongshan, Haishang) and the resultant flour sieved to obtain a uniform size of 400 µm and packaged for further analysis.

Table 1: Formulation Table of Bread Production from Wheat and Fermented Plantain Composite Flour.

Samples	Flour Formulations	Wheat	Fermented Plantain	Total (%)
ZOM 1	Wheat+Fermented Plantain	90	10	100
ZOM 2	Wheat+Fermented Plantain	80	20	100
ZOM 3	Wheat+Fermented Plantain	70	30	100
ZOM 4	Wheat (commercial product)	100	-	100

Bread Dough Formation and Specific Volume Determination

The bread dough was produced based on the Straight dough as described by maneju et al. (2011). This method involves the addition of all ingredients; Wheat/ Composite flour (100g), sugar (12.5g), salt (2.5g), yeast (0.5 Mc Farland standard), fat (20g) and water (50mL) at the mixing stage, then kneading to form the dough. Specific volume was measured by dividing the volume by the weight; Specific volume =v/wt (cm3/g).

Production of Bread Using Composite Flour Blends

The bread samples were produced in batches by mixing and kneading manually each of the above flour blends with the principal bread ingredients in a stainless-steel bowl. After thorough kneading in each case, the dough was allowed to ferment and develop for an hour at 35^0C before being knocked back and then moulded into cylindrical shape. After moulding in each case, the dough was placed in a well-oiled baking pan where it was proofed for 30 minutes at room temperature before it was baked in an oven preheated and set at 210^0C. After baking, the dough was brought out in each case from the oven and immediately de-panned by knocking out. The knocked-out bread was allowed to cool, weighed and measured before being packed in a polyethylene bag and stored at room temperature (Ayo et al., 2014).

Proximate Composition Analysis

Proximate compositions of breads were determined as described by AOAC (2012). The carbohydrate content was determined by the difference (100-the sum of the content of protein, fat, ash, and moisture), while the energy value was calculated using the Atwater factor (fat × 9 + carbohydrate × 4 + protein × 4 kcal/100 g).

Amino Acid Analysis

The amino acid profiles of the breads were determined using the High-performance liquid chromatography (HPLC) method as previously described by Alagbe and Malomo (2024). Briefly, food sample was placed in hydrolysis ampoule, then dried under vacuum using a Savant SpeedVac. Approximately 100 µL of 6 N HCL was placed in the lower part of the ampoule, freezed in a dry ice/ethanol bath, attached to a vacuum system via ¼" ID x 5/8" OD Tygon tubing, then slowly thawed and evacuated to <150 mtorr. Oxygen/methane flame was used to seal the neck of the tube at the constriction. After hydrolysis and acid removal, samples that contained 0.5-10 µg of protein were reconstituted with 60-200 µg of Na-S sample buffer and the amino acid composition then finally analyzed. The cysteine and methionine contents were determined after performic acid oxidation and the tryptophan content was determined after alkaline hydrolysis.

Anti-inflammatory activities

Inhibition of protein denaturation

The inhibition of protein denaturation was evaluated by the method of Alagbe and Malomo (2024) with slight modification. 500 µL of 1% bovine serum albumin was added to 100 µL of plant extract. This mixture was kept at room temperature for 10 min, followed by heating at 51°C for 20 min. The resulting solution was cooled down to room temperature and absorbance was recorded at 560 nm. Acetyl salicylic acid was taken as a positive control.

Determination of anti-proteinase

The test was performed according to the modified method of. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1ml test sample of different concentrations (100 – 500 µg/ml). The mixture was incubated at 37 ^{o}C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank (Alagbe & Malomo, 2024).

Nitric oxide (NO) scavenging activity

The NO scavenging activity of sample was determined by adding 400 µL of 100 mM sodium nitroprusside, 100 µL of PBS (pH - 7.4) and 100 µL of different concentration of plant extract (Alagbe & Malomo, 2024). This reaction mixture was kept for incubation at 25 °C for 150 min. To 0.5 mL of above solution, 0.5 mL of Griess reagent was added (0.1 mL of sulfanilic acid and 200 µL naphthylethylenediamine dichloride (0.1%) w/v)). This was kept on incubation at room temperature for 30 min, and finally absorbance is observed at 540 nm.

Anti-lipoxygenase and anti-cyclogenase activity

The anti-lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme (Alagbe & Malomo, 2024). Test samples were dissolved in 0.25 ml of 2 M borate buffer pH 9.0 and added 0.25 ml of lipoxidase enzyme solution (20,000U/ml) and incubated for 5 min at 25 ^{0}C. After which, 1.0 ml of linoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234 nm. Indomethacin was used as reference standard. The percentage inhibition was calculated from the following equation,

$$\%\ \text{inhibition} = [\{\text{Abs control} - \text{Abs sample}\}/\text{Abs control}] \times 100$$

The anti-cyclooxygenase activity was measured using the assay mixture containing TrisHCl buffer, glutathione, hemoglobin & enzyme. The assay started by the addition of arachidonic acid and terminated after 20 min incubation at 37ºC by addition of 0.2 ml of 10% TCA in 1N HCl, mixed and 0.2 ml of TBA was added and contents heated in a boiling water bath for 20 min, cooled and centrifuged at 1000 rpm for 3 min. The supernatant was measured at 632 nm for COX activity.

In vivo glycaemic index and Measurement of blood glucose response

This procedure was carried out according to the methods of Oluwajuyitan et al. (2020). Briefly, thirty (30) Wistar Albino rats of body weights between 140-150 g were divided into 5 groups (5 rats/group) including the control group, and the rats were housed individually in metabolic cages in a climate-controlled environment with free access to feed and water. The rats were allowed to acclimatize to the new environment for 7 days. After the adaptation period, the animals were reweighed and fasted for 12 h (overnight fasting). The blood glucose of the animals was taken at zero time from the tail vein before feeding them with 2.0 g of the extruded snacks food samples and glucose (control), which were consumed within 25 min. After the consumption, the serum glucose levels of the animals were measured using an automatic glucose analyzer ('Accu-chek Active' Diabetes monitoring kit; Roche Diagnostic, Indianapolis, USA) at 0, 15, 30, 60, 90 and 120 min. The glycaemic response was determined as the Incremental Area under the Blood Glucose Curve (IAUC) measured geometrically from the blood glucose concentration-time graph ignoring area beneath the fasting level (Wolever et al., 1991).

Blood glucose curves were constructed from blood glucose values of animals at time 0, after 15, 30, 60, 90 and 120 min intervals after consumption of the glucose (control) and experimental food samples of each group. The Incremental Area Under the Curve (IAUC) was calculated for reference food (glucose) by the trapezoidal rule in every rats in each group separately as the sum of the surface of trapezoids between the blood glucose curve and horizontal baseline going parallel to x-axis from the beginning of blood glucose curve at time 0 to the point at time 120 min to reflect the total rise in blood glucose concentration after eating the reference food (glucose). The Incremental Area under the Curve (IAUC) from the animals fed with the formulated food samples was similarly obtained. The glycemic Index (GI) for each diet was calculated by ratio of Incremental Area Under 2 h of blood glucose response or Curve (IAUC) for each diet to the IAUC for glucose solution standard according to the method of Wolever et al. (1991) using the following equation:

$$\text{GI} = \frac{\text{Incremental area under 2h blood glucose curve for food samples (2 .0g)}}{\text{Incremental area under 2h blood glucose curve for glucose (2.0g)}} \text{ x } 100$$

Ethical clearance

The study protocol was approved and ethic clearance given by the Ethical Committee for Laboratory Animals of School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Nigeria (FUTA/SAAT/2023/043).

Evaluation of sensory attributes of bread samples

The formulated food samples and the control sample were coded and presented to 30 untrained panelists. The panel members were assigned individually to well illuminate laboratory booths and the cookies were served, coded with random three digits. Water at room temperature was provided for mouth rinsing in between successive evaluation. Sample attributes (appearance, texture, taste, aroma, mouth feel etc.) were rated on a scoring scale of 1 to 9, where 1 = dislike extremely and 9 = like extremely. Panelists made their responses on score sheets which were designed in line with the test procedures (Alagbe et al., 2024).

Statistical analysis

All determinations were carried out in triplicates and errors were recorded as standard deviation from the mean. Data were subjected to analysis of variance using SPSS (version 21, USA), while means were separated using New Duncan Multiple Range Test (NDMRT) and significance was accepted at 95% confidence level.

RESULTS AND DISCUSSION

Proximate Composition of Bread

The proximate composition of bread produced from wheat and fermented plantain flour is presented in Table 2. The moisture content ranges from 6.31% to 8.58% across the samples. The control (ZOM4, 100% wheat flour) has the highest moisture content at 8.58%, while ZOM3 (70% wheat + 30% fermented plantain) has the lowest at 6.31%. This trend suggests that increasing the proportion of fermented plantain flour generally decreases the moisture content of the bread. A study by Adeola and Ohizua (2018) found similar results, where the incorporation of plantain flour in composite bread led to a decrease in moisture content. This could be attributed to the higher water absorption capacity of wheat flour compared to plantain flour.

The crude fat content varies from 7.26% to 13.38%. Interestingly, ZOM3 (70% wheat + 30% fermented plantain) has the highest fat content at 13.38%, while the control (ZOM4) has the lowest at 7.26%. This indicates that the addition of fermented plantain flour increases the fat content of the bread. Adebayo-Oyetoro et al. (2016) reported similar findings, where the fat content of composite bread increased with higher plantain flour substitution. This could be due to the natural lipid content in plantain flour.

Protein content ranges from 13.75% to 23.16%. ZOM3 (70% wheat + 30% fermented plantain) has the highest protein content at 23.16%, while the control (ZOM4) has the lowest at 13.75%. This suggests that the fermented plantain flour contributes significantly to the protein content of the bread. A recent study by Oladunmoye et al. (2022) also observed an increase in protein content with the addition of plantain flour in composite bread. This could be attributed to the protein content of plantain and possible protein enrichment during the fermentation process.

Ash content varies from 1.43% to 3.14%. ZOM2 (80% wheat + 20% fermented plantain) has the highest ash content at 3.14%, while the control (ZOM4) has the lowest at 1.43%. This indicates that the addition of fermented plantain flour increases the mineral content of the bread. Ayo-Omogie and Ogunsakin (2013) reported similar findings, where the ash content of composite bread increased with plantain flour substitution, suggesting higher mineral content in plantain flour compared to wheat flour.

Fiber content ranges from 1.89% to 3.51%. ZOM3 (70% wheat + 30% fermented plantain) has the highest fiber content at 3.51%, while the control (ZOM4) has the lowest at 1.89%. This demonstrates that fermented plantain flour significantly contributes to the fiber content of the bread. A study by Arun et al. (2020) also found that incorporating plantain flour in bread increased its fiber content, which can have potential health benefits such as improved digestion and gut health.

Carbohydrate content varies from 50.96% to 67.09%. The control (ZOM4) has the highest carbohydrate content at 67.09%, while ZOM3 (70% wheat + 30% fermented plantain) has the lowest at 50.96%. This suggests that increasing the proportion of fermented plantain flour decreases the overall carbohydrate content of the bread. Anyasi et al. (2018) reported similar findings, where the carbohydrate content of composite bread decreased with increased plantain flour substitution. This could be due to the higher protein, fat, and fiber content contributed by the plantain flour.

The incorporation of fermented plantain flour in wheat bread significantly affects its nutritional composition. The composite bread samples (ZOM1, ZOM2, and ZOM3) generally show higher protein, fat, ash, and fiber content compared to the control (ZOM4), while having lower moisture and carbohydrate content. These changes in nutritional profile could potentially contribute to the antioxidant and antidiabetic properties of the composite bread. The increased fiber and protein content, along with the reduced carbohydrate content, may be particularly relevant to the antidiabetic potential of the composite bread. Recent studies have shown that high-fiber, high-protein diets can help in managing blood glucose levels and improving insulin sensitivity (Hu et al., 2022). The fermentation process of the plantain flour may enhance the bioavailability of nutrients and potentially increase the antioxidant capacity of the bread. A study by Oboh et al. (2019) found that fermentation of plantain flour increased its antioxidant properties and enzyme inhibition potential, which could be beneficial for managing diabetes.

Table 2: Proximate Composition of Bread Samples (mg/100 g)

Sample	Moisture	Crude Fat	Crude Protein	Ash	Crude Fiber	CHO
ZOM1	7.75±0.11	9.07±0.03	17.09±0.10	2.50±0.06	2.11±0.21	61.48±12
ZOM2	6.86±0.14	9.12±0.12	20.57±0.01	3.14±0.14	2.40±0.05	57.91±0.08
ZOM3	6.31±0.31	13.38±0.80	23.16±0.05	2.68±0.01	3.51±0.05	50.96±0.14
ZOM4	8.58±0.23	7.26±0.12	13.75±0.01	1.43±0.01	1.89±0.05	67.09±0.14

Key: ZOM 1 = 90% wheat flour + 10% fermented plantain flour; ZOM 2 = 80% wheat flour + 20% fermented plantain flour; ZOM 3 = 70% wheat flour + 30% fermented plantain flour; ZOM 4 = 100% wheat flour (control).

Amino Acid Profile of Bread

The amino acid profile of bread produced from wheat and fermented plantain flour is presented in Table 3. Leucine increases with higher plantain flour content (7.10 mg/100g in ZOM4 to 8.31 mg/100g in ZOM3). Leucine is crucial for protein synthesis and glucose homeostasis. A study by Zhang et al. (2019) highlighted leucine's role in improving insulin sensitivity and glucose metabolism. Lysine is higher in composite breads (2.89-3.61 mg/100g) compared to the control (2.20 mg/100g). Lysine is often limiting in cereal-based diets. Its increase in composite breads is significant for nutritional quality. Soares et al. (2019) reported that lysine supplementation could improve glucose homeostasis in diabetic models. Methionine increases with plantain flour addition (1.37 mg/100g in ZOM4 to 2.53 mg/100g in ZOM3). Methionine plays a role in antioxidant defense. A recent study by Martínez et al. (2022) suggested that methionine restriction might have beneficial effects on metabolic health and longevity. Glutamic Acid decreases with increasing plantain flour (25.42 mg/100g in ZOM4 to 16.82 mg/100g in ZOM3). While glutamic acid decreases, other NEAAs increase, balancing the overall NEAA content. Arginine significantly higher in composite breads (2.66-5.83 mg/100g) compared to control (2.14 mg/100g). Arginine has been associated with improved insulin sensitivity and endothelial function. A study by Monti et al. (2021) highlighted arginine's potential in managing diabetes complications. Aspartic Acid increases with plantain flour addition (4.65 mg/100g in ZOM4 to 7.93 mg/100g in ZOM3). Aspartic acid is involved in the urea cycle and gluconeogenesis.

Total Essential Amino Acids (TEAA) slightly higher in composite breads (35.74-36.8 mg/100g) compared to control (35.85 mg/100g). This suggests that the composite breads maintain or slightly improve the essential amino acid profile. Total Non-Essential Amino Acids (TNEAA) increases with plantain flour addition (47.59 mg/100g in ZOM4 to 50.89 mg/100g in ZOM3). Total Amino Acids (TAA) is highest in ZOM3 (87.48 mg/100g) and lowest in ZOM4 (83.44 mg/100g), indicating an overall improvement in amino acid content with plantain flour addition.

These changes in amino acid profile could contribute to the nutritional, antioxidant, and antidiabetic potentials of the composite bread. The increased content of essential amino acids, particularly lysine, in the composite breads enhances their nutritional value. Lysine is often limiting in cereal-based diets, so its increase is significant. A study by Adeola et al. (2017) on plantain-wheat composite flour similarly found improved essential amino acid profiles. The increase in sulfur-containing amino acids like methionine and cysteine in some composite bread samples could contribute to enhanced antioxidant capacity. These amino acids are precursors to glutathione, a potent antioxidant. Research by Tamanna and Mahmood (2015) highlighted the antioxidant properties of various amino acids and their potential health benefits. Several amino acids increased in the composite breads have been associated with improved glucose metabolism and insulin sensitivity. For instance: Leucine, shown to stimulate insulin secretion and improve glucose uptake (Zhang et al., 2019). Arginine, associated with improved insulin sensitivity and endothelial function (Monti et al., 2021). Lysine, may improve glucose homeostasis in diabetic models (Soares et al., 2019). A recent review by Chartrand et al. (2017) discussed the potential of various amino acids in managing diabetes and its complications. The fermentation process of plantain flour might also contribute to these potential benefits. Fermentation can enhance the bioavailability of nutrients and produce bioactive peptides. A study by Obi et al. (2023) on fermented plantain flour found that fermentation increased the antioxidant activity and altered the amino acid profile favorably.

Table 3: Amino Acid Profile of Bread Samples (mg/100 g)

Amino Acids	ZOM1	ZOM2	ZOM3	ZOM4	AVERAGE	STD	LSD(P<0.05)
Leucine	7.35	7.92	8.31	7.10	7.67	0.43	1.00
Isoleucine	5.22	5.63	6.79	4.81	5.61	0.12	1.00
Lysine	2.89	3.61	3.49	2.20	3.05	0.55	0.13
Methionine	1.96	2.40	2.53	1.37	2.07	0.14	1.00
Valine	4.65	4.86	4.47	4.10	4.52	0.10	1.00
Cystine	0.64	0.38	0.43	1.72	0.80	0.68	1.00
Phenylamine	7.53	7.18	6.29	8.60	7.40	0.34	1.00
Tyrosine	5.50	4.82	4.28	5.95	5.14	0.23	0.08
Alanine	4.34	5.11	6.64	4.0	5.02	0.15	1.00
Arginine	2.66	4.17	5.83	2.14	3.70	0.32	1.00
Aspartic Acid	5.68	6.27	7.93	4.65	6.13	0.51	1.00
Glutamic Acid	23.81	21.17	16.82	25.42	21.81	1.45	1.00
Histidine	2.97	4.96	6.13	3.31	4.34	0.87	1.00
Glycine	3.75	4.10	4.98	3.0	3.96	0.44	1.00
Serine	4.11	3.34	2.56	5.07	3.77	0.37	1.00
TEAA	35.74	36.8	36.59	35.85	36.26	2.59	1.00
TNEAA	47.32	49.12	50.89	47.59	48.73	4.11	1.00
TAA	83.06	85.92	87.48	83.44	84.99	6.70	1.00

TEAA = Total Essential Amino Acid, **TNEAA** = Total Non-Essential Amino Acid, **TAA** = Total Amino Acid, **LDS** = Least Significant Difference. **Key:** ZOM 1 = 90% wheat flour + 10% fermented plantain flour; ZOM 2 = 80% wheat flour + 20% fermented plantain flour; ZOM 3 = 70% wheat flour + 30% fermented plantain flour; ZOM 4 = 100% wheat flour (control).

Anti-Inflammatory Properties of Bread

Protein denaturation management activities of bread

Protein denaturation is a process in which biological proteins lost their tertiary structure and secondary structure (necessary for biological function) by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. The oxidative stress has been known to be inducing inflammation through protein denaturation (Leelaprakash and Dass mohan, 2011). Hence, inhibition of protein denaturation may play an important role in the anti-inflammatory activity of the food products (Kumar et al., 2013). As part of the investigation on the mechanism of the anti-inflammation activity of foods, the ability of the bread to inhibit protein denaturation was thus studied and presented in Fig 1 with the ranging values from 26.1 to 77.4 µg/ml. Sample ZOM 3 had the corresponding similarity of inhibition capacity (~26 µg/ml) against protein denaturation when compared to diclofenac, a common and known non-steroidal anti-inflanmatory drug that has inhibition power of ~21.6 µg/ml. Notably, the inhibition capacity of ZOM 3 (~26 µg/ml) is higher and better than the capacity (73.3 and 77.4 µg/ml) of the sample ZOM 1 (90% wheat flour + 10% fermented plantain flour) and ZOM 4 (100% wheat) control sample. This is because a high inhibition or better anti-inflammatory capacity is directly related to a low IC50. That is the lower the IC50 of any bioactive compound, the better and higher would be its performing actions.

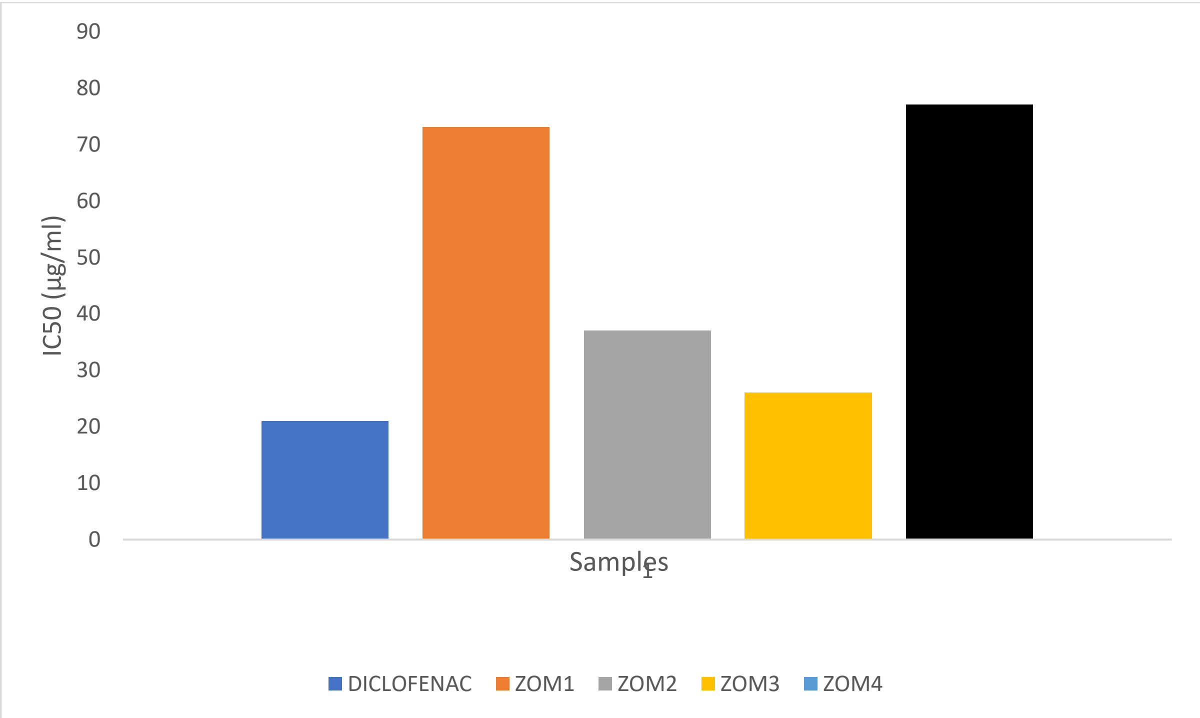

Figure 1: Inhibition of protein denaturation activity of different Bread samples at 50% level of inhibition concentration (IC_{50})

Key: ZOM 1 = 90% wheat flour + 10% fermented plantain flour; ZOM 2 = 80% wheat flour + 20%

fermented plantain flour; ZOM 3 = 70% wheat flour + 30% fermented plantain flour; ZOM 4 = 100% wheat flour (control).

Proteinase (trypsin) inhibitor management activities of bread

A trypsin inhibitor is a protein and a type of serine protease inhibitor (serpin) that reduced the biological activity of trypsin by controlling the activation and catalytic reactions of proteins. Trypsin is an enzyme involved in the breakdown of many different proteins, primarily as part of digestion in humans and other animals such as monogastrics and young ruminants (Silverman et al., 2001; Cohen et al., 2019). The ability of the bread to inhibit trypsin inhibitor was is presented in Fig 2 with the IC50 values ranging from 25.2 to 76.1 µg/ml. As earlier observed from the results presented in Fig 4.1, the same trend was also observed in the results presented in Fig 4.2, whereby sample ZOM 3 is having the highest inhibition (IC50; ~25.2 µg/ml) value. The enhanced activity observed for sample ZOM 3 might have been as contributions from its high protein, improved amino acid profiles and better antioxidant properties when compared to other samples.

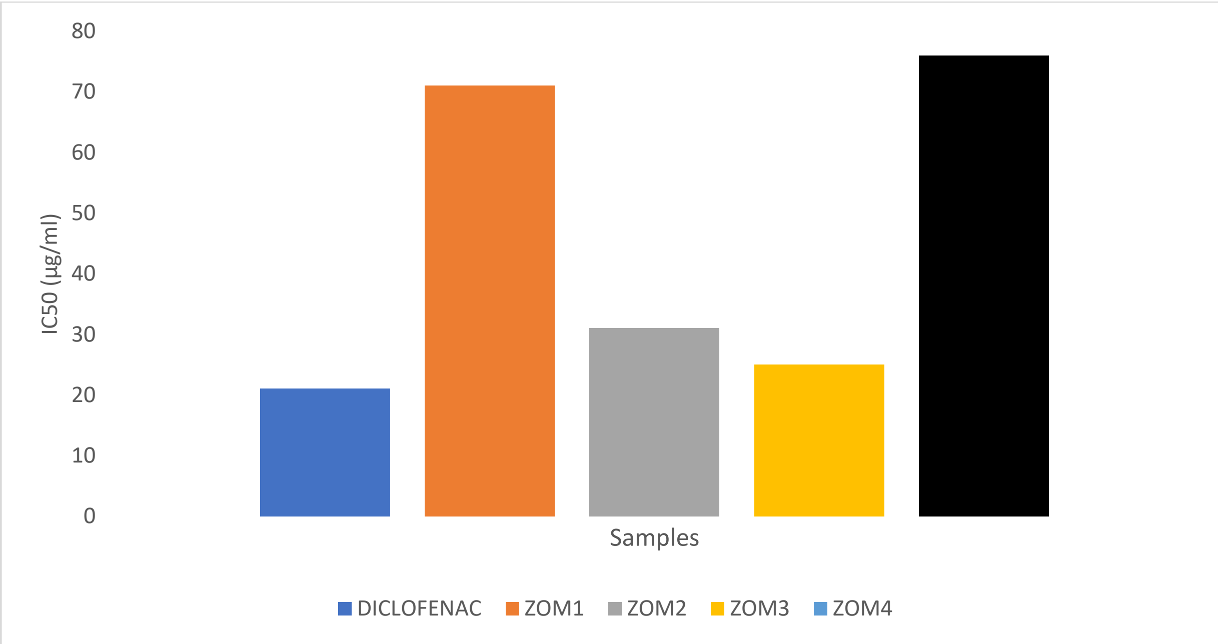

Figure 2: Inhibition of proteinase (trypsin) activity of different Bread samples at 50% level of inhibition concentration (IC_{50})

Key: ZOM 1 = 90% wheat flour + 10% fermented plantain flour; ZOM 2 = 80% wheat flour + 20% fermented plantain flour; ZOM 3 = 70% wheat flour + 30% fermented plantain flour; ZOM 4 = 100% wheat flour (control).

Membrane stabilization activity of bread

Membrane stabilization is a tendency to restore pathologically altered membrane permeability, permitting normal ionic transport through membranes of the heart muscle cells and protecting the heart from arrhythmia. Membrane stabilization is a method through which local anesthetics worked by blocking the propagation of action potentials across nerve cells, thereby producing a nerve block. Hence, the membrane stabilization activities of the breads are presented in Fig 3. The result revealed that the values ranged from 30.0-79.7 µg/ml with significant ($p<0.05$) highest and least values reported for ZOM 3 and ZOM 4, respectively. However, sample ZOM 1 and ZOM 4 (control sample) had the similar but least value (IC50; 75.2 and 79.7 µg/ml) when compared to the formulated samples and diclofenac.

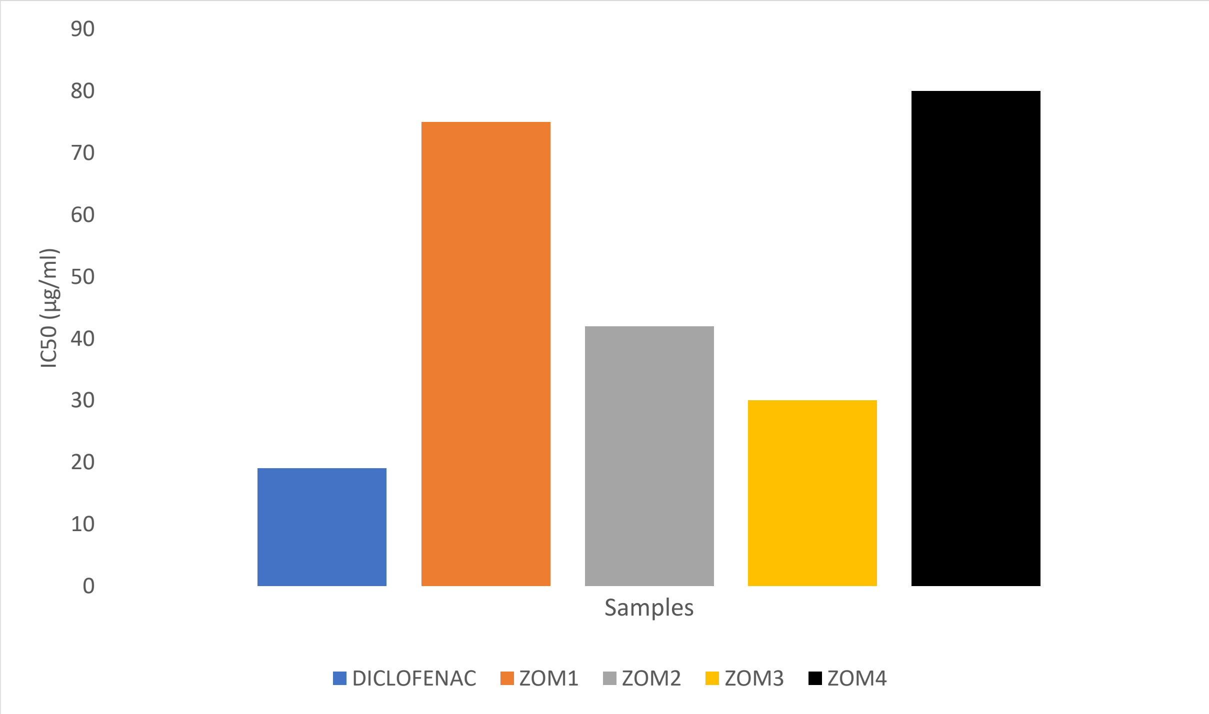

Figure 3: Membrane stabilization activity of different Bread samples at 50% level of inhibition concentration (IC_{50})

Key: ZOM 1 = 90% wheat flour + 10% fermented plantain flour; ZOM 2 = 80% wheat flour + 20% fermented plantain flour; ZOM 3 = 70% wheat flour + 30% fermented plantain flour; ZOM 4 = 100% wheat flour (control).

Nitric oxide (NO) scavenging management activities of bread

The activation of macrophages, neutrophil granulocytes and many other immune cells by lipopolysaccharide (LPS) during bacterial infection or by cytokines being IL-1, TNF-α or IFN-γ, induced overexpression of NOS that enhanced overproduction of nitric oxide (NO) (Coleman, 2001). NO is a signalling agent that possessed a crucial role in the pathogenesis of inflammation (Boora et al., 2014), thus its quenching would prove the important anti-inflammatory of such food products. Study had shown the NO to boost the expression of cyclooxygenase (COX-2) thereby leading to increased prostaglandin formation that is implicated in pathogenesis of different inflammations (Marrassini et al., 2011; Alam et al., 2015). The NO scavenging ability of the bread is presented in Fig 4 and significantly ($p<0.05$) ranged from 19.3 to 79.5 µg/ml % with significant difference. Interestingly, the activity observed in sample ZOM 3 showed higher and better scavenging ability (IC50; 19.3 µg/ml) than diclofenac (IC50; 22.31 µg/ml) as well as (30.1 µg/ml) of ZOM 2.

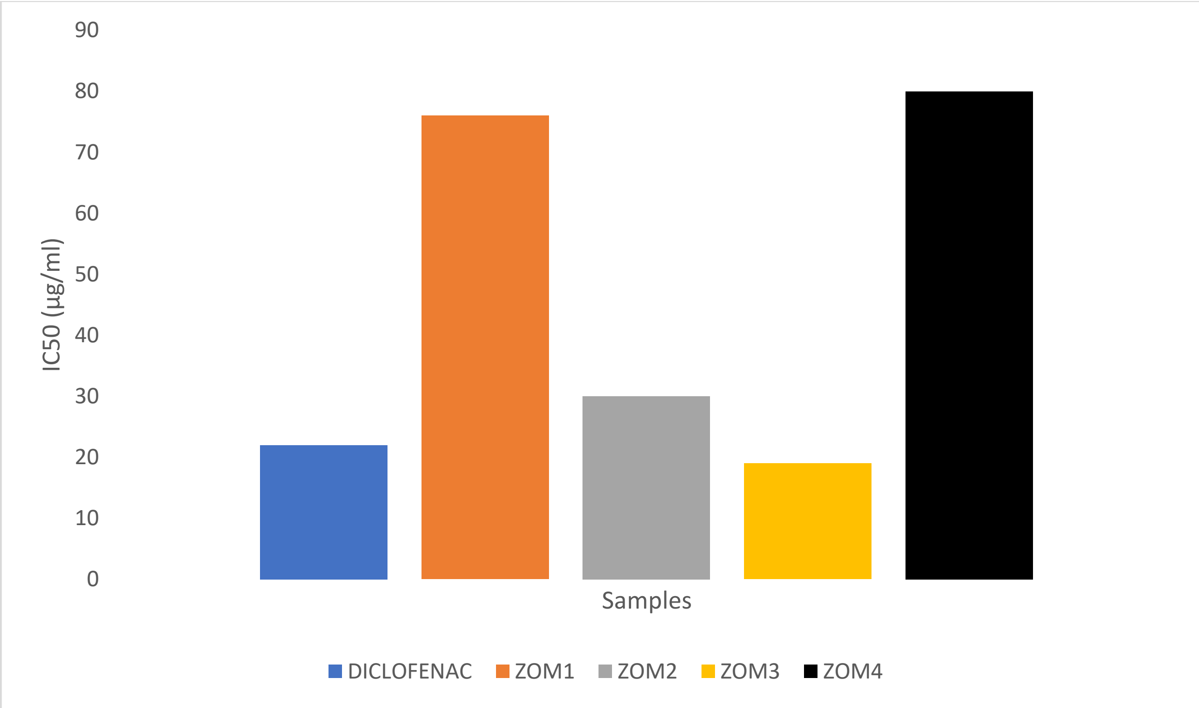

Figure 4: Nitric oxide (NO) scavenging activity of different Bread samples at 50% level of inhibition concentration (IC_{50})

Key: ZOM 1 = 90% wheat flour + 10% fermented plantain flour; ZOM 2 = 80% wheat flour + 20% fermented plantain flour; ZOM 3 = 70% wheat flour + 30% fermented plantain flour; ZOM 4 = 100% wheat flour (control).

Anti-lipoxygenase and anti-cyclooxygenase and activities of bread

Lipoxygenases are the key enzymes in the biosynthesis of leukotrienes, an agent that played an important role in several inflammatory diseases, such as arthritis, asthma, cancer, and allergic diseases (Rackova et al., 2007). The mechanism of anti-inflammation involved a series of events of arachidonic acid metabolism, which involved cleaving of the acid from the membrane phospholipids upon appropriate stimulation of neutrophils, and converting it to leukotrienes and prostaglandins through lipoxygenase and cyclooxygenase pathways, respectively (Akinwumi & Oyedapo, 2015). Therefore, the antilipoxygenase and anticycloxygenase activites of the bread significantly ($p<0.05$) ranges are obtained as IC50; 16.01-73.60 and 19.20-73.60 µg/ml, respectively, as shown in Fig 5 and 6 with samples ZOM 3 and ZOM 4 having the highest and least activity, respectively. Study had shown the catalyzed deoxygenation of polyunsaturated fatty acids by lipoxygenase to produce cis, trans-conjugated diene hydroperoxides, such as leukotrienes, which were essential mediators in a variety of inflammatory events (Khasawneh, 2011). Previous study also revealed the ability of plant protein-based foods to block or interfere with the cascade process of arachidonic acid metabolism by inhibiting lipoxygenase activity, thereby serving as scavengers of various reactive free radicals, which were produced during arachidonic acid metabolism (Trouillas, 2003).

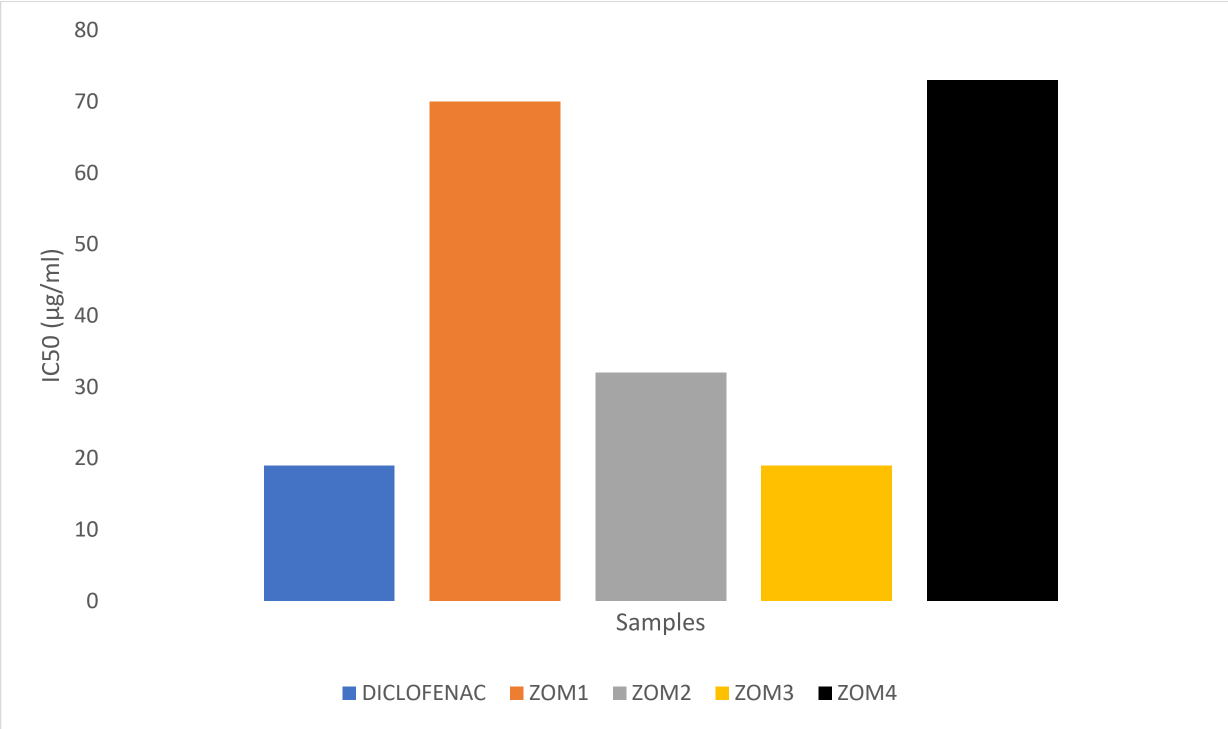

Figure 5: Anti-lipoxygenase activity of different Bread samples at 50% level of inhibition concentration (IC_{50})

Key: ZOM 1 = 90% wheat flour + 10% fermented plantain flour; ZOM 2 = 80% wheat flour + 20% fermented plantain flour; ZOM 3 = 70% wheat flour + 30% fermented plantain flour; ZOM 4 = 100% wheat flour (control).

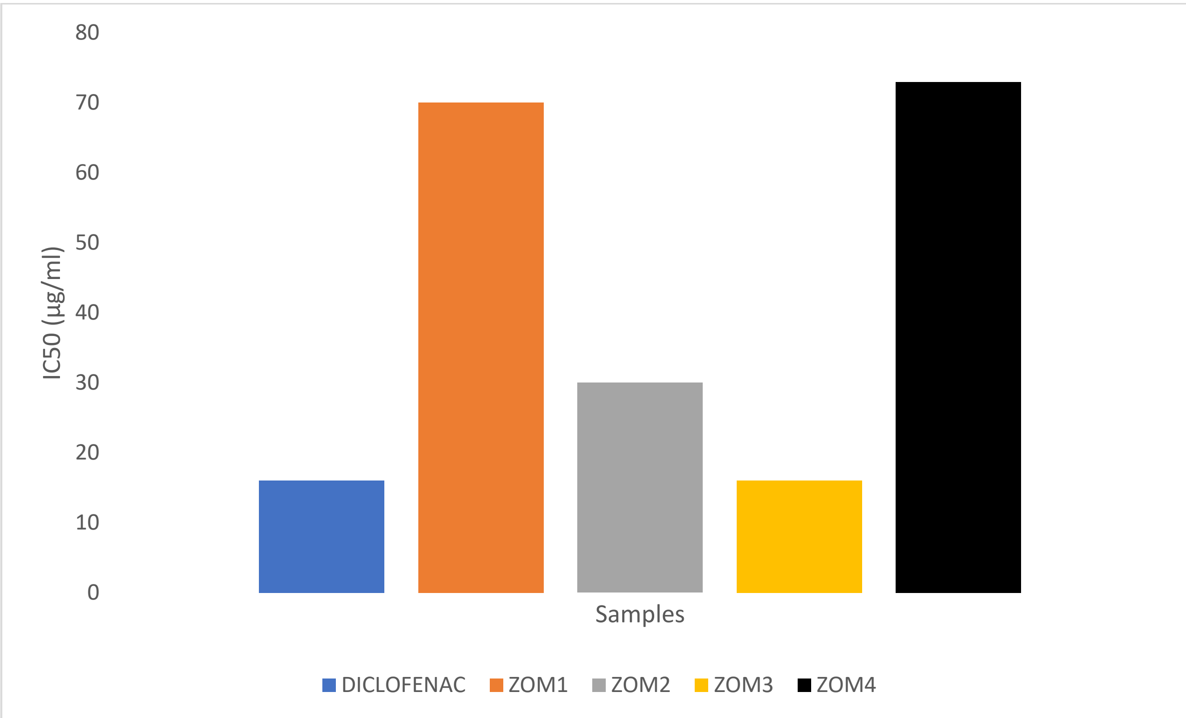

Figure 6: Anti-cyclooxygenase activity of different Bread samples at 50% level of inhibition concentration (IC_{50})

Key: ZOM 1 = 90% wheat flour + 10% fermented plantain flour; ZOM 2 = 80% wheat flour + 20% fermented plantain flour; ZOM 3 = 70% wheat flour + 30% fermented plantain flour; ZOM 4 = 100% wheat flour (control).

Glycemic index of bread in Wistar rats

The glycemic index (GI) is a value of the effect of consumption of certain food on 2 h postprandial blood glucose response with respect to an equivalent carbohydrate portion of referenced sample (Lestari et al., 2020). GI provided a ranking on a scale from 0 to 100, where foods with GI of ≥70 are classified as high GI, 56-69 as moderate GI, and ≤55 as low GI (Huang and Miskelly, 2017).

The glycemic index (GI) of different bread samples containing varying proportions of wheat and fermented plantain flour is presented in figure 7. Sample ZOM 4 (100% Wheat Flour - Control) mimics glucose closely, peaking at 135 mg/dL at 15 minutes and declining steadily to 95 mg/dL at 120 minutes. This confirms the high GI of wheat-based bread. Sample ZOM 1 (90% Wheat, 10% Plantain Flour) shows a lower glycemic response than ZOM 4, peaking at 130 mg/dL at 15 minutes and reducing to 90 mg/dL at 120 minutes. The slight incorporation of plantain flour reduces the GI compared to the control. Sample ZOM 2 (80% Wheat, 20% Plantain Flour) exhibits a further reduced peak (126 mg/dL at 15 minutes) and a gradual decline to 70 mg/dL by 120 minutes, indicating a more pronounced effect of the plantain flour in lowering the glycemic response. Sample ZOM 3 (70% Wheat, 30% Plantain Flour) shows the lowest GI, peaking at 91 mg/dL and reducing to 55 mg/dL at 120 minutes, reflecting the significant impact of higher plantain flour content in reducing GI.

The GI value of certain food depended on the amount and composition of carbohydrates as well as the fat and protein content of the food, acidity, particle size, cooking methods (Eleazu, 2016). It was observed that GI of each food consumed by each rat varied at time 0 to 120 mins. After the consumption of food, glucose concentrations changed to a greater degree in capillary blood samples than in venous blood samples. Therefore, capillary blood may be a more relevant indicator of the physiological consequences of high GI foods (Foster-Powell et al., 2002).

This result demonstrates that incorporating fermented plantain flour into wheat bread lowers its glycemic response. This trend suggests that increasing the percentage of fermented plantain flour in bread formulation reduces the glycemic index, which could be beneficial for individuals managing blood sugar levels, such as those with diabetes. Recent studies support these findings, indicating that incorporating resistant starches or fiber-rich flours (such as plantain) into wheat-based products can lower their glycemic index (Famakin et al., 2016). Fermented plantain flour, in particular, adds resistant starch and dietary fibers that slow down carbohydrate absorption. In a study conducted in 2022 on plantain flour-enriched bread, it was reported that resistant starch improved postprandial glycemic response, confirming the benefits of composite flours in glycemic control. This data highlights the potential of composite flours like fermented plantain for functional foods aimed at enhancing nutritional value and managing chronic conditions like diabetes.

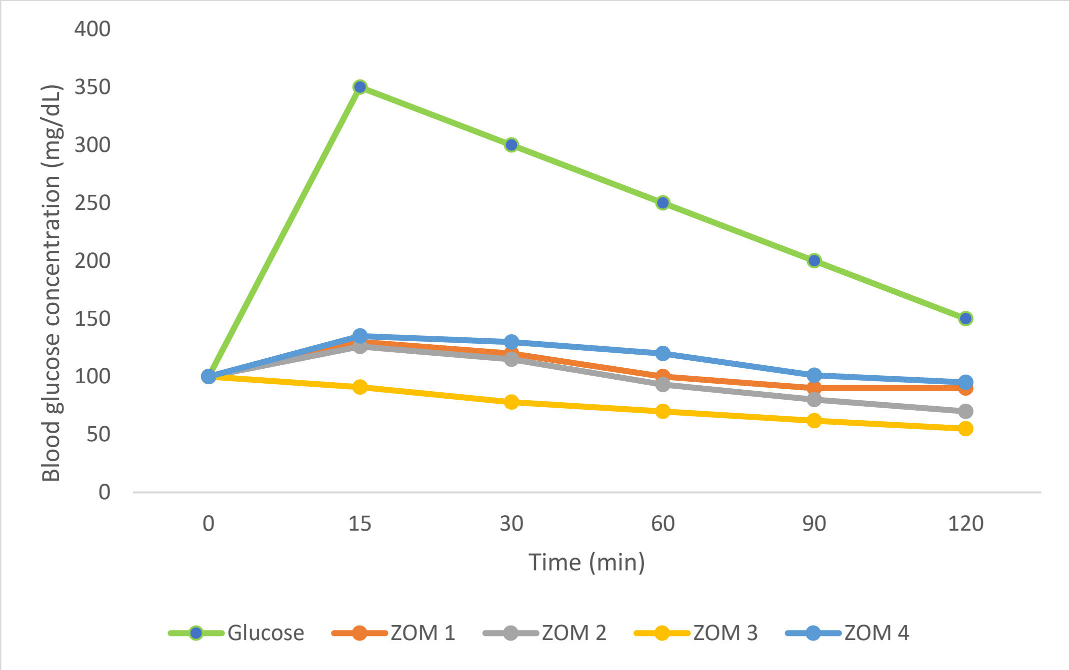

Figure 7: In vivo glycemic index of different Bread samples

Key: ZOM 1 = 90% wheat flour + 10% fermented plantain flour; ZOM 2 = 80% wheat flour + 20% fermented plantain flour; ZOM 3 = 70% wheat flour + 30% fermented plantain flour; ZOM 4 = 100% wheat flour (control).

Glycemic load of bread in Wistar rats

The glycemic load (GL) is known as the glycemic response to a consumed food which depended on the total amount of carbohydrates consumed (Lestari et al., 2020). The GL calculated for how much of carbohydrate is in the food and how each gram of carbohydrate in the food increased blood glucose levels. However, the GL is categorized as low (≤20), moderate (20-25) and high (≥25) by previous findings (Barclay et al., 2008; Campbell, 2011).

The glycemic load (GL) of bread from composite flour blends and control sample is presented in Fig 8, which showed lowest GL (25.1) for sample ZOM 3 when compared to others (37.14-51.26). GL are previously grouped into high (>20), medium (10-19) and low (<10) categories (Kindo, 2011; Jariyah et al., 2018). The data indicate that increasing the proportion of fermented plantain flour in the bread results in a reduced glycemic load. ZOM 3, with the highest percentage of fermented plantain (30%), has the lowest glycemic load (25.1), while the control sample (100% wheat flour) has the highest GL (GL = 51.26). This trend aligns with the glycemic index findings, where the inclusion of plantain flour significantly reduces the overall glycemic impact of the bread.

Glycemic load (GL) accounts for both the quality (glycemic index) and quantity of carbohydrates in a food, offering a more comprehensive view of its impact on blood sugar. Studies show that substituting wheat flour with fibers or resistant starch-rich alternatives, like plantain flour, can lower both GI and GL. A 2021 study found that composite flours with resistant starch significantly reduced the glycemic load of wheat products, making them suitable for managing blood sugar levels in individuals with diabetes. Incorporating plantain flour, especially in fermented form, introduces more resistant starch, which slows carbohydrate digestion and reduces the glucose load. This suggests that breads formulated with fermented plantain flour may be beneficial for individuals looking to manage their dietary glycemic load.

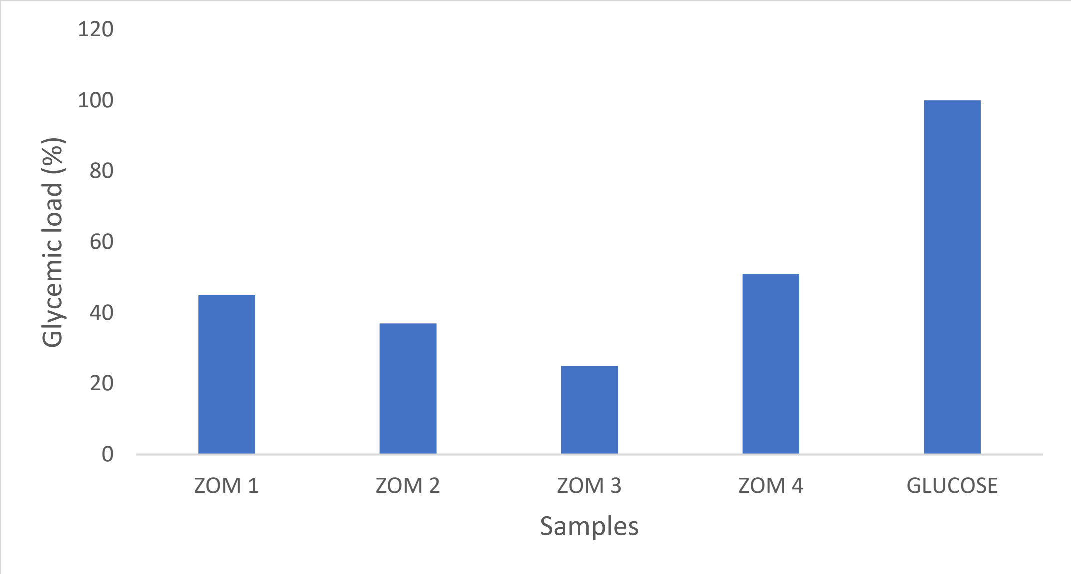

Figure 8: In vivo glycemic load for different Bread samples

Key: ZOM 1 = 90% wheat flour + 10% fermented plantain flour; ZOM 2 = 80% wheat flour + 20% fermented plantain flour; ZOM 3 = 70% wheat flour + 30% fermented plantain flour; ZOM 4 = 100% wheat flour (control).

Sensory attributes of bread

Table 4 showed the sensory qualities of bread produced from wheat and fermented plantain flour. The control sample (ZOM 4, 100% wheat flour) received the highest overall acceptability score (9.00±1.32). However, it's noteworthy that all composite flour samples also received high acceptability scores, ranging from 8.12 to 8.67. This suggests that the incorporation of fermented plantain flour up to 30% can produce bread that is still highly acceptable to consumers. This finding aligns with recent research by Adeola and Ohizua (2018), who reported that composite breads made with up to 20% plantain flour were well-accepted by consumers.

The control sample scored highest in these categories, but the composite flour samples also performed well. Interestingly, ZOM 1 (10% fermented plantain flour) scored very close to the control in these attributes. This suggests that at lower substitution levels, the sensory properties are minimally affected. While the control sample scored highest, ZOM 1 and ZOM 2 (10% and 20% fermented plantain flour, respectively) also received high scores for flavour and taste. This indicates that the fermented plantain flour contributes positively to the bread's flavour profile at these substitution levels.

All samples scored well in mouthfeel, with the composite flour samples not far behind the control. This suggests that the addition of fermented plantain flour doesn't significantly compromise the texture of the bread. There's a general trend of slightly decreasing scores as the percentage of fermented plantain flour increases from 10% to 30%. However, even at 30% substitution (ZOM 3), the scores remain reasonably high across all attributes. These findings are consistent with recent studies on composite flours in bread making. For instance, Adebayo-Oyetoro et al. (2020) reported that bread made with up to 20% plantain flour substitution was comparable to wheat bread in sensory attributes.

The high acceptability of this composite flour breads could be attributed to the fermentation process of the plantain flour. Fermentation has been shown to improve the flavour and aroma of composite flour products. This is supported by work from Alcantara et al. (2021), who found that fermentation of non-wheat flours can enhance the sensory properties of composite bread. This result demonstrates the potential of using fermented plantain flour as a partial substitute for wheat flour in bread production. This is particularly relevant in the context of food security, gluten reduction, and utilization of local crops. The high acceptability scores for the composite flour breads suggest that this could be a viable option for diversifying bread products while maintaining consumer acceptance.

Table 4: Sensory Evaluation of Bread Samples

Sample	Colour	Flavour	Taste	Appearance	Aroma	Mouth feel	Overall Acceptability
ZOM 1	8.32±1.44	8.53±1.31	8.50±1.42	8.61±1.27	8.62±1.12	8.62±1.41	8.21±1.33
ZOM 2	8.10±1.20	7.60±1.21	8.00±1.30	8.11±1.30	8.50±1.10	8.31±1.25	8.67±1.27
ZOM 3	8.33±1.51	7.31±1.37	7.83±1.62	7.88±1.24	8.03±1.21	8.10±1.20	8.12±1.50
ZOM 4	9.00±1.82	9.00±1.41	9.00±1.38	9.00±1.40	9.00±1.12	9.00±1.33	9.00±1.32

Key: ZOM 1 = 90% wheat flour + 10% fermented plantain flour; ZOM 2 = 80% wheat flour + 20% fermented plantain flour; ZOM 3 = 70% wheat flour + 30% fermented plantain flour; ZOM 4 = 100% wheat flour (control).

CONCLUSION

This study demonstrates that the incorporation of fermented plantain flour into wheat bread significantly impacts its nutritional, functional, and therapeutic properties while maintaining acceptable sensory characteristics. The composite bread with 30% fermented plantain flour (ZOM3) exhibited the most promising results across multiple parameters. This formulation showed enhanced protein content (23.16%), improved fiber content (3.51%), and a more balanced amino acid profile compared to the control, with notably higher levels of essential amino acids like leucine (8.31 mg/100g) and lysine (3.49 mg/100g).

The anti-inflammatory properties of the composite breads were particularly noteworthy, with ZOM3 demonstrating superior performance in protein denaturation inhibition (IC50 ~26 µg/ml), membrane stabilization (IC50 30.0 µg/ml), and nitric oxide scavenging activity (IC50 19.3 µg/ml), comparable to or better than the reference drug diclofenac. Additionally, ZOM3 showed remarkable anti-lipoxygenase (IC50 16.01 µg/ml) and anti-cyclooxygenase (IC50 19.20 µg/ml) activities, suggesting potential therapeutic applications in managing inflammatory conditions.

The glycemic response studies revealed that increasing the proportion of fermented plantain flour effectively lowered both the glycemic index and glycemic load of the bread. ZOM3 exhibited the lowest glycemic load (25.1) compared to the control (51.26), indicating its potential suitability for individuals managing blood glucose levels. Importantly, sensory evaluation demonstrated that while the control sample received the highest overall acceptability score (9.00±1.32), all composite bread formulations maintained high acceptability scores (8.12-8.67), suggesting good consumer acceptance even at 30% plantain flour substitution.

These findings suggest that fermented plantain flour can be successfully incorporated into wheat bread up to 30% substitution level to create a functional food product with enhanced nutritional value, improved health-promoting properties, and acceptable sensory characteristics. This development has implications for both food security and public health, offering a viable approach to reducing wheat flour dependency while producing bread with potential therapeutic benefits. Future research could explore the mechanisms behind the observed anti-inflammatory properties and investigate the long-term health impacts of consuming these composite breads in clinical trials.

Acknowledgements

We acknowledge the support of the laboratory staff of the Departments of Food Science and Department of Biochemistry of Federal University of Technnology Akure, as well as Nutrition and Dietetics Department of Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Funding

This study received no external funding.

Conflict of interest

The authors declared no conflict of interest.

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