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Functional Dough Meal from Unripe Plantain (Musa paradisiaca), Soymeal, Moringa (Moringa Oleifera) Seed and Marugbo (Clorendendrum volubile) Leaf Blends: Antioxidant, in Vitro Anti-Diabetic and Anti-Hypertensive Potentials

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Abstract

Diabetes mellitus and hypertension are prevalent conditions globally, with their occurrence becoming more frequent as individuals age. This study aimed to evaluate the in vitro anti-diabetic and anti-hypertensive potential of dough meal from plantain (Musa paradisiaca), soymeal, moringa (Moringa oleifera) seed and marugbo (Clerodendrum volubile) leaf. The flour blends, categorized as A: (70% unripe Plantain, 20% Soymeal, 5% Moringa, 5% Marugbo), B: (75% unripe Plantain, 15% Soymeal, 5% Marugbo Leaf, 5% Moringa), C: (80% unripe Plantain, 15% Soymeal, 5% Moringa), and D: (80% unripe Plantain, 15% Soymeal, 5% Marugbo), as well as the controls (CERO: Cerolina and PLT: 100% Unripe Plantain flour), were thoroughly evaluated in terms of their glycaemic index and load, alpha-amylase inhibition, alpha-glucosidase inhibition, antioxidants, and ACE inhibition. The results showed that the samples A, B, C, and D demonstrated higher levels of antioxidant activity. Additionally, the dough meal samples A, B, C, and D exhibited increased alpha-amylase (33.03%, 30.04%, 28.29%, 29.32%) and alpha-glucosidase inhibitory activities (32.53%, 30.21%, 27.43%, 28.13%) in comparison to the control samples CERO and PLT (25.04% and 23.54%) and (26.09% and 24.87%), respectively. The study concluded that sample A had enhanced in vitro anti-diabetic and anti-hypertensive potentials compared to the control samples and other experimental samples and it can serve as a healthy food option for diabetic and hypertensive patients.

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Introduction

Diabetes mellitus and hypertension are prevalent conditions globally, with their occurrence becoming more frequent as individuals age [1]. The global incidence of diabetes mellitus is steadily increasing without any indication of a turnaround or reversal. Arising data from the World Health Organization (WHO) signifies that at about 80% increase has been prevalent of diabetes between the years 1980 and 2014 (WHO, 2024), and the International Diabetes Federation (IDF) also approximates that in the year 2019, at about 9.3% of the global adult population between the ages of 20 -79 years suffered from diabetes (IDF, 2019).

Hypertension is a formidable health issue and is noted to impact over 1.13 billion individuals across the globe. In 2015, women aged 18 years and above constituted approximately 20% of the affected population, while men accounted for approximately 24% [2]. The incidence of hypertension is on a steady rise in both developing and developed nations, particularly in urban centres and townships, due to lifestyle changes which are namely dietary patterns and insufficient physical activity, as well as a dearth of appropriate knowledge regarding hypertension and its associated comorbidities [3,4]. Nonetheless, oxidative stress, inflammation, immunomodulation, and endothelial dysfunction are widely recognized as the primary pathological mechanisms associated with hypertension [3].

In the present day, numerous scholars are devoting substantial attention to various dietary regimens (dietary approach), with a particular emphasis on micro and macronutrients, in conjunction with modified lifestyle habits and antihypertensive medications. Micronutrients (minerals/vitamins) possess a central function in the regulation of blood pressure, as they facilitate the performance of macronutrients and also enhance the antihypertensive properties of certain antihypertensive agents [4]. While there is not a universally standardized diet for individuals with diabetes, the dietary needs of people managing diabetes are frequently shaped by factors like socioeconomic status, religious convictions, and cultural norms; nonetheless, the focus of managing diabetes through dietary measures aims to enhance overall well-being by ensuring an optimal nutritional state, achieving effective glycaemic control, and averting both immediate and lasting complications associated with diabetes [5].

In the context of the use of dietary management in alleviating diabetes and hypertension, the surging worldwide incidence of these two co-morbidities has spurred a search for natural remedies and functional foods, which has garnered increasing momentum. The integration of plantain, soymeal, Moringa seed, and Marugbo leaf into a dough meal offers a promising pathway for tackling these pressing health issues.

Dough meal is a common food product in many African countries, especially Nigeria, where it is consumed as a staple food. However, dough meal is mainly starchy and lacks the biochemical diversity required for normal healthy living [6]. Therefore, there is a need to improve the nutritional and functional properties of dough meal by incorporating other ingredients that have beneficial effects on human health. Plantain is a rich source of dietary fibre, especially when the peel is included, which can help lower blood glucose and cholesterol levels [6]. Soymeal is a high-quality protein source that can also reduce the risk of cardiovascular diseases and diabetes [7].

Moringa seed and leaf are known for their antioxidant, anti-inflammatory, anti-diabetic and anti-hypertensive activities, as well as their high content of vitamins, minerals and phytochemicals [8]. Marugbo leaf is a traditional medicinal plant that has been reported to have antidiabetic, antihyperlipidemic and antihypertensive properties [7]. The combination of these ingredients in appropriate proportions may result in a dough meal that has improved quality characteristics, such as colour, texture, aroma and taste, as well as enhanced anti-diabetic and anti-hypertensive potential. The study aims to evaluate the physicochemical, functional, sensory and antioxidative properties of the dough meal from different blends of plantain, soymeal, moringa seed and marugbo leaf flours.

Dough meal is a type of food product that is prepared by mixing different flours with water and cooking them on a hot plate or in an oven [9]. Dough meal can be enriched with various ingredients to improve its nutritional and functional quality. This study aims to formulate dough meal from plantain, soymeal, moringa seed and marugbo leaf flours and evaluate its physicochemical, sensory, in vitro anti-diabetic and angiotensin converting enzymes

inhibition properties. Plantain, soymeal, moringa seed and marugbo leaf are widely consumed in Nigeria as staple foods and medicinal plants Akinjsola et al. However, their nutritional and functional properties have not been fully explored, especially about diabetes and hypertension. Diabetes and hypertension are two major chronic diseases that affect millions of people worldwide and pose significant challenges to health care systems (World Health Organization, 2020). Therefore, there is a need to develop novel food products that can provide health benefits and prevent or delay the onset of these diseases.

The main objective of the study was to evaluate the invitro antidiabetics and ACE inhibition potential of dough meal from plantain (Musa paradisiaca), Soymeal, moringa (Moringa Oleifera) seed and marugbo (Clorendendrum volubile) leaf.

Materials and Methodology

Source of Raw Materials

Unripe plantain (Musa spp) and Moringa seed (Moringa Oleifera) were obtained from a local farm near the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. Soycake was obtained from ROM mills, Ibadan, Nigeria, and Marugbo leaf was obtained from Oja Oba Market in Akure, Ondo State. The raw materials were authenticated at the Department of crop science and Pest Management, Federal University of Technology, Akure. All reagents used for the study were of analytical grade.

Composite Flour Sample Preparation

The mature unripe plantain, soycake were processed into flour using the method described by Olugbuyi [7]. The unripe plantain was peeled, sliced, washed with water, and dried in a forced-air oven at 60 ºC for 8 hours. It was then milled using a Malex Excella heavy duty laboratory blender (Model B11, Kenwood Electric, Matiala, New Delhi) sieved and packaged for further analysis. Soybean cake was cleaned to remove extraneous particles and oven-dried at 60 ^{0}C for 2 h, milled using Philips laboratory blender (HR2811 model, Matiala, New Delhi), and sieved through a 200 µm mesh sieve before packaging in a polythene bag. Moringa seed and marugbo leaf were sorted and cleaned. They were milled using a Malex Excella heavy duty laboratory blender (Model B11, Kenwood Electric, Matiala, New Delhi) sieved and packaged for further analysis.

Formulation Of the Composite Flour Blends and Dough Meal

Twelve (12) composite blends comprising unripe plantain flour, soymeal, Marugbo leaf flour and Moringa seed flour were initially obtained using the optimal mixture model design of Response surface methodology (Design Expert 9.0), The independent variables were unripe plantain flour (70.00 – 80.00%), soymeal (15.00 – 20.00%), marugbo leaf (0 - 5.00%), and Moringa leaf (0 – 5.00%) while the dependent variable were crude protein and fibre contents. Thereafter, four samples were selected based on their highest fibre and protein. The experimental samples are; A (70% Plantain, 20% Soymeal, 5% Marugbo and 5% Moringa), B (75% Plantain, 15% Soymeal, 5% Marugbo and 5% Moringa), C (80% Plantain, 15% Soymeal, 5% Marugbo), D (80% Plantain, 15% Soymeal, and 5% Marugbo) while PLT (100% Plantain flour) and CER (Cerolina) were used as positive and negative control respectively. The optimised flours blend, i.e., A, B, C, D and, 100% Plantain flour and cerolina (a commercial antidiabetic flour) were prepared into dough meal separately by stirring the flour (500 g) in 1.5 litres of boiling water (100 °C, 20 min) [6].

Determination of Antioxidant Activities of Composite Flour Samples Dough meal Samples

DPPH radical scavenging assay

The scavenging effect of the composite flour samples on 2, 2- Diphenyl-1-picryhydrazyl (DPPH) free radical was measured according to the method of Aderinola [10]. Each sample (10 mg) was dissolved in 1 mL of buffer (0.1 M sodium phosphate buffer, pH 7.0 containing 1% (w/v) Triton X-100). DPPH was dissolved in methanol to a final concentration of 100 µM. Protein fractions (100 µl) were mixed with 100 µL of the DPPH solution in the 96-well plate to a final assay concentration of 1 mg/mL and incubated at room temperature in the dark for 30 min. The absorbance values of the blank, Glutathione (GSH) (control) and samples were measured at 517 nm. The control consisted of sodium phosphate buffer in place of the protein fractions sample while Glutathione (GSH) was used as the positive control. The percent DPPH radical scavenging activity of the samples was determined using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{A_{517}\text{ of sample}}{A_{517}\text{ of blanc}}\right) \times 100 \quad \text{Eqn. 3.9}$$

Ferric-Reducing Antioxidant Property (Frap)

The ferric reducing power of the protein fraction samples was determined according to the modified method of

Sun et al. Experimental sample or Glutathione (GSH) was dissolved in 0.2 M phosphate buffer, pH 6.6; an aliquot (250 µL) was mixed with 250 µL of the buffer and 250 µL of 1% potassium ferricyanide solution. The mixture was thoroughly mixed using a vortex machine and heated at 50 °C for 20 min. After incubation, 250 µL of 10% trichloroacetic acid (TCA) was added followed by 50 µL of 0.1% ferric chloride dissolved in double distilled water and then 200 µL of double distilled water was added. The solution was allowed to stand for 10 min at room temperature, after which it was centrifuged at 1000 ×g for 10 min. An aliquot (200 µL) of the supernatant was transferred to a clear bottom 96-well plate and the absorbance was measured at 700 nm.

Determination of Total Phenol Content

The total phenol content (TPC) was determined by Folin–Ciocalteu assay using gallic acid as standard [11]. Fifty microliter of aqueous extract solution containing 0.5 mg of aqueous extract was dispensed into a test tube, 50 µL of distilled water and 500 µL of Folin–Ciocalteu reagent was added respectively into the test tube and shaken thoroughly, after 3 min, 400 µL of 7.5% sodium carbonate solution was added and the mixture was incubated at 45 ^{O}C in a water bath for 40 min. Absorbance was measured at 765 nm against blank. The same procedure was repeated to all standard gallic acid solution (0.1 mg/mL). The blank is a mixture of 100 µL of distilled water, 500 µL of Folin-Ciocalteu reagent and 400 µL of 7.5% sodium carbonate. The total phenolic content was expressed as gallic acid equivalent per gram of sample (mg of GAE/g sample) through the calibration curve of gallic acid and calculated as follows;

$$\text{Total phenolic content } \left(\text{mg GAE}/\text{g}\right) = \frac{\text{Abs.}_{\text{sample}} \times \text{Conc.}_{\text{standard}} \left(\frac{\text{mg}}{\text{ml}}\right)}{\text{Abs.}_{\text{standard}} \times \text{Conc.}_{\text{sample}} \left(\frac{\text{mg}}{\text{g}}\right)} \quad \text{Eqn. 3.10}$$

Determination of Total Flavonoid

Total flavonoid content was determined by aluminium chloride colorimetric assay Chekol and Desta, with slight modification. About 500 µL of methanol was added to 10 mL flask containing 500 µL of aqueous extract. To this 50 µL 10% $AlCl_3$ and 50 µL of 1M CH_3COOK was added respectively. The total volume was made up to 2500 µL with distilled water. The solution was then incubated at room temperature for 30 min. Absorbance was read against blank at 415 nm with spectrometer. (JENWAY 6305, United Kingdom). The flavonoid was calculated using quercetin as standard.

$$\text{Total flavonoid content } \left(\text{mg QE}/\text{g}\right) = \frac{\text{Abs.}_{\text{sample}} \times \text{Conc.}_{\text{standard}} \text{ (mg/ml)}}{\text{Abs.}_{\text{standard}} \times \text{Conc.}_{\text{sample}} \text{ (mg/g)}} \quad \text{Eqn. 3.11}$$

$\text{Abs}_{\text{standard}}$ is the absorbance of the solution containing 500 µL quercetin, About 50 µL 10% $AlCl_3$ and 1M CH_3COOK. Blank is the mixture of 500 µL of distilled water, 500 µL of methanol, 50 µL distilled water and 1M CH_3COOK.

Metal Chelating Activity of Composite Flour Samples

Metal chelating effects on ferrous ions was carried out calorimetrically according to Liao [12]. The absorbance was measured at 562 nm. Mixture without extract was used as the control. A lower absorbance indicates a higher ferrous ion chelating capacity. The percentage of ferrous ion chelating ability was calculated using the following equation:

Iron chelating activity (Inhibition %) = (Ac - As)/As x 100

Where: Ac is the absorbance of the control reaction and as the absorbance in the presence of the plant extracts.

Determination of Alpha-Glucosidase Inhibitory Activity on Dough Meal Samples

The effect of the dough meal samples on alpha-glucosidase activity was determined according to the method described by Kim et al. using α -glucosidase from Saccharomyces cerevisiae. The substrate solution p-nitrophenylglucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, and pH 6.9. 100 µL of α-glucosidase (0.3 U/mL) was pre-incubated with 50 µL of the sample for 10 min. Then 50 µL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. The -glucosidase activity was determined by measuring the yellow-coloured paranitrophenol released from pNPG at 405 nm.

Determination of Alpha –Amylase Inhibition Activity on Dough Meal Samples

The method used involved estimating the amount of reducing sugar produced by the activity of each enzyme on buffered starch. α - amylase was assayed as reported by De Moraes. (1995). The substrate for assay was 0.5 ml of 0.5 % soluble starch, buffered with 0.2 ml of 0.1 M sodium acetate (pH 5.6). Crude enzyme extract (0.3 ml) was added to the mixture, mixed and incubated at 40 ºC for 30 min in a water-bath. DNSA (colorimetric) method

as used by Miller (1959) was thereafter employed for estimation of reducing sugars produced. One ml of DNSA solution was added to the mixture and boiled for 5 min. Four ml of distilled water was introduced after cooling before absorbance is read at 540 nm in spectrophotometer. Blank that consisted of 0.3 ml distilled water, 0.5 ml of 0.5 % soluble starch, 0.2 ml of buffer was subjected to similar treatments.

Angiotensin-I Converting Enzyme (Ace) Activity of Dough Meal Samples

The ACE activity was determined as described by Ijarotimi [7]. The lung tissue homogenate (50 µL) was mixed with Tri's buffer (50 µL) and then incubated (37 °C, 10 min). To the mixture, 8.3 mmol/L hippuryl-histidyl-leucine (HHL) (150 µL) substrate was added and the mixture was re-incubated (37 °C, 30 min), and the reaction was finally terminated by adding 1.0 mol/L HCl (250 µL). To the mixture, 0.5 mL ethyl acetate was added and then centrifuged (3000 × g, 10 min) to extract hippuric acid, and then the supernant was poured into a test tube and allowed to evaporate at room temperature in a vacuum for 2 h. The extracted hippuric acid was then dissolved in distilled water (1.0 mL), and absorbance reading was measured at 228 nm using a UV–visible spectrophotometer. The ACE activity was calculated (Units/mg).

Statistical Analysis

All data were carried out in triplicates. Statistical analysis was carried out using statistical package for social science (SPSS) version 21.0 for windows and the results were presented as mean (±SD), and statistical difference between the means was determined using one-way analysis of variance (ANOVA) while means were separated at $p<0.05$ significant difference using Duncan Multiple Range Test (DMRT).

Results and Discussion

Antioxidant Properties of Composite Flour Samples from Unripe Plantain, Soymeal, Moringa Seed and Marugbo

The results of the antioxidant composition of the composite flour samples from unripe plantain, soymeal, moringa seed and marugbo leaf indicate that the different blends have varying effects on the antioxidant activity of the flours. The phenol content of the samples ranged from 226.45 to 258.45 mg GAE/100 g, with sample D (80% unripe Plantain, 15% Soymeal, 5% Marugbo) having the highest value and sample C (80% unripe Plantain, 15% Soymeal, 5% Moringa) having the lowest. Phenols are important phytochemicals that have antioxidant and anti-inflammatory properties Baker. The flavonoid content of the samples ranged from 24.00 to 30.58 mg QE/100 g, with sample D also having the highest value and sample B having the lowest.

Table 1: Antioxidant Properties of Composite Flour Samples from Unripe Plantain, Soymeal, Moringa seed and Marugbo

Samples	Phenol (mg/g)	Flavonoid (mg/g)	DPPH (%)	FRAP (mg/g)	OH (%)	FE2+(%)
A	254.53±1.28^{b}	25.31±0.13^{b}	70.42±0.12^{a}	75.69±0.28^{a}	52.74±0.37^{d}	55.02±0.93^{b}
B	245.17±1.71^{c}	24.00±0.19^{c}	70.11±0.08^{ab}	75.10±0.77^{a}	61.25±0.96^{b}	53.04±0.58^{c}
C	226.45±0.85^{d}	24.41±0.00^{c}	69.91±0.28^{ab}	66.30±0.66^{b}	71.25±0.83^{a}	56.74±1.04^{ab}
D	258.45±0.85^{a}	30.58±0.10^{a}	68.85±0.12^{b}	79.54±0.18^{a}	54.74±0.10^{c}	58.18±0.07^{a}
CERO	115.94±1.71^{f}	16.68±0.58	43.34±0.16^{d}	27.52±4.27^{d}	48.88±0.14^{e}	45.28±0.39^{d}
PLT	160.18±0.64^{e}	17.28±0.12^{d}	64.49±1.34^{c}	56.79±1.58^{c}	52.33±0.21^{d}	46.37±0.81^{d}

*Values with different superscript on the same column are significantly different ($p<0.05$)

Key;

A: (70% unripe Plantain, 20% Soymeal, 5% Moringa, 5% Marugbo)

B: (75% unripe Plantain, 15% Soymeal, 5% Marugbo Leaf, 5% Moringa)

C: (80% unripe Plantain, 15% Soymeal, 5% Moringa)

D: (80% unripe Plantain, 15% Soymeal, 5% Marugbo); CERO: Cerolina

PLT: 100% Unripe Plantain flour

Flavonoids are a subclass of phenols that have various biological activities, such as scavenging free radicals, chelating metal ions, and modulating enzyme activity [13]. The DPPH radical scavenging activity of the samples ranged from 43.34 to 70.42%, with sample A (70% unripe Plantain, 20% Soymeal, 5% Moringa, 5% Marugbo) having the highest value and CERO having the lowest. DPPH is a stable free radical that can be used to measure the antioxidant potential of plant extracts or foods [14].

The FRAP assay measures the ferric reducing ability of plasma, which reflects the antioxidant capacity of foods or biological fluids [15]. The FRAP values of the samples ranged from 27.52 to 79.54 mM Fe2+/100 g, with sample D having the highest value and CERO having the lowest. The hydroxyl radical scavenging activity of the samples ranged from 48.88 to 71.25%, with sample C having the highest value and sample A having the lowest. Hydroxyl radicals are highly reactive and can cause oxidative damage to biomolecules, such as lipids, proteins, and DNA [16]. The iron chelating activity of the samples ranged from 45.28 to 58.18%, with sample D having the highest value and CERO having the lowest. Iron chelation is another mechanism of antioxidant action that can prevent the formation of hydroxyl radicals from Fenton reaction [14].

The results of the antioxidant composition of flour samples from unripe plantain, soymeal, moringa seed and marugbo leaf indicate that the samples have different levels of phenolic, flavonoid, DPPH, FRAP, OH and FE chelation activities. The sample D, which contains 80% unripe plantain, 15% soymeal and 5% marugbo leaf, has the highest phenolic, flavonoid, FRAP and FE chelation activities among the four blends. The sample C, which contains 80% unripe plantain, 15% soymeal and 5% moringa seed, has the highest OH activities. The sample A, which contains 70% unripe plantain, 20% soymeal, 5% moringa seed and 5% marugbo leaf, has the highest value of DPPH among the four blends. This implies that this blend has the highest ability to donate hydrogen atoms and reduce the stable DPPH radical [14].

The commercial sample (CERO) and the 100% unripe plantain flour (PLT) have the lowest antioxidant activities in all the assays. These results suggest that the addition of soymeal, moringa seed and marugbo leaf to unripe plantain flour can enhance its antioxidant potential and provide health benefits to consumers.

Antioxidants are compounds that can prevent or delay oxidative damage caused by free radicals in biological systems. Oxidative stress is associated with various chronic diseases such as diabetes, cancer, cardiovascular diseases and neurodegenerative disorders Santos-Sanchez. Therefore, consuming foods rich in antioxidants can help protect against these diseases and improve human health [17].

The results showed that the experimental flour blends had higher antioxidant properties than the commercial sample and the pure unripe plantain flour. The result also showed that the proportion of unripe plantain, soymeal, moringa and marugbo leaf in the flour blends affected their antioxidant composition. The differences in antioxidant activity among the samples may be attributed to the different proportions of unripe plantain, soymeal, moringa seed, and marugbo leaf in each blend. Therefore, by combining these ingredients in different ratios, different levels of synergistic effects may occur on the antioxidant activity of the flours enhancing the antioxidant potential of the flour and providing health benefits to consumers [18].

Antioxidant Properties of Dough Meal from Unripe Plantain, Soymeal, Moringa Seed and Marugbo

The results of the antioxidant composition of dough meal from unripe plantain, soymeal, moringa seed and marugbo leaf show that the different blends have varying effects on the antioxidant activity of the product. Phenolic compounds are known to have antioxidant properties due to their ability to scavenge free radicals and chelate metal ions [19]. The phenolic content was highest in sample B (145.53 mg GAE/g), followed by sample D (132.25 mg GAE/g), Sample A (127.38 mg GAE/g) and Sample C (94.50 mg GAE/g). The commercial sample (CERO) and the 100% unripe plantain flour (PLT) had lower phenolic content than the blends, with 73.61 mg GAE/g and 57.97 mg GAE/g respectively. This suggests that the addition of soymeal, moringa seed and marugbo leaf enhanced the phenolic content of the dough meal as these ingredients are rich sources of phenolic compounds [20,21]. The higher proportion of unripe plantain in sample C (94.50 mg GAE/g) may have reduced the phenolic content, as unripe plantain has a lower phenolic content [22].

Flavonoids are a subclass of phenolic compounds that have antioxidant activity due to their ability to donate hydrogen atoms and electrons to free radicals [23]. The flavonoid content, which is another measure of the antioxidant capacity, was also highest in sample D (80% unripe Plantain, 15% Soymeal, 5% Marugbo) with 13.11 mg QE/g, followed by sample A (70% unripe plantain, 20% soymeal, 5% moringa, 5% marugbo) with 12.45 mg QE/g, sample B (10.73 mg QE/g) and sample C (9.36 mg QE/g). The commercial sample (CERO) and the 100% unripe plantain flour (PLT) had lower flavonoid content than the blends, with 6.92 mg QE/g and 9.93 mg QE/g respectively.

Table 2: Antioxidant Properties of Dough Meal from Unripe Plantain, Soymeal, Moringa seed and Marugbo

Samples	Phenol (mg/g)	Flavonoid (mg/g)	DPPH (%)	FRAP (mg/g)	OH (%)	FE2+(%)
A	127.38±0.80^{c}	12.45±0.04^{a}	60.19±0.04^{a}	28.88±0.01^{a}	43.76±0.13^{e}	47.71±1.48^{b}
B	145.53±0.85^{a}	10.73±0.38^{b}	57.63±0.04^{b}	29.08±0.73^{a}	48.70±0.21^{b}	41.53±0.20^{c}
C	94.50±1.28^{d}	9.36±0.19^{c}	40.14±0.08^{c}	23.35±0.17^{b}	56.48±0.26^{a}	51.82±0.07^{a}
D	132.25±0.85^{b}	13.11±0.52^{a}	57.99±0.39^{b}	29.18±0.27^{a}	47.44±0.22^{c}	43.89±0.47^{c}
CERO	73.61±1.28^{e}	6.92±0.19^{d}	23.29±0.08^{d}	18.78±1.44^{c}	22.37±0.57^{f}	41.14±2.36^{cd}
PLT	57.97±0.85^{f}	9.93±0.00^{c}	39.76±0.12^{c}	22.22±0.34^{b}	45.55±0.16^{d}	38.28±0.39^{d}

*Values with different superscript on the same column are significantly different ($p<0.05$)

Key;

A: (70% unripe Plantain, 20% Soymeal, 5% Moringa, 5% Marugbo)

B: (75% unripe Plantain, 15% Soymeal, 5% Marugbo Leaf, 5% Moringa)

C: (80% unripe Plantain, 15% Soymeal, 5% Moringa)

D: (80% unripe Plantain, 15% Soymeal, 5% Marugbo)

CERO: Cerolina

PLT: 100% Unripe Plantain flour

This indicates that the addition of soymeal, moringa seed and marugbo leaf increased the flavonoid content of the dough meal.

DPPH is a stable free radical that can be used to measure the radical scavenging activity of antioxidants by measuring the decrease in absorbance at 517 nm [23]. The DPPH radical scavenging activity, which reflects the ability of the dough meal to neutralize free radicals, was also highest in sample A (60.19%), followed by sample B (57.99%), sample D (57.63%) and sample C (40.14%). The commercial sample (CERO) and the 100% unripe plantain flour (PLT) had lower DPPH radical scavenging activity than the blends, with 23.29% and 39.76% respectively. This implies that the addition of soymeal, moringa seed and marugbo leaf improved the DPPH radical scavenging activity of the dough meal.

FRAP is a method that measures the ferric reducing antioxidant power of antioxidants by measuring the increase in absorbance at 593 nm [23]. The FRAP assay, which measures the ferric reducing antioxidant power of the dough meal, was also highest in sample D (29.18 mM Fe2+/g), followed by Sample B (29.08 mM Fe2+/g), sample A (28.88 mM Fe2+/g) and sample C (23.35 mM Fe2+/g). The commercial sample (CERO) and the 100% unripe plantain flour (PLT) had lower FRAP assay than the blends, with 18.78 mM Fe2+/g and 22.22 mM Fe2+/g respectively. This shows that the addition of soymeal, moringa seed and marugbo leaf enhanced the FRAP assay of the dough meal.

OH, is a method that measures the hydroxyl radical scavenging activity of antioxidants by measuring the decrease in absorbance at 532 nm [23]. The hydroxyl radical scavenging activity, which evaluates the ability of the dough meal to scavenge hydroxyl radicals, was highest in sample C (56.48%), followed by sample B (48.70%), sample D (47.44%) and sample A (43.76%). The commercial sample (CERO) and the 100% unripe plantain flour (PLT) had lower hydroxyl radical scavenging activity than the blends, with 22.37% and 45.55% respectively. This indicates that the addition of soymeal, moringa seed and marugbo leaf increased the hydroxyl radical scavenging activity of the dough meal.

FE Chelation is a method that measures the metal chelating activity of antioxidants by measuring the decrease in absorbance at 562 nm [23]. The iron chelating activity, which assesses the ability of the dough meal to chelate iron ions, was highest in sample C (51.82%), followed by sample A (47.71%), sample D (43.89%) and sample B (41.53%). The commercial sample (CERO) and the 100% unripe plantain flour (PLT) had lower iron chelating activity than the blends, with 41.14% and 38.28% respectively. This suggests that the addition of soymeal, moringa seed and marugbo leaf improved the iron chelating activity of the dough meal.

The results showed that all the blends had higher antioxidant activity than the commercial sample and the 100% unripe plantain flour in all the assays performed. The results also showed that different blends had different effects on different antioxidant parameters, suggesting that there is an interaction between the components of each blend that affects their antioxidant potential. Furthermore, the results showed that the dough meal from unripe plantain, soymeal, moringa seed and marugbo leaf is a good source of natural antioxidants that can be used for various applications [24].

Alpha Amylase and Alpha Glucosidase Potential of Dough Meal Samples from Unripe Plantain, Soymeal, Moringa Seed and Marugbo

The results of the alpha amylase and alpha glucosidase activities of the dough meal samples indicate that the addition of soymeal, moringa and marugbo leaf to unripe plantain flour enhanced the antidiabetic potential of the dough meal by enhancing the enzymatic hydrolysis of starch and glucose [25].

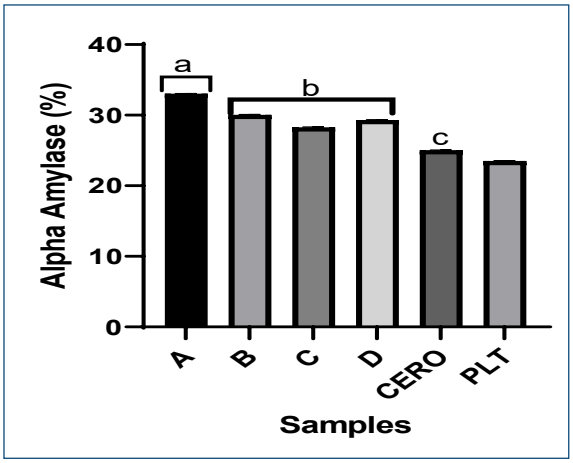

Figure 1: Alpha Amylase inhibition Potential of Dough meal Samples from Unripe Plantain, Soymeal, Moringa Seed and Marugbo

*Bars with different superscript are significantly different ($p<0.05$)
Key;
A: (70% unripe Plantain, 20% Soymeal, 5% Moringa, 5% Marugbo)
B: (75% unripe Plantain, 15% Soymeal, 5% Marugbo Leaf, 5% Moringa)
C: (80% unripe Plantain, 15% Soymeal, 5% Moringa)
D: (80% unripe Plantain, 15% Soymeal, 5% Marugbo)
CERO: Cerolina
PLT: 100% Unripe Plantain flour

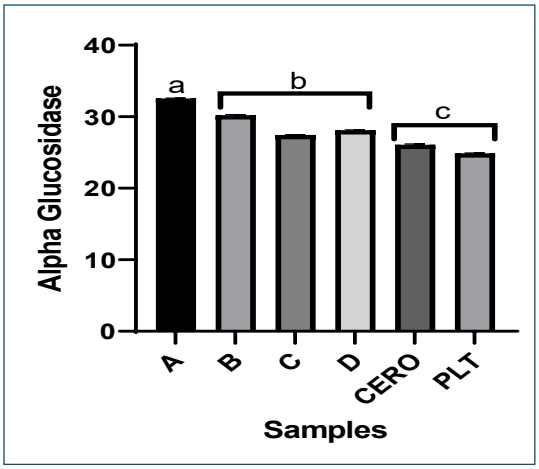

Figure 2: Alpha Glucosidase inhibition Potential of Dough meal Samples from Unripe Plantain, Soymeal, Moringa Seed and Marugbo

*Bars with different superscript are significantly different ($p<0.05$)
Key;
A: (70% unripe Plantain, 20% Soymeal, 5% Moringa,

5% Marugbo)
B: (75% unripe Plantain, 15% Soymeal, 5% Marugbo Leaf, 5% Moringa)
C: (80% unripe Plantain, 15% Soymeal, 5% Moringa)
D: (80% unripe Plantain, 15% Soymeal, 5% Marugbo)
CERO: Cerolina
PLT: 100% Unripe Plantain flour

Alpha amylase and alpha glucosidase are enzymes that break down starch and disaccharides into glucose, respectively, and their inhibition can reduce postprandial hyperglycemia in diabetic patients Proença. Both enzymes are involved in the digestion of carbohydrates and can be inhibited by some functional foods. Therefore, the lower the enzyme activity, the higher the antidiabetic potential of the dough meal [25]. The dough meal samples A, B, C and D had higher alpha amylase (33.03%, 30.04%, 28.29%, 29.32%) and alpha glucosidase inhibitory activities (32.53%, 30.21%, 27.43%, 28.13%) than the control samples CERO and PLT (25.04% and 23.54%) and (26.09% and 24.87%) respectively, suggesting that the phytochemicals present in soymeal, moringa and marugbo leaf may have synergistic effects with the phenolic compounds and resistant starch in unripe plantain flour [26]. Among the dough meal samples, sample A had the highest alpha amylase inhibitory activity (33.03%) and the highest alpha glucosidase inhibitory activity (32.53%). This may be attributed to the different proportions of soymeal, moringa and marugbo leaf in each sample, as these ingredients have been reported to possess varying degrees of antidiabetic properties [20,21]. Therefore, the dough meal produced from blends of unripe plantain, soymeal, moringa and marugbo leaf may have potential benefits for the management of diabetes mellitus.

Glycemic Index and Glycemic Load of Dough Meal Samples from Unripe Plantain, Soymeal, Moringa Seed and Marugbo

The glycaemic index (GI) and glycaemic load (GL) are indicators of the postprandial blood glucose response to carbohydrate-containing foods. Foods with low GI and GL are considered to have potential benefits for the prevention and management of diabetes mellitus [21]. The glycaemic index (GI) is a measure of how quickly a carbohydrate-containing food raises blood sugar levels after consumption [26].

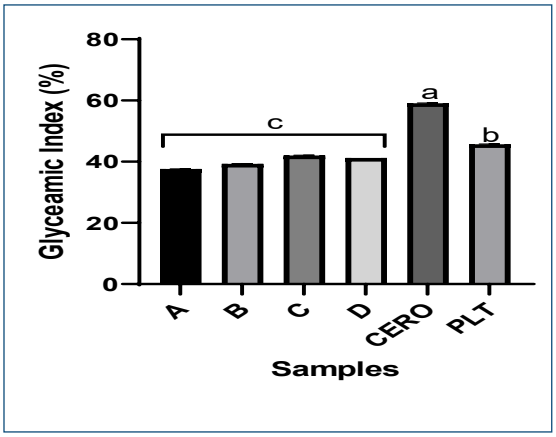

Figure: 3 Glyceamic Index of Dough meal Samples from Unripe Plantain, Soymeal, Moringa Seed and Marugbo

*Bars with different superscript are significantly different ($p<0.05$)
Key;
A: (70% unripe Plantain, 20% Soymeal, 5% Moringa, 5% Marugbo)
B: (75% unripe Plantain, 15% Soymeal, 5% Marugbo Leaf, 5% Moringa)
C: (80% unripe Plantain, 15% Soymeal, 5% Moringa)
D: (80% unripe Plantain, 15% Soymeal, 5% Marugbo)
CERO: Cerolina
PLT: 100% Unripe Plantain flour

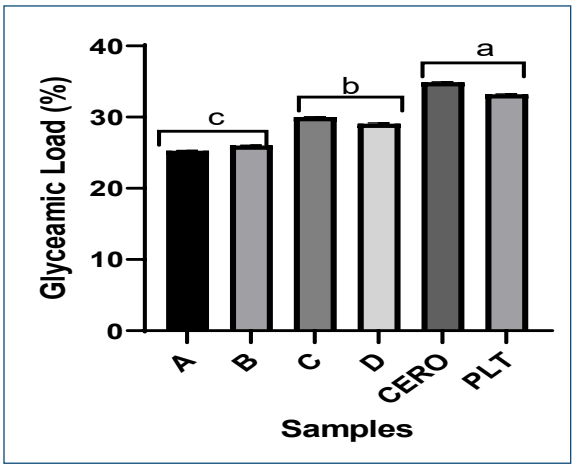

Figure 4: Glyceamic Load of Dough meal Samples from Unripe Plantain, Soymeal, Moringa Seed and Marugbo
*Bars with different superscript are significantly different ($p<0.05$)
Key;
A: (70% unripe Plantain, 20% Soymeal, 5% Moringa, 5% Marugbo)
B: (75% unripe Plantain, 15% Soymeal, 5% Marugbo Leaf, 5% Moringa)

C: (80% unripe Plantain, 15% Soymeal, 5% Moringa)
D: (80% unripe Plantain, 15% Soymeal, 5% Marugbo)
CERO: Cerolina
PLT: 100% Unripe Plantain flour

The glycaemic load (GL) is a measure of both the quantity and quality of carbohydrates in a food, taking into account the portion size and the GI. Foods with a low GI (55 or less) are digested more slowly and cause a lower and more gradual rise in blood sugar levels than foods with a high GI (70 or more) (Vlachos et al., 2020). Foods with a low GL (10 or less) have a minimal impact on blood sugar levels, while foods with a high GL (20 or more) have a significant impact [27].

The results showed that the dough meals had significantly lower GI and GL values than the control samples (cerolina and 100% unripe plantain flour). The lowest GI and GL values were observed for sample A (70% unripe plantain, 20% soymeal, 5% moringa, 5% marugbo), having the lowest values of 37.64% and 25.28%, respectively followed by sample B (75% unripe plantain, 15% soymeal, 5% marugbo leaf, 5% moringa), sample D (80% unripe plantain, 15% soymeal, 5% marugbo) and sample C (80% unripe plantain, 15% soymeal, 5% moringa). The differences in GI and GL among the dough meals could be attributed to the variations in the proportions of unripe plantain, soymeal, moringa and marugbo, which have different effects on the starch digestibility and glucose absorption [28].

This suggests that adding more soymeal, moringa and marugbo to the unripe plantain could further reduce the GI and GL of the dough meal. The possible mechanisms for this effect could be the higher protein, fibre and phytochemical content of these ingredients, which could slow down the digestion and absorption of carbohydrates in the dough meal [29]. More so, this indicates that the dough meal has a lower impact on blood glucose levels and insulin response than the other samples. The results further shows that the dough meals have potential antidiabetic properties due to their low GI and GL, and could be used as alternative foods for diabetic patients [30].

ACE Inhibition Potential of Doughmeal from Unripe Plantain, Soymeal, Moringa Seed and Marugbo

The results below show the ACE inhibition potential of Dough meal from unripe plantain, soymeal, moringa seed and marugbo. ACE inhibition potential is a measure of how well a substance can block the activity of angiotensin-converting enzyme (ACE), which is involved in blood pressure regulation and fluid balance. The higher the ACE inhibition potential, the more effective the substance is in lowering blood pressure [31].

The results show that blend A (70% unripe Plantain, 20% Soymeal, 5% Moringa, 5% Marugbo) had the highest ACE inhibition potential (42.89%), followed by blend B (75% unripe Plantain, 15% Soymeal, 5% Marugbo Leaf, 5% Moringa) with 40.23%. Blend C (80% unripe Plantain, 15% Soymeal, 5% Moringa) and blend D (80% unripe Plantain, 15% Soymeal, 5% Marugbo) had similar ACE inhibition potentials of 38.98% and 38.84%, respectively. The commercial sample (CERO) had an ACE inhibition potential of 31.45%, while the pure unripe plantain flour (PLT) had an ACE inhibition potential of 33.14%. These results suggest that adding soymeal, moringa seed and marugbo leaf to unripe plantain flour can enhance its antihypertensive effect by increasing its ACE inhibition potential.

The results indicate that all the Dough meal samples had higher ACE inhibition potential than the commercial sample (CERO) and the unripe plantain flour (PLT), suggesting that they have better antihypertensive properties. Among the Dough meal samples, A had the highest ACE inhibition potential, followed by B, C and D. This implies that adding moringa and marugbo to unripe plantain and soymeal enhances the antihypertensive effect of Dough meal.

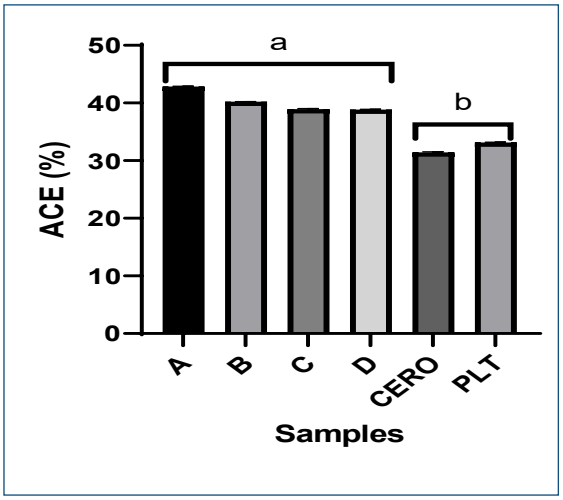

Figure 5: Angiotensin Converting Enzyme Inhibition Potential of Dough meal from Unripe Plantain, Soymeal, Moringa Seed and Marugbo
*Bars with different superscript are significantly different ($p<0.05$)
Key:
A: (70% unripe Plantain, 20% Soymeal, 5% Moringa, 5% Marugbo)
B: (75% unripe Plantain, 15% Soymeal, 5% Marugbo Leaf, 5% Moringa)
C: (80% unripe Plantain, 15% Soymeal, 5% Moringa)
D: (80% unripe Plantain, 15% Soymeal, 5% Marugbo)
CERO: Cerolina
PLT: 100% Unripe Plantain flour

The antihypertensive effect of Dough meal may be attributed to the synergistic action of the four components, which are rich in antioxidants, phytochemicals and dietary fibre that can lower blood glucose and blood pressure levels by inhibiting carbohydrate digestion and absorption, reducing oxidative stress and inflammation, and modulating vascular tone and endothelial function. Unripe plantain contains resistant starch, which can lower blood glucose and insulin levels. It also contains phenolic compounds, flavonoids and tannins, which have been reported to have ACE inhibitory activity [26]. Soymeal is rich in protein, isoflavones and saponins, which have also been shown to inhibit ACE and lower blood pressure by modulating the renin-angiotensin system [32].

Meanwhile, Moringa seed contains a peptide called niazinin, which has potent ACE inhibitory activity and antihypertensive effect in animal models Aderinola et al. Further, Marugbo leaf contains alkaloids, terpenoids and steroids, which have been found to have vasodilatory and antihypertensive effects in rats. It is rich in polyphenols, tannins and saponin, which can scavenge free radicals and inhibit ACE activity [11].

Therefore, Dough meal from unripe plantain, soymeal, moringa seed and marugbo leaf may have synergistic effects on lowering blood pressure by inhibiting ACE activity and reducing oxidative stress. The results indicate that Dough meal from different blends of unripe plantain, soymeal, moringa seed and marugbo leaf has a high ACE inhibition potential that can contribute to its antihypertensive effect. The results also suggests that each component of the samples has its own mechanism of action that can synergize with the others to produce a better outcome.

Conclusion

The study revealed that the dough meal produced from the blend of plantain, soymeal, moringa and marugbo leaf had higher protein, fat, ash, crude fibre and mineral contents than the 100% unripe plantain dough meal. The experimental dough meal samples also had higher water absorption capacity, oil absorption capacity, swelling capacity when compared with the commercial sample. The dough meal samples had lower pasting properties than the 100% unripe plantain flour dough. The dough meal samples had higher antioxidant activity, lower glycaemic index and lower glycaemic load than the control samples (100% unripe plantain and cerolina). The dough meal samples also had higher alpha amylase inhibition, alpha glucosidase inhibition and ACE inhibition than the control samples. The sensory evaluation showed that the dough meal had acceptable colour, taste, texture and overall acceptability. However, sample A showed superior nutritional, in vitro anti-diabetic and hypertensive property. Therefore, the study concluded that samples A which is the dough meal from 75% plantain, 15% soymeal, 5% moringa and 5% marugbo leaf had improved nutritional, functional as well as enhanced in vitro anti-diabetic and anti-hypertensive potentials compared to the control samples and other experimental samples [33-36].

Recommendations

The dough meal from plantain, soymeal, moringa and marugbo leaf can be used as a functional food for the management of diabetes and hypertension, as it has high antioxidant, antidiabetic and antihypertensive activities. The dough meal can also serve as a good source of protein, dietary fibre, minerals and phytochemicals, which are beneficial for human health and nutrition. The dough meal flours can be stored in airtight containers at room temperature for up to six months without significant deterioration in quality and functionality. Further studies are needed to evaluate the bioavailability and efficacy of the dough meal in vivo, as well as its safety and toxicity.

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