



Research Article

Proximate, Mineral, Amino Acid Composition, and Bioactive Properties of Dough Meals Supplemented with African Walnut Flour

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Abstract: This study investigated the impact of African walnut flour supplementation on the nutritional and bioactive properties of dough meals formulated with varying proportions of plantain flour. The proximate composition, mineral content, amino acid profile, color characteristics, antioxidant and anti-diabetic properties were analyzed for five different formulations: 100% plantain flour (P100), 95% plantain flour with 5% African walnut flour (PAW95), 90% plantain flour with 10% African walnut flour (PAW90), 85% plantain flour with 15% African walnut flour (PAW85), and 80% plantain flour with 20% African walnut flour (PAW80). The proximate analysis revealed significant variations, with moisture content ranging from 53.71% to 71.89%, ash content from 0.77% to 1.28%, and protein content from 3.38% to 11.14%. Notably, PAW80 showed the highest protein content. Mineral analysis showed that calcium, potassium, and magnesium levels varied significantly across the samples, with the highest calcium content observed in PAW80 (32.55 mg/kg) and the highest potassium content in PAW95 (47.80 mg/kg). Amino acid analysis indicated that essential amino acids, particularly leucine, valine, and lysine, were present in higher concentrations in the supplemented samples compared to P100. Antioxidant assays indicated that PAW80 had the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) (67.22%) and hydroxyl (73.66%) free radical scavenging activities. The *in vitro* anti-diabetic assays revealed that α -amylase and α -glucosidase inhibitory activities were enhanced with higher walnut flour supplementation. These findings suggest that African walnut flour can significantly enhance the nutritional quality and bioactive properties of plantain-based dough meals, and thus make it a functional food with potential health benefits.

Keywords: African walnut flour, plantain flour, nutritional composition, antioxidant activity, anti-diabetic properties

1. Introduction

In addition to the burden of food insecurity in developing countries resulting in stunted growth and under development, the challenges of non-communicable diseases also continue to increase. Several factors have been responsible for this trend, including both economic and political instabilities as well as impacts of climate change, conflicts and wars [1-2]. One of the ways to address this is through the development of food products with enhanced nutritional profile as well as potential with health-promoting ability. In this regard, several studies have been carried out focusing mainly on the nutritional improvement of locally available indigenous foods [3-5]. Diabetes mellitus is a long-term metabolic condition marked by high blood sugar levels, resulting from either insufficient insulin production, inadequate insulin response, or a combination of both factors [6]. The effects of diabetes weigh heavily on individuals, families, and healthcare systems with epidemiological data revealing a concerning upward trend in its prevalence

over the past decade. The worldwide prevalence of diabetes affects about 537 million individuals, with a significant proportion of 24 million people residing in the African Region, which comprises 48 countries, including Nigeria, under the International Diabetic Foundation's (IDF) African region umbrella. With the prevalence of diabetes estimated to be 4.3 percent from 2011 to 2021, the number of diabetics in Nigeria increased from 48% to 52% [7]. Urbanization, sedentary lifestyles, and poor dietary habits increased this epidemic [8]. The linkage between diet and health is no longer questionable as, most consumers are now deliberate in their food choices, especially for plant-based food due to their health promoting properties.

The African walnut, scientifically classified as *Tetracarpidium conophorum*, is also recognized by various names, including the conophor tree, conophor nut, black walnut, and Nigerian walnut [9]. This plant holds cultural significance, with different regions in Nigeria referring to it by distinct names, such as *Asala* (Yoruba), *Ukpa* (Ibo), *Okwe* (Edo), *Ekporo* (Efik, Ibibios-Cross River and Akwa Ibom), *Gwandi and Bairi* (Hausa). It is abundant in all cocoa-producing states in Nigeria, particularly in the south [9]. Plantain (*Musa parasidiaca*), is an important crop and a staple for many in Africa Central and South America [10]. Plantains are rich in dietary fiber, minerals (potassium and phosphorus), vitamins (A, B1, B2, B6, and C), and phenolic compounds [11]. In Nigeria, plantains are processed into a variety of products, including *Elubo* (dried unripe plantain flour), *dodo* (crispy fried slices made from ripe plantain pulp), chips (crunchy fried snacks made from half-ripe plantain pulp). Plantain can be added to yam and can be pounded to a sticky paste eaten with soup [12]. The market demand for unripe plantain flour has increased greatly because of its health benefits [11]. Consumers now prefer low-glycemic food products with resistant starch, which has low digestion rates and resistance to α -amylase in the stomach, resulting in reduced energy intake by intestinal cells [13].

Composite flour can serve as functional food products with an improved nutritional profile compared to the main flour because different crops with varied nutritional compositions are combined. Traditional foods are a significant aspect of a country's culture, history, identity, religion and heritage, as well as key components of dietary patterns [14-15]. In recent years, there has been growing interest in exploring the nutritional and health benefits of traditional foods, particularly those derived from plant-based sources [5, 16]. Dough meal is a staple food in Nigeria that is prepared by mixing different flours with water and cooking them on a hot plate. It is a traditional thick paste commonly consumed in Nigeria [17]. Despite the cultural importance of dough meal, its low protein content is a concern for the nutritional well-being of consumers. The utilization of plantain and African walnut in composite flour formulation is promising. This may improve the protein content of dough meals as well as reduce the adverse glycemic effects associated with the consumption of high-carbohydrate dough meals. This study is therefore aimed at determining the nutritional antioxidant and anti-diabetic properties of dough meal from plantain-African walnut composite flour.

2. Materials and methods

2.1 Source of materials

Unripe Plantain and matured African walnuts were purchased from a farm in Oba-Akoko, Ondo-State, Nigeria. The chemicals/reagents used were purchased from Pascal Scientific Limited (Akure, Ondo state, Nigeria) and were of analytical grade.

2.2 Methods

2.2.1 African walnut flour processing

African walnut flour was prepared using a modified method of Awofadeju et al. [18]. First, the walnut seeds were sorted and cleaned to remove any damaged or rotten seeds. The remaining seeds were then boiled for 45 min, cooled, and de-shelled. The de-shelled nuts were cut into smaller pieces (3 mm) to facilitate drying, and then oven-dried at 65 °C for 72 h to reduce the moisture content. The dried nuts were subsequently milled in a Marlex blender and defatted using n-hexane. Finally, the defatted flour was ground in a Marlex Excella grinder at the highest speed to obtain a homogeneous flour. The resulting flour was stored in an airtight zip-lock bag and kept at 4 °C until further use.

2.2.2 Plantain flour processing

Plantain flour was prepared using the method described by Adeleke and Odedeji [19]. Green plantain fingers were washed to remove any dirt or debris, then peeled and sliced into uniform pieces using a dicing equipment. The slices were oven-dried at 65 °C until they reached a constant weight, indicating complete dryness. The dried slices were then milled into a smooth flour using an attrition machine. The flour was sifted through a 0.25 mm mesh sieve to produce a fine, uniform powder. The plantain flour was stored in an airtight container at ambient temperature until needed for further use.

2.2.3 Formulation of composite flours

Plantain flour was mixed with African walnut flour at varying proportions of 100:0, 95:5, 90:10, 85:15 and 80:20 in a Kenwood blender. The blends were kept in a ziplock bag at room temperature pending their use.

2.2.4 Preparation of dough meal from the composite flour blends

To prepare the dough meal, the composite flour blend was first sieved and then gradually added to hot water (100 °C) while stirring vigorously with a flat wooden spoon. Initially, about half of the composite flour was added to the boiling water to form a non-smooth gel or paste. The remaining flour was then slowly added to the boiling paste, stirring constantly and thoroughly to ensure a smooth consistency and prevent the formation of lumps. The resulting smooth gel or thick paste was cooked for 10 min to obtain the dough meal.

2.2.5 Extraction of samples

The dry dough meal was milled into flour and used to prepare extracts for biochemical analyses. A concentration of 10 mg/mL was achieved by measuring the required amount of flour and placing it in 2 mL microcentrifuge tubes. Distilled water was added, and the mixture was vortexed for approximately one min and then left to hydrate for 10 min. After brief mixing (15 s), the mixture was centrifuged at 10,000 g for 10 min using a KX3400C model centrifuge from KENXIN Intl. Co. The resulting supernatant was used for analysis.

2.2.6 Determination of color characteristics of dough meal

The color of the dough meal prepared from the flour blend was evaluated by determining L* brightness; 100 = white, 0 = black), a* (+, red;- , green) and b* (+, yellow;- , blue) parameters by means of a color measuring instrument (ColorTec-PCM, model SN 3000421, USA).

2.2.7 Determination of mineral content

Mineral analysis was performed using the method described by AOAC [20]. The ash was dissolved in 3 mL of 3 M HCl and then diluted to 100 mL with 0.36 M HCl in a standard flask. The mineral element concentrations were subsequently measured using atomic absorption spectroscopy (AAS) on a Buck Scientific 210 VGP spectrophotometer (Bulk Scientific Inc., USA). Phosphorus content was determined using colorimetry, specifically the phosphomolybdate method, on a JENWAY 6100 spectrophotometer. A blank (control) sample was prepared and analyzed in parallel to ensure accuracy.

2.2.8 Determination of proximate compositions

The proximate compositions (crude protein, crude fat, total ash, crude fiber and moisture) were determined using standard methods [20]. Carbohydrate content was determined by difference.

2.2.9 Determination of amino acid composition of the dough meal

The amino acid composition of the dough meal was analyzed using high performance liquid chromatography

(HPLC), as previously reported [21]. The sample was hydrolyzed with performic acid and sodium metabisulphite, and then heated with hydrochloric acid. After cooling, the mixture was diluted, filtered, and partially dried. The residue was dissolved in sodium carbonate buffer and frozen for dansylation. The sample was then dansylated, incubated overnight, and analyzed using HPLC, with results reported as g/100 g of protein.

2.2.10 Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability

The DPPH free radical scavenging ability of the dough meal extract was determined according to a previously reported method [22]. In summary, 1 mL of the extract was mixed with 1 mL of a 0.4 mM methanolic solution of DPPH. After incubating in the dark for 30 minutes, the absorbance was measured at 517 nm using a Healicom 721S spectrophotometer (China). A control sample containing methanol instead of the extract was also prepared. The radical scavenging ability of the sample was then calculated as:

$$\% \text{ DPPH} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

2.2.11 Determination of 2, 2'-azino-bis (3-ethylthiazoline-6-sulphonic acid) (ABTS) scavenging ability scavenging ability

The ABTS radical scavenging ability of the dough meal was assessed using a method previously described [23]. ABTS radicals were produced by combining a 7 mM ABTS aqueous solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark for over 15 h. The absorbance of the resulting solution was adjusted to 0.7 nm with ethanol. Thereafter, two hundred microliters of the ABTS solution were mixed with twenty microliters of the extract, and the absorbance was measured at 734 nm using a Healicom 721 S spectrophotometer (China). The percentage of ABTS radical scavenging ability of the extract was calculated using the following formula:

$$\% \text{ ABTS} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

2.2.12 Determination of ferric reducing antioxidant power (FRAP)

The extract's reducing capability was evaluated following the method of Aderinola [24]. Briefly, 0.25 mL of the extract was combined with 0.25 mL of 200 mM sodium phosphate (pH 6.6) and 0.25 mL of 1% potassium ferricyanide. This mixture was then heated at 50 °C for 20 min. Then 0.25 mL of 10% trichloroacetic acid was added and mixed at 2,000 rpm for 10 min. Then, 1 mL of supernatant was mixed with 1 mL of distilled water. The absorbance of 0.1% FeCl₃ and was measured at 700 nm using a Healicom 721 S screen (China). Ascorbic acid (0.01 mg/ml) was used as standard.

2.2.13 Determination of hydroxyl radical scavenging ability

The hydroxyl radical scavenging ability of the dough meal extract was assessed using a method from a prior study [5]. Briefly, varying amounts (0-100 µL) of freshly prepared sample extract were added to a reaction mixture containing 400 µL of 0.1 M phosphate buffer (pH 7.4), 120 µL of 20 mM deoxyribose, 40 µL of 20 mM hydrogen peroxide, and 40 µL of 500 µM FeSO₄. The volume was adjusted to 800 µL with distilled water. After a 30-minute incubation at 37 °C, the reaction was stopped by adding 0.5 mL of 2.8% trichloroacetic acid, followed by 0.4 mL of 0.6% thiobarbituric acid solution. The tubes were then incubated in boiling water for 20 min. Absorbance was recorded at 532 nm, and the hydroxyl radical scavenging ability of the samples was determined using the following formula:

$$\% \text{ OH inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

2.2.14 Determination of α -amylase inhibition property

The α -amylase inhibitory activity of the dough meal extract was evaluated using a slightly modified version of a previously described method [25]. Equal volumes (100 μ L) of the sample extract or distilled water (as a control) and the enzyme solution (dissolved in 0.02 M phosphate buffer, pH 6.9, with 0.006 M sodium chloride) were combined and incubated at 28 °C for 10 min. Following this, 200 μ L of a 1% starch solution (prepared in the same buffer) was added, and the reaction mixture was incubated at room temperature for an additional 10 min. The reaction was terminated by adding 1 mL of dinitrosalicylic acid reagent, and the mixture was heated in a boiling water bath for 5 min. After cooling to room temperature, the mixture was diluted with distilled water at a ratio of 1:5 (sample to distilled water, v/v). Absorbance was then measured at 540 nm using a Healicom 721 S spectrophotometer (China). The percentage inhibition of α -amylase by the sample was calculated using the following formula:

$$\alpha - \text{amylase inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

2.2.15 Determination of α -glucosidase inhibitory ability

The effect of the extracts on α -glucosidase activity was determined using the method described by Aderinola et al. [23]. A solution containing the substrate p-nitrophenylglucopyranoside (pNPG) was prepared with 20 mM phosphate buffer at pH 6.9. Initially, 100 μ L of α -glucosidase (0.3 U/mL) was pre-incubated with 50 μ L of the sample for 10 min. Subsequently, 50 μ L of 3.0 mM pNPG, dissolved in 20 mM phosphate buffer (pH 6.9), was added to initiate the reaction. The mixture was then incubated at 37 °C for 20 min. The reaction was stopped by adding 2 mL of 0.1 M Na_2CO_3 . Glucosidase activity was assessed by measuring the yellow-colored p-nitrophenol released from pNPG at 405 nm.

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

2.2.16 Sensory evaluation of dough meal prepared from flour blends

Thirty-five panelists familiar with dough meal participated in the organoleptic evaluation of the dough meal made from blends of plantain and African walnut flour. A 9-point hedonic scale was used, where 9 indicated “like very much” and 1 indicated “dislike very much”. The dough meal was assessed for color, aroma, appearance, texture, mouthfeel, taste, and overall acceptability.

2.3 Statistical analysis

The data generated from triplicate determinations were subjected to one-way Analysis of Variance (ANOVA) using the Statistical Package for Social Sciences (SPSS, version 20) software. Means were separated using Duncan’s New Multiple Range Test (DNMRT) at $p < 0.05$, and the results were expressed as mean \pm standard deviation.

3. Results and discussion

3.1 Proximate composition of the dough meal

The proximate composition of the dough meal produced from plantain and African walnut is presented in Table 1. The moisture content ranged between 53.71 and 71.89%. With dough meal prepared by mixing flour in excess water (hot), the high moisture content may be expected. The total ash (0.77-1.28%) decreases significantly from PAW 80 to P100, which shows a reduction in the mineral content as the proportion of plantain flour increases. This could be due to differences in the mineral composition of the two flours. Fat contents were generally low (0.01-0.02%) and constant among all the samples, with a slight decrease in PAW95 and P100. The slight increase in samples with higher concen-

tration of African walnut may be due to the higher fat content of African walnut since African walnut is an oilseed crop. Dietary fiber is important for digestive health and can influence the glycemic response of foods. The crude fiber contents ranged between 1.00 and 1.03%. There was a slight increase and with higher supplementation, with PAW95 (1.03%) having the highest and PAW80 (1.00%) the lowest. Supplementation with African walnut significantly increased the crude protein contents compared to the control. Specifically, the crude protein content increased from 3.38% (control) to 11.14% (PAW80). With the very low crude protein content of the control, an increase of over 200% is significant and may help to improve the nutritional profile of the developed dough meal. The low carbohydrate content of the dough meal (19.23-32.84%) is due to the already high moisture content of the sample. The results obtained in this study are similar to the reported for stiff dough supplemented with *Moringa* leaf [26]. These authors also obtained high moisture contents (70.29-74.29%), increased protein contents (3.52-10.36%) and relatively low carbohydrate contents (13.98-18.66%).

Table 1. Proximate composition of the dough meal (%)

Samples	PAW80	PAW85	PAW90	PAW95	P100
Moisture	53.71 ± 0.00 ^e	62.02 ± 0.00 ^d	64.44 ± 0.02 ^b	71.89 ± 0.13 ^a	63.18 ± 0.06 ^c
Ash	1.28 ± 0.00 ^a	1.14 ± 0.00 ^b	1.05 ± 0.00 ^c	0.77 ± 0.00 ^e	1.03 ± 0.00 ^d
Fat	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.01 ± 0.00 ^b	0.01 ± 0.00 ^b
Fiber	1.00 ± 0.00 ^c	1.01 ± 0.00 ^b	1.01 ± 0.00 ^b	1.03 ± 0.00 ^a	1.02 ± 0.00 ^a
Protein	11.14 ± 0.01 ^a	9.94 ± 0.02 ^b	8.68 ± 0.11 ^c	7.08 ± 0.11 ^d	3.38 ± 0.18 ^e
Carbohydrate	32.84 ± 0.01 ^a	25.87 ± 0.03 ^c	24.81 ± 0.13 ^d	19.23 ± 0.03 ^e	31.38 ± 0.24 ^b

Results are mean of triplicate determinations. Values followed by different superscripts in the same row are significantly different at $p \leq 0.05$
 Keys: P100: 100% plantain flour, PAW95: 95% plantain flour + 5% African walnut flour, PAW90: 90% plantain flour + 10% African walnut flour, PAW85: 85% plantain flour + 15% African walnut flour, PAW80: 80% plantain flour + 20% African walnut flour

3.2 Mineral composition of the dough meal

The mineral element result of the sample is shown in Table 2. Micronutrients are essential nutritional composition in diets and feeds because they are involved in regulating various metabolic and physiological processes in the body. Their deficiency has been implicated in the onset of some diseases [23, 27]. The levels of evaluated mineral elements-Ca, K, Mg, Na, P, Zn, Mn and Fe varied significantly among the samples. The calcium content ranged between 21.20 and 32.55 mg/kg. These values are significantly higher than the range of 9.46-11.98 mg/kg reported by Anajekwu et al. [11] for varieties of hybrid plantain cultivars but lower than 43.4 mg/kg reported by Taiwo and Kehinde [28]. Calcium plays a crucial role in various functions of the body. These include facilitating enzyme secretion and fat metabolism, regulating muscle contractions and nerve impulses, supporting bone formation and development, aiding in eggshell formation and blood clotting, promoting muscle growth and contraction, maintaining a healthy heart function, and enabling the passage of nutrients in and out of cell walls. Calcium is essential for maintaining overall physical health and function, from muscle and nerve function to bone health and cellular processes [28].

Potassium is a major micronutrient in plantain [29-30]. It is essential for maintaining overall physical health and function, from heart and muscle function to nerve and cellular processes. It plays a vital role in various bodily functions, including regulating heart rhythm and blood pressure, supporting muscle contractions and nerve impulses, and aiding in muscle growth and recovery among others [31-32]. The content of magnesium obtained in this study ranged from 41.40 to 47.80 mg/kg. Slight variations were observed among the samples. The content of magnesium increased slightly with the addition of African walnut. Sodium is an essential electrolyte that helps regulate fluid balance inside and outside of cells. It plays a key role in maintaining blood pressure and volume [23, 26]. It is also a crucial mineral for proper nerve function, as it helps generate electrical impulses necessary for nerve signaling. It aids in muscle contraction and overall cellular function by balancing fluid levels and supporting proper cellular hydration. The sodium content of the samples

(31.60-34.60) mg/kg is considerably lower than 462 mg/kg reported by Karim et al [26]. This may be due to cultivar type as well as method of determination. Phosphorus is a critical mineral for the formation and maintenance of healthy bones and teeth. It is a major component of hydroxyapatite, the mineralized matrix of bones and teeth, contributing to their strength and structure [26]. Phosphorus is also involved in energy production, as it is part of ATP (adenosine triphosphate), which cells use to store and transfer energy. Also, phosphorus is important for the synthesis of DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), which are essential for cell growth and repair, and help maintain acid-base balance in the body [23]. There were significant differences among the phosphorus contents of the samples (5.49-23.81 mg/kg). Specifically, the phosphorus contents of the samples increased with increasing concentration of African walnut. This may be expected with phosphorus being one of the major macro minerals in African walnut [33]. Its use in other food products has also increased the phosphorus content of the samples such as in cookie production [34]. Zinc's presence suggests that the dough may enhance nerve function and reproductive health, while also regulating vitamin activity, red and white blood cell formation, heart health, and normal growth [35]. Contrary to the previous trends in mineral concentration, control sample had higher zinc content (1.39 mg/kg) when compared to the supplemented samples (0.5-1.48 mg/kg). Depending on variety and geographical location, the Zn content in walnut may vary widely, ranging from 17.98-56.80 mg/100 g [36]. The Zn content for the control sample is also higher than 0.08 mg/100 g previously reported by Oyeyinka and Afolayan [37] for plantain flesh. Therefore, supplementing food with African walnut could be recommended as a beneficial source of minerals essential for human metabolism and cellular function [34, 36]. Manganese is crucial for the pituitary gland and brain function; it supports hepatorenal function, combats anemia, and promotes growth. In this study, the content of manganese was slightly increased at 20% supplementation with African walnut flour. These values are significantly lower than 3.7-5.7 mg/100 g reported for raw and processed walnut [38]. Iron is a vital micro mineral that is a core component of hemoglobin, the protein in red blood cells responsible for transporting oxygen from the lungs to tissues throughout the body. Adequate iron levels are essential for preventing anemia, which can lead to fatigue and weakness [26]. Moreover, iron is involved in various enzymatic processes, including energy production and immune function. It supports cognitive development and plays a role in DNA synthesis and repair [28]. The iron contents for flours, which also increased with increasing concentrations of African walnut flour, ranged between 1.01 and 1.99 mg/kg.

Table 2. Mineral composition of the dough meal (mg/kg)

Samples	PAW80	PAW85	PAW90	PAW95	P100
Ca	32.55 ± 0.10 ^a	29.80 ± 0.20 ^c	21.20 ± 0.20 ^d	30.80 ± 0.20 ^b	30.30 ± 0.30 ^{bc}
K	42.50 ± 0.10 ^c	41.40 ± 0.10 ^e	43.55 ± 0.10 ^b	47.80 ± 0.10 ^a	42.05 ± 0.10 ^d
Mg	21.25 ± 0.20 ^a	18.85 ± 0.10 ^b	15.50 ± 0.10 ^c	13.80 ± 0.20 ^e	14.80 ± 0.00 ^d
Na	32.05 ± 0.10 ^d	31.60 ± 0.10 ^e	33.60 ± 0.10 ^b	34.60 ± 0.01 ^a	32.95 ± 0.01 ^c
P	23.81 ± 0.25 ^a	16.58 ± 0.00 ^b	9.60 ± 0.45 ^c	7.97 ± 1.24 ^c	5.49 ± 0.25 ^c
Zn	1.15 ± 0.01 ^a	1.48 ± 0.45 ^a	0.500 ± 0.30 ^a	1.03 ± 0.02 ^a	1.39 ± 0.50 ^a
Mn	0.12 ± 0.01 ^c	0.09 ± 0.01 ^c	0.09 ± 0.01 ^d	0.09 ± 0.01 ^b	0.09 ± 0.01 ^a
Fe	1.75 ± 0.02 ^b	1.99 ± 0.02 ^a	1.59 ± 0.02 ^c	1.01 ± 0.02 ^e	1.09 ± 0.01 ^d
Na/K	0.75	0.76	0.77	0.72	0.78

Results are mean of triplicate determinations. Values followed by different superscripts in each row are significantly different at $p \leq 0.05$

Keys: P100: 100% plantain flour, PAW95: 95% plantain flour + 5% African walnut flour, PAW90: 90% plantain flour + 10% African walnut flour, PAW85: 85% plantain flour + 15% African walnut flour, PAW80: 80% plantain flour + 20% African walnut flour.

3.3 Amino acid profiles of the dough meal

The amino acid composition of the dough meal samples is shown in Table 3. The nutritional composition of food plays a critical role in human health, with amino acids serving as fundamental building blocks for protein synthesis and various physiological functions. Stiff dough meal is a local delicacy made from plant sources that are generally of

low/poor protein quality. Major crops used are cassava, yam and plantain. Therefore, one of the major objectives of this study in addition to creating value addition for African walnut is to improve the protein quality of the dough meal. Although it is a local food, it is also consumed by younger people, who are still in the developmental stages, especially among those with low purchasing power. This study showed that African walnut is a rich source of essential amino acids (EAA), particularly leucine, valine, phenylalanine and threonine, which are essential for muscle growth, tissue repair, and overall metabolic function. The contents of these EAA are higher than the recommended values from FAO/WHO (Food and Agriculture Organization/World Health Organization). Plant-derived foods have been reported to contain high a content of glutamic acid [21, 39-40] and this was also observed in this study.

Table 3. Amino acid composition of the dough meal (g/100 g protein)

Samples	PAW80	PAW85	PAW90	PAW95	P100	FAO/WHO
Essential Amino Acids						
Leucine	7.65	7	7.24	6.07	3.06	6.6
Valine	6.64	5.61	3.92	3.42	2.4	3.5
Phenylalanine	5.76	5.05	4.35	2.22	2.04	2.8
Threonine	4.75	4.24	3.41	4.02	2.05	3.4
Lysine	4.48	4.08	3.45	3.05	2.81	5.8
Isoleucine	3.6	2.72	2.06	1.6	1.31	2.8
Histidine	2.2	1.98	1.5	0.96	0.7	1.9
Methionine	1.66	1.39	1.2	0.8	0.56	2.2
Tryptophan	0.74	0.63	0.5	0.34	0.32	1.1
Total	37.48	32.7	27.63	22.48	15.25	30.1
Non-Essential Amino Acids						
Glutamic	9.54	8.02	7.04	5.15	4.09	
Arginine	8.43	8.00	6.28	4.90	4.04	
Aspartic	6.20	5.95	5.37	4.28	3.35	
Serine	4.75	4.24	3.41	4.02	2.05	
Tyrosine	3.78	3.10	2.58	2.06	1.38	
Alanine	3.60	3.15	2.62	2.12	1.40	
Cysteine	2.97	2.91	1.94	1.45	0.85	
Glycine	2.92	2.52	2.19	1.81	1.40	
Proline	2.44	2.13	1.73	1.32	0.81	
TAA	82.11	72.72	60.79	49.59	34.62	
TArAA	10.28	8.78	7.43	4.62	3.74	
TSAA	4.63	4.30	3.14	2.25	1.41	
P-PER	2.61	2.38	2.55	2.07	0.78	
P-BV	60.84	59.94	33.73	25.54	2.98	

TAA = total amino acids, TArAA= total aromatic amino acids, TSAA = total sulphur-containing amino acids, P-PER=predicted protein efficiency ratio, P-BV = predicted biological value

Keys: P100: 100% plantain flour, PAW95: 95% plantain flour + 5% African walnut flour, PAW90: 90% plantain flour + 10% African walnut flour, PAW85: 85% plantain flour + 15% African walnut flour, PAW80: 80% plantain flour + 20% African walnut flour.

The essential amino acid content is a key indicator of protein quality. Our results show that the total essential amino acid content varied between 15.25 mg/g protein to 37.48 mg/g protein for the control and PAW80 samples, respectively. The inclusion of African walnut significantly improved the amino acid profile of the samples, including the EAA contents. At the 20% African walnut supplementation, most of the samples had a minimum of 200% increase in the contents of EAA compared to the control sample. This may be expected because plantain is mainly a carbohydrate crop. However, the inclusion of African walnut could increase the contents of EAA profiles beyond 30.1 g/100 g protein recommended by FAO/WHO may impart additional benefits to the consumers. Moreover, the enhanced protein quality of dough meal from the composited flour is also supported by the predicted protein efficiency ratio (P-PER) and the predicted biological value (P-BV) for the samples. While both PER and BV are used to measure the quality of a protein, PER measures the potential of the protein to support growth while BV measures the proportion of the absorbed protein that becomes incorporated into the protein of the animals' body [41-42].

3.4 Color characteristics of the dough meal

The color characteristics of dough meal obtained from the composite flour blend are presented in Table 4. Color plays an important role in the quality assessment of any food product and is therefore a major factor used by consumers to accept or reject the product [43]. Generally, all dough meal samples showed a moderate degree of lightness L^* (49.65-59.41), low degree of redness a^* (6.26-8.10), low degree of yellowness b^* (2.62-6.78) values, low degree of c^* (6.79-10.56) and slightly increased h^* (22.70-39.90). The lightness, redness, yellowness, and color intensity values from the P100 sample differed significantly ($p < 0.05$) from that of the doughmeals samples. The observed difference in lightness (L^*) is likely a result of enzymatic browning, a reaction that took place during the drying process of the flour, leading to a darker color. Moreover, the color of the dough meal may have been affected by Maillard reaction, which involves chemical reaction between proteins and reducing sugars during the cooking process [43-44]. Observation of color changes was also reported in previous similar studies on flour or dough meal [43-44]. The dough meal from PAW95, PAW90, PAW85, and PAW80 were slightly lighter than the P100. The P100 (100% plantain) dough meal exhibited the most pronounced brown color, likely due to partial enzymatic browning occurring in the plantain slices during the drying process and non-enzymatic browning in the flour [45]. It was previously noted that plantain flour can undergo non-enzymatic browning during storage, leading to a degradation of its color [3].

Table 4. Color characteristics of the dough meal

Samples	L^*	a^*	b^*	c^*	H^*
PAW80	59.41 ± 0.26 ^{ab}	8.10 ± 0.01 ^a	6.78 ± 0.04 ^b	10.56 ± 0.03 ^{ab}	39.90 ± 0.13 ^a
PAW85	59.60 ± 0.34 ^a	7.90 ± 0.02 ^b	7.13 ± 0.09 ^a	10.64 ± 0.08 ^a	42.10 ± 0.32 ^b
PAW90	59.02 ± 0.45 ^b	8.22 ± 0.06 ^c	6.31 ± 0.01 ^c	10.36 ± 0.04 ^b	37.48 ± 0.21 ^c
PAW95	55.78 ± 0.11 ^c	7.79 ± 0.12 ^c	5.49 ± 0.23 ^d	9.53 ± 0.24 ^c	35.17 ± 0.71 ^d
P100	49.65 ± 0.01 ^d	6.26 ± 0.25 ^d	2.62 ± 0.01 ^e	6.79 ± 0.02 ^d	22.70 ± 0.16 ^e

Results are mean of triplicate determinations. Values followed by different superscripts in each column are significantly different at $p \leq 0.05$
 Keys: P100: 100% plantain flour, PAW95: 95% plantain flour + 5% African walnut flour, PAW90: 90% plantain flour + 10% African walnut flour, PAW85: 85% plantain flour + 15% African walnut flour, PAW80: 80% plantain flour + 20% African walnut flour

3.5 Antioxidant properties of the dough meal

Antioxidants are natural compounds found in some foods that help neutralize free radicals in the body. They scavenge free radicals generated in the body due to various metabolic processes [46]. Plants and their by-products are rich in array of antioxidants which have been found to be of health benefit to humans [39,47]. DPPH was chosen as the assay method due to its excellent stability, simplicity, feasibility, and its capability to form stable radicals. Figure 1 (A-D) shows that the dough meal from the composited flour had better free radical scavenging ability compared to the

control samples. The impact of the degree of supplementation with African walnut flour was also pronounced as the free radical scavenging abilities of the sample increased with increased African walnut concentration in the samples. In this study, sample PAW80 had the highest DPPH value (67.10%). The increase in DPPH scavenging activity may indicate the extent of antioxidant efficacy of the sample. Higher DPPH is an indication of higher free radical scavenging ability while lower DPPH is an indication of lower free radical scavenging ability [28]. Other antioxidant assays also showed similar trends to DPPH result, whereby the values for the dough meal from composited flour had better free radical scavenging ability compared to the control sample. The range of values for ABTS were 19.27-26.11%, OH (36.42-73.25%) and FRAP (1.60-5.44 mg vit C/g of sample). The results of the free radical scavenging assay in this study suggest that the dough has a better ability to scavenge free radicals compared to the control.

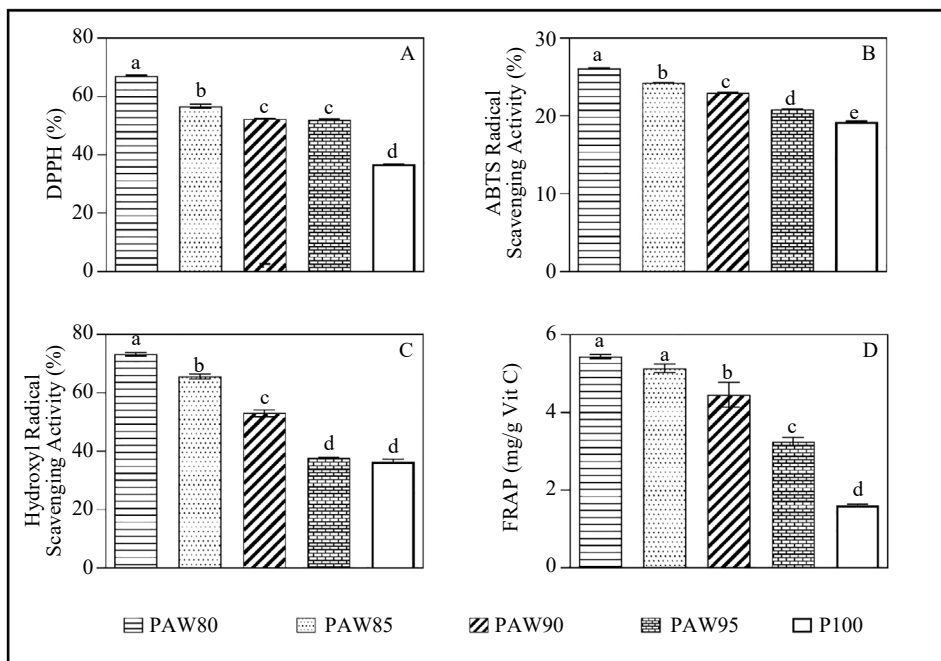


Figure 1. Antioxidant properties of the dough meal

Results are mean values of triplicate determinations. Bars with different alphabets are significantly different at $p \leq 0.05$

Keys: P100: 100% plantain flour, PAW95: 95% plantain flour + 5% African walnut flour, PAW90: 90% plantain flour + 10% African walnut flour, PAW85: 85% plantain flour + 15% African walnut flour, PAW80: 80% plantain flour + 20% African walnut flour

3.6 In vitro inhibition of α -amylase and α -glucosidase by the dough meal

Managing T2DM can be achieved effectively by slowing down glucose absorption, which is made possible by inhibiting the activity of carbohydrate-digesting enzymes [33]. The key enzymes responsible for carbohydrate digestion are α -amylase and α -glucosidase [42]. These enzymes break down carbohydrates into glucose, with α -amylase acting on carbohydrates and α -glucosidase converting starch and disaccharides into glucose [48]. By inhibiting these enzymes, glucose absorption is slowed, leading to a reduced postprandial increase in plasma glucose levels [33]. As a result, researchers have sought to identify safe and effective inhibitors of α -glucosidase and α -amylase from natural sources to develop functional foods for diabetes management [16, 43-44]. The in vitro evaluation of the dough meal samples against α -glucosidase and α -amylase was performed, and the results (Figure 2 A&B) demonstrated that Sample PAW80 exhibited the highest α -glucosidase inhibitory capacity (62.41%), followed by sample PAW85 (58.93%), sample PAW90 (47.91%), sample PAW95 (38.47%) and the least was sample P100 (34.58%), respectively. Similarly, sample PAW80 also displayed the highest α -amylase inhibitory activity with inhibitory ability of 39.53% followed by sample PAW 85, PAW90, and PAW95 with 37.33%, 30.35%, and 24.37%, respectively. Also, sample P100 had the least α -amylase activity (21.91%).

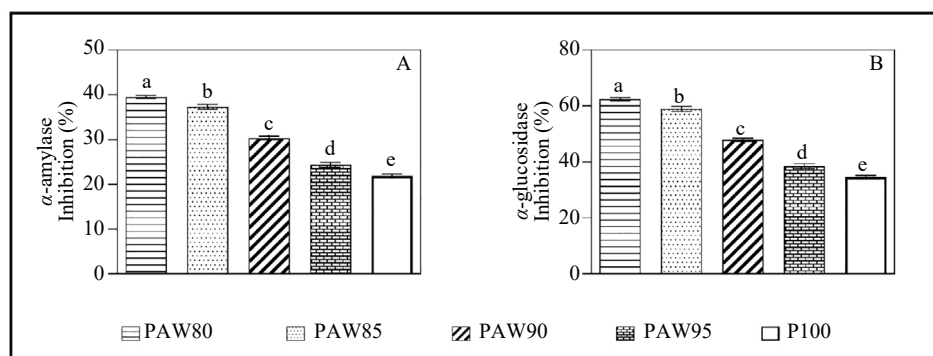


Figure 2. Invitro α -amylase and α -glucosidase of the dough meal

Results are mean values of triplicate determinations. Bars with different alphabets are significantly different at $p \leq 0.05$

Keys: P100: 100% plantain flour, PAW95: 95% plantain flour + 5% African walnut flour, PAW90: 90% plantain flour + 10% African walnut flour, PAW85: 85% plantain flour + 15% African walnut flour, PAW80: 80% plantain flour + 20% African walnut flour

African walnut has been reported to demonstrate significant health promoting properties, including antidiabetic activities [23, 49-50]. Therefore, the inclusion of African walnut flour in the plantain-based dough meal may have a significant impact on the enzymatic activity involved in carbohydrate digestion. The enhanced ability of the composited flour to inhibit the carbohydrate-digesting enzymes compared to the control samples may be due to African walnuts being rich in dietary fiber in addition to the presence of other bioactive phytochemical such as phenols and flavonoids [47, 51-52]. The low antidiabetic potential observed in the P100 dough meal sample shows the importance of dietary diversity in modulating metabolic processes. Plantain, while a staple carbohydrate source in many diets, may lack certain bioactive compounds or nutrients present in African walnuts that contribute to enhanced enzymatic activity.

3.7 Quality acceptability of the dough meal

Table 5. Quality acceptability of the dough meal

Samples	Appearance	Texture	Aroma	Taste	Overall Acceptability
P100	8.1714 \pm 0.75 ^a	8.1714 \pm 0.71 ^a	8.2286 \pm 0.65 ^a	8.2286 \pm 0.73 ^a	8.7273 \pm 0.55 ^a
PAW95	7.0286 \pm 0.95 ^b	6.8571 \pm 1.24 ^{bc}	7.1429 \pm 1.19 ^b	7.5429 \pm 0.70 ^b	6.8571 \pm 1.03 ^b
PAW90	7.0286 \pm 1.15 ^b	7.0286 \pm 1.07 ^b	7.2857 \pm 0.89 ^b	7.0571 \pm 0.80 ^c	6.7714 \pm 0.49 ^b
PAW85	6.6286 \pm 0.69 ^{bc}	6.7143 \pm 0.89 ^c	6.9429 \pm 0.80 ^b	6.6286 \pm 0.84 ^d	6.3429 \pm 0.53 ^c
PAW80	6.3429 \pm 1.06 ^c	6.4286 \pm 0.97 ^c	6.8857 \pm 0.67 ^b	6.6000 \pm 0.81 ^d	6.3714 \pm 0.49 ^c

Results are mean of triplicate determination \pm standard deviation. Values within the same column with different superscripts are significantly different at $p \leq 0.05$

Keys: P100: 100% plantain flour, PAW95: 95% plantain flour + 5% African walnut flour, PAW90: 90% plantain flour + 10% African walnut flour, PAW85: 85% plantain flour + 15% African walnut flour, PAW80: 80% plantain flour + 20% African walnut flour.

The sensory quality assessment of dough meals from the composite flour blends is presented in Table 5. P100 dough meal was generally rated as the best in terms of appearance, taste, texture (mouldability) and overall acceptability. Sensory assessment and consequent quality acceptability of product is subjective. Therefore, higher consumers' preference for P100 dough meal may be due to their familiarity with dough meal from P100, including its texture and aroma. The obtained values reflect consumers' preference for a known traditionally-based food product rather than newly-introduced ones. Food selection by consumers is often influenced by cultural preferences (beliefs, sensory attributes or personal liking, status or level of education and exposure and level of exploring new things/discoveries) [53]. Although incorporating African walnut into the plantain flour for dough meal production is new in

terms of the organoleptic properties (appearance, taste, texture and mouldability) and the degree of its acceptability slightly decreased with an increase in the concentration of African walnut, it is likely that its acceptability will increase as consumers become more aware of its superior nutritional profile.

4. Conclusion

This study revealed that blending African walnut with plantain in the production of dough meals has a substantial impact on their antioxidant, amino acid, and antidiabetic qualities. Among the various dough meal sample formulations, sample (PAW 80) containing 80% plantain and 20% African walnut was the most promising, showing significant improvement in antioxidant activities, essential amino acid profiles, as well as the potential to offer antidiabetic properties when compared to other samples. Although formulated dough meal from African walnut resulted in decreased sensory and quality acceptability, it can be concluded that inclusion of African walnut in dough meal production is feasible and has the potential to offer both improved nutritional and antidiabetic properties compared to the control sample.

Authors' contribution

Conceptualization: Taiwo Aderinola; Formal analysis: Peace Mayomi; Methodology: Taiwo Aderinola, Peace Mayomi, Project administration: Peace Mayomi; Supervision: Taiwo Aderinola; Writing-original draft: Peace Mayomi; and Writing-review & editing: Taiwo Aderinola.

Conflict of interest

The authors declare that there are no competing interests

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