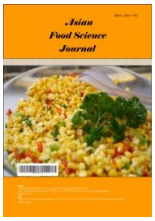

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Fermentation and Exrusion Effects on the *In Vitro* Proteinand Starch Digestibility of Unripe Plantain and Pigeon Pea Blends

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Authors' contributions

This work is a collaborative effort among the authors. The etiquette and first draft of the manuscript was drafted by authors SEO, AOO designed the study. Second draft and corrections were performed by authors AOA, JOA managed the literature searches. Authors POG and IMA managed the analyses of the study. Authors BOBA-M, IMA performed the statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated effects of fermentation and extrusion on the *in vitro* protein andstarch digestibility of unripe plantain and pigeon pea blends. The blended samples were set-up in three arrangements (A=100g unripe plantain; B= 70g unripe plantain: 30g pigeon pea; C= 50g unripe plantain: 50g pigeon pea) and divided into four batches (i.e. first batch = preconditioned and fermented; second batch = extruded; third batch = fermented and extruded; and fourth batch = unfermented/unextruded). Semi-solid state method of fermentation was deployed to ferment blended samples for 96 hours. The pH, temperature and total titratable acidity (TTA) of these samples were evaluated. Fifteen microorganisms comprising 9 bacteria, 2 yeasts and 4 molds were isolated and identified as; *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Lactobacillus plantarum, Lactobacillus fermentum, Leuconostocmesenteroides, Lactobacillus mali, Streptococcus lactis, Saccharomyces cerevisiae,*

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Candida utilis, Aspergillusniger, Aspergillusfumigatus, Aspergilluscandidus, and *Mucorhiemalis.* There were notable variation in the values of pH and total titratable acidity (TTA) during fermentation. The processes of fermentation and extrusion significantly amplified the *in vitro* starch digestibility of the flour blends with fermented extruded samples (51.03±0.02 to 55.19±0.02mg/ml) unlike theraw flour blends (36.77±0.20 to 41.26±0.003mg/ml).The *in vitro* protein digestibility significantly increased with the extruded fermented samples (12.73±0.17 to 15.45±0.06mg/ml) and lowest forraw flour blends (4.57±0.29 to 5.98±0.37mg/ml). Hence, it can be concluded based from the available information from this study that fermentation and extrusion increase the *in vitro* starch digestibility and protein digestibility of unripe plantain and pigeon pea blends.

Keywords: Unripe plantain; pigeon pea; fermentation; extrusion; in vitro starch digestibility and protein digestibility.

1. INTRODUCTION

Fermentation and extrusion improve reduces the water-binding capacity of cereal flour, thus improved the nutritional value. This allows the fortified to have a free-flowing consistency even with high proportion of flour. Extrusion has been reported as an effective processing treatment to increase the nutrient value of cereals [1]. In the developing world, fermentation is one of the oldest technologies used for food processing and preservation. Fermentation reduces antinutrient properties of foods. It can be described as a desirable process of biochemical modification of primary food products brought about by microorganisms and their enzymes [2]. Extrusion cooking technology is as a heat-treatment process in which raw materials areacted upon mechanically while passing through compression screws and is forced through a die or other restrictions [3].

Plantain (*Musa paradisiaca)*, a gigantic perennial crop, grown in many tropical(and subtropical countries) of the world [4]. Plantains are staple food that provides 60 million people with 25% calories [5]. Plantain is a source of starchy staple food for a considerable percentage of Nigeria. Mature plantain pulp is valuable in iron, potassium and vitamin A but short in protein and fat [6]. Unripe plantain meal is consumed by diabetics primarily to lower postprandial glucose level, hence, a substitute for carbohydrate-rich foods with a high glycemic index that hastendency todevelop diabetes and obesity with increasedconsumption [7].

Pigeon peas are leguminous shrubby herb, with trifoliate leaves, yellow flowers and flattened pods that is much cultivated especially in the tropics [8]. Pigeon pea is well adapted to the tropical regimes. One of the best solutions to protein energy malnutrition in developing countries is supplementing cereals with protein rich legumes. Pigeon pea flour has been tested and found to be suitable as a protein source for supplementing cereal food products due to its high level of protein, iron and phosphorus [9].

The problem of malnutrition is predominant in Nigeria due to deficiency of protein and calories and protein-calories sources of vegetable origin have been proposed as a solution to this problem [10]. This research investigates the effects of fermentation and extrusion on the *in vitro* starch digestibility and protein digestibility of unripe plantain and pigeon pea flour blends.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Green matured unripe plantain and pigeon pea seeds used for this study were obtained from Oja Oba, Akure metropolis in Ondo state, Nigeria. These samples were identified and authenticated by a botanist.

2.2 Processing of Unripe Plantain Flour

The unripe plantain was sorted for maturity and cleaned by washing with water. The clean unripe plantains was peeled and sliced thinly into 2 mm diameter and sun dried for 72 hours. The dried unripe plantain was then fed into a Bentall attrition mill (Model 200L090). The milled flour was sieved with 0.25 mm mesh sieve into fine flour and kept in an air tight container.

2.3 Processing of Pigeon Pea Flour

Pigeon pea seeds were cleaned by sorting out dirt and stones. The cleaned pigeon pea seeds were coarsely milled to separate the coat from the cotyledon. The husk was separated from the

seed by blowing air through rotary lobe into it. The dehulled pigeon pea seeds were milled into fine flour using an attrition mill after which it was sieved through 0.25 mm mesh. The pigeon pea flour was kept in an airtight container.

2.4 Formulation of Pigeon Pea-plantain Blends

The unripe plantain and pigeon pea flours were formulated into three samples
Sample A (100:0) = 100% unripe plantain flour
Sample B (70:30) = 70% unripe plantain flour and 30% pigeon pea flour
Sample C (50:50) = 50% unripe plantain flour and 50% pigeon pea flour

2.5 Fermentation and Extrusion of the Flour Blends

A batch of the flour blend was fermented using semi- solid state fermentation for 96 hours. 70 ml of sterilized water was added to 100 g of each sample in cleaned containers and properly sealed. The fermentation process was terminated by oven drying at 60°C for 24 hours. Two batches of samples were subjected to extrusion cooking. The first batch consists of the unfermented blends. The blends were hydrated and preconditioned by adding 50 ml of water to 1000 g of the sample and manually mixed in a sterile bowl to ensure even distribution of water. The samples were extruded using a Brabender 20DN single screw laboratory extruder (Brabender OHG, Duisburg, Germany). The second batch of the samples consists of the fermented samples. The fermented samples were also extruded using a Brabender 20DN single screw laboratory extruder (BrabenderOHG, Duisburg, Germany). The samples were extruded at 100°C, 20 revolution per minute and the feeding rate of 30 kg/h. All the extrudates were air-dried for 12 hours after which they were stored at 32°C in sterile polyethylene bags and kept in properly labeled air-tight containers. The control which consists of the raw blends that were neither fermented nor extruded was kept in air-tight containers.

2.6 Microbiological Analysis of the Samples

Bacteria and fungi were evaluated using nutrient agar (NA) and potato dextrose agar (PDA) respectively while De Man Rogosasharpe agar was used to isolate lactic acid bacteria. Microorganisms were enumerated by using appropriate serial dilution and pour plate techniques. The bacterial culture was incubated at 37°C for 18 to 24 hours, fungal plates were inverted and incubated at 24°C for 48 to 72 hours. De Man Rogosasharpe agar plates were incubated at 32°C for 18 to 24 hours anaerobically. The organisms were characterized based on biochemical and morphological observations according to the methods of Fawole and Oso [11] and Cheesbrough [12].

2.7 Determination of pH and TTA

The pH of all fermenting samples was determined at 24 hours interval using a pocket size pH meter. One (1) gram of the sample was dissolved in 10 ml of distilled water and filtered. The pH meter was calibrated with buffer solutions of pH 4, 7 and 9, this was followed by dipping the electrode of the pH meter into the sample solution and the observed pH was read and recorded in triplicates. The total titratable acidity of the fermenting samples was determined at 24 hours interval. Two(2) grams of macerated sample was weighed into a beaker. 20 ml of distilled water was added to it, it was mixed and filtered. 10 ml of the filtrate was measured into a beaker and 2 drops of phenolphthalein indicator was added into it. This was titrated with 0.1 M sodium hydroxide (NaOH) solution and the titre value was read. Total titratable acidity was expressed as percent (%) lactic acid. The acidity was calculated as: TTA= Titre value × 9 mg/100. The pH and TTA of the samples were carried out according to the method described by AOAC [13].

2.8 Determination of *in vitro* Starch Digestibility

In vitro starch digestibility was determined by using 1% carboxymethyl cellulose (CMC) in sodium acetate buffer (pH 5.5) as substrate. Zero point two(0.2) ml of the sample (enzyme) solution was added to 0.2 ml of the substrate solution and incubated at 37°C for 30minutes. Zero poin five(0.5) ml of 3.5 %dinitrisalicylic acid (DNSA) was added and heated for 5 minutes in a water bath. The solution was allowed to cool and 10 ml of distilled water was added. The same procedure was carried out on the substrate without addition of the enzyme solution. The absorbance was read at 540 nm [14]. The absorbance at 540 nm was extrapolated from

glucose standard curve to obtain the amount of glucose liberated.

Unit of cellulose activity = µ mole glucose / 30minutes.
Unit of cellulose activity/Min = µ glucose per minute
Specific activity (unit/mg) =µ mole glucose/min/mg.

2.9 Determination of *In vitro* Protein Digestibility

In vitro protein digestibility of each sample was evaluated using a sequential pepsin and pancreatin digestion model according to the method of Chavan*et al*. [15] and Nunes*et al*. [16]. One gram(1g) of the sample was suspended in 60 ml of 0.1M HCl at pH of 1.0 containing 6mg of pepsin, followed by gentle shaking for 15 minutes at 37°C. The resulting solution was neutralized with 0.5M NaOH to pH 7.0 and treated with 16mg of pancreatin in 30 ml of 0.1M phosphate buffer (pH 8.0). The mixture was then shaken for 24 hours at 37°C in a water bath. The undigested solid was separated by filtration. The protein content of the undigested solid and initial protein content of the sample was determined using the Kjedahl method [13]. *In vitro* protein digestibility was expressed as percentage as indicated below:

In vitro protein digestibility (%) = A-B

Where A= % protein in the samples before digestion
B = % protein after enzyme digestion.

2.10 Statistical Analysis

Statistical analyses of the data were done using SPSS statistical software (SPSS for window version 20). Data obtained as mean standard deviations were analyzed by Analysis of Variance (ANOVA), followed by Duncan's New Multiple Range Test($P \leq 0.05$) to determine the significant differences between the mean values.

3. RESULTS

3.1 Microorganisms Identified

A total number of fifteen (15) microorganisms comprising nine (9) bacteria, two (2) yeasts and four (4) molds were isolated and identifiedduring fermentation of unripe plantain and pigeon pea flour blends. They include;*Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Lactobacillus plantarum, Lactobacillus fermentum, Leuconosto cmesenteroides, Lactobacillus mali, Streptococcus lactis, Saccharomyces cerevisiae, Candida utilis, Aspergillusniger, Aspergillus fumigatus, Aspergilluscandidus,* and *Mucorhiemalis.*

3.2 Changes in pH During Fermentation of Unripe Plantain and Pigeon Pea Flour Blends

Variations in pH during the fermentation of unripe plantain and pigeon pea blends are shown in Fig. 1. Sample A gradually decreased from 5.80±0.00 to 5.10±0.03, Sample B decreased from 6.0±0.00 to 5.30±0.00, and sample C, decreased from 5.90±0.00 to 5.20±0.00.

3.3 Changes in Total Titratable Acidity During Fermentation of Unripe Plantain and Pigeon Pea Flour Blends

Titratable acidity (TTA) during fermentation of unripe plantain and pigeon pea blends are seen in Fig. 2. Sample A had TTA of 1.20±0.00 at 0 hour; this increased to 2.20±0.00 and 4.40±0.00 at 24 hours and 48 hours and increased slightly to 4.5±0.00 at 72 hours and finally decreased to 3.70±0.00 at 96 hours. Sample B increased from 1.00±0.00 at 0 hour and increased to 2.20±0.00 at 24 hours, decreased slightly to 2.00±0.00 at 48 hours and increased to 6.60±0.00 at 72 hours and finally decreased to 3.6±0.00 at 96 hours. Sample C at 0 hour increased from 1.10±0.00 to 2.30±0.00 at 24 hours and increased to 3.60±0.00 at 48 hours, decreased to 2.60±0.00 at 72 hours and finally decreased to 3.6±0.00 at 96 hours.

3.3.1 *In vitro* protein digestibility of unripe plantain and pigeon pea blends

The *in vitro* protein digestibility of the samples is shown in Fig. 3. Significant ($P \leq 0.05$) values were obtained among the raw samples A to C. Raw flour blends had the lowest values ranged from 4.57±0.29 to 5.98±0.37mg/ml in samples A to C. There was significant ($P \leq 0.05$) increase in the *in vitro* protein digestibility of extruded unfermented, and fermented unextruded samples. Extruded fermented samples had the highest values ranging from 12.73±0.17 to 15.45±0.06mg/ml.

3.3.2 *In vitro* starch digestibility of unripe plantain and pigeon pea blends

The *in vitro* starch digestibility of the samples is represented in Fig. 4. Significant ($P \leq 0.05$) values were obtained among the samples. Raw flour blends had the least values ranging from 36.77±0.20 to 41.26±0.003mg/ml. Fermented extruded blends had the highest values ranging from 51.03±0.02 to 55.19±0.02mg/ml.

4. DISCUSSION

The fifteen microorganisms (15) that were present in the fermenting media is similar to the findings of Ojokoh and Udeh [17] that legume supplemented products had a greater microbial diversity and higher microbial populations.

As fermentation progressed, the pH of the samples decreased. The lowering of pH may be as a result of the activities of microorganisms on the fermentable medium which led to the hydrolysis of complex organic compounds of the medium thereby resulting into the production of acid and ethanol. The acids produced brought about a decrease in pH and increase in total titratable acidity which consequently resulted in low microbial load. Related results were reported by Hassan et al. [18] and Ojokoh and Udeh [17]. However, the result of this research suggests that it is a lactic acid type where pH of fermenting media decreases with increase in total titratable acidity (TTA).

The *in vitro* starch digestibility of the samples - raw flour blends had the reduced values while fermented extruded blends had the highest values, this agrees with the findings of Singh et al. [19] when the effect of fermentation on the starch digestibility, resistant starch and some physicochemical properties of sorghum flour were assessed.

Protein digestibility studies conducted showed that processing (fermentation and extrusion) increased *in vitro* protein digestibility as was stated by Raihanatu*et al.* [20]. Wedad*et al.* [21] also reported significant increase in the *in vitro* protein digestibility of fermented sorghum. The improvement in the *in vitro* protein digestibility caused by fermentation could be attributed to the partial degradation of complex storage proteins to more simple and soluble products [15] [22]. It could also be attributed to the degradation of tannins, polyphenols and phytic acid by microbial enzymes. Enhanced proteolytic activity during fermentation is generally associated with improved protein digestibility, which increases amino nitrogen by partial breakdown of proteins peptides and amino acid [23]. The results obtained in this study agrees with Mohiedeen *et al.* [24] who reported that fermentation is found to improve the *in-vitro* protein digestibility of the two maize cultivars and this could be attributed to the partial degradation of complex storage proteins into simpler and soluble products.

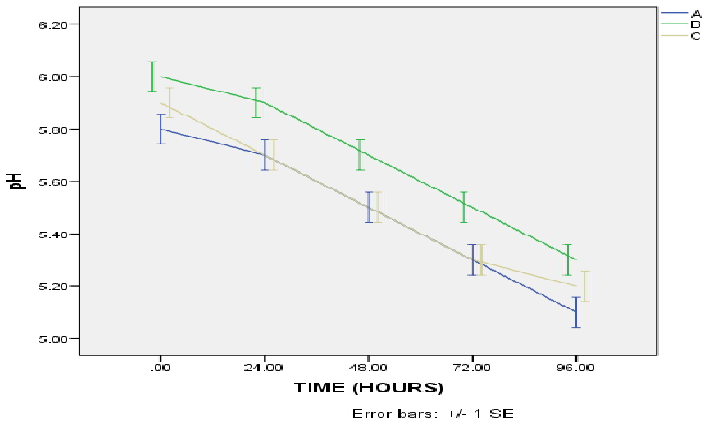

Fig. 1. pH variation during fermentation of Unripe plantain and pigeon pea blends
KEYS: A= 100g Unripe Plantain flour; B= 70g unripe plantain flour and 30g pigeon pea flour; C= 50g unripe plantain flour and 50g pigeon pea flour

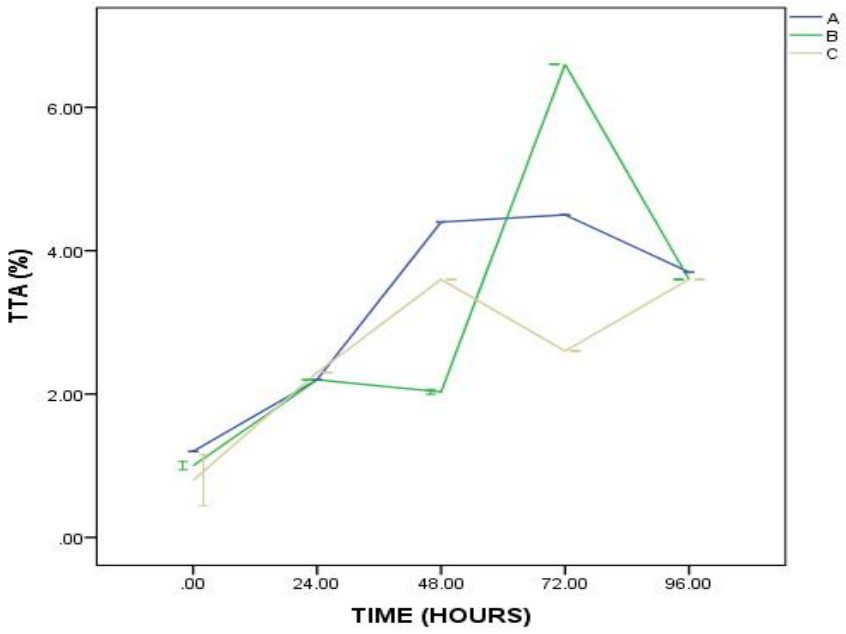

Fig. 2. Total titratable acidity variation during fermentation of unripe plantain and pigeon pea blends

KEYS: A= 100g Unripe Plantain flour; B= 70g unripe plantain flour and 30g pigeon pea flour; C= 50g unripe plantain flour and 50g pigeon pea flour

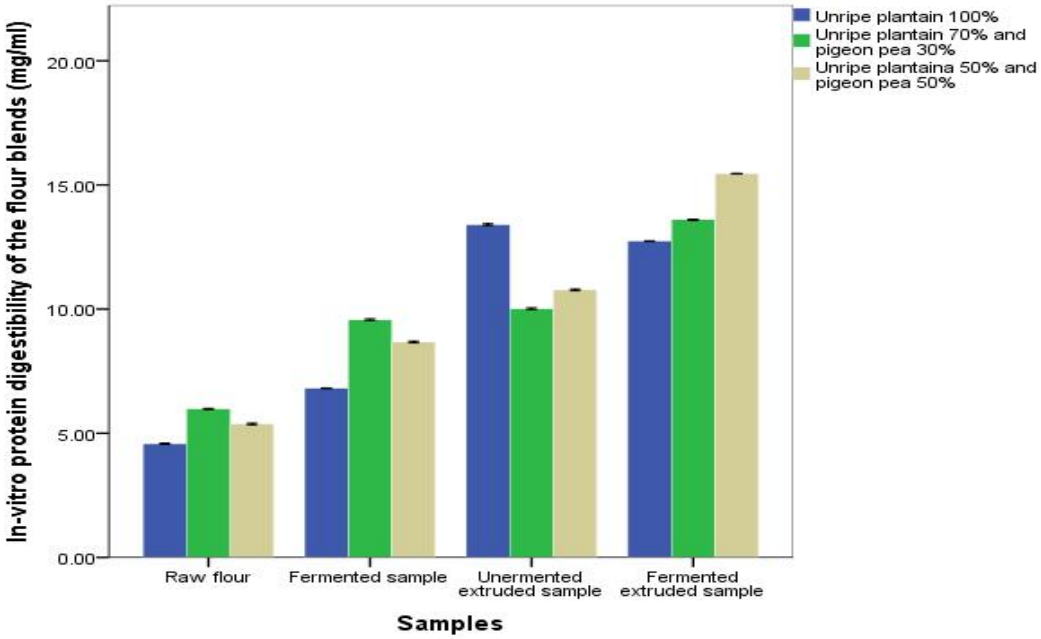

Fig. 3. *In vitro* protein digestibility of the flour blends

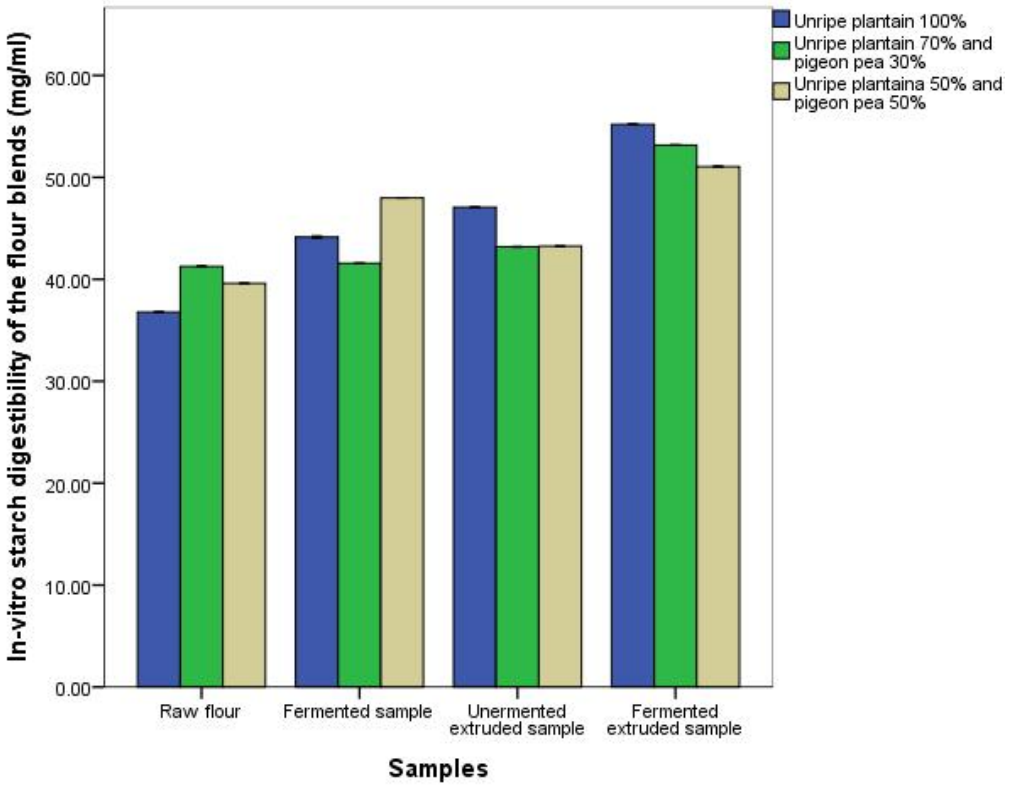

Fig. 4. *In vitro* starch digestibility of the flour blends

5. CONCLUSION

This investigation shows that the blending of unripe plantain and pigeon pea has the potential of producing enriched complementary food for improving the health of malnourished children of developing countries. From the results of this research, it is evident that fermentation and extrusion will produce acceptable products and will go a long way to increase the nutritional, starch and protein digestibility of unripe plantain and pigeon pea.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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