Original Research Article

EFFECTS OF FERMENTATION AND STEAMING ON THE PROXIMATE AND ANTINUTRITIONAL PROPERTIES OF PIGEON PEA FLOUR

ABSTRACT

Pigeon pea is one of the underutilized legumes despite its high nutritional quality. The effect of fermentation and steaming on the quality attributes of commonly consumed legumes have been established but not fully on pigeon pea. This study therefore investigated the effects of fermentation and steaming on the quality properties of pigeon pea flour with the aim of optimizing these processing conditions. Pigeon pea was obtained from Oja-oba market, Ilorin, Kwara state and was identified in Botany Department, University of Ibadan. Response Surface Methodology (RSM) based on 3-level full factorial of the Random sample design was selected to optimize the effects of fermentation and steaming on the quality parameters of pigeon pea flour resulting experimental runs. The quality parameters of the treated pigeon pea flour: proximate (protein, ash, moisture, crude fibre, fat, carbohydrate) and antinutritional factors (Tannin, phytate, trypsin inhibitor and saponin) were determined to see the effects of fermentation and steaming using standard laboratory procedures. Numerical optimization technique was used to obtain the optimum processing conditions for the treated sample. Data were analyzed by ANOVA analysis. The values for moisture content, protein content, fat content, fiber content, ash content, and Nitrogen Free Extract of fermented pigeon pea flour ranged between 10.07 – 14.11%, 23.53-26.51%, 0.86 – 2.89%, 0.97 – 1.34%, 3.35 – 4.43%, and 51.39 – 58.04%, respectively. The antinutritional levels of the pigeon pea flour were reduced as shown from the results, which indicated that pigeon pea flour could be utilized effectively for the production of complementary, confectionary foods or supplemented in legume-cereal based diets.

Keywords: Pre-treatment, Fermentation, steaming, pigeon pea, nutritional properties, antinutritional properties

INTRODUCTION

Pigeon pea (*Cajanus cajan* (L.) ranks fifth in importance among edible legumes in the world (Rachie and Wurster, 2007). Pigeon pea is a locally available, affordable and under-utilized grain legume of the tropics and sub-tropics (Pal *et al.*, 2011). Pigeon pea varieties have protein content in the range of 23 – 26 % (Akporhonor *et al.*, 2006). The protein content is comparable with those of other legumes like cowpea and groundnut which have been used in complementing maize. It is rich in mineral content and fibre content. Pigeon pea grows well in Nigeria but the hard-to-cook phenomenon and the presence of antinutrients have limited its utilization (Fasoyiro *et al.*, 2010a).

Reports showed that pigeon peas contain 17.9-24.3% protein, 20-28.7% carbohydrate, 1.2-8.10% crude fibre and 0.6- 3.8% fat. Pigeon pea is also a good source of calcium, phosphorus, magnesium, iron, and sulphur etc. In spite of its high nutritional qualities, pigeon is not popular in the Western and Northern states of Nigeria. It has no industrial use as at now. Like most tropical legumes, pigeon pea contains anti-nutritional substance, such as trypsin inhibitors and tannins which affects its utilization, especially the raw seeds. They also contain flatulent factors which are present in oligosaccharides such as starchyose, raffinose and verbascose (Amarteifio *et al.*, 2002).

Besides its nutritional value, pigeon pea also possesses various medicinal properties due to the presence of a number of polyphenols and flavonoids. It is an integral part of traditional folk medicine in India, China and some other nations (Saxena *et al.*, 2010). In India, leaves of pigeon pea are used for curing wounds, sores, abdominal tumors and diabetes (Odeny, 2007). Fresh seeds are used to help from incontinence of urine in males, while immature seeds are

suggested for treatment of kidney ailments. Scorched seeds are added to coffee to relieve from headache and vertigo (Saxena *et al.,* 2010). Dried roots of pigeon pea are used as an alexeritic, anti-helminthic, expectorant, sedative and therapeutic agents (Saxena *et al.,* 2010).

Fermentation gives the food longer keeping quality; helps develop flavour and decreases antinutritional factors in foods (Onimawo and Akubor, 2012). Utilization of pre-treated pigeon pea flour for non-traditional and traditional food product is expected to bring out food products of high nutritional value with less anti-nutritional factors and increase the utilization of pigeon pea among the elite and non- elites. Therefore, this study is aimed at evaluating the effects of selected pre-treatment methods on the properties of pigeon pea (*Cajanus cajan* (L.)

Steaming operation works by boiling water continuously, causing it to vaporize into steam; the steam then carries heat to the nearby food, thus cooking the food. The food is kept separately from the boiling water but has direct contact with the steam, resulting in a moist texture of the food. This differs from double boiling, in which food is not directly exposed to steam or pressure cooking, which uses a sealed vessel, but which is capable of pressure steaming or submerging (Subramanian, 2009).

MATERIALS AND METHODS

Materials

The brown pigeon pea (*Cajanus cajan*) used for this research was obtained from Oja-Oba Central market at Ilorin Kwara state and was identified at Botany Department, University of Ibadan, Oyo State. The equipment used were oven, bowls, sieve, attrition mill, trays, mixer, knives, and spoons which were obtained from the Department of Food Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Methods

Experimental Design

The experiment was designed using Response Surface Methodology; Design expert software (version 13.0) to study the effects of fermentation and steaming on the quality of Pigeon pea flour. Fermentation and steaming was selected as pre-treatment while the factors considered were fermentation temperatures of 27 – 30 ^{o}C for time interval of 2-5 days according to Akindahunsi, (2004) and steaming temperatures of 95 – 100 ^{o}C for time interval of 10 – 30 minutes using the modified method of Fasoyiro *et al.* (2010b).

Production of fermented pigeon pea seed into flours

Pigeon pea seeds were fermented using the modified method of Adebowale and Maliki (2011). Raw pigeon pea seeds were sorted by hand picking the dirts, stones and other extraneous materials and one kilogram (1 kg) was weighed. It was washed thoroughly with clean water and thereafter soaked in water (1:3 w/v) at a formulated temperature and time produced by the experimental runs for fermentation to take place. The soaked water was changed on daily basis. At the end of the soaking period, the soaked water was discarded and the pigeon pea rinsed. The fermented seeds were dehulled using mortar and pestle, and the seed coat was separated from the seeds and the dehulled seeds were dried in a hot air oven at 60^{0}C for 8 hours. The dried fermented samples were ground into flour using laboratory blender (Kenwood Blender model BL335). The fine powdered fermented pigeon pea flour was sieved using mesh sieve size of 60 microns to remove dirts and was stored at room temperature (27±2 ^{o}C) in an air tight high density polythene bag for further analysis.

Production of Steamed pigeon pea into Flour

Pigeon pea were steamed using the modified method of Fasoyiro *et al.* (2010b). Pigeon pea was sorted after which one kilogram (1 kg) was weighed and thoroughly washed with clean water. The pigeon pea seeds were placed on perforated stainless steel sieve and steamed over boiling water at formulation temperature (95 ^{o}C, 97.5 ^{o}C and 100 ^{o}C) and time (10, 20 and 30 minutes) produced by the experimental runs. The sample was rapidly cooled using cold water. The steamed seeds were dehulled using mortar and pestle, and the seed coat was removed and the dehulled seeds were oven dried at a temperature of 60 ^{o}C until constant moisture content of was achieved. It was milled and sieved using a mesh sieve size of 60 microns and the flour was stored in an air tight polythene

bag and stored under ambient temperature for further use.

ANALYSES

Determination of proximate composition of fermented and steamed pigeon pea flour samples

Proximate composition of the fermented and steamed pigeon pea flour samples (moisture, protein, fat, ash, crude fibre and carbohydrate) were analyzed according to the official methods of analysis described by the Association of Official and Analytical Chemist (AOAC, 2012).

Anti-nutritional factors determination of the fermented and steamed pigeon pea flour samples

a) Saponin content

The spectrophotometric method of Brunner (1994) was used for Saponin determination. Two (2) g of the finely grinded sample was weighed into a 250 ml beaker and 100 ml of Isobutyl alcohol or (But-2-ol) was added. Shaker was used to shake the mixture for 5 hours to ensure uniform mixing. The mixture was filtered with No 1 Whatman filter paper into 100 ml beaker containing 20 ml of 40% saturated solution of magnesium carbonate ($MgCO_3$). The mixture obtain again was filtered through No. 1 Whatman filter paper to obtain a clean colorless solution. 1 ml of the colorless solution was taken into 50 ml volumetric flask using pipette, 2 ml of 5% iron (iii) chloride ($FeCL_3$) solution was added and made up to the mark with distill water. It would be allow standing for 30min for the color to develop. The absorbance is read against the blank at 380 nm. The saponins content was determined by difference and calculated as a percentage of the original sample thus:

$$\%\ \textbf{Saponin} = \frac{W_1 - W_2}{\textit{Weight of samples}}\ X\ \ 100$$

Where: W^1 = *Weight of evaporating dish,* W^2 = *Weight of dish and s*

b) Phytate content determination

The phytate contents of the samples were determined using the method of Inuwa *et al.* (2011). Two (2) g of each finely ground flour sample was soaked in 20ml of 0.2N HCL and filtered. After filtration, 0.5 ml of the filtrate was mixed with 1 ml ferric ammonium sulphate solution in a test tube, it was boiled for 30 minutes in a water bath, cooled in ice for 15 minute and centrifuge for 15 min. one millimeter of the supernatant was mixed with 1.5 ml of 2.2-pyridine solution and the absorbance measured in a spectrophotometer at 519 nm. The concentration of phytic acid was obtained by extrapolation from a standard curve using standard phytic acid solution.

c) Oxalate content

Titration method described by Inuwa *et al.*, (2011) was used to determine the oxalate content. One gram of the sample was weigh into 100 ml conical flask where 75 ml 3 N_2SO_4 was added and stir intermittently with a magnetic stirrers for 1 hr. It was filtered using whatman No. 1 filter paper. From the filtrate, 25 ml was taken and titrated while hot (80 – 90 ^oC) against 0.1 N $KMnO_4$ solution until a faint pink color persisted for at least 3 seconds.

d) Trypsin inhibitor

The method Onimawo and Akubor, (2012) was used in the determination of the trypsin inhibitor. 0.5 g of the sample was dispensed in 50 m/s of 0.5 m/s sodium chloride solution and shaken for 30 minutes at room temperature. The mixture was centrifuged and supernatant was used as the extract. Assay for trypsin activity involved mixing a portion (1ml) of the extract with 90 m/s of 0.03% trypsin substrate in a test tube containing 1 ml of 0.6% trypsin enzymes solutions after mixing, the mixture was allowed to stand for 15 min before it absorbance was read at 410 nm in a spectrophotometer. A control, which consists of 1 ml enzyme solution in 9m/s of trypsin substrate but not extract, was set up as described above. The absorbance for the control was now measured. Trypsin inhibitor will be calculated by using the formula:

$$\%\ \textbf{Fibre} = \frac{1\ x\ au - as\ x\ vf}{w\ 0.01\ va}\ \ X\ \ 100$$

3.15

Where:

w = *weight of sample*
au = *Absorbance of sample at* 410 nm
as = *Absorbance of control*
vf = *Total extract volume*
va = *Volume of extract analyzed*

e) Tannin content of the samples

The method Onimawo and Akubor, (2012) was used in the determination of the tannin. About 0.2 g of finely ground sample was weighed into a 50 ml sample bottle. 10 ml of70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 hours at 30^{o}C. Each solution was then centrifuge and the supernatant store in ice. 0.2 ml of each solution was pipetted into the test tube and 0.8 ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. 0.5 ml of Folincio cateau reagent was added to both sample and standard followed by 2.5 ml of 20% $Na_2CO_3$the solution was then vortexes and allowed to incubate for 40 minutes at room temperature, its absorbance was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve.

Statistical analysis

The data collected was subjected to statistical analysis using Statistical Package for Social Science (SPSS) version 21.0. Analysis of variance (ANOVA) was used to determine the differences at 5% level of significance. In cases where differences occurred, the means was separated using turkey's test according to Rattanathaanalerk *et al*. (2005).

RESULTS AND DISCUSSION

Effect of fermentation as pre-treatment on proximate composition of pigeon pea flour

The proximate composition of the fermented pigeon peas was assessed and compared for different fermentation temperatures and fermentation days (table 1). For responses where the same letter appears in the same column, there is no significant different, the standard error was also determined and presented. The two level of the central composite design (CCD) was selected to optimize the effect of fermented pigeon pea flour on quality parameters (proximate composition).

The values for moisture content, protein content, fat content, fibre content, ash content and NFE (Nitrogen Free Extract) were 10.07 – 14.11, 23.53 - 26.51, 0.86 – 2.89, 0.97 – 1.34, 3.35 – 4.43, and 51.39 – 58.04%, respectively. Pigeon pea samples fermented at 30 ^{o}C for 5 days and 30 ^{o}C for 3.5 days had the highest levels of protein (26.51%) and ash (4.43%). The Pigeon pea sample fermented at 30°C for five (5) days had the lowest value of fat content (0.86). Significant difference ($p<0.05$) was observed among the entire samples' of the moisture contents. However, no significant difference ($p > .05$) was shown in the moisture content of the pigeon pea fermented at 30, 27.50 and 25 ^{o}C for 3.5 and 2 days respectively; whereas 30, 27.50 and 25 ^{o}C for 5 days were different ($p<0.05$) significantly from each other. Again, after two days of fermentation at 30 and 25 ^{o}C, the fermented pigeon pea flour sample showed no significant difference ($p<0.05$) in terms of moisture, fat, fibre and NFE content. Regardless of the fermentation conditions used in this study, the protein content of the pigeon pea flour sample showed a significant difference at ($p < .05$).

Moisture content, fat content and fibre content all increased as fermentation temperature and fermentation day increase. As the period or days of the fermentation process increased, a decline in the value of NFE was observed, while an increase in the protein content and ash content was also observed. As fermentation temperature and duration increases, ash content decrease.

The low moisture content observed in the fermented pigeon flour samples is desirable since it inhibits the growth of pathogenic microbes, resulting in a longer shelf life for the flour product. It has been commonly observed that high moisture content in any food product promotes the growth and multiplication of microorganisms, resulting in food deterioration. This always reduces the shelf life of food products (Adepeju *et al.,* 2015; Ahaotu *et al.*, 2021; Arukwe, 2021). The fermentation of pigeon pea by Igbabul *et al.* (2012) and Torres *et al.* (2006) demonstrated a comparable increase in moisture contents observed in this study.

Furthermore, the body uses protein for synthesis, tissue and cell maintenance, growth and healthy life. Nkhata *et al.* (2018) and Anaemene and Fadupin, (2022) claim that it is unpredictable how fermentation will influence a

food's protein content. In this study, the pigeon pea was found to have a high protein content, which is important for diets in developing countries like Nigeria where many people cannot afford protein-rich meals, especially fish and meat, due to cost and poverty. In this study, it was discovered that the pigeon pea's crude protein content increased significantly during the fermentation process. According to Onweluzo and Nwabugwu (2009), fermented grains have greater protein content.

Protein levels in this study may have increased due to the hydrolysis of lipids and carbohydrates for energy, which resulted in a loss of dry matter. However, Pranoto *et al*. (2013) reported that complex proteins can be broken down by microorganisms into peptides and amino acids, which could have increased the amount of protein in the processed pigeon pea. Furthermore, protein synthesis by fermenting microorganisms may be responsible for the increase in protein brought on by fermentation pre-treatment.

This result was consistent with the findings of Oshodi *et al*. (1999), who observed an increase in the protein content of beniseed flour made from fermented beniseed. According to Uwaegbute *et al*. (2000), the increase in protein value caused by fermentation could be linked to net protein synthesis by fermenting bacteria, which may have resulted in the production of some amino acids during protein synthesis.

This increase in protein offers nutrient value to fermented pigeon pea flours. Furthermore, the effects are time-dependent, as highlighted by Onweluzo and Nwabugwu (2009) and Anaemene and Fadupin, (2022). They reported that twenty-four (24) hours of fermentation reduced crude protein content in both pigeon pea and millet flours, but a significant increase occurred after seventy-two (72) hours. This is comparable to what was observed in this study, when a slight decrease was found within the first two days of fermentation. Again, the decrease in crude protein observed during fermentation was attributed to a growth in the number of microorganisms that utilizes protein for metabolism.

Microorganisms hydrolyse proteins during fermentation, releasing free amino acids that can be utilized to synthesize new proteins. These findings are consistent with the findings of Onweluzo and Nwabugwu (2009), who claimed that fermentation process had no significant effect on the total protein content or amino acid composition of the substrates. In addition, the increase in protein content during the fermentation period may indicate the net protein synthesis that occurs when fermenting seeds, which may have produced some amino acids during protein synthesis (Igbabul *et al.,* 2014).

In this study, the fat content increased and slightly decreased. Legumes store energy in the form of starch rather than fat, the low-fat content of the samples in this study was expected. Low fat values are beneficial for extending the shelf life of food since excessive fat content raises the risks of rancidity, according to Ilelaboye and Jesusina (2021). A product's shelf stability may be negatively impacted by a high fat content due to the development of rancidity. According to Mbaeyi-Nwoha and Obetta (2016), fermentation increased millet's fat content while decreasing pigeon pea's fat content. The increase in fat content as fermentation temperature and days increased could be explained by increased activity of lipolytic enzymes, which hydrolysed fat to glycerol and fatty acids.

Similar findings were observed by Onweluzo and Nwabugwu (2009). Similar to fermented pigeon pea, the decrease in fat content may be explained by lipolytic enzymes that are released during fermentation and hydrolyse fat to glycerol and fatty acids, as previously mentioned. There is a chance that some of the microorganisms have lipolytic properties. According to Olasupo *et al*. (2016), nearly 97% of the proteolytic Bacillus species engaged in the fermentation of some bean seeds were also lipolytic. It however appears that the rate of these lipolytic reactions notably declined as fermentation progressed, since the reduction in fat content, aside being significant after the first day of fermentation, remained almost the same throughout the rest of the period.

The findings of this study are compatible with the findings of Adebowale and Maliki (2011) and Balogun *et al*. (2021), who showed a decrease in the fat content of pigeon pea seed flour with increasing fermenting period. Additionally, the observed increase in fat in the flour samples may have been as a result of fermenting microbes releasing some non-lipid extractable compounds. The increase in crude fat may have influenced the considerable increase in metabolizable energy found in the flour samples after 72 hours of fermentation. Another study (Chinma *et al.,* 2009) reported that fermentation decreased fat content. The

decreased fat content of fermented pigeon pea flour could be due to increased lipolytic enzyme activity, which hydrolysed fat components into fatty acid and glycerol.

Fibre helps in lowering the blood cholesterol level and slows down the process of absorption of glucose, thereby helping in keeping the blood glucose level in control. Fiber also has useful role in providing roughages and aids digestion (Bolaji *et al.,* 2021). It also reduces the risk of cardiovascular disease but the increase in consumption might have contributed to the reduction in the incidence of certain diseases such as diabetes, coronary heart diseases, colon cancer and various digestive disorders (Onyango *et al.,* 2020). The fibre content increased in this study as the fermentation temperature and days increased.

This result contradicts the findings of Onweluzo and Nwabugwu (2009), who reported that crude fibre (2.2%) levels in pigeon pea decreased to 1.4%. It has also been claimed that fiber degradation by some fermenting bacteria may have contributed to the decrease in fibre content (Liang *et al.,* 2008). According to Nwanekezi *et al*. (2017) the increase in fibre observed in this study could be attributed to the depletion of carbohydrate and fat contents of the flour as a result of the fermentation process, which increases the ratio of crude fibre in the flours. Previously, Igbabul *et al.* (2014) reported that fermentation increases the crude fibre content of Afzelia *africana*.

In general, a food product's ash content indicates the amount of minerals contained in the product (Adepeju *et al*., 2015). Total ash in pigeon pea flour was significantly reduced as fermentation days increased in this study. However, Adebowale and Maliki (2009) reported over 20% increase. The variable factor could be the difference in fermentation process. Again, the observed decrease in ash was attributed to potential losses of dry matter and volatiles during fermentation (Nnam and Obiakor, 2003). Furthermore, the increase in ash content of fermented pigeon pea flours is consistent with the findings of Enujiugha *et al*. (2003) and Chinma *et al.* (2009).

The decrease in fermented pigeon pea carbohydrate content was consistent with the findings of Falmata *et al.* (2014) and Mbaeyi-Nwoha and Obetta (2015). This could be because of increased activity of microbial enzymes such alpha amylases, which degraded the complex carbohydrate into simple sugars (Sade, 2009). Fermentation is a metabolic process in which a carbohydrate, such as starch or sugar, is converted into alcohol or acid by an organism. As a result, the NFE, which is essentially carbohydrate, is the most desired substrate on which the microbe acts and its concentration will decrease as the fermentation process progresses. The decrease in NFE with fermentation is projected to result in a decrease in flour gross energy (Abd El-Hack *et al.,* 2018).

Effect of fermentation as a pre-treatment on anti-nutritional properties of pigeon pea

Table 2 shows the values for Tannin, Phytate, T-Inhibitor and Saponin contents of the fermented flour samples at various temperatures and periods. The values of these responses were observed to be in the ranges 51.95 – 93.96 mg/100g, 4.62 – 6.13 mg/100g, 86.72 – 275.21mg/100g and 2.18 - 4.32 % respectively. The lowest tannin (51.96 mg/100g) and phytate (4.62 mg/100g) contents were observed in samples fermented at 25 ^{o}C for 5 days and 3.5 days, respectively. The result of ANOVA further reveal that the response surface model generated were found insignificant ($p<0.05$) for all the parameters.

Anti-nutritional factors are compounds or substances produced by the normal metabolism of species in natural food stuffs that serve to diminish nutrient intake, digestion, absorption, and utilization, as well as have a number of other adverse effects (Abbas and Ahmad, 2018). They include phytic acid, tannins, polyphenols, trypsin, chymotrypsin, a-amylase inhibitors, and hemagglutinin activity (Alonso *et al.,* 2000), as well as flatulence-causing oligosaccharides (Udensi *et al.,* 2007). Additionally, these constituents have an impact on appetite, nutrient absorption, metabolism and the bioavailability of certain minerals. Most legumes contain tannin, a significant anti-nutritional factor. It is characterized by a bitter polyphenolic compound that binds to proteins and a number of other organic compounds, including alkaloids and amino acids, to form precipitates (Redden *et al.,* 2005). Numerous reports like (Uzoechina, 2007) had explained how tannins affect the availability of nutrients. By forming insoluble complexes with proteins and carbohydrates, tannins reduce their digestibility and flavour. They decrease the minerals' bioavailability as well (Osagie, 1998).

According to Ene-Obong (1995) and Onwurafor *et al*. (2014), fermentation has the potential to reduce tannin contents. The activity of seed-associated enzymes may be responsible for the reduced tannin content in the fermented product. In addition, Ojha *et al.* (2018) revealed that phytate acyl hydrolases and microbial activity were responsible for the decrease in tannin content. Reduction in tannin content and were attributed to the rearrangement and depolymerization of the tannin structure, according to Adebo *et al.* (2018). This is due to the fermentation medium's acidic environment, reduced extractability, self-polymerization, interactions between tannin and other macromolecules (such starch and AAs), and the potential of LABs to metabolize tannins. In line with these research' findings, the fermentation process causes the production of enzymes like tannase, which reduces and/or eliminates tannins (Espinosa-Páez *et al.*, 2017).

According to recent research, the hydrolysis of ester and peptide bonds by tannase during the fermentation of legumes reduces tannins and yields glucose and gallic acid (Omojokun and Jokoh, 2020). A lower pH, as that obtained during the fermentation of legumes, facilitates this enzymatic breakdown of tannins. Some studies have suggested that the water solubility of tannins, which would result in their leaking out into the fermenting media like all other polyphenolic compounds, may also contributes to their reduction during fermentation (Omojokun and Jokoh, 2020).

The observed tannin decrease with increased fermentation days are in close agreement with the research findings of Onweluzo and Nwabugwu (2009). Phytic acid is one of the most important anti-nutritional factors for human health and nutrition (Kumar *et al.*, 2010). Phytic acid is present in legume seeds at the harvest stage and has a negative charge, which causes it to interact with minerals such as iron, zinc, calcium and magnesium to form an insoluble complex (Abbas and Ahmad 2018).

It also interacts with proteins and starch to form complexes (Oatway *et al.*, 2001). The observed decreases in phytate with increasing fermentation days are in agreement with Khetarpaul and Chauhan's (1990) report for fermented black gram. Mulimani *et al*. (2003) also observed that fermentation reduced the phytic acid content of soybean by one-third. The observed reduction in phytate levels was attributed to phytase activity during fermentation. Phytase dephosphorylates phytate through a series of processes that results in the synthesis of inositol and phosphoric acid, according to Fardiaz and Markakis (1981). Certain minerals, such as phosphorus, are known to be released throughout the process, boosting their availability, they limit protein digestion in the body (Balogun *et al.*, 2019).

Mosha and Gaga (1999) claim that an inhibitor is an organic compound that can reduce enzyme activity by binding to the enzyme or rendering it unavailable to substrate, interfering with enzyme production, or influencing a hormone, which alters the level of enzyme activity. They are naturally occurring chemical substances that interact with proteolytic enzymes, specifically trypsin, leaving them inactive for protein digestion. As a result, the inhibitors lower protein bioavailability and contribute to the low nutritional quality of human diets.

Plants are rich in trypsin inhibitors. The fermentation conditions employed in this study, however, decreased these inhibitors in pigeon pea seeds. The saponin reduction was most likely induced by enzymatic hydrolysis, whereas the fermentation reduction was driven by microbial degradation (Nwanekezi *et al*., 2017). Fermentation, for example, has been shown to lower the anti-nutritional content of bean flours (Edema and Sanni, 2006).

Effect of steaming as a pre-treatment on proximate properties of pigeon pea

The steamed pigeon pea flour characteristics with different steaming temperature and steaming time were determined and compared using one way ANOVA (Table 3). The moisture content, protein content, fat content, fiber content, ash content and NFE were found in the range 9.86 -11.44 %, 21.48-23.89 %, 1.70 - 3.94%, 1.08 -1.83 %, 1.94 -3.15 %, and 59.76 - 62.59 %, respectively. The highest protein (23.89%), fiber (1.83%) and ash content (3.15 %) were detected in pigeon pea sample that were steamed at a temperature of 95 ^{o}C for 30 mins, and 97.5 ^{o}C for 10 min, and 95 ^{o}C for 10 mins, respectively. The least value (1.70) of fat content was found in pigeon pea flour sample steamed at 95 ^{o}C for 30 min. The significance of each coefficient was determined using Fisher's P-value.

The moisture content was observed to decrease and then increase with increase in steaming

temperature. A decrease was observed in protein content as the steaming temperature and the time increased. Fat content was observed to increase with increase in steaming temperature but remain unchanged as the steaming time progressed, however, an increase in the fibre content was observed as the steaming temperature increased whereas a slight decrease was observed as the steaming time increased. Meanwhile, the ash content was observed to decrease with increase in steaming temperature but remain unchanged with increase in steaming time. NFE, on the other hand, was observed to increase with increase in steaming temperature but experience as slight decrease as the steaming time increased.

The decrease in the moisture content of steamed pigeon pea flour samples was due to increase in temperature and increase in surface due to continuous stirring. Increase in surface area relate to increase in moisture lost therefore, decrease in moisture content of the sample can be attributed to this factor. This result agrees with the observation by Eke-Ejiofor and Kporna (2019) and Akajiaku *et al.* (2014) who reported a decrease in the moisture content of cowpea. Reduced moisture content of the sample ensures the inhibition of microbial growth, hence an important factor in food preservation. This may ensure high storage quality of the food product (Sakpo and Osundahunsi, 2016).

Reduction in protein content could be attributed to several factors such as leaching, the formation of insoluble complexes with tannins (Adamczyk *et al*., 2017) and thermal denaturation that leads to degradation or reaction with other components. Fat content usually plays a role in the shelf-life stability of food products (Mixer *et al.*, 2011). High-fat content could impact negatively on the shelf stability of a product due to rancidity development (Olatunde *et al.*, 2019).

Fat plays a significant role in the shelf life of food products and as such relatively high fat content could be undesirable especially in baked food products. This is because fat can promote rancidity in foods, leading to development of unpleasant and odorous compounds. The result obtained for steamed sample agrees with the findings by Ogundele *et al.* (2015) who reported 1.91% for steaming pudding.

High fibre is of great benefit to the body, as it helps to maintain bowel integrity, lower blood cholesterol level, and control blood sugar level. The consumption of this product has potentials to provide an appreciable amount of fibre to the body for proper functioning of the digestive and excretory systems. Crude fibre is known to aid the digestive system of human indicating that the snacks could attract good acceptability by many people as well as health organizations. The increase may be due to formation of protein-fibre complex as similarly reported by Mittal *et al.* (2012).

Ash content may be associated with the amount of mineral present in a sample, and hence the decreased crude ash recorded in this study for steamed sample apparently suggests a reduction in mineral content. The decrease in the ash content of the steamed sample may be attributed to leaching losses, especially during steaming process (Ouazib *et al.*, 2015). Ash content of steamed pigeon pea flour obtained from this study is in accordance with the findings by Ehimen *et al.* (2017) and Akusu and Kinn-Kabari (2012) who reported 1.17% and 1.39% respectively for moi-moi prepared using steamed cooking method. The decrease in Nitrogen Free Extract (NFE) recorded in the steamed sample was a result of leaching of some nutrients into the boiling water (Enwere, 2016; Olu *et al.*, 2017).

Effect of steaming as a pre-treatment on anti-nutritional properties of pigeon pea flour

Table 4 showed the values for tannin, phytate, T-Inhibitor, and saponin contents of the steamed samples at various temperatures and periods. The values of these responses were observed to be in the ranges 31.93-62.00 mg/100g, 4.59-5.87 mg/100g, 25.34-75.11 mg/100g, and 1.95-3.38%, respectively. The lowest tannin (31.93 mg/100g) and phytate (4.59 mg/100g) contents were observed in samples steamed at 97.5 ^{o}C for 30 and 20 minutes, respectively.

The tannin content was observed to steadily increase with increase in steaming temperature as steaming time increased. However, the phytate content was observed to steadily decrease with increase in steaming temperature and steaming time increase. Also, the T-Inhibitor content was observed to decrease with increase in steaming temperature but remained unaffected with respect to the steaming time increase. On the other hand, the saponin content was observed to decrease with increase in steaming temperature and steaming time.

Tannins have adverse effects on biological systems because they form insoluble complexes with protein. Thus, tannins decrease protein digestibility and palatability (Hossain and

Becker, 2001). Furthermore, other adverse effects related to tannins include damage caused to the intestinal tract, toxicity of tannin absorbed from the gut and interference with the absorption of iron leads to a potential carcinogenic effect (Osagie and Eka, 1998). The decrease in tannin content of the samples as the steaming period progressed could be attributed to the destruction of the contents as the heat treatment process progressed (Awika and Duodu, 2016) or formation of insoluble complexes between phytate and other components, such as protein and minerals (Siddhuraju and Becker, 2001). Yadav *et al.* (2011) also reported similar findings of the decreased amount of tannin content upon steaming of pearl millet samples.

Phytic acid in climbing beans reduces protein digestibility and availability of minerals by chelating mineral cations such as Zn^{2+}, Fe^{2+}, Ca2+ and interacts with proteins to form insoluble complexes (Oatway *et al.*, 2001). On the other hand, when phytate is used at low level (0.1-0.9%) as reported by Yoon *et al.* (1983), it contributes to anti-carcinogenic and antioxidant properties and reduces blood cholesterol response (Minihane and Rimbach, 2002). In addition, phytate also could be used in reducing triglycerides and prevention of renal stone development (Kumar *et al*, 2010).

The decrease in the phytate contents under the steaming conditions is similar to the observation of Yadav *et al* (2011) on steamed pearl millet samples and Maheesh *et al* (2015) in a 52% reduction in phytate content of beans, 56.50 % in sunflower and 47.90 % in rice grains. This decrease in phytate during heat treatment would be the result of their degradation by heat due to their thermolabile nature (Puwandari *et al.*, 2021). However, López-Martínez *et al.* (2017) observed an increase in phytic acid content of faba bean seeds upon cooking and attributed the increase to the heat-stable nature of phytic acid in faba bean seeds.

Trypsin inhibitors are anti-nutritional factors that inhibit the action of trypsin and pepsin in the gut and subsequently prevent absorption of protein (Tanwilson *et al.*, 1987). Steaming is known to possess pronounced effect on reduction of trypsin inhibitors (Kaur *et al.*, 2012). The reduction in Trypsin inhibitors on steaming might be because of its heat-labile nature. Similar observation was observed by Kaur *et al.* (2012) on cereal bran. Moist heat proved most effective than dry heat in destroying trypsin inhibitor activity (Carlini and Udedibie, 1997).

A study conducted by Barakat *et al.* (2015) showed that when chickpea flour is incorporated into broccoli-based bars, it loses 18–59% of its total saponin content. Studies have shown the potential to reduce the risk of cardiovascular diseases and obesity in humans when consuming a diet rich in saponins. Therefore, chickpea is a desired source of saponins in the preparation of nutraceutical and functional foods (Chan *et al.* 2014; Güçlü-Üstündag˘ and Mazza, 2007). The reduction in the saponin content of the samples upon steaming could be due to either the destruction of these compounds or chemical rearrangements such as the binding of phenolics with other organic substances (Volf *et al.* 2014) or the leaching of water-soluble phenolics into the cooking water. Similar observation was also reported by Xu and Chang (2009).

Conclusion

This study examined the effects of fermentation and steaming on the proximate composition and antinutritional factors of pigeon pea flour. The central composite design (CCD) of RSM has been used successfully to optimize the pigeon pea flour. The relationship between the independent variables and the dependent variables (proximate and anti-nutrients composition) investigated showed that there were significant differences in all the parameters investigated. The results obtained have shown that fermentation and steaming significantly improved the nutritional quality and reduced the antinutritional properties. Owing to this, its effective utilization in complementary, confectionary foods or other legume-supplemented diets is feasible. The optimum conditions for the variables investigated for producing fermented pigeon pea flour with suitable quality characteristics were fermentation temperature of 27 ^{o}C for 3.5 days and steaming at 100 ^{o}C for 20minutes.

Contribution to Knowledge

At the end of this research, the most effective fermentation and steaming methods for processing high quality pigeon pea flour was established. The effects of the fermentation and steaming processes on the proximate composition and antinutritional properties of the

pigeon pea flour was determined and established. The optimum processing variables were also established.

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Table 1: Effect of fermentation as pre-treatment on proximate composition of pigeon pea

Fermentation Temperature (^{0}C)	Fermentation Days	Moisture Content	Crude Protein	Crude Fat	Crude Fibre	Total Ash	NFE
			Proximate (%)				
27.50	3.50	10.07±0.18^{a}	23.53±0.01^{a}	2.89±0.15^{f}	1.23±0.03^{bc}	4.26±0.02^{e}	58.04±0.33^{e}
30.00	3.50	10.73±0.26^{b}	23.81±0.06^{b}	2.61±0.03^{e}	1.29±0.03^{cd}	4.43±0.01^{f}	57.13±0.38^{d}
27.50	2.00	10.77±0.16^{b}	25.52±0.05^{ef}	2.32±0.04^{d}	1.21±0.04^{b}	4.40±0.00^{f}	55.79±0.11^{c}
25.00	3.50	11.16±0.06^{b}	24.90±0.09^{c}	1.54±0.09^{b}	0.99±0.06^{a}	3.60±0.04^{b}	57.82±0.09^{de}
25.00	5.00	11.86±0.12^{c}	25.30±0.02^{d}	1.84±0.07^{c}	1.30±0.02^{cd}	3.35±0.03^{a}	56.37±0.06^{c}
27.50	5.00	12.67±0.18^{d}	25.40±0.02^{de}	1.54±0.09^{b}	0.97±0.01^{a}	3.40±0.02^{a}	56.04±0.28^{c}
30.00	2.00	13.51±0.66^{e}	26.51±0.05^{h}	1.01±0.03^{a}	1.16±0.02^{b}	4.03±0.06^{c}	53.80±0.72^{b}
25.00	2.00	13.40±0.05^{e}	25.55±0.06^{f}	0.87±0.04^{a}	1.21±0.03^{b}	4.14±0.04^{d}	54.54±0.35^{b}
30.00	5.00	13.82±0.04^{ef}	25.68±0.06^{g}	0.86±0.03^{a}	1.34±0.04^{d}	4.22±0.04^{e}	54.10±0.06^{b}
27.50	3.50	14.11±0.03^{f}	26.49±0.08^{h}	2.61±0.03^{e}	1.22±0.02^{b}	4.23±0.03^{de}	51.39±0.13^{a}

Values are means ± Standard Deviation (SD)
Different letters in the same column represent significant differences ($p < 0.05$)
NFE= Nitrogen Free-Extract

Table 2: Effect of fermentation temperature and fermentation time on anti-nutritional properties

Fermentation temperature (℃)	Fermentation days	Tanin content (mg/100)	Phytate content (mg/100)	Trypsin Inhibitor (Mg/100)	Saponin content (%)
27.50	3.50	74.261±0.17^{f}	4.92±0.10^{c}	102.39±0.18^{b}	3.71±0.25^{cd}
30.00	3.50	79.686±0.02^{g}	5.81±0.05^{f}	150.24±0.85^{e}	2.18±0.04^{a}
27.50	2.00	79.891±0.06^{g}	5.06±0.08^{cd}	201.24±0.97^{h}	2.36±0.06^{a}
25.00	3.50	93.96±0.07^{h}	4.62±0.02^{b}	148.70±1.01^{e}	3.91±0.22^{de}
25.00	5.00	51.95±0.18^{a}	5.74±0.10^{e}	134.82±0.25^{c}	3.58±0.04^{c}
27.50	5.00	53.85±0.08^{b}	5.45±0.01^{e}	176.83±0.33^{f}	3.06±0.17^{b}
30.00	2.00	60.15±0.52^{c}	5.45±0.09^{e}	275.21±0.20^{i}	4.08±0.05^{ef}
25.00	2.00	63.67±0.30^{e}	4.96±0.10^{c}	197.49±0.73^{g}	4.01±0.08^{e}
30.00	5.00	61.97±0.19^{d}	5.18±0.02^{d}	86.72±1.66^{a}	4.08±0.02^{ef}
27.50	3.50	74.60±0.46^{f}	4.13±0.09^{a}	102.89±0.41^{b}	3.77±0.05^{d}

Values are means ± standard deviation (SD)
Different letters in the same column represent significant differences ($p < 0.05$)

Table 3: Effect of steaming process as a pre-treatment on proximate composition of pigeon pea

		Proximate composition (%)					
Steaming Temp (℃)	Steaming time (mins)	Moisture Content	Crude Protein	Crude Fat	Crude Fibre	Total Ash	NFE
95.00	20	9.86±0.4	22.04±0.10	2.60±0.01	1.11±0.23	2.73±0.34	62.59±0.07
97.50	20	9.88±0.02	22.23±1.45	2.79±0.76	1.29±0.44	2.35±0.87	61.47±0.89
95.00	10	10.05±1.24	22.18±0.56	2.33±0.99	1.43±0.21	3.15±0.99	61.95±0.56
97.50	30	10.51±0.45	21.92±0.87	3.94±0.65	1.25±0.56	1.94±0.5	61.89±0.34
100.00	30	10.84±-0.2	21.88±0.98	2.12±0.43	1.08±-1.05	2.06±0.03	62.02±0.21
100.00	10	10.54±0.2	22.12±0.45	2.42±0.56	1.12±1.80	2.61±0.07	61.19±0.11
97.50	10	10.81±0.02	21.48±0.12	2.42±0.32	1.83±0.34	1.95±0.06	62.45±0.66
95.00	30	10.78±0.01	23.89±0.43	1.70±0.14	1.32±0.78	2.53±0.31	59.76±0.02
100.00	20	11.44±1.45	22.58±0.67	2.54±0.16	1.28±0.73	2.40±0.02	60.54±0.04
97.50	20	9.87±1.56	22.51±1.54	2.51±0.48	1.31±0.78	2.31±0.07	62.52±0.76

Values are means ± standard deviation (SD)
Different letters in the same column represent significant differences ($p < 0.05$)
NFE = Nitrogen free extract

Table 4: Effect of steaming process as a pre-treatment on anti-nutritional properties of pigeon pea

Steaming temp (^{o}C)	Steaming time (mins)	Tannin (mg/100g)	Phytate (mg/100g)	T-Inhibitor (mg/100g)	Saponin (%)
95.00	20	34.27±0.36^{b}	5.38±0.02^{b}	47.50±0.88^{e}	2.09±0.04^{a}
97.50	20	50.19±0.46^{f}	4.84±0.24^{a}	46.58±0.43^{e}	2.71±0.06^{c}
95.00	10	47.65±0.10^{e}	5.87±0.03^{c}	74.94±0.19^{g}	3.02±0.11^{d}
97.50	30	31.93±0.01^{a}	4.60±0.02^{a}	25.34±0.69^{a}	1.95±0.05^{a}
100.00	30	42.25±0.52^{c}	5.29±0.02^{b}	40.21±0.59^{c}	3.35±0.02^{e}
100.00	10	60.00±0.02^{h}	4.66±0.10^{a}	47.07±0.06^{e}	2.81±0.09^{c}
97.50	10	56.03±0.19^{g}	5.27±0.12^{b}	28.69±0.27^{b}	2.80±0.11^{c}
95.00	30	62.00±0.08^{g}	4.91±0.17^{a}	75.11±0.08^{g}	3.38±0.04^{e}
100.00	20	46.13±0.29^{d}	5.52±0.14^{b}	49.10±0.37^{f}	2.48±0.03^{b}
97.50	20	50.07±0.07^{f}	4.59±0.21^{a}	43.99±0.12^{d}	3.09±0.03^{d}

Values are means ± standard deviation (SD)
Different letters in the same column represent significant differences ($p< 0.05$)