

Effects of Combined Processing Methods on Protein Qualities of Pigeon Pea (*Cajanus cajan*) Flour Samples Using Rat Feeding

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Abstract: Combined effects of different processing methods of pigeon pea on the protein qualities of formulated diets using rat feeding were investigated. Eight sets of diets were formulated from pigeon pea flour sample that was soaked (control i.e. diet S₀PPF), soaked and boiled (diet S₀BPPF), soaked and fermented (diet S₀FPPF), soaked, boiled and fermented (diet S₀BFPPF), soaked and sprouted (diet S₀S_pPPF), soaked, sprouted and boiled (diet S₀S_pBPPF), soaked, sprouted and fermented (diet S₀S_pFPPF) and soaked, sprouted, boiled and fermented (diet S₀S_pBFPPF). The formulated diets were subjected to proximate analysis. A 14-day rat feeding experimental study was conducted to assess the combined effects of sprouting, boiling and fermentation of pigeon pea on the protein utilization in rats. The albino rats were divided into eight sets for consumption of the eight sets of diets. The formulated diets proximate results showed that the proteins are above that recommended by PAG (1971) for weaning foods. The feed intake and weight gain/loss results showed that rats fed diet S₀S_pFPPF had the highest feed intake and highest percentage weight gain (11.62g and 20.3%) followed by rats fed on diet S₀S_pBFPPF. Protein qualities result showed that rats fed diet S₀S_pFPPF had the highest nitrogen intake, nitrogen balance, apparent digestibility, PER, NPU and BV. Therefore, combined soaking/sprouting/fermentation and soaking /sprouting /boiling/fermentation are the best methods of processing pigeon pea.

Keywords: Protein Quality, Formulated Diets, Rat Feeding, Feed Intake, Weight Gain

1. Introduction

Legumes are rich in protein and can be used to substitute for the animal protein that has become scarce and unaffordable by the poor majority of the populace. Legume flour has been reported to be very useful and can be utilized to overcome the problem of shortage of animal protein [23]. Nutritionists have equally advocated for increased consumption of food legumes [21]. Many nutritious foods are produced in Nigeria from legumes like cowpea that are widely cultivated. The popularity of cowpea tends to obscure the importance of other legumes [7].

Pigeon pea is a legume commonly referred to as “fiofio” in the south eastern part of Nigeria. Studies have shown that it

has high protein content and its protein is rich in lysine – a sulphur containing amino acid which is limited in other legumes and cereals.

Malnutrition is becoming an endemic dietary problem in Nigeria [19]. This is characterized by protein-energy malnutrition (PEM) especially among children in the low income family that cannot afford animal protein. Therefore, there is need for a high quality protein food to feed children at weaning age in order to help alleviate protein-energy malnutrition. But pigeon peas are known to contain anti-nutritional factors that limit their nutritional quality and digestibility. It has long become necessary to develop improved methods for detoxifying pigeon pea seeds so that

their potential for use in improving protein consumption in human diets and alleviating the problem of protein malnutrition can be achieved. Different researchers have worked on effect of individual processing techniques such as boiling [11], sprouting [14] and fermentation [6] on the nutritional values of some legumes. There has not been any published work to evaluate the combined effects of sprouting, boiling and fermentation on the protein quality of pigeon pea flour using rat feeding.

2. Materials and Methods

2.1. Materials Collection

The pigeon pea (*Cajanus cajan*) seeds for this research were purchased from Ahiaohuu market in Aba, Abia State. The chemicals used were obtained from Food Science and Technology laboratory, Imo State University, Owerri and National Root Crops Research Institute, Umudike, Umuahia. The animals for the nutritional study were obtained from the Veterinary department, University of Nigeria, Nsukka.

2.2. Sample Preparation

Sixteen kilograms of pigeon pea seeds were sorted to remove dirt and other foreign particles after which they were washed. The grains were then soaked in water (1:3v/v) for 3 hours using a large container and the water drained off.

2.2.1. Production of Soaked Pigeon Pea Flour (Control)

Two (2) kilograms of the soaked grains were dehulled and dried in an oven at 60°C for 7 hours. The dried seeds were milled into flour using disc attrition mill (Asiko All, Addis Nigeria). The flour was then sieved with standard sieve (1.0mm mesh) and packaged in polyethylene bag for further studies.

2.2.2. Production of Soaked and Boiled Pigeon Pea Flour

Two (2) kilograms of the soaked grains were dehulled and boiled in water for 1 hour at 100°C. The water was drained and the seeds dried in an oven at 60°C for 7 hours. The dried seeds were milled into flour using disc attrition mill (Asiko All, Addis Nigeria). The flour was then sieved with standard sieve (1.0mm mesh) and packaged in polyethylene bag for further studies.

2.2.3. Production of Soaked and Fermented Pigeon Pea Flour

Two (2) kilograms of the soaked grains were dehulled, crushed, wrapped in plantain leaves and allowed to ferment for 4 days. After fermentation, the grains were dried in an oven at 60°C for 7 hours. The fermented dried cotyledons were milled into flour with disc attrition mill (Asiko All, Addis Nigeria) and standard 1.0mm mesh sieved before packaging in polyethylene bag for further studies.

2.2.4. Production of Soaked, Boiled and Fermented Pigeon Pea Flour

Two (2) kilograms of the soaked grains were boiled with water for 1 hour and the water drained off. The grains were wrapped in plantain leaves and allowed to ferment for 4 days as described by [17]. After fermentation, the seeds were dehulled and dried in an oven at 60°C for 7 hours. The dried seeds were milled into flour using disc attrition mill (Asiko All, Addis Nigeria). The legume flour were sieved with standard sieve (1.0mm mesh) and packaged in polyethylene bag for further studies.

2.2.5. Production of Soaked and Sprouted Pigeon Pea Flour

Sprouting was carried out according to the method described by [3]. Eight (8) kilograms of the soaked grains were spread in a single layer on a moistened jute bag and allowed to germinate (sprout) at room temperature for 3 days. During this time, the grains were sprayed with water at intervals of 12 hours until the last day of sprouting. After sprouting, the seeds were dehulled and rootlets removed. Then the cotyledons were divided into four portions of 2kg each. Then the portion (2kg) for the production of soaked and sprouted pigeon pea flour were dried in an oven at 60°C for 7 hours and milled into flour using a disc attrition mill (Asiko All, Addis Nigeria) and 1.0 mm mesh sieved before packaging into polyethylene bag for further studies.

2.2.6. Production of Soaked, Sprouted and Boiled Pigeon Pea Flour

Two (2) kilograms of the sprouted pigeon pea seeds were boiled for 1 hour with water at 100°C, drained and dried in an oven at 60°C for 7 hours. The dried sprouted boiled cotyledons were milled with disc attrition mill (Asiko All, Addis Nigeria), sieved with standard sieve (1.0mm mesh) and packaged in polyethylene bag for further studies.

2.2.7. Production of Soaked, Sprouted and Fermented Pigeon Pea Flour

Two (2) kilograms of the sprouted dehulled grains were wrapped in plantain leaves and allowed to ferment for 4 days as described by [17]. After fermentation, the cotyledons were dried in an oven at 60°C for 7 hours and milled into flour with disc attrition mill (Asiko All, Addis Nigeria) and standard 1.0mm mesh sieved before packaging in polyethylene bag for further studies.

2.2.8. Production of Soaked, Sprouted, Boiled and Fermented Pigeon Pea Flour

Two (2) kilograms of the sprouted dehulled grains were boiled in water for 1 hour at 100°C and the water drained off. The sprouted, dehulled and boiled cotyledons were wrapped in plantain leaves and allowed to ferment for 4 days as described by [17]. After fermentation, the cotyledons were dried in an oven at 60°C for 7 hours. The dried sprouted-boiled-fermented grains were milled into flour with disc attrition mill (Asiko All, Addis Nigeria) and standard 1.0mm

mesh sieved before packaging in polyethylene bag for further studies.

3. Experimental Diet Formulation

Eight sets of diets were prepared from the pigeon pea flour samples processed by different methods. The diets were used for nutritional studies by feeding them to Albino rats. The diets were formulated using soaked pigeon pea flour (S₀PPF) (control); soaked and boiled pigeon pea flour (S₀BPPF); soaked and fermented pigeon pea flour (S₀FPPF); soaked, boiled and fermented pigeon pea flour (S₀BFPPF); soaked and sprouted pigeon pea flour (S₀S_pPPF); soaked, sprouted and boiled pigeon pea flour (S₀S_pBPPF); soaked, sprouted and fermented pigeon pea flour (S₀S_pFPPF) and soaked, sprouted, boiled and fermented (S₀S_pBFPPF) pigeon pea flour. The ingredients which were included in the formulation of the diets were corn starch, vegetable oil, common salt, dried pumpkin leaves (ugu) and vitamins/minerals premix. Equal weights of the respective ingredients were used for the diets formulations.

The ingredients for each diet were thoroughly mixed in the dried form. Equal volume of water (75cl) was added in each of the diets. Each diet was formed into dough with help of the added water. The respective doughs were moulded manually into pellets and dried for 7h at 60°C in an oven (Model – MC – 1110K Gallenkamp). The dried pellets were packed in well labeled polyethylene bags and stored at room temperature for rat feeding experiment.

4. Animal Feeding Experiment

Fifty six (56) male weaned rats of the albino Wistar strain with average initial weights range of 48 – 55g were obtained from the Veterinary department of University of Nigeria, Nsukka. The rats were divided into eight groups of seven rats each based on their body weight and each group was fed each of the diets. Each group of rats was housed in a metabolic wire-mesh-bottomed cage equipped to separate urine and faeces. The eight cages were placed in a room at a range of temperature 25°C - 33°C. The rats were weighed before they were allowed to have access to the diets. Drinking water was provided with nipple drinkers and the test diets were supplied in troughs. The experimental animals received feed and water ad libitum. Carmine red was used as fecal marker at the beginning and end of the experiment. The rats were fed for 11 days plus the period of acclimatization of 3 days. Food intake and weight of the animals were recorded every other day for 11 days after the 3 days of acclimatization. Weighed diets (100g) for each group were given daily and unconsumed or left over diet were collected. Food intake and body weight gain/loss were recorded. Complete daily collection of faeces and urine were done during the 5 - day nitrogen balance study for each group of rats. Urine were collected in sample bottles, preserved in 0.1 N HCl to prevent loss of ammonia and stored in a refrigerator until analyzed

for urinary nitrogen. Faeces of rats in the eight groups were pooled for each group, dried at 85°C for 4 hours, weighed and ground into fine powder and stored for faecal nitrogen determination. The concentration of nitrogen in the test diet, faeces and urine were estimated by the Kjeldahl [2] method. The parameters that were determined and calculated were food intake, weight gain/loss, biological value (BV), apparent digestibility (AD), net protein utilization (NPU), protein efficiency ratio (PER), nitrogen balance, nitrogen intake, faecal nitrogen, digested nitrogen and urinary nitrogen.

5. Results and Discussion

5.1. Proximate Composition of Diets Formulated from Pigeons Pea Flour Samples Processed by Different Methods

The results of the proximate composition of formulated diets from pigeon pea flour samples processed by different processing methods are shown in Table 1. The result showed that the protein contents ranged from 12.0–19.20% (Table 1). The Protein Advisory Group (PAG) guidelines of [24] recommended a minimum protein content of at least 15% (on a dry weight basis), fat levels of up to 10%, moisture 5% to 10% and total ash not more than 5% for infants and preschool children [10]. The protein values for some of the formulated diets produced with these pigeon pea flours S₀S_pFPPF, S₀S_pBFPPF, S₀S_pPPF and S₀FPPF fall within the acceptable range of recommendations given with exception of the diets formulated with these pigeon pea flours S₀PPF, S₀BPPF, S₀BFPPF and S₀S_pBPPF which were below the standard for protein. This implies that some diets from some of the pigeon pea flours were higher in protein content than the diet from the standard pigeon pea flour (S₀PPF). Protein is necessary for growth and repair of worn out tissues.

The fat content of the formulated diets ranged from 5.55 – 6.50% and were below the standard (10%). This could be an advantage in the diets are to be stored as they will have longer shelf-life due to lower fat contents. All fats and fat containing foods contain some unsaturated fatty acids and hence are potentially susceptible to oxidative rancidity [15]. Also, the low fat content of the formulated diets was due to lower vegetable oil incorporated into the diets.

The ash content of the formulated diets ranged from 3.10 – 3.55% (Table 1). All the formulated diets in this study contained total ash which was not more than 5% as recommended for infants or preschool children. Ash is a measure of quantity of mineral in a food.

The carbohydrate contents of the formulated diets ranged from 65.23 – 75.0%. The carbohydrate contents of all the formulated diets were higher than the percentage recommended by [9] which is 58%. This implied that the carbohydrate content of the formulated diets from the pigeon pea flour samples were higher than the recommended Standard. Carbohydrate serves as sources of energy.

Table 1. Proximate Composition of Diets Formulated from Pigeon Pea Flour Samples Processed By Different Methods.

Diets	Protein %	Ash %	Fat %	Fiber %	Carbohydrate %
S ₀ PPF	12.25±0.02 ^g	3.10±0.01 ^c	6.50±0.01 ^a	4.30±0.01 ^f	73.85±0.01 ^b
S ₀ BPPF	12.0±0.0 ^h	2.75±0.01 ^d	6.25±0.01 ^c	4.00±0.01 ^h	75.0±0.01 ^a
S ₀ FPPF	16.15±0.03 ^c	3.30±0.0 ^{bc}	6.33±0.01 ^b	5.45±0.01 ^b	68.77±0.01 ^f
S ₀ BFPPF	14.50±0.01 ^e	2.80±0.01 ^d	6.11±0.01 ^c	4.33±0.0 ^e	72.26±.01 ^d
S ₀ S _p PPF	15.10±0.01 ^d	3.40±0.06 ^{ab}	6.20±0.0 ^d	5.40±0.01 ^c	69.9±0.01 ^c
S ₀ S _p BPPF	14.00±0.01 ^f	3.10±0.0 ^c	6.0±0.01 ^f	4.10±0.0 ^g	72.80±0.01 ^c
S ₀ S _p FPPF	19.20±0.01 ^a	3.55±0.0 ^a	5.80±0.0 ^g	6.22±0.01 ^a	65.23±0.01 ^h
S ₀ S _p BFPPF	18.95±0.01 ^b	3.20±0.0 ^{bc}	5.55±0.01 ^h	5.20±0.01 ^d	67.1±0.01 ^g
LSD	0.128	0.22	0.009	0.01	0.009

Values are means ± Standard deviations from the means (on dry weight basis). Means with different letter within a column are significantly different (p<0.05). LSD= Least significant difference.

Key:

S₀PPF diet=Diet formulated from soaked pigeon pea flour (control)

S₀BPPF diet=Diet formulated from soaked and boiled pigeon pea flour

S₀FPPF diet=Diet formulated from soaked and fermented pigeon pea flour

S₀BFPPF diet=Diet formulated from soaked, boiled and fermented pigeon pea flour

S₀S_pPPF diet=Diet formulated from soaked and sprouted pigeon pea flour

S₀S_pBPPF diet=Diet formulated from soaked, sprouted and boiled pigeon pea flour

S₀S_pFPPF diet=Diet formulated from soaked, sprouted and fermented pigeon pea flour

S₀S_pBFPPF diet=Diet formulated from soaked, sprouted, boiled and fermented pigeon pea flour

5.2. Average Daily Feed in Take of the Formulated Diets Per Rat and the Percentage Weight Gain

The average daily intake of the feeds per rat fed on diets formulated from pigeon pea flour samples are shown in Table 2. The average feed intake/rat/day ranged from 7.19 – 11.62g. There were significant differences (p<0.05) in the daily feed-intakes of the rats. The highest daily feed- intake (11.6g) was recorded for the group of rats fed on S₀S_pFPPF diet followed by group of rats fed on S₀S_pBFPPF diet (9.7g). The least feed intake per day perrat (7.19g) was recorded for the group of rats fed the diet produced from the control flour sample, S₀PPF. This showed that diet formulated from combined soaked/sprouted/fermented pigeon pea flour (S₀S_pFPPF) was consumed most by the experimental rats while the diet formulated from soaked pigeon pea flour, control (S₀PPF) was the least consumed. This could be because the S₀S_pFPPF diet formulated with sprouted/fermented pigeon pea flour was more palatable to the rats. [4] had noted that feed intake is associated with nitrogen source, palatability, flavour and essential amino acids rather than nitrogen levels. This result showed that S₀S_pFPPF diet made from sprouted/fermented pigeon pea flour had the necessary nutrients required by the rats for growth. It is important to note that food intake determine the level of nitrogen intake in the body. Therefore, increased food intake as a result of feeding the rats with S₀S_pFPPF and S₀S_pBFPPF diets was an indication of increased nitrogen intake. The amount of food consumed also influence nitrogen retention or nitrogen balance.

There were significant differences (p<0.05) in the percentage weight gain among the rats fed with the various diets which were formulated from the pigeon pea flour samples (Table 2). The percentage weight gain for the rats ranged from 8.2 – 20.3%. The highest percentage weight gain

(20.3%) was recorded for rats that were fed on the diet that was formulated from flour sample S₀S_pFPPF followed closely by the rats fed on the diet that was formulated from flour sample S₀S_pBFPPF with percentage weight gain of 17.3%. The lowest percentage weight gain (8.2%) was recorded for rats fed on diet made from control flour sample S₀PPF. This result showed that weight gain was influenced by the quantity of diet consumed and the quality of the protein constituents of the diets as shown in the results (Table 1). Meaning that flour sample S₀S_pFPPF had highest protein in terms of quantity and quality and that the use of combined processes (sprouting and fermentation) in production of pigeon pea flour was responsible for the high quantity and high quality of the flour protein. Protein is required for growth, healthy living and maintenance or production of tissues and cells of the body.

The rats fed on the diet that was formulated from combined sprouted/fermented pigeon pea flour had increased growth more than the other experimental rats on the other diets formulated from the pigeon pea flours that were prepared with other processing methods. This is an indication that the diet S₀S_pFPPF (formulated from combined sprouted/fermented pigeon pea flour sample) contained good quality amino acids which the rats utilized for growth more than the diets formulated from sprouted, fermented, boiled, and sprouted/boiled/fermented or soaked flour samples. [5] opined that proteins are essential part of animals' diet because they cannot synthesize all the amino acids and must obtain these essential amino acids from food. This result was in agreement with the report of [25] who observed that the rats that consumed higher quantity of diet had increased weight gain while the rats that consumed the least diet also gained the least weight. [1] also reported that growth rate in experimental rats was influenced by their feed intake. The result equally showed that rats fed on the control diet formulated from soaked pigeon

pea flour (S₀PPF) and those diets that the pigeon pea seeds were boiled (except S₀S_pBFPPF) recorded less growth when compared to rat fed on the other formulated diets. This was evidenced by the low percentage weight gain recorded for the group of rats fed on them. This might be due to the lower protein content recorded for them in the proximate

composition analysis of the formulated diets (Table 1). The high weight gain recorded for the diets formulated with sprouted/boiled/fermented flour sample (S₀S_pBFPPF) could be because the high level protein generated by the combined sprouting and fermentation processes was not drastically reduced by the boiling process.

Table 2. Average Feed Intake Per Day and Percentage Weight Gain Per Rat.

Group	Total feed taken by each group of rats (g)	Average Feed Intake in 11 days/ rat (g)	Average Feed Intake/ rat/day (g)	Total weight gain (g)	Percentage weight gain (%)
S ₀ PPF diet	609.05 ± 0.03 ^h	87.01 ± 0.01 ^b	7.91 ± 0.02 ^f	4.1 ± 0.01 ^f	8.2 ± 0.01 ^h
S ₀ BPPF diet	679.65 ± 0.02 ^d	97.09 ± 0.01 ^b	8.83 ± 0.02 ^d	6.1 ± 0.01 ^e	12.2 ± 0.02 ^g
S ₀ FPPF diet	675.44 ± 0.02 ^c	96.49 ± 0.03 ^b	8.77 ± 0.01 ^d	7.5 ± 0.0 ^c	14.7 ± 0.02 ^c
S ₀ BFPPF diet	637.50 ± 0.01 ^g	91.07 ± 0.02 ^b	8.28 ± 0.01 ^c	7.5 ± 0.0 ^c	13.5 ± 0.03 ^f
S ₀ S _p PPF diet	674.38 ± 0.01 ^f	96.34 ± 0.03 ^b	8.76 ± 0.02 ^d	7.2 ± 0.01 ^d	14.3 ± 0.02 ^d
S ₀ S _p BPPF diet	702.01 ± 0.01 ^c	100.29 ± 0.04 ^{ab}	9.12 ± 0.02 ^c	7.2 ± 0.01 ^d	13.8 ± 0.01 ^e
S ₀ S _p FPPF diet	894.63 ± 0.02 ^a	127.80 ± 0.02 ^a	11.62 ± 0.01 ^a	10.6 ± 0.02 ^a	20.3 ± 0.0 ^a
S ₀ S _p BFPPF diet	746.90 ± 0.02 ^b	106.7 ± 0.03 ^{ab}	9.7 ± 0.02 ^b	9.2 ± 0.02 ^b	17.3 ± 0.01 ^a
LSD	0.0209	7.198	0.0189	0.011	0.0135

*Mean daily feed intake of seven rats, means with different superscript letter within a column are significantly different (p<0.05)

S₀PPF diet: Rats Fed on Feeds Formulated From Soaked Pigeon Pea Flour

S₀BPPF diet: Rats Fed on Feeds Formulated From Soaked and Boiled Pigeon Pea Flour

S₀FPPF diet: Rats Fed on Feeds Formulated From Soaked and Fermented Pigeon Pea Flour

S₀BFPPF diet: Rats Fed on Feeds Formulated From Soaked, Boiled and Fermented Pigeon Pea Flour

S₀S_pPPF diet: Rats Fed on Feeds Formulated From Soaked and Sprouted Pigeon Pea Flour

S₀S_pBPPF diet: Rats Fed on Feeds Formulated From Soaked, Sprouted and Boiled Pigeon Pea Flour

S₀S_pFPPF diet: Rats Fed on Feeds Formulated From Soaked, Sprouted and Fermented Pigeon Pea Flour

S₀S_pBFPPF diet: Rats Fed on Feeds Formulated From Soaked, Sprouted, Boiled and Fermented Pigeon Pea Flour

5.3. Protein Qualities of Pigeon Pea Flour Samples Processed by Different Methods Using Rat Feeding

The results of the protein quality of pigeon pea flours processed using different methods are shown in Table 3. The nitrogen intake ranged from 11.52 – 24.79g. There were significant differences (p<0.05) in the nitrogen intakes of the experimental rats. The highest nitrogen intake (24.79%) was recorded for the rats fed with diet that was formulated with soaked, sprouted and fermented pigeon pea flour (S₀S_pFPPF) and they were followed by rats fed with diets formulated from soaked, sprouted, boiled and fermented pigeon pea flour - S₀S_pBFPPF (24.45g), rats fed on diets formulated with soaked and fermented pigeon pea flour - S₀FPPF (21.78g) and rats fed on diets formulated from soaked and sprouted pigeon pea flour – S₀S_pPPF (19.98g). The lowest nitrogen intake (11.52g) was recorded for the rats fed with the control diet formulated from soaked pigeon pea flour (S₀PPF) and second, third and fourth lowest nitrogen intakes were recorded for the rats fed on diets formulated with soaked, sprouted and boiled pigeon pea flour (S₀S_pBPPF), rats fed on diets formulated with soaked, boiled and fermented pigeon pea flour (S₀BFPPF) and rats fed on diets formulated with soaked and boiled pigeon pea flour (S₀BPPF) with nitrogen intake values of 16.85g, 17.59g and 17.94g respectively. This result showed that the nitrogen intake of the rats fed the diet processed with the pigeon pea flour which was subjected to combined soaking/sprouting/fermentation recorded the

highest nitrogen intake. And there was general increase in the nitrogen intake of all the rat groups fed the respective diets that were formulated from the pigeon pea flours which were respectively processed by sprouting, fermentation and combined sprouting/fermentation processing methods. The reason for the increments in nitrogen intake could be due to the increased nitrogen consumption as these processes individually increased the protein (nitrogen) content of the flours their diets were formulated with. The diet prepared with S₀S_pFPPF flour sample contained the highest protein percent and highest food intake was recorded by the rats fed with this diet. This was followed by the diet prepared with S₀S_pBFPPF flour sample (Tables 1 and 2).

The result equally showed that the rats fed on the control and the boiled diets with exception of S₀S_pBFPPF had low nitrogen intake. This might be due to low nitrogen consumption by the rats from the diets. The rats fed on these diets equally recorded low food intake and low weight gain (Table 2).

The faecal nitrogen content of the group of rats fed with formulated diets from pigeon pea flour processed with different methods ranged from 1.18 – 1.35g. There were significant differences (p<0.05) in the faecal nitrogen content of the rats fed on the diets with the exception of rats fed on diet S₀BPPF and S₀S_pBPPF which were similar. The highest faecal nitrogen (1.35g) was recorded for rats fed on the control diet (S₀PPF) and this was significantly different (p 0.05) from the faecal nitrogen of rats fed on the other diets. The least faecal nitrogen (1.18g) was recorded for rats fed on diet S₀S_pFPPF followed by rats fed on diet S₀S_pBFPPF

(1.20g). The rats fed on fermented and sprouted diets equally recorded low faecal nitrogen while the rats fed on the other boiled diets recorded higher faecal nitrogen. The faecal nitrogen of the rats indicates the level of digestibility of the diets. The low faecal nitrogen recorded for rats fed on diets formulated with S₀S_pFPPF, S₀S_pBFPPF, S₀FPPF and S₀S_pPPF flour samples showed that they were more digestible while the high faecal nitrogen recorded for rats fed on diets

formulated with S₀PPF (control), S₀BPPF, S₀BFPPF and S₀S_pBPPF flour samples showed that they were less digestible. [22] stated that high faecal nitrogen of rats indicates low nitrogen digestibility and utilization. The low faecal nitrogen recorded for rats fed on diets formulated with S₀S_pFPPF, S₀S_pBFPPF, S₀FPPF and S₀S_pPPF flour was significant in their high nitrogen retention or nitrogen balance.

Table 3. Protein Quality Characteristics of Rat Diets Formulated with the Pigeon Pea Flour Samples.

Diets	Nitrogen Intake	Fecal Nitrogen	Digested Nitrogen	Urinary Nitrogen	Nitrogen balance	Apparent Digestibility	Protein Efficiency	Net Protein Utilization	Protein Biological value
	g	g	g	g	g	%	Ratio	%	%
S ₀ PPF	11.52± 0.02 ^h	1.35± 0.001 ^a	11.38± 0.02 ^h	0.70± 0.02 ^a	10.03± 0.03 ^h	90.74± 0.02 ^h	3.46± 0.02 ^g	87.07± 0.02 ^h	88.14± 0.02 ^h
S ₀ BPPF	17.94± 0.02 ^c	1.32± 0.002 ^b	17.58± 0.02 ^c	0.60± 0.02 ^b	17.20± 0.02 ^c	92.19± 0.03 ^g	5.82± 0.02 ^d	90.85± 0.03 ^g	91.91± 0.02 ^g
S ₀ FPPF	19.98± 0.01 ^d	1.26± 0.001 ^c	21.25± 0.01 ^c	0.39± 0.02 ^f	20.74± 0.03 ^c	93.81± 0.02 ^b	6.01± 0.02 ^c	95.73± 0.04 ^c	97.81± 0.01 ^c
S ₀ BFPPF	17.59± 0.02 ^f	1.30± 0.001 ^c	17.01± 0.01 ^f	0.53± 0.01 ^d	16.49± 0.02 ^f	93.30± 0.01 ^c	5.59± 0.02 ^c	93.76± 0.02 ^c	97.19± 0.03 ^c
S ₀ S _p PPF	21.78± 0.03 ^c	1.28± 0.001 ^d	19.58± 0.02 ^d	0.44± 0.01 ^e	19.14± 0.01 ^d	93.43± 0.02 ^d	6.0± 0.0 ^c	95.23± 0.01 ^d	97.56± 0.01 ^d
S ₀ S _p BPPF	16.85± 0.02 ^g	1.32± 0.003 ^b	16.66± 0.04 ^g	0.56± 0.01 ^c	16.37± 0.02 ^g	92.83± 0.02 ^f	5.18± 0.03 ^f	93.17± 0.02 ^f	96.79± 0.02 ^f
S ₀ S _p FPPF	24.79± 0.01 ^a	1.18± 0.002 ^g	24.11± 0.02 ^a	0.15± 0.01 ^h	23.44± 0.03 ^a	94.70± 0.02 ^a	6.21± 0.02 ^a	97.75± 0.0 ^a	98.57± 0.02 ^a
S ₀ S _p BFPPF	24.45± 0.02 ^b	1.20± 0.001 ^f	22.10± 0.03 ^b	0.26± 0.02 ^g	21.65± 0.02 ^b	93.50± 0.03 ^c	6.10± 0.03 ^b	96.68± 0.02 ^b	98.44± 0.03 ^b
LSD (p<0.05)	0.022	0.0013	0.0396	0.0167	0.026	0.022	0.021	0.023	0.022

Mean + Standard deviation (7 albino rats). Means with different letter within a column are significantly different (p<0.05).

LSD= Least significant difference.

Key:

S₀PPF diet = diet formulated from soaked pigeon pea flour (control)

S₀BPPF diet= diet formulated from soaked and boiled pigeon pea flour

S₀FPPFdiet =diet formulated from soaked and fermented pigeon pea flour

S₀BFPPF diet = diet formulated from soaked, boiled and fermented pigeon pea flour

S₀S_pPPF diet= diet formulated from soaked and sprouted pigeon pea flour

S₀S_pBPPF diet= diet formulated from soaked, sprouted and boiled pigeon pea flour

S₀S_pFPPF diet = diet formulated from soaked, sprouted and fermented pigeon pea flour

S₀S_pBFPPF diet = diet formulated from soaked, sprouted, boiled and fermented pigeon peaflour

There were significant differences (p<0.05) in the digested nitrogen values which ranged from 11.38 – 24.11g. The highest digested nitrogen value (24.11g) was recorded for rats fed on diet S₀S_pFPPF followed by those fed on diet S₀S_pBFPPF (22.10g), rats fed on diet S₀FPPF (21.25g) and S₀S_pPPF (19.58g) respectively. The lowest digested nitrogen (11.38g) was recorded for rats fed on the control diet (S₀PPF) followed by rats fed on diet S₀S_pBPPF (16.66g), rats fed on diet S₀BFPPF (17.01g) and rats fed on diet S₀BPPF (17.58g) respectively. Rats fed on diet S₀S_pFPPF which was formulated with sprouting/fermentation pigeon pea flour sample had the highest digested nitrogen followed by those fed on diets S₀S_pBFPPF, S₀FPPF and S₀S_pPPF. Rats fed on diet S₀S_pFPPF equally had high nitrogen intake and low faecal nitrogen followed by rats fed on diet S₀S_pBFPPF. The highest digested nitrogen recorded for diet S₀S_pFPPF was due to its highest nitrogen intake which was higher than those of the other diets. Furthermore, the highest digested nitrogen in diet S₀S_pFPPF probably was due to its highest apparent digestibility. [8] Had reported some improvement in the protein digestibility of sprouted and fermented blends. They attributed this to the activities of proteolytic enzymes during sprouting and fermentation which resulted to degradation of the protein into simpler proteins, polypeptides and amino acids, thus enhancing digestibility of the diets. Conversely,

the lower digested nitrogen recorded for diet S₀PPF (control) and other boiled diets might be due to their low nitrogen intake of rats fed with these diets.

The urinary nitrogen ranged from 0.15 – 0.70g. There were significant differences (p<0.05) in the urinary nitrogen of rats fed on the formulated diets. The highest urinary nitrogen (0.70g) was recorded for rats fed on diet S₀PPF (control) followed by rats fed on diet S₀BPPF (0.60g), rats fed on diet S₀S_pBPPF (0.56g) and rats fed on diet S₀BFPPF (0.53g) respectively. The lowest urinary nitrogen (0.15g) was recorded for rats fed on diet S₀S_pFPPF (0.15g) followed by rats fed on diet S₀S_pBFPPF (0.26g), rats fed on diet S₀FPPF (0.39g) and rats fed on diet S₀S_pPPF (0.44g) respectively. The low urinary nitrogen observed for rats fed the diets which were formulated with pigeon pea seeds which were either subjected to sprouting, fermentation or combination of sprouting and fermentation processes could be due to their high digested nitrogen. The low faecal and urinary nitrogen released by rats fed the above diets might be the reason for the high nitrogen balance, apparent digestibility, net protein utilization and biological value. [20] Reported that low urinary nitrogen and low faecal nitrogen indicates high protein quality of fed diets. On the other hand, high urinary nitrogen recorded for rats fed on the control diet, S₀PPF and the other boiled diets might have led to their low digested

nitrogen values, low nitrogen intake, low apparent digestibility, etc.

There were significant differences ($p < 0.05$) in the nitrogen balance of rats fed on the formulated diets. The highest value of nitrogen balance (23.44g) was recorded for rats fed on diet S₀S_pFPPF followed by those of rats fed on diet S₀S_pBFPPF (21.65g), diet S₀FPPF (20.74g) and diet S₀S_pPPF (19.14g) respectively. The lowest nitrogen balance (10.03g) was recorded for rats fed on diet S₀PPF (control), diet S₀S_pBPPF (16.37g), diet S₀BFPPF (16.49g) and diet S₀BPPF (17.20g) respectively. The rats with the highest nitrogen retention (nitrogen balance) were the group fed on diet formulated from pigeon pea flours which were processed by combination of sprouting and fermentation processes (S₀S_pFPPF). This shows that diet S₀S_pFPPF induced higher nitrogen retention in the experimental rats than the other diets because it had better pattern of essential amino acids. Furthermore, rats fed on diet S₀S_pFPPF recorded the highest protein in the proximate analysis, highest food intake (nitrogen intake) and highest percentage weight gain in the experimental rats which reflected in its high nitrogen balance. Conversely, rats fed the control diet, S₀PPF and those fed the other boiled blends with exception of S₀S_pBFPPF recorded low nitrogen balance. This might be due to their lower digestible nitrogen, nitrogen intake and apparent digestibility.

There were significant differences ($p < 0.05$) in the apparent digestibility of the formulated diets. The apparent digestibility ranged from 90.74 – 94.70%. The highest value (94.70%) was recorded for rats fed on diet S₀S_pFPPF which was significantly ($p < 0.05$) higher than those of rats fed on diet S₀S_pBFPPF (93.50%), diet S₀FPPF (93.81%) and diet S₀S_pPPF (93.43%) respectively. The lowest apparent digestibility (90.74%) was recorded for rats fed on diet S₀PPF (control) which was significantly ($p < 0.05$) lower than those of rats fed on diets S₀BPPF (92.19%), S₀S_pBPPF (92.83%) and S₀BFPPF (93.30%) respectively. This result showed that diets S₀S_pFPPF and S₀S_pBFPPF had the highest apparent digestibilities. Digestibility is a measure of protein hydrolysis. These diets (S₀S_pFPPF and S₀S_pBFPPF) also recorded the least in antinutritional contents. The high protein digestibility of these diets might be due to their low levels of antinutrients (tannins and trypsin inhibitors) found in them since trypsin inhibitors and tannins are said to be responsible for the poor digestibility of dietary proteins [18]. The high apparent digestibility recorded for the diet (S₀S_pFPPF) formulated with combined sprouted/fermented pigeon pea flour accounted for the good growth performance of the rats in the feeding experiment. On the other hand, the low apparent digestibility recorded for the boiled diets (S₀BPPF) and control diet (S₀PPF) could be due to higher presence of trypsin inhibitors and haemagglutinins which hampers protein digestibility and decreased nutrient absorption respectively.

There were significant differences ($p < 0.05$) in the protein efficiency ratio of the groups of rats fed with the formulated diets. The highest protein efficiency ratio (PER) (6.20) was recorded for rats fed on diet S₀S_pFPPF followed by rats fed

on diets S₀S_pBFPPF (6.10), S₀FPPF (6.01) and S₀S_pPPF (6.0) respectively. There were no significant differences ($p < 0.05$) in PER of rats fed on diets S₀FPPF and S₀S_pPPF. The lowest PER (3.46) was recorded for rats fed on diet S₀PPF (control) followed by rats fed on diets S₀S_pBPPF (5.18), S₀BFPPF (5.59) and S₀BPPF (5.82) respectively. PER are influenced by food intake and body weight gain. Rats fed with diet S₀S_pFPPF recorded the highest PER and the group of rats had the highest food intake and percentage weight gain (Table 3). This was closely followed by rats fed with diet S₀S_pBFPPF. The high PER exhibited by rats fed on diets S₀S_pFPPF and S₀S_pBFPPF indicated that combined sprouting/fermentation process generated an improved pattern of amino acids which was utilized by the rats for the synthesis of tissue protein. The rats fed the control diet (S₀PPF) and boiled diets (with exception of S₀S_pBFPPF) recorded low PER compared to rats fed the other diets. This could be due to low protein in the diet, their low food intake and low average percentage weight gain. The Protein Advisory Group (PAG) guidelines recommended a PER of not less than 2.3 for weaning food [24]. All the diets in this study exceeded the PAG recommendation for protein. This implies that the test diets for this study were high in protein.

There were significant differences ($p < 0.05$) in the net protein utilization (NPU) of the rats fed on the formulated diets. The NPU ranged from 87.07 – 97.75%. The highest NPU (97.75%) was recorded for rats fed on diet S₀S_pFPPF followed by rats fed on diets S₀S_pBFPPF (96.68%), S₀FPPF (95.73%) and S₀S_pPPF (95.23%) respectively. The lowest NPU (87.07%) was recorded for rats fed on diet S₀PPF (control) followed by rats fed on diets S₀BPPF (90.85), S₀S_pBPPF (93.17%) and S₀BFPPF (93.76%) respectively. The result of rat feeding showed that diet (S₀S_pFPPF) formulated from pigeon pea flour that was processed by combination of sprouting and fermentation processing methods had the highest net protein utilization. This means that diet S₀S_pFPPF had better pattern of amino acids followed by diet S₀S_pBFPPF. This result was in agreement with the report of [8] who reported high NPU for combined sprouted/fermented sorghum, cowpea and groundnut blends. The result also showed that the NPU values of the control diet (S₀PPF) and the boiled diets (except S₀S_pBFPPF) were lower than the NPU of the diets whose flours were produced by sprouting, fermentation and combined sprouting/fermentation processes. The reason for this might be due to deficiency in essential amino acids of the diets. The diet from combined sprouted/fermented pigeon pea flour had better growth promoting quality on the experimental animals than the other diets.

There were significant differences ($p < 0.05$) in the biological value (BV) of the rats fed on the formulated diets. The BV ranged from 88.14 – 98.57%. The highest BV (98.57%) was recorded for rats fed on diet S₀S_pFPPF followed by those fed on diets S₀S_pBFPPF (98.44%), S₀FPPF (97.81%) and S₀S_pPPF (97.56%) respectively. The lowest BV (88.14%) was recorded for rats fed on diet S₀PPF (control) followed by rats fed on diets S₀BPPF (91.91%), S₀S_pBPPF (96.79%) and S₀BFPPF (97.19%) respectively. The results

showed that rats fed on diet S₀S_pFPPF which was formulated from combined sprouted/fermented pigeon pea flour had the highest BV followed by diet S₀S_pBFPPF. The reason for their high biological values could be because of the high protein content of the flours they were formulated from (Table 1) and the diets' high apparent digestibility values. This result is in agreement with the work of [8] who reported high BV for combined sprouted/fermented sorghum, cowpea and groundnut blends. BV measures the efficiency of utilization of absorbed nitrogen [13]. According to [16], a protein material is said to be of good nutritional quality when its biological value (BV) is high (i.e. 70 – 100%). Protein quality is a measure of the balance of the amino acid that are absorbed and utilized for growth and other purposes [12]. The BV of all the diets studied were higher than the recommended value of 75% for children by [24]. The BV of diet S₀S_pFPPF was the highest recorded for this study followed by diet S₀S_pBFPPF. BV is usually higher than NPU. The very high BV for the diets produced from combined sprouted/fermented flours indicate adequate complementation of amino acid in the diet and it is therefore recommended as the best processing method for pigeon pea flour.

6. Conclusion

This study indicated that the combined soaking/sprouting/fermentation and the combined soaking/sprouting/boiling/fermentation improved digestibility and protein qualities of the pigeon pea flour samples. This was further proved by the high growth performance of the experimental animals, the high PER, NPU and BV. Therefore, the combined soaked/sprouted/fermented and combined soaked/sprouted/boiled/fermented formulated diets served better for weaning the albino rats than the other diets and are recommended as the best methods of processing pigeon pea flour.

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