PHYSICOCHEMICAL PROPERTIES AND AMINO ACID PROFILE OF EXTRUDED PRODUCTS FROM PEARL MILLET AND GERMINATED PIGEON PEA

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Abstract
This study was carried out to evaluate the proximate, functional properties, anti-nutritional factors, amino acid and sensory evaluation of extruded products from pearl millet and germinated pigeon pea. The pearl millet and germinated pigeon pea flour were prepared at different mixing ratios: Sample A (70% pearl millet flour and 30% germinated pigeon pea), B (80% pearl millet flour and 20% germinated pigeon pea) and C (90% pearl millet flour and 10% germinated pigeon pea). The formulated flours were extruded using Brabender laboratory single- screw extruder, at barrel temperature (120^{o}C), feed moisture content 8% and screw speed (70 rpm). The following range of values were obtained for carbohydrate (55.6-57.6%), protein (11.7-14.6%), fat (11.5-12.7%), moisture (12.50-13.78%), fibre (2.23-2.61%) and ash (1.75-2.05%) content of the different proportion of pearl millet and germinated pigeon pea flour. The carbohydrate, protein and fibre contents of extruded products increased while moisture, fat and ash contents reduced. The functional properties of the extruded products increased when compared with the flours except for oil absorption capacity. The saponin and tannin levels were reduced possibly due to germination. Lysine was the highest essential amino acid obtained while the highest amino acid value was glutamic acid. It was observed from sensory evaluation that sample MPP1 (70% pearl millet and 30% germinated pigeon pea) was more acceptable to the panelists. This product can serve as a potential source of nutrients to prevent protein energy malnutrition, which may help in growth and development of children.

Keywords: *Pearl millet, anti-nutritional, germination, extrusion, amino acid*

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1. INTRODUCTION

Extrusion cooking is one of the contemporary food processing technologies applied for preparation of a variety of snacks, speciality and supplementary foods (Harper and Jansen, 1985). It reduces the anti-nutritional factors, renders the product microbiologically safe and enhances the consumer acceptability (Nibedita and Sukumar, 2003). Pearl millet (*Pennisetum glaucum*) is the most widely grown type of millet which produces the largest seeds, and it is the variety most commonly used for human consumption (Mariac et al., 2006; ICRISAT, 2007). Nutritionally pearl millet is comparable and even superior to major cereals with respect of energy value, proteins, fat and minerals (Sehgal and Kwatra, 2006). It makes an important contribution to human diet due to high levels of calcium, iron, zinc, lipids and high quality proteins. Pigeon pea (*Cajanus cajan*) is a nutritionally important food crop of the leguminous family found in the tropical and subtropical regions of the world (Singh and Eggum, 1984; Rampersad *et al*., 2003). It is rich in protein, essential amino acids and minerals, and it can be used to supplement other foods (Etonihu et al., 2009). Pigeon pea flour is an excellent component in the snack industry and has been recommended as an ingredient to increase the nutritional value of pasta without affecting its sensory properties (Torres *et al*., 2007). Germination of legumes promote breakdown of macromolecules thereby enhances the starch and protein digestibility, and improves contents of amino acids (Martins-Cabrejas *et al*., 2003). Some research has been carried out using sorghum and pigeon pea as flaked breakfast (Mbaeyi-Nwaoha and Onweluzo, 2013), millet and pigeon pea as weaning food (Onweluzo and Nwabugwu, 2009), Viralarani et al (2016) worked on pearl millet based extruded product. This work aimed at producing extruded products from pearl millet and germinated pigeon pea.

2. MATERIALS AND METHODS

Pearl millet, pigeon pea, strawberry flavor and sugar were purchased in Ondo market, Ondo State, Nigeria and all chemicals were of analytical grades.

Production of pearl millet flours

Pearl millet grains were cleaned, washed with water twice, then oven dried for 12hrs at 60 ^{o}C and was milled with attrition, was then packed in air tight polythene bag and kept in freezer for further use.

Production of germinated pigeon pea flours

Pigeon peas were sorted, cleaned, soaked for 8 h at room temperature. The steeped water was removed and spread on a jute bag, then covered with a jute bag. It was germinated for 72 h water was sprinkled on it at every 6hrs interval. The germinated pigeon pea was washed, oven dried at 60 ^{o}C for 24 hr and milled with attrition mill. It was then stored in an air tight polythene bag and kept in the freezer for further use (Sodipo and Fashakin, 2011).

Table 1: Formulation composition (%) of pearl millet and germinated pigeon pea flour

Samples	Pearl millet flour	Germinated pigeon pea flour
A	70	30
B	80	20
C	90	10

Keys:
Sample A: Pearl millet flour 70% and Germinated pigeon pea flour 30%
Sample B: Pearl millet flour 80% and Germinated pigeon pea flour 20%
Sample C: Pearl millet flour 90% and Germinated pigeon pea flour 10%

Extrusion Processing

The formulation mixture of pearl millet and germinated pigeon pea before extrusion is shown on Table 1. Prior to extrusion processing, 3g of sugar and 0.5g of strawberry were added to 100g of composite flours which were then preconditioned to adjust the feed moisture content to 8%. The moistened flours were kept for 48 h for preconditioning in air tight polythene bags to equilibrate moisture. After 48 h of conditioning, flours were fed into feeder hopper that contained screw auger to transport materials at uniform rate into the barrel. The single screw extruder consists of one screw in the barrel to transport the ingredients through its three zones viz. feeding zone, kneading zone and cooking zone. The temperature of cooking zone was maintained at 120 ^{o}C and screw speed was 70 rpm. The material was finally extruded and cut into pieces by a cutter. Then, extrudates were collected in a plastic tray, packed in the polythene bags and sealed.

Proximate Analysis

Moisture, fat, protein, ash, crude fibre and carbohydrate by difference were carried out by AOAC (2005). Energy was determined by Atwater factor.

Functional Properties

Bulk density was determined by Onimawo and Akubor (2005), 10 g of the sample was weighed into 100 ml graduated measuring cylinder, the initial volume was recorded. The measuring cylinder was tapped on the table continuously until the volume became constant, the final volume was recorded and the bulk density (g/ml) was calculated. Swelling capacity was carried out by Dhingra *et al.* (1992), water absorption capacity by Abbey and Ibeh (1988), oil absorption capacity (Sefa-Dedeh et al., 2004).

Anti-nutritional factors

Tannin and saponin were determined by the method described by Nwosu (2010).

Amino acid analysis

Amino acid content was determined using Technicon amino acid analyser (TSM-1 Technicon Instrument, Basingstoke, Hampshire, UK) after hydrolyzing the samples with 6 M HCl at 110 ^{0}C for 24 h (Bassler and Buchholz, 1993).

Sensory evaluation

The samples were coded and served to a twenty member untrained panelist using nine point Hedonic scale. Each panelist was given coded samples and sample was evaluated for appearance, taste, aroma, texture, and the overall acceptability of the sample.

Statistical analysis

The results obtained were subjected to analysis of variance (ANOVA) using SPSS version 20, means were separated by Duncan Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

Proximate composition of Pearl millet and germinated pigeon pea flour blends and extruded product

Table 2 shows the proximate composition of pearl millet and pigeon pea flour blends. The moisture, protein, ash, crude fibre, fat and carbohydrate were within the range of (12.35-12.85), (11.70-14.60), (1.75-2.05), (2.23-2.61), (11.50-12.70) and (55.60-57.60) respectively. It was observed that protein increases as the percentage of pigeon pea increased. Fat and carbohydrate levels had no significant difference. The sample A was with the highest value in moisture and crude fibre level. The moisture level was high and this is not desirable because of microbial growth. This is not in agreement with Mbaeyi and Onweluzo (2010) that the moisture level was within range of 7.05- 9.50% for the sprouted flour blend of sorghum and pigeon pea. The fat content ranged from 11.50-12.70%. The ash content ranged from 1.75- 2.05%, this is similar to report of Fasasi (2009) that processed pearl millet ranged from 1.9-2.7%. Sample A had lowest value of carbohydrate and energy. This may be due to this sample contain lowest proportion of millet flour.

The proximate composition of extruded products is shown on Table 3. It was shown that the moisture content was reduced compared to flour within the range of 2.01-3.56%. It indicated that all the samples exhibit good keeping quality and will have long shelf life because higher moisture content indicates in susceptibility to spoilage and thus reduces shelf life. This was in accordance to the work done by Onweluzo and Nwabugwu (2009) which discovered low moisture content by using fermented millet and pigeon pea. Sample MMP2 had the highest protein value while the protein decreased in other samples. This may be due to extrusion process that was done. The fat content reduced in extruded product within the ranges of 7.80-9.40%. This is in agreement with Khan et al (2015) which observed that there was decreased in fat content of extruded rice based compared to flour. There was no significant difference in the crude fibre value even though sample MPP3 had the highest value. The high level of crude fibre in food enhances digestibility. The ash content of extruded product was a little bit reduced except for sample MPP1 compared to the flour sample. But the carbohydrate and energy values were increased within the range of 66.47- 72.83% and 401.38- 416.74 Kcal/100g respectively.

Table 2: Proximate composition of pearl millet and germinated pigeon pea flour blends

Sample	Moisture (%)	Protein (%)	Crude fibre (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Energy (Kcal/100g)
A	13.85± 0.25^{a}	14.60± 0.17^{a}	2.61±0.03^{a}	11.50± 1.30^{a}	1.75± 0.75^{c}	55.60± 1.30^{a}	384.06± 7.18^{b}
B	12.50 ± 0.40^{b}	13.00± 0.09^{b}	2.23± 0.30^{c}	12.70± 0.50^{a}	2.05± 0.05^{a}	57.50± 1.07^{a}	396.38± 0.58^{a}
C	13.78± 0.42^{a}	11.70± 0.63^{c}	2.51± 0.05^{b}	12.50± 0.30^{a}	1.90± 0.10^{b}	57.60± 1.19^{a}	389.72± 0.44^{b}

Values are means in triplicate determinations; different superscripts in the same column are significantly different (P< 0.05)

Keys:

Sample A: Pearl millet flour 70% and Germinated pigeon pea flour 30%

Sample B: Pearl millet flour 80% and Germinated pigeon pea flour 20%

Sample C: Pearl millet flour 90% and Germinated pigeon pea flour 10%

Table 3: Proximate composition of extruded product

Sample	Moisture (%)	Protein (%)	Crude fibre (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Energy (Kcal/100g)
MPP1	2.01± 0.50^{c}	12.66± 0.16^{c}	2.50±1.50^{a}	7.80± 1.30^{b}	2.20± 0.47^{a}	72.83± 1.28^{a}	412.15± 0.26^{a}
MPP2	3.56 ± 0.16^{a}	16.56± 0.06^{a}	3.00± 2.00^{a}	9.40± 0.80^{a}	1.00± 0.00^{b}	66.47± 1.09^{b}	416.74± 0.15^{a}
MPP3	2.81± 0.16^{b}	13.98± 0.03^{b}	6.00± 3.00^{a}	8.70± 0.30^{ab}	1.73± 0.12^{a}	66.79± 1.12^{b}	401.38± 0.09^{a}

Values are means in triplicate determinations; different superscripts in the same column are significantly different ($P < 0.05$)
Keys:
Sample MPP1: Pearl millet flour 70% and Germinated pigeon pea flour 30% extruded product
Sample MPP2: Pearl millet flour 80% and Germinated pigeon pea flour 20% extruded product
Sample MPP3: Pearl millet flour 90% and Germinated pigeon pea flour 10% extruded product

Functional properties of Pearl millet and germinated pigeon pea flour blends and extruded product

The functional properties of flour blends are shown in Table 4. The bulk density was ranged within 0.60-067 g/ml. This is in agreement with Sodipo and Fashakin (2011) that reported high bulk density in using germinated maize, cowpea and pigeon pea. The swelling capacity, water absorption capacity and oil absorption capacity were within the ranged of 1.13-1.68 g/ml, 2.00-2.75g/ml and 1.25-2.50g/ml respectively. Swelling capacity is the volume of expansion of molecule in response to water uptake which is retained until colloidal suspension is achieved or until further expansion and uptake is prevented intermolecular forces in the swelled particle (Houssou and Ayernor, 2012). Water absorption capacity of food is an index of the maximum amount of water that it can take up and retain. The high value of water absorption capacity had been attributed to lose structure of starch polymers while low value water absorption capacity to the compactness of molecular structure (Sanni et al., 2006). High water absorption capacity may leads to product cohesiveness (Houssou and Ayenor, 2012). High oil absorption capacity of the food product improves the mouth feel and flavour retention.

The functional properties of extruded products are shown in Table 5. The bulk density, swelling capacity and water absorption capacity values were increased in all of extruded samples compared to blended flour samples except for oil absorption capacity. Bulk density of sample MPP1 would be an added advantage in the preparation of complementary food (Akubor and Obiegbuna, 1999; Onwuelzo and Nnabuchi, 2009). Bulk density is an important parameter that determines easy packaging and transportation of flour (Shittu et al., 2005). Comparing with flakes produced from sorghum and pigeon pea (Mbaeyi and Onwuelzo, 2013) with this research work the bulk density is similar. There was low swelling capacity ranged from 3.18-4.48 ml/g which is similar to report of Sodipo and Fashakin (2011) that low swelling capacity were observed in the complementary diets of maize, cowpea and pigeon pea. The water absorption capacity and oil absorption capacity ranged from 2.00-3.00 ml/g, 1.00-2.00 ml/g respectively.

Table 4: Functional properties of pearl millet and germinated pigeon pea flours.

Sample	Bulk density g/ml	Swelling capacity ml/g	Water absorption capacity ml/g	Oil absorption capacity ml/g
A	0.67±0.01^{a}	1.38±1.36^{a}	2.75±0.25^{a}	2.00±1.00^{a}
B	0.64±0.00^{a}	1.68±0.45^{a}	2.50±0.50^{a}	1.50±0.50^{a}
C	0.60±0.01^{b}	1.13±0.10^{a}	2.00±0.00^{b}	1.25±0.25^{a}

Values are means in triplicate determinations; different superscripts in the same row are significantly different ($P< 0.05$)
Keys:
Sample A: Pearl millet flour 70% and Germinated pigeon pea flour 30%
Sample B: Pearl millet flour 80% and Germinated pigeon pea flour 20%
Sample C: Pearl millet flour 90% and Germinated pigeon pea flour 10%

Table 5: Functional properties of extruded product.

Sample	Bulk density (g/ml)	Swelling capacity ml/g	Water absorption capacity ml/g	Oil absorption capacity ml/g
MPP1	0.70±0.14[c]	3.30±1.34[b]	3.00±1.28[a]	1.00±0.36[b]
MPP2	0.72±0.20[b]	3.18±1.20[b]	2.00±1.09[a]	2.00±0.20[a]
MPP3	0.77±0.06[a]	4.48±1.00[a]	3.00±0.84[a]	1.25±0.12[b]

Values are means in triplicate determinations; different superscripts in the same row are significantly different (P< 0.05)
Keys:
Sample MPP1: Pearl millet flour 70% and Germinated pigeon pea flour 30% extruded product
Sample MPP2: Pearl millet flour 80% and Germinated pigeon pea flour 20% extruded product
Sample MPP3: Pearl millet flour 90% and Germinated pigeon pea flour 10% extruded product

Anti-nutritional factor of extruded product

It was revealed from Table 6 the level of antinutritional factor. The low saponin value may be as a result of the germination of pigeon pea. Saponin increases permeability of small intestinal mucosa cells thereby inhibits the transportation of nutrients (Jimoh et al., 2011). The sample MPP1 with highest pigeon pea had the lowest level of tannin (0.24 mg/100g). The reduction in tannin level is in accordance with the report of Anuonye et al (2012) that observed low level of tannin after extrusion of plantain and pigeon pea. Tannins have been reported to form insoluble complexes with proteins and reduce their digestibility, palatability and utilization of food proteins (Delumen and Salament, 1980; Uzeochina, 2007).

Table 6: Anti-nutritional factor of extruded product

Samples	Saponin (mg/100g)	Tannin (mg/100g)
MPP1	0.97±0.32[a]	0.24±0.67[c]
MPP2	0.62±0.28[b]	0.34±0.04[b]
MPP3	0.85±0.14[b]	0.44±0.26[a]

Values are means in triplicate determinations; different superscripts in the same row are significantly different (P< 0.05)
Keys:
Sample MPP1: Pearl millet flour 70% and Germinated pigeon pea flour 30% extruded product
Sample MPP2: Pearl millet flour 80% and Germinated pigeon pea flour 20% extruded product
Sample MPP3: Pearl millet flour 90% and Germinated pigeon pea flour 10% extruded product

Amino acid profile of extruded product

Table 7 shows the amino acid profile of the extruded products. The sample with higher percentage of germinated pigeon pea was higher in glycine, glutamic acid, alanine, cysteine, lysine and histidine compared with other samples. All samples had high values for leucine. Findings by some authors showed that high leucine might be a factor contributing to the development of pellagra as observed in maize (Aremu *et al*., 2011; FA0, 1995; Belavady and Gopalan, 1969). Therefore, the Leucine Isoleucine ratios would be considered more essential; conversely, samples in this study had low values (1.57-1.82) for Leu/Ile. As a whole, result showed that samples contained all essential amino acids which were significantly higher than acceptable standards of recommended daily allowance. From result obtained for predicted nutritional qualities, the percentage of total essential amino acid (TEAA) to total non-essential amino acid (TNEAA) ranged from 0.57 to 0.83. The percentage ratios of TEAA to total amino acid (TAA) in the samples ranged from 36.44 to 45.34. Oshodi *et al*. (1993) reported 43.6 % ratio TEAA to TAA in pigeon pea flour while the result obtained by Aremu *et al*. (2011) for different ratios of maize ogi fortified with *Kerstingiella geocarpa* seed flour was slightly comparable to results obtained for extruded products from millet flour and germinated pigeon pea. Furthermore, the percentage ratios of TNEAA to TAA in the samples ranged from 54.66 to 63.56. Of much importance to this study is the ratio of TEAA to TAA. It was observed that sample MPP2 and MPP3 had values which were significantly above 39 % considered to be acceptable for model protein diet for infants, 26 % for children and 11 % for adults according to reports by FAO/WHO (1991). It is expected that those two samples *i.e* MPP2 and MPP3 can sufficiently make available the daily essential amino acids needed by the consumers for developments.

Table 7: Amino acid profile (mg/100g) of the extruded product

Parameters	MPP1	MPP2	MPP3	*RDA
Non-essential amino acids				
Aspartic acid	9.41	8.80	9.64	-
Tyrosine	2.67	2.84	1.94	-
Serine	4.68	5.27	5.74	-
Glycine	8.62	2.50	4.47	-
Glutamic acid	13.79	10.29	9.38	-
Proline	5.62	5.98	5.86	-
Alanine	7.05	5.13	5.29	-
Cysteine	9.23	2.23	1.91	-
Arginine	6.25	7.65	7.72	2
Essential amino acids				
Valine	4.78	7.33	5.91	3.5
Leucine	7.58	7.74	8.08	6.6
Isoleucine	4.17	4.94	4.97	2.8
Methionine	2.78	3.21	3.92	2.2
Phenylalanine	4.21	4.21	3.50	2.8
Lysine	7.99	7.74	7.20	5.8
Histidine	2.50	1.97	2.49	1.9
Threonine	4.58	4.91	5.19	3.4
Tryptophan	ND	ND	ND	
Predicted nutritional qualities				
TEAA+His+Arg/TAA %	46.99	52.27	52.03	-
TEAA/TNEAA	0.57	0.83	0.79	-
TEAA/TAA %	36.44	45.34	44.27	-
TNEAA/TAA %	63.56	54.66	55.73	-
TSAA(Meth + Cys)	12.01	5.44	5.83	-
ArEAA (Phe + Tyr)	6.88	7.05	5.44	-
Leu/Ile	1.82	1.57	1.62	

where:

- MPP1 = Pearl millet flour: 70%; Germinated pigeon pea flour: 30% extruded product.
- MPP2= Pearl millet flour: 80%; Germinated pigeon pea flour: 20% extruded product.
- MMP3 = Pearl millet flour: 90%; Germinated pigeon pea flour: 10% extruded product.
- ND= not determine * Recommended daily allowance (WHO/FAO 1991).

Sensory evaluation of extruded product

The result of sensory evaluation of the extruded product is presented on Table 8. It was shown that was no significant difference in this sample MPP1 and MPP2 for crunchiness, aroma and overall acceptability. Even though, there were significant differences for taste, appearance and texture may be due to the different level of addition of pigeon pea. Sample MPP1 was rated highest in taste, appearance, crunchiness, aroma, texture and overall acceptability. This implies that it was most acceptable sample.

Table 8: Sensory evaluation of extruded product

Sample	Taste	Appearance	Crunchiness	Aroma	Texture	Overall acceptability
MPP1	6.95 ± 1.87^{a}	5.65 ± 2.15^{a}	6.30 ± 1.38^{a}	7.80±1.28a	6.15 ± 1.63^{a}	7.30 ± 1.55^{a}
MPP2	6.30 ± 2.02^{b}	5.10 ± 2.21^{b}	5.90 ± 1.44^{a}	7.65 ± 1.30^{a}	5.40 ± 1.63^{ab}	6.35 ± 1.84^{a}
MPP3	5.80 ± 2.33^{b}	4.65 ± 2.30^{c}	5.30 ± 1.52^{b}	7.45 ± 1.39^{b}	4.70 ± 1.39^{c}	5.15 ± 1.56^{b}

Values are means in triplicate determinations; different superscripts in the same row are significantly different (P< 0.05)

Keys:
Sample MPP1: Pearl millet flour 70% and Germinated pigeon pea flour 30% extruded product
Sample MPP2: Pearl millet flour 80% and Germinated pigeon pea flour 20% extruded product
Sample MPP3: Pearl millet flour 90% and Germinated pigeon pea flour 10% extruded product

4. CONCLUSION

It was shown from the study that pearl millet and germinated pigeon pea flour blends were significantly different from extruded product in proximate composition and functional properties. The germination of pigeon pea contributed to reduction of saponin and tannin level of the extruded product. Sample MPP1 had the highest value in glycine, glutamic acid, alanine, cysteine, lysine and histidine, and it was most acceptable sample in sensory evaluation. Therefore, this extruded product from pearl millet and germinated pigeon pea may serve as complementary diet for the children.

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