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Effect of soy protein supplementation on the nutritive value of 'gari' (farina) from *Manihot esculenta*

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Abstract

The supplementary role of soy protein on cassava (*Manihot esculenta*) "gari" or farina was studied in 35 day-old albino rats of the Wistar strain ($n = 6$ per group) for 10 days. Gari was supplemented with soy beans at 10 and 15% soy protein levels to produce "soy gari" and the performances of rats fed the test diets compared with those on a 10% casein diet. Protein efficiency ratio (PER), Net protein utilization (NPU), true digestibility (TD) and biological values (BV) were the parameters used to assess the nutritional performance of the diets based on weight gains and nitrogen balance. The PER for 10% casein, 10 and 15% soy protein-supplemented gari groups were 2.31, 1.85 and 1.94 respectively, while the corresponding values for NPU were 71.9, 65.8 and 64.1, respectively. The TD values for the 10% casein, 10 and 15% soy protein-fed rats were 97.2, 91.9 and 90.0, respectively, while the corresponding values for BV were 73.9, 70.9 and 71.5. The PER, NPU, TD and BV for 10% casein diet were significantly superior ($P < 0.05$) to those of the soy gari diets which gave NPU, TD and BV values 89–97% of those of the casein diet. There were no significant differences ($P > 0.05$) in these parameters between the 10 and 15% soy protein supplementation. Soy gari is safe to the consumer, because the procedures involved in processing soy beans and cassava ensure the elimination of toxic anti-nutritional factors known to impact negatively on nutrient availability, metabolic processes and growth. It is suggested that the consumption of soy gari at 10% soy protein supplementation be promoted in gari-eating areas, to ameliorate the endemic problems of protein deficiency disorders.

Keywords: Soy bean; Soy protein; Supplementation; Nutritive value; *Manihot esculenta*

1. Introduction

In tropical and sub-tropical areas of the world, feeding of the fast-growing population has continued to pose a serious problem. These areas are also characterized by a shortage of animal protein and incidences of protein energy malnutrition (Hernández, Montalvo, Sonsa, & Sotela, 1996). These regions are, however, rich in starchy tubers, such as yam, cocoyam and cassava.

Cassava (*Manihot esculenta*, Crantz) is a tuber that is grown throughout the tropics and sub-tropics, where it contributes a considerable proportion of the total caloric intake (Okezie & Kosikowski, 1982). It is the staple food for more than half of the Nigerian population (Anonymous, 1992; Steinkraus, 1983). It is processed into various products that are useful as human and animal foods, including "gari" (Lancaster, Igram, Lim, & Coursey, 1982). Gari (fried, fermented cassava flour) is the most popular cassava product consumed in West Africa and the most important item in the diet of millions of Nigerians (International Institute of Tropical Agriculture, 1990; Kordylas, 1990). It forms a significant part of the diet in many other countries, such as Ghana, Cameroun, Zaire and Brazil where it is called "Farinhade moniaca" (Lancaster et al., 1982). Although cassava is high in linamarin (Steinkraus, 1983; Vasconcelos, Twiddy, Wetby, & Reilly, 1990), about 83% of the total cyanogenic glucosides (linamarin and lotaustralin) are detoxified during processing of the tuber into gari and 98% of the cyanide is lost when gari is cooked to "eba" (Mahungwu, Yamaguchi, Almazan, & Hatin, 1987). However, cassava and its products are low in protein, deficient in essential amino acids and therefore, have poor qualitative and quantitative protein content (Oyenuga, 1968). Thus, continuous dependence on gari

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without supplementation with meat, fish and/or other protein-rich sources would result in protein deficiency. However, because of the high cost of animal proteins, the majority of the population cannot afford such supplementation of gari. Ignorance about the importance of protein also contributes to the low protein intake. There is therefore, a need to search for cheaper but good quality protein sources that are readily available for the supplementation of gari. Soy bean, a protein-rich legume with a good essential amino acid profile, is potentially useful for this purpose.

Soy beans (*Glycine max* Merr), an inexpensive high quality protein source, are readily available in many countries where starchy tubers are consumed in large quantities. In comparison to most other legumes, soy beans are much higher in protein (35% of energy; Messina, 1995). Soy bean oil is 61% polyunsaturated and 23.4% monounsaturated (Gunstone, Harwood, & Padley, 1986). The processing of soy beans to inactivate antitryptic and other antinutritive factors, to aid their potential use as a protein supplement in low protein foods and diet, has been widely explored (Eka, 1979). Furthermore, the amino acid profile of soy protein, and other non-protein components present in soy, confers a hypocholesterolemic effect which has been observed in humans and animals (Potter, 1995; Potter et al., 1993).

The aim of this study was to supplement gari with soy beans to form "soy-gari" and to evaluate the role of soy protein in enhancing the nutritive value of gari.

2. Materials and methods

2.1. Preparation of "soy-gari"

Samples of soy beans (*Glycine max*, Merr) of white variety (TG×536-02D) were bought from the Nigerian Cereals Institute, Ibesikpo, Akwa Ibom State, Nigeria, between June and July. The soy beans were cleansed by removing stones, sticks and damaged beans and then washed using plain tap water. The soy bean seeds were dehulled by soaking in plain tap water (1:10 w/v seed to water ratio) at room temperature (28°C) for 12 h, followed by hand-rubbing (within two palms) to remove the testa.

The floating testa on the soak water were removed by decanting until no testa were present. The soak water was decanted, before boiling. Boiling of the dehulled seeds was done for 30 min, with plain tap water (three times the weight of dry seeds) to inactivate the trypsin inhibitor. The boiled samples were then dried in a hot air-circulating oven (Stuart scientific HT Oven Size 1, Surrey, England) at 60°C to constant weight. They were then ground in a mill (National Supergrinder MK 830N, Japan) to form soy flour.

Cassava (*Manihot esculanta* Crantz) was bought from Uyo Market, Uyo, Akwa Ibom State, Nigeria. The tubers were washed using plain tap water and peeled using a kitchen knife. The peeled tubers were washed using plain tap water and grated in a cassava grater (Slawd Peters Engineering, Nigeria). Grated cassava was then fermented for 3 days and the liquor squeezed out using a hydraulic press (Blitz HPL 652, Germany).

It was sieved to removed fibre waste and fried, adding 0.3% palm oil. The gari thus formed was cooled and packaged in air-tight plastic containers. Soy-gari was prepared by adding the soy flour (32.1% protein) to the gari (2.8% protein) to produce soy protein at levels of 10 and 15% (Table 1).

2.2. Chemical analyses

The chemical compositions of the soy flour, gari and soy-gari were determined. The methods for sample treatment and analyses were the standard procedures recommended by Association of Official Analytical Chemists (AOAC, 1990). Moisture content was assayed by loss in weight on drying at 60°C in a hot air circulating oven (Stuart Scientific HT Oven Size 1, Surrey, England). The ash was determined by incineration of known weights of the samples in a muffle furnace (Gallenkamp Size 3, England). The crude fat was determined by exhaustively extracting a known weight of sample in petroleum ether (boiling point, 40–60°C) in a Soxhlet extractor. The ether was volatilized and the dried residue quantified gravimetrically and calculated as percentage of fat. Protein ($N \times 6.25$) was determined by the Kjeldahl method. Crude fibre was determined after digesting a known weight of fat-free sample in refluxing 1.25% sulphuric acid and 1.25% sodium hydroxide. The available carbohydrate was obtained by the difference method (subtracting the percent crude protein, fat, fibre and ash from 100% dry matter).

2.3. Rat studies

Albino rats of the Wistar strain (weanlings at 21–27 days old) were obtained from the Animal House of the College of Medical Sciences, University of Calabar, Nigeria and fed commercial rat mash (Pfizer Livestock Feed Ltd., Ikeja, Nigeria) until they were 35 days old. They were divided into four groups of six 6 rats each (with body weights evenly distributed across all groups).

The rat studies were performed essentially as reported by Ayatse, Eka, and Ifon (1985). Rats were housed individually in stainless steel screen bottom cages. One group was fed the basal diet, another the casein (reference) diet and the third and fourth groups soy gari based diets at 10 and 15% soy protein supplementation (Table 1). The animals had free access to food and drinking water. The animal house temperature was

Table 1
Composition of diets

Component (g/kg dry wt.)	Diets			
	Basal non-protein	Casein (reference)	Soy-gari I (10% soy protein)	Soy-gari II (15% soy protein)
Sucrose	100	100	100	50
Corn starch	700	600	75	29
Cellulose	50	50	50	40
Corn oil[a]	100	100	100	50
Mineral mix[b]	40	40	40	40
Vitamin mix[c]	10	10	10	10
Casein (fat free and vitamin free)	–	100	–	–
Soy flour	–	–	312	468
Gari	–	–	313	313
Protein content (g/100g dry wt.)	0.90	9.04	10.88	15.87

[a] Corn oil was added last, after vitamin mixture.
[b] Mineral mixture used has composition suggested by ICN Nutritional Biochemicals (1979/1980).
[c] Vitamin mixture: ICN Nutritional Biochemicals (1979/1980).

28±2°C during the experiment. Lighting regimen was about 13 h: 11 h of light and dark.

The weights of the animals were recorded every day throughout the duration of the experiment. The first 3 days were considered to be an acclimatization period during which no record of food consumption or faeces collection was made.

Total collection of faeces from individual rats was carried out during the 7 days of the experiment. Records of daily food consumption were also kept during the collection period. Total faeces from each rat were oven-dried (Stuart Scientific Oven 251D, England) at 60°C for 24 h, weighed and ground in a mortar. The faeces were analyzed for total nitrogen. The nitrogen levels and hence, protein intakes by individual rats were calculated using the records of food consumption.

At the end of the experimental period, the rats were sacrificed by chloroform inhalation and incisions made into the skull, thoracic and body cavities. The carcass of each rat was dried in a hot air-circulating oven (Stuart Scientific HT Oven, England) at 70°C to constant weight. It was then ground in a mortar and digested in concentrated sulphuric acid. After about 1 h, the slurry was cooled and made up to 250 ml with distilled water. Triplicate 25-ml portions of each slurry were taken for nitrogen determination (of the carcass) by the Kjeldahl procedure (AOAC, 1990).

2.4. Assessment of diets

Food intake, protein intake, weight gain, carcass nitrogen and faecal nitrogen were obtained to calculate biological parameters. The criteria used in the assessment of the diets were: protein efficiency ratio (PER), net protein utilization (NPU), true digestibility (TD), and biological value (BV). PER determination employed the concept introduced by Osborne, Mendel and Ferry (1919). The carcass nitrogen technique of Miller and Bender (1955) was used to determine NPU and the TD of the dietary nitrogen. The BV was computed by dividing NPU by TD (Bender & Haizelden, 1957; Campbell, 1963).

2.5. Statistical analysis

Results were expressed as the means±S.D. Differences between any two means were compared using the Student's *t*-test and considered significant at $P<0.05$.

3. Results

The proximate composition of soy flour, gari and "soy-gari" are given in Table 2. The protein contents of the prepared soy flour and gari were 32.1± 0.10 and 2.77±0.25%, respectively, while those of the 10 and 15% soy protein-based diets were 10.9±0.16 and 15.9±0.50%, respectively.

The results of the biological performance of the rats fed the various diets are summarized in Table 3. The PER, NPU, TD and BV show that the reference diet (casein) was significantly superior ($P<0.05$) to both soy-gari diets but the values in the 10 and 15% soy protein supplemented groups were not significantly different ($P<0.05$) from each other; that is, enhancement of nutritional value of gari by 10% supplementation was not significantly improved ($P>0.05$) by a further supplementation to 15% soy protein level.

Table 4 shows the parameters used in assessing the nutritive value of soy gari compared with corresponding values in the reference (casein) diet. There were impressive improvements in the nutritional parameters as a result of supplementation, with most parameters having values between 89 and 97% relative to those of the casein diet. The biological performance of the 10 and 15% soy protein-supplemented gari-based diets were comparable with each other.

4. Discussion

The proximate composition of the soy bean flour and gari used in this study (Table 2) was comparable with values reported for whole soy beans (Adeojo & Oguntunde, 1993; Faryna, 1982; Oyenuga, 1968) and cassava farina or gari (Oke, 1966; Oyenuga, 1968).

The biological performance of the rats fed soy-gari is indicative of soy-gari's potential as a source of high quality protein foods. Because of its low protein content (less than 3%) and poor quality of the protein (deficient in methionine, lysine, tryptophan and phenylalanine), gari is expected to be unable to support growth. However, supplementation resulted in the ability to support growth and an impressive biological performance, with NPU values greater than 64%: approximately 90% of the NPU of the casein diet; the other parameters for assessment of nutritional value resulted in over 80% in that of the casein diet (Tables 3 and 4).

The protein intake of the casein group (4.27±0.82 g) was lower than those of soy-gari groups (8.36±1.20 and 9.55±1.70), yet the biological performance of the rats consuming the casein diet showed a better utilization of protein as evidenced by the superior PER, NPU, TD and BV values. This may be explained by the inferior essential amino acid profile of soy gari compared with casein. Both cassava and soy beans are limiting in methionine (Faryna, 1982; Oyenuga, 1968). However, the relatively high performance of rats eating soy gari in spite of this limitation may be attributable to the high protein score of about 70% for soy protein (Faryna, 1982). Also, the quantitatively high protein content of soy beans may compensate for the slight methionine deficiency.

Leaf protein concentrate (LPC) is another cheap plant protein source that has been advocated for the supplementation of starchy tubers and foods (Umoh & Oke, 1977). The performance of rats fed LPC-supplemented cassava flour resulted in NPU and BV values that were 72 and 73.5% of casein diets, respectively (Umoh & Oke, 1977). The current research, using soy protein supplemented cassava farina, gave corresponding values of over 90% relative to casein (Table 4). Thus, LPC is

Table 2
Proximate composition of soy flour, gari and soy gari (per 100g dry wt.)[a]

Parameters	Soy flour	Gari	Soy-gari I (10% soy protein)	Soy-gari II (15% soy protein)
Moisture content (g)	12.25±0.35	13.75±0.35	10.75±0.35	11.50±0.61
Crude protein (g)	32.1±0.10	2.77±0.25	10.9±0.16	15.9±0.50
Ash (g)	4.82±0.07	2.03±0.04	5.17±0.61	5.58±0.50
Carbohydrate (g)	35.3±0.05	92.45±0.14	64.9±0.67	60.2±1.83
Crude fibre (g)	7.02±0.01	2.44±0.06	6.65±0.37	6.53±0. 51
Fat content (g)	20.76±0.65	1.31±0.01	12.37±1.30	11.85±1.21

[a] Means of three determinations ± S.D.

Table 3
Biological performance of rats fed on casein and soy gari diets[a]

Parameter	Diets[b]			
	Basal non-protein	Casein (reference)	Soy-gari I (10% soy protein)	Soy-gari II (15% soy protein)
Weight gain (g)	−9.32±4.42	9.91±1.32	15.53±4.10	19.20±4.30
Protein intake (g)	–	4.27±0.82	8.36±1.20	9.85±1.70
Nitrogen intake (g)	–	0.71±0.10	1.35±0.16	1.59±0.20
Carcass nitrogen (g)	1.11±0.03	1.62±0.20	1.98±0.10	2.13±0.09
Fecal nitrogen (g)	0.08±0.01	0.10±0.02	0.19±0.01	0.24±0.05
PER	–	2.31±0.20	1.85±0.30	1.94±0.21
NPU (%)	–	71.9±2.42	65.8±2.10	64.13±1.40
TD (%)	–	97.2±1.70	91.9±2.10	89.99±1.90
BV (%)	–	73.9±2.15	70.9±1.32	71.53±2.52

PER, protein efficiency ratio; NPU, net protein utilization; TD, true digestibility; BV, biological value.
[a] Means± SD (6 rats per group).
[b] The 10% casein diet was superior ($P<0.05$) to both soygari diets. But there was no significant difference ($P>0.05$) between the performance of rats on soy-gari I and soy-gari II.

Table 4
Summary of the improvement in nutritive value of gari supplementation with soy bean protein

Parameter	Value of parameter of experimental diet relative to that of the casein diet		
	Casein	Soy-gari I (10% soy protein)	Soy-gari II (15% soy protein)
PER	100	80	84
NPU	100	92	89
TD	100	95	93
BV	100	96	97

PER, protein efficiency ratio; NPU, net protein utilization; TD, true digestibility; BV, biological value.

an inferior supplement for starchy foods compared with soy beans.

The food digestibility (about 90%) shows that the protein is easily digestible, an indication that the anti-tryptic factors that are known to be present in soy beans were effectively inactivated by the procedures used during soy-gari preparation. The food NPU and BV confirm the utilizability of the readily digestible soy gari protein.

There were no significant variations ($P > 0.05$) in the biological performance of the 10% compared with 15% soy protein-supplemented diets. This suggests that supplementation above 10% soy protein only causes minimal alterations in the nutritive value of soy-gari. It is, therefore, suggested that 10% soy protein supplementation (which can be obtained by mixing one part soy bean flour to three parts gari) should be used for soy-gari preparation.

There was marked improvement in the nutritional potential of gari by supplementation with soy beans to produce soy-gari, as evidenced by the NPU, TD and BV values. Therefore, soy-gari may be particularly useful for infants and children who are most vulnerable to protein energy malnutrition (PEM) because of their greater protein needs for growth. Protein intake in the predominantly starch foods area is usually further worsened because animal protein sources are out of reach to the majority of the population. Soy-gari is, therefore, recommended as a local food supplement to supply protein needs and eradicate PEM. Soy beans needed for its preparation are cheap and readily available in gari-consuming areas. The preparation of soy-gari is simple and requires no sophisticated equipment. Soy-gari is palatable, indistinguishable from gari, and can be consumed with customary or local soups without changing the cultural food habits of the people. The only cheaper plant protein source that has been advocated for the supplementation of starchy products is LPC. This has an inferior amino acid profile, gives lower improvement in nutritive value, is likely to face cultural acceptability problems and is technically more difficult to prepare than soy-gari.

5. Conclusion

Cassava farina (gari) has been supplemented with soy beans to produce soy-gari and the nutritional assessment of the soy gari has been studied. The results show that the nutritive value of gari is improved by soy bean supplementation to values as good as 90% of casein protein. The use of properly processed soy beans (by soaking in water, dehulling and heat treatment) for the supplementation of gari (fried fermented cassava flour) is a safe means of upgrading a staple food poor in protein content. The procedures involved in the processing of soy beans (into soy flour) and cassava (into gari) ensure that toxic anti-nutritional factors (lipoxygenases, protease inhibitors, haemaglutinins and cyanogenic glucosides), which have a negative effect on palatability, nutrient availability/utilization, metabolic processes and growth are eliminated (Anderson & Wolf, 1995; Kakade, Hoffa, & Liener, 1973; Mahungwu et al., 1987).

It is suggested that soy-gari be promoted in gari consuming areas of the tropics to alleviate nutritional protein deficiency disorders.

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