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Protein Quality and Anti-Nutrient Contents of Biscuits Produced From High Quality Cassava Flour (HQCF) and Rice Bran (RB) Blends

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ABSTRACT

Purpose: *This study was aimed at evaluating the protein quality and antinutrient contents of biscuits produced from blends of high-quality cassava flour and rice bran.*

Design methodology approach: *Biscuits were developed from blends of high- quality cassava flour (HQCF) and rice bran (RB) in the ratio of 100:0, 90:10, 80:20, 70:30 and 60:40. The 100% wheat flour sample served as control. The amino acid profile, protein value and anti-nutrient contents of biscuits developed were evaluated.*

Findings: *The total essential amino acids (EAA) and non- essential amino acids (NEAA) of the sample blends were very low, lower than the FAO reference patterns and the control though they increased significantly ($p<0.05$) with increased rice bran substitution. The biological value (BV), net protein utilization (NPU) and protein efficiency ratio (PER) increased with increase in rice bran supplementation from 16.67 to 88.89 %, 4.55 to 31.10 % and 1.38 to 2.68 respectively. These values were significantly lower than those of 84.87%, 66.41% and 3.50 respectively obtained for the control and 87.63%, 74.56% and 2.62 respectively for casein. The phytates and oxalates ranged from 0.07-1.18 and 0.16-2.30 % respectively indicating that the amino acids in the samples will not be interfered with. Significant differences $p<0.05$ existed among the samples.*

Originality value: *Inclusion of rice bran to high quality cassava flour improved the protein quality of the biscuit samples. This implies that blending HQCF and RB at up to the ratio of 100:40 can serve as a substitute to wheat flour.*

Keywords

Amino acid profile, Protein quality, Phytates, High quality cassava flour and rice bran.

Introduction

Biscuits are prominent ready-to-eat baked snack among the people, globally. The association of wheat consumption with such health problems as celiac disease makes it pertinent to utilize composite flour in biscuit manufacture [1].

High quality cassava flour (HQCF) is simply unfermented cassava flour and is one of the major products from cassava roots traded in the world food market. The future of Nigeria as one of the leading HQCF exporting countries is particularly bright as its export capacity had risen drastically. High quality cassava flour HQCF has also continued to find wider applications in food, feed and chemical industries due to low- cost, high quality and supply reliability (Guardian Nigeria News, 23 December 2025) (Accessed 27/04/2026). Gbenga Akinfenwa [2] reported that the Nigeria cassava investment accelerator (NCIA) estimated the actual HQCF inclusion in composite flour to be 1%, equivalent to a market value of up to US$35m. One of the most popular uses of HQCF worldwide is in the manufacture of baked products mounting up to 10 -35 % [3] (Abass Adebayo @a.abass@cgiar.org

Accessed 27/04/26). Nevertheless, cassava flour is low in protein [4]. Fortification with rice bran, a cheap and relatively available high protein source will certainly expand the spectrum of cassava flour utilization in the food industry. The starch of cassava flour is capable of forming strong gel and hence cassava flour could be used as a composite flour of good binding property [5].

Rice bran (RB), a nutrient-rich by-product of milled rice, is a soft brown powder comprising about 8 % by weight of the paddy rice (ResearchGate https://www.researchgate.net Accessed 27/04/2026) [6].

In view of the huge cost of wheat importation on the country's foreign reserve, the federal government in 2012, advocated for the partial replacement of wheat flour with 10 % inclusion of locally produced cassava (Manihot esculenta) flour in all baked products. The country stands to save over N300 billion annually [7].

With the recent government promotion of rice production and processing through its policy on the ban of imported rice, there is every need to explore other ways of utilizing the additional quantities of rice bran that would be generated. Rice bran, a rice industrial by- product is presently considered as waste. About 90 % is discarded and the remaining 10 % is fed to livestock [8]. It can be up graded and used for human food for value addition.

The use of cassava flour fortified with rice bran in producing biscuits will not only reduce the economic burden of importing wheat, but also reduce the production cost of biscuits. Biscuits are becoming everyday snack mothers give to their children especially the younger ones when going to school.

Therefore, the purpose of this work is to estimate the protein quality and anti-nutrient content of biscuit produced from high quality cassava flour fortified with rice bran as a way of valorization of rice bran in the improvement of nutritional value of biscuit.

Materials and Methods

Sources of raw materials

Freshly milled rice bran was obtained from Ebony Agro Industries Limited, a world class rice mill at Ekpa Omaka Industrial Cluster Ikwo, Ebonyi state.

High quality cassava flour was obtained from Thai Farm International, Ososa, Ogun State.

Margarine, sugar, salt, sodium bicarbonate (baking powder), milk and flavorings (vanilla) were purchased from Abakaliki main market, Ebonyi State.

Sample Preparation

Rice Bran Stabilization

The freshly obtained rice bran was stabilized in an oven at a temperature of 120 ºC for 15 seconds. The stabilized bran was sieved to obtain a consistent particle size (0.50mm) and also to remove impurities such as hulls and endosperms. The bran was packaged in polyethylene bags for future use [9].

Formulation of flour blends

Five flour blends were prepared by mixing high quality cassava flour with rice bran flour in the ratio of 100:0, 90:10, 80:20, 70:30, 60:40 and 100 % wheat flour as control as shown in Table 1. The blended flours were packaged in polyethylene bags and stored at room temperature from where samples were drawn for biscuit production.

Table 1: Formulated flour blends.

Sample	HQCF%	RBF%	WF%	Total%
A	-	-	100	100
B	100	-	-	100
C	90	10	-	100
D	80	20	-	100
E	70	30	-	100
F	60	40	-	100

HQCF-High quality cassava flour, RBF-Rice bran flour, WF-Wheat flour A-100% WF (Control), B-100% HQCF, C-90% HQCF+ 10%RBF D-80% HQCF + 20%RBF E-70% HQCF + 30% RBF and F-60% HQCF + 40% RBF.

Biscuit Production

The biscuits were prepared according to the procedure described by Mtswen. The basic ingredients used were 380 g of flour blend, 100 g margarine, 225 g of granulated cane sugar, 21 g of beaten whole fresh egg, 3.75 g of salt, 1.8 g of baking powder and required amount of water (15-20 ml) as shown in Table 2. The dry ingredients were weighed and mixed thoroughly in a bowl by hand for 5 min. The margarine rubbed-in until uniform. The egg was added and dough was formed. The dough was then kneaded thoroughly in a mixer for 5 min. The kneaded dough was rolled thinly on a sheeting board to a uniform thickness (8.0 mm) and cut out using a round scorn cutter to a diameter of 35 mm. The dough pieces were baked on greased pans at 160 °C for 20 min in an oven. The prepared biscuits were cooled at room temperature (30±2°C) and packed in high density polyethylene bags.

Table 2: Material composition for biscuit production**.**

Materials	Quantity (g)
Formulated flour blend	380
Margarine	100
Sugar	225
Eggs	21(whole, fresh)
Salt	3.75
Baking powder	1.8

Analysis

Total HCN determination

Preliminary determination of Total hydrogen cyanide HCN (part per million) in the high- quality cassava flour was carried out using the enzymatic picrate paper kit developed by Bradbury et al. [10].

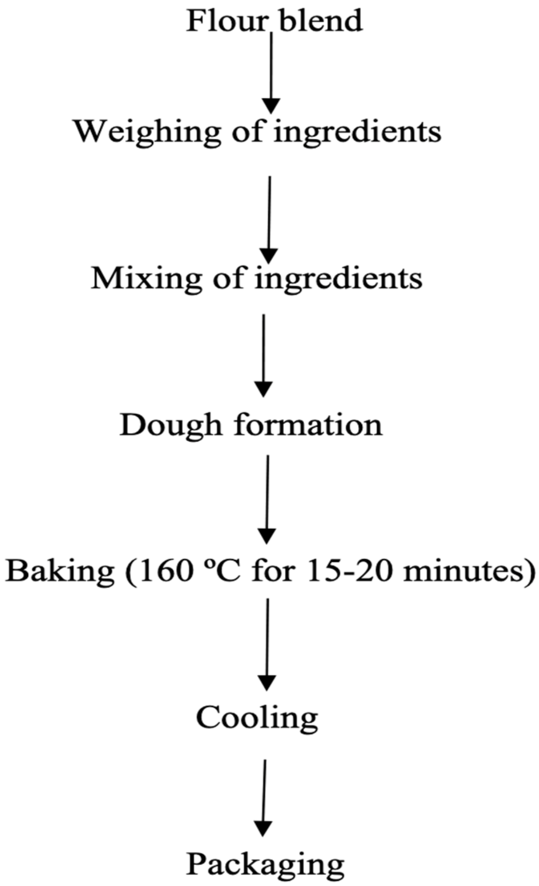

Figure 1: Flow chart for the production of biscuits.
Source: Mtswen [9]

Determination of Amino Acid Profile

The Amino acid profile of the samples was determined using the method described AOAC [11]. Chloroform and methanol were mixed in the ratio of 2:1. The solution was used to de-fat the flours. Amino acid analyzer was used to analyze and determine the amino acid contents of the samples.

Determination of protein quality using bioassay

The method as described by Ijeomah [12] was used.

Determination of protein efficiency ratio (PER)

Male weanly rats of albino strain of average weight (42-46) were grouped into seven groups of six rats each. The test biscuits were fed to the rats ad libitum for 28 days, period of acclimatization inclusive. The PER was estimated using the average protein consumed and body weight gain of the different groups.

Determination of biological value (BV) and net protein utilization (NPU)

At the end of the feeding period, nitrogen balance study was conducted for 7 days. Feacal marker (0.2 % carmine red) was used at the beginning and end of the 7-day study. Every day, the individual rats feaces in each group were collected together, dried for 3 h at 85^{o}C and weighed. These were ground into powder and stored in a plastic bottle. These were used for feacal nitrogen (FN) determination. Sample bottles containing 0.1 N HCL (to prevent ammonia loss) were used to collect the urine and stored in a fridge. These were used for urinary nitrogen (UN) determination. Estimation of these (BV and (NPU)) were done using these datae.

Determination of Anti-nutrients

Determination of Phytate

The method described by Ukpong et al. [13] was used. About 2.4 % hydrochloric (HCL) was used to extract 0.5 g of the sample. An aliquot was prepared by mixing 0.7 M NaCl (sodium chloride) and 0.1 M NaOH (sodium hydroxide), 2 ml mixed with the extract and filtered in a 200-400 mesh resin. A modified Wade reagent and distilled water were used to mix the filtrate, votex mixed for 5 s, centrifuged for 10 min and the supernatant read in a spectrophotometer (Genway 6305, England) at 500 nm wavelength. A standard curve was prepared wiyh sodium phytate where phytate concentration was read off. Phytate content was calculated using the equation

$$\text{Phytate (mg)} = \frac{A \times B}{C}.$$

Where A = concentration (mg/g) from the standard curve; B = dilution factor; C = weight of the sample.

Determination of oxalate

Method described by Ukpong et al. [13] was employed for the determination. Two grams of the sample flour was dissolved in 200 ml of HCl and the extract titrated with 0.1 M $KMnO_4$ (potassium permanganate) and the resultant constituent analyzed in spectrophotometer (Genway 6305, England) at 490 nm wavelength. Oxalate concentration was extrapolated from the standard curve. This was expressed as Oxalate mg/100g.

Statistical Design and Analysis

Data obtained were analyzed using statistical software (SPSS version 16). Analysis of variance was used to evaluate significant differences *($p<0.05$)* and means were separated using Duncan's multiple range test.

Results and Discussion

Protein quality of the samples

Cassava Flour Cyanide Content

Hydrogen cyanide content of the purchased high- quality cassava flour was found to be 1.23 mg/kg. This agreed with the manufacturer's claim of 1.23 mg/kg.

Amino Acid Composition of the Biscuits Samples

The essential amino acid (EAA) and non- essential amino acid (NEAA) contents of the samples are presented in Table 3 and 4 respectively. The total amount of EAA and NEAA obtained for the control sample (A) made from 100 % wheat flour was found to be (21.33 and 24.34) and for sample (B) made from 100 % high quality cassava flour (5.73 and 5.62) were relatively higher and lower than those of other samples. There were significant differences ($p<0.05$) in the EAA

Sample A (Control)= 100% Wheat flour biscuit, B= 100% High quality cassava flour biscuit, C= 90% High quality cassava flour: 10% Rice bran biscuit, D= 80% High quality cassava flour:20% Rice bran biscuit, E= 70% High quality cassava flour: 30% Rice bran biscuit and F= 60%High quality cassava flour: 40% Rice bran biscuit.

And NEAA of the samples. The EAA and the NEAA of all the individual amino acids were below the FAO/WHO reference pattern. The result of the protein quality evaluation is shown in Table 5. Variations existed in the quantity of protein consumed by the different groups of rats as a result of the differences in the protein contents of the samples. Rats fed with the biscuits containing 40 % rice bran consumed the highest amount of protein (7.25 g) while those fed with biscuits produced with 0 % rice bran consumed the least (3.97 g). Rats fed with 0% rice bran sample had low ($p<0.05$) food intake (50.93 g) and weight gain (5.48 g) in relation to those fed with other rice bran containing samples. Probably, 0 % rice bran sample was not palatable. This may be as a result of the fact that the rice bran fortified samples contained fat which made them palatable for fat is known to make food palatable [12]. Rats fed biscuits produced with 40% rice bran consumed the highest amount of food (77.01 g) and rats fed biscuits that contained 10 % rice bran consumed less (59.95 g) relative to those fed other blended samples. Rats fed blended samples had high weight gain 10 % HQCF/RB (11.76 g), 20 % HQCF/RB (13.72 g), 30 % HQCF/RB (16.38 g) and 40 % HQCF/RB (19.43 g) except for those fed the unblended sample (5.48 g). This is an indication that the blended samples promote growth better than the unblended sample.

Protein efficiency ratio (PER)

The protein efficiency ratio (PER) of the biscuit sample ranged from 1.38 in the unblended sample (0 % RB) to 3.50 in sample containing 100 % wheat flour (control). The PER (2.68) of sample that contained 40 % rice bran was higher ($p<0.05$) compared to the other blended samples. This suggests superiority of the sample over others. The PER of the samples were higher than the 2.0 recommended by Onimawo and Akubor [14] for a good quality protein

Biological value (BV) and Net protein utilization (NPU)

The group of rats fed biscuits containing 40% rice bran had higher nitrogen intake (1.16 g) and digested nitrogen (0.42 g) in relation to rats fed other blended samples. Rats fed with 40 % rice bran inclusion had relatively high feacal nitrogen (1.02 g) loss

Table 3: Essential amino acid content of the samples.

			Samples				
Amino Acids g/100g	A(Control)	B	C	D	E	F	Recommended FAO/WHO Amino Acids provisional pattern (1973)
Lysine	2.91	0.87	1.34	1.36	1.38	1.42	4.5
Threonine	2.69	0.67	1.72	1.75	1.78	1.83	2.6
Valine	2.97	0.92	1.39	1.44	1.46	1.48	4.2
Methionine	2.47	0.38	1.79	1.86	1.88	1.93	2.2
Isoleucine	2.88	0.80	1.38	1.44	1.47	1.88	4.2
Leucine	2.29	1.14	1.05	1.15	1.75	1.34	4.8
Phenylalanine	2.88	0.78	1.87	1.90	1.92	1.95	2.8
Tryptophan	2.24	0.17	1.39	1.44	1.46	1.49	1.1

Values are means of duplicates determinations± standard deviation, means with different superscripts in the same column are significantly different ($p<0.05$).

Table 4: Non-essential amino acid profile of biscuits samples.

	SAMPLES						
Amino Acids	A (Control)	B	C	D	E	F	FAO/WHO (1991) Reference Pattern). (g/100g protein)
Histidine	$1.39^{a}\pm0.01$	$0.28^{e}\pm0.02$	$1.43^{cd}\pm0.01$	$1.47^{bc}\pm0.02$	$1.49^{c}\pm0.01$	$1.59^{b}\pm0.01$	2.80
Arginine	$2.42^{a}\pm0.02$	$0.30^{e}\pm0.03$	$1.43^{d}\pm0.01$	$1.48^{c}\pm0.00$	$1.52^{c}\pm0.02$	$1.58^{b}0.02$	5.20
Aspartate	$2.43^{a}\pm0.01$	$1.21^{d}\pm0.03$	$1.83^{c}\pm0.02$	$1.87^{c}\pm0.01$	$1.88^{bc}\pm0.00$	$1.93^{b}\pm0.02$	7.70
Serine	$2.98^{a}\pm0.00$	$0.60^{e}\pm0.02$	$1.15^{d}\pm0.02$	$1.27^{c}\pm0.01$	$1.37^{bc}\pm0.01$	$1.44^{b}\pm0.03$	7.00
Glutamate	$2.55^{a}\pm0.02$	$1.34^{e}\pm0.02$	$1.30^{d}\pm0.00$	$1.35^{d}\pm0.01$	$1.40^{c}\pm0.01$	$1.49^{b}\pm0.02$	14.70
Proline	$2.28^{a}\pm0.01$	$0.11^{f}\pm0.01$	$1.31^{e}\pm0.01$	$1.43^{d}\pm0.02$	$1.53^{c}\pm0.02$	$1.65^{b}\pm0.01$	10.70
Glycine	$2.43^{a}\pm0.01$	$0.29^{e}\pm0.02$	$1.48^{d}\pm0.02$	$1.53^{c}\pm0.01$	$1.65^{b}\pm0.01$	$1.68^{b}\pm0.01$	2.20
Alanine	$2.83^{a}\pm0.01$	$0.68^{e}\pm0.00$	$1.85^{d}\pm0.01$	$1.88^{d}\pm0.01$	$1.91^{c}\pm0.01$	$1.94^{b}\pm0.01$	6.10
Cystine	$2.33^{a}\pm0.01$	$0.30^{d}\pm0.04$	$1.35^{c}\pm0.01$	$1.39^{c}\pm0.01$	$1.43^{b}\pm0.01$	$1.48^{b}\pm0.01$	3.0
Tyrosine	$2.70^{a}\pm0.01$	$0.51^{f}\pm0.01$	$1.76^{e}\pm0.01$	$1.91^{d}\pm0.01$	$1.91^{c}\pm0.01$	$1.27^{b}\pm0.01$	1.10

Values are means of duplicates determinations± standard deviation, means with different superscripts in the same column are significantly different ($p<0.05$). Sample A (Control)= 100% Wheat flour biscuit, B= 100% High quality cassava flour biscuit, C= 90% High quality cassava flour: 10% Rice bran biscuit, D= 80% High quality cassava flour:20% Rice bran biscuit, E= 70% High quality cassava flour: 30% Rice bran biscuit and F= 60%High quality cassava flour: 40% Rice bran biscuit.

indicating low nitrogen digestibility and utilization compared to rats fed with blended samples which had low feacal nitrogen loss. Biscuit samples containing 10 % and 20 % rice bran substitution showed similar *(p>0.05)* nitrogen intake (0.95 g). Biscuit samples produced with high quality cassava flour only had lower *(p<0.05)* retained nitrogen (RN) (0.04 g), biological value (BV) (16.67 g), net protein utilization (NPU) (4.55 g) and digestibility (27.29 g) than the blended samples. The higher BV for rats fed blended samples 10 % (72.73 %), 20 % (79.31 %), 30 % (88.89 %) and 40 % (88.10 %) rice bran respectively suggests better protein sources than non- blended sample. Significant variation *(p<0.05)* was observed in NPU and digestibility among the samples. The low digestibility obtained for the samples may probably be due to the fact that the samples were not boiled because heat is known to improve protein digestibility which consequently affected the protein utilization (NPU) [15]. The biological value (BV) of the Casein sample had the highest biological value followed by the whole wheat flour while biscuits made from the whole high quality cassava flour had the lowest biological value. The biological value of the biscuits increased with increase in rice bran substitution but however decreased at the highest level of rice bran (40 %) substitution. Biological value is a measure of the efficiency of utilization of absorbed nitrogen [16].

The values of the samples used for the study are relatively low to this value, which is an indication that all the available proteins were not properly utilized for the growth and maintenance of the rats. The net protein utilization (NPU) of biscuits increased with addition of rice bran but however decreased with the highest rice bran (40 %) substitution. The values obtained from the biscuits samples produced from high quality cassava flour and rice bran blended flours for NPU ranged from 4.55 to 31.10 % were far below the findings of Okpala and Okoli [16] who reported values for NPU range from 88.53 to 95.54 % for cookies produced from pigeon pea, sorghum and cocoyam flour blends. The values obtained were below 70 % recommended for good protein [17].

Net protein utilization is a measure of both digestibility and biological value of amino acids absorbed from food [16,18].

Values are means of triplicate determinations ± standard deviation. Means with different superscripts on the same row are significantly different (p≤0.05). Sample A (Control): 100% wheat flour biscuit, Sample B: 100% high quality cassava flour biscuit, Sample C: 90% high quality cassava flour: 10% rice bran biscuit, Sample D: 80% high quality cassava flour: 20% rice bran biscuit, Sample E: 70% high quality cassava flour: 30% rice bran biscuit, Sample F: 60% high quality cassava flour: 40% rice bran biscuit, FI: Food intake, BWG: Body weight gain, PC: Protein consumed, PER: Protein efficiency ratio, NI: Nitrogen intake, FN: Feacal nitrogen, DN: Digested nitrogen, UN: Urinary nitrogen, RN: Retained nitrogen, BV: Biological value, NPU: Net protein utilization.

Anti-nutrients content of samples

The anti-nutrients content of the biscuits is shown in Table 6.

Phytate

The phytate content of the biscuits ranged from 0. 07 to1.18 mg. Sample F made from 60 % cassava flour and 40 % rice bran had the highest mean value and sample B had the lowest mean

Table 6: Anti-nutrients content of samples (mg/100g).

Samples	Phytates	Oxalates
A (control)	0.23 a ±0.01	0.46 e ±0.03
B	0.07 f ±0.00	0.16 f ±0.01
C	0.86 e ±0.02	1.27 d ±0.00
D	0.93 d ±0.03	1.74 c ±0.01
E	0.98 c ±0.06	1.96 b ±0.03
F	1.18 b ±0.04	2.30 a ±0.01

Values are means of duplicates determinations± standard deviation, means with different superscripts in the same column are significantly different (p≤0.05).

Sample A (Control): 100% Wheat flour, B: 100% High Quality Cassava flour, C: 90% High Quality Cassava flour: 10% Rice bran, D: 80% High Quality Cassava flour: 20% Rice bran, E: 70% High Quality Cassava flour: 30% Rice bran and F: 60% High Quality Cassava flour: 40% Rice bran.

Table 5: Protein Quality of Samples.

	Samples						
Parameters	**A**	**B**	**C**	**D**	**E**	**F**	**Casein**
Protein (%)	10.25b	7.79g	8.31f	8.84e	9.38d	9.41c	10.00a
FI (g)	80.03a	50.93g	59.95f	64.97e	70.99d	77.01b	71.15c
BWG (g)	28.70a	5.48g	11.76f	13.72e	16.38d	19.43b	18.65c
PC (g)	8.20a	3.97g	4.98f	5.74e	6.66d	7.25b	7.12c
PER	3.50a	1.38g	2.36f	2.39e	2.46d	2.68b	2.62c
N I (g)	1.31a	0.88g	0.95e	0.95e	1.07d	1.16b	1.14c
FN (g)	0.28f	0.64e	0.80d	0.92c	0.98b	1.02a	0.17g
DN (g)	1.03a	0.24f	0.22g	0.29e	0.36d	0.42c	0.97b
UN (g)	0.16a	0.16a	0.15b	0.03f	0.09e	0.14c	0.12d
RN (g)	0.87a	0.04g	0.16f	0.23e	0.32d	0.37c	0.85b
BV (%)	84.47d	16.67g	72.73f	79.31e	88.89a	88.10b	87.63c
NPU (%)	66.41b	4.55g	23.16f	24.21e	29.91d	31.10c	74.56a
Digestibility (%)	78.62b	27.29g	31.84e	30.53f	33.65d	35.30c	85.44a

Significant difference ($p<0.05$) existed in the phytate content of the samples. Increase in rice bran levels increased the phytate concentration in the biscuits. High levels of phytic acid interferes with mineral utilization in the body by binding the divalent minerals (iron, zinc, magnesium and calcium) implying non availability of these nutrients in the samples [13]. This report concours with Oboh et al. [19]. Phytate content of the samples increased with increase in rice bran supplementation. The values obtained in this study were lower than that reported for Ocimum basillieum (260 mg/100g) by Udosen and Ukpanah [20]. The low values of phytate found in the blends implies no interference and high bioavailability of the nutrients. Liu et al. [21] reported anti-cancer property and protective action in carcinogenesis of phytic acid. According to Makkar [22], the presence of phytic acid in dietary sources is a potential antioxidant.

Oxalate

The oxalate contents of the biscuits ranged from 0.16 mg in the sample containing 0 % rice bran and 100 % high quality cassava flour (HQCF) to 2.30 mg in the sample that contained 40 % rice bran. There were significant differences ($p<0.05$) in the oxalate contents of the samples. Increase in rice bran substitution increased the oxalate concentration in the biscuit samples. According to Ukpong et al. [13], oxalate chelates calcium to render it unavailable. The oxalate content increased significantly ($p<0.05$) with increase in the rice bran supplementation. This agrees with Egbedike et al. [23] who reported increase in oxalate content of water yam /rice bran flour blend with increase in rice bran substitution. Satinder et al. [24] reported high oxalate value for rice bran. The oxalate value obtained for this study was lower than the maximum daily recommended intake of 50-60 mg/100g [25] implying no risk of renal calcium absorption because higher oxalate diet can increase the risk of renal calcium absorption [26].

Antinutrients are concentrated in the seed coats of legumes and hull/husks of cereals. Removal of the hull/husk of the paddy rice during milling accounted for the low levels of these antinutrients in the rice bran and the blends.

Conclusion

The study showed that rice bran generally improved the nutritional quality of biscuits produced from cassava and rice bran flour blends. Biscuit sample made from 60 % high quality cassava flour and 40 % rice bran (F) was generally acceptable among the test samples.

However, the protein efficiency ratio (PER) of the blended samples was higher than 2.0 recommended value for good quality protein, biological value (BV) high, net protein utilization (NPU) low and digestibility average in relation to the standards for high quality proteins.

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