Research Article

Amino Acid Profiles, Total Nitrogen Contents, and Computed-Protein Efficiency Ratios of *Manihot esculenta* Root and *Dioscorea rotundata* Tuber Peels

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Tuberous roots of cassava and yam are major sources of dietary carbohydrate to human, alternative sources of energy in livestock feeds, and sources of starch in small-scale industries. Investigations on amino acid profiles, total nitrogen contents, and computed-protein efficiency ratios (C-PER) of tuberous root peels of *Manihot esculenta* Crantz and *Dioscorea rotundata* Poir. were carried out. Amino acid analysis was carried out using ion-exchange chromatography methods. Total nitrogen content was measured using the micro-Kjeldahl methods. The C-PER was calculated using regression equation. The concentrations of amino acids detected in the cassava peels ranged from 0.54 to 6.54 g/100 g protein, whereas those of yam peels were between 0.37 and 6.25 g/100 g protein. The total amino acid concentration of the cassava peels was not significantly ($p > 0.05$) higher than that of the yam peels. Essential amino acid scores showed that Phe + Tyr and Met + Cys were the most abundant and limiting amino acids, respectively, in cassava and yam peels. The percentage nitrogen content and C-PER of the cassava peels were significantly ($p < 0.05$) higher than those of the yam peels. The cassava and yam peels were not sources of good quality proteins. Therefore, the use of cassava or yam peels as livestock feeds should be supplemented with other sources rich in good quality proteins.

1. Introduction

L- α -Amino acids are the primary sources of nitrogen atom for biological systems. They are precursors for the biosynthesis of nitrogenous compounds such as haem, purines, urea, pyrimidines, hormones, neurotransmitters, biological active peptides, and proteins [1, 2]. Out of over 300 naturally occurring amino acids, precisely 20 amino acids are utilized in biologic systems to form vast arrays of protein molecules [1, 3]. Nutrition scientists have shown that humans and other mammals lack the capability to synthesize around 10 of the 20 L- α -amino acids present in proteins in amounts adequate to support infant growth or to maintain well-being in adulthood. Consequently, diets for human and livestock must contain adequate quantities of these nutritionally

essential amino acids, whereas the remaining nutritionally nonessential amino acids are readily biosynthesized from metabolic pathways involving amphibolic intermediates [2]. Amino acids such as Leu, Ile, Trp, Lys, Phe, and Tyr are termed ketogenic because they are precursors for the synthesize ketone bodies, namely, acetone, acetoacetate, and β -hydroxybutyrate, while Arg, Gln, His, Pro, Ile, Met, Thr, Val, Phe, Tyr, Asp, Asn, Ala, Cys, Gly, Ser, and Trp are termed glucogenic because they can be metabolized to glucose and glycogen. However, Ile, Trp, Tyr, and Phe are both ketogenic and glucogenic, whereas Lys and Leu are strictly ketogenic [4].

The dietary quantity and quality of proteins depend on the source of the food material. Dietary protein may exhibit varied physiochemical properties in terms of their

digestibility and bioavailability as well as corresponding biologic value [5]. From nutritional standpoint, protein efficiency ratio (PER) defines the quotient between quantity of protein consumed and corresponding body weight gained by the animal. By derivation, computed-protein efficiency ratio (C-PER), as previously reported [6], describes a useful parameter for the evaluation of protein quality [7]. For the most part, the animal proteins are considered superior to those of plants because they can maintain body positive nitrogen balance by supplying all the essential amino acids even as the only source of nitrogen in the diet [8]. Additionally, plant proteins are generally not as well digested and assimilated when compared to animal proteins [9]. Nevertheless, studies have shown that proteins derived from plant products such as corn germ, soya bean, wheat germ, and yeast provide approximately the same proportion of amino acids as are supplied by animal proteins [10].

Overall, tuberous roots of *Manihot esculenta* Crantz (cassava) and *Dioscorea rotundata* Poir. (yam) are major sources of dietary carbohydrate to human, alternative sources of energy in livestock feeds, and sources of starch in small-scale industries [11–15]. Report according to Okigbo [16] noted that cassava root peels contained slightly more protein than was present in the starchy parenchyma portion of whole root. Some cassava-based products known by their local names include *abacha*, *fufu*, *farinha*, *lio-lio*, *tapioca*, and *garri*. The tropical belt in African produces more cassava roots than the rest of the world put together, with production level that scaled over 230 million metric tonnes in 2010 [17].

A transverse section of cassava root reveals three distinct layers [18–20]. The outermost layer or peridermal region of the cassava root weighs about 0.5–2.0% of the total wet root weight. The cortical parenchyma measures between 1 and 2 mm thick and contains most of the cyanogenic glycosides present in cassava root. The early step of processing cassava root into various products involves manual removal of its outer cover using a knife.

Yam tuber is usually cylindrical in shape and weighs 3–5 kg. However, the shape and size may vary due to genetic and environmental factors [19]. Although there are over 200 species of yams, only 10 species are sources of staple food in the tropics [21, 22]. In 2005, five million hectares in about 47 countries worldwide produced 48.7 million metric tonnes of yams, of which 97% of the produce was in Sub-Saharan Africa [23]. Yams are often eaten boiled, fried, and roasted or pounded into white paste or dark brown paste called *Amala* in southern Nigeria, which is a popular local delicacy in Yoruba land made from yam powder. Like other tuber and root crops, processing of yam tubers begins with removal of the outer cover using a knife. The nutrient composition and energy values of yam varieties have been described elsewhere [24]. The transverse section of a mature yam tuber as described [19, 25] reveals an outer portion or corky periderm and inner cortex beneath the periderm, which contains small quantity of stored starch. The meristematic layer is comprised of thin-wall of cells from which sprouts are initiated. The ground tissues are repositories to the vascular bundles and vast number of starchy cells. The yam peels are mostly comprised of the corky periderm, cortex, and meristematic layer.

One of several measures to overcoming the major challenges of food security in Nigeria involves maximum utilization of food crops, in which the by-products and wastes generated during the processing stage are transformed into useful and consumable products. In animal husbandry, cassava and yam peels are cheap sources of feeds for livestock [15, 26, 27]. Ruminants digest the fibre content of the peels using mutualistic microorganisms into methane, CO₂, acetic, and propionic and butyric acids, which are absorbed by the animal (host) as a major source of energy [28]. However, in the agro-based small-scale industries, the impact of cassava peels on environmental pollution [29–32] underscores the necessity to convert these wastes to useful products, which serves to increase the food and economic values of cassava roots and, by extension, yam tubers. Accordingly, investigations on the amino acid profile, total nitrogen content, and C-PER of tuberous root peels of *M. esculenta* and *D. rotundata* were carried out in order to establish their collective potential to serve as readily available sources of dietary amino acids and quality proteins for maintenance of body positive nitrogen balance.

2. Materials and Methods

2.1. Collection of Cassava and Yam Samples. Matured and healthy roots of the “bitter” cassava variety (*M. esculenta*) and “white” yam variety (*D. rotundata* Poir.) were harvested during the wet season, on 16 August, 2015, from Ofkaja Farm at Uruagu-Nnewi, Anambra State (latitude 6°20'N; longitude 7°00'E), Nigeria, which lies on the rainforest belt. The cassava roots and yam tubers were transported to the laboratory within 24 h, identified, and authenticated by Dr. F. N. Mbagwu at the Herbarium of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The samples have voucher numbers IMSUH 076 and IMSUH 116 for the yam tubers and cassava roots, respectively.

2.2. Peeling and Drying. The cassava roots and yam tubers were washed under continuous current of tap water for 5 min to remove soil matter and thereafter mopped dry using blotting papers. The outer cover of the cassava roots and yam tubers were manually removed using a stainless kitchen knife. The cassava and yam peels were collected separately on stainless-steel trays and oven-dried (Gallenkamp Oven 300 plus series, England) at 150°C for 24 h. The samples were heated at such a temperature because denaturation of proteins by heat affects primarily the hydrogen bonds without breaking the covalent bonds in the polypeptide [4]. The dried peels were cooled to ambient room temperature ($T = 25 \pm 5^\circ\text{C}$), ground into powder, and stored in sterile glass jars with screw caps until used for further analyses.

2.3. Analysis for Amino Acid Composition. Amino acid analysis was carried out using ion-exchange chromatography (IEC) as described by Spackman et al. [33], Ibegbulem et al. [34], and Ibegbulem and Belonwu [35]. The samples were defatted, acid digested before dispensing the digest into the amino acid analyzer. Briefly, the dried cassava and yam peels were defatted according to standard methods [36]. A quantity (6 g) of the pulverized cassava peels was weighted

and transferred into an extraction thimble. The extraction of the lipid soluble matter of the peels was carried out using chloroform/methanol (2:1; v/v) mixture in a Soxhlet extraction apparatus. Then, 4 g of the defatted pulverized peels was transferred into a glass ampoule. A volume (8 mL) of 6 N HCl was added to the sample and oxygen expelled from the vicinity of the sample/acid mixture by passing gaseous nitrogen into the glass ampoule. The ampoule was sealed over a Bunsen burner flame, transferred into an oven (Gallenkamp Oven 300 plus series, England), preset at $105 \pm 5^\circ\text{C}$, and allowed to stand for 22 h. Thereafter, the ampoule was allowed to cool to ambient room temperature, the content released by breaking the tip and filtered using Whatman No 52 filter paper [37]. The filtrate was evaporated to dryness in a hot air oven (Gallenkamp Oven 300 plus series, England). Finally, the residue was dissolved in 5 mL acetate buffer (pH = 2.0) and stored in a plastic tube at freezing temperature of -4°C until used for analyses. A volume of 10 μL of the digest was dispensed into the cartridge of Technicon Sequential Multi-sample (TSM) amino acid analyzer (Technicon Instruments Corporation, New York). The automated analysis lasted for 76 min. The entire procedure was repeated for the pulverized yam peels. The concentration of each free amino acid was proportional to the peak area marked out by an integrator attached to the TSM analyzer.

2.4. Resolution of Asp, Asn, Glu, and Gln Contents of Sample.

The contents of Asp and Asn as well as Glu and Gln were resolved as described by Ibegbulem [39] and Ibegbulem et al. [34] using the ratio of 5.3/4.3 for Asp to Asn and 6.3/4.2 for Glu to Gln. Amino acids like Asp, Asn, Glu, and Gln have average percentage occurrence of 5.3, 4.3, 6.3, and 4.2, respectively, in 1150 proteins of known amino acid sequences (Nelson and Cox, 2008). The total Glu (Glx) and total Asp (Asx) contents estimated for the cassava and yam peels were 6.54 and 6.00, respectively, and 6.25 and 5.81, respectively.

2.5. Computation of Amino Acid Groupings. The computation of the total amino acid (TAA), total essential amino acid (TEAA), total nonessential amino acid (TNEAA), total acidic amino acid (TAAA), and total basic amino acid (TBAA) of a sample was calculated as described by Ibegbulem et al. [34]. Total glucogenic amino acid (TGAA) was calculated by summing up the concentrations of Arg, Gln, His, Pro, Met, Thr, Val, Asp, Asn, Ala, Cys, Gly, and Ser while the total ketogenic amino acids (TKAA) were calculated by summing up the Lys and Leu contents of the samples. Trp was not used in the computations because it is normally destroyed during such chemical analysis [4, 34, 35].

2.6. Percentage Amino Acid/TAA Ratio. This was calculated as the ratio of the concentration of the amino acid to TAA multiplied by 100.

2.7. Chemical Scores of Essential Amino Acids. The essential amino acid (EAA) score of an EAA was calculated as the ratio of the concentration of that EAA (mg/g protein) to that of its desired concentration (mg/g protein) in a reference food

TABLE 1: Amino acid profile (g/100 g protein) of cassava and yam peels.

Amino acid	Cassava peels	Yam peels	Mean	SD	%CV
Lys ^c	2.42	2.36	2.30	0.03	1.26
His ^c	1.08	0.95	1.02	0.07	6.40
Arg ^c	3.23	3.15	3.19	0.04	1.25
Thr ^c	2.27	2.16	2.22	0.06	2.70
Met ^c	0.54	0.37	0.46	0.09	19.57
Ile ^c	3.13	1.70	2.42	0.72	29.75
Phe ^c	2.82	2.29	2.56	0.27	10.55
Leu ^c	4.17	3.58	3.88	0.30	7.75
Val ^c	3.10	3.16	3.13	0.33	0.95
Asp ^b	3.31	3.21	3.26	0.05	1.53
Asn ^b	2.69	2.60	2.65	0.05	1.89
Ser ^b	1.59	1.41	1.50	0.09	6.00
Glu ^b	3.92	3.75	3.84	0.09	2.34
Gln ^b	2.62	2.50	2.56	0.06	2.34
Pro ^b	1.62	1.39	1.51	0.12	7.95
Gly ^b	2.40	2.41	2.41	0.00	0.00
Ala ^b	3.31	3.31	3.31	0.00	0.00
Cys ^b	0.55	0.41	0.48	0.07	14.50
Tyr ^b	1.99	1.90	1.95	0.05	2.56

Values are means of triplicate determinations; c = essential amino acids; b = nonessential amino acids; SD = standard deviation; CV = coefficient of variation.

protein [34, 35, 40]. The FAO/WHO/UNU [38] amino acid scores were used as standard reference values.

2.8. Total Nitrogen Content. The total nitrogen contents of the cassava and yam peels were measured using the micro-Kjeldahl methods as previously described [36].

2.9. Computation of Protein Efficiency Ratio. The C-PER was calculated using the regression equation described by Alsmeyer et al. [6]:

$$\text{C-PER} = -0.684 + 0.456 [\text{LEU}] - 0.047 [\text{PRO}]. \quad (1)$$

2.10. Statistical Analysis. The results were expressed as mean $\pm$ SD, statistically analyzed using one-way ANOVA, and the level of significance was set at $p < 0.05$. The data were also analyzed using the percentage coefficient of variation (%CV).

3. Results

The concentrations of the various amino acids detected in the cassava peels ranged from 0.54 to 6.54 g/100 g protein, whereas those of yam peels were between 0.37 and 6.25 g/100 g protein (Table 1). Furthermore, the results in Table 1 showed that cassava and yam peels contained comparatively high concentrations of Leu and Glu, respectively, whereas Met and Cys concentrations were relatively low. There was no disparity in amino acids concentrations between the cassava and yam peels with respect to Gly

TABLE 2: Average amino acid groupings of cassava and yam peels and some ratios.

Groups	Cassava peels	Yam peels	Mean	SD	%CV
TAA (g/100 g protein)	46.76	42.61	44.69	2.08	4.65
TNEAA (g/100 g protein)	24.00	22.89	23.45	0.56	2.39
TEAA (g/100 g protein)	22.76	19.72	21.24	1.52	7.16
TAAA (g/100 g protein)	7.23	6.96	7.10	0.14	1.97
TBAA (g/100 g protein)	6.73	6.46	6.60	0.14	2.12
TGAA (g/100 g protein)	28.31	27.03	27.67	0.64	2.31
TKAA (g/100 g protein)	6.59	5.94	6.27	0.33	5.26
TEAA/TAA ratio	0.49	0.46	0.48	0.12	25.00
TNEAA/TAA ratio	0.51	0.54	0.53	0.12	22.64
TNEAA/TEAA ratio	1.05	1.86	1.46	0.40	27.40
TAAA/TBAA ratio	1.07	1.08	1.08	0.01	0.93
TGAA/TKAA ratio	4.30	4.55	4.43	0.03	0.68
%Pro/TAA ratio	3.46	3.26	3.36	0.10	2.98
%Gly/TAA ratio	5.13	5.66	5.40	0.27	5.00

and Ala. In contrast, the variability in Ile concentrations between the cassava and yam peels was relatively very high as exemplified by the %CV = 29.75. Additionally, %CV of His, Met, Ser, Leu, Pro, Cys, and Phe concentrations between the cassava and yam peels were moderately high.

The total amino acids (TAA) concentration of the cassava peels was not significantly ($p > 0.05$) higher than those of the yam peels (Table 2). Likewise, the total nonessential amino acids (TNEAA) concentration of the cassava peels was not higher than those of the yam peels.

Table 2 showed that the yam peels exhibited relatively lower concentrations of total essential amino acids (TEAA) than those of the cassava peels. The ratios of TEAA or TNEAA to TAA, TNEAA to TEAA, and %Gly/TAA between cassava and yam peels were significantly different ($p < 0.05$). However, the ratios of either TAAA to TBAA, TGAA to TKAA, and %Pro/TAA between cassava and yam peels were not significantly different ($p > 0.05$).

The values of the TEAA, TKAA, and TEAA/TAA ratios of the cassava peels were significantly ($p < 0.05$) higher than those of the yam peels, whereas the TNEAA/TAA and TNEAA/TEAA ratios of the yam peels were significantly ($p < 0.05$) higher than those of the cassava peels (Table 2). The average amino acid concentrations, in the cassava and yam peels, according to their groups were in the order: TGAA > TNEAA > TEAA > TAAA > TBAA > TKAA.

The Met + Cys, Ile, Leu, and Phe + Tyr essential amino acid scores of the cassava peels were significantly ($p < 0.05$) higher than those of the yam peels (Table 3). The TEAA score of cassava peels was significantly ($p < 0.05$) higher than those of the yam peels.

Table 4 showed that the percentage nitrogen content (%N) and C-PER value of the cassava peels were significantly higher than those of the yam peels.

TABLE 3: Essential amino acid scores of cassava and yam peels relative to provisional amino acid scoring pattern.

Amino acid	*Standard value	Amino acid scores (mg/g protein)				
		Cassava peels	Yam peels	Mean	SD	%CV
Lys	55	0.44	0.43	0.44	0.01	2.27
Thr	40	0.57	0.54	0.56	0.02	3.57
Met + Cys	35	0.31	0.22	0.27	0.05	18.52
Ile	40	0.78	0.43	0.61	0.18	29.51
Leu	70	0.60	0.51	0.56	0.05	8.93
Val	50	0.62	0.63	0.63	0.01	1.59
Phe + Tyr	60	0.80	0.70	0.75	0.05	6.66
Total	360	4.12	3.46	3.79	0.33	8.71

SD = standard deviation; CV = coefficient of variation; *standard value of essential amino acid scores according to FAO/WHO/UNU [38].

TABLE 4: Percentage nitrogen contents and computed-protein efficiency ratios of cassava and yam peels.

Parameter	Cassava peels	Yam peels	Mean	SD	%CV
%N	3.33 ± 0.07 ^a	1.17 ± 0.09 ^b	2.25	0.08	6.40
C-PER	1.14	0.88	1.01	0.13	12.87

%N = percentage nitrogen; C-PER = computed-protein efficiency ratio; SD = standard deviation; CV = coefficient of variation. Data on same row with different superscript letters are significantly different ($p < 0.05$).

4. Discussion

Previous reports had shown that tuberous roots of cassava and yam contain relatively low amounts of proteins that were

within the range of 1–4% [41, 42]. However, protein content of the tuberous roots may vary considerably among different species and cultivar depending on climatic, edaphic, and growth conditions as well as level of maturity at harvest [42–44]. The outcome of the amino acid profiles of the present study (Table 1) showed that Leu and Glu were, comparatively, the most abundant amino acids in cassava and yam peels, respectively. The relative high concentrations of Glu in yam peels conformed to previous reports on the distribution of the amino acid in two cultivars of *D. rotundata* tubers undergoing storage [42] and matured *M. esculenta* roots [45]. Earlier reports had noted that the total nitrogen content of cassava roots accounted for about 50% of the crude protein content, whereas the other 50% comprised free amino acids, which were predominantly Glu and Asp, and nonprotein components such as nitrite, nitrate, and cyanogenic compounds [44, 46, 47]. The level of disparity in amino acids contents between cassava and yam peels were such that Cys, Met, Phe, and Ile concentrations exhibited double-digit variability, whereas those of Ser, His, Leu, and Pro exhibited single-digit variability as exemplified by their corresponding %CVs (Table 1). Nevertheless, the protein quality of cassava peels was better than those of the yam peels (Table 4) due to its higher Pro and Leu contents (Table 1) as expressed in (1) and defined by C-PER.

The ratios of TAAA to TBAA and TGAA to TKAA between cassava and yam peels (Table 2) suggested that their proteins were more negatively charged and that more than fourfold of the ketogenic amino acids contents can be used to synthesize glucose and glycogen. The %Pro/TAA ratios and %Gly/TAA ratios indicated that cassava and yam peels contained globular proteins. Fibrous proteins like collagen contain 33% Gly and 13% Pro, whereas globular proteins like haemoglobin contain 4% Gly and 5% Pro [48].

The aromatic amino acids were the most abundant essential amino acids in the peels (Table 3) but fell short of meeting adequate nutritional requirements by 20 and 30% for cassava and yam peels, respectively. Conversely, the present findings showed that Met + Cys had the least essential amino score in the tuberous roots peels of cassava and yam, respectively, exemplified by their relatively low levels (Table 1). This result corroborated previous reports [42, 46, 49, 50]. The sulphur-containing amino acids, Met + Cys, fell short of meeting adequate nutritional requirement by 69 and 78% in cassava and yam peels, respectively. Previous reports showed that Leu had the lowest essential amino acid score in heat-treated fresh palm wines from *Raphia hookeri* and *Elaeis guineensis* [34], whereas Thr had the lowest essential amino acid score in process-line cocoa nibs and processed cocoa cake samples [50]. However, the vagaries of intermediary metabolism, especially in tuberous roots during storage or sprouting, may prompt wide variability in their amino acids concentrations and distribution [42]. Since the peels contained all the essential amino acids, their amino acids contents can be used to synthesize proteins with strikingly different properties and activities unlike those of palm kernel oil which did not contain essential amino acids like Ile, Thr, and Val [35]. The corrections for the sulphur-containing amino acids in the peels were calculated to be 1/0.31 or

3.23 times and 1/0.22 or 4.55 times the protein contents of cassava and yam peels, respectively. Specifically, their protein contents were 20.81 and 7.31% for the cassava and yam peels, respectively, if their %N contents (Table 4) are multiplied by the conversion factor of 6.25. These were indications that the cassava peels were of better protein quality than the yam peels, especially since it contained more essential amino acids. However, earlier reports [43, 51, 52] had indicated that yam species such as *D. dumetorum* (bitter yam) and *D. trifida* contained comparatively higher protein concentrations, which, by implication, contained higher quantity of TAA compared with *D. rotundata*. Incidentally, yam species noted to contain relative high protein concentrations which were correspondingly rich in alkaloids [41].

The higher percentage of nitrogen in cassava peels than that of the yam peels (Table 4) may be connected to the presence of comparatively more nonprotein nitrogen elements derived from nitrite, nitrate, cyanogenic glycosides, and hydrocyanic acid (HCN) in cassava roots [46], since the total amino acids concentrations of cassava and yam peels exhibited no significant difference (Table 2). The C-PER of cassava peel was higher than that of yam peels, suggesting that it is more nutritious. However, the C-PER indices of cassava and yam peels were lower than those of whole body, flesh, and exoskeleton of *Sudananautes africanus africanus* (West African freshwater male crab) and other animal proteins [53, 54]. The minimum threshold index of C-PER for good quality protein was reported to be 1.50 [10]. Plant proteins that have been noted to be of good quality in this regard include those from groundnuts: C-PER = 2.62 [55], pigeon pea: C-PER = 1.82 [56], raw and heat-treated fruits of *Canarium schweinfurthii* (African elemi): C-PER = 1.69–2.10 [57], and millet *ogi*: C-PER = 1.62 [58]. Therefore, the findings of the present study did not qualify proteins from cassava and yam peels to be of good quality by virtue of their C-PER indices. Nonetheless, cassava and yam peels were better protein sources than palm kernel oil [35, 59]. However, the use of cassava or yam peels as livestock feeds are usually supplemented with other sources that are rich in good quality proteins or are subjected to biofortification and protein enrichment as previously described [12, 26, 32, 46, 60–62].

5. Conclusion

The present study showed that cassava and yam peels had relatively low protein content. The amino acid profile of the peels of cassava roots and yam tuber indicated that Leu and Glu were the most abundant amino acids, whereas Met and Cys were the limiting amino acids. Cassava peels contained higher levels of nitrogenous elements than yam peels. The C-PER indices did not qualify proteins from cassava and yam peels to be of good quality. Accordingly, the use of cassava or yam peels as livestock feeds should be supplemented with other sources rich in good quality proteins or subjected to biofortification and protein enrichment.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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