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Varietal Composition and Functional Properties of Cassava (*Manihot esculenta, Cranzt*) Leaf Meal and Leaf Protein Concentrates

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Abstract: Cassava leaf samples harvested from local and genetically improved cassava varieties were processed into cassava leaf meal (CLM) and cassava leaf protein concentrate (CLPC) using the low cost village-level fractionation scheme. Chemical and physicochemical analyses were carried out to determine the proximate and amino acids composition and to also ascertain the functional properties of the CLMs and CLPCs. The protein content of the CLPC was high at 470gkg^{-1} DM comparable with other conventional protein sources. Crude fibre content was 20gkg^{-1} DM. The crude fat was high at 216gkg^{-1} DM and nitrogen free extract low at 159gkg^{-1} DM. The amino acid profile of the CLPC showed a favourable balance of both essential and non-essential amino acids especially for lysine, leucine, valine and tryptophan at 6.80, 9.65, 6.30 and 2.31 g/16gN, respectively. The limiting amino acid appeared to be methionine at 2.48g/16gN. The gross energy value was also noteworthy at 52.4MJkg^{-1}. The water absorption capacity (WAC) of CLM averaged 409.6% while that of CLPC averaged 181.5%. Fat absorption capacity (FAC) was 48.3% in CLM and 33.4% in CLPC. Fat emulsion capacity (FEC) value for CLM was 27.4% and 32.5% for CLPC. Fat emulsion stability (FES) values were 41.2% and 42.9% for CLM and CLPC, respectively. The foaming capacity values were 17.7% and 32.1% in the CLM and CLPC, respectively. Foaming stability and least gelation concentration were 4.3cm^3 after 30min and 9.0 for CLM as compared with 10.2cm^3 and 12.5% for CLPC. The nutritive potential, low-cost and the simplicity of the production technology make CLPC attractive as a source of protein in local food production system as a practicable and ameliorative intervention strategy for the endemic protein under-nutrition in most developing regions.

Key words: Village-level fractionation scheme, amino acid profile, cassava leaf

Introduction

The green vegetable has long been recognized (Byers, 1961; Oke, 1973) as the cheapest and most abundant potential source of proteins because of its ability to synthesize amino acids from a wide range of virtually unlimited and readily available primary materials such as water, CO_2 , atmospheric N_2 (as in legumes). For example, cassava leaves, as by-products of cassava roots harvest are (depending on the varieties), rich in proteins, minerals, vitamins B_1, B_2, C and carotenes (Eggum, 1970; Ravindrian and Blair, 1992; Adewusi and Bradbury, 1993; Aletor and Adeogun, 1995).

Paradoxically, cassava leaves (with an all-year-round availability in this region), although very rich in proteins (Aletor and Fasuyi, 1997) have remained under researched and consequently under-utilized. Similarly, large tonnages of cassava leaves are currently discarded as wastes after harvesting the roots. While small quantities of cassava leaves may be consumed either as condiments in human diets or as supplements to non-ruminant diets, the consumption of enough quantities of the leaves to meet protein needs by these species is impracticable. The impracticability derives from the high fibre and water content (bulkiness) of the leaves.

Consequently, it has been suggested (Oke, 1972; Aletor and Fasuyi, 1997) that with mechanical separation of the fibre, fibre-free proteins from leaves [(i.e. leaf protein concentrates (LPC)] represent a viable option in ameliorating the endemic problems of protein under-nutrition through the development and utilization of this non-conventional protein resource. The present report presents the proximate composition, energy values, amino acid profile, and the functional properties of cassava leaf meals and leaf protein concentrates. These parameters are important determinants of the food value or supplemental potential of this alternative protein resource especially in low-N foods/feed.

Materials and Methods

Leaf meal preparation and leaf protein concentrate (LPC) production: Leaf samples were harvested from local or genetically improved cassava varieties: Local variety *(Ege Oda)*; MS 6; TMS 30555 and TMS 30572 in fresh conditions from cultivated plots on the campuses of the Federal University of Technology, Akure and

Federal College of Agriculture, Akure. Both campuses are located in the humid tropical rainforest with average rainfall ranging between 1150-2000m with utisols as the predominant soil type. The harvested leaves were divided into 2 portions. One portion was sun-dried, and milled to pass through 0.5 mm sieve for functional properties studies, while the other in fresh condition was pulped with a pulping machine followed by pressing with a screw press. The flow-chart for the low-cost, village-level fractionation scheme as adapted from Fellows (1987) is shown in Fig 1. The separated leaf juice was heated in batches to 80-90^{o}C for about 10 mins to coagulate and pasteurize the leaf protein. The protein coagulum was separated from the fraction by filtering through cloth or pillow cases followed by pressing with screw-press as described for gari making (Aletor, 1993). The LPC was then washed with water and repressed. The product was then pulverized and spread in the sun to dry prior to analyses.

Chemical and Physicochemical Analyses

Proximate and amino acids composition: Proximate composition of the cassava LPCs were determined by AOAC (1990) method, while the amino acids were determined after 6M HCl acid hydrolyses, as previously described (Aletor, 1987). Tryptophan was determined chemically by the basic hydrolysis of proteins as described by Miller (1967). The gross and digestible energy values were computed by method of Ng and Wee (1989).

Determination of functional properties of the leaf meals and LPCs: The protein solubility (PS) of these products were determined as described by Oshodi and Aletor (1993); the water absorption capacity (WAC) and fat emulsion stability (FES) were determined by the procedure of Beuchat (1977); the fat absorption capacity (FAC) was determined as described by Sosulski (1962). Similarly, the lowest gelation concentration (LGC), foaming capacity (FC) and foaming stability of the products were determined using the techniques of Coffman and Garcia (1977).

Data analysis: All data were means for duplicate determinations. Mean values within the cassava leaf varieties were assigned coefficients of variation (Steel and Torrie, 1960).

Results and Discussion

Table 1 presents the mean values for the proximate constituents, calculated gross and digestible energies of the cassava leaf protein concentrates. The crude protein ranged from 424±0.1gkg^{-1} amino acid profile DM in MS6 to 500±0.0gkg^{-1} DM in TMS 30572 with a low coefficient of variation (CV) of 7.4%. The mean crude fibre content was 20±0.5 gkg^{-1} DM with a range of 1.4±0.1 gkg^{-1} DM in the local variety to 2.6 ± 0.0 gkg^{-1} DM in TMS 30555 and a CV of 25%. The ether extract (crude fat) was high with mean values ranging from 194 ± 0.4 gkg^{-1} DM in MS 6 to 228 ± 0.4 gkg^{-1} DM in TMS 30555. The nitrogen free extract (carbohydrate) was generally low with a mean value of 159 ± 5.2 gkg^{-1} DM (range, 11.5 - 23.5 gkg^{-1} DM). Gross energy averaged 52.4 ± 17.8 MJkg^{-1} DM (range, 50.0 - 53.9 MJkg^{-1}; CV = 3.4%) while digestible energy averaged 46.1 ± 18.8 MJkg^{-1} (range, 43.3 - 47.6 MJkg^{-1}; CV = 4.1%).

The amino acid profiles of the CLPCs from the different cassava varieties are shown in Table 2. Amino acid content of CLPCs from the different varieties were similar as indicated by the generally low coefficients of variation. The CLPCs generally had favourable balance of both essential and non-essential amino acids except methionine in which it was marginal (mean, 2.48 ± 0.05 g/16gN; range 2.41- 2.53g/16g N). Of high nutritional significance, is the good balance in lysine (6.8 ± 0.08g/16gN), leucine (9.65 ± 0.1g/16g N), valine (6.30 ± 0.22g/16gN) and tryptophan (2.31 ± 0.07g/16gN) which are usually the limiting amino acids especially, in cereal- or root tuber-based practical diets.

Functional Properties of CLMs and CLPCs indicating their water absorption capacity (WAC), fat (oil) absorption capacity (FAC), emulsion capacity and emulsion stability for the leaf meals and their corresponding CLPCs are shown in Tables 3 and 4, respectively. The WAC of the leaf meals averaged 409.6 ± 8.6% (range, 400.5 - 417.0%) while values for the LPCs averaged 181.5 ± 45.4% (range 118.0 - 225%; CV = 25.0%). The WAC of the leaf meals was about twice the values of the CLPCs. Whereas there were high varietal differences in WAC in the CLPCs (CV = 25.0%), such differences were minimal in the leaf meals. The FAC in the leaf meals varied from 48.3 ± 2.4% in the local variety to 60.8 ± 1.2% in TMS 30555 while the CLPCs values ranged from 19.2 ± 1.2% in MS 6 to 40.8 ± 1.0% in the local variety. Like the WAC values, the FAC values of the leaf meals were generally higher than those of the corresponding CLPCs. Similarly, there were higher varietal differences for FAC in the CLPCs than in the leaf meals.

The mean fat emulsion capacity and emulsion stability for the leaf meals (Table 3) were 27.4 ± 1.1% (range, 26.3 - 28.8%) and 41.2 ± 9.3% (range, 27.3 - 46.9%), respectively. These values were similar to those of the corresponding CLPCs (Table 4) which averaged 32.5 ± 8.3% (range, 25.0 - 40.8%) and 42.9 ± 2.9% (range, 38.8 - 45.8%), respectively. The mean values for foaming capacity, foaming stability and least gelation concentration in the leaf meals (Table 5) were generally lower than those of their corresponding CLPCs (Table 6). For example, foaming capacity averaged 17.7 ± 2.3% in the leaf meal compared with 32.1±7.7% in the CLPCs. Similarly, average values for foaming stability and least gelation concentration of leaf meals were 4.3 ± 0.2 cm^{3}

Table 1: Proximate Composition (gkg-1 DM) and Energy values ($MJkg^{-1}$) of cassava leaf protein concentrates (Means, n = 2)

Cassava varieties	Crude protein	Crude fibre	Ether extract	Ash	Nitrogen Free extract	Gross energy	Diges-tible energy
MS 6	42.4±0.2	2.3±0.4	19.4±0.4	7.3±0.1	23.5±0.5	520.0	433.6
TMS 30555	46.4±0.7	2.6±0.0	22.8±0.4	6.8±0.1	14.7±0.4	539.9	466.6
TMS 30572	50.0±0.3	1.8±0.0	21.6±0.3	9.3±0.0	11.5±0.7	536.2	467.4
Local (Ege Oda)	49.3±0.3	1.4±0.1	22.4±0.5	7.9±0.1	14.1±0.2	500.8	476.3
Mean	47.0	2.0	21.6	7.8	15.9	524.3	461.0
Standard Deviation	3.5	0.5	1.5	1.1	3.2	17.8	18.8
Coefficient of variation (%)	7.4	25.0	6.9	14.1	32.7	3.4	4.1

Table 2: Amino acid profile (g/16gN) of cassava leaf protein concentrates

Amino acids	MS 6	TMS 30555	TMS 30572	Local (Ege Oda)	SD		CV (%)
Alanine	6.12	6.34	6.51	6.40	6.34	0.16	2.5
Aspartic acid	9.98	7.10	7.31	7.12	7.87	1.4	17.7
Arginine	5.96	6.18	6.38	6.01	6.13	0.19	3.1
Glycine	5.61	5.81	6.10	5.85	5.84	0.20	3.4
Glutamic acid	11.38	11.52	10.85	11.62	11.34	0.34	3.0
Histidine	2.63	2.85	2.90	2.68	2.77	0.13	4.7
Isoleucine	5.52	5.50	5.74	5.66	5.61	0.11	2.0
Lysine	6.69	6.82	6.88	6.80	6.80	0.08	1.2
Methionine	2.48	2.51	2.53	2.41	2.48	0.05	2.0
Cystine	1.30	1.33	1.30	1.08	1.25	0.11	8.8
Meth.+Cys.	3.78	3.84	3.83	3.49	3.74	0.17	4.5
Leucine	9.56	9.60	9.78	9.64	9.65	0.10	1.0
Serine	4.87	5.00	5.30	5.14	5.08	0.18	3.5
Threonine	4.90	5.11	5.40	4.72	5.03	0.29	5.7
Phenylalanine	6.30	6.52	6.22	6.01	6.26	0.21	3.4
Valine	6.20	6.35	5.98	6.49	6.30	0.22	3.5
Tyrosine	4.71	4.91	5.00	4.73	4.84	0.14	2.9
Tryptophan	2.36	2.30	2.37	2.21	2.31	0.07	3.0

Table 3: Water and oil absorption capacity, Emulsion capacity and Emulsion stability of cassava leaf meal

Cassava varieties	Water absorption capacity (%)	Oil absorption capacity (%)	Fat emulsion capacity (%)	Fat emulsion stability (%)
TMS 6	417.0 ± 1.4	59.9 ± 0.1	26.3 ± 0.3	27.3 ± 0.1
TMS 30555	400. ± 2.1	60.8 ± 1.2	26.9 ± 0.3	45.8 ± 0.4
TMS 30572	417.0 ± 1.4	58.3 ± 2.3	28.8 ± 0.0	44.6 ± 0.5
Local (Ege Oda)	404.0 ± 2.8	48.3 ± 2.4	27.5 ± 0.0	46.9 ± 0.9
Mean	409.6	56.8	27.4	41.2
SD	8.6	5.7	1.1	9.3
CV(%)	2.1	10.0	4.0	22.6

Means are for duplicate determinations.

and 9.0 ± 2.0%, respectively as compared with the respective average values of 10.2 ± 4.0 cm3 and 12.5 ± 3.5% for the CLPCs. The varietal differences in these functional attributes were generally higher in the CLPCs than in the leaf meals as indicated by the coefficients of variation (Tables 5 and 6).

The protein solubility as a function of pH for the leaf meals and their corresponding CLPCs are depicted in Fig. 2 and 3, respectively. The leaf meal (Fig. 2) showed moderate and identical solubilities at both acid and alkaline media. Minimum solubilities (isoelectric points) for the leaf meals were reached at between pH 4 and 5.The CLPCs (Fig. 3) also showed moderate and identical solubilities at both acid and alkaline media reaching minimum solubility (isoelectric point) at between pH 4 and 6. Protein solubility generally tended

Table 4: Water and oil absorption capacities, Emulsion capacity and Emulsion stability of Cassava leaf protein concentrates

Cassava varieties	Water absorption capacity (%)	Oil absorption capacity (%)	Emulsion capacity (%)	Emulsion stability (%)
MS 6	118.0 ± 1.4	19.2 ± 1.2	25.6 ± 0.8	43.4 ± 0.4
TMS 30555	195.0 ± 7.1	34.2 ± 1.2	25.0 ± 0.0	45.8 ± 2.4
TMS 30572	187.5 ± 3.5	39.2 ± 1.2	38.7 ± 1.9	43.4 ± 0.9
Local (Ege Oda)	225.5 ± 0.7	40.8 ± 1.0	40.8 ± 1.2	38.8 ± 0.5
Mean	181.5	33.4	32.5	42.9
SD	45.4	9.8	8.3	2.9
CV(%)	25.0	29.3	25.5	6.8

Means are for duplicate determinations.

Table 5: Foaming capacity, least gelation concentration and foaming stability of cassava leaf meal

Cassava varieties	Foaming capacity (%)	Least gelation concentration (%)	Foaming stability (cm^3) (After 30 mins)
TMS 6	21.0 ± 0.3	8.0 ± 0.1	4.1 ± 0.1
TMS 30555	17.5 ± 0.5	8.0 ± 0.9	4.2 ± 0.3
TMS 30572	16.6 ± 0.3	8.0 ± 0.2	4.4 ± 0.6
Local (Ege Oda)	15.8 ± 0.3	12.0	4.4 ± 0.6
Mean	17.7	9.0	4.3
SD	2.3	2.0	0.2
CV(%)	13.0	22.2	4.7

Means are for duplicate determinations.

Table 6: Foaming capacity, least gelation concentration and foaming stability of cassava leaf protein concentration

Cassava varieties	Foaming capacity (%)	Least gelation concentration (%)	Foaming stability (cm^3) (After 30 mins)
TMS 6	33.5 ± 0.3	14.0 ± 0.9	8.0 ± 0.0
TMS 30555	35.0 ± 0.3	8.0 ± 0.5	16.4 ± 0.6
TMS 30572	38.9 ± 1.1	12.0 ± 0.5	8.0 ± 0.0
Local (Ege Oda)	21.0 ± 0.3	16.0 ± 0.6	8.5 ± 0.6
Mean	32.1	12.5	10.2
SD	7.7	3.4	4.1
CV(%)	24.0	27.2	40.2

Means are for duplicate determination.

to higher at the alkaline medium than acid medium for both the leaf meal and CLPCs.

The potential of cassava CLPCs as food/feed or alternative protein resources is amply demonstrated by the results of the proximate constituents. For example, the mean crude protein content of 47.0 ± 3.5 g/100g DM (range, 42.4 - 50.0g/100g DM) were generally higher than those reported for most tropical legumes (FAO, 1970; Ologhobo, 1980; Aletor and Aladejimi, 1989). Also, the protein content were generally higher than those reported for LPCs from other cassava varieties, *Eupatorium* or *Panicum* species by Eggum (1970), but similar to the LPCs from *Solanum* and *Amaranthus* species (Oke, 1973) and leguminous browse species. The leaf protein extraction process led to enhanced crude protein, crude fat, gross energy and, a concomitant decrease in crude fibre and ash values in the final product as indicated in an earlier report by Aletor and Fasuyi (1997) for the proximate constituents of these same leaf species.

Like the proximate constituents, the amino acid profile (Table 2) of the CLPCs from these cassava varieties compared with, and in many instances, surpassed those of high quality proteins such as fish, egg, meat or meat. Similarly, the amino acid values were in close agreement with those reported for CLPCs from other cassava varieties (Eggum, 1970), *Solanum* and *Amaranthus* species (Oke, 1973) and some leguminous browse species. For example, the lysine, leucine, valine and tryptophan content of the CLPCs were higher than those reported for soya, fish or egg by FAO (1973). The sulphur-containing amino acids - methionine (range, 2.41-2.53g/16gN) and cystine (range, 1.08-1.33) were limiting. Consequently, dietary formulations involving cassava LPCs would need supplemental methionine or, in the alternative, the CLPCs should be fed in

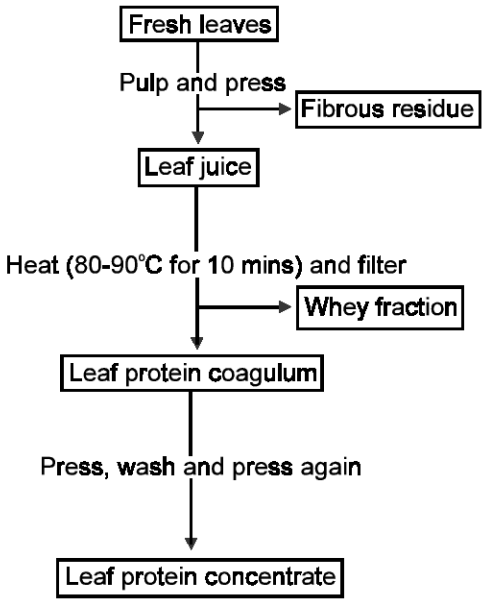

Fig. 1: Flow-chart of Leaf Protein Concentrate(LPC) production (Adapted from Fellows, 1987).

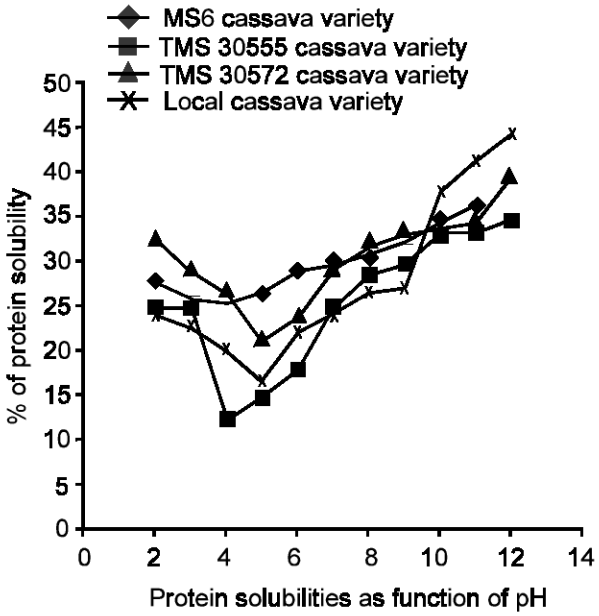

Fig. 2: Protein Solubility of Cassava leaf meals (CLM) as a function of pH

combination with feed resources higher in S-amino acids for complementarily and hence, favourable amino acid balances. Similarly, these CLPCs with high lysine content, will be of high supplemental value to cereals and tubers which are generally deficient in lysine. It is also of interest that CLPCs from these cassava varieties have identical

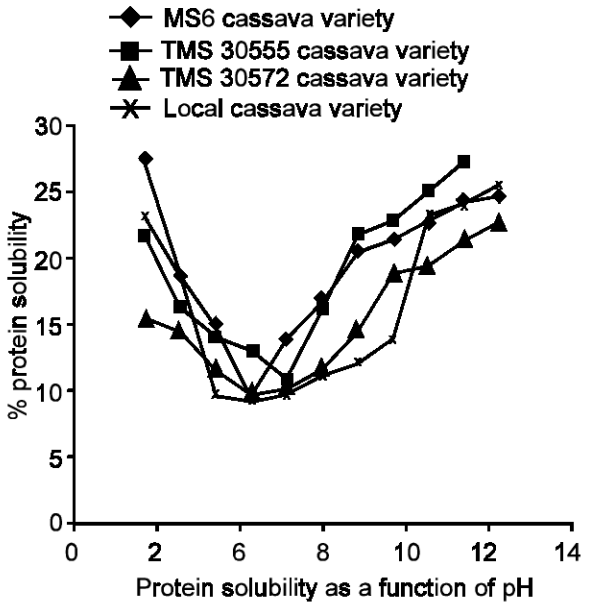

Fig. 3: Protein Solubility of cassava leaf protein concentrates as a function pf pH

crude protein content and amino acid profile suggesting that they may have similar protein quality.

With regard to functional properties, the water absorption capacity (Table 4) of the CLPCs was generally higher than 130% reported for soya bean (Lin *et al.*, 1994); 134% reported for African yam bean by Adeyeye *et al.* (1994) but similar to 200-288.8% reported for melon by Ige *et al.* (1984). The WAC of the leaf meals were much higher than these values. The WAC is a critical property of proteins in viscous foods like soups, gravies, dough and baked products (Adeyeye *et al.* 1994). Given the high crude protein content, the favourable amino acid profile and WAC of these CLPCs, they may be quite suitable in the formulation of these foods. Also, these CLPCs may be suitably incorporated into low-protein traditional foods such as maize gruel (palp), cassava and yam flours to enhance their nutritive value.

Fat (oil) absorption capacity of the leaf meal and CLPCs were generally lower than the 84.4 and 207% reported respectively, for soya and sunflower flours by Lin *et al.* (1994) while values for fat (oil) emulsion capacity were higher than 11.7% (wheat) and 18% (soya) reported by the same workers. Kinsella (1976) opined that FAC was a critical determinant of flavour retention, while fat (oil) emulsion capacity and emulsion stability are important attributes of additives for the stabilization of fat emulsions in the production of such foods as sausages, soups and cakes. The ability of proteins to aid the formation and stabilization of emulsions is important in many applications including mayonnaise, milks, comminuted meats and salad dressings (Adeyeye *et al.*, 1994).

The foaming capacity of the leaf meals and CLPCs (Table 5 and 6) was considerably lower than those of soya flour (70%) and sunflower (230%) reported by Lin *et al.* (1974). The foaming stability of these leaf products were also low when compared with legume products. Foaming stability is an important determinant of the suitability of a whipping agent in food systems. The least gelation concentration of the CLPCs (12.5 ± 3.4%) was comparable to those of pigeon pea (12% w/v) and lupin flour (14% w/v) reported by Sathe *et al* (1984), Oshodi and Ekperigin (1989) but lower than (36% w/v) reported for fluted pumpkin by Fagbemi and Oshodi (1991). Protein gel formation provides the matrix for holding water, flavours, sugars and ingredients hence it is an important consideration in food product development. The present results suggest that cassava CLPCs may be useful in the production of curd or, as an additive to other materials for gel formation in food products.

Protein solubility profiles of these products as a function of pH (Fig. 2 and 3) indicate their higher solubility at both acid and alkaline media with minimum solubility (isoelectric point) at between pH 4 and 6. The solubility profile of the CLPCs was quite similar to that reported for the African yam bean flour (Adeyeye *et al.*, 1994). The solubility pattern suggest that these cassava leaf products may be useful in the formulation of acid or alkaline foods such as protein-rich carbonated beverages.

Conclusion: The analytical data on crude protein, crude fat, gross energy and amino acid profiles of the cassava leaf products, clearly suggest their high potentials as cheap source of alternative proteins for human and, or animals. Equally of nutritional interests, are the favourable balance in most of the essential amino acids (especially lysine) and several desirable functional attributes of the proteins such as water absorption capacity, least gelation concentration and solubility characteristics.

Consequently, it is suggested that these products (especially the CLPCs with low fibre content) may be used to enhance the protein value of low-nitrogen traditional staples such as flours from cereals, and tubers including cassava flour. Because of the simplicity of the technology involved in leaf protein concentrate production, its incorporation into local food production systems is recommended as a practicable, sustainable and ameliorative intervention strategy for the endemic protein under-nutrition in this region.

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