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Nutritive and Physico-Chemical Characteristics of the Leaves and Leaf Protein Concentrates from Two Edible Vegetables: A Comparative Evaluation

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Abstract: The leaves and Leaf Protein Concentrates (LPCs) from two edible vegetable species; *Amaranthus hybridus* and *Manihot esculenta* were analysed for their chemical and physico-chemical characteristics. The LPCs were produced from these species using village-level, low-cost fractionation techniques. The leaves and their corresponding LPCs were subsequently characterized with respect to their proximate composition, mineral constituents, polyphenolic content and functional properties. On the average, the leaves contained 29.5 g 100 g^{-1} DM crude protein (range, 28.0-30.9 g 100 g^{-1} DM) and 7.6 g 100 g^{-1} DM crude fibre (range, 6.4-9.0 g 100 g^{-1} DM). Gross energy averaged 459.0±22.6 kcal 100 g^{-1}. The leaf protein extract on the average contained 38.5 g 100 g^{-1} DM crude protein (range, 35.2-41.7 g 100 g^{-1} DM); 1.0±0.6 g 100 g^{-1} DM crude fibre; 7.7±2.0 g 100 g^{-1} DM fat; 6.9±1.8 g 100 g^{-1} DM ash and 554.5±113.8 kcal 100 g^{-1} gross energy. Ca, Mg, Na, K were most abundant minerals in the leaves and leaf protein concentrate while Cu and Zn were the least abundant. The polyphenols, as tannic equivalent, were low in the protein extracts. The Fat Absorption Capacity (FAC) varied from 16.7±1.2% in *A. hybridus* to 19.3±1.2% in *M. esculenta* while the Water Absorption Capacity (WAC) varied from 158.0±1.2% in *M. esculenta* to 226.7±% in *A. hybridus* with a CV of 25%. The emulsion capacity and stability were similar between the two species as indicated by the low Coefficients of Variation (CV) of 15.4 and 14.2%, respectively. The foaming capacity and stability did not also vary widely between the LPCs as indicated by low CVs. All the samples had varying solubilities with multiple maxima and minima with changes in pH. Based on the analytical data, the nutritional potentials of these underutilized protein resources were discussed and the need for their incorporation into low-Nitrogen foods was recommended.

Key words: Comparative Evaluation · Nutritive · Physico-Chemical · Leaf Protein

INTRODUCTION

The rapid population growth in most African countries (Nigeria inclusive) has led to serious food crises, especially among the vulnerable groups such as the weaning, pre-school children, pregnant or nursing mothers, etc. This class of people are particularly prone to dietary protein, mineral and vitamin inadequacies [1,2]. The dietary inadequacies which arise mainly from the high cost of animal proteins (milk, egg and meat) have, in some developing countries resulted in kwashiorkor, marasmus, infant blindness, mortality and morbidity [3-6]. Cheap protein sources from edible leaf protein concentrates of *Amaranth hybridus* and *Manihot esculenta* Agbede and Aletor [7] have been identified as having the potentials to mitigate the endemic incidence of protein undernutrition, especially among the resource-poor persons in Sub-Saharan Africa.

Cassava leaves (with all all-year-round availability in this region), although very rich in protein Aletor and Adeogun [8] have remained under researched and consequently under utilized. Similarly, large tonnages of cassava leaves are currently discarded as wastes after harvesting the roots. Although small quantities of these 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, especially by humans is impracticable due to high fibre and water (bulkiness) of the leaves.

It was therefore the objectives of this study, to characterize the leaves and the corresponding LPCs from these edible vegetables with respect to their proximate and mineral content, gross energy and nutritionally valuable mineral content. The functional properties of the LPCs were also determined.

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MATERIALS AND METHODS

The 2 edible vegetable samples – *Amaranthus hybridus* and *Manihot esculenta* – were purchased in early March from the open market in fresh conditions. The vegetable stalks were removed and the leaves rinsed with distilled water before division into two portions. One portion was sun-dried while the other half was used for LPC production.

Leaf Protein Concentrate (LPC) Production: Leaves of *A. hybridus* and *M. esculenta* were harvested fresh between October and November (at the beginning of dry season), from the campus of the Federal University of Technology, Akure. They were weighed and washed prior to pulping with a pulping machine. The flow-chart or fractionation scheme adapted from Fellow [9] (Fig. 1) involved the pulping and separation of the leaf juice followed by heating to 80-90°C for 10 min. to coagulate and pasteurize the leaf protein.

Siphoning the hot whey using a rubber hose facilitated the rapid cooling of the LPC. The protein coagulum was thereafter filtered with a muslin bag and pressed with a screw press as described by Aletor [10]. The LPcs from *A. hybridus* and *M. esculenta* were greenish in colour, but the green colour was deeper in LPCs from *M. esculenta* than *A. hybridus*. The LPCs were then pulverized, sun-dried and milled using a laboratory hammer mill (Dietz, Dettingen-Teck, West Germany). They were then kept in airtight containers and deep-frozen prior to chemical and physico-chemical analysis.

Chemical Analysis: The proximate composition of these materials were determined as descried by Pearson [11] while nitrogen was by micro-kjeldahl method of AOAC [12]. The minerals were analysed after first dry-ashing at 550°C in a Muffle furnace and dissolved in deionised water to standard volume. Na and K were determined by flame photometry and phosphorous by vanadomolybdate method of AOAC [12]. Mg, Ca, Zn, Mn, Fe and Cu were determined using an atomic absorption spectrophotometer, AAS. The gross energy was computed from the proximate constituents as described by Ng and Wee [13].

Quantification of Tannin and Phytin: Finely milled leaves and their corresponding LPCs (200 mg in 10 cm^3 of 70% aqueous acetone) were extracted for 2 h at 30°C in a water-bath using a Gallenkamp orbital shaker (Electro Ltd. Avon. UK) at 120 r.p.m. Pigments and fat were first removed from the samples by extracting with diethyl ether containing 1% acetic acid. Thereafter the total polyphenols (as tannic equivalent) were determined in 0.05 cm^3 aliquots in test tubes by the addition of distilled water to make it to 1.0 cm^3, followed by the addition of 0.5 cm^3 Folin Ciocalteau reagent (Sigma, St Louis, Mo, USA) and then 2.5 cm^3 sodium carbonate solution. The tubes were vortexed and the absorbance recorded at 725 nm after 40 min as described by Makkar and Goodchild [14]. The amount of total polyphenols (as tannic equivalent) was thereafter calculated from the standard curve.

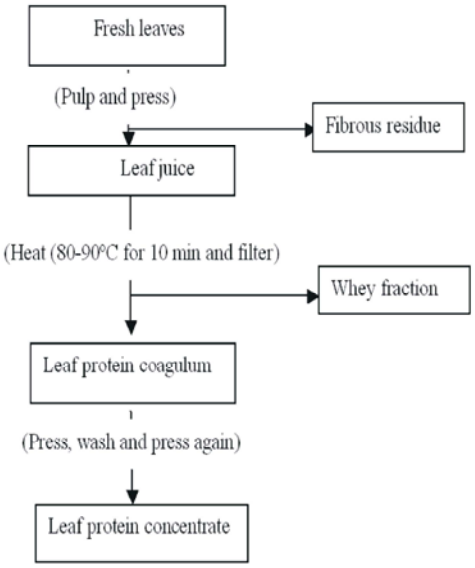

Fig. 1: Flow-chart of Leaf Protein Concentrate (LPC) production

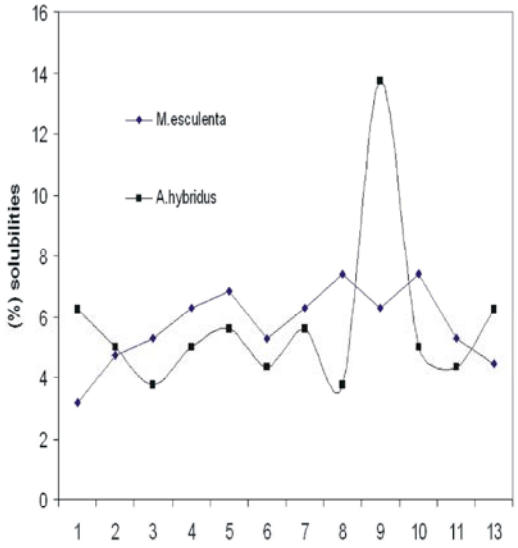

Fig 2: Protein solubilities as a functionof pH

For the quantification of phytin, 8 g each of finely ground leaf meal and LPC was soaked in 200 cm^3 of 2% hydrochloric acid and allowed to stand for 3 h. The extract was thereafter filtered through two layers of hardened filter paper. Filtrate of 50 cm^3 was pipetted in triplicate into 400 cm^3 capacity beakers before the addition of 10 cm^3 0.3% ammonium thiocyanate solution as an indicator and 107 cm^3 distilled water to obtain the proper acidity (pH 4.5). The solution was then titrated with a standard iron chloride ($FeCl_3$) solution containing 0.00195 g Fe L^{-1} until a brownish yellow colour persisted for 5 min. Phytin-phosphorus was determined and phytin content was calculated by multiplying the value of phytin-phosphorus by a factor of 3.55 [15]. Each milligram of iron is equivalent to 1.19 mg of phytin-phosphorus.

Determination of the Functional Properties of the LPCs: The Protein Solubilities (PS) of the LPC were determined as described previously [10,16] while the Water Absorption Capacity (WAC) and fat emulsion stability were determined by the procedure of Beuchat [17]. The Fat Absorption Capacity (FAC) was determined as described by Sosulki [18]. Similarly, the Lowest Gelation Concentration (LGC), Foaming Capacity (FC) and foaming stability of the LPCs were determined using the techniques of Coffman and Garcia [2].

Data Analysis: All data used were means of triplicate (n = 3) determinations. The Coefficients of Variation (CV) between the edible vegetable species were determined Steel and Torrie [19].

RESULTS AND DISCUSSION

The crude protein of the leaf (Table 1) ranged from 28.0 to 30.9 g 100 g^{-1} DM; crude fat from 6.4 to 9.0 g 100 g^{-1} and fibre from 7.4 to 7.6 g 100 g^{-1} DM in *A. hybridus* to *M. esculenta*, respectively. The LPCs on the other hand, contained on average 38.5±4.6; 7.7±2.0; 1.0±0.6; 6.9±1.8 g 100 g^{-1} crude protein, crude fat, crude fibre and ash, respectively. Crude protein was higher in *M. esculenta* than in *A. hybridus*. The mean gross energy values were 459.0±22.6 and 554.5±113.8 kcal 100 g^{-1} for the leaf meals and LPCs, respectively. The values for the protein extracts from these vegetables surpass those reported by Agbede and Aletor [20] for differently processed *Canavalia ensiformis* and *Mucuna pururiens* seeds flours. Subject to a high level of intake and amino acid supplementation, it is conceivable that quite a large proportion of animal protein requirement could be met by feeding these vegetable proteins.

The phytin and polyphenols (Table 2) were generally low in all the samples. The low levels of anti-nutrients point to their potentials as food sources. High phytate diets are discouraged for monogastric animals, including man, due to lack of endogenous phytase-an enzyme which breaks down phytin to release phosphorus for metabolism [21]. Of the major minerals (Table 3), Ca, Mg, Na and K were the most abundant in the leaf meal and LPCs while Cu and Mn were the least abundant. This indicated that the more nutritionally important minerals involved in the skeletal structure of the animal body – Ca, P and Mg Agbede and Aletor [7] are well furnished by these vegetable species.

Table 1: Proximate composition (g 100-1 DM) and gross energy (kcal 100-1) of leaf meal and leaf protein concentrates of some edible vegetables (n=3)

	Dry matter		Crude protein		Crude fat		Crude fibre		Ash		NFE		GE	
Plant Sp.	LM	LPC	LM	LPC	LM	LPC	LM	LPC	LM	LPC	LM	LPC	LM	LPCs
Amaranth hybridus	96.6±0.1	95.0±0.2	28.0±0.1	35.2±0.9	6.4±0.5	9.1±0.2	7.4±0.4	1.4±0.2	7.4±0.2	5.6±0.4	46.1±0.5	43.6±0.4	474.9	635
Manihot esculenta	94.3±0.2	97.7±0.4	30.9±0.2	41.7±0.4	9.0±0.3	6.3	7.8±0.1	0.5±0.1	1.5±0.1	8.1±0.2	34.9±0.2	59.0±0.1	443	474
Mean	95.1	96.4	29.5	38.5	7.7	7.7	7.6	1	9.5	6.9	40.5	57.3	459	554.5
SD	1.6	1.9	2.1	4.6	1.8	2	0.3	0.6	2.9	1.8	7.4	10.9	22.6	113.8
CV (%)	1.7	2	7.1	11.9	23.3	26	3.9	60	30.5	26.1	19.5	21.2	49.1	20.5

LM: Leaf Meal; LPC: Leaf Protein Concentrates; NFE: Nitrogen Free Extract; GE: Gross Energy; CV: Coefficient of Variation

Table 2: Phytin, phytin-phosphorus and polyphenotic contents of leaf meal and leaf protein concentrates of some edible vegetables

	Phytin (µg 100 g-1)		Phytin phosphorus (µg 100 g-1)		Polyphenols (as tannic acid, g 100 g-1 DM)	
Vegetables	LM	LPC	LM	LPC	LM	LPC
Amaranth hybridus	220	160	180	130	0.8	0.6
Manihot esculenta	120	160	130	100	1.5	0.5
Mean	170	160	155	115	1.15	0.6
SD	70.7	0	35.4	21.2	0.5	0.1
CV (%)	41.6	0	22.8	18.4	43.5	18.2

LM: Leaf Meal; LPC: Leaf Protein Concentrates; NFE: Nitrogen Free Extract; GE: Gross Energy; CV: Coefficient of Variation

Table 3: Major and trace mineral components (mg kg-1) of leaf meal and leaf protein concentrates of some edible plant species (n=3)

	Major minerals										Minor minerals							
	Ca		Na		K		P		Mg		Mn		Fe		Cu		Zn	
	LM	LPC	LM	LPC	LM	LPC	LM	LPC	LM	LPC	LM	LPC	LM	LPC	LM	LPC	LM	LPC
Amaranth hybridus	766.1	457.1	405.7	321.1	421.8	112.2	266.4	224.1	257.3	137.1	8.7	1	97	67.7	ND	ND	203.7	93.5
Manihot esculenta	136.1	287.5	174.7	362.8	156.3	299.8	60.7	110.2	125.2	259.3	0.1	1.7	9.5	18.5	0.3	ND	77.8	118
Mean	451.1	372.3	290.2	341.5	289.1	206	163.6	167.2	191.3	198.2	4.4	1.35	53.3	43.1	-	-	140.8	105.8
SD	445.5	126.7	163.3	30.2	187.7	132.7	145.5	80.5	93.4	86.4	6.08	0.5	61.9	34.8	-	-	89	17.3
CV (%)	98.8	32.2	56.3	8.84	65	64.4	88.9	48.1	48.8	43.6	38.2	37	116.1	80.7	-	-	63.2	16.4

LM: Leaf Meal; LPC: Leaf Protein Concentrates; NFE: Nitrogen Free Extract; GE: Gross Energy; CV: Coefficient of Variation

Table 4: Functional properties (%) of some edible leaf protein concentrates (n = 3)

Vegetables	Fat absorption capacity	Water absorption capacity	Emulsion capacity	Emulsion stability at 1 h	Foaming capacity	Foaming stability at 1 h	Least gelation concentration
Amaranth hybridus	16.7±1.2	226.7±1.2	46.6±0.3	44.5±0.2	4.8±0.3	2.1±0.1	5.0±0.1
Manihot esculenta	19.3±1.2	158.0±1.2	56.9±0.1	55.4±0.3	4.9±0.1	2.0±0.1	8.0±0.0
Mean	18	192.4	51.6	50	0.9	2.1	6.5
SD	1.9	48.6	7.3	7.7	0.1	0.1	2.1
CV (%)	10.6	25.3	14.2	15.4	2	4.8	32.3

CV: Coefficient of Variation

Table 4 shows the cumulative mean values (%) of fat absorption, water absorption, emulsion capacity, emulsion stability, foaming capacity and foaming stability of the two different leaf protein concentrates to be 18.0±1.9%, 192.4±48.6, 51.6±7.3, 50.0±7.7,4.9±0.1 and 2.1±0.1%, respectively. These values compared well with those reported for some legumes and defatted fluted pumpkin (*T. occidentalis*) seed flours by Fagbemi *et al.* [22]. This suggested that the LPC may be a good emulsifying agent and may be useful as a meat additive in sausage production, salad dressing preparation and in pie fillings. The solubilities of the LPCs varied with pH (Fig. 2) with multiple maxima and minima. *A. hybridus* had minimum solubility at pH 4 and maximum solubility at pH 1 and 12 while *M. esculenta* had minimum solubility at pH 1 and maximum solubility at pH 8 and 11. Results on functional properties of these leaf protein concentrates clearly indicate their potentials for the development of different food products or systems.

CONCLUSION

Given the relatively high nutrient constituents and the desirable physico-chemical (functional) attributes of the LPCs, it is suggested that they may be used in enhancing the nutritive value of low-nitrogen foods such as maize gruel (ogi) or other high carbohydrate foods commonly consumed by resource-poor citizens. They may also find important uses as binders or extenders in food systems.

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