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PROTEIN CONTENT AND AMINO ACID COMPOSITION OF CASSAVA LEAF

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Abstract—On the basis of leaf dry wt, the protein content of six varieties of cassava varied from 29.3 to 38.6% and the estimated leaf protein production ranged from 242 to 953 kg per ha. On the basis of fr. wt of leaf, the total amino acids ranged from 8.42 to 9.4% while the essential amino acids averaged 4.21% and the sulphur-containing amino acids only 0.25%. The amino acid composition profiles for the six varieties was similar.

INTRODUCTION

For centuries, cassava (*Manihot esculenta* Crantz) has been widely cultivated in the tropics for its starch [1]. The economic importance of cassava tubers as dietary carbohydrate and as an industrial source of hydrocarbon has increased greatly in recently years [2]. Cassava plants also produce a good crop of leaves which are a rich source of proteins. Ramos-Ledon *et al.* [3] have reported protein levels ranging from 2.92 to 7.76% of leaf fr. wt. Furthermore, cassava leaves serve as vegetables in many parts of the tropics [1, 4] but their potential as a source of proteins in terms of quantity and quality was not recognised until recently [5].

We have analysed the total leaf protein and amino acid composition of six varieties of special interest: Medan, Black Twig, CS-1, CS-3, CS-4 and CS-5. Medan is a sweet variety, while Black Twig is a high tuber-yielding bitter variety which also serves as a reference variety for field studies at the Malaysian Agricultural Research and Development Institute (MARDI). CS-1, CS-3, CS-4 and CS-5 are recently derived clones [6]. Protein values of mature and young leaf samples and leaf protein yield per ha was estimated.

RESULTS AND DISCUSSION

The mean leaf protein content varied from one variety to another, ranging from 29.3% dry wt in CS-3 to 38.6% dry wt in CS-1 (Table 1). Black Twig was second with a protein content of 37.8% dry wt, while CS-4, CS-5 and Medan had an average protein content of 33.6% dry wt. Moisture content varied from 73.6% fr wt in Medan, to 83.1% fr. wt in CS-4; the average value was 77.7%. The values were high when compared to those of young leaves which averaged only 73.2% fr. wt (Table 2). This could be due to errors in bulk handling.

Black Twig gave a good yield of leaf protein (381 g/plant), while the lowest level was recorded in CS-4 (79 g/plant). In terms of leaf protein per ha of cultivated land, Black Twig would yield 953 kg proteins and CS-4 only 242 kg. For comparison, spinach yields *ca* 400 kg protein per ha after 3–4 months [7]. The wide variation in cassava leaf protein production between varieties was due to two factors; (1) the quantity of leaves produced by the plant and (2) the protein content of the leaves leaves. For example, CS-1 had a high leaf protein content (38.6% dry wt) compared to CS-5 (33.3% dry wt), but the amount of leaves produced per plant by CS-1 was only 340 g dry wt whereas in the case of CS-5, it was 660 g dry wt per plant. The protein production in CS-1 was therefore only 131 g protein per plant while that for CS-5 was 220 g protein per plant. However, cassava plants are grown commercially at a spacing of 1 × 1 m [8]. Although 4× more plants could be thus grown per ha, the leaf yield per plant decreases markedly.

The young leaves of the six varieties were also analysed. The moisture content ranged from 70.6% fr. wt

Table 1. Mature leaf and leaf protein yield of cassava varieties

Variety	Moisture content* (% fr. wt)	Leaf no.* per plant	Leaf weight* per plant (kg dry wt)	Leaf protein content (% dry wt)	Protein prod. per plant (kg)	Protein yield per hectare (kg)
Black Twig	80.8	649	1.01	37.8	0.381	953
CS-5	75.6	536	0.66	33.3	0.220	549
CS-1	79.1	246	0.34	38.6	0.131	328
Medan	73.6	411	0.38	33.1	0.126	314
CS-3	74.1	802	0.35	29.3	0.102	256
CS-4	83.1	330	0.28	34.6	0.097	242

* Data supplied by MARDI [8].

Table 2. Moisture, ash, sulphur and protein contents of young cassava leaf

Variety	Moisture (% fr. wt)	Ash (% fr. wt)	Sulphur (% dry wt)	Total protein	
				Dry wt leaf (%)	Fr. wt leaf (%)
Black Twig	79.9	1.06	0.94	39.4	8.10
CS-1	73.7	1.37	0.62	37.8	8.97
CS-3	70.6	1.34	1.13	37.4	8.90
CS-5	74.2	1.22	0.78	34.6	9.26
CS-4	70.6	1.34	0.84	33.8	9.33
Medan	77.3	1.53	0.87	32.5	7.35

leaf in CS-4 to 79.9% fr. wt leaf in Black Twig (Table 2). The ash content in the six varieties averaged 1.36% fr. wt while sulphur was 0.86% dry wt. The protein content of these leaves did not vary much from those collected at the time of harvesting of the tubers. The protein content ranged from 32.5% dry wt in Medan to 39.4% dry wt in Black Twig. CS-1 and CS-3 contained an average of 37% dry wt protein while that in CS-5 and CS-4 were *ca* 34% dry wt. Although Black Twig had a protein value of 39.4% dry wt, the leaf protein value, based on fr. wt was only 8.1%, whereas CS-4 which had a protein content of 33.8% dry wt, had a protein content of 9.33% fr. wt.

Out of 126 species of other higher plants, 63% have leaf protein contents of less than 3% fr. wt, 30% have protein contents ranging from 4 to 5% fr. wt and only 7% have protein contents greater than 6% fr. wt [9]. Species that have the highest leaf protein content are *Sesbania grandiflora* (8.7% fr. wt), *Acacia* sp. (8% fr. wt) and *Carica papaya* (7% fr. wt); this shows that cassava can be classified as a high leaf protein plant.

The amino acid composition of the six varieties did not show any marked varietal differences. The individual amino acids as a percentage of total amino acids indicated a similar profile for all the varieties studied. Leu, Asp, and Glu exceeded 10% of the total amino acids for the six varieties while Ile, Lys, Thr, Phe, Val, Ala and Gly averaged 5% of the total amino acids, except for cysteine which was less than 1% of the total amino acids. The Medan variety had the lowest His value but the highest Asp and Trp when compared with the rest, while Black Twig had the highest Val value and CS-3 the lowest Leu. A recent report [10] on amino acid composition of cassava from Zaire also showed little variation between varieties, implying that there was no genotypic variations.

The amino acid content of cassava leaf is shown in Table 3. The 6 varieties of cassava yielded an average total amino acid of 8.8% fr. wt leaf. The highest value was 9.4% fr. wt leaf in CS-4 and the lowest was 8.34% fr. wt leaf in Black Twig. The amino acid composition indicated not only that the sulphur containing amino acids were low in quantity, ranging from 0.17% fr. wt in CS-1 to 0.33% fr. wt in CS-3, but also a low level of His which ranged from 0.01% fr. wt in Medan to 0.35% fr. wt in CS-1. Trp was also low, ranging from 0.12% fr. wt in CS-1 to 0.35% fr. wt leaf in Medan. The essential amino acids (Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, Met and Phe) were present in quantities ranging from 3.87% fr. wt in Black Twig to 4.43% fr. wt in CS-3. They averaged 47.8% of the total amino acids present in the leaf. Cysteine and Tyr are considered to be semi-essential because of their sparing effects on Met and Phe [9].

Compared with the amino acid content (expressed as a percentage of the total amino acids) of raw hen egg (*Gallus domesticus*), oat grain (*Avena sativa*), dried soybean seed (*Glycine max*), rice grain (*Oryza sativa*) and raw leaf of spinach (*Spinach oleracea*) [9], cassava leaf had a higher percentage of Ile, Leu, Thr, Trp and Asp but a lower percentage of cysteine, Arg, Glu and Ser while the other amino acids were comparable. The S-containing amino acids in cassava leaf protein (2.84% total amino acid) was lower than that for hen egg (5.48% total amino acid), rice (3.77% total amino acid) and oat grain (3.52% total amino acid) but was equal to that of dried soybean (2.71% total amino acid). However, it was more than that for spinach leaf which was only 1.06% total amino acid. The total Phe and Tyr for cassava leaf protein was 8.98% total amino acid. This was slightly lower than the value for hen egg (9.34%) and rice (9.99%) but more than that for oat grain (7.98%), dried soybean seed (8.23%) and was equal to that for spinach leaf (8.99%). Furthermore, the total essential

Table 3. Amino acid content of freshly harvested young cassava leaf varieties (% fr. wt leaf)

Amino acid	Medan	Black Twig	CS-1	CS-3	CS-4	CS-5
Ile	0.50	0.43	0.47	0.56	0.59	0.61
Leu	1.00	0.84	0.88	0.81	0.89	0.86
Lys	0.70	0.54	0.61	0.59	0.60	0.59
Met	0.21	0.19	0.10	0.25	0.14	0.10
Cys/2	0.10	0.07	0.07	0.08	0.08	0.09
Total S-containing amino acids	0.31	0.26	0.17	0.33	0.22	0.19
Phe	0.36	0.39	0.39	0.51	0.50	0.46
Tyr	0.31	0.23	0.35	0.42	0.41	0.36
Total Phe + Tyr	0.67	0.62	0.74	0.93	0.91	0.82
Thr	0.34	0.32	0.46	0.51	0.47	0.51
Trp	0.35	0.25	0.12	0.14	0.15	0.21
Val	0.33	0.61	0.43	0.56	0.55	0.59
Arg	0.47	0.35	0.48	0.34	0.37	0.42
His	0.07	0.22	0.35	0.26	0.30	0.24
Ala	0.40	0.51	0.53	0.45	0.61	0.52
Asp	1.36	1.12	0.93	1.19	1.13	1.15
Glu	0.95	1.04	1.09	1.07	1.14	1.12
Gly	0.35	0.47	0.44	0.52	0.61	0.48
Pro	0.46	0.34	0.34	0.34	0.43	0.26
Ser	0.28	0.42	0.38	0.31	0.43	0.46
Total essential amino acids	4.20	3.87	3.88	4.43	4.38	4.38
Total amino acids	8.34	8.42	8.42	8.91	9.40	9.03

amino acid for cassava leaf protein was 47.8% total amino acid which was similar to that for hen egg (49.7%) and greater than that for oat grain, rice, soybean seed and spinach leaf.

The total amino acid content of cassava leaf (8.80% fr. wt leaf) was less than that for hen egg (13.0% fr. wt), oat grain (13.9% fr. wt) and dried soybean seed (36.8% dry wt) but greater than that for rice (8.21% fr. wt) and several times higher than that for spinach leaf (1.89% fr. wt). Although the S-containing amino acids were 0.25% fr. wt leaf in cassava leaf, this was nevertheless greater than in spinach leaf (0.02% fr. wt). It was *ca* the same as that for rice (0.31% fr. wt) but less than that for oat grain (0.49% fr. wt) and hen egg (0.71% fr. wt). The level of the essential amino acids found in cassava leaf was 4.21% fr. wt which was slightly lesss than that for hen egg (6.42% fr. wt), oat grain (5.48% fr. wt) and greater than that for rice (3.40% fr. wt) and spinach leaf (0.72% fr. wt) [9].

Cassava leaf is not only rich in proteins but also has a good complement of the essential amino acids. However, in cassava, toxicity is caused by cyanogenic glucosides, namely linamarin and lotaustralin which may be hydrolysed by β-glucosidase or acid to liberate hydrogen cyanide [11]. The cyanogen is distributed throughout the plant, but the concentration varies between varieties and also with the climate, edaphic and cultural conditions. The cyanide content of the young cassava leaf can range from 174 to 622 ppm [12]. However, the problem of cyanide toxicity could be overcome by drying, heating and soaking in water [11].

EXPERIMENTAL

Sample collection. 6 varieties of cassava were grown at MARDI at a spacing of 2 × 2 m in similar soil conditions. The tubers were harvested after 9 months and leaves were collected during this harvesting period for analysis. Young cassava leaves were collected by random sampling. Fresh leaves were used for all analyses.

Moisture and ash content determination. Moisture of young leaf samples was determined by drying 2 g fr. wt leaf at 100° for 24 hr. The loss in wt was reported as moisture. Moisture content for leaves collected at tuber harvesting was determined in bulk. Ashing was performed with 2 g dried sample in a porcelain dish with slow heating for 2–3 hr to constant wt.

Total N determination. The micro-Kjeldahl technique was used and the factor 6.25 was used to convert total N to protein [13]. About 2 g fr. wt leaf was digested in 3 ml H_2SO_4 (N-free) in the presence of 0.5 g catalyst for 5 hr.

Total S determination. The turbidimetric method [14] was used. *Ca* 50 mg of dried leaf sample was digested in 4 ml HNO_3 at 100° until clear. The digest was cooled and 3 ml of 72% $HClO_4$ was added and warmed for 25 min and boiled for 30 min. 0.2 ml was used for S determination.

Amino acid analysis. Acid hydrolysis was performed with 0.5 g fr. wt leaf in 2 ml 6N HCl in a sealed tube *in vacuo* at 100° for 22 hr. The hydrolysate was centrifuged and the supernatant neutralised with buffer [15] in the ratio of 1:2. 0.5 ml of this hydrolysate was applied for analysis on the short column. For analysis on the long column, the neutralised hydrolysate was diluted ×6 with Na–citrate buffer pH 2.2 and 1 ml of this was applied to the column.

Analysis of amino acids by ion-exchange chromatography. [16]. The short column (22 × 0.9 cm) was packed with M81 resin (equivalent to PA 35) and operated at 50°. Na–citrate buffer (pH 5.26) with flow rate of 45 ml hr^{-1} was used to elute the sample. The long column (55 × 0.9 cm) was filled with M.72 resin (equivalent to PA 28) and operated at 50°. Na-citrate buffer (pH 3.25) (flow rate 42 ml/hr) was used for elution. After 146 min (equivalent to collecting 115 ml) Na–citrate buffer pH 4.25 was used (42 ml/hr). A total vol of 300 and 200 ml resp was collected from the short and long columns. Fractions (1 ml) were assayed using freshly prepared ninhydrin reagent [17].

Determination of tryptophan. Alkaline hydrolysis was carried out with 0.5 g fr. wt leaf in 2 ml 4N $Ba(OH)_2$ in evacuated, sealed tubes for 50 hr at 110° [18]. The hydrolysate was neutralised with H_2SO_4 and centrifuged. 50 μl of the neutralised hydrolysate was added to 2.45 ml of 8M urea and titrated with 0.02M-*N*-bromosuccinimide. The titration was followed by scanning from 250 to 350 nm [18].

Determination of cysteine. In the modified method [19] the cysteine and cystine were determined as cysteine. Acid hydrolysate of 1 ml (prepared earlier) was heated with 1 ml of hydrazine hydrate in a sealed tube at 120° for 18 hr. The cysteine was then determined as sulphide with the bismuth reagent.

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