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Food Safety and Amino Acid Balance in Processed Cassava "Cossettes"

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Processed cassava (*Manihot esculenta* Crantz) roots provide more than 60% of the daily energy intake for the population of the Democratic Republic of Congo. Insufficiently processed cassava roots in a diet deficient in sulfur amino acid have been reported to cause the irreversible paralytic disease konzo, afflicting thousands of women and children in the remote rural areas of Bandundu Province. "Cossettes" (processed cassava roots) purchased in several markets of Kinshasa were analyzed for their content of cyanogens, free amino acids, and total protein amino acids. Residual cyanogen levels were below the safe limit recommended by the codex FAO/WHO for cassava flour (10 mg kg^{-1}). The amino acid score was evaluated. Lysine and leucine were the limiting amino acids. Methionine content was very low and contributed about 13% of the total sulfur amino acids. Dietary requirements for sulfur amino acids need to be adjusted for the loss caused by cyanogen detoxification.

KEYWORDS: ***Manihot esculenta*; konzo; cyanogens; methionine requirement; amino acid score; cyanide detoxification; food safety**

INTRODUCTION

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) is the major staple food consumed by the population of the Democratic Republic of Congo (DRC) (*1*). Processed cassava roots provide more than 60% of the daily energy intake (*2*).

Sweet varieties of cassava roots may be consumed directly, but bitter varieties with high content of cyanogenic glycosides are traditionally processed to reduce toxicity and to improve palatability and storability. Linamarin is the main cyanogenic glycoside (about 97%), and lotaustralin is present in a much smaller amount (*3*−*5*). Many varieties of processed cassava roots with different local names are known: cossettes, chikwangue, fufu, malemba, luku, ntuka, etc.

The processing methods generally adopted include peeling, soaking, fermentation, boiling or cooking, drying, and pounding/milling. "Cossettes", which are the most popular cassava product in DRC, are obtained by soaking or immersing fresh bitter cassava roots (whole or peeled) in a stream or stationary water (near a stream) for at least 3 days to allow them to ferment until they become soft. The fermented roots are then taken out, peeled, and sundried on mats, racks, roofs of houses, etc. Depending on the weather, sundrying takes 2−5 days (*3*). The dried fermented cassava roots are the so-called "cossettes" (**Figure 1**). This form of cassava product is preferred because it can be stored for a long period and can be traded over much greater distances (*1, 6*). When the roots are soaked and dried for a shorter period because of insufficient food supply or poor agro-ecological conditions, the remaining cyanogen content can be much higher than that after normal processing. High intakes of dietary cyanogens from poorly processed cassava roots in a diet deficient in sulfur amino acids have been implicated in the causation of konzo (*7*).

Konzo is a toxico-nutritional disease characterized by abrupt onset of permanent spastic paralysis of both legs (paraparesis) in a formerly healthy person. Konzo affects mainly women and children and has been reported from remote rural areas of Central African Republic, DRC, Mozambique, and Tanzania. DRC is the most affected country, and konzo has been reported from remote rural areas of Bulungu, Kahemba, Masi-Manimba, and Popokabaka in Bandundu Province (*8, 9*).

Besides the high content of cyanogens, cassava roots are also known to be poor in protein content (*3*). Proteins are a necessary part of the daily diet because the human body does not store protein as it does carbohydrates and fats. Furthermore, 9 of the 20 protein amino acids are either not synthesized at all by the body or can only be synthesized in insufficient amounts. Humans must obtain those 9 amino acids from dietary sources. These are known as the dietary essential amino acids, and they include histidine, isoleucine, leucine, tryptophan, lysine, methionine, phenylalanine, threonine, and valine. Failure to receive an adequate dietary supply of essential amino acids leads to retarded growth and development in children and to disease and body deterioration in adults (*10*).

The objective of this study is to determine residual cyanogen in different samples of cossettes to check the safety, to quantify the daily intake of cyanogen, and to estimate the amount of sulfur amino acids required for their detoxification. Free and

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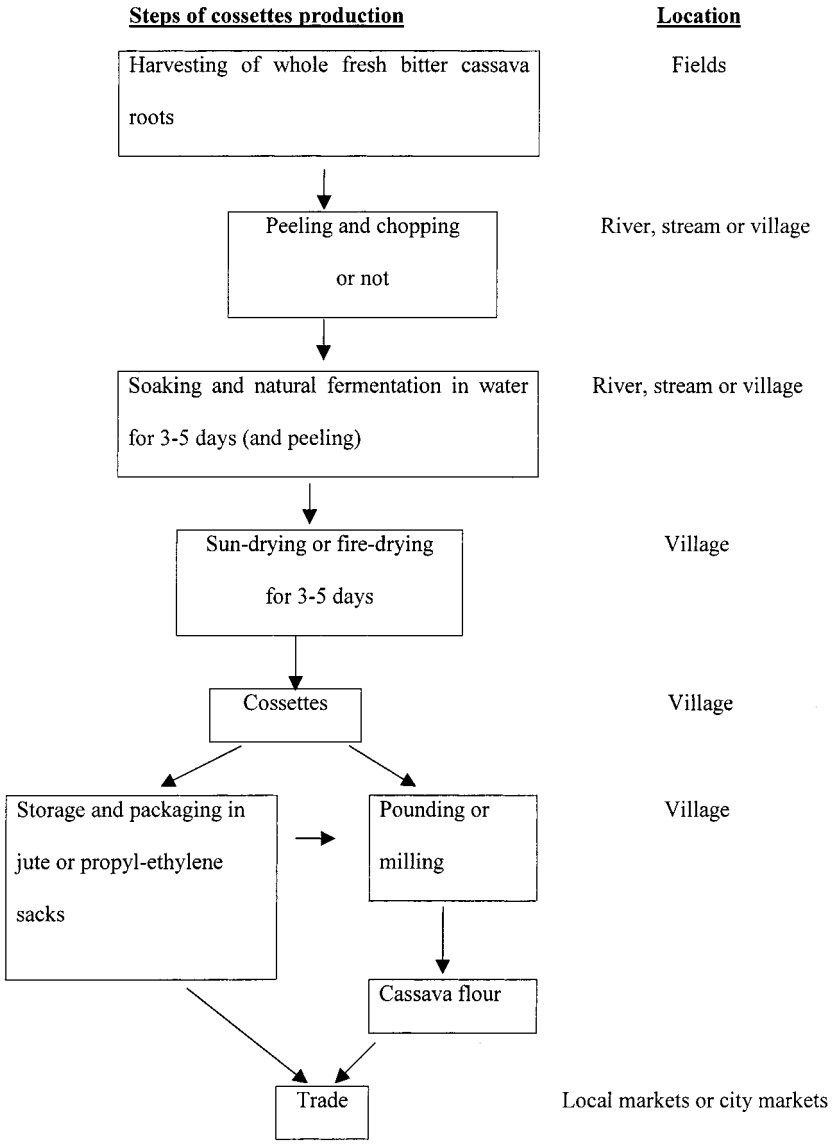

Figure 1. Flow diagram of cassava cossettes processing.

total protein amino acids profiles of cossettes are developed to evaluate the dietary protein quality and to compare with the amino acid requirements of children and adults. Nonprotein amino acids have been reported to be present in many commonly eaten foods, and these compounds have the ability to interfere with a wide range of fundamental biochemical processes and cause disease (*11, 12*). Neurolathyrism, which shares clinical similarities with konzo, has been associated with the overconsumption of grass pea (*Lathyrus sativus* L., Fabaceae) which contains a neurotoxic nonprotein amino acid, β-oxalylaminoalanine (BOAA), or its synonym β-*N*-oxalyl-α,β-L-diaminopropionic acid (ODAP) (*8, 13, 14*). Therefore, the presence of any inherent potentially toxic nonprotein amino acid in cossette samples is also examined.

MATERIALS AND METHODS

Plant Materials. Cossettes were purchased in five different markets (Ngaba, Lemba, Livulu, RondPoint, and Matete) of Kinshasa, the capital of DRC. The cossettes in those markets are supplied by Bandundu province where konzo has been reported. Depending on the size, they are sold in bulk of about 10 pieces of roots. About 500 g (2 or 3 pieces) of the cossettes from each market was finely ground with an electric small laboratory grinder (Culatti) with 200-μm sieve prior to sampling and analyses.

Cassava flour from Cameroon was purchased from an exotic food shop in Antwerp, Belgium for comparison. Cameroon is a part of central Africa where cassava is processed in the same manner as it is processed in DRC.

Determination of Cyanogens. A simple picrate paper kit developed by Egan et al. (*15*) and improved by Bradbury et al. (*16*) was used for the determination of all forms of cyanogens in the cassava products. Protocol B1 was followed for the determination of total cyanogens and acetone cyanohydrin + HCN/ CN^-.

Total Cyanogens. A 100-mg portion of sample was placed on top of a 21-mm diameter Whatman 3 MM filter paper disk containing 1 M phosphate buffer at pH 8 and linamarase, in a flat-bottomed plastic bottle (supplied in the kit). Millipore filtered deionized water (0.5 mL) was added, and a yellow picrate paper attached to a plastic strip was immediately inserted into the vial. The vial was closed immediately with a screw lid and allowed to stand at room temperature for 24 h. The plastic backing sheet was removed carefully from the picrate paper, and the paper was immersed in 5.0 mL of deionized water for about 30 min. The absorbance of the solution was measured at 510 nm, using cuvettes of 1-cm light path against a blank, which contained a yellow solution produced by a picrate paper not exposed to HCN/ CN^-.

The total cyanogens content (expressed in ppm) was calculated by the simple equation

$$\text{total cyanogens content} = 396 \times \text{absorbance}$$

Other samples were prepared as above but without cassava flour, using square linamarin papers equivalent to 50 and 400 ppm, to serve as controls.

Acetone Cyanohydrin + HCN/ CN^-. This analysis was done as the above procedure. However, 200 mg of guanidine hydrochloride was added after the addition of the phosphate buffer pH 8 filter paper disk. The incubation time was 3 h.

The amount of linamarin was calculated by the following equation:

$$\text{linamarin content} = \text{total cyanogens} - (\text{acetone cyanohydrin} + \text{HCN/ } CN^-)$$

Determination of Amino Acids. An HPLC gradient system with precolumn phenylisothiocyanate (PITC) derivatization (*17*) was used to analyze free amino acids. Total protein amino acids were determined after sample hydrolysis.

Extraction of Free Amino Acids. A 50-μL portion of D,L-allylglycine (100 μmol/mL) was added to finely ground sample (5 g) as internal standard. The samples were then extracted in 3 vol 70% ethanol and stored overnight at 4 °C. The extracts were centrifuged (34800*g*, 20 min) and the pellets were washed twice with 70% ethanol. The supernatants were pooled and concentrated under vacuum and stored in a deep freezer at −20 °C.

Sample Hydrolysis. The flour sample was hydrolyzed under vacuum in 6 M HCl following the AOAC 982.30 E procedure (*18*).

Amino Acid Analysis. Aliquots of extract or hydrolysate were concentrated and dried under vacuum (37 °C, 20 mmHg), then a coupling reagent (methanol/water/triethylamine, 2:2:1, v/v) was added, and the solution was mixed and dried immediately under vacuum during 10 min. After this, PITC reagent (methanol/triethylamine/water/PITC, 7:1:1:1, v/v) was added and allowed to stand at room temperature for 20 min before drying under vacuum. PITC derivatives were dissolved in buffer A (0.1 M ammonium acetate, pH 6.5) and filtered through a 0.22-μm Millipore membrane.

A 20-μL portion of sample was injected into an HPLC (Waters model 991 equipped with photodiode array detector) using a gradient system of buffer A (100−0%) and buffer B (0.1 M ammonium acetate containing acetonitrile and methanol, 44:46:10, v/v, pH 6.5) (0−100% after 50 min). The operating temperature was 43 °C. A reverse-phase column from Alltech (Alltima C 18, 5 μm, 250 × 4.6 mm) was used. The absorbance at 254 nm was recorded and used for calculations. A standard protein amino acid mixture (food hydrolysate A 9656, Sigma) was derivatized as above and used as standard for calculations. The results were analyzed by Millennium software (Waters, version 1.10)

Tryptophan Determination. A rapid and simple acid ninhydrin method described by Gaitonde and Dovey (*19*), and adapted for colorimetric determination of tryptophan by Sodek et al. (*20*), was used. Cossettes samples were partially defatted by suspension in 20 vol acetone and stirring occasionally for 0.5 h. After filtration, the powder was left to air-dry. Portions (500 mg) of defatted cassava cossettes were extracted in a centrifuge tube with 2.0 mL of 70% ethanol for 0.5 h at room temperature. The mixture was occasionally stirred and homogenized with a glass rod. NaOH (5 mL, 0.5%) was then added and extraction continued for another 1 h. After centrifugation, a clear supernatant was collected and 0.2 mL of it was taken for tryptophan assay. The acid ninhydrin method using reagent b (250 mg of ninhydrin dissolved in 10 mL of formic acid/hydrochloric acid, 3:2, v/v) was followed for the determination of tryptophan in the samples. Readings were made against a reagent blank in a spectrophotometer (Shidmazu, UV-1601) at 390 nm using cuvettes of 1-cm light path. Sample blanks contained a similar aliquot of extract together with reagent b without ninhydrin.

After subtracting the absorbance value of the sample blank, the tryptophan content was read off a standard curve. Lysozyme (Grade I from egg white; Sigma Chemical Co.) was used to construct the standard curve. Tryptophan values obtained from this graph were then corrected for tyrosine interference according to Zahnley and Davis (*21*).

Statistics. The results were computed and compared by analysis of variance using the software package SPSS 9.0 for Windows. Significant differences among means were confirmed using the Tukey honestly significant differences set at 95% confidence interval ($P < 0.05$). Data are expressed as means ± standard deviation.

Table 1. Cyanogens Content in Cassava Cossettes (mg HCN equivalent kg^{-1} dry weight)[a]

cossettes	total cyanogens	acetone cyanohydrin + HCN/ CN^-	linamarin
Matete ($n = 3$)	$2.772^{b} \pm 0.396$	$0.264^{a} \pm 0.280$	$2.508^{c} \pm 0.457$
Cameroon ($n = 3$)	$9.372^{c} \pm 0.229$	$0.792^{a} \pm 0.280$	$8.580^{d} \pm 0.229$
Lemba ($n = 3$)	$1.716^{a} \pm 0.229$	$0.396^{a} \pm 0.457$	$1.320^{a,b} \pm 0.229$
RondPoint ($n = 3$)	$1.452^{a} \pm 0.229$	$0.528^{a} \pm 0.229$	$0.924^{a} \pm 0.229$
Livulu ($n = 3$)	$1.584^{a} \pm 0.396$	$0.132^{a} \pm 0.229$	$1.452^{a,b,c} \pm 0.229$
Ngaba ($n = 3$)	$2.904^{b} \pm 0.457$	$0.792^{a} \pm 0.280$	$2.112^{b,c} \pm 0.243$

[a] Values are means ± standard deviation. [a,b,c]Same superscript within a column means no significant difference ($P > 0.05$).

RESULTS AND DISCUSSION

The six samples of cassava cossettes had residual cyanogens below 10 mg HCN equivalent kg^{-1} as shown in **Table 1**. This is the recommended safe limit set forth by the C*odex alimentarius* (*22*). The highest level was found in the Cameroon sample (9.37 mg HCN equivalent kg^{-1}), and the lowest level was found in the RondPoint sample (1.45 mg HCN equivalent kg^{-1}), showing a 6.5-fold variation, with a significant difference between Cameroon and all other samples ($P < 0.05$). No significant difference was found between Matete and Ngaba samples, and among RondPoint, Livulu, and Lemba samples, but those last samples were significantly different from Matete and Ngaba samples ($P < 0.05$).

Enzymatic determination of the cyanogenic glycoside linamarin, the major source of cyanide in cassava, showed a variation of almost 10 fold between 0.924 and 8.58 mg HCN equivalent kg^{-1}. Again, the Cameroon sample was significantly higher than all other samples ($P < 0.05$). The linamarin content of RondPoint was significantly different from that of Ngaba and Matete, and also Lemba was different from Matete ($P < 0.05$). No significant difference was found for the content of acetone cyanohydrin + HCN/ CN^- between samples ($P > 0.05$), this varied 6-fold between 0.13 and 0.79 mg HCN kg^{-1} in the cassava cossettes examined.

The fresh bitter cassava roots typically used in the region have total cyanogen levels of 100 to 500 mg HCN equivalent kg^{-1} root, and even up to 1500 mg HCN kg^{-1} (*23*−*25*). Although the original content of the fresh roots from which the cossettes were prepared is not known, it is obvious that the processing and handling of the material resulted in a reduction of total cyanogen of at least 10−30-fold, up to 150−500-fold, giving a final result within the recommended safe limit set at 10 mg HCN equivalent per kg of dry weight. The processing and handling included soaking, sundrying, storage, and transportation to the open markets in Kinshasa where the cossettes were sold in jute sack or in bulk. The Cameroon sample bought in Europe was packed in a plastic foil for industrial transportation.

The low levels of glycosides in the flour from cossettes can be explained by continued cell desintegration and enzymatic activity of the linamarase from the cytoplasm hydrolyzing the cyanogens from the disrupted vacuoles during soaking and throughout the 4 days of drying before moisture fell to low levels in these big root pieces (*26*). It can be assumed that even in short-soaked cossettes, more cyanohydrins might be lost during storage and transportation over the long distance from Bandundu to the markets in Kinshasa than might be lost when the cossettes are consumed locally in the Bandundu area. This might explain

Table 2. Estimated Daily Cossettes and Total Cyanogens Intake

subjects	daily energy required[a](kcal)	60% daily energy required(kcal)	daily cossettes intake[b](g)	daily cyanogens from cossettes[c] (mg)	
				Matete samples	Livulu samples
children 1–3 yr	1360	816	241	0.7	0.4
children 7–9 yr	2190	1314	389	1.1	0.6
adult female (moderately active)	2200	1320	390	1.1	0.6
adult male (moderately active)	3000	1800	532	1.5	0.8

[a] From FAO/WHO/UNU (*31*). [b] 100 g cassava provides 338 kcal (*31*). [c] Total cyanogens (**Table 1**) × daily cossettes intake (**Table 2**).

Table 3. Free Protein Amino Acids Content in Cassava Cossettes (mg g^{-1} dry weight)[a]

free protein amino acids	cossettes					
	Matete ($n = 4$)	Cameroon ($n = 3$)	Lemba ($n = 3$)	RondPoint ($n = 3$)	Livulu ($n = 3$)	Ngaba ($n = 3$)
Aspartic acid	$0.005^{a} \pm 0.003$	$0.012^{b} \pm 0.000$	$0.030^{c} \pm 0.001$	ND	$0.051^{d} \pm 0.005$	$0.044^{d} \pm 0.007$
Glutamic acid	$0.013^{a} \pm 0.000$	$0.079^{d} \pm 0.003$	$0.048^{c} \pm 0.001$	$0.012^{a} \pm 0.001$	$0.082^{d} \pm 0.006$	$0.025^{b} \pm 0.001$
Serine	$0.002^{a} \pm 0.000$	$0.008^{a,b} \pm 0.005$	$0.015^{b,c} \pm 0.003$	$0.003^{a} \pm 0.002$	$0.029^{d} \pm 0.001$	$0.023^{c,d} \pm 0.007$
Glycine	$0.007^{a,b} \pm 0.000$	$0.016^{b,c} \pm 0.003$	$0.025^{c} \pm 0.003$	$0.006^{a} \pm 0.003$	$0.051^{d} \pm 0.002$	$0.005^{a,b} \pm 0.009$
Histidine	ND**	$0.017^{a} \pm 0.000$	$0.048^{a} \pm 0.050$	ND	$0.245^{b} \pm 0.015$	ND
Arginine	ND	ND	ND	ND	ND	ND
Threonine	$0.370^{a} \pm 0.119$	$1.106^{b} \pm 0.056$	$1.135^{b} \pm 0.212$	$0.072^{a} \pm 0.005$	$2.171^{c} \pm 0.344$	$1.523^{b} \pm 0.264$
Alanine	$0.046^{a} \pm 0.029$	$0.151^{a,b} \pm 0.016$	$0.223^{c} \pm 0.047$	$0.025^{a} \pm 0.000$	$0.284^{b} \pm 0.116$	$0.270^{b} \pm 0.065$
Proline	$0.039^{b} \pm 0.002$	$0.064^{c} \pm 0.007$	$0.098^{e} \pm 0.004$	$0.017^{a} \pm 0.000$	$0.298^{f} \pm 0.008$	$0.081^{d} \pm 0.005$
Tyrosine	$0.014^{b} \pm 0.002$	$0.031^{c} \pm 0.002$	$0.077^{d} \pm 0.001$	$0.004^{a} \pm 0.000$	$0.145^{e} \pm 0.005$	$0.087^{d} \pm 0.007$
Valine	$0.025^{a,b} \pm 0.000$	$0.043^{a,b} \pm 0.001$	$0.076^{b,c} \pm 0.043$	$0.010^{a} \pm 0.001$	$0.124^{c} \pm 0.010$	$0.056^{a,b} \pm 0.000$
Methionine	ND	ND	ND	ND	ND	ND
Cysteine	ND	ND	ND	ND	ND	ND
Isoleucine	$0.012^{b} \pm 0.000$	$0.016^{b} \pm 0.001$	$0.070^{d} \pm 0.005$	$0.003^{a} \pm 0.002$	$0.107^{e} \pm 0.006$	$0.027^{c} \pm 0.003$
Leucine	$0.023^{a} \pm 0.001$	$0.044^{a} \pm 0.001$	$0.139^{a,b} \pm 0.002$	$0.007^{a} \pm 0.000$	$0.541^{b} \pm 0.000$	$0.081^{a,b} \pm 0.003$
Phenylalanine	$0.015^{a,b} \pm 0.000$	$0.043^{a,b} \pm 0.000$	$0.089^{c} \pm 0.004$	$0.006^{a} \pm 0.004$	$1.925^{d} \pm 0.060$	$0.075^{b,c} \pm 0.003$
Lysine	$0.010^{b} \pm 0.000$	$0.024^{c} \pm 0.002$	$0.036^{d} \pm 0.003$	$0.005^{a} \pm 0.003$	$0.112^{e} \pm 0.003$	$0.018^{c} \pm 0.002$
Asparagine	ND	$0.003^{a} \pm 0.000$	$0.002^{a} \pm 0.003$	$0.096^{a} \pm 0.083$	ND	$0.010^{b} \pm 0.001$
Glutamine	$0.006^{a,b} \pm 0.000$	$0.024^{c,d} \pm 0.000$	$0.015^{b,c} \pm 0.003$	$0.002^{a} \pm 0.001$	$0.032^{d} \pm 0.002$	$0.160^{e} \pm 0.010$
Tryptophan	$0.027^{a,b} \pm 0.000$	$0.221^{c} \pm 0.011$	$0.038^{a,b} \pm 0.026$	$0.006^{a} \pm 0.003$	$0.005^{a} \pm 0.004$	$0.063^{b} \pm 0.008$

[a] Values are means ± standard deviation. ND = not detected. [a,b,c,d,e] Same superscript within a row means no significant difference ($P > 0.05$).

the absence of cases of konzo in urban consumers, while the crippling disease konzo is prevalent in remote rural areas of Bandundu Province (*6*, *27*, *28*). Even the shortcut-processed cassava products from Bandundu area sold in Kinshasa do not cause clinical symptoms of cyanide exposure (*28*).

Oke (*29*) reported HCN contents of 1.0 mg/100 g in cossettes from DRC, and O'Brien et al. (*24*) found a variation in cyanogens content of fermented cassava roots ranging between 0 and 11.3 mg kg^{-1} in villages of Cameroon.

In populations with cassava roots as their main staple food, a basic daily energy need of 1500 kcal can be satisfied with 500 g dry weight cassava root products. Adult consumers would then be exposed to approximately less than 5 mg HCN equivalent per day, compared to the Codex Alimentarius safe level of 10 mg HCN equivalent per kg dry weight (*30*). If cossettes as staple food provide 60% of the daily dietary energy intake of residents in DRC, calculated from the FAO/WHO energy requirements (*31*), then for Matete samples about 0.7 mg HCN equivalent is present in the 241 g of cassava cossettes to be consumed daily by children (1−3 years old), and about 1.5 mg HCN equivalent is present in the 532 g of cassava cossettes to be consumed daily by a moderately active adult man to meet their energy requirements of 816 and 1800 kcal, respectively (**Table 2**).

The free amino acid pattern of cossettes samples is summarized in **Table 3**. The concentrations of free amino acids were, in general, very low. Arginine and sulfur amino acids (methionine and cysteine) were not found. Histidine was not found in Matete, RondPoint, or Ngaba samples. No asparagine was detected in Matete or Livulu samples.

The Livulu sample showed the highest amount in total free amino acids (6.2 mg/g of dry weight cossettes), and RondPoint had the lowest (0.27 mg/g of dry weight cossettes). This represents about a 23-fold variation among the few samples examined; duration of soaking and flow rate of water during soaking leading to leaching out can probably explain this finding. Threonine was quantitatively the most important free amino acid in five of the samples examined, but in the RondPoint sample asparagine was the most abundant (**Figure 2**). Alanine ranked second except in Livulu, RondPoint, and Cameroon. No known potentially toxic nonprotein amino acids were detected in our samples.

Table 4 presents the total protein amino acids profiles in cassava cossette samples. The overall average of the total protein amino acids was 23.7 mg/g dry weight cassava cossettes in which essential amino acids represent 54.6% and the sulfur containing amino acids represent 9.7%. Alanine was the major protein amino acid in all samples except in RondPoint, where glutamic acid was the most important. This finding is in agreement with some studies done with fresh cassava roots (*23*, *32*, *33*). This suggests that during post-harvest processing, the loss of protein is negligible, whereas the loss of cyanogens is considerable.

The Livulu sample had the highest total protein amino acid content (27.3 mg/g of dry weight cassava cossettes) with 54.2% of essential amino acids. Lemba contained the highest essential amino acids proportion (63.5%), and RondPoint had the lowest (50.4%).

Leucine and lysine, the purely ketogenic amino acids, were the limiting amino acids in our samples (**Table 5**). Leucine was

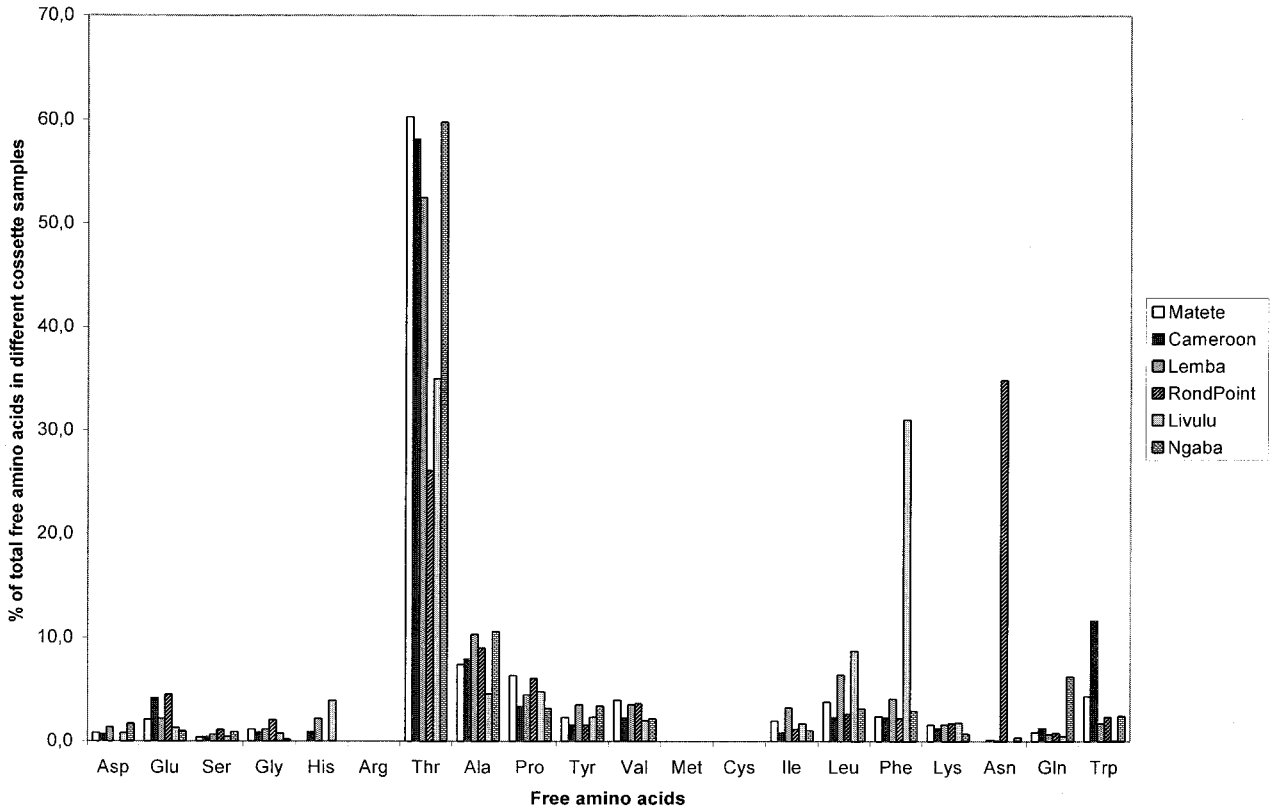

Figure 2. Free protein amino acids in cassava cossettes samples.

Table 4. Total Protein Amino Acids Content in Cassava Cossettes (mg g^{-1} dry weight)[a]

total protein amino acids	cossettes					
	Matete ($n = 4$)	Cameroon ($n = 4$)	Lemba ($n = 4$)	RondPoint ($n = 4$)	Livulu ($n = 3$)	Ngaba ($n = 4$)
Aspartic acid	$0.200^{a} \pm 0.022$	$0.234^{a} \pm 0.044$	$0.351^{a,b} \pm 0.074$	$0.480^{b,c} \pm 0.120$	$0.403^{b} \pm 0.049$	$0.564^{c} \pm 0.041$
Glutamic acid	$1.074^{a} \pm 0.257$	$1.255^{a} \pm 0.079$	$1.343^{a} \pm 0.209$	$2.016^{b} \pm 0.186$	$1.702^{a,b} \pm 0.589$	$2.270^{b} \pm 0.291$
Serine	$0.700^{a} \pm 0.157$	$0.688^{a} \pm 0.058$	$0.749^{a} \pm 0.112$	$1.053^{b} \pm 0.023$	$0.970^{a,b} \pm 0.272$	$0.691^{a} \pm 0.066$
Glycine	$1.342^{a} \pm 0.175$	$1.254^{a} \pm 0.231$	$1.423^{a,b} \pm 0.164$	$1.921^{b} \pm 0.173$	$1.388^{a} \pm 0.413$	$1.217^{a} \pm 0.111$
Histidine	$1.218^{a,b} \pm 0.177$	$1.744^{b} \pm 0.140$	$1.082^{a} \pm 0.038$	$1.203^{a} \pm 0.113$	$1.111^{a} \pm 0.580$	$1.060^{a} \pm 0.121$
Arginine	$0.796^{a} \pm 0.073$	$0.715^{a} \pm 0.024$	$0.744^{a} \pm 0.085$	$1.029^{b} \pm 0.071$	$1.034^{b} \pm 0.089$	$0.796^{a} \pm 0.092$
Threonine	$1.787^{ab} \pm 0.638$	$2.239^{abc} \pm 0.711$	$3.177^{bc} \pm 0.807$	$0.945^{a} \pm 0.335$	$3.508^{c} \pm 0.907$	$3.493^{c} \pm 0.594$
Alanine	$5.124^{c} \pm 0.869$	$4.309^{bc} \pm 0.675$	$3.349^{b} \pm 0.334$	$0.962^{a} \pm 0.059$	$5.329^{c} \pm 0.968$	$5.628^{c} \pm 0.316$
Proline	$1.494^{ab} \pm 0.072$	$1.345^{a} \pm 0.154$	$1.664^{ab} \pm 0.269$	$1.930^{b} \pm 0.264$	$1.679^{ab} \pm 0.269$	$1.498^{ab} \pm 0.110$
Tyrosine	$1.410^{a} \pm 0.064$	$1.272^{a} \pm 0.190$	$1.035^{a} \pm 0.506$	$1.343^{a} \pm 0.186$	$1.035^{a} \pm 0.506$	$1.191^{a} \pm 0.346$
Valine	$0.961^{a} \pm 0.287$	$1.153^{a} \pm 0.485$	$3.306^{b} \pm 0.363$	$1.104^{a} \pm 0.449$	$1.733^{a} \pm 0.783$	$1.374^{a} \pm 0.343$
Methionine	$0.162^{a} \pm 0.028$	$0.108^{a} \pm 0.029$	$0.399^{a} \pm 0.079$	$0.372^{a} \pm 0.110$	$0.302^{a} \pm 0.368$	$0.398^{a} \pm 0.243$
Cysteine	$1.828^{a} \pm 0.070$	$2.183^{a} \pm 0.231$	$2.157^{a} \pm 0.168$	$1.975^{a} \pm 0.440$	$1.853^{a} \pm 0.250$	$2.061^{a} \pm 0.999$
Isoleucine	$0.740^{ab} \pm 0.148$	$0.554^{a} \pm 0.237$	$1.604^{b} \pm 0.556$	$0.609^{a} \pm 0.303$	$0.612^{a} \pm 0.241$	$0.914^{ab} \pm 0.539$
Leucine	$0.660^{a} \pm 0.108$	$0.648^{a} \pm 0.261$	$1.717^{b} \pm 0.692$	$0.699^{a} \pm 0.527$	$0.652^{a} \pm 0.112$	$0.760^{a} \pm 0.321$
Phenylalanine	$0.900^{a} \pm 0.104$	$0.783^{a} \pm 0.234$	$0.842^{a} \pm 0.063$	$0.708^{a} \pm 0.202$	$2.314^{b} \pm 0.568$	$0.523^{a} \pm 0.363$
Lysine	$0.497^{a} \pm 0.123$	$0.527^{a} \pm 0.092$	$0.531^{a} \pm 0.256$	$0.530^{a} \pm 0.108$	$0.751^{a} \pm 0.124$	$0.691^{a} \pm 0.164$
Tryptophan	$0.741^{a} \pm 0.004$	$0.754^{a} \pm 0.022$	$0.877^{a} \pm 0.188$	$0.780^{a} \pm 0.026$	$0.907^{a} \pm 0.018$	$0.740^{a} \pm 0.012$

[a] Values are means ± standard deviation. [a,b,c] Same superscript within a row means no significant difference ($P > 0.05$).

the first limiting amino acid in Livulu and Ngaba with an amino acid score of 0.36 and 0.45, respectively. Lysine was the first limiting amino acid in the other samples with an amino acid score varying from 0.35 to 0.47.

Results of Firmin and Kamenan (*32*) showed sulfur amino acids (methionine + cysteine) in fresh cassava roots and leucine in fermented pulp of cassava roots as first limiting amino acids, respectively. Yeoh and Truong (*34*) found sulfur amino acids, leucine, and lysine, to be the limiting amino acids in different cultivars of cassava roots studied. Bradbury and Holloway (*23*) reported large differences in amino acid composition between different cultivars of cassava roots examined, and there was no essential amino acid which was clearly the first limiting amino acid. Nevertheless, on the average, histidine was the first limiting amino acid and leucine was the second limiting amino acid.

Although sulfur amino acids were not the limiting amino acids in our samples, we should notice that the proportion of methionine represented on average only 13% (4.7−16.2%) of total sulfur amino acids, and cysteine represented 87% (83.8−95.3%) of total sulfur amino acids. Methionine normally supplies part of the body's needs for cysteine. With cysteine-free diets, methionine can supply all of the body's needs for cysteine. Cysteine can spare methionine and a certain proportion of dietary methionine is converted to cysteine (*35*).

Rose and Wixon (*36*) demonstrated the influence of cysteine on the methionine requirement for an adult man by determining

Table 5. Amino Acid Scoring Patterns of Different Cossette Samples

	FAO/ WHO[a]	amino acid scores[b]					
essential amino acid (EAA)	children (2–5 years)	Matete	Lemba	Livulu	Ngaba	RondPoint	Cameroon
Threonine	3.4	2.43	3.54	3.78	3.97	1.42	3.05
Cysteine + Methionine	2.5	3.68	3.88	3.16	3.80	4.80	4.24
Valine	3.5	1.27	3.58	1.81	1.52	1.61	1.66
Isoleucine	2.8	1.22	2.17	0.80	1.26	1.11	0.91
Leucine	6.6	0.46	0.99	0.36	0.45	0.54	0.45
Tyrosine + Phenylalanine	6.3	1.69	1.14	1.95	1.05	1.66	1.37
Histidine	1.9	2.97	2.16	2.14	2.16	3.24	4.25
Lysine	5.8	0.40	0.35	0.47	0.47	0.47	0.37
Tryptophan	1.1	3.11	3.02	3.02	2.60	3.62	3.17
first limiting amino acid		Lysine	Lysine	Leucine	Leucine	Lysine	Lysine
second limiting amino acid		Leucine	Leucine	Lysine	Lysine	Leucine	Leucine

[a] Recommended amino acid scoring pattern from FAO/ WHO/ UNU (*31*). [b] Amino acid score = mg of amino acid in 1 g of test protein per mg of amino acid in 1 g of reference Protein (*42*).

Table 6. Essential Amino Acid (EAA) Requirements and Estimated Daily Intake from Cossette Samples

	suggested patterns of requirement[a] (mg AA/day)			estimated daily EAA (from cossettes) intake[b] (mg)							
				Matete samples				Livulu samples			
EAA	child >1 yr	adult female	adult male	child 1–3 yr	child 7–9 yr	adult female	adult male	child 1–3 yr	child 7–9 yr	adult female	adult male
Thr	1000	305	500	431	695	697	951	845	1365	1368	1867
Cys + Met	-	550	1100	-	-	776	1059	-	-	840	1146
Met	800	-	-	39	63	-	-	73	117	-	-
Val	900	650	800	232	374	375	511	418	674	676	922
Ile	1000	450	700	178	288	289	394	147	238	239	326
Leu	1500	620	1100	159	257	257	351	157	254	254	347
Tyr + Phe	-	1120	1100	-	-	901	1229	-	-	1306	1782
Phe	800	-	-	217	350	-	-	558	900	-	-
Lys	1600	500	800	120	193	194	264	181	292	293	400
Trp	250	157	250	179	288	289	394	219	353	354	483

[a] From Altman and Dittmer (*31*). [b] Daily cossettes intake (**Table 2**) × amino acid (**Table 4**).

the conditions that supported a zero or slightly positive nitrogen (N) balance. They observed that cysteine alone, without methionine, resulted in a negative N balance. A near zero N balance was observed with a diet containing 0.8 g of methionine, whereas the N balance was negative with a 0.7-g methionine diet. Higher levels of methionine resulted in a positive N balance. They concluded that oversupply of cysteine could give a positive N balance with lower intake of methionine, but even then the intake of methionine remains essential. This statement illustrates the limiting of the ability of cysteine to spare methionine. Although cysteine can fulfill a large fraction of our requirement for sulfur amino acids, according to Altman and Dittmer (*37*), in the combination cysteine + methionine, 30−50% of the total requirement for adults may be furnished by cysteine and 50−70% may be furnished by methionine.

The expected daily methionine and sulfur amino acids intake provided by cassava cossettes consumption, which in the case of DRC represents 60% of daily energy intake, are compared with the suggested amino acid patterns requirement (**Table 6**). It can be concluded that children of 1 to 9 years old cannot expect to meet the methionine requirement, whereas adults can meet the sulfur amino acid requirement. Sulfur amino acids are required for cyanide detoxification in the human body (*23, 38*). A daily supply of about 1.2 mg of dietary sulfur from S-containing amino acids is needed by the human body to detoxify 1.0 mg of HCN (*25*). When the body is regularly exposed to cassava cyanogens, the increased synthesis of rhodanese (the enzyme responsible for cyanide detoxification in the human body by forming thiocyanate) makes extra demands on the body's reserves of sulfur amino acids. If this demand is prolonged, as it is in the regular consumption of cassava root insufficiently processed, and the diet is inadequate, the synthesis of many proteins vital for bodily functions may be impaired and lead to the development of protein deficiencies and other diseases (*25, 39, 40*). Other food components of the diet should contribute to a better-balanced amino acid composition of the diet, especially the level of sulfur amino acids. In the case of lathyrism, a neurodegenerative disease with clinical symptoms similar to those of konzo, Lambein et al. (*41*) have suggested that the ratio of cereals (rich in methionine) to *Lathyrus* seeds (rich in lysine and low in sulfur amino acid) may be a determining factor in the etiology. In the regions neighboring the konzo-affected areas in Bandundu, where traditionally corn or millet flour is mixed with cassava as a staple food, no cases of konzo have been reported. This may corroborate our views as to the importance of methionine for a healthy balanced diet.

Hence, the recommended daily methionine allowance should be reconsidered and given separately from total S-amino acid requirement.

We can conclude that the processed cassava roots available on the markets in Kinshasa have cyanogens content within the safe limit recommended by FAO/ WHO. Traditional transport in jute sacks appears to contribute to reducing residual cyanogens in the cossettes, whereas transport in airtight wrapping probably prevents the release of cyanide.

No potentially toxic nonprotein amino acids were detected in this study.

The dietary requirements for sulfur amino acids need to be adjusted for the loss caused by cyanide detoxification. The total sulfur amino acids availability does not give a correct value for the requirement of the essential amino acid methionine. In the case when cassava is consumed as a staple food, the low methionine content may aggravate the risk for cyanide toxicity and konzo disease, even when the cysteine present covers the dietary requirement for sulfur amino acids.

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