cogent food & agriculture

FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Nutritional and anti-nutritional composition of cassava leaf protein concentrate from six cassava varieties for use in aqua feed

A. Oresegun[1], O. A. Fagbenro[2], P. Ilona[3] and Edah Bernard[1]*

Received: 20 April 2015
Accepted: 19 January 2016
First Published: 01 February 2016

*Corresponding author:
Edah Bernard, Department of Biotechnology/Fish Nutrition, Nigeria Institute for Oceanography and Marine Research (N.I.O.M.R), 3- Wilmot Point Road, Victoria Island, Lagos P. M. B. 12729, Nigeria
E-mail: bernardnda@yahoo.com

Reviewing editor:
Fatih Yildiz, Middle East Technical University, Turkey

Additional information is available at the end of the article

Abstract: Leaves from six varieties of cassava (*Manihot esculenta* crantz) cultivated in Nigeria were harvested and analysed for their nutritional composition and anti-nutritional factors using standard analytical techniques. The leaves were further processed into cassava leaf protein concentrate. Results obtained indicated highest crude protein levels, β-carotene levels and lipid levels of 48.85 ± 0.45, 816.92 ± 8.80 and 13.27 ± 0.06, respectively, in variety 419 and lowest crude protein levels and β-carotene levels of 40.19 ± 0.08 and 298 ± 5.74 in variety 326. However, ash, moisture and carbohydrate levels for all six varieties were relatively the same. Mineral compositions for all varieties were also fairly similar. Hydrogen cyanide levels were significantly low ($p > 0.05$) in variety 419 with a value of 0.98 ± 0.05. However, phytate and tannin levels for all six varieties were not significantly different ($p > 0.05$). It can be concluded from this study that cassava variety 419 had the highest potential for use in aqua feed production.

Subjects: Agriculture; Agriculture and Food; Fisheries Science; Oceanography

Keywords: fish feed; cassava leaves; anti-nutritional factors; variety 419; β-carotene

ABOUT THE AUTHORS

Nigeria produces more cassava than any other country in the world. It was estimated that in 2010, Nigeria's production of cassava reached 37.5 million tonnes and that the country has consistently been ranked as the world's largest producer of cassava since 2005 (FAOSTAT, 2012). Its production requires minimum labour and inputs, and remains the most important food security crop for millions of Nigerians.
One hectare of cassava plantation contains between 7,000 and 8,000 stands. Cassava leaf takes about 6–8% of the weight of each stand. Therefore, an established weight of leaves per hectare is 0.69–0.92% MT. This implies some 2.6–3.5 million MT of leaves are unutilized in Nigeria. In addition, their low production cost is emphasized, since the leaves are rather considered as residues and they do not compete with the roots, which are the main commercial product from cassava. This is where value addition comes in.

PUBLIC INTEREST STATEMENT

This research work was carried out to add value to the millions of cassava leaves discarded as waste annually in Nigeria.

1. Introduction

Cassava is extremely reliable to grow, especially on sloping rain-fed soils of low fertility, survives drought periods and grows well with limited supplies of water. In addition, it is tolerant of acid soils and yields well on marginal soils without excessive use of costly inputs. These qualities have endeared cassava to resource-poor farmers (Phuc et al., 2000).

In Nigeria, considerable amount of cassava leaves are generated annually and readily available as a by-product at the time of harvesting the roots. While cassava leaf protein is low in sulphur and amino acids (Kobawila, Louembe, Keleke, Hounhouigan, & Gamba, 2005), the content of most other essential amino acids is higher than in soya bean meal (Ly & Samkol, 2001). The high protein content and a relatively good profile of essential amino acids are reasons for believing that cassava leaves could be a potential protein source for monogastric animals.

Cassava leaves are a significant source of potential alternative protein resource for both humans and animals (Fasuyi, 2005). The leaves, depending on the varieties, are rich in minerals, proteins, vitamin and carotenes (Adewusi & Bradbury, 1993). However, it also has some anti-nutritional and toxic substances. These substances interfere with digestibility and uptake of the nutrients, and they might present toxic effects depending on the amount in which they are consumed (Wobeto, Corrêa, Abreu, Santos, & Pereira, 2007).

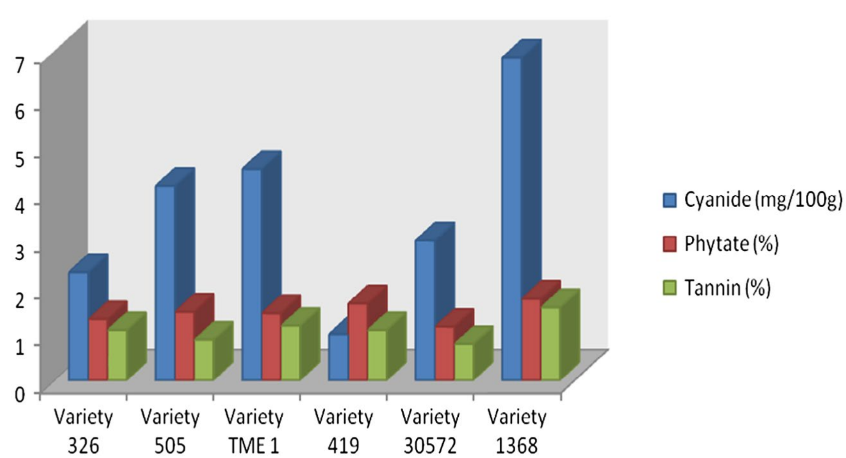
Cassava and cassava by-products are widely used and represent the basic diet of about 500 million people in the world (Chavez et al., 2000). The use of the cassava leaf meal (CLM) may supply a positive balance for the nutritional quality because they present higher contents of proteins, vitamins, minerals and fibre (Corrêa et al., 2004; Ngudi, Kuo, & Lambein, 2003). In addition, their low production cost is emphasized, since the leaves are rather considered as residues and they do not compete with the roots, which are the main commercial product from cassava. The main objectives of this research are to investigate the nutritional composition and anti-nutritional factors of six varieties of cassava leaf and their protein concentrate. This will also provide knowledge on the nutritional potential of cassava and its use in the aqua feed industry (Figure 1).

2. Materials and methods

2.1. Collection and processing of cassava leaves for cassava leaf protein concentrate

Leaves of six cassava varieties namely variety 326, variety 505, variety 419, variety TME 1, variety 1,368 and variety 30,572 were freshly collected from the International Institute for Tropical Agriculture (IITA) Ibadan Nigeria and transported in plastic bags. The harvested leaves were weighed and washed to pulping with a leaf pulping machine. This was followed by crushing in water (1:3 w/v at a pH 8.5) and filtered as described by Fellows (1987). The separated leaf juice was then heated at

Figure 1. Anti-nutritional factors of six varieties of cassava.



about 90–100°C for 10 min to coagulate leaf protein concentrate. The coagulated protein fraction was thereafter separated by a method of filtration and subsequently dried at atmospheric temperature. The dried cassava leaf protein concentrate (CLPC) was then milled using a laboratory hammer miller and kept in an airtight container prior to chemical analysis.

2.2. Nutritional composition of cassava leaves

The proximate composition of variety 326, variety 505, variety 419, TME 1, variety 1,368 and variety 30,572 were determined for moisture, ash, crude fat, total carbohydrate and total β-carotene as described by (Association of Official Analytical Chemist Official [AOAC], 2000). Thereafter, the nitrogen was determined by the Micro-Kjeldahl method.

2.3. Determination of mineral content

Mineral composition after wet digestion with a mixture of sulphuric acid, nitric and perchloric acid was determined using the atomic absorption spectrometer (AAS) (Buch Scientific, East Norwalk, CT 06855, USA) for calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), cupper (Cu) and zinc (Zn), while potassium (K) and sodium (Na) were determined using flame photometry (Table 3).

2.4. Anti-nutritional factor analysis

Variations in the chemical composition and inherent anti-nutritional substances in the different cassava varieties may restrict usage. The principal problems that could undermine its potential include high fibre content and anti-nutrients typified mainly by cyanide, tannin and phytate (Table 2).

2.5. Statistical analysis

The entirely randomized statistical design was used, on a factorial scheme of 6 + 1 + 3, which is, six varieties, a specific plant age and three replicates. Data were analysed by descriptive analysis and one-way analysis of variance (ANOVA). SPSS (version 16.0) statistical software package (SPSS, Chicago, USA) was employed in the analysis. Differences were considered significant at an alpha level of 5%.

3. Results and discussion

The nutritional content and anti-nutritional factors of the six varieties of cassava were investigated. The CLPC was prepared under environmental conditions. The variance analysis and significant differences were observed at 5% probability, by the *F*-test, in all other parameters studied. Table 1 shows the result of the chemical composition for the different CLPC in percentages owing to their nutritional values with variety 419 recording the highest percentage in crude protein (CP) concentration with a value of 48.85 ± 0.45, while variety 505 had the lowest CP concentration of 40.19 ± 0.42 and differs significantly ($p < 0.05$) from the rest of the varieties.

Average values for moisture content in the CLPC (g 100 g^{-1}) were highest in variety 505 with a value of 9.55 ± 0.1, while variety 30,572 had the lowest moisture content with a value of 6.27 ± 0.20. The high values of ash content (5.56 ± 0.03) in variety 30,572 indicate a high mineral content in it than in variety 1,368 with an ash content of 3.4 ± 0.01. The values do not correspond with that reported by Frederick (2008).

Total β carotene levels were equally highest in variety 419 with a value of 816.92 ± 8.80 but show a remarkable decrease in variety TME 1 and variety 30,572 with equal concentrations of 298 ± 5.74. This study demonstrates a considerable variation in the chemical composition among leaves of different cassava varieties which is in agreement with earlier reports (Ly & Samkol, 2001; Ravindran, 1990). The variation may be due to differences in plant development as well as genetic make-up of the different varieties. Table 2 shows the anti-nutritional factors which include cyanide, phytate and tannin concentrations for the different CLPCs.

Toxicity problems that affect nutritive value of cassava leaves can be reduced by traditional preparation methods such as drying, pounding and long periods of boiling (Ajibade, Balogun, Afolabi, &

Table 1. Chemical composition of six different varieties of cassava

Cassava variety	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Total carbohydrate (%)	Total β carotene (μg/g)
Variety 326	43.95 ± 0.08[b]	6.33 ± 0.01[b]	12.13 ± 0.06[b]	3.76 ± 0.01[c]	33.84 ± 0.11[d]	327.06 ± 19.6[d]
Variety 505	40.19 ± 0.42[d]	9.55 ± 0.1[a]	7.68 ± 0.02[d]	3.88 ± 0.02[b]	38.71 ± 0.32[a]	636.92 ± 9.02[b]
Variety 419	48.85 ± 0.45[a]	6.71 ± 0.13[b]	13.27 ± 0.06[a]	3.87 ± 0.04[b]	27.31 ± 0.56[e]	816.92 ± 8.80[a]
Variety TME 1	42.19 ± 0.03[c]	6.29 ± 0.20[b]	10.25 ± 0.07[c]	5.53 ± 0.03[a]	35.74 ± 0.03[c]	298 ± 5.74[d]
Variety 1,368	44.49 ± 0.35[b]	9.46 ± 0.27[a]	5.59 ± 0.02[e]	3.4 ± 0.01[d]	37.07 ± 0.09[b]	303.74 ± 2.50[c]
Variety 30,572	42.22 ± 0.02[c]	6.27 ± 0.20[b]	10.18 ± 0.0[7]	5.56 ± 0.03[a]	35.16 ± 0.03[c]	298 ± 5.74[d]

Notes: Mean in the same column followed by different superscript differ significantly ($p < 0.05$).

Kupolati, 2006; Aletor & Adeogun, 1995; Fasuyi, 2005; Lewis & Fenwick, 1987). Sun-drying is an inexpensive effective method of preserving surplus micronutrient-rich foods (Tontisirin, Nantel, & Bhattacharjee, 2002). Variety 1,368 had the highest anti-nutritional factors with cyanide, phytate and tannin concentrations of 6.85 ± 0.0, 1.73 ± 0.0 and 1.55 ± 0.0, respectively. Cyanide concentrations were lowest in variety 419 with a value of 0.98 + 0.05. Variety 30,572 recorded the lowest phytate and tannin concentrations of 1.14 + 0.0 and 0.77 + 0.0 respectively. These levels are within the range reported by (Awoyinka, Abegunde, & Adewusi, 1995 and Câmara & Madruga, 2001) with cyanide levels ranging from 5.3 to 80 mg/100 g dry matter (DM). However, values were not significantly different between groups of varieties ($p > 0.05$). This variation is probably due to the genetic differences among the cultivars, the plant ages, the leaf maturity and soil fertility.

The CLPC from the analysed six varieties could well be classified as non-toxic because generally their cyanide contents fell below 10 mg 100 g^{-1} powder (Ikediobi, Onyia, & Eluwah, 1980). The lethal dose (LD) of cyanide oscillates from 0.5 to 3.5 mg kg^{-1} human body weight (Wogan & Marletta, 1993). However, it is worth emphasizing that the chronic toxicity is due to the consumption of lower cyanide doses at longer timer intervals (Osuntokun, 1981).

This study further revealed a considerable variation in the mineral composition among the six different cassava varieties. Table 3 shows the average mineral contents in DM. Iron content in variety 419 was highest (458.08) compared to variety 505 with a value of 321.32. These results are in line with the result analysed by Kadashi (2005). Iron is required for the synthesis of haemoglobin and myoglobin, which are oxygen carriers in the blood.

In conclusion, among the varieties investigated in this study, variety 419 had the lowest contents for most anti-nutrients (cyanide, phytate and tannin inhibitors) and consequently the highest content for most nutritional values like the CP value, while on the other hand, varieties with most highest anti-nutritional contents tends to exhibit the lowest nutritional contents indicating an inversely

Table 2. Anti-nutritional factors of CLPC of six different varieties of cassava

Cassava variety	Cyanide (mg/100 g)	Phytate (%)	Tannin (%)
326	2.30 ± 0.19[c]	1.30[c]	1.07[b]
505	4.13 ± 0.09[b]	1.46[b]	0.86[c]
TME 1	4.49 ± 0.01[b]	1.43[b]	1.16[b]
419	0.98 ± 0.05[d]	1.63[a]	1.07[b]
30,572	2.97 ± 0.00[c]	1.14[c]	0.77[c]
1,368	6.85 ± 0.00[a]	1.73[a]	1.55[a]

Notes: Mean in the same column followed by different superscript differ significantly ($p < 0.05$).

cogent • food & agriculture

Table 3. Mineral composition of six different varieties of cassava

Cassava variety	Ca (%)	Mg (%)	K (%)	Na (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
326	0.68	0.02	0.13	32.78	76.69	356.96	22.33	36.25
505	0.9	0.03	0.15	33.45	124.21	321.32	18.34	62.06
TME 1	0.85	0.05	0.14	33.35	152.71	371.05	20.7	56.32
419	0.71	0.03	0.16	32.75	77.85	458.08	17.03	52.99
30,572	1.2	0.07	0.12	33.18	141	343.06	23.04	68.95

proportional relationship between anti-nutritional factors and nutritional contents of the six varieties. From this study, therefore, variety 419 exhibited highest potential for use both for human consumption, fish feed production and livestock feed production. Though, the other varieties analysed might also be relevant in the feed industry at a non-toxic amount.

Funding
The authors received no direct funding for this research.

Competing interests
The authors declare no competing interest.

Author details
A. Oresegun[1]
E-mail: koresegun@yahoo.com
O. A. Fagbenro[2]
E-mail: fagbenro_dapo@yahoo.com
P. Ilona[3]
E-mail: p.ilona@cgiar.org
Edah Bernard[1]
E-mail: bernardnda@yahoo.com
[1] Department of Biotechnology/Fish Nutrition, Nigeria Institute for Oceanography and Marine Research (N.I.O.M.R), 3 – Wilmot Point Road, Victoria Island, Lagos P. M. B. 12729, Nigeria.
[2] Department of Fisheries and Wildlife, Federal University of Technology, Akure P.M.B. 704, Nigeria.
[3] Harvest Plus Country Manager for Nigeria, International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

Citation information
Cite this article as: Nutritional and anti-nutritional composition of cassava leaf protein concentrate from six cassava varieties for use in aqua feed, A. Oresegun, O. A. Fagbenro, P. Ilona & Edah Bernard, *Cogent Food & Agriculture* (2016), 2: 1147323.

References

Adewusi, S. R. A., & Bradbury, J. H. (1993). Carotenoids in cassava: Comparison of open-column and HPLC methods of analysis. *Journal of the Science of Food and Agriculture, 62*, 375–383. http://dx.doi.org/10.1002/(ISSN)1097-0010

Ajibade, S. R., Balogun, M. O., Afolabi, O. O., & Kupolati, M. D. (2006). Sex differences in the biochemical contents of *Telfairia occidentalis* Hook F. *Journal of Food Agriculture and Environment, 4*, 155–156.

Aletor, V. A., & Adeogun, O. A. (1995). Nutrient and anti-nutrient components of some tropical leafy vegetables. *Food Chemistry, 53*, 375–379. http://dx.doi.org/10.1016/0308-8146(95)99830-S

Association of Official Analytical Chemist Official. (2000). *Association of Official Analytical Chemist Official methods of analysis* (15th ed.). Washington, DC: Author.

Awoyinka, A. F., Abegunde, V. O., & Adewusi, S. R. A. (1995). Nutrient content of young cassava leaves and assessment of their acceptance as a green vegetable in Nigeria. *Plant Foods for Human Nutrition, 47*, 21–28. http://dx.doi.org/10.1007/BF01088163

Câmara, F. S., & Madruga, M. S. (2001). Cyanic acid, phytic acid, total tannin and aflotoxin contentes of a Brazilian (Natal) multimistura preparation. *Revista de Nutrição, Campinas, 14*, 33–36.

Chavez, A. L., Bedoya, J. M., Sanchez, T., Iglesias, C., Ceballos, H., & Roca, W. (2000). Iron, carotene, and ascorbic acid in cassava roots and leaves. *Food and Nutrition Bulletin, 21*, 410–413. http://dx.doi.org/10.1177/156482650002100413

Corrêa, A. D., et al. (2004). Remoção de polifenóis da farinha de folhas de mandioca. *Ciência e Tecnologia de Alimentos, 24*, 159–164. http://dx.doi.org/10.1590/S0101-20612004000200001

Fasuyi, A. O. (2005). Nutrient composition and processing effects on cassava leaf (*Manihot esculenta*, crantz) antinutrients. *Pakistan Journal of Nutrition, 4*, 37–42.

Fellows, P. (1987). Village-scale leaf fractionation in Ghana. *Tropical Science, 27*, 77–84.

Frederick, D. O. (2008). *Hog and hominy: Soul food from Africa to America* (pp. 1-2:1-24). New York, NY: Columbia University Press.

Ikediobi, C. O., Onyia, G. O. C., & Eluwah, C. E. (1980). A rapid and inexpensive enzymatic assay for total cyanide in cassava (*Manihot esculenta,* Crantz) and cassava products. *Agricultural and Biological Chemistry, 44*, 2803–2809. http://dx.doi.org/10.1271/bbb1961.44.2803

Kadashi , Y.D. (2005). *Vitamin and minerals nutritional foods in Nigeria*. Kaduna: De-peak.

Kobawila, S. C., Louembe, D., Keleke, S., Hounhouigan, J., & Gamba, C. (2005). Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. *African Journal of Biotechnology, 4*, 689–696.

Lewis, J., & Fenwick, G. R. (1987). Glucosinolate content of *brassica* vegetables: Analysis of twenty-four cultivars of calabres (green sprouting broccoli, *Brassica oleracea* L. var. *botrytis* subvar. *cymosa Lam.*). *Food Chemistry, 25*, 259–268. http://dx.doi.org/10.1016/0308-8146(87)90012-4

Ly, J., & Samkol, P. (2001). The nutritive value of ensiled cassava leaves or young Mong Cai pigs fed high levels of protein. *Livestock Research for Rural Development, 13*, 5 p.

Ngudi, D. D., Kuo, Y.-H., & Lambein, F. (2003). Cassava cyanogens and free amino acids in raw and cooked leaves. *Food and Chemical Toxicology, 41*, 1193–1197. http://dx.doi.org/10.1016/S0278-6915(03)00111-X

Osuntokun, B. O. (1981). Cassava diet, chronic cyanide intoxication and neuropathy in Nigerian Africans. *World Review of Nutrition and Dietetics, 36*, 141–173. http://dx.doi.org/10.1159/000393156

Phuc, B. H. N., & Lindberg, J. E. (2001). Ileal apparent digestibility of amino acids in growing pigs given a cassava root meal diet with inclusion of cassava leaves, leucaena leaves and groundnut foliage. *Animal Sciences, 72*, 511–517.

Ravindran, V. (1990). Cassava leaf meal. In: P. A. Thacker, R. N. Kirkwood (Eds.), *Non-traditional feed sources for use in swine production* (pp. 91–101). Boston, MA: Butterworths.

Tontisirin, K., Nantel, G., & Bhattacharjee, L. (2002). Food-based strategies to meet the challenges of micronutrient malnutrition in the developing world. *Proceedings of the Nutrition Society, 61*, 243–250. http://dx.doi.org/10.1079/PNS2002155

Wobeto, C., Corrêa, A. D., Abreu C. M., Santos, C. D., & Pereira H. V. (2007). Antinutrients in the cassava (*Manihot esculenta* crantz) leaf powder at three ages of the plant. *Food Science and Technology (Campinas), 27*, 6 p.

Wogan, G. N., & Marletta, M. A. (1993). Componentes perjudiciales o potencialmente perjudiciales de los alimentos. In O. R. Fennema, Química de los alimentos, (2nd ed., pp. 775–811). Zaragoza: Acribia.