

Nutritional Quality of Enriched Bitter-Cassava Flour Porridge (*Manihot esculenta***) for Feeding Infants and Young Children (6-23 months) in South Sudan**

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ABSTRACT

Micronutrient deficiencies affect 40-60% of infants (6-11 months) and 20-40% of young children (12-23 months) in developing countries, including South Sudan, where 25% are vitamin A deficient and many others lack iron. These deficiencies are partly due to diets low in vitamin A and iron, such as those based on cassava, especially bitter high-cyanide varieties. This study aimed to improve the nutritional quality of bittercassava flour "*Lenga Tome*" for feeding of infants and young children (6-23 months). Four porridge composites were formulated with varying ratios of cassava flour, spinach, carrots, and green grams. The nutritional contribution of these composites to iron and provitamin A was evaluated. Results showed that the enriched flour had significantly low cyanide content (<10 ppm), high contributions to the recommended dietary allowances for iron and provitamin A, and increased bioavailability of these nutrients. The iron contribution ranged from 98.08-199.26%, while provitamin A contribution ranged from 78.24-340.44%. The study concludes that the enriched cassava-based porridge composites, with their low cyanide content and high bioavailability of key micronutrients, could be used to substitute use of plain bitter-cassava in child feeding and help alleviate iron and vitamin A deficiencies among infants and young children in South Sudan.

Keywords: Bioavailability, Iron, Provitamin A, Biter cassava "*Lenga Tome*".

INTRODUCTION

Iron (Fe) and vitamin A deficiencies (VAD) impair about 40-60% and 20-40% of infants and young children (IYC) 6-23months in developing countries respectively [1,2]. VAD affects about 25% of children under five [3], coupled with high iron deficiency (ID) prevalence in South Sudan [4]. ID in infancy and early childhood is associated with neurocognitive, motor, and behavioral defects [5] and several features such as xeropthalmia and impaired resistance to infections [6]. One of the contributing factors of ID and VAD in IYC is consumption of foods low in Fe and provitamin A (PvA),

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including cassava roots [7].

Globally, cassava is ranked third largest source of carbohydrate [8], feeding over 500 million people in tropical Africa [9]. In South Sudan alone, it is the most widely cultivated crop, covering 21% of the major food production, with a consumption rate of 98% especially in Equatoria [10] where it is a staple food for the people of Kajo-Keji [11]. The root contains majorly carbohydrate (20–31%), moisture (60–65%) with low contents of vitamins and minerals [12]. More so, it provides less protein (1-5%), of low quality due to low levels of essential amino acids (lysine and leucine), and Sulphur-containing amino acids (methionine and cysteine) [13]. Nevertheless, the root is rich in calcium, vitamin C, thiamine, riboflavin, nicotinic acid; amylopectin (70%) and amylose (20%) with digestibility of over 75% [13].

Like some other foodstuffs, cassava contains antinutrients including phytates, tannins and cyanide [14]. Of particular concern are cyanogenic glucosides (linamarin and lotaustralin) whose contents are less (< 100mg/kg) in sweet varieties but high (100-500mg/kg) in the bitter ones [15]. On hydrolysis, these cyanides release hydrogen cyanide (HCN), which is toxic [16], a concern in the feeding of IYC. The acute toxicity of HCN retards growth in children by damaging the tissues of the central nervous system [17], manifesting in form of neurological symptoms including paralysis [18], that reduces productivity in adulthood. High doses weaken synthesis of thyroid hormones and compete for iodine uptake by the glands, reducing its absorption [19]. This triggers iodine deficiency which at high level impairs mental ability and increases mortality rate [20].

In the olden days, in Kajo-Keji, the dry cassava chips used to be blended with other staple grains such as finger millet (*Eleusine coracana*) and sorghum (*Sorghum bicolor*) and ground, which solved the problem of low protein and micronutrient content in the flour. To date, these grains have faced production and supply challenges which has affected their availability. Production has reduced due to high labor requirements, limited processing facilities and poor marketing infrastructure, among others [21]. These same factors have made the grains very expensive, doubling the cost of cassava [22]. As a result, the entire population has no choice but to consume plain cassava even in use as complementary foods.

Families that feed on plain cassava, & lack protein from animal source, seeds and nuts are at risk of Severe Acute Malnutrition (SAM) [23]. Despite advances made to enrich cassava for example, through biofortification with vitamin A, such practices have not yet penetrated South Sudan. The country relies on industrially fortified flours from Uganda, Kenya and North Sudan. Over-reliance on such imports has inherent nutritional risks in the long run [24], besides coming with costs attached to purchase of premixes, making it unsustainable to the local population. There is need to adopt more sustainable methods of using the locally available enhancers, which are culturally acceptable by the populations. The current study thus, aimed at enriching cassava flour of the IBV "*Lenga Tome*" with green gram, spinach and carrot powders to establish the ability of the formulation to provide for Fe and PvA in amounts close to the RDA for IYC (6-23months).

MATERIALS AND METHODS

Raw materials

The raw materials included cassava (*Manihot esculenta*– Crantz) roots, green grams (*Vigna radiata*), spinach (*Spinacia oleracia*), and carrots (*Deuces carota*). Inclusion of these ingredients was based on the literature that they contain substantial quantities of Fe, PvA, protein and energy [25, 26, 27]. Besides, these ingredients are abundant in South Sudan [28] but have not been utilized in the formulation of complementary foods for IYCF.

The freshly harvested cassava (*M.esculenta* – Crantz) roots, spinach (*S.oleracia*) leaves, carrots (*D.carota*) and the dry green grams (*V.radiata*) were procured from South Sudan. About 25kg of dry green grams were purchased from Wudu market-Kajo-Keji County. About 15kg each of spinach and carrots were purchased from a farmer at Muludyang, Likamerok boma-Kajo-Keji County, while 50kg of the roots of IBV cassava "*Lenga Tome*", known for its high cyanide content [29], were purchased from a farmer at Kudaji, Longira boma-Kajo-Keji County.

Preparation of raw materials

The green grams were processed into flour following the method of Puranik and others [30]. The grains were sorted, washed, soaked in water for eight hours, germinated for 24hours, dried in oven at 65oC for 5hours, dehulled, cleaned, milled with a high-speed blender (1800W, Model: YT-6198) and sieved through a 500µm sieve (B.S. 410) to form a clean flour.

The spinach leaves were processed into powder following the method of Gupta and others [31] with slight modifications. The leaves were sorted, washed, steam blanched, dried in an oven (Model: HDN225ELAD200HYD, SR 96L002, Widnes Cheshire, England) at 65oC for 7hours, milled using a HighSpeed Blender (1800W, Model: YT-6198) and sieved through a 500µm sieve (B.S. 410), packed in clean dry polyethylene bags and sealed till use.

The carrots were processed into flour according to the method of Reyes De Corcuerose and others [32]. The roots were peeled, grated, steam blanched at 71oC for 4minutes, and soaked in water for 15minutes to stop further cooking. They were dried for 4days in a solar dryer (constructed using plain wire, gauge 10 for roofing and a black dampproof membrane (gauge 100) covering the entire dryer). The dried samples were milled into flour using a High-Speed Blender (1800W, Model: YT-6198), sieved through a 500µm sieve (B.S. 410), packed in clean dry polyethylene bags and sealed till use.

The roots of the IBV "*Lenga Tome*" were washed, peeled,

soaked in clean tap water for three days, grated using a greater (Kitchen ware series, 100% high quality), squeezed using clean cloth, sun dried for three days and milled into flour using a high speed blender (1800W, Model: YT-6198) and sieved through a 500µm sieve (B.S. 410) to make clean flour and stored in sealed dry polyethylene bags till use.

Formulation of the porridge composites

A generative concept 4-Ed creative software, version 8.01.01 was used to formulate composites ensuring appreciable percentage contribution for energy, protein, Fe and PvA to the RDA of IYC (6-23months) as indicated (Table 1). The treatment levels were adjusted on the software ensuring the intended factors (nutrients) corresponded to the ones derived from the literature including USDA National Nutrient Database for Standard Reference [33,34].

Table 1. The expected nutrient composition (% contribution) of the composites to the RDAs for iron, PvA, energy and protein per two servings in a day as predicted by concept 4-Ed creative software

Composites	Ingredient (levels)				Nutrient composition (expected % contribution)								
					Energy (kcal)		Protein (%)			Iron $(mg/100g)$		PvA $(\mu$ g/100g)	
					Age (Months)		Age (Months)			Age (Months)		Age (Months)	
	Cassava (%)	Green gram (%)	Spinach (%)	Carrot (%)	$06-11$	$12 - 23$	06-08	$09-11$	$12 - 23$	$06-11$	$12 - 23$	$06-11$	$12 - 23$
F ₃	40	25	5	30	32.66	29.69	12.74	9.56	11.74	11.02	17.67	47.5	71.23
F ₄	55	30	10	5	39.37	35.79	15.75	11.81	14.51	12.85	20.6	17.21	25.81
F8	30	30	15	25	33.14	30.13	15.45	11.59	14.23	13.55	21.72	50.25	75.38
F ₉	25	25	25	25	28.69	26.05	13.75	10.3	12.65	12.69	20.34	59.96	89.93
* CoN	100	$\bf{0}$	$\mathbf{0}$	$\mathbf{0}$	32	29.09	2.59	1.94	2.39	1.45	2.33	0.17	0.25

**Control (No ingredients added; only cassava flour)*

Preparation of the porridge composites

The composites were prepared following WHO [34], with modifications. The choice for porridge was influenced by its likelihood to be accepted since similar foods are fed to IYC. For preparation, 2.5 heaped table spoons (tbs) of each composite (25g), was prepared in 146.20mL of water, making a thick porridge of 125mL for 6-11months old, then, five (5) heaped tbs of each porridge composites (equivalent to 50g), were prepared in 292.40mL of water making a thick porridge of 250mL for 12-23 months old. These measured 0.25 of the South Sudanese gamma cup, an equivalent of 0.38 of the apiliga cup (the South Sudanese common cup for measurement), and 0.5 gamma cup, an equivalent of 0.75 of apiliga cup; respectively. Intake was measured for two servings per day (Table 2).

For nutritional quality and safety of the porridge composites, the following were done;

Determination of cyanogenic glucosides (cyanides)

Both flours of the control and laboratory processed (fermented) cassava roots were analyzed for cyanide content following Essers [35] with modifications from Piero and others [36].

Determination of percentage contribution of porridge composites to energy, protein, iron and PvA

The percentage contribution of the porridge composites for the nutrients was calculated based on the RDA of the 375µgRE of vitamin A for infants (6-11months) and 400µgRE for children of 12-23 months [34]; 9.3mg of iron for infants (6-11months) and 11.6mg for children of 12-23 months; age group serving portions of 125mL of porridge for infants (6-11months) and 250mL for children of 12-23months [37]; the nutrient densities predicted by the software, except energy which was calculated based on general Atwater factors [38] as; Energy (Kcal) = $(4$ Kcal/g protein \times g protein + (9 Kcal/g fat × g fat + (4 Kcal/g carbohydrate × g carbohydrates.

Determination of total iron content

Total iron content was determined by the open wet digestion and spectroscopic methods using Genesys 2 UV-VIS Spectrometer, model TM2 [39].

Determination of phytate content

Phytate content was determined following Abulude [40] and Adeolu [41]. The results were multiplied by a factor of 1.95 to obtain Phytin phosphorus and then by a factor of 3.55, to convert into phytate.

Determination of inhibitory effect of phytate on iron bioavailability

The molar ratio of phytate: mineral iron (P: Fe) was used to determine the bioavailability of the mineral Fe. The inhibitory effect of phytate on bioavailability of the mineral iron was determined following Shimi and Hasnah [42] , with modifications recommended by Hallberg et al.[43].

Determination and quantification of carotenoids and β-carotene (provitamin A)

The total carotenoids were extracted using acetone and petroleum ether following Darwin et al. [44], with modifications. Water was used instead of NaCl because water and acetone are able to mix chemically. Carotenoids were quantified for β-carotene using a HPLC system (Agilent technologies 1200 series, Waldbronn, Germany) on dry weight basis. Quantity was determined by integrating the peak area against the standard curve prepared from a known concentration of all- trans-β-carotene. Validation was done based on the requirement of Thompson et al.[45] to obtain a linearity of 0.99925 at 2, 6, 10, 14, 18, 25 and 30 ppm of levels and three replicates to ensure accuracy.

Determination of provitamin A (PvA) content

Using the quantified content of β-carotene above, the PvA content was calculated based on the concept of the retinol equivalence (RE) of the joint FAO/WHO46 consultation experts on human minerals as;

Determination of PvA bioavailability of porridge composites

The PvA bioavailability was determined by the amount of β-carotene quantified from HPLC assay. The β-carotene μ g/g) was converted to RE following FAO/WHO [46]

conversion factors for the ingested PvA carotenoids to that of retinol. The value of the RE was converted to μmol/L (the standard SI unit of serum retinol) and compared with the threshold (0.06 μmol/L), which is the limit of detection of provitamin A (LODPvA). The comparison of the LODPvA (PvA bioavailability) value to that of the current study was to deduce if β-carotene (PvA) is bioavailable in the formulations with a limit $\geq 0.06 \mu$ mol/L. The LODPvA determines the adequacy of β-carotene or PvA bioavailability [47].

RESULTS

Cyanide content of flours of control and fermented bitter cassava roots of "*Lenga Tome***"**

Results of cyanide content of flours of the control and fermented (laboratory processed) of "*Lenga Tome*" are presented in Fig. 1. There was significant reduction (P<0.05) in cyanide content of the fermented roots flour compared to the control flour. The mean cyanide content of the unfermented cassava roots flour was 15.51ppm while that of the fermented roots was about 4.38ppm, implying that laboratory processing reduced cyanide by about 70%.

Figure 1. Cyanide content of flours of fermented (lab processed) and unfermented roots of cassava "*Lenga Tome*" (CoN) and of the laboratory processed ones (Treatment).

Percentage contribution of porridge composites to the RDA for iron, PvA, Protein and energy for IYC (6-23months)

All the composites apart from F4 (the sample that had highest cassava content compared to the counterparts), contributed highly to the RDAs of infants in both age groups. Results (Table 3), indicated that, two servings of 125mL of porridge could contribute up to 78.24-181.57% of PvA (RE)

while 250mL of it could contribute up to 146.71-340.44% of PvA (RE). These same amounts could contribute about 98.08-124.27% and 157.27-199.26% of iron to the RDAs of infants of 6-11months and children of 12-23months respectively; about 59.38-93.30%, 44.54-69.98%, and 54.69-85.94% of protein requirements for infants 6-8, 9-11 and children of 12-23months respectively; 22.44-26.08% of energy requirement for infants (6-11months) and 20.41- 23.71% for children 12-23months.

Table 3. The percentage contribution of porridge composites from bitter cassava "*Lenga Tome*" to the RDA of energy, protein, iron and provitamin A for IYC (6-23months)

The values given are means ± Stand deviations of data from triplicate samples. Values in the same columns with the same superscript

were not significantly different (P>0.05). The % contribution of the porridge composites of the bitter cassava "Lenga Tome" were

calculated per two servings; 25g and 50g of composites for infants (6-11 months) and children (12-23 months) respectively according to FAO, (2007); WHO, (2000) guidelines on family foods for breastfed children

Phytate content of the porridge composites and the control

Results of the phytate content are presented in Figure 2. The phytate content was in the range of 19.85-26.54mg/100g

while the control had 11.63 mg/100g. There was no significant difference (P>0.05) in the phytate content among the composites. However, the control samples had the least phytate content of about 11.63mg/100g and, significantly (P<0.05) lower than any values of the composites.

Figure 2. Phytate content of the porridge composites from bitter cassava "*Lenga Tome*"

Total iron content of the porridge composites and the control

in F9. There was no significant difference (P>0.05) observed between composites F9 and F4, while composites F3 and F8 differed significantly (P<0.05). As expected, the iron content of the control was significantly lower than any of the composites, with a value of about 31.69mg/100g of cassava.

The composites exhibited varying total iron content as indicated (Figure 3), the highest value being 46.23mg/100g

Figure 3. Iron content of the porridge composites and control from bitter cassava "*Lenga Tome*".

The inhibitory effect of phytate on iron bioavailability of porridge composites and control

The molar ratio of phytate to the mineral iron (P: Fe) in the composites, ranged from 0.04 - 0.06 (Table 4). The molar

ration of F3 and F9 was not significantly different (P>0.05), whereas that of F4 and F8 differed significantly. The efficacy of bioavailability increased with decrease (percent) of spinach and green grams.

The values given are the means ± Stand deviations of data from triplicate samples. Values in the same columns with the same superscript are not significantly different (P>0.05). Mole ratio, P: Fe ≥1 reveals poor bioavailability of iron in the porridge composite flours.

β-carotene content of porridge composites

The results of β-carotene content are presented (Figure 4). The results indicate that β-carotene of the composites was in the range of 70.27-163.09µg/g. The β-carotene

value of F4 was significantly (P<0.05) lower than of others. However, there were no significant differences (P>0.05) in the β-carotene content observed among the other porridge composites. The control sample (plain cassava flour) did not have β-carotene hence the values are not presented.

Figure 4. The β-carotene of porridge composites.

The Provitamin A (PvA) content of porridge composites The results of the mean PvA content are presented (Figure 5). PvA content varied from 11.74 - 27.70µg/g RE. The PvA

content of F4 was significantly lower (P<0.05) than that of F3, F8 and F9. However, the PvA contents of F3, F8 and F9 were not significantly different (P>0.05).

Figure 5. The Provitamin A (PvA) content of the porridge composites.

PvA bioavailability of porridge composites and the control

The results of bioavailability of PvA are presented (Table

5). Composite F3, F8 and F9 exhibited appreciably high bioavailability (LODPvA) with no significant difference (P>0.05) compared to F4. The control sample showed no bioavailability.

Table 5. Provitamin A bioavailability(LODPvA) of porridge composites

Values given are means ± Stand deviations of data from triplicate samples. Values in the same columns with the same superscript were not significantly different (P>0.05). Porridge composite with LODPvA<0.06µmoles/L has poor bioavailability of PvA (Manssens & Escobar, 2015)

DISCUSSION

The cyanide content of fermented roots was within the safe levels of the <10 ppm [48]. These results were in agreement with the findings of Iwuoha et al. [49] in their study on detoxification effect of fermentation on cyanide content. This therefore confirms that fermentation is an effective method for detoxifying cassava roots for human consumption hence recommended for processing the bitter cassava roots of the "*Lenga Tome*" intended for making porridge for IYC (6-23months).

All the composites apart from F4 (the sample that had highest cassava content) contributed highly to the RDAs of infants of both age categories based on Wang et al. [50]. Therefore, when an infant of age 6-11months and a child of 12-23months consume 125mL and 250mL of porridge; respectively, it would lead to a percentage contribution to their RDAs indicated in Table 3.

Overall, the phytate content (Figure 3) was low compared to the values (40-78.80mg/100g) reported by Ayele et al. [51] on the wheat supplement enriched with cassava and soybean flours. The difference could be attributed to the type of ingredients used in the formulations. Lowering antinutritional factors increase the bioavailability of iron since phytate has been implicated in making nutrients unavailable [52]. However, the control samples had the least phytate content (Figure 3) and, which was significantly (P<0.05) lower than other values of the composites, implying that "*Lenga Tome*" generally have low levels of phytates.

Although the control sample presented lowest Fe content, the value was higher than what is reported so far in the literature (1.11mg/100g and 0.01 mg/100g) for local bitter cassava varieties [53]. The difference could be due to environmental variations, soil properties, the ability of the mineral uptake by the plant species and differences in the varieties [54]. The current study therefore reveals that, "*Lenga Tome*" has appreciable amounts of Fe, making it a better choice for use in product development for IYCF than other varieties. The Fe content of the porridge composites compared favourably well to the values (6.19-8.10mg/100g) reported for cassavabased composite crackers developed as supplements for primary school children [55].

The mean molar ratio of phytate to the mineral iron (P: Fe), which defines the bioavailability of mineral iron ranged from 0.04-0.06. The ratios were below the critical value (P: Fe >1) set by Hallberg et al, [43]. It can therefore be concluded that the porridge composites of "*Lenga Tome*" contain Fe which is free of the inhibitory effect of the phytate hence is bioavailable though at varying levels. The efficacy of bioavailability increased with percent decrease of spinach and green grams, indicating that spinach and green grams possess high content of phytate.

The results of β-carotene content compare favorably well to the content (0.40-1.60µg/g) of β-carotene reported so far [56] in cassava flakes (gari) produced from yellow cassava varieties. The difference could be from the methods used in processing of the ingredients and recipe formulation. Among the composites, F8 presented significantly (P<0.05) higher β-carotene content compared to other formulations. There was no significant difference (P>0.05) observed among the other porridge composites however, F4 presented extremely low content, possibly due to the lower level of carrots and spinach flour in the composite (5% and 10% respectively). The control experiment (plain cassava flour) did not indicate any β-carotene content which concurs with the report on dried fermented plain cassava flour [57]. Indeed, IYC who receive their complementary food from plain cassava flour are deprived of this precursor of PvA. That is why enrichment of cassava flour with ingredients that are rich in this pigment is necessary.

Apart from composite F4, whose bioavailability (LODPvA) was low certainly due to its low levels of spinach and carrot flours [58] and that of control (100% plain cassava flour), the LODPvA in composites F3, F8 and F9 compared favourably well to the threshold (0.10µmoles/L) designed for the assessment of vitamin A status in foods [59]. The bioavailability values were also above the limit of detection (< 0.06 µmoles/L) of PvA in the Caco-2 cell lines [60], implying that the nutrient is bioavailable upon consumption of the porridge made from the composites. The porridges (except that from F4) are expected to contribute highly to the recommended nutrient density of the plant-based diet of the 500µgRE [46], making them suitable for IYCF.

CONCLUSION

This study shows that nutritious food (porridge composites) can be formulated from bitter cassava "*Lenga Tome*" (M. esculenta – Crantz), spinach, carrots and green grams. Majority of the composites in the study showed good limits of PvA and Fe bioavailability and high percentage contributions, implying that blending ratios of a given food has influence on the nutritional quality of the products. Fermentation by soaking is strongly recommended as a means of reducing the cyanide content in "*Lenga Tome*". The justification of this study generally lies in the application of a low-cost technology in the enhancement of the nutritional composition of the commonly grown bitter cassava varieties, using available and culturally acceptable crops. Considering the difficult conditions in South Sudan, these crops can be used as ingredients for reducing under nutrition especially of Fe and vitamin A among infants and young children in the South Sudanese communities consuming better cassava.

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CONFLICT OF INTEREST

All authors have no conflict of interest.

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