ENRICHMENT OF FLOUR OF INDIGENOUS BITTER CASSAVA “LENGA TOME”
FOR FEEDING INFANTS AND YOUNG CHILDREN (6-23 MONTHS) IN KAJO-KEJI
COUNTY- SOUTH SUDAN

BY

BOJO OPENZI KONYO

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Declaration

I Bojo Openzi Konyo declare that the work presented here is from my own research and has not been published or submitted for any other academic award in any University or Institution of higher learning.

Signed .................................................................

BOJO OPENZI KONYO Researcher

Date .................................................................

This dissertation has been submitted with the approval of the following supervisors;

Signed .................................................................

Dr. HEDWIG AČHAM (PhD)

Department of food Technology and Nutrition, Makerere University.

Date .................................................................

Prof. ARCHILEO N. KAAYA (PhD)

Department of Food Technology and Nutrition, Makerere University.

Signed .................................................................

Date .................................................................
Dedication

This work is dedicated to my parents, beloved late father, Pompilio Konyo Mogga and my mother Mary Konga Kwoji who set up the foundation of my education.
Acknowledgment

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<td>Infants and Young Children</td>
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<tr>
<td>BM</td>
<td>Breast Milk</td>
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<td>SAM</td>
<td>Severe Acute Malnutrition</td>
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<tr>
<td>MAM</td>
<td>Moderate acute malnutrition</td>
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<td>RDA</td>
<td>Recommended Dietary Allowance</td>
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<td>TUL</td>
<td>Tolerable upper limit</td>
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<td>CFs</td>
<td>Complementary Foods</td>
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<td>PA</td>
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<td>Pp</td>
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<td>TCC</td>
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<td>CRBPii</td>
<td>Cellular Retinoic acid Binding Protein II</td>
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<tr>
<td>RE</td>
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</tr>
<tr>
<td>FD</td>
<td>Freeze- Dryer</td>
</tr>
<tr>
<td>SD</td>
<td>Solar- Dryer</td>
</tr>
<tr>
<td>MSD</td>
<td>Modified Solar Dryer</td>
</tr>
<tr>
<td>BDPM</td>
<td>Black Damp-Proof Membrane</td>
</tr>
<tr>
<td>WDPM</td>
<td>White Damp-Proof Membrane</td>
</tr>
<tr>
<td>SnD</td>
<td>Sun Dryer</td>
</tr>
<tr>
<td>OvD</td>
<td>Oven Dryer</td>
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<tr>
<td>GAM</td>
<td>Global Acute Malnutrition</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>FAO</td>
<td>Food and Agricultural Organization</td>
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<tr>
<td>PvA</td>
<td>Provitamin A</td>
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<td>CBCFs</td>
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<tr>
<td>CoN</td>
<td>Control</td>
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<td>NIRS</td>
<td>Near-Infrared Spectroscopy</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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<tr>
<td>RVA</td>
<td>Rapid Viscos Analyzer</td>
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<tr>
<td>Cgls</td>
<td>Cyanogenic glucosides</td>
</tr>
<tr>
<td>MOE</td>
<td>Margin of exposure</td>
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<tr>
<td>BMDL</td>
<td>Benchmark dose lower limit</td>
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<tr>
<td>NaCRRI</td>
<td>National crops resources research institute</td>
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<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
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ABSTRACT
Cassava (*Manihot esculenta* - Crantz) is a root crop that feeds over 500 million people in the tropics though is poor in protein, vitamins and minerals. This study sought to enrich cassava flour of the South Sudanese indigenous bitter variety (IBV) “Lenga Tome” from Kajo-keji County for feeding IYC (6-23 months). A batch of cassava roots was heaped and the other one soaked to ferment. Their flours were analyzed for cyanide content. Composites F3, F4, F8 and F9 were enriched in the ratio of soak fermented cassava flour: Spinach: Carrot: Green grams as 40:5:30:25%, 55:10:05:30%, 30:15:25:30%, 25:25:25:25% respectively. The control was 100% plain flour of the unfermented roots. Porridges were made from the composites and rated for their acceptability using a 9-point hedonic scale from 1 (disliked extremely) to 9 (liked extremely). The % contribution of composites for nutrients was estimated per two servings of 125ml of porridge for infants (6-11 months) and 250ml for children (12-23 months). Composites were quantified by HPLC for Provitamin A (PvA) to estimate the Limit of Detection of Provitamin A (LODPvA) or bioavailability based on Retinol Equivalence (RE) factor. Composites were digested in 2% HCl for Phytate (P) and in conc. HNO3 and H2SO4 for iron (Fe) content. Mole ratio P: Fe was used to estimate Fe bioavailability. Soak fermented flours had significantly low (P<0.05) cyanide (4.38ppm). Composite F3 was qualified for making nutrient dense porridge (CBNDP) for the IYC in the community. The % contribution of Freeze Dried (FD) composites to RDA of Fe for infants (6-11 months) was 98.1-124.3% mg/100g and 157.3-199.0% mg/100g for children (12-23 months). The % contribution of FD composites for PvA was 266.9-434.7% RE for infants (6-11months) and 500.4-815.1% RE for children (12-23months). The Modified Solar Dried (MSD) composites contributed 78.3-181.6% RE of PvA for infants (6-11months) and 146.7-340.5% RE of PvA for children (12-23months). The LODPvA was significantly high (P<0.05) in all composites (0.1-0.2 µmol/L) except for F4 (0.0 µmol/L) of the MSD. The P: Fe ratio was significantly (P<0.05) as low as 0.0-0.6. The high acceptability of the porridge, its high % contribution for iron and provitamin A to the RDA for IYC (6-23months) and bioavailability of the nutrients predict reduction of iron and vitamin A deficiencies in Kajo-Keji, South Sudan as a whole and other parts of the world that will have access to the product.

**Key words:** Composites, bioavailability, % contribution, Provitamin A, iron, complementary food (CF), Infants and young children (IYC)
CHAPTER ONE: INTRODUCTION

1.1. BACKGROUND

Cassava \textit{(Manihot esculenta - Crantz)} is a tuberous root crop that feeds over 500 million people around the tropics (Ogunnaike \textit{et al.}, 2015). It is particularly preferred because it yields well under marginal soil conditions, and is tolerant to drought (Kolawole & Ayodeji, 2007; Adeniji, 2013). The crop is the third largest source of carbohydrates for human nutrition in the world especially in Africa (Kombate \textit{et al.}, 2017). In South Sudan, it is the most widely grown crop, covering 20.8\% of the major food crops and consumed by 98\% of the households in Equatoria as a daily diet (Ntawuruhunga \textit{et al.}, 2007).

The crop contains majorly carbohydrate (20–31\%), moisture (60–65\%) and has low content of vitamins and minerals (Makanjuola \textit{et al.}, 2012). On the other hand, the crop is rich in calcium, vitamin C, thiamine, riboflavin, nicotinic acid; amylopectin (70\%) and amylose (20\%) with digestibility of over 75\%. Nevertheless, the root provides less protein (1-5\%), whose nutritional value is further reduced by its low levels of the essential amino acids such as lysine and leucine, and sulphur-containing amino acids such as methionine and cysteine (Stupak, 2008).

Like other crops, cassava contains antinutrients including phytates, tannins and cyanides (Oresegun \textit{et al.}, 2016). Of particular concern are the cyanides or cyanogenic glucosides (linamarin and lotaustralin) whose contents lie < 100mg/kg in sweet varieties, and found ranging from 100-500mg/kg in the bitter ones (Ezeigbo \textit{et al.}, 2015). On hydrolysis, the cyanides release hydrogen cyanide (HCN), the toxin (Jaszczak \textit{et al.}, 2017). Cyanides in cassava is of concern in feeding Infants and Young Children (IYC). The acute toxicity of HCN retards growth in children as it damages the tissues of their central nervous system (Bolarinwa \textit{et al.}, 2016). This manifests into neurological symptoms such as paralysis (Nhassico \textit{et al.}, 2008), reducing their productivity in adulthood. Also at high doses, HCN weakens the synthesis of thyroid hormones and competes for iodine uptake by the glands, reducing its absorption (Worki \textit{et al.}, 2017). This triggers iodine
deficiency where at its high level impairs mental ability and increases mortality rate among IYC (Chuot et al., 2014).

Traditionally, at the household, the dry cassava chips are used to be blended with other staple grains such as finger millet (Eleusine coracana) and sorghum (Sorghum bicolor) in Kajo-Keji since times past. However, today, such grains have faced supply challenges and the dwindled production has affected their availability and profitability. Farmers have reduced their production due to associated costs including high labor requirement, limited processing facilities, poor marketing infrastructure, among others (Mitaru et al., 2006). On the other hand, these same factors have also made the grains very expensive, doubling the cost of cassava (FAO/WFP, 2012). As a result, the entire population has been left with no choice other than resorting into consuming plain cassava flour and using it as complementary food for infant and young child feeding (IYCF).

Families that feed on plain cassava, in the absence of animal proteins, seeds and nuts are prone to Severe Acute Malnutrition (SAM) (Muhimbula & Issa-zacharia, 2010). Also, relaying on this food as staple as for the case of Kajo-Keji (Ajak & Kursat, 2017) predisposes children to cases associated with zinc, iron, and vitamin A deficiencies (Gegios et al., 2010). One in seven children suffer from vitamin A deficiency in the county, coupled with wide spread cases of anemia due to inadequate consumption of vitamin A and iron reach foods, lack of iron and vitamin A supplements in health units and pharmacies (Frankenberger et al., 2007 ; Mija-tesse, 2010).

Advances have been made to enrich cassava through biofortification for example with vitamin A. However, such a practice is not being explored in South Sudan. The African pilot studies invented feasibility of fortification of local foodstuff, but there is cost attached to buying of premixes. The South Sudanese rural populations should therefor adopt to the use of local but nutrient rich enhancers like cassava leaves, sweet potato leaves, spinach (Spinacia oleracia), amaranth leaves, soybean, cowpea, green gram (Vigna radiata), grain amaranth and other roots such as carrots (Daucus carota) to attain nutrition security. The WHO, (2018) study indicated a feasibility of fortifying wheat flour with vitamins B_{12}, Vitamin A, folic acid, iron and zinc to curb on their deficiencies. However, this is lucking in South Sudan, the country depends on readily fortified flours imported from Uganda, Kenya and North Sudan. Over reliance on such imports has inherent nutritional risks and is certainly unsuitable in the long run (Ebert, 2014). Therefore, the current
study sought to enrich cassava flour with green grams (*V.radiata*), Spinach (*S.oleracia*) and Carrot (*D.carota*) to enhance the dietary intake of iron, vitamin A, protein and energy for IYC (6-23 months) in the rural settings.
1.2. Problem statement

The food security situation is precarious in South Sudan; 60% of the population do not consume sufficient foods that provide nutritious diet, of which 47% of the population gain a daily average of 1318 kcal, which is fewer than the RDA of 1717 kcal per person/day for the country (WFP, 2012). Vitamin A deficiency affects about 25% of children under five (Mater et al., 2016); Vitamin A deficiency in children ≥20% is a state of emergency (WHO, 2010). Cases of anemia are also wide spread due to inadequate consumption of iron rich foods, and due to malaria and helminthic infection (Bayoumi et al., 2016). Among other forms, micronutrient malnutrition has led into high Global Acute Malnutrition (GAM) rate of 23%, leaving 31% of children under five stunted, 23% wasted and 28% under weighed in the Country (South Sudan MoH, 2018). Kajo-keji contributes a lower GAM rate of about 5.5% to the national average (OCHA, 2016). However, GAM rate of 5-9% plus aggravating factors, defined by food ration below the mean energy, protein and fat requirement is an indication of risk (alert), which calls for targeted supplementary feeding program for the malnourished individuals in the population (Reginald et al., 2014). Micronutrient deficiencies and growth faltering adversely affect children under five especially infants after introduction of complementary foods (Amagloh et al., 2012). Food blending is a promising strategy of combating micronutrient malnutrition among vulnerable groups (Jain, 2013). In the scarcity of finger millet (Eleusine coracana) and sorghum (Sorghum bicolor), due to their growing disappearance, micronutrient rich ingredients such as Green gram (V.radiata) with 7.3mg/100g iron, 94 I.U/100g of Vitamin A and protein of 24.0% (Shital & Patil, 2013); Spinach (S.oleracia) with 60-90mg/100g of iron and 9420 I.U vitamin A (Sharma et al., 2013; Tewani et al., 2016); and Carrot (D.carota) with 2805 I.U of Vitamin A and 1.667mg/100g Iron (Olalude et al., 2015) which are highly grown and consumed in Kajo-Keji by other age groups, could be used for blending with cassava to adequately meet the requirements of the 9.3mg/day of iron for infants 6-11 months and the 11.6mg/day of iron for the children 12-23 months (Brannon & Taylor, 2017); and could as well cater for the 375µg RE/day of vitamin A for infants 6-11 months and 400µg RE/day for the children 12-23 months (WHO, 2004b). This Research seeks to counteract the problem of the low protein, iron and vitamin A in high cassava consuming areas by incorporating V. radiata, S.oleracia, and D.carota into the cassava flour, making it suitable for feeding infant and young children.
1.3. General objective of the study

To enhance the nutritive value of cassava flour of the indigenous bitter variety (IBV) “Lenga Tome” for feeding infant and young children (6-23 months) in Kajo-Keji County.

1.4. Specific Objectives

1. To develop cassava based composite flours from IBV “Lenga Tome” by incorporating green grams, spinach & carrots for feeding infants and young children (6-23months)
2. To assess the most suitable composites of the IBV “Lenga Tome” for making nutrient dense porridge for feeding infants and young children (6-23months)
3. To target composites of the IBV “Lenga Tome” that deliver appropriate percentages of iron and provitamin A to the recommended dietary allowances (RDA) for infants and young children (6-23 months)
4. To assess the bioavailability of iron and provitamin A in the cassava based composite flours of the IBV, “Lenga Tome”

1.5. Hypothesis

1. Cassava based composite flours developed from the indigenous bitter variety (IBV) “Lenga Tome”, green grams, spinach and carrots are suitable for feeding infants and young children (6-23 months)
2. Cassava based composite flours of the IBV “Lenga Tome” are suitable for making nutrient dense porridge for feeding infants and young children (6-23 months)
3. Cassava based composite flours of the IBV “Lenga Tome” can deliver appropriate percentages of iron and provitamin A to the recommended dietary allowance (RDA) for infants and young children (6-23 months)
4. Iron and provitamin A are bioavailable in the cassava based composite flours of the IBV “Lenga Tome”
1.6. Justification for the study

This study was proposed to prevent the malnutrition rate (GAM) of 5.5% in Kajo-keji (OCHA, 2016) from escalating further since GAM rate of 5-9% plus aggravating factors, defined by food ration below the mean energy, protein and fat requirement is an indication of risk which requires intervention (Reginald et al., 2014).

The common economic activity of the people of Kajo-Keji is Agriculture, they grow food crops such as maize, beans, grand nuts, green grams, cabbages, spinach, tomatoes, onions and carrots including Simsims and sunflowers as cash crops with cassava being the staple food crop (Ajak & Kursat, 2017). About 65% of the farmers grow cassava on large scale in Kajo-keji compared to its native counties of Morobo and Yei (22% and 14% ) respectively (Abt Associates Inc., 2013). Populations that depend on cassava as staple food are at risk of inadequate dietary protein intake with children facing a number of consequences including stunting, wasting, underweight and compromise in their body immune system (Stephenson et al., 2010).

When cassava is blended with green gram (V. radiata), spinach (S.oleracia) and carrot (D. carota), we expect improvement in the dietary intake of provitamin A, iron, protein and energy of the rural poor especially infants and young children. We also expect increase in production of these food crops and provision of capacity to deliver the most needed products in a cost-effective manner, reinforcing the benefits that sustainably improve nutrition.
2.1. Nutritive value of cassava

The nutritive value of cassava root especially the normal white variety is hampered by its low protein and micronutrient content, more especially β-carotene, making it unsuitable for use as complementary food (Onabanjo et al., 2008; Ugwu, 2009). The biofortified cassava varieties are yet to yield tangible results especially in the East Africa region though more conventional breeding schemes are being employed. More effort is still needed because many small scale farmers in the rural communities still have negative attitude towards adapting such improved cassava varieties due to lack of awareness (Salum, 2016). Furthermore, the improved cassava varieties still have low coverage in the communities because they are still scarce. In South Sudan, the only two improved cassava varieties (TME 14 and NASE 14) imported from Uganda and from other International Institute of Tropical Agriculture (IITA) lines are still limited to a few farmers’ associations (Abt Associates Inc., 2013). Additionally, the preference of the local bitter varieties due to their attributes of fast maturing, production of quality flours and resistance to drought and pest as the case of “Lenga Tome”; the bitter variety in Kajo-keji, is a limiting factor towards adapting the newly introduced varieties.

The local bitter varieties are reported to have associated with a number of anti-nutritional factors including cyanogenic glucoside (Cgl) or cyanogen (Table 2.1). Among these anti-nutritional factors, the Cgl has the most devastating effect to humans because it is lost in small percentage (25.3%) after fermentation as compared to phytate, tannin and oxalate of 77%, 96.7% and 67.85% respectively (Ojha et al., 2017). The Cgl exists as 93% linamarin (α-hydroxyisobutyryl-3-onitrile-β-D-glucopyranoside) and 7% lotaustralin (methyl-linamarin) which are β-glucosides of acetone cyanohydrin and ethyl-methyl cyanohydriins respectively (Salvador, 2015; Haque & Bradbury, 2004). The total cyanide content of such β-glucosides ranges from 1-5050 mg hydrogen cyanide (HCN) equivalence/kg (ppm) in the root parenchyma and 900-2000 mg HCN equivalence/kg (ppm) in the root cortex (peel) of fresh material of all cassava varieties (Burns et al., 2012).

HCN is toxic to human and has affinity to bind with mineral ions (Fe²⁺, Mn³⁺and Cu²⁺) hence inhibiting their utilization (Essack et al., 2017). Also, this mineral ions are functional groups of
many enzymes including cytochrome oxidase (Salvador, 2015); in excess, HCN forms complex with the heme ion \(\text{Fe}^{2+}\) of the cytochrome oxidase, it reacts well with such ions after binding with protein to form a stable cytochrome oxidase-heme complex, which in turns inhibits protein from being utilized (Cooper & Guy, 2008) hence restraining the enzyme from carrying out its function of oxidative phosphorylation during Krebs cycle where oxygen is utilized to generate the essential energy source in form of adenosine triphosphate (ATP) (Bhattacharya, 2000) which causes distress in the entire human system (Essack et al., 2017).

Dietary exposure to cyanide toxicity impairs early child neurocognitive development even in absence of its clear clinical symptoms such as paralysis (Kashala et al., 2018). These medical conditions are more prevalent in populations that depend on monotonous dietary consumption of insufficiently processed roots of bitter cassava, coupled with protein deficiency in sulphur containing amino acids (Nzwalo & Cliff, 2011) that are reported responsible of converting the HCN to urinary excretable thiocyanide through a sulphur-dependent rhodanese-mediated detoxification pathway in the human body (Tshala et al., 2013).

Many methods of removing HCN in cassava are being employed including wet and solid-state fermentation which involve soaking/grating and heaping of the cassava roots respectively (Nwokoro, 2011). Soaking was reported to reduce HCN by 94.7\% (8.45mg/kg) (Iwuoha et al., 2013) under the influence of \textit{Corynobacterium manihot}, \textit{Lactobacillus plantarum} and \textit{Leuconostoc mesenteroides} and \textit{Klebsiella aerogenes} in the cassava root that produce organic acids, including Dehydro acetic acid in the process which causes retting (softening) of the root hence inducing leaching of the HCN into the water (Iwuoha et al., 2013; Guira et al, 2016; Ogunnaike et al., 2015). Grating the root tissue of cassava was also reported to causing significant loss of HCN by 81.3\% (28.70mg/kg), as it exposes the active cites of the substrate (Linamarin), bringing it into contact with the linamarase enzyme to act upon, enabling it to disintegrate to yield cyanohydrin which then spontaneously dissociates to HCN at pH of $\geq$7 (Attah et al., 2013; Orjiekwe et al., 2013) Orjiekwe et al., 2013; Iwuoha et al., 2013); (Figure 1).

Study by Tivana et al., 2007 also reported reduction in HCN by 17 mg/kg during solid-state (heap) fermentation though was associated with hurdles caused by mold growth which produce mycotoxins particularly penicilllic acid (25-184µg/kg) and Aflatoxin B$_1$ (AFB$_1$) (6-194µg/kg) in cassava products (Njumbe et al., 2014). These mycotoxins are heat stable and pose deleterious
effect on the central nervous, pulmonary, cardiovascular, and digestive system depending on the type and amount of toxin ingested. They also impair body immune response and growth in children (Adjovi et al., 2015). In high doses, aflatoxin especially AFB\textsubscript{1} causes acute liver cancer and cirrhosis, leading into death of both human and animals (Ogodo & Ugbo, 2016; Wu et al., 2014). As reported by Sowley, (2016), there has been no data that quantify the health effect of aflatoxin in human to enforce standards for aflatoxin exposure to benefit the rural poor, however, according to WHO, (2002), as cited by Murashiki et al., (2017), the tolerable upper limit of aflatoxin B\textsubscript{1} is 2µg/kg body weight/day and 4 µg/kg body weight/day total aflatoxin (B1, B2, G1 and G2) (Sowley, 2016). Its acutely toxic and lethal dose (of AFB\textsubscript{1}) in human ranges from 20-120 µg/kg body weight/day when consumed over a period of 1-3 weeks. Staple food which is contaminated with aflatoxin of 1mg/kg or above is also associated with acute aflatoxicosis (FAO/WHO, 2017).

To stay at a safer site of food contamination of aflatoxin, the risk characterization of aflatoxin should be based on margin of exposure (MOE) calculated from its benchmark dose lower limit (BMDL) of 170ng/kg body weight/day divided by the toxin exposed (Adetunji et al., 2017). The MOE of aflatoxin for infants and young children is 0.12 and 0.3µg/kg body weight/day respectively. Increased dietary diversity and improved drying practices is invented as an effective avenue of reducing toxins and increasing dietary intake of nutrients that counteract the toxicity of aflatoxin and HCN in food (Wu et al., 2014).

The standard dietary safe level of HCN in cassava flour varies according to the national legislation of a given country, like in Indonesia, the acceptable limit of total cyanide in cassava flour is 40 ppm (Cardosoa et al., 2005). However, the World Health Organization WHO, (2004) had set a safe limit of 10ppm total cyanide in cassava flour and has been adapted in New Zealand and Australia (FSANZ, 2009). Many other countries including South Sudan are yet to adapt recommended limit of HCN in cassava or cassava based products especially of the bitter varieties (FAO/WHO, 2013). The acceptable international codex standard of sweet cassava variety is being set at 50 ppm already (FAO/WHO, 2013). These safe limits of cyanide would protect dietary exposure to cyanide toxicity. However, it should not be neglected that the toxic effect of cyanide on human body also depends on a number of other factors including body size, health status of the individual, the dose of the cyanide consumed, and the time over which it is ingested (Burns et al.,
The extreme harmful dose of HCN for human is being established ranging from 0.5-3.5 mg/kg body weight (Klaus et al., 2016).

Obueh & Kolawole, (2016) reported the proximate composition of sweet and bitter cassava varieties, of which, the bitter one presented low moisture, crude protein, fat, crude fibre and ash contents except for reducing sugar (Table 2.2), the mineral composition had varying proportions though was of low amount in both varieties (Table 2.3). In terms of essential amino acids, the bitter varieties were also registered with the lowest levels (Table 2.4).

The high levels of anti-nutrients including cyanogenic glucoside, deficiency in mineral, protein and amino acids coupled with the high contents of calories qualify cassava as a driver of malnutrition especially in infants and young children (Salvador et al., 2014; (NEACŞU, 2014). However, according to Noorfarahzilah et al., (2014), blending food with legume provides protein-reached product with improved and well balanced amino acid profile. Also, (Joko et al., 2016) reported reduction of HCN to 2.50mg/kg in fortified flour compared to plain flours of the native (7.75mg/kg) and modified (3.88mg/kg) cassava.

![Enzymatic hydrolysis of linamarin](image)

**Figure 2.1 Enzymatic hydrolysis of linamarin; Source (Orjiekwe et al., 2013)**
Table 2.1. Anti-nutritional factors in sweet and bitter cassava varieties.

<table>
<thead>
<tr>
<th>Anti-nutrient</th>
<th>Cassava variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sweet</td>
</tr>
<tr>
<td>Hydrogen cyanide (mg HCN/100g)</td>
<td>7.58</td>
</tr>
<tr>
<td>Oxalate (mg/100g)</td>
<td>1.30</td>
</tr>
<tr>
<td>Tannin (%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Phytate (mg/100g)</td>
<td>53.70</td>
</tr>
<tr>
<td>Alkaloid (mg/100g)</td>
<td>0.27</td>
</tr>
<tr>
<td>Lignin (mg/100g)</td>
<td>0.13</td>
</tr>
<tr>
<td>Saponin (%)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

The values circled in red are of the anti-nutrient that was identified to cause more anti-nutritional effect to the infants and young children; (IYC 6-23 months) hence was of concern in this study. Source (Obueh & Kolawole, 2016)

Table 2.2. Proximate composition of sweet and bitter cassava varieties

<table>
<thead>
<tr>
<th>Proximate (%)</th>
<th>Cassava variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sweet</td>
</tr>
<tr>
<td>Moisture</td>
<td>8.04</td>
</tr>
<tr>
<td>Crude protein</td>
<td>23.52</td>
</tr>
<tr>
<td>Fat</td>
<td>10.01</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>5.66</td>
</tr>
<tr>
<td>Ash</td>
<td>0.44</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>33.79</td>
</tr>
</tbody>
</table>

Source (Obueh & Kolawole, 2016)
### Table 2.3. Mineral composition of sweet and bitter cassava varieties

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Sweet (mg/l)</th>
<th>Sweet (mg/100g)</th>
<th>Bitter (mg/l)</th>
<th>Bitter (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (Ca)</td>
<td>224.57</td>
<td>22.457</td>
<td>187.53</td>
<td>18.753</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>35.17</td>
<td>3.517</td>
<td>28.67</td>
<td>2.867</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>259.64</td>
<td>25.964</td>
<td>338.66</td>
<td>33.866</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>319.87</td>
<td>31.987</td>
<td>413.96</td>
<td>41.396</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>593.73</td>
<td>59.373</td>
<td>402.37</td>
<td>40.237</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>75.49</td>
<td>7.549</td>
<td>124.55</td>
<td>12.455</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>20.85</td>
<td>2.085</td>
<td>16.84</td>
<td>1.684</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>3.31</td>
<td>0.331</td>
<td>3.97</td>
<td>0.397</td>
</tr>
<tr>
<td>Aluminium (Al)</td>
<td>1.78</td>
<td>0.178</td>
<td>2.04</td>
<td>0.204</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>0.83</td>
<td>0.0829</td>
<td>1.05</td>
<td>0.105</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>3.96</td>
<td>0.3959</td>
<td>3.71</td>
<td>0.371</td>
</tr>
</tbody>
</table>

*The values circled in red are of the nutrient of public health concern for infants and young children; IYC (6-23 months) that had been targeted in this study. Source (Obueh & Kolawole, 2016)*

### Table 2.4 Amino Acid profile of sweet and bitter cassava varieties

<table>
<thead>
<tr>
<th>Amino acid g/100g</th>
<th>Sweet</th>
<th>Bitter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>12.75</td>
<td>8.27</td>
</tr>
<tr>
<td>Histidine</td>
<td>3.57</td>
<td>1.87</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.15</td>
<td>1.85</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.73</td>
<td>0.92</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.15</td>
<td>1.72</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.39</td>
<td>1.97</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.11</td>
<td>4.24</td>
</tr>
<tr>
<td>Leucine</td>
<td>18.70</td>
<td>16.71</td>
</tr>
<tr>
<td>Valine</td>
<td>13.90</td>
<td>8.72</td>
</tr>
</tbody>
</table>

*Source (Obueh & Kolawole, 2016)*
2.2. Malnutrition and its consequences in infants and young children

Malnutrition kills about 2.6 million children each year, million more survive, but suffer lifelong physical and cognitive impairment because they did not meet the nutrients they deserve early in life (Save the Children, 2012). This accounts for about 7.3% of the global disease burden with iron and vitamin A ranking among the top 15 leading causes of the global disease burden, affecting 90% of the food-insecure, poor and vulnerable people in developing countries (Wimalawansa, 2013). Most of the cases of Severe Acute Malnutrition (SAM) in children are being associated with lack of breastfeeding practices, lower household dietary diversity scores, lower consumption of fish, dairy products, seeds and nuts, fresh fruits as well as green leafy vegetables (Alou et al., 2017).

Such cases will persist unless the “nutrition sensitive” agricultural concept is practiced along the whole food chain, including promoting home gardening with special consideration to indigenous crops since most of them provide adequate micronutrients especially provitamin A, vitamin C and iron (Keding et al., 2013). Most of such indigenous foods are perishable hence need appropriate processing methods such as drying to ensure constant supply during period of scarcity (Rensburg et al., 2004). Under nutrition during complementary feeding is also attributed to inappropriate consistency (food is too thin or too thick); too few essential amino acids; and too few calories among non-breast feed infants. These children have high nutrient needs to support growth and development especially the breast feed infants who consume relatively small amount of food other than BM; they need a nutrient dense complementary foods (CFs).

2.3. Complementary foods and guidelines

The period of transition from exclusive breast feeding to consuming a variety of family foods in addition to breast milk is regarded the period of complementary feeding particularly between (6-23 months) of age (Mburu et al., 2011). This 18- month interval covers the largest part of the 1000 days, from pregnancy to the first 2 years after birth, now considered as the critical window of opportunity for preventing under nutrition and its long term effect Arabena et al., (2015). It is also referred to as the “sensitive period” for the development of healthy habits of eating where the
infant discovers variety of foods and flavors (Cosmi et al., 2017). Such foods and liquids along breast milk (BM) are introduced because the BM alone is no longer sufficient to meet the nutritional requirements of infants (WHO, 2000). In this stage, most of the infants have reached a neurological stage of development (stage where they are able to chew, swallow, digest and excrete the food). Is also during this stage that distinct growth faltering and major cases of stunting occur, coupled with high incidence of infection which increases nutritional needs (Dahie & Heyle, 2017). A diversified health diet including high quality fortified nutrient dense foods in the period of complementary feeding is required to meet nutrient requirement to reduce stunting (Bloem et al., 2013).

WHO, (2009) recommended 600 kcal and 700 kcal of energy for healthy breastfeed infants of 6-8 and 9-11 months respectively and 900 kcal of energy for the young children 12-23 months per day, of which, CFs should provide energy of 200 kcal for 6-8 months, 300 kcal for 9-11 months and 550 kcal for the 12-23 months per day (Figure 2.2a). The protein requirements for infants 6-8 and 9-11 months is 9.1g and 9.6g per day respectively and 10.9g for children 12-23 months per day; CFs should provide 1.9g (21%) of protein for infants 6-8 months, 4.0g (42%) for infants 9-11 and 6.2g (57%) for the children 12-23 months per day. The protein requirement is often met when the energy intake is sufficient enough except if there is predominant consumption of foods that are low in protein such as sweet potato and cassava (Acheng, 2014).

The WHO, (2004b) of the United Nations, as cited by Brannon & Taylor, (2017) recommended 9.3 and 11.6 mg of iron for infants 6-11 and children 12-23 months, 375µg RE and 400µg RE of vitamin A for infants 6-11 and children 12-23 months respectively. The total dietary intake needed from CFs should however provide 97% of iron, 86% of zinc, 81% of phosphorus, 76% of magnesium, 73% of sodium and 72% of calcium during 9-11 (Dewey, 2001). When enough food is consumed to satisfy energy needs, the need for protein (or other nutrient) will be fulfilled if the ratio of protein (or of other nutrient) to energy is appropriate (WHO, 2007).

The contribution from BM to the overall nutrient intake is remarkable, in a well-nourished mother, it contains generous amount of Vitamin A, B, C, folate, Iodine and Selenium. However, iron and zinc concentrations are very low relative to need, and since the average energy intake from CFs is lowest for infants 6-8 months (200 kcal per day), the target nutrient densities from CFs for this age
group tend to be highest for example 4.5 mg iron/100 kcal and 1.14 mg Zn/100 kcal. On the other hand, the target nutrient densities from CFs for infants 9-11 months is low because the average expected energy intake from CFs increases to about 300 kcal/day, while the need for iron at 12-23 months is lowest and for zinc is constant because expected energy intake increases further to 550 kcal/day (Dewey, 2013b); Figure 2.2b. Therefore, it is more challenging to meet the micronutrient needs during the second six month of life of a breast-fed infants. Thus, at households, infants should be provided with the most nutrient-dense foods, though the reverse is true always, especially in low-income countries where they are usually fed with nutrient-poor, watery porridges (Dewey, 2013a). Infants from 6-8 months should feed on fairly thick porridge in addition to mashed foods, 9-11 months should feed consistently thick porridge with chopped/mashed foods, and children 12-23 months should continue with consistently thick porridge and chopped/mashed foods (FAO, 2007; WHO, 2009) (Table 2.5).

2.2(a) 2.2(b)

Figure 2. 2 (a). Energy required by age from complementary food and 2.2(b). Gap to fill by complementary foods for a breastfed child 12-23 months; Source (WHO, 2009).
<table>
<thead>
<tr>
<th>Age group (months)</th>
<th>Texture of complementary food (porridge)</th>
<th>Daily meal or complementary food (porridge)</th>
<th>Quantity of flour for one meal</th>
<th>Quantity of cooked food (porridge) per serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-8</td>
<td>Start with fairly thick porridge in addition to mashed foods</td>
<td>2-3 meals / day plus frequent breastfeeding</td>
<td>Starting with 2-3 heaped tablespoons with gradual increase to 1/2, then to 3/4 cup</td>
<td>1/2 - 3/4 of the cup</td>
</tr>
<tr>
<td>9-11</td>
<td>Give consistently thick porridge with chopped/mashed foods</td>
<td>3-4 meals / day plus breast feeding, depending on the infant’s appetite. 1-2 snacks could be offered between meals</td>
<td>3-4 heaped tablespoons</td>
<td>3/4 to 1 cup</td>
</tr>
<tr>
<td>12-23</td>
<td>continue with consistently thick porridge and chopped/mashed foods</td>
<td>3-4 meals/day plus breast feeding, depending on the child’s appetite. 1-2 snacks could also be offered between meals</td>
<td>5-6 heaped tablespoons</td>
<td>1-1 1/4 cup of consistently thick</td>
</tr>
</tbody>
</table>

To prepare porridges of recommended thickness, the following measurements should be noted: 1 cup of composite is prepared in 200ml of water; 1 tablespoonful of flour is equals to 10g of flour; 3 tablespoons of flour are equal to 1/3 cup; Source (FAO, 2007; WHO, 2009).
2.3.1. Complementary food developed from cereals

The adequacy of complementary diet to meet the IYCs’ RDA depends on the nutrient content of the ingredients used in the formulation of such a complementary food (Onabanjo et al., 2008). The best way to ascertain essential nutrients in sufficient amount is by using culturally accepted food that are affordable in the community and blended with nutrients that are commonly missing in the diet (Wimalawansa, 2013). In low income countries, the diet of the infants and young children is usually dominated by Cereals-based porridge with low nutrient density and poor mineral bioavailability (K. G. Dewey, 2013a). In this scenario, the child has to consume a large volume to meet the nutrients and energy requirement yet the amount of food that a child consumes depends on the capacity of the child’s stomach which is usually 30ml /kg of the child’s body weight (WHO, 2009). The bulkiness of the cereal based diet can therefore be a limiting factor.

If the infant consumes a typical amount of porridge; 100-200 kcal/day, the amount of other nutrient-rich foods that can be consumed are limited since the total energy needs from CFs are only 200-550kcal/ day. Cereal flour also contains relatively little protein with an average of about 10-12 % dry weight and lacks essential amino acids such as lysine and threonine (Shewry & Halford, 2002 ; Garcia Del Moral et al., 2017). Lysine has antiviral properties thus enables buildup of body immune system and threonine involves in maintenance process such as intestinal mucus renewal and immune protein synthesis (Boyvin et al., 2017). Also such flours are poor in dietary source of both Vitamin A and bioavailable iron (Fadzelly, 2014; Srivastava et al., 2015).

2.3.2. Cereal-legume blended complementary foods

Consuming complementary food from lysine and threonine-rich foods such as legumes is essential for effective early nutritional management of children (Noorfarahzilah, M. et al., 2014). However, legumes are also deficient in sulphur containing amino acids like methionine and cysteine that are incorporated into protein to provide defence against reactive oxygen and nitrogen species such as hydroxyl radical (OH), Hydrogen peroxide (H2O2), Superoxide (O2−), Nitric oxide (NO−), Peroxynitrite (ONOO−) that are toxic to the human body (Mukwevho et al., 2014).
Even the cereal-legume based diets are of intense concern in regards to bioavailability of iron (Fe\(^{2+}/^{3+}\)), Zinc (Zn\(^{2+}\)), and Calcium (Ca\(^{2+}\)) due to presence of high content of phytate (myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate; InsP\(_6\) ) in this food which bind these minerals hence limiting their absorption by the child (Kumar et al., 2010). The negatively charged phytatic salts have very low solubility under the pH condition of the upper gastrointestinal tract where absorption of the minerals take place. They tend to precipitate with increasing pH along the intestine forming insoluble aggregate in the insoluble precipitate in the pH range of 2.5-8.0. The solubility of the inositol phosphate and bioavailability of the minerals, decreases as the level of phosphorylation increases (Troesch, B., Jing, H., Arnaud, L., & F., 2013) (Troesch et al., 2013).

The high content of phytate in the cereals and legumes also limits the bioavailability of phosphorus, because 80% of it in such diets is bound up in the phytate (its storage form for the plant) which is excreted unabsorbed (Nielsen et al., 2013). Such concern has been neglected by many nutrition researchers since there is no simple biomarker of phosphorus deficiency. However, it is a key constituent of body tissues, its inadequate dietary intake can limit the deposition of lean body mass of the IYC hence limiting their growth (Dewey, 2013).

The inhibitory effect of the phytate on the cereals and legumes can be reduced by soaking and germination to activate the endogenous Phytases that are specific for the metabolic break down of Phytate (Afify et al., 2011). The Phytases hydrolyze the phosphate esters in the phytate, releasing phosphorus and therefore making it available for absorption while rendering the antinutritional chelating effect of the phytate vestigial. This in turns, releases the chelated cations (McKie & Barry, 2016). Beside reduction in the phytate, Chingakham et al., (2015) also reported increase in dietary quality of protein from 9-12\%, and decrease in trypsin inhibitor activity by 28-55\% along increase in in-vitro protein digestibility by 8-20\% after soaking and germinating cowpeas for three and 24 hours at 25\(^{0}\)C respectively.

Efforts are being made to study the relationship between the contents of phytate in a meal and the extent to which it inhibits iron absorption or bioavailability in human. One of the pioneer work was done by Hallberg et al., (1989) on two types of wheat rolls A and B. Roll A had no phytate and B was assigned a known amount of phytate but all were fortified with 4.1mg of iron (FeSO\(_4\)) and labelled with isotopes 55Fe and 59Fe respectively. Roll A and B were served with water
(150ml) to the subjects on alternate morning after an overnight fasts on four consecutive days in the order BAAB or ABBA. Subjects were tested for their blood two weeks after the last roll was served to determine the concentration of $^{55}\text{Fe}$ and $^{59}\text{Fe}$. The total retention of $^{59}\text{Fe}$ was measured by the whole body counting at the same time whereas the total retention of $^{55}\text{Fe}$ was calculated from the ratio $^{55}\text{Fe}$: $^{59}\text{Fe}$ in the red blood cells. A solution containing 10 ml of HCl/L, 3mg of iron (FeSO$_4$) and 30mg ascorbic acid labelled with $^{59}\text{Fe}$ was used as reference in the entire study. The reference solution was rinsed twice with water and served to the subjects each with two doses of 55.5 kBq $^{59}\text{Fe}$ on two consecutive morning after overnight fasts. The iron absorption was measured as a relative absorption of the two tracers ($^{55}\text{Fe}$ and $^{59}\text{Fe}$) in the blood samples of the subjects and as an absolute absorption of the whole body counting $^{59}\text{Fe}$. The subjects were not allowed to eat any food or drink any water for the next 3 hours after the reference dose. Now, the ratio of A: R of absorption of non heme iron from meal (A) to that of meal (R) of the reference dose is the expression of the bioavailability of non heme iron in the meal. In their study, the decrease in iron absorption was marked as more phytate was added. The decrease in iron absorption was already significant when 2mg of phytate was added and the rate of decrease was marked at a lower dose level of 2-10mg phytate. From 10-250mg phytate, the rate of decrease of iron absorption was lower but strongly significant. The 10mg dose level was found corresponding to a ratio of 1mole phytate: 1mole iron (P: Fe). This ratio is being used today as a threshold or critical value in plant based diets by many nutrition researchers to estimate the bioavailability of Fe, where P: Fe > 1 indicates poor bioavailability (Lazarte et al., 2015).

In a study of single meal diet, Lim et al., (2013) pointed similar results that 2mg and 250mg of phytate reduced iron absorption by 18% and 82% respectively. The method seem more promising in a single meals than in composite meals because there might be some factors that interact with the phytate. However, in the previous study by Hallberg et al., (1989), 25ml of phytate was found inhibiting iron with the same magnitude whether or no meat was present, meaning, the inhibition would be of the same magnitude in composite meals. Therefore the critical value would be useful in the context of the current study because the phytate to iron (P: Fe) ratio is being used for the purpose of determining iron bioavailability (Yankey et al., 2011).
2.3.3. Root-based complementary food and its nutritive value

Roots or tuber based complementary diets are known of having lower phytic content (3-20%) compared to cereal-legume base diets (Amagloh et al., 2012). Based on the fact that the roots of cassava (Manihot esculenta – Crantz) like of the potato (Solanium tuberosum) and yam (Dioscorea species) have sufficient quantities of ascorbic acid; 15-45mg/100g (Montagnac et al., 2009 ; Ukpabi et al., 2014), this cassava based complementary food would be a diet of relatively low phytate, though low protein and minerals (Iron, Zinc and Vitamin A) and potential toxicity of cassava are other challenges (Gegios et al., 2010 ; Burns et al., 2010). Ascorbic acid is a famous enhancer of iron uptake (Tatiana et al., 2015), it has the property of reducing the insoluble Fe$^{3+}$ into the soluble Fe$^{2+}$ hence reducing its capacity of forming insoluble complexes with phytates or tannins making it more available for absorption (Nielsen et al., 2013). However, iron a times exceeds the tolerable upper limit (TUL) of the 45mg/day in adults and 40mg/day in children (Gallicchio, 2014).

Children under five are particularly prone to iron toxicity and of other accidental poisoning because of their activity level, curiosity and oral phase of development (Baranwal & Singhi, 2003). In case of overdose, iron corrodes organs such as gastrointestinal tract, heart, liver, lungs, and kidneys leading to acute hemorrhagic gastric, excessive fluid loss, bleeding and shock (Yosier et al., 2018). Large doses of iron also saturates the iron-binding protein such as Cytochrome oxidase (Kotze et al., 2009), causing electron shunt which uncouples oxidative phosphorylation leading to anaerobic metabolism and thus metabolic acidosis (Baranwal & Singhi, 2003). When serum iron level exceeds the iron binding capacity of the body, the unbound Fe$^{2+}$ induces production of reactive oxygen species such as ·OH, H$_2$O$_2$, and O$_2^-$ in the cell (Esparza et al., 2015), leading to lipid peroxidation and cellular damage (El-Beltagi & Mohamed, 2013; Brannon & Taylor, 2017). However, such effect is minimal in cassava based product because the cells of cassava roots have the affinity of synthesizing antioxidants such as Superoxide dismutase, catalase and peroxidases to trap, mop and inhibit the actions of such highly reactive oxygen and nitrogen species (Ndidi & Akeem, 2011).

Nevertheless, cassava has about 57 protein digestibility corrected amino acid score, one of the lowest on any staple foods, with lysine and leucine being the limiting amino acid (Stephenson et al., 2015).
This justifies that feeding children on monotonous cassava diet deprives them from acquiring adequate dietary protein with essential amino acids which subsequently impair their growth (Millward, 1999).

2.4. Beta carotene bioconversion and bioavailability in human body

Vitamin A is the essential nutrient that enhances general growth, maintenance of visual function, regulation of differentiation of epithelial tissues, immunity and embryonic development (Tang et al., 2012). It is derived from diet either as preformed Vitamin A; retinyl esters, retinol and retinoic acid from animal source or as provitamin A carotenoid mainly β-carotene, α-carotene, and β-cryptoxanthin of which β-carotene exhibits the major provitamin A constituent of carotenoids containing foods found in fruits and vegetables (Spiegler et al., 2012).

In developed countries, provitamin A carotenoid accounts for < 30% of daily vitamin A intake whereas the preformed vitamin A accounts for >70% vitamin A intake per day; in developing countries, the reverse is true, provitamin A carotenoids in fruits and vegetables account for > 70% vitamin A intake per day (Dias, 2013). Although widely consumed, carotenoids are not readily solubilized in the digestive fluid due to their high hydrophobicity of C₄₀ isoperenoid carbon skeleton (Kotake-Nara & Nagao, 2011). The high fibre content of fruits (16%) and vegetables (30-40%) also offers resistance to enzymatic digestion (Dhingra et al., 2012), reducing carotenoid bioavailability by 33-43% (Haskell, 2012). Their release from the food matrix for human accessibility is hampered by other foods especially row vegetables because of the hard structure of the plant cell wall. They undergo several steps before uptake by the intestinal epithelial cells. However, processing techniques that disrupt their matrixes such as mild cooking (blanching) and homogenization can accelerate their release and bioavailability (D’Evoli et al., 2013). However, the level of absorption (β-carotene) differs according to the specific type of food as was 5-26% for spinach, 7-65% for carrots and 12% for broccolli (Haskell, 2012; Grune et al., 2010). Its bioconversion (β-carotene) in human body takes place mainly in the intestine, Figure 2.3. When taken into the enterocyte, it is converted to retinal (retinaldehyde) by the action of β-carotene 15, 15’monooxygenase (BCMO1), the retinal formed is then converted to retinol and subsequently to retinol esters which either binds to the cellular retinoic acid binding protein (CRBP11) or
incorporated completely with dietary fats and cholesterol into chylomicrons and carried (secreted) to the lymphatic system then into duodenum (Lindqvist et al., 2007; Ambrosio et al., 2011).

The ratio of β-carotene consumed to the vitamin A amount derived from β-carotene dose denotes the β-carotene-to-Vitamin A conversion factor or β-carotene equivalence of vitamin A (Lin et al., 2000). The vitamin A activity of the carotenoid in the diet is expressed as retinol equivalent of 1µg of retinol which equals to 1 Retinol Equivalent (RE) ; 1µg β-carotene equals to 0.167µg RE and 1µg of other provitamin A carotenoids is equal to 0.084µg RE (FAO/WHO, 2001).

It is quite challenging to study β-carotene conversion and absorption as vitamin A in human at physiologic doses because of the complexities that usually occur during labelling the β-carotene in plants and measuring the recovered content in the feces (Haskell, 2012). Some of these challenges are associated with the human genetic characteristic. Individuals who possess heterozygous mutant gene T170M of BCMO1 in their bodies do have high level of serum β-carotene (14.8µmole/L) due to abnormality of conversion of β-carotene to retinol by the enzyme (Ambrosio et al., 2011). The β-carotene content remains high in their bodies though they consumed foods that are deficient in the pigment. However, a study by Tang, (2010) reported success in measuring the conversion efficiency of carotenoids in a food based intervention study using paired Deuterated- retinol Dilution (DRD) test on children with marginal to normal vitamin A status who were fed on green-yellow and light colored vegetables. The results before and after showed stability in vitamin A body stores in children fed on green-yellow vegetables. Also Manssens & Escobar, (2015) have made study of β-carotene (provitamin A) bioavailability feasible by detecting the limit of β-carotene in the Caco-2 cell line after feeding it with a mixture of Dulbecco’s Modified Eagle’s medium and 4.5g/L glucose and Glutamax. The medium was supplemented with fetal bovine serum (10%), non-essential amino acids solution (1%) and penicillin-streptomycin (1%) to further the cell growth and prevent it from contamination. The Caco-2 cells were grown on a semi-permeable membrane of 0.4µm polyester to allow movement of the compounds including the β-carotene. The growth and permeability of the cells were monitored and evaluated by Trans Epithelial Electrical Resistance (TEER) and Lucifer yellow assay methods. The Caoc-2 cells were seeded at an initial concentration of about 7.5 × 10⁴ cells/cm² and the transport experiment accomplished in 21 days. Thereafter, the Caco-2 cell monolayers were washed twice
with fetal bovine serum (FBS) and 10% ethanolic FBS added twice to dissociate the cell monolayers. The cell medium culture and the cells were then extracted and quantified by HPLC. A calibration curve was acquired by extracting a known volume of β-carotene in the cell culture medium. The limit of quantification (LOQ) and limit of detection (LOD) of β-carotene in the cell culture medium was found at a level of 0.6 μmol/L (0.5µg/ml) and 0.06 μmol/L (0.05 µg/ml) respectively. The LOD of β-carotene or provitamin A is the range that determines the adequacy of β-carotene or provitamin A bioavailability (Zhu et al, 2006). Meaning, a plant based diet with beta carotene with critical value or limit of detection < 0.06 μmol/L indicates poor provitamin A bioavailability. Therefore, in the current study, it is prudent to quantify the carotenoids for β-carotene (provitamin A) and converted to μmol/L using the concept of the retinol equivalence (RE) introduced by the joint FAO/WHO, (2001) expert group on human vitamin and mineral requirement if it is bioavailable with a score of ≥ 0.06 µmole/L suitable for IYCF.

Figure 2.3. General scheme for the uptake and bioconversion of dietary carotenoids in human; Source (Ambrosio et al, 2011)
2.5. Body immune response to under and over consumption of preformed retinol and provitamin A, and the recommendations

The body immune system and its components are equally sensitive to both excess and deficient minerals and vitamins (Mahassni & Nahla, 2013). Inadequate dietary intake of vitamin A (preformed retinol) manifests into clinical vitamin A deficiency characterized by several ocular features such as Xerophthalmia and general impaired resistance to infection. Other than that, it has also been discovered that there exists an association between serum retinol and biochemical indicators of iron nutrition. A study by (Mejia, 1992) revealed that hematological values are positively associated with vitamin A deficiency, as was assessed by conjunctival impression cytology (CIC) in Micronesian children who were also found suffering from PEM at the same time. In that study, children with abnormal CIC had significantly lower hematocrit than did children with normal cytology. On the other hand, Edem, (2010) discovered that iron deficiency impairs mobilization of liver retinol. However, in a study of influence of iron on vitamin A nutritional status on Mexican children, supplementation with iron was associated with significant increase in retinoic binding protein by 5.4mg/L, transthyretin by 33mg/L and serum retinol by 0.27µmol/L (Oliveira et al., 2008).

The distribution of serum retinol values in the population together with the prevalence of individuals with serum retinal values below a certain cut-off point reveals a significant information about vitamin A status of a given population (NCEH/DLS, 2012). Serum retinol concentration ≤ 0.70 µmol/L (200µg/L) define public health problems involving vitamin A deficiency among children (6-71 months) as mild (2-9%), moderate (10-19%), or severe (≥ 20%). Then Population with serum retinol status of 0.75 ≤ 1.05µmol/L (300µg/L) in children indicates adequacy of the retinol (WHO, 2011). The WHO, (2004b) of the United Nations in a join expert consultation with FAO, recommended vitamin A intake of 375µg RE/day for infants (6-11 months) and 400µg RE/day for children (12-59 months ) as the sufficient and safer doses. However, Intakes a times exceed the RDA, or even the upper limit (600 µg RE/day) especially in non-breastfed infants Vidailheta et al., (2016), and 10000 μg RE/day in young children (EVM, 2003).

High doses of preformed vitamin A or retinoid derivatives can cause hypervitaminosis A which results into complications associated with embryonic malformation, reduced born mineral density
and increased risk for heap fracture, Osteoporosis, exostosis, and growth retardation in children (Blomhoff et al., 2003). However, hypervitaminosis A does not occur after increases intake of provitamin A carotenoids such as β-carotene. Its conversion to retinol is feedback regulated, it decreases when adequate vitamin A has been reached (Conaway et al., 2013). However, effect of supplementation of diet with 3mg/day of provitamin A is being reported by Haskell, (2012) to decrease intestinal β-carotene conversion to vitamin A in human.

2.6. Effect of drying fruits and vegetables on β-carotene retention and bioavailability

The drying method used for a given food material determines the quality of the product (Asekun et al., 2006). The content of β-carotene and its bioavailability is influenced by thermal processing (blanching and drying) of vegetable. Blanching stabilizes the β-carotene by inactivating the enzymes peroxidase and lipoxidase that are heat stable to act for the entire dehydration process until the substrate becomes inert to any catalytic activity (Nurhuda et al., 2013), while the application of the dry heat is to remove off the water from the ingredient (Urbonaviciene et al., 2012).

The chemical conversion of β-carotene during drying is somewhat complicated, however, the rate constant of the reaction depends on the temperature, concentration of the reactants and water activity of the drying material (Driscoll, 2014). The water serves as solvent for the chemicals of nutritional significance present in the food (Vaclavik & Christian, 2014). Removing the water increases the chemical concentration which the nutrient loss depends upon, the nutrient content therefore increases as dehydration progresses (Goula et al., 2010). The commonly used drying methods in food industry are freeze drying (FD) and Oven drying (OvD), which are tailored towards specific ingredient, the availability of the dryer itself, the cost of dehydration and the targeted nutrient in the product (Sagar & Kumar, 2010). For fruits and vegetable, the most preferred one is the FD since it retains more nutrients because the drying process takes place at lower temperature compared with the other drying methods (Sarangam & Chakraborty, 2015).

Taking into consideration the high capital and running cost associated with FD because of the slow drying rate hence high energy consumption coupled with maintenance of the system (Ciurzyńska
& Lenar, 2011), sun-drying (SnD) and OvD are more frequently preferred for preserving food in developing countries (Tengweh et al., 2017). More so, OvD at 45°C and air drying preserve or improve food flavor better than freeze-dryer (Abascal et al., 2005). Also, in terms of nutrient retention, as investigated by Kiharason et al., (2017) on pumpkin (Cucurbita moschata), it was found that, in the various drying scenarios, 74.8425, 62.9875 and 27.1750µg/g of β-carotene content was retained by OvD, enhanced solar dryer (ESD) and open SnD respectively.

The above evidence was in agreement with the findings of Kiremire et al., (2010), where OvD emerged the best in terms of β-carotene retention followed by solar dryer (SD) and lastly SnD which was noted to had caused a loss of about 86.5% of the nutrient. Beta (β)-carotene is more degradable on exposure to light, other food components, oxygen and heat through oxidation, isomerization and/ or free radical formation (Pénicaud et al., 2011).

The use of SD has been encouraged to overcome the high cost and running capital of FD but the fact that most of the sensitive nutrients like β-carotene that can be lost through the light that usually penetrates the entire SD has been neglected. More so, constructing the modern SD with solar collectors, dry chambers with special materials such as visqueen is still unaffordable to the vulnerable communities in developing countries such as Kajo-keji in South Sudan (Sontakke & Sanjay, 2015). This study had therefore suggested a simple, nutrient retentive modified solar dryer (MSD) for fruits and vegetables to address these concerns.

The choice of a given drying method in the current study was also based on the knowledge behind the retention potential of a given dryer for the targeted nutrient, that is why solar dryer but of the modified type used for spinach and carrot was based on the results obtained by Chege et al., (2014) showing that iron (71.85mg/100g) and β-carotene (40.11mg/100g) were retained after solar drying of Amaranth (A.cruentus) leaves. More so, the results obtained in the experiment set by Boateng, (2013) to study the effect of drying methods on nutrient retention revealed that oven drying also retained appreciable content of iron (42% wt ) than microwave (22% wt) in Basil (Ocimum viride) leaves, meaning, both solar and oven dryers can retain iron in good contents.
3.1. Study materials

3.1.1. Study materials, their source and the reason of inclusion in the diet

The row materials involved in this study including the IBV cassava “Lenga Tome” (*Manihot esculenta*-Crantz), green grams (*V.radiata*), spinach (*Soleracia*), and Carrots (*D. carota*) were procured right from farmers within Kajo-keji County- South Sudan. This is because the level of minerals in the vegetable is usually affected by a number of factors including genetic properties of the crop species, climatic conditions and soil characteristics (Martínez-Ballesta *et al.*, 2010). The selection of these raw materials was also based on the basic knowledge of their mineral contents of the nutrients of public health concern including vitamin A, iron as well as protein and energy they contain.

Cassava is the staple food for the people of Kajo-keji, and the local bitter variety; “Lenga Tome” is preferred by the local people because of its attributes associated with fast maturing, high and good flour production, resistance to drought and pests. Therefore, the bitterness and dominance of “Lenga Tome” variety in the preparation of complementary food (porridge) for IYC (6-23 months), in spite of its low vitamin and mineral content were the triggers of its inclusion.

Green grams, spinach and the carrots were selected because they are widely grown in Kajo-Keji and other parts of South Sudan (Grosskinsky & Gullick, 2000) and not included in the complementary food for the IYC (6-23months). So they were included in this diet to promote their utilization and consumption by this age group. Also, Carrots, apart from their availability and being a good source of nutrients especially provitamin A carotenoid, were included in this diet as a colorant to mash the dark green colour of the spinach and green gram such that the composite flours look more appealing to the IYC.
3.2. Processing of the raw materials

During food processing, food materials may be combined with a number of ingredients to formulate a product which is then subject into series of unit operations either sequentially or simultaneously (Sung et al., 2014). Food processing enhances nutrient content, safety and prolongs self-life of the product (Ann et al., 2016)

3.2.1. Processing of spinach (*S.oleracea*) to flour

Processing spinach in this study was done according to the method reported by Gupta et al., (2015) with modification. The leaves were sorted and washed with clean water to remove any foreign body and other disease causing Organisms; steam blanched at 80°C for 5 minutes to inactivate enzymes especially Peroxidase and Catalase that are heat resistant to cause deterioration of the quality of the product during storage (Gupta et al., 2008; Reyes De Corcuera et al., 2004). The content was divided into two batches, one of the batches was dried in the MSD for 96 hours (4 days) and the other one was spread evenly in an Aluminum plate and dried in an oven dryer (Leader Engineering (Heat control), Model: HDN225ELAD200HYD, SR 96L002, Widnes Cheshire, England) at a temperature of 65°C for 7 hours, blended using High Speed Blender (1800W, Model: YT-6198), sieved through a 500µm sieve (B.S. 410) and packed in clean dry, air tied bags ready for use (Figure 3.1)
Figure 3.1. Processing of Spinach (S. oleracia) flour; Source: Gupta et al., (2015).

3.2.2. Processing of green grams (Vigna radiata) to flour

Processing of green grams in this study was done according to the method adopted from Puranik et al., (2011) with modification. The green grams were graded, washed and soaked in water at 22-25°C for 8 hours under ambient laboratory condition; drained and allowed to germinate under a wet cotton wool in a Petridis for 24 hours and spread in an Aluminium plate and dried in an oven at 65°C for 5 hours; dehulled to remove the antinutritional factors that are mostly located in the seed coat (hull) and to improve on the protein digestibility of the product as well as reducing its cooking time (Nakitto et al., 2015); winnowed to separate the hulls from the cotyledons, blended using High Speed Blender (1800W, Model: YT-6198), sieved through a 500µm sieve (B.S. 410) and packed in clean dry bags ready for use (Figure 3.2).
3.2.3. Processing of carrots (*D. carota*) to flour

The row carrot roots were washed, peeled and grated to a 0.3 cm³ using a stainless grater (Kitchen ware series, 100% high quality); steam blanched at 71°C for 4 minutes to modify its texture and colour, remove trapped air, shorten its drying time (Ondrej *et al*., 2017) and to inactivate enzyme such as peroxidase, lipoxidase and catalase that are more resistant to heat and would cause deterioration to the quality of the product during storage (Goula *et al*., 2010; Reyes De Corcuerose *et al*., 2004). The sample was then soaked for 15 minutes in cold water which was left overnight to cool in order to stop further cooking. The content was divided into two portions, one of it was dried for 96 hours (4 days) in the MSD and the other one dried in a freeze dryer (FD) at a
temperature of -30 to 25°C for 50 hours at a constant pressure of 0.4 mbar and were separately blended using High Speed Blender (1800W, Model: YT-6198), sieved through a 500 µm sieve (B.S. 410) and packed in clean dry air tied bags ready for use (Figure 3.3).

![Diagram of carrot processing]

**Figure 3.3. Processing of Carrots (*D. carota*) flour. Source (Reyes De Corcuerose *et al.*, 2004)**

### 3.2.4. Processing of Cassava (*Manihot esculenta – Crantz*) to flour

Cassava roots are bulky and have high perishability that, they deteriorate within three-four days (Kolawole *et al.*, 2010). They are therefore consumed either immediately or are processed into a form with better storage attributes. The unit operation employed during cassava processing is tailored towards achieving a desired final product (Kolawole, 2007). Also, the devastating effect of the cyanide plays a role towards selecting an appropriate processing method of reducing its toxicity in the root, as such, many traditional methods including fermentation (wet and solid-state) and drying are commonly preferred in the African rural communities including Kajo-keji in South Sudan (Theodory *et al.*, 2014).
3.2.4.1. Traditional processing of cassava flour in Kajo-Keji County

The freshly harvested better cassava roots of the IBV “Lenga Tome” were peeled, heaped on a clean ground, covered with plant leaves and allowed to ferment for four days to reduce the bitterness due to cyanogen. They were then opened and scraped using a knife or any piece of sharp wood for any soil or growing mold, crushed between two stones and spread on a mat to dry in the sun for three days (Appendix 2a-d). The dried chips were then milled using a wooden harmer, sieved through a 50-100 mesh screen and packed in a clean dry bags ready for use.

3.2.4.2. Laboratory processing of cassava flour (Soak fermentation)

The wet (soak) fermentation has been one of the traditional methods of processing cassava in many African countries. It has undergone some modifications according to method described by Hongbété et al., (2009). This study had some few modifications to the preceding method as well. The cassava roots were thoroughly washed to remove the adhering soils and the outer layer peeled off using stainless knives to avoid contamination, sliced into cubes of 10cm$^3$ to reduce the cyanide content at even rate. The cubes were soaked in bucket containing clean tap water for three days to reduce the level of cyanide and to prevent colour change; they were washed again, grated using a stainless grater (Kitchen ware series, 100% high quality), squeezed using a clean cloth, spread on a clean carpet, dried in the sun for four days, blended using High Speed Blender (1800W, Model: YT-6198), sieved through a 500µm sieve (B.S. 410) and packed in clean dry, air tied bags ready for use (Figure 3.4)
3.2.5. Drying of spinach and carrots by modified solar dryer

The Modified Solar Dryer (MSD); Appendix 3a-c was constructed using plain wire (gage 10); for roofing and a black damp-proof membrane (BDPM) (gage 100) covering the entire dryer to prevent the samples from exposure to light and to absorb enough heat needed for drying the samples. Below the dryer was a pallet covered with a white damp-proof membrane (WDPM) (gage 100) on which the samples were spread for drying. The WDPM serves to reflect the excessive heat from the samples and equilibrate it with that from BDPM. The selection of the BDPM and the WDPM was based on the fact that black bodies are good absorbents of heat and the white ones reflect light.
This method was employed to retain the carotenoids in the samples since carotenoids are heat labile that highly react to light, air and any prooxidants or associated compounds especially β-carotene (Mezzomo & Ferreira, 2016). The study obligated to avoid any unfavorable change in this pigment due to such effects by modifying the indirect solar dryer described by Kumar et al, (2015). The samples were spread on the pallet and the MSD was closed completely, left to stand for about 96 hours for the samples to dry. Samples were then removed and blended using a High Speed Blender (1800W, Model: YT-6198), sieved through a 500µm sieve (B.S. 410) and packed in clean dry, air tied bags ready for use.

The MSD might encounter some limitations such as longer period of drying the samples especially during rainy season. It is also too small which may require some expansion such that it can dry samples of large quantities.

3.3. Experimental design/Treatments

Nine treatment levels; Cassava Based Composite Flours (CBCFs) were formulated using concept 4-Ed creative software, version 8.01.01 to derive rations of cassava (Manihot esculenta Crantz), green grams (V.radiata), spinach (S.oleracia), and Carrots (D. carota) flours as indicated in table 3.1. The % compositions of these treatment levels were varied to see the effect on the intended factors (iron, Provitamin A carotenoid (β-carotene), protein and energy) such that the values obtained correlate with the ones obtained from other literatures including USDA National Nutrient Database for Standard Reference, (2016) and Hui et al, (2011) to meet the RDAs for IYC according to WHO, (2009) guidelines. Four treatment levels (CBCFs) out of the nine were selected based on the predication of the software for the intended factors and were mixed using a High Speed Blender (1800W, Model:YT-6198) to ensure homogeneity in the ratio of cassava (soak fermented):Spinach (blanched and dried by OvD and MSD):Carrot (sliced and dried by FD and MSD):Green gram (soaked, germinated, oven dried and dehulled) as 40:5:30:25% (F3), 55:10:05:30% (F4), 30:15:25:30% (F8), 25:25:25:25% (F9) respectively and 100% plain cassava of the unfermented roots was used as control. Each treatment level was separately prepared in triplicate to rule out the random error or improve the precision of model parameter estimate (Singer et al., 2007).
Table 3.1. The effect of varying % composition of the treatment levels (Composites) on the intended factors (nutrients)

<table>
<thead>
<tr>
<th>Composites</th>
<th>Cassava (%)</th>
<th>Spinach (%)</th>
<th>Carrot (%)</th>
<th>Green gram (%)</th>
<th>Energy (Kcal)</th>
<th>Protein (mg/100g)</th>
<th>Iron (µg/100g)</th>
<th>β-carotene (µg/100g)</th>
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<tr>
<td>F1</td>
<td>50</td>
<td>15</td>
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<td>15</td>
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<td>F2</td>
<td>45</td>
<td>10</td>
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<td>20</td>
<td>153.55</td>
<td>5.75</td>
<td>1.79</td>
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<tr>
<td>F3</td>
<td>40</td>
<td>05</td>
<td>30</td>
<td>25</td>
<td><strong>163.30</strong></td>
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<td>55</td>
<td>10</td>
<td>05</td>
<td>30</td>
<td><strong>196.85</strong></td>
<td><strong>8.27</strong></td>
<td><strong>2.39</strong></td>
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<td>60</td>
<td>15</td>
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<td>25</td>
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<td><strong>7.21</strong></td>
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<td>0</td>
<td>160.00</td>
<td>1.36</td>
<td>0.27</td>
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</table>

The figures circled in red were the intended factors after varying the treatment levels. The factors were compared with values from USDA National Nutrient Database for Standard Reference, (2016); Hui et al, 2011. They were chosen among the nine because they were close to their RDAs for the Infants and Young Children (6-23 months) hence were used for calculating the predicted % contribution to the RDAs for this age group in table 4.3, page 57 based on WHO, (2000); FAO, (2007) guidelines on family foods for breastfed children.

3.3.1. Blending the cassava flour with flours of green grams (V. radiata), Spinach (S.oleracia), and Carrot (D. carota)

The development of CBCFs were done as designed in section 3.3. The flours were rationed using concept 4-Ed creative software, version 8.01.01 to predict the overall nutrient densities and blended. The nutrient values obtained were eventually used for calculating their expected % contribution to the RDA for iron, provitamin A, protein and energy for the IYC (6-23 months) per two servings of 25g composite (125ml of porridge) for infants (6-11 months) and 50g composite.
(250ml of porridge) for the young children (12-23 months) according to WHO, (2000) and FAO, (2007) guidelines on family foods for breastfeed children as presented in Table 3.1.

Due to the cost attached to the analysis of the product, only FD composites were analyzed for all the parameters chosen for this study. The MSD composites were only analyzed for Beta (β)-carotene which was also used in same way as the FD composites to establish the Provitamin A (PvA) content, and its limit of detection or bioavailability (Zhu et al., 2006). The study was also aimed at comparing the performance of the MSD composites in terms of β-carotene (provitamin A) retention and bioavailability relative to the FD composites before recommending the modified solar dryer (MSD) to the rural communities for use.

3.4. Determination of parameters

3.4.1. Determination of cyanogenic glucosides in cassava flour

Flours from both heap (traditional) and soak (laboratory) fermented cassava roots were analyzed for the cyanide contents to assess how superior the method adapted for this study (the soak fermentation) was over the commonly used method in Kajo-keji (heap fermentation) in terms of cyanide reduction. The cyanide content was determined according to the method of Essers, (1995) with additions from Piero et al., (2015). Four (4) g of heaped and soaked fermented cassava flour were gently swirled separately in 25ml of cold 0.1M ortho phosphoric acid in a closed 50ml falcon tube. The homogenous mixture was centrifuged at 6000rpm for 15 minutes at 4°C to get the clear supernatant where 0.1ml of it was added to 0.4ml of orthophosphoric buffer (pH 7.0) in a test tube, followed by 0.1ml of linamarase enzyme solution. The sample was then incubated at 30°C for 15minutes in a water bath after which 0.6ml of 0.2M NaOH was added and the sample incubated for 15minutes at room temperature. Thereafter, 2.8ml of buffer (pH 6.0) was added followed by 0.1ml of chloramine T solution and mixed on a shaker. After 5mins, 0.6ml of colour reagent (sodium hydroxide/isonicotinic acid/barbituric acid) was added and mixed well. The sample was incubated at room temperature once again for 10minutes and measured spectrophotometrically after 20mins at a λ of 605nm and the cyanide contents calculated as total cyanide, free cyanide and HCN contents of the samples using the formula;
Extraction factor = sample weight (g) + extraction media (g)

\[
\frac{\text{Sample weight (g)}}{\text{Sample weight (g)}}
\]

Cyanide content (sample solution) (mg/l) =

\[
\frac{\text{Absorbance of sample – Y-intercept of the standard}}{\text{Slope of the standard}}
\]

Cyanide content (mg/kg fwt) = dilution factor (sample) × extraction factor × cyanide content (sample solution).

Cyanide content (mg/kg dwt) =

\[
\frac{\text{Cyanide content (mg/kg fwt) ×100}}{\text{Sample dry weight}}
\]

Therefore, cyanogenic glucosides was calculated as (total cyanide – free cyanide) and cyanohydrin as (free cyanide – HCN)

3.4.2. Carotenoid extraction and quantification of cassava based composite flours for β-carotene content

The total carotenoids were extracted using acetone and petroleum ether and quantified using High Performance Liquid Chromatography (HPLC) according to the method described by (Darwin et al., 2011) with modification. Water was used to separate the Acetone - petroleum phase instead of 0.1M NaCl solution. This modification was based on the fact that water and acetone chemically mix, forming a strong acetone-water complex in the ratio of 1:1 where the water is hydrogen-bonded to the carbonyl oxygen of the acetone (Monakhova et al., 2014). As such, the two components were able to dissociate from the petroleum ether phase and because water is also cost-effective than the NaCl solution, the study opted to it. Five grams (5g) of each CBCFs were homogenized for 1 minute with 10ml Acetone: Petroleum ether (1:1) and shaken followed by centrifugation (Eppendorf; CL-Falcon) at 3000rpm, for 10minutes at 4°C. The supernatant was collected in an empty Eppendorf, covered well and kept in dark. The residue was extracted once again (Three times) till it became colorless. The supernatants were then combined with 10ml of
distilled water and the petroleum ether phase containing the carotenoids separated from the lower aqueous-acetone phase. From the petroleum ether solution, carotenoids were quantified for β-carotene using the HPLC where aliquot (15ml) was consistently picked, partially dried by rotavaporation and completely dried with nitrogen. Immediately before injection, the dry extract was dissolved in 1ml of methanol: methyl tert butyl Ether (1:1) HPLC grade and filtered through 0.22μm PTFE syringe filter. Carotenoids were separated and quantified using YMC carotenoid S-5 C30 reversed-phase column (4.6×150mm): particle size 5µm) with an YMC carotenoid S-5 column guard (4.0×23mm) in a HPLC system (Agilent technologies 1200 series, Waldbronn, Germany) with a cooling auto-sampler unit at 4°C to prevent the injector solvent from evaporation and the carotenoid from heat degradation and from DAD detector of wave length 450nm. Peaks were identified by comparing the retention time and special characteristics against a pure β-carotene standard and available literature. Quantity was determined by integrating the peak area against the standard curve prepared with a known concentration of all- trans-β-carotene. The total carotenoid content was quantified for the total β-carotene content on fresh and dry weight basis. Before any determination according to Darwin et al., (2011), the method was previously validated according to Thompson et al., (2002) requirement. This method had a linearity of 0.99925 at 2, 6, 10, 14, 18, 25, 30 PPM of levels and three replicates, implying that it was highly accurate.

3.4.3. Determination of provitamin A content of cassava based composite flours

Using the quantified content of β-carotene in section 3.7, the provitamin A content of cassava based composite flours (CBCFs) was calculated based on the concept of the retinol equivalence (RE) introduced by a joint FAO/WHO, (2001) consultation experts on human minerals and vitamins who established a relationship among food sources of vitamin A as;

\[ 1 \mu g \text{ retinol} = 1\text{RE} \]
\[ 1\mu g \beta\text{-carotene} = 0.167\mu g\text{RE} \]
\[ 1\mu g \text{other provitamin A carotenoids} = 0.084\mu g\text{RE} \]
3.4.4. Determination of phytate content of cassava based composite flours

The Phytate content in the composite flours was determined following standard procedure according to Abulude, (2007) and Adeolu, (2013). Four grams (4g) of each composites were digested with 100ml of 2% Hydrochloric acid for 3hrs and then filtered. Twenty five mills (25ml) of the filtrate was placed in a 250ml conical flask and 5ml of 0.3% ammonium thiocyanide (NH₄SCN) solution was added as indicator followed by 53.5ml of distilled water to give it the proper acidity (PH 4.5). The content was titrated with ferric chloride solution which contained 0.00195g of iron per ml of FeCl₃ solution until a brownish yellow colour persisted after 5mins. The results was multiplied by a factor of 1.95 to obtain Phytin phosphorus and the results also multiplied by factor 3.55 converting it to phytate (P).

3.4.5. Determination of total iron content of cassava based composite flours

Determination of total iron content in the composites was done by open wet digestion and spectroscopic method using Genesys 2 UV-VIS Spectrometer, model TM2 according to Mandal & Malenica, (2015). One gram (1g) of each composites were heated in a mixture of concentrated nitric and sulphuric acid for 4hrs at a temperature 110-130°C. The samples were filtered and the residues retained and dissolved in a mixture of nitric and hydrochloric acid of concentrations 0.05ml/L, 1:1 v/v. Samples were transferred into a 100ml volumetric flask and topped to the mark with same mixture of acids. Digestion of each mixture was done in triplicate. Two mills (2ml) of the solution was picked and added to it 1ml of nitric acid followed by 1.2ml potassium thiocyanide of the same concentration, it was mixed well and absorbance read at λ 481nm.

3.4.6. Determination of percentage contribution of cassava based composite flour to the Recommended Dietary Allowances (RDAs) of nutrients for IYC (6-23 months)

The % contribution of cassava based composite flours (CBCFs) was calculated according to WHO, (2009) and FAO, (2007) guidelines on family foods for breastfed children and Brannon & Taylor, (2017) recommendation on mineral supplements for infancy and young children. The calculation was based on the recommended dietary allowances of the IYC, age group serving portions of the
composites and predicted nutrient densities by the software except energy which was calculated from general Atwater factors according to Schakel et al., (1997) as:

\[
\text{Energy (Kcal)} = (4 \text{ Kcal/g protein} \times \text{g protein} + (9 \text{ Kcal/g fat} \times \text{g fat} + (4 \text{ Kcal/g carbohydrate} \times \text{g carbohydrates}).
\]

Following the above guidelines, 2.5 heaped table spoon of composites (25g) each, were prepared in 146.21 ml of water, making a thick porridge of 125 ml for infants (6-11 months). This measures 0.25 of the Ugandan tumpeco or South Sudanese gamma cup, an equivalent of 0.38 of the apiliga cup (the South Sudanese common cup for measurement). Then, 5.0 heaped table spoons of composites (50g) each, were prepared in 292.42 ml of water making a thick porridge of 250 ml which measures 0.5 tumpeco or gamma cup, an equivalence of 0.75 of apiliga cup. Diet was calculated per two servings in a day.
Table 3. 2. The expected percentage contribution of Cassava Based Composite Flours (CBCF) to the RDA of iron and Provitamin A, including energy and protein for IYC (6-23 Months)

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<th>Vitamin A (µg/100g)</th>
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The figures circled in red were the intended factors after varying the treatment levels. They were chosen among the nine treatments because they contributed favorably to their RDAs for the Infants and Young Children (6-23 months). The expected % contribution of the CBCFs 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.
3.4.7. Determination of nutrient bioavailability of cassava based composite flours

3.4.7.1. Determination of provitamin A bioavailability of cassava based composite flours

In this study, provitamin A bioavailability was established by quantifying the carotenoids for β-carotene using HPLC as described in section 3.4.2. The β-carotene in μg/g was converted to retinol equivalence (RE) based on the FAO/WHO, (2001) recommended conversion factors for the ingested provitamin A carotenoids to that of retinol as stated in section 3.4.3. The value of the RE was then converted to μmol/L (the standard SI unit of serum retinol) and compared with the threshold hold (0.06 μmol/L) which is the limit of detection of provitamin A (LODPvA) established by Manssens & Escobar, (2015) as described in section 2.4. The comparison is to deduce if β-carotene (provitamin A) is bioavailable in this diet with a limit of ≥ 0.06 μmol/L. The LODPvA determines the adequacy of β-carotene or provitamin A bioavailability (Zhu et al, 2006)

3.4.7.2. Determination of inhibitory effect of phytate on iron bioavailability of cassava based composite flours

The inhibitory effect of phytate on bioavailability of the mineral iron of cassava based composite flours was determined based on the methods stated by Shimi & Hassnah, (2013); Norhaizan & Nor, (2009). The content of the phytate (P) and of the mineral iron (Fe) were converted into moles by dividing the weight of P and Fe with their molecular weights (P: 660g/mol; Fe: 56/mol); the mole ratio of P: Fe was then obtained after dividing the moles of P with the mole of Fe. The molar ratio of P: Fe in the diet actually quantifies the inhibitory effect of the phytate on the mineral absorption (Sanz-Penella et al., 2013). The molar ratio of P:Fe > 1 is critical (Hasan et al., 2016), it indicates poor bioavailability of Fe in the diet (Lazarte et al., 2015).

3.4.8. Proximate analysis of cassava based composite flours

The proximate analysis (moisture, protein, fat, fibre, ash, and carbohydrate) of the composites was done using Near-Infrared Spectroscopy (NIRS) according to the method described by Masoum et
al., (2012) and Wang et al., (2014). NIRS is a rapid, non-destructive technique with the capacity to distinguish & quantify mixed diet (Lei & Bauhus, 2010; Locher et al., 2005). The samples were scanned in reflectance mode over the NIR spectral range of 1000-2500nm using a monochromatic instrument Fourier Transform Near-Infrared (FT-NIR) system (FOSS NIRS TM DS2500, SR; 91793020, Part No. 60066162, 24 VDC, Max 100VA, Fuse; 5.0A, 2016, Denmark). 20g of every composite was filled in a sample holder which was fitted in the measurement cell and scanned. The spectra of each composite was obtained by collecting their average of 64 individual spectral scans, each of which was done in triplicate.

3.4.9. Determination of pasting characteristics of cassava based composite flours

The pasting properties of the composite flours was evaluated using a Rapid Viscos Analyzer (RVA) according to the method reported by Reungmaneepaitoon, (2009). Three grams (3g) of cassava based composite flours (CBCFs) each was weighed into a dried empty aluminum canister and mixed with 25ml of distilled water. The slurry was thoroughly mixed as the canister was firmly fitted into the RVA. The slurry was then heated from (50 – 95)oC at a rate of 12oC per minute with constant stirring at 160rpm at 95oC for 2.5 mins (Breakdown), then cooled to 50oC at a rate of 13oC per minute (Setback) and held for 2 minutes (holding time). The total cycle was 12.5minutes and Peak Viscosity, set back, peak time, and pasting temperature was read from the pasting profile with the help of thermocline for windows software connected to a computer.

3.4.10 Sensory evaluation of the Cassava Based Nutrient Dense Porridge

The color, flavor, texture and nutritional value of a product are crucial factors to consumer acceptance and preference of a given product (Barrett et al., 2010), thus porridges of the CBCFs were subjected into sensory evaluation. The cassava based nutrient dense porridges (CBNDPs) were cooked according to the method recommended by FAO, (2007) with modification. Two cups of water (400 ml) was boiled in a clean saucepan. Then 100g CBCFs, each were mixed in another 200 ml clean water to make a smooth paste and added to the boiling water, mixed well until it became smooth. The smooth paste was brought to boiling and continued with cooking for 20 minutes. The ready porridge was then kept in a well coded thermos vacuum flask. A panel of 22
males and 8 females that composed of internship students and some members of the nutrition unit of the NaCRRRI-Uganda were selected to represent the caregivers of IYC (6-23 months). They were given a training for one hour on how to go about the exercise and were also briefed about the ingredients that constituted the products. However, to minimize biases that might affect the validity or accuracy of the test, samples were “blind coded”. Each sample was given a randomly chosen three digit number which bears no meaning. Panelists were only provided with enough information about the product in the training. They were availed with safe drinking water to rinse their mouths before evaluating successive porridges. Porridges of different composite flours alongside the plain cassava (control) were presented at once at room temperatures to each panel member in plastic disposable cups. They were tested while rating the sensory attributes including appearance, aroma, mouth feel, after taste and overall acceptability using a nine-point hedonic scale rating from 1 (disliked extremely) to 9 (liked extremely) (Wichchukit & Mahony, 2014). The results of the sensory evaluation was used in the selection of the most suitable composite for making nutrient dense porridges (CBNDP) for IYC in the community.

3.4.10.1. Final composite selection and quality evaluation of cassava based nutrient dense porridge

With reference to the above sensory evaluation exercise on the CBNDPs, the best composite was selected for sustainability of the product. The sample was screened for consumer acceptability, nutritional quality and physiochemical properties as well as its contribution to the RDA for Fe, provitamin A, protein and energy for the IYC (6-23 months).

3.4.11. Statistical analysis

All the experimental analysis in this study were carried out in triplicates and all data analyzed using Minitab software (version 16, @ 2010 Minitab Inc. state college, Pennsylvania) to determine difference in quality attributes. Means were obtained for each composite and one way analysis of variance (ANOVA) was used to test for the difference among the composites at 95% level of significance (P ≤ 0.05).
CHAPTER FOUR: RESULTS AND DISCUSSION

4.1. The cyanide content of the heap and soak fermented cassava flours

The mean cyanide contents of flours from both heap (traditional) and soak (laboratory) fermented cassava roots and the control are presented in Figure 4.1. Both treatments showed significant reduction (P<0.05) in the cyanide content as compared with the control. The range of their cyanide contents were within the safe level of the <10 ppm (WHO, 2004). The results were in agreement with the findings of Iwuoha et al., (2013) in the study of detoxification effect of fermentation on cyanide content of cassava roots. This therefore indicates that fermentation is an effective method for detoxifying cassava roots for human consumption hence is recommended for processing the IBV “Lenga Tome” for making porridge for IYC (6-23months).

![Figure 4.1. Cyanide content of solid (Heap) and Wet (Soak) fermented cassava flours](image)

4.2. The β-carotene content of cassava based composite flours

The formulated cassava based composite flours are shown in Appendix 5. The β-carotene content of the freeze dried (FD) and modified solar dried (MSD) composites was ranging from 239-390
µg/g and 70.3-163.1 µg/g as presented in figures 4.2 (a) and 4.2 (b) respectively. Among the freeze dried composites, F8 was found with significantly high (P<0.05) β-carotene content, whereas composites F3, F4 and F9 were not significantly (P>0.05) different though with adequate contents as well; Figure 4.2 (a). In similar way, composite F8 of the modified dried composites displayed significantly (P<0.05) high content of β-carotene. There was also no observed significant (P>0.05) difference among the rest of the composites of this same batch except, F4 whose content had reduced tremendously; figure 4.2 (b). The control experiment (plain cassava flour) did not show any β-carotene content, coinciding with what was reported on the β-carotene content of the dried fermented cassava flour by Gouado et al., 2008). Indeed IYC who receive their complementary food from plain cassava flour of the indigenous white varieties are deprived of this precursor of provitamin A. That is why blending of cassava flour with ingredients that are rich in this pigment is necessary for the survival of this vulnerable group, the IYC (6-23 months).

The slight reduction of β-carotene content of the modified solar dried composites could be attributed to the temperatures in the MSD that seemed to be high because the roofing material used for construction of the MSD was a black one (the black damp-proof membrane). Black body is reported capable of absorbing and emitting radiation completely at any wavelength (Zwinkels, 2015). Drying samples in high temperatures exposes them to hot air which eventually reduces the β-carotene content (Goula et al., 2010). The loss of the pigment in samples could be due to its highly unsaturated structure, which renders it electrically rich by delocalization of π electrons, causing degradation through isomerization and oxidation (Pénicaud et al., 2011b). However, despite the loss of the β-carotene in the drying process, the MSD was able to retain a certain content which was sufficient for making nutrient dense porridge upon blending with the plain cassava flour; figure 4.2 (b).
4.2. The β-carotene content of Freeze Dried Composites and 4.3(b). The β-carotene content of modified Solar Dried Composites

Figure 4.2 (a). Beta carotene content of Freeze Dried Composites and 4.3(b). The β-carotene content of modified Solar Dried Composites

4.3. The Provitamin A content of cassava based composite flours

The mean Provitamin A content of the freeze dried and modified solar dried composites varied from 40.0-65.2 and 11.7-27.7 RE (µg/g) respectively (figure 4.3a-b). Composite F8 of the freeze dryer exhibited significantly (P<0.05) high content of retinol equivalent. There was no significant (P>0.05) difference observed among composites F4, F3, and F9 in this same batch. On the other hand, composites F8, F3 and F9 of the modified solar dryer were not significantly (P>0.05) different with F4 presenting the lowest means of retinol equivalent. Overall, though β-carotene retained in the modified solar dried composites was slightly lower than the one retained by freeze dried composites, both contents, leaving alone of the F4 of MSD, were expected to contribute favorably to the recommended nutrient density for plant based diet of the 500µg RE (FAO/WHO, 2001), hence are suitable for IYCF.
Figure 4.3 (a). The Provitamin A content RE (µg/g) of Cassava based composites. 4.3 (b). The Provitamin A content RE (µg/g) of Modified Solar Dried Campsites

4.4. The phytate content of cassava based composite flours

The results showing means of phytate content are displayed in figure 4.4. The range of phytate content was from 19.8-26.5mg/100g. Composite F8 presented slightly high content of phytate however, it was not significantly (P>0.05) different from the rest of the composites. The slight increase could be attributed to the high % flour of the spinach and green grams in the composite. The control (plain cassava flour) showed significantly (P<0.05) lower content of phytate. The results obtained was lower than the one (40.0-78.8mg/100g) reported by Ayele et al., (2017) on the wheat supplement enriched with cassava and soybean flours. The difference could be from the types of ingredients, processing techniques used and the quantities of treatment levels used in formulating the recipes. The presence of phytate in food is known of blocking the absorption of mineral ions such as Fe^{2+}, Zn^{2+} and Ca^{2+} (Gupta et al., 2013). It would therefore be expected that, lowering this antinutritional factor should increase the bioavailability of such mineral ions in the composites since phytate has been implicated in making these mineral ions unavailable (Adebawale et al., 2015).
4.5 The total iron content of cassava based composite flours

The composites exhibited varying iron contents as shown in figure 4.6. The content varied from 31.7-46.2mg/100g. There was no significant (P>0.05) difference observed between composites F9 and F4, while composites F3 and F8 differed significantly (P<0.05). Though the control presented the lowest iron content compared to the treatments, the value was higher than what is known so far in the literature for iron content in local bitter cassava varieties; 1.11 mg/100g (Sule et al., 2017) ; 0.01 mg/100g (Manano et al., 2018). The difference could be from environmental variations since micronutrient content of the plant depends on the soil property where it is being grown, the ability of the mineral uptake of the plant species and differences in the cultivars (Lindstrom et al., 2012 ; Atefeh, 2013). The current study has therefore revealed that, the IBV “Lenga Tome” has appreciable amount of iron content, making it a better choice for use in product development especially for IYCF. The iron content of the composites compared favorably to the value (6.19-8.10mg/100g) reported for cassava based composite crackers that were developed as supplements for primary school children (Mosha et al., 2010). The serving size of composites of the current study was adjusted to meet the recommended daily dose of iron supplement of the 10-
12.5 mg/day (Patsy & Taylor, 2017) for IYC (6-23 months) to avoid inadequacy or toxicity of iron for this age group in the population.

![Iron content of Cassava based Composite flours](image)

**Figure 4.6. Iron content of Cassava based Composite flours**

4.6. Percentage contribution of cassava based composite flours to RDA of IYC (6-23 months) for iron, provitamin A, protein and energy per 100g

The results for the % contribution of cassava based composite flours (CBCFs) to the recommended dietary allowance of IYC (6-23 months) for the mineral iron, provitamin A, energy and protein are shown in table 4.1.

4.6.1. Iron

The mean % contribution of the composites to the RDA of iron for infants (6-11 months) per two servings of 2.5 table spoons (tbs) or 25g of composite (125ml) of porridge was in the range of 98.1-121.6% and for young children (12-23 months) per two servings of 5tbs (50g) of composite or 250ml of porridge was in the range of 157.3-195.0%. Composite F4 and F9 were not
significantly different (P>0.05), and had the highest % contribution. Composites F3, F8 and the control were significantly different (P<0.05). The % contribution of composites for the mineral iron for IYC (6-23 months) was neither below the RDA nor above the tolerable upper limit (TUL) of the 40mg (215%) per day for these age groups (Gallicchio, 2014). The composites are therefore expected to mitigate the cases of iron deficiency among IYC (6-23 months) in the community. The IYC are also expected to have less/no cases associated with metabolic acidosis such as lipid peroxidation and cellular damage that would have arisen due to electron shunt as a result of consuming large doses of mineral iron in food.

4.6.2. Provitamin A (Retinol Equivalent)

In terms of provitamin A (Retinol Equivalent), the mean % contribution of freeze dried composites to the RDA for infants (6-11 months) per two servings of 2.5tbs (25g) of composite or 125ml of porridge ranged from 266.9-434.7%. While for children (12-23 months) per two servings of 5tbs (50g) of composite or 250ml of porridge was in the range of 500.4-815.1%. For the case of modified solar dried composites, their % contribution was found ranging from 78.2-181.6% and 146.7-340.4% for infants (6-11 months) and young children (12-23 months) respectively.

Among the freeze dried composites, F8 had significantly (P<0.05) high % contribution compared to F3, F4 and F9 that were not significantly (P>0.05) different. Similarly, composite F8 of the modified solar dryer contributed significantly (P<0.05) high % of provitamin A to the RDA of IYC (6-23 months). Composites F3 and F9 were not significantly (P>0.05) different and F4 showed the lowest % contribution. Nevertheless, many scientific investigations have reported blood retinol responses in populations with low vitamin A intake after β-carotene supplements (Saskia et.al., 1995). However, the adequate and safe content of β-carotene (provitamin A)-RE that could combat vitamin A deficiency after increased consumption of provitamin A carotenoids from vegetable remains a mystery.

In a study of plasma response of children to β-carotene supplementation, a dose of 6mg of β-carotene (1000 RE) per day caused an increase in plasma β-carotene by 0.59 and 0.60 µmol/L after 10 and 20 days respectively (Bulux et al., 1994). On the other hand, dietary supplementation with 3mg of β-carotene (500 RE) per day is reported to impair further conversion of intestinal β-
carotene to retinol in humans (Haskell, 2012). Also, vitamin A deficient (< 0.7 µmol/L) anemic children were feed with 509 RE from fruits and 684 RE from vegetable, and their serum retinol increased by 0.12 and 0.07µmol/L respectively (Tang, 2010b). However, 0.07µmol/g liver retinol is the minimum acceptable level in IYC (Lindsay & Haskell, 2001). Meaning, the adjusted serving sizes of the composites (porridges) of the IBV “Lenga Tome” in the current study apart from that of F4, are expected to increase the serum retinol without impairing the conversion of the intestinal β-carotene (provitamin A) to curb the prevailing vitamin A deficiency among the IYC (6-23months) upon consumption.

4.6.3. Energy

The mean % contribution of cassava based composite flours to the RDA of infants (6-11months) and young children (6-23months) for energy was in the range of 22.4-26.1% and 20.4-23.7% respectively (Table 4.1). Composite F4 displayed significantly (P<0.05) high % contribution of energy probably due to its high % flour of cassava. Cassava provides high energy because of the high starch content of its carbohydrates that is composed of 85% amilopeptin and 17% amilo dil (Montagnac et al., 2009; Bertoft, 2017). This also accounts for the high % contribution of the entire composites for energy as compared to the 6-18% contribution of the porridge from whole maize flour, finger millet flour, sardines, groundnut and salt composite prepared for IYC of age 6-23months (Kulwa et al., 2015). Energy dense food is essential for children to top up their energy stores to facilitate daily activities, and to prevent them from snaking on foods that are high in fat and / or sugar (Bartolo, 2014). Familiarizing children to high sugars exposes them to dietary trajectory for sweetness hence risks for dental caries and obesity (Diep et al., 2017). On this basis, porridges of cassava based composite flours of the IBV “Lenga Tome” would make a better complementary food for IYC (6-23months).

4.6.4. Protein

The mean % contribution of cassava based composite flours to the RDA of protein for infants of age 6-8, 9-11 and children 12-23months was in the range of 59.4 -99.3, 44.5-69.9, and 54.7-85.9% respectively. Composite F9 contributed significantly (P<0.05) high % of protein compared to the
other composites. The % contribution of the composites decreased with increase in the level of cassava flour in the treatments. This surely indicates that high consumption of plain cassava flour deprives IYC from protein. However, the values of all composites in regards to % contribution compared favorably to the one (21.2-29.2%) reported by Nkuba et al., (2017) on banana and maize based porridges for IYC (6-23months). Despite that, the % of the protein contributed for infants (6-8months) was higher than expected (21%). There is growing evidence that high protein intake during first two years of life is a risk factor for development of overweight and obesity later in life (Tang, 2018). More so, if protein intake is higher during infancy (more worse before 4 months) especially when undiluted cow milk is added to the infant formula at this age, the glomerulus filtration rate of the IYC cannot cope up with the renal solute load. The solute load increases the glomerulus filtration rate beyond normal, resulting into hypernatremia where the infants lost a lot of body fluids relative to electrolyte content (Kim & Greer, 2014). The excessive loss of the electrolytes due to hypernatremia contributes to neurological morbidity in IYC who are undergoing treatment (Jagadish, 2008). Nevertheless, RDA of protein (21%) for infant of age 6-8months is the value expected of any complementary food being plant or animal based. Therefore since this complementary food is purely plant based diet, there is no sight effect expected because plant protein is less digestible than of animal (Tanya & Guyda, 2007).
Table 4.1. The percentage contribution of the formulated Cassava Based Composite Flours (CBCFs) to the RDA of iron, provitamin A, Protein and energy for IYC (6-23months)

<table>
<thead>
<tr>
<th>Composites</th>
<th>Cassava (g)</th>
<th>Spinach (g)</th>
<th>Carrot (g)</th>
<th>Grams</th>
<th>%flours</th>
<th>% Contribution of composites to the RDA of IYC (6-23 months) per two serving in a day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Energy (kcal)</td>
<td>Protein (%)</td>
<td>Fe (mg/100g)</td>
<td>Provitamin A RE(µg/g) (FD)</td>
<td>Provitamin A RE(µg/g) (MSD)</td>
<td></td>
</tr>
<tr>
<td>Age (Months)</td>
<td>Age (Months)</td>
<td>Age (Months)</td>
<td>Age (Months)</td>
<td>Age (Months)</td>
<td>Age (Months)</td>
<td></td>
</tr>
<tr>
<td>F3 40 05 30 25</td>
<td>22.4 ± 0.32d</td>
<td>61.6 ± 1.35c</td>
<td>112.1 ± 0.56ab</td>
<td>277.5 ± 145.06b</td>
<td>171.3 ± 30.73a</td>
<td></td>
</tr>
<tr>
<td>F4 55 10 05 30</td>
<td>26.1 ± 0.23b</td>
<td>59.4 ± 0.91d</td>
<td>121.6 ± 0.43a</td>
<td>319.9 ± 137.30b</td>
<td>78.3 ± 11.73b</td>
<td></td>
</tr>
<tr>
<td>F8 30 15 25 30</td>
<td>23.5 ± 0.23c</td>
<td>84.2 ± 0.80b</td>
<td>98.1 ± 0.98bc</td>
<td>434.7 ± 31.30a</td>
<td>181.6 ± 47.69a</td>
<td></td>
</tr>
<tr>
<td>F9 25 25 25 25</td>
<td>23.1 ± 0.28d</td>
<td>93.3 ± 3.00a</td>
<td>124.3 ± 47.29a</td>
<td>266.9 ± 36.71b</td>
<td>176.1 ± 39.87a</td>
<td></td>
</tr>
<tr>
<td>Co 100 0 0 0</td>
<td>27.7 ± 0.14a</td>
<td>29.1 ± 2.01c</td>
<td>85.2 ± 0.56c</td>
<td>0.00 ± 0.00c</td>
<td>0.00 ± 0.00c</td>
<td></td>
</tr>
</tbody>
</table>

Note: Values with different superscripts are significantly different.
The values given were the means ± Stand deviations of data of triplicate determinations. Values in the same columns with the same superscript were not significantly different (P>0.05). The % contribution of the CBCFs were based 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.

4.7. The bioavailability of provitamin A and iron in cassava based composite flours

4.7.1. Provitamin A bioavailability

The mean bioavailability or limit of detection of provitamin A (LODPvA) of the freeze dried (FD) and modified solar dried (MSD) composites was found ranging between 0.1-0.2µmoles/L and 0.0-0.1µmoles/L respectively (Table 4.2). Composite F8 of the freeze dried composites had significantly (P<0.05) higher LODPvA. Composites F3, F4, and F9 had no significant (P>0.05) difference. However, F4 of the modified solar dryer had significantly (P<0.05) lower LODPvA, while no significant difference was observed between composite F8 and F9. The lowest LODPvA of the F4 composite could be due to the lowest level of flour of carrot (5%) and spinach (10%) assigned to it. However the LODPvA of the composites in this study apart from F4 was within the acceptable limit of the 0.1µmoles/L designed for the assessment of vitamin A status in foods (Enriqueue & DEL PILAR , 2004). The LODPvA was also found lying above the 0.06 µmoles/L, the value which determines the adequacy of β-carotene or provitamin A bioavailability (Manssens & Escobar, 2015; Zhu et al, 2006). Therefore, the composite flours (porridges) of the IBV “Lenga Tome” had adequate and bioavailable provitamin A for the IYC (6-23months) in the community.
Table 4.2. Provitamin A Bioavailability (LODPvA μmoles/L) of cassava based composite flours

<table>
<thead>
<tr>
<th>Composites</th>
<th>g/g-mole FD</th>
<th>g/g-mole MSD</th>
<th>LODPvA μmoles/L FD</th>
<th>LODPvA μmoles/L MSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F3</td>
<td>0.5 ± 0.12b</td>
<td>0.3 ± 0.03a</td>
<td>0.2 ± 0.04b</td>
<td>0.1 ± 0.01a</td>
</tr>
<tr>
<td>F4</td>
<td>0.5 ± 0.06b</td>
<td>0.1 ± 0.01b</td>
<td>0.2 ± 0.02b</td>
<td>0.0 ± 0.00b</td>
</tr>
<tr>
<td>F8</td>
<td>0.7 ± 0.03a</td>
<td>0.3 ± 0.04a</td>
<td>0.2 ± 0.01a</td>
<td>0.1 ± 0.01a</td>
</tr>
<tr>
<td>F9</td>
<td>0.5 ± 0.03b</td>
<td>0.3 ± 0.03a</td>
<td>0.1 ± 0.01b</td>
<td>0.1 ± 0.01a</td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.00c</td>
<td>0.0 ± 0.00c</td>
<td>0.0 ± 0.00c</td>
<td>0.0 ± 0.00c</td>
</tr>
</tbody>
</table>

The values given were the means ± Stand deviations of data of triplicate determinations. Values in the same columns with the same superscript were not significantly different (P>0.05). Composite with LODPvA <0.06 μmoles/L indicates poor bioavailability of provitamin A (Manssens & Escobar, 2015)

4.7.2. Iron bioavailability

The mean molar ratios of phytate to the mineral iron (P: Fe), which defines iron bioavailability was found ranging between 0.0-0.6 in all composites (Table 4.3). The ratio was below the critical (P: Fe >1) as was based on the recommendation of Hallberg et al., (1989) on iron inhabitation in human. These ratios were also low compared to the one (4.9) and (3.8-8.7) reported by Makori et al., (2017) and Tufa et al., (2016) on cereal and sorghum based complementary flours for feeding IYC (6-23months) respectively. The difference could be attributed to the unit operations involved in preparation of the recipe. Ratio P: Fe >1 is known of reducing absorption of iron in the diet (Hasan et al., 2016). It could therefore be concluded that iron was free from the inhibitory effect of phytate in cassava based composite flours of the IBV “Lenga Tome” hence is available upon consumption by IYC (6-23months) though at varying magnitudes. Composites F3 and F9 displayed significantly (P<0.05) high level of bioavailability (with low P: Fe mole ratios). There was significant difference between composite F4 and F8. The efficacy of bioavailability increased with decrease in % flour of spinach and green grams. Meaning, the higher concentration of phytate could be from spinach and green grams though had no effect on the mineral bioavailability herein. Other evidence that supports bioavailability of the mineral iron is based on the fact that the composites had high contents of provitamin A. During digestion, provitamin A and iron are known
of forming complex that act as a chelating agent at a pH 6.0 in the human intestinal epithelial cells that prevents the inhibitory effect of phytate and polyphenol on the absorption of non-heme iron (Germano et al., 2011).

**Table 4.3. Iron bioavailability (Mole ratio P: Fe) of cassava based composite flours**

<table>
<thead>
<tr>
<th>Composites</th>
<th>Moles of P</th>
<th>Moles of Fe</th>
<th>Mole ratio of P:Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>F3</td>
<td>0.0 ± 0.004ab</td>
<td>0.7 ± 0.002ab</td>
<td>0.0 ± 0.007bc</td>
</tr>
<tr>
<td>F4</td>
<td>0.0 ± 0.005a</td>
<td>0.8 ± 0.001a</td>
<td>0.1 ± 0.006ab</td>
</tr>
<tr>
<td>F8</td>
<td>0.0 ± 0.008a</td>
<td>0.7 ± 0.003bc</td>
<td>0.1 ± 0.012a</td>
</tr>
<tr>
<td>F9</td>
<td>0.0 ± 0.009a</td>
<td>0.8 ± 0.157a</td>
<td>0.0 ± 0.006bc</td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.006b</td>
<td>0.6 ± 0.002c</td>
<td>0.6 ± 0.002c</td>
</tr>
</tbody>
</table>

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

**4.8. Proximate composition of cassava based composite flour**

The results of proximate composition of the composites for moisture, protein, carbohydrates, energy, fat and fibre contents are shown in (Table 4.4).

**4.8.1 Moisture**

In regards to moisture content, it ranged between 9.8-11.2%. Composite F4 was recorded with significantly (P<0.05) high content, while no significant (P>0.05) difference was observed between composite F3 and F9. Composite 8 displayed the lowest means. However, all the composites had moisture contents that lie within the acceptable limit of ≤ 14% for flour (Carter & Galloway, 2015; Edet et al., 2017). Nevertheless, in context of IYCF, these moisture contents were above the maximum level recommended by Codex standard for infants’ formulae of the 5% in all composites (Kotb et al., 2016). Therefore, the composites need to be consumed within shortest time possible. This is because high moisture content in infants’ formulae reduces its self-life span.
by increasing microbial degradation activity resulting into bad odor and unacceptable taste. Consuming such a microbial degraded formulae may cause complications to the health of IYC (Bamidele et al., 2007)

4.8.2. Protein

The mean protein content of the composites was found ranging from 12.5-19.6%. Composite F9 presented significantly (P<0.05) higher content, while no significant (P>0.05) difference was observed in composites F3, F4 and F8. The protein content recorded in all composites was comparatively high than the one reported for complementary meal (0.5-1.7%) by Kulwa et al., (2015). The protein content increased with increase in the level of flour of the green grams. This was in agreement with Jyotsna et al., (2011) report that the protein content (10.1-14.0%) of the wheat flour increased with increase in the level of green gram flour. In this study, all composites have their values close to the threshold expected of complementary foods; (21%) for infants of 6-8 months, (42%) for infants of 9-11 months, and 57% for young children 12-23 months (WHO, 2009). If protein content of complementary food is too low especially for already malnourished IYC, their growth and recovery is restricted (Kim et al., 2009). On the other hand, if the intake exceeds requirement, the surplus content is metabolized into energy which is not an energy efficient process. Excess protein also produces urea, adding to the renal solute load which is harmful to the already malnourished children due to risks associated with negative water balance and hypernatraemic dehydration (WHO, 2005b). Also, high dietary protein was confirmed to induce satiety and subsequently reduced energy intake due to alteration of leptin, a metabolic hormone which carries physiological function of informing the CNS about the amount of energy which is stored to regulate satiety and energy expenditure (Vu et al., 2017). This can be dangerous to the malnourished IYC especially those undergoing treatment. Therefore cassava based composites flour of the IBV “Lenga Tome” is also expected to carry functional role upon consumption by the IYC (6-23months).
4.8.3. Fat

The mean fat content of the composites was 1.7-3.4% with composites F8 and F9 displaying high contents that were not significantly (P>0.05) different. Composites F3 and F4 were significantly different. The overall fat content of the study was lower compared to the fat content (1.9-8.8%) of wheat-groundnut protein concentrate flour blend (Ocheme et al., 2018). The lower value could be due to sprouting of the green grams since fats are degraded by sprouting (Murugkar et al., 2013). Fats are the major energy providers. They contribute about 35% of the energy required and 90% of the energy retained from milk during the first 6 months of life (Delplanque et al., 2015). Upon consumption of cereal or cereal-legume based complementary foods, the infant energy reserve declined substantially by 30% during 17months, and drastically till 15% of it is left by the second year of life (Uauy & Dangour, 2009). However, there still remain a controversy about optimal level of fat in complementary foods and in diets for IYC. Nevertheless, following the prospective study on dietary fat intake in non-breastfed infants, Koletzko et al., (2000) found after feeding infants of ages 1, 4, 6, and 12 months with foods of respective energies of 44.8%, 42.9%, 37.4%, and 35.7% that, nutrients were within recommended values and weight gain was normal after calculation. Meaning, there is no reason of modifying total fat intake at this age. Their finding was in agreement with some of the recommendations from high income countries that, there should be no restrictions on fat intake for infants of age 6-11 months (Kim et al, 2009). However, according to WHO, (2003), fat intake of 30-40% of total energy is prudent for children (12-23months) because diet high in fats also reduces nutrient density. Except, if the diet is predominantly plant based, then 10-20g of fat or oil should be added to it per day to give extra energy and 5g (one teaspoon) of fat or oil should also be added if diet is of animal source (Kim et al, 2009). On this basis, the level of dietary energy of the composites encountered in this study showed that, the dietary fat content was suitable for feeding IYC (6-23months).

4.8.4. Carbohydrate

The mean carbohydrate content of the composites varied from 30.4-48.9%. Composite F4 had significantly (P<0.05) higher content compared to F3, F8 and F9 that also differed significantly (P<0.05). The overall contents of the composites were lower compared to the value (73.8-85.9%)
recorded for wheat-breadfruit-cassava starch composites (Ajatta et al., 2016). However, the proposed threshold of carbohydrates for infants (6-11months) is 40% of the total energy whereas for children (12-23months) is 55% of the energy (Stephen et al., 2012). Therefore, the range of carbohydrate content in the composites of the IBV “Lenga Tome” was within the recommended level for IYCF. Their porridges could therefore make a better complementary food for the IYC (6-23months). High carbohydrate is found of causing displacement of other nutrients in the foods as well as those one already in the body stores to enable their proper oxidation to liberate their calories as energy, causing malnutrition in IYC (Dinicolantonio & Berger, 2016).

4.8.5. Fibre

The mean dietary content of fibre in the composites was ranging from 2.1-5.1%. Composite F9 presented significantly (P<0.05) high dietary content compared to composites F3, F4 and F8 whose means also differed significantly (P<0.05). Generally, the dietary fibre (DF) registered in these composites was low as compared to 7.2-9.9% for cassava-cereal-legume blends (Padmaja & Sajeev, 2008). Decrease in the fibre content of the composites was recorded with decrease in flour of spinach and increase in the flour of green grams. This was in contrast with findings of Venkatachalam et al., (2017) that fibre content decreased with green gram content in composite of wheat and green gram flour. The difference could be attributed to the processing (dehulling) of green grams since the highest content of the fibre is highly concentrated in the hull (Eashwarage et al., 2017). Nevertheless, Meals that contain high DF are very mouth filling, difficult to swallow and reduce hunger quickly after ingestion. Such meals increase intraluminal concentration (viscosity), slow gastric emptying and create a mechanical barrier to enzymatic hydrolysis of macronutrients such as starch in the human intestinal tract (Dreher, 2015). The high fiber in these foods causes direct displacement of the energy dense foods and increases food bulk (volume) and satiety signals thus reducing chances of intake of other nutrients. However, adequate intake (AI) of DF is also cited essential for proper gut function and laxation and is also related to reducing risks of other diseases including cardiovascular, cancer and diabetes in humans (Slavin, 2013; Ministry of Health, 2007). However, no recommended AI of dietary fibre for infants (6-11months) is known so far but children (12-23months) are being recommended for AI of 10g per day (EFSA NDA Panel, 2013). Therefore servings of cassava based composites (porridges) of the IBV “Lenga
“Tome” for IYC (6-23months) is expected to provide AI of dietary fibre that could not deprive them from the other nutrients.

4.8.7. Energy

The mean energy density (ED) of the composites was in the range of 44.9-52.2Kcal with composite F4 displaying significantly high ED. Composites F3, F8 and F9 differed significantly (P<0.05). The ED of the composites in the current study was lower than 367-371Kcal of cassava-wheat composites reported by Girma et al., (2015). The difference could be from the methods of processing and type of enhancers used. The ED was also below the one expected of complementary food (CF) of the 200Kcal for infants (6-8months), 300Kcal for infants (9-11months) and 550Kcal for children of age 12-23months (WHO, 2009). However, it was above the recommended minimum density of the 0.8Kcal/g (Abeshu et al., 2016) expected of CF. Food for IYCF must provide adequate energy because low dietary energy causes bulkiness to the food and the IYC could not be able to eat sufficient amounts. They have limited gastric capacities and have energy requirements per unit body weights of about three times higher than for the adult humans (Kim et al., 2009). Their intensity of body functions are therefore much higher as well as the basal metabolic rate (1.5-2), though the maximum activity level is considerably lower than of the adults (Valentin & Tambovtsева, 2012). This accounts for the reason as to why the child’s body existence is always stressful. High energy dense food is prerequisite especially for the malnourished (wasted) children because they have increased energy requirements for catch up growth. There is therefore no wonder that the composite flours of the IBV “Lenga Tome” could cater for such body demands of IYC (6-23months).
Table 4. Proximate composition of cassava based composite flours per 20g

<table>
<thead>
<tr>
<th>Composites</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Fibre (%)</th>
<th>Carbohydrate (%)</th>
<th>Energy (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F3</td>
<td>10.4 ± 0.14c</td>
<td>12.9 ± 0.14c</td>
<td>2.9 ± 0.11d</td>
<td>2.1 ± 0.17d</td>
<td>36.4 ± 0.46c</td>
<td>44.9 ± 0.32e</td>
</tr>
<tr>
<td>F4</td>
<td>11.2 ± 0.08b</td>
<td>12.5 ± 0.10d</td>
<td>1.7 ± 0.55c</td>
<td>2.5 ± 0.04c</td>
<td>48.9 ± 0.39b</td>
<td>52.2 ± 0.23b</td>
</tr>
<tr>
<td>F8</td>
<td>9.8 ± 0.22d</td>
<td>17.7 ± 0.08b</td>
<td>3.4 ± 0.04a</td>
<td>3.6 ± 0.05b</td>
<td>33.5 ± 0.22d</td>
<td>47.0 ± 0.23c</td>
</tr>
<tr>
<td>F9</td>
<td>10.5 ± 0.15c</td>
<td>19.6 ± 0.32a</td>
<td>3.4 ± 0.05a</td>
<td>5.1 ± 0.09a</td>
<td>30.4 ± 0.18e</td>
<td>46.2 ± 0.28d</td>
</tr>
<tr>
<td>Control</td>
<td>12.6 ± 0.7a</td>
<td>6.1 ± 0.21c</td>
<td>1.5 ± 0.02d</td>
<td>1.6 ± 0.05c</td>
<td>59.8 ± 0.34a</td>
<td>55.5 ± 0.14a</td>
</tr>
</tbody>
</table>

The values were the means ± Standard deviations of data of triplicate determinations. Values in the same columns with the same superscript were not significantly different (P>0.05)
4.9. Pasting characteristics of cassava based composite flours

Knowing the pasting characteristic of the composite flour is particularly importance in food industry as it provides nutritionist with better understand of the effect of blending on such flour (Qazi et al., 2014). The mean pasting characteristics of the composites for viscosity load (trough viscosity, break down viscosity, final viscosity, set back viscosity) and pasting temperature is presented in Table 4.5.

4.9.1. Viscosity load

Generally, the viscosity load, peak (17.3-89.6 RVA), trough (2.9-23.0 RVA), breakdown (14.3-66.6 RVA), final (3.6-23.1RVA), and setback (0.0 -0.9RVA ) of the composites of IBV “Lenga tome” is comparatively lower than of the maize based composites (peak ; 944.0-3552.7 RVA), trough (567-1842.3 RVA), breakdown (377.0-1717.3 RVA), Final (963.0-3926.7 RVA), Setback (396.0-2084.7 RVA) reported by Jude-Ojei et al., (2017). The difference could be attributed to the high protein contents (12.49-19.59%) of the composites of the IBV “Lenga tome”. High protein restricts starch granules to be embedded within its rigid matrix which subsequently limits access of the starch granules to water hence restricting the swelling power (Tharise et al., 2014). Starch swelling power could also be restricted by resistant starch (Abioye et al, 2017); a starch fraction which resists digestion in the intestinal tract, though is valuable in human nutrition. The longer the time the resistant starch takes in the human intestinal tract, the more the mineral absorption takes place (Polesi et al., 2017) before it is fully digested in the colon (Zheng et al., 2016).

In the context of IYCF, the lower the viscosity load of the porridge, the more suitable it is. This is because porridge that forms viscous paste during cooking requires excessive dilution with water before it is suitable for consumption. However, diluting the porridge to reduce the viscosity results into reduction of energy and nutrients in the porridge (Amagloh et al., 2013). Since the infants and young children have small gastric capacity to consume the diluted porridge in large amounts to meet their energy and nutrient requirements, they eventually become malnourished (Temesgen,
Therefore, to improve on the nutritional status of IYC (6-23 months) in the community, cassava based porridge of the IBV “Lenga tome” could be used as an alternative complementary food for IYC of this age group.

4.9.2. Pasting temperature

Pasting temperature is the temperature at which the first increment in the viscosity is noticed in the amylogram (Mufumbo et al., 2011). It indicates the minimum temperature required for cooking the porridge (Nkundabombi et al., 2015; Romee et al., 2013). In this study the pasting temperature was found ranging from 72.5-76.6°C. This value was found higher than the one (71.0-72.3%) stated by (Enyinnaya et al., 2011) for cassava starch and soy protein concentrate blends. The difference could be from the ingredients (spinach, green grams and carrots) involved in formulating the composites. Porridge with high pasting temperature indicates high water-binding potential, high gelatinization tendency and low swelling power of the starch-based flour due to high degree of association of the starch granules (Awolu et al., 2017). High pasting temperatures cause irreversible changes to the porridge due to gelatinization of starch granules including melting of starch crystallites, starch solubilization and leaching of starch granules (Schirmer et al., 2015). This triggers rapid digestion of starch which subsequently cause abrupt increase in blood glucose level after intake (Polesi et al., 2017). However, in IYCF, starch should be provided in a readily digestible form to ensure that its energy value is realized (Abeshu et al., 2016). Children with small blood volume are particularly at risk of hyperkalemia due to both volume or size considerations and the development of renal function of IYC (Palmieri, 2017). Therefore, cassava based composite flour of the IBV “Lenga Tome” could be one of the suitable complementary food for IYCF.
Table 4.5 Pasting properties of cassava based composite flours

<table>
<thead>
<tr>
<th>Pasting properties</th>
<th>Composites</th>
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<tr>
<td></td>
<td>F3</td>
<td>F4</td>
<td>F8</td>
<td>F9</td>
<td>Control</td>
</tr>
<tr>
<td>Peak viscosity $V_P$ (RVU)</td>
<td>33.9 ± 1.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>89.6 ± 1.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.8 ± 0.95&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>17.3 ± 0.55&lt;sup&gt;d&lt;/sup&gt;</td>
<td>271.3 ± 14.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Trough viscosity $V_T$ (RVU)</td>
<td>3.2 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.0 ± 0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.4 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.9 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>159.3 ± 4.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breakdown viscosity $V_B$ (RVU)</td>
<td>30.8 ± 1.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66.6 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.4 ± 0.85&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>14.3 ± 0.51&lt;sup&gt;d&lt;/sup&gt;</td>
<td>112.0 ± 10.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final viscosity $V_F$ (RVU)</td>
<td>4.1 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.1 ± 0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.1 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.6 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>165.1 ± 4.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Setback viscosity $V_S$ (RVU)</td>
<td>0.9 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.6 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.8 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pasting temperature (°C)</td>
<td>73.7 ± 0.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.5 ± 0.39&lt;sup&gt;d&lt;/sup&gt;</td>
<td>75.5 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.6 ± 42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.9 ± 0.38&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*The values were means of data of triplicate determinations. Values in the same columns with the same superscript were not significantly different (P>0.05)*
4.10. Sensory evaluation and overall acceptability of cassava based composite flours

The sensory attributes of a given food product are the most important aspects that influence its preference, selection and acceptability by the child (Izdehar, 2016). The results for the sensory evaluation of cassava based nutrient dense porridge (CBNDPs) of the IBV “Lenga Tome” for appearance, aroma, mouth feel, taste and overall acceptability are presented in table 4.6.

4.10.1. Appearance

The most important attribute of any food’s appearance is its color (MacDougall, 2003). Color is one of the visual cues that influences the gustatory, olfactory, oral-somatosensory, and the overall multisensory flavor percept of the food (Spence et al., 2010). It stimulates the appetite of the child to accept the food (Dias et al., 2012) or can even instigate neophobia, meaning, it influences food preference and nutrient intake (Decosta et al., 2017). In this study, it was observed that fortifying the cassava flour with flours of green grams (V.radiata), spinach (Soleracia) and carrots (D.carota) had altered the color of cassava flour and eventually the CBNDPs. The porridges were rated and found that, the one of composite F3 was highly preferred in same way as of the control. No significant (P>0.05) difference was observed between porridges of F4 and F8, while that of F9 was rated the lowest (disliked slightly). Therefore, composite F3 is expected to contribute tremendously in busting the nutrient density of the IYC in the community.

4.10.2. Aroma

Concerning the aroma, porridges of composites F3, F4, and F8 were not significant (P>0.05) different and were slightly liked, whereas composite F9 scored the lowest means of acceptance as was slightly disliked. Aroma (smell) and taste of the food are the important sensory attributes that bring out the appreciable flavor (Barrett et al., 2010). Therefore, food that are formulated for IYCF should be of appealing smell because these children especially the non-breastfed ones were not exposed to the scent of different foods of the maternal diet in the breast milk, they cannot therefore accept food easily (Monte & Giugliani, 2004). Breast milk contains all the nutrients including fats,
carbohydrates, proteins, vitamins and minerals that IYC need especially during the first six months of life (WHO, 2009). Therefore, IYC who are frequently breastfed generally receive plenty of these nutrients, however, the non-breastfed ones need to obtain such nutrients from other sources including formulated foods (WHO, 2005a). It should therefore be noted that composites F3, F4 and F8 are the best candidates for IYCF in this case.

4.10.3. Taste

Children are predisposed to prefer high energy dense foods to reject newly formulated ones and learn the associations between food flavors and the post-ingestive consequences of eating (Cosmi et al., 2017). However, the ability of food to suppress appetite post-ingestion depends on the ore-sensory experience of eating (McCrickerd & Forde, 2015). Infants do accept certain tastes at specific times during early life (Marion et al., 2015), and is difficult for them to change from one formula to another at later infancy once they are acquainted with a specific formula taste in their early infancy (Harris & Mason, 2017). That is why sensory evaluation is important at any stage a child is introduced to a newly formulated complementary food. The porridges of composites of the IBV “Lenga Tome” were rated and found that composite F3 was liked slightly and was the highest in acceptability as compared to the other composites (F4, F8 and F9). Porridge of the control was recorded with significantly (P<0.05) high means of acceptance. This therefore justifies that caregivers could also take time to adapt into a newly introduced product in similar way as the IYC, based on the literature. It can therefore be concluded that food which is appealing to the caregiver is also appealing to the IYC. This therefore gives a ground that the IYC would get use to the porridge of IBV “Lenga Tome” when they are continuously feed on.

4.10.4. Mouth feel

Mouth feeling refers to as the tactile (feel) aspect of texture (mechanical and surface) perception of the food during time of placing in the mouth until it is masticated and swallowed (Stokes et al., 2013). Feeding IYC (6-23 months) with varied textures of home formulated foods predicts the nutrient intakes of such foods by this age group even when they are 7 years, than when they are fed on ready-made foods (Coulthard et al., 2010). Therefore, sensory properties of food offered
during period of complementary influence initial food acceptance as well as the patterns of food intake later in life. In this study, the acceptability of CBNDPs made from various composites in terms of mouth feel revealed that, composite F3 scored the highest means and was not significantly (P>0.05) different from the control. Also, no significant difference was observed between F4 and F8, while F9 was registered with the lowest means as being disliked slightly. Therefore the preference of composite F3 was an indication that the food will increase the intake of the concerned nutrients for the IYC (6-23 months) in the community.

4.10.5. Overall acceptability

With respect to overall acceptability, porridge of composite F3 was found with the highest level of acceptance (liked moderately) and was not significantly (P>0.05) different from that of control. There was no significant difference between porridges of composites F4 and F8 (they were liked slightly) whereas that of composite F9 was registered with significantly (P<0.05) lower level of acceptance (was disliked slightly). Therefore, composite F3 was qualified for making nutrient dense porridge (CBNDP) for the IYC in the community.

Table 4.6. Mean scores of sensory attributes and overall acceptability of cassava based nutrient dense porridges from composites

<table>
<thead>
<tr>
<th>Composites</th>
<th>Appearance</th>
<th>Aroma</th>
<th>Taste</th>
<th>After taste</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>F3</td>
<td>7.47 ±153\textsuperscript{a}</td>
<td>6.43 ±168\textsuperscript{a}</td>
<td>6.23 ±23\textsuperscript{ab}</td>
<td>6.23 ±23\textsuperscript{ab}</td>
<td>7.37 ±1.16\textsuperscript{a}</td>
</tr>
<tr>
<td>F4</td>
<td>5.57 ±1.98\textsuperscript{b}</td>
<td>5.87 ±2.05\textsuperscript{a}</td>
<td>5.33 ±2.23\textsuperscript{bc}</td>
<td>5.33 ±2.23\textsuperscript{bc}</td>
<td>6.33 ±1.67\textsuperscript{b}</td>
</tr>
<tr>
<td>F8</td>
<td>6.07 ±1.91\textsuperscript{b}</td>
<td>5.70 ±2.00\textsuperscript{a}</td>
<td>5.03 ± 2.25\textsuperscript{cd}</td>
<td>5.03 ± 2.25\textsuperscript{cd}</td>
<td>5.57 ±191\textsuperscript{b}</td>
</tr>
<tr>
<td>F9</td>
<td>4.50 ±2.66\textsuperscript{c}</td>
<td>4.63 ±2.55\textsuperscript{b}</td>
<td>4.10 ±2.11\textsuperscript{d}</td>
<td>4.10 ±2.11\textsuperscript{d}</td>
<td>4.60 ±2.67\textsuperscript{c}</td>
</tr>
<tr>
<td>Control</td>
<td>7.57 ±1.33\textsuperscript{a}</td>
<td>6.37 ±2.03\textsuperscript{a}</td>
<td>7.03 ±1.65\textsuperscript{a}</td>
<td>7.03 ±1.65\textsuperscript{a}</td>
<td>7.53 ±1.46\textsuperscript{a}</td>
</tr>
</tbody>
</table>

The values given were the means ± Stand deviations of the data. Values in the same columns with the same superscript were not significantly different (P>0.05). The scores of sensory quality attributes; 1=disliked extremely, 2=Disliked very much, 3=disliked moderately, 4=disliked slightly, 5=neither liked nor disliked, 6=Liked slightly, 7=liked moderately, 8=liked very much, 9=liked extremely
CHAPTER FIVE

5. RECOMMENDATION AND CONCLUSION

5.1 Conclusion

It was found in this study that cassava roots of the South Sudanese indigenous bitter variety (IBV) “Lenga Tome” from Kajo-Keji County can make better flour with low cyanide and appreciable color after soaking (laboratory) fermentation for formulating composites suitable for feeding IYC (6-23 months). Nutrients of public health concerns (iron and provitamin A), and protein, that are insufficient in the cassava, are being improved by blending with the green grams (V.radiata), spinach (S.Oleracia) and carrots (D.carota). The heat labile nutrient (provitamin A) was retained in high amount using a modified solar dryer established in this study for use by the rural poor populations. Among the formulated composites, F3 was rated the most suitable composite for making cassava based nutrient dense porridge (CBNDP) in the community for IYC (6-23 months) because of its good appearance, aroma (smell) and taste. Similarly, this composite was among those that had high % contribution of iron, provitamin A, protein and energy to the RDA of IYC (6-23 months). Its adaptation can be of great importance in reducing malnutrition among the age group, though the green color of the spinach had reduced its acceptability to a lesser extent. The antinutritional factors particularly phytate that is implicated in making nutrients such as iron, zinc and calcium unavailable in plant based diets was reduced to the extent that it cannot interfere with the bioavailability of iron in the composites of IBV “Lenga Tome”. Provitamin A was found bioavailable as well. However, while adapting this product in the community for IYCF, the unit operation of the current study must be adhered to, including rationing of the treatment levels and serving sizes of the composites (porridge) for the IYC (6-23 months) to avoid toxicity due to cyanide and nutrients especially iron.
5.2. Recommendation

Since blending cassava flour with flours of green grams (*V.radiata*), spinach (*S.Oleracia*) and carrots (*D.carota*) had increased the density of the targeted nutrients (iron, provitamin A, protein and energy in the composites, it can be recommended that utilization of these ingredients in the formulation of complementary foods should be promoted in the communities. The rural populations should use the Modified solar dryer (MSD) for drying foods especially those targeted for the heat labile nutrients such as provitamin A to reduce the incidence of micronutrient malnutrition.

Composite F3 of the IBV “Lenga Tome” is recommended for preparing CBNDPs for IYC (6-23 months). Composites F8 and F9 can also make good porridges for IYCF however, more improvement is needed in terms of color to stimulate the appetite and aroma (smell) to bring out appreciable flavor of the porridge for the IYC to accept. All composites apart from F4 delivered appropriate % contribution of iron, provitamin A, protein and energy to the RDA of IYC (6-23 months), therefore composite F4 is not recommended for making porridge for the age group.

Iron and provitamin A were bioavailable in all composites apart from F4, however this master’s dissertation only used proxy indicator for determining bioavailability of the nutrients. More investigation will have to be conducted to assess this subject thoroughly especially by use of *in vitro* Caco-2 cell model for the nutrient bioavailability of these composites, and in long ran, results have to be confirmed with *in vivo* experiments and real human study for this product to gain a stronger fame in use for IYCF.
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APPENDICES

Appendix 1. Map of South Sudan showing food security situation of the states, counties and location of Kajo-keji. Source; (WFP, 2012)
Appendix 2. Traditional processing of cassava flour in Kajo-keji county-South Sudan

2(a) The freshly uprooted cassava root, 2(b). Peeling of the cassava root, 2(c). Scraping of soil or mold on the heap fermented cassava roots, 2(d). Sun-drying of the chips from heap fermented cassava roots

Appendix 3. The Modified Solar Dryer (MSD) for fruits and vegetables

3(a) Bojo was constructing the MSD, 3(b). Bojo was spreading the vegetable (slices of carrots) on the pallet for drying, 3(c). The drying process of the vegetables
Appendix 4. The Nine-point hedonic scale for testing the sensory attributes of the Cassava Based Nutrient Dense Porridges (CBNDPs); Source (Wichchukit & Mahony, 2014).

<table>
<thead>
<tr>
<th></th>
<th>DISLIKE EXTREMELY</th>
<th>DISLIKE VERY MUCH</th>
<th>DISLIKE MODERATELY</th>
<th>DISLIKE SLIGHTLY</th>
<th>NEITHER LIKE NOR DISLIKE</th>
<th>LIKE SLIGHTLY</th>
<th>LIKE MODERATELY</th>
<th>LIKE VERY MUCH</th>
<th>LIKE EXTREMELY</th>
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(a)  

(b)  

Rating Scale: 

- **Like the least** or **dislike the most**
- **Neither like nor dislike**
- **Like the most**
Appendix 5: Photographs of Cassava Based Composite Flours (CBCFs); FD composites enriched with green grams (*Vigna radiata*), Spinach (*Spinacia oleracia*), Carrots (*Daucus carota*)