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**Research Article**

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# Formulation and Iron Bioavailability in Complementary Foods Processed with Traditional Foodstuffs in Eastern of the Democratic Republic of Congo

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## *ABSTRACT*

Consumption of low iron bioavailability foods is the main contributing factor of iron deficiency for young children, a major public health problem especially common in our low income African countries. This study aimed at utilizing traditional foodstuffs to develop nutrient dense and high iron bioavailability complementary foods in Eastern D.R. Congo. Nutrient and anti-nutrient contents analysis were carried out using appropriate methods. The complementary foods were formulated by extrusion cooking and ground into flour that could be reconstituted into porridge for child feeding as recommended by Codex alimentarius. In-*vitro* iron availability was measured as Fe (II) bioavailability obtained by a method combining in-*vitro* protein digestion and dialysis (IVPD-dialysis). Phytic acid content was done by HPLC analysis method of phytic acid with modifications. The foods were formulated to meet the *Recommended Dietary Allowance (*RDA) for 6 month olds. Three (3) formulated complementary foods (CF0221, CF0322 and CF0423) composed of four (4) selected traditional foodstuffs: non-germinated amaranth grain (*Amaranthus cruentus*), maize (*Zea mays*), termites (*Macrotermes subhylanus*) and *dagaa* fish (*Rastreneobola argentea*) result in this investigation. Complementary foods developed and precooked products contained 914.5-1234.0 mg/100g phytic acid and <5% bioavailability of non-heme iron. Phytate/iron molar ratio was beyond the critical limits for all the foods. The foods contained up to 3.3% bioavailable iron after pepsin digestion and up to 2.5% bioavailable iron after pepsin + pancreatin digestion. This study provides evidence that Eastern D.R. Congo has traditional foodstuffs which are nutrient dense with bioavailable iron content. It is therefore recommended that both traditional animal and plant foods be exploited. Facilitation to commercialize and patent the process and products should be done to enable full exploitation.

**Keywords:** food processing, traditional ingredients, weaning food, nutritional value, anti-nutrient, malnutrition, anemia

## Introduction

Iron deficiency is a major public health problem especially common in countries with low income.The most affected are South Asia and Africa with 1.62 billion people affected worldwide, according to the World Health Organization (WHO) 1993-2005 databases. The deficiency or nutritional anemias are due mainly in tropical zones to iron and/or folic acid deficiency (Aubry and Gaüzère, 2015). 43% children under 5 years old in the world, and 67% children under 5 years in sub-Saharan Africa, suffer for anemia (Stevens et al., 2013). This disease corresponds to a ferritin level of less than 10µg/L, a Mean Corpuscular Volume (MCV) less than 73-75 femtoliters (fL), a coefficient of

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transferrin saturation of less than 10-12%, confirmed by measurement of serum iron (Sazawal et al., 2006; Beutler and West, 2005). The children's anemiais associated with a high risk of morbidity and mortality. For infants and young children, anemia causes general weakness and affect the motor and mental development if it is not quickly corrected (Stoltzfus et al., 2001). These adverse consequences are partly irreversible and result in absences in class, repetition, and lower school performance (Bobonis et al., 2006; Soemantri et al., 1985).

The medical literature points out that even if the cause of iron deficiency anemia in young children may be multifactorial, food consumption in low iron bioavailability of a hand is probably the main contributing factor and a second.Under-nutrition, which is the results from not getting enough protein, energy or other micronutrients and vitamins: iron, folic acid, vitamins A, B6, B12, C, etc. is also one of the specific causes of anemia (Aubry and Gaüzère, 2015; Earl and Woteki, 1993; Yip, 1989).

Equally, inadequate food intake is one key cause of malnutrition. Usually, in resource poor settings, the availability of nutritionally sound complementary foods is a major limitation in ensuring optimum feeding of infants and young children after 6 months of age when breastfeeding alone becomes inadequate (Dewey, 2005). In addition, complementary foods often fail to meet the nutritional requirements of infants (WHO, 2008) because they are mostly plant foods with low energy and nutrients concentration and contain anti-nutrient factors such as phytic acid, tannins and phenolics that limit the absorption of essential micronutrients (Towo et al., 2006; Gibson *et al.,* 1998a). Mineral deficiencies are likely to occur if these foods are processed in a way that does not ensure enhanced bioavailability of the micronutrients (Towo et al., 2006).

Protein-energy malnutrition and micronutrient deficiencies begin during weaning and/or immediately thereafter as most foods used for weaning do not provide adequate amounts of energy, protein and micronutrients (Mosha and Bennink, 2005). In Africa, traditional weaning foods are based on starchy cereals such as maize (*Zea mays*), sorghum (*Sorghum bicolor* L), finger millet (*Eleucine coracona*) and rice (*Oryza sativa*) or non-cereals such as cassava (*Manihot esculenta*), round potato (*Solanum tuberosum*), sweet potato (*Ipomea batatas*), yams (*Dioscorea* spp.) and plantains (*Musa paradisiaca sapientum*). These foods have been widely associated with nutrient deficiencies among pre-school age children (Walker, 1990).

Developing nutrient dense, low-cost weaning foods from the locally available food ingredients has been strongly recommended as a viable and sustainable approach to address the problem of nutrient deficiency in developing countries (WHO/UNICEF, 1998; Dijkhuizen, 2000; WHO, 2002; Dewey and Brown, 2003; Lutter, 2000; Mensah and Tomkins 2003; Lutter 2003; Lutter and Dewey, 2003). Such foods would provide reliable options for many families and can thus contribute immensely in ameliorating protein-energy and micronutrient deficiencies among older infants and young children (WHO/UNICEF, 1998; Dewey and Brown, 2003). These nutrient dense, low cost foods must be stable during storage so as to allow ample time for transportation, storage, and marketing while still maintaining their nutritional and sensory wholesomeness (King and Burgess, 1993). Foods should also be devoid of harmful germs and other microorganism-derived chemicals that might pose a health risk. They should also not include any other dangerous or harmful compounds in quantities that might endanger the health of newborns or young children (Codex, 1991).

The potential of utilizing traditional foodstuffs and traditional food processing techniques in developing nutrient dense complementary foods is a viable one. Amaranth grain has in the recent past been promoted as a healthy food for lactating mothers and infants in Eastern D.R. Congo after years of neglect (Muyonga et al., 2008). Consumption of termites and *dagaa* fish is a common practice in Eastern D.R. Congo and exploiting the resource in improving infant and child feeding is both viable and acceptable. The two types of food are rich in minerals and essential fatty acids.

A major contributor of the widespread childhood micronutrients deficiency in Eastern D.R. Congo is inadequate complementary feeding both in quantity and quality due to over reliance on cereals for complementary feeding. Recent second national Demographic and Health Survey 2013-2014 shows that 72% of infants are weaned using cereal based complementary foods. These foods have high bulk density and high anti-nutrient content and therefore do not offer adequate nutrient density and bioavailable minerals. The complementary foods are also lacking in animal source foodstuffs partly due to high costs, limitations on processing techniques and insufficient knowledge on nutritional and health benefits (EDS, 2014). With civilization, indigenous processing methods which are affordable and likely to enhance nutrient density and bioavailability have been neglected. A strategy to combine plant and animal foodstuffs in development of complementary foods will result in adequate nutrient density and high mineral bioavailability (Kinyuru, 2012).

WHO recommends the use of traditional foodstuffs in complementary feeding. Eastern D.R. Congo in general and the territories of South Kivu province in particular havediverse traditional plant and animal foodstuffs most of which are nutrient rich (Ombeni, 2015; Ombeni and Munyuli, 2016; Matabaro et al., 2017). Animal foodstuffs have high mineral content; the minerals are more digestible and have been found to improve bioavailability of minerals in plant foodstuffs. Appropriate indigenous processing techniques will reduce anti-nutrients thus further improve mineral bioavailability. The foodstuffs are culturally acceptable and affordable in Eastern D.R. Congo and uptake of the developed foods will be high (Ombeni, 2015). Traditional grains in Eastern D.R. Congo have anti-nutrients and reducing them is necessary before processing to complementary foods. Germinating amaranth grain may reduce phytic acid further improving mineral bioavailability. Termite and *dagaa fish* can be utilized in processing nutrient dense and acceptable complementary foods (Ombeni and Munyuli, 2016).

This study is therefore aimed at optimizing a process to develop a safe complementary food with high *in-vitro* iron bioavailability, because traditional foods are rich in nutrients and maybe utilized in development of nutrient dense complementary foods.

# Materials and Methods

## Study design

The study was cross-sectional, with random sampling to allow for generalisation of findings. The methodological protocol closely followed a multidisciplinary strategy that combined botanical inventory collection of voucher plant specimens, and taxonomic assessment, as well as semi-structured and informal interviews (Martin, 1995; Grenier, 1998), which resulted in a list of Bashi, Barega and Bafuliro (local) names of the different foods mentioned. Secondary data on the nutritional content of the foods was obtained from food composition tables.

## Field survey

This field survey targeted 50 women of child bearing age identified using a snow balling technique beginning with a Community Health Worker (CHW). The study was carried out in five selected communities of South Kivu province namely: Walungu, Kabare, Kalehe, Mwenga and Uvira. The purpose of the study was explained to the selected mothers and oral consent to participate in the study was sought. Only consenting mothers were included in the study. Some respondents' homes and agricultural plots were chosen at random for food species surveys. Scrubs, thickets, grasslands, kitchen gardens, farmlands, built-up regions, hedges, and wastelands were all used to harvest traditional food species.

## Key informant interviews

Six (6) key informants were purposefully chosen with the assistance of interviewees, elders, and local administrators, each representing a hamlet within the study area. The interviewer read out the names of items and asked why they were eaten. The survey tool provided a list of all plant and animal foods identified during the field study mentioned above. The goal was to discover why the previously mentioned foods were consumed and to establish whether consumption of these foodstuffs was associated with nutritional and/or health benefits. The informants used phrases like "increase of blood levels," "addition of energy," and "enhancement of breast milk by lactating mothers" frequently. Food availability and preparation methods were investigated.

## Sampling design

Traditional foodstuffs were obtained from the local markets within Eastern DR Congo following the field survey. However, sample collection spread beyond South Kivu province to the wider Eastern DR Congo in order to get a more representative picture of the foods in Eastern DR Congo. Six samples of foodstuff weighing 0.25kg – 2kg each were sampled randomly from the local markets. Amaranth and maize samples were packed in kraft paper bags and sealed to avoid loss and transported to the laboratory. Insect and *dagaa* fish samples were packed in 300 gauge zip-lock polythene bags, packed in cool boxes lined with frozen ice packs and transported by airplane to the food analysis laboratory at Jomo Kenyatta University of Agriculture and Technology, Nairobi-Kenya.

A 200 g portion of each sample was milled to a fine powder within 24 hours of reception at the laboratory. They were then stored at -20°C until analysis. Edible portions of each foodstuff were analyzed in duplicate. Proximate composition (moisture, protein, fat, ash, dietary fibre, available carbohydrates); iron, zinc and calcium content; fat composition; phytic acid, tannins and total free polyphenols in plant foods were analysed. Whole grains were analysed while the insects were de-winged before analysis.

## Description of food optimization methodology

Amaranth grain (*Amaranthus cruentus*), commonly called "Lengalenga", white maize (*Zea mays*), *dagaa* fish (*Rastreneobola argentea*) "Ndagara, Kabuchungu"' and winged termite (*Macrotermes subhylanus*) commonly called "Iswa (Lega, Kiswahili), Bushoun'gwé (Mashi)" formed the basis forthe formulations of complementary foods (Photos 1, 2, 3, 4).

The selection of amaranth grain among othertraditional plants consumed in Eastern D.R. Congo was done according to Kinyuru (2012) from Kenya and Mbemba (2013) from Bandundu-D.R. Congo, because it has lower phytic acid levels than other plant foods evaluated. This means that amaranth may be processed into complementary foods with high iron bioavailability compared to phytates. For termite and *dagaa* fish, as being an animal food is a source of more bioavailable heme iron (Kinyuru, 2012). Inclusion of edible termites and *dagaa* fish during this processing of complementary foods is therefore a strategy to improve non-heme iron bioavailability abundant in plant foodstuffs. These animal foodstuffs according to Mbemba (2013) are also major sources of iron and zinc among other minerals.

**Photo 1.** Amaranth plant (head loaded with grains) (*Amaranthus cruentus*)

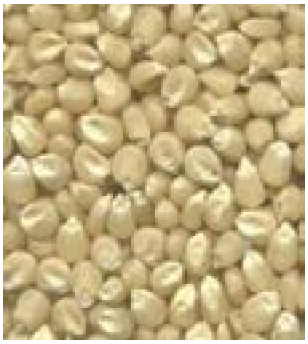


**Photo 2.** White maize grain (*Zea mays*)

**Photo 3.** Dried *dagaa* fish (*Rastreneobola argentea*)

**Photo 4.** Winged termites (*Macrotermes subhylanus*)

It is hoped that being animal foods, they may enhance non-heme iron bioavailability (Sorensen et al., 2017a). Therefore varying amounts of amaranth grain with termite and *dagaa* fish may achieve high iron content, low phytic acid content and high iron bioavailability.

Extrusion cooking has significant nutrient retention owing to high temperature and short time required in addition to retaining natural color, flavor and reducing microbial load of the food (Fellows, 2000; Bhandari et al., 2001). Singh et al., (2007) reported that extrusion cooking has marginal effect on phytic acid and therefore its effect on phytic reduction was not expressly evaluated in this study.

Pathogenic bacteria are a major health hazards in animal source foods such as fish (Owaga et al., 2009). The challenge is complicated by the increased manual handling during harvesting and drying of the *dagaa* fish and termite. Good harvesting practices and heat pre-treatment of the foodstuffs werenecessary to reduce the contamination. Blanching was utilized in order to reduce microbial contamination. The content of each ingredient was determined by manual iteration to optimize iron and zinc contents balanced with a moderate energy and protein content using MS Excel (2007 version). Combinations of these ingredients were incorporated into the original draft set of complementary foods to produce alternative sets of complementary foods by varying food amounts. Three foods, CF0221, CF0322 and CF0423 were formulated (Table 1). Energy content of selected ingredients were obtained from tropical Africa food composition tables by Sehmi, (1993) from Kenya and Lukmanji et al., (2008) from Tanzania (constituent of our Secondary

were formulated to meet the RDA for 6 month olds as follows; energy 400 Kcal, Protein 9.1 g, iron 9.3 g, zinc 4.6 g and calcium 400g (Codex 1991; WHO, 1998; FAO/WHO, 2002; FAO/WHO, 2004).

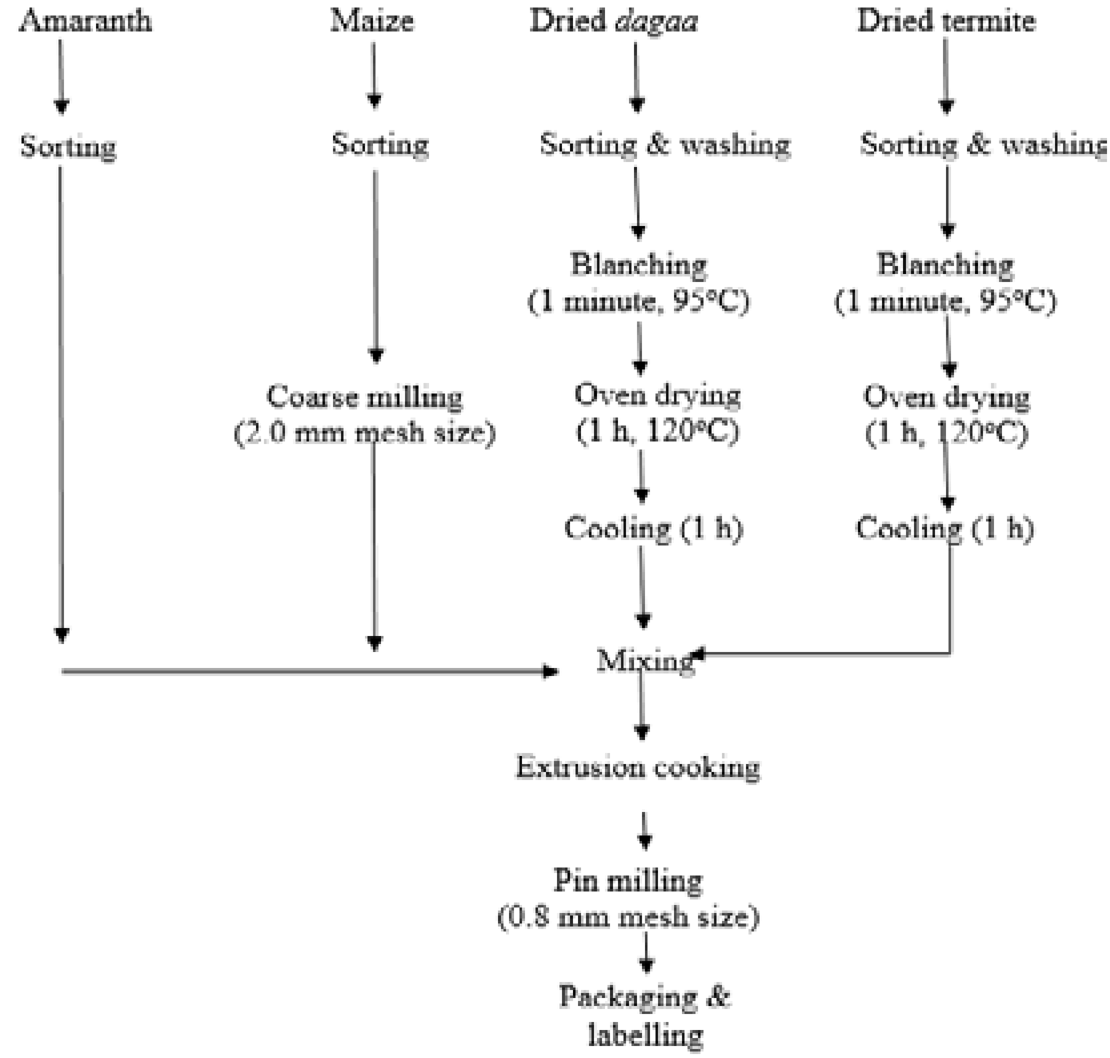
data) while other nutrients values were results of analysis in this research study (Primary data). The foods

**Table 1.** Formulations for CF0221, CF0322 and CF0423 complementary foods (%)

| Product | Amaranth grain | Maize | Dagaa fish | Termite | Soy bean oil | Sugar |
|---|---|---|---|---|---|---|
| CF0221 | 73.0 | 15.0 | 3.0 | 8.0 | 0.6 | 0.4 |
| CF0322 | 71.0 | 15.0 | 3.0 | 10.0 | 0.6 | 0.4 |
| CF0423 | 66.0 | 15.0 | 3.0 | 15.0 | 0.6 | 0.4 |

## Preparation of ingredients and processing of complementary foods

Amaranth grains were sorted to remove extraneous materials; *dagaa* fish and termites were sorted to remove pebbles and other extraneous materials, washed in clean water and blanched for 1 minute. A single layer of the blanched fish and termite was then spread on aluminium trays and oven dried for 1 hour at 120°C with continuous turning. The mixture was processed by extrusion cooking and ground into flour that could be reconstituted into porridge for child feeding as recommended by Codex (1991). Extrusion was carried out at All-grain co. LTD, Kenya, with a barrel length/diameter ratio of 25 using a locally fabricated extruder. The extrusion conditions were as follows: Feed moisture content 12-14.4%, moisture injection 9%, feed rate 12.5 kg/h, screw-speed 300 rev/min, barrel temperatures 70°C (zone 1), 100°C (zone 2), 127°C. The process flow for production of the complementary foods was as shown (Figures 1).



**Figures 1.** Flow diagram for processing of complementary foods

## Moisture, protein and mineral content

Moisture content was assayed by the drying method, crude fat by Soxhlet extraction method and crude protein by semi-micro-Kjeldhal method (AOAC, 1996). Nitrogen values obtained were converted to crude protein using a factor of 6.25. Crude ash was calculated by incinerating at 550°C in a muffle furnace. Dietary fibre was measured using the Prosky enzymatic gravimetric technique (AOAC, 1995). The difference between 100 and the total of the percentages of water, protein, fats, ash, and dietary fibre was used to compute the available carbohydrate value.

The quantification of iron, zinc and calcium was done by atomic absorption spectrometry (AAS) (Shimadzu AA-6200, Tokyo, Japan) according to AOAC methods (AOAC, 1996). Quantification was done using external standards (SigmaAldrich Chemie, Steinheim, Germany).

## In vitro non-heme iron bioavailability in complementary foods

This was determined according to a method described by Sorensen *et al.*, (2007a) with slight modifications. In-*vitro* iron availability was measured as Fe (II) bioavailability obtained by a method combining in-*vitro* protein digestion and dialysis (IVPD-dialysis). IVPD imitates the conditions in the duodenum and the proximal jejunum of the human gastrointestinal tract.

The reagents used were: Sodium taurocholate (500 mg/mL), porcine pepsin (885 units/mg protein; 5 mg/mL in 50 mM acetate buffer pH 4.5), porcine pancreatin (at least 3xUSP specification; 7.5 mg/mL in 1 mM HCl), trichloroacetic acid (TCA), PIPES buffer (piperazine - N, N'-bis [2-ethane-sulphonic acid] disodium salt; 75 mM, pH 6.1), HEPES buffer (N-2-hy droxylethylpiperazine- N'-2-ethanesulphonic acid, sodium salt; 0.3 M, pH 9.9), tris-glycine buffer (trishydroxymethylaminomethane (Trizma Base) (10.4 g/L), glycine (6.9 g/L), and EDTA (Tritriplex III) (1.2 g/L) in water and adjusted to pH 8), SDS solution (7.25 g/ L), and Ellmans reagent (5,5' dithio-bis-(2-nitrobenzoic acid; 0.35 g/L) were diluted in the tris-glycine buffer . All solutions and ferrozine chromogen solution (3-(2- pyridyl)-5,6 bis(4-phenyl sulfonic acid)1,2,4-triazine, disodium salt; 5 mg/ mL in water) were prepared from reagents purchased from Sigma - Aldrich (St. Louis, MO). The used dialysis tubing with a molecular weight cut-off of 6000–8000 Da was from Spectrapore®, Millipore. Slurry of the food was prepared by homogenizing with water. The homogenate was prepared to contain 50 mg protein/mL of slurry. To 7.5 mL of the homogenate, 10 mL 0.1 M HCl was added resulting in pH 2.5. The remaining 13.5 mL of homogenate were incubated with pepsin (1.40 mL) for 1 h in a 37°C water bath. Subsequently, aliquots (6.6 mL) were drawn and mixed with 3.4 mL water resulting in the pepsin digests. Finally, the residual assay mixtures were mixed with $NaHCO_3$ (2 mL, 600 mM), NaOH (100 mL, 1 M), pancreatin solution (2.15 mL), and sodium taurocholate (500 mL) resulting in pH 7, and further incubated for 1 hour at 37°C, resulting the pepsin/pancreatin digests. Dialysis was performed by placing a dialysis bag containing 1 mL of PIPES buffer in 5 mL of the different digests and continuing dialysis until equilibrium was obtained for 4 hours at 37°C with stirring.

Fe (II) bioavailability and iron-reducing capacity were measured immediately after dialysis by determining iron in the retentate and dialysate of pepsin-digested and pepsin/pancreatin-digested samples from IVPD dialysis using a micro-scale method based on reaction with chromogen ferrozine. The assay was adapted to microtiter plates. Fe (II) content was analysed by adding 100 mL non-reducing protein precipitate (TCA, 1 g; HCl, 37%, 1 mL , adjusted to 10 mL with water) to aliquots of dialysate and retentate, respectively (200 mL). The detection range of the microassay was between 4 mM and 0.5 mM Fe. The aliquots to which were added either non- reducing or reducing protein precipitate were left over night at room temperature followed by centrifugation (2575 g; 10 min). The supernatants and $FeCl_3$ dilutions (100 mL) in double determinations were placed in microtiter wells (96-well ELISA plates), HEPES (0.3 M; 200 mL) and ferrozine solution (5 mg/mL; 25 mL) were added and the absorbance done using the standard curve was used for sensitive estimation of iron content and oxidative status measured at 570 nm (ELISA reader EL340 Microplate) immediately after ferrozine addition for the quantification of Fe (II) and after 1hr for determination of total (Fe (II) + Fe (III)) iron content. This colorimetric assay has been optimised for the application in microtiter plates with the measurement of absorbance at 570 nm for sensitive estimation of iron content and oxidative status (Sorensen *et al.*, 2007b). Bioavailable Fe (II) and reducing capacity were calculated. Reducing capacity of Fe (II) is a measure of the soluble but not bioavailable iron.

% Bioavailability of Fe (II) = [(Fe (II) in dialysate (mM) x 1total volume (mL))/*total (Fe (II) + Fe (III)) (μmol) x 100.
% reducing capacity of Fe (II) = (Fe (II) in dialysate (mM) x dialysate volume (mL)] + [Fe (II) in retentate (mM) x retentate volume (mL))/*total (Fe (II) + Fe (III)) (μmol)] x 100

*Dialysate and retentate

## Phytic acid content

Determination of phytic acid was done by HPLC analysis method of phytic acid according to Camire and Clydesdale, (1982) with modifications as follows, 50 mg of sample was weighed into a 125 mL Erlenmeyer flask and 10 mL of 3% $H_2SO_4$ added. The flasks were shaken at a moderate speed on a shaker for 30 minutes at room temperature before being filtered with rapid filter paper (Shaker Model KS 250 basic, Germany). After 5 minutes in a boiling water bath (BWB), 3 mL of $FeCl_3$ solution (6mg ferric iron per mL in 3% $H_2SO_4$) was added. A 45-minute BWB heating was used to complete the precipitation of the ferric phytate complex. The supernatant was removed after 10 minutes of centrifugation at 2500 rpm. The precipitate was washed with 30mL distilled water, centrifuged and the supernatant discarded. Three (3) mL of 1.5N NaOH was added to the residues and the volume brought to 30mL with distilled water. Heating was done for 30 minutes in a BWB to precipitate the ferric hydroxide. Cooled samples were centrifuged and the supernatant transferred into a 50 ml volumetric flask. The precipitate was rinsed with 10 mL distilled water, centrifuged and the supernatant added to the contents of the volumetric flask. A sample of 20 µL of the supernatant was injected into a HPLC fitted with a 50377 RP-18 (5 µm) column at an oven temperature of 30 °C and a refractive index detector. The mobile phase was 0.005 N sodium acetate in distilled water and flowing at a flow rate of 0.5 µL/minute. Quantification was done using external standards (Merk IV, multi element standard solutions).

## Predicting mineral availability by phytate/mineral molar ratio

The mole of phytate and minerals was obtained and molar ratio was calculated by dividing the mole of phytate with the mole of minerals (Norhaizan and Nor, 2009).

## Determination of tannin

Tannin content was determined colorimetrically (Singleton and Rossi, 1965) with modifications as follows, 20g of samples were homogenized with 40 mL water and filtered. The filtrate was centrifuged at 4°C, 12000 rpm for 2 minutes. Then 0.1ml aliquot of the solution was mixed with 5.0ml of 0.2 N Folin-Ciocalteu reagent and 4.0mL off saturated sodium carbonate. After 2 hours, the absorbance was read at 765 nm in a spectrophotometer (UV mini 1240, Kyoto, Shimadzu, Japan). The concentration was quantified from a standard curve prepared using gallic acid.

## Determination of total free polyphenols

Total free polyphenol content was determined colorimetrically (Singleton and Rossi, 1965) with modifications as follows, 20 g sample was homogenized with 40 mL of water and filtered. The filtrate was centrifuged at 4°C, 12000 rpm for 2 minutes. A 0.1 ml aliquot of the solution was mixed with 5.0 mL of 0.2 N Folin-Ciocalteu reagent and 4.0mL off saturated sodium carbonate. After 2 hours, the absorbance was read at 765 nm in a spectrophotometer (UV mini 1240, Kyoto, Shimadzu, Japan). The concentration was quantified from a standard curve prepared using tannic acid.

## Approval of the study and Ethical consideration

Before the data collection of this study, ethical approval was obtained from the accredited-Local Ethic Committee ('LEC'): South Kivu Provincial Health Inspection (IPS) to conduct the research, approval number IRB/LEC/IPS-SK-43/18. The mothers were not forced to take part in the study and the purpose of the study was explained to obtain informed consent and further permission obtained from the local ethic committee, the chief and other local administrators. All mothers gave oral consent.

## Statistical analysis

All results are given as mean ± SD; n refers to the number of observations. The data was analysed by Student's t-test for paired or unpaired variates and $p<0.05$ was considered significant using SAS (2004) software.

# Results and Discussion

## Perceptions of nutritional, health benefits and availability of selected traditional foods

Perceptions on health and nutritional benefits of the traditional foods are summarised in Table 2. The most popular advantages included energy provision and 'enhancing' blood flow in the human body. Other health advantages included avoiding stomach discomfort, bloating, and constipation. The nutritional content of *Amaranth spp.* was supposed to enhance breast milk in breastfeeding women, according to popular belief. The winged termite (*Macrotermes subhylanus*) and dagaa fish (*Rastrineobola argentea*) were supposed to have body building properties, improve health, and 'increase' blood. A high iron level may be connected with 'increasing' blood.

Most of the items described were available in moderate to high quantities. However, jute mallow and oxalis availability wereevaluated as poor, making gathering sufficient quantities for product development difficult. The winged termites have been reported to be extensively accessible throughout the year, with dagaa fish in abundance. A significant informant bemoaned the loss in termite availability and linked this food resource's decline to increasing farming, particularly when pesticides were used.

[1] Source: National food composition tables and the planning of satisfactory diets in Kenya (Sehmi, 1993); [2] Source: Tanzania food composition tables (Lukmwanji *et al.*, 2008); - Common names or values missing

**Table 2.** Perceived nutritional and health benefits, availability of selected foodstuffs according to key informants and their iron and zinc contents

| Common name | Scientific name | Perceived nutritional & health benefits | Availability of the food | Iron[*1] (mg/100g) | Zinc[2] (mg/100g) |
|---|---|---|---|---|---|
| **Amaranth grain** | *Amaranthus cruentus L.* | Increases breast milk in mothers; Increases energy & blood; Reduces constipation | High | 21.0 | - |
| **White maize** | *Zea mays L.* | Source of energy | High | 4.5 | 1.8 |
| ***Dagaa fish*** | *Rastrineobola argentea* (Pellegrin) | Body building ; Promotes good health | High | 7.0 | 5.2 |
| **Winged termite** | ***Macrotermes subhylanus*** (Rambur) | Body building; Increases blood | Moderate | 21.0 | 2.5 |

Review of secondary data (Table 2) indicated that the selected foodstuff amaranth grain had the highest iron content (21.0 mg/100g). Winged termite had the highest iron content (21.0 mg/100g) than dried dagaa fish the animal source food selected. White maize had significant iron (4.7 mg/100g) and high energy content (Sehmi, 1993). Termites have high iron (Sehmi, 1993) and zinc (Lukmanji et al., 2008) content. Termites emerged to be widely consumed within the study area especially during the rainy season. *Dagaa fish* had iron content of 7.0 mg/100g (Sehmi, 1993).

Recently, much attention has been focused on determining the nutritional value of various insects ingested by the Bashi, Barega, and Bafuliro of South Kivu province. Some insects have been found to be a rich source of minerals, fat soluble vitamins and even highly digestible proteins (Muvundja et al., 2013; Ombeni and Munyuli, 2016) and also in Western Kenya (Christensen et al., 2006; Ayieko and Oriaro, 2008; Kinyuru et al., 2010a; Kinyuru et al., 2010b). Research has shown that consumption of edible larva of Westwood (*Cirina forda*) in Nigeria does not pre-dispose neurotoxicity or hepatotoxicity to study animals (Akinnawo et al., 2002). This can be further complemented by the fact that no toxicity disaster associated with consumption of insects has been reported by the consuming communities in South Kivu.

## Proximate composition

Table 3 presents the proximate composition of the selected traditional foods to make the complementary food. Amaranth grain had the highest protein (18.5 g/100g) and fat content (14.4 g/100g) than white maize. This was higher than amaranth varieties grown in Uganda, 12.0-13.0 g/100g protein content (Muyonga et al., 2008). Consumption of 100g of amaranth grain would provide 200% of Recommended Daily Allowance protein for 6 month old infants (WHO, 1998). The carbohydrates in amaranth grain consist primarily of starch made up of both glutinous and non-glutinous fractions (Muyonga et al., 2008; Teutonico and Knorr, 1985). Due to the unique size and composition of amaranth starch, the starch may exhibit distinctive characteristics which could be of benefit to the development of complementary foods (Singhal and Kulkarni, 1990a,b). Maize was found to compare with samples grown elsewhere within the tropical Africa (Sehmi, 1993; Muyonga et al., 2008). Of interest however, was the high carbohydrate level in amaranth and meaning they can be exploited as energy source foods for infant.

The fat content of the termites (44.8 g/100g) was lower than the values reported by Sehmi, (1993) for an unspecified dried termite (53.4 g/100g). The values were also slightly higher than those reported by Ombeni and Munyuli, (2016) for an unspecified dried termite Isopteria (41.63 g/100g) and those of *Nausitermes* spp. reported by Oyarzun *et al.* (1996) at 40.23%.

The protein content of the studied winged termite (39.3 g/100g) was within the range reported for an unspecified dried termite (35.7 g/100g) in NFCT (Sehmi, 1993). Consumption of 100g of termites would provide 3 - 4 times more protein than RDA for 6 month old infants (WHO, 1998). The protein content exhibited by the insects analysed in this study was significantly higher than that of red meats reported by Williams (2007) and therefore insects may offer an alternative source of protein to counteract the protein malnutrition in the Democratic Republic of the Congo. Protein profiles of insects indicate high protein quality, beneficial for human nutrition (Ombeni and Munyuli, 2016; Ramos-Elorduy et al., 1997; Verkerk et al., 2007, Kinyuru et al., 2010b), especially in an otherwise plant-dominated diet, typical in Eastern D.R. Congo. High fibre content was also found in the selected plant foods white maize and amaranth grain with 5.9 g/100g and 7.1 g/100g respectively and this may pose a challenge in utilizing these grains in complementary foods. The dietary fibre in termites (6.3 g/100g) is largely composed of chitin whose effect on the nutrients in a complementary food is not known.

**Table 3.** Proximate composition of selected traditional foodstuffs (g/100g)

| Foodstuff | Protein | Fat | Total ash | Dietary fibre | Available Carbohydrate |
|---|---|---|---|---|---|
| **Amaranth grain** | 18.5 ± 0.4 | 14.4 ± 0.6 | 2.5 ± 0.1 | 7.1 ± 0.1 | 63.8 ± 1.5 |
| **White maize** | 8.2 ± 0.2 | 4.8 ± 0.8 | 1.1 ± 0.1 | 5.9 ± 1.1 | 79.8 ± 1.0 |
| ***Dagaa fish*** | 58.1 ± 0.6 | 26.6 ± 0.9 | 11.1 ± 0.1 | 3.0 ± 1.0 | 11.0 ± 1.3 |
| **Winged termite** | 39.3 ± 0.1 | 44.8 ± 2.8 | 7.5 ± 0.1 | 6.3 ± 1.1 | 1.8 ± 0.7 |

Values are mean ± SD on dry weight basis, *n*= 6

## Calcium, iron* and zinc

Calcium, iron and zinc content were the minerals of interest in the selected traditional foods as shown in Table 4. Amaranth had the highest iron and zinc content than white maize. Muyonga et al., (2008) reported 17.0 mg/100g iron and 3.7 mg/100 g zinc content in amaranth harvested in Eastern Uganda. Amaranth grains contain twice the level of calcium found in milk, five times the level of iron in wheat (Becker et al., 1981).

This could however be explained by regional variations. Consumption of 100 g of amaranth grain would provide 1.6 times more iron than the RDA for 6 month old infants (WHO, 1998) consuming 100 g of the termites will provide 5-7 times more iron than RDA.

The most notable finding was the high calcium content in dagaa fish, as well as zinc and iron in the winged termite. Consumption of 100 g of dagaa fish would provide 8 times more calcium than the RDA for 6 month old infants (WHO, 1998). The high levels of calcium, iron and zinc of this insect and dagaa fish collected for this study are in line with previous studies in Africa (Christensen et al., 2006; Onigbinde and Adamolekun, 1998; Kinyuru et al., 2010a; Kabahenda et al., 2011). The bioavailability of the minerals from the fish and the termite is likely to also be higher than from the plant foods (Christensen et al., 2006) since animals contain heme iron which is more bioavailable

**Table 4:** Mineral composition of selected traditional foodstuffs (mg/100g)

| Foodstuff | Calcium | Iron* | Zinc |
|---|---|---|---|
| **Amaranth grain** | 12.2 ± 0.1 | 15.0 ± 0.1 | 3.4 ± 0.1 |
| **White maize** | 43.6 ± 3.0 | 9.4 ± 0.1 | 0.9 ± 0.1 |
| ***Dagaa* fish** | 3505 ± 6.2 | 25.7 ± 0.2 | 12.0 ± 0.9 |
| **Winged termite** | 58.7 ± 1.2 | 53.3 ± 1.4 | 8.1 ± 2.8 |

Values are mean ± SD on dry weight basis, n= 6

**Interested mineral in this study

## Anti-nutrient content

Phytic acid measured as myoinositol hexa-inositol phosphate (IP6), tannins and total free polyphenols composition of the traditional foods are reported in Table 5.

Maize had the lowest phytic acid content (318.5 mg/100g) indicating its appropriateness in complementary food development. Amaranth grain had highest phytic acid (1285 mg/100g).The tannin (2.2 mg/100g) and total free polyphenols (69.0 mg/100g) were highest in white maize. Other researchers have reported a wide variation of phytic acid content in amaranth grain. Becker et al., (1981) reported 200 mg/100g in *Amaranthus caudatus*, while 1.3

Other researchers have reported a wide variation of phytic acid content in amaranth grain. Becker et al., (1981) reported 200 mg/100g in *Amaranthus caudatus*, while 1.3 g/100g (1300 mg/100g) was reported in unspecified amaranth grain specie (Egli et al., 2004) and 300 mg/100g in *Amaranthus cruentus* (Colmenares and Bressani, 1990). Kanensi et al., (2011) reported 10 mg/100g phytic acid in *Amaranthus cruentus* from Kenya. This means phytic acid content in amaranth should be ascertained for the specific grain species in focus before utilization.

High phytic acid in both selected traditional plant foodstuffs pose a major challenge in formulation of food rich in bioavailable calcium, iron and zinc (Garcia-Estepa et al., 1999; Norhaizan and Nor, 2009). Phytic acid binds the minerals and results to insoluble salts with poor bioavailability of minerals in plant foods (Rhou and Erdman, 1995).

Therefore, a process to reduce the phytic acid content is essential in lowering the anti-nutrients during the processing of a complementary food using either amaranth grain. Phytic acid/mineral molar ratios were calculated and the findings presented as shown in Table 5.

All the samples analysed were beyond the critical limits for phytate/iron ratio and phytate/calcium ratio while phytate/zinc ratios in amaranth was beyond the critical limits (Norhaizan and Nor, 2009). This means that bioavailability of the minerals is low in this food if consumed in their current form. The ratio is an indicator of bioavailability of the minerals with respect to the set critical limits for each mineral.

The bioavailability of added and intrinsic non-heme iron is largely determined by the solubility of the iron in the upper gastrointestinal tract (Sorensen et al., 2007a). Dietary phytates inhibit non-heme iron absorption (Miller, 1996). Zinc and phytic acid form insoluble complexes and the negative effect of such complexes on zinc absorption can be predicted by phytate-to–zinc molar ratios when the dietary zinc intake is close to the requirement (Oberleas and Harland, 1981). Calcium-zinc-phytate complexes generated in the gut are significantly less soluble than phytate complexes formed by either ion alone, exacerbating the inhibitory action of phytate on zinc absorption (Egli et al., 2004).

## Moisture, protein, iron and phytic acid content in the developed foods

Moisture, protein, Iron and phytic acid content of the developed foods is shown (Table 6). The moisture content was within safe moisture content of below 12% to ensure a stable product in pre-cooked complementary foods (WFP, 2010).

**Table 5:** Anti-nutrient content of selected traditional plant foodstuffs (mg/100g)

| Foodstuff | Phytic acid | Tannins | Total free polyphenols | Phytate/ Iron ratio | Phytate/ Zinc ratio | Phytate/ Calcium ratio |
|---|---|---|---|---|---|---|
| **Amaranth grain** | 1285 ± 206.3 | 7.1 ± 1.4 | 30.3 ± 1.4 | 7.2 ± 1.1 | 36.3 ± 5.4 | 6.3 ± 1.0 |
| **White maize** | 318.5 ± 11.2 | 12.2 ± 0.2 | 69.0 ± 2.9 | 2.8 ± 1.0 | 2.8 ± 1.0 | 34.7 ± 1.2 |
| ***Critical limits**** | | | | 1 | 15 | 0.24 |

Values are mean ± SD on dry weight basis, *n*= 6

*Source: Norhaizan and Nor (2009)

**Table 6:** Moisture, protein, iron, phytic acid and molar ratio in the developed foods

| Food | Moisture (g/100g) | Protein (g/100g) | Iron (mg/100g) | Phytic acid (g/100g) | PA/Fe* molar ratio |
|---|---|---|---|---|---|
| **CF0221** | 8.0 ± 0.3$^b$ | 17.8 ± 1.2$^b$ | 16.2 ± 0.1$^b$ | 1234 ± 22.4$^a$ | 3.6 ± 0.3$^a$ |
| **CF0322** | 8.0 ± 0.1$^b$ | 17.5 ± 1.4$^b$ | 16.5 ± 0.1$^b$ | 1110 ± 31.6$^b$ | 3.2 ± 0.1$^b$ |
| **CF0423** | 8.8 ± 0.6$^b$ | 18.9 ± 0.8$^a$ | 17.0 ± 1.0$^a$ | 914.5 ± 15.6$^c$ | 2.5 ± 0.1$^c$ |

*Critical limit = 1

Mean ± standard deviation; *n*=6

Values on the same column with different superscripts are significantly different ($p<0.05$)

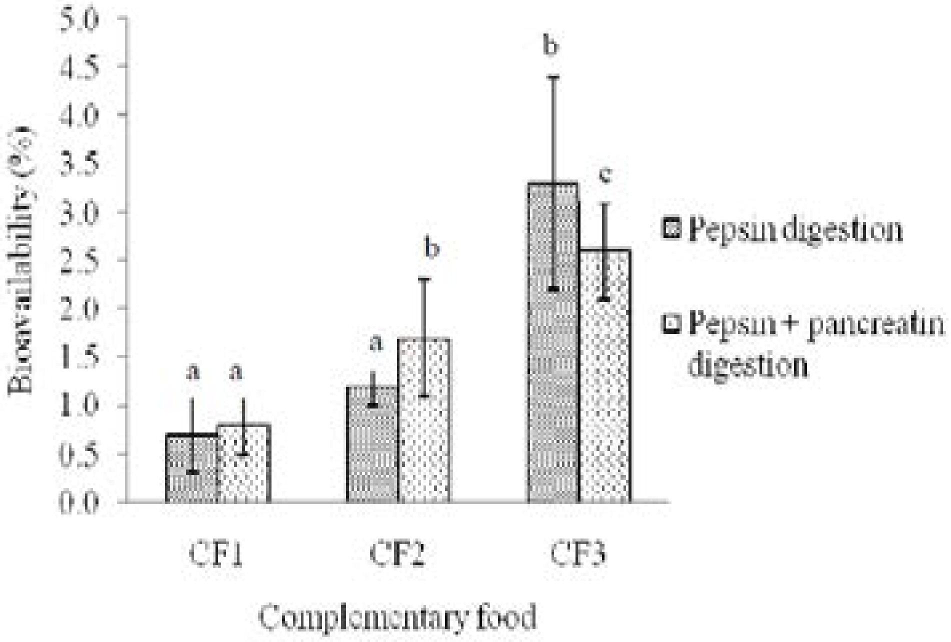


**Fig. 2.** In-vitro non-heme iron bioavailability of the complementary foods
Values on the same variable with different letters are significantly different (p<0.05)

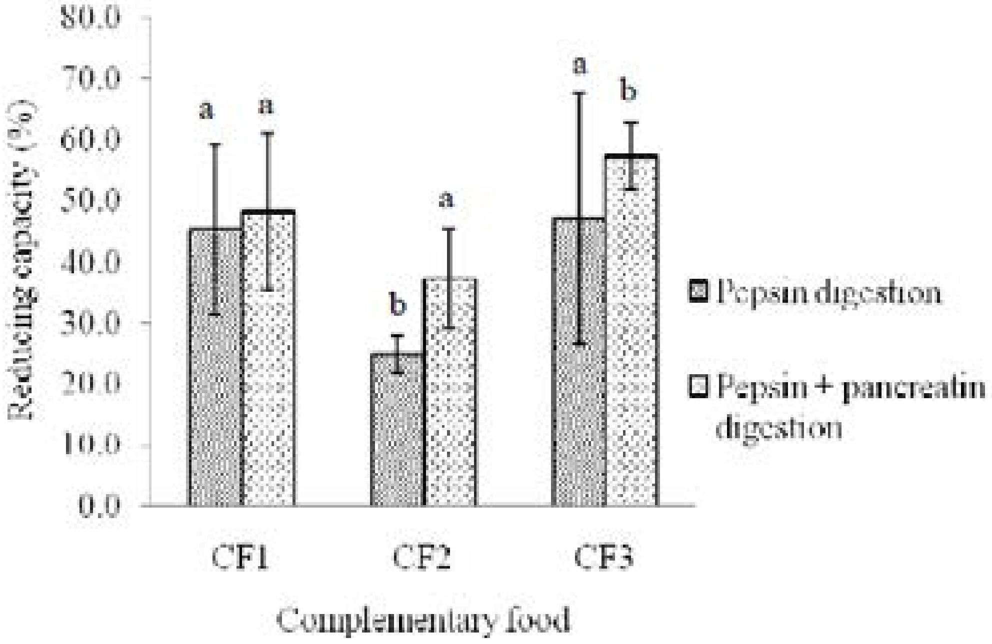


**Fig. 3:** Reduction capacity of the complementary foods
Values on the same variable with different letters are significantly different (p<0.05)

It is postulated that majority of iron absorption in vivo occurs in duodenum and the proximal part of jejunum. Gunshin et al. (2001) and Sorensen et al. (2007a) found that iron bioavailability should be higher on pepsin digestion than on pepsin+ pancreatin digestion. However, in this study only CF0423 showed such a tendency.

The findings of this study indicate that presence of termite in a food matrix increases Fe (II) bioavailability though the results are not significant at some of the levels of termite used in this study (Figure 2). This means that though the termite may enhance, there is possibility of an inhibitor in the food matrix that is prohibiting the gains. It is therefore important to significantly reduce the phytic acid before addition of iron enhancers such as animal source foods. Increasing the termite content in the formulation may be a strategy to further improve bioavailability of the iron but that is likely to affect the processability, storage and consumer acceptability properties due to the high fat and protein content of termite. The animal source foods in the formulation not only enhanced bioavailability of non-heme iron, but also contributed heme iron resulting in high amount of readily bioavailable iron in the foods.

The capacity to reduce Fe (III) to Fe (II) in the samples was higher in the pepsin+ pancreatin digested than pepsin digested samples in all the foods (Figure 3). Reducing capacity was not significantly different between CF0221 and CF0423 upon pepsin. High reducing capacity shows that a significant amount of Fe (II) is present in the digested samples in a non-bioavailable but yet soluble form, which could be polymeric soluble aggregates or Fe (II) complexes with phytates. Means of making this iron bioavailable should therefore be explored during processing of a complementary food with these ingredients so as to raise the level of available iron. A process that breaks complexes formed between iron and inhibiting food components such as phytates may also be employed.

# Conclusion

The findings of this study provide evidence that Eastern D.R. Congo has traditional foodstuffs which are nutrient dense and have hight bioavailable iron content. Availability of the animal foodstuffs within the study area suggests that they maybe be incorporated in cereal based foods to develop high energy, iron and zinc content complementary foods. Amaranth grain had the highest protein content (18.55 g/100 g) than white maize while *dagaa* fish had the highest protein content (58.1 g/100 g) than winged termite selected animal foodstuffs. Iron content ranged between 9.4–15.0 g/100 g among the plant foods and 25.7 g/100g and 53.3 g/100 g in *dagaa* fish and termite respectively. The animal foodstuffs had higher level of zinc (8.1-12.0 g/100 g) compared to the cereal grains (0.93–3.48 g/100 g). High anti-nutrient content in amaranth was found. Amaranth grain had the highest phytic acid content (1285.4 g/100 g) compared to maize. The foods analysed met the RDA for protein, fat, iron, zinc, and calcium. Formulation of complementary foods (CF0221, CF0322 and CF0423) composed of four (4) selected traditional foodstuffs: non-germinated amaranth grain (Amaranthus cruentus), maize (*Zea mays*), termites (*Macrotermes subhylanus*) and *dagaa* fish (*Rastreneobola argentea*). Nutrient and anti-nutrient contents and functional properties consumer of the three foods wereevaluated. After that, a process to optimize inclusion of termites and *dagaa* fish was developed and precooked products obtained. Phytate/iron molar ratio was beyond the critical limits for all the foods. The foods contained up to 3.3% bioavailable iron after pepsin digestion and up to 2.5% bioavailable iron after pepsin+ pancreatin digestion. Incorporation of termites and *dagga* fish in processed complementary foods containing of phytic acid does not translate into large mineral bioavailability and therefore techniques to further reduce the inhibitor are necessary. An indigenous process such as germination maybe evaluated in order to reduce phytic acid before processing the grains into complementary foods. All the foods were shelf stable for up to 6 months. Both animal and plant traditional foods should be exploited in development of nutrient dense complementary foods and appropriate pre-processing steps such as germination should be incorporated as means to reduce anti-nutrients in traditional grains before processing to complementary foods. Methods to further improve acceptability qualities of the foods should be evaluated. The foods formulated should be subjected to a controlled clinical trial to determine their efficacy in maintenance of good nutrition.

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## Conflicts of Interest/Competing interests

The authors declare no conflict of interest and competing interests.

## References

Akinnawo, O.O., Abatan, M.O., Ketiku, A. O. (2002). Toxicological study on the edible larva of Cirina forda (Westwood). *African Journal of Biomedical Research,* 5:43–46. http://www.bioline.org.br/pdf?md02008

AOAC (1995). Association of Official Analytical Chemists. Official Methods of Analysis (1995), 16th ed. Arlintong, VA. http://lib3.dss.go.th/fulltext/scan_ebook/aoac_1995_v78_n3.pdf

AOAC (1996). Association of Official Analytical Chemists. Official Methods of Analysis, 16th ed. Gaithersburg, Maryland. http://lib3.dss.go.th/fulltext/scan_ebook/aoac_1995_v78_n3.pdf

Aubry, P. Gaüzère B-A. (2015). Anémies carentielles ou nutritionnelles. Diplôme de médicine tropicale des pays de l'océan Indien. Medecine tropicale, 6 pp. http://medecin-etropicale.free.fr

Ayieko, M.A. Oriaro, V. (2008). Consumption, indigenous knowledge and cultural values of lake flies species within the Lake Victoria region. African Journal of Environment and Technology, 2(10):282-286. http://www.academic-journals.org/AJest

Ayieko, M.A., Kinyuru, J.N., Ndong'a, M.F., Kenji G.M. (2012). Nutritional Value and Consumption of Black Ants (Carebara vidua Smith) from the Lake Victoria Region in Kenya. Advance Journal of Food Science and Technology, 4(1):39-45. https://maxwellsci.com/print/ajfst/v4-39-45.pdf

Bahizire, N.E. (2018). Anemia in rural area South Kivu: contribution of malaria and iron deficiency. These de Doctorat en Sciences de la santé Publique (2017-2018), Universite libre de Bruxelles, Ecole de Sante Publique, Bruxelles. https://doi.org/10.4269/ajtmh.17-0030

Becker, T., Wheeler, E.L., Lorenz, K., Stafford, A.E., Grosjean, O.K., Betschart, A.A. Saunders, R.M. (1981). A compositional study of amaranth grain. Journal of Food Science, 46:1175-1180. https://doi.org/10.1111/j.1365-2621.1981.tb03018.x

Beutler, E. West, C. (2005). Hematologic differences between African-Americans and whites: the roles of iron déficiency and α-thalassemia on hemoglobin levels and mean corpuscular volum. Blood, 106:740-745. https://doi.org/10.1182/blood-2005-02-0713

Bhandari, B., D'Arcy, B. Young, G. (2001). Flavour retention during high temperature short time extrucion cooking process: a review. International Journal of Food Science and Technology, 36:453–461. https://doi.org/10.1046/j.1365-2621.2001.00495.x

Bobonis, G. J., Miguel, E. & Puri-Sharma, C. (2006). Anemia and school participation. Journal of Human Resources, 41(4):692-721. https://doi.org/10.3368/jhr.XLI.4.692

Byavu, N., Henrard, C., Dubois, M. & Malaisse, F., (2000). Phytothérapide traditionnelle des bovins dans les élevages de la plaine de la Ruzizi. Biotechn. Agron. Soc. Environ., 4(3):135-156. https://popups.uliege.be/1780-4507/text/v4n3/135.pdf

Camire, A. L. and Clydesdale, F. M. (1982). Analysis of phytic acid in foods by HPLC. Journal of food science, 47:575-578. https://doi.org/10.1111/j.1365-2621.1982.tb10126.x

Christensen, D. L., Orech, F. O., Mungai, M. N., Larsen, T., Friss, H. & Aagaard Hansen, J. (2006). Entomophagy among the luo of Kenya: A potential mineral source?. International Journal of Food Science and Nutrition, 57(3/4):198-203. https://doi.org/0.1080/09637480600738252

Codex Alimentarius (1991). Standard for Processed Cereal-Based Foods for Infants and Children, Codex Stan 74. FAO, Rome. https://www.ibfan.org/wp-content/uploads/2019/05/STANDARD-FOR-PROCESSED-CEREAL-BASED-FOODS-FOR-INFANTS-AND-YOUNG-CHILDREN.pdf

Colmenares, S. & Bressani, R. (1990). Effect of Germination on the Chemical Composition and Nutritive of Amaranth Grain. Cereal Chemistry, 67(6):519-522. https://www.cerealsgrains.org/publications/cc/backissues/1990/Documents/67_519.pdf

Dewey, K. G. & Brown, K. H. (2003). Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. Food and Nutrition Bulletin, 24:5-28. https://doi.org/10.1177/156482650302400102

Dewey K.G. (2005). Infant nutrition in developing countries: what works?. Lancet, 365:1832-1834. https://doi.org/10.1016/S0140-6736(05)66427-6

Dijkhuizen, P. (2000). Processed complementary foods in the World Food Program. Food and Nutrition Bulletin, 21:62–64. https://doi.org/10.1177/156482650002100110

Earl, R. & Woteki, C. E, éd. (1993). Iron deficiency anemia: recommended guidelines for the prevention, detection, and management among U.S. children and women of childbearing age. Washington, DC: National Academy Press. https://doi.org/10.17226/2251

EDS-RDC-Enquête Démographique et de Santé (2014). Deuxième enquête démographique et de santé en

République démocratique du Congo (EDS-RDC II 2013-2014); 2013–4. https://dhsprogram.com/pubs/pdf/FR300/FR300.pdf

Egli, I., Davidsson, L., Zeder, C., Walczyk, T. & Hurrell, R. (2004). Dephytinization of a complementary foods based on wheat and soy increases zinc, but not copper apparent absorption in adults. Journal of Nutrition, 134:1077-1080. https://doi.org/10.1093/jn/134.5.1077

FAO/WHO (2002). Joint FAO/WHO Expert Consultation. Vitamin and mineral requirements in human nutrition. World Health Organization, Geneva, Switzerland. https://apps.who.int/iris/handle/10665/42716

FAO/WHO (2004). Joint FAO/WHO Expert Consultation. Human energy requirements. Food and Agriculture Organisation, Rome, Italy. https://www.fao.org/3/y5686e/y5686e.pdf

Fellows (2000). Food Processing Technology: Principles and Practice. Pp. 177-182. Cambridge: Woodhead Publishing Ltd. https://www.elsevier.com/books/food-processing-technology/fellows/978-0-08-100522-4

Garcia-Estepa, R. M., Guerra-Hernandez, E. and Garcia-Villanova, B. (1999). Phytic acid content in milled cereal products and breads. Food Research International, 32:217-221. https://doi.org/10.1016/S0963-9969(99)00092-7

Gibson, R. S., Yeudall, F., Drost, N., Mitimuni, B. & Cullinan, T. (1998a). Dietary interventions to prevent zinc deficiency. American Journal of Clinical Nutrition, 64:484S–487S. https://doi.org/10.1093/ajcn/68.2.484S

Gibson, R. S., Ferguson, E. L. & Lehrfeld, J. (1998b). Complementary foods for infant feeding in developing countries: their nutrient adequacy and improvement. European Journal of Clinical Nutrition, 52:764-770. https://doi.org/10.1038/sj.ejcn.1600645

Gibson, R.S., Bailey, K.B., Gibbs, M. & Ferguson, E.L. (2010). A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. Food and Nutr Bulletin, 31(2):S134-S146. https://doi.org/10.1177%2F15648265100312S206

Grenier, L. (1998). Working with Indigenous Knowledge: A Guide for Researchers. IDRC, Ottawa. https://www.idrc.ca/en/book/working-indigenous-knowledge-guide-researchers

Gunshin, H., Allerson, C. R., Polycarpou-Schwarz, M., Rofts, A., Rogers, J. T., Kishi, F., Hentze, M. W., Rouault, T. A., Andrews, N. C. & Hediger, M. A. (2001). Iron-dependent regulation of the divalent metal ion transporter. FEBS Letters, 509:309-316. https://doi.org/10.1016/S0014-5793(01)03189-1

Kabahenda, M. K., Amega, R., Okalany, E., Husken, S. M. C. & Heck, S. (2011). Protein and micronutrient composition of low-value fish products commonly marketed in the Lake Victoria region. World Journal of Agricultural Sciences, 7(5):521-526. https://hdl.handle.net/20.500.12348/1196

Kanensi, J. O., Ochola S., Gikonyo, N. K. & Makokha A. (2011). Optimization of the period of steeping and germination for amaranth grain. Journal of Agriculture Food and Technology, 1(6):101-105. https://ir-library.ku.ac.ke/handle/123456789/5793

Kinyuru, J. N., Kenji, G. M., Njoroge, M. S. & Monica, A. (2010a). Nutritional potential of longhorn grasshopper (Ruspolia differens) consumed in Siaya District. Journal of Agriculture, Science and Technology, 12(1):32 - 47. http://ir.jkuat.ac.ke/handle/123456789/2520

Kinyuru, J. N., Kenji, G. M., Njoroge, S. M. & Ayieko, M. (2010b). Effect of processing methods on the in vitro protein digestibility and vitamin content of edible winged termite (Macrotermes subhylanus) and grasshopper (Ruspolia differens). Food Bioprocess and Technology, 3(5):778–782. https://doi.org/10.1007/s11947-009-0264-1

Kinyuru, J. N. (2012). Development and evaluation of complementary foods based on traditional foodstuffs in Western Kenya. PhD thesis in Food Science and Nutrition in the Jomo Kenyatta University of Agriculture and Technology, Nairobi-Kenya, 115pp. http://ir.jkuat.ac.ke/handle/123456789/1350?show=full

Kinyuru, N. J., Silvenus O. Konyole, Glaston M. Kenji, Christine A. Onyango, Victor O. Owino, Bethwell O. Owuor, Benson B. Estambale, Henrik Friis & Nanna Roos (2012). Identification of traditional foods with public health potential for complementary feeding in western Kenya. Journal of Food Research, 1(2):148-158. https://doi.org/10.5539/jfr.v1n2p148

King, F. S. & Burgess, A. (1993). Nutrition for Developing Countries, 2nd edn, ELBS with Oxford University Press, London. https://global.oup.com/academic/product/nutrition-for-developing-countries-9780199685226

Lukmanji, Z., Hertzmark, E., Mlingi, N., Assey, V., Ndossi, G. & Fawzi, W. ( 2008). Tanzania Food Composition Tables. MUHAS- TFNC, HSPH, Dar es Salaam, Tanzania. https://cdn1.sph.harvard.edu/wp-content/uploads/sites/30/2012/10/tanzania-food-composition-tables.pdf

Lutter, C. K. (2000). Processed complementary foods: summary of nutritional characteristics, methods of production and distribution, and costs. Food and Nutrition Bulletin, 22:95–100. https://doi.org/10.1177%2F156482650002100118

Lutter, C. K. (2003). Macro-level approaches to improve the availability of complementary foods. Food and

Nutrition Bulletin, 24:83–103. https://doi.org/10.1177%2F156482650302400105

Lutter, C. K. & Dewey, K. G. (2003). Proposed nutrient composition of fortified complementary foods. Journal of Nutrition, 133:3011S–3020S. https://doi.org/10.1093/jn/133.9.3011S

Martin, G. J. (1995). Ethnobotany: A methods manual. WWF, UNESCO & RBG, Kew, Chapman and Hall. https://www.routledge.com/Ethnobotany-A-Methods-Manual/Martin/p/book/9781844070848

Mbemba Fundu Di Luyindu, T. (2013). Aliments et denrées alimentaires traditionnels du Bandundu en R.D. Congo. Répertoire et composition en nutriments. Paris, L'Harmattan RDC., 325 pp. http://www.editions-harmattan.fr/catalogue/couv/aplat/9782343017419.pdf

Mensah, P. & Tomkins, A, (2003). Household-level technologies to improve the availability and preparation of adequate and safe complementary foods. Food and Nutrition Bulletin, 24:104–125. https://doi.org/10.1177%2F156482650302400106

Miller, D. D. (1996). Effects of cooking and food processing on the content of bioavailable iron in foods. In: Micronutrient Interactions: Impact on Child Health and Nutrition. ILSI, Washington DC, USA. https://www.yumpu.com/en/document/view/13267681/micronutrient-interactions-impact-on-child-health-and-idpasorg

Muvundja, F. A., Uwikunda, S. H., Mande, P., Alungalu, F. G., Balagizi, K. I. & Isumbisho, M. P. (2013). Valorisation de la chenille comestible Bunaeopsis aurantiaca dans la gestion communautaire des forêts du Sud-Kivu (République Démocratique du Congo) , VertigO, Hors-série 17, septembre 2013, consulté le 22 avril 2015. http://vertigo.revues.org/13929.

Muyonga, J. H., Nabakabya, D., Nakimbugwe, D. & Masinde D. (2008). Efforts to promote amaranth production and consumption in Uganda to fight malnutrition. In Robertson, G.L. & Lupien, J.R. (Eds), Using Food Science and Technology to Improve Nutrition and Promote National Development. International Union of Food Science and Technology, 2-10. https://alimentosprocessados.com.br/arquivos/ciencia-e-tecnologia/Using-Food-Science-and-Technology-to-Improve....pdf

Mosha, T. C. E. & Bennink, M. R. (2005). Protein digestibility-corrected amino acid scores, acceptability and storage stability of ready-to-eat supplementary foods for pre-school age children in Tanzania. Journal of the Science of Food and Agriculture, 85:1513–1522. https://doi.org/10.1002/jsfa.2147

Norhaizan, M. E. & Nor, F. A. A. (2009). Determination of phytate, iron, zinc, calcium contents and their molar ratios in commonly consumed raw and prepared food in Malaysia. Malysian Journal of Nutrition, 15(2):213–222. https://nutriweb.org.my/mjn/publication/15-2/j.pdf

Oberleas, D. & Harland, B. F. (1981). Phytate content of foods: effect on dietary zinc bioavailability. Journal of American Diet Association, 79:433 –436. https://doi.org/10.1016/S0002-8223(21)39390-7

Ogoye-Ndegwa, C. and Aagaard-Hansen, J. (2003). Traditional gathering of wild vegetables among the luo of western kenya - A nutritional anthropology project. Ecology of Food and Nutrition, 42:69-89. https://doi.org/10.1080/03670240303114

Ombeni, B. J. (2015). Evaluation de la valeur nutritionnelle des Aliments Sauvages Traditionnels consommés par les communautés rurales (Bashi, Barega et Bafulero), province du Sud-Kivu en République Démocratique du Congo. Mémoire Online, Institut Supérieur des Techniques Médicales de Bukavu, 134 pp. https://www.memoireonline.com/03/17/9672/valuation-de-la-valeur-nutritionnelle-des-aliments-sauvagestraditionnels-consommes-par-les-diff.html

Ombeni, J. B. & Munyuli, B. M. (2016). Évaluation de la valeur nutritionnelle des aliments sauvages traditionnels (Règne Animalia) intervenant dans la sécurité alimentaire des communautés rurales du Sud-Kivu (République Démocratique du Congo). Geo-Eco-Trop, 40(2):115-132. http://www.geoecotrop.be/uploads/publications/pub_402_03.pdf

Onigbinde, A. O. & Adamolekun, B. (1998). The nutrient value of Imbrasia belina Lepidoptera: Saturnidae (madora). Central African Journal of Medicine, 44:125- 127. https://pubmed.ncbi.nlm.nih.gov/9810409/

Owaga, E., Onyango C. A., Njoroge C. K. (2009). Assessment of insect contamination, acid insoluble ash content and colour characteristics of traditionally sun-dried and oven-dried dagaa (Rastrineobola argentea). Journal of Applied Biosciences, 24:1497 – 1507. http://www.m.elewa.org/JABS/2009/24/4.pdf

Owaga, E. E., Onyango, C. A. & Njoroge, C. K. (2010). Influence of selected washing treatments and drying temperatures on proximate composition of dagaa (Rastrineobola argentea), a small pelagic fish species. African Journal of Food Agriculture, Nutrition and Development, 10(7):2834 - 2836. https://www.ajol.info/index.php/ajfand/article/view/59031

Oyarzun S.E.; Graham J. & Eduardo V. (1996). Nutrition of the tamandua: Nutrient composition of termites and stomach contents from wild tamanduas (Tamandua tetradactyla). Zoo Biology, 15:509-524. https://doi.org/10.1002/(SICI)1098-2361(1996)15:5%3C509::AID-ZOO7%3E3.0.CO;2-F

Ramos-Elorduy J., Moreno, J. M. P., Prado, E. E., Perez, M. A., Otero, J. L. & Larron, D. G. O. (1997). Nutritional value of edible insects from the State of Oaxaca, Mexico. Journal of Food Composition and Analysis, 10:142-57. https://doi.org/10.1006/jfca.1997.0530

Rhou, J. R. & Erdman, J. V. (1995). Phytic acid in health and disease. CRC Critical Reviews in Food Science and Nutrition, 35:495-508. https://doi.org/10.1080/10408399509527712

Sazawal, S., Black, R. E., Ramsan, M., Chwaya, H. M., Syoltzfus, R. J., Dutta, A. et al. (2006). Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting community-based, randomised, placebocontrolled trial. Lancet, 307:133-143. https://doi.org/10.1016/S0140-6736(06)67962-2

Sehmi, K. J. (1993). National Food Composition Tables and the Planning of Satisfactory Diets in Kenya. Government Printers, Nairobi. http://www.fao.org/3/i8897en/I8897EN.pdf

Silvenus, O., Konyole, Kinyuru, N. J., Bethwell, Owuor, O., Glaston, M., Kenji, Christine A. Onyango, Benson B. Estambale, Henrik Friis, Nanna Roos, Victor O. Owino (2012). Acceptability of amaranth grain-based nutritious complementary foods with dagaa fish (Rastrineobola argentea) and edible termites (Macrotermes subhylanus) compared to corn soy blend plus among young children/mothers dyads in western Kenya. Journal of Food Research, 1(3):111-120. https://www.ccsenet.org/journal/index.php/jfr/article/view/16809

Singh S., Shirani G. & Wakeling L. (2007). Nutritional aspects of food extrusion: a review. International Journal of Food Science and Technology, 42:916 – 929. https://doi.org/10.1111/j.1365-2621.2006.01309.x

Singleton, V. L. & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16:144 - 158. https://www.ajevonline.org/content/16/3/144

Singhal, R. S. & Kulkarni, P. R. (1990a). Utilization of Amaranthus paniculatas (Rajgeera) starch in salad dressing. Starch/Starke, 42:52-53. https://doi.org/10.1002/STAR.19900420206

Singhal, R. S. & Kulkarni, P. R. (1990b). Studies on applicability of Amaranthus paniculatas (Rajgeeraa) starch for custard preparation. Starch/Starke, 42:102-103. https://studylib.net/doc/13445073/

Soemantri, A. G., Pollitt, E. & Kim, I. (1985). Iron deficiency anemia and educational achievement. American Journal of Clinical Nutrition, 42(6):1221-1228. https://doi.org/10.1093/ajcn/42.6.1221

Sorensen, A. D., Sorensen, H., Bjergegaard, C., Andersen, K. E., Sondergaard, I., Sorensen, S. & Bukhave, K. (2007a). Matrix effects of lupine (Lupinus luteus L.) and rapeseed (Brassica napus L.) products on in-vitro non-heme iron availability from pork meat. Journal of Food Composition and Analysis, 20:515-522. https://zh.booksc.eu/book/3687433/e3858a

Sorensen, A. D., Sorensen, H., Sondergaard, I. & Bukhave, K. (2007b). Non-heme iron availability from pork meat: Impact of heat treatments and meat protein dose. Meat Science, 76:29 – 37. https://doi.org/10.1016/j.meatsci.2006.10.008

Stevens, G. A., Finucane, M. M., De-Regil, L. M., Paciorek, C. J., Flaxman, S. R., Branca, F., Peña-Rosas J. P., Bhutta, Z. A. & Ezzati, M., (2013). Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: A systematic analysis of population-representative data », The Lancet Global Health, 1(1):e16-e25. https://doi.org/10.1016/S2214-109X(13)70001-9

Stoltzfus, R. J., Kvalsvig, J. D., Chwaya, H. M., Montresor, A., Albonico, M., Tielsch, J. M., Savioli, L. & Pollitt, E. (2001). Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: Double blind, placebo controlled study. British Medical Journal, 323:1389. https://doi.org/10.1136/bmj.323.7326.1389

Teutonico, R. A. & Knorr, D. (1985). Amaranth: composition, properties and applications of a rediscovered food crop. Food Technology, 39(4):49–60. https://www.eap.mcgill.ca/CPAT_1.htm

Towo, E., Celestin, M., Godwin, D. N. & Kimboka, S. (2006). Effect of phytate and iron-binding phenolics on the content and availability of iron and zinc in micronutrients fortified cereal flours. African Journal of Food Agriculture, Nutrition and Development, 6(2):1123-1127. https://doi.org/10.4314/ajfand.v6i2.71756

Verkerk, M. C., Tramper, J., Trijp, J. C. M. & Martens, D. E. (2007). Insect cells for human food. Biotechnology Advance, 25:198-202. https://doi.org/10.1016/j.biotechadv.2006.11.004

Walker, A. F. (1990). The contribution of weaning foods to protein energy malnutrition. Nutritional Review and Research, (3):25–47. https://doi.org/10.1079/NRR19900005

WFP (2010). WFP handbook: Fortified Blended Food – Good Manufacturing Practice and HACCP Principles (A handbook for processors in partnership with the UN World Food Programme). http://foodquality.wfp.org

WHO (1998). Complementary feeding of young children in developing countries: a review of current scientific knowledge. WHO/ NUT/98.1, Geneva, Switzerland. https://apps.who.int/iris/handle/10665/65932

WHO/UNICEF (1998). Complementary Feeding of Young Children in Developing Countries: a review of the Current Scientific Knowledge. WHO Publications, Geneva. https://apps.who.int/iris/handle/10665/65932

WHO (2002). Infant and Young Child Nutrition: Global Strategy on Infant and Young Child Feeding, World Health Assembly Document WHA55/15, WHO Press, Rome. https://apps.who.int/gb/archive/pdf_files/WHA55/ea5515.pdf

WHO (2008). Indicators for assessing infant and young child feeding practices. WHO/NUT/2008.1, Geneva, WHO. http://whqlibdoc.who.int/publications/2008/9789241596664_eng.pdf

WHO (1993-2005). Anemia". http://www.who.int/vmnis/database/anaemia/anaemia_status_summary/fr/.

Williams, P. (2007). Nutritional composition of red meat. Nutrition & Dietetics, 64(4):113–119. https://doi.org/10.1111/j.1747-0080.2007.00197.x

Yip, R. (1989). The changing characteristics of childhood iron nutritional status in the United States. In: Filer LJ Jr, éd. Dietary iron: birth to two years. New York, NY: Raven Press, pp. 3761.https://agris.fao.org/agris-search/search.do?recordID=US910194