



Anti-nutrient, vitamin a and mineral composition of germinated/pre-gelatinized maize complementary food substituted with cowpea and carrot flour

Anesthesia C Ojiako, Patience Chisa Obinna-Echem*

Department of Food Science and Technology, Rivers State University, NKpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

Abstract

This research evaluated anti-nutrient, vitamin A and mineral composition of complementary food from germinated/pre-gelatinized maize flour blended with cowpea and carrot flour. Processed maize, cowpea and carrot flour were blended in the ratio of A - 95:5:0, B - 90:5:5, C - 85:10:5, D - 80:15:5, E- 75:20:5, F - 70:25:5 and G - 60:30:5. Un-supplemented maize served as control. Analysis were carried out using standard analytical methods. Anti-nutrient content of the formulated complementary food blend ranged from 2.86 - 4.54, 4.63 - 6.45 and 138.73 - 154.10 mg/100g respectively, for oxalate, phytate and tannins. Vitamin A varied significantly ($P \leq 0.05$) from 375 - 730 $\mu\text{g}/\text{RE}/100\text{g}$ for the control and sample G respectively. These values meet 94 -182% of the WHO recommended safe intake of Vitamin A (400 $\mu\text{g}/\text{RE}/\text{Day}$) for infants. The trace elements iron and zinc varied significantly ($P \leq 0.05$) from 1.35 - 4.10 and 0.21 - 2.67 mg/100g respectively, while the macro-elements calcium, magnesium and phosphorus ranged from 9.66 - 49.35, 158.40 - 212.66, and 10.39 - 22.76 mg/100g respectively. The addition of cowpea and carrot flour to the germinated/pre-gelatinized maize flour increased the anti-nutrient, vitamin A and mineral content of the complementary food blend. The increase in cowpea flour resulted in significant increase in all the components analyzed. Sample G with 30% cowpea and 5% carrot addition had significantly ($P \leq 0.05$) the highest values while the control had the least. Samples with higher cowpea inclusion (20 - 30%) had higher anti-nutrient content, therefore, Samples B and C with 5 and 10% addition of cowpea is recommended as their vitamin A content will meet >100% of the daily requirement and sufficient amount of the mineral needs.

Keywords: maize, germination, pre-gelatinization, cowpea, carrot, anti-nutrient, mineral, vitamin A

Introduction

Maize (*Zea mays*) is an important cereal crop in Nigeria, second to sorghum in the number of people it feeds (Sule *et al.*, 2014) ^[32]. It is commonly used in the preparation of infant complementary foods after fermentation, and also a major component of adult main meals. Maize is a rich source of carbohydrate providing energy and fatty acids from its lipid rich germ. Maize is also a good source of many minerals: magnesium, phosphorus, potassium, calcium, manganese, zinc, iron, copper, and sodium (Sule *et al.*, 2014; Okafor *et al.*, 2018) ^[32, 22].

Legumes play important roles in the diet of many people in Africa and Asia and are major sources of proteins, carbohydrates, vitamins and minerals (Famata *et al.*, 2013) ^[14]. The legumes commonly used in complementing cereals are cowpea, chicken peas, soy beans, kidney, lentils, mung beans, peanuts, peas, pigeon pea and vetches (Peter and Carroll, 2003) ^[24]. Cowpea (*Vigna unguiculata* (L) Walp.) is a source of protein in many diets in Nigeria that is the largest cowpea producer in the world (Kormawa *et al* 2002) ^[18]. The protein content of cowpea is about 2 to 4 times greater than cereals and tubers (Jayathilake *et al.*, 2018) ^[16]. However, it is low in protein digestibility and limiting in sulphur containing amino acids such as methionine and cysteine. For which it is used as a compliment to cereals that are limiting in lysine. Cowpea is a good source of minerals: sodium, potassium, magnesium, calcium, phosphorus, zinc and iron (Famata *et al.*, 2013) ^[14]. It contains some anti-nutrient that forms complexes with essential dietary minerals, vitamins and precipitate proteins making them biologically unavailable for absorption (Diouf *et al.*, 2020) ^[13].

Carrot (*Daucus carota L.*) is one of the most consumed vegetables rich in β - carotene a precursor of Vitamin A and also contains vitamins, minerals, phenolic compounds, and other antioxidant micronutrients (Nicolle *et al.*, 2004) ^[20]. The intake of some essential nutrient are insufficient in human diet. Carrot with its rich nutrient content especially the pomace containing about 50% of β -carotene can be profitably utilized for supplementation in the preparation of different products like cake, bread, biscuits and preparation of several types of functional products (Sharma *et al.*, 2011) ^[31]. Its inclusion in infant complementary food could enhance its mineral and vitamin content.

Minerals are essentially required for various functions in the human body, and their deficiency can lead to various health problems. Most of the minerals in plants are found in the bound form with anti-nutrient factors. Anti-nutrients are vastly present in plants, they are highly reactive natural or synthetic compounds such as phytic

acid, oxalate, saponin, tannin, protease, lipase and amylase inhibitors etc., that can impact a negative effect by interfering with the absorption of nutrients, some also have some health benefits (Popova and Mihaylova 2019)^[25]. For example, dietary phytic acid binds strongly with calcium, magnesium, iron, copper and zinc preventing their absorption while oxalate binds with calcium. To increase the nutritional quality of the plant food, different processing methods are employed for the bioavailability of the minerals through the reduction of the anti-nutrient factors.

Various pre-processing methods contribute to reduction in anti-nutrient content of cereals and legumes. Germination has been reported to cause a reduction in anti-nutrient factors in maize due to the endogenous enzyme activities in anti-nutrient factor degradation during germination (Anaemene and Fadupin 2020)^[4]. Soaking is another pre-treatment for legumes, it is commonly used in many homes. Reports have shown a reduction of 23.7% of phytate in cowpea by soaking in water for 24 h (Razafitsalama 2006)^[28], while soaking with sodium chloride led to a reduction of 41.7%. Although some essential minerals were also lost during the process (Diouf *et al.*, 2020)^[13]. Boiling and roasting have also been reported to reduce anti-nutrient factors in cereals and legumes. The reduction in legumes is attributed mostly to the removal of the testa and leaching in the process water (Makinde and Abolarin 2020)^[19]. The pre-processing methods can however lead to loss of some essential dietary elements (Anaemene and Fadupin 2020; Diouf *et al.*, 2020)^[4, 13].

Complementary foods are foods given to infants and children other than breast milk. Adequate nutrition is required for proper growth and development of the infants and children. Thus, the food given in addition to breast milk should be able to meet the daily requirement of essential nutrients for the children. Cereal-based complementary foods are commonly used in Nigeria. They are mostly made of maize, sorghum, millet or available root crops. The cereal-based complementary foods do not contain adequate nutrients to meet the daily requirement of the infants and children (WHO 2001)^[34]. This has led to the inclusion of other plant based materials to improve upon the nutrient content of cereal-based foods. Legumes are widely used to complement the protein content of cereals while fruits and vegetables enhance mineral and vitamin contents. This study was therefore aimed at evaluation of anti-nutrient, vitamin A and mineral content of complementary food from germinated/pre-gelatinized maize flour blended with cowpea and carrot flour.

Materials and Methods

Maize, Cowpea and Carrot Samples

The yellow variety of maize (*Zea mays*) and cowpea (*Vigna unguiculata*) were purchased from Mile 3 market in Port Harcourt, Rivers State. Carrot tubbers (*Dacus carota*) were purchased from fruit garden in Port Harcourt, Rivers State, Nigeria

Sample Preparations

Germinated/Pre-gelatinized Maize Flour Preparation

The steps for the preparation of the germinated and pre-gelatinized maize flours were according to the methods described by Traoré *et al.*, (2004 and Obinna-Echem *et al.*, 2019)^[33, 21] respectively. Briefly, the maize grains after sorting and cleaning were soaked in distilled water at room temperature overnight and then spread out on a humidified thick towel for 3 days to sprout. The germinated grain was heated at 70°C for 15 min, oven dried in hot air oven (Gallenkamp, UK) overnight at 70°C, milled, sieved with 0.2 mm sieve, and packaged in a well-labeled transparent polyethylene bag.

Roasted Cowpea Flour Preparation

Cowpea seeds were cleaned, sorted, and about 4 kg was soaked in excess distilled water for 10 – 15 min for the manual removal of the seed coats. Dehulled seeds were washed in water, drained and roasted in hot air oven at 150°C for 30 min, cooled at room temperature, milled using hammer mill and sieved with 0.2 mm sieve to obtain the flour that was packaged in a dry air tight plastic container.

Carrot Flour Preparation

Fresh carrots were washed and the outer layers scraped using a hand scraper. About 3 kg of the carrot were grated, dried at 70°C overnight and blended with hammer mill and sieved with 0.2mm sieve to obtain carrot flour that was packaged in a well labelled plastic container. The prepared flours were preserved in a deep freezer until required for use.

Formulation of the germinated/pre-gelatinized maize flour complementary food with cowpea and carrot flour.

Table 1, showed the supplementation of the germinated and pre-gelatinized maize flour with different proportions of the cowpea and carrot flours.

Table 1: Formulation Ratios for the Complementary Food from Germinated/Pre-Gelatinized Maize Flour with Cowpea and Carrot Flour

Sample	Control	Formulations						
		A	B	C	D	E	F	G
Germinated/Pre-gelatinized Maize Flour	100	95	90	85	80	75	70	65
Cowpea Flour	0	5	5	10	15	20	25	30
Carrot Flour	0	0	5	5	5	5	5	5

Analysis of the anti-nutrient composition of complementary food from blends of germinated/pre-gelatinized maize flour with cowpea and carrot flours.

Oxalate determination

Oxalate was determined following the method described by Inuwa *et al.*, (2011) [15]. Oxalate was determined titrimetrically after precipitation as calcium oxalate and titration against 0.05 M standard potassium permanganate (KMnO₄). Oxalate was calculated as equivalent of KMnO₄ (1 ml of the 0.05 M of KMnO₄ = 2.2 mg of oxalate) (Aina *et al.*, 2012) [1].

Phytate determination

The amount of phytic acid in the sample was determined titrimetrically after the hydrolysis of 2 g of the sample with hydrochloric (HCl) and filtration. Trtitration was against standard iron III chloride solution (containing 0.00195 g of iron per milliliter) until a brownish yellow colour persisted for 5 min using 5 ml of 0.3% ammonium thiocyanate solution as indicator. Phytic acid (%) was be calculated as titre x 0.00195 x 1.19 x 100. (Aina *et al.*, 2012) [1].

Tannin determination

Tannin was determined spectrophotometrically. Aqueous extract of 1 g of the sample was incubated with 1.0 ml folin - dennis reagent and 2.5 ml of saturated Na₂CO₃ solution for 90 min at room temperature. A set of standard tannic acid solution was treated in the same manner. The absorbance of the sample with that of a blank and a set of standard tannic acid solution (20, 40, 60, 80, 100 µg/ml) treated as the sample was measured at 250 nm. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample (Chandran and Indira, 2016) [12].

Determination of Vitamin A content of complementary food from blends of germinate/pre-gelatinize maize flour with cowpea and carrot flours

Vitamin A content of the flour blends was determined as described by Aremu and Nweze, (2017) [7]. The β-carotene content was determined spectrophotometrically at a wavelength of 436 nm after extraction with methanol and two times separation using hexane and sodium sulphate respectively. The vitamin A was calculated using the retinol equivalent (1 µg β-carotene = 0.167 µg RE) (WHO/FAO, 2004) [35].

Determination of Mineral content of complementary food from blends of germinate/pre-gelatinize maize flour with cowpea and carrot flours

The calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), and zinc (Zn) content of the flour samples were evaluated according to the standard methods of AOAC, (2012) [5] using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES Varian 725-ES, Melbourne, Australia). The ICP-OES was operated under the following conditions: plasma, auxiliary and nebulizer flow rate of 15, 1.50, 0.68 L min⁻¹ respectively at a viewing height of 8 mm, replication read time and instrument delay time of 4 and 10 s respectively. The measured spectra analyte lines in nanometre (nm) were: Ca: 317.933, Mg: 285.213, P: 213.618, Fe: 238.204 and Zn: 213.857. Validation of the analytes' concentration was performed using standard stock solution made up of 1, 4, 10 and 20 mg/l of the macro elements: Ca, Mg, P, and 0.01, 0.04, 0.1 and 0.2 mg/l of the micro elements: Fe, and Zn respectively. Calibration curves with linearity of 0.998 for each of the macro and micro elements were obtained from the four different concentrations of the standards stock solutions. Results were expressed in mg/kg of dry matter.

Statistical Analysis

Data were statistically analyzed using Minitab (Release 18.1) Statistical Software English (Minitab Ltd. Coventry, UK). Statistical differences and relationship among variables were evaluated by analysis of variance (ANOVA) under general linear model and Tukey pairwise comparisons at 95% confidence level.

Results and Discussion

1. Anti-nutrient content of complementary food from blends of germinated/pre-gelatinized maize flour with cowpea and carrot flours.

Shown in Figure 1 is the oxalate, phytate and tannin content of complementary food from blends of germinated/pre-gelatinized maize flour with cowpea and carrot flours.

Anti-nutrients are part of the plant protective mechanisms and they can limit the amount of nutrients available for utilization by animals. Their gradual accumulation in the body can be poisonous. Several processing treatments have been reported to reduce anti-nutrient factors in foods. There was significant ($P \leq 0.05$) increase in the anti-nutrient content with increase in addition of cowpea flour. Oxalate content varied significantly ($P \leq 0.05$) from 2.86 - 4.54 mg/100g. The control sample had significantly ($P \leq 0.05$) the least oxalate content that did not vary from Sample A with only 5% cowpea flour. The decreased oxalate content of the control can be attributed to the processing of the maize. According to the report of Anaemene and Fadupin, (2020) [4] germination resulted in decrease in oxalate content of raw maize by about 93% (from 590.00 to 42.50mg/100g). Sample B with 5% cowpea and carrot flour had oxalate content significantly ($P \leq 0.05$) higher than Sample A without carrot flour. This implies that the addition of 5% carrot flour also had effect on the oxalate content of the samples. Raw carrot has been reported to have an oxalate content of 49 mg/100g (Akhtar *et al.*, 2011) [2]. Sample G with 30% cowpea flour and 5% carrot flour had significantly ($P \leq 0.05$) the highest oxalate content of 4.54 mg/100g. Legumes such as beans have been reported to contain 158 mg/100g of oxalate (Chai and Liebman, 2004) [11]. Oxalate content of plants can be reduced by boiling soaking and dehulling. The low level of oxalate in this complementary food is good as high oxalate content has negative effect on mineral availability. High content of oxalate can lead to increased risk of renal calcium absorption that is implicated in kidney stone (Bello *et al.*, 2008) [9].

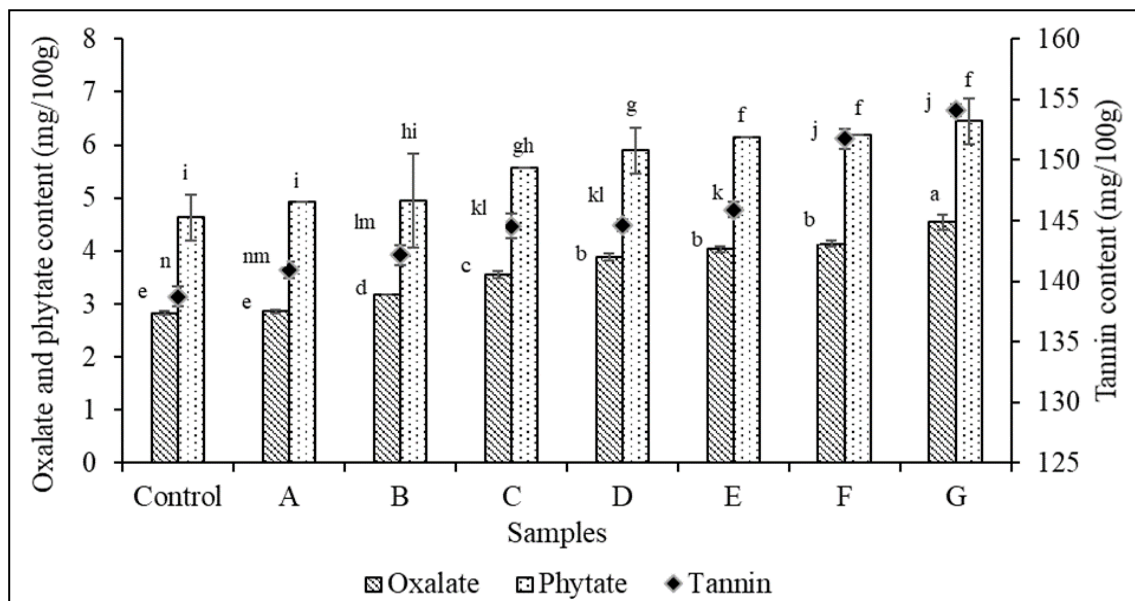


Fig 1: Oxalate, Phytate and Tannin Content (mg/100 g) of Complementary Food from Blends of Germinated/Pre-Gelatinized Maize with Cowpea and Carrot Flours Bars and marker are means \pm standard deviation of duplicate determination Means with different superscript for each parameter are significantly ($P \leq 0.05$) different. Control – 100% Maize flour, A – 95% Maize, 5% Cowpea flour, B – 90% Maize, 5% Cowpea, 5% Carrot flour, C – 85% Maize, 10% Cowpea, 5% Carrot flour, D – 80% Maize, 15% Cowpea, 5% Carrot flour, E – 75% Maize, 20% Cowpea, 5% Carrot flour, F – 70% Maize, 25% Cowpea, 5% Carrot flour, G – 65% Maize, 30% Cowpea, 5% Carrot flour

Phytate in the samples ranged from 4.63 - 6.45 mg/100g. The control had significantly ($P \leq 0.05$) the least phytate content that did not differ from those of Sample A, B, C and D with 5, 10 and 15% cowpea flour and 5% carrot flour respectively. Sample G had the significantly ($P \leq 0.05$) the highest phytate content that did not vary significantly ($P > 0.05$) from Sample E and F. The increase in phytate content could be due to the addition of the cowpea flour. The various pre-processing methods contribute to reduction in phytate content of cereals and legumes. Germination resulted in about 91% of reduction in phytate content of maize (967.50 - 85.50 mg/100g) (Anaemene and Fadupin, 2020) [4], the decrease, is due to the endogenous enzyme activities in the degradation of phytate during germination. Pre-gelatinization may have also reduced the anti-nutrient content through leaching in the cooking water. Soaking in tap water 4 - 24 h was found to decrease the phytate content of cowpea by 1.48 - 37.59%. The low levels of phytate in the formulated complementary food is good to avoid chelation with important minerals and vitamins. Phytic complexes are indigestible and will decrease the bioavailability of the vitamins and minerals in the body for absorption (Bello *et al.*, 2008) [9].

The tannin content of the sample ranged from 138.73 – 154.10 mg/100g for the control and Sample G respectively. The control and Sample A and B did not differ significantly ($P \leq 0.05$), in their tannin content, sample C, D and E did not vary in their tannin content, so was Sample F and G. Tannin imports an astringent taste that affects that affects palatability, reduce food intake and consequently body growth (Bello *et al.*, 2008) [9]. They inhibit enzymatic activities, bind and precipitate protein hence interfering with protein digestion and absorption and lowers absorption of minerals and vitamins from the gastrointestinal tract, although several health benefits are also derived from tannins (Raut *et al.*, 2016) [27].

2. Vitamin A content of complementary food from blends of germinated/pre-gelatinized maize flour with cowpea and carrot flours.

The vitamin A content of the complementary food ranged from 375 - 730 $\mu\text{g}/\text{RE}/100\text{g}$. There was significant increase in the vitamin A content of the samples with increase in cowpea and the addition of carrot.

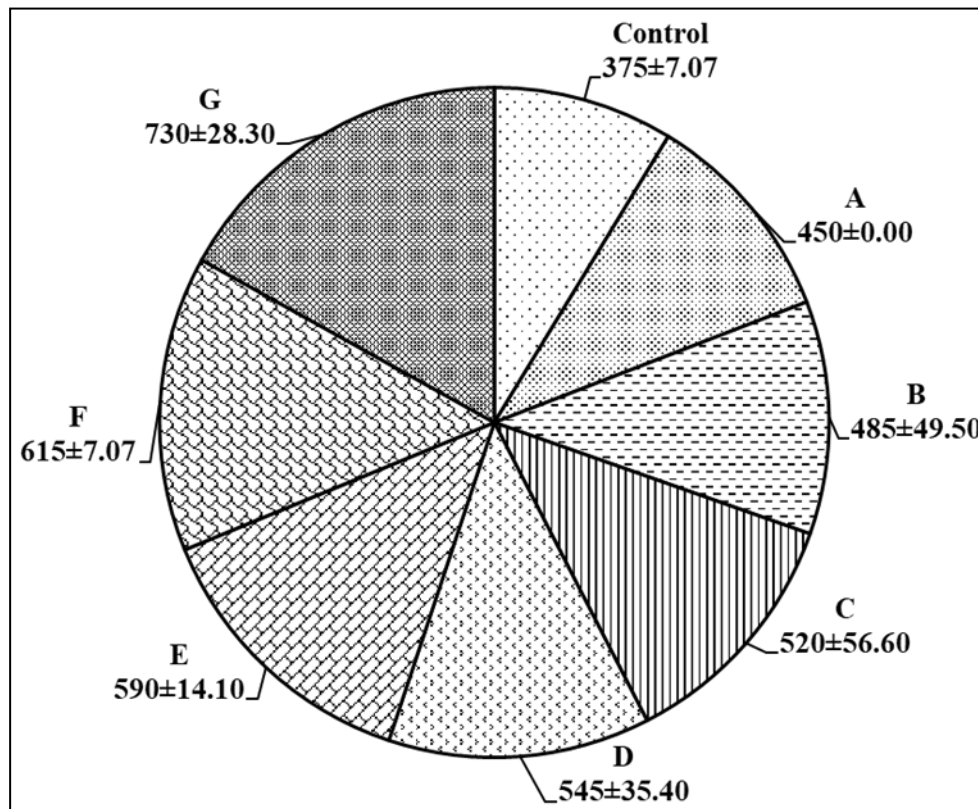


Fig 2: Vitamin A Content ($\mu\text{g}/\text{RE}/100\text{g}$) of Complementary Food from Blends of Germinated/Pre-Gelatinized Maize Flour with Cowpea and Carrot Flours Each section represents means \pm standard deviation of duplicate determination Values with different superscript are significantly ($P \leq 0.05$) different. Control – 100% Maize flour, A – 95% Maize, 5% Cowpea flour, B – 90% Maize, 5% Cowpea, 5% Carrot flour, C – 85% Maize, 10% Cowpea, 5% Carrot flour, D – 80% Maize, 15% Cowpea, 5% Carrot flour, E – 75% Maize, 20% Cowpea, 5% Carrot flour, F – 70% Maize, 25% Cowpea, 5% Carrot flour, G – 65% Maize, 30% Cowpea, 5% Carrot flour

As shown in Figure 2, the control had significantly ($P \leq 0.05$) the least vitamin A content, sample G had the highest while there was no significant ($P > 0.05$) difference between sample E and F. This was similar with result obtained by Bello *et al.*, (2020) [8] in complementary foods from maize-carrot pigeon pea flour blends. The inclusion of cowpea and carrot flours is responsible for the increase in vitamin A content of the samples. The inclusion of carrot in maize flour resulted in increase in vitamin content of maize carrot flour blends from 0.34 mg/l in raw maize to 0.62 and 0.80 mg/l with 20 and 40 % carrot inclusion (Joshua *et al.*, 2021) [17]. One way to increase the vitamin A content of infant complementary food and thereby increase its intake is by the inclusion of high carotenoid food in their diet (Roshana and Mahendran, 2019) [29]. Cowpea and carrot are rich in β -carotene a precursor of vitamin A (Sharma *et al.*, 2011; Owade *et al.*, 2019) [31, 23]. Infants of 6 months of age require about 300 μg of vitamin A per day to accumulate adequate liver stores and about 125 $\mu\text{g}/\text{day}$ to prevent clinical symptoms of deficiency from developing Allen and Haskell (2001) [3]. According to WHO/FAO (2004) [35], the mean daily requirement of vitamin A for infants between 7 – 12 months and 1 – 3 years is 190 and 200 $\mu\text{g}/\text{RE}/\text{Day}$ while the recommended safe intake is 400 $\mu\text{g}/\text{RE}/\text{Day}$. The vitamin A content (375 - 730 $\mu\text{g}/\text{RE}/100\text{g}$) of the maize, cowpea and carrot complementary food will meet 197 - 384 and 188 -365% of the mean daily requirement and 94 – 182% of the recommended safe intake respectively. Vitamin A plays a crucial role in vision, protein synthesis, and synthesis of RNA which is significant in growth and reproduction. Excess vitamin A is stored in the liver but intakes of 10 times more than normal can be toxic (Berdanier and Zempleni, 2009) [10]. The vitamin A content of this complementary food from geminated/pre-gelatinized maize with cowpea and carrot flours meets 125 – 243 % of the 300 μg required for adequate liver accumulation, this is not in excess of 10 times that can cause hypervitaminosis.

3. Mineral content of complementary food from blends of germinated/pre-gelatinized maize flour with cowpea and carrot flours.

The result of the mineral composition of the flour blends (Table 2) revealed significant ($P \leq 0.05$) differences between the control sample (100% maize) and the test samples with cowpea and carrot flour additions. There

was increase in the mineral content with increase in cowpea addition which is in line with the report by Shakpo and Osundahunsi (2016) [31].

Table 2: Mineral Content (mg/100 g) of Complementary Food from Blends of Germinated/Pre-Gelatinized Maize Flour with Cowpea and Carrot Flours

Samples	Calcium	Magnesium	Phosphorus	Iron	Zinc
Control	9.66±0.22 ^f	158.42±0.09 ^g	10.39±0.12 ^c	1.35±0.00 ^c	0.21±0.00 ^d
A	12.14±0.02 ^e	165.51±0.42 ^f	11.45±0.06 ^c	1.71b±0.12 ^c	0.26±0.01 ^d
B	13.26±0.13 ^{de}	167.72±0.01 ^f	11.47±0.03 ^c	2.12b±0.00 ^c	0.38±0.06 ^d
C	14.29±0.60 ^d	175.94±2.16 ^e	11.49±0.00 ^c	2.42±0.16 ^{bc}	0.73±0.04 ^c
D	18.94±0.03 ^c	181.31±0.93 ^d	19.19±0.70 ^b	2.44±0.13 ^b	1.95±0.02 ^{ab}
E	21.36±0.64 ^{bc}	185.86±0.63 ^c	19.37±0.08 ^b	3.52±0.11 ^a	1.01±0.00 ^c
F	22.36±0.03 ^b	194.82±0.69 ^b	21.30±0.70 ^a	3.62±0.07 ^a	1.47±0.06 ^{ab}
G	49.35±0.00 ^a	212.66±0.74 ^a	22.76±0.78 ^a	4.11±0.38 ^a	2.67±0.21 ^a

Values are means ± standard deviation of duplicate determination

Values with different superscript within a column are significantly ($P \leq 0.05$) different.

Control – 100% Maize flour

A – 95% Maize, 5% Cowpea flour

B – 90% Maize, 5% Cowpea, 5% Carrot flour

C – 85% Maize, 10% Cowpea, 5% Carrot flour

D – 80% Maize, 15% Cowpea, 5% Carrot flour

E – 75% Maize, 20% Cowpea, 5% Carrot flour

F – 70% Maize, 25% Cowpea, 5% Carrot flour

G – 65% Maize, 30% Cowpea, 5% Carrot flour

The calcium content of the germinated/pre-gelatinized maize, cowpea and carrot complementary food ranged from 9.66 – 49.35 mg/100g. Control sample had significantly ($P \leq 0.05$) the least calcium content and sample G had the highest. The calcium content of sample G (49.35mg/100g) was comparable with the calcium content of maize, carrot and pigeon pea blends (Bello *et al.*, 2020) [8] and maize, cowpea flour blends (Shakpo and Osundahunsi, 2016) [31], but others were lower. This may be due to differences in the varieties of maize and legume used. The calcium content in 100 g of the maize, cowpea and carrot complementary food will meet about 2 -12% of the WHO/FAO, (2004) [35] recommended calcium intake of 400 mg/day for infants between 7 -12 months and 2 - 9% for those aged 1 – 3 years. This implies that other calcium sources are to accompany this complementary food to meet up the daily requirement. The addition of milk to complementary gruel for families that can afford it will enhance the calcium content. Calcium is an essential micro nutrient in infants and young children for building bones and teeth, functioning of muscles and nerves, blood clotting and for immune defense (Bello *et al.*, 2020) [8].

Magnesium content of the germinated/pre-gelatinized maize, cowpea and carrot flour varied significantly ($P \leq 0.05$) from 158.40 - 212.66 mg/100g. Magnesium plays a crucial role in many biochemical reactions involving phosphate transfer in the body, is essential in the structural stability of nucleic acid, intestinal absorption and the relaxing of muscles along the airway to the lung which grants asthma patients easier breath (Bello *et al.*, 2008; Rashida *et al.*, 2014) [9], [26]. Several conditions such as severe diarrhea, migraines, hypertension, cardiomyopathy atherosclerosis and stroke has been attributed to the deficiency of Magnesium in the body (Rashida *et al.*, 2014) [26]. According to WHO/FAO (2004) [35], the Recommended Nutrient Intake (RNI) for infants between 7 – 12 months and 1 - 3 years respectively, is 6.0 and 5.5 mg/Kg. The magnesium content of this maize, cowpea and carrot complementary food will meet 293 – 393% and 264 – 354% of the RNI for infants between 7 – 12 months and 1 - 3 years with assumed body weight of 9 and 12 kg respectively. Excess magnesium (above the upper limit of 65 mg for children aged 1 – 3 years) is known to cause hypermagnesaemia, nausea, hypotension, and diarrhea, but the absorption efficiency of magnesium from other foods other than the breast milk is only about 50%, of which the large amount of magnesium in the complementary food may not pose any problem.

The germinated/pre-gelatinized maize, cowpea and carrot complementary food blend had phosphorus content of 10.39 – 22.76 mg/100g for the control and sample G respectively. This is higher than values for maize, carrot and pigeon pea blends (Bello *et al.*, 2020) [8]. Phosphorus is useful in bone mineralization, intermediary metabolism and a crucial part of nucleic and genetic materials and aids in calcium uptake in the body (Berdanier and Zemleni, 2009) [10], hence it is essential for bone formation, metabolism, growth and development.

The germinated/pre-gelatinized maize, cowpea and carrot complementary food had zinc content in the ranged of 0.21 - 2.67 mg/100g. Control sample had significantly ($P \leq 0.05$) the least calcium content and sample G had the highest. The zinc content of Sample A and B did not vary significantly ($P > 0.05$) from that of the control while sample C to G had zinc content significantly ($P \leq 0.05$) higher than the control. Zinc is very essential to all life forms. It is present in all body tissues and fluids, involves in the metabolism of macro- and micro-molecules as an essential component of enzymes, contributes to the maintenance of cell and organ integrity, polynucleotide transcription and genetic expression (WHO/FAO, 2004) [35]. The central role of zinc in cell division, protein

synthesis, and growth makes it very important for infants and children. The Recommended Nutrient Intake (RNI) of zinc for infants between 7 – 12 months is 2.5, 4.1 and 8.4 mg/day for high, moderate and low bioavailability, while for ages 1 – 3 years it is 2.4, 4.1 and 8.3 mg/day (WHO/FAO, 2004) [35]. About 10 – 107, 6 – 65 and 3 – 31% of the RNI will be met by the zinc content of 100 g of the maize, cowpea and carrot complementary food for infants 7 – 12 months and 11 – 111, 6 – 65 and 3 – 32% for ages 1 – 3 years respectively, for high, moderate and low bioavailability. In terms of high bioavailability, samples D, F and G were found to meet >70, 50 and 100% of the RNI.

Iron is a trace mineral that is an essential component of the red blood cells, a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, and a transport medium for electrons within cells (WHO/FAO, 2004; Appel, 1999) [35, 6]. According to WHO/FAO, (2004) [35] the iron intake required for growth for infants between 6 - 12 months and 1 - 3 years is 0.55 and 0.27 mg/day respectively. The iron content of the germinated/pre-gelatinized maize cowpea and carrot complementary food blend ranged from 1.35 - 4.10 mg/100g for the control and sample G respectively. These values will meet about 245 - 747 and 500 - 1522% respectively, of the required iron intakes for growth of infants between 6 - 12 months and 1 - 3 years. The iron content of the complementary food blend is therefore very high but iron toxicity would not be an issue as diets that contain whole grain cereals and legumes contain only non-heme iron which is poorly absorbed, also the presence of zinc reduces iron absorption by 30 - 50% (Berdanier and Zemleni, 2009) [10]. Toxicity is characterized by damage to the intestine with bloody diarrhea, vomiting, acidosis and sometimes liver failure (Berdanier and Zemleni, 2009) [10].

Conclusion

The study revealed significant ($P \leq 0.05$) differences in the anti-nutrient, vitamin A and mineral content of the control sample (100% maize) and the test samples with cowpea and carrot flour additions. The addition of cowpea and carrot flour to the germinated/pre-gelatinized maize flour increased the anti-nutrient, vitamin A and mineral content of the complementary food blend. The increase in cowpea flour resulted in significant increase in all the components analyzed. Sample G with 30% cowpea and 5% carrot addition had significantly ($P \leq 0.05$) the highest values while the control had the least. Considering the high content of anti-nutrient factors in samples with higher cowpea (20 – 30%) inclusion, Samples B and C with 5 and 10% addition of cowpea is recommended as their vitamin A content will meet >100% of the daily requirement and sufficient amount of the mineral needs.

Acknowledgements

The authors appreciate the technical assistance of Dr Friday Owuno of the analytical laboratory of Food Science and Technology, Rivers State University, Port Harcourt.

References

1. Aina VO, Sambo B, Zakari A, Haruna MSH, Umar H, Akinboboye RM, *et al.* Determination of Nutritional and Anti-Nutrient Content of *Vitis vinifera* (Grapes) Grown in Bomo (Area C) Zaria, Nigeria. *Advance Journal of Food Science and Technology*, 2012;4(6):445-448.
2. Akhtar MS, Israr B, Bhatti N, Ali A. Effect of Cooking on Soluble and Insoluble Oxalate Contents in Selected Pakistani Vegetables and Beans, *International Journal of Food Properties*, 2011;14(1):241-249. DOI: 10.1080/10942910903326056
3. Allen LH, Haskell M. Vitamin A requirements of infants under six months of age. *Food and Nutrition Bulletin*, 2001;22(3):214-234.
4. Anaemene DI, Fadupin GT. Effect of Fermentation, Germination and Combined Germination-Fermentation Processing Methods on the Nutrient and Anti-nutrient Contents of Quality Protein Maize (QPM) Seeds. *Journal of Applied Science and Environmental Management*, 2020;24(9):1625-1630.
5. AOAC. *Official Methods of Analysis of the Association of Analytical Chemists*. 19th ed. Washington, DC, USA., 2012.
6. Appel LJ. Nonpharmacologic therapies that reduce blood pressure: A fresh perspective. *Clin. Cardiol.*, 1999;22:1111-1115.
7. Aremu O, Nweze CC, Determination of vitamin A content from selected Nigerian fruits using spectrophotometric method. *Bangladesh Journal of Scientific and Industrial Research*, 2017;52(2):153-158.
8. Bello FA, Akpaoko NA, Ntukidem VE. Formulation and assessment of nutritional functional and sensory attributes of complementary foods from maize-carrot-pigeon pea flour blends. *Journal of Scientific Research and Report*, 2020;26(2):90-99.
9. Bello MO, Falade OS, Adewusi SRA, Olawore NO. Studies on the chemical compositions and anti-nutrients of some lesser known Nigeria fruits. *African Journal of Biotechnology*, 2008;7(21):3972-3979.
10. Berdanier CD, Zemleni J. *Advanced Nutrition Macronutrients and Micronutrients and Metabolism*. CRC. Press Taylor and Francis Group, New York., 2009
11. Chai W, Liebman M. Assessment of oxalate absorption from almonds and black beans with and without the use of an extrinsic label. *Journal of Urology*, 2004;172:953-957.
12. Chandran KC, Indira G. Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of *Strobilanthes Kunthiana* (Neelakurinji). *Journal of Medicinal Plants Studies*, 2016;4(4):282-286.

13. Diouf A, Sarr F, Ndiaye C, Ayessou NC, Fall SM. Improving Nutritional Quality of Cowpea (*Vigna unguiculata*) by Soaking Process. *International Journal of Food Science and Nutrition Engineering*,2020:10(1):37-41. DOI: 10.5923/j.food.20201001.03
14. Famata AS, Modu S, Mida HM, Hajjagana L, Shettima AY, Hadiza A, Chemical composition and mineral element content of two cowpea (*Vigna unguiculata* l. walp.) varieties as food supplement. *International Research Journal of Biochemistry and Bioinformatics*,2013:3(4):93-96.
15. Inuwa HM, Aina VO, Gabi B, Aimola I, Toyin A, Comparative Determination of Antinutritional Factors in Groundnut Oil and Palm Oil. *Advanced Journal of Food Science and Technology*,2011:3(4):275-279.
16. Jayathilake C, Visvanathan R, Deen A, Bangamuwage R, Jayawardana BC, Nammic S, *et al.* Cowpea: an overview on its nutritional facts and health benefits. *Journal of the Science of Food and Agriculture*,2018:98(13):4793-4806. DOI: 10.1002/jsfa.9074 *J Sci Food Agric* (2018)
17. Joshua ZP, Mariam IS, Goje EA, Suleiman MM, Dallhatu RY. Formulation and evaluation of maize (*Zea mays*) flour fortified with carrot (*Daucus carota*) powder. *Science World Journal*, 2021, 16(3).
18. Kormawa PM, Chianu JN, Manyong VM. Cowpea demand and supply patterns in West Africa: the case of Nigeria., 2002, 376 -386. <https://www.researchgate.net/publication/237536245>
19. Makinde FM, Abolarin OO. Effect of Post-Dehulling Treatments on Anti-Nutritional and Functional Properties of Cowpea (*Vigna Unguiculata*) Flour. *Journal of Applied Science and Environmental Management*,2020:24(9):1641-1647.
20. Nicolle C, Simon G, Rock E, Amouroux P, Rémésy C. Genetic variability influences carotenoid, vitamin, phenolic and mineral content in white, yellow, purple, orange and dark-orange carrot cultivars. *Journal of the American Society for Horticultural Science-JASHS*,2004:129(4):523-529.
21. Obinna-Echem PC, Barber LI, Jonah CP. Effect of germination and pre-Gelatinization on the proximate composition and pasting properties of maize Flour a base ingredient for cereal based infant complementary food. *Journal of Biotechnology and Food Science.*,2019:7(3):30-37.
22. Okafor UI, Adebunkola MO, Adewale OO, Mobolaji OB, Adeyeye SAO, Nutritional composition and antinutritional properties of maize ogi cofermented with pigeon pea. *Food Science and Nutrition*,2018:6:424-439.
23. Owade JO, Abong G, Okoth M, Mwangombe AW. A review of the contribution of cowpea leaves to food and nutrition security in East Africa. *Food Science and Nutrition*,2019:8(1):36- 47.
24. Peter HG, Carroll PV, Legumes importance and constraints to greater use. *Plant Physiology*,2003:131:872-877.
25. Popova A, Mihaylova D. Antinutrients in Plant-based Foods: A Review. *The Open Biotechnology Journal*,2019:13:68-76. DOI: 10.2174/1874070701913010068
26. Rashida P, Mohammed A, Satter SA, Jabin NA, Foridul I, Kamruzzaman M, *et al.* Studies on the Development and Evaluation of Cereal Based Highly Nutritive Supplementary food for young children. *International Journal of Innovation and Applied Studies*,2014:9(2):974-984.
27. Raut NA, Dhore PW, Saoji SD, Kokare DM, Chapter 9 - Selected Bioactive Natural Products for Diabetes Mellitus, Editor: Atta-ur-Rahman, *Studies in Natural Products Chemistry*, Elsevier,2016:48:287-322. ISSN 1572-5995, ISBN 9780444636027, <https://doi.org/10.1016/B978-0-444-63602-7.00009-6>.
28. Razafitsalama N. Evolution of antinutritional factors of seeds of two varieties of voandzou, mara and fotsy, during germination. DEA dissertation of Biochemistry applied to the sciences of food and nutrition). Faculty of Science: University of Antananarivo., 2006.
29. Roshana MR, Mahendran T. Nutritional and sensory evaluation of carrot flour-incorporated complementary food mixtures for infants. *Sri Lanka Journal of Food and Agriculture*,2019:5(2):27-32. DOI: <http://doi.org/10.4038/sljfa.v5i2.74>
30. Shakpo IO, Osundahunsi OF. Effect of cowpea enrichment on the physico-chemical, mineral and microbiological properties of maize: cowpea flour blends. *Research Journal of Food Science and Nutrition*,2016:1(2):35-41. Doi.org/10.31248/RJFSN2016.007
31. Sharma KD, Swati K, Thakur NS, Attri S. Chemical composition, functional properties and processing of Carrot: A review. *Journal of Food Science and Technology*,2011:49(1):22-32.
32. Sule EI, Umoh VJ, Whong CMZ, Abdullahi IO, Alabi O. Chemical and nutritional value of maize and maize products obtained from selected markets in Kaduna State, Nigeria. *African Journal of Food Science and Technology*,2014:5(4):100-104. DOI: <http://dx.doi.org/10.14303/ajfst.2014.029>
33. Traoré T, Mouquet C, Icard-Vernière C, Traoré A, Trèche S. Changes in nutrient composition, phytate and cyanide contents and alph-amylase Activity during cereal malting in small production units in Ouagadougou (Burkina Faso). *Food Chemistry*,2004:88:105-114.
34. WHO. Complementary feeding: report of the global consultation, and summary of guiding principles for complementary feeding of the breastfed child. Convened jointly by the Department of Child and Adolescent Health and Development and the Department of Nutrition for Health and Development, WHO Library Cataloguing-in-Publication Data, Geneva., 2001.
35. WHO/FAO. World Health Organization and Food and Agriculture Organization of the United Nations Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation, Bangkok, Thailand, 21–30 September, 1998. © World Health Organization and Food and Agriculture Organization of the United Nations., 2004.