# **Effects of Germination and Fermentation on Quality Characteristics of Infant Complementary Foods from Acha (***Digitaria exilis***) and Iron Beans (***Vigna Unguiculata)*

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#### *Abstract*

*The effects of germination and fermentation on quality characteristics of infant complementary foods from Acha (Digitaria exilis) and iron beans was investigated. Acha grains was cleaned, parboiled, oven-dried, milled and sieved to 0.6µm particle size flour. Similarly, 30kg grains after soaking in water (1:3w/v) % for 4 hr. was resoaked in 0.2% formaldehyde for 20 mins. drained and spread on moistened jute bag then overlayed with another wet bag and germinated for 5 days at room temperature. Green malt was ovendried, derooted and milled into flour. Another 30kg of acha grains was pretreated as above, fermented for 72 hr. then washed, dried and milled to flour. Iron beans were cleaned, soaked in 0.5% NaHCO3, washed, decorticated, oven-dried and milled to flour. Infant complementary foods were formulated using varying levels of acha flour (70%), germinated acha flour (35 – 70%), fermented acha flour (35 – 70%) and iron bean (30 – 50%), respectively. Amylase activities, proximate composition, mineral content, vitamin, functional properties, anti-nutrient, amino acid and sensory properties of the formulated foods were evaluated. About 0.71mg/100ml glucose was extracted in the germinated samples after 24 hr while 0.76 mg/100ml and 0.78mg/100ml was extracted on day 2 and 3, respectively. The 50:50 germinated acha: iron bean flour, 50:50 fermented acha: iron bean flour and 70:30 germinated acha: iron bean flour had 12.5, 12.03 and 11.7% proteins respectively. The K, Ca, Mg, Fe, Cu and P contents of the acha-iron bean complementary infant foods ranged from 528 – 895 mg/100g, 70.6 – 109.7 mg/100g, 89.1 – 168.6mg/100g, 0.36 – 1.75mg/100g and 275 – 82.5mg/100g respectively. Contents of vits A, B1, B<sup>2</sup> and C were generally low. The water absorption capacity, bulk density, solubility (%) and swelling power ranged from 1.8 – 3.5mg/g, 0.96 – 1.1 g/g, 10.0 – 18.4% and 8.9 – 13.2 (g/g) respectively. The oxalate, phytate, tannin and HCN contents of the flours were generally low. Essential amino acids contents were; Leucine (6.8 – 7.99), lysine (5.3 – 5.96), isoleucine (3.4 – 4.5), phenylalanine (3.7 – 4.5), valine (4.1 – 4.9), arginine (5.8 – 6.6), Alanine (4.2 – 14.3) and threonine (3.1 – 3.2), as compared to cerelac (control) and the FAO reference pattern. The 70:30 germinated acha: iron bean gruel was rated significantly (* $p \le 0.05$ *) higher than other tested samples for the colour, taste, texture, flavor, and overall acceptability characteristics. Germination and fermentation thus enhanced the nutritive value, physicochemical, functional properties and sensory acceptability of acha-iron bean infant complementary foods.* 

*Keywords: Germination, Fermentation, Physicochemical, Nutritional, Anti-nutrient, Acha, Iron Beans.*

#### **1. Introduction**

In developing countries, complementary infant foods are mainly prepared from cereals such as maize, rice, sorghum, wheat, acha, etc. The primary sources of nutrition for weaning children in these nations are cereals and legumes, either consumed separately or in combination (Okoye *et al.,* 2010). Breastfeeding by itself cannot satisfy an infant's nutritional needs after six months (Dewey and Brown, 2003). Infants are exposed to semisolid food gruels during this developmental stage as a method to introduce them to the family diet. The World Health Organization (WHO) recommends that supplemental feeding be provided in a timely, sufficient, appropriate, and sufficient amount (WHO, 2003). It is generally known that infants weaned from breast milk can find supplemental nutrition in traditional staples like grains (Ikujenlola, 2014). In Nigeria, the majority of kids are weaned on ogi, a fermented corn gruel (Elemo *et al*., 2011). The bulkiness and low nutrient density of these handmade supplemental foods are two key issues. Additionally, young babies' stomachs cannot hold so much of these low-nutrient diets. The majority of these meals are very starchy, making it impossible for newborns to consume and benefit from there nutrient containts. Consumption of these starchy gruels which are inadequate in protein, energy, essential amino acids, and micronutrients has been the major cause of nutrient related illnesses, weak immunological response, and retarded body growth in infants (National Nutritional and Health Survey (NNHS) [Nigeria] 2015; Nwosu *et al.,* 2014; Nnam, 2000).

Numerous researches have demonstrated that the bulkiness issue has been resolved utilizing a variety of processing techniques, including germination. In many cereal-producing regions, germination is a widespread procedure in which grains are germinated to develop amylolytic qualities that lower the high dietary bulk in the products ((Murugkar *et al*., 2013). The application of fermentation improves the nutritional composition, sensory qualities, and digestibility of the foods for infants (Ojokor *et al*., 2020) In most developing nations, numerous brands of proprietary weaning foods have been created and marketed in addition to indigenous supplementary newborn foods (Okafor *et al.,* 2008). When they are available, these commercial supplemental foods are frequently too expensive and out of the price range of the majority of Nigerian households, especially in the rural areas (Ikujenlola, 2014). Thus, affordable cereals that are readily available locally must be used to their fullest extent in order to create dishes that are low in viscosity, rich in calories, and nutrient dense (Ozumba *et al.,*2002; Elemo *et al.,* 2011)

Acha (*Digitaria exilis*) contains about 9 to 11%, 84 to 86% carbohydrate. 3.3 to 3.5% lipids and about 1.1% minerals (Cruz *et al*., 2011) Its protein is reported to be high in leucine (9.8%), methionine (5.6%), and valine (5.8%) (Ballogou *et al*; 2013). Because of the nutritional value, acha is highly recommended for diabetic patients by doctors (Philipband Itodo, 2006). The Northern region of Nigeria has an abundance of acha, however there is little knowledge on how it could be used in complementary food preparations (Ikujenlola, 2014). Like other cereals, Acha lacks lysine (Ikujenlola, 2014). The problem of malnutrition in infants can be solved by introducing weaning food formulation from acha *(D .exilis* ) and iron beans.

Beans (*Vigna unguiculata)* has been reported to be the most important food legume in dry Savanna of tropical Africa (AATF, 2005). It is consumed by millions of people in the tropics especially Africa (AATF,2005). Nigeria is the world's largest producer of cowpea (FAO/STAT 2015). Iron beans (big seed) is one of the many varieties of beans consumed in Nigeria. Iron beans contains about 26 % crude protein (Otitoji *et al* ;2015; Uduak, 2018). Beans also contain a considerable quantity of soluble fiber, with one cup of cooked beans containing nine to thirteen grams. Compared to cereal grains, the protein in beans is higher in the amino acids lysine and tryptophan. As a result, bean seed is appreciated as a cereal nutritional additive.

#### **2. Materials and Methods**

Acha grains and Iron beans (big seeds) were obtained from Mile 1 market Port Harcourt, Rivers State. The analytical grade reagents used for analysis were products of the British Drug Houses (BDH) England and were of analytical grade.

#### **2.1 Preparation of acha flour**

Acha flour was prepared by sorting, cleaning, washing and boiling of grains for 5 minutes, and then draining. The drained grains were oven dried at 60◦C for 12 hours before milling and sieving into flour using 0.4 -0.6  $\mu$ m sieve (Fig 1).



### **Fig 1: Production Sequence of Acha flour**

# 2.2 Preparation of germinated acha flour

Acha grains were germinated according to the method described by Elkhalifa (1998) with slight modification as shown in (Fig 2). The acha grains were steeped in water for 4 h. The wet grains were subsequently soaked in 1-2 volumes of 0.2 % formaldehyde solution for 40 mins to retard mold growth during germination. The soaked grains were then washed with sterile water and then soaked in water for 20 min to remove residual formaldehyde. The grains were subsequently drained and spread over jute bag and covered with another jute then placed in a dark room. The grains were allowed to germinate at room temperature for 5 days with constant spraying of water and revolving of bed mat to prevent drying out and excessive rooting. Samples were withdrawn at 24 hr. interval for 5 days and oven dried, derooted, ground and sieved to 0.4µm flour, then assayed for amylase activity. (Fig 2)





# **Fig 2: Production Sequence of Germinated acha flour**

### **2.3 Preparation of fermented acha flour**

Fermented acha flour was prepared as described by Chikwendu*, et al*., (2014), with slight modification. Acha grains were cleaned manually, washed, soaked in cold water at 1:3 w/v (%) and allowed to ferment for 72 h at room temperature. The fermented acha was then washed and oven dried at 60℃ for 12 h then milled and sieved to a 0.4-0.6µm flour size (Fig 3). The flour was packed in polythene bags for further analysis.



#### **Fig 3: Production Sequence of Fermented acha flour**

#### **2.4 Production of iron bean flour**

The iron bean seeds were sorted, cleaned, winnowed, and washed. The cleaned seeds were then soaked for 24 hours with  $0.5\%$  NaHCO<sub>3</sub> with six hourly change of water, then washed, drained, and dried at 60◦C for 12 h before milling and sieving into flour, using the method of Yahya *et al.* (2022), with modification. The flour was packed in polythene bags for further analysis

## **2.5 Sample formulation**

The complementary foods were formulated using the following flour blends (Table 1)

	Level of Substitution $(\% )$				
<b>Sample</b>	Acha <b>Flour</b>	<b>Germinated</b> <b>Acha Flour</b>	<b>Fermented</b> <b>Acha Flour</b>	<b>Iron Beans</b>	
A (commercial gruel; cerelac)					
B	70			30	
		70		30	
D			70	30	
E		50		50	
F			50	50	
G		35	35	30	

**Table 1: Recipe for formulation of acha - iron beans complimentary foods.**

## **2.6 Estimation of Amylase Activity**

Germinating acha grains were taken at 24 hours intervals and dried at 60◦C, then ground in a blender to fine flour. Amylase activity was determined by the method described by Bernfeld (1955) as modified by Elemo *et al*., (2011). Two grams of the germinated acha sample was suspended in 10ml of double distilled water for 1 h at room temperature with occasional shaking and centrifuged (10,000 g, 10 min). The supernatant was then taken for the enzyme assay. One milliliter of the above extract was incubated for 1 h with 0.5 ml of 1% soluble starch and 0.5 ml of 0.1 M acetate buffer,  $P^{H}$  4.5 in a water bath. Then 2 ml of dinitrosalicylic acid (DNS) was added and boiled for 20 min in a boiling water bath. After cooling, the volume was made to 25 ml with distilled water and the absorbance of the solution was read at 550 nm in a UV2100 Recording Spectrophotometer (Shimadzu, Kyoto, Japan)

# **2.7 Proximate Composition**

Percentage moisture, Ash, fat, protein and crude fiber were determined using AOAC (2012) standard method, while Carbohydrate content was determined by difference; % available carbohydrate =  $100 - (% \text{moisture} + % \text{ Ash} + % \text{ Fat} + % \text{ crude protein} + % \text{ crude fibre}).$ 

### **2.7 Determination of mineral content**

The following minerals Ca, Mg, Cu, Fe, P and K were determined using the Atomic Absorption spectrophotometer (Buck – Scientific – 210 Vap, USA) after preparation of their mineral solutions.

# **2.7.1 Preparation of Mineral Solution**

Mineral solution was prepared using AOAC (2012) standard procedure.

### **2.7.2 Determination of vitamin A (β-carotene)**

The B-Carotene was determined by soaking 1g of the sample in 5ml of methanol for 2 h at room temperature under dark condition in order to get a complete extraction. The B-Carotene layer was separated u using hexane through separating channel. The volume was made up to 10ml with hexane and then this layer was passed through sodium sulphonate through a funnel in order to remove any moisture from the layer. The absorbance of the layer was measured at 436mm using hexane as a blank (Ranganna, 1999). The beta-carotene was calculated using the formula:

W

## Calculation:

Beta Carotene  $(UG/100g) = \frac{Absorbance \times V \times D \times 100 \times 100 (436nm)}{V}$ 

Where  $V = Total Volume of extract$ 

 $D = D$ ilution Factor

 $W =$  Sample Weight

 $Y =$  Percentage dry matter content of the Sample

To express the vitamin A activity of carotenoids in diets on a common basis, a joint FAO/WHO

Expert Group (FAO/WHO) in 1967 introduced the concept of the Retinol Equivalent (RE) and

established the following relationships among food sources of vitamin A.

 $1\mu$ g = 1 RE method used. Variation in ecological growth conditions

1µg β-carotene = 0.167 µg RE like variety and environmental aspects may also be

 $1\mu$ g other pro-vitamin A carotenoids = 0.084  $\mu$ g RE contributing factors.

### **2.7.3 Determination of vitamin B<sup>1</sup> (Thiamine)**

The vitamin B1 (Thiamin) content of the samples was determined according to the method described by Okwu and Josiah, (2006). One gram (1g) of each sample was homogenized with 10ml ethanoic potassium hydroxide (KOH) and then filtered into a 100ml conical flask. 1ml the filtrate was pipette and the colour developed by addition of 2ml of 1% potassium dichromate and absorbance was read at 360nm. A blank solution was also prepared.

The thiamine content (Vitamin B1) was calculated as follows:

Calculation:

 $Mg/Kg = \frac{A}{Rg}$  $\frac{AB3 \times VL}{EC \times 1 \times WC}$ Where:  $Abs = Absorbance$  $Vt = Volume of extract from which aliquot was taken$  $I =$  Curvette Thickness or Path length EC= Extinction coefficient

# **2.7.4 Determination of Vitamin B<sup>2</sup>**

Vitamin B2 content was determined by the method described by Okwu and Josiah (2006). One gram (1g) of the sample was extracted with 20ml of 50% ethanol and shaken for one hour. This was filtered into a 100ml flask. 2ml of the extract was pipette into 50ml volumetric flask. 2ml of 5% potassium permanganate and 2ml of 30%  $H_2O_2$  (Hydrogen Peroxide) was added and allowed to stand over a hot water bath for 30 min. 0.5ml of 40% sodium sulphate was added. This was made up to 10ml mark and absorbance read at 510nm using spectrophotometer.

Vitamin B2 Content was calculated as follows. Calculation:

 $Mg/Kg = \frac{E}{E}$ Where  $Abs = Absorbance$  $Vt = Volume of extract from which aliquot was taken$  $L =$  curvette Thickness or path length  $EC = Extinction co-efficient$ 

### **2.7.5 Determination of Vitamin C**

The Vitamin C content of the samples were determined using AOAC (2012) standard procedure.

## **2.8 Functional Properties**

Functional properties of the blends such as bulk density, water absorption capacity and swelling index were determined according to the standard procedures;

# **2.8.1 Bulk Density**

The method of Akpapunam and Markakis (1981) was used. A 10 ml-graduated cylinder was gently filled to mark with the sample. The filled cylinder was gently tapped on a laboratory bench about 10 times until there was no further diminution of the sample level after filling to the 10 ml mark. The procedure was adopted for each of the sample and the bulk density was calculated using the formula:

Bulk density  $(g/ml) = \frac{1}{v}$ packed weight of sample

## **2.8.2 Water Absorption Capacity**

The method described by Elkhalifa *et al*. (2005) was used to determine the water absorption capacity of the flour samples. Five millilitres of water were added to 1.0 g of the sample in a centrifuge tube. The mixture was sonicated for 1 min to disperse the sample and the suspension was allowed to stand for 30 min. The suspension was then centrifuged after standing at 3500 rpm for 30 min and the water absorbed was calculated using the formula:

Water absorbed (ml/g) =  $\frac{a}{a}$ 

where  $a = initial volume of water$  $b =$  final volume of water

# **2.8.3 Swelling Power and Solubility**

The swelling power and solubility were carried out using method Crosbie (1991). One gram of the sample was weighed and transferred into a 100ml conical flask. 15ml of distilled water was added to the sample and mixed. The sample suspension was sent to a shaker bath (Gallenkamp, UK) set at  $100^{\circ}$ c for 1 h. The Conical flask and its content were cooled under running water, transferred into weighed centrifuge tube. This was centrifuged at 3000rpm for 30 min using a digital control centrifuge (L-600, China). After centrifuging the swelling volume was read off the graduation on the centrifuge tube the height of the swollen sediment. The clear supernatant was transferred into a previously dried and weighed moisture can and was dried in the oven (DHG-9140A, China), cooled and weighed to get the solubility. The weight of the centrifuge tube and swollen sediment was also taken to calculate for swelling power.

Weight of soluble matter x 100 Solubility  $(\%) =$ Sample weight

Swelling power  $(\%) = \frac{\text{Weight ofswelling sediment}}{\text{x 100}}$ Sample weight

# **2.9 Determination of Antinutrients**

### **2.9.1 Oxalate Determination**

The oxalate content of the samples was determined according to the method described by Munroe, (2000). One gram (1g) of each sample was weighed into a 250ml conical flask. 75 ml of  $3N H<sub>2</sub>SO<sub>4</sub>$  was added to each sample and then filtered using a Whatman No 1 fitter paper. 25ml of filtrate was then pipette into a beaker and 2 drops of methyl red indicator was added. The solution was then heated to boil and filtrated while hot against  $0.05m$  KMnO<sub>4</sub> until a faint pink colour persist for at least 30 seconds.

The Oxalate content is calculated by taking 1ml of  $0.5m$  KMnO<sub>4</sub> as equivalent to 2.2mg oxalate.

Calculation

Oxalate  $(mg/100g) = \frac{\text{Titre value} \times 2.2}{\text{T}}$  $\frac{u \cdot u}{w}$  x DF X 100

Where  $2.2mg =$  mass equivalent oxalate value of 1ML of 0.05m KMnO<sub>4</sub> solution.

 $DF = Dilution Factor$ . That is total volume of sample divided by volume of portion used for titration.

 $W =$  Sample weight in g.

# **2.9.2 Tannin Content Determination**

The tannin content of the formulated infant complementary food blends was determined using the method described by Galvao *et al.* (2018). 0.5mg of joy well blended sample was weighed into a flask.

10mls of distilled water was added and the mixture was agitated and then allowed to stand for 30min at room temperature. The solution was then centrifuged at 2500 rpm for 15min. 1ml of the supernatant was measured into 10ml volumetric flask. 0.5ml of folin Ciocalteu reagent was added followed by 1ml of saturated  $Na<sub>2</sub>CO<sub>3</sub>$  solution, the solution was diluted to 20ml with distilled water and incubated for 30 min at room temperature. Absorbance was read at a wavelength of 725nm.

Tannic acid content is calculated as shown below:

Tannic Acid content  $(Mg/Kg) = \frac{\text{Concentration contained in mg/L} \times \text{Volume of sample} \times \text{DF}}{g/Kg}$ Sample weight

# **2.9.3 Phytate Content Determination**

Phytic acid content was determined according to the method of Russel, (1980). 0.5g of the sample was weighed into 250ml conical flasks. 25ml of 2% concentrated HCL was added, and the solution was allowed to soak for 3 hr. and then filtered. 12.5ml of the filtrate was pipette into 250ml beaker and 26.75ml of distilled water was added to improve acidity. 2.5ml of 0.3% ammonium thiocyanate solution was added as indicator. The solution was titrated with standard Iron III Chloride (FeCl<sub>3</sub>) solution which contain 0.00195g iron/ml until a brownish yellow colour appear and persist for 5 min. Phytic acid content was calculated as follows:

Phytic acid  $g/kg = \frac{0.00195 \times \text{volume of FeCl3} \times \text{DF} \cdot \text{Consumed}}{25.000195 \times \text{volume of FeCl3} \times \text{DF} \cdot \text{Consumed}} \times 100$ sample WT

# **2.9.4 Hydrogen Cyanide Content Determination**

**Extraction of cyanide sample**: This extraction was carried out according to Wang and Filled method.

One gram (1g) of sample was weighed into a conical flask and dissolved with 20ml of distilled water. The cyanide extraction was allowed to stay overnight. The extract was filtered, and the filtrate used for the cyanide determination.

# **Preparation of Alkaline Picrate Solution**

One gram (1g) of picrate and 5g sodium carbonate were dissolved in a volume of minimally warm water and the volume made up to 200ml with distilled water.

# **Procedure for Cyanide Determination**

One Milliliter (1ml) of the filtrate was measured into a corked test tube and 2ml of alkaline picrate was added and incubated in a water bath at  $80^{\circ}$ c for 5 min. After colour development (Reddish brown colour), absorbance of the corked test tube was read in spectrophotometer at 490mm. Absorbance of the blank containing only 1ml distilled water and 2ml alkaline picrate solution was read. The cyanide content was extrapolated from a cyanide standard curve.

### **Preparation of Cyanide Standard Curve**

Different concentrations of KCN solution containing 5 to 50 µg cyanide in a 500ml conical flask was prepared. 25ml of 1N HCL was Prepared. 25ml of 1n HCl was added. To 1ml standard solution in a corked test tubes was added 4ml alkaline picrate and circulated in a water bath for 5min. After the colour development (reddish brown colour) absorbance of the corked test tube was read in spectrophotometer at 490nm. A cyanide standard curve was prepared using different concentrations.

# **2.10 Amino Acid analysis**

Quantification of amino acids of the samples was carried out using the method outlined by Wang and Cavins (1989), with little modifications where necessary. Precise volume of 100cm3 was measured into 250ml quick fit round bottom flask and dried using rotary evaporator. A clean spatula was used to remove the dried sample and defatted in Soxhlet extraction apparatus using chloroform methanol mixture (2:1). Known weight indicated in the result sheet was hydrolyzed, filtered through nonabsorbent cotton wool or glass wool.

## **2.10.1 Nitrogen Determination**:

A small amount (115mg) of ground sample was weighed, wrapped in Whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na2SO4), copper sulphate (CuSO4) and selenium oxide (SeO2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Six pieces of anti-bumping granules were added. The flask was put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10m1) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected.

The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured end point.

Percentage Nitrogen =  $\frac{(a-b)x 0.01}{W}$ Where:



# **2.10.2 Hydrolysis of the sample**

A known weight (mentioned in the calculation sheet) of the defatted sample was weighed into glass ampoule. 7ml of 6NHCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 1050C± 50C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. It should be noted that tryptophan is destroyed by 6N HCL during hydrolysis. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

#### **2.10.3 Loading of the hydrolysate into analyser and calculation of amino acid concentration**

The amount loaded was 60 microliters. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyse free acidic, neutral, and basic amino acids of the hydrolysate. An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

### **2.11 Sensory Evaluation**

The formulated complementary food blends were served to a 20 semi- trained panelist made up nursing mothers who were familiar with the sensory attributes of complementary infant foods such as colour, aroma, texture, taste and over all acceptability. A nine (9) point hedonic scale was designed to measure the degree of preference of the samples. (Iwe, 2007).

## **2.12. Statistical Analysis**

All the analyses were carried out in duplicate. Data obtained were subjected to Analysis of variance (ANOVA), differences between means were evaluated using Tukey's multiple comparison test and LSD. Significance accepted at  $P \le 0.05$  level. The statistical package in SPSS version 25 computer program was used.

### **3. Results and Discussion**

# **3.1 Amylase Activity of Germinated Acha Flour**

From the result in Figure 4, day 3 germination showed optimum amylase activity. Amylase activity of the extracts increased with increasing germination time reaching a maximum on day 3 and declined thereafter. This result corroborated with earlier report by Elkahia and Bernhali (2010) and Elemo *et al.* (2011) that in germinated sorghum, amylase activity reached a maximum on the  $3<sup>rd</sup>$  day and decreased steadily thereafter. Amylase activity in grains has been found to break down starch to maltose and with germination, its activity increases thereby lowering the viscosity of the food. (Correoa *et al*.,2008).



**Fig 4: Evaluation of Amylase Activity of Germinated Acha Flour**

# **3.2. Proximate Composition**

Proximate composition of the formulated complementary infant food blends from acha and iron beans compared to the control (cerelac) and RDA reference pattern are shown in Table 2. The moisture content of the samples ranged from  $8.3 - 8.60$ . The results indicated that the moisture content of the formulated blends met the minimum recommendation by FAO/WHO (1998) of less than ten  $(10)$  patterns indicated for processed cereal foods  $(5 - 10\%)$ recommended by protein advisory group (FAO/WHO/UNICEF, PAG ,2007). Oduro et al., (2007) and Morris *et al*., (2004) reported that low moisture content can increase the concentration of nutrients in foods and make it more available. The moisture content of Cerelac (control) was significantly lower than that of the moisture contents of the tested samples. The Lower the moisture content of food, the higher it's keeping quality. Low moisture indicates good shelf life when properly packed and stored (Etudaiye *et al*., 2009). The higher the moisture contents of food the lower the shelf life stability (Ajatta *et al* 2016).

The ash content of the tested samples ranged from 1.6 -2.4 %. The importance of the ash content is that it indicates the mineral portion of a food (Owiredu *et al*., 2013). The ash contents of the 70:30 germinated acha flour: iron bean flour and 50: 50 fermented acha flour: iron bean flour compared favorably with the ash content of Cerelac (control). CODEX (1999) reported that the ash content of a complementary food should be less than 5 %. All the formulated complementary food samples, therefore, meet this standard.

The protein contents of the tested samples differed significantly  $(p<0.05)$  from the protein content of Cerelac (control). The values ranged from 9.62 to 12.50mg/100g compared to the 14mg/100g recorded for the cerelac control and the  $13 - 14$  mg/100g RDA for infants  $0 - 1$ years. Sample E (50 parts germinated acha flour: 50 parts iron bean flour) and sample F (50 parts fermented acha flour: 50 parts iron bean flour) had the highest protein content amongst the formulated complementary infant food blends (12.03 and 12.50 mg/100g, respectively). These values compare favorably with that of Cerelac (14mg/100g) and the RDA for infants 0 - 1 years. The values obtained for the protein contents of samples E and F are similar to those reported by Elemo *et al*., (2011) for germinated sorghum and steamed cooked cowpea weaning food formulations. The reason for the high protein content of samples E and F may be adduced to higher iron bean content. Majekodunmi and Olapade ,2018 reported similar result for complementary food from acha and cowpea flour. Almeida *et a.,* (1993) also reported an increment in protein content of millet based complementary foods with addition of cowpea. Protein is important, especially during the weaning period to prevent protein energy malnutrition (PEM), which is usually observed among children in developing countries (Achidi *et al*., 2016).

Fiber contents of the tested samples ranged from 1.4 -3.2 %. The fiber contents of the samples were below the 5% range recommended by FAO/WHO (1989). The low fiber contents of the samples could be of nutritional advantage in a complementary food formula because it will improve the quality of the complementary diet (Oroniran *et al.,* 2017). Also, it has been reported that when the fiber content of a complementary food is above 5%, it can reduce the nutrient density of the food by adding bulk and may cause gastrointestinal irritation as well as reduce the availability of minerals such as calcium, magnesium, iron, zinc, and copper. Fibre plays a role in the increased the utilization of nitrogen and absorption of some other micronutrients (Obinna-Echem *et al*., 2018).

The lipid content of the acha-iron bean complementary foods ranged from 1.50 to 4.0 %. The lipid contents of samples C (70:30 germinated acha flour: iron bean flour) and G (35:35:30 germinated acha flour: fermented acha flour: iron bean flour) were significantly  $(p<0.05)$ higher, with values of 4.00 and 3.99 % respectively. The lowest lipid content was recorded for sample B (1.5%) The lipid contents of the blends were below the RDA value of 10 to 15 mg/100g. The low-lipid content of the blends resulted to the low energy level of the blends. Fat is important in the diets of infants and young children as it provides essential fatty acids, facilitates absorption of fat-soluble vitamins, enhances dietary energy density and sensory qualities and the prevention of undesirable weight gain in infants (Obinna-Echem *et al*., 2018). However, Adegunwa, *et al* (2014) reported that low fat content in a dry product will help in increasing the shelf life of the sample by decreasing the chances of rancidity and also contribute to low energy value of the food product.

The carbohydrates also contribute to the energy values of each formulation. From the results obtained, sample B had the highest carbohydrate value followed by sample D. Sample A(cerelac control) had the least carbohydrate value. The high carbohydrate contents of these weaning food formulation make them ideal for babies since they require energy for their rapid growth. Carbohydrate contributes a lot towards energy in complementary foods. Its content could be high but must be digestible enough for infants and young children to obtain the energy required or needed (CODEX, 1999). WHO recommended intake of carbohydrate for infants is  $60g/day$  for 0 -6months old and 85 g/day for  $7 - 12$  months old children, this agreed with the carbohydrate content of 70.3% for the formulated complementary foods. The energy content of the Acha-iron beans complementary infant foods ranged from 357.1 to 365.3 Kcal/100g. The energy contents of the formulated blends compared favorably to cerelac control. However, the energy content of the formulated acha -iron bean blends did not meet the RDA for infants  $0 - 1$  years (400 -425 Kcal/100g). The number of kilocalories (often termed "calories") needed per unit of a person's body weight expresses energy needs (Lawrence *et al*., 2005).





Values are means  $\pm$  standard deviation of duplicate samples

Mean values bearing different superscripts in the same column differ significantly  $(p<0.05)$ 

**Key**:  $A = 100\%$  cerelac flour,

 $B = 70\%$  acha flour and 30% iron bean flour.

 $C = 70\%$  germinated acha flour and 30% iron beans flour.

D =70% fermented acha flour and 30% iron beans flour.

 $E = 50\%$  germinated acha flour and 50% iron beans flour.

F =50% fermented acha and 50% iron beans flour.

G= 35% germinated acha flour, 35% fermented acha flour and 30% iron bean flour

### **3.3 Mineral Content of Acha-iron Beans Complementary Infant Foods**

The potassium and magnesium contents of the formulated complementary blends were higher than the RDA of infants 0 -1 years (Table 3). These results are higher than those reported by Okeke and Chikwendu *et al.,* (2015) for fermented African yam bean and acha flour blends. The calcium content of the formulated blends was significantly lower  $(p<0.05)$  than that of the control and did not meet the RDA for infants 0 -1 years. Similarly, significantly lower phosphorus contents were recorded for the formulated complementary blend (B, C, D, E, F and G) compared to the control (sample A). The results of the calcium and phosphorus contents for the formulated blends were also found to be lower than the result for calcium and phosphorus as reported by Enoch *et al.,* (2019) for low -cost infant's diet from five locally available foodstuffs. However, the magnesium contents of the blends were found to be higher than the magnesium contents reported by Enoch *et al.,* (2019). Children develop birth defects and inability to learn properly among other long-term disabilities when minerals are in inadequate supply (Okafor *et a*l.,2018; Szalai *et al.,* 2001).

The low levels of iron, calcium and phosphorus in the complementary infant food blends when compared with the control (cerelac) could be attributed to the fact that commercial weaning foods are usually fortified with micro and macro nutrients in order to meet the FAO/WHO guidelines in infant complementary food formulations (Ijarotimi and Keshinro, 2022). Hence, the result of this study suggests that the formulated foods need to be fortified with calcium and phosphorus as they are essential for the formation of strong bones and teeth as well as iron and other essential macro and micro elements.





Values are means  $\pm$  standard deviation of duplicate samples

Mean values bearing different superscripts in the same column differ significantly  $(p<0.05)$ 

### **3.4 Vitamin Contents of Acha-iron beans Complementary Infant Foods**

There were significant differences  $(p<0.05)$  between the complementary blends and the control in vitamin A, Vitamin  $B_1$ , vitamin  $B_2$  and vitamin C contents (Table 4). Sample F had significantly higher vitamin A content among the complementary blends. Similarly, sample D has a significantly higher ( $p<0.05$ ) vitamin B1 content than the other formulated samples. Significantly higher ( $p<0.05$ ) vitamin B2 content was recorded for samples D and G for the complementary blends. Samples B, D and F had significantly higher  $(p<0.05)$  vitamin C content than samples C, E and G. Hence, the formulated complementary infant food blends (B, C, D, E, F and G) did not compare favourably with the vitamin A, vitamin B1, vitamin B2 and vitamin C contents of the control (cerelac) and did not meet 1/3 RDA for infants 0-1 years.



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Values are means  $\pm$  standard deviation of duplicate samples

Mean values bearing different superscripts in the same column differ significantly  $(p<0.05)$ 

#### **3.5 Functional Properties**

From the result in Table 5, The highest water absorption capacity was recorded in sample B (3.49mg/g). The water absorption capacities of the formulated blends compared favorably with that of the control (cerelac). The significance of water absorption capacity is that such products can be used where lower water absorption capacity is desirable especially in thinner gruels with high caloric density per unit volume Oronira *et al.,* (2017).

All the formulated samples had low bulk densities but did not compare favorably with the cerelac (control). The low bulk densities implied that the gruel or porridge made from these diets will have a lower dietary bulk. This is important in complementary foods as high dietary bulk limits the caloric and nutrient intake per feed per child, and infants sometimes are unable to consume enough to satisfy their energy and nutrient requirements. Omueti *et al*., 2009).

Swelling index (power) recorded for the acha-iron beans complementary foods ranged from 1.3 to 13.20 g/g. The values recorded for the formulated blends were significantly higher  $(p<0.05)$  than the value recorded for the cerelac control. The implication of low swelling power to the quality of the complementary food is that thinner gruels with low viscosity will be produced while samples with higher swelling power will produce thick viscous gruels which is as a result of their high carbohydrate content. (Oninoran *et al.*, 2017). According to WHO, appropriate weaning food is the one which produces a gruel that is neither too thin or too thick (when it is thick, it will be difficult for the infant to ingest and digest because of limited gastric capacity) for the infant to consume. (Oninoran *et al*., 2017).

The result of the % solubility for the formulated infant complementary foods ranged from 10.03 to 18.42. These results did not compare favorably with the % solubility recorded for the control (cerelac). Solubility is an index of protein functionality such as denaturation and its potential applications. The higher the solubility, the higher the functionalities of the protein in the food.

	<b>Water</b>			<b>Swelling</b>
<b>Sample</b>	Abs. $(mg/g)$	<b>Bulk Density(g/g)</b>	Solubility (%	Power $(g/g)$
$\mathbf{A}$	$1.9^b \pm 0.0$	$0.6^{\circ}$ ±0.0	$39.3^a \pm 0.14$	$1.3^d \pm 0.3$
B	$3.5^a \pm 0.4$	$1.10^b \pm 0.1$	$10.0^{\text{g}}\text{\textsterling}0.0$	$9.2^{\circ}$ ±0.3
$\mathbf C$	$1.9^b \pm 0.1$	$0.99^{ab}$ ± 0.0	$14.0^{\rm d}$ ±0.0	$9.98^b \pm 0.0$
D	$1.8^b \pm 0.3$	$1.1^{ab}$ ±0.0	$11.0^{\text{f}}\pm0.0$	$13.2^a \pm 0.3$
E	$1.8^b \pm 0.0$	$1.05^{ab}$ ± 0.07	$17.3^{\circ}$ ±0.42	$8.9^{\circ}$ ±0.14

**Table 5: Functional Properties of Acha-iron Beans Complementary Infant Foods** 



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Values are means  $\pm$  standard deviation of duplicate samples

Mean values bearing different superscripts in the same column differ significantly  $(p<0.05)$ 

#### **3.6 Antinutrient Content**

Tannin contents of the acha-iron beans complementary foods ranged from 0.165 to 1.265 mg/Kg. Tannin content was highest in sample C (1.265mg/Kg) compared to tannin content in samples C, D and B (0.165, O,38 and 0.52 mg/100g respectively), resented in Table 6. The results are similar to the reports by Okojoh et al., (2020) for fermented sorghum and cowpea blends.

Oxalate content in the samples (A, B, C, D, E, F and G) were not significantly different (p<0.05) giving a yield of 0.01, 0.02, 0.0085, 0.0075, 0.015, 0.06 and 0.0085 mg/100g respectively. (Table 4). These values are lower than the values for oxalate reported by Okojoh et al., (2020) for fermented sorghum and cowpea blends.

Phytate content of the samples A, B, C, D, E, F and G were found to be significantly different at ( $p<0.05$ ). The values ranged from 2.45 to 4.86 g/Kg. This report does not agree with the findings of Ojokoh *et a*l., (2020) who reported values of 0.24 to 0.58 for fermented sorghum and cowpea blends.



#### **Table 6: Antinutrient contents of Acha -iron beans Complementary Infant Foods**

Values are means  $\pm$  standard deviation of duplicate samples

Mean values bearing different superscripts in the same column differ significantly  $(p<0.05)$ 

#### **3.7 Amino Acid Content of Acha -iron beans Complementary Infant Foods**

The amino acid pattern of the acha-iron bean complementary foods compared to the FAO reference pattern. The levels of essential amino acids leucine, lysine, phenylalanine, valine, and threonine in the formulated complementary infant foods were significantly higher  $(p<0.05)$  when compared to the FAO reference values while the concentration of the remaining amino acids was comparable to the FAO reference values (FAO/WHO/UNICEF,

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2007). This suggests that the protein content of the formulated diet is of high quality. Lysine is essential for children as it is critical for bone formation, it is involved in hormone production, lowers serum triglyceride levels (Gersten, 2013). Arginine is thought to be conditionally essential for children up to 5years old and the elderly 60 and up while histidine is essential for children up to 5 years of age (Sowers, 2009). The amino acid pattern of the formulated blends also compared favorably to the control (cerelac).





Values are means  $\pm$  standard deviation of duplicate samples Mean values bearing different superscripts in the same row differ significantly  $(p<0.05)$ **FAO=** FAO reference preterm

### **3.8 Sensory Properties**

The colour scores of the acha-iron bean complementary foods differed significantly ( $p<0.05$ ). Sample C (50parts germinated acha flour: 50 parts iron bean flour) compared favourably with sample A (cerelac control). The lowest score for colour was recorded for sample D (4.4) followed by sample F (5.0).

There were variations in the texture scores of the acha -iron bean complementary foods. the highest texture score was recorded for sample A followed by sample C. sample C compared favorably to the cerelac control in terms of texture. The lowest score for texture was recorded for sample B.

The taste score for sample A (cerelac control) was significantly higher than the taste score for samples B, C, D, E, F and G. Sample G has a taste score of and this compared favorably to sample A (cerelac control)

The flavors of the acha-iron beans complementary infant foods were influenced by the proportion of iron bean flour in the formulated samples. The lowest score for flavor was recorded for samples E (50 parts germinated acha flour:50 parts iron bean flour) and F (50 parts fermented acha flour: 50 iron bean flour). Sample C (70 parts germinated acha flour: 30 parts iron bean flour) compared favorably to sample A (cerelac control). In terms of overall acceptability, sample A had the highest score. Sample C compared favorably to the cerelac control followed by sample G.

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<b>Samples</b>	<b>Colour</b>	<b>Taste</b>	<b>Texture</b>	<b>Flavor</b>	<b>Overall</b> <b>Acceptability</b>			
A	$8.3^a \pm 0.73$	$8.25^a \pm 0.64$	$8.20^a \pm 0.77$	$7.95^a \pm 1.15$	$8.2^a \pm 0.03$			
$\mathbf{B}$	$5.0^{\rm d}$ ±1.17	$4.50^{\circ}$ ± 0.95	$4.80^{\rm d}$ ±1.15	$5.65^{\circ}$ ± 1.49	$5.2^{\circ}$ ±0.09			
$\mathcal{C}$	$7.05^b \pm 1.32$	$6.8^{b} \pm 1.06$	$7.1^b \pm 0.97$	$6.95^b \pm 1.39$	$5.3^{\circ}$ ±0.08			
D	$4.4^{d}$ ±1.39	$4.45^{\circ}$ ± 1.39	$6.25^{\circ}$ ± 1.25	$5.40^{\circ}$ ± 1.09	$5.1^{\rm d} \pm 0.03$			
E	$6.2^{\rm c}$ ± 1.32	$3.7^{\rm d}$ ± 1.30	$5.7^{\circ}$ ± 1.08	$4.5^d \pm 0.61$	$5.0^{\rm e}{\pm}0.01$			
$\mathbf{F}$	$5.0^{\rm d}$ ± 1.08	$4.35^{\text{cd}} \pm 0.67$	$6.3^{\circ}$ ± 1.22	$4.5^{\rm d}$ ±0.76	$5.0^{\rm e}{\pm}0.00$			
G	$6.65^{\rm bc}$ ± 1.23	$7.35^b \pm 1.04$	$5.85^{\circ}$ ±1.31	$5.85^{\circ}$ ±0.67	$6.4^b \pm 0.14$			
<b>LSD</b>	0.85	0.75	0.8	0.9	0.113			

**Table 8: Sensory Characteristics of Infant gruel prepared with Acha – Iron bean Flour**

Values are means  $\pm$  standard deviation of duplicate samples

Mean values bearing different superscripts in the same column differ significantly  $(p<0.05)$ 

**Key**:  $A = 100\%$  cerelac flour,

 $B = 70\%$  acha flour and 30% iron bean flour.

 $C = 70\%$  germinated acha flour and 30% iron beans flour.

D =70% fermented acha flour and 30% iron beans flour.

 $E = 50\%$  germinated acha flour and 50% iron beans flour.

 $F = 50\%$  fermented acha and 50% iron beans flour.

G= 35% germinated acha flour, 35% fermented acha flour and 30% iron bean flour

#### **4: Conclusion and Recommendation**

The 70:30 (w/w) % germinated acha: iron bean had 11.7 % protein, 4.0% lipid, 3.2 % crude fiber, 2.4 % ash and 70.4 % carbohydrate. It had comparable contents of Leucine (6.9), Lysine (5.9), isoleucine (3.8), phenylalanine (4.1), Tryptophan (1.2), Valine (4.4), Arginine (5.94), Histidine (2.98) and Alanine 4.4 g/100g with the control. Similarly, it was scored significantly  $(p<0.05)$  highly for colour, tastes, flavour, texture, and overall acceptability. Thus, the 70:30 germinated acha: iron bean is recommended for use in infant food formulation. The 50:50 (w/w) % germinated acha: iron bean, 50:50 fermented acha: iron bean and 35:35:30 germinated acha: fermented acha: iron bean had 12.5%, 12.03% and 9.62% protein, respectively.

Germination and fermentation significantly (p≤0.05) increased the protein, lipid, and carbohydrate contents of the complementary foods. The acha – iron bean complementary infant foods had low contents of vitamins (A, B1, B2 and C,) and minerals (iron, calcium, and phosphorus). Thus, the need for supplementation or fortification of the complementary infant food by addition of food rich in calcium, iron, vitamin A, B vitamins and ascorbic acid. Soaking of iron beans in 0.5% NaHCO3 for 24 hours for easy dehulling is recommended.

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