Effect of Processing Method on Proximate, Mineral and Anti-nutrientscompositionof complementary foods produced from Maize, Soybeanand Pumpkin Seed

Abstract

Background: Complementary foods are formulated food mixtures meant to be fed along with breast milk for infants from 6months until completely weaned off of breast milk.

Objective: This study is therefore designed to assess the effect of processing method on nutrients and anti-nutrient composition ofdeveloped complementary foods.

Methods: Raw materials were purchased at OwenaMarket in Ondo statewhile a commercial complementary food was purchasedAyofemshopping mall and supermarket, Akure Ondo state. Maize, soy beans and pumpkin seeds were separately subjected to fermentation, sprouting and toasting, milled into powder and packaged. The flours were formulated into three (3) different samples using the ratio 70:20:10 for maize, soybean and pumpkin seed respectively. The complementary were labeled as follows, sample A(70% fermented maize:20% fermented soybeans:10%fermented pumpkin seed) B(70% roasted maize: 20% roasted soybeans: 10% roasted pumpkin) and C(70% sprouted maize: 20%sprouted soybeans: 10% sprouted pumpkin)were formulated. Samples of the complementary food were subjected to chemical and instrumentation using standard methods.Analysis of variance (ANOVA) was performed using Statistical Package for Social Science (SPSS) version 23. Difference is considered statistically significant at $P<0.05$.

Results: Findings shows that sample B was significantly ($P<0.05$) lower in moisture content (8.18%) but higher than the control sample D (2.5%). The control sample was higher in crude fibre (4.5%), Ash (3.0%). Protein was significantly ($P<0.05$) higher in sample A (18.39%) while sample B(11.33%) has the highest fat and energy (410kcal) content. Sample D had the highest carbohydrate (65.0%) compared to other samples. Beta-carotene content varies from 0.203mg to 0.461mg.The oxalate, cynaideand phytate content was significantly ($P<0.05$) higher in sample C (21.350mg), 4.637mg and B (7.296mg) respectively. The mineral content of the samples is significantly different from one another. Sample A was significantly (P<0.05) lower in Ca (16.02mg), Mg (26.979), K (62.185), and P (125.12mg),while Sample Cwas significantly ($P<0.05$) higher in Ca (27.034), Mg (42.996mg), K (569.069mg), and P (281.162) respectively. The Iron (Fe) content was significantly ($P<0.05$) higher in sample A(14.008mg).

Conclusion: This study had shownthat fermentation, roating and sprouting improved the nutrieints composition of the formulated complementary foods and can replaced the control sample for feeding infant age 6months and above.

Keywords: Complementary food, processing methods, minerals, Phytochemical, pumpkin seed

Introduction

Breast milk has been established as the most ideal food for infants during the first six months of life (UNICEF, 2009; WHO, 2010). It contains all the essential nutrients and immunological

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

factors and infant requires sustaining optimal health and growth. However, after six months the breast milk nutrient becomes insufficient to meet the nutritional requirements for the transitions of the infant. Therefore, complementary foods are introduced and they typically cover the period from the age of six to twenty four months in developing countries (WHO/OMS, 2010).

Complementary foods are foods purposely prepared and given to infants with additional to breast milk when breast milk nutrients become insufficient to provide their calorie; macronutrients need (Adu-Afarwuah et al., 2016). Complementaryfoods play a vital role on child growth, development and needs of an infant when breast milk alone is no longer sufficient (Temesgan, 2013). Good quality complementary food must be density in nutrients, with low viscosity, bulk density and appropriate texture along with a consistency that allows easy consumption (Bala Subramanian et al., 2014).

InAfricanations the high cost of fortified commercial nutritious complementary food is always, if not beyond the reach of most Nigerian families (Mshelizaet *et al.*, 2018). Such families often depend on inadequately processed traditional foods consisting mainly of un-supplemented cereal porridge made from maize, sorghum and millet (Mshelizaet *et al.*, 2018). This poor quality of complementary foods and improper complementary feeding practices predispose infants to malnutrition, infections and mortality. Some of these staple crops have a high energy density and often lack other macronutrients such as protein and micronutrient (Roa and Annadana, 2017).

Maize is a popular complement food base in sub-Saharan Africa. It is made into a thin gruel and it contain low amount of the essential amino acid such as lysine (Nuss *et al.*, 2011). Traditional complementary foods from maize commonly given to infant are not enough to meet the daily nutrients, energy and micronutrient requirements and this has been the major causes of malnutrition in infants and young children in developing countries (WHO, 2003; Anigo, et al., 2010).

In developing countries, it's a non fact that animal source of protein is inadequate to meet the rapid population growth considering it cost and available which is a major of food insecurity and intense research efforts are currently directed towards identification and evaluation of food grains which normally have considerable high protein content (Mshelizaet *et al.*, 2018). The addition of legumes to start based foods is reported to improve the protein content and provide the deficient amino acid in complementary foods (Egounlety, 2002; Ademulegunet *al.*, 2021).

Enrichingcomplementary foods with soybeans and Pumpkin seeds is a convenient, inexpensive and highly effectiveway to upgrade the quality of traditional complementary foods and to provide the nutrition a growing childneeds and soybeans works together with cereals to achieve an overall increase in the value of the protein (Msheliza et al., 2018). Likeother seeds are rich in functional components such as vitamin E, pro -vitamin D and are good source of magnesium, potassium, phosphorus as well as other minor minerals such as ,zinc, magnesium, iron, calcium, sodium and copper (Amen et al.,2019). They are also used for fortification up to 10%, which increases protein, lysine, mineral contents, total sulfur amino acids, chemical score, protein digestibility crude fat and ash of the final product than using 100% wheat flour (El-soukkary, 2001;Brozinc*et al.*, 2016; Koh, *et al.*, 2018; Amin *et al*., 2019).

The processing of grains could be in the form of milling (dry and wet milling), thermal processing, germination, fermentation, roasting or cooking, soaking,autoclaving or sterilization and enzymatic treatment. Study had shown that processing method of grains had some significant effects on the Viscosity, dietary bulkiness and its nutrient density (Ademulegun*et al*., 2021).

Formatted: Font: Italic

The high cost of commercial complementary foods coupled with household food insecurities and global economic meltdown now demands for effective strategies for improving the nutritional status of infants and young children by promoting the use of high-quality complementary foods which could be of better nourishment, low in dietary bulkiness and viscous at cottage level production. Therefore, this study was designed toassessthe effect of processing method on proximate, mineral and anti-nutrients composition of complementary foods produced from maize, soybean and pumpkin seed

Materials and Methods

Procurement of Raw Materials

The raw materials basically used are; dried yellow maize, Pumpkin seed and soybean. The raw materials were purchased from Owena Market, Idanre Local Government Area of Ondo State. The methods of processing of the samplesinto flour by employing in this study are fermentation, sprouting and toasting. The commercial complementary food was purchased Ayofem shopping mall and supermarket, Akure Ondo state.

Production of fermented Maize Flour

The yellow maize was sorted by removing stones and other contaminants. It was followed by cleaning the sorted maize in clean water, steeping into water for three (3) days for fermentation to occur in line with the method described by Ademulegun*et al* (2021). The fermented maize was washed with clean water and wet-milled using attrition milling machine. The wet-milled fermented maize was then sieved using muslin cloth and was allowed to settle down over night to form slurry. The maize slurry was put in clean muslin cloth and squeezed to remove water. This was air dried for three (3) days, milled and packaged in air tight container and kept in a refrigerator prior to analysis (Fig 1)Ademulegun*et al*. (2021).

Formatted: Font: Italic

Production of sprouted maize flour

The yellow maize was sorted by removing broken maize, stones and other contaminants. It was followed by cleaning and soaking for 72hrs at ambient temperature. The hydrated maize was drained, spread thinly on a wet thin foam placed inside a tray and covered with another layer of wet thin foam (Ademulegun*et al*., 2021). The grains were germinated for four (4) days with constant watering between intervals. After four (4) days, the un-terminated maize was discarded while the sprouted maize was sun dried for three (3) days. After sun drying, it was de-vegetated before milling into flour, sieved and packaged in air tight container and kept in a refrigerator prior to analysis (Fig 1)

Formatted: Font: Italic

Formatted: Highlight

Production of toastedmaize flour

The yellow maize was sorted by removing stones and other contaminants. The sorted maize was toasted for fifteen minutes (15mins). The toasted maize was allowed to cool and attrition mill machine was used to mill it into fine particles. It was sieved and packaged in air tight container and kept in a refrigerator prior to analysis (Fig 1)

Formatted: Highlight

Production of fermented soybean flour

The soybean was sorted by removing stones and contaminants. It was followed by cleaning the sorted soybean in clean water. It was followed by steeping it in water for three (3) days for fermentation to occur. The fermented soybean was washed with clean water and wet-milled using attrition mill. The wet-milled fermented soybean was then sieved using muslin cloth and was allowed to settle down over night to form slurry. The soybean slurry was put in clean muslin cloth and squeezed to remove water. This was sundried for three (3) days, milled and packaged in air tight container and kept in a refrigerator prior to analysis (Fig 2)

Formatted: Highlight

Preparation of sprouted soybean flour

The Soybean seeds were cleaned by hand, sorted and floated to remove broken grains and extraneous materials. The grains were soaked for 24hours at ambient temperature. The hydrated grains were drained and spread thinly on a wet jut bag and covered with another layer of jut bag. The seeds were germinated for three days with watering every six (6) hours. Un-germinated grains were discarded while the sprouted ones were oven dried ($50^{\circ}C$ for 12 hours) and de-vegetated before milling to flour, sieved and packaged in air tight container and kept in a refrigerator prior to analysis (Fig 1)

Preparation of Toasted Soybean Flour

The soybean seeds were sorted and cleaned. The grains were soaked for 24hours at ambient temperature and dehulled and boiled for 2hours, drained and oven dried ($50^{\circ}C$ for 12 hours) Soybean seeds were toasted using frying pan on a cooking gas for 30minutes until golden brown coloration was achieved. The soybean was milled using attrition milling machine in ratio to fine particle, sieved and packaged in air tight container and kept in a refrigerator prior to analysis (Fig 1)

Production of fermented pumpkin seeds

The pumpkin fruits were broken in other to extract the pumpkin seeds. The extracted pumpkin seeds were sun dried for two (2) hours and dehulled. Cleaning of the dehulled pumpkin seeds were done with clean water and it was sliced into small pieces and fermented for 72hours. The fermented pumpkin seeds were washed with clean water and wet milled using attrition milling machine. The wet-milled fermented pumpkin seed was then sieved using Muslin cloth and allowed to settle down over night to form slurry. The pumpkin seeds slurry was put in a clean muslin cloth and squeezed to remove water. This was sundried for three (3) days, milled and packaged into fine particles.

Preparation of Toasted pumpkin seeds

The pumpkin fruits were removed from the seed pod. It was sundried for 2hrs, dehulled and sliced into small pieces. The sliced pumpkin seeds were sundried for 48hrs and it was toasted for about fifteen minutes (15mins). The toasted pumpkin seed was allowed to cool and attrition milling machine was used to mill it into fine particles. It was sieved and packaged.

Preparation of sprouted pumpkin seeds

The pumpkin fruits were removed from the seed pod. The seeds were arranged in layers of a saw-dust inside a medium rubber basket. It was wetted daily for 8day. The sprouted pumpkin seeds were sorted, washed and dehulled. The dehulled pumpkin seeds were sliced and sundried for 3days. It was milled and packaged.

Formulation of maize-soybean complementary food blends

Table 1 shows the flour formulation of the maize –soybean flour complementary food in 70:20: 10 ratios respectively.

Table 1: Formulation of Maize-Soybeans- pumpkin seed complementary foods

Samples	Maize (%)	Soybean (%)	Pumpkin seed (%)
A	70% fermented maize flour	20% fermented soybean	10% sprouted pumpkin seed
B	70% toasted maize flour	20% tasted soybean	10% sprouted pumpkin seed
C	70% sprouted maize flour	20% sprouted soybean	10% sprouted pumpkin seed

Pumpkin Seed Fruit ↓ **Breaking** ↓	**Pumpkin fruit seed** ↓ **Breaking**	**Pumpkin fruit seed** ↓ **Breaking** ↓

Extracting ↓ Sun drying (2hrs) ↓ Dehulling ↓ cleaning ↓ Steeping (72hrs) ↓ Washing ↓ Wet milling ↓ Sieving ↓ Settling (overnight) ↓ De-watering ↓ Sun drying (72hr) ↓ Milling ↓ Packaging ↓ Fermented Pumpkin Seed Flour	↓ Extracting ↓ Sun drying(2hrs) ↓ Dehulling ↓ Slicing ↓ Sun drying (24hrs) ↓ Toasting ↓ Cooling ↓ Milling ↓ Sieving ↓ Packaging ↓ Toasted Pumpkin Seed Flour	Extracting ↓ Arranging ↓ Wetting ↓ Sprouting (8days) ↓ Sorting ↓ Washing ↓ Dehulling ↓ Slicing ↓ Sun drying (3days) ↓ Milling ↓ Packaging ↓ Sprouted Pumpkin Seed Flour

Figure 1: Flow chart for the production of pumpkin seed flour using different processing method

Soybean ↓	Soybean ↓	Soybean ↓

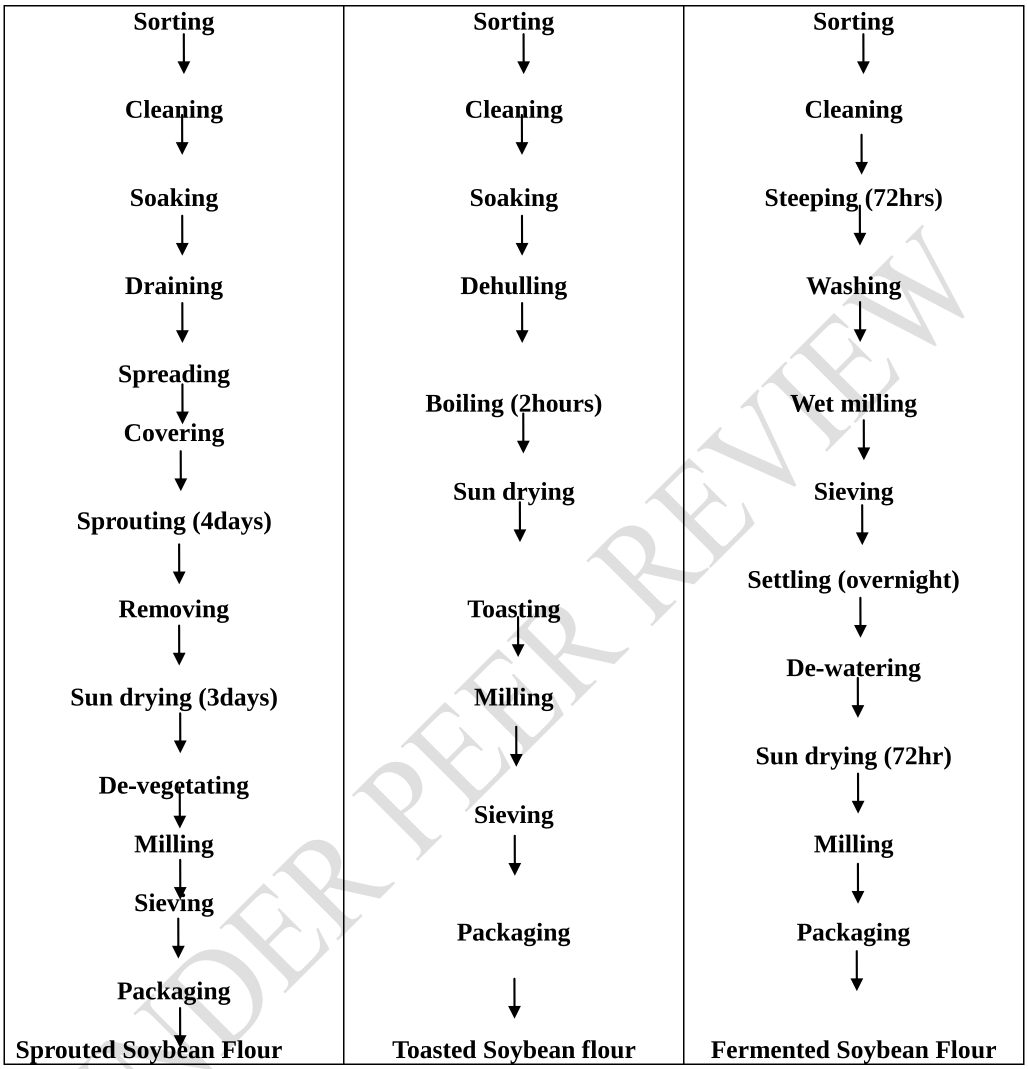

Figure 2: Flow chart for the production of soybean flour using different processing method

Yellow Maize grain	**Yellow Maize**	**Yellow Maize Grain**
↓	↓	↓
Sorting	**Grain**	**Sorting**
↓	↓	↓
cleaning	**Sorting**	**Cleaning**
↓	↓	↓
Steeping (72hrs)	**Toasting**	**Soaking**
↓	↓	↓
Washing	**Cooling**	**Draining**
↓	↓	↓
Wet milling	**Milling**	**Spreading**
↓	↓	↓
Sieving	**Sieving**	**Covering**
↓	↓	↓
Settling (overnight)	**Packaging**	**Sprouting (4days)**
↓	↓	↓
De-watering	**Toasted Maize Flour**	**Removing**
↓		↓
Sun drying (72hr)		**Sun drying (3days)**
↓		↓
Milling		**De-vegetating**
↓		↓
Packaging		**Milling**
↓		↓
Fermented Maize Flour		**Sieving**
		↓
		Packaging
		↓
		Sprouted Maize Flour

Figure 3: Flow chart for the production of maize grain flour using different processing method

Proximateanalysis of the formulated complementary foods

Theash, protein, crude ~~fibre~~fiber,fat and moisture contents of the formulated complementary foods were determined using the standard methods described by AOAC (2012). Total carbohydrate was calculated as the difference between 100 and the sum of the percentages of ash, protein, crude ~~fibre~~fiber,fat and moisture (Ferris *et al*., 1995). The sample energy value was calculated from the percentages of crude protein, total carbohydrates, and total fat. The conversion factors used were 9kcal/g for total fat, 4kcal/g for protein and carbohydrates respectively (Buchholz & Schoeller, 2004).

Formatted: Font: Italic

Determination of Beta Carotene

2g of the sample was weighed into a 250ml volumetric flask, 50ml of petroleum ether: Acetone (2:1v/v) mixture was added to the extract the ß-Carotene. The flask containing the mixture was placed on a shaker to shake at 200rpm for 20min to ensure uniform mixing at room temperature. The mixture was later centrifuged at 4000rpm for 10min and the supernatant collected and made up to 50ml with the solvent mixture. The supernatant was transferred to a 250ml separatory funnel to separate the organic layer (upper layer). The aqueous layer was discarded and the organic layer was transferred into the 50ml volumetric flask and made up with solvent mixture for reading of ß-carotene. Working standard of ß-carotene of range 0-50ppm or /ml were prepared from stock Beta carotene solution of 100ppm concentration. The absorbances of samples as well as working standard solutions were read on a Cecil 2483 UV Spectrophotometer at a wavelength of 450nm against blank.

$$\text{B-carotene } (\mu g/100g) = \frac{\text{Absorbance of sample x Average Gradient x Dilution Factor}}{10000}$$

Determination of Phytate

This was determined using McCance-Widdowcon method as modified by (Wheeler and Ferrel). 2g of the defatted samples were extracted with 3% Triochloroacetic acid (TCA) was precipitated with 4ml of ferric chloride solution. The precipitated ferric phytate was converted to ferric hydroxide with 4ml 1.5M sodium hydroxide each and was then dissolved in hot 40ml 3.2M ~~HN03~~ $HNO_3$and then diluted with 20ml of 1.5M KSCN. The iron was determined colorimetrically.

Formatted: Subscript

Cyanide determination

The measurements of total cyanide contents in the samples were performed according to the auto-enzymatic method.The samples (1g) were incubated in 20 ml acetate buffer (pH 5.5) at

37°C for 18 hours. After incubation, the samples were cooled at room temperature (20°C) and then, 4 ml of NaOH were added. The samples were placed into a distillation flask round bottom containing distilled water. The flask was heated at 100°C in a digital temperature-controlled dry bath and its central neck was connected to the condenser glass directly attached to two ~~bubbler~~bubblers in series containing each 10ml of 0.1MK_2CO_3. After, which 10 ml of H_2SO_4 were added to the flask by one of the two side necks to facilitate evaporation of HCN in sample. In the second lateral neck, a very thin tube was inserted, directly immersed into the solution and allowing the entry of air.The air flow was controlled by vacuum pump placed after the second bubbler. During distillation, the HCN released was constantly carried through the glass condenser and trapped in the first bubbler. The second is considered as a control measure. After 15 minutes, the solution of the first bubbler was collected in a flask (50 ml) and made to volume with 0.1 M K2CO3. The anion CN- was measured using a polarograph (Metrohm E 506 with Stand Polarecord 663 VA Stand) in differential pulse mode. Oxygen dissolved in the solution was removed by bubbling nitrogen (inert gas) for 5 minutes. Finally, the registration of the polarogram was done in an unstirred solution. The residual cyanide contents in the samples measurements were performed according to the method described above with slight modification by adding the commercial linamarase.

Determination of Oxalate

This was determined using Dye method.2g each of the samples was extracted with dilute HCl, 10ml concentrated ammonia and then precipitated with calcium chloride as calcium oxalate. The precipitate was then washed with 25ml of hot 25% H_2SO_4 and dissolved in hot water and titrated with 0.05M KMnO4 to determine the concentration of oxalate (Abaza *et al*., 1968).

Determination of mineral contents of theformulated complementary foods

The mineral contents were determined in a dilute solution of the ash samplesaccording to the method outlined in AOAC (2012) by Atomic Absorption Spectrophotometer (AAS) (210 bulks scientific) for Calcium, Copper, Magnesium, Iron, phosphorus, Zinc while potassium was by flame photometry and phosphorus was determined by colorimetric method.

Statistical Analysis

Statistical analysis of the data was carried out using the one-way Analysis of Variance (ANOVA) technique (SPSS 17.0 for windows), and the differences were separated using Duncan's Multiple Range Test (DMRT) at a level considered to be significant at $p < 0.05$

Formatted: Subscript
Formatted: Subscript
Formatted: Subscript
Formatted: Subscript
Formatted: Subscript
Formatted: Subscript
Formatted: Font: Italic

Result

Proximate composition of complementary food

The moisture content of the formulated sample was significantly lower in sample B(8.18%), but, higher than the 2.5% of the control sample. The protein content ranged from 14.09 to 18.3%. Sample C had the least protein(14.09%) content lower than the Sample D while sample had the highest protein content (18.39%).The fat content of formulated blends revealed that sample Bwas significantly (*P*<>0.05) higher in fat(11.33%)than any other sample formulated including the control. The crude fibre content was low in the formulated samples and they were all significantly (*P*<>0.05), lower than the control sample (4.50%) and ranged between 1.35%and 1.69% for sample A and C respectively. Ash and carbohydrate content of the samples were significantly (*P*<>0.05) higher in the control sample respectively. Sample A had the least energy (357kkcal) content while sample B (419kcal) had the highest energy content and it was significantly(*P*<>0.05) higher than any other samples including the control sample.

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Table 2: Proximate composition of complementary food

Parameter	Sample A	Sample B	Sample C	Sample D
Moisture (%)	10.58±0.023^{b}	8.18±0.035^{c}	12.15±3.348^{a}	2.50±0.01^{d}
Crude protein (%)	18.39±0.045^{a}	16.68±1.769^{b}	14.09±0.020^{d}	15.0±0.01^{c}
Crude fat (%)	4.49±0.010^{d}	11.33±0.045a	11.00±0.035^{b}	10.0±0.01^{c}
Crude fibre (%)	1.35±0.005^{d}	1.48±0.020^{c}	1.69±0.020^{b}	4.50±0.01^{a}
Total ash (%)	0.59±0.015^{d}	0.68±0.015^{b}	2.18±0.015^{b}	3.00±0.01^{a}
NFE (%)	64.58±0.255^{b}	62.630±0.007^{d}	63.31±0.025^{c}	65.0±0.01^{a}
Energy (Kcal)	357.03±1.035^{c}	419.38±37.97^{a}	408.36±.135^{b}	410.10±0.101^{b}

Values are mean ± standard deviation of triplicate analyses. Values with the same superscript in the same column are statistically not significant at (P<0.05). Key:A (70% fermented maize: 20% fermented soybeans: 10% fermented pumpkin seed) B (70% roasted maize: 20% roasted soybeans: 10% roasted pumpkin) and C (70% sprouted maize: 20%sprouted soybeans: 10% sprouted pumpkin: D commercial complementary food

Minerals content of complementary food

The Calcium content of the commercial complementary food (sample D) (600mg/100g), was significantly (P<>0.05) higherthan any of the formulated samples. The formulated samples had an appreciable amount of magnesium. Although, sample A was significantly (P<>0.05) lower that sample B and C. Potassium content of the commercial complementary food (sample D) (635mg/100g), was significantly (P<>0.05) higher than any of the formulated samples. Sample A had least potassium (62.185mg/100g). Phosphorus was significantly (P<>0.05) high in sample C (281.162mg/100g) while sample A had the least value (125.12mg/100g). All the formulated samples had an appreciable amount of iron higher than the control sample (6.0mg/100g). There was no significant difference ($P<0.05$)between sample A (6.238mg/100g) and sample D (7.0mg/100g) which the control in terms of zinc content. Copper content of the sample B was significantly (P<>0.05) higher in the than any other samples.

Table 3: Minerals content of complementary food

Parameter	Sample A	Sample B	Sample C	
Ca (mg/100g).	16.020±0.445[c]	23.526±0.149[b]	27.034±0.141[a]	600.0±0.010[a]
Mg (mg/100g).	26.979±0.045[b]	42.916±0.030[a]	42.996±0.039[a]	NA
K (mg/100g).	62.185±0.032[d]	544.754±0.355[c]	569.067±0.167[b]	635.0±0.010[a]
P (mg/100g).	125.120±0.012[c]	256.337±0.122[b]	281.162±0.058[a]	NA
Fe (mg/100g).	14.008±0.358[a]	11.311±0.019[b]	13.730±0.093[a]	6.00±0.010[a]
Cu (mg/100g)	1.051±0.004[b]	1.431±0.003[a]	0.464±0.008[c]	NA
Zn (mg/100g).	6.283±0.022[a]	3.133±0.006[b]	2.322±0.025[c]	7.00±0.010[a]

Values are mean ± standard deviation of triplicate analyses. Values with the same superscript in the same column are statistically not significant at (P<0.05). Key: A (70% fermented maize: 20% fermented soybeans: 10% fermented pumpkin seed) B (70% roasted maize: 20% roasted soybeans: 10% roasted pumpkin) and C (70% sprouted maize: 20%sprouted soybeans: 10% sprouted pumpkin: D commercial complementary food

Beta-carotene and anti-nutrients content of complementary foods

The samples were low in beta-carotene compared to the commercial complementary food (sample D) (24.6mg/100g). On the anti-nutrient composition, sample C had the highest value of oxalate (21.35mg/100g) while sample B had 12.18mg/100g.Phytate was significantly (P>0.05) higher in sample B (7.29mg/100g) sample A had the least value(7.48mg/100g) and significant difference(P>0.05) existed samples.Cyanide (4.637mg/100g) was higher in sample C than any other formulated samples.

Table 4: Beta-carotene and anti-nutrients content of complementary foods

Parameter	Sample A	Sample B	Sample C	Sample D
Beta-carotene(mg/100g)	0.203±0.002[c]	0.416±0.003[b]	0.461±0.003[a]	
Oxalate(mg/100g)	7.483±0.035[b]	12.187±5.277[b]	21.350±0.37[a]	
Phytate(mg/100g)	6.690±0.112[b]	7.296±0.0014[a]	6.748±0.109[b]	
Cyanide(mg/100g)	0.439±0.003[c]	2.016±0.028[b]	4.637±0.043[a]	

Values are mean ± standard deviation of triplicate analyses. Values with the same superscript in the same column are statistically not significant at (P<0.05). Key: A (70% fermented maize: 20% fermented soybeans: 10% fermented pumpkin seed) B (70% roasted maize: 20% roasted soybeans: 10% roasted pumpkin) and C (70% sprouted maize: 20%sprouted soybeans: 10% sprouted pumpkin: D commercial complementary food

DISCUSSION

The moisture content of all the formulated complementary food samples reported in thisstudy were within therecommended moisture contents of dried foods (Ndife*et al.,* 2011; Igyor*et al.,* 2011). High moisture content in food has been shown to encourage microbial growth (Sanni and Oladapo,2008). The values obtained in this study were within the range reported to have no adverse effect on the quality attributes of the product (Yusufu*etal.,*2013). Bolarinwa et al., (2015) reported that the lower the moisture contents of a product, the better the shelf stability of the food product. However, low residual moisture content in food is advantageous in that, microbial proliferation is reduced and storage life may be prolonged, if stored in appropriate packaging materials undergood environmental conditions ().Protein is one the major classes of macronutrients and is needed for tissue replacement, deposition of lean body mass, growthand development and in infancy, it's obvious (UNICEF, 2009).The high levels of protein find in these samples could be as a result fermented and roasted soybean which promotes and doesn't destroy lysine and tryptophan. All the food samples had high amount of protein, this agreed with previous study of Ademulegun et al. (2021). The increased in the protein level was as a result of the inclusion of pumpkin seed and soybean. Theprotein was higher when compared to the Recommended Daily Allowance (9.1-13g per day) for infants within the age bracket of 6-24 months (National Academy of Science, 2005). The protein values were higher than that reported by Ojinaka et al. (2013), which ranged from (3.9-3.97%). The formulated samples had an appreciable amount of fat similar to that of the commercial complementary food (control). This might be due to the effect of roasting and sprouting on soybean. Studies had shown thatroasting and sprouting increase Fat content of food (Okoye & Ene, 2018). Fat is important in the diet of infant and young children because it provides essential fatty acid, facilitates absorption of fatsoluble vitamin, enhance dietary energy, density and sensory quality (FAO 2013). It has been recommended that, during the complementary feeding period (6 – 12 months) a child's diet should derive 30–40% of energy from fat (Michaelsen and Robertson, 2000). According to a joint WHO/FAO/UNU The energy requirements for a 6-month-old female involved in moderate physical activity is 340kJkg1 body weight (WHO, 2007), an infant weighing 7.34 kg would 2495 kJ of energy daily. The fat composition of this complementary food will only meet 2.5 – 16.4% of the energy requirement. This however can be enhanced with available oil to increase the recommended fat ratio. The samples had varying ash content from 2.8 – 1.45 % for sample C and G respectively. The general trend observed in this study was that of an increase with increase in soy flour substitution. Fat is also important in the diets of infants and young children as it provides essential fatty acids like Omega-3 and Omega-6 polyunsaturated fatty acids (PUFA"s) that are needed in the body forproper neural development(Mariam,2005).

The crude fibre content was significantly (p>0.05) higher in the sample D (control)than in the formulated samples. The fibre content of the formulated samples was very low. This might be due to fermentation and roasting method which had been proved to reducefibre content. This low fibre content was also reported byArawande and Borokini, (2010), in a similar study on complementary food made from maize, soy bean, and carrot.Fibre plays a role in the increased the utilization of nitrogen and absorption of some other micronutrients. Complementaryfood should contain low fibre as high fibre can lead to high water absorption and displacement of nutrient and energy needed for the growth of children less than two years (Klim and Joseph 2001; Michaelsen and Robertson, 2000). The ash content of a food material could be used as an index for estimating the mineral constituents of the food (). In this study, Sample C had highest value of ash content with significant difference (p>0.05). Thismaybe due to the effect ofsprouting. Study had shown thatsproutingincrease the ash content of food ().The ash content obtained in this study was lower than the ash content (3.47-4.53%) of complementary food prepared from maize, sesame and crayfish flour blends reported by Fasuan et al., (2017). This might be due to the inclusion of animal protein in their sample. The nitrogen Free energy content also known as the carbohydrate wasabundant in all the formulated complementary foods and similar to the carbohydrate content of the commercial complementary foodthe levels of carbohydrate in all the complementary food samples are nutritionally adequate as children require energy to carry out their rigorous playing and other activities as growth continues. The

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

values obtained in this study were in ranged of the carbohydrate content (55.86-70.00%) of complementary food formulated from fermented maize, soybean and carrot flours reported by Barber et al. (2017). The energy value ranged from 357.03to 392.51 kcal with All sample had no significant difference ($P>0.05$) respectively. This might be due no effect on energy in any method of processing of food. The energy values of the formulated samples met the FAO/WHO (1991) specification guidelines for young children complementary food formulations.

The loss in the level of calcium, Magnesium, Potassium, and Phosphorus in the processed samples may be attributed to the loss in the ash contents during fermentation and it is due to reduction of antinutritional factor. Similar works by Oyarekua and Eleyinmi, 2014; Chamba et al., 2021 reported that more than 50% of the ash in sorghum was leached out of the steep water and washed away. A decrease in the levels of Ca, P, Zn, and Fe levels in processed samples were reported (Ijarotimi & Keshinro, (2013). Iron (Fe) in the fermented product were recorded to be higher than products from toasting and sprouting, though the value for sprouted product were similar. The changes that occurs during fermentation may be as a result of enzymatic activities (Van *et al.*, 2010). The activity of desirable micro-organisms and/or their enzymes on foods during fermentation leads to biochemical changes that significantly modify food products. Teucher *et al.*, (2004), reported that the low weight organic acids produced during fermentation are to enhance iron and zinc. The copper of the fermented product was reported to be an intermidiary between the toasted and sprouted product, this maybe as a result of enzymic activities during fermentation. The Zn content of the fermented product was reported to be the highest compared to the results from toasted and sprouted products. The effect of toasting in this study is reported that there is significant increase in the minerals (i.e Ca, Mg, K, P, Fe, Cu and Zn) content which agrees with the work of Oboh *et al.*, (2010) stating that there was increase in the minerals (Ca, Zn, Mg and Na) content after roasting yellow and white maize. This may be due to increase in the ash content of the toasting processing method. This study also shows that sprouting has the highest mineral (Ca, Mg, K, P & Fe) content compared to toasting and fermentation, though the iron (Fe) from fermentation were similar with that of sprouting. This could be as a result of sprouts from the grains which is a unique food safety concern due to the ease of microbiological contamination and inherent ability of the sprouting process to support microbial growth (Symes & Goldsmith, 2015).

The beta carotene content of fermented maize, fermented soybean and fermented pumpkin seed were lower compared to the flours from toasted and sprouted maize, soybean and pumpkin seed respectively, this might be due to the fact that fermentation reduces the antinutritional factors, which was similar to the work reported by Correia *et al.* (2005). This study shows that phytate and cyanide were reduced by fermentation, Huffman *et al.*(2004) also reported that phytic acid or phytate and cyanide compounds are removed during fermentation.

Sprouting, germination and malting are similar processing method, it involes soaking, germination under controled conditions and drying of cereals, grains or pulses (Hubner & Arendt, 2013). Germination induces biochemical modification that lead to improved nutritional quality in food. This study revealed that the results from sprouting is higher in oxalate, beta carotene and cyanide but reduces in phytate, it may be as a result of their concentration in the testa, which is more in sprouted seeds. Saleh *et al.*, (2013) also reported that germination may be attributed to reduction in anti-nutrients such tannin and phytic acid that may have form complexes with protein.

Toasting/roasting is a dry heat treatment process that leads to irreversible structural changes in food (Awopetu *et al.*, 2017). Normally roasting is suppose to reduce some of the phytochemicals because of the heat treatment and some may not reduce because they maybe resistance to heat. In this study, the phytate increases more in roasting than other processing methods (fermentation & sprouting) this may be that phytate is heat resistant that was why it was not reduced by heat but reduces by fermentation and sprouting.

Conclusion

This study revealed that complementary foods formulated from maize, soybean and pumpkin seed which was processed using three (3) different processing method (fermentation, sprouting and toasting) can be consumed by children from 6months of age. The results carried out shows that the processing method of sprouting increases majority of the minerals (Ca, Mg, k, P and Fe) but there was reduction in Cu and Zn. The minerals composition of the blended complementary food can help in the growth and development of children in addition to regular breast feeding. Since maize, soybean and pumpkin seed are easily available and affordable raw material, it can be used by mothers as home-based complementary food.

Ethical approval

Ethical approval reference number RUGIPO/NUD/2023/100 was obtained for the study from the Ethic committee of the department of Nutrition and Dietetics Rufus Giwa Polytechnic, Owo, Ondo State

References

1. UNICEF, (2009).Tracking Progress on Child and Maternal Nutrition. A survival and development priority: New York, NY 10017, USA.
2. World Health Organization. (2010). Summary of guiding principles for complementary feeding of the breastfed children Geneva, Switzerland.
3. World Health Organization (2000). World Health Organization: Complementary feeding: Family foods for breast feeding children, WHO, Geneva.
4. Temesgen, M. (201). Nutritional Status of Ethiopian Weaning and Complementary Foods: A Review. Sci. Rep. 2(2), 66-80.
5. Adu-Afarwuah, S.; Lartey, A.; Dewey, K.G. (2016). Meeting Nutritional needs in the first 1000days: A place for small-quantity lipid-based Nutrient supplements. Ann.Ny Acad. Sci. 1392(1), 18-29.
6. Rao, C.K.; Annadana, S. (2017). Nutrient Biofortification of staple food crops: Technologies, products and prospects; John wiley and Sons: Hoboken, NJ, 113-183.
7. Nuss, E.T.; Tanumihardjo, S.A. (2011). Quality protein maize for Africa; closing the protein inadequacy gap in vulnerable populations. Advance Nutrition. 2(3), 217-224.
8. WHO (2003). Guiding principles for complementary feeding of the breastfed child. (WH/A56/INFO.DOC.) Geneva, Switzerland.
9. WHO/FAO/UNU (World Health Organization/Food and Agriculture Organization of the United Nations /United Nations University). (2007). Protein and amino acid requirements in Human Nutrition. Report of a joint WHO/FAO/UNU/ Expert consultation, WHO Technical Report series, Geneva. 935.
10. Anigo, K.M., Ameh, D.A., Ibrahim, S. &Danbauchi, S. S. (2010). Nutrient composition of complementary food gruels formulated from malted cereals, soybean and groundnut for use in North-western Nigeria. African journal of Food Science. 4(3), 65-72
11. Igyor, M.A., Yusuf, P.A. &Senger, I.A. (2011). Evaluation of Phytochemical and Functional and Sensory Properties of Fermented fura powder supplemented with soy." *Nigerian Food Journal*, 28: 113-121.
12. Egounlety, M. (2002). Production of legume-fortified weaning Foods. Food Res. Int. 35(2-3), 233-237.
13. Ademulegun, T.I, IbidayoAlebiosu, Adedayo, O.E, Abraham, M.L., **Olanrewaju, O.I** (2021). Formulation and assessment of nutrient contents of complementary foods from fermented, sprouted and toasted maize-soybean blend. *Journal of Dietitian Association of Nigeria Vol 12 (1) pp 83-91*
14. D. Broznic, G. Canadi Juresic, C. Milin. (2016). Involvement of α-, γ- and δ- tocopherol isomers from pumpkin (Cucurbita pepo L.) seed oil or oil mixtures in the biphasic DPPH disappearance kinetics. Food Technol. Biotechnol. 54(2), 200-210.

15. W.Kok, U. Uthumporn, A. Rosma, A. Irfan, Y. Park. (2018). Optimization of fermented pumpkin-based beverage to improve Lactobacillus mali survival and α-glucosidase inhibitory activity: a response surface methodology approach. Food Sci Hum. Wellness. 7(1), 57-70.
16. Egbujie, A.E&Okoye, J. I (2019). Chemical and sensory evaluation of complementary foods produced from sorghum, African yam bean and crayfish flours. *International Journal of Food Science and Nutrition* 4(3) 114-119
17. Mosha T., Laswais, Tetens I (2000). Nutritional composition and micronutrient status of homemade and commercial weaning foods consumed in Tanzania. *Plant Foods Human Nutr*ition, 55(3): 185-205.
18. Muhimbula, H., Zacharia, I. &Kinabo, J. (2011). Formulation and sensory evaluation of complementary foods from local, cheap and readily available cereals and legumes in Iringa, Tanzania. *African Journal of Food Science.* 5 (1): 26 – 31.
19. Kikafunda J.K., Walker A.F., Tumwine J.K. (2003).Weaning Foods and Practices in Central Uganda: A cross-sectional study. *African Journal Food Agriculture, Nutrition and Devel*opment, 3 (2); pp. 1-13.
20. Adelekan, D.A. (2003). Childhood Nutrition and Malnutrition in Nigeria. *Journal of Nutrition*, 19 (2), pp. 179-181.
21. Ikujenlola, V.A. &Fashakin, J.B. (2005).The physiochemical properties of complementary diet prepared from vegetable proteins. *Journal of food, Agriculture environment*; 3 (3 & 4), 20-22.
22. Abiose, S.H.,Ikujenlola, A.V., and Abioderin, F.I. (2015). Nutritional quality Assessment of Complementary Foods Produced from Fermented and Melted Quality Protein Maize Fortified with Soybean Flour. *Poland Journal Food Nutrition Sciences* .65 (1), pp.49-56.
23. Ojinaka, M.C, Ebinyasi, C.S, Ihemeje, A and Okorie, S.U (2013).Nutritional evaluation of complementary food formulated from blends of soybean flour and ginger modified with cocoyam starch. *Advanced journal of food science and technology* 5(10)1325-1335
24. Yusufu, P.A., Egbunu, F.A., Egwujeh, S.I.D., Opega, G.L. and Adikwu, M.O. (2013). Evaluation of Complemtnary food prepared from sorghum, African yam bean (*Sphenostytisstenocarpa)* and mango mesocarp flour blends. *Pakistan Journal Nutrition 12(2): 205-208.*
25. Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., De Onis, M., Ezzati, M., Grantham-McGregor, S., Katz, J., Martorell, R. &Uauy, R. (2013). Maternal and Child Under-nutrition and Overweight in Low-Income and Middle-Income Countries." *Lancet* 382 (9890): 427–451.
26. Okoye, J. I. and Ene, G. I (2018). Evaluation of Nutritional and Organoleptic Properties of Maize-Based Complementary Foods Supplemented with Black Bean and Crayfish Flours. *Global Advanced Research Journal of Food Science and Technology.* 6(1) pp. 001-009
27. Inyang C.U and Idoko C.A (2006). Assessment of the Quality of "Ogi made from malted millet. *African Journal biotechnology*. 5; 2334-2337.

28. Okoye, J. I. and Ene, G. I (2018). Evaluation of Nutritional and Organoleptic Properties of Maize-Based Complementary Foods Supplemented with Black Bean and Crayfish Flours. *Global Advanced Research Journal of Food Science and Technology.* 6(1) pp. 001-009
29. Onabanjo, O.O, Akinyemi C.O, and Agbon C.A (2009). Characteristics of complementary foods produced from sorghum, sesame, carrot and crayfish. *Journal of Natural Sciences, Engineering and Technology* 8 (1):71-83
30. Ademulegun, T. I. &Koleosho, A.T. (2012). Effect of processing method on the nutrients composition of maize/Soy complementary food. ISSN: 2278-3008. Volume 4, Pp 39-43.
31. Lopez, Y., Godon, D. I. & Field, M. L. (2014). Release of phosphorus from phytate by natural lactic acid fermentation. *J. Food Sci.* 48: 933 – 54.
32. AOAC (2012). Official Methods of Analysis,19^{th} ed. Association ~~Of~~of Official Analysis Chemist, Washington
33. Ferris DA, Flores RA, Shanklin CW, Whitworth MK (1995). Proximate analysis of food service wastes. *Applied Engineering in Agriculture* 11:567–7225.
34. Buchholz AC, Schoeller DA (2004). Is a calorie a calorie? *America Journal ofClinical Nutrition* 79; 899S–906S.
35. Nnam, N.M (2002). Evaluation of complementary foods based on maize, groundnut, paw paw and mango flour blends. *Nigerian Journal of Nutritional sciences* 23 (1&2). 8-18
36. Barber L.I, Obinna-Echem, P.C, Ogburia, E.M (2017). Proximate Composition, Micronutrient and Sensory Properties of complementary food from fermented maize, soybean and carrot flours. *Sky J. Food Sci. 6(3): 033-039.*
37. Onimawo I.A &Offurum M, (2015). Formulation and nutrient composition of maize pap fortified with groundnut flour. *Nigerian journal of nutritional sciences*, 36(2) 64-71
38. Okudu, H.O., Ojinnaka, M.C. & Kalu, M.O. (2017). "Chemical and sensory properties of complementary food produced from sorghum (sorghum bicolor), Pigeon pea (Cajanuscajan) and carrot (Daucuscarrota) blends. *African journal of food science and Technology*, 8 (4), pp.050-055.
39. Akubor PI (2008). Evaluation of Physiochemical and Sensory Properties of Soybean-Sweet potato supplementary food. *Journal of chemical society of Nigeria* 33: 112-121
40. Onoja, U.S. and Obizoba, I.C. (2009). Nutrient composition and organoleptic attributes of gruel based on fermented cereal, legume, tuber and root flour. *Journal of tropical Agriculture Food Environment and Extension; 8: 162-168.*
41. Fasasi O.S. (2009). Proximate, Anti-nutritional Factors and Functional Properties of Processed pearl millet (Pennisetumglaucum). *Journal of Food Technology*. 7(3): 92-97
42. Ijarotimi S.O and Olufunke O.K (2011). Determination of Nutrient Composition and Protein Quality of Potential Complementary Foods Formulated from the Combination of Fermented Popcorn, African Locust and Bambara Groundnut Seed Flour
43. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002/2005) and Dietary Reference Intakes for Water, Potassium,

Sodium, Chloride, and Sulfate (2005). The report may be accessed at www.nap.edu. Copyrighted by the National Academy of Science

44. Ojinaka, M.C, Ebinyasi, C.S, Ihemeje, A and Okorie, S.U (2013).Nutritional evaluation of complementary food formulated from blends of soybean flour and ginger modified with cocoyam starch. *Advanced journal of food science and technology* 5(10)1325-1335

45. Amadi, J.A.C, Asinobi C.O, Elo-Ilo, J.C, Afam-anene, O.C, Obiakor-okeke, P.N and Akujuobi C.I (2017). Effect of Nutrition Education of Mothers on the Anthropometric indices of preschoolers and mothers nutrition knowledge in umuguma, imo state. Nigeria. *Journal of Dietitian Association of Nigeria* 8: 52-59
46. Matilda, A. Kjartensandnes (1993). Effect of processing (sprouting and /fermentation) on sorghum and maize: II. Vitamins and amino acid composition. *Biological utilization of maize protein* volume 48 (2) 201-204
47. United Nations Children Fund (UNICEF)(2010). How to feed a baby after 6 months. A brochure developed by University research Co., LLC in Tanzania, Kenya, Malawi and Uganda. NuLifeProgram: USAID Cooperative agreement 717-A-00-08-00006-00.
48. Amankwah, E.A, Barimah J, Acheampong, R, Addai, L.O. Nnaji, C.O (2009). Effect of Fermentation and Malting on Viscosity of Maize-Soyabean Weaning Blends. *Journal of Nutrition* 8 (10): 1671-1675
49. World Health Organization (2010). Summary of guiding principles for complementary feeding of the breastfed children Geneva, Switzerland
50. Akinola O.O, Opreh, O.P. & Hammed, I.A. (2014). Formulation of local ingredient based complementary food in south-west Nigeria". *Journal of Nursing and Health science* 3; 2320-1940
51. Mahan, L.K. & Escott-Stump, S. (2008). Krause's Food Nutrition Therapy: Nutrition during infancy, 12th ed. Saunders Elsevier pub.pg 199-221.
52. Hounhouigan, D.J., Jansen, J.M.M., Nout, M.J.R., Nago, M.C., &Rombouts, F.M. (1991). Production and quality of maize-based fermented dough in Benin urban area. In: Westby A, Reilly PJA, editors. Traditional African Foods Quality and Nutrition. Stockholm: IFS. Pp. 9-8.
53. Rusydi, M.R.M., Noraliza, C.W, Azrina, A. &Zulkhairi, A. (2011). Nutritional changes in germinated legumes and rice varieties. *International Food Research Journal*, 18:705-71335.
54. Arawande, J.O &Borokini, F.B (2010). Comparative study on chemical composition and functional properties of the three Nigeria legumes (*jack beans, pigeon pea and cowpea). Journal of Emerging Trends in Engineering & Applied sciences* 1 (1)89-95
55. Butt, S.M. and Batool, R. (2010). Nutritional and Functional Properties of Some Promising Legumes Protein Isolates. *Pakistan Journal of Nutrition*; 9, 373-379
56. Klim, F., Isaac, O. & Joseph, J.O. (2001). Feeding & Nutrition and Infant and Young Children; Energy and micronutrients: WHO *European series*, 87, 45-80.

57. Michaelsen, K.F., Weaver, L., Branca, F. & Robertson, A (2000). Feeding and nutrition of infants and young children-Guidelines for the WHO European Region, with emphasis on the former soviet countries. WHO Regional Publications, *European series*,87,45-80
58. Bolarinwa IF, Olajide JO, Oke M.O, Olaniyan S.A and Grace FO (2016). Production and quality evaluation of complementary food from malted millet, plantain and soybean blends. International Journal of Scientific and Engineering Research, 7(5): 663-674
59. Oyarekwa, M.A. Adeyeye, E.I. (2009). Comparative evaluation of the nutritional quality, functional properties and amino acid profile of co- fermented maize/cowpea and sorghum/cowpea ogi as infant complementary food. *Asian Journal of Clinical Nutrition,* 1 (1), 31- 39
60. Anigo, K.M, Ameh, D.A, Ibrahim S, Daubauch, S.S (2010). Nutrient composition of complementary gruels fermented from malted cereals, soybean and groundnut for use in North Western Nigeria. *African Journal of Food Science.* 4(3):65-67.
61. Ibironke SI, Fashakin JB and Badmus OA (2012). Nutritional evaluation of complementary food developed from plant and animal sources. *Nutrition and Food Science* ; 42(2):111-120
62. Wardlaw GM, Hampl JS. Perspective in Nutrition, 7th ed. McGraw-Hill press, New York, 2007, 1-758.
63. Abaza, R.H, Blake J.T, Fisher, E. J (1968). Oxalate determination: Analytical problems encountered with certain plants species. Journal of association of official analytical chemist 51(5)963-967
64. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002/2005) and Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005). The report may be accessed at www.nap.edu. Copyrighted by the National Academy of Science
65. 33. Ojinaka, M.C, Ebinyasi, CS, Ihemeje, A & Okorie, SU. Nutritional evaluation of complementary food formulated from blends of soybean flour and ginger modified with cocoyam starch. Advanced journal of food science and technology 2013, 5(10)1325-1335