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EFFECT OF FERMENTATION TIME AND BLENDING RATIO ON NUTRIENTS AND S OME ANTI NUTRIENT COMPOSITION OF COMPLEMENTARY FLOUR.

Milkesa Feyera[1], Solomon Abera [2], Melesse Temesgen [3]
[1] *Melkassa Agricultural Research, P.O.BOX 436, Adama, Ethiopia*
[2,3] Department of Food Science and Technology, Haramaya Institute of Technology, P.O.BOX 138, Dire Dawa Ethiopia.

KEYWORDS*: Most complementary foods used in low income households are often characterized by low nutrient density, poor protein quality, low mineral bioavailability, and low energy. Fermentation and blending are an easily applicable approach to improve the nutrient content and mineral bioavailability of such complementary foods. Therefore, this study aimed to develop nutritionally adequate complementary flour with a safe level of antinutrients. Three fermentation times (0, 24, and 36 hr) and four blends of composite flour consisting of maize, haricot bean, and cooking banana, respectively, in the proportion of 65:20:15, 60:30:10, 50:35:15, 30:60:10 were used in the formulation with 100% maizeas control. Fermentation causes significant (P<0.05) reduction in moisture from 9.00 to 6.59%, ash from 2.31 to 1.75%, crude fiber from 5.05 to 3.21%, iron from 5.14 to 4.21mg/100g, calcium from 54.42 to 43.75 mg/100g, condensed tannin from 46.93 to 31.32 mg/100g, phytate from 70.24 to 36.99 mg/100. On the other hand anincrement of crude fat from 5.12 to 6.43%, protein from 13.44 to 14.98%, total carbohydrate from 70.14 to 72.07%, energy from 380.39 to 398.83 Kcal/100g, zinc from 4.41 to 5.24 mg/100g, Vitamin C from 3.99 to 5.75 mg/ml were recorded as fermentation time increased. The proximate composition of composite flour ranged from 1.19 to 2.87% for ash, 6.93 to 7.50% for moisture, 5.31 to 6.23% for crude fat, 2.71 to 5.71% for crude fiber, 9.35% to 18.28% for protein, 66.04 to 77.16 % for carbohydrate and 385.11 to 395.28 Kcal/100g for energy value. Substitution of haricot bean and cooking banana to maize flour increased in (mg/100g) calcium content from 27.80 to 61.43, iron from 3.50 to 5.69, zinc from 3.07 to 6.24, phytate from 2.04 to107.21 and condensed tannin from 1.44 to 74.60 in formulated composite flour. Vitamin C content of complementary flour increased from 2.97 to 5.55 mg/ml as cooking banana proportion level increased. Based on the present finding fermentation and substitution of maize with haricot bean and cooking banana could be, recommended in the production of nutritious complementary food for older infants and young children.*
KEYWORDS*:* Proximate composition, antinutrients, minerals, composite flour

INTRODUCTION

In developing countries, one of the greatest problems affecting millions of people, particularly children, is lack of adequate protein intake, micronutrient deficiency, and limited energy-dense food in terms of quality and quantity (Braveman and Gruskin, 2003). The reason for this is the fact that most common complementary food used to feed an infant and young children in developing countries are starchy gruels produced from cereals or tubers (WHO, 1998). According to Birhanu (2013), protein-energy malnutrition and micronutrient deficiencies are the most common forms of undernutrition in Ethiopian children. Vitamin A deficiency, Iodine deficiency disorders, and Iron deficiency anemia are also common among the prevalent malnutrition problems in Ethiopia (CSA, 2011).

Complementary feeding interventions were suggested in reducing malnutrition and promoting adequate growth and development (Martorell *et al.*, 1994). Complementary foods in the developing countries are known to be of low nutritive value and are characterized by low protein, low energy density, and high bulk because they are usually cereal–based. Several studies have reported that the complementary food made from cereal given to infants in many developing countries including Ethiopia are deficient in macronutrients (protein, carbohydrates and fat, leading to protein-energy malnutrition), micronutrients (minerals and vitamins, leading to specific micronutrient deficiencies) or both (Ijarotimi, 2008). These nutritional deficiency in cereal can be corrected by several ways, one of which is complementation with grain legumes or oilseeds and fortification with fruits and vegetables. The prospects of blending tubers, roots, and fruits with cereals and legumes are receiving considerable attention worldwide for production of promising nutritive potential of household food products (Nnam, 2002).These products would be relatively cheap, nutritious and affordable to the rural poor to alleviate protein-energy malnutrition. Maize (*Zea mays L., Poaceae*), which is the most important cereal in the world has an average chemical composition of 10.3% protein, 60.5% starch, 1.2% sugar, 2.5% crude fiber and other substances (Addo-Quaye *et al*., 2011). It also contains a high level of dietary fiber (12.19%) but, low in trace minerals and ascorbate (Hornick and Weiss, 2011). Maize protein content varies in common varieties from about 8 to 11% of the kernel weight (FAO, 2014). The protein is relatively fair in the sulfur-containing amino acids, methionine, and cystine, but, low in lysine and very low in tryptophan (Okoh, 2014). Those ingredients particularly cereals (maize) is low in protein. Complementation of cereals with locally available legume that is high in protein increases the protein content of cereal-legume blends.

Legumes are particularly important locally available raw material as a protein source for developing countries where animal protein is in short supply. Beans are very important food crops in many parts of Eastern and Southern Africa (Broughton *et al.*, 2003). It is one of the best means of mitigating food and nutrition problems experienced in most urban and rural areas in many developing countries like Ethiopia. The nutritional composition of haricot bean contributes greatly towards a balanced and healthy diet. There is an increasing demand for the haricot bean in the worldwide, because of its significance for human nutrition as a source of proteins, complex carbohydrates, vitamins, and minerals (Bennink, 2005). Fruit and vegetables are valuable sources of vitamins and minerals. They could provide significant quantities of micronutrients, when blended with cereal and legumes diets. Cooking banana is an important food crop in the humid forest and mid-altitude agro-ecological zones of Sub-Saharan Africa. It is one of the major sources of carbohydrate in Asia, Oceania, Africa and Central Americas (Engberger *et al*., 2006).

Cooking banana is source of protein, fiber, carbohydrate, vitamins, and minerals (iron, zinc, selenium, magnesium, calcium, phosphorus, and potassium) (Adeniji *et al*., 2006). Vitamin A (fresh ripe cooking banana) is an antioxidant that plays a valuable role in visual cycle, maintaining healthy mucosa and enhances skin complexion. Vitamin B6 (pyridoxine) in cooking banana is very important in the treatment of neuritis, anemia and decrease homocysteine (one of the causative factors for coronary artery disease and stroke episodes) in the body. While a very high level of vitamin C help to develop resistance against infectious agents and scavenge harmful oxygen-free radicals, but if boiled and cooked the level of this vitamin is drastically reduced (Umesh Rudrappa, 2017). Some ready-to-eat complementary food products formulated from locally available cereal -legumes food commodities, can meet the macro nutritional needs of infants and children (Solomon, 2005). However, certain aspects like the digestibility and bio-availability of the macronutrients in these local diets are limited due to the presence of antinutrients and food toxicants. These anti-nutrients and food toxicants limit the full utilization of cereal-legume based food mixtures by humans (Hsu *et al.*, 2006). Different food processing techniques are used to reduce food toxicants and other antinutrients to safe levels. Fermentation, dehulling, drying and milling are economic domestic food processing techniques used at homes to improve and increase nutrient density, acceptability, quality, availability, flavor, aroma and palatability (Hotz and Gibson, 2007). They also reduce bulk, viscosity, and antinutrients (Nnam, 2002). Fermentation is biological process, which has the potential to improve the nutrient availability in foods. It helps in the introduction of probiotic bacteria so by consuming fermented foods, beneficial bacteria and enzymes are being added to overall intestinal flora for important health benefits (Kalui *et al*., 2010). The breakdown of some of the sugars and starches in food during fermentation makes for easy digestibility of fermented foods (Michaelsen *et al*., 2000). Other advantages of fermentation include the increase in the availability of vitamins and minerals and the removal of some natural compounds that interfere with the absorption of nutrients (Towo *et al*., 2006). According to Ojokoh *et al*. (2015), fat, fiber and carbohydrate contents of the flour blends decreased with fermentation, while protein contents increased. Therefore, this study aimed to dev elop nutritionally adequate complementary flour with safe level of antinutrients.

MATERIALS AND METHODS

Experimental Location

The experiment was conducted at Melkassa Agricultural Research Center of Ethiopian Institute of Agricultural Research, Ethiopia. The Center is geographically located at latitude of 8˚24'N, longitude of 39˚21' E and at altitude of 1,550 meters above sea level. It is situated at about 107 km from Finfine, capital city of Ethiopia and 17 km of south east from Adama town of Oromiya Regional state. Raw materials were prepared and processed in Food Science and Postharvest Technology laboratory of Melkassa Agricultural Research Center. Proximate (moisture and ash content) were performed in Food Science and Postharvest Technology laboratory of Melkassa Agricultural Research Center. Crude fiber and protein content were determined in Food Science and Nutrition laboratory of Debrezeit Agricultural Research Center. Micronutrient analyses (calcium , iron, zinc and Vitamin C) and condensed tannin were conducted in the laboratory of the School of Food Science, Postharvest Technology and Process Engineering of Haramaya University. Phytic acid (Phytate) and crude fat were analyzed at Ethiopian Public Health Institute (EPHI).

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Experimental Materials

Maize (*Zea mays* L.), Haricot bean (*Phaseolus vulgaris* L.) and Cooking banana (*Musa spp)* of kno wn variety were collected from Melkassa Agricultural Research Center of Ethiopian Institute of Agricultural Research, Ethiopia. All raw materials collected were not treated with post harvest pesti cides. Maize (Melkassa -2 variety), Haricot bean (Awash -1 variety) and cooking banana (Matoke variety) were used in the experiment.

Experimental Design and Plan

The experiment was organized in a factorial arrangement consisting of two factors, fermentation time and blending ratio. Three fermentation time and four blending ratio were applied to produce complementary flour. The details of treatment combination were performed as indicated below. Fermentation has three levels (0 h, 24 h and 36 h) and blending ratio has five level. In this study 100% maize flour was used as control. The experiment was organized in the total of fifteen treatments as shown in the table below.

Table 1. Experimental plan:

Factor 1 Blending Ratio	Factor 2 Fermentation time		
	F0	F1	F2
B1	B1*F0	B1*F1	B1*F2
B2	B2*F0	B2*F1	B2*F2
B3	B3*F0	B3*F1	B3*F2
B4	B4*F0	B4*F1	B4*F2
B5	B5*F0	B5*F1	B5*F2

Where, F0 = Fermentation for 0 h, F1 = Fermentation at room temperature for 24 h, F2 = Fermentation at room temperature for 36 h, B1= 100% Maize flour, B2= 65:20:15, B3 = 60:3 0:10, B4 = 50:35: 15, B5 = 30:60:10, Maize, Haricot bean and Cooking banana, respectively.

Sample Preparation

Preparation of maize flours

Maize grain about six kilogram was sorted, cleaned and abundantly washed by immersion in cold tap water stirred by hand and screened out of the water to remove impurities. The kernels then sun dried and milled using cyclone milling machine with 30-1060 model into flour to sieve size of 0.5 mm. Finally, the maize flour was packaged and stored at room temperature in glass bottles (container) until used for formulation and experimental analysis.

Processing of haricot bean flour production

Haricot bean seeds, about seven kilogram were sorted by removing dirt and broken beans. The clean beans were soaked in tap water (1:5 w/v) for 12 h at room temperature (28 ± 2^{o}C). Soaked haricot bean seeds were dehulled manually by rubbing between palms. The dehulled beans were spread on a tray and dried in hot air oven drying with 3010 - 019EN55014/EN55014S/N model at 60^{o}C to a constant weight. The dried haricot bean seeds were subjected to roasting. Haricot bean roasting was carried out following the method described by Oraka and Okoye (2017). Accordingly dried beans were roasted in an open frying pan with constant stirring on a electric stove using a moderate heat (130-140 ^{0}C) for 15 –20 min. The roasted beans were milled into flour, packed in a polythene bag and sealed. The beans flour were kept in the refrigerator before used for food formulation and experimental analysis

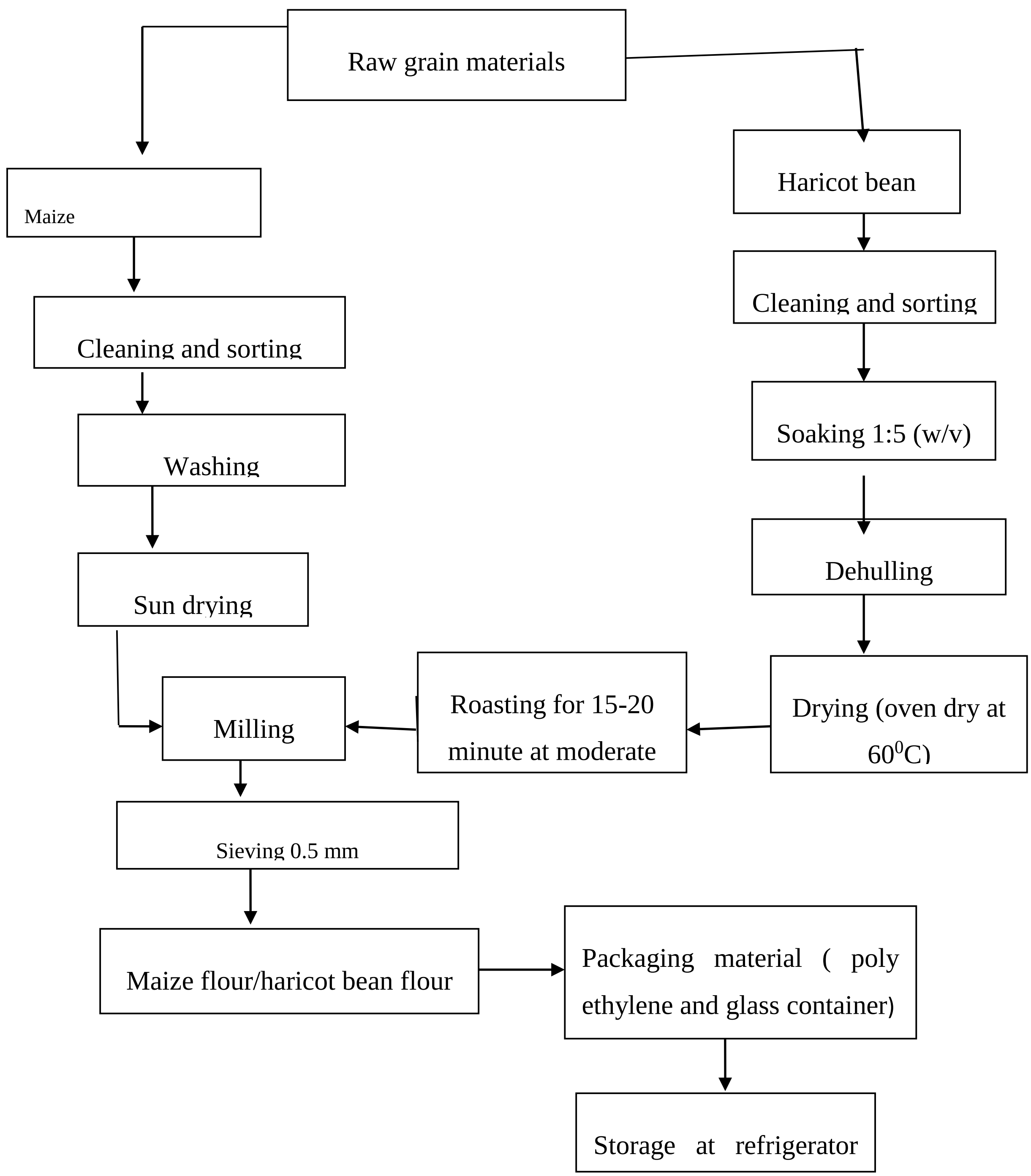

Figure 1. Flow diagram for maize and haricot bean flour processing

Cooking banana flour production

Healthy, clean and well-matured bunch was selected and representative fingers from the middle bunch of the Matoke cooking banana variety were collected. Harvested matured bananas from five bunch were washed using tap water to remove surface contaminants such as soil, dust, debris, and others. Cleaned cooking banana was blanched at $80^{o}C$ for five minutes (Ng alani, 1989). Cooking banana flour production procedures such as peeling of the fruits with the hands and cutting the pulp into small pieces were applied. Then small pieces of cooking banana were allowed to dry in a laboratory using oven dry method. Dried cooking banana was milled into sieve size of 0.5 mm flour using laboratory milling machine and placed in polyethylene bag until used for experiments.

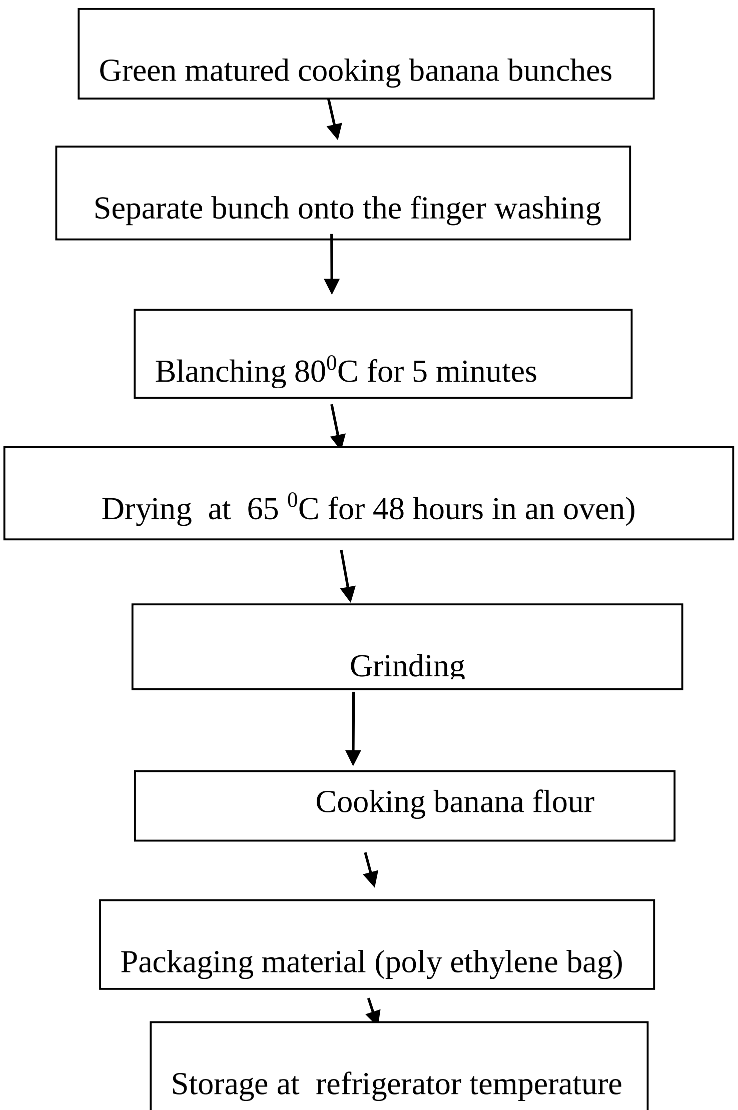

Figure 2. Cooking banana flour production flow chart (Ngalani, 1989)

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Fermentation
The fermentation was carried out according to Griffith *et al.* (1998). Flour obtained from each raw materials were mixed with distilled water independently in the ratio of 1:4 (w/v) to make a dough. It was allowed to ferment in a cleaned plastic container covered with plastic material at room temperature for 24 hr and 36 hr fermentation period. The dough was fermented by adding 5% of ersho, starter culture obtained from previously fermented maize and haricot bean to start the fermentation process. The fermented dough was dried in laboratory oven dry at 70^0C for 16 hr. The dried slurries were milled to a fine powder using a laboratory miller machine to 0.5 mm sieve size and kept at 4 $^{\mathbf{0}}$C for nutrient composition, anti-nutrient analysis (Antony and Chandra, 1998).

Formulation of Complementary Flour
The formulation was estimated using nutrient composition of raw materials to obtain a product that could have an energy level of 400 Kcal/100g, and protein content at least 15g/100g on dry matter basis to meet the specified guidelines for complementary flour for older infant and young children (Codex Alimentarius Commission, 1991). In addition, recommended micronutrients composition at 50% supply of fortified complementary food per 100g: 250 mg calcium, 5.8 mg iron, and 4.15 mg zinc were considered (WHO/FAO, 2010). The rest 50% was expected to be supplied from breast milk. Formulation scope targeted to age group after six month to five years of old.

The conducted parameters were proximate composition, some antinutrients, minerals contents and vitamin C. Moisture content determined by (AOAC, 2005), ash with official method of (AOAC, 2005), crude fat by (AOAC, 2005), crude fiber by (AOAC, 2000), crude protein by (AOAC, 2000), total carbohydrate determined using procedure described by Joshi *et al*., 2015) and gross energy value according to procedure described by Guyot *et al.*, (2007). Analysis of condensed tannin was carried out according to the modified Vanillin-HCl methanol method as described by Price *et al*. (1980) while Phytic acid was determined by the procedure of Lucas and Markakas (1975). Iron, calcium and zinc of the sample were determined according to official method of (AACC,2000). Vitamin C (Ascorbic acid) content of the flour sample was estimated by titration method (AOAC, 1996) using 2,6-dichlorophenol indo-phenol dye solution that gets reduced to a colorless form by ascorbic acid in alkaline solutions.

Data Analysis
The collected data was analyzed using two way analysis of variance (ANOVA). SAS Statistical software (statistix 10) was used for statistical data analysis. The critical difference at $P< 0.05$ was estimated and used to find the significant difference among the sample mean.

RESULTS AND DISCUSSION

Nutrient Composition of Raw Materials
The data in Table 2 showed the proximate composition of raw materials. The average value of moisture content exhibited between 7.64 to 9.68%. The moisture content 9.68% of maize was higher than 7.64 and 8.16 of cooking banana and haricot bean flour, respectively. Generally, higher values

of ash (3.96%), crude fiber (4.93%), and crude protein (23.68%) were recorded for haricot bean flour. The value of ash, crude fiber, and crude protein recorded for haricot bean flour in the current study was in agreement with the finding of Mathewos *et al.* (2014) reported same parameters for haricot bean.

The higher (5.87%) crude fat and energy (384.31 Kcal/100g) were recorded for maize flour, whereas the higher (83.50%) carbohydrate value was noted for cooking banana flour. The average ash content, crude fiber and total carbohydrates of cooking banana obtained in this study were consistent with the respective parameters reported by Abiodun *et al*. (2015) for plantain used during processing of plantain - cowpea based complementary foods. The crude fat result of maize flour 5.87% obtained in this finding is barely greater than 5.40% of crude fat reported by Shiriki *et al*. (2015) for maize. The energy value 384.31 kcal/100g recorded for maize in this work is close to the energy value of 381.85 kcal/100g reported by Obinna *et al*. (2018) for pregelatinized maize flour.

Table 2. Proximate composition of raw materials used in the study in (%)

Raw materials	Moisture	Ash	Crude fat	Crude fiber	Protein	Total carb ohydrate	Energy value (kcal/100g)
Haricot Bean	8.16	3.96	2.96	4.93	23.68	61.24	366.32
Cooking Banana	7.64	3.82	1.84	1.97	3.65	83.05	363.36
Maize	9.68	1.58	5.87	3.73	9.23	73.64	384.31

Minerals and Antinutrients of Raw Materials

The result of the mineral contents of raw materials was indicated in Table 3. The higher values of calcium (121.43 mg/100g) were recorded for cooking banana flour. The lowest (34.54 mg/100g) was recorded for maize flour. Haricot bean flour had a higher value of iron and zinc, which were 7.37 and 6.25 mg/100g, respectively, as compared to other materials used in this study. This is because beans are excellent sources of iron, magnesium, zinc, and potassium (USDA, 2016). Data indicated in Table 3 showed that substantial content of both condensed tannin and phytate were present in haricot bean flour. These occurrences of higher antinutrients in haricot bean contributed by the presence of high accumulated antinutrient compounds in most legume seeds as reported by Liener (1994) for soybean foods.

Table 2. Minerals and antinutrients of raw sample in (mg/100g)

Raw materials	Ca	Fe	Zn	Tannin	Phytate
Haricot bean	75.38	7.37	6.25	162.54	240
Cooking Banana	121.43	3.92	3.54	0.16	ND
Maize	3 4.54	3.60	2.94	1.82	2.28

ND = Not detectable

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Effect of Fermentation Time and Blending Ratio on Proximate Composition of Complementary Flour.

Moisture content

Moisture content of samples varied significantly (P<0.05) from 6.92 to 7.61% for composite flour as shown in Table 4. Fermentation time significantly reduce (P<0.05) the moisture contents of samples from 9.02% for unfermented samples to 6.29% for 24 hr fermented samples Table 4. This might have been due to over drying of fermented flour slurry. The obtained moisture content of whole samples in this study were in acceptable limits lower than 10%, which is applicable for long term storage of flour. Low moisture content play important role in preventing growth and development of mould as well as reducing moisture dependent biochemical reaction (Onimawo and Akubor, 2012).

Ash content

Ash content represent total mineral level present in a given food staff (Fouzia , 2009). As can be referred from the Table 4, the total ash content of the blends ranged from (1.59 to 2.87%) for 65:20:15 and 30:60:10 maize, haricot bean and cooking banana composite flour, respectively, were significantly (P<0.05) higher than that of sample B1(100% maize flour) (1.19%). Total ash content in all the developed blends of complementary food increased with an increased in the percentage of cooking banana and haricot bean flour. This is due to presence of higher mineral level in cooking banana and haricot bean flour than maize flour (Table 3). Total ash content obtained in this finding was closely related to ash content of complementary blends of maize, soyabean and plantain flour (1.00% to 3.05%) reported by Noah (2017). Total ash content of complementary food sample fermented for 0, 24 and 36 hr were 2.30%, 1.83% and 1.75%, respectively.

This indicate fermentation time has a significantly (P<0.05) decreasing effect on the ash content of both the blends and maize flour. The result of total ash content reduction due to fermentation time was agrees with report of Igbabul *et al*. (2014), who reported total ash content of defatted coconut flour decreased from 2.76 % at 0 to 1.02 at 72 hr. Similar observation reported by Michodjehoun *et al*. (2005) indicate that decreased in ash content during fermentation of ‘‘Gowe’’, a traditional food made from sorghum, millet or maize from 1.8 to 1.7 % on dry basis. This is due to the fact that decreased in ash content during fermentation process is attributable to the leaching of soluble mineral element from the maize and haricot bean flour into fermenting water. The higher and lower value of ash obtained in this finding was meet recommended level of ash that could be not greater than 5% for older infants and young children by FAO/WHO (2010).

Crude protein

Protein is important for tissue replacement, disposition of lean body mass and growth. Protein content in all the developed blends of formulated complementary food increased with an increased in the percentage incorporation of haricot bean flour. Increased in protein content of the blends are due to blending effect of high protein contained of haricot bean flour. Sample formulated from 30:60:10 of maize, haricot bean and cooking banana flour had significantly (P < 0.05) the highest protein content of 18.28% while the control had significantly (P < 0.05) the lowest 9.34% protein

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content. Protein content of complementary blends obtained in this study was higher than protein content (7.68 – 8.56%) reported by Amankwah *et al.* (2009) for maize-soybean complementary blends. The protein content of complementary blends obtained in this study was similar to protein content, which varied from 10.64 to 15.86% reported by Noah (2017) for maize, soybean and plantain blends.

As indicated in Table 4, protein content of complementary food fermented at 0, 24 and 36 hr were 13.44%, 14.07% and 14.98 %, respectively. The obtained protein content of fermented complementary food was increased as fermentation duration increased. Increment in protein content during fermentation is due to microflora enzymes activity, which has ability to hydrolyses bonds among bound protein antinutrient and enzyme to release free amino acids for synthesis of new protein (Hotz and Gibson, 2007). The other similar report by Oyango (2005) revealed that increase in protein content after fermentation has been attributed to the increase in nitrogen content released, when microorganisms used carbohydrates for energy. Protein digestibility raised during fermentation can be related to enzymatic properties of fermenting microflora that brings about the breakdown of protein (Yousif *et al*., 2000). Protein content of most of composite flours obtained in this study was in consistent with recommended protein content, which is at least 15g/100g on dry matter basis by Codex Alimentarius Commission (1991) in the complementary food specified for infant and young children.

Crude fat content

Fat is important in the diet of older infant and young children because it provides essential fatty acid, facilitates absorption of fat soluble vitamin, enhance dietary energy, density and sensory quality (FAO, 2001). A significant increase ($P < 0.05$) in fat content was observed in all developed complementary food samples. The highest mean value of crude fat content of prepared complementary flour as influenced by fermentation time was 6.43% for sample fermented at room temperature for 36 hr, while the lowest mean value of 5.12% was observed for un fermented (0 hr) samples. This indicate that fermentation process resulted significant increasing effect on crude fat content of the complementary ingredients. This is in conformity with the findings of Chikwendu *et al.* (2014), who reported an increased in lipid content of pearl millet fer mented for periods of 24 h, 48 h and 72 hours. This is might be due to the removal of soluble carb ohydrates and use of nondigestible carbohydrates during fermentation by fermenting microorganis m which could concentrate the fat content.

There were significant differences ($P<0.05$) among crude fat content of complementary blends .As can be shown in Table 5 the higher and lower crude fat content of the formulated complementary blends were 6.23% for 60:30:10 and 5.31% for 30:60:10,maize,haricot bean and cooking banana flour, respectively. Crude fat increased as the inclusion level of maize flour increased. This could be due to superior quantity of crude fat in maize flour than in other ingredients used during complementary blend formulation Table 2. The obtained result of crude fat in the current study was slightly higher than the fat content (4.82 to 5.25%) reported by Noah *et al.* (2017) for complementary blends formulated from maize, soybean and plantain flour.

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Table 4. Effect of fermentation time and blending ratio on proximate composition of complementary flour (%)

Fermentation time (hr)	Moisture	Ash	Crude fat	Crude fiber	Protein	Tot. Carbohydrate	Energy value (kcal/100g)
0	9.00[a]	2.31[a]	5.12[c]	5.05[a]	13.44[b]	70.14[b]	380.39[c]
24	6.29[b]	1.82[b]	5.64[b]	3.71[b]	14.07[ab]	72.07[a]	395.39[b]
36	6.59[b]	1.75[b]	6.43[a]	3.21[c]	14.98[a]	70.24[b]	398.83[a]
LSD	0.4701	0.5153	0.13	0.1112	1.3136	0.63	1.08
CV	8.98	4.92	6.10	7.63	12.40	2.43	0.76
Sample code	Moisture (%)	Ash (%)	Crude fat (%)	Crude fiber (%)	Protein (%)	Tot. Carbohydrate (%)	Energy value (Kcal/100g)
B1	6.93[b]	1.19[e]	5.38[c]	3.40[c]	9.35[d]	77.16[a]	394.42[ab]
B2	7.62[a]	1.59[d]	5.80[b]	2.71[d]	12.66[c]	72.33[b]	392.20[bc]
B3	7.11[b]	1.88[c]	6.23[a]	3.41[c]	14.54[b]	70.21[c]	395.28[a]
B4	7.48[a]	2.26[b]	5.93[ab]	4.72[b]	15.99[b]	68.33[d]	390.67[c]
B5	7.50[a]	2.87[a]	5.31[c]	5.71[a]	18.28[a]	66.04[e]	385.11[d]
LSD	0.17	0.34	0.16	0.14	1.69	0.81	1.4
CV	8.98	4.92	6.10	7.63	12.40	2.43	0.76

Means within the same column followed by the same letter are not significantly different (P>0.05), a>b>c>d>e, CV = Coefficient of variance, LSD = Least significant difference, hr = hour,B1=100% Maize flour, B2 = 65:20:15, B3 = 60:30:10, B4 = 50:35:15,B5 = 30:60:10 ,Maize, Haricot bean and cooking banana, respectively.

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Crude fiber

FAO/WHO (1998) has recommended 10-15g/day intake of crude fiber for older infants and young children. High fibre content is known to increase viscosity of food. This is particularly worth considering in infant feeding because highly viscous foods would reduce food intake. Fibre plays a role in the increase of the utilization of nitrogen and absorption of some other micronutrients. The result of crude fiber showed that significant (P<0.05) reduction as fermentation time increased. The highest (5.05%) crude fiber obtained in unfermented sample followed with the lowest value (3.21%) recorded for the sample fermented for about 36 hr. The possible reduction in crude fiber content during fermentation process could be attributed to the partial solubilisation of cellulose and hemi cellulosic type of material in fermentation water by activities of microbial enzymes.

A significant difference (P<0.05) in crude fiber content was observed in most of complementary blends. The highest mean value of crude fiber content of prepared composite flour as affected by blending ratio was 5.71% for composite sample formulated from 30:60:10 of maize, haricot bean and cooking banana flour, respectively, while the lowest mean value of 2.71% was noted for 65:20:15 maize, haricot bean and cooking banana samples, respectively. Increment in crude fiber content of complementary blends could be attributed to the increased incorporation level of haricot bean flour, which is rich in fiber. The result of crude fiber content obtained in the present study was closely related to crude fiber content (2.27-3.11%) reported by Ezeokeke and Onuoha (2016) for complementary food formulated from maize, soybean and banana fruit. The low fibre content obtained in the current study is in agreement with the report that revealed food used for complementary feeding should contain low fiber as high fiber can lead to high water absorption and displacement of nutrient and energy needed for the growth of children less than two years (Klim *et al*., 2001).

Total Carbohydrate

There were significant differences (P<0.05) in the carbohydrate content of complementary flour due to fermentation time as indicated in Table 4. The mean carbohydrate content of complementary flour subjected to different fermentation time set at 0, 24 and 36 hr was 70.14, 72.07 and 70.24%, respectively. The result showed that carbohydrate content decreased as fermentation time increas ed from 24 to 36 hr. This is possibly due to the microbial degradation of carbohydrates and the d ecreasing effect of nondigestible carbohydrates during the process of fermentation. According to Ejigui *et al*. (2005), the decrease in total carbohydrates calculated by difference could be due to degradation of principal substances by fermenting microorganisms, particularly starch and soluble sugars that brought occurrence of subsequent reduction in starch content.

The total carbohydrate content of the blends ranged from 66.04 to 72.33 % for composite flour formulated from 30:60:10 and 65:20:15 of maize, haricot bean and cooking banana, respectively, were significantly (P<0.05) lower than 77.16 % of sample B1 (100% maize flour). This is obviously might be associated with the high accumulation of carbohydrates in maize Table 2. Trends of decreasing total carbohydrate content of blends were attributed to decreased

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percentage incorporation of maize flour as blending ratio progressed. Total carbohydrate content of complementary blends in this study was in agreement with the total carbohydrate content (69.63 - 73.32%) reported by authors Ezeokeke and Onuoha (2016) for maize, soyabean and banana based complementary blends.
More over the total carbohydrate content of developed complementary blends in the current study was slightly higher than total carbohydrate content (61.76%) reported by Bolaji *et al.* (2010) for ogi prepared from maize and soybean blends. This could be due to the blending effect of accumulated carbohydrates in cooking banana flour. The total carbohydrate result obtained in this study is consistent with range of recommended carbohydrate content ≥65 g/100g of complementary food products for older infants and young children (WHO/FAO, 2003).

Energy value

The energy content is the reflection of the presence of carbohydrate, protein and fat in infant diet. The average energy value of formulated complementary flour fermented at different fermentation time, 0, 24 and 36 hr were 380.39, 395.39 and 398.83 kcal/100g, respectively. The result indicated that energy value increased as fermentation time increased. This is probable due to the fact that increasing effect of fermentation on the value of protein and fat content of the composite flours. In the present study, the energy values were found to be significantly greater than recommendation of WHO (2003) for an infant complementary food in developing countries, which ranged from 200 to 300 kcal/day. In other observation, the obtained energy value of the current study was within the range of the recommended daily allowance of energy which is varied over 370 to 420 kcal/100gm as described by Walker (1990) for complementary foods in developing countries.

The value was also in the range with world food program (WFP) specification minimum requirement of 380 Kcal/100g energy. The total energy value of the composite flour which ranged from 395.28 to 385.11 kcal/100g for 60:30:10 and 30:60:10, maize, haricot bean and cooking banana based composite flour, respectively, were slightly difference from energy value 394.42 kcal/100g of 100% maize flour sample. The energy value in the composite flour varied due to variation in protein and fat contents of the composite samples. The result showed that energy values obtained in the present study fell between 385.11 to 395.28 kcal/100g and are in consistent with energy value 360.43 –405.00 kcal/100g reported by Obinna *et al*. (2018) for complementary food produced from blends of malted pre-gelatinized maize flour, soy flour, and carrot flour. The current result of energy value (385.11- 395.28 kcal/100g) was also closely related to specified guidelines of energy recommendation (400 kcal/100g) for complementary food supplemented to infant and young children (Codex Alimentarius Commission, 1991).

Effect of Fermentation Time and Blending Ratio on Mineral content Zinc

Zinc is an essential element that promotes healthy immune system functioning and protects y oung infants and old children against infectious diseases. It fuels the productions of infection fi ghting white blood cells and ensures the body's cell grow and repair themselves properly (Penny, 2004). Significant differences were observed in zinc content of all composite flour fermented at different fermentation time (Table 5). Fermentation cause increment in zinc content from 4.41 mg/100g for unfermented composite flour followed by 5.24 mg/100g for 36 hr fermented composite flour. The increasing effect of the zinc concentration of composite flour as fermentation period increased in the present study was in agreement with the research finding of Nout *et al*. (1997), who report increment of soluble zinc in several folds as the result of phytate level reduction during fermentation by activity of phytase enzyme formed.

Highly significant differences were observed in zinc concentration among the formulated composite flours. The obtained zinc concentration presented in the Table 5 indicated that the highest 6.24 mg/100g of zinc concentration recorded for composite flour consisting of 30:60:10 maize, haricot bean and cooking banana flour, respectively, while the lowest 3.07 mg/100g of zinc concentration recorded for 100% maize flour sample. This showed that the zinc concentration of formulated complementary flour increased as the incorporation level of haricot bean flour increased. Zinc values of the current study were comparable with the zinc values of the composite samples which ranged from 2.74 to 4.55 mg/100g reported by Ezeokeke and Onuoha (2016) for complementary food formulated from maize, soybean and banana fruit. Zinc concentration of the processed complementary food found in this work were exist in the range 2.4 to 10 mg/100g that reported by FAO/WHO (2010). For exception of the zinc concentration found in control sample (100% maize flour), all value of zinc concentration obtained in this finding were above 4.15 mg/100g which is 50% supply of fortified complementary food recommended by WHO/FAO (2010) for older infants and young children.

Iron

Based on the present research work, unfermented composite flour sample contained 5.14 mg/100g of iron whereas 24 and 36 hr fermented composite flour contained 4.43 mg/100g and 4.21 mg/100g iron concentration, respectively. Statistically, fermentation time had a significant influence on the iron content of the composite flour used in this study. As presented in Table 5 recorded iron content of complementary blends 5.69 mg/100g for 30:60:10 maize, haricot bean and cooking banana flour, respectively, and 4.24 mg/100g for 65:20:15 of maize, haricot bean and cooking banana flour, respectively, were significantly higher than 3.50 mg/100g that of control sample 100% maize flour. This might be due to the blending effects of the increasing percentage of haricot beans flour inclusion. Research finding reported by Beebe *et al*. (1999) state that legumes in general and beans, in particular, contain appreciable quantities of iron and other minerals. The values of iron obtained in these findings were above the range of WFP (2014) specification which is 3.25 mg/100g for the manufacture of corn soya blend for older infants.

Calcium

Calcium is an essential nutrient that plays a vital role in blood clotting, as well as providing rigidity to the skeleton by virtue of its phosphate salts. The fermentation time had shown significant effects ($P<0.05$) on the calcium content of all developed composite flour Table 5. Fermentation had shown a decreasing effect in the calcium content from 54.42 mg/100g for composite sample fermented for 0 hr to 46.08 mg/1000g and 43.75 mg/100g for 24 and 36 hr fermented composite flour, respectively. Significant differences ($P<0.05$) were observed among complementary flours

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with respect to their blending proportions. The result of calcium shown in Table 5 indicated that the highest 61.43 mg/100g calcium content was noted for 30:60:10 maize, haricot bean and cooking banana flour, respectively, composite flours and the lowest 27.80 mg/100g calcium content noted for control sample (100% maize flour). Calcium content of composite flour in the present study was within the range of 34.08 – 65.02 mg/100g reported by Meseret (2011) for weaning food formulated from quality protein maize and soya bean blends. However, the obtained calcium content in this work was higher than 22 mg/100g value reported by Lalude and Fashakin (2006) for Nutrend – commercial weaning food from Nigeria.
Table 5. Effect of fermentation time and blending ratio on mineral composition of complementary flour on dry weight basis in (mg/100g)

Fermentation time(hr)	Zn	Fe	Ca	Vitamin C (mg/mL)
0	4.41^{b}	5.14^{a}	54.42^{a}	3.99^{c}
24	5.36^{a}	4.43^{b}	46.08^{b}	5.44^{b}
36	5.24^{a}	4.21^{b}	43.75^{c}	5.75^{a}
LSD	0.19	0.25	0.43	0.08
CV	5.22	7.26	1.20	2.30
Blending Ratio	Zn	Fe	Ca	Vitamin C (mg/mL)
B1	3.07^{e}	3.50^{d}	27.80^{d}	3.97^{d}
B2	4.76^{d}	4.24^{c}	48.21^{c}	5.62^{a}
B3	5.24^{c}	4.22^{c}	47.83^{c}	4.95^{c}
B4	5.71^{b}	5.30^{b}	55.14^{b}	5.22^{b}
B5	6.24^{a}	5.69^{a}	61.43^{a}	5.55^{a}
LSD	0.25	0.32	0.55	0.11
CV	5.22	7.26	1.20	2.30

Means within the same column followed by the same letter are not significantly different ($P>0.05$), a>b>c>d>e, CV = Coefficient of variance, LSD = Least significant difference, hr = hour, B1=100% Maize flour, B2 = 65:20:15, B3 = 60:30:10, B4 = 50:35:15,B5 = 30:60:10 ,Maize, Haricot bean and cooking banana, respectively. Zn = Zinc, Ca = Calcium, Fe = Iron

Vitamin C

The level of vitamin C in developed fermented composite flour significantly ($P<0.05$) increased as fermentation time increased. The highest vitamin C content 5.75 mg/mL was recorded in composite flour fermented for 36 hr followed by 5.44 and 3.99 mg/mL recorded for composite flour fermented for 24 and 0 hr, respectively. Table 5 showed the vitamin C content of the composite blends ranged from 4.95 to 5.62 mg/mL for composite blends consists 60:30:10 and 65:20:15 of maize, haricot bean and cooking banana, respectively, were significantly ($P<0.05$) higher than 3.97 mg/mL of 100% maize flour sample. Vitamin C content in all the developed composite blends of complementary flour increased with increased in the percentage incorporation of cooking banana flour. This is possibly might be due to presence of higher vitamin C level in cooking banana flour.

Effect of Fermentation Time and Blending Ratio on Condensed Tannin and Phytate

Condensed tannin

Statistical analysis of tannin levels of the fermented and unfermented flour showed that there were significant differences (P < 0.05) in the tannin content of the developed complementary flour. The mean value of tannin content of composite flour as affected by fermentation time was 46.93 mg/100g for unfermented flour sample, 33.98 mg/100g for 24hr fe rmented sample and 31.32 mg/100g for 36 hr fermented one as shown in Table 6. The result showed that tannin content decreased as fermentation time increased from 0 to 36 hr. The reduction in the condensed tannin contents after fermentation could be due to leaching of the condensed tannin into the fermentation water. Reduction in tannin contents due to fermentation also caused by the activity of polyphenol oxidase or tanniase of fermenting microflora on tannins (Fagbemi *et al.*, 2005). This is in agreement with Gernah *et al*. (2011), who study the effect of fermentation on some chemical and physical properties of maize.

Reduction in tannin contents has numerous benefits, which includes reducing the risk of bowel irritation, kidney irritation, liver damage, irritation of the stomach and gastrointestinal pain. According to Onweluzo *et al*. (2009), fermenting microorganisms are responsible for the cleavages of tannin-protein, tannic acid-starch and tannin iron complexes thereby releasing the free nutrients, which will invariably improve the availability of the nutrients. The tannin content of the blends ranged from 26.15 to 74.60 mg/100g for 65:20:15 and 30:60:10 of maize, haricot bean and cooking banana flour, respectively, were significantly (P<0.05) higher than 1.44 mg/100g that of sample 100% maize flour. This is obviously might be attributed to the high accumulation of condensed tannin in haricot bean flour. Trends of increasing tannin content of composite blends were attributed to increased percentage incorporation of haricot bean flour.

Phytic acid

Phytic acid is the primary storage compound of phosphorus in cereals, legumes, nuts, and oilseeds. The intake of phytate has a direct correlation with the poor iron and zinc status commonly seen in complementary food of preschool children after 6 months of age in low-income countries (Abdel *et al.*, 2010). Fermentation time was a significant (P<0.05) factor for the reduction of phytate content of the composite blends. As fermentation time increased from 0 to 36 hr the phytate content was decreased from 70.24 to 36.99 mg/100g. This is possibly due to formation of optimum pH conditions during fermentation for enzymatic degradation of phytate, which is present in cereals in the form of complexes with polyvalent cations such as iron, zinc, calcium, magnesium, and proteins.

It has been also suggested that the loss of phytate during fermentation could be a result of the activity of native phytase and/or the fermentative microflora (Abdelhaleem *et al*., 2008). Such a reduction in phytate may increase the amount of soluble iron, zinc, calcium several folds (Blandino *et al.*, 2003). Phytic acid reduction by 25-29.77% in the current study was higher than the 5-18% reported by Anshu and Neelam (2002), during fermentation of wheat flour containing spices mixture and leguminous stuffing at 35 °C and 40 °C for 18 and 24 hr for bread production. Possible cause of higher reduction percentage of phytate in the present study could be due to extension of fermentation duration. The phytate content of maize 2.04 mg/100g was significantly ($P<0.05$) lower than 107.21 mg/100g of composite flour produced from 30:60:10 of maize, haricot bean and cooking banana, respectively. This sound variation in the phytate content of composite blend majorly contributed from haricot bean flour.

Table 6. Effect of fermentation time and blending ratio on tannin and phytic acid content of composite flour on dry weight basis in (mg/100g)

Fermentation time (hr)	Tannin	Phytate
0	46.93[a]	70.24[a]
24	33.98[b]	49.33[b]
36	31.32[c]	36.99[c]
LSD	2.08	1.26
CV	7.44	3.24
Sample code	Tannin	Phytate
B1	1.44[e]	2.04[e]
B2	26.15[d]	37.01[d]
B3	38.39[c]	53.84[c]
B4	46.46[b]	60.83[b]
B5	74.60[a]	107.21[a]
LSD	2.69	1.63
CV	7.44	3.24

Means within the same column followed by the same letter are not significantly different ($P>0.05$), a>b>c>d>e, CV = Coefficient of variance, LSD = Least significant difference, hr = h our, B1=100% Maize flour, B2 = 65:20:15, B3 = 60:30:10, B4 = 50:35:15,B5 = 30:60:10 ,Maize, Haricot bean and cooking banana, respectively.

CONCLUSION AND RECOMMENDATION

Most nutrient composition of complementary flour in this study were meet nutrient requirement s for older infants and young children. The study showed that fermentation significantly improv ed some proximate composition, minerals, vitamin C and decreased anti nutritional factors in developed complementary flour. Crude protein, crude fat, vitamin C and energy value of composite flour were enhanced as fermentation time increased. The higher value of protein, crude fat, vitamin C and energy in all developed composite flour were recorded for 36 hr fermented samples regardless of blending ratio. While higher values of ash content, crude fiber, moisture and carbohydrate contents were recorded for unfermented composite flour irrespective to blendin g ratio. Both antinutrients, condensed tannin, and phytic acid were significantly reduced in the c omposite flour fermented for 36 hr.

Majority of developed complementary blends brought significant increment of crude fiber, crude protein, energy, carbohydrate, ash, crude fat, calcium, iron, zinc and vitamin C contents. Both condensed tannin and phytate content of complementary flour increased as incorporation level of haricot bean increased. Generally, cereals-legume blends with the addition of cooking banana flour resulted in an increased in some essential nutrients desirable for older infants and young children. Furthermore, composite flours had enhanced mineral contents (Calcium, iron and zinc) as a result of the combination of fermentation time and blending ratio. Finally, based on major finding of this work, further study is recommended to increase the amount of calcium, and vitamin C content by increasing the amount of cooking banana flour in complementary flour prepared in this study. Determination of invitro protein and starch digestibility of complementary flour should require further study.

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