Effect of fermentation on physicochemical and antinutritional factors of complementary foods from millet, sorghum, pumpkin and amaranth seed flours

[1*]Simwaka, J.E., [1,2]Chamba, M.V.M., [1]Huiming, Z., [1,3]Masamba, K.G. and [1]Luo, Y.

[1]*State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, 214122 Wuxi, Jiangsu Province, China*
[2]*Department of Physics and Biochemical Sciences, University of Malawi – The Polytechnic, Private Bag 303, Chichiri, Blantyre 3, Malawi*
[3]*Department of Food Science and Technology, Lilongwe University of Agriculture and Natural Resources, Bunda College Campus, P.O Box 219, Lilongwe, Malawi*

Abstract

In this study, effect of natural fermentation using different fermentation time (0-36 h) on physicochemical and functional properties as well as antinutritional factors on complementary foods formulated from pumpkin seeds, amaranth, finger millet and sorghum grains was investigated. High protein content above WHO/FAO recommended levels for weaning foods was observed in sorghum-pumpkin (SP) (24.5%), millet-pumpkin (MP) (23.6%), Sorghum-amaranth-pumpkin (SAP) (22.8%) and millet-amaranth-pumpkin (MAP) (17.6%), which varied with fermentation time ranging from 0 - 36 h. Results on protein digestibility, showed that MAP had the highest value of 92.8% followed by MP (92.3%), SP (86.9%), SAP (78.7%), (SA) (88.2%) and (MA) (79.0%); while fermented SP for 36 h registered the highest starch digestibility of 77.7%, followed by SAP (74.8%), SA (74.6%), MAP (70.1%) and MA (69.3%). Total polyphenols, tannins and phytates decreased after fermentation. Functional properties such as Water Absorption Index (WAI), Water Solubility Index (WSI), Oil Absorption Capacity (OAC), Bulk Density (BD) and Least Gelation Concentration (LGC) were within the acceptable ranges for complementary foods with WSI showing an increase after fermentation while WAI and OAC decreased and LGC observed at 20% flour concentration after fermentation. These results showed that pumpkin seed, amaranth, finger millet and sorghum flours, in their blended form have potential to formulate a complementary food with high protein content and reduced antinutritional factors. In this way, utilization of cereals not commonly used for complementary foods, pseudo cereals and oil seeds in infant foods can be enhanced to address nutritional related cases especially in developing countries.

Introduction

Pumpkin seeds have been mostly considered agro-industrial waste and discarded, although a few people regard them as food source at domestic scale (Patel, 2013). Apart from large amount of oil, pumpkin seeds have high protein content with a well-balanced amino acid composition, with their lysine content quite higher compared to that of wheat (Giami *et al.*, 1999). In most African countries such as, Tunisia, pumpkin seeds are mainly salted and roasted then consumed as snack (Rezig *et al.*, 2013). 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). Supplementation of wheat flour with defatted pumpkin seed flour at 5-15% level produces cookies with increased crude protein, calcium, sodium, potassium and phosphorus contents. Cookies with 15% pumpkin seeds are nutritionally comparable to a diet based on casein (Giami *et al.*, 1999). Amaranth is a wild cereal-like plant of the Amaranthaceous family. It contains about 15% protein and 60% starch. Amaranth is considered a good source of high quality balanced protein due to its high content of lysine, methionine and cystein, as well as dietary fiber and minerals (Marcone, 1999). In recent years, there has been an increasing interest in the use of amaranth flour for blending with wheat and maize flours not only to enhance nutritive value of the final products, but also as a functional food due

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*Corresponding author.
Email: joysimwaka20@yahoo.co.uk

to its cholesterol-lowering effect observed in animal model (Mendonça *et al.*, 2009).

Sorghum and finger millet are well known to largely contribute to food security in semi-arid and tropical regions due to their productivity under hard and drought conditions, short growing season, and resistance to pests and diseases. Sorghum and finger millet are a major source of protein, carbohydrate and calorie in the diets of large population but their bioavailability is considered to be low due to presence of antinutritional factors like phytic acid, polyphenols and tannins (Singh *et al.*, 2012). A combination of cereals such as sorghum and finger millet with soy or pulses has enhanced their protein quantity and quality (Devi *et al.*, 2014). Soy bean protein has been the most used plant protein at industrial scale, leaving out other plant proteins that could alternatively be used (Atuonwu and Akobundu, 2010). Fermentation has been reported to be effective in decreasing the level of antinutritional factors in cereals and improves their starch and protein digestibility, amino acid balance as well as nutritive value (Belton and Taylor, 2004; Singh *et al*, 2012).

The use of pumpkin seed and amaranth flours with cereals such as wheat, corn, rice, and oats has been reported (Sanchez-Marroquin *et al.*, 1986; Giami *et al.*; 2003; Ikujenlola *et al.*, 2013). However, there is limited research on the use of pumpkin and amaranth seed flours with sorghum and finger millet or their blend in the formulation of complementary foods. The aim of this study therefore, was to evaluate the effect of fermentation on physicochemical and functional properties as well as antinutritional factors of complementary foods formulated from different ratios of flour blends of millets, pumpkin and amaranth seed flours. It is our expectation that the developed complementary foods can be used as infant foods and thereby contribute to increased utilization of these least commonly used cereals and oil seeds.

Materials and Methods

Materials

Sorghum was purchased from Baokang Company in Harbin city, People's Republic of China. Amaranth seeds and finger millet were purchased from the local market in Wuxi city, China. Dehulled pumpkin seed kernels were purchased from Grain Mill Firm in Hohhot City, Inner Mongolia, China. All chemicals used were of analytical grade.

Sample preparation

All materials, except the pumpkin seeds, were carefully cleaned and ground in a portable mill to pass through a 60 mesh wire screen, packed in polyethylene bags and stored at 4°C until use. The dehulled pumpkin seed kernels were first milled in a portable mill to pass through a 100 mesh wire then suspended in n-Hexane in the ratio of 1:4 (w/v) to remove the fat. The slurry was shaken for 8 h and vacuum filtered. The resultant defatted flour was air dried to remove the remaining n-Hexane and milled again to pass through a 60 mesh wire screen. The final flour was then packed in polyethylene bags and stored at 4°C until use.

Sample formulations

The samples were formulated in line with WHO recommended ratios of about 70:30 (cereal: legume) for satisfying macronutrient requirements in infant formulas (FAO/WHO, 1991). Six formulations based on prior analysis were formulated: (1) Sorghum-Amaranth (SA), 60:40; (2) Millet-Amaranth (MA), 60:40; (3) Sorghum-Pumpkin (SP), 60:40; (4) Millet-pumpkin (MP), 60:40; (5) Sorghum-Pumpkin-Amaranth (SAP), 50:30:20; and (6) Millet-Pumpkin-Amaranth (MAP), 50:30:20.

Fermentation

One part of each flour blend was fermented according to method of Usha and Chandra (1998), using natural fermentation at 37°C. Different fermentation times were used and ranged from 0 to 12, 18, 24, 30 and 36 h. The slurry was mixed and dried at 70°C for 16 h or more in a hot air oven. The dried slurry was milled into flour to pass through a 60 mesh wire screen and stored at 4°C until analysis.

Proximate composition

Proximate composition of the raw and fermented composite flours was conducted as follows: Moisture, ash and fat were analyzed using standard methods of analysis (AOAC, 2000). Crude protein was determined by micro-Kjeldahl method with the conversion factor of $N \times 6.25$. Carbohydrate content was estimated by subtracting the sum of percentages of moisture, crude fat, crude protein and ash contents from 100 (Joshi *et al.*, 2015). Total energy was determined by the formula, [(% available carbohydrates $\times$ 4) + (% protein $\times$ 4) + (% fat $\times$ 9)].

Bulk Density

Bulk density was determined according to the method of Anderson *et al.* (1969) as modified by (Kaur and Singh, 2005). The flour samples were transferred into a 10 ml graduated cylinders that were previously weighed. The cylinder was tapped

gently at the bottom on a laboratory bench several times until no diminution of the sample level was observed. Bulk density was calculated as the weight of the sample per unit volume of the sample (g/ml). Measurements were done in triplicate.

Least gelation concentration

Least gelation concentration was determined by the method of (Sathe and Salunkhe., 1981). Test tubes containing suspensions of 2, 4, 6, 8, 10, 12, 16, 18 and 20 g/100 ml of material in 5 ml distilled water were heated for 1 h in boiling water, followed by rapid cooling under running tap water. Tubes were further cooled at 4°C for 2 h. Least gelation concentration was defined as the concentration above which the sample did not fall down or slip when test tubes were inverted.

Water absorption and water solubility index

Water absorption index (WAI) and water solubility index (WSI) were determined according to the method of Anderson et al. (1969) as modified by (Kaur and Singh, 2005). Sample, 2.5 g was dispersed in 30 ml distilled water using a glass rod and heated in a water bath at 90°C for 15 min. The resultant paste was cooled to room temperature and transferred to pre-weighed centrifuge tubes and then centrifuged at 3000 rpm for 10 min. The supernatant was decanted for determination of its solid content into a pre-weighed evaporating dish and the sediment was weighed. Weight of dry solids was recovered by evaporating the supernatant overnight at 110°C in an oven. WSI and WAI were calculated using the following the equation:

WAI= Weight of sediment/weight of dry solids

WSI= Weight of dissolved solids in supernatant × 100/weight of dry solids

Oil absorption index

Oil absorption capacity (OAC) was determined according to the method of Lin *et al.* (1974) as modified by Kaushal *et al.* (2012). Sample (0.5 g) was mixed with 6 ml of corn oil in pre-weighed centrifuge tube. The contents were stirred for 1 min with a thin brass wire to disperse the sample in the oil. After a holding period of 30 min, the tubes were centrifuged for 25 min at 3000 rpm. The separated oil was removed with a pipette and the tubes were inverted for 25 min to drain the oil prior to reweighing. Oil absorption capacity was expressed as gram of oil bound per gram of the sample on a dry basis. OAC was calculated by the equation:

$$OAC \ (g/g) = (W_2 - W_1) / W_0$$

Where: W_2 = Weight of centrifuge tube after drawing oil,

W_1 = Centrifuge tube weight + sample weight, and

W_0 = original sample weight, dry basis.

Protein digestibility

Protein digestibility was determined using the method of Saunders *et al.* (1973) as modified by Elmaki *et al.* (1999). A sample 0.2 g was placed in 50 ml centrifuge tube, then, 15 ml of 0.1 M HCL containing 1.5 mg pepsin were added and the tube was incubated at 37°C for 3 h. The suspension was neutralized with 0.5 M NaoH and treated with pancreatin in 7.5 ml of 0.2 M phosphate buffer, PH 8.0 containing 0.005% sodium azide. The mixture was gently shaken and incubated at 37°C for 24 h. After incubation, the sample was treated with 10% trichloroacetic acid and centrifuged at 5000 rpm for 20 min at room temperature. Nitrogen in the sample was determined by Kjeldahl method.

Starch digestibility

Digestibility of starch was determined following the method of Singh *et al.* (1982). A suitable amount of defatted meal (50 mg) was dispersed in 1.0 ml of 0.2 M phosphate buffer, PH 6.9. Pancreatic amylase (40000 U/ml,) was added to the sample suspension and incubated at 37°C for 2 h. A 2 ml of 3,5 dinitrosalicyclic acid reagent was quickly added and the mixture was heated for 5 min in a boiling water bath. After cooling, the solution was made up to 25 ml with distilled water. After sample filtration, absorbance was read at 550 nm using UV Spectrophotometer (T6 ultraviolet Spectrophotometer, Beijing General instrument company Ltd). The blank was run simultaneously by incubating sample with 3,5 dinitrosalicylic acid before addition of the enzyme solution. Maltose was used as standard and results were expressed as mg of maltose released per gram of sample.

Total polyphenols

Total polyphenols were determined by the method of Dykes *et al.* (2006) with some modifications. 0.2 g flour was extracted in 25 ml of 0.1 M HCL in methanol (v/v) for 2 h using a magnetic stirrer at a low speed. A 0.5 ml of the extract was mixed with 2.5 ml folin-ciocalteu reagent (10%). Within 2-8 min, a 2 ml of 7.5% Na_2CO_3 was used and the mixture vortexed. The sample was incubated in the dark for 15 min and absorbance read at 760 nm by UV Spectrophotometer (Ultraviolet Spectrophotometer,

Beijing General Instrument Company Ltd). A standard curve of Gallic acid from 0-60 µg/ml was used.

Tannins

Tannins were determined according to the method by Price *et al*. (1978). A sample (0.2 g) was extracted with 10 ml acidified methanol for 20 min in rotating screw cap culture tubes. After centrifugation at 3000 rpm for 10 min, a 1.0 ml of supernatant was dispersed in each culture tube. Triplicate of each sample was run with a blank. The samples were incubated at 30°C, and 5.0 ml of Vanillin reagent was added at 1.0 min interval. After 20 min, absorbance was read at 500 nm UV Spectrophotometer (Ultraviolet Spectrophotometer, Beijing General instrument company Ltd). A standard curve using catechin from 0-1 mg/ml was prepared.

Phytic acid

The method of Haug and Lantzsch (1983), modified by Onyango *et al.* (2005) was used to measure phytates. A sample of 0.1 g was extracted with 10 ml 0.2 M/l HCl by shaking for 1 h before centrifugation at 5000 rpm for 15 min. Then, 0.5 ml of the supernatant was pipetted into a test tube before adding 1 ml acidic ammonium iron III sulphate deodecahydrate (0.2 g) in 100 ml 2 M/l HCL, and made up to 1000 ml with distilled water. The sample was boiled for 30 min then rapidly cooled to 25°C in water bath. A 2 ml of 2,2 bypiridine solution in thioglycolic acid was added and the contents mixed. Absorbance was read after 1 min at 519 nm using UV Spectrophotometer (Ultraviolet Spectrophotometer, Beijing General instrument company Ltd), against distilled water. A standard curve was prepared using sodium phytate to give final phytate phosphorus concentration of 6-80 µg/ml.

Statistical analysis

Variations among means were determined by a One way Analysis of Variance (ANOVA) with Duncan's multiple range test, using SPSS 19.0 software (SPSS Statistics 17, Chicago, Illinois).The significant differences were tested at $p<0.05$.

Results and Discussions

Crude protein content

Results for proximate composition of the complementary foods from different flour blends are shown in Table 1. Figure 1 shows effect of fermentation on crude protein content. Protein content of samples ranged from 8.9 - 24.5% in unfermented samples and 9.0% - 25.8% in 36 h fermented samples. Protein content was lowest in MA and highest in SP at ($p<0.05$). Protein content in unfermented SP, MP, MAP and SAP were comparable to weaning food formulated from blends of rice, soybean, carrot, whole egg and maltodextrin (Mohamed and Huiming, 2007) and those from wheat, soy protein concentrate, whey protein concentrate, green gram flour fortified with vitamins and minerals (Khanam *et al*., 2013). Protein values for unfermented SP, MP, MAP and SAP were within the FAO/WHO recommended values of >15% (FAO/WHO, 1991). Crude protein content increased up to 18 h of fermentation after which some samples started showing a decrease except SP, MP and MAP, which continued to increase up to 30 h with SP having the highest protein content. After 36 h of fermentation, crude protein content started decreasing. Lactic acid bacteria use sugar as a substrate for growth. Different amounts of sugar content in different samples may have affected the growth cycles of lactic acid bacteria, thereby contributing to fluctuations in protein content in different samples at different fermentation time. A fluctuation in protein content during 24 h fermentation of sorghum and pearl millet was reported by Ibrahim *et al.* (2005). The increase in protein content after fermentation has been attributed to the increase in nitrogen content released when microorganisms utilize carbohydrates for energy (Oyango, 2005). A similar observation was reported by Pranoto (2013), where proteolysis of protein during fermentation resulted in producing peptides and amino acids. A decrease in protein content as fermentation continues is attributed to amino acids being metabolized to ammonia and flavor compounds due to accumulation of lactic acid activities (Pranoto, 2013). The high levels of protein content in blends with pumpkin mixtures is attributed to high levels of protein content in defatted pumpkin seed flour as reflected in our preliminary work. Several authors have also reported an increase in protein content of products containing pumpkin seed flour (El-Soukkary, 2001; Giami *et al*., 2003; Norfezah *et al.,* 2011).

Ash content

Ash content ranged from 2.23 - 3.93% in unfermented samples and 1.7 - 3.9% in fermented samples. Ash content significantly decreased ($p<0.05$) from 2.2 - 1.7% in SA and 3.3-2.8% in MA after fermentation. The decrease in ash content during fermentation has been reported to either leaching of soluble minerals into water during fermentation or metabolic activities of microorganisms (Nnam, 2001). In this study, it was speculated that both the

Table 1. Effect of fermentation on proximate composition

Sample	Protein (g/100g)		Carbohydrate by difference (g)		Total Energy (kcal/100 g)		Ash (g/100g)	
	NF	F	NF	F	NF	F	NF	F
SA	9.74 ± 0.27[b]	10.54 ± 0.22[c]	71.33 ± 1.81[e]	69.51 ± 0.09[e]	354.62 ± 9.58[a, b, c]	348.19 ± 0.87[a, b]	2.23 ±0.01[b]	1.78 ±0.08[a]
MA	8.99 ± 0.20[a]	9.02 ± 0.65[a]	70.48 ± 0.71[e]	70.74 ± 0.16[e]	357.08 ± 2.10[b, c, d]	347.32±1.75[a]	3.34 ±0.46[d]	2.82 ±0.06[c]
SP	24.52 ±0.52[h]	25.85 ± 0.93[i]	49.51 ± 0.16[a]	52.38 ± 2.46[b, c]	386.72 ± 3.11[h]	360.59 ± 11.66[c,d,e]	3.09 ±0.02[c]	3.22 ±0.03[d]
MP	23.61 ±0.26[g]	24.17 ± 0.09[h]	50.75 ±1.68[a, b]	48.95 ± 0.08[a]	385.93 ± 7.50[h]	375.88 ±2.11[f, g]	3.88 ±0.06[e]	3.90 ±0.06[e]
SAP	22.75 ±0.32[f]	23.62 ± 0.05[g]	51.94 ± 0.72[b, c]	51.94 ± 0.05[b, c]	385.11 ± 3.29[h]	364.71 ± 1.10[d, e]	3.25 ±0.03[d]	3.34 ±0.05[d]
MAP	17.61 ±0.16[d]	20.55 ± 0.16[e]	57.86 ± 0.19[d]	53.67 ± 0.60[c]	381.36±1.10[g, h]	369.42 ± 1.20[e, f]	3.93 ±0.02[e]	3.99 ±0.03[e]

Values with different superscript letters within the same parameter are significantly different (p<0.05, mean ± SD, n=3)
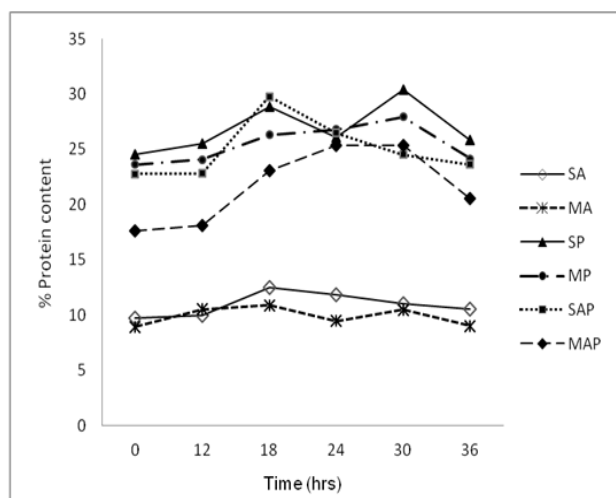NF: Non-fermented; F: fermented

Figure 1. Effect of fermentation on changes in crude protein content

increase in synthetic activities of microorganisms during metabolic processes and leaching might have contributed to increase and decrease in ash content. An increase in ash content has been reported to possibly indicate a higher mineral content (Bassey *et al.*, 2013).

Fat content

Results on fat content showed that values in fermented samples were slightly lower, ranging from 3.1 - 8.0% compared to those in unfermented samples which ranged from 3.3 - 8.8%. A significant decrease (p<0.05) in fat content was observed in all samples. A study by Afify *et al.* (2012) revealed a reduction in oil content after cooking, germination and fermentation of three white sorghum varieties and attributed the reduction to biochemical and physiological changes occurring during germination and fermentation. Fat levels decreased in fura from germinated grains due to increased activities of the lipolytic enzymes which hydrolyses fats to fatty acids and glycerol (Inyang, 2008). FAO/WHO (1991) recommends a total fat

percentage of up to 10% in complementary foods as long as it does not compromise with keeping qualities of food.

Carbohydrate and energy content

Results on carbohydrate and energy contents are shown in Table 1. Carbohydrate content by difference ranged from 49.51% in SP to 71.33% in SA. Fermentation caused a decrease in carbohydrate content in all samples except in MA and SP. Microbial activity requires energy and nutrient during fermentation hence a decrease in carbohydrates, which are the main source of energy. This was confirmed in decrease in energy content at the end of 36 h of fermentation period. For energy content, SA and MA were significantly lower in unfermented samples than the rest in the same category. After fermentation, there was energy reduction in all samples and were all significantly different, with MP being the highest (375.8 kcal/100 g). Energy content of all flour blends fell below the recommended values of WHO/UNICEF for 9-11 months babies, who require 451 kcal/day but within the range of 6-8 months, who require 269 kcal/day. However, according to the US longitudinal data, the energy value of the samples were within the recommended range for 6-8 months (202 kcal/day) and 9-11 months (307 kcal/day). Consumption of this meal twice a day with other foods would meet the energy requirements of both 6-8 and 9-11 months old children (Dewey and Brown, 2003).

Phytates

Results for different anti-nutritional factors are presented in Table 2. Results showed that all unfermented samples contained higher contents of phytates, compared to fermented samples with SAP being the highest (128.48 μg/ml phytate phosphorus, PAP). These were significantly reduced (p<0.05) to 78.26 μg/ml PAP after fermentation. The rest of

Table 2. Effect of fermentation on anti-nutritional factors

Sample	Phytates (µg/ml PAP)		Tannins (mg/ml Catechin)		Polyphenols (µg/ml Gallic acid)	
	NF	F	NF	F	NF	F
SA	88.26 ± 3.17[d]	63.45 ± 0.74[b]	0.59 ± 0.02[a]	0.45 ± 0.08[a]	18.30 ± 0.13[a]	16.63 ± 0.06[a]
MA	77.17 ± 1.95[c]	52.74 ± 1.88[a]	0.69 ± 0.08[a]	0.59 ± 0.04[a]	23.45 ± 0.40[b]	18.16 ±1.01[a]
SP	118.32 ± 1.88[g]	94.60 ± 6.51[e]	0.99 ± 0.12[d]	0.83 ± 0.22[b, c, d]	56.22 ± 1.73[e]	33.30 ± 1.14[c]
MP	114.60 ± 0.84[f, g]	111.10 ± 0.74[f]	0.93 ± 0.01[c, d]	0.73 ± 0.04[b, c, d]	41.83 ± 2.25[d]	40.64 ± 2.36[d]
SAP	128.48 ± 0.34[h]	78.26 ± 1.07[c]	0.96 ± 0.01[b, c]	0.76 ± 0.18[b, c, d]	43.11 ± 0.07[d]	33.16 ± 0.40[c]
MAP	115.69 ± 0.34[f, g]	94.76 ± 0.90[e]	0.80 ± 0.08[b, c, d]	0.75 ± 0.18[b, c, d]	42.97 ± 0.13[d]	39.21 ± 0.06[d]

Values with different superscript letters within the same parameter are significantly different ($p<0.05$, mean ± SD, n=3)

NF: Non-fermented; F: Fermented

the samples followed the same trend. This showed that fermentation had a significant effect on the content of anti-nutritional factors. Phytic acid has been recognized as a major inhibitor of iron and zinc absorption. Combinations of sorghum and amaranth (SA), and finger millet with amaranth (MA), gave lowest levels of phytates while combinations of pumpkin gave the highest levels of phytates. Antinutritional factors are proteins in nature and pumpkin flour had the highest content of protein. This may explain why combinations of pumpkins had higher levels of phytates. In raw materials, phytates were highest in pumpkin seed flour, about 103 µg/ml compared to amaranth (96.79 µg/ml) confirming the fact that phytates are protein in nature. After fermentation, reduction in phytates was more pronounced in SAP with 50.22% and lowest in MP with 3.5%. Reduction in other samples was from 20-24%. A study by Usha and Chandra, (1998) on fermentation of finger millet flour using endogenous grain micro flora reduced phytates up to 20%. Fermentation is known to cause a greater reduction in phytic acid than other nutrients because of the low pH. The optimal PH for cereal phytase is around 5.0, where phytase will have high activity during fermentation (Towo *et al*., 2006). At this low PH, phytase activity hydrolyses phytic acid to inorganic phosphate and inositol (Greiner *et al.*, 2000). Microbial phytase in microorganisms hydrolyzes phytic acid during fermentation and account for the reduction in phytic acid in the fermented product (Khetarpaul and Chauhan, 1989).

Total phenols

Polyphenolic compounds were measured in terms of Gallic acid equivalent (GAE) and the results are also presented in Table 2. Significantly higher amount of polyphenols ($p<0.05$) were found in SP before fermentation (56.2 µg/ml GAE) and in MP (40.6 µg/mlGAE) after fermentation. Polyphenols decreased after fermentation in all samples. The decrease was more pronounced in SP (22.9%) than in other samples. Reduction was least in MP and SA at 1.1% and 1.6% respectively. Reduction in polyphenols was reported due to the activity of polyphenoloxidase of the fermenting microflora (Dhanker and Chauhan, 1987). Although polyphenols are considered good for human health, they are also known to interfere with the availability of minerals such as iron and bind and precipitate macromolecules including protein and carbohydrates, thereby reducing their digestibility in foods (Ferguson, 2001).

Total tannins

Tannins were measured in terms of Catechin equivalent (CE). The highest tannin levels were found in unfermented SP (0.99 mg/ml CE) followed by SAP, MAP, MA and SA. There were no significant differences ($p<0.05$) between SA and MA before and after fermentation in their tannin levels, however, a slight decrease in tannin was observed in all samples after fermentation. The decrease ranged from 0.05 to 0.16%. Elyas (2002) reported no changes in tannin content of fermented dough for millet after 36 h of fermentation at room temperature. A decrease in tannin can be attributed to tannase activity by lactobacillus during fermentation that breakdown tannin complex with protein (Molin, 2008).Tannins are reported as a large part of polyphenols that strongly inhibit iron absorption by forming insoluble chelates (Roos *et al*., 2013). This explains why total polyphenols were found to be higher than total tannins. The type of sorghum that was used in this study was type 1 sorghum with no pigmented testa and contained low levels of phenols and tannins according to Dykes and Rooney, (2006). This was

Table 3. Effect of fermentation on functional properties

Sample	BD (g/ml)		WSI (g/g)		WAI (g/g)		OAC (g/g)	
	NF	F	NF	F	NF	F	NF	F
SA	0.74 ± 0.03^c	0.75 ± 0.01^c	1.87 ± 0.15^a	$4.77 \pm 1.06^{c,d}$	4.91 ± 0.16^f	$4.15 \pm 0.07^{a,b,c,d}$	0.85 ± 0.05^a	0.85 ± 0.01^a
MA	0.75 ± 0.02^c	0.68 ± 0.04^b	3.04 ± 0.19^b	$5.73 \pm 0.54^{d,e}$	$4.67 \pm 0.08^{d,e,f}$	3.74 ± 0.04^a	0.85 ± 0.02^a	0.91 ± 0.02^a
SP	0.51 ± 0.01^a	0.68 ± 0.00^b	4.40 ± 0.04^c	$4.77 \pm 0.45^{c,d}$	$4.38 \pm 0.31^{b,c,d,e}$	$4.26 \pm 0.01^{a,b,c,d}$	0.79 ± 0.56^a	0.75 ± 0.02^a
MP	0.53 ± 0.01^a	0.65 ± 0.02^b	$4.45 \pm 0.21^{c,d}$	$6.53 \pm 0.14^{e,f}$	$4.53 \pm 0.11^{c,d,e,f}$	$4.02 \pm 0.08^{a,b,c}$	1.15 ± 0.08^a	0.91 ± 0.02^a
SAP	0.55 ± 0.01^a	0.66 ± 0.01^b	$3.49 \pm 0.49^{b,c}$	10.57 ± 0.92^f	$4.38 \pm 0.31^{b,c,d,e}$	$4.23 \pm 0.03^{a,b,c,d}$	1.01 ± 0.01^a	0.81 ± 0.03^a
MAP	0.54 ± 0.02^a	0.66 ± 0.01^b	$4.68 \pm 0.25^{c,d}$	7.00 ± 0.83^e	$4.80 \pm 0.28^{e,f}$	$3.84 \pm 0.34^{a,b}$	1.08 ± 0.01^a	0.88 ± 0.01^a

Values with different superscript letters within the same parameter are significantly different ($p < 0.05$, mean $\pm$ SD, n=3)
NF: Nonfermented; F: Fermented

confirmed by a prior chlorox test of tannins, which showed that sorghum had no tannins.

Bulk density

Table 3 present results on bulk density, Water solubility index, water absorption index, oil absorption capacity and least gelation concentration. Bulk density (BD) of powders is defined as the mass of particles that occupies a unit volume of the container (Barbosa-Canovas and Yan, 2003) and it plays an important role in storage, transportation and marketing. The bulk densities of the fermented and unfermented samples are presented in Table 3. In general, the unfermented samples had significantly lower BD ($p < 0.05$) compared to their fermented counterparts. This is contrary to the findings of El-khalifa *et al.* (2005) where BD decreased after fermentation or germination. Bulk density of food is reported to depend on combined effects of interrelated factors such as intensity of attractive inter-particle forces, particle size and number of contact points. That is, a change in any one of the powder characteristics may result in significant change in the powder bulk density (Al-Kahtani and Abou-Arab, 1993). Results of bulk density of fermented samples however, were consistent with findings from other authors (Khanam *et al.*, 2013). Low bulk density is desirable not only for weaning foods, but also offers an advantage of less volume during packaging, therefore, economical.

Water solubility index

Table 3 shows Water Solubility Index (WSI). Solubility of flour blends ranged from 1.87 g/g to 4.68 g/g in unfermented samples. After fermentation, the WSI of all samples increased significantly ($p < 0.05$) with SAP having the highest form from 3.49 – 10.57 g/g, followed by MAP (4.68 – 7.00 g/g). The WSI values observed in this study were in line with those of Itagi and Singh, (2012) in multigrain composite

mixes. Water solubility index is related to the presence of soluble molecules and is a measure of starch degradation. Lower WSI means there is minor degradation of starch and leads to less numbers of soluble molecules in a food (Hernández-Díaz *et al.*, 2007). Amaranth has generally been reported to have high solubility index (Menegassi *et al.*, 2011). In our preliminary work, higher solubility index was observed in amaranth, followed by pumpkin, sorghum and finger millet. Thus, this explains why SAP had higher solubility than MAP. An increase in WSI observed in all samples was an indication that molecules of the flour particles were significantly degraded by the fermentation process making them more readily available for digestion.

Water absorption index

Results for water absorption index (WAI) are presented in Table 3. Water absorption index plays an important role in the food preparation as it influences other functional and sensory properties (Sreerama *et al.*, 2012). In general, fermentation significantly decreased ($p < 0.05$) the WAI in all the samples. This observation is in agreement with the findings of Singh *et al.* (2012) who also reported a decreasing trend in water absorption capacity during a sequential natural fermentation of selected cereals. A similar trend was observed by El-khalifa *et al.* (2005) on effect of fermentation on the functional properties of sorghum flour. Sreerama *et al.* (2012) further observed that polar amino acid residues of proteins have affinity for water molecules hence differences in WAI may be attributed to different content of these amino acids as well as differences in carbohydrate composition in the samples. According to Singh *et al.* (2012), lower water absorption index is desirable for making thinner gruels.

Table 4. Effect of fermentation on *in-vitro* protein digestibility

Time (h)	SA	MA	SP	MP	SAP	MAP
0	56.58 ± 1.10[a,b]	50.26 ± 3.00[a]	60.96 ± 0.00[a]	88.73 ± 8.27[b]	66.59 ± 0.00[b]	58.11 ± 0.00[a]
12	70.18 ± 4.39[c]	62.35 ± 4.16[b]	78.16 ± 7.00[b]	75.17 ± 2.10[a]	61.52 ± 3.33[b]	67.71 ± 4.84[b]
18	51.40 ± 2.02[a]	54.83 ± 2.32[a,b]	70.85 ± 2.32[b,c]	74.92 ± 7.25[a]	69.13 ± 1.47[b]	82.29 ± 2.90[c]
24	60.18 ± 5.63[b]	58.49 ± 2.67[b]	86.68 ± 2.56[d]	78.43 ± 3.27[a]	74.41 ± 1.65[c]	80.55 ± 2.64[c]
30	70.11 ± 2.29[c]	57.08 ± 4.82[a,b]	78.74 ± 4.26[c]	87.76 ± 3.13[b]	86.78 ± 2.72[e]	80.45 ± 1.00[c]
36	88.21 ± 0.00[d]	79.02 ± 5.95[c]	86.92 ± 5.12[d]	92.32 ± 3.85[b]	78.72 ± 0.00[d]	92.75 ± 2.26[d]

Values with different superscript letters within the same column are significantly different (p<0.05, mean ± SD, n=3)

Oil absorption capacity

Results show that fermentation at 36 h decreased oil absorption capacity (OAC) in selected samples. MA however, showed an increase in OAC and there was no change observed in SA. These results are in agreement with those of Udensi and Okoronkwo, (2006) who reported a decrease in oil binding capacity of Mucuna bean protein isolate after fermentation. Oil absorption capacity is important for retaining flavor and mouth feel of foods (Kinsella, 1976) hence a decrease in oil absorption capacity may affect flavor and mouth feel of fermented foods.

Least gelation concentration

Results showed that least gelation concentration (LGC) of the unfermented flour blends commenced at 16%. At 18%, most samples had gelled. When the blends were fermented for 36 h, the LGC of the blends showed no gelation up to 20%. A study by Obatolu and Cole (2000), in complementary blends of soybean and cowpea with malted or unmated maize did not show any gel up to 28% concentration. The high concentration range was attributed to high globulin fraction in soybean. Pumpkin seed flour has been reported to contain albumin and globulin fractions as major seed proteins constituting about 58.6% of the total protein (Giami *et al.*, 1999). After all, globulin levels increased after fermentation (Giami *et al.*, 1999), explaining why there was no gelation up to 20% flour concentration in fermented samples.

Effect of fermentation on changes in in-vitro protein digestibility (IVPD)

Table 4 shows the effects of fermentation on in-vitro protein digestibility. In-vitro Pepsin-Pancreatin digestibility of the samples ranged from 56.58-76.60% in unfermented samples and from 75.78 - 92.32% in fermented samples. Fluctuation in IVPD was observed with increased fermentation time. However, protein digestibility was highest after 36 h of fermentation except in SAP, which had its highest digestibility (86.78%) at 30 h and later decreased to 78.72% at 36 h. Highest digestibility was observed in MAP (92.75%), followed by MP (92.32%) at 36 h. All changes were significantly different (p<0.05). Proteolytic activity of lactic flora during fermentation results in increased availability of amino acid and peptides thereby producing more soluble protein (Elkhalifa *et al.,* 2005). Mugula *et al.* (2003) observed hydrolysis of protein and tannin during fermentation of sorghum increased IVPD of sorghum flour due to proteolytic activity. A high correlation coefficient between reduction in phytate, phenols, tannins and antitryptic activity and increase in IVPD in fermented finger millet flour was observed by (Usha and Chandra, 1998).

Effect of fermentation on starch digestibility

The starch digestibility in non-fermented samples ranged from 53.28% in MAP to 77.65% in SP and were significantly different (p<0.05). After fermentation, a significant increase was observed in all samples ranging from 3.29 - 16.84% in MP. The increase was least in SP and highest in SAP. An increase in starch digestibility by 43% was reported by Onyango *et al.* (2005) in Uji production. Fermentation is reported to cause proteolysis of the protein matrix surrounding the starch granules thereby releasing starch and making it available for hydrolysis by amylase. Fermentation also breaks down large starch granules into smaller granules which are more susceptible to enzymatic hydrolysis due to higher specific area that is exposed to the enzymes leading to increase in starch digestibility (Singh *et al.*, 2010). Fermentation improved starch digestibility in all samples.

Conclusion

The results have shown that fermentation greatly improved physicochemical, functional properties, protein and starch digestibility and decreased the antinutritional factors of complementary foods from sorghum and finger millet blended with pumpkin seed alone and also with amaranth. Protein digestibility was optimal at 36 h fermentation period. Blends of amaranth with sorghum (SA) and finger millet (MA) alone produced a low level protein content food but with excellent physical properties. A combination of sorghum or finger millet with pumpkin and/or amaranth (SP, MP, SAP, and MAP) was excellent both in protein content and physical properties. It is therefore concluded that appropriate processing of these raw materials could produce a complementary food with improved nutritional value for infant feeding.

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