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RESEARCH ARTICLE

Amino Acid Profile, Mineral, Pasting, Thermal and Protein Solubility Characteristics of Sorghum-Finger millet based Complementary Food as affected by Fermentation

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Abstract

In this study, sorghum-finger millet based complementary food incorporated with amaranth and pumpkin seed flour was formulated and the effect of fermentation on some of its characteristics was investigated. It was observed that fermentation significantly increased pasting temperature and decreased peak viscosity, breakdown and setback of the formulated foods. The foods had a final viscosity range of 831-2202 cP in non-fermented samples and 719-2135 cP in fermented samples. Formulated foods had a recommended consistency range of 1000–3000 cP except for Millet-Amaranth-Pumpkin, fermented Sorghum-Pumpkin and fermented Sorghum-Amaranth-Pumpkin. Protein solubility exhibited lowest solubility at isoelectric pH of 4 for non-fermented samples and between pH 4 and 6 for fermented samples. It was further observed that fermentation increased protein solubility. Threonine and lysine were found to be the most limiting amino acids in almost all samples. Results on mineral content showed potassium and magnesium to be the highest and higher than the reference levels set by FAO/WHO. Furthermore, high zinc levels were observed and calcium was highest in Millet-Amaranth-Pumpkin. Although the amino acid profile was found to be low, the formulated complementary foods meet other relevant nutritional requirements ideal for complementary foods.

Keywords: Sorghum-finger millet, pasting temperature, protein solubility, isoelectric pH, amino acid profile.

Introduction

Complementary foods are foods other than breast milk or infant formula, which are introduced to an infant of over six months old for provision of adequate nutrients. These foods are supposed to be nutrient dense because after the six months, infants have high nutritional requirements relative to body size and consume small amounts of food at a time. A deficient intake of certain mineral content in the diet leads to diseases and abnormal development (Camara *et al*., 2005). The incidence of acute malnutrition in low and middle-income countries is more prominent in the first years of life when children have a high demand for nutrients and there are limitations in the quality and quantity of their diets, including inadequate breastfeeding practices (Black *et al*., 2008). A prolonged breastfeeding without appropriate complementary feeding is another crucial contributory factor for malnutrition among young children (Dahiya and Kapoor, 1995). Complementary foods with a high energy and nutrient dense should be provided just at the right time. Waterlow and Payne (1975) previously reported that the most problematic age is about 9-12 months, when an already considerable nutritional demand coincides with a limited stomach capacity. World Health Organization (2003), indicates that a good quality weaning food should have high nutrient density, low bulk density, low viscosity and appropriate texture with high energy, protein and micronutrient contents and low consistency that allows easy consumption (Kim *et al*., 2003). Other authors have previously reported that the total energy intake for a child weighing 7 kg is calculated as 3500 kJ, 14 g proteins and a liquid consistency of 1000-3000 cP for easy swallowing (Nout and Ngoddy, 1997). Moreover, ingredients must be locally available and acceptable like staple cereals or starchy tubers and their anti-nutritional factors be minimized by adequate processing.

In developing countries where malnutrition remains a major health problem in infants, considerable efforts to improve health and nutritional status of growing children have focused on the production of nutritious, locally sourced complementary foods (Makinde and Ladipo, 2012). In such countries, most traditional weaning foods are made from cereals, roots and tubers, which are usually low in nutritive value (Sanni *et al*., 1999). Gibson and Ferguson (1998) reported that cereals and tubers or starchy roots are used as a basis for complementary food and fed as early as one month of age, thereby displacing breast milk. Early introduction of these complementary foods compromise the bioavailability of trace minerals especially zinc and iron (Bell *et al*., 1987). The presence of phytic acid, dietary fibre and polyphenols in cereals and legumes and tubers inhibit absorbance of some major trace minerals (Gibson and Ferguson, 1998).

It has been further reported that vitamin and mineral deficiencies particularly Iron, Vitamin A and Zinc affect more than two billion people worldwide with young children being highly vulnerable because of rapid growth and inadequate diet (Kim *et al*., 2003). However, a number of commercial formula on the market, which provide balanced weaning foods are too expensive and not within the reach of the poor, hence the suggested strategies to develop weaning and supplementary food from inexpensive locally available cereals and pulses to combat protein energy malnutrition among children of low socio-economic group (Dahiya and Kapoor, 1995). Sorghum and finger millet are drought resistant cereals with low nutritive quality especially for infants. Amaranth and pumpkin seed flours are highly nutritious than cereals but they are underutilized resources and often considered as waste. Fermentation improves nutritional characteristics of food. The aim of this study, therefore, was to evaluate the effect of fermentation on the amino acid profile, mineral content, protein solubility, pasting and thermal characteristics of sorghum-finger millet based complementary food incorporated with amaranth and pumpkin seed flours.

Materials and methods

Raw materials: Finger millet and amaranth were purchased in the local market of Wuxi city, Jiangsu Province, China. Sorghum was purchased from Baokang Company in Harbin city, People's Republic of China. Pumpkin seeds were purchased from Grain Mill Firm in Hohhot City, Inner Mongolia.

Chemical reagents: All chemical reagents used were of analytical grade purchased from Sigma-Aldrich Co. Ltd. and Sinopharm Chemical Reagent Co., Ltd. Shanghai, China.

Sorghum-finger millet complementary food formulations: Six formulations from prior analysis were formulated as follows: (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 37ºC. Different fermentation times were used and ranged from 0 to 12, 18, 24, 30 and 36 h. The slurry was mixed and dried at 70ºC 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 4ºC until analysis.

Mineral analysis: Minerals were determined using dry ashing according to the method of AOAC (2000). Samples (2 g) were ashed at 500ºC in a muffle furnace for 6 h or more until ash was light grey or white. Ash was dissolved in 5 mL of 1 N nitric acid and transferred to 50 mL volumetric flask. Crucible was washed several times with 1 N nitric acid to ensure complete removal of the ash and filtered using Whatman filter paper No. 541. Analysis was done using Atomic Absorption Spectrophotometer (Varian Spectr 220). The minerals analyzed included Zinc, Iron, Potassium, Calcium, Magnesium and Sodium.

Amino acid analysis: Samples (300 mg) were hydrolyzed with 6 M HCl at 110ºC for 24 h under nitrogen atmosphere. Amino acids were analyzed using Reverse phase high performance liquid chromatography (RP-HPLC; Agilent 1100 model, Agilent technologies, USA) with the following conditions: column: (240 x 4.6 mm), 5 µm ODS Hypersil; Column temperature: 40ºC; Mobile phases A comprised of sodium acetate/ triethylamine/tetrahydrofuran (500:0.12:2.5; v/v/v), pH adjusted to 7.20. Mobile phase B comprised of sodium acetate/methanol/acetonitrile (1:2:2; v/v/v). Flow rate: 1.0 mL/min. Tryptophan was determined after alkaline hydrolysis. Amino acid composition was reported as g/100 g of protein.

Pasting characteristics: Pasting characteristics were determined using a Rapid Viscosity Analyzer (RVA4500, Perten Instruments Australia Pvt. Ltd., NSW, Australia) according to AACC method 76-21 (AACC, 2000). Approximately 3.5 g of sample was added to about 25 g of distilled water (adjusted to correct for sample moisture content) and total content made up to 28 g in an aluminum canister. The mixture was then homogenized and introduced to heating and cooling cycle, which involved an initial equilibration at 50°C for 1 min, heating to 95°C for 3 min 42 sec, holding at 95°C for 2 min 30 sec, cooling to 50°C for 3 min 48 sec, and holding at 50°C for 2 min. Each analysis was done in duplicate and viscosity was expressed in cP.

Protein solubility: Protein solubility was done according to the method of Bera and Mukherjee (1989) with some modifications. A sample (1 g) was suspended in 40 mL of distilled water and pH adjusted from 2.0 to 12.0 with either 0.1/1 M HCl or 0.1/1 M NaOH. Total volume was made up to 50 mL. The suspensions were then shaken for 1 h at room temperature (Lab-Line Environ-Shaker; Lab-Line Instrument, Inc., Melrose Park, IL, USA) before centrifuging for 30 min at 4000 × g. The supernatants were assayed for protein using micro-Kjeldahl method. Percentage protein solubility was calculated as:

$$\% = \frac{PS(g)}{PIS(g)} \times 100$$

Where, PS is the amount of protein in supernatant and PIS is the Protein in initial sample.

Table 1. Mineral content of the formulated sorghum-finger millet complementary foods.

Mineral (mg/100 g)	SP	MP	SAP	MAP	FAO/WHO
Zinc	4.33	5.38	3.58	4.35	4.10
Iron	5.15	5.83	6.93	6.68	9.30
Potassium	557.50	587.50	482.50	635.00	400.00
Sodium	6.70	6.98	8.73	7.53	350.00
Magnesium	282.50	282.50	252.50	310.00	54.00
Calcium	257.50	397.50	335.00	495.00	400.00

Table 2. Amino acid profile in sorghum-finger millet complementary foods.

Amino acid (mg/g)	SA	MA	SP	MP	SAP	MAP	FAO/WHO/UNU (mg/g)
Histidine	3.18	2.89	7.09	5.92	5.08	6.13	20
Threonine	4.05	3.82	8.38	7.12	6.48	7.16	31
Valine	6.93	6.41	17.49	15.17	13.01	14.95	43
Methionine + cysteine-s	7.63	7.03	7.04	13.77	4.98	14.05	27
Phenylalanine + tyrosine	8.72	7.18	24.04	19.35	16.64	19.59	52
Isoleucine	5.67	4.93	13.61	11.53	9.88	11.67	32
Leucine	12.62	8.17	26.90	20.20	17.00	22.51	66
Lysine	4.69	4.71	10.24	8.42	7.65	8.99	57
Tryptophan	1.18	1.07	3.52	2.74	3.14	2.51	8.5
Non-essential amino acid							
Aspartic acid	11.00	9.46	27.02	22.32	19.34	23.01	-
Glutamic acid	24.53	20.26	62.66	50.96	43.43	53.50	-
Serine	5.85	5.39	12.20	10.09	9.59	10.56	-
Glycine	8.10	8.49	16.09	14.07	12.88	14.82	-
Arginine	7.20	7.11	36.28	31.25	25.84	28.87	-
Alanine	8.98	5.72	17.62	13.26	11.42	15.19	-
Total	120.32	102.64	290.16	246.18	206.36	253.51	-

Sorghum-Amaranth (SA), Millet-Amaranth (MA), Sorghum-Pumpkin (SP), Millet- pumpkin (MP), Sorghum-Pumpkin-Amaranth (SAP), Millet-Pumpkin-Amaranth(MAP).

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 discussion

Results of this study are based on 36 h fermentation.
Mineral profile: Results for the mineral content of the sorghum-finger millet based complementary foods are presented in Table 1. Minerals were determined in four of the six samples. Selection of the four samples was based on those with high ash values. The results showed that iron and sodium were limiting in all the samples. Calcium levels were highest in MAP followed by MP, SAP and SP. Fermentation is well known to increase minerals and amino acid levels in food samples. It degrades anti-nutritional factors like phytates and lead to availability of calcium, magnesium, phosphorus, zinc and iron (Greffeuille *et al.*, 2011). Finger millet is a rich source of calcium (300 to 350 mg/100 g grain) and a good source of phosphorus and iron (Shahidi and Chandrasekara, 2013), hence higher levels of calcium in MP and MAP could be attributed to high calcium levels in millet. World Health Organization defines iron, zinc and calcium in complementary foods as problem micronutrient because their concentrations normally fall below the calculated requirements for breast-fed infants. From the results, SP, MP and MAP met the required zinc levels for one meal in a day. It is assumed that twice consumption of any of these formula meals by children between 9-11 months would provide adequate levels of these determined minerals.

Amino acid profile: Results for the amino acid profile of the complementary foods are presented in Table 2. The results indicate that SA and MA samples had the lowest total levels of all amino acids. SP showed higher levels of the amino acids. However, when compared with recommended values of FAO/WHO/UNU for complementary foods, all samples fell below recommended values of essential amino acids in complementary foods in children between 9-11 months. Similar observations were found in a study by Mohammed and Zhou (2007), where an extruded diet formulated from rice, soybean, carrot, whole egg and maltodextrin had amino acids below the required values of young children. Fermentation has been reported to increase individual amino acids but may also decrease some during the process. The decrease is attributed to microbes utilizing the amino acids for growth and the increase is attributed to ability of these microbes to synthesize some amino acids (Sanni *et al.*, 1999). Even though crude protein content was higher in Sorghum-Pumpkin (SP), Millet- pumpkin (MP), Sorghum-Pumpkin-Amaranth (SAP) and Millet-Pumpkin-Amaranth (MAP), their protein quality was low, being plant proteins. An alternative to improve the quality of these proteins would be to include animal protein.

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Table 3. Digestible indispensable amino acid score (DIAAS) of sorghum-finger millet complementary foods.

Essential amino acid	FAO/WHO reference pattern (mg/g)	SA	MA	SP	MP	SAP	MAP
Histidine	20	0.14	0.11	0.31	0.27	0.20	0.29
Threonine	31	0.11	0.10	0.24	0.21	0.17	0.21
Valine	43	0.14	0.12	0.35	0.32	0.24	0.32
Methionine + Cysteine	27	0.25	0.22	0.23	0.47	0.15	0.48
Phenylalanine + Tryptophan	52	0.15	0.11	0.40	0.34	0.25	0.35
Isoleucine	32	0.16	0.12	0.37	0.33	0.24	0.34
Leucine	66	0.17	0.10	0.35	0.28	0.20	0.32
Lysine	57	0.21	0.07	0.16	0.14	0.11	0.15
Tryptophan	8.5	0.12	0.10	0.36	0.30	0.29	0.27
DIAAS (% lowest ratio)		Threonine 11.49	Lysine 6.53	Lysine 15.63	Lysine 13.59	Lysine 10.61	Lysine 14.67

Table 4. Pasting characteristics of sorghum-finger millet complementary food.

Sample	Pasting temperature	Peak viscosity	Trough	Breakdown	Final viscosity	Setback
SA	81.90 ± 0.57^{a}	1512 ± 4.25^{g}	1001 ± 6.22^{e}	511 ± 4.75^{e}	2202 ± 3.03^{d}	1200 ± 6.25^{f}
MA	83.15 ± 0.071 ^{a}	1554.50 ± 6.36^{e,f}	823 ± 2.12^{e}	228 ± 0.00^{c}	1347 ± 0.00^{b}	525 ± 0.00^{c}
SP	81.53 ± 0.04^{a}	1489.50 ± 9.19^{e}	887 ± 8.49^{f}	602.50 ± 0.71^{f}	1825 ± 3.41^{c}	937.50 ± 2.92^{e}
MP	80.90 ± 0.28^{a}	1408.50 ± 5.861^{e}	724.50 ± 9.02^{c,d}	684 ± 6.77^{g}	1169.50 ± 8.91^{b}	445 ± 9.89^{c}
SAP	82.55 ± 0.28^{a}	964 ± 4.24 $^{c, d}$	642 ± 2.83^{a,b,c,d}	322 ± 1.41^{d}	1161 ± 5.55^{b}	519 ± 2.73^{c}
MAP	82.75 ± 1.69^{b}	924 ± 1.41^{b,c,d}	561 ± 2.83^{a}	363 ± 2.82^{d}	831 ± 5.66^{a}	270 ± 2.83^{a,b}
FSA	92.90 ± 0.28^{d,e}	1429.50 ± 6.36^{e}	1274 ± 2.82^{h}	155.50 ± 3.54^{a,b}	1778 ± 6.00^{c}	504 ± 5.65^{c}
FMA	88.73 ± 0.04^{b,c}	1080 ± 4.28^{d}	1368 ± 4.24^{h}	186.50 ± 6.07^{b,c}	2135 ± 2.62^{d}	767 ± 6.87^{d}
FSP	94.93 ± 0.25^{e}	694 ± 4.24 ^{a}	599.50 ± 3.54 $^{a, b}$	94.50 ± 0.71^{a}	719.50 ± 2.12^{a}	120 ± 1.41^{a}
FMP	91.30 ± 0.28^{c,d}	829 ± 14.23$^{a, b, c}$	736 ± 9.89 $^{d, e}$	93 ± 5.66^{a}	1150 ± 9.56^{b}	414 ± 9.68^{b,c}
FSAP	92.68 ± 0.04^{d,e}	749 ± 4.24 $^{a, b}$	618 ± 7.07 $^{a, b, c}$	131 ± 1.31^{a,b}	889.50 ± 2.01^{a}	271.50 ± 4.95^{a,b}
FMAP	87.35 ± 3.75^{b}	791 ± 8.32 $^{a, b, c}$	685 ± 2.82 $^{b, c, d}$	104 ± 0.71^{a}	1166 ± 1.31^{b}	479 ± 4.50^{c}

Mean values with different superscripts within the same column indicate significant difference ($p<0.05$). (F: Fermented).

Digestible indispensable amino acid score (DIAAS): Digestible indispensable amino acid score is used to estimate the available protein intake. DIAAS assesses the quality of a single ingredient or individual foods to decide whether the food needs complementation or adjust its safe levels of intake (FAO, 2011). DIAAS is calculated as a percentage value of digestible indispensable amino acid content in 1 g protein of food in comparison with 1 g of reference protein or amino acid scoring pattern. The results as presented in Table 3 showed that amino acids were limiting and there is need for complementation. The most limiting amino acids were threonine and lysine. MA showed the most limiting levels of lysine. Cereals are generally limited in lysine, threonine and tryptophan content (Usha and Chandra, 1998).

Effect of fermentation on pasting characteristics: Results of pasting characteristics are indicated in Table 4. The pasting characteristics measured included pasting time, peak viscosity, trough, breakdown, final viscosity, setback and peak time. It was found that all were significantly ($p<0.05$) affected by fermentation. Pasting temperatures for non-fermented samples were 80.90, 81.53, 81.90, 82.55, 82.75 and 83.15ºC in MP, SP, SA, SAP, MAP and MA respectively. However, in fermented samples, an increase in pasting temperature was observed. Pasting temperatures increased to 91.30, 94.93, 92.90, 92.68, 87.35 and 88.73ºC respectively.

A study by Chakrabarthy *et al*. (1972) reported that cooking time positively correlated with protein content. In a related study, it was reported that rice proteins played a significant role in pasting by possibly encasing the starch granules and regulating their swelling and resistance to shear at high temperature (Likitwattanasade and Hongsprabhas, 2010). More protein is associated with forming a thicker barrier around the starch granules, thereby slowing water uptake by each granule. In this study, the high pasting temperatures would be associated with high protein levels, which increased after fermentation. An addition of soy protein isolate in wheat grits increased pasting temperature from 64.4 to 65.6ºC (Manohar *et al*., 2011) and an incorporation of soy protein concentrate was found to increase pasting temperature of corn starch (Li *et al*., 2007) and this was attributed to the reduction of available water for starch swelling due to the presence of soy concentrate. A decrease in peak viscosity, breakdown and setback was also observed in fermented samples. Peak viscosity in non-fermented samples varied from 924 to1554 cp whereas after fermentation it ranged from 749-1429 cp. Peak viscosity indicates the water-binding capacity of starch or a mixture in a product correlates it with the final product quality and indicates the ability of starch to swell freely before their physical breakdown (Ingbian and Adegoke, 2007). Viscosity indicates associations among leached starch molecules, primarily amylose (Funami *et al*., 2008).

Table 5. Thermal properties of non-fermented and fermented samples.

Sample	To (ºC)	Td (ºC)	ΔH (J/g)
SA	67.62 ± 0.05 ^{a}	70.59 ± 0.10^{a}	1.26 ± 0.03$^{b, c}$
MA	71.30 ± 0.02^{b}	74.52 ± 0.02^{b}	2.14 ± 0.07^{d}
SP	67.98 ± 0.06^{a}	69.78 ± 0.02^{a}	0.31 ± 0.07^{a}
MP	71.32 ± 0.06^{b}	74.34 ± 0.00^{b}	1.66 ± 0.13$^{c, d}$
SAP	68.45 ± 0.05^{a}	70.42 ± 0.00^{a}	0.26 ± 0.05^{a}
MAP	71.90 ± 0.02^{b}	74.84 ± 0.00^{b}	1.32 ± 0.01$^{b, c}$
FSA	78.01 ± 0.03$^{d, e}$	80.00 ± 0.02^{d}	0.84 ± 0.05^{a}
FMA	78.42 ± 0.01^{f}	81.25 ± 0.00^{e}	2.85 ± 0.06^{e}
FSP	73.94 ± 0.00^{c}	74.81 ± 0.11^{b}	0.33 ± 0.03^{a}
FMP	75.51 ± 2.73^{d}	76.77 ± 3.36^{c}	3.09 ± 1.15^{e}
FSAP	76.95 ± 0.07^{e}	78.93 ± 0.04^{d}	0.33 ± 0.03^{a}
FMAP	76.92 ± 0.03^{e}	79.11 ± 0.00^{d}	3.79 ± 0.15^{f}

Mean values with different superscripts within the same column indicate significant difference ($p<0.05$).

High values of breakdown are associated with high peak viscosities, which relate to the degree of swelling of the starch granules during heating. More starch granules have a high swelling capacity, which results into a higher peak viscosity (Ragaee and Abdel-Aal, 2006). The non-fermented samples exhibited higher peak viscosities (1554, 1412, 1489, 1408, 964 and 924 cP) in comparison with their corresponding fermented samples (1,429, 530, 694, 829, 749 and 791 cP). Breakdown was significantly reduced after fermentation ($p<0.05$). Breakdown is the measure of the degree of disintegration of granules or paste stability (Dengate, 1984). At breakdown, the swollen granules disrupt further and amylose molecules leach into solution and setback reveals the gelling ability or retro gradation tendency of amylose (Zaidul *et al*., 2007). Reassociation between starch molecules especially amylose results in to formation of a gel structure, which increases the final viscosity. Low setback values indicate low rate of starch retro-gradation and syneresis (Ragaee and Abdel-Aal, 2006). From these results, high values in both non-fermented and fermented samples were observed in setback than those in breakdown, confirming the findings of the earlier studies. Fermentation was observed to increase peak time and reduce peak viscosity, trough, breakdown and setback, although they were variations within the samples due to their different compositions. Fermentation, therefore, influenced enzymatic hydrolysis of the starch resulting into higher soluble starch fractions, which lead to increase in viscosity during setback.

Thermal properties: Results of thermal properties of both non-fermented and fermented samples are presented in Table 5. Fermentation significantly influenced onset and decomposition temperatures at $p<0.05$. The fermented samples had higher values of onset and decomposition temperature, with the mean onset temperature of 76.61ºC that fell between 73.94 and 78.66ºC and a mean enthalpy value of 1.7546 J/g (0.3 J/g minimum and maximum of 4.40 J/g); whereas in non-fermented samples, mean onset temperature was 69.76ºC (between 67.59 and 71.92ºC) and mean enthalpy values of 1.1583 J/g (minimum of 0.21 J/g and maximum of 2.21 J/g).

Mean decomposition temperature of fermented samples was 78.44°C between (74.73-78.44°C), whereas; mean decomposition temperature of non-fermented sample was 74.84ºC (between 69.77-72.41ºC). Similar results regarding the influence of fermentation on thermal properties were reported by Amadou *et al.* (2014) in foxtail millet. Growth of Bacillus species or acid produced by lactic acid bacteria is associated with hydrolyzing the amorphous regions of starch granules, leaving an increased proportion of crystalline structure in the starch granules, which absorb more heat thereby increasing enthalpy (Lu *et al*., 2005). Temperature for gelatinization of starch is important in defining the proportion of energy required during cooking (Zavareze and Dias, 2011). In this study, the results indicated that fermented samples would require more energy than the non-fermented samples during cooking. The effect of fermentation on thermal properties was similar to those of pasting characteristics, indicating that fermentation caused hydrolysis of starch granules.

Protein solubility profile: Protein solubility profile was determined in both non-fermented and fermented samples as a function of pH from 2 to 12 and the results are presented in Fig. 1 and 2. Protein solubility is the most important functional property because it affects other properties such as emulsification ability and foam and gel formations (Sreerama *et al*., 2012). It was observed that minimum solubility occurred at pH 4 (7.27-10.03%). There was a decrease in solubility from pH 2 (11.46-19.45%). An increase in solubility was observed again at pH 6 and rapidly at from pH 7 with highest solubility percentage at pH 12 (50.30-92.73%). A number of authors have previously reported the same trend of protein solubility, which markedly decreases near the isoelectric point, usually between pH 4 and pH 6 (Wani *et al*., 2013). The same trend was reported by Yalcin and Celik (2007) for hull-less barley flour in distilled water and various salt concentrations. Solubility in distilled water was higher at pH 10 and 11 and minimum at around pH 4. The solubility values at pH 4 and 6 were close to each other. In general, the results show a trend of solubility typical of plant flours as reported by other researchers.

Fig. 1. Protein solubility in non-fermented sorghum-finger millet samples.

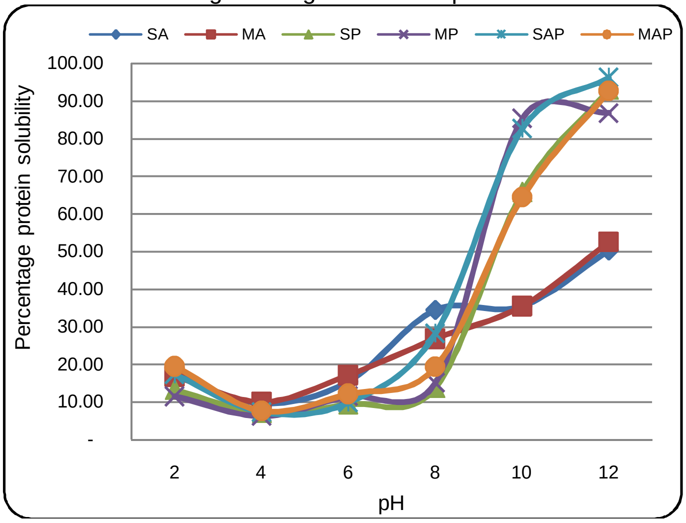
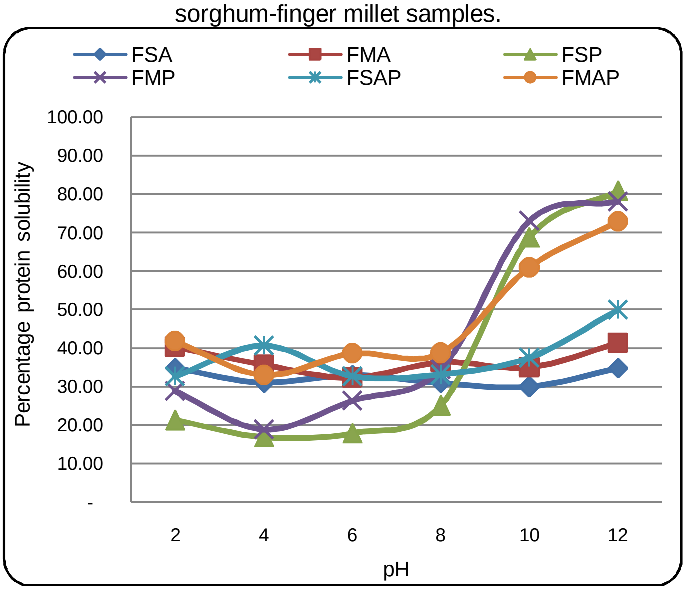


Fig. 2. Protein solubility in fermented sorghum-finger millet samples.



Minimum solubility of protein in fermented samples was observed at pH 4 and 6. Four of the samples: SA, SP, MP MAP had minimum solubility at pH 4 while MA and SAP had minimum solubility at pH 6. Kinsella (1979) reported that protein solubility characteristics are influenced by factors such as origin, processing conditions, pH, ionic strength and the presence of other ingredients. At pH 2, solubility percentages were in the range of 21.28-41.83% which was higher than those in non-fermented samples. However, at highest pH (12), solubility in the fermented samples was lower (34.79-80.93%) than in non-fermented samples (50.30-96.31%). Elkhalifa (2005) reported that fermentation shifted solubility of sorghum protein profiles by 2 pH units and the proteins were more soluble at the isoelectric pH. A similar trend of lower solubility at highest pH was also reported, where, beyond pH 6, solubility was not as high as in the unfermented samples. This was attributed to exposure of some hydrophobic groups in the fermented samples, which cause reduction in solubility (Elkhalifa *et al*., 2005). This confirm the fact that protein solubility is affected by changes of pH and ionic strength of the medium (Yalcin and Celik, 2007).

Conclusion

The findings in this study revealed that fermentation significantly affected protein solubility, thermal and pasting characteristics of sorghum-finger millet based complementary food. The pasting temperature and protein solubility increased as a result of fermentation. Thermal properties were similar to pasting properties, which indicate starch hydrolysis during fermentation. Lysine and Threonine were the least amino acids. Potassium and magnesium were highest minerals while iron and sodium were the least. Fermentation, therefore, plays a significant role in enhancing the nutritional content of the formulated cereal foods. From the results, sorghum-pumpkin (SP), millet-pumpkin (MP), sorghum-amaranth-pumpkin (SAP) and millet-amaranth-pumpkin (MAP) would be preferred for further processing due to their higher nutrient content compared to others. An addition of animal protein would greatly enhance the quality of the formulated foods because of its high quality amino acids.

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