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Preparation and Nutritional Composition of a Weaning Food Formulated from Germinated Sorghum (*Sorghum bicolor*) and Steamed Cooked Cowpea (*Vigna unguiculata* Walp.)

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ABSTRACT

Weaning food was produced from sorghum and cowpea based on a malted technology with a view to determining the amylase activity, nutritional composition/properties and its ability to meet the Recommended Dietary Allowance. Malted sorghum flour was produced (steeping, germination, drying, toasting, grinding and sieving) and steamed cooked cowpea flour was produced. Both were blended in ratio 2:1 to get malted weaning food (GSC), unmalted sorghum and steamed cooked cowpea in same ratio was also produced (USC). Optimum amylase activity of sorghum was determined, proximate composition, amino acid, vitamin and mineral contents were analysed. Seventy two hours gave optimum amylase activity with reduced dietary bulk in GSC due to decrease in viscosity. Germination had no significant effect on protein contents, 12.07 g (GSC) and 12.57 g (USC), samples met 1/3 RDA protein requirement for 1-3 years old child. Germination significantly increased essential amino acid except sulphur amino acids and tryptophan. GSC had amino acid value that approximate FAO reference pattern except for threonine that was also the limiting amino acid. Vitamin A ie beta-carotene (267.0 IU/100 g), thiamin (0.24 mg/100 g) and ascorbic acid (5.0 mg/100 g) were increased from 197.0 IU/100 g (Vit. A), 0.16 mg/100 g (thiamin) and 2.73 mg/100 g (ascorbic acid) in GSC. Phosphorus and iron contents also increased from 91.65 mg/100 g and 4.01 mg/100 g to 100.0 mg/100 g and 6.40 mg/100 g, respectively. Weaning food based on germinated sorghum had improved/superior nutritional values compared to the ungerminated. Germination significantly increased essential amino except for Histidine, sulphur amino acid and tryptophan. It also increased phosphorus, iron, vitamin A (β-carotene) thiamine, riboflavin, niacin and ascorbic acid.

Key words: Amylase activity, supplementary food, chemical score, vitamin, minerals, RDA

INTRODUCTION

The growth of the infant in the first or second years is very rapid and breast feeding alone will not meet the child nutritional requirement. After about four months of age the child needs supplementary feeding (Achinewhu, 1987; Ijarotimi and Famurewa, 2006). As a result many brands of preparatory weaning foods has been developed and marketed in most developing countries including Nigeria (Adeyemi *et al.*, 1989; Okafor *et al.*, 2008). In these countries, the child is usually weaned into a porridge prepared from cereal flour such as maize which is characterized by bulky, high viscosity and low energy density per unit volume of the food, thus necessitating frequent feedings to meet the daily energy requirements of the child. Apart from energy, the food is usually inadequate in other nutrients, leading to widespread protein energy malnutrition and its complications, during the weaning period (WHO, 2000; Ijarotimi and Famurewa, 2006; Ugwu, 2009).

Nigeria is no exception, as most children are weaned onto Ogi, a gruel made from fermented corn (Ugwu, 2009). The situation is even more critical as prices of commercial weaning food products are too expensive for many mothers (Njoki and Fallar, 2001; Ijarotimiand Famurewa, 2006; Inyang and Offiong, 2010).

Thus the need to have maximum utilization of commonly available cheap cereals to formulate weaning foods that would be low in viscosity, high in caloric density and with adequate necessary nutrients (Onofiok and Nnayelugo, 1998; Ozumba *et al*., 2002).

In Nigeria, many attempts to produce weaning food which are quite rich in protein and other nutrient by combination of cereals with various sources of rich protein from animal, legumes and oil seeds has been reported (Ozumba *et al*., 2002; Okafor *et al*., 2005, 2008; Okafor and Ozumba, 2006).

However, the problem of high viscosity persists and more work is necessary with various combinations. Our primary concern in this study is to formulate a diet that would be low in viscosity and high in energy/nutrient dense using existing technology. Among the various existing traditional technologies that could be used for the preparation of low bulk weaning foods, germination of cereals and legumes has been shown to be advantageous (Desikachar, 1981).

Germination has been extensively studied (Ozumba *et al*., 2002) and it was found useful/ advantageous in increasing energy and nutrient density of infant diets. During the process of germination, enzymes released from the scutellium digest some of the starch into dextrin-maltose which of course does not swell when cooked into gruel (Ebrahim, 1983). The flour prepared from sprouted grain, therefore, can be used in greater amount to give the same viscosity as flour from ungerminated grain, thereby obtaining more nutrients and energy (Ozumba *et al*., 2002; Elkhalife and Bernhardt, 2010). Germination/ malting of cereals and legume have been shown to be generally advantageous as it also improves the nutritional qualities of cereals and legumes (Lorenz, 1980; Pedersen *et al*., 1989; Correia *et al*., 2008).

Hence, this study was conducted to prepare a weaning food formulation using locally available and cheap raw material, *viz*., sorghum and cowpea, based on a malting technology. The nutritional properties of the formulation was evaluated and compared with commercially available weaning foods as well as its ability to meet Recommended Dietary Allowance (RDA) for children, it reviewed the contribution of the food formula to the RDA of infants for energy and protein, protein quality using amino acid composition (chemical score) and contribution of the weaning formula to RDA of vitamin A (beta-carotene), Thiamin, Riboflavin, Niacin, Ascorbic acid and minerals especially, Fe, Ca and P.

MATERIALS AND METHODS

Germination of seeds: The criterion used in determining optimum germination condition for sorghum was maximum amylase activity.

Preparation of raw materials: The seeds of sorghum (*Sorghum bicolor)* and cowpea (*Vigna unguiculata* walp.) were obtained from the local market in Lagos. This research project was carried out at the Department of Biochemistry, Lagos State University and Lagos, Nigeria from September, 2007 to January, 2008.

Germination procedure: Whole seeds of sorghum weighing 500 g were washed and soaked in volume of water three times the weight of the seed for 4 h. The soaked grains were then put into a wide container with filter paper to allow for germination at room temperature (27°C) for 5 days. Sample grains were taken at 12 h intervals and assayed for amylase activity.

Amylase determination: Germinating sorghum grain samples taken at 12 hourly intervals were dried at 35°C to a moisture content of about 10% and ground in a blender to fine flour passing through a sieve.

A 0.5 g of the flour was extracted with 10 mL of 0.2 M NaCl for 30 min and the Amylase activity was then determined in this extract by the method of Chrispeels and Varner (1967).

Preparation of the sorghum-cowpea mixture

Germinated sorghum flour: Well picked and clean sorghum grains weighing 500 g were stepped in triple the volume of water for 4 h. Then allowed to germinate under the trial conditions for 72 h (pre-determined maximum activity for amylase). The washed germinated seeds were dried in the oven at 35°C for a total of about 10 to 12 h. Then the grains were cleaned of sprouts and hulls by hand rubbing and winnowing, after which they were toasted in a shallow pan at 80°C until a uniform light brown product. The dried grains were ground to fine flour by passing through a fine sieve.

Steamed cowpea flour: Well cleaned cowpea grains weighing 500 g were soaked for 1 h and dehulled by hand rubbing. The dehulled seeds were steamed for 45 min in a saucepan until tender. The water was boiled to dry and the seeds were dried in an oven at 56°C for 10 h overnight. The dried seeds were ground using a blender and sieved into fine flour.

Formulation of weaning food: The germinated sorghum flour and steamed cooked cowpea flour thus prepared were blended at a ratio of 2:1, respectively. The resultant product labeled (GSC) was packed in plastic containers and stored in freezer until analyses.

Preparation of ungerminated formula (Control): This was prepared from ungerminated sorghum flour and steamed cooked cowpea and blended in ratio 2:1 as described above and labeled (USC).

Analysis: Proximate composition of the weaning food formulation was determined using the official methods of AOAC (1990). Food energy was determined by direct calorimetry with the Ballistic Bomb calorimeter using Benzoic acid as standard.

Amino acid analysis was carried out by hydrolysis of the sample with 6N HCl (AOAC, 1980). Amino acids were separated in the hydrolysate with the Technicon amino acid analyzer. Tryptophan was determined by the method of Sato *et al.* (1984). About 100 mg sample was hydrolysed with 10 mL of 4.2 N Na0H at 110°C for 20 h and tryptophan was analysed in the technicon amino acid analyzer.

Mineral content was determined by a wet-ashing method (AOAC, 1990). Estimation of the minerals was by atomic absorption spectro-photometry using a Perkin-Elmer 290 instrument.

Vitamin A in the form of β-Carotene (AOAC, 1980), involved extraction of powdered samples with acetone and hexane. Thiamine was also by the standard method (AOAC, 1980), while Riboflavin and Niacin were analysed by colorimetric method of Snell and Snell (1953). Ascorbic acid was analysed by the method of Roe and Hall (1939) by extraction with 0.5% oxalic solution.

Statistical analysis: The data generated were subjected to analysis of variance using the SPSS statistical package version (10.00) 2000 edition. Significant of treatment means was tested at 5% level of probability using Duncan Multiple Range Test (DMRT).

RESULTS

Proximate composition: Sample GSC and the market brand weaning food had significantly ($p>0.05$) lower moisture content than sample USC. Equally, sample GSC had significantly higher carbohydrate value 77.40% than sample USC with 74.44%. However, the samples USC and GSC did not differ significantly in their protein contents 12.52% (USC) and 12.70% (GSC), fat 2.86 and 2.20%, Ash 1.60 and 1.70% and crude fibre 1.52 and 1.66% for sample USC and GSC, respectively.

Amino acid analysis: The amino acid analysis (Table 2) showed that sample GSC had higher values of most of the essential amino acid; isoleucine (24), leucine (74), lysine (40), sulphur containing amino acids (38), total aromatic amino acid (142), threonine (17), valine (36) and tryptophan (13) than sample USC; the result also indicated that threonine was the first limiting amino acid with chemical score, 37.

Vitamin analysis: Table 3 shows the vitamin content of the formulation. Sample GSC had significantly ($p<0.05$) higher vitamin A (β-carotene) 267.40 IU/100 g, Naicin 2.90 mg/100 g than USC. The two samples did not differ significantly ($p>0.05$) in their thiamine and riboflavin contents.

Mineral composition: Table 4 revealed sample USC and GSC did not differ significantly ($p>0.05$) in their calcium content (32.63-33.39 mg/100 g), sodium 32.64-32.70 mg/100 g, potassium 506.10-508.60 mg/100 g, magnesium 96.98-97.50 mg/100 g and zinc 3.68-4.10 mg/100 g. However, sample GSC had significantly ($p<0.05$) higher content of phosphorus 100.00 mg/100 g and Iron 6.40 mg/100 g than sample USC with 91.65 mg/100 g and 4.01 mg/100 g, respectively.

DISCUSSION

Figure 1 shows that 72 h to be germination period that yield optimum amylase activity. After 72 h, there was no appreciable change in amylase activity. This result agrees with result of Elkhalifa and Bernhardt (2010) that in germinated sorghum, amylase activity reached a maximum on the 3rd day and decreased steadily thereafter but in variance with that of Okoli *et al.* (2010) who reported maximum activity of 2 and 5 days for different sorghum cultivars. Amylase activity in grains have been found to break down starch to maltose and with germination, its activity increases, thereby lowering the viscosity of the food (Correia *et al.*, 2008).

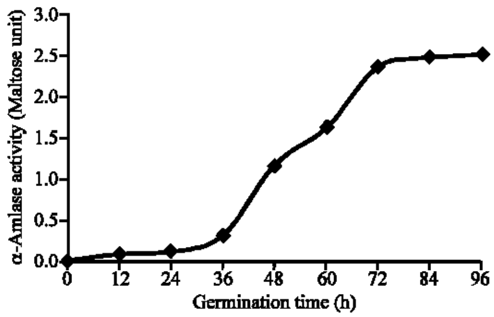

Fig. 1: Activity curve of α-amylase during germination

Table 1: Proximate composition of weaning food formulation from germinated and ungerminated sorghum and cooked cowpea compared to recommended daily allowance (RDA)

Nutrient per 100 g	Ungerminated weaning food	Germinated weaning food	Commercial weaning food	PAG (1971)	SON (1988)	⅓ RDA 7-12 1-3 months years
Moisture (g)	7.06b	4.40a	4.00a	5-10 (max)	5-10 (max)	- -
Protein (g)	12.52a	12.70a	16.00b	20 (min)	14-17 (min)	8.30 8.6
Fat (g)	2.86a	2.20a	9.00b	10 (max)	10 (max)	9.10 ND
Ash (g)	1.60a	1.70a	2.30a	10-May	10-May	- -
Crude fibre (g)	1.52a	1.66a	5.00b	5 (max)	5 (max)	-5.75
Carbohydrate (g)	74.44b	77.40a	63.7c	-	-	28.79 39.40
Energy (kcal/100 g)	373.58	387.7	400	400	350-400	- -

Mean values with same subscripts in a row are not significantly different (p>0.05)

The proximate composition of GSU and USC weaning foods compared to standard and ⅓ RDA are shown on Table 1. The moisture content of USC was 7.06% and GSC was 4.40%, respectively. There was no significant difference (p>0.05) in the protein content of USC (12.52 g) and GSC (12.70 g). It provides ⅓ RDA of protein required for infants 7-12 months and 1-3 years old. These findings were similar to those reported by Malleshi *et al.* (1989) for germinated and roller dried weaning foods. Sample USC had fat content 2.86 and GCS 2.20 g, which didn't meet ⅓ RDA for fat for both young and older infants 1-3 years. The Ash content of the USC and GSC were 1.60 and 1.70 g, respectively, while the carbohydrate contents were 74.4 g for USC and 77.4 g for GSC, respectively and provide ⅓ RDA for both 7-12 months and 1-3 years groups. The energy content of USC (373.58 kcal/100 g) and GSC (387.58 kcal/100 g), it compared favorably well with the standard set by SON (1988) and PAG (1971).

Table 2 shows the amino acid pattern of the USC and GSC formulations compared to FAO reference pattern. GSC gave efficient complementation of amino acid, which met the FAO reference pattern except for threonine. A significant increase in the isoleucine, leucine, lysine, total aromatic amino acid, valine and threonine was noted in GSC, compared to the USC. Also slight increase in tryptophan, sulphur containing amino acid were noticed, this increase in amino acids could be presumably due to increase in amino acid during germination process, that are produced in excess of the requirements and tend to accumulate in the free amino acid pool (Chen *et al.*, 1975). Wang and Field (1978) and Marero *et al.* (1988) also reported significant increase in lysine, methionine and tryptophan of germinated sorghum, maize and rice compared to the ungerminated grains. The chemical score indicated that threonine is the first limiting amino acid.

Table 2: Amino acid composition of germinated and ungerminated weaning food compared to FAO reference pattern

Essential amino acids (16 g N2)	Ungerminated weaning food	Germinated weaning food	FAO/WHO reference pattern	Chemical score
Histidine	21	20	18	143
Isoleucine	20	24	25	69
Leucine	70	74	44	95
Lysine	37	40	34	77
Methionine + Cystine (i.e., total sulphuramino acids)	36	38	22	131
Total aromatic amino acids	137	142	38	225
Threonine	13	17	25	37*
Valine	32	36	31	72
Tryptophan	11	13	6.5	153

+ Source: PAG (1971), *Threonine is the first limiting amino acid

Table 3: Vitamin content of formulated weaning foods compared to the recommend daily allowance

Vitamins per 100 g	Ungerminated formula	Germinated formula	Commercial brand diet	⅓ RDA 7-12 1-3 months years
Vitamin A (IU)	191.20a	267.40b	1500.00c	83.00 83.00
Thiamin (mg)	0.21a	0.24a	0.80b	0.16 0.23
Riboflavin (mg)	0.19a	0.28ab	0.30b	0.16 0.23
Niacin (mg)	1.89a	2.90b	4.0c	2.00 3.00
Ascorbi acid (mg)	2.73a	5.0b	5.0b	10.00 11.60

Mean in same row with same subscript are not significantly different (p>0.05)

The result (Table 3) shows there were significant difference (p<0.05) between the USC and GSC in Vitamin A (in form of β-carotene), Niacin, Riboflavin and Vitamin C contents. GSC had significantly higher quantities of these Vitamins than USC and it met ⅓ RDA for Vitamin A (β-carotene), Thiamin, Riboflavin and Niacin. However, 50% of ⅓ RDA ascorbic acid were provided in 7-12 months and 43% in 1-3 years. Increase in Vitamins in germinated cereals/legume blends has been observed by Marero *et al.* (1988), he reported the increase in concentration of niacin, thiamin (3-55%), riboflavin (22-221%) and other vitamins in certain grains legume after germination. Equally drastic increase in vitamin C in seeds of 29-86 folds were reported by Hamilton and Vandertoep (1979), Hsu *et al.* (1980) and Nnam (2000) for different cereals/legumes. The increased ascorbate level during germination is attributed to the activity of ascorbinase that synthesize ascorbate from hydrolysed glucose (Nnam, 2000).

The result (Table 4) indicate that germination significantly increased phosphorus and iron contents but had no significant effect on the calcium, potassium, magnesium, manganese and sodium contents. This result is similar to those of Luhila and Chipulu (1987), Marero *et al.* (1988) and Sulieman *et al.* (2007), that reported increase in iron and phosphorus and decrease in Calcium on germination of various cereals/legumes but invariance with that of Oloya (2004) with increase in Fe, P, Cal, mn and mg in cajanus cajan. Increase in phosphorus content from 91.65 mg/100 g-100 mg/100 g and 4.01 mg/100 g to 6.40 mg/100 g in iron was recorded in GSC. The increase in phosphorus could be presumably due to the increased activities of the enzyme phytase; during germination it hydrolyses the bond between protein-enzyme-mineral to free phosphorus (Nnam, 2000; Sulieman *et al.*, 2007; Samia *et al.*, 2007). Except for calcium and

Table 4: Mineral contents of formulated weaning food compared to recommended daily allowance (RDA)

Minerals (mg/100 g)	Ungerminated	Germinated	Commercial brand diet	⅓ RDA 7-12 1-3 months years
Macro-elements				
Calcium	32.63a	33.39a	390.00b	100.00 152.09
Phosphorus	91.65a	100.00b	260.00c	100.00 139.02
Sodium	32.64a	32.70a	220.00b	120.00 333.30
Potassium	506.10a	508.609	570.00b	212.00 900.10
Micro-elements				
Magnesium	96.98a	97.50a	-	22.72 24.24
Manganese	1.37a	1.43a	-	0.21 0.36
Zinc	3.69a	4.10a	7.00b	2.5 2.50
Iron	4.01a	6.40b	10.0c	3.3 2.12

Mean in same row with same subscript are not significantly different ($p > 0.05$)

sodium, all the other minerals provided ⅓ RDA requirements for these nutrients in both the 7-12 months and 1-3 years groups.

CONCLUSIONS

The weaning food formulation based on germinated sorghum and cooked cowpea had improved nutritional qualities. They were good sources of protein, energy, B- vitamins, iron and phosphorus. Germination significantly increased the essential amino acids of the formula except for Histidine, sulphur containing amino acid and tryptophan.

It also increased the contents of the micro-nutrient such as phosphorus, iron, β-carotene (vitamin A), thiamine, riboflavin, niacin and ascorbic acid in the formulation.

However, supplementation or fortification of this weaning food by addition of food rich in calcium and vitamin A e.g., fish, crayfish, shrimps and carrot should be explore to improve and increase these micro-nutrients and also enhance the protein quality.

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