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Full Length Research Paper

Influence of germination and fermentation on chemical composition, protein quality and physical properties of wheat flour (*Triticum aestivum*)

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The present study investigated effects of germination and fermentation on protein quality of the wheat flour. The wheat seeds were obtained locally and were divided into three portions and processed as raw wheat flour (RWF), germinated wheat flour (GWF) and fermented wheat flour (FWF) respectively. The samples were analysed for chemical and protein qualities using standard methods. Protein content (g/100g) varied between 10.77±0.66 (RWF) and 13.70±0.30 (FWF). Mineral composition of RWF, GWF and FWF had potassium as the highest while zinc (RWF and FWF) and nickel (GWF) were the least. For amino acid, glutamic acid was the most abundant; while cysteine (RWF and FWF) and methionine (GWF) were the least. Total essential amino acid plus histidine and arginine range between 26.4% (FWF) and 37.9% (GWF). For protein efficiency ratio (PER), RWF had the highest value (1.99), while GWF had the least (0.86), while for the essential amino acid index (EAAIs), RWF was higher than that of GWF and FWF. Similarly, the biological value of RWF (31.8%) was higher than those of GWF (29.4%) and FWF (29.1%) sample respectively. The anti-nutrient compositions of the wheat flour samples were low, while phytate:zinc, phytate:calcium, (Ca)(phytate):zinc and phytate:iron molar ratios were lower than the critical values. Bulk density (BD) ranged between 0.80±0.04 and 0.86±0.02, swelling capacity (SC) 0.0±0.03 and 1.23±0.21, water absorption capacity (WAC) 315 and 415% and least gellation 3.33±1.10 and 14.6±1.16%. The finding concluded that the employed processing methods, particularly fermentation, influenced the chemical composition of the wheat flour in terms of protein content and reduction of anti-nutrient composition.

Key words: Germinated wheat flour, fermented wheat flour, nutritional quality.

INTRODUCTION

Cereals such as rice, wheat and maize are members of the grass family *Graminae* and are particularly important because of their role as staple food for human nutrition, and their incorporation into various products is of great economic importance in many countries of the world (Pena et al., 2006). Cereals, particularly wheat, are important components of the daily diet, providing carbohydrates, proteins, dietary fibers and vitamins to less privilege in many countries.

The main wheat producing countries are United State of America, China, Russia, India, Pakistan, Canada, Argentina, Australia and some countries of European Union (FAO, 2009). Wheat is highly consumed in various forms like breads, biscuits, cookies, cakes, pasta, noodles (Hussain et al., 2004; Pena et al., 2006); and it is the major sources of dietary energy and protein for the survival of the people (Aslam et al., 1982; Hussain et al., 2004; Moore et al., 2006). A number of studies have indicated protective role of whole grain foods against several nutritional related diseases such as type 2 diabetes (Murtaugh et al., 2003; Pereira et al., 2002), cardiovascular diseases (Jacobs and Gallagher, 2004) and certain cancers (Larsson et al., 2005).

Scientific studies have reported that wheat contain appreciable amount of nutrient and anti-nutritional factors; and that majority of these anti-nutrients are concentrated in the aleurone layer and only 10% in the embryo (Cheryan, 1980; Muahamad et al., 2010). Evidence has shown that there are many factors, such as genetics, environmental fluctuations, type of soils, year and fertilizer application that can affect the anti-nutrient composition of cereal grains (Muahamad et al., 2010). These anti-nutritional factors reduce the nutritional quality of cereals and their removals through traditional processing techniques enhance utilization of the cereal as human food.

Processing techniques such as germination and fermentation have been found to improve the quality of cereals due to chemical changes that enhance organoleptic response (Nout, 1992), contents of free sugars, protein and vitamins, as well as bioavailability of minerals (Zamora and Fields, 1979; Chavan and Kadam, 1989a, b; Egli, 2001; Helland et al., 2002; Ochanda, et al., 2010), and results in the breakdown of some of the anti-nutritional endogenous compounds (Ahmed et al., 2006). In many instances, usage of only one method may not impart the desired removal of anti-nutritional compounds and a combination of two or more methods is required (Hassan et al., 2007).

Germination has profound effect on nutritional quality of the cereal (Chavan and Kadam, 1989b). Germination is a natural biological process of plants by which the seeds come out of latency stage (Sangronis and Machado, 2007). During germination, certain changes occur in terms of quantity and type of nutrients within the seed. These changes can vary depending on the type of vegetable, the variety of seed and the condition of germination (Bau et al., 1997; Dhaliwal and Aggarwal, 1999). An increase in bioavailability of minerals and weight has been observed during seed germination. Germinated seeds are good source of ascorbic acid, riboflavin, choline, thiamine, tocopheroles and pantothenic acid (Sangronis and Machado, 2007).

Fermentation is a metabolic process serving for some microorganisms to get energy through digestion of simple fermentable sugars, mostly glucose and fructose. It serves as a means of providing a major source of nourishment for large rural populations and contributes significantly to food security by increasing the range of raw material which can be used in the production of edible products. Fermentation enhances the nutrient of foods through biosynthesis and bioavailability of vitamins (Zamora and Fields, 1979; Gabriel and Akharaiyi, 2007), essential amino acids and improving the protein quality and fibre digestibility (Gabriel and Akharaiyi, 2007).

In view of improving food security and nutritional wellbeing of the people that relies on wheat as their staple food; thus, this present study aimed at improving the nutritional quality of wheat through germination and fermentation processing techniques. A number of studies have reported on nutritional benefits of germination and fermentation technique in terms of increasing the mineral and protein content and reducing the anti-nutrient composition of food products (Sripriya et al., 1997; Abdel Rehman et al., 2005; Hassan et al., 2007; Kouakou et al., 2008). The specific objectives of this study were to investigate protein quality, antinutritional factors and physical properties of wheat flour **(***T. aestivum***).** The outcomes of this study would be important for food and nutrition policy maker in terms of preventing protein energy malnutrition in Nigeria and other developing countries.

MATERIALS AND METHOD

Materials procurement

The wheat grains used in this study were procured in January, 2012 from Erekesan market, Akure, Ondo State, Nigeria. The chemicals used for the analyses were of analytical grade and they were purchased from Sigma Chemical Company, St. Louis, MO, USA. The chemical analyses of the wheat flour samples were carried out in the Food Science and technology laboratory, Federal University of Technology, Akure, Nigeria. The samples were divided into three batches of 1 kg each. These constituted samples for germination and fermentation while one batch that was untreated constituted the control.

Processing of wheat flour

Raw wheat flour

One kilogram of wheat grains was weighed sorted and oven dried (Galenkamp, size 3, hotbox, London, UK) at 60°C for 20 h. The dried wheat grain was milled in attrition mill, sieved through 150 wire mesh and packed in polyethylene nylon, put in covered container and stored at room temperature prior to analysis (Figure 1)

Germinated wheat flour

One kilogram of wheat grains were weighed, sorted, steeped in distilled water for 3 h at room temperature and then completely drained of steep water using sieves. The drained wheat grain were then spread on a moistened jute sack and allowed to germinate at room temperature (4 days).The germinated grains were manually washed with distilled water, drained and oven dried at 60°C for 7 to 8 h. The dried grains were milled using attrition mill, sieved and packaged in polyethylene bags, put in an airtight container and stored under room temperature prior to analysis (Figure 1).

Fermented wheat flour

One kilogram of wheat grains were weighed, sorted, and soaked in distilled water for 2 days at room temperature. The soaked grains were washed, drained and wet milled using a Philips laboratory blender (HR2811 model), sieved with muslin cloth and fermented for 3 days at room temperature. The excess water was decanted and a moist paste that was drained to reduce the moisture content obtained. The drained paste was oven dried in hot air at 60°C for 6 to 7 h, after which it was re- milled, sieved and packaged in polyethylene bags put in a airtight container and stored at room temperature prior to analysis (Figure 1).

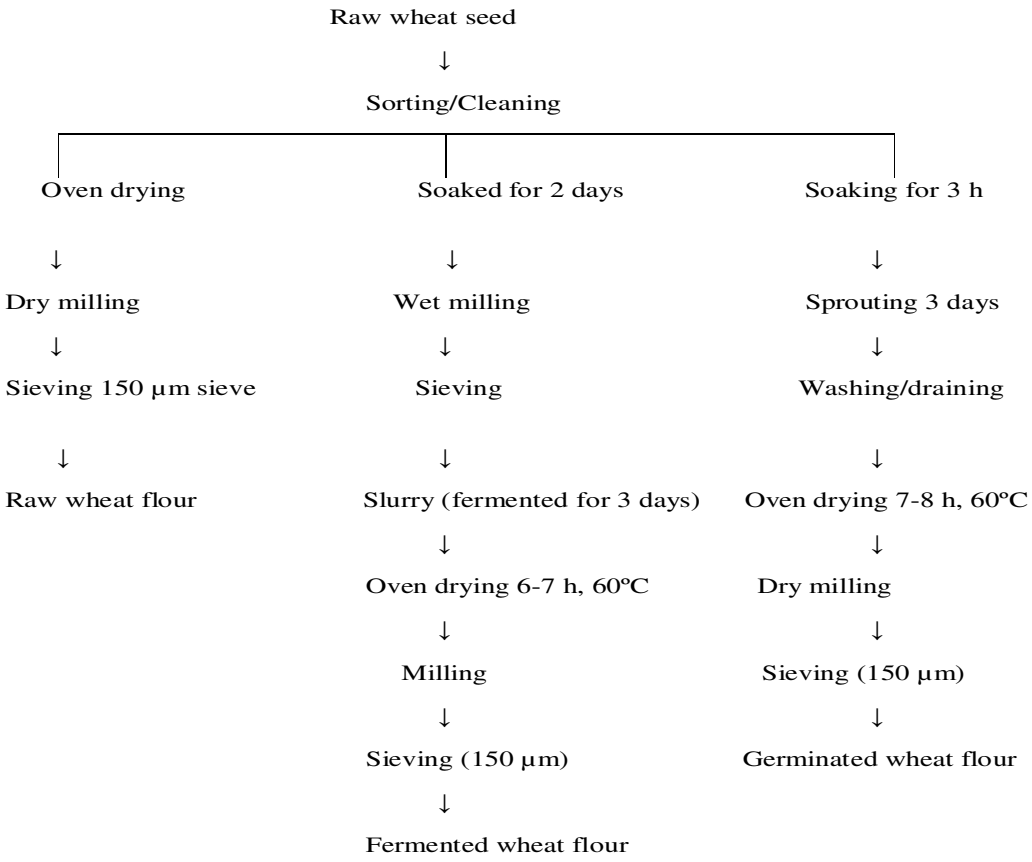

Figure 1. Flowchart of processing wheat seeds using different processing methods.

Nutrient composition analysis

Proximate analyses

Proximate compositions of the food sample were determined in triplicate using the standard procedures of Association of Official Analytical Chemists (AOAC) [2005]. Moisture content, crude protein (Kjeldahl method), crude fat (solvent extraction), crude fibre and ash were determined using standard methods (AOAC, 2005). The carbohydrate content was determined by difference, that is, addition of moisture, fat crude protein, ash and crude fibre, which was subtracted from 100% .This gave the amount of nitrogen free extract otherwise known as carbohydrate.

%Carbohydrate = 100 - (%Moisture + %Fat + %Ash + %Crude fibre + %Crude protein)

Energy value (kcal.)

The sample calorific value was estimated [in kcal/g] by multiplying the percentages of crude protein, crude fat and carbohydrate with the recommended factors (2.44, 8.37 and 3.57, respectively) as proposed by Martin and Coolidge [1978].

Mineral analyses

The standard method described by Association of Official Analytical Chemists was used for mineral content analysis of the samples (AOAC, 2005). The samples were ashed at 550°C. The ash was boiled with 10 ml of 20% hydrochloric acid in a beaker and then filtered into a 100 ml standard flask. This was made up to the mark with deionized water. The minerals were determined from the resulting solution. Sodium [Na] and Potassium [K] were determined using the standard flame emission photometer. NaCl and KCl were used as the standards (AOAC, 2005). Phosphorus was determined calorimetrically using the spectronic 20 [Gallenkamp, UK] Kirk and Sawyer [1991] with KH_2PO_4 as the standard. Calcium [Ca], Magnesium [Mg] and Iron [Fe] were determined using Atomic Absorption Spectrophotometer [AAS Model SP9]. All values were expressed in mg/100 g.

Amino acid analysis

Sample preparation for amino acid analysis*:* About 2.5.0 g of each sample were weighed into the extraction thimble and the fat extracted with chloroform/methanol (2:1v/v) mixture using a Soxhlet apparatus (AOAC, 2005). The extraction lasted for 5 to 6 h.

Hydrolysis of samples: Thirty milligram of defatted sample was weighed into glass ampoule. A portion of 7 ml of 6 M HCl added and oxygen expelled by passing nitrogen gas into the sample. The glass ampoule sealed with a Bunsen flame and put into an oven at 105 ± 5°C for 22 h. The ampoule was allowed to cool; the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. Each residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in a plastic specimen bottle, and kept in the deep freezer.

Amino acid analysis: Amino acid analysis was by ion exchange chromatography (IEC) (FAO/WHO 1991) using the Technicon Sequential Multisample (TSM) Amino Acid Analyser (Technicon Instruments Corporation, New York). The period of analysis was 76 min for each sample. The gas flow rate was 0.50 ml/min at 60°C with reproducibility consistent within ±3%. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of two replicates. Norleucine was the internal standard used.

Determination nutritional quality of the samples: Nutritional qualities of the flour samples were determined on the basis of the amino acid profiles. The Essential Amino Acid Index [EAAI] was calculated using the method of Labuda et al. (1982) according to the following equation:

$$EAAI = \sqrt[9]{\frac{[\text{Lys } x \text{ Threo x Val x Meth x Isoleu x leu x Phynylal x Histi x Trypt}]a}{[\text{Lys } x \text{ Threo x Val x Meth x Isoleu x leu x Phynylal x Histi x Trypt}]b}}$$

Where: [lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine and methionine]$_a$ in test sample and [lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cystine]$_b$ content of the same amino acids in standard protein [%] [egg or casein] respectively. Nutritional index of the food samples were calculated using the following formula:

$$\text{Nutritional index [\%]} = \frac{\text{EAAI x \%protein}}{100}$$

Biological value was calculated according to Mune-Mune et al. (2011) using the following equation:

BV = 1.09 × Essential amino acid index [EAAI] - 11.7

The Protein Efficiency Ratio [PER] was estimated according to the regression equations as given below (Mune-Mune et al., 2011):

PER = -0.468 + 0.454(LEU) - 0.105(TYR)

Amino acid scores (%) was calculated using the following formula:

$$\text{Amino acid scores (\%)} = \frac{\text{Value of essential amino acid in diet (g/100 g protein)}}{\text{FAO Ref. value for essential amino acids}} \times 100$$

Determination of anti-nutritional composition of the samples

Phytic acid determination

Phytic acid was extracted from each 3 g flour sample with 3% trichloro-acetic acid by shaking at room temperature followed by high speed centrifugation as described by Wheeler and Ferrel (1971). This method depends on an iron to phosphorus ratio of 4: 6. Five grams of the test sample was extracted with 3% tri-chloro acetic acid. The phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding sodium hydroxide. The precipitate was dissolved in hot 3.2 N HNO and the colour read immediately at 480 nm[3]. The standard solution was prepared from Fe[NO_3]$_3$ and the iron content was extrapolated from a Fe(NO_3)$_3$ standard curve. The phytate concentration was calculated from the iron results assuming a 4: 6 iron:phosphorus molecular ratio. The phytic acid was estimated by multiplying the amount of phytate-phosphorus by the factor 3.55 based on the empirical formula $C_6P_6O_{24}H_{18}$.

Tannin content determination

Tannin contents were determined by the modified vanillin-HCl methods (Burns, 1971; Price et al., 1978). A 2 g sample was extracted with 50 ml 99.9% methanol for 20 min at room temperature with constant agitation. After centrifugation for 10 min at 653 rpm, 5 ml of vanillin-HCl [2% vanilli and 1% HCl] reagent was added to 1 ml aliquots and the colour developed after 20 min at room temperature was read at 500 nm. Correction for interference light natural pigments in the sample was achieved by subjecting the extract to the conditions of the reaction, but without vanillin reagent. A standard curve was prepared using catechin [Sigma Chemical, St. Louis, MO] after correcting for blank and tannin concentration was expressed in g/100 g.

Oxalate content determination

Oxalate was determined by AOAC (2005) method. One gram of the sample was weighed into 100 ml conical flask. A portion of 75 ml of 3 M H_2SO_4 was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 h and then filtered using Whatman No.1 filter paper. The sample filtrate [extract] (25 ml) was collected and titrated against hot [80 to 90°C] 0.1 N $KMnO_4$ solution to the point when a faint pink colour appeared that persisted for at least 30 s. The concentration of oxalate in each sample was obtained from the calculation: 1 ml 0.1 permanganate = 0.006303 g oxalate.

Trypsin inhibition activity determination

The trypsin inhibition activity was assayed in terms of the extent to which an extract of the defatted flour inhibited the action of bovine trypsin [EC 3.4.21.4] on the substrate benzoyl-DL-arginine-p-nitrianilide [BAPNA] hydrochloric (Kakade et al., 1969). The samples [1 g each] were extracted continuously at ambient temperature (30±2°C) for 3 h with 50 ml, 10 mM NaOH using a mechanical shaker [GallenKamp orbital shaker Surrey, UK]. The pH of the resulting slurry was adjusted to 9.4 to 9.6 with 1 M NaOH. After extraction, the suspension was shaken and diluted with distilled water such that 1 cm^3 of the extract produced trypsin inhibition of 40 to 60% at 37°C. The respective dilutions were noted. Consequently, TIA was calculated in terms of mg pure trypsin [Sigma type lll, lot 20H0868]:

$$\text{TIA} = \frac{2.632 \text{ DA mg pure trypsin inhibited g - 1 sample}}{\text{S}}$$

Where D is the dilution factor, A is the change in absorbance at 410 mm due to trypsin inhibition per cm^3 diluted sample extract and S is the weight of the sample.

Functional properties

Water/oil absorption capacity

Water and oil absorption capacities of the flour samples were determined by Beuchat (1977) methods. One gram of the flour was mixed with 10 ml of water or oil in a centrifuge tube and allowed to stand at room temperature (30 ± 2°C) for 1 h. It was then centrifuged at 200 × g for 30 min. The volume of water or oil on the sediment water measured. Water and oil absorption capacities were

Table 1. Mean (± SEM) of proximate composition of raw, germinated and fermented wheat flour.

Nutrient/sample	Raw wheat flour	Germinated wheat flour	Fermented wheat flour
Moisture (g/100 g)	13.20 ± 0.46[a]	13.23 ± 1.51[a]	12.67 ± 0.29[a]
Protein (g/100 g)	10.77 ± 0.66[b]	13.50 ± 0.49[a]	13.70 ± 0.30[a]
Fat (g/100 g)	1.93 ± 0.36[a]	1.53 ± 0.33[a]	1.13 ± 0.03[a]
Ash (g/100 g)	0.97 ± 0.18[a]	0.97 ± 0.36[a]	0.77 ± 0.16[a]
Fiber (g/100 g)	1.70 ± 0.34[a]	1.93 ± 0.18[a]	1.13 ± 0.31[a]
Carbohydrate (g/100 g)	84.63 ± 0.43[a]	82.13 ± 0.49[b]	83.27 ± 0.66[ab]
Energy (Kcal.)	398.83 ± 2.41[a]	396.17 ± 1.05[a]	398.13 ± 1.53[a]

*Mean values with the same superscript in a row are not significantly different (P>0.05).

calculated as ml of water or oil absorbed per gram of flour.

Bulk density

A 50 g flour sample was put into a 100 ml measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density (g cm^{-3}) was calculated as weight of flour [g] divided by flour volume (cm^3) (Okaka and Potter, 1979).

Least gelation property

Least gelation property was determined using the method described by Coffman and Garcia [1977]. Sample suspensions of 2 to 16% were prepared in distilled water. Each of aliquot dispersion (10 ml) was transferred into a test tube and heated in a boiling water bath for 1 h, cooled rapidly in a cold water bath, and allowed to cool further at 4°C for 2 h. The least gelation concentration was determined when the sample from the inverted test tube did not slip or fall.

Swelling capacity

This was determined with the method described by Leach et al. (1959) with modification for small samples. One gram of the flour sample was mixed with 10 ml distilled water in a centrifuge tube and heated at 80°C for 30 min. This was continually shaken during the heating period. After heating, the suspension was centrifuged at 1000×g for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as: swelling power = weight of the paste / weight of dry flour.

Determination of protein solubility

The protein solubility of wheat flour was studied using the Were et al. (1997) method. Approximately 0.5 g each of the samples was suspended in 10 ml different salts concentrations. The solubility at natural pH was first determined, that is no acid or alkali was added and so solubility in this case was based on the normal pH of the sample in solution. The suspension was centrifuged at room temperature for 30 min at 3500rpm. The suspension obtained was filtered and the protein of filtrate was determined by biuret method with standard Bovine Serum Albumin (BSA). The Biuret method is a convenient assay for large numbers of samples of relatively soluble protein unlike the Kjeldahl method which is not a rapid and convenient assay though useful for the determination of the amount of protein in crude mixtures.

For the quantitative determination of standard protein in Biuret method, 1 g of BSA was dissolved in 100 ml distilled water in a volumetric flask. Five tubes were set up containing fractions of the BSA solution in this order: - 0.0, 0.5, 1.0, 1.5 and 2.0 ml, and they were made up to 2 ml by adding in the order 2.0, 1.5, 1.0, 0.5 and 0.00 ml of distilled water and the tubes were left to stand for 30 min. The solution from the tube containing 2.0 distilled water and 8.0 ml Biuret solution was used as the blank to standardize the UV spectrophotometer at 450nm (spectronic 20 Bausch and Lomb). The absorbance of each of the other tubes was equally taken. A standard curve was drawn for absorbance against protein concentration curve.

The determination of protein of the filtered supernatant in each sample was carried out in this way, 1.0 ml of the filtrate was pipette into a test tube and 8 ml of Biuret solution was added. The tube was let to stand for 30 min after which the absorbance was taken. The readings obtained were used to plot a graph of protein concentration for the flour samples.

Statistical analysis

The data were analysed using SPSS version 15.0. The mean and standard error of means (SEM) of the triplicate analyses of the samples were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means of proximate composition, minerals, antinutritional factors, amino acid profile, fatty acids and functional properties; while the means were separated using the new Duncan multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Proximate composition of wheat flour

The proximate composition of wheat flour samples is presented in Table 1. The moisture content of germinated wheat flour was the highest (13.23 ± 1.51 g/100g), while that of fermented wheat flour was the lowest (12.67 ± 0.29 g/100g). These values were within the range reported by other investigators (Reihaneh and Jamuna, 2007; Rasha et al., 2011; Amagloh et al., 2012). However, investigations have shown that low moisture content of food samples is a desirable phenomenon, since the microbial activity is reduced (Oyenuga, 1968). Low moisture content in food samples increased the storage periods of the food products (Alozie et al., 2009); while high moisture content in foods encourage microbial

Table 2. Mineral composition of raw, germinated and fermented wheat flour (mg/100g).

Nutrient/sample	Raw wheat flour	Germinated wheat flour	Fermented wheat flour
Calcium	101[c]	151[a]	120[b]
Potassium	133.0[c]	290[a]	186[b]
Manganese	40.55[a]	39.95[a]	41.50[a]
iron	0.30[c]	0.55[b]	1.50[a]
Zinc	0.10[b]	0.02[c]	0.50[a]
Sodium	9.1[c]	19.7[a]	10.9[b]
Magnesium	8.50[c]	8.64[a]	8.57[b]
Phosphorous	9.0[c]	14.7[a]	9.8[b]
Copper	0.20[b]	0.00[c]	1.00[a]
Nickel	0.30[b]	0.01[c]	0.50[a]
Na/K	0.67[b]	0.69[a]	0.59[c]
P/Ca	0.86[a]	0.97[a]	0.82[b]

Mean with similar alphabets belong to the same homogenous subset and are not significant.

growth; hence, food spoilage (Temple et al., 1996).

The protein content of processed wheat samples varied between 10.77 ± 0.66 g/100g for raw wheat flour (RWF) and 13.70 ± 0.30 g/100g of fermented wheat flour (FWF). However, the protein content of both germinated and fermented wheat flour samples were significantly similar and higher than that of raw wheat flour (RWF) sample ($p<0.05$). This observation agreed with other scientific findings that processing techniques such as germination and fermentation improved the nutritional quality of the food products, particularly in terms of protein content (Enujiugha et al., 2003; Fasasi, 2009). In this present study, the protein content of fermented wheat flour was higher when compared with the remaining wheat samples; this finding could be attributed to the fact that during fermentation step the micro-organisms in food utilized the carbohydrate content in the food sample to synthesis amino acid needed for their growth and development (Obizoba, 1988).

Carbohydrate and energy values of the wheat flour samples range between 82.13 ± 0.49 to 84.63 ± 0.43 g/100g and 396.17 ± 1.05 to 398.83 ± 2.41 Kcal, respectively. The carbohydrate content and energy values of germinated sample were lower than those of raw and fermented wheat flour samples; this observation could be due to the utilisation of fat and carbohydrate for biochemical activities of the germinating seeds (Wang et al., 1997).

Mineral composition of raw, germinated and fermented wheat flour

The mineral composition of raw, germinated and fermented wheat flour is shown in Table 2. The mineral composition of raw wheat flour showed that potassium had the highest value while zinc had the lowest value. For germinated wheat flour, potassium was the highest while nickel was the lowest. Also, for that of fermented wheat flour, potassium was the highest while zinc had the lowest value. In comparison, the mineral composition of germinated wheat flour sample was higher in calcium, potassium, sodium, magnesium and phosphorous than fermented and raw wheat flour sample respectively. It was observed in this study that germination and fermentation processing techniques improved the mineral composition of the flour samples except in manganese, zinc copper and nickel than that of raw wheat flour sample ($p<0.05$). This observation could be attributed to bio-synthesis and activities of micro-organism during germination and fermentation processes (Zamora and Fields, 1979; Bau et al., 1997; Dhaliwal and Aggarwal, 1999; Gabriel and Akharaiyi, 2007). Nutritionally, the ratio of Ca/P of the flour samples range between 0.82 for fermented wheat flour and 0.97 for germinated wheat flour. This finding indicates that the wheat flour samples would serve as good sources calcium and phosphorous, which are considered essential for bone and teeth formation and development in children. However, It is evident that food products containing a Ca/P ratio of >1.0 is rated good, while <0.5 is rated poor (Nieman et al., 1992). The ratio of Na/K of the food samples range between 0.59 for fermented wheat flour and 0.69 for germinated wheat flour. This indicates that consumption of wheat flour is suitable for hypertensive patients. Potassium has a beneficial effect on sodium balance. A high intake of potassium has been reported to protect against increasing blood pressure and other cardiovascular risks (Langford, 1983; Cappuccio and McGregor, 1991). Hence, the sodium to potassium (Na/K) ratio in the body is of great concern for the prevention of high blood pressure. A Na/K ratio less than one is recommended in the diets of people who are prone to high blood pressure (Langford, 1983; Cappuccio and

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Copper	0.20[b]	0.00[c]	1.00[a]
Nickel	0.30[b]	0.01[c]	0.50[a]
Na/K	0.67[b]	0.69[a]	0.59[c]
P/Ca	0.86[a]	0.97[a]	0.82[b]

Mean with similar alphabets belong to the same homogenous subset and are not significant.

McGregor, 1991).

Amino acids profile and nutritional quality of wheat flour samples

The amino acids profile and nutritional quality of wheat flour samples are presented in Tables 3 to 5. The result showed that glutamic acid was found to be the most abundant in raw (25.88 g/100g), germinated (20.57 g/100g) and fermented (29.23 g/100g) wheat flour; while cysteine was the least for raw (1.27 g/100g) and fermented (0.94 g/100g), and methionine (1.31 g/100g) for germinated wheat flour. The range values of total non-essential amino acid, conditionally amino acid and essential amino acid of the wheat samples were between 38.93 to 40.52 mg/100g, 14.77 to 21.91 mg/100g and 21.13 to 28.45 mg/100g, respectively. In comparison, the total amino acid composition of germinated wheat sample was higher than fermented wheat flour sample, but the value was lower than that of raw wheat sample; and this is due to the breaking down of other nutrients like carbohydrate to synthesis amino acids that the germinating seeds needed for its biochemical activities and growth. Scientific studies have documented that the protein content of sprouted or germinated plant increased due to degradation of nutrient like carbohydrate and fat in the synthesising of protein (Enujiugha et al., 2003; Fasasi, 2009).

The result of nutritional quality of the raw germinated and fermented wheat flour samples food samples is presented in Table 4. The percentage of total essential amino acid including histidine and arginine showed that germinated wheat four had the highest value (37.9%) followed by raw (35.0%) and fermented wheat flour has the least value (26.4%). The values of protein efficiency ratio (PER) of the wheat flour samples were 1.99, 0.86 and 1.61 for the raw, germinated and fermented wheat flour sample, respectively. Essential amino acid indices (EAAIs) of the raw sample had the highest value, while fermented flour sample had the lowest value. Similarly, the biological value of raw wheat flour sample (31.8%) was higher when compared with germinated (29.4%) and fermented wheat flour (29.1%) sample respectively. This observation shows that consumption of wheat flour alone without complement with other protein-based foods like legumes may not adequately meet the nutritional needs of it consumers. Scientifically, it is well known that a protein-based food material is of good nutritional quality when its biological values (BV) is high (70 to 100%) and also when the essential amino acid index (EAAI) is above 90% and to be useful as food when the values is around 80% and to be inadequate for food material when its EAAI is below 70% (Oser, 1959).

In this present study, it is observed that the PER, BV and EAAI values of germinated and fermented flour samples were low when compared with the raw wheat flour sample and also with the values reported by Oser (1959). These observations could be attributed to the complex metabolic process during germination and fermentation processing methods. For instance, it is well documented that during these processing techniques the lipids, carbohydrates and storage proteins within the seed are broken down in order to obtain the energy and amino acids necessary for the micro-organisms' and plant's development (Ferreira et al., 1995; Jachmanian et al., 1995; Podesta and Plaxton, 1994; Ziegler, 1995).

The calculated amino acid scores (Table 5) of the essential amino acids indicated that fermented wheat flour sample contained the lowest amounts of essential amino acids, while that of germinated wheat flour samples compared well with the raw flour sample. Lysine was found to be the limiting essential amino acid in the raw, germinated and fermented wheat flour samples. This

Table 3. Amino acid composition (mg/100 g protein) of raw, germinated and fermented popcorn flour.

Amino acids	Raw wheat flour	Germinated wheat flour	Fermented wheat flour
Non essential amino acids			
Alanine	3.48^{b}	3.72^{a}	2.25^{c}
Aspartic acid	5.81^{b}	9.98^{a}	4.75^{c}
Serine	5.35^{a}	4.66^{b}	3.18^{c}
Glutamic acid	25.88^{b}	20.57^{c}	29.23^{a}
Total	40.52	38.93	39.41
Conditionally essential amino acids			
Proline	8.93^{a}	4.98^{c}	6.59^{b}
Glycine	3.81^{b}	4.14^{a}	2.15^{c}
Arginine	4.14^{b}	7.92^{a}	2.99^{c}
Cysteine	1.27^{b}	1.32^{a}	0.94^{c}
Tyrosine	3.71^{a}	3.55^{b}	2.10^{c}
Total	21.86	21.91	14.77
Essential amino acids			
Lysine	3.23^{b}	3.49^{a}	2.29^{c}
Threonine	2.78^{a}	2.52^{b}	2.28^{c}
Valine	3.65^{b}	4.08^{a}	1.78^{c}
Methionine	2.20^{a}	1.31^{c}	1.59^{b}
Isoleucine	3.48^{a}	3.49^{a}	2.30^{b}
Leucine	6.29^{a}	6.10^{b}	5.06^{c}
Phenylalanine	4.24^{a}	4.69^{a}	3.56^{b}
Histidine	2.58^{a}	2.30^{b}	2.27^{b}
*Tryptophan	ND	ND	ND
Total	28.45	27.98	21.13

Mean values with the same superscript in a row are not significantly different (P>0.05).

Table 4. Calculated nutritional quality of raw, germinated and fermented wheat flour.

Parameter	Raw wheat flour	Germinated wheat flour	Fermented wheat flour
TEAA+His+Arg/TAA%	35	37.9	26.4
TEAA/TAA%	31.3	31.3	28.0
TNEAA/TAA%	68.7	68.7	71.9
TSAA(Meth+Cys)	3.45	2.62	2.52
ArEAA (Phe+Tyr)	7.93	8.02	5.64
TEAA/TNEAA	0.46	0.46	0.39
PER (g/100g)	1.99	0.86	1.61
EAAI (%)	39.9	37.7	37.4
BV (%)	31.8	29.4	29.1
Nutritional index	4.29	5.09	5.12

observation agreed with the report of Davidson et al. (1980), who reported that cereals are deficient in essential amino acids like lysine and tryptophan.

Anti nutritional factor in wheat flour

The anti-nutrient composition of raw, germinated and fermented wheat flour samples is presented in Table 6. The phytic composition of the wheat flour ranged from 0.412 ± 0.01 to 0.597 ± 0.03 mg/100g, tannin content from 0.011 ± 0.00 to 0.037 ± 0.01 mg/100g, oxalate from 0.255 ± 0.01 to 0.855 ± 0.01 mg/100g; and trypsin inhibitor was between 0.707 ± 0.09 to 0.980 ± 0.02 mg/100g. The tannin, oxalate and trypsin composition of germinated wheat flour sample were higher and significant different from that of raw and fermented wheat flour samples ($p<0.05$), respectively. However, the values

Table 5. Amino acid scores of germinated and fermented wheat flour with reference to FAO/WHO standard.

Essential amino acid for infant	FAO/WHO REF.	Amino acid scores		
		Raw wheat flour	Germinated wheat flour	Fermented wheat flour
Lysine	5.8	55.52*	60.17*	39.31*
Threonine	3.4	81.47**	73.53**	67.06
Valine	3.5	102.86	116.29	50.57**
Methionine	2.2	99.55	59.09	72.27
Isoleucine	2.8	124.29	124.29	81.79
Leucine	6.6	95.30	92.27	76.52
Phenylalanine	2.8	151.07	160.00	126.79
Histidine	1.9	135.26	120.53	118.95
Tryptophan	1.1	ND	ND	ND
Arginine	2	204.00	395.50	149.00

Source: FAO/WHO 1991. ND (Not Detected); *First limiting amino acid; **second limiting amino acid.

Table 6. Anti nutritional factor of raw, germinated and fermented wheat flours (mg/g).

Sample	Raw wheat flour	Germinated wheat flour	Fermented wheat flour
Phytic	0.597^{a} ±0.03	0.412^{b} ±0.01	0.474^{b} ±0.03
Tannin	0.028^{b} ±0.00	0.037^{a} ±0.01	0.011^{c} ±0.00
Oxalate	0.585^{b} ±0.01	0.855^{a} ±0.01	0.255^{c} ±0.01
Trypsin	0.742^{b} ±0.00	0.980^{a} ±0.02	0.707^{c} ±0.09

*Mean with similar alphabet belong to the same homogenous subset and are not significantly different from each other at 5% statistical level.

Table 7. Relationship between phytate and bioavailability of selected minerals (zinc, iron and calcium) (mol/kg).

Parameter	Phytate: Zinc	Phytate: Calcium	(Ca)(Phytate): Zinc	Phytate: Iron
Raw wheat	2.109	0.128	0.532	6.055
Germinated wheat	10.045	0.061	3.792	2.360
Fermented wheat	0.648	0.166	0.194	1.861
*Critical values	>15.0	>0.24	>200	>1.0

*Sources: phytate: calcium > 0.24 (Morris and Ellis, 1985), phytate : iron > 1 (Hallberg et al., 1989), phytate : zinc >15 (Turnlund et al.,1984; Sandberg et al., 1987; Morris and Ellis, 1989), phytate : calcium/zinc > 200 (Davies et al., 1985; Bindra et al., 1986; Gibson, 2006).

were comparable to other reports (Mbithi-Mwikya et al., 2001; Ibrahim et al., 2002; Anju and Khetarpaul, 2008; Syed et al., 2011). Scientific studies have established that processing methods such as cooking, dehulling, soaking, fermetation and germination, improve the nutritional quality of food products by reducing or eliminating the anti-nutrient composition of the food products (Oboh et al., 2000; Mbithi-Mwikya et al., 2001; Ibrahim et al., 2002; Anju and Khetarpaul, 2008; Syed et al., 2011).

Calculation of phytate and zinc, iron and calcium molar ratios to predict their bioavailability

The molar ratio of phytate and zinc, iron and calcium to predict their bioavailability is shown in Table 7. The phytate:zinc molar ratio range between 0.648 to 10.045 mol/kg, phytate:calcium 0.061 to 0.166 mol/kg, (calcium)(phytate):zinc 0.194 to 3.792 mol/kg; while that of phytate:iron was between 1.861 to 6.055 mol/kg. In comparison, the Phytate:Zinc, Phytate:Calcium,

Table 8. Functional properties of raw, germinated and fermented wheat flour.

Sample	Raw wheat flour	Germinated wheat flour	Fermented wheat flour
Bulk density (g/cm^3)	0.80^c ±0.04	0.85^{ab} ±0.01	0.86^a ±0.02
Swelling capacity	0.03^b ±0.03	1.23^a ±0.21	0.35^b ±0.03
Water Absorption capacity (%)	405^b ±5.0	315^c ±5.0	415^a ±5.0
Least Gelation (%)	14.67^a ±1.20	3.33^c ±1.16	8.67^b ±1.16

* Mean with similar alphabets belongs to the same homogenous subset and are not significantly different from each other at the 5% statistical level.

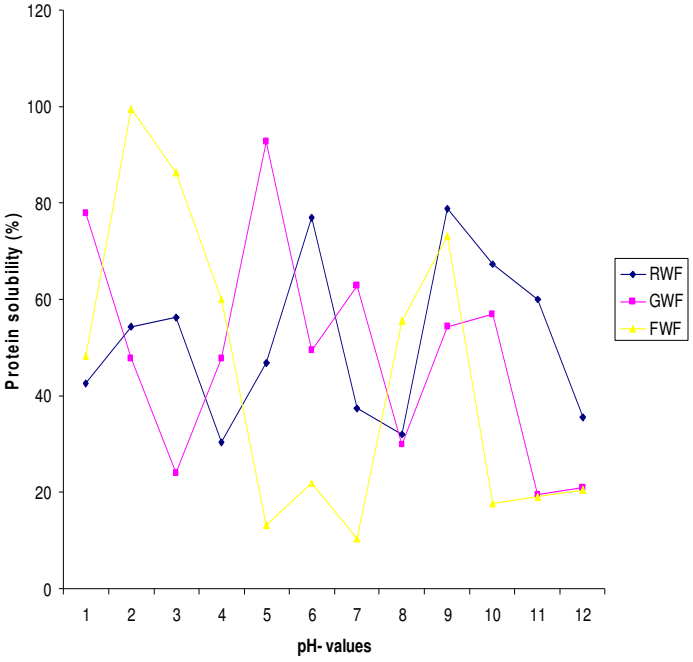

Figure 2. Protein solubility of the raw wheat flour (RWF), germinated wheat flour (GWF) and fermented wheat flour (FWF).

(Ca) (Phytate):Zinc and Phytate:Iron molar ratios of the wheat flour samples were lower than the critical values reported by other investigators (Morris and Ellis, 1985; Davies et al., 1985; Bindra et al., 1986; Gibson et al.,1991; Gibson, 2006). The inhibitory effect of phytate on zinc, iron and calcium absorption has been quantified by the molar ratios of phytate to zinc, iron and calcium in the diet. The ratio greater than these critical level, that is, 15.0, 0.24, 200 and 1.0 have been associated with biochemical and/or clinical evidence of zinc calcium and iron deficiency (Morris and Ellis, 1985; Davies et al., 1985; Bindra et al., 1986; Gibson et al., 1991; Gibson 2006). Evidence has shown that zinc, iron and calcium are essential trace elements for human nutrition (Kono and Yoshida, 1989); and that children are more vulnerable to sub-optimal of these trace elements with adverse effects on their growth rate and cognitive development (Hambidge et al., 1985). Phytic acid may reduce the bioavailability of dietary zinc, iron and calcium by forming insoluble mineral chelate at physiological pH.

Functional properties of raw, germinated and fermented wheat flour

The functional properties of wheat flour samples are shown in Table 8. The Bulk density (BD) ranged between 0.80 ± 0.04 and 0.86 ± 0.02 for raw and fermented wheat flour sample respectively. Swelling capacity (SC) ranged between 0.0 ± 0.03 for raw sample and 1.23 ± 0.21 for germinated sample. For water absorption capacity (WAC) fermented wheat flour sample had the highest value (415%); while germinated had the lowest value. For least gellation, raw wheat sample had the highest value (14.6%); while fermented sample had the lowest value (31.5%).

Figure 2 shows the protein solubility of the raw, germinated and fermentation of wheat flour. The minimum protein solubility of the raw wheat flour was at pH 4, however at this pH the protein content in the food was precipitated out; while the maximum protein solubility was at pH 10. For germinated wheat flour, the minimum protein solubility was at pH 11 and maximum at pH 5; while that of fermented wheat flour sample the minimum protein solubility was at pH 7 and the maximum protein solubility was at pH 2. This observation implies that the protein content of both the germinated and fermented wheat flour samples could only be fully utilized in acidic medium. Hence, the protein content of the wheat flour samples would be fully solubilised and digested at the acidic environment (pH 2) of the stomach, which is the starting point of protein digestion in man. Also, the protein solubility was determine in order to know at what pH the protein would be more soluble, which is very important during food production system to know the state of the food if they are acidic or basic food.

Fermented wheat flour sample has the highest bulk density of 0.86 ± 0.02 g/cm^3, while raw wheat flour (0.80 ± 0.04 g/cm^3) has the least value. The value of bulk density obtained for germinated and fermented wheat flour sample in this study were higher than 0.62 g/cm^3 reported for tigernut flour, 0.54 g/cm^3 for African breadfruit kernel flour and 0.71 g/cm^3 reported for wheat flour (Akubor and Badifu, 2004; Oladele and Aina, 2007). The bulk density value is of importance in packaging

(Snow, 1974). The lower loose bulk density implies that less quantity of the food samples would be packaged in constant volume thereby ensuring an economical packaging. However, the packaged bulk densities would ensure more quantities of the food samples being packaged, but less economical. Nutritionally, loose bulk density promotes easy digestibility of food products, particularly among children with immature digestive system (Osundahunsi and Aworh, 2002; Gopaldas and John, 1991**)**. Water absorption capacity (WAC) of fermented wheat flour (415%) was higher than germinated wheat flour (315%) and raw wheat flour (405%) sample. For the swelling capacity, the value for germinated wheat flour sample was 1.23, while that of raw and fermented wheat flour were 0.03 and 0.35, respectively.

The water absorption capacity values obtained for germinated and fermented wheat flour sample were higher than raw conophor flour (Odoemelam, 2003), but comparable to that of African yam bean flour (Eke and Akobundu, 1993). Water absorption capacity is an index of the maximum amount of water that a food product would absorb and retain (Mosha and Lorri, 1987; Marero et al., 1988**)**. This result suggests that wheat flour may find application in baked products. Studies have shown that the microbial activities of food products with low water absorption capacity would be reduced (Giami and Bekeham, 1992). Hence the shelf-life of such product would be extended.

Least gelation property of the wheat flour samples range from 3.33 ± 1.16 to 14.67 ± 1.20%. It was observed that the gelation property of germinated and fermented wheat flour were comparatively lower than that of raw wheat flour sample. This implies that the flour samples have good gelating ability. Scientific studies have shown that foods that form gels at low concentrations are not ideal for infant diet, because they would require a lot of dilution in an attempt to improve digestibility in relation to volume (Ezeji and Ojimelukwe, 1993; Obatolu and Cole, 2000).

Conclusion

This study established that germinated wheat flour sample had better nutritional parameters like mineral content, total essential amino acid, biological value and lower functional properties than fermented wheat flour sample. However, further study is required on the suitability of this processed flour in food production.

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