Physicochemical, Antioxidants and Pasting Properties of Some Grains as Affected by Germination

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Abstract
Changes in the nutritional, anti-oxidant and pasting properties of yellow maize, sorghum vulgare and Pennisetum glaucum were investigated after soaking in distilled water for 12hrs and geminated for 24hrs. The proximate composition was determined and the antioxidant potential evaluated by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, flavonoids and poyphenol were accessed. Among the cereal tested, high total protein content and the DPPH scavenging activity were observed in yellow maize. The germination processes has increases the protein content with yellow maize having the highest protein content (12.80%) and sorghum vulgare the least value (10.96%) the flavonoid content in sorghum vulgare and Pennisetum glaucum; increased on germination but reduces in the yellow maize. The germination condition was shown to reduce the phenol content but the cereals exhibited high reducing power activity and the ferric reducing power ability was higher in the yellow maize. The pasting property reveals that sorghum vulgare was more viscous that the other cereal and that germination reduce the gelling property in the grains. The study suggests that the cereal grain have high protein content and good antioxidant activity this could make them useful as natural antioxidant, and in preparation of functional food

Key words: *Cereal grain; protein content; pasting property; Antioxidant activity.*

INTRODUCTION

Cereals are the staple food in many parts of Africa, Asia, Central America and Arab countries. About 300million people rely on cereals for their sustenance. The products of milling includes maize grits, meal flour, protein (gluten feed), corn steep liquor and ready-to-eat breakfast cereal 'corn flakes' made from maize grits. Presently, the use of staple foods (sorghum, millet, maize, cassava) as a partial replacement for wheat flour in the production of different snack foods is receiving attention; as most developing countries are now encouraging the addition of locally available flour as a substitute for wheat flour thereby increasing the addition of their local staple food crops as well reduce the cost of wheat flour importation (Kent, 1983). Nutritionally, maize is relatively poor cereal when it comes to the quality of its protein; this is because it has limiting amounts of two essential amino acids lysine and tryptophan Azevedo *et al.*, (1997). Maize grains are rich in vitamin A, C and E, carbohydrates, essential minerals, dietary fibre and contains 9% protein according to Halley, (1983).

Germination of cereals is extensively used in weaning and geriatric foods. Germination generally improves the nutrient content and digestibility of foods. It can bring about a two fold increase in bioavailability of iron, whereas malting of millet brings about a five –ten fold increase in the same (Xu, Dong and Zhe, 2005). Several work has been done on germination as an alternative to genetic engineering in improving the nutritive values (as, vitamins and minerals etc); functional (temperature and enthalpy, gelatinization, pasting characteristics,

swelling and solubility) and chemical (pH, amylase, total starch etc) properties of maize particularly in the developing countries. (Oluwamukomi *et al.,* 2003; Obasi *et al.,* 2009; Eneche, 2009 and Gernah *et al.,* 2011).

Most weaning foods are prepared from cereals or starchy roots, commonly reconstituted with water, such become viscous when reconstituted and are difficult to feed infants due to the small stomach capacity of infants, they cannot consume adequate amounts of bulky foods, resulting in inadequate intakes of vital nutrients. Germination is known to reduce the viscosity of food through amylolytic breakdown of starch reducing bulk. Grains provide a wide range of nutrient sand phyto-chemical that may work synergistically to optimize human health. It is believed that their content of antioxidant compounds is a key to such protection. Hence this work is designed to evaluate the nutritional, pasting and antioxidant properties of some commonly consumed grains as affected by germination to complement diet of babies as well as those suffering from antioxidant deficient diseases and consequently to some extent serves as a defense system as ageing sets in.

EXPERIMENTAL PROCEDURES

Zea maize (yellow), *Sorghum vulgare* and *Pennisetum glancum* (millet) used for experiment were purchased from kings market, Ado-Ekiti. Nigeria. Cleaned samples were soaked in distilled water for 12hrs and allowed to sprout by spreading on a platform covered with wet cotton wool for 24hrs.The sprouted seeds was washed and oven dried at 60^{o}C for 6hrs. It was milled into flour using attrition mill (globe p44 chima). Each flour sample was passed through 0.5mm mesh size sieve. The samples were packaged in an air tight polyethylene bags store in plastic containers with lids and stored in a cool dry place for the next analysis. All reagents used were of analytical grade.

SAMPLE ANALYSIS

Proximate Analysis

The samples were estimated for their moisture content, ash, fat and protein content according to AOAC (2010). The pasting properties were determined by using Rapid Visco Analyzer (RVA) using the RVA pasting method (Newport Scientific, 1998). The sample was turned into slurry by mixing 3g (14% moisture basis) with 25ml of water inside the RVA test canister which was then lowered into the system. The slurry was heated from 50 to 95^{o}C and cooled back to 50^{o}C within 12hrs, rotating the can at a speed of 160rpm with continuous stirring of the content with a plastic paddle. Parameter estimated were peak viscosity, final viscosity, setback viscosity, pasting temperature and time to reach peak viscosity.

DETERMINATION OF FLAVONOIDS

Aluminum chloride colorimetric method was used for flavonoids determination according to the procedure reported by Chang *et al*., (2002). Each plant extracts (0.5 ml of 1:10g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30min; and the absorbance of the reacting mixture measured at 415nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA).

DETERMINATION OF PHENOL CONTENT

Phenolic content of the sample was determined using the method of Makkar *et al*., (1993). A dilute extract of the sample extract (0.5ml of 1:10 gml^{-1}) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5ml, 1:10 diluted and distilled water) and aqueous Na_2CO_3 (4ml, 1M). The mixtures were allowed to stand for 15min and the total phenols were determined by colorimetry at 750nm. Total phenol values are expressed in

terms of gallic acid equivalent (mg/g of dry mass), as a common reference compound.

IN VITRO ANTIOXIDANT ACTIVITY ASSAY

DPPH Radical Scavenging Assay

The radical scavenging effect of cereal proteins on DPPH free radical was measured according to the procedure of Yu *et al*., (2002). To 1mg/ml of cereal sample, 2 ml of 0.1mM of DPPH in 95% ethanol was added; the solution was mixed well and kept for 45min at room temperature. The absorbance of the solution was measured at 517nm. Ascorbic acid was used as the positive control. A lower absorbance represents a higher DPPH radical scavenging activity. The scavenging effect was represented by the following equation.

DPPH radical scavenging activity (%) = (Ac- As)/ (Ac) X 100

(Ac)= Absorbance of the control; (As) = Absorbance of the sample

REDUCING POWER ASSAY

Reducing power of the cereal protein was estimated according to Duh *et al*., (2001). To 2.5ml of cereal sample (1mg/ml) in phosphate buffer (0.2 M, pH 7.6), 2.5 ml of 1% potassium ferricyanide was added. The reaction mixture was incubated at 50^0C for 20min; equal volume of 10% TCA was added to the reaction mixture, mixed by vortex and centrifuge at 1000rpm for 10min at 4^0C, the supernatant with 2.5ml of distilled water and 0.5 ml of 0.1% of $FeCl_3$ were mixed. The mixture was kept for incubation at room temperature for 30min. the absorbance of the solution was measured at 700nm. Ascorbic acid was used as standard and reducing power activity was expressed as μg AAE/mg of sample and increasing absorbance indicators greater reducing power.

Statistical analysis

All work was done in triplicates and the data presented are means ± S.D of three independent determinations. Significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

Chemical Composition

The proximate analysis reveals a low ash content (1.63 - 2.18%), lower in sorghum than millet and maize. Sprouting does not significantly increase the ash content of the cereal grains. The protein content which ranged (10.12 - 12.80%) in the grain reveals that sprouting increases the crude protein, the increase which might be due to syntheses of enzymes or compositional change following degradation of other constituents; however lower levels of protein content were observed in sorghum (10.12 – 10.96%). Higher level of protein was observed in maize as affected by sprouting (Table 1). The moisture content (8.26 – 9.84%) was relative for dried products which indicate storability extension.

Table 1: Chemical composition of some commonly consumed grain as affected by sprouting.

Grains	Moisture (%)	Ash (%)	Fibre (%)	Fat (%)	Protein (%)	Carbohydrate (%)
Zea maize	9.84±0.01	2.08±0.05	2.31±0.05	4.45±0.06	11.15±0.05	70.17 ± 0.08
Sprouted	9.79±0.02	2.18 ±0.03	2.11 ±0.07	3.51 ±0.05	12.80 0.07	69.63 ± 0.09
Sorghum vulgare	8.61±0.03	2.03 ±0.07	1.12 ±0.07	5.22 ±0.04	10.12 0.08	72.91 ± 0.04
Sprouted	8.26±0.02	2.12 ±0.06	0.85 ±0.08	4.76 ±0.07	10.96 ± 0.06	73.07 ± 0.06
Pennisetum glaucum	9.26±0.05	1.67 ±0.05	1.84 ±0.06	3.35 ±0.05	12.07 ± 0.07	71.83 ± 0.05
Sprouted	8.79±0.04	1.63 ±0.03	2.02 ±0.09	3.32 ±0.08	12.37 ± 0.06	71.88 ± 0.03

Values are mean of triplicate determination ± standard deviation

PASTING PROPERTIES

Changes in the viscosity of starch suspension as the result of temperature changes were measured with the RVA as summarized in Table 2. Cereals forms paste when reconstituted with hot water hence its amylographic viscosities are important in assessing the suitability of its application as functional ingredients in food and other industrial products (Aviara *et al*., 2010). The peak viscosity ranged from 68.00RVU – 195.00RVU, with sorghum having least and millet the highest. The peak viscosity of maize is higher than sorghum and sprouting has improved the peak viscosity of the samples. Peak viscosity is an indicative of the strength of paste, which are formed from gelatinization during processing in food applications. It also reflects the extent of granule swelling (Liang and King, 2003). Trough values ranged from 25.00RVU -134.00RVU while breakdown viscosity ranged from 32.00RVU -151.00RVU. Breakdown and Trough viscosities reflect the stability of the paste. This shows that millet sample will be more stable than sorghum and maize becomes less stable.

Final and setback viscosities ranged from 49.00RVU – 190.10 RVU and 24.00RVU – 126.70 RVU respectively. These viscosities increase on sprouting of the cereals. This indicates that the final viscosities are important in determining the ability of the sample materials to form gel during processing while setback indicates gel stability and potential for retrogradation (Liang and King 2003). Generally time taking to attain peak viscosity ranged from 3.89min – 7.00min. While pasting temperature ranged between 62^{o}C – 83.15^{o}C. The attainment of the pasting temperature is essential in ensuring swelling, gelatinization and subsequent gel formation during processing; lower pasting temperature as shown by sprouted sorghum indicates faster swelling properties than maize and millet. There were significant differences ($p< 0.05$) in all the pasting properties of the cereal grains.

Table 2: Pasting properties of some locally consumed grains as affected by sprouting.

Sample	Peak viscosity (RVU)	Trough viscosity (RVU)	Breakdown viscosity (RVU)	Final viscosity (RVU)	Setback viscosity (RVU)	Peak Time (min)	Pasting Temp (^{0}C)
Zea maize	135.00±0.3	98.00 ± 0.8	40.00 ± 0.5	603.00± 0.6	505.00± 0.9	7.00±0.04	75 ± 3
Sprouted	153.00±0.6	121.00±0.4	32.00 ± 0.6	453.00± 0.8	332.00 ±0.8	7.00±0.06	72 ± 4
Sorghum vulgare	188.00±0.5	34.00 ± 0.6	354.00± 0.9	901.00±0.7	12.67 ± 0.7	5.20 ± 0.8	83 ± 5
Sprouted	195.00±0.8	44.00 ± 0.7	151.00± 0.5	95.00 ± 0.9	51.00 ± 0.6	4.00 ± 0.4	79 ± 6
Pennisetum glaucum	68.00 ± 0.8	25.00 ± 0.8	43.00 ± 0.4	49.00 ± 0.6	24.00 ± 0.7	3.87±0.02	65 ± 5
Sprouted	68.00 ± 0.7	25.00 ± 0.7	43.00 ± 0.7	49.00 ± 0.8	24.00 ± 0.9	3.87±0.04	62 ± 3

Values are mean of triplicate determinations ± standard deviation

Table 3: Antioxidant activities of some locally consumed grains as affected by sprouting.

Grains	Flavonoid (mg/100g)	Phenol content (mg/100g)	DPPH scavenging activity (%)	Reducing power activity (μgAAE/mg)
Zea maize	55.60 ± 0.06	4.11 ± 0.07	48.20 ± 0.08	92.8 ± 0.09
Sprouted	44.90 ± 0.08	1.93 ± 0.06	39.18 ± 0.06	76.8 ± 0.07
Sorghum vulgare	11.09 ± 0.08	8.70 ± 0.03	21.75 ± 0.07	74.2 ± 0.06
Sprouted	15.30 ± 0.03	6.28 ± 0.02	39.23 ± 0.06	82.5 ± 0.08
Pennisetum glaucum	10.27 ± 0.04	11.09 ± 0.02	20.32 ± 0.05	54.6 ± 0.07
Sprouted	31.10 ± 0.05	6.12 ± 0.04	27.24 ± 0.07	39.1 ± 0.05

The values presented mean ± standard deviation

It has been recognized that yellow maize has considerable flavonoids, which have effects on human nutrition. The flavonoid content in yellow maize 44.90-55.60mg/100g higher than in millet 3.11-10.27mg/100g and sorghum 11.09-15.30mg/100g according to Table 3. By this study, the high content of flavonoid in the cereals can explain its high radical scavenging activity and sprouting of has improved the flavonoid content.

The total phenolic content was reduced on sprouting which may be attributed to the polyphenol oxidase based enzymatic hydrolysis Jood *et al.,* (1987). The total phenolic content was higher in millet (11.09) and least in maize (4.11). Sprouting reduces the phenolic content as shown in Table 3. Oxidation reactions produces free radicals which start chain reaction that damage cell, and antioxidant terminates these chain reaction by removing the free radicals which is intermediates and inhibit other oxidation reaction by been oxidized themselves according to Guard (2000). Phenolic content of these cereals have good antioxidant activity according to Sreeramulu *et al.,* (2009).

The antioxidant activity of the cereals were expressed as (%) DPPH radical scavenging activity with higher values, indicating that the samples analyzed has potential as an anti oxidation food products. According to Varady *et al*., (2003), sorghum contains high antioxidants and the wax surrounding sorghum contains impact on human cardiac health. The antioxidant activity in the cereals sample ranged 20.30-48.20% with maize the highest and least value in millet 20.30%. Sprouting enhances the antioxidant activity but reduces only in maize.

The reducing power activity of the cereal was observed to be high in maize 7.68-9.28μg AAE/ mg and least in millet 3.91μg AAE/mg. However, Allhoorn *et al*., (2005) reported that reducing property can be an anti oxidation defense mechanism; this is possibly through the ability of the antioxidant compound to reduce transition metals, this suggest that these cereals with high antioxidant parameters could be useful ingredients in ageing.

CONCLUSION

This study could be concluded that germination has improved the protein content of the cereal as well as the antioxidant properties of the grain. The cereals exhibit free radical scavenging activities which could make them useful in therapeutics for health benefits for ageing people. Yellow maize and sorghum has more antioxidant potential than *Pennisetum glaucum*. However sprouting has improved the nutritional composition of the grains; this could encourage the use of germination of the cereals (yellow maize and sorghum) before its utilization in order to enhance the antioxidant capacity.

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