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ASSESSMENT OF PHYSICO-CHEMICAL AND PHYTOCHEMICAL PROPERTIES OF WHITE FONIO (Digitaria exilis) FLOUR

Egbebi, A.O.[1*], Muhammad A.A[2]
*[*1]Department of Biological Sciences, Afe Babalola University, AdoEkiti, Nigeria;*
[2]Department of Biological Sciences, Afe Babalola University, AdoEkiti, Nigeria

Corresponding Author:-

Abstract:-

White variety of Digitaria exilis seeds were obtained and milled into flour. Assessments of physicochemical and phytochemical properties of the sample were determined. The sample maintained high percentage of proximate compositions except for crude fiber with 0.385%. Digitaria exilis is rich in potassium with 38.78mg/100mg and has low calcium of 0.83%/100mg. Water Absorption Capacity and Oil Absorption Capacity with the functional properties of 242.00% and 260.00% respectively were high which makes it useful for bakery products. It helps in flavor retention, improvement of palatability oil and improves mouth feel. The pasting properties of the sample were also analyzed with Peak viscosity of 12.00RVU, Trough value of 10.00RVU, Breakdown 2.00RVU, and final viscosity of 16.00RVU. The pasting temperature was however found to be nil. There is a presence of phytochemicals in the sample, which fight against some diseases in the body and which occur naturally in cereal grains. The phytochemicals present are alkaloids, saponins, tannins, steroids, terpenoids, and flavonoids. Phlobatannin and anthraquinone were not found to be present in Digitaria exilis.

Keywords:-*Fonio, flour, Digitaria exilis, phytochemicals, cereals*

INTRODUCTION

Cereals constitute the major sources of energy, proteins, vitamins, and minerals for the world population. The cereal grains of economic importance are the cool-season crops like wheat, barely, and rye, and warm-season cereals like rice, maize, sorghum and millet (Ayodele and Mohammed, 2011). Traditional cereals like Fonio grains have played a central role in the emergence and development of traditional agricultural nutrition and indigenous medicine in the West African savannah (Adoukonou *et al*., 2007*)*.

Cereals are important sources of many nutrients for populations in all regions of the world but especially for people living in undeveloped countries where the lack of protein-rich foods and economic constraints compel them to rely on cereals (Coda *et al*., 2010). Fonio, though considered one of the oldest West African indigenous cereals, has for a long time been neglected by scientific research and development programs. Fonio species belong to the Poaceae family, sub-family of Panicoideae tribe of Paniceae and the genus *Digitaria Haller.* The genus *Digitaria Haller* comprises 230-325 annual and perennial grass species with a wide geographic distribution in the tropics and subtropics (Adoukonous- sogbadja, 2010). In local agriculture of West Africa, hundreds of Fonio landraces exist and derive from traditional selection. White Fonio (*Digitaria exilis)* is the most diverse and widely cultivated species in West Africa and is called Acha, Irovaga, Feningue, Findi, Kansambahon, and Ova in Nigeria, Benin, Burkina Faso, Guinea, Mali, and Togo, respectively. Fonio grains are extraordinarily tiny with weight of 0.5-0.6g. Some authors have shown that the general anatomical structure of the Fonio grain was similar to that of other cereals like rice (Cruz *et al*., 2011).

Traditionally, Fonio is a useful diet for those suffering from diabetes or for women after delivery (Jidean, 1999). Fonio is one of the most nutritious and best tasting of African cereals. It is sometimes regarded as 'grain of life' as it provides food early in the farming season when other crops are yet to mature for harvest (Ibrahim, 2001). Among the native crops of Africa, Fonio is selected as target for biotechnology because of its exceptional culinary and nutritional properties. In Nigeria, Fonio products are currently recommended as choice of carbohydrate for diabetes patients. Today, it is the most expensive grain crop in Nigeria, providing the resources for poor farmers with enough to alleviate their poverty. The recent news of Fonio groomed to conquer the Europe market is another indication of the importance of Fonio for food security and economy revival of Africa (Aslafy, 2003). The objective of this study is to assess the physiochemical and phytochemical properties of white Fonio (*Digitaria exilis*) flour.

MATERIALS AND METHODS

Sample Collection

Digitaria exilis (Acha or Fonio) used in this study was purchased at Bokkos market in the city of Plateau State (Jos) in North-central part of Nigeria.

Sample Preparation

The seeds were cleaned to remove unwanted or extraneous materials such as chaff and stones that may be present. The seeds were then dried and milled into flour before being packaged in an airtight container.

Determination of Proximate Composition

The proximate analysis of the sample was carried out using the standard procedure of Association of Official Analytical Chemists (AOAC, 2005).

Moisture Content Determination

Moisture content was determined by the method described by AOAC (2005) using the air over method. Sterile petri dish was weighed and the weight recorded as W_1. Two grams (2g) of sample was then weighed into the petri dish and the weight was taken as W_2. The sample was then dried at 105ºC for 3 hours and transferred into a desiccator, cooled for an hour and then weighed until constant weight was attained. Calculation:

$$\%\text{Moisture Content} = \frac{loss\ in\ weight}{weight\ of\ sample\ before\ drying} \text{ X } 100$$

$$\%\text{M.C} = \frac{W3-W1}{W2-W1} \text{ X } 100$$

Where:

W_1 is weight of petri dish

W_2 is weight of petri dish and sample

W_3 is weight of petri dish and sample after drying

Total Ash Content Determination

The total ash content was determined by using the procedure of AOAC (2002). About 2g of the sample was weighed into a clean crucible weighed W (and together as W_2). The crucible was then placed into a muffle furnace chamber at 6000ºC until the sample turned into ashes. The crucible was removed from the furnace, cooled in desiccators and allowed to cool to room temperature and reweighed as W_3. Calculation:

$$\%\text{Ash} = \frac{W3-W1}{W2-W1} \text{ X } 100$$

$$\% \text{ Organic matter} = 100 - \%\text{Ash}$$

Where:
W_1 is the weight of crucible
W_2 is the weight of the crucible and sample before drying
W_3 is the weight of the crucible and sample after drying

Crude Protein determination

The total crude protein was determined using the micro kjeldhal method AOAC (2005). 0.2g of the sample was weighed into a kjeldhal tablet. The mixture was digested to obtain a clear solution. The digest was cooled and 75ml distilled water was added, and then followed by 50ml of sodium hydroxide solution. The ammonia formed in the mixture was subsequently distilled into 25ml 2% boric acid solution containing 0.5ml of methyl red indicator. The distillate collected was then titrated against 0.1M of HCL. Blank titration was also carried out on the reagent and the nitrogen in the sample was calculated. The nitrogen content was multiplied by 6.25 (conversion factor) to obtain crude protein content. Calculation:

$$\%\text{Nitrogen} = \frac{titrate\ value\ X\ N\ X\ 0.014}{weight\ of\ sample}\ X\ 100$$

$$\text{Crude Protein} = \%\text{nitrogen X } 6.25$$

Where: N is the total Nitrogen and 6.25 is the conversion factor

Crude Fiber Content Determination

Crude fiber was determined using a method described by AOAC (2005). Two grams (2g) of sample was weighed (W_1) which was extracted with n-hexane. This was transferred into a 1liter flask. Sulphuric acid (1.25% of 200ml) was added and the flask was placed on a hot plate and boiled for 30mins. The content was filtered and the residue was washed with 70ml of distilled water. The residue was removed and 200ml of boiling 1.25% sodium hydroxide (NAOH) was added and boiled for 30mins. The content was filtered and the residue was washed with distilled water. The residue was then transferred to a dish and dried at 130°C for 30mins cooled in desiccators and weighed (W_2). This was then ignited at 600°C, cooled and reweighed (W_3). Calculation:

$$\% \text{ Crude fiber} = \frac{W2-W3}{W2-W1} \text{ X } 100$$

W_1 is the sample weight
W_2 is the sample weighed with the dish after drying
W_3 is the sample weighed with the dish after being ignited

Crude Fat Determination

Crude fat was determined by the method described by AOAC (2002). Crude fat was determined by using soxhlet apparatus. Approximately 3g of sample was put into a thimble and extracted with nhexane for about 6 hours. The solvent was removed from the extracted oil by evaporation. The oil was further dried in a hot-air oven at 100°C for 30mins to remove residual organic solvent and moisture. This was cooled in desiccators and weighed. The quantity of the oil was expressed as percentage of the original sample used. Calculation:

$$\% \text{ Crude fat} = \frac{W4-W3}{W2-W1} \text{ X } 100$$

Where:
W_1 is the weight of thimble
W_2 is the weight of thimble and sample
W_3 is the weight of round bottom flask
W_4 is the weight of the round bottom flask and the residual oil

Carbohydrate Content Determination (by difference)

Carbohydrate content was determined by subtracting the value of the analyzed components i.e. moisture content, protein, crude fat, ash content, and crude fiber. 100 - %(crude protein + total ash + crude fiber + crude fat + moisture content)

DETERMINATION OF PHYTOCHEMICAL OR ANTI-NUTRITIONAL FACTORS

Tannin Determination

About 0.2g of finely ground sample was weighed into a 50ml sample bottle. I0ml of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 hours at 30°C. The solution was then centrifuged and the supernatant stored in ice. 0.2ml of the solution was pipetted into the test tube and 0.8ml of distilled water was added. Standard tannin acid solution was prepared from a 0.5mg/ml of' the stock and solution made up to 1ml with distilled water. 0.5ml of folin ciocateau reagent was added to both sample and the standard followed by 2.5ml of 20% Na_2Ca_3. The solutions were then vortexed and allowed to incubate for 40 minutes at room temperature. The absorbance was read at 725mm against a reagent blank concentration of the same solution from and a standard tannic acid curve was prepared (Makkar and Godchild, 1996).

Determination of Phytate
Phytate was determined according to the method of wheeler and ferret (1971). 4g of the sample was soaked in 100ml of 2% HCL for 3 hours and then filtered through a No. 1 Whatmann filter paper. 25ml was taking out of the filtrate and placed inside a conical flask and 5ml of 0.3% of ammonium thioeyanate solution was added as indicator. After which 53.5ml of distilled water as added to give it the proper acidity and this was titrated against 0.00566g per milliliter of standard ion (iii) chloride solution that contain about 0.00195g of iron per milliliter until a brownish yellow coloration persist for 5 minutes.

Determination of Oxalate
Oxalate determination was determined by soaking 1g of the sample in 75ml of 1.5N H_2SO_4 for 1 hour and then filter through a No. 1 Whatmann filter paper. 25ml was taking out of the filtrate and placed inside a conical flask and this was titrated hot (80-90^oC) against 0.1$KMNO_4$ until a pink color that persists for 15 seconds is noticed (Day and Underwood, 1986).

Determination of Saponin
The spectrophotometric method of Brunner (1994) was used for saponin determination. 2g of the finely grinded sample was weighed into a 250ml beaker and 100ml of isobutyl alcohol or was added to it. A shaker was used to shudder the mixture for 5 hours to ensure uniform mixing. The mixture was filtered with No. 1 Whatmann filter paper into 100ml beaker containing 20ml of 40^oC-saturated solution of magnesium carbonate ($MgCO_3$). The obtained mixture was filtered again to obtain a clean colorless solution. 1ml of the colorless solution was taken into 50ml volumetric flasks using a pipette. 2ml of 5% iron (iii) chloride solution was added and made up to the mark with distilled water. It was allowed to stand for 30 minutes for the color to develop. The absorbance was read against the blank at 380nm.

Determination of Total Phenol
The total phenol content of the extract was determined by the method (Singleton *et al*., 1999). 0.2ml of the extract was mixed with 2.5ml of 10% folin ciocalteaus reagent and 2ml of 7.5% sodium carbonate. The reaction mixture will be subsequently incubated at 45^oC for 40 minutes and the absorbance measured at 700nm in the spectrophotometer. Garlic acid will be used as standard phenol.

Determination Total Flavanoid
The total flavonoid content of the extract was determined using a colorimeter assay developed by Bao J.Y., 2005. 0.2ml of the extract was added to 0.3ml of 5% $NaNO_3$ at zero time. After 5 minutes, 0.6ml of 10%$AlCl_3$ was added to the mixture, followed by the addition of 2.1ml of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

PHYTOCHEMICAL SCREENING
Alkaloid Determination
About 0.5g of the extract was stirred with 5ml of 1% aqueous HCl solution on a water bath. 1ml of the filtrate was treated with a few drops of dragendorf reagent. Blue-black turbidity was taken as preliminary evidence for the presence of alkaloid.

Anthraquinone Determination
Borntrager's test was used for the detection of anthraquinone. 0.5mg of the extract was shaken with 10ml of benzene, filtered, and 5ml of 10% ammonia solution added to the filtrate. The mixture was shaken and presence of pink, red, or violet color in the ammonia layer indicated the presence of free anthraquinone.

Steroid Determination
About 20ml of acetic anhydride was added to 0.5mg of the extract and filtered. 2ml of the conc. H_2SO_4 was added to the filtrate. There was a color change from violet to blue or green, indicating the presence of steroids.

Trepenoid Determination
About of 0.5g of the extract was mixed with 20ml of chloroform and filtered 3ml of conc. H_2SO_4 was added to the filtrate to form a layer. A reddish-brown color at the interface was observed which indicated the presence of trepenoid.

Cardiac Glycosides
The following tests were carried out to test for cardiac glycosides:
Legal's test: The extract was dissolved in pyridine and a few drops of sodium nitroprusside and 20% NaOH were added. A deep red coloration that faded to a brownish yellow indicated the presence of cardiac acid.

Lieberman's test: About 20ml of acetic anhydride was added to 0.5g of the extract and 2ml of conc. H_2SO_4 was added to the filtrate. There was a color change from violet to blue or green that indicated the presence of steroids nucleus. (i.e. aglycone portion of the cardiac glycosides).

Salkowski's Test: About 0.5g of the extract was mixed with 20ml of chloroform and 3ml conc. H_2SO_4 was added to the filtrate to form a layer. A reddish-brown color at the interface was observed and indicated presence of steroidal ring.

Keller-Killiani's Test: About 0.5g of the extract was dissolved in 2ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then mixed with 1ml of conc. H_2SO_4 and a brown color change at the interface indicated the presence of a deoxy sugar.

DETERMINATION OF FUNCTIONAL PROPERTIES

Least Gelation Capacity Determination (LGC)

Least gelation capacity was determined by the method described by Coffman and Garcia (1977). Ten suspensions of the flour were blended 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, and 20% w/v) in 5ml of distilled water was prepared in test tubes. The test tubes containing the suspensions were heated for 1 hour in boiling water bath followed by rapid cooling under running tap water. The test tubes were then cooled for an hour. The least gelation concentration was determined by:

$$\text{Least Gelation Concentration\%} = \frac{weight\ of\ sample}{5ml\ of\ water}\ X\ 100$$

Bulk Density Determination

The method of Ukpabi and Ndimele (1990) was used:

1. **Losses:** About 11-14g of sample was weighed and poured gently into a 23ml-measuring cylinder in a separate cylinder. The level of the sample in the cylinder was traced out in ml and the density calculates

$$\text{Density} = \frac{mass}{volume}$$

2. **Packed:** about 12-16g of each sample was weighed and poured into a 23ml-measuring cylinder. The sample was pressed down into the cylinder. The volume was determined and the density calculated.

Water Absorption Capacity Determination

The method of Ukpabi and Ndimele (1990) was used. About 1g of the sample was dispensed in 10ml of distilled water. The content was then stirred for 2-3 minutes using a magnetic stirrer. The sample was then poured into a 50ml centrifuge tube and then centrifuged in a cyclo-mixer at 3500rpm for 30 minutes.

At the end of the centrifuging, the sample in the tube was allowed to stabilize and the supernatant of the tube was carefully drained into a graduated cylinder of 10ml. The volume of the supernatant was noted and the density of water was assumed to be 1hm/ml/

Determination of Oil Absorption Capacity

About 1g of the sample was dispersed in 10mls of oil. The content was then stirred for 2-3 minutes using a magnetic stirrer. The sample was then poured into a 50ml centrifuge tube and then centrifuged in a cyclo-mixer at 3500rpm for 30minutes. At the end of the centrifuging, the sample in the tube was allowed to stabilize and the supernatant of the tube was carefully drained into a graduated cylinder of 10ml. the volume of the supernatant was noted and the density of oil was assumed to be 1gm/ml. Calculation:

$$\text{Weight} = \frac{D\ X\ volume\ of\ bound\ oil}{weight\ of\ sample}$$

Mineral Content Analysis

Standard method obtained from AOAC (2005) was used. The samples were digested prior to mineral determination by weighing 0.5g of sample into a digestion tube and 20ml of nitrite/hydrochloric acid was added. It was digested at 150^{o}C until a clear flame was obtained and washed into a standard 50ml volumetric flask and distilled water was added up to the mark.

Atomic absorption spectrophotometer was used for the analysis of Ca, Fe, and Zn by UV visible spectrophotometer and Na and K were determined using a flame photometer. The standard for each metal using suitable metal salt of each metals were prepared

Determination of Pasting Characteristics

Pasting characteristics was determined with a rapid viseo analyzer (RVA). 2.5g of sample was weighed into a dried empty canister, then 25ml of distilled water was dispensed into the canister containing the sample. The solution was thoroughly mixed and the canister was well fitted into the RVA. The slurry was heated to about 95^{o}C with a holding time of 2 minutes followed by cooling to 50^{o}C with 2 minutes holding time. The rate of heating and cooling were at a constant rate of 11.25^{o}C per minutes.

Peak viscosity, trough, breakdown, final viscosity, set back, peak time, and passing temperature, were read from the pasting profile with the aid of thermocline for windows software connected (Newport Scientific, 1998). The viscosity was expressed in terms of rapid viso units (RVO), which is equivalent to 10 centipoises.

RESULTS

Table 1: Proximate Composition of White Fonio flour

Composition	White Fonio (%)
Moisture Content	10.40
Ash Content	8.26
Crude Protein	11.71
Fat Content	2.86
Crude fiber	0.38
Carbohydrate	66.38

Table 2: Mineral Elements of White Fonio flour

Element	Concentration (mg/100g)
Sodium	1.65
Potassium	38.78
Calcium	0.83
Zinc	1.60
Iron	3.82

Table 3: Functional Properties of White Fonio flour

Properties	White Fonio
Bulk density (g/dm^3)	0.775
Water absorption capacity (%)	242.00
Oil absorption capacity (%)	260.00
Least gelation determination (%)	6.00

Table 4: Phytochemical screening of White Fonio flour

Screening	White Fonio flour
Alkaloid	+
Saponin	+
Tannin	+
Pholobatannin	--
Steroid	+
Terpanoid	+
Anthraquinone	--
Flavoid	+

Cardiacglycosides

Lieberman test	+
Salkowski	+
Legal test	+
Keller killiani test	+

Table 5: Phytochemical determination of White Fonio flour

Phytochemical	White Fonio flour (mg/g)
Oxalate	0.585
Saponin	33.455
Phenol	7.332
Phytate	27.192
Flavanoid	3.228
Tannin	0.646

Table 6: Determination of pasting properties of white Fonio flour

Properties	White Fonio flour
Peak Viscosity	12.00
Trough	10.00
Breakdown	2.00
Final viscosity	16.00
Setback	6.00
Peak time	6.80
Pasting temperature	--

DISCUSSION

Table 1 shows the proximate composition of *Digitaria exilis* flour. The results showed high levels of protein (11.71%) compared to the report by Irving and Jideani, 1997, with crude protein 8.05%. the main source of human protein comes from cereal grains because they constitute the basic food in many developing countries. The carbohydrate by difference was 66.38%, which is moderate in Fonio grain. Carbohydrates are present in food products in form of monosaccharides, oligosaccharides, and polysaccharides. Carbohydrates are major components in cereals and constitute the main energy sources used by the human organism. *Digitaria exilis* flour has a high level of ash content. The fat content is however a relatively minor constituent in cereal grains (but is significant in human nutrition as energy source and for essential fatty acids). The moisture content of Fonio was found to be high (10.40%) compared to other grains and this could lead to deterioration of the grain during storage.

Table 2 shows results for mineral elements of White Fonio flour. The flour has high potassium, moderate iron, low sodium and zinc, and lesser calcium. Grains with high potassium and calcium concentrate might be beneficial to people with hypertension and blood pressure (Birkett, 1998).

Table 3 shows the functional properties of white Fonio flour, which helps to effect the gluten formation in the flour. The bulk density of the flour was found to be 0.775g/dm^3; water absorption capacity, 242%; oil absorption capacity 260%; and least gelation determination, 6%. The bulk density of a food material is important in relation to packaging. Oil absorption capacity is useful in structure interaction in foods especially in flavor retention, improvement of palatability, and extension of shelf life particularly in bakeries (Adebowale and Lawal, 2004).

The phytochemical screening and phytochemical determination of white Fonio flour are shown in Tables 4 and 5 respectively. The phytochemical screening showed the presence of alkaloid, saponin, steroid, trepenoid, flavonoid, and cardiacglycosides, all in little amounts.

Table 6 shows the pasting properties of the flour. The peak viscosity, which is the ability of flour to well freely during physical breakdown during mixing, is 12.00RVU. The trough, which is the minimum viscosity value in the constant temperature phase of the RVA profile and measures the ability of dough to withstand breakdown during cooling after baking or frying, is 10.00 RVA. The final viscosity if 16.00 RVA and is used to define the particular quality of flour and indicate the stability of the backed paste in actual use.

CONCLUSION AND RECOMMENDATION

From the results obtained, it can be concluded that white Fonio flour (*Digitaria exilis*) contains mineral elements, proximate compositions, phytochemical and functional properties that makes it suitable for consumption. Fonio grains contain nutritional properties, are gluten free, and rich in protein. Fonio is light an easy to digest and can be included in many different cereal based recipes, making it an attractive ingredient or health products for those with gluten intolerance. White Fonio has received some attention and show impressive future and huge potential for wider use. The National Agriculture Research Extension and Liaison services should enlighten local farmers on the advantages of Fonio grains that consumers can benefit from.

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