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Assessment of *in vitro* Digestibility Kinetics of Resistant Starch in Some Under-Utilized Legumes and Cereal

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ABSTRACT

Normally digestible starch is a starch that digest in the small intestine. Partial starch is digested in the small intestine but not completely, in it some amount of starch used to escape digestion while resistant starch normally withstand digestion in the small intestine of healthy human beings. The study is aimed at determining the in vitro digestibility kinetics of resistance starch derived from underutilized legumes and millet). This is done by determining the proximate composition of underutilized legumes (Bambara groundnut and African yam Beans) and millet. S and analyzing the digestibility kinetics of both modified and unmodified resistant starch using alpha amylase. The result of proximate composition of cereal and legumes for modified and unmodified samples are thus; Moisture content is 6.33± 0.41 and 8.50± 0.35, Crude fiber is 2.83± 0.53 and 3.50± 0.35, the Fat content is 1.33± 0.41 and 1.67± 0.41, the Ash content is 1.17± 0.18 and 2.50± 0.35, the Protein content is 8.75± 0.03 and 13.12± 0.25 while the Carbohydrate content is 63.52± 0.56 and 57.83± 0.52. Moreso, the Proximate Composition of modified and unmodified bambara groundnut are as follows: the Moisture content has it as 15% ± 0.5 and 6.8% ± 1.0, the fats content is 1% ± 0.0 and 6.5% ± 0.0, the Fibre content is 4% ± 1.0 and 5.5% ± 0.5, the protein content is 17.50% ± 0.1 and 22.76%±0.0, 22.76%±0.0 the Carbohydrate content is 60.5% and 64.94%. The digestibility of both modified and unmodified millet is. From the result the modified and unmodified sample for Reducing sugar (mg/g) is 0.55± 0.09 and 2.7 ± 0.06, the Amylose (%) is 35.10 ± 1.81 and 28.41 ± 0.20, the Amylosepectin (%) is 64.9 ± 1.81 and 48.41 ± 0.80, the Starch damage (%) is 73.04 ± 0.02 and 1.8 ± 0.05. while the Starch undamaged (%) is 56.25 ± 0.08 and 62.40 ± 0.20. The study showed that millet is a good source of starch that will help to reduce poverty level and enhanced food security. It equally adds to knowledge by revealing the digestibility kinetics of modified millet starch to the unmodified starch and enlighten the benefits of millet starch and its uses in relation to enhance nutritional security.

Keywords: in vitro digestibility, Bambara groundnut and African yam Beans) and millet proximate analysis.

INTRODUCTION

Background to the Study

Legumes refers to any plant from the fabaceaae family that would include its leaves, stems, and pods. when used as a dry grain for human consumption, the seeds are also called pulses. Legumes are grown agriculturally, primarily for human

consumption, for livestock forage and silage; and as soil-enhancing green manure. There are a number of underutilized legume crops (marama bean, bambara groundnut, cowpea, pigeon pea, African yam bean e.t.c. Due to their high drought tolerance and excellent nutritional profile comparable to commercially available legume crops like (soybean, peanut, and navy bean) these could yam bean, and lablab) in Africa, which due to their rich nutritional profile, high adaptability to adverse climatic conditions, and ability to grow in poor soils are highly advantageous for sustainable cultivation. Among these, African yam bean (sphenostylis stenorcapa) and many other nutritionally important underutilized legumes crops potentially provide sustainable food and feed resources in the future. The richness of these crops in proteins can support the global protein demand in future to partially or completely replace other animal proteins in the human diet. However, in spite of having huge potential for sustainable agriculture, the marama bean is still in wild, while the African yam bean is cultivated by small landholders as a subsistence crop. Intensive agronomic, genetic, and food research is required to move these crops out of obscurity and to use their potential as cash crops.

Starch that are consumed by humans can be categorized into three different groups such as digestible starch, partial digested starch, and resistant (*Englyst & Cummings 1787*). The starch digested in the small intestine is known as the Digestible starch. The Partial starch is just like the name imply is digested in the small intestine but not completely in the intestine. From it some amount escape digestion. The resistant starch completely withstand digestion in the small intestine of healthy human beings. Resistant starch has positive impact on health especially in obesity control. (*Slen, et al., 2015).*

Starch, a long chain carbohydrate, is the food of many plants that is found in potatoes, wheat, rice and other foods, and differ in appearance depending on its source (Abbas et al., 2012; Alcazar-Alay and Meireles, 2015). Starch consists of a large number of the polymers amylose and amylopectin units joined together by glycosidic bonds (Perez and Bertoft, 2010).

Native starch has limited uses in the food industry, as it produces weak-bodied, cohesive, rubbery paste when heated and undesirable gel when cooled. It also shows strong tendency towards decomposition and retrogradation, and becomes unstable with changes in temperature, pH and shear forces (Berski et al., 2011). Native starches are often modified to improve specific properties such as solubility, texture, adhesion and tolerance to the heating temperature used in industrial processes (Miyazaki et al., 2006; Sweedman et al., 2013; Alcazar-Alay and Meireles, 2015).

Modified starches have been produced with a variety of characteristics and applications using physical, chemical and enzymatic methods. The techniques alter the starch

Polymer, making it highly flexible and changing its physicochemical properties and structural attributes to increase its value for food and non-food industries (Lopez et al., 2010). Physical methods, however, involve the use of heat and moisture, while chemical modifications involve the introduction of functional groups into the starch molecule using derivatization reactions (e.g., etherification, esterification, crosslinking) or involve breakdown reactions (e.g., hydrolysis and oxidation) (Singh et al., 2007). Chemical modifications generate significant changes in retrogradation and paste properties (Lopez et al., 2010; Yousif et al., 2012; Sweedman et al., 2013; Yadav et al., 2013). Starch molecules from different origins could interact to produce attributes unique to the starch blends (Eun et al., 2009). The transformation of starch during manufacturing depends on the temperature and mixture ratio during processing (Londe-Petit et al., 2001).

Nutritional quality of food, including mineral element contents, are affected by modification processes. Minerals are inorganic substances found in body tissues

and fluids, and help in the maintenance of certain physicochemical processes which are essential to life (Soetan et al., 2010). The body requires different amounts of each mineral, which depends on their age, sex, physiological state (e.g. pregnancy) and sometimes their state of health.

Minerals are involved in the formation of bones and teeth, and are components of enzyme systems which are involve in normal nerve function. Calcium, for instance, is required in large quantity for building and maintenance of bone, and normal function of nerves and muscles. Iron is an important component of the cytochromes that function in cellular respiration. Magnesium, copper, zinc, iron, and manganese are important co-factors found in the structure of certain enzymes and are necessary for some biochemical reactions (Zamberlin et al., 2012). Sodium and potassium, among other minerals, are important in the maintenance of osmotic balance between cells and the interstitial fluid. However, excessive intake of some minerals can upset homeostatic balance and cause toxic effects. Excess sodium intake is associated with high blood pressure and excess iron can cause liver damage (Gergely et al., 2014).

Different studies (Singh and Srivastava, 2006; Chetan and Malleshi, 2007; Shashi et al., 2007; Bwai et al., 2014) have been carried out on starches. Functional properties of FMS modified with different starches have been reported by Tukura et al. (2016). However, information on the proximate and mineral contents of the starches are scarce, therefore, the research was carried out to determine these contents in the modified FMS starches.

Resistant starch (RS) is a degradation product that escapes from digestion in the small intestines of healthy individuals. Not all starch you eat gets digested sometimes a small part of it passes through the tract unchanged in other words it's resistant to digestion this type of starch is called resistant starch. Resistant starch occurs naturally in foods but can also be added as part of dried raw foods, or used as additive in manufactured foods. However, Millets are small-seeded cereals belonging to the poaceae family commonly known as the grass family. Botanically known as panicum miliaceum, millet is called Gero/dawa in Hausa, Achara in igbo and Okababa in Yoruba. Having excellent nutritional quality, they are comparable or superior to the commonly consumed cereals like wheat and rice (Ragaee, et al., 2006). Despite its superior nutritional quality, it has received less attention compared to the major cereals. They are gradually gaining importance in the North American and European countries due to it gluten-free and hypoglycemic property.

A few studies have focused on the nutrient quality of millet however documentation on the other types is limited. Millets are also preferred to be decorticated to improve sensory quality and bioavailability of nutrients (lestienne, et al., 2005; Shobana and Malleshi 2007). Decortication removes the germs and pericarp reducing the anti-nutrients but at the same time resulting in decrease of fibre, lipid, mineral and phenolic acid (Lestienne, et al., 2005; Shabana and Malleshi 2007). However, limited information exists in the comparison of nutrient composition in whole and decorticated millets. Millets are known to have low glycemic index as suggested by some in vivo studies however all of these studies have mainly focused on millet product from composite flour (Anju and Sarita 2010; Thathola et al., 2010 Shukla and Srivastava 2011). Starch digestibility studies on millet flour have been rarely done. Dietary fibre, phenolics and lipids which are mainly lost during decortication may affect in vitro starch digestibility (Singh et al. 2010; Venn and Mann 2013). Removal of protein and lipid or both has shown to significantly increase the expected glycemic index. (Annor et al., 2013a). However, there are few of no studies to compare the in vitro starch digestibility kinetics of resistant starch derived from millet from whole and decorticated grains.

The first part of the study evaluated the nutrient composition and in vitro starch digestibility kinetics of resistant starch.

MATERIALS AND METHODS

Description of the Study Area

This study was carried out in Jalingo Local Government area of Taraba state, Nigeria. The state has sixteen local government areas. The northern part of the state consists of; Ardo Kola, Lau, Karim Lamido, Yorro, Zing and Jalingo. Taraba state has an estimated population of 2 million people according to the 2006 population census. The state is located at $6^0$30' and $9^0$36' north and longitude $9^0$10 and $11^0$50' east. The state geographically consists of an undulating landscape dotted with few mountain features. Jalingo is one of the most successful local government areas in the state, it is also the most populated with a high range of development. Jalingo lies in the savanna-covered foothills of the Shebshi Mountain about 25 miles southeast of the Benue River. It is a market town, has a government dairy farm and is connected by road with Yola and Wukari. It has population inhabitants of 660,213 and a population density of 3456.6/km^2 (8,952.6/sq mi). Geographically it is located between latitude 8.9, and longitude 11.3667, 8^0 54'0" North, 11^0 22' 0" East. It holds the prime position of being the headquarters of the Muri Emirate Council and the capital city of Taraba state.

The study area has a land mass of about 19,100 hectares, 191.00km^2, the climate, soil and hydrology of the study area provide a conducive atmosphere for the cultivation of the most stable food crops, grazing land for animal and fresh water for fishing as well as forestry. The study area has an annual rainfall of 1200mm with an annual temperature of about 20^0C-29^0C. It is characterized by dry and rainy seasons common to tropical regions. The local government is situated on rich agricultural land, suitable for the cultivation of the following crops; Rice, Maize, Sorghum, Assorted Fruit Trees and vegetables, most of the people in Jalingo engage in agricultural activities. The ethnic groups of the study area are; Kona, Mumuye, Fulani, Jenjo and Yandang among others. Hausa and English language are the predominant languages in the study area as a medium of communication and little Fulani for social interactions

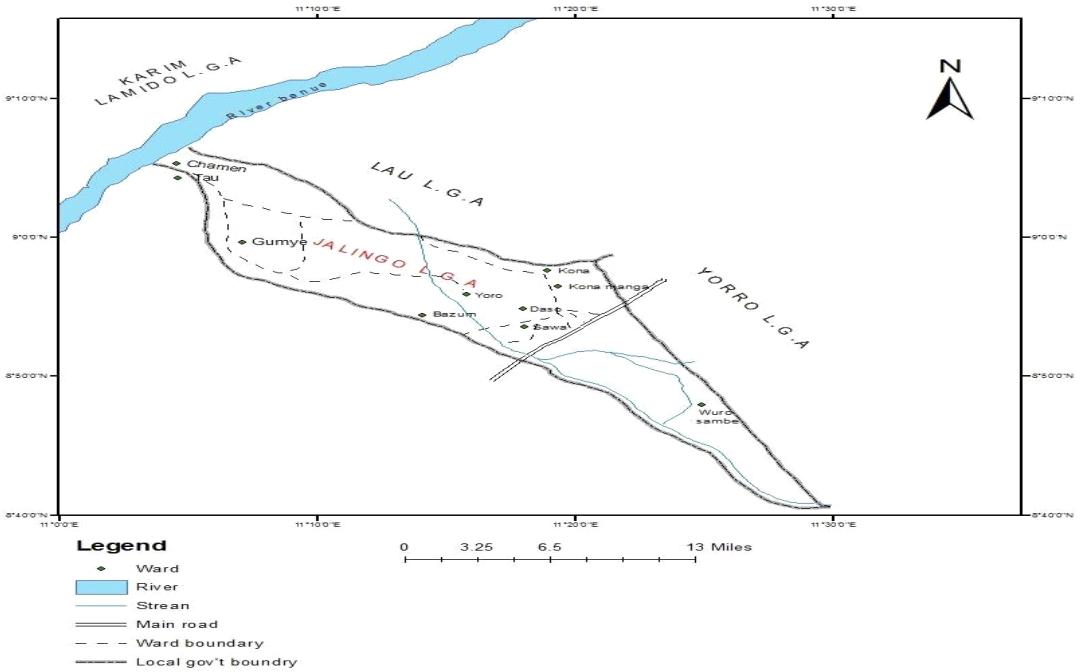


Fig 1: Map of Jalingo Showing the Study Area

The materials used are:

Muffle furnace, drying oven, blender, Centrifuge, volatile matter furnace, Beakers, weighing balance, x-ray diffractometer, glass slide, SEM (scanning electronic microscope).

Solvent and Reagent

Sodium sulphite, sulphuric acid, cupper anhydrous, sodium sulphite, sodium hydroxide, silicon (Si) standard, standard for recalibrating XRD machine, distilled water, boric acid, acetone, diethyl ether, ethanol, nitric acid, hydrochloric acid.

Sample Collection and Preparation/ Millet Seed Identification

Bambara groundnut was gotten from a local market at Ardo-kola local government area of Taraba State. The seed was screened to eliminate defective ones. Water was added to the sample and was left overnight. The seed coat was manually removed, the sample was divided into two; which is modified and unmodified. The modified was grinded using blender. Sodium sulphite was added to the modified portion and oven dried at 45°C while the unmodified was dried in an oven at 45°C also. It was grinded to fine powder using mortar and pestle. Both modified and unmodified flour was stored separately in a polythene bag and was kept in a refrigerator at 4°C prior to use.

However, in the case millet, it was identified by a botanist in biological science department. The defective millet seeds were separated and discarded. The seeds were screened and sieved to remove defective once and eliminate dust particles before further processes.

Millet seed was grinded into whole flour and sieved to remove excess bran. The semi-refined flour was soaked overnight in distilled water containing 0.01g/100g sodium azide to inhibit microbial growth. The soaked flour was screened through 60 and 150 mesh British standard sieved. The process was repeated until no more starch could be separated. The slurry obtained was washed several times with distilled water and centrifuged. The upper layer (protein) of the residue was removed with a spatula and discarded. The pH of the slurry was adjusted to 9.5 with diluted NaOH (0.1N), stirred for 15 min, and washed several times with distilled water to remove alkali. The lower layer (starch) was suspended in distilled water and stirred for 5hrs followed by centrifugation. It was washed until a neutral pH was reached. The steps were used in order to ensure we remove protein completely. The process was repeated twice to ensure complete protein removal. The crude starch was purified by suspending in NaCl (0.1N): Toluene (1:1) and stirred for 3hrs, followed by centrifugation. It was washed further for several times with distilled water to ensure better purification. Moreso, alcohol was added to the starch and stirred for 3hrs followed by centrifugation. The process was repeated with acetone. The white prime starch obtained were air dried and stored in air-tight polythene bags for further analysis.

CHEMICAL ANALYSIS

Proximate analysis of legume starch and flour were carried out based on the methodology of described by [9]. The moisture, Ash, Protein, fat, and carbohydrate contents were determined according to AOAC method, (2006) while the crude content was determined using to AOAC, (2000). All proximate analyses of the samples will be carried out in triplicate.

IN-VITRO DIGESTIBILITY WITH PANCREATIC AMYLASE AND ALPHA

In-vitro starch digestibility of the sample was determined using pancreatic amylase and alpha glucosidase (sigh et al. 1982). 50 mg of the sample was dispersed in 1ml of 0.2M phosphate buffer PH 6.9. 20mg of the enzymes was dissolve in 50 ml of the same buffer, and 0.2 ml of the and sample and the enzymes were added, 1ml of DNSA reagent was added into the mixture. The mixture was heated for 5minutes in a boiling water bath. The solution was allowed to cool and the absorbance were read at 540 nm against the blank containing buffer. The standard solution used was maltose. The experiment

was performed in triplicates and the set of data generated were analyzed statistically with Microsoft excel 2016 and recorded as the mean value after calculating the standard deviation (mean ± standard deviation).

Determination of Crystallinity Using X-ray Diffractometer (XRD)

The XRD (x-ray diffraction) machine was calibrated using silicon standard. The file ID was filled and scan it was scan from 2.0 degrees to 35.0 degrees with a step size of 0.03 degrees and dwell time of 0.5seconds. It was scan with the axes coupled. The sample was poured onto double stick tape mounted on glass slide at appropriate height on slide (aluminum holder was used to check tape heights). The slide was labeled with sample number using tape and indelible marker. The sample number was entered into XRD machine to identify electronic output. The sample was put into the XRD machine (Angstrom ADX2700) then analyzed.

Determination of morphology using scanning electronic microscope

A holder was selected large enough to hold the sample, carbon paint/tape was used to ensure a good conductive system. The carbon paint used was dried under the IR lamp for 2-3minutes. The microscope (SEM; product phenom, model prox, model number no: 800 - 07334) was set to the following settings;

X-control 25mm
Y-control 35mm
Stage height (z-control) 39mm
Rotation 000 graduates
Accelerating voltage (63) off (unit)
Filament (65) fully CCN

The specimen exchange chamber isolation valve undergo rotation, such that valve was completely open such that it could not be pulled any further. It was ensured there was light on inside the SEM illuminating the stage, the holder was inserted into the stage then the sample was analyzed.

Total utilized carbohydrates

The total carbohydrates content was determined by difference (Nascimento and canteri, 2018).

%carbohydrates=100/% - (%moisture +\ %protein + \%fat +\ % ash)

RESULT AND INTERPRETATION

Table 1: Proximate composition analysis of modified and unmodified millet sample

% content	Modified sample	Unmodified sample
Moisture content	6.33± 0.41	8.50± 0.35
Crude fiber	2.83± 0.53	3.50± 0.35
Fat content	1.33± 0.41	1.67± 0.41
Ash content	1.17± 0.18	2.50± 0.35
Protein content	8.75± 0.03	13.12± 0.25
Carbohydrate content	63.52± 0.56	57.83± 0.52

Proximate Composition

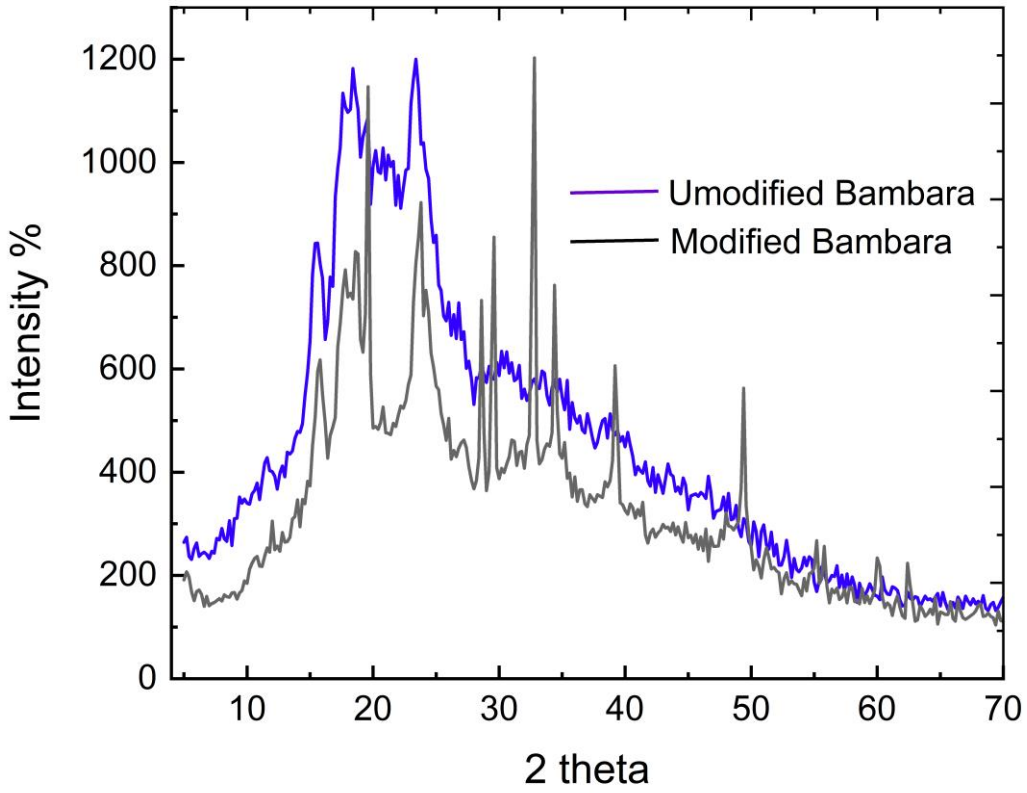
Samples	Moisture	Fat	Fiber	Protein	Ash	Carbohydrate
Modified sample	15% ± 0.5	1% ± 0.0	4% ± 1.0	17.50% ±	0.1 2.0% ± 0.2	60.5%
Unmodified sample	6.8% ± 1.0	6.5% ±	5.5% ± 0.5	22.76% ±	3.0% ±	64.94%

Note: Data are mean ± standard deviation of triplicate determinations.

Table 2: Determination of In-vitro digestibility.

	Modified Sample	Unmodified sample
Reducing sugar (mg/g)	0.55± 0.09	2.7 ± 0.06
Amylose (%)	35.10 ± 1.81	28.41 ± 0.20
Amylosepectin (%)	64.9 ± 1.81	48.41 ± 0.80
Starch damage (%)	73.04 ± 0.02	1.8 ± 0.05
Starch undamaged (%)	56.25 ± 0.08	62.40 ± 0.20

Crystallinity of modified and unmodified bambara groundnut starc



Sharp, high-intensity peaks in XRD pattern typically indicate the presence of well order crystalline material. The position of the peaks, represented as 2 theta (where theta is the diffraction angle) corresponds to the interatomic spacing within the crystal lattice. The intensity of the peaks is related to the number of atoms contributing to the diffraction and their arrangement, which influences the scattering strength. (*Muhammad Aug 2025).* The width of the peaks is inversely proportional to the crystal size. A thinner peak corresponds to a bigger crystal. A broader peak means that there may be smaller crystal defect in the crystalline structure. However, from fig.3 shows that both modified and unmodified sample have high peaks at 1190 on intensity and 15 on 2 theta, also 1200 on intensity and 25 on 2 theta for the unmodified. The modified sample have high peaks at 170 on intensity and 20 on 2 theta, also 1200 on intensity and 32 on 2 theta. Which mean that both modified and unmodified bambara groundnut starch has well order crystalline material. If fig. 3 is carefully observed it shows that the modified sample has thinner peaks which corresponds to bigger crystal. The unmodified have broader peaks which corresponds to smaller crystal. This result also shows that the modification process of the bambara groundnut starch did not destroy the crystallinity of the sample, rather reduced the crystal size. This result is in agreement with the report of Oyenyinka, et al., 2015).

Morphology of Modified and Unmodified Bambara groundnut Starch

fig.4a **fig.4b**

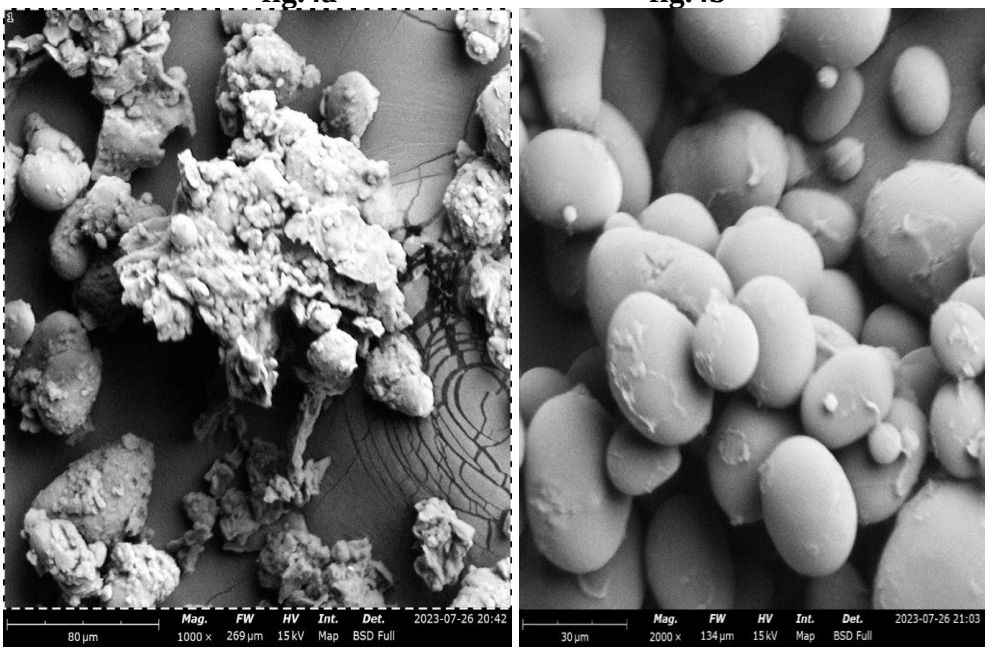
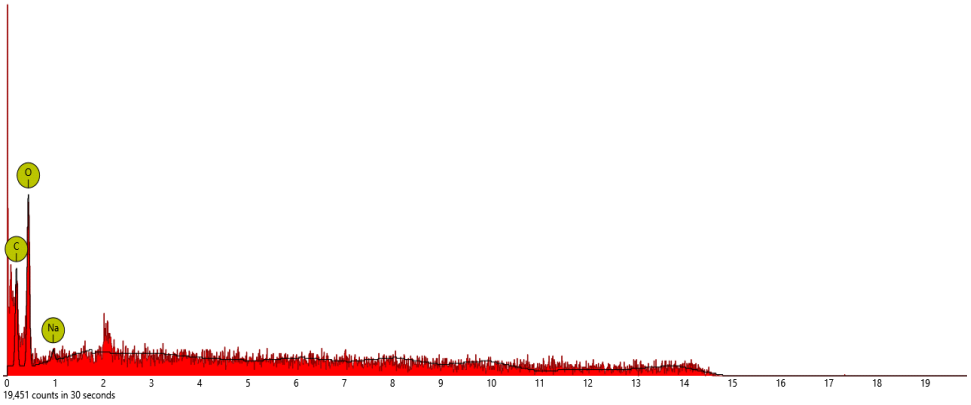


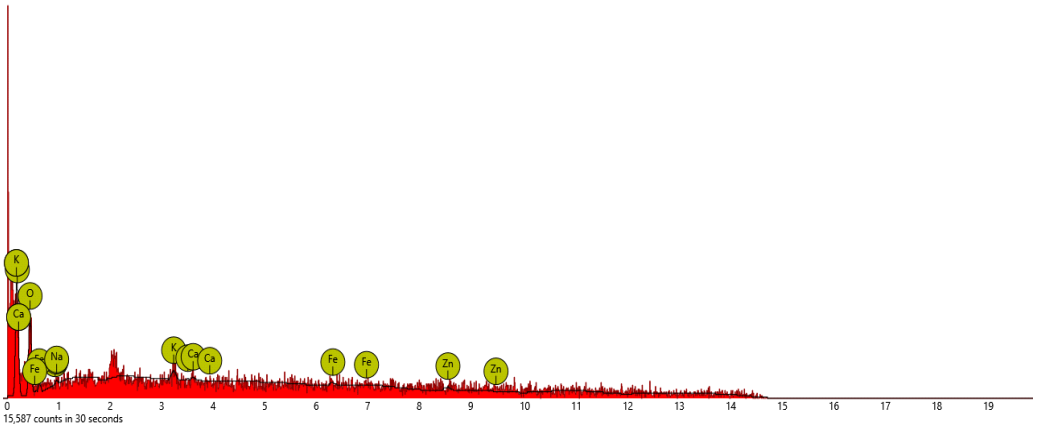
Fig. 4a Unmodified sample; the amorphous form characterizes the starch bambara groundnut
Fig. 4b Modified sample; the spherical form characterizes the starch bambara groundnut

Micrograph in fig. 4a showed a spherical form with no pores while that fig 4b has an amorphous morphology. The difference in morphology could be associated to method of sample preparation employed. I.e. the modification process alters the morphology of the original sample.

Table 3: Elemental composition of modified bambara groundnut Starch



Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	82.00	82.68
6	C	Carbon	12.68	9.60
11	Na	Sodium	5.33	7.72



Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	70.55	61.87
6	C	Carbon	19.95	13.13
30	Zn	Zinc	3.83	13.73
11	Na	Sodium	2.19	2.75
19	K	Potassium	1.90	4.06
26	Fe	Iron	1.12	3.43
20	Ca	Calcium	0.46	1.02

Heavy Metal Content of modified and unmodified bambara groundnut starch

Table 4: Unmodified sample

Element	Signal Abs	Rsd%	Conc. Mg/L	Corrected conc. Mg/L
Mn	- 0.001	14.9	0.0609	0.0609
Pb	0.002	19.3	0.1235	0.1235
Fe	0.006	8.6	0.2488	0.2488
Cd	- 0.007	3.8	0.0359	0.0359
Cu	0.001	15.3	0.0276	0.0276

Table 5: Modified sample

Element	Signal Abs	XRD %	Conc. Mg/L	Corrected conc. Mg/L
Mn	- 0.005	16.5	0.0219	0.0219
Pb	0.005	10.9	0.2732	0.2732
Fe	0.009	6.1	0.3479	0.03479
Cd	- 0.007	7.2	0.0367	0.0367
Cu	- 0.001	20.9	- 0.0021	- 0.0021

Abbreviations: Abs; absorbance, XRD; relative standard deviation, conc; concentration, mg/L milligram per liter.

Note: data are mean ± standard deviation of triplicate determinations.

The average moisture content of unmodified and modified bambara groundnut starch are shown in Table below. The result showed that the moisture content of the modified greatly increase compared to that of the unmodified sample.

Protein content

The average protein content of modified and unmodified bambara groundnut starch are shown in Table 1. The result showed that the protein content of the unmodified greatly increase, which means that the chemical added to the modified sample alter the composition of its nutrient. However, the modified sample has about 17.50% protein content and the unmodified sample has about 22.76%. Which is in agreement with the report of (Belewu et al., 2008) which says that bambara groundnut has about 17-25% protein depending on its variety.

Fiber content

The average fiber content of both modified and unmodified sample are shown in Table 1. However, the result showed that the fiber content of modified groundnut is about 4% which is less than that of unmodified sample which is about 5.5% (P >0.5). This result shows that there is more fiber content in the unmodified sample. This result agrees with the report Xin Lintan et al., (Dec 10, 2020)

Fat content

The average fat content of modified and unmodified bambara groundnut starch are presented in Table 1. The result showed that the fat content of modified sample was less compared to that of unmodified. However, the result of the unmodified sample agrees with the report of (Belewu et al., 2008)

Ash content

The average ash content of modified and unmodified bambara groundnut starch are presented in Table 1. The result showed that modified sample had lesser ash content compared to that of unmodified sample. The result of the ash content of the unmodified is in agreement with the report of Grace Ime et al., 2022 which says that bambara groundnut has about 3.2% of ash. The slight difference from the just obtained result could also be attributed to climate different and soil texture.

Carbohydrates content

The carbohydrates content of both modified and unmodified bambara groundnut starch are represented in Table 1. The result showed that carbohydrates content of both modified and unmodified bambara groundnut starch are represented in Table 1. The result showed that carbohydrates of unmodified sample have a greater carbohydrates content than that of modified sample. However, this is in agreement with the report of Belewu et al., 2008 which shows that carbohydrates occur about 63% in bambara groundnut. This shows slight difference with the result obtained from this analysis which could be attributed to variety difference which could be also due to climate different and soil texture.

Proximate Composition

Moisture content

The moisture content was determined according to AOAC (2010) official method number 925.10. Briefly, 2g sample was weighed in previously heated at 130 ± 3°C, cooled and weighed Then the dish and its content were heated in an oven at 130 ± 3°C for 1 hr. Finally, cooled in a desiccator to room temperature and weighed; the moisture content was then obtained from the weight loss by difference. This was done for both modified and unmodified samples.

Crude protein content

Protein was determined using the kjeldhal flask (JINOTECH), according AOAC official method 920.87 (2010). Briefly, bambara starch (1.0g) mixed with a catalyst (K_2SO_4 and $CuSO_4.5H_2O$) and digested in 12ml concentrated sulfuric acid at 420°C for 60min to liberate the organically bound nitrogen in form of ammonium sulfate. The ammonia in the digested ammonium sulfate then distilled off with reagent (50 ml distillation water, 50 ml NaOH, and 30ml H_3BO_3) added and then automatically titrated with standard hydrochloric acid (0.2N). Finally, a conversion factor of 6.25 used to convert from total nitrogen content to determine crude protein of the samples.

Fiber content

Crude fiber content was determined by the Weeknd's scheme as adopted by Bekele and Bekele (2018). About 1g of the dried sample was boiled for 40 min in 50ml dilute sulfuric acid (2.5%) and filtered. The residue then washed with distilled water and again boiled in 50ml of 2.5% of sodium hydroxide for 40 min. The residue consecutively washed with 20ml ethanol (99.8%) and 20ml diethyl ether two times, followed by washing with 20ml acetone three times. The insoluble residue consisted of crude fiber, and wash was dried in oven and weighed (w1). This residue was burned in an oven at 250°C for 6hrs, 30min (w2) and the weight difference (w1 - w2) taken as crude fiber.

Ether extract

The ether extract (taken as fat content) in the sample was determine by diethyl ether method, as described by Bekele and Bekele (2018). The dried bambara starch was extracted with diethyl ether for 1hr. Then, the extract was dried in an oven at 105°C for 3hrs. This extract was weighed and taken as crude fat content

Ash content

The ash content was determined using AOAC (2010) method number 923.03. A 2g sample was weighed put into crucible and ignited in an oven at 250°C until ash results, and the sample then placed in desiccator at room temperature and weighed. The remaining weight in the crucible was determined by difference and taken as ash content.

DISCUSSION

The average moisture content of unmodified and modified bambara groundnut starch showed that the moisture content of the modified greatly increase compared to that of the unmodified sample. The finding on the average protein content of modified and unmodified bambara groundnut starch also revealed that the protein content of the unmodified greatly increase, which means that the chemical added to the modified sample alter the composition of its nutrient. However, the modified sample has about 17.50% protein content and the unmodified sample has about 22.76%. Which is in agreement with the report of (Belewu, et al., 2008) which says that bambara groundnut has about 17-25% protein depending on its variety.

In respect to the average fiber content of both modified and unmodified sample, the result showed that the fiber content of modified groundnut is about 4% which is less than that of unmodified sample which is about 5.5% (P >0.5). This result shows that there is more fiber content in the unmodified sample. This result agrees with the report Xin Lintan et al., 2020) According to the average fat content of modified and unmodified bambara groundnut starch, the result showed that the fat content of modified sample was less compared to that of unmodified. However, the result of the unmodified sample agrees with the report of (Belewu, et al., 2008)

Based on the average ash content of modified and unmodified bambara groundnut starch, the result showed that modified sample had lesser ash content compared to that of unmodified sample. The result of the ash content of the unmodified is in agreement with the report of Grace Ime et al., 2022 which says that bambara groundnut has about 3.2% of ash. The slight difference from the just obtained result could also be attributed to climate different and soil texture. The carbohydrates content of both modified and unmodified bambara groundnut starch are represented, the result showed that carbohydrates content of both modified and unmodified bambara groundnut starch are represented in Table 1. The result showed that carbohydrates of unmodified sample have a greater carbohydrates content than that of modified sample. However, this is in agreement with the report of Belewu, et al., 2008 which shows that carbohydrates occur about 63% in bambara groundnut. This shows slight difference with the result obtained from this analysis which could be attributed to variety difference which could be also due to climate different and soil texture.

Modified starch exhibits low percentage amylose and amylosepectin content. A high value of amylose amylosepectin ratio indicate low glycemic index (Dipnaik and Kokare, 2017). Frie et Al., (2003) reported that the rate of hydrolysis of starch is fast which contains high amount of amylopectin. The amylose -amylopectin ratio was determined in all cultivars viz. millet. It was observed that the amylose and amylopectin ratio was found maximum in pant Basmati 2 followed by pant Dhan 19.

Modified and unmodified starch from millet show that the modified has higher damage starch than the unmodified starch which implied that vast numbers of inter-molecular bonds in the unmodified starch as reported by Okkada K. Negeshi Y., Nagao S.., studies on heavy ground floor using roller Mills I.

Some heavy metals are not naturally present in food samples but concentration depends on the soil where they are grown and it has been established that some of them are necessary for life. However, when the concentration of the heavy metals is very high beyond certain tolerable limits, they become toxic. Iron is reported to be very important for normal functioning of the central nervous system. Iron also facilitates the oxidation of carbohydrates, protein and fats. The daily intake of iron is about 16 to 18mg for men and 12mg for some in child bearing age and 8mg for women in their menopause.

However, the iron content present in the modified and unmodified sample is about

0.3479 mg/L and 0.2488 mg/L respectively, which is far lesser than the required daily dosage. If one is looking for a food with high iron than bambara groundnut is not the choice of food to go for. Cadmium is a toxic heavy metal that can be harmful to both humans and their environment. It can cause a variety of health problems if it's ingested or inhaled, and it can accumulate in the body over time. Exposure to cadmium can also leads to kidney damage, respiratory problems and even cancer. The food and drug administration (FDA) set limit on the amount of cadmium that is allowed in food. The FDA's current limit is 0.1mg/L and 0.5mg/L in food that are high in calcium.

However, from the result obtained in table 2 and 3 shows that there is no harm associated with consuming bambara groundnut grown within this research area because the cadmium present in the samples is below the allowed limit given by FDA. The FDA sets limit for lead (Pb) is 0.1mg/L and 0.5.mg/L for foods that are high in calcium. But even at these levels, lead can still pose a health risk, especially for children. The FDA has proposed lowering the limits for leads in foods, because it is dangerous especially for children developing their brains and nervous system. This is because children's bodies are still growing and developing, so they absorb more lead than adults do. They also tend to put their hands in their mouth and eats things they find on the ground which can increase their exposure lead. And because their nervous system is developing, lead can interfere with that process and cause lifelong problems with thinking, behavior and learning. However, from table 2 and 3 shows that lead content in the modified sample is in agreement with the FDA allowed limits for Pb in food. But not that of the modified sample since it is beyond the tolerable limits it may cause negative effect on consumption.

Copper is another essential heavy metal, and it's required for the body to make red blood cells and connective tissue it's also important for maintaining healthy nerves, bones and immune system function. But too much copper can cause health problems like nausea, vomiting, diarrhea abdominal pain and liver damage. Copper deficiency can cause a variety of symptoms including anemia, low white blood cell count, low platelet count, neurological problems and osteoporosis. Copper is an anti-microbial agent. This means that copper can kill bacteria and too much of copper can also be toxic. However, the result obtained from table 2 and 3 shows that copper present in both modified and unmodified sample is less than the required daily intake. If one is looking for a food with high copper content, then bambara groundnut is definitely not the choice of food to consider.

Manganese is an essential heavy metal, but it can also be toxic in large doses. Small amount of manganese is necessary for the body to make enzymes, but too much of it can damage the nervous system and the kidney. It's found in variety of food including whole grain, leafy green vegetables, nuts and seeds. Some people are exposed to high levels of manganese through drinking water that comes from wells in areas with manganese-rich rock. The wealth health organization recommends a daily intake of 2-5mg for adults. Some researchers suggested that manganese intake over 11mg per day could harmful, so it's important to pay attention to the levels in your diet. However, the manganese content in bambara groundnut is less than the required daily intake; result is shown in table 2 and 3 which is in agreement with the report of Aremu et al., 2006)

CONCLUSION

In conclusion bambara groundnut can be a notable source of resistant starch as earlier discussed, it has a lot of nutritional and health benefits. The resistant starch derived from bambara groundnut is known to have so many health benefits such as anti-inflammatory and anti-tumor actions. This resistant starch also has high nutritional value. However, it's also rich with metals such copper which enables the body make red blood cells, manganese which is

responsible for the production enzymes in the body and iron which is responsible for normal functioning of the central nervous system. Bambara groundnut could be an underutilized legume but it's also of great importance.

RECOMMENDATION

Based on the findings the research on this study recommends the use of furnace for ashing because it takes lesser time to yield desired result. This study also recommends further research, such as employing the crystallinity of the sample to study the chemical and physical properties

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REFERENCES

1. Abbas KA, Sahar KK. 2012. Modified starches and their usages in selected food products: A Review study. J Agric Sci. 2(2): 90 - 100.
2. Adrinna Bojarck, syluria skapska, Anin Mousavi khanegjack, Krystain Marszaclak, (2022): publication; journal of functional foods.
3. Alcazar-Alay SA, Meireles MAA. 2015. Physicochemical properties, modifications, and applications of starches from different botanical sources. Food Sci Technol. 35(2): 34 - 42. http://dx.doi.org/10.1590/1678-457X.6749.
4. Aleskaa Ristic, (2018): medicinal properties of resistant starch,Aravin Underutilized legumes.
5. A.M Brikelt, l. L Brown, (2008): Technology of functional creal and legumes products
6. Aremu, (2006): assessment of heavy metal content in some selected agricultural products. Department of chemistry, Nassarawa state university.
7. Association of Official Analytical Chemists. 2000. Official methods of analysis of AOAC International. 17th edition. P. Culliff, AOAC International Arlington, Virginia, USA 22 - 24.
8. Babu BV, Ramana T, Radhakrishna TM 1987. Chemical composition and protein in hybrid varieties of finger millet. Indian J Agric Sci. 57(7): 520 - 522.
9. Berski W, Ptaszek A, Ptaszek P, Ziobro R, Kowalski G, Grzesik M, Achremowicz B. 2011. Pasting and rheological properties of oat starch and its derivatives. *Carbohydrate Polymers*, 83(2): 665 - 671. http://dx.doi.org/10.1016/j.carbpol.2010.08.036.
10. Bhatt A, Singh V, Shrotria, PK, Baskheti DC. 2003. Coarse grains of Uttaranchal: Ensuring sustainable food and nutritional security. Indian Farmer's Digest, 34-38.
11. Bwai MD, Afolayan M, Odukomaiya D, Ikokoh P, Orishadipe A. 2014. Proximate composition, mineral and phytochemical constituents of *Eleusine coracana* (finger millet). Int J Advan Chem. 2(2): 171 - 174.
12. Chiemela Enyuinaya chinma, (2022): structure, in vito starch digestibility and physicochemical properties of starch isolated from germinated bambara groundnut.
13. Diane F. Birt, Terri Boylston and Elizabeth M. whitely, (2013): resistant starch: promise for improving health.
14. Dr Mack Weisskopf of the Harcerd T. H chan, (2011): school of public health blow manganese exposure and cognitive function
15. Englyst & Cummings, (1987): digestion of polysaccharides of potato in the small intestine of man. American journal of clinical nutrition, 45, 423 – 431
16. FAO 1982; Italian, (2019): journal underutilized legumes in West Africa
17. Gideon G, Doss R. 2003. Nutritive evaluation of extruded and enzyme supplemented small millets in broiler chicken. Ph.D. Thesis, University of Agricultural Science, Bangalore, India. pp.201.
18. H. N senfur, M. D Matlock, (2018): Nutrition and environment impact of resistant starch M.Gulzar, A Manmmar, (2016): underutilized protein resources from African legumes. University of Pretoria, Hatfield 2015 Protoria South Africa.
19. Mathanghi SK, Sudha K. 2012. Functional and phytochemical properties of finger millet *(Eleusine coracana)* for health. Int J Pharm Chem Biol Sci. 2(4): 481 - 438.
20. Mist, Jang, (2015): Technology of functional legumes. A functional food

journal on; Resistant start h type and health benefits
21. Miyazaki MR, Hung PV, Maeda T, Morita N. 2006. Recent advances in application of modified starches for bread making. Trends Food Sci Technol. 17: 591 - 599.
22. Muazu J, Musa H, Isah AB, Bhatia PG, Tom GM. 2011. Extraction and characterization of *kaffir* potato starch. A potential source of pharmaceutical raw material. J. Natural Products Plant Resources, 1(2): 41 - 49.
23. Muhammad Kashif (2023): Adul wali Khan University Marden Phytochemical constituent and proximate Composition of Bambara groundnut and pigeaon pea underutilized in Calabar cross river state.
24. Perez S, Bertoft E. 2010. The molecular structures of starch components and their contribution to the architecture of starch granules: a comprehensive review. Starch/Staerke, 62(8): 389 - 420. http://dx.doi.org/10.1002/star.201000013.
25. Samson A. Oyenyinka, Aminat O. Abdulsalam, (2021): Total phenolic content, antioxidant, anti-inflammatory and anti-microbial potential of Bambara groundnut (Vigna substreranea) seed extract.
26. Samson A. Oyenyinka, Suren Sing, Patric Adebola, (2015): physicochemical properties of starches with variables amylose content extracted from bambara groundnut genotypes.
27. Saeid A, Hoque S, Kumar U, Das M, Muhammad N, Rahman MM, Ahmed M. 2015. Comparative studies on nutritional quality of commercial wheat flour in Bangladesh. Bangladesh J Sci Ind Res. 50(3): 181-188.
28. Saldivar S. 2003. Cereals: dietary importance. In B. Caballero, Trugo, L. and Finglas, P. (Eds.), Encyclopedia of Food Sciences and Nutrition, Reino Unido: Academic Press, Agosto, London. pp. 1027 - 1033.
29. Seetharam A. 2001. Annual Report 2000-01 All India coordinated small millets improvement project, Bangalore.
30. Shashi BK, Sharan S, Hittalamani S, Shankar AG, Nagarathna TK. 2007. Micronutrient composition, anti-micronutrient factors, and bioaccessibility of iron in different finger millet (*Eleusine coracana*) genotype. Karnataka. J Agric Sci. 20(3): 583 - 585.
31. Singh J, Kaur L, McCarthy OJ. 2007. Factors influencing the physico-chemical, morphological, thermal, and rheological properties of some chemically modified starches for food applications. A review. Food Hydrocoloids, 21: 1 - 22.
32. Singh P, Srivastava S. 2006. Nutritional composition of sixteen new varieties of finger millet. J Community Mobilization Sust Develop. 1(2): 81-84.
33. Sira EE, Amaiz ML. 2004. A laboratory scale method for isolation of starch from pigmented sorghum. J Food Engineer. 64: 515519.
34. T.Adenyi Afolabi, (2017): invitrodisgistability of hydrothermally modified bambara groundnut (Vigna substreranea) starch and flour. Department of chemistry Federal University Abeokuta, Nigeria
35. Tukura BW, Obelle FN. 2016. Functional characterization of blended starch. Int J Biochem Biophys Molecul Biol. 1(1): 25-30http://dx.doi: 10.11648/j.ijbbmb.20160101.14
36. Xinlin Tan, (2020): bambara groundnut: An underutilized leguminous crop for global food security and nutrition
37. Yadav BS, Guleria P, Yadav RB. 2013. Hydrothermal modification of Indian water chestnut starch: Influence of heat-moisture treatment and annealing on the physicochemical, gelatinization, and pasting characteristics. Food Sci Technol. 53(1): 211 - 217.
38. Yousif EI, Gadallah MGE, Afaf MS. 2012. Physico-chemical and rheological properties of modified corn starches and its effect on noodle quality. Annals Agric Sci. 57(1): 19 - 20.

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