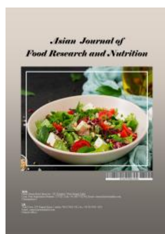

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Anti-Nutrients Composition of Starch Isolated from Red and White Sorghum Cultivars Subjected to Different Steeping Time

A. B. Zubair [a*], M. O. Ojo [a], F. A. Femi [a], Y. M. O. Maxwell [a], L. R. Isah [b] and J. O. Owheruo [c]

[a] Department of Food Science and Technology, Federal University of Technology, P.M.B. 65, Minna, Niger State, Nigeria.
[b] Department of Food and Home Science, Kogi State University Anyigba, Kogi State, Nigeria.
[c] Department of Food Science and Technology, Delta State University of Science and Technology, Ozoro, Delta State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Sorghum is a food crop that serves as a major source of calories to a substantial number of populations living in semi-arid tropics of Africa, Asia and other countries of the world. Steeping processing techniques improves nutritional qualities, palatability and consumer acceptability of foods crops. The effects of steeping periods on the anti-nutrient composition of starch isolated from two varieties of sorghum (red and white) were investigated. Sorghum steeped for period of 6, 12, 18 and 24 h with water were processed into starch and sample from unsteeped sorghum was used as

**Corresponding author: E-mail: b.zubair@futminna.edu.ng;*

control. The starch samples were analysed for anti-nutrients components using standard analytical procedure. Anti-nutrients investigated varied with cultivars and increased steeping time. Hydrogen cyanide observed was in the range of 1.34 to 1.86 mg/kg, tannin 1.30 to 4.82 mg/g, saponin 6.10 to 13.05 mg/g, oxalate 0.43 to 0.90 mg/g, phytate 2.15 to 9.06 mg/g, cardiac glycoside 9.30 to 13.35 mg/g, terpenoid 13.50 to 18.99 mg/g and alkaloid 3.41mg/g to 5.03 mg/g. The objective of the research is to determine the anti-nutrients composition of starch isolated from red and white sorghum. Significant difference was not observed between starch from the two cultivars of sorghum in most of the parameters investigated. However, the red cultivar recorded higher values in some of the anti-nutrients investigated. The anti-nutrient levels significantly reduce with increase in steeping time thus improving bioavailability of mineral element and other nutrients found in sorghum. The mixture of two varieties (red and white) could find applications in food formulations.

Keywords: Anti-nutrients; starch; steeping; sorghum.

1. INTRODUCTION

Sorghum is regarded as one of the vital cereal crops in the world and the most grown in Sub-Saharan Africa [1]. “It is a versatile crop serving as a staple food for millions of people and regarded as an important source of calories and different nutrients” [2]. “Sorghum is generally high in carbohydrate, low in both quantity and quality protein as well as limiting in lysine, threonine, methionine, and tryptophan” [3]. “The colour of the grains varies from white to dark brown depending on the type of phenolic pigments present in them. The seed coat contains abundant polyphenolic compounds that combine with other flavonoids (anthocyanins, anthocyanidins, e.t.c.) to give it various colours” [4]. “The germ fraction of sorghum rich in minerals, protein, and lipids as well as B-group vitamins: thiamine, niacin and riboflavin” [5]. “Despite an impressive array of nutrients in sorghum, sorghum-based foods product has continued to be nutritionally deficient and organoleptically inferior due to the presence of anti-nutrients that make them to be unavailable for human absorption” [6].

“Starch is the most abundant carbohydrate in food plants that is deposited in plant parts in the form of small granules or cells ranging from 1 up to 100 µm” [7]. “They are usually processed by wet milling, whereby the seed or tuber is milled, followed by the separation of the main constituents such as starch, fiber and protein” [8]. “Starch granules contain two polymers namely linear polysaccharide (amylose 20 to 25%) and highly branched polysaccharide (amylopectin 75 to 80%). Anti-nutritional factors are primarily associated with compounds or substances of natural or synthetic origin that interfere with the absorption of nutrients thereby reducing nutrient intake, digestion, and utilization as well may produce adverse effect” [9]. “The presence of anti-nutrients in food limits the digestibility of proteins and carbohydrates through inhibition of their respective proteolytic and amylolytic enzymes” [6]. Steeping is a unit operation that help to facilitates processing of sorghum or other crops whereby the cereals or the legumes are kept inside water for a specific period [10]. Adequate steeping of sorghum is needed development of value-added products like a highly nutritious and easy-to-digest whole-grain food with robust flavor [11]. Steeping of sorghum significantly reduce anti-nutrient content of sorghum leading to an improvement in protein digestibility and other protein quality characteristics [12]. Knowledge of anti-nutrient in sorghum during processing is required so that the potential of sorghum can be evaluated and to develop processing method that will remove completely or reduce these undesirable components to improve the nutritional quality of cereals-based food products. Hence, the objective of the study is to determine the effect of different steeping time on the anti-nutrient component of starch isolated from white and red varieties of sorghum.

2. MATERIALS AND METHODS

Red and white sorghum grains were purchased from a local market in Akure, Ondo State, Nigeria. All chemicals used were of analytical grade.

2.1 Sample Preparation

Sorghum grains were subjected to manual cleaning and sorted to remove the husks, stem, damaged and discolored seeds by winnowing, hand picking and washing with tap water. The modified method described by Singh, et al. [8] was used in the production of the starch. Two

Hundred and Fifty grams (250 g) of the sorghum was washed with tap water, steeped for 6, 12, 18, 24 h and thereafter subjected to a wet milling process, while control sample was only washed and wet milled without steeping. The slurry obtained was filtered through a 100 µm mesh screen and the filtrate allowed to sediment overnight, decanted and reslurried twice before final decantation. The resultant starch cake was oven dried at 50 ^{0}C for 6 h, milled and sieved through 0.25 µm mesh screen to get a fine sorghum starch powder.

2.2 Sample Analyses

2.2.1 Determination of alkaloid content

The alkaloid content was determined gravimetrically as described by Uchenna and Otu [13]. Five grammes of the sample was weighed (w_1) and dispersed in 50 ml of 10% acetic acid solution in ethanol. The mixture was well shaken and allowed to stand for 4 h before it was filtered. The filtrate was evaporated to one quarter of its original volume on hot plate and concentrated ammonium hydroxide was added drop wise to precipitate alkaloids. A pre-weighed filter paper (w_2) was used to filter off the precipitate and washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was dried in an oven at 60 ^{0}C for 30 min, transferred into desiccator to cool and reweighed (w_3) until a constant weight was obtained. The alkaloid content was determined by weight difference of the filter paper and expressed as percentage of the sample weight analyzed.

$$\% \text{ Alkaloid} = \frac{W_3 - W_2}{W_1} \times 100$$

2.2.2 Determination of Terpenoid Content

Terpenoid content was determined as described by Uchenna and Otu [13]. Approximately 0.5 g of the sample was weighed into a 50 ml conical flask, followed by the addition of 20 ml chloroform: methanol 2:1, shaken thoroughly and allowed to stand for 15 min at room temperature. The suspension was centrifuge at 3000 rpm, the supernatant discarded, and the precipitate was re-washed with 20 ml chloroform: methanol 2:1 and re-centrifuge again. The precipitate was dissolved in 40 ml of 10% SDS solution. About 1 ml of 0.01 M ferric chloride was added and allowed to stand for 30 min before taking the absorbance at 510 nm.

2.2.3 Determination cardiac glycosides

The cardiac glycoside was determined using the procedure described by Uchenna and Otu [13] was used; 10 ml of the sample extract was pipetted into a 250 ml conical flask. About 50 ml chloroform was added and shaken on vortex mixer for 1 h, the mixture was filtered into 100 ml conical flask. Thereafter 10 ml of pyridine and 2 ml of 29% of sodium nitro prusside were added and shaken thoroughly for 10 min, 3 ml of 20% NaOH was added to develop a brownish yellow color and absorbance was read at 510 nm.

2.2.4 Determination of saponin content

The spectrophotometric method described by Aiwonegbe et al. [14] was used. Two grammes of the sample was weighed into a 250 ml beaker and 100 ml of isobutyl alcohol added. The mixture was shaken for 5 h to ensure uniform mixing. The mixture was filtered with filter paper into 100 ml beaker containing 20 ml of 40% saturated solution of magnesium carbonate ($MgCO_3$). The mixtures obtained was again filtered through filter paper to obtain a clean colorless solution. About 1 ml of the colorless solution was transferred into 50 ml volumetric flask, 2 ml of 5% iron (iii) chloride ($FeCl_3$) solution was added and made up to the mark with distill water. It was allowed to stand for 30 min for color development and absorbance was read against the blank at 380 nm.

2.2.5 Determination of tannin content

Tannin content was determined as described by Aiwonegbe et al. [14]. About 0.2 g of the sample was weighed into a 50 ml sample bottle. Approximately 10 ml of 70% aqueous acetone was added and properly covered, the bottle was place in an ice bath shaker and shaken for 2 h at 30 ^{0}C. The solution was centrifuge and the supernatant store in ice, 0.2 ml of the solution was pipetted into the test tube with the addition of 0.8 ml of distilled water. Standard tannic acid solutions were prepared from a 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. 0.5 ml of Folinciocateau reagent was added to both sample and standard followed by 2.5 ml of 20% Na_2CO_3 the solution was then vortexed and allow to incubate for 40 min at room temperature, absorbance was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve prepare.

2.2.6 Determination of cyanide content

The cyanide content was determined as described by Aiwonegbe et al. [14]. Four grammes of the sample was soaked in a mixture of distilled water (40 ml) and orthorphosphoric acid. The sample was thoroughly mixed and stored, left at room temperature overnight to set free all bounded hydrocyanic acid. The resulting mixture was transferred into distillation flask and a drop of paraffin was added to the broken chips. The flask was fitted to other distillation apparatus and distilled. About 45 ml of the distillate was collected in the receiving flask containing 4 ml of distilled water and 0.1 g of sodium hydroxide pellets. The distillate was transferred into 50 ml volumetric flask and made up to mark with distilled water, 1.6 ml of 5% potassium iodide was added and titrated against 0.01 M Ag $(NO3)_2$.

Endpoint indicated by faint but permanent turbidity and total HCN content in mg/kg was calculated as:

HCN content = 13.5 X TV/M
TV = titre value
M = mass of sample

2.2.7 Determination of oxalate content

Total oxalate content was assayed using the method described by Aiwonegbe et al. [14]. Oxalate was precipitated as insoluble calcium oxalate which was collected by centrifugation. The precipitate was dissolved in an excess hot dilute H_2SO_4 and titrated against standardized $KMnO_4$. Two grammes of the sample was weighed into 250 ml beaker, 150 ml distilled water and 55 ml 6 M HCl added. Two drops of alcohol were added, and the mixture boiled for 15 min, cooled, and transferred quantitatively into 500 ml volumetric flask, diluted to volume with distilled water and mixed again. The mixture was allowed to stand overnight, mixed thoroughly, and filtered with filter paper. About 25 ml of the filtrate was pipetted into 50 ml flasks and 5 ml tungsto phosphoric acid added, mixed, and allowed to stand for 5 h. The mixtures were filtered, and 20 ml of the filtrate was pipetted into centrifuge tube followed by the addition of ammonium hydroxide solution drop wise until a pH of 4.5 was achieved using indicator paper. About 5 ml acetate buffer (pH 4.5) was added to maintain a constant pH. The mixture was allowed to stand overnight at room temperature after which it was centrifuged for 15 min at 1700 rpm to compact the precipitate. Supernatant was carefully decanted, and calcium oxalate precipitates washed three times with centrifugation and decantation using cold washing liquid. The precipitate was re-dissolved in 5 ml dilute H_2SO_4 (1.9 v/v), the diluted H_2SO_4 also served as the blank solution. The mixture was heated in a boiling water bath for 15 min and the hot solutions titrated with 0.01 N $KMnO_4$ until a persistent pink colour was obtained. Oxalate content was calculated as follows:

Mg oxalate/100g sample = ml of 0.01 N $KMnO_4$ / Weight of sample × 1350

Where

1350 = 0.45 (mg oxalic acid equivalent to 1 ml 0.01 N $KMnO_4$) × [(30/20) × (50/25)

dilution factors] × 100 (to convert to 100g sample).

2.2.8 Determination of phytate content

Phytate content was determined according to the method described by Aiwonegbe et al. [14]. Four grammes of sample was soaked in 100 ml of 2% HCl for 3 h, filtered with filter paper, 25 ml was taken, transferred into conical flask and 5 ml of 0.3% of ammonium thiocyanate solution was added as indicator. After which 53.5 ml of distilled water was added to give it the proper acidity and this was titrated against 0.00566 g per milliliter of standard iron (iii) chloride solution that contain about 0.00195 gram of iron per milliliter until a brownish yellow coloration persist for 5 min. The amount of phytic acid was calculated thus:

Phytic acid (mg/g) = Titre value × 8.24

2.3 Statistical Analysis

All the experiments were carried out in triplicate. Data obtained were subjected to analysis of variance (ANOVA) and the means were separated by lowest standard deviation test (SPSS version 16) and significant level of 5% was accepted.

3. RESULTS AND DISCUSSION

The anti-nutrients composition of starch isolated from red and white sorghum cultivar is presented in Table 1. Hydrogen cyanide recorded was in the range of 1.34 mg/kg to 1.86 mg/kg, tannin content was in the range of 1.30 mg/g to 4.82

Table 1. Anti-nutrient composition of starch isolated from red and white sorghum cultivar subjected to different steeping time

Variety	Steeping time (h)	Hydrogen cyanide (mg/kg)	Tannin (mg/g)	Saponin (mg/g)	Oxalate (mg/g)	Phytate (mg/g)	Alkaloids (mg/g)	Terpenoid (mg/g)	Cardiac glycoside (mg/g)
Red	0	1.86±0.01[a]	4.82±0.01[a]	13.05±0.06[a]	0.90±0.01[a]	9.06±0.01[a]	5.03±0.06[a]	18.99±0.01[a]	13.35±0.21[a]
	6	1.83±0.01[ab]	2.72±0.02[c]	12.44±0.62[bc]	0.85±0.01[a]	8.94±0.08[ab]	4.92±0.35[ab]	17.75±0.35[b]	12.21±0.01[b]
	12	1.66±0.01[c]	2.21±0.04[d]	8.73±0.01[d]	0.71±0.01[b]	6.20±0.28[c]	3.80±0.05[c]	16.14±0.11[c]	10.95±0.07[c]
	18	1.48±0.01[d]	1.82±0.01[e]	6.57±0.01[e]	0.56±0.01[cd]	3.30±0.00[d]	3.58±1.21[d]	14.04±0.04[d]	10.20±0.28d[e]
	24	1.34±0.01[e]	1.69±0.01[e]	6.15±0.07[e]	0.44±0.06[e]	2.40±0.28[e]	3.42±0.35[e]	13.70±0.14[d]	9.50±0.42[f]
White	0	1.83±0.01[ab]	3.50±0.14[b]	12.90±0.14[ab]	0.88±0.03[a]	9.02±0.02[ab]	4.90±0.00[b]	18.70±0.14[a]	13.15±0.07[a]
	6	1.82±0.02[b]	2.10±0.14[d]	12.30±0.42[c]	0.76±0.01[b]	8.70±0.14[b]	4.83±0.42[b]	17.60±0.57[b]	12.05±0.07[b]
	12	1.64±0.01[c]	1.70±0.14[e]	8.50±0.14[d]	0.61±0.01[c]	6.10±0.14[c]	3.82±0.28[c]	16.00±0.01[c]	10.70±0.14[cd]
	18	1.47±0.01[d]	1.40±0.14[f]	6.30±0.14[e]	0.54±0.01[d]	3.32±0.03[d]	3.65±0.71[d]	13.95±0.07[d]	10.10±0.14[e]
	24	1.34±0.01[e]	1.30±0.14[f]	6.10±014[e]	0.43±0.04[e]	2.15±0.07[e]	3.41±0.14[e]	13.50±0.14[d]	9.30±0.42[f]

Values are mean ± standard deviation of triplicate determinations. Means in the same column with different superscript are significantly different ($p≤0.05$).

mg/g, saponin content was in the range of 6.10 mg/g to 13.05 mg/g, oxalate content was in the range of 0.43 mg/g to 0.90 mg/g, phytate content was in the range of 2.15 mg/g to 9.06 mg/g, alkaloid content was in the range of 3.41% to 5.03 mg/g, terpenoid content was in the range of 13.50 mg/g to 18.99 mg/g and cardiac glycoside between 9.30 mg/g to 13.35 mg/g. All anti-nutrients investigated showed significant decrease ($p \leq 0.05$) as the steeping time increased. This finding is in line with the observation of [15] who reported that grains showed significant decrease in anti-nutritional composition when subjected to steeping and this could be considered as an effective way to reduce the risk of mineral deficiency among populations especially in developing countries where unrefined cereals are highly consumed. The reduction observed in the anti-nutrient because of the steeping could be attributed to leaching of the anti-nutrients in the steeping water considering a change in colour of steep water. In addition to leaching, increased enzymatic hydrolysis could have facilitated the reduction of the anti-nutrients [15]. The result obtained for tannin agreed with the observation of Melaku, et al. [5] who reported that white colored sorghum contained less tannin than the red colored sorghum. Reduction in tannin content could be attributed to the leaching of polyphenol into the water because tannin are polyphenolic compounds that are readily soluble in water and mostly located in the seed coat [16]. Hemalatha, et al. [17] reported that steeping substantially reduced the tannin content in most food grains. “Phytate might have been significantly affected by the endogenous enzyme like phytase activated during steeping. Phytase degrade phytate into inorganic phosphorous and inositol and its intermediate forms” [18]. Phytate are the principal storage form of phosphorous and are particularly abundant in cereals and legumes.

The oxalate content range of 0.43 mg/g to 0.90 mg/g recorded in this research was similar to the range of 0.36 mg/g to 0.57 mg/g reported by Opeyemi, et al. [16] for germinated sorghum flour. The rate of reduction in tannin because of steeping was less than that of phytate possibly because while phytate is degraded by enzyme, tannin is only leached out [19]. Combined effects of physical and enzymatic actions on the sorghum grains during steeping significantly reduced the concentration of antinutrients.

4. CONCLUSION

Significant reduction was observed in the level of the anti-nutrient composition with an increase in steeping time thereby improving the bioavailability of mineral in the resultant product. There was no significant difference in the starch from the two cultivars of sorghum (red and white) in most of the parameters investigated. However, the difference observed in some results could be attributed to isolation method. Mixture of two varieties (red and white) could find better applications in food formulations in the food industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sobowale SS, Adebo OA. Mulaba-Bafubiandi AF. Production of extrudate pasta from optimal-sorghum-peanut flour blend and influence of composite flours on some quality characteristics and sorption isotherms. Trans. Royal Soc. S. Afr. 2019; 74:268–275.
2. Odunmbaku LA, Sobowale SS, Adenekan MK, Oloyede T, Adebiyi JA, Adebo OA. Influence of steeping duration, drying temperature, and duration on the chemical composition of sorghum starch. Journal of Food Science and Nutrition. 2018;6:348–355.
3. Eckhoff SR, Watson SA. Corn and sorghum starches production. In Starch: Chemistry and Technology, 3 ed. J. BeMiller and RWhistler, Eds. Academic Press:New York; 2009.
4. Gilani GS, Cockell KA. Sepehr E. Effects of anti-nutritional factors on Protein digestibility and amino acid availability in foods. Journal of AOAC International. 2005;88:967-968.
5. Melaku U, West CE, Habtamu F. Content of Zinc, Iron, Calcium and their absorption inhibitors in food commonly consumed in Ethiopia. Journal of Food Composition and Analysis. 2005;18:803-817.
6. Elkhier MKS, Hamid AO. Effect of malting on the chemical constituents, anti-Nutrition factors and ash composition of two sorghum cultivars (feterita and tabat) grown in Sudan. Research Journal of Agriculture and Biological Sciences. 2008; 4:500-504.

7. Onimawo IA, Onofun AM. Some nutritional contents and functional properties of sorghum toasted soybean blends. Nigeria Journal of Food Science. 2003;22:18-22.
8. Singh H, Sodhi NS, Singh N. Characteristics of starches separated from sorghum cultivars grown in India. Journal of Food Chemistry. 2010;119:95-100.
9. Gemede HF, Ratta N. Anti-nutritional factors in plant foods: Potential health benefits and adverse effects. International Journal of Nutritioin and Food Science. 2014;3(4):284-9.
10. Zubair AB, Osundahunsi OF. Effect of steeping period on the physicochemical and pasting properties of sorghum starch. Applied Tropical Agriculture. 2016;21(3): 12-18.
11. Zhang G, Xu Z, Gao Y, Huang X, Yang T. Effects of germination on the nutritional properties, phenolic profiles, and antioxidant activities of buckwheat. Journal of Food Science. 2015;80:H1111–H1119.
12. Dewar J, Joustra SM, Taylor JRN. Accepted methods of sorghum malting and brewing analysis. SIR Food Science and Technology Pretoria, South Africa; 1995.
13. Uchenna JA, Otu O. Nutritional and phytochemical properties of wild black plum (*Vitex doniana*) Seed from Ebonyi State. International Journal of Horticulture. Agriculture and Food Science (IJHAF). 2019;3(1):32-36.
14. Aiwonegbe AE, Iyasele JU, Izevbuwa NO. Proximate composition phytochemical and antimicrobial screening of methanol and acetone extracts of *Vitex doniana* fruit Pulp. Ife Journal of Science. 2018;20(2): 207-212.
15. Hassan AB, Ahmed IAM, Osman NM, Eltayeb MM, Osman GA, Babiker EE. Effect of processing treatments followed by fermentation on protein content and digestibility of pearl millet (*Pennisetum typhoideum*) cultivars. Pakistan Journal of Nutrition. 2006;5(1):86–89.
16. Opeyemi OA, Stephen AA, Oluwatooyin FO. Effect of malted sorghum on quality Characteristics of wheat-sorghum-soybean flour for potential use in confectionaries. Food and Nutrition Sciences. 2016;7: 1241–1252.
17. Hemalatha S, Platel K, Srinvesank K. Influence of germination and fermentation on bioaccessibility of zinc and iron from food grains European. Journal of Clinical Nutrition. 2007;342348:61-63.
18. Idris WH, Hassan AB, Babikar EE, Eltinay AH. Effect of malt pre-treatment on anti-nutritional factors and HCl extractability of minerals of sorghum cultivars. Pakistani Journal of Nutrition. 2005;4:396-401.
19. Ugwu FM, Oranye NA. Effects of some processing methods on the toxic components of African breadfruit (*Treculia africana*). African Journal of Biotechnology. 2006;5:2329-2333.

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