

Effect of steeping time on the anti-nutrients composition of starch from red and white sorghum cultivars

Zubair, A. B.*, Femi, F. A., Maxwell, Y. M. O. and Jiya, M.J.

Department of Food Science and Technology, Federal University of Technology, P. M.B. 65, Minna, Niger State, Nigeria.

*Corresponding author. Email: b.zubair@futminna.edu.ng; Tel: +2348035889779.

Copyright © 2023 Zubair et al. This article remains permanently open access under the terms of the [Creative Commons Attribution License 4.0](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

Received 11th March, 2022; Accepted 10th April, 2022

ABSTRACT: Sorghum (*Sorghum bicolor*) is a staple food that provides a major source of calories to large segments of the population living in semi-arid tropics of Africa and Asia. Steeping is an age-long process among all the available food processing techniques known to improve nutritional qualities, palatability and consumer appeal of sorghum. The study investigate the effects of steeping periods on the anti-nutrient composition of starches from two varieties of sorghum (red and white) were investigated. Sorghum grains steeped for 6, 12, 18 and 24 hours with water were processed into starch and sample from unsteeped sorghum served as 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 (34.10 to 50.31%). There was no significant difference between starches from the two cultivars of sorghum (red and white) 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 thereby improving the bioavailability of mineral and other nutrients present in sorghum. Mixture of two varieties (red and white) could find applications in food formulation.

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

INTRODUCTION

Sorghum is the fifth most important cereal crop in the world after rice, wheat, maize and barley, and the most grown cereal in Sub-Saharan Africa, after maize (Sobowale *et al.*, 2019). Sorghum remains one of the most versatile cereal crops on the continent, serving as a staple and main meal for millions of people (Schober and Bean, 2008). It is an important source of calories, variety of nutrients and beneficial food components (Ogunmbaku *et al.*, 2018). It is generally high in carbohydrate, low quantity and quality protein and is limiting in lysine, threonine, methionine and tryptophan (Eckhoff and Watson, 2009). Sorghum grains vary from white to dark brown depending on the phenolic pigments present. The seed coat contains abundant number of polyphenolic compounds which combine with other flavonoids (anthocyanins, anthocyanidins, e.t.c.) to

give it various colours (Gilani *et al.*, 2005). The germ fraction of sorghum is rich in minerals (ash), protein and lipids as well as B-group vitamins: thiamine, niacin and riboflavin ((Umeta *et al.*, 2005). The endosperm consists mainly of starch granules, storage proteins and cell-wall materials ((Umeta *et al.*, 2005).

Starch is the most abundant carbohydrate reserve in plants and it is deposited in plant parts in the form of small granules or cells ranging from 1 up to 100 μm (Tharanathan, 2005). Starch is commonly processed by wet milling, the seed or tuber is milled, followed by the separation of the main constituents such as starch, protein and fiber (Singh *et al.*, 2010). Starch granules are composed of a mixture of two polymers: an essentially linear polysaccharide called amylose and a highly

branched polysaccharide called amylopectin. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin by weight. Despite an impressive array of nutrients in sorghum grains, sorghum-based foods have continued to be nutritionally deficient and organoleptically inferior due to the presence of anti-nutritional factors such as tannin, saponin, phytic acid, polyphenol and trypsin inhibitors which bind these food ingredients into complexes making them unavailable for human nutrition (Elkhier and Hamid, 2008).

Anti-nutritional factors are primarily associated with compounds or substances of natural or synthetic origin, which interfere with the absorption of nutrients, and act to reduce nutrient intake, digestion, and utilization and may produce other adverse effect (Gemedede and Ratta, 2014). Anti-nutrients are found in their highest concentrations in grains, beans, legumes and nuts, but can also be found in leaves, roots and fruits of certain varieties of plants (Jiru *et al.*, 1995). The presence of anti-nutritional factors limits the digestibility of proteins and carbohydrates by inhibiting respective proteolytic and amylolytic enzymes (Elkhier and Hamid, 2008). Anti-nutritional factors equally determine the bioavailability of divalent mineral elements which play key roles as enzyme stabilizers, transport co-factors in metabolic pathways and other key physiological functions (Elsheikh *et al.*, 2000).

Steeping is an important unit operation that facilitates processing of sorghum. Hence, the need for adequate steeping of sorghum for development of value added products from the grains like a highly nutritious and easy-to-digest whole-grain food with robust flavor (Zhang *et al.*, 2015). Steeping of sorghum have been shown to significantly reduce anti-nutrient content thereby, leading to an improvement in protein digestibility and other protein quality characteristics including percentage of protein, nitrogen solubility index and content of the limiting amino acid lysine (Dewar *et al.*, 1995). Kazanas and Fields (1981) reported an improvement in the *in-vitro* digestibility of protein and starch during soaking of sorghum while Chavan *et al.* (1988) indicated an improvement in the composition and content of essential amino acids and an increased absorption of mineral such as zinc, iron, potassium, magnesium and calcium respectively in sorghum. Soaking of sorghum have been reported to leads to a modification (increase/decrease) of inherent metabolites and constituents, activation of enzymes, decrease in pH levels, increased metabolic activities and microbial actions with detoxification and degradation of contaminants (Adebo *et al.*, 2019). Knowledge of anti-nutrient in sorghum during processing is required so that the potential of sorghum can be evaluated and also the needs to develop processing method or steeping time that will removes completely or reduce these undesirable components to improve the nutritional quality of cereals. Hence, the need to investigate the effect of steeping time on anti-nutrient content of sorghum starch. The objective of this study is therefore, to determine the effect of steeping periods on the anti-nutrient component of starch from two varieties of sorghum (red and white).

MATERIALS AND METHODS

Sorghum (*Sorghum bicolor*) grains were purchased from Oja-Oba market in Akure, Ondo State, Nigeria. All reagent (Acetic acid, ethanol, ammonium hydroxide, chloroform, methanol, isobutyl alcohol, distilled water, orthophosphoric acid, hydrogen chloride) use were of analytical grade. Equipment used includes filter paper, conical flask, beaker, centrifuge machine, pH metre, measuring cylinder and distillation flask.

Sample preparation

Sorghum grains were cleaned manually and sorted to remove the husks, stem, damaged and discoloured seeds which were achieved by winnowing, hand picking and washing with tap water. The method of Singh *et al.* (2010) was used for the starch production. About 250 g of sorghum grains were washed with tap water, steeped for 6, 12, 18, 24 hours and wet milled; while control sample was milled without steeping. The slurry was filtered through a 100 µm mesh screen and the filtrate was allowed to sediment overnight, decanted and reslurried twice before final decantation. The starch cake was oven dried at 50°C for 6 hours, milled after drying and sieved through 0.25 µm mesh screen to get the sorghum starch.

Sample analyses

Alkaloid content was determined gravimetrically using the method outlined by AOAC (2005). Terpenoid and cardiac glycosides content was determined using the method described by Sofowara (1995). Saponin content was determined using method of Brunner (1994). Tannin content was determined according to the method described by Marker and Goodchild (1996). Cyanide content was determined using the method of AOAC (1990). Total oxalate content was determined using the method outlined by AOAC (2005). Phytate content was determined using the method of Kubmarawa *et al.* (2008).

Statistical analysis

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

RESULTS AND DISCUSSION

Anti-nutrients content of starch from red and white sorghum cultivar is presented in Table 1. Hydrogen cyanide content was in the range of 1.34 to 1.86 mg/kg, tannin content was in the range of 1.30 to 4.82 mg/g, saponin content was in the range of 6.10 to 13.05 mg/g,

Table 1. Anti-nutrient content of starch from red and white sorghum cultivar.

Variety	Steeping time (h)	Hydrogen cyanide (mg/kg)	Tannin (mg/g)	Saponin (mg/g)	Oxalate (mg/g)	Phytate (mg/g)	Alkaloids (%)	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	50.31±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}	49.25±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	38.04±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	35.81±1.21 ^d	14.04±0.04 ^d	10.20±0.28 ^d ^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	34.25±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}	49.00±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	48.30±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	38.20±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	36.50±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±0.14 ^e	0.43±0.04 ^e	2.15±0.07 ^e	34.10±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).

oxalate content was in the range of 0.43 to 0.90 mg/g, phytate content was in the range of 2.15 to 9.06 mg/g, alkaloid content was in the range of 34.10 to 50.31%, terpenoid content was in the range of 13.50 to 18.99 mg/g and cardiac glycoside ranged between 9.30 to 13.35 mg/g. All anti-nutrients investigated showed significant decrease (p≤0.05) with an increase in steeping time. This finding is in line with the observation of Hassan *et al.* (2006) who reported that when sorghum grains are subjected to steeping, they showed notable decrease in anti-nutritional and these has been 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 of the anti-nutrient as a result 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 (Bishnoi *et al.*, 1994). The result obtained in this research for tannin was in line with the observation of Shadad (1989) who

reported that white colored sorghum contains less tannin than the red colored sorghum. Reduction in tannin content could be due to the leaching out of polyphenol into steeping water under the influence of concentration since tannin are polyphenols and polyphenolic compounds which are readily soluble in water and mostly located in the seed coat (Singh, 2010). Hemalatha *et al.* (2007) reported that soaking/steeping significantly reduces the tannin content in some food grains such finger millet. Phytate may 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 (Idris *et al.*, 2005). Phytate are the principal storage form of phosphorous and are particularly abundant in cereals and legumes (Reddy *et al.*, 1989). The reduction in phytate content occasioned by increase in steeping time is in agreement with the finding of Vidal-Valverde *et al.* (1994) who reported that steeping of lentils greatly reduced the phytate content.

The oxalate content range of 0.43 to 0.90 mg/g recorded in this research was within the range of

0.36 to 0.57 mg/g reported by Aluge *et al.* (2016) for germinated sorghum flour. Calcium is released from oxalate complexes and iron from protein-tannin complexes (Umata *et al.*, 2005). The rate of reduction in tannin as a result of steeping was less than that of phytate possibly because while phytate is degraded by enzyme, tannin is only leached out (Ugwu and Oranye, 2002). Hence, combined effects of physical and enzymatic actions on the sorghum grains during steeping significantly reduce the concentration of antinutrient present.

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. There was no significant difference between starches 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 formulation.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest.

REFERENCES

- Adebo, O. A., Kayitesi, E., & Njobeh, P. B. (2019). Reduction of mycotoxins during fermentation of whole grain sorghum to whole grain ting (a Southern African Food). *Toxins*, 11(3), 180.
- Aluge, O. O., Akinola, S. A., & Osundahunsi, O. F. (2016). Effect of malted sorghum on quality characteristics of wheat-sorghum-soybean flour for potential use in confectionaries. *Food and Nutrition Sciences*, 7(13), 1241-1252.
- AOAC (2005). *Official Methods of Analysis*, Association of Official Analytical Chemist 17th Edition Washington.
- Bishnoi, S., Khetarpaul, N., & Yadav, R. K. (1994). Effect of domestic processing and cooking methods on phytic acid and polyphenol contents of pea cultivars (*Pisum sativum*). *Plant Foods for Human Nutrition*, 45(4), 381-388.
- Brunner, J. H. (1994). Direct spectrophotometer determination of Saponin. *Animal Chemistry*, 34, 1314-1326.
- Chavan, U. D., Chavan, J. K., & Kadam, S. S. (1988). Effects of fermentation on soluble proteins and in vitro digestibility of sorghum, green grain and sorghum green gram blends. *Journal of Food Science*, 53(5), 1574-1575.
- Dewar, J., Joustra, S. M., & Taylor, J. R. N. (1995). Accepted methods of sorghum malting and brewing analysis. SIR *Food Science and Technology* Pretoria, South Africa.
- Eckhoff, S. R., & Watson, S. A. (2009). Corn and sorghum starches: production. In: Be Miller, J., & Whistler, R. (eds.). *Starch: Chemistry and Technology* (third edition). Academic Press: New York. Pp. 373-439.
- Elkhier, M. K. S., & Hamid, A. O. (2008). Effect of malting on the chemical constituents, antinutrition factors and ash composition of two sorghum cultivars (feterita and tabat) grown in Sudan. *Research Journal of Agriculture and Biological Sciences*, 4(5), 500-504.
- Elsheikh, E. A. E., Fadul, I. A., & El Tinay, A. H. (2000). Effect of cooking on anti-nutritional factors and in vitro protein digestibility (IVPD) of faba bean grown with different nutritional regimes. *Food chemistry*, 68(2), 211-212.
- Gemedede, H. F., & Ratta, N. (2014). Antinutritional factors in plant foods: Potential health benefits and adverse effects. *International Journal of Nutrition and Food Sciences*, 3(4), 284-289.
- Gilani, G. S., Cockell, K. A., & Sepehr, E. (2005). Effects of antinutritional factors on protein digestibility and amino acid availability in foods. *Journal of AOAC international*, 88(3), 967-987.
- Hassan, A. B., Ahmed, I. A. M., Osman, N. M., Eltayeb, M. M., Osman, G. A., & Babiker, E. E. (2006). Effect of processing treatments followed by fermentation on protein content and digestibility of pearl millet (*Pennisetum typhoideum*) cultivars. *Pakistan Journal of Nutrition*, 5(1), 86-89.
- Hemalatha, S., Platel, K., & Srinivasan, K. (2007). Influence of germination and fermentation on bioaccessibility of zinc and iron from food grains. *European Journal of Clinical Nutrition*, 61(3), 342-348.
- Idris, W. H., Hassan, A. B., Babiker, E. E., & El Tinay, A. H. (2005). Effect of malt pretreatment on antinutritional factors and HCl extractability of minerals of sorghum cultivars. *Pakistan Journal of Nutrition*, 4(6), 396-401.
- Jiru, K., & Urga, K. (1995). Forms and contents of oxalate and calcium in some vegetables in Ethiopia. *Ethiopian Journal of Health Development*, 9(1), 13-18.
- Kazanas, N., & Fields, M. L. (1981). Nutritional improvement of sorghum by fermentation. *Journal of Food Science*, 46(3), 819-821.
- Kubmarawa, D., Khan, M. E., Punah, A. M., & Hassan, M. (2008). Phytochemical screening and antimicrobial efficacy of extracts from *Khaya senegalensis* against human pathogenic bacteria. *African Journal of Biotechnology*, 7(24), 4563-4566.
- Marker, A. O. S. & Goodchild, A. V. (1996). Qualification of tannin. A laboratory manual. *International Centre of Agricultural Research in Dry Areas* (ICRDA). Aleppo Syria, IV. 25pp.
- Odunmbaku, L. A., Sobowale, S. S., Adenekan, M. K., Oloyede, T., Adebisi, J. A., & Adebo, O. A. (2018). Influence of steeping duration, drying temperature, and duration on the chemical composition of sorghum starch. *Food Science & Nutrition*, 6(2), 348-355.
- Reddy, N. R., Pierson, M. D., Sathe, S. K., & Salunkhe, D. K. (1989). *Occurrence, distribution, content and dietary intake of phytate*. CRC press, Boca Raton, FL, U.S.A. P. Pp. 39-56.
- Schober, T. J., & Bean, S. R. (2008). Sorghum and maize. In: Arendt, E. K., & Bello, F. D. (eds.). *Gluten-free cereal products and beverages*. Elsevier: Amsterdam, The Netherlands. Pp. 101-118.
- Shadad, M. E. (1989). Proximate composition, tannin content and protein digestibility of sorghum cultivars grown in Sudan. M. Sc. Thesis. Faculty of Agriculture, University of Khartoum, Sudan.
- Singh, H., Sodhi, N. S., & Singh, N. (2010). Characterisation of starches separated from sorghum cultivars grown in India. *Food chemistry*, 119(1), 95-100.
- Sobowale, S. S., Adebo, O. A., & Mulaba-Bafubandi, A. F. (2019). Production of extrudate pasta from optimal sorghum-peanut flour blend and influence of composite flours on some quality characteristics and sorption isotherms. *Transactions of the Royal Society of South Africa*, 74(3), 268-275.
- Sofowara, A. (1995). *Medicinal plants and traditional medicine in Africa*. Spectrum books Ltd, Ibadan, Nigeria. p. 289.
- Tharanathan, R. N. (2005). Starch—value addition by modification. *Critical Reviews in Food Science and Nutrition*, 45(5), 371-384.
- Ugwu, F. M., & Oranye, N. A. (2006). Effects of some processing methods on the toxic components of African breadfruit (*Treculia africana*). *African Journal of Biotechnology*, 5(22), 2329-2333.
- Umata, M., West, C. E., & Fufa, H. (2005). Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. *Journal of Food Composition and Analysis*, 18(8), 803-817.
- Vidal-Valverde, C., Frias, J., Estrella, I., Gorospe, M. J., Ruiz, R., & Bacon, J. (1994). Effect of processing on some antinutritional factors of lentils. *Journal of Agricultural and Food Chemistry*, 42(10), 2291-2295.
- Zhang, G., Xu, Z., Gao, Y., Huang, X., Zou, Y., & Yang, T. (2015). Effects of germination on the nutritional properties, phenolic profiles, and antioxidant activities of buckwheat. *Journal of Food Science*, 80(5), H1111-H1119.