

Original Research Article

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Antinutrients, *In Vitro* Digestibility and Antioxidant Activity of Sorghum Grain and Flour of Two Different Varieties

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ABSTRACT

Sorghum commonly is eaten with the hull (the outer layer of the grain), which retains the majority of the nutrients. Sorghum has excellent chemical and physical properties, which make it a grain of good quality for processing different types of products. The nutrient composition of sorghum grain indicates that it is a good source of carbohydrates, fibre, protein, vitamins and minerals. Sorghum contains about 70 per cent starch, so is a good energy source. Its starch consists of 70 to 80 per cent amylopectin, a branched-chain polymer of glucose, and 20 to 30 per cent amylose, a straight-chain polymer. *In-vitro* protein digestibility of wheat (control), HC-308 and HJ-513 was 71.12, 69.66 and 69.86 per cent, respectively which did not vary among them. *In-vitro* starch digestibility of wheat (control), HC-308 and HJ-513 i.e. 32.98, 31.46 and 32.39 (mg maltose released/g meal) was found, which too did not vary. Phytic acid content of HC 308 (565.6 mg/100 g) and HJ-513 (587.6 mg/100g) did not differ but both had significantly higher ($p \leq 0.05$) phytic acid content when compared to that of wheat (control) (469.3 mg/100 g). Polyphenol content of HC-308 (757.95mg/100g) and HJ-513 (752.95mg/100g) did not vary. However, both sorghum varieties had higher polyphenol contents when compared to that of wheat (control) i.e. 466.33 mg/100g. Total phenolic contents of two sorghum varieties viz. HC-308 and HJ-513 and wheat (control) varied i.e. 7.03, 7.13 and 5.26 mg GAE/g, respectively. DPPH free radical scavenging activity of HC 308 and HJ-513 had antioxidant activity of 50.10 and 52.55 per cent, respectively which were higher than antioxidant activity of wheat control (42.58%).

Keywords

Antinutrients, *In vitro* digestibility and Antioxidant activity

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Introduction

Sorghum is the fifth most important cereal crop in the world after rice, wheat, corn and barley. It is the main cereal food for over 750 million people living in semi-arid tropical regions of Africa, Asia and Latin America.

The largest producers are the United States (almost 17% of world production), with yields obviously much higher, followed by India, Nigeria, China, Mexico, Sudan and Argentina. India is the third largest producer of sorghum in the world with its 2016-17 crop forecast at 5.5 million tonnes, up from 4.4 million tonnes.

In Sorghum, the cultivation area is showing a decreasing trend and the reduction is to the extent of 41.81 per cent from the year 2008-09 to 2014-15 and a decline 74.71 per cent was recorded over the past four decades in the country. The crop is primarily produced in Maharashtra and southern states of Karnataka and Andhra Pradesh. These, three states together account for close to 80 per cent of the all-India production. Madhya Pradesh, Gujarat and Rajasthan are the other states producing sorghum. In many semi-arid countries of Africa and Asia, grains occupy an important place in the food and feed. It is one of the major cereal crops produced and consumed after rice (*Oryza sativa*) and wheat (*Triticum aestivum*). Sorghum grains are used by these people (especially farmers), who often do not have the means to feed themselves with food sources of energy, rich in protein, vitamins, minerals. Sorghum grains are rich in energy and non-energy nutrients (Ramatoulaye *et al.*, 2016). Sorghum commonly is eaten with the hull (the outer layer of the grain), which retains the majority of the nutrients. Sorghum has excellent chemical and physical properties, which make it a grain of good quality for processing different types of products. The nutrient composition of sorghum grain indicates that it is a good source of carbohydrates, fibre, protein, vitamins and minerals. Sorghum contains about 70 per cent starch, so is a good energy source. Its starch consists of 70 to 80 per cent amylopectin, a branched-chain polymer of glucose, and 20 to 30 per cent amylose, a straight-chain polymer.

It is a gluten-free, high protein and cholesterol-free source of a variety of essential nutrients i.e. iron, zinc, manganese and copper. Sorghum has the potential for high levels of iron (more than 70 ppm) and zinc (more than 50 ppm) in the grain. It is rich in B-complex vitamins like thiamine, riboflavin, niacin, pantothenate, and vitamin B₆ which

play key role in energy metabolism. Sorghum's high-energy content and ready supply of B-complex vitamins are a perfect combination for energy utilization.

Depending upon the variety, sorghum provides good to excellent sources of phytochemicals such as phenolic acids, anthocyanins, phytosterols and policosanols etc. and antioxidants which are believed to help lower the risk of cancer, diabetes, heart disease and some neurological diseases. The wax surrounding the sorghum grain contains compounds, policosanols that may have an impact on human cardiac health.

As with other foodstuffs, certain nutritional inhibitors and toxic substances are associated with sorghum grains as well which lower its nutritional value. The antinutritional effect of tannin and phytate in sorghum has been demonstrated by many researchers. The tannin-protein interaction in sorghum involves hydrogen bonding and hydrophobic interactions. Sorghum prolamins (proline-rich proteins) bind strongly to sorghum tannins and these results in reduced protein digestibility (Abdelhaleem *et al.*, 2008). Like all grain species, sorghum contains phytic acid which binds minerals and reduces their availability to the consumer. However, these antinutrients could be eliminated or reduced by processes such as soaking, dehulling, popping, germination and fermentation involved in processing of sorghum during product development (Ugwu and Oranye, 2006). During the value addition and application of suitable processing methods, the utilization of sorghum, being a cheap protein and mineral source, can be greatly enhanced. As per the 2012 report of the statistical appraisal on children in India, the prominent findings relevant to nutritional status is described as 48 percent of children under age five years are stunted which indicates that, half of the country's children are chronically

malnourished. Acute malnutrition, as evidenced by wasting which results in a child being too thin for his or her height. Approximately 20 percent of children less than five years in the country are wasted which indicates that, one out of every five children in India is wasted. Nearly 45 percent of children under five years of age are underweight. Prevalence of stunting and underweight was highest in age group 11 to 13 years, whereas prevalence of wasting was highest in age group of 5 to 7 years. With the revelation of the above findings, it is reassured that malnutrition is not the result of a single cause; the problem is multifaceted, the causes acting singly or in combination with other complex factors like poverty, purchasing power, health care, ignorance on nutrition and health education, female illiteracy, social convention etc. Cereal grains form a major source of dietary nutrients for all people, particularly those in the developing countries. The present study was planned with the following specific objective

To study the anti nutrients in vitro protein digestibility, starch digestibility and antioxidant activity two varieties of sorghum

Materials and Methods

Procurement of raw material

The locally available varieties of sorghum *i.e* HC 308 and HJ 513 were procured from the Department of Genetics and Plant Breeding, CCSHAU, Hisar. *In vitro* Digestibility

***In vitro* protein digestibility**

In vitro protein digestibility was carried out by the modified method of Mertz *et al.*, (1983).

Reagents

Pepsin reagent: 0.1 M KH_2PO_4 (pH 2.0) containing 0.2 % pepsin; 13.6 g potassium

phosphate was dissolved in 1 litre of water, pH of the solution was adjusted to 2.0 and 2 g pepsin (Sigma Chemical Company, USA.) was dissolved in the buffer.

TCA (50 %): 50 g Trichloroacetic acid was dissolved in water and volume was made to 100 ml.

Procedure

250 mg of sample was weighed and transferred to a centrifuge tube. To it 20 ml of pepsin reagent was added. The tube was stoppered and arranged in a shaker-incubator maintaining the water temperature at 37°C for 3 h. The centrifuge tube was removed and cooled. 5 ml of TCA (50 %) was added and the contents were centrifuged at 10,000 rpm for 10 min at room temperature and filtered. Ten ml of aliquot was taken and dried in hot air oven and digested for nitrogen determination by Micro Kjeldahl method (AOAC, 2000). Digested protein of sample was determined. Protein digestibility was calculated by the following formula:

$$\text{Protein digestibility (\%)} = \frac{\text{Digested protein}}{\text{Total protein}} \times 100$$

***In vitro* starch digestibility**

In vitro starch digestibility was assessed by employing method of Singh *et al.*, 1982.

Reagents

Pancreatic amylase: Twenty mg pancreatic amylase (Sigma Chemical Company, USA) was dissolved in 50 ml phosphate buffer (pH 6.9).

0.2 M Disodium hydrogen phosphate: Dissolved 35.598 g disodium hydrogen phosphate in distilled water and volume was made to one litre.

0.2 M Potassium dihydrogen phosphate: Dissolved 27.28 g potassium dihydrogen phosphate in distilled water and volume was made to one litre.

Phosphate buffer (pH 6.9): Added 50 ml 0.2 M Potassium dihydrogen phosphate to 46.8 ml 0.2 M sodium hydrogen phosphate and volume was made upto 200 ml.

Di-nitrosalicylic reagent: 3,5-dinitrosalicylic acid (10 g), sodium potassium tartarate (300 g) and sodium hydroxide (16 g) were dissolved in carbon dioxide free water and volume was made to 1 litre. The reagent was stored in brown bottle and protected from carbon dioxide.

Standard maltose solution: Maltose monohydrate (100 mg) was dissolved in distilled water and volume was made up to 100 ml.

Estimation

Fifty mg defatted sample was dispersed in 1.0 ml 0.2 M phosphate buffer (pH 6.9). 0.5 ml pancreatic amylase was added and incubated in water bath at 37°C for 2 h with occasional shaking of the test tubes. After incubation, 2 ml dinitrosalicylic reagent was quickly added and heated for 5 min in a boiling water bath. After cooling, the solution was made to 25 ml with distilled water and filtered through an ordinary filter paper prior to measurement of absorbance at 550 nm. A blank was run simultaneously by incubating the sample without enzyme. Dinitrosalicylic reagent was added before addition of the enzyme solution. Values were expressed as mg maltose released/g defatted sample. Standard curve was prepared by taking 0.5 to 4.0 mg maltose from standard maltose solution. The starch digestibility was calculated as:

$$\text{In vitro starch digestibility} = \frac{\text{Concentration from graph (mg)}}{\text{Weight of sample (g)}}$$

Anti-nutritional factors

Phytic acid

Phytic acid content was determined by the method of Davies and Reid (1979).

Reagents

Nitric acid (0.5M): HNO₃ 69.5 % (15.96 ml) (AR grade, sp. gr.1.42) was diluted to 500 ml with distilled water.

Ferric ammonium sulphate: Ferric ammonium sulphate (215 mg) was dissolved in distilled water. To it few drops of HCl were added and volume was made to 500 ml with distilled water.

Ammonium thiocyanate: Ammonium thiocyanate (10g) was dissolved in distilled water and volume was made to 100 ml.

Iso-amyl alcohol Sodium phytate: Sodium phytate (5.5 % H₂O, 97 % purity and containing 12 Na/mole) was dissolved in 100 ml of 0.5 M HNO₃, which gave a solution containing 20 mg phytic acid in 100 ml or 200 µg phytic acid/ml.

Extraction

To 500 mg sample, 20 ml 0.5 M HNO₃ was added in a conical flask and shaken continuously for 3 h on shaker at room temperature. The contents were centrifuged at 800 rpm for 15 min. Supernatant was used for estimation of phytic acid.

Procedure

To a test tube, 0.5 ml HNO₃ extract was taken and volume was made to 1.4 ml with water.

To it, 1 ml ferric ammonium sulphate solution was added, the contents were thoroughly mixed and placed in boiling water bath for 20 min. immediately the tubes were cooled to room temperature under tap water.

Five ml iso-amyl alcohol was added to it, the contents were mixed vigorously and to it, 0.1 ml ammonium thiocyanate solution was added. The tubes were shaken well and centrifuged at 300 rpm for 10 min. Colour intensity in the alcohol was read exactly after 15 min of addition of ammonium thiocyanate at 465 nm against iso-amyl alcohol blank. For plotting a standard curve, 0.2 to 1.2 ml standard phytate solution containing 40- 240 µg phytic acid was taken and made to 1.4 ml with water.

The phytic acid was calculated by the formula:

$$\text{Phytic acid (mg/100g)} = \frac{M \times V \times 100}{W \times V1 \times 1000}$$

Where,

M = concentration of sample for graph

W = weight of sample

V = volume of extract made

V1 = volume of aliquot taken

Polyphenols

Total polyphenols were extracted by the method of Singh and Jambunathan (1981). Defatted sample (500 mg) was refluxed with 50 ml methanol containing one per cent HCl for 4 h. The extract was concentrated by evaporating on a hot water bath and brought its volume to 25 ml with methanolic - HCl. The amount of polyphenolic compounds was estimated as tannic acid equivalent according to Folin-Danis procedure (Swain and Hills, 1959).

Reagents

Folin-Denis reagent: To 750 ml water, 100 g sodium tungstate, 20 g phosphomolybdic acid and 50 ml phosphoric acid were added and heated and then refluxed for 2 h. It was cooled and diluted to one litre.

Tannic acid (stock solution): 100 mg of tannic acid was dissolved in water and made upto one litre. In order to have working standard solution, 20 ml stock solution was further diluted to 100 ml with water.

Saturated aqueous sodium carbonate solution: Dissolved 350 g sodium carbonate in one litre hot distilled water at 70°C to 80°C, cooled and filtered through glass wool.

Procedure

Test solution (1.5 ml) was diluted with distilled water to 8.5 ml in a graduated test tube. After thorough mixing, added 0.5 ml Folin- Denis reagent and the tubes were well shaken. Exactly after 3 minutes, one ml of saturated sodium carbonate solution was added and the tubes were thoroughly shaken again. After an hour, the absorbance was read at 725 nm on UV- VIS Spectrophotometer-118 using a suitable blank. If the solution was cloudy or precipitates appeared, it was centrifuged before readings were taken. A standard curve was plotted by taking 0.5 ml to 4.0 ml working tannic standard solution containing 10 µg to 80 µg tannic acid.

$$\text{Polyphenols (mg/100g)} = \frac{M \times V \times 100}{W \times V1 \times 1000}$$

Where,

M = Concentration of extract elute obtained from graph

V = Volume made of extract (ml)

W = Weight (g) of the sample

V1 = Volume of extract aliquot taken (ml)

Antioxidant activity

Total phenolic content

Total phenolic contents were estimated by the method of Singleton and Rossi (1965).

Reagents

Folin-Ciocalteu reagent (1N)

Sodium carbonate (Na₂CO₃): Dissolved 200 g anhydrous sodium carbonate in 800 ml water and brought to a boil. After cooling, added a few crystals of sodium carbonate and let it sit for 24 h at room temperature. Filtered through Whatman # 1 filter paper and added water to

make the volume to 1 liter.

Standard gallic acid

Preparation of calibration curve using gallic acid as standard

Ten mg of standard gallic acid was accurately weighed and dissolved in 100ml distilled water in a volumetric flask (100 µg/ml of stock solution). From the above stock solution, 0.1 to 1 ml aliquots were pipetted out into 25 ml volumetric flask. Ten ml of distilled water and 1.5 ml of Folin-Ciocalteu reagent were added and diluted accordingly to the label specification to each of the above volumetric flasks. After 5 min., 4 ml of 20 percent sodium carbonate solution was added and volume was made upto 25 ml with distilled water. Absorbance was recorded after 30 min. at 765 nm and calibration curve of absorbance verses concentration was plotted.

Table.1 Phytic acid (mg/100g) and polyphenol contents (mg/100g) of sorghum varieties (on dry matter basis)

Parameters		Sorghum varieties		Wheat (control)	CD (p≤0.05)
		HC 308	HJ 513		
Antinutrient content	Phytic acid	565.6±8.59 ^a	587.6±11.62 ^a	469.3±5.24 ^a	42.54
	Polyphenol	757.95±35.30 ^a	752.95±35.53 ^a	466.33±2.40 ^a	79.05

Values are mean ±SE of three independent determinations
Similar superscripts in the column indicate that they do not differ significantly (p ≤0.05)

Table.2 *In vitro* protein (%) and starch (mg maltose released/g meal) digestibility of sorghum varieties (on dry matter basis)

Parameters		Sorghum varieties		Wheat (control)	CD (p≤0.05)
		HC 308	HJ 513		
IVD	Protein	69.66±1.40 ^a	69.86±2.21 ^a	71.12±0.15 ^a	NS*
	Starch	31.46±0.29 ^a	32.39±0.22 ^a	32.98±0.22 ^a	NS*

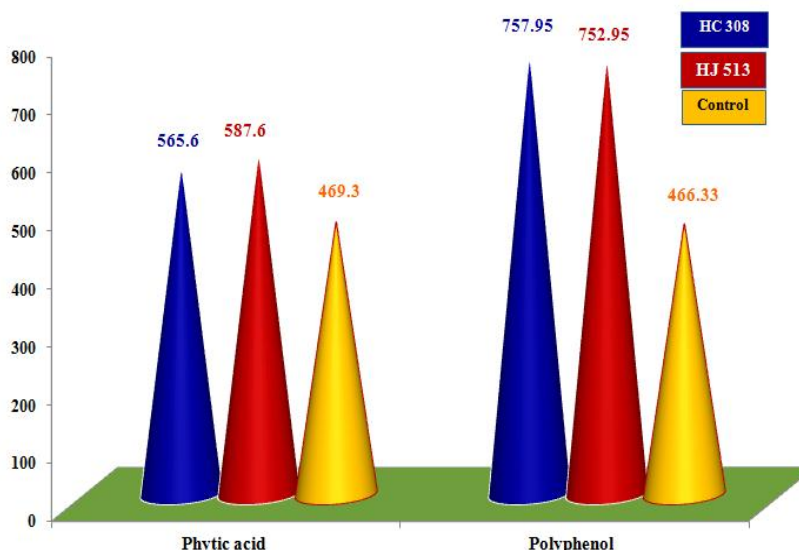
Values are mean ±SE of three independent determinations
Similar superscripts in the column indicate that they do not differ significantly (p ≤0.05)
*Not significant

Table.3 Total phenolic contents (mg GAE/g) and DPPH free radical scavenging activity (%) of sorghum varieties (on dry matter basis)

Antioxidant activity	Sorghum varieties		Wheat (Control)	CD (p≤0.05)
	HC 308	HJ 513		
Total phenolic content (mg GAE/g)	7.03±0.01 ^a	7.13±0.02 ^a	5.26±0.18 ^b	0.40
DPPH free radical scavenging activity (%)	50.10±2.97 ^a	52.55±1.52 ^a	42.58±0.69 ^b	7.48

Values are mean ±SE of three independent determinations
 Similar superscripts in the column indicate that they do not differ significantly (p ≤0.05)

Fig.1 Phytic acid (mg/100g) and polyphenol contents (mg/100g) of sorghum varieties (on dry matter basis)



Procedure

One g of sample was added to 15ml of methanol (50%) and extracted for three times by maceration of 2 hours. Then it was filtered and volume was made 50 volumetric flask with methanol (50%). One ml aliquot of the sample was taken in a test tube and diluted with 10ml of distilled water.

Then, 1.5 ml Folin-Ciocalteu reagent was added and allowed to incubate at room temperature for 5 min, then 4 ml of 20% (w.v) Na₂CO₃ was added, adjusted with distilled water up to the mark 25ml, agitated and left to stand for 30 min at room temperature. Absorbance of sample was measured at 765nm. Quantification was done on the basis

of a standard curve of gallic acid. Results were expressed as µg gallic acid equivalent (GAE) and percentage w/w.

(DPPH) 2, 2'- Diphenyl-1-picrylhydrazyl free radical scavenging activity (RSA)

The DPPH free radical scavenging activity of sample extracts was evaluated by the DPPH method of Hatano *et al.*, (1988).

Reagents

DPPH: 2.5mg/1 in methanol

Procedure

DPPH is a stable free radical that shows a maximum absorption at 517 nm. When DPPH

encounters proton donating substance such as antioxidant and a radical species, the absorbance at 517 nm disappears because the DPPH radical is scavenged. On the basis of this principle, the radical scavenging effect of each fraction was measured. For this, in 0.2 ml of methanol extract, 3.0 ml of DPPH was added and mixed thoroughly for 5 min.

A control was also made containing 0.2 ml of solvent (methanol) instead of extract (at zero min). The absorbance of the sample as well as control was measured at 517 nm after 30 min. of incubation in dark at room temperature using the UV-VIS double beam Spectrophotometer Model 2203 against a blank containing respective solvent. Three replications were carried out for each sample. The percent of DPPH radical discoloration of the sample was calculated according to the equation (%) discoloration:

$$\text{DPPH free radical scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where,

(A_{control}) = absorbance for the control

(A_{sample}) = absorbance for the sample

Results and Discussion

Antinutrients

Data pertaining to antinutrients content of sorghum varieties is presented in Table 1, Phytic acid contents of HC 308 (565.6 mg/100gm) and HJ 513(587.6 mg/100g) did not differ significantly ($p \leq 0.05$) but both had significantly higher phytic acid content when compared to that of wheat (control) 469.3 mg/100 g (Fig. 1).

Polyphenol content of HC 308 (757.95mg/100g) and HJ 513(752.95mg/100g) followed the same trend and did not vary significantly ($p \leq 0.05$). However, both

had higher polyphenol content when compared to that of wheat (control) 466.33 mg/100g.

In vitro digestibility

Data pertaining to *in vitro* digestibility in sorghum varieties is presented in Table 2. *in vitro* protein digestibility of wheat control cereal bar, HC 308 and HJ 513 was found to be 71.12, 69.66 and 69.86 per cent, respectively which did not vary significantly ($p \leq 0.05$) among themselves. Similar trend was noticed for *in vitro* starch digestibility of wheat (control), HC 308 and HJ 513 which too did not vary significantly ($p \leq 0.05$) among themselves and ranged from 31.46 to 32.98mg maltose released per g.

Antioxidant activity

The data related to antioxidant activity is given in Table 3. Total phenolic contents of two varieties viz. HC 308 and HJ 513 were found to be similar. However, wheat (control) depicted significantly ($p \leq 0.05$) lower 5.26 mg GAE/g of total phenolic content in comparison to that of HC 308 and HJ 513.

HC 308 and HJ 513 had DPPH free radical scavenging activity of 50.10 and 52.55 per cent, respectively which did not vary significantly. However, both the varieties had significantly ($p \leq 0.05$) higher DPPH free radical scavenging activity in comparison to that of wheat (control) 42.58 percent.

In-vitro protein digestibility of wheat (control), HC-308 and HJ-513 was 71.12, 69.66 and 69.86 per cent, respectively which did not vary among themselves. *In-vitro* starch digestibility of wheat (control), HC-308 and HJ-513 i.e. 32.98, 31.46 and 32.39 (mg maltose released/g meal) was found, which too did not vary. Phytic acid content of HC 308 (565.6 mg/100 g) and HJ-513 (587.6

mg/100g) did not differ but both had significantly higher ($p \leq 0.05$) phytic acid content when compared to that of wheat (control) (469.3 mg/100 g). Polyphenol content of HC-308 (757.95mg/100g) and HJ-513 (752.95mg/100g) did not vary. However, both sorghum varieties had higher polyphenol contents when compared to that of wheat (control) i.e., 466.33 mg/100g. Total phenolic contents of two sorghum varieties viz. HC-308 and HJ-513 and wheat (control) varied i.e. 7.03, 7.13 and 5.26 mg GAE/g, respectively. DPPH free radical scavenging activity of HC 308 and HJ-513 had antioxidant activity of 50.10 and 52.55 per cent, respectively which were higher than antioxidant activity of wheat control (42.58%).

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