Effect of popping on sorghum starch digestibility and predicted glycemic index

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Abstract Effect of popping on carbohydrate, protein, phytic acid and minerals of three varieties (pop sorghum, maldandi and red sorghum) of sorghum were studied. Significant changes ($p \leq 0.05$) in the starch degradability including total and soluble amylose content, and resistant starch occurred due to popping; in-vitro protein digestibility along with the content of albumin proteins increased. Starch characteristics had substantial differences among these three varieties which are based on the nature of endosperm and amylose content. Phytic acid content had a reduction of 20%–25% after popping. Glycemic index (GI) determined from kinetic study of enzymatic hydrolysis of sorghum starch was between 85 and 92; the rate constant for hydrolysis for these three varieties were in the range of 0.025 and 0.029 min^{-1} . Popping helped to control phytic acid content in sorghum and enhanced protein as well as starch digestibility.

Keywords Digestibility · Glycemic index · Phytic acid · Popping · Sorghum

Introduction

Sorghum is an important staple food in semi-arid regions worldwide. It is considered to be native to tropical Africa and continues to be a leading cereal grain in the most areas of the continent. The world production of sorghum is about

70 MT (FAO/WHO 2005) and its popularity is due in part to its ability to produce reasonable yields in warmer, drier regions and is primarily a source of dietary energy, with significant levels of phytochemicals, which impart tangible health benefits (Awika and Rooney 2004). The colour of sorghum grain may be white, yellow, red or brown. Certified food grade sorghum varieties are white but red variety has been recently developed for food use as well. Pigmented or red sorghums are preferred in some African cultures because of the characteristic colour. However, the red variety is still considered as an inferior grade and thus used as a feed rather a food commodity. Conventionally, sorghum is pulverized and the meal is used for the preparation of traditional foods, and to a small extent, also processed to prepare snack products (Murthy et al. 1988). Sorghum products including expanded snacks, cookies and ethnic foods are gaining popularity in Japan (Awika and Rooney 2004), also used to a small extent in the USA to substitute for wheat in products for people allergic to wheat gluten (Awika and Rooney 2004). Utilization of sorghum in the whole grain form is not being exploited as sorghum protein lacks the gluten part, which reduces its use in bakery, pasta and snack foods. Popping of sorghum is a cost effective process to obtain flour with improved starch characteristics and represents an unexploited opportunity to design novel and healthier cereal-based food products (Baskaran et al. 1999). Food and feed products may contain a number of antinutritional factors that may adversely affect protein digestibility and amino acid availability (Gilani et al. 2005). Different released var., hybrids, improved var. and animal feed var. of sorghum were studied for physical, cooking and popping characteristics (Sankarapandian 2000). Effect of storage on popping quality of sorghum grains was studied by Gupta and Ratna (1995). Effect of grain moisture content on popping quality of sorghum was

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studied by Singh and Srivastava (1993). A comprehensive investigation was undertaken to determine effects of various processing treatments on in vitro digestibility of carbohydrates from cereals and legumes and products made there from (Kelkar et al. 1996). Gaul and Rayas (2008) studied the effect of moisture content and tempering method on the functional and sensory properties of popped sorghum. The effects induced by cooking, dry heating, popping, fermentation and germination on sorghum proteins were studied by Correia et al. (2010). Popping is one such inexpensive processing method that can be employed to make different sorghum varieties suitable for human consumption for use in functional foods. Although published studies (Parker et al. 1999; Duodu et al. 2001; Kayode et al. 2007) have indicated the use of low polyphenolic white sorghum due to its non-intrusive flavour and colour, information on the use of coloured sorghum varieties is scarce.

The objectives of the present work are thus to study the effect of popping on (a) physicochemical, nutritional and anti-nutritional properties of three varieties of sorghum (pop sorghum, *maldandi* and red sorghum) differing in their endosperm nature and pigment and (b) starch and protein digestibility.

Materials and methods

Three varieties of sorghum namely pop sorghum, common variety (*maldandi*) and red sorghum were procured from the local market in Mysore, India, and cleaned. α -Amylase (from *Bacillus amyloliquefaciens*) and amyloglucosidase (from *Aspergillus niger*) were obtained from Sigma Chemicals (St, Louis, Mo, USA). Pepsin (pig stomach mucosa, 1:10000 units) and glucose oxidase-peroxidase kit were obtained from Megazyme International, Ireland. All other solvents/chemicals used were of analytical grade and were obtained from Merck, Mumbai, India.

Popping of samples Initial trials indicated that the pop sorghum variety can offer complete popping of individual grains; the other two varieties (*maldandi* and red sorghum) require a minimum moisture content of 16% for complete popping. The pop sorghum variety was dried in a tray drier at 40 °C until the sample attained about 12% moisture content. Red sorghum and *maldandi* was sprayed with water to adjust to 16% moisture followed by moisture equilibration for 48 h in sealed condition and stored at 4 °C. A hot air laboratory model corn popper (Model # NPC-1212, Nova, China) was used for popping of samples. Fifteen grams of each variety of sorghum were popped while maintaining a temperature of 240 ± 5 °C. The popped

grains were removed by the hot air and corresponding time was considered as the popping time. The separation of popped from unpopped grains was done by using appropriate sieves, and popping yield was noted as the percentage of the grain popped. Both native and popped samples of all three varieties of sorghum were ground to flour using a laboratory model coffee grinder; particle passing through a 60-mesh British Standard (BS) sieve was used for further analysis. All popping trials were repeated thrice.

Total starch (TS) and resistant starch (RS) Total starch was determined according to Holm et al. (1988) after removal of fat from the samples by petroleum ether. Glucose concentration was determined by using a glucose oxidase-peroxidase kit. Resistant starch was determined by using the Megazyme kit following the procedure by Niba and Hoffman (2003). Glucose solution (1 mg/0.1 ml) was used as a standard. All experiments were repeated thrice.

Amylose content Amylose from defatted sorghum samples was estimated in triplicates using the method of Sowbhagya and Bhattacharya (1971) using potato amylose as a standard.

In-vitro kinetics of starch digestion These were analysed following the procedure suggested by Goni et al. (1997). Fifty milligram of sample was dispersed in 10 ml of KCl-HCl buffer. The rate of starch digestion was expressed as a percentage of the starch hydrolyzed at different times (30, 60, 90, 120, 150 and 180 min). Each sample was analysed in triplicates.

Kinetic parameters of starch hydrolysis were calculated from the derived generalized equation (Eq. 1) where any change in the quality parameter (C) with time t is represented as

$$\frac{dC}{dt} = k C^n \quad \dots \quad (1)$$

where, C corresponds to the percentage of starch hydrolysed at time t, k is the kinetic rate constant and n is the order of change. For a first order change, we get

$$-\frac{dC}{dt} = k C \quad \dots \quad (2)$$

If equilibrium condition is attained during reaction/change, then C equals C_s at $t \rightarrow t_\infty$, where C_s is the equilibrium percent of starch hydrolysed after 180 min. Then Eq. 2 is written (Bhattacharya et al. 1997) as:

$$-\frac{dC}{dt} = k(C - C_s) \quad \dots \quad (3)$$

At $t=0$, C is equal to C_o , and hence Eq. 3 becomes

$$\ln \frac{C - C_s}{C_o - C_s} = -k t \quad \dots \quad (4)$$

In a situation when the initial value is zero i.e. $C_o = 0$, $\ln \frac{C - C_s}{-C_s} = -k t$

$$\text{Thus, } C = C_s(1 - \exp^{-kt}) \quad \dots \quad (5)$$

Hence, Eq. 5 can be conveniently used to calculate the rate constant k from the slope of $\ln \frac{C_s - C}{C_s}$ versus t . The parameter C_s was estimated for each sample based on the data obtained from the in-vitro hydrolysis procedure. Glycemic index (GI) was estimated based on the kinetics of in-vitro starch hydrolysis. The area under the hydrolysis curve (AUC) was calculated using the Eq. 6

$$\text{AUC} = [C_s(t_f - t_o) - (C_s/k)\{1 - \exp[-k(t_f - t_o)]\}] \quad \dots \quad (6)$$

where, t_o is the initial time and t_f is the equilibration time of starch hydrolysis. Hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each sample by the corresponding area of a reference sample (fresh white bread) (Goni et al. 1997); these authors indicated that hydrolysis index is a good a predictor of glycemic response. Expected GI was thus estimated using the Eq. 7.

$$\text{GI} = 39.71 + (0.549\text{HI}) \quad \dots \quad (7)$$

Total protein and soluble protein The micro-Kjeldahl method was employed to determine the total nitrogen and the crude protein ($N \times 6.25$) (AOAC 2002). Soluble proteins in sorghum samples were determined by the method followed by Cagampang and Kirleis (1984) where proteins were extracted from the flour by stirring 200 mg of the flour with 10 ml of distilled water in a 15 ml screw-capped centrifuge tube for 2 h at 4 °C. The suspension was

centrifuged at 3000 rpm at 0 °C for 10 min, and the clear supernatant was assayed for protein content by the method described by Lowry et al. (1951).

In-vitro protein digestibility The in-vitro protein digestibility procedure, as described by Walter and Mark (1964) with minor modification, was employed. To 100 mg of sample, pepsin (pig stomach mucosa) was added along with 10 ml of KCl-HCl buffer at pH 2 at 37 °C and incubated for 2 h. Later, it was neutralized with 0.2 N NaOH followed by addition of pancreatic enzyme from pig pancreas and incubated for 2 h. The undigested protein residue was then recovered by precipitation and filtration through Whatman # 4 filter paper as followed by Parker et al. (1999). The micro-kjeldahl method was employed to determine the total nitrogen and the crude protein ($N \times 6.25$) from the undigested protein residue (AOAC 2002). Protein digestibility was calculated as the difference between total nitrogen and nitrogen content of undigested protein residue.

Iron and zinc content Ash sample was wet digested using nitric acid (5:1, v/v). The total amount of iron and zinc in the digested samples of native and popped sorghums was determined by atomic absorption spectrophotometer (Atomic Absorption Flame Emission Spectrophotometer, AA6701F, Shimadzu) according to the method of AOAC (2002).

Phytic acid content Phytic acid was determined according to the procedure of Haug and Lantzsch (1983) and was expressed as $\mu\text{g/g}$ of the sample.

Statistical analysis One way and two-way analysis of variance (factorial) with replicates were used to analyse the significant difference among the results of samples. Multiple comparisons were made for all experiments using least significant difference test (Duncan's Multiple Range

Table 1 Total starch, resistant starch, digestible starch and amylose contents of sorghum samples

Sorghum samples	Treatment	Popping yield (%)	Total starch (%)	Resistant starch (%)	Amylose content	
					Total (%)	Soluble (%)
Pop sorghum	Native	—	76.3±1.8 ^a	3.4±0.4 ^b	22.2±1.0 ^b	10.5±0.5 ^a
	Popped	100±0.2 ^c	78.3±1.5 ^a	2.0±0.3 ^a	17.4±1.7 ^a	14.2±0.7 ^c
Maldandi	Native	—	73.5±1.2 ^a	4.3±0.7 ^c	24.7±1.3 ^c	10.8±0.8 ^a
	Popped	30.3±5.0 ^a	74.3±1.0 ^a	3.1±0.5 ^b	21.2±0.7 ^b	12.6±0.9 ^b
Red sorghum	Native	—	75.5±1.5 ^a	3.9±0.6 ^c	24.5±1.5 ^c	10.8±1.3 ^a
	Popped	55.5±5.5 ^b	76.2±1.7 ^a	2.9±0.3 ^b	21.5±1.2 ^b	12.4±1.1 ^b

Expressed as dry basis

Values are mean ± SD of three observations

Means of the same column followed by different letters differ significantly ($p \leq 0.05$) according to least significant difference

Table 2 Kinetic parameters, hydrolysis index (HI) and estimated glycemic index (GI) of popped sorghum samples

Sorghum samples	Equilibrium percentage of starch hydrolyzed C_s (%)	Rate constant k (min^{-1})	Calculated HI (-)	Estimated GI (-)
Pop sorghum	82.0 ± 2.2^c	0.029 ± 0.001^b	95.6 ± 4.5^c	92.2 ± 2.5^b
<i>Maldandi</i>	76.8 ± 1.2^b	0.026 ± 0.001^a	85.3 ± 3.1^b	86.6 ± 1.5^a
Red sorghum	73.0 ± 1.7^a	0.025 ± 0.001^a	82.9 ± 2.1^a	85.2 ± 2.2^a

Values are mean $\pm$ SD of three observations

Means of the same column followed by different letters differ significantly ($p \leq 0.05$) according to least significant difference

Test) at a probability (p) of 0.05 and the results are expressed as mean $\pm$ standard deviation (SD).

Results and discussion

Starch characteristics The total starch, resistant starch and the amylose contents of the three varieties of sorghum along with their popping yield are shown in Table 1. Pop sorghum variety shows 100% popping yield possibly due to the highest starch content whereas *maldandi* variety shows the least (only 30%). The total starch content of the native (raw) sorghum varieties ranges from 73.5% to 76.3% (dry basis). The total amylose and resistant starch content significantly ($p < 0.05$) decreases after popping while soluble amylose increases for all the varieties. Puffing was promoted by gelatinisation as well as lipid-amylose complexation (Mahanta and Bhattacharya 2010). As puffing of the grains is expected to disrupt the sub-cellular structure of the endosperm, the penetration of digestive enzymes becomes easier, and consequently, increases the starch content marginally. Unprocessed sorghum contains native starch which is resistant to digestion (Topping and Clifton 2001) and hence the resistant starch content is high. Severe heat treatment like autoclaving has been reported to reduce the resistant starch content (Skrede et al. 2001).

Starch digestibility The results of in-vitro starch digestion are summarized in Table 2 in addition to kinetic parameters of starch hydrolysis. The starch hydrolysis curves of popped samples (Fig. 1) indicate the attainment of equilibrium beyond 150 min; the pop variety shows highest starch digestibility while red variety exhibits the least value. Pop sorghum shows significantly ($p < 0.05$) higher equilibrium percentage of starch hydrolysed (C_s) and rate constant (k) followed by *maldandi* and red varieties (Table 2). The magnitude of GI is highest for the pop sorghum (92) followed by *maldandi* (87) and red sorghum (85) (Table 2). Application of popping treatment decreases resistant starch content leading to an increase in starch digestibility. The *maldandi* variety fails to provide complete popping meaning that certain parts of the grain do not expand and

the cell walls remain intact (Parker et al. 1999); this leads to restricted action of amylolytic digestion process in popped samples. Moreover, starch rich in amylose generally retrogrades fast to offer a less digestible product (Voragen 1998); this phenomenon is possibly responsible for the presence of higher resistant starch content in *maldandi* and red sorghum compared to pop variety. Gelatinisation of starch has been reported to influence starch digestibility (Niba and Hoffman 2003). The high GI for the pop sorghum is possibly due to an increase in soluble amylose content due to popping.

Protein characteristics The total protein content of the three varieties shows non-significant differences on popping; however, albumin or the soluble protein content shows significant increase in all the varieties (Table 3) due to popping with a larger variation noticeable for pop and red sorghum varieties; a higher increase is observed in pop sorghum which is concomitant to the increase in soluble protein content (Table 3). Starch granule in raw cereals is usually surrounded by a protein matrix making it inaccessible to the digestive enzymes. During popping starch granules explodes to yield a foam like structure to disrupt starch-protein matrix; this modified structure can undergo

Fig. 1 In-vitro starch hydrolysis for different varieties of sorghum after popping. Values are mean and standard deviation of three observations

Table 3 Total protein, albumins and protein digestibility in sorghum after popping

Sorghum samples	Treatment	Total protein (%)	Albumins $\mu\text{g/g}$ (%)	Protein digestibility (%)
Pop sorghum	Native	9.7 ± 0.7^a	175.0 ± 2.5^c	70.2 ± 1.5^c
	Popped	9.7 ± 0.5^a	220.0 ± 3.2^f	73.5 ± 1.1^d
<i>Maldandi</i>	Native	9.8 ± 0.6^a	192.0 ± 4.5^d	67.1 ± 1.3^b
	Popped	9.7 ± 0.4^a	200.0 ± 5.3^e	69.9 ± 1.2^c
Red sorghum	Native	9.7 ± 1.0^a	135.0 ± 3.0^a	65.0 ± 1.3^a
	Popped	9.8 ± 0.8^a	168.0 ± 4.2^b	67.9 ± 1.5^b

Expressed as dry basis

Values are mean $\pm$ SD of three observations

Means of the same column followed by different letters differ significantly ($p \leq 0.05$) according to least significant difference

easy gelatinisation, becomes accessible to digestive enzymes and improves digestibility.

The proteins of sorghum are less digestible compared to other cereals due to the exogenous and endogenous factors. In the corneous endosperm of native sorghum, the non-kafirins (albumins, globulins and glutelins) effectively “glue” the bodies into a matrix surrounding the starch granules. However, sorghum proteins contain a higher proportion of cross-linked fractions of kafirins and are more hydrophobic that explain their higher propensity to form disulphide bonds (Wong et al. 2009). Perhaps the disruption of cell wall and damage to these gluing substances make the soluble proteins available for digestion by pepsin to enhance protein digestibility.

Phytic acid The phytic acid content is higher in the red sorghum compared to the other two varieties (Fig. 2). The phytic acid content also reduces to an extent of 20%–25% after popping which is close to earlier reported values of 17.9%–37.5% (Mahgoub and Elhag 1997) for African sorghum after heat treatment. Processing treatments have been reported to reduce phytic acid content and improves protein digestibility (Knuckles et al. 1985). Popping helps to control phytic acid content in sorghum by which the

medical and health benefits of the food may be preserved (Liu et al. 2005). Phytic acid is also considered as a phytochemical and antioxidant. The available phytic acid can help in bringing down the peroxidation levels and thereby increases the shelf-life of product.

Total iron and zinc content The changes in the iron and zinc content of the sorghum varieties on popping are presented in Table 4. The iron and zinc content of three varieties are not statistically different and these elements decreases marginally due to popping.

Conclusions

Popping treatment decreases resistant starch leading to an increase in starch digestibility. A starch rich in amylose is responsible for the presence of higher resistant starch content in *maldandi* and red sorghum compared to pop variety. The high GI for the pop sorghum is due to higher soluble amylose content after popping. Popping treatment reduces phytic acid content and improves protein digest-

Fig. 2 Effect of popping on phytic acid content in sorghum Values are mean and standard deviation of three observations

Table 4 Effect of popping on changes in the iron and zinc content in sorghum samples

Sorghum samples	Treatment	Fe (mg/100 g)	Zn (mg/100 g)
Pop sorghum	Native	3.9 ± 0.5^a	1.6 ± 0.3^a
	Popped	3.5 ± 0.3^a	1.5 ± 0.2^a
<i>Maldandi</i>	Native	4.4 ± 0.4^a	1.8 ± 0.3^a
	Popped	3.4 ± 0.4^a	1.6 ± 0.2^a
Red sorghum	Native	4.9 ± 0.5^a	1.9 ± 0.5^a
	Popped	4.6 ± 0.3^a	1.7 ± 0.4^a

Expressed as dry basis

Values are mean $\pm$ SD of three observations

Means of the same column followed by different letters differ significantly ($p \leq 0.05$) according to least significant difference

ibility. High digestibility of popped sorghum may be part of the rationale for the traditional use of flour from popped sorghum as easily-digested food for young and elderly people.

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