



Effect of Heat Moisture Treatment on Resistant Starch Content of Sorghum Flour and Sorghum Starch

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ABSTRACT

Sorghum flour is a gluten-free ingredient and can be used to prepare foods for celiac patients. In addition, sorghum flour is a good source of fiber in the form of resistant starch. The objectives of this research were to develop an effective process to increase resistant starch content of sorghum flour. Samples of white sorghum flour with different moisture contents (20%, 25% and 30%) were treated at a temperature of 100°C for 4 h. Samples after heat treatments were tested for resistant starch. The sample treated with 30% moisture at 100°C for 4 h had high resistant starch (RS) content (30.2% compared with 24.6% of the native sample). The same heat-moisture treatment on isolated sorghum starch showed high resistant starch (RS) content (10.8% compared with 8.2% of 20% moisture content). In conclusion, heat-moisture treatments were successful in increasing resistant starch content of sorghum flour to retain starch functionality in food product applications. Sorghum flour with increased resistant starch content after heat treatment was evaluated and compared with normal sorghum flour for starch digestibility using the Integrated Total Dietary Fiber method. The amylose content the soluble amylase content of both of sorghum starch and sorghum flour had decreased significantly during HMT.

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INTRODUCTION

Starch is classified based on the rate of digestion as rapid digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). RS is the portion of the starch that resists enzymatic digestion in the small intestine (Englyst et al., 1992; Sajilata, Singhal, & Kulkarni, 2006) and can be classified into five different types: RS1 is resistant because of physical barriers that prevent enzymes from accessing and digesting starch; RS2 is granular resistant starch that cannot be digested completely due to its granular structure; RS3 is retrograded starch formed after cooking and cooling in which the amylose is re-arranged into a crystal form and is not digestible; RS4 is chemically modified starch such as cross-linked starch; and RS5 is a new RS category based on amylose-lipid complex (Hasjim et al., 2010). Because RS can escape digestive enzymes in the small intestine, RS can positively affect blood glucose (Behall, Scholfield, Hallfrisch, & Liljeberg-Elmstahl, 2006) and insulin levels (Behall et al., 2006; Maki et al., 2012), and improve colonic health by increasing fecal bulk and short chain fatty acid (SCFA) (Jenkins et al., 1998).

Different methods have been used to improve RS content of cereal starches and flours including chemical and thermal treatments. Thermal treatments, including heat-moisture treatment, also have been used successfully to increase RS content of different starches. Heat-moisture treatment requires high temperature and low moisture content (less than 30%). Heat-moisture treatments has been reported to affect the physicochemical properties and functionality of different starches including sorghum (Olayinka, Adebowale, & Olu-Owolabi, 2008; Singh, Chang, Lin, Singh, & Singh, 2011; Sun, Han, Wang, & Xiong, 2014), normal and waxy potato (Jiranuntakul, Puttanlek, Rungsardthong, Pancha-Arnon, & Uttapap, 2011; Varatharajan, Hoover, Liu, & Seetharaman, 2010; Kim & Huber, 2013), normal and waxy rice, normal and waxy corn (Jiranuntakul et al., 2011), and millet (Amadou, Gounga, Shi, & Le, 2014). Both decreased and increased susceptibility to α -amylase of different starches after heat-moisture treatments were reported by Hoover & Vasanthan (1994). Heat-moisture treatment with low moisture content (<30%) and high temperature (80-140 °C) has been shown to improve SDS and RS content of corn, pea, and lentil starches (Chung, Liu, & Hoover, 2009). The combination of acidic conditions and heat-moisture treatment on corn starch resulted in higher level of boiling-stable RS when applied to normal and high-amylose corn

starches (Brumovsky & Thompson, 2001; Lin, Singh, Wen, & Chang, 2011). Heat-moisture treatment combined with mildly acidic conditions (pH 5 to 6.5) was shown to increase levels of thermally-stable RS content of potato starch (Kim & Huber, 2013). A combination of heat-moisture treatments and phosphorylation on high-amylose corn starch was shown to result in high RS content that can survive cooking (Sang & Seib, 2006). Enzymatic modification combined with hydrothermal treatments can be used in the formation of RS in red kidney bean starch (Reddy, Suriya, & Haripriya, 2013). In summary, different methods can be used to increase RS content of starches, but the process usually involves adding chemical groups to the starch, or using specialty materials (e.g. high amylose cornstarch).

Sorghum is widely grown in the semiarid tropics of Africa and Asia, and constitute a major source of carbohydrates and proteins for people living in these regions. Although the use of sorghum as human food is widespread, the technology for processing these grains into consumable products is still far from adequate compared with other major cereal grains such as maize, wheat and rice (Lazaro & Favier, 2000). Since sorghum grain can be one of the important foodstuffs under local circumstances, it can be a new source for starch industry. The sorghum starch isolated from the grain may be important in supplying material for the food industries. The sorghum starch is like corn starch in many functional properties, likewise it can be a good substitute for corn starch (Perez, Lares, & Gonzalez, 1997).

Sorghum flour is an excellent material for heat-moisture treatment to obtain high resistant starch content. Compared with corn starch, sorghum starch in flour is known to be less digestible (Elkin et al., 2002; Rooney & Pflugfelder, 1986). In sorghum flour, protein exists mostly on the outside of starch granules, forming a physical barrier on the surface of starch granules, and making the starch granules inaccessible for enzymatic digestion. This could be an advantage of sorghum flour in preparing RS. (Hamaker, Kirleis, Mertz, & Axtell, 1986; Zhang & Hamaker, 2003).

Starch is the major component of sorghum (75%) and it is unique among carbohydrate classes because it occurs naturally as discrete particle/granules (Watson, 1984). Granules are composed of an essentially linear polysaccharide called amylose, and a highly branched polysaccharide called amylopectin. Starch is used widely in the food industry as a thickening, stabilizing, and gelling properties, which makes it an excellent ingredients for the manufacturing of various food products, paper and textile industries (Slattery, Kavakli, & Okita, 2000; Wurzburg, 1999). The native starch has a limited application, in order to get a wider application the starch has to be modified physically by heat-moisture treatment (HMT). HMT is a process that involves heating of starch at elevated temperature with incubation of starch granules in varying moisture levels between 18% and 27% for a certain period of (upto 16 h) at temperature of 110 °C (Kweon, Haynes, Slade, & Levine, 2000). The objective of this study was to determine the impact of HMT on resistant starch content of white sorghum starch

MATERIALS AND METHODS

Sample

White sorghum grains were collected from Indian Institute of Millet Research, rajendranagar, Hyderabad, Telangana. The grains were cleaned and they appeared to be free of mold or weathering. All chemicals used were of loba grade.

Starch isolation

100g of the sorghum grains was suspended in water with the ration 1:5 and the pH adjusted to 8 with 0.2%w/v of NaOH solution for 24 h. Then it was stirred for 30min and the grains were screened and washed with distilled water until they were unpigmented. Wet milling procedure of Watson, Sawners, Wakely, and Williams (1955) was used for the isolation with some modification. The grains were blended with distilled water for 45 min while the slurry obtained after blending was re-suspended in 51 ml of distilled water and the pH was adjusted to 8.0 using 0.5M NaOH solution. The pH was maintained between 8.0 and 8.5 and the mixture was manually stirred for 30 min. The suspension obtained was screened through a 75 and 80 mm sieve and centrifuged for 30min and was thoroughly washed four times with distilled water. The starch slurry was allowed to settle for 45min and water was decanted. This was then allowed to dry at room temperature for 48 h.

Heat treatments of sorghum flour

Heat-moisture treatment of sorghum flour

Appropriate amounts of distilled water were added to 15 g of sorghum flour to adjust moisture content (wet basis) to 20%, 25% and 30% and the samples were whisked to thoroughly distribute the water into the flour. Sorghum flour with moisture content of 20%, 25% and 30% was sealed in canning jars and heated for 4 h at 100°C to prevent moisture loss and heated for 4 h. After heat-moisture treatment, flour samples were transferred to Petri dishes for drying and moisture equilibration overnight at room temperature.

Heat treatments of sorghum starch

Similar to sorghum flour, sorghum starch was heated for 4 h at 100°C with moisture contents of 20%, 25% and 30%. Samples after heat treatments were cooled, dried (if needed), and left on the bench top overnight for moisture equilibration. Samples of sorghum starch before and after heat treatments were analyzed for resistant starch.

Chemical composition such as moisture content, protein, and fat was determined according to AOAC standard method (AOAC, 2005). Moisture was determined using hot air oven method carried out at 100-105°C for 3 hours (AOAC, Method 945.43). Protein content (% N x 6.25) was obtained by Kjeldahl method (AOAC, Method 950.36). Fat content was obtained by Soxhlet Extraction Method utilizing petroleum ether 40°C-60°C (AOAC, Method 922.06). Resistant starch content was determined by (AOAC, 1990). The sorghum flour was tested total starch content and was carried out using the method of Dubois *et al.* (1956). All analyses were carried out in triplicate and average value was recorded for further analysis.

Resistant starch

Sorghum flour was subjected to a Newer method for measuring RS, including AOAC 985.29 (Prosky, Asp, Schweizer, Devries, & Furda, 1992) and AOAC 991.43 (Lee, Prosky, & Devries, 1992), employ thermally stable α -amylase to digest starch at boiling temperatures. In these methods, after starch digestion, samples are treated with protease and amyloglucosidase to remove protein and digestible starch, and RS is precipitated, filtered, dried, and corrected for protein and ash content.

Procedure

500mg of sample was dispersed in 25ml of 0.08M phosphate buffer. 0.05ml of α - amylase was added and kept in water bath at 95°C for 15min and cooled to room temperature then Ph was adjusted to 7.5 by adding 5 ml of 0.205N NaOH. 0.05ml of protease(50mg/ml of protease in phosphate buffer) and kept in water bath at 60°C for 30 min. Then Ph was adjusted to 4.3 by adding 0.325N HCL. 0.05ml of amyloglucosidase was added and placed in water bath at 60°C for 30 min. Then 4 ml of 95% ethanol was added and kept over nigh. Then precipitates were collected on a filter paper by washing with 78% ehanol (120 ml), absolute ethanol (10 ml), acetone(10 ml).

Resistant starch= Insoluble residue weight / sample weight* 100

Amylose content and soluble Amylose content

The apparent amylose content of sorghum starch and sorghum flour were estimated by using the method of Sun and Wang *et al.* (2013). The starch (20 mg, dry weight basis) was dissolved in 90% dimethylsulfoxide (8 mL) in 10 mL screw-cap reaction vials. The contents of the vials were vigorously agitated for 20 min and then heated in a water bath (with intermittent shaking) at 85°C for 15 min. The vials were then cooled to ambient temperature and the contents diluted with water to 25 mL in a volumetric flask. The diluted solution (1.0 mL) was mixed with water (40 mL) and 5 mL of iodine (I₂)/potassium iodide (KI) solution (0.0025 M I₂ and 0.0065 M KI) and the final volume was 50 mL. The solution was allowed to stand for 15 min at ambient temperature prior to absorbance measurements at 600 nm. A standard curve of amylase standard was plotted for estimating the content of amylose and amylopectin for sorghum starch and sorghum flour. The apparent amylose content of sorghum starch and sorghum flour was calculated using .

The apparent amylose content (%)= $A \cdot 100 / (W \cdot 5) \cdot 100$

where A = weight of the apparent amylose found in standard curve according to the absorbance of apparent amylose;

W= weight of dried sample (sorghum starch and sorghum flour).

The next step of the experiment involved accurately weighing about 100 mg of starches or flours and placed it in a 100 mL conical flask. The starches or flours were wetted with 1 mL distilled alcohol, and then about 50 mL distilled water were added. The flask was then covered with a bulb stopper, heated for 20 min in a boiling water bath with occasional shaking, then cooled in ambient temperature water to room temperature. Boiled and cooled distilled water was then added to the content in a volumetric flask such that the resulting content had a volume of 100 mL. The mixture was then filtered through a Whatman No. 4 filter paper and the first portion was rejected. About 20 mL of the extract were then transferred into a graduated 50 mL glass-stoppered cylinder and 7 mL of petroleum ether (boiling range, 60-80 °C) were added. The cylinder was shaken intermittently for 10 min and let to stand for 10-15 min. The ether layer was then suctioned off with a water suction device. The extraction with petroleum ether was repeated. 5 mL of the extracted solution were pipetted into a 100 mL volumetric flask and about 50 mL of distilled water (boiled and cooled) and 2 mL of iodine solution were added, and then the volume (100 mL) was made up with distilled water (boiled and cooled). An iodine blank was prepared by adding 2 mL iodine solution to 100 mL with ordinary distilled water. The solutions were read after approximately 20 min in a spectrophotometer such as the Spectronic 20 or the Zeiss Spekol, at 630 nm against the blank. The soluble amylose content and insoluble content of sorghum starch and sorghum flour were calculated using Eqs. (2) and (3):

The soluble amylose content(%) = $R/A * a/r * 1/5 * 100$

where, A = the absorbance of standard amylose; R = the absorbance of soluble amylose; a = weight of standard amylose; r = weight of dried samples (sorghum starch and sorghum flour).

The insoluble amylose content(%) = $\frac{1}{4} CAA - CSA$

where, CAA: the content of apparent amylose; CSA: the content of soluble amylose (sorghum starch and sorghum flour).

Swelling power (SP) and solubility (% SOL)

The swelling power (SP) and solubility (% SOL) of the starch samples were determined by a method of Adebooye and Singh (2008) with a slight modification. Approximately 800 mg (db) of sample was cooked in about 80 mL of water at different temperatures of 55, 65, 75, 85 and 95 °C for 30 min, respectively. Then they were cooled to room temperature and centrifuged at 3000 rpm (AnkeLXJ-IIB Centrifuge, Shanghai, China) for 15 min. The supernatant was decanted carefully and kept and the residue was weighed for SP determination. The supernatant was then poured out from the tube to a glass dish (of known weight). Afterwards, the dish was dried at 105 °C to constant and weighed. All measurements were done in triplicates. The swelling power and percent solubility were calculated.

Effect of temperature on swelling power and solubility

A starch sample 1.0 g was accurately weighed and quantitatively transferred into a clear dried test tube and re-weighed (W1). The starch was then dispersed in 50 cm³ of distilled water. The resultant slurry was heated at the desired temperatures 60, 70, 80, and 90 °C for 30 min in a water bath. The mixture was cooled to 30± 2°C and centrifuged (500 rpm, 15 min). Aliquots (5 ml) of the supernatant were dried to a constant weight at 110°C. The residue obtained after drying the supernatant represented the amount of starch solubilized in water. Solubility was calculated as g per 100 g of starch on a dry weight basis. The residue obtained from the above experiment (after centrifugation) with the water it retained was quantitatively transferred to the clean dried test tube used earlier and weighed (W2).

Swelling of starch = $W2 - W1 / \text{weight of starch}$.

RESULTS AND DISCUSSION

Sorghum flour composition

The flour as received had a moisture content of 10% (wet basis), protein content of 12.5% (dry basis), starch content of 80% (dry basis), and RS content 5% (starch basis).

While heat-moisture treatments increased RS levels. Samples of sorghum flour with 20%, 25% and 30% MC heated for 4 h at 100°C were found to have a significantly higher RS content (26.8%, 25.8% and 30.2% respectively) compared with RS content of untreated sorghum flour (24.6%). The increase in RS content of heat-treated sorghum flour was larger than that previously reported by (Chung et al., 2009) for corn, pea, and lentil starches. Thermo-stable RS contents increase after heat-moisture treatment of potato starch under mildly acidic pH, where amylopectin molecules are hydrolyzed under acidic conditions and re-associate to become resistant to enzyme digestion (Kim & Huber, 2013). Heat-moisture treatment alone (e.g. without pH adjustment or enzymatic pretreatment) is not very effective at increasing RS content of other starches compared with sorghum flour.

Sorghum starch

RS contents of native sorghum starch and sorghum starch that was heat-treated for 4 h are shown in Table No 2 While heat-moisture treatments increased RS levels. Samples of sorghum starch with 20%, 25% and 30% MC heated for 4 h at 100°C were found to have a significantly higher RS content (8.2%, 9.2% and 10.8% respectively) compared with RS content of untreated sorghum starch (5.2%).

RS content increased for samples heated at 100°C and 30% MC. With the majority of the protein removed during the isolation process, isolated sorghum starch did not show a significant increase in RS content compared with the same heat treatment on sorghum flour. Thus, the change in RS content of heat treated sorghum flour cannot be explained only by changes in starch properties. RS contents of native sorghum flour and sorghum starch, as well as heat treated sorghum flour and sorghum starch at 100°C, 4h, and 30% MC, are shown in Table 1. Interestingly, isolated starch from heat treated sorghum flour had a high RS content.

RS content of the same sample can vary from method to method because of the differences in techniques to digest and measure starch (Maningat, Seib, & Bassi, 2013). For example, evaluating cross-linked wheat starch with the integrated TDF method (AOAC2009.01) results in low RS content (23.9%) compared with Englyst method (81.7%) (Shukri, Seib, Maningat, & Shi, 2013).

Solubility, swelling power

The contents of solubility, swelling power and amylose content of sorghum starch and sorghum flour are presented in Table 1 and 2. The solubility and swelling power of sorghum starch and sorghum flour decreased significantly after HMT than the native sample. The solubility of sorghum flour was higher than that of sorghum starch, but the swelling power of sorghum flour was lower than that of sorghum starch. Therefore, HMT had a far greater effect on solubility and swelling power of flour than that of starch. The amylose content of sorghum starch and sorghum flour had no significant change during HMT.

The amylose content of sorghum starch and sorghum flour had no significant change during HMT, but the soluble amylose content of both decreased evidently with the increased moisture content of HMT. After a 25% moisture content HMT treatment, soluble amylose contents of sorghum flour and sorghum starch were decreased from 9% and 14% to 8% and 9%, respectively (Table 1 & 2).

4. Conclusion

Heat-moisture treatment can effectively increase RS content of sorghum flour by modifying sorghum protein, presumably through increases in cross-linking. Changes in sorghum starch after heat-moisture treatment were minimal. Unlike with sorghum flour, the same heat-moisture treatment of isolated starch did not result in increased RS content at the same level. This study provides insight into how sorghum starch changes during heat-moisture treatment, providing information for further modification to increase or decrease digestibility of sorghum starch without affecting starch structures and functionality. Amylase and soluble amylase content of sorghum flour and sorghum starch had decreased after HMT.

Table 1

	Sorghum flour				
	Resistant starch content	Solubility	Swelling power	Amylose	Soluble Amylose
Native sample	24.6	28	13	20	10
20%	26.8	23	9	18	9
25%	25.8	20	7	17	8
30%	30.2	19	5	17	8

Table 2

	Sorghum starch				
	Resistant Starch Content	Solubility	Swelling Power	Amylose Content	Soluble Amylose Content
Native sample	5.2	24	15	26	16
20%	8.2	16	13	25	14
25	9.2	14	12	24	10
30	10.8	12	10	24	9

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