Effect of pepsin and SO₂ treatments on yield and properties of starch from Tabat and Wad-baco sorghum cultivars

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Abstract This study aims to evaluate the effect of pepsin and SO₂ treatments on the yield and physicochemical-, functional-, and color properties of starches isolated from Tabat and Wad-baco sorghum cultivars. Results revealed that starch yield from treatments B (steeping for 7 h; 3 h in distilled water, 4 h in 0.1 M sodium acetate buffer, pH 4.5), BE (steeping for 7 h; 3 h in distilled water, 4 h in 0.1 M sodium acetate buffer, pH (4–4.5) with 250 mg pepsin), BES (steeping for 7 h; 3 h in distilled water, 4 h in 0.1 M sodium acetate buffer, pH 4.5 with 250 mg pepsin and 0.2% SO₂), and S (steeping for 7 h; 3 h in distilled water, 4 h in 0.2% SO₂) were significantly higher than that from the control (A; steeping for 24 h in 0.2% SO₂) in both cultivars. The combination of pepsin and SO₂ treatment (BES) produced an increased starch yield of 78.90% and 85.56% from Tabat and Wad-baco, respectively. Micrographs showed hydrolysis of the protein matrix attached to the starch by pepsin, which created holes on the surface of starch granule. Gelatinization temperature decreased in the treated starches from both cultivars compared to control. Water- and fat- absorption capacities were higher in the starch from all treatments compared to control. Generally,

enzyme treatment improved the yield and functional properties of starches from both cultivars.

Keywords Sorghum (Tabat, Wad-baco) · Starch · Pepsin enzyme · Functional properties · Starch yield · Morphological properties

Introduction

In plants, starch represents the main polysaccharide reserve and its granules are found in various sizes and shapes depending on the botanical source. Amylose and amylopectin are the major components in starch granules and the arrangement and interaction between these molecules in starches has great influence on the functional and physicochemical properties of starch (Boudries et al. 2009). The quality of starch-based products is affected by these properties and is crucial in determining their suitability for use in food systems. The ability of starch to form a viscous paste when heated in water followed by cooling makes it suitable for various uses in both food and non-food industries (Nguyen et al. 1998). Common sources of starch used in food systems include corn, potato, and wheat (Boudries et al. 2009). However, the use of sorghum starch in food systems has been given a little or no attention. Sorghum is cultivated annually in Sudan as a staple food. Gadarif State (eastern Sudan) is the most important region for sorghum production in Sudan and Tabat and Wad-baco cultivars are among the most popular sorghum cultivars grown.

Starch is the most important carbohydrate polymer due to its functionality in food systems (Taylor et al. 2006). Sorghum is very rich in starches that comprise 60–80% of sorghum kernels and has been recommended for several

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industrial applications (Elkhalifa et al. 2004). Starch plays a central role in the nutritional and physicochemical attributes of finished food products. Starch is versatile and a raw material for producing many products. Since 1930s, carbohydrate chemists have developed numerous products that have greatly expanded starch use and utilization. Starch is a raw material for multiple industrial products. It has several applications in pharmaceutical industry and is also used as an adhesive in paper manufacturing. In addition, starch can be fermented to produce alcohol and is a raw material for the production of ethanol, a high performance fuel that considered environmentally safe. However, sorghum use in food systems is limited by the relatively low grain starch yield due to the presence of strong covalent bonds between the protein matrix and starch granules in the grain.

Several methods are used for isolation and purification of sorghum starch at both traditional and industrial levels. Traditionally, starch is isolated from sorghum by steeping the grains in water for 24–96 h followed by wet milling (Wang et al. 2000). However, the traditional method is time consuming and starch yield is often low and poorly digestible. Physical, chemical and biological methods have been designed and successfully used to reduce steeping time and improve starch yields. Fernandes et al. (2009) and Mandal et al. (2017) reported that power ultra-sonication (high-intensity ultrasound) processes change the structure of plant tissues, causing breakdown of the cell wall and forming microchannels, thereby increasing tissue porosity. The effects of sonication might reduce steeping time and increase starch yield and purity by breaking bonds between the protein matrix and starch granules in sorghum. Pepsin treatment during steeping has also been reported to break down sorghum protein matrix. Mezo-Villanueva and Serna-Saldivar (2004) reported that pepsin treatment reduced the amount of inseparable gluten-starch complexes to zero, thereby increasing sorghum starch yield. The effect of pepsin and cell wall hydrolyzing enzymes in improving starch yield and decreasing starch protein during wet milling of sorghum have been reported by Perez-Carrillo and Serna-Saldivar (2006). They also reported a significant increase in starch recovery following the addition of pepsin enzyme, while the use of cell-wall-degrading enzymes alone did not have a significant effect (Perez-Carrillo and Serna-Saldivar 2006). The effect of SO₂ treatment on starch yields from cereal grains has also been reported. Yang et al. (2005) reported a significant increase in yield and starch properties of SO₂ treated wet-milled corn starch. However, studies on the combined effect of pepsin and SO₂ treatments on the yield and purity of sorghum starch are scarce. Therefore, the main aim of the current study is to investigate the impact of pepsin and SO₂ treatments on

starch yield and properties from two Sudanese sorghum cultivars (Tabat and Wad-baco).

Materials and methods

Materials

Two sorghum varieties (*Sorghum bicolor* L. Moench) Tabat and Wad-baco were used. Tabat and Wad-baco were respectively obtained from Alfao Research Station and local market in Gadarif, Sudan. Pepsin enzyme (EC 3.4.23.1) and other chemicals were purchased from Sigma-Aldrich (Sigma Chemical Co., MO, USA). Samples were cleaned, transported to the laboratory, and kept at 4 °C prior to use.

Extraction of sorghum starch by different methods

Wet milling procedure as described by Steinke and Johnson (1991) was used with slight modifications. One hundred grams of sorghum grains were soaked at 4 °C for 24 h in 180 mL of distilled water containing 0.2% sulfur dioxide to inhibit microbial growth. Thereafter, the grains were washed several times with distilled water, wet-milled in a Warring blender (DS-1, BIAO, China) for 3 min, and screened through a 180 micron sieve (Tyler standard screen scale, Ohio, USA). The process of wet-milling was repeated until no further starch could be separated; this sample was labelled A and used as the control. For the treatment, a two stage modified procedure described by Johnston and Singh (2001) was used. Four hundred grams of grains were placed in 2000 mL Erlenmeyer flasks with 720 mL distilled water. Grains were soaked for 3 h at 48 °C. Thereafter, the water was drained, the grains were washed with both tap and distilled water, then milled in an equal volume of water using a Warring type blender. The slurry was divided into four portions and mixed with either 10 mL of 1 M sodium acetate buffer alone (sample B), 10 mL of 1 M sodium acetate buffer containing 250 mg pepsin (sample BE), 10 mL of 0.2% of SO₂ solution (sample S), or 10 mL of 1 M sodium acetate buffer containing 250 mg pepsin and 0.2% of SO₂ (sample BES). Samples were incubated at 48 °C for 4 h and mixed at 30-min intervals. The starch suspensions were centrifuged at 2000 g for 5 min (Dentrifu- oversize, serial No.A080-5, Shanghai food package, Machinery Branch Corp., China). Then, the starches were suspended in water, centrifuged, and the tailings were scraped with a spatula. The last step was repeated until a white prime starch (free of tailing) was obtained, and the starch was air dried for 18 h. The dried starch was re-dispersed in water and wet sieved through 150 micron sieve (Tyler standard screen scale, Ohio, USA).

The sieved slurries were centrifuged at 2000 g for 5 min, the water decanted and the starch air dried and kept for analysis.

Starch yield and purity

Starch yield was calculated for the ratio of extracted starch to grain amount as follows:

$$\text{Starch yield\%} = \frac{\text{Starch weight}}{\text{Grain weight}} \times 100$$

Starch purity (expressed as residual protein) was determined as the protein content of isolated starch using the Kjeldahl method (AOAC 2000)

Amylose content of sorghum starches

A rapid calorimetric method was used for estimating amylose content of the starch (William et al. 1975).

Determination of pH

The pH of the starch samples was measured according to the method reported by Aviara et al. (2010). Twenty milliliter of distilled water was added to 2 g of starch sample in a measuring flask, suspended, and kept for about 1 h at room temperature and pH was measured using a glass electrode pH meter (Hanna pH211, HANNA Instruments, USA). The measurement was taken three times and the average pH of the starch determined.

Titrateable acidity

Total starch acidity was determined according to AOAC (2000). Ten grams starch was weighed into a 250 mL beaker, 150 mL distilled water was added and mixed, and then the mixture was filtered through Whatman No. 1 filter paper. The filtrate was then titrated against 0.1 M NaOH using 0.3 mL phenolphthalein indicator (prepared by dissolving 0.5 g phenolphthalein in 100 mL of 50% alcohol). Titrateable acidity was expressed as lactic acid.

Titrateable acidity =

$$\frac{\text{Titre (mL)} \times N(\text{NaOH}) \times \text{dilution factor} \times \text{equivalent weight} \times 100}{\text{Weight of sample taken (g)} \times \text{volume taken for estimation} \times 1000}$$

where N = Normality of NaOH

Water and fat absorption capacities

The methods of Lin and Zayas (1987) were used for determining water absorption capacity (WAC) and fat absorption capacity (FAC). Briefly, 1 g sample was suspended in either 10 mL distilled water or 10 mL oil and

stirred occasionally for 1 h in a previously weighed centrifuge tube. The dispersion was then centrifuged for 30 min at 1500 g, the supernatant decanted and the tube weighed. The WAC was reported as weight of water (g) retained in the sample, while weight of oil (g) retained was reported as FAC.

Bulk density

The bulk density (BD) was determined using the method of Waring and Kinsella (1976). Ten grams starch sample was transferred to a 50 mL graduated cylinder and the cylinder gently tapped about 10 times. The volume of sample was recorded and BD expressed as g/mL.

Dispersibility

Dispersibility was determined according to the method described by Kulkarni et al. (1991). Three grams starch was dispersed in distilled water in a 50 mL stoppered measuring cylinder. Distilled water was added to reach a volume of 30 mL and the mixture was stirred vigorously and then allowed to settle for 3 h. The volume of settled particles was subtracted from 30 and divided by 30 and multiplied by 100 and reported as a percentage of dispersible starch.

Gelatinization temperature

Gelatinization temperature (GT) was measured according to Abdalla et al. (2009) by suspending 1 g starch in 100 mL distilled water and heating and then the temperature required for gelatinization was measured using a thermometer.

Viscosity

The method of Quinn and Beuchat (1975) was used to evaluate the viscosity of a 1% aqueous solution using a HAAK viscotester 6 plus (Type 3870100, Thermo Electron Corporation, Karlsruhe, BW, Germany), spindle No. 2, speed 60 rpm at 31 °C and viscosity was expressed in centipoises (cps). Viscosity was also measured for hot slurries heated to 70 °C for 15 min.

Color test

Color was determined using a Lovibond system as described by Aldogsabi (2009). Briefly, 10 g sample was weighed and placed in a Lovibond sample cell and the color was read after the sample color was adjusted with the color of the apparatus cell on a Lovibond Tintometer type D (The Tintometer Ltd., Salisbury, UK).

Scanning electron microscopy

Scanning electron microscopy (SEM) of starches was done by sprinkling the starch sample on adhesive tape, attaching the tape to specimen studs, and coating with gold JFC-1500 (JEOL, Tokyo, Japan). Photomicrographs were taken at $600\times$, $1000\times$, and $4000\times$ magnification using the SEM apparatus at an accelerating voltage of 10 kV.

Results and discussion

Effect of enzyme treatments on the yield, purity and physicochemical properties of sorghum starches

Starch yield for various treatments of Tabat and Wad-baco cultivars are shown in Table 1. The results revealed that starch yield from treatments (B, BE, BES, and S) were significantly ($p < 0.05$) higher than A in both cultivars. The starch yield of pepsin treated (BE) sorghum was 82.53% for Wad-baco and 76.53% for Tabat cultivars. These values were significantly ($p < 0.05$) higher than those of Tabat (75.99%) and Wad-baco (69.73%) starches obtained by buffer treatment (B) alone, indicating the beneficial effect of enzyme addition. In addition, the combination of pepsin and SO_2 treatments (BES) gave a higher yield of 78.90% and 85.56% for Tabat and Wad-baco respectively, which was better than all other treatments. However, addition of SO_2 alone showed significantly ($p < 0.05$) lower yield when compared to the enzyme treatment. These results were in agreement with those reported by Johnston and Singh (2005) who found that the addition of a combination of bromelain and SO_2 gave a higher yield of corn starch than the addition of SO_2 alone. Also, Perez-Carrillo and Serna-Saldivar (2006) reported a significant increase in starch yield from maize and sorghum treated with protease and SO_2 . Additionally, incorporation of multiple enzymes during sorghum steeping resulted in an increased starch yield compared with the SO_2 solution only (Wang et al. 2000). It is worth mentioning that in addition to improved starch yield from all four treatments, namely B, BE, BES, and S, the two stage modified procedure resulted in a reduced steeping time that could decrease energy cost and capital investment required for commercial use.

The results of starch purity expressed as residual protein content are shown in Table 1. The residual protein content was significantly ($p < 0.05$) varied between the treatments of the two cultivars. With exception of BES treatment, residual protein content of Wad-baco starch was the highest under all treatments suggesting a tight binding between protein and starch in Wad-baco cultivar. This could be due to the difference in the genetic background

that contributed to the variation in protein and starch structure and composition. Tightly packed protein-starch matrices is a general characteristic of pigmented and waxy sorghums (Chew-Guevara et al. 2015) and this could explain why the residual protein was higher in Wad-baco (a pigmented sorghum) compared to Tabat (a white sorghum). Treatment with pepsin alone resulted in 0.30% residual protein (99.7% purity) for Tabat, which lies within the acceptable limit of starch quality. Combined with high yield, a 0.42% residual protein (99.6% purity) was obtained for Wad-baco with pepsin treatment. Similarly, Chew-Guevara et al. (2015) reported that applying protease treatment increased the purity of red sorghum starch to 98.75%. The SO_2 and pepsin treatments disrupt the protein matrix, which results in starch release during subsequent milling and increased starch yield. The addition of SO_2 breaks down the sulfur bond of sulfur containing amino acids while the addition of pepsin hydrolyzes peptide bonds, breaking down protein into smaller peptide molecules (even down to amino acid units), opening the protein matrix and increasing starch yield and purity.

The amylose and amylopectin content of sorghum starch extracted by B, BE, BES, S, and A treatments are presented in Table 1. Amylose content ranged from 19.45–25.13% and 19.71–23.82% in Tabat and Wad-baco starches, respectively. Amylose values of B and BES from Tabat cultivar were significantly ($p < 0.05$) higher than values reported in other treatments for both cultivars. Amylose values were within the range reported by Benmoussa et al. (2006) (19.2–22.4%) for sorghum genotypes grown in USA, and the range reported by Singh et al. (2010) (11.2–28.5%) for sorghum grown in India, while Boudries et al. (2009) reported values of 24.8% for white sorghum and 27.1% for pigmented sorghum grown in Algeria. The average of amylose content of most sorghum starches is in the range of 20–30% (Udachan et al. 2012). Amylopectin ranged from 74.87–80.55% and 76.18–80.29% for Tabat and Wad-baco starches, respectively, which were similar to the values (81.28–89.2%) found in four Indian sorghum starches (Udachan et al. 2012). Slight changes in amylose/amylopectin ratio and branched chain distribution of amylopectin have tremendous effects on a wide range of functional characteristics of starch and particularly pasting properties (Singh et al. 2014).

The pH ranged from 6.00–6.75 and 6.31–6.78 for Tabat and Wad-baco starches, respectively (Table 1). The pH of Tabat starches extracted by various treatments was significantly ($p < 0.05$) different compared to the control and significant differences among the four treatments were observed in B and BES samples. The pH of Wad-baco starches obtained by the various treatments were significantly ($p < 0.05$) different compared the control sample. Titratable acidity ranged from 0.20–0.46% and 0.18–0.31%

Table 1 Effect of enzyme treatments on the yield, residual protein, amylose, amylopectin, pH, and acidity of sorghum starch extracted by different methods

Extraction method	Yield (%)		Residual protein (%)		Amylose (%)		Amylopectin (%)	
	Tabat	Wad-baco	Tabat	Wad-baco	Tabat	Wad-baco	Tabat	Wad-baco
B	75.99 ^c ± 1.56	69.73 ^e ± 201	0.30 ^e ± 0.03	0.42 ^a ± 0.00	25.13 ^a ± 1.50	23.82 ^{ab} ± 0.08	74.87 ^d ± 1.50	76.18 ^{cd} ± 0.08
BE	76.53 ^{bc} ± 1.44	82.53 ^a ± 0.61	0.30 ^e ± 0.00	0.42 ^a ± 0.03	19.45 ^d ± 2.51	20.70 ^{cd} ± 2.54	80.55 ^a ± 2.51	79.30 ^{ab} ± 2.54
BES	78.90 ^b ± 3.18	85.56 ^a ± 0.14	0.35 ^c ± 0.03	0.32 ^d ± 0.00	24.22 ^a ± 0.82	19.71 ^d ± 1.02	75.78 ^{cd} ± 0.82	80.29 ^a ± 1.02
S	74.07 ^d ± 1.94	78.13 ^b ± 1.10	0.25 ^f ± 0.00	0.36 ^b ± 0.03	22.67 ^{abc} ± 0.71	22.14 ^{bcd} ± 0.13	77.33 ^{bcd} ± 0.71	77.86 ^{abc} ± 0.13
A (control)	59.32 ^f ± 1.56	57.41 ^f ± 2.62	0.32 ^d ± 0.00	0.30 ^e ± 0.00	22.78 ^{abc} ± 1.27	19.82 ^d ± 1.90	77.22 ^{bcd} ± 1.27	80.18 ^a ± 1.90
Lsd _{0.05}	3.206 ^{**}		0.0005386 [*]		2.556 [*]		2.556 [*]	
SE±	1.087		0.0001826		0.8666		0.8666	
Extraction method	pH-value		Titratable acidity (%)					
	Tabat	Wad-baco	Tabat					
B	6.62 ^a ± 0.06	6.78 ^a ± 0.09	0.20 ^e ± 0.06					
BE	6.04 ^{de} ± 0.05	6.68 ^{bc} ± 0.21	0.38 ^{ab} ± 0.06					
BES	6.75 ^a ± 0.06	6.31 ^{bc} ± 0.10	0.21 ^c ± 0.06					
S	6.00 ^e ± 0.12	6.43 ^b ± 0.13	0.46 ^a ± 0.03					
A (control)	6.20 ^{cd} ± 0.02	6.66 ^a ± 0.06	0.21 ^c ± 0.09					
Lsd _{0.05}	0.1703 [*]		0.1204 [*]					
SE±	0.05774		0.04082					

Mean values (± SD) having different superscript(s) in columns and rows are significantly different according to DMRT; *significant at $p \leq 0.05$; **significant at $p \leq 0.01$; A (control): steeping for 24 h with 0.2% SO₂; B: steeping for 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5); BE: steeping for 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5) + 250 mg pepsin enzyme; BES: steeping for the 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5) + 250 mg pepsin enzyme + 0.2%SO₂; S: steeping for 7 h with 3 h in distilled water and 4 h in 0.2%SO₂

for Tabat and Wad-baco starches, respectively. Among the treated samples of Tabat cultivar, BE and S were significantly ($p < 0.05$) different from the control. However, there were no significant ($p < 0.05$) differences between the four treatments and the control of Wad-baco cultivar.

Effect of enzyme treatment on the functional properties of sorghum starches

Functional properties [WAC, FAC, BD, dispersibility (Disp) and gelatinization temperature (GT)] of sorghum starches from two sorghum cultivars are presented in Table 2. WAC for Tabat starches varied significantly ($p < 0.05$) from 60.67% to 71.33% and for Wad-baco starches from 67.33% to 76.33%. WAC of Wad-baco starches at all treatments was higher than that of Tabat starches ($p < 0.05$) which might be due to the variation in the starch granule structure, the degree of hydrogen and covalent bonds between starch chains, amylose/amylopectin ratio, and availability of water binding sites. WAC was higher in starches extracted by all treatments compared to that extracted by wet-milling alone indicating that the treatments enhanced the WAC sorghum starches. Significant ($p < 0.05$) differences were observed in Tabat BE samples compared to the other samples, while starch from Tabat B had the lowest WAC ($p < 0.05$). Also, there were significant ($p < 0.05$) differences in WAC among the four treatments, however, the WAC in BES and S treatments were not different ($p > 0.05$) in both cultivars. The BE and S treatments for Wad-baco showed significantly ($p < 0.05$) higher values of WAC in comparison to the rest of the treated starches. These results were lower than the values of 96.0% and 97.4% reported for Dadar and Parbhani sorghum cultivars (Udachan et al. 2012). A water binding capacity of 79.19% was reported for white sorghum starch (Aviara et al. 2010), while lower values of 56% and 58.5% were reported for Feterita and Dabar sorghum cultivars (Abdelnour 2001). WAC is an indication of the amount of water available for gelatinization, and is useful in determining the ability of a starch to hold water. Low water binding capacity is attributable to tight starch polymer association (Soni et al. 1990) while high water binding capacity is an indication of loose association of native starch polymers or low lipid content (Nwokocha and Ogunmola 2005).

FAC of Tabat starches varied from 20.33% to 25.00% and that of Wad-baco varied from 24.33% to 27.33% (Table 2). No significant ($p > 0.05$) variation was observed in FAC between the two types of sorghum cultivars or among the various treatments.

BD of sorghum starch varied from 0.48–0.57 g/mL and 0.45–0.52 g/mL for Tabat and Wad-baco, respectively

Table 2 Effect of enzyme treatments on the functional properties of sorghum starch extracted by different methods

Extraction method	WAC (%)		FAC (%)		BD (g/mL)		Disp. (%)		GT (°C)	
	Tabat	Wad-baco	Tabat	Wad-baco	Tabat	Wad-baco	Tabat	Wad-baco	Tabat	Wad-baco
B	60.67 ^d ± 2.52	73.33 ^a ± 2.52	24.33 ^{ab} ± 2.52	26.33 ^a ± 2.08	0.5167 ^e ± 0.01	0.5100 ^f ± 0.00	85.00 ^b ± 0.00	76.67 ^e ± 0.00	57.33 ^{abcd} ± 1.53	51.33 ^e ± 0.58
BE	71.33 ^{ab} ± 3.06	75.00 ^a ± 2.65	25.00 ^a ± 3.61	27.33 ^a ± 0.58	0.5300 ^c ± 0.00	0.5167 ^e ± 0.01	83.30 ^c ± 0.00	90.00 ^a ± 0.00	58.50 ^{ab} ± 1.50	54.83 ^{cd} ± 0.29
BES	67.33 ^{bc} ± 3.51	76.33 ^a ± 2.08	23.67 ^{ab} ± 2.08	27.33 ^a ± 0.58	0.4833 ^g ± 0.01	0.4500 ^h ± 0.00	83.30 ^c ± 0.00	83.30 ^c ± 0.00	56.00 ^{bcd} ± 1.32	50.33 ^e ± 1.53
S	66.67 ^{bc} ± 2.31	74.00 ^a ± 3.61	24.67 ^a ± 2.52	24.33 ^{ab} ± 3.06	0.5533 ^b ± 0.01	0.5167 ^e ± 0.01	83.30 ^c ± 0.00	76.67 ^e ± 0.00	57.67 ^{abc} ± 1.53	55.00 ^{cd} ± 2.00
A (control)	62.33 ^{cd} ± 1.53	67.33 ^{bc} ± 2.89	20.33 ^b ± 1.53	25.00 ^a ± 2.00	0.5700 ^a ± 0.00	0.5233 ^d ± 0.01	81.67 ^d ± 0.00	76.67 ^e ± 0.00	59.00 ^a ± 1.00	54.67 ^d ± 2.84
Lsd _{0.05}	4.654 [*]		3.834 [*]		0.0003586 [*]		0.0005386 [*]		2.666 [*]	
SE±	1.578		1.30		0.0001826		0.0001826		0.9037	

Mean values (± SD) having different superscript(s) in columns and rows are significantly different according to DMRT; *significant at $p \leq 0.05$; A (control): steeping for 24 h with 0.2% SO₂; B: steeping for 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5); BE: steeping for 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5) + 250 mg pepsin enzyme; BES: steeping for the 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5) + 250 mg pepsin enzyme + 0.2% SO₂; S: steeping for 7 h with 3 h in distilled water and 4 h in 0.2% SO₂; WAC, water absorption capacity; FAC, fat absorption capacity; BD, bulk density; Dis., dispersibility; GT, gelatinization temperature

(Table 2). BD of Tabat starch at all treatments was higher than that of Wad-baco starch. Treated samples showed lower DB values compared to control samples from both cultivars. The BD of Tabat and Wad-baco starches at various treatments was significantly ($p < 0.05$) different. A significant difference in BD was also observed between the treated samples and the control in starches of both cultivars. Obtained values were lower than 0.58 g/mL reported by Ganorkaz and Kulkarni (2013) and higher than 0.32 g/mL reported by Otutu et al. (2014) for sorghum starches.

Dispersibility of sorghum starches varied from 81.67–85.00% and 76.67–90.00% for Tabat and Wad-baco, respectively (Table 2). The Disp. of Tabat starch extracted by the four treatments was significantly ($p < 0.05$) higher compared to the control. Also, the Disp. of Wad-baco starch was significantly ($p < 0.05$) higher compared to the control, with the exception of B and S treated samples. These results concur with reported results for two rice and two legume varieties (Ashogbon and Akintayo 2013). Also, values of 86% and 84% were reported for potato and cassava starches, respectively (Eke-Ejiofor and Owuno 2014). Higher dispersibility is associated with improved starch reconstitution properties, which have a marked effect on gel quality (Eke-Ejiofor and Owuno 2014). In this study, higher dispersibility of the Tabat and Wad-baco starches extracted using different treatments demonstrating great reconstitution ability of these starches to form a fine and consistent paste.

GT of sorghum starches ranged from 56.00–59.00 °C and 50.33–55.00 °C for Tabat and Wad-baco respectively (Table 2). The highest GT was found in Tabat starch at control treatment while the least was observed in BES and

B treated Wad-baco starch ($p < 0.05$). GT of Tabat starches was higher than that of Wad-baco starches at all treatments ($p < 0.05$). This could be due to the differences in the genetic makeup which might lead to the variation in amylopectin branch chain lengths and distributions (Singh et al. 2010). Significant variations ($p < 0.05$) in GT were observed in starches isolated using different treatments compared to the control suggesting the influence of extraction conditions on the GT of starches. GTs between 67–73 °C and 71–81 °C have been reported for starch extracted from South African and Indian sorghum cultivars, respectively (Taylor et al. 2006), and at 73.2 °C for Korean waxy sorghum (Choi et al. 2004). Many factors, particularly amylopectin unit chain lengths and distribution have been reported to influence GT of starches (Singh et al. 2010). Longer the length of the amylopectin molecules resulted higher the GT of starches (Taylor et al. 2006). According to Moorthy (2002), higher GT could be indicative of starch stability. In the present study, GT values compared favorably with values reported in previous studies. Ubwa et al. (2012) reported comparable GT values of 60 °C, 64 °C and 66 °C for mixed, red, and white sorghum cultivars, respectively. In addition, Udachan, et al. (2012) reported GTs of 68 °C and 64 °C for Dadar and CSH-9 sorghum cultivars, respectively.

Viscosities of Tabat and Wad-baco sorghum starches extracted by different treatments are presented in Table 3. Viscosity which was measured at 31 °C and 70 °C was significantly ($p < 0.05$) varied between treated samples and control. The highest viscosity was reported at 31 °C for B (83.93 cP) from Tabat cultivar and A (96.53 cP) from Wad-baco cultivar. Differences in viscosity between

Table 3 Effect of enzyme treatments on viscosity (cps) of sorghum starch extracted by different methods

Extraction method	Viscosity (c.ps)			
	Before heating		After heating	
	Tabat	Wad-baco	Tabat	Wad-baco
B	83.93 ^b ± 27.97	55.20 ^f ± 33.41	125.70 ^c ± 25.89	94.23 ^f ± 32.75
BE	72.87 ^c ± 13.28	81.37 ^b ± 19.09	103.90 ^c ± 19.97	114.90 ^d ± 33.23
BES	64.40 ^d ± 33.65	56.80 ^{ef} ± 22.27	135.10 ^b ± 26.46	92.63 ^f ± 7.64
S	60.37 ^{de} ± 25.36	73.37 ^c ± 42.44	114.40 ^d ± 29.14	106.20 ^e ± 26.27
A (control)	57.70 ^{ef} ± 9.54	96.53 ^a ± 23.76	86.70 ^g ± 54.74	144.30 ^a ± 28.73
Lsd _{0.05}	45.59**		52.12**	
SE±	15.45		17.67	

Mean values (± SD) having different superscript(s) in columns and rows are significantly different according to DMRT; **significant at $p \leq 0.01$; A (control): steeping for 24 h with 0.2% SO₂; B: steeping for 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5); BE: steeping for 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5) + 250 mg pepsin enzyme; BES: steeping for the 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5) + 250 mg pepsin enzyme + 0.2%SO₂; S: steeping for 7 h with 3 h in distilled water and 4 h in 0.2%SO₂

Table 4 Effect of enzyme treatments on color characteristics of sorghum starch extracted by different methods

Extraction method	Color					
	Redness		Yellowness		Blueness	
	Tabat	Wad-baco	Tabat	Wad-baco	Tabat	Wad-baco
B	0.27 ^{ef} ± 0.06	0.33 ^{de} ± 0.06	0.47 ^{bc} ± 0.06	0.63 ^b ± 0.15	0.60 ^a ± 0.00	0.30 ^{cd} ± 0.10
BE	0.20 ^f ± 0.00	0.37 ^{de} ± 0.06	0.33 ^{cde} ± 0.06	0.37 ^{cde} ± 0.12	0.50 ^{ab} ± 0.10	0.57 ^{ab} ± 0.06
BES	0.63 ^b ± 0.06	0.73 ^a ± 0.06	0.40 ^{cd} ± 0.20	0.27 ^{cde} ± 0.06	0.30 ^{cd} ± 0.00	0.43 ^{bc} ± 0.06
S	0.33 ^{de} ± 0.06	0.40 ^d ± 0.10	0.47 ^{bc} ± 0.06	0.23 ^{de} ± 0.15	0.30 ^{cd} ± 0.17	0.27 ^d ± 0.06
A (control)	0.30 ^{de} ± 0.00	0.50 ^{ab} ± 0.00	0.17 ^e ± 0.06	1.17 ^a ± 0.15	0.53 ^{ab} ± 0.06	0.60 ^a ± 0.10
Lsd _{0.05}	0.09329*		0.2015*		0.1425*	
SE±	0.03162		0.06831		0.0483	

Mean values (± SD) having different superscript(s) in columns and rows are significantly different according to DMRT; *significant at $p \leq 0.05$; A (control): steeping for 24 h with 0.2% SO₂; B: steeping for 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5); BE: steeping for 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5) + 250 mg pepsin enzyme; BES: steeping for the 7 h with 3 h in distilled water and 4 h in 0.1 M sodium acetate buffer pH (4–4.5) + 250 mg pepsin enzyme + 0.2%SO₂; S: steeping for 7 h with 3 h in distilled water and 4 h in 0.2%SO₂

control and treatment groups could be due to the effect of enzymes and chemicals added during steeping on starch polymer association. However, after heating at 70 °C the viscosity increased to 135.10 cP for Tabat BES and 144.30 cP for Wad-baco A samples. The increase in viscosity after heating may be due to swelling and gelatinization of starch granules. Cooling after heating increased the viscosity, while increased temperature and shear rate decreased it; such properties are important for industrial mixing and pumping operations (Sang et al. 2008).

The color parameters of starch isolated from Tabat and Wad-baco sorghum cultivars using different experimental conditions are presented in Table 4. The redness value of starch obtained using different experimental conditions ranged from 0.20 to 0.63 for Tabat and 0.33 to 0.73 for Wad-baco, while yellowness ranged from 0.17 to 0.47 for Tabat and 0.23 to 1.17 for Wad-baco. Blueness values were in the range of 0.30–0.60 for Tabat and 0.27–0.60 for Wad-baco. The values of redness, blueness and yellowness of Wad-baco starch are higher than those of Tabat at A, B and BE treatments, whereas, yellowness of Tabat starch at BES and S treatments and the blueness at S treatment are higher compared to Wad-baco starch. The highest redness, yellowness and blueness of Wad-baco starch could be due to the difference in endosperm color between the two cultivars as the endosperm of Wad-baco is dark-yellow while that of Tabat is white. The highest redness was observed in starches of BES treatment of both cultivars, while the lowest values were seen in starches of BE treatment of Tabat and B treatment of Wad-baco. The highest redness value of starches of BES treatment of both cultivars may be attributed to the combined effect of enzyme and SO₂ in breaking down the starch matrix and thereby releasing

proteins. A strong positive correlation between a* (indicator of redness and greenness) and protein content of corn starch has recently been reported (Singh et al. 2014). The highest yellowness and blueness values were observed in Wad-baco starch under control treatment and in Tabat starch under B treatment, while the least values were found in Wad-baco starch with S treatment, and Tabat starch at A and S treatments. Yellowness and redness value of sorghum starches in this study were lower than that reported for starches of white and red sorghum (Chew-Guevara et al. 2015; Boudries et al. 2009) and corn (Singh et al. 2014). The differences between these studies could be due to the variation in the genetic makeup, extraction methods, and starch matrix compositions. The low redness, yellowness and blueness values confirmed the high purity of the treated starches from the two cultivars. High purity of treated starches can provide many opportunities for both food and industrial applications.

Morphological properties of starches extracted by enzymatic treatment (BE) and control (A) from Tabat and Wad-baco cultivars are presented in Fig. 1. The results in Fig. 1a showed the scanning electron micrograph (SEM) of Tabat control (A) sample, while Fig. 1b is the enzymatically treated (BE) sample from Tabat cultivar. Figure 1c represents SEM of Wad-baco control (A) sample, while Fig. 1d is the enzymatically treated (BE) starch from Wad-baco cultivar. The morphological properties were studied to determine the effect of pepsin enzymes on sorghum starch granules. Starch granules in the control and treated samples from both varieties varied in shape and size. Generally, the starch granules showed polygonal and irregular shapes, while indentations or holes were observed on the surface of enzymatically treated starches from both

Fig. 1 Scanning electron micrograph of sorghum starch granules. **a** Tabat control (wet milling for 24 h), **b** Tabat treated with 250 mg pepsin, **c** Wad-baco control, **d** Wad-baco treated with 250 mg pepsin. Arrows showed the holes in the starch granules as results of enzyme action

cultivars. Olayinka et al. (2013), Sang et al. (2008) and Singh et al. (2010) reported similar observations for different sorghum cultivars. The starch granules from control of both cultivars were surrounded by a thin layer of protein matrix. The effect of the pepsin treatment on sorghum starch granules is very obvious (Fig. 1b, d). Extremely large holes were observed on the granule surface of enzymatically treated starches from both cultivars. The holes may be due to hydrolysis of the protein matrix attached to the starch by pepsin. Generally, enzyme treatment reduced the granular size of starches from both Tabat and Wad-baco varieties.

Conclusion

This study concluded that pepsin opened the protein matrix surrounding starch granules, as evident in SEM images of sorghum starch granules, which concomitantly increased starch yield compared to normal wet milling. Steeping with pepsin and SO₂ improved starch color more than steeping in pepsin alone. Significant effects of pepsin and SO₂ treatments on Tabat and Wad-baco starches were observed, especially on decreased steeping time, BD, GT, WAC, FAC, viscosity, and increased yield. This study has revealed the potential benefits of pepsin and SO₂ treatments on various properties of Tabat and Wad-baco sorghum cultivars. The study has also shown that pepsin and SO₂ treatments could be used to improve the use of Tabat

and Wad-baco starches in food systems since high WAC, FAC, viscosity and lower GT are desirable properties in the system.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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