Effect of Annealing on Morphological and Functional Properties of Pakistani White Sorghum (Sorghum *bicolor*) Starch

Tahira Mohsin Ali* and Abid Hasnain#

Department of Food Science and Technology, University of Karachi, 75270, Karachi, Pakistan

Abstract: The present study reports the effect of annealing on the morphological and functional properties of starch isolated from white sorghum grains. Scanning electron micrographs of native white sorghum starch (NWSS) revealed the presence of spherical and polygonal shaped granules with random distribution of surface pores. The diameter of surface pores ranged between 213-275 nm in NWSS. The size of small and large granules in NWSS ranged between 5-7 µm and 11-21 µm, respectively. There was no significant difference in the size and shape of NWSS and annealed white sorghum starch (AWSS) granules. However, the diameter of surface pores doubled after annealing. The AWSS showed reduced swelling power, solubility and water binding capacity. Pasting temperature increased from 73.4°C in NWSS to 75.8°C in AWSS. The pasting profile of annealed starches showed reduction in peak viscosity, breakdown viscosity, cold paste viscosity and set back viscosity. Paste clarity improved on annealing while, the extent of reduction in percent transmittance after 72 hours of storage was higher for NWSS suggesting use of annealed starch in high clarity products.

Keywords: White sorghum, morphological properties, surface pores, functional properties.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) locally known as “Jowar” is a non celiac cereal grain [1]. The crop is used as animal feed in developed countries and as food in developing countries of Asia and Africa [2]. The annual production of this grain in Pakistan as recorded in the year 2013 was 125,500 tonnes covering 200,000 hectares of land [3]. This grain grows both in dry and wetlands of Baluchistan and Punjab provinces of Pakistan. Unique physiological properties of sorghum increase its importance as a food security crop. Like maize and sugarcane it uses C4 malate cycle for photosynthesis and thereby efficiently utilizes sunlight [4]. It also has high drought tolerance and can tolerate hot and dry weather conditions, making it the second most important crop for sub tropical and semi arid areas of Africa. Another important physiological attribute of sorghum is its ability to grow at varying altitudes ranging from sea level to elevations in excess of 300 m [5]. The major nutrient of sorghum is starch, which accounts for 75-79 % of the grain weight [6]. Sorghum starch is composed of 20-30% amylose and its gelatinization temperature is slightly higher than corn starch [7].

In India, African and Mexico, this crop is widely utilized in different products. But in Pakistan this crop has remained largely under-utilized. The aim of the present study was to isolate starch from sorghum grains and add value to it via annealing; a physical modification. The study also investigated the effect of annealing on the morphological and functional properties of white sorghum starch.

MATERIALS AND METHODS

Cleaned and sound white sorghum grains from a single cultivar, free from molds and insects were procured from local market. All chemicals used were reagent grade.

Isolation of Starch

Starch isolation from white sorghum grains was carried out in two steps (a) steeping (b) wet milling. Cleaned grains were steeped in 0.5% lactic acid and 0.2% SO_2 solution for 24 hours at 50 °C. The steeped grains were then washed twice for the complete removal of steeping solution. Grains were then coarsely ground and blended in a waring blender for 5 minutes at 8000 rpm. The slurry thus obtained was adjusted to pH 8.5 using 0.5 M NaOH solution and was allowed to stand for 30 minutes. It was then sieved through a stack of 80, 170 and 270 wire mesh sieves from top to bottom. The overs from the sieves were reblended in a waring blender for 2 minutes followed by sieving. This step was repeated twice. The starch slurry thus collected was homogenized using a homogenizer (Polytron PT 2100, Kinematica Inc., USA) for 60 seconds and starch was allowed to sediment at 4ºC. The protein layer was scraped off using a spatula. The sedimented starch was washed with water and homogenized again to remove the residual protein. The washed starch was then dried at 45 ºC in a forced air oven.

*Address correspondence to this author at the Department of Food Science and Technology, University of Karachi, 75270, Karachi, Pakistan; Tel: +92-21-34823115; Fax: +92-21-99243206; E-mail: tahira.mohsin@uok.edu.pk

#Co-author: E-mail: abidhasnain@uok.edu.pk

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Annealing

One-step annealing of starch was carried out by the method of Jayakody *et al.* [8] with some modifications. Starch (100g) dry basis was adjusted to 75% moisture by adding appropriate amount of distilled water in a glass container. The sealed glass container was then placed in a thermostatically controlled water bath at 55 °C for 72 hours. Starch slurry was allowed to sediment. The sedimented starch was then air dried at 25 °C. The dried starch was passed through a 100 wire mesh sieve and stored in polyethylene bags at 4°C prior to use.

Morphological Properties

Granular morphology of native and annealed white sorghum starches were studied through scanning electron microscopy (SEM) by sprinkling a thin layer of starch on an aluminum specimen holder by a double sided tape. The starch was then coated with gold and examined under 1000x and 16,000x magnifications.

Functional Properties

Swelling Power and Solubility

The method reported by Bello Perez *et al.* [9] with some modifications was employed to determine the swelling power and solubility of starches. A starch suspension (1% drybasic, w/v) was prepared in centrifuge tubes and heated at 50, 60, 70, 80 and 90°C for 30 minutes with intermittent shaking after every 5 minutes. The tubes were then cooled followed by centrifugation at 3500 rpm for 15 minutes. The supernatant was decanted and residue was weighed. The precipitate was used to determine moisture content to evaluate by difference the solubilized solids (2 hours at 130°C).

Water Binding Capacity (g/g)

Water binding capacity of starch was determined by the method of Bryant and Hamaker [10] with some modifications. The 1% (w/v) slurry of starch was prepared in a preweighed centrifuge tube at room temperature. It was then heated in a water bath to 50, 60, 70, 80, and 90 °C for 15 minutes with shaking after every 5 min. The mixture was then cooled to room temperature and centrifuged at 3500 rpm for 15 minutes. The supernatant was decanted and the tubes were allowed to drain off for 10 min at a 45° angle. The gain in weight was used to calculate the water binding capacity of starch.

Microviscoamylography

The starch slurry was prepared by dispersing 8 g (dry basis) sorghum starch in 100 mL of distilled water and was subjected to heating in a microviscoamylograph (Model 803201, Brabender, Germany) equipped with 300 cmg sensitivity cartridge from 30-95 °C at 1.5 °C/min. The slurry was held at 95 ºC for 10 minutes and subsequently cooled back to 40 ºC at 1.5 ºC/min and held at this temperature for 10 minutes. The speed of rotating bowl was 75 rpm. The following parameters were recorded from resulting graph namely pasting temperature, peak viscosity, breakdown viscosity, cold paste viscosity and setback viscosity.

Paste Clarity

Starch suspension 1% (w/v) in a screw capped tube was placed in a boiling water bath for 30 minutes with vortexing every 5 minutes. After cooling to room temperature percent Transmittance (%T) was measured at 650 nm against water as a blank using JASCO V-670 spectrophotometer. The samples were then refrigerated at 6 °C and (%T) was measured after 24, 48 and 72 hours.

Syneresis

A starch suspension (2%) w/v was heated in a boiling water bath for 30 minutes in a temperature controlled water bath, followed by rapid cooling. The starch sample was then stored for 24, 72 and 120 hours at 4°C. Syneresis was measured as percentage amount of water released after centrifugation at 4000 rpm for 10 minutes.

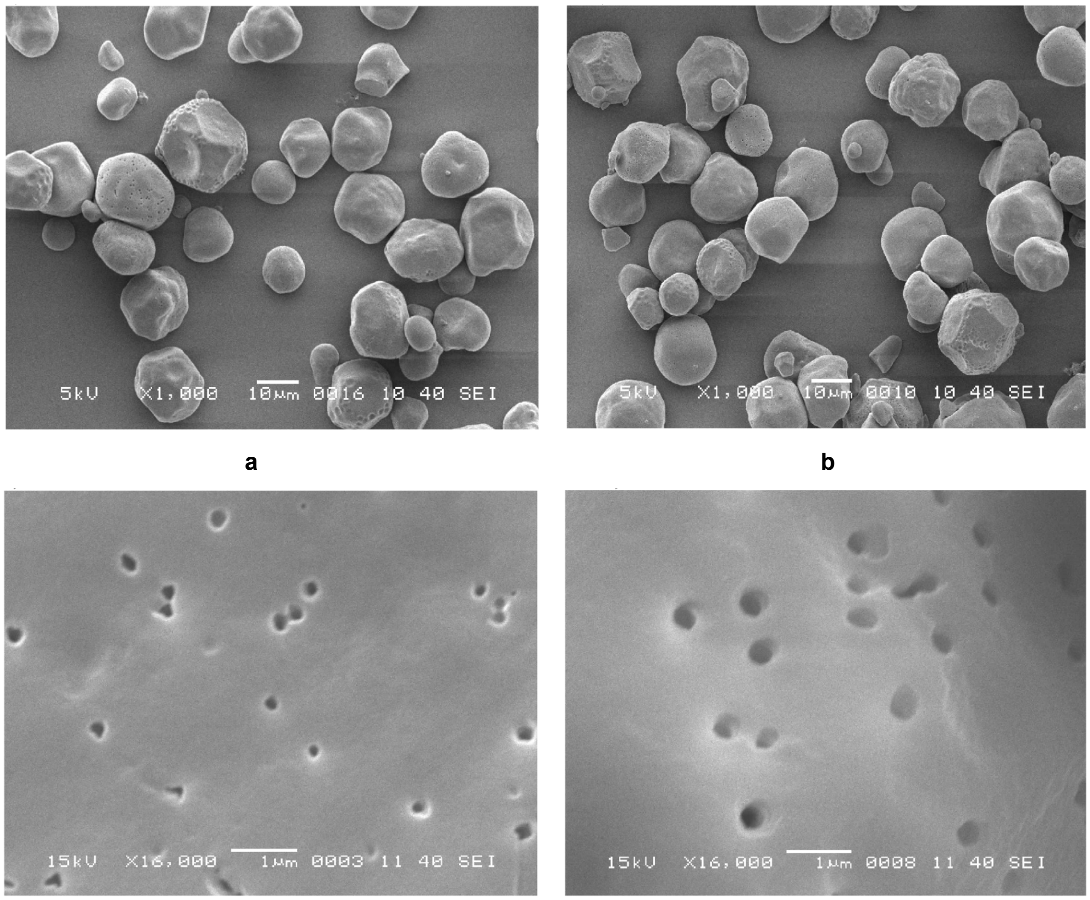
Statistical Analysis

Mean values of all analysis were reported along with standard deviations. An analysis of Student's t-test was performed using SPSS software (SPSS version 11, Inc., USA).

RESULTS AND DISCUSSION

Morphological Characteristics

The scanning electron micrographs of NWSS (Figure **1a**) and AWSS (Figure **1b**) showed a mixture of large (polygonal and spherical) and small (mostly spherical) starch granules under 1000x magnification. The size (diameter) of native starch granules varied between 5-7 μm for small and 11-21 μm for large sized



a b c d

Figure 1: Scanning electron micrographs of native and annealed white sorghum starches. **a**) NWSS 1000x, **b**) AWSS 1000x, **c**) NWSS 16000x, & **d**) AWSS 16000x.

granules. Annealing did not significantly alter the size and shape of starch granules. Similar results were reported for annealed yam starches [8] and bread wheat starches [11]. The small annealed starch granules were 5-7 µm in diameter whereas, the size of large AWSS granules ranged from 10-21 µm in diameter. When starch granules were observed under 16000x magnification, spherical pores were clearly visible both in native and annealed starch granules. Pores were randomly distributed on the granule surface. Several other researchers [12-14] also reported the presence of surface pores on sorghum starch granules. The diameter of pores in NWSS ranged from 213-275 nm (Figure **1c**). The diameter of pores doubled after annealing ranging from 425- 560 nm (Figure **1d**). Waduge *et al*. [15] reported a slight increase in pore size post annealing in barley starches. However, the diameter of pores was not reported, perhaps because the granular morphology was examined at a lower magnification of 3000x. Pores are basically surface opening to radial tube like channels [16]. External pores are connected to internal cavity at hilum via these channels [17]. Region around hilum is believed to be the least organized area of starch granule and is most susceptible to acid hydrolysis, gelatinization and enzymatic attack [18-20]. Channels that are basically void spaces [13] provide direct access to chemical reagents into this weakly organized part of starch granules. The expansion or increase in the diameter of pores after annealing could be the result of reorientation or reorganization of the weakly organized region around hilum as water which acts as a plasticizer during annealing will reach this region directly through channels causing alpha-glucan chain mobility above glass transition temperature. As pores, channels and hilum are all inter-connected, therefore reorganization in the region around hilum could alter the microstructure of starch.

Functional Properties

Swelling Power and Solubility

Swelling power and solubility of native and annealed starches are presented in Table **1**. Both

Table 1: Effect of Temperature on the Swelling Power and Solubility of Native (NWSS) and Annealed (AWSS) White Sorghum Starches[a]

Starch	50°C	60°C	70°C	80°C	90°C
Swelling Power (g/g)					
NWSS	3.0±0.2^{a}	2.8±0.1^{a}	5.6±0.3^{a}	10.9±0.2^{a}	11.8±0.1^{a}
AWSS	2.5±0.3^{a}	2.8±0.1^{a}	3.2±0.6^{b}	9.9±0.5^{b}	10.6±0.2^{b}
Solubility (g/100g)					
NWSS	1.5±0.07^{a}	1.7±0.2^{a}	2.2±0.1^{a}	5.9±0.04^{a}	6.4±0.04^{a}
AWSS	1.40±0.04^{a}	1.0±0.2^{a}	1.5±0.1^{b}	4.7±0.36^{b}	5.6±0.06^{b}

[a]All values are mean of triplicate determinations. Means ± standard deviation within a column with different superscripts are significantly different at P ≤ 0.05.

swelling power and solubility increased with the rise in temperature. Gelatinization of starch granules with the increase in temperature is responsible for the increased swelling power and solubility. However, AWSS exhibited significantly reduced swelling power and solubility at 70°C, 80°C and 90 °C compared to NWSS owing to the structural changes in starch granules on annealing. The reduced swelling power on annealing has also been reported for African yam bean starch [21], for normal, waxy, and high amylose bread wheat starches [11], Srilankan yams [8] and peas [22]. The reduction in swelling power on annealing is due to crystallite perfection, reduced hydration of amorphous region due to increased interaction between AM-AM (amylose-amylose) chains and AM-AP (amylose-amylopectin) chains, intra-granular bonding and V-amylose lipid complex formation in annealed starches [23]. The reduced solubility may be due to the reduction in leaching of amylose as a result of increased interaction between amylose chains within the starch granules on annealing.

Water Binding Capacity

Water binding capacity (WBC) of NWSS and AWSS is presented in Table **2**. The WBC is the measure of hydrophilic tendency of starch granules. The results showed no significant difference in WBC of NWSS and AWSS from 50 °C to 60 °C. But thereafter, a rapid increase in WBC was observed with the rise in temperature for both the starches. However, the highest increase in WBC was observed close to the pasting temperature between 70°C-80°C. Similar results have been reported for square banana starch by De la Torre-Gutiérrez *et al.* [24]. This rapid increment in WBC is due to the solubilization of starch granules on gelatinization. With the rise in temperature, intermolecular hydrogen bonds are disrupted initially in the amorphous region, thus exposing more hydroxyl groups and thereby increasing the number of available water binding sites. Water binding capacity of AWSS was significantly less than NWSS at 70°C, 80°C and 90°C, indicating that the number of available water binding sites is comparatively less numerous in AWSS. Reduced WBC in AWSS could be due to interaction between AM-AM chains and AM-AP chains on annealing. Such interactions reduce the number of available water binding sites, as increased number of hydroxyl groups engage in the formation of hydrogen bonds between the polymeric chains.

Microviscoamylography

Pasting profile of the starch slurry was generated through a controlled heating-mixing and cooling cycle in a Micro-Viscoamylograph. Pasting parameters of NWSS and AWSS are presented in Table **3**. AWSS had a significantly higher pasting temperature as compared to NWSS, whereas peak viscosity, cold paste viscosity, breakdown and setback values were significantly reduced post annealing. Similar results for annealed starches have been reported by Adebowale

Table 2: Effect of Temperature on Water Binding Capacity (g/g) of Native (NWSS) and Annealed (AWSS) White Sorghum Starches[a]

Starch	50°C	60°C	70°C	80°C	90°C
NWSS	1.3±0.08^{a}	1.2±0.07^{a}	2.1±0.04^{a}	7.3±0.04^{a}	8.3±0.11^{a}
AWSS	1.2±0.03^{a}	1.2±0.03^{a}	1.3±0.06^{b}	6.8±0.18^{b}	7.7±0.06^{b}

[a]All values are mean of duplicate determinations. Means ± standard deviation within a column with different superscripts are significantly different at P ≤ 0.05.

and Lawal [25] and Lan *et al.* [11]. High pasting temperature and reduced breakdown in AWSS indicate improvement in granular stability of white sorghum starch on annealing. As granular stability improves more energy is required for the structural disintegration of starch granules, thus elevating the pasting temperature. The increased interaction between AM-AM chains and AM-AP on annealing may be responsible for increased resistance to breakdown of AWSS granules.

Table 3: Pasting Profile of Native (NWSS) and Annealed (AWSS) White Sorghum Starches[a]

	NWSS	AWSS
Pasting Temperature (°C)	73.4±0.0[a]	75.8±0.1[b]
Peak Viscosity (BU)	277.0±4.2[a]	242.0±4.2[b]
Breakdown[b](BU)	93.50±0.7[a]	76.0±1.4[b]
Cold Viscosity (BU)	490.5±0.7[a]	426.0±8.5[b]
Setback[c] (BU)	289.5±2.1[a]	267.5±2.1[b]

[a]Values are mean of duplicate determinations. Means ± standard deviation within a row with different superscripts are significantly different at P ≤ 0.05.
[b]Breakdown= Peak Viscosity-Viscosity at the end of 95 °C holding period.
[c]Setback= Viscosity at the end of 40°C holding period - Viscosity at the end of 95 °C holding period.

The viscosity development of starch in dilute aqueous suspensions depend on the extent of starch swelling [26]. The reduced peak viscosity of AWSS is due to the restricted swelling power of annealed granules as compared to their native counterparts (Table **1**). Setback is the measure of the tendency of starch granules to retrograde. Linear amylose chains have higher tendency to reassociate as compared to amylopectin chains with highly branched structure. Annealing reduced the set back viscosity of white sorghum starches. The decreased set back values on annealing could be attributed to the reduction in the leaching of linear amylose molecules as evident by decreased solubility (Table **1**) of AWSS.

Paste Clarity

Percent transmittance (%T) of NWSS and AWSS is presented in Table **4**. Paste clarity of both the starches significantly reduced with the increase in number of storage days. Similar time dependent reduction in paste clarity of starch has been reported by [27]. However the extent of reduction in (%T), which is a measure of retrogradation tendency was higher in NWSS compared to AWSS. The extent of reduction in (% T) for NWSS and AWSS is 33.57 % and 22.05 %, respectively after 72 hours of storage. Increase in opacity of starch paste on storage is due to the reassociation of linear amylose chains at low temperature. The reduced tendency of retrogradation in AWSS could be attributed to the reduction in amylose leaching . The pasting profile generated through Micro-Viscoamylography also showed decreased set back values for AWSS, thus revealing the fact that annealing reduces the tendency of starch molecules to retrograde and thereby improves the low temperature stability of AWSS granules.

Table 4: Effect of Storage Time on Paste Clarity (%T) of Native (NWSS) and Annealed (AWSS) White Sorghum Starches[a]

Storage time	NWSS	AWSS
0 hours	8.5±0.0[a]	28.9±0.0[b]
24 hours	6.7±0.2[a]	28.3±0.4[b]
48 hours	6.7±0.3[a]	27.1±0.7[b]
72 hours	5.7±0.3[a]	22.5±0.02[b]

[a]Values are mean of triplicate determinations. Means ± standard deviation within a row with different superscripts are significantly different at P ≤ 0.05.

Annealing significantly improved paste clarity of sorghum starch which could be attributed to reduced amylose leaching as evident by decreased solubility of AWSS. The interaction between leached out amylose and amylopectin molecules increase the turbidity of starch pastes through formation of junction zones which reflect significant amount of light. [28].

Syneresis

Percent syneresis in starch gels prepared from native and annealed white sorghum starches is presented in Table **5**. Syneresis in annealed starch gels was significantly higher than NWSS gels after 24, 72 and 120 hours of refrigerated storage. The extent of syneresis in starch gels is influenced by the arrangement of starch chains in crystalline and amorphous regions in ungelatinized starch granules [28]. The increase in % syneresis in AWSS gels could be due to the structural rearrangement on annealing. Secondly, reduced water binding capacity (Table **2**) could be another factor contributing to higher syneresis [29] in AWSS gels. However, increase in % syneresis on storage, which is a measure of retrogradation tendency was found to be 0.71% and 0.39% in NWSS and AWSS gels, respectively after 120 hours of storage Lower solubility of AWSS implies decreased amylose leaching as this linear component of starch mainly contributes to the solubility of starches [30]. Syneresis on storage is due to interaction between leached out amylose and amylopectin chains through formation of junction zones. Therefore, reduced amylose leaching in AWSS during starch gel preparation could have attributed to reduction in percent increase in syneresis after 120 hours of storage.

Table 5: Effect of Storage Time on % Syneresis of Native (NWSS) and Annealed (AWSS) white Sorghum Starches[a]

% Syneresis	NWSS	AWSS
24 hours	81.1±0.05[a]	83.6±0.13[b]
72 hours	81.0±0.84[a]	83.9±0.09[b]
120 hours	81.7±0.71[a]	84.0±0.05[b]

[a]Values are mean of duplicate determinations. Means ± standard deviation within a row with different superscript letters are significantly different at P ≤ 0.05.

CONCLUSION

The present study revealed that annealing doubled the diameter of pores present on the surface of white sorghum starches as observed in scanning electron micrographs. Functional properties were also affected post annealing. Swelling power, solubility and WBC decreased after annealing. AWSS showed improvement in thermal and shear stability as evident by increase in pasting temperature, and reduction in breakdown viscosity. Paste clarity improved after annealing whereas % syneresis was found to be higher for AWSS gels.

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