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Effect of Steeping Period on the Physicochemical and Pasting Properties of Sorghum Starch

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ABSTRACT

The effects of steeping periods on the proximate composition, amylose content, functional and pasting properties of sorghum starch were investigated. Sorghum grains steeped for 6, 12, 18 and 24 h with water were processed into starch and sample from unsteeped sorghum served as control. The starch samples were evaluated for proximate composition, amylose content, functional properties and pasting properties using standard procedures. Moisture content (8.47-13.75%), total ash (0.99-1.29%), crude fat (4.64-8.92%), Crude protein (7.76-15.82%), crude fibre (1.05-1.63%), carbohydrate (76.62-79.31%), amylose content (18.9-23.60%), water absorption capacity (1.71-2.81 g/g), oil absorption capacity (1.31-1.79 g/g), bulk density (0.61-0.75 g/cm^3), swelling index (0.71-0.99 g/ml), least gelation (14-20%), foaming capacity (21.90-22.58%), foaming stability (82.00-83.67%), Peak viscosity (111.56-170.30 RVU), trough (83.55-126.85 RVU), breakdown (16.27-46.85 RVU), final viscosity (128.21-248.55 RVU), setback (55.58-125.07 RVU), peak time (4.97-6.26 min) and pasting temperature (83.66 ^{o}C-85.9 ^{o}C). Steeping of sorghum grains enhances its nutritional attributes, functional and pasting properties of sorghum based foods. The effect of steeping on shelf stability of sorghum starch could be further investigated.

Key words:sorghum, starch, proximate composition, amylose, functional properties and pasting properties

INTRODUCTION

Sorghum (*Sorghum bicolor*) is a staple food that provides a major source of calories to large segments of the population living in semi-arid tropics of Africa and Asia (Miladinov *et al*., 2004). Sorghum is processed into a variety of traditional foods including fermented and non-fermented products such as unleavened bread, porridges, cookies, cakes, cereal extracts, malted alcoholic and non-alcoholic beverages, 'tuwo', 'akamu', 'kunu', 'ingera', 'kisra' and 'koko' (Onimawo and Onofun, 2003). The nutritive value of sorghum is similar to that of maize; it is generally high in carbohydrate, low quantity and quality protein which is limiting in lysine, threonine, methionine and tryptophan (Eckhoff and Watson, 2009). The grains are composed of three main parts: seed coat (pericarp-testa), germ (embryo) and endosperm (storage tissue). Sorghum grains vary from white to dark brown depending on the phenolic pigments present. The seed coat contains abundant amount of polyphenolic compounds which combine with other flavonoids (anthocyanins, anthocyanidins, e.t.c.) to give it various colours (Gilani *et al*., 2005). The germ fraction of sorghum is rich in minerals (ash), protein and lipids as well as B-group vitamins: thiamine, niacin and riboflavin (Melaku *et al*., 2005). The endosperm consists mainly of starch granules, storage proteins and cell-wall materials (Melaku *et al*., 2005). Sorghum has many uses beyond food; the most intriguing is its use for biofuel. The stems of certain types yield large amounts of sugar almost like sugarcane. Thus, sorghum is a potential source of alcohol fuels for powering vehicles or cooking.

Starch is the most abundant carbohydrate reserve in plants and it is deposited in plant parts in the form of small granules or cells ranging from 1 up to 100 µm (Tharanathan, 2005). Starch plays a major role in our daily life as it is an important component in our diet (Schwartz and Whistler, 2009). Starch can be present in many different plant organs including seeds (e.g. corn, wheat, rice, sorghum, barley or peas), tubers and roots (e.g. potato, sweet potato, cassava) (Jobling, 2004). Starch is commonly processed by wet milling, the seed or tuber is milled, followed by the separation of the main constituents such as starch, protein and fiber (Singh *et al*., 2010). Starch granules are composed of a mixture of two polymers: an essentially linear polysaccharide called amylose and a highly branched polysaccharide called amylopectin. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin by weight. The functional properties of most starch depend on the amylose and amylopectin constituent (Holmes, 2005).

MATERIALS AND METHODS

Sorghum (*Sorghum bicolor*) grains were purchased from Ojo-Oba market in Akure, Ondo State, Nigeria. All chemicals used were of analytical grade. Analysis of proximate composition, amylose content and functional properties were carried out at the Food Science and Technology Department Laboratory, Federal University of Technology, Akure while analysis of pasting properties were carried out at the Central Research Laboratory Federal University of Technology Akure.

Preparation of Sample

Sorghum grains were cleaned manually and sorted to remove the husks, stem, damaged and discolored seeds which were achieved by winnowing, hand picking and washing with tap water. The method of Singh *et al*. (2010) was used for starch isolation. About 250 g of sorghum grains were washed with tap water, steeped for 6, 12, 18, 24 h and wet milled; while control sample was milled without steeping. The slurry was filtered through a 100 µm mesh screen and the filtrate was allowed to sediment overnight, decanted and reslurried twice before final decantation. The starch cake was oven dried at 50 °C for 6 h, milled after drying and sieved through 0.25 µm mesh screen to get the sorghum starch.

Determination of proximate composition

Moisture content, crude protein, crude fat, total ash and crude fibre contents of the samples were determined using the standard methods of AOAC (2005).

Determination of amylose content

Amylose content was determined according to the method of Juliano (1971). About 0.1 g of the starch sample and 70% amylose standard were weighed into different test tubes, 1 ml of 95% ethanol and 9 ml of 1M NaOH were added and mixed using American scientific vortex mixer. The test tubes were heated in a boiling water bath for 10 min to gelatinize the starch after which they were allowed to cool. Approximately 1 ml was taken from the extracts into another test tube and made up to 10 ml with 9 ml distilled water after which 0.5 ml was taken from the 10 ml diluent into another test tube and 0.1 ml acetic acid solution and 0.2 ml iodine solution were added and made up to 10 ml with 9.2 ml of distilled water. It was allowed to stand for 20 min for colour development into dark blue complex. The test tube was vortexed and the absorbance was read on the spectrophotometer at 620 nm.

% Amylose content =

$$\frac{\%\,Amylose\ of\ standard\ x\ Absorbance\ of\ samples}{Absorbance\ of\ standard}\ x\ 100$$

% Amylopectin content = 100 – (%) amylose content

Determination of functional properties

Bulk density was determined using the method described by Okaka and Porter (1999), water absorption capacity (WAC) and oil absorption capacity (OAC) were determined by methods described by Okezie and Bello (1988), swelling index were determined using the method of Takashi and Seib (1988), foam capacity and stability determined by the method of Coffman and Garcia (1977).

Determination of the Pasting Properties of Samples

The pasting properties of the sample were determined using a Rapid Viscosity Analyser (RVA model 3D for windows).

Statistical Analysis

All experiments were carried out in triplicate. Mean and standard deviation were calculated for each treatment. Data obtained were subjected to analysis of variance (ANOVA) and the means were separated by lowest standard deviation test (SPSS version 16). Significant level was accepted at 5%.

RESULTS AND DISCUSSION

Proximate composition of sorghum starch is shown in Table 1. Moisture content was in the range of 8.47%-13.75%. The significant increase ($p \leq 0.05$) in moisture content could be indication of rapid water uptake by viable grains during steeping. As steeping proceeds, seed absorbs water rapidly as a result of the increasing number of cells within the seed becoming more hydrated (Adebowale *et al*., 2012). This hydration process activated a wide array of enzyme system in hydrolyzed and solubilized food reserves during steeping. Moisture content of a food is indicative of the dry matter in the food, predicts its shelf life and determines the type of micro-organism that can thrive in it (Adebowale *et al*., 2012). Sanni *et al*. (2006) reported that the lower the initial moisture content of a product to be stored, the better the storage stability of such product.

Total ash content was in the range of 0.99%-1.29%. The significant decrease ($p \leq 0.05$) in total ash content may be due to leaching of the inorganic elements into the steeping medium. Results obtained in this study is in line with observations of Pontieri *et al*. (2011) who reported 0.77-1.39% total ash in different sorghum varieties. Total ash content could be used as an index of total mineral matter present in foods because ash is the inorganic residue remaining after the water and organic matter are removed by heating (Sanni *et al*., 2008). Crude fat content was in the range of 4.52%-8.92%. The significant decrease ($p\leq0.05$) in crude fat content could be as a result of the biochemical and physiological changes occurring (breakdown of complex compound into more simpler form by lipolytic enzymes e.g hydrolysis of fat to glycerol and fatty acids) during steeping and such changes require energy hence part of the seed oil were utilized to produce such energy (El-Beltagi and Mohamed, 2010). Crude protein was in the range of 7.76%-15.82%. The significant increase in crude protein content could be due to the alteration of other components (starch, lipid, ash, crude fibre) which might have altered the proportion of the protein on dry weight basis during steeping. This increase is also reported to be due to conversion of bioactive ingredient which also resulted in ionic leaching (Mathanghi and Sudha, 2012). Crude fibre content was in the range of 1.05%-1.63%. The significant increase in crude fibre content could be due to the increase in bran matter and building up of dry matter as a result of breakdown of starch during steeping. The result are in agreement with observation of Pontieri *et al*. (2011) who reported 0.99-1.71% crude fibre in different sorghum varieties. Crude fibre consists mainly of cellulose, lignin and hemicellulose. Carbohydrate content ranged between 76.62%-82.03%. Carbohydrate content decrease significantly ($p\leq0.05$) due to the increase and decrease in other component of the starch (moisture, protein, ash, fibre and fat content). The decrease could also be attributed to the increase in alpha amylase activity (Lasekan, 1996). The alpha amylase breaks down complex carbohydrate to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of steeping thereby resulting to loss in dry matter and volatiles.

Amylose and amylopectin content of sorghum starch is shown in Table 2. Amylose content was in the range of 18.97%-23.60% and amylopectin was in the range of 76.40%-81.03%.The significant decrease ($p\leq0.05$) in amylose content could be attributed to the activity of α-amylase enzyme which increased during steeping and hydrolyzes amylose and amylopectin to dextrin's and maltose (Hotz and Gibson, 2007). Pasting and functional properties of starch depend on the type and amount of amylose contained in the starch (Richard *et al*., 1991). The retrogradation tendency of starch depends on the amylose/amylopectin ratio. Generally, the higher the concentration of amylose in a given starch/flour, the higher its tendency towards retrogradation. The respective proportions of amylose/amylopectin determine their behaviors during the cooking process and suitability for certain industrial applications such as the manufacture of protective films and thickening agent.

Table 1: Effect of variation of steeping on proximate composition (%) of sorghum starch (dry basis)

Parameter	Steeping time (h)				
	0	6	12	18	24
Moisture	8.53±0.01e	9.89±0.01d	10.23±0.04c	12.20±0.00b	13.70±0.00a
Ash	1.27±0.00a	1.14±0.00b	1.05±0.01c	1.02±0.00cd	0.99±0.00d
Fat	8.92±0.28a	7.90±0.33bc	6.59±0.06d	4.68±0.01e	4.64±0.01e
Protein	9.70±0.01e	11.82±0.01d	13.84±0.01bc	15.14±0.00ab	15.82±0.00a
Fibre	1.08±0.01c	1.15±0.06c	1.33±0.14b	1.42±0.07b	1.62±0.00a
Carbohydrate	79.31±0.26b	78.10±0.28c	77.18±0.25de	77.60±0.06e	76.62±0.01f

Values are means ± standard deviation of triplicate determinations. Means on the same row with different superscript are significantly different ($p\leq0.05$).

Table 2: Effect of variation of steeping on amylose and amylopectin content of sorghum starch (%)

Parameter	Steeping time (h)				
	0	6	12	18	24
Amylose	22.24±0.01a	21.81±0.01b	20.57±0.01c	20.51±0.01c	18.97±0.01d
Amylopectin	77.76±0.01d	78.19±0.01c	79.43±0.01b	79.49±0.01b	81.03±0.01a

Values are means ± standard deviation of triplicate determinations. Means on the same row with different superscript are significantly different ($p\leq0.05$).

Table 3: Changes in functional properties of sorghum starch as influenced by variation in steeping time

Parameter	Steeping time (h)				
	0	6	12	18	24
Bulk density (g/cm^3)	0.75±0.01a	0.73±0.01ab	0.71±0.01b	0.67±0.01c	0.61±0l.01d
Water absorption capacity (g/g)	1.71±0.01d	1.81±0.01d	2.15±0.01c	2.60±0.14ab	2.81±0.01a
Oil absorption capacity (g/g)	1.31±0.01e	1.40±0.01d	1.59±0.01c	1.71±0.04b	1.79±0.01a
Swelling index (g/ml)	0.73±0.01d	0.82±0.03c	0.90±0.03b	0.97±0.01a	0.98±0.03a
Foaming capacity (%)	22.58±0.01a	22.26±0.01b	22.21±0.00b	22.10±0.07c	21.95±0.07de
Foaming stability (%)	83.65±0.01a	83.35±0.07b	82.96±0.01c	82.35±0.07d	82.05±0.07e

Values are means ± standard deviation of triplicate determinations. Means on the same row with different superscript are significantly different (p≤0.05).

Table 4: Pasting properties of sorghum starch as affected by variation in steeping time

Parameter	Steeping time (h)				
	0	6	12	18	24
Peak (RVU)	161.18±0.01a	133.51±0.01b	128.52±0.03bc	115.24±0.02d	111.56±0.02d
Trough (RVU)	123.25±0.00a	106.42±0.00b	85.07±0.02e	90.21±0.01d	95.30±0.00c
Breakdown (RVU)	37.93±0.01ab	27.09±0.01c	43.46±0.05a	25.03±0.01cd	16.27±0.02d
Final Velocity (RVU)	247.34±0.01a	229.34±0.01b	145.26±0.01c	135.45±0.07de	128.21±0.01f
Setback (RVU)	124.09±0.01a	122.93±0.01a	85.34±0.01b	67.33±0.01c	55.58±0.01d
Peak Time (min)	4.97±0.01f	5.13±0.01e	5.66±0.01d	5.76±0.01c	6.26±0.01a
Pasting Temp (^{o}C)	83.66±0.01f	84.15±0.01d	85.05±0.07c	85.50±0.14b	85.95±0.07a

Values are means ± standard deviation of triplicate determinations. Means on the same row with different superscript are significantly different (p≤0.05).

Functional properties of sorghum starch are shown in Table 3. Bulk density ranged between 0.61 g/cm3-0.75 g/cm3. The significant decrease (p≤0.05) in bulk density could be as a result of reduction in weight of the starch owing to the breakdown of complex denser compounds attributed to enzymatic activities during steeping (Gernah *et al*., 2011). Bulk density is a measure of heaviness of a flour sample and is generally affected by particle size. It is important for determining packaging requirement, material handling and application in wet processing in food industry (Karuna *et al*., 1996; Ajanaku *et al*., 2012). Lower bulk density enhances easy mixing, storage, packaging and transportation as a result of reduction in mass-volume ratio. Water absorption capacity was in the range of 1.71 g/g-2.81 g/g. The significant increase (p≤0.05) in water absorption capacity could be due to high protein content and presence of more hydrophilic carbohydrate such as soluble sugars with good water holding capacity (Ocheme and Chinma, 2008). Water absorption capacity of food product is an index of the maximum amount of water a product can absorbs and retains, it is important to soften and increase digestibility (Ijarotimi and Keshinro, 2012). According to Okaka and Porter (1997), water holding capacity depends on the water binding capacity of food components. High value of water absorption capacity is attributed to lose structure of starch polymer while low value indicates the compactness of the structure (Adebowale *et al*., 2005; Oladipupo and Nwokocha, 2011). An advantage of high water absorption capacity is that it facilitates easy digestion while the disadvantage is a relatively high water activity which could cause food spoilage (Ocheme *et al*., 2010).

Oil absorption capacity was in the range of 1.31 g/g-1.79 g/g. Oil absorption capacity also followed the same trend; the increase could be due to solubilization and dissociation of protein resulting to exposure of non-polar constituents from within the protein molecules (Deepali *et al*., 2013). High oil absorption capacity makes flours/starches suitable in enhancement of flavor and mouth feel when used in food preparations. (Balogun and Olatidoye, 2010). Food with good oil binding ability can be used as meat replacers and extenders. Swelling index was in the range 0.71 g/ml-0.99 g/ml. The significant increased (p≤0.05) in swelling index could be attributed to increase in soluble solids brought about by the breakdown of lipid, fibre and larger amount of amylose-lipid complex that could inhibit the swelling of starch granules (Ocheme *et al*., 2015). Fats may complex with starch and limit swelling (Zobel, 1984). This report is in line with the result obtained in this study because the swelling index increases as the fat content decrease. Swelling index is a measure of the hydration capacity of starch and is expressed as the weight of centrifuged swollen granules divided by the weight of the original dry starch used to make the paste (Shimelis *et al*., 2006). Least gelation was in the range of 14%-20%. The significant increase ($p \leq 0.05$) in least gelation could be attributed to the altered carbohydrate composition of the grains during steeping. Foaming capacity and stability was in the ranged of 21.95%-22.58% and 82.00%-83.67% respectively. Foaming capacity and stability showed a significant decrease ($p \leq 0.05$) with an increased steeping time. This could be as a result of denaturation of protein molecules during steeping. Brou *et al*. (2013) reported that native protein provide higher foam capacity than denatured protein. Foam is a colloid of gas bubbles trapped in a solid or liquid. Foam formation and stability are dependent on protein type, pH, surface tension, viscosity and processing method. Food material with good foaming capacity and stability are useful in the formulation of aerated food.

Pasting properties of sorghum starch are presented in Table 4. The peak viscosity was in the range of 111.56-170.30 RVU. The significant decrease ($p \leq 0.05$) in the value of peak viscosity could be attributed to disruption of the starch granules and leaching of amylose molecules during steeping. Peak period is commonly accompanied by a breakdown in viscosity sometimes called trough. The rate of viscosity reduction depends on the temperature and degree of shear applied to the mixture and the nature of the material itself. Trough was in the range of 83.55-126.85 RVU. High trough value recorded at initial stage of steeping shows that the starch will have low cooking loss and superior eating quality (Bhattacharya *et al*., 1999). Trough is an indication of breakdown or stability of starch gel during cooking, it is also a minimum viscosity value in the constant temperature phase of the RVA profile and measures the ability of paste to withstand breakdown during cooling (Newport Scientific, 1998). Breakdown ranged between 16.27-46.85 RVU. The significant decrease ($p \leq 0.05$) in breakdown could be attributed to increase and decrease in the peak viscosity and trough value since breakdown is the difference between the peak viscosity and trough value. Breakdown is a measure of susceptibility of cooked starch granules to disintegration and has been reported by Beta *et al*. (2000) to affect the stability of the starch products. The higher the breakdown value, the higher the ability to remain undisrupted when subjected to long period of constant high temperature and ability to withstand breakdown during cooking.

Final viscosity ranged between 128.21-248.55 RVU. The significant decrease ($p \leq 0.05$) in final viscosity could be attributed to starch degradation caused by the actions of alpha and beta-amylase during steeping (Fitzgeral *et al*., 2003). An indication of high final viscosity is that such starch can form viscous and firm gel after cooking and cooling than the sample with slightly lower value (Adeyemi , 1989). Setback was in the range of 55.58-125.07 RVU. Setback value is an index of the tendency of cooked starch to harden on cooling due to amylose retrogradation (Adeyemi, 1989). Higher setback value is synonymous to reduced dough digestibility while lower setback value during the cooling of the paste indicates lower tendency for retrogradation (Sandhu *et al*., 2007). Setback is sometimes measured as the difference between final viscosity and peak value which has been related to firmness and the amylose content. Peak time was in the range of 4.97 min-6.26 min. Pasting temperature was in the range of 83.66 ^{o}C-85.95 ^{o}C. The peak time and pasting temperature showed significant increase ($p \leq 0.05$) with increase in steeping time.

CONCLUSION

The study showed the functional and pasting properties of starch from sorghum grains steep in water at different periods of 6, 12, 18 and 24 h. The moisture content, crude protein content and crude fibre content show significant increase while the ash content, fat content and carbohydrate content showed significant decrease with increase in steeping time. The amylose content decreased significantly with increasing steeping time. The pasting properties of the starches determined by RVA showed that starch from sorghum steeped for longer time had lower pasting characteristics (peak viscosity, trough, breakdown, final viscosity and setback value) except peak time and pasting temperature which are higher with longer steeping time. The functional properties (bulk density and foaming properties) decreased with an increased steeping time while water absorption capacity, oil absorption capacity and swelling index show significant increase with an increase in steeping time. Generally, the results indicated that steeping at different period had a significant effect on the parameter studied.

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