Effect of chemical modifications on thermal, rheological and morphological properties of yellow sorghum starch

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Abstract Starch isolated from yellow sorghum grains was subjected to chemical modifications like acetylation, hydroxypropylation and benzylation. Proximate compositions of these, such as crude protein, crude fat, moisture content and ash content were determined. The effects of modifications on thermal, rheological and morphological properties of yellow sorghum starch were investigated. Differential Scanning Calorimetry studies showed that the gelatinization temperature and enthalpy of modified yellow sorghum starches decreased when compared to that of native starch. The results showed that peak, hot pasting, final, breakdown and setback viscosities were significantly reduced except in hydroxypropylated starch that showed increase in breakdown and setback viscosities. Scanning electron microscopy pictures showed that the modified starch granules had disrupted surfaces compared to native starch granules; hydroxypropylated starch showed the presence of slight fragmentation and a distinct groove in their central core region. The extent of granule disruption was observed to be higher for hydroxypropylated starch than other modified starches.

Keywords Starch · Chemical modifications · Thermal · Rheological properties · Scanning electron microscopy

Introduction

Starch is the major polysaccharide in plants and is in the form of granules that exist naturally within the plant cells. Starch granules can be separated into two distinctly different components: amylose and amylopectin. Starch contributes greatly to the textural properties of many foods and has many industrial applications as a thickener, colloidal stabilizer, gelling agent and water retention agent. Interest in new value-added products such as chocolate, peanut butter, oleoresin, dextrins and adhesives to the industry has resulted in many studies being carried out on the morphological, rheological, thermal and textural properties of corn and potato starches (Evans and Haisman 1979; Kim et al. 1995; Lii et al. 1996). Native starch has limited industrial applications due to high tendency of retrogradation, shear resistance and thermal resistance; therefore there is the need for its modification. Starch modification, involves the alteration of the physical and chemical characteristics of the native starch to improve its functional characteristics, and also it can be used to tailor the starch to particular process through derivatization such as etherification, esterification and decomposition (acid hydrolysis and oxidation of starch). Chemical modification involves the introduction of functional groups into the starch molecule resulting in markedly altered physico-chemical properties. This modification alters the gelatinization, pasting and retrogradation properties (Choi and Kerr 2003; Perera et al. 1997; Liu et al. 1999a, 1999b). In acetylation, hydrophilic hydroxyl groups are substituted with hydrophobic acetyl groups. It makes starch more hydrophobic and prevents the formation of hydrogen bonding between hydroxyl groups and water molecules. The acetylation treatment eliminates retrogradation and will effect stabilization of the starch sol.

In hydroxypropylation, hydroxypropyl groups introduced into the starch chains are capable of disrupting inter and intra

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molecular hydrogen bonds, thereby weakening the granular structure of starch, leading to an increase in motional freedom of starch chains in amorphous regions. Hydroxypropylation inhibits retrogradation and enhance freeze/thaw stability.

Benzylation imparts hydrophobic character to starch, and also it weakens the internal hydrogen bonding and helped starch swelling at relatively low temperature. It decreases starch solubility, pasting viscosity and clarity.

The effect of chemical modifications on thermal behavior of starches can be studied by differential scanning calorimetry (DSC). During gelatinization, DSC measures the extent of disruption of primary hydrogen bonds that stabilize the double helix within the starch granules and quantifies the heat energy (Biliaderis 1990). In rapid visco-analyzer (RVA),

swelling of starch is a function of temperature and is continuously recorded as a change in viscosity. At the peak viscosity, some of the granules are broken due to stirring, with continuous stirring, these granules rupture leading to reduction in viscosity. On cooling starch molecules re-associate to form a gel, which the firmness depends on the extent of interference; these are altered by modifications. Scanning electron microscopy (SEM) has played an important role in improving the understanding of the granular structure of starches. It has been used to detect structural changes caused by chemical modifications in most substituted regions of starch granules.

The aim of this study was to determine the effect of chemical modifications on thermal, morphological and rheological properties of yellow sorghum starch.

Materials & methods

Materials

Yellow sorghum grains were collected from Institute of Agricultural Research Samaru, Zaria, Nigeria. The grains were handpicked and they appeared to be free of mold or weathering. All chemicals used were of Analar grade.

Isolation of starch

Sorghum starch was isolated as this: 1 kg of the sorghum grains was suspended in water ratio 1:5 differently. The pH of each solution was adjusted to 8 with (0.2% w/v) of NaOH solution for 24 h. Then it was stirred for 30 min and the grains were screened and washed with distilled water until they were unpigmented. Wet milling procedure of Watson et al. (1955) with minor modification was employed. The grains were blended with distilled water in a blender for 45 min while the slurry obtained after blending was re-suspended in 50 ml of distilled water and the pH was adjusted to 8.0 using 0.5 M 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 μm and 80 μm mesh sieve and centrifuged for 30 min and was thoroughly washed four times with distilled water. The starch slurry was allowed to settle for 45 min and water was decanted before drying in air for 48 h at 30 ± 2.0 °C. It was stored in polythene bag prior analysis.

Chemical composition

Standard Association of Official Analytical Chemistry method, AOAC (1996) was adopted for estimating moisture content, ash content, crude protein and crude fat.

Starch acetylation

Starch acetylation was prepared according to the method of Sathe and Salunkhe (1981). 100 g of starch were dispersed in 500 ml of distilled water; it was magnetically stirred for 20 min. The pH of the slurry obtained was adjusted to 8.0 using 1 M of NaOH. 10.2 g acetic anhydride was added over a period of one hour while maintaining a pH range of 8.0–8.5. The reaction proceeded for 5 min after the addition of acetic anhydride. The pH of the slurry was reduced to 4.5 using 0.5 M HCl. It was filtered, washed for four times with distilled water and air-dried of 30 ± 2.0 °C for 48 h.

Starch hydroxypropylation

According to the procedure of Leeg and Luten (1971), 200 g, (dry basis) of the sample was weighed into 600 ml screw cap jars, a solution of NaOH (2.6 g) and Na_2SO_4 (30 g) in distilled water (240 ml) was added at room temperature. The jar with the sample was placed in a water bath at 40 °C and 20 ml of propylene oxide was added and the suspension in the jar was thoroughly mixed and tightly closed.

The reaction continued with shaking for 24 h at 40 °C. The starch suspension was then neutralized to pH 5.5 with dilute H_2SO_4 (1 mol/L). The starch cake was washed with distilled water until it tested negative to sulfate ions when BaCl_2 was added. All hydroxypropylated suspension was freeze - dried until the moisture content was reduced to 10–12 g/100 g.

Starch Benzylation

Starch Benzylation was prepared by the method of Berkhout and Guns (1973). 100 g of starch was weighed into 500 ml conical flask, a solution of 100 g Anhydrous Na_2SO_4 and NaOH (3–8 g) were dissolved in 150 ml of warm distilled water at 50 °C and 5–50% of benzyl chloride was added and stirred at 65 °C for 14 h. Concentrated Na_2SO_4 in the solution prevented the alkali from making the starch granules swell during the reaction. The reaction was stopped by neutralizing the mixture to pH 6 with 1 mol/L HCl. The starch was filtered,

and washed five times with 150 ml distilled water and finally with 95% ethanol (150 ml). The starch residue was then dried overnight in a 40 °C conventional oven.

Differential scanning calorimetry and retrogradation studies

Thermal transition of starches was measured using a differential scanning calorimeter. The DSC was equipped with thermal analysis software and pyres windows (Perkin – Elmer). With a microsyringe, 6.0 µl distilled water was added to 2.0 mg (dry weight basis) of starch in one of the DSC pans. It was sealed, reweighed and kept at 30 ± 2 °C for 24 h to ensure equilibration of the starch sample and water. The samples were scanned at a temperature range of 30 °C – 140 °C, at the rate of 10 °C/min, using the empty pan as the reference. The heated pans were then cooled immediately and kept at 4 °C in a refrigerator for 24 h, following which they were kept for six days at 30 ± 2 °C, to make complete storage days of two and seven, respectively. During the periods of storage, the samples were scanned under the same condition as the first scanning. Indium and Zinc were used for calibration while an empty pan was used as the reference scale. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and Enthalpy changes (ΔH_{ij} /g) for gelatinization and retrogradation were determined.

Pasting properties

Rapid Visco-Analyzer Model 3D (RVA: Newport Scientific Pty.Ltd., Warriewood, Australia) was used to determine the pasting properties of the 10% starches suspension (3 g in 30 ml of distilled water). The slurry underwent a controlled heating-and-cooling cycle under constant shear where it was held at 50 °C for 1 min, heated from 50 to 95 °C at 12 °C/min and the sample held at 95 °C for 2.5 min, and held at 50 °C for 5 min.

Granule morphology

Granule morphology of the starches was studied using scanning electron microscopy (SEM). The sample was sputter-coated with Au/pd. using a vacuum evaporator (Edwards, Milano, Italy) and examined using a scanning electron microscope (model 500, Philips, Eindhoven, The Netherlands) at 10 kV accelerating voltage using the secondary electron technique.

Statistical analysis

Analyses were done in triplicate. Analysis of variance was done to calculate significant differences in treatment means, and LSD ($p < 0.05$) was used to separate the means SAS (1988).

Results and discussion

Chemical composition

The chemical composition of the yellow sorghum starch is presented in Table 1. The crude protein content of the native starch was 4.34% and it was lower than 8.55% and 7.7% reported for wheat flour and rice starch respectively (Anderson 1987). The low value showed the purity of the starch as indicated by the low nitrogen and low ash levels. The native starch was rich in starch with 87.59% carbohydrate content. The percentage yield of yellow sorghum starch (78.5%) was higher than the percentage yields of 68–75% reported for white and dark sorghum starches by Elevina et al. (1997). Moisture contents ranged from 7.55% to 9.90%, and these values were lower than the value reported by Davila and Camelo (2001). This might be due to different method used. In addition, these low values were advantageous in terms of shelf life and keeping the quality of the starch. Chemical modifications reduced the values for non-starch components such as (protein, ash, fat and moisture content). The low non-starch components of the starch make them valuable in some industrial applications. For instance, low protein content of the modified yellow sorghum starch make them important in the production of syrups and also in confectionery industry with high glucose content compared to the native starch. Reductions observed in fat, protein and moisture contents could also be attributed to various degradative processes during modifications of the starch. Furthermore, low moisture and fat contents in modified starch play a vital role in long storage but high moisture levels could be deleterious because it would enhance microbial growth.

Gelatinization studies

Gelatinization thermograms of native and modified starches are presented in Figs. 1a–d. The thermogram depicts the onset temperature, T_o , the peak temperature, T_p and conclusion temperature T_c . Onset gelatinization temperature for native yellow starch was 69.69 °C. The value agreed with previous values reported in the literature on corn and waxy maize (Lim et al. 2001). The ΔH gel provides an overall measure of crystallinity (quality and quantity) and is an indicator of the loss of molecular order within the granule Cooke and Gildey (1992). The result showed that gelatinization enthalpy reduced from 1.697 J/g in native yellow sorghum starch to 1.609, 1.578 and 1.459 J/g in acetylated, hydroxypropylated and benzylated respectively. Tester and Morrison (1990) had earlier reported that the gelatinization of a starch is controlled in part by the molecular structure of amylopectin (perfection and ordering of amylopectin crystallites, length of the external ‘A’ chains of amylopectin, extent of branching, starch composition) (amylose/amylopectin ratio, lipid complexed amylose

Table 1 Proximate analysis of native, acetylated, hydroxypropylated and benzylated yellow sorghum starch

Sample	Moisture (%)	Protein (%)	Crude fat (%)	Ash (%)	Carhydr(%)
YNS	9.90 ± 0.60 ^a	4.34 ± 0.26 ^a	1.05 ± 0.05 ^a	0.62 ± 0.07 ^b	86.09 ± 0.09
ACETY	8.77 ± 0.07 ^a	1.78 ± 0.20 ^c	0.37 ± 0.02 ^b	0.48 ± 0.05 ^a	88.00 ± 0.09
HPY	8.45 ± 0.05 ^c	2.02 ± 0.04 ^a	0.40 ± 0.35 ^b	0.32 ± 0.10 ^a	87.11 ± 0.31
BENY	7.55 ± 0.04 ^c	1.28 ± 0.10 ^c	0.32 ± 0.05 ^b	0.70 ± 0.03 ^b	90.10 ± 0.06

Each value represents the mean of three determinations ±standard deviation.

Samples followed by the same letter are not significantly different ($p < 0.05$).

YNS Yellow native sorghum starch, ACETY Acetylated yellow sorghum starch, HPY Hydroxypropylated yellow sorghum starch, BENY Benzylated yellow sorghum starch

chains and granule architecture, which changes crystalline to amorphous ratio).

The chemical modifications; acetylation, hydroxypropylation and benzylation reduced gelatinization enthalpy of the starch. This suggested that little energy will be needed to gelatinize the starch molecule. Acetylation decreased the gelatinization temperatures and enthalpy of gelatinization. Weakening of the starch granules by acetylation leads to early rupture of the amylopectin double helices, which accounts for the lower values of T_o , T_p and T_c . Kaur et al. 2004; Singh et al. 2004; Sodhi and Singh (2005) reported a similar result for maize and rice starches. Upon hydroxypropylation, the reactive groups introduced into the starch chains were capable of disrupting

inter and intra-molecular hydrogen bonds, leading to an increase in accessibility by water that lowers the temperature of gelatinization (T_o , T_p , T_c). The decrease in ΔH gel on hydroxypropylation suggested that hydroxypropyl groups disrupt double helices within the amorphous regions of the granule. Consequently, the number of double helices that unravel and melt during gelatinization will be lower in hydroxypropylation. Benzylation (substitution of side chains with benzyl group) resulted in the inhibition of ordered structure of starch paste, thus retarding retrogradation and reducing its gelatinization temperature. Therefore tendency, towards retrogradation was greatly reduced by acetylation, hydroxypropylation, and benzylation.

Fig. 1 DSC thermogram of **a** native, **b** acetylated, **c** hydroxypropylated, **d** benzylated yellow sorghum starch

Pasting properties

The pasting properties of native and modified starches of yellow sorghum are presented in Table 2. The native yellow sorghum starch (YNS) had pasting temperature of 81.90 °C. The pasting temperature between 72 and 87 °C and 75–93 °C are reported for dark and white sorghum starches (Elevina et al. 1997). The result revealed that chemical modifications reduced the pasting temperature (T_p) of the native starch when compared with modified starches. This suggested that chemical modification processes increased the strength of intragranular binding forces, thus making the starch respond to heat and lose birefringence at lower temperature compared to the unmodified starch.

The peak viscosity (P_v), hot paste viscosity (H_v), breakdown value (BD), final viscosity (F_v) and set back value (SB) were also reduced after modifications. Conversely, hydroxypropylated starch increased peak viscosity; because the hydrophilic group caused a loosening of the network, allowing additional water into the starch micelle (a cluster of starch granules). The micelle remained intact, holding the granules together in enormously swollen net works; thus the increase in hydration volume resulted in higher viscosity. A similar result was reported by Anil and Harold (2007). The pasting temperature and peak viscosity were significantly lower than those of the native starch in benzylated starch. Decrease in the pasting temperature showed that the starch chains become readily dissociated presumably because of the steric hindrance of the big benzyl substituents. Also the hydrophobic benzyl groups weakened the internal hydrogen bondings and improved starch swelling at relatively low temperature. There was reduction in setback values of the chemically modified starches. (Set-back value is a measure of starch retrogradation.). This suggested that hydroxypropylation, acetylation and benzylation reduced the starch tendency to retrograde as indicated by the reduction in setback values. It showed that electrostatic repulsion among the starch

molecules leads to a reduction in reassociative tendency in starch gels, and this resulted in a reduction in starch viscosity.

In this study, breakdown value (BD) is defined as the difference between peak viscosity and hot paste viscosity Jian and Manfred (1999). It is a measure of the fragility of the starch (Bhandari and Singhal 2002) and decreases following modifications. The decreased was more significant in acetylated starches than native starch due to high shearing conditions in the Rapid Visco Analyzer (RVA) and unit is Rapid Visco Unit (RVU). Sirirat et al. (2005) reported a similar result with the Thai- purple canna starch. Hydroxypropylated starch increased breakdown value (compared with the native starch) due to the degradation that occurs during the modification process.

Granule Morphology

Scanning electron micrographs (SEM) of native and modified yellow sorghum starches are presented in Figs. 2a–d. The micrographs revealed round and polygonal shapes for the starch granules with heterogeneous sizes. Surface pores on granules of corn, sorghum and millet are openings to channels that penetrate in a radial direction through the granules (Huber and

Table 2 Pasting properties of native and chemically modified yellow sorghum starch.

	YNS	ACETY	HPY	BENY
Peak Viscosity, PV (RVU)	419.33	112.17	475.83	265.50
Hot Pasting Viscosity, HV, (RVU)	272.17	82.25	126.25	160.20
Breakdown (PV–HV) (RVU)	147.17	29.92	349.58	105.50
Final Viscosity, FU (RVU)	421.92	100.92	318.75	301.21
Setback (FU–HV) (RVU)	149.75	18.67	192.50	141.01
Pasting temperature (°C)	81.90	80.45	79.20	77.15

Results are means of triplicate determinations

YNS Yellow native sorghum starch, ACETY Acetylated yellow sorghum starch, HPY Hydroxypropylated yellow sorghum starch, BENY Benzylated yellow sorghum starch

(a) native

(b) acetylated

(c) hydroxypropylated

(d) benzylated

Fig. 2 Scanning electron micrograph of **a** native, **b** acetylated, **c** hydroxypropylated, **d** benzylated yellow sorghum starch ($\times 3000$ magnification)

Bemiller 1997). This suggested that the concentration of H_3O^+ in the granule may also be influenced by granule porosity. However at $\times 3000$ magnification, the big granules observed had indentations and clearly showed the damage due to modifications. In hydroxypropylated yellow sorghum starch, many of the less affected modified granules with propylene oxide developed a depression leading to slight fragmentation and the formation of a deep groove in the central core region along the highly affected granules. The affected granules appeared as folded structures with their outer side drawn inwards, giving the appearance of a doughnut. The results agreed with those reported by (Kim et al. 1992), who suggested that most of the structural changes take place at the relatively less organized central region of the starch granule, i.e., where.

the hydroxypropyl groups are most densely deposited.

The “pushing apart effect”, arising from the bulky hydroxypropyl groups especially in the central region of the granule, might lead to an alteration in the granule morphology upon hydroxypropylation. Kaur et al. (2004) reported a similar observation for hydroxypropylated potato starch. Acetylation treatment has also been found to alter the granule morphology, although to a lesser extent. Rough appearance with indentations was observed on the granule surfaces of yellow sorghum starch. The surface granules of the benzylated yellow sorghum starch was fused and deformed with more pores. This suggested that the highly affected granules were in a gelatinized form, had loosened their boundaries and fused together to form a gelatinized mass.

Conclusion

Chemical modifications reduced low-non starch components which make them useful in some industrial applications. Retrogradation tendency was also reduced after modifications, which serves as an advantage in production of foods like bread which undergo staling easily and sauces which undergo loss of viscosity. The changes observed in the thermal, morphological and rheological properties of the yellow sorghum starch after modification may provide a crucial basis for understanding the efficiency of the starch modification process on an industrial scale. Starch with desirable functional properties could play a significant role in improving the quality of different food products.

References

- Anderson S (1987) Paste properties of wheat flour and rice starches. *Starch/Stärke* 42(10):10–15
- Anil G, Harold C (2007) Effect of hydroxypropylation and alkaline treatment in hydroxylpropylation on some structural and physicochemical properties of heat-moisture Treatment wheat, potato and waxy maize starches. *J Carbohydr polym* 68:305–313
- AOAC (1996) Association of Official Analytical Chemists. Official Methods of analysis. (15th edition) Washington DC
- Berkhout F, Guns J (1973) Process for the manufacture of hydrophobiogranules starch ethers. BPT. 1309321
- Bhandari PN, Singhal RS (2002) Effect of succinylation on the corn and amaranth starch pastes. *J Carbohydr polym* 48:233–240
- Choi SG, Kerr WL (2003) Effects of chemical modification of wheat starch on molecular mobility as studied by pulsed 1H NMR. *Lebensm-Wiss Tech* 51:1–8
- Cooke D, Gildey MJ (1992) Loss of crystalline and molecular order during starch gelatinization: origin of the enthalpic transition. *J Carbohydr Res* 277:103–112
- Davila O, Camelo M (2001) Physicochemical and functional characterization of Baby lima bean (*Phaseolus Lunatus*) starch. *J Carbohydr polym* 45:122–126
- Elevina E, Mary L, Zurima M (1997) Characterization of starch isolated from white and dark Sorghum. *Starch/Stärke* 49:103–106
- Evans ID, Haisman DR (1979) Rheological of gelatinized starch suspensions. *J Texture Stud* 17:253–257
- Huber RC, Bemiller JN (1997) Visualization of channels and cavities of corn and sorghum starch granules. *J Cereal Chem* 74:537–541
- Jian YQ, Manfred KS (1999) Characterization of *Amaranthus cruentus* and *chenopodium quinoa* starch. *Starch/Stärke* 51:116–120
- Kaur L, Singh N, Singh J (2004) Factors influencing the properties of hydroxypropylated potato starches. *J Carbohydr polym* 55:211–223
- Kim HR, Hermansson AM, Eriksson CE (1992) Structural characteristics of hydroxypropyl potato starch granules depending on their molar substitution. *Starch/Stärke* 44:111–116
- Kim SY, Wiesenborn DP, Orr PH, Grant LA (1995) Screening potato starch for novel properties using differential scanning calorimetry. *J Food Sci* 60:1060–1065
- Leeg DC, Luten JB (1971) Study on the In vitro digestibility hydroxypropyl starches by pancreatin. *Starch/Stärke* 23:430–432
- Lii CY, Tsai ML, Tseng KH (1996) Effect of amylose content on the rheological properties of rice starch. *J of Cereal Chem* 72:393–400
- Lim ST, Chang EH, Chang HJ (2001) Thermal transition characteristics of heat-moisture treated corn and potato starches. *J Carbohydr polym* 46:107–115
- Liu H, Ramsden L, Corke H (1999a) Physical properties and enzymatic digestibility of hydroxypropylated ae,wx and normal maize starch. *J Carbohydr polym* 40:175–182
- Liu H, Ramsden L, Corke H (1999b) Physical properties of cross-linked and acetylated normal and waxy rice starch. *Starch/Stärke* 51:249–252
- Perera C, Hoover R, Martin AM (1997) The effect of hydroxypropylation on the structure and physicochemical properties of native defatted and heat- moisture treated potato starches. *J of Food Res Inter* 30: 235–247
- SAS (1988) SAS/STAT user’ guide, release 6.03, SAS Institute Inc., Cary NC
- Sathe SK, Salunkhe DK (1981) Isolation, partial characterization and modification of the great Northern Bean (*Phaseolus vulgaris* L.) starch. *J Food Sci* 46:617–621
- Singh N, Chawla D, Singh J (2004) Influence of acetic anhydride on physicochemical, morphological and thermal properties of corn and potato starch. *J of Food Chem* 86:601–608
- Sirirat S, Chureerat P, Vilai R, Dudsadee U (2005) Paste and gel properties of low-substituted acetylated caura starches. *J Caborhyd polym* 42:420–431
- Sodhi NS, Singh N (2005) Characteristic of acetylated starches prepared using starches separated from different rice cultivars. *J Food Eng* 70: 117–127

Tester RF, Morrison WR (1990) Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipids. *J. Cereal Chem* 67:551–557

Watson SA, Sawners EH, Wakely RD, Williams CB (1955) Peripheral cells of the endosperms of grain sorghum and corn and their influence on starch purification. *J Cereal Chem* 32:165–182