1 Title: Characterization of hydrothermal and acid modified proso millet starch

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Abstract

The aim of this study was to characterize modified proso millet (*Panicum miliaceum*) starch in order to explore its prospect as an ingredient for food applications. The current study determined the effect of hydrothermal modification (HTM) at 30 g/100 g moisture level and acid modification (AM) with HCl on extracted proso millet starch physicochemical and functional properties. Amylose content reduces with AM while HTM showed negligible effect. HTM starch had higher water binding capacity (WBC) whereas AM starch showed reduction in WBC. Additionally, the solubility and swelling power of HTM starch decreased with increase in temperature, and in AM starch solubility increased sharply but swelling power increases at 80 °C but significantly ($P < 0.05$) reduces at 90 °C. HTM caused increase in gelatinization temperature with a mean value of 87.17 °C compared to 78.61 °C in native starch. AM reduced onset (69.71 °C) and gelatinization temperature (77.26 °C), and it increased the range (26.56 °C) significantly ($P < 0.05$) with no effect on ΔH_G . Pasting profiles of native proso millet starch changed significantly ($P < 0.05$) upon modifications and reduction in peak viscosity was observed in both modifications. AM reduced the holding strength, final viscosity, setback and breakdown whereas HTM reduced only breakdown and no change was observed in other parameters.

Keywords

Acid modification; Hydrothermal modification; Proso millet; Starch

1. Introduction

Interest in millet utilization has increased due to the various rediscovered health benefits and also due to its increasing use as non-gluten ingredient in food applications (Zhu, 2014). Millet has many advantages over other cereals such as higher resistance to pest and diseases, adaptability to a wide range of climatic conditions and grows well in high temperatures and dry conditions (Saleh, Zhang, Chen, & Shen, 2013). Besides having agronomic advantages, millets have better amino acid composition and high nutritive value which is comparable to that of major cereals such as wheat, corn and rice (Klopfenstein & Hosney, 1995; Parameswaran & Sadasivam, 1994). Millet is widely consumed as food in African countries, China and Indian subcontinent, however it is not part of human diet in USA and mainly used for animal and bird feed (Lyon, 2008). Proso millet is the major variety of millet grown in the US with a total production of 418,145 tons in 2013 (FAO, 2013). Proso millet, considered an underutilized grain in USA, can serve as source of starch as it is reported to contain 60 - 67% starch (Santra, 2013). Due to the vast application of starches in food systems, different sources with good functional properties are being explored.

Starch is a naturally renewable, inexpensive and biodegradable material which is used to alter the textural properties of several foods (Radley, 1976). It has various industrial applications such as thickener, binder, encapsulating agent, stabilizer and gelling agent (Radley, 1976). However, it is the modified starch that is used mostly in industrial applications due to undesirable characteristics of native starch upon cooking whereas modification improves gelling tendency, clarity and texture (Bemiller, 1997). Starch modification alters physical and chemical properties to improve functionality of native starch (Hermansson & Svegmarm, 1996). Hydrothermal modification (HTM) involves controlled application of heat and moisture, which

causes physical modification of starches without gelatinization and damage to the starch granules with respect to size, shape or birefringence (Stute, 1992). Acid modification (AM) of starch is a chemical modification process involving hydrolysis of starch using hydrochloric acid, which breaks the glycosidic linkages of α -glucan chains, changing the structure and characteristics of native starch (Hoover, 2000). AM is used to modify physicochemical properties of native starch for applications in various industries such as food and textile (Radley, 1976). Acid hydrolysis is widely used for production of starch gum candies, paper, cationic and amphoteric starches (Wurzburg, 1986). Understanding the properties, and potential uses of proso millet starch significantly contributes to the further expansion of millets as alternative functional crop (Zhu, 2014). The present study was undertaken to explore the behavior of native and modified starches as affected by different modifications methods. Most studies on millet starch have focused on pearl millet and other major millet varieties but no work has been done to investigate the effect of hydrothermal and acid modification on proso millet starch.

2. Materials and methods

2.1.Raw materials

Proso millet flour was purchased from Bob's Red mill (Milwaukie, OR, USA) and stored at ambient temperature (24-28 °C). All chemicals used for the analyses were of analytical grade (Sigma Aldrich, St. Louis, MO, USA).

2.2.Starch Isolation

Starch was isolated using alkaline steeping method (Sira & Amaiz, 2004; Wang & Wang, 2001). Proso flour (100 g) was steeped in 200 mL of 0.1 g/100 g NaOH for 18 h. Mixture was blended for 2 min using waring blender and passed through a sieve (U.S. 100 sieve size) and centrifuged at 2000 rpm for 15 min. The top layer was carefully decanted and the bottom layer was re-

slurried and washed thrice with 0.1 g/100 g NaOH, while removing the upper layer carefully every time. The starch was washed with deionized water, then treated with 0.1 mol/L HCl to pH 6.5, and washed with deionized water four times, centrifuged, dried in an oven at 45 °C for 48 h.

2.3. Acid modification

Millet starch was modified according to the method described by Wang & Wang (2001). HCl (0.14 mol/L) was added to 40 g starch and kept in water bath for 8 h at 50 °C and thereafter, 1 mol/L NaOH was used to adjust the pH to 6.5. Starch slurry was washed thrice with deionized water and then dried in an oven at 45 °C for 24 h.

2.4. Hydrothermal modification

Millet starch, conditioned to 30 g/100 g moisture content (dry basis) was added in glass bottle and kept at 4 °C for 12 h to equilibrate the moisture. Starch sample in sealed glass bottle was then heated for 3 h at 110 °C. The bottle was occasionally shaken to distribute the heat evenly and then cooled and dried for 4 h at 45 °C (Collado, Mabesa, Oates, & Corke, 2001).

2.5. Physico-chemical analysis

Moisture, protein, fat, ash were determined using AOAC standard methods (AOAC, 2005). Amylose content were determined using AACCI method 61-03.01 (AACC, 1997). Starch sample (100 mg) was mixed with 1 ml of 95 g/100 g ethanol and 9 ml of 1 mol/L NaOH and then transferred to 100 ml volumetric flask. Flasks were kept at room temperature for 10 min then heated in boiling water bath for 10 min and cooled for 2 h at room temperature. The resulting mixture was diluted to 100 ml using distilled water and mixed vigorously. An aliquot of starch solution (5 ml) was pipetted into 100 ml volumetric flask containing 50 ml distilled water. 1.0 mL of acetic acid (1 mol/L) and 2 mL iodine solution were added and diluted to 100 ml. After 20

min, absorbance was measured at 620 nm using blank to zero the spectrometer (EVO 60 ThermoFisher scientific, Waltham, MA USA). Standard curve was developed using standard amylose and amylopectin blends and used to measure amylose content.

2.6. Thermal properties

Degree of gelatinization was determined using differential scanning calorimetry (DSC – Q20, TA instruments, New Castle, Delaware, USA). Starch sample (10 mg, dry basis) was weighed into high volume stainless steel pans, followed by addition of 20 µl of distilled water. The pan was hermetically sealed and equilibrated at 4 °C for 24 h. Samples were kept at room temperature for one hour prior to scanning from 10 to 150 °C at 10 K/min (Krueger, Knutson, Inglett, & Walker, 1987).

After gelatinization, the samples were kept at 4 °C for 10 d and then reheated at the rate of 10 K/min from 10 °C to 150 °C to determine retrogradation properties.

2.7. Pasting properties

Pasting characteristics were determined using Discovery Hybrid Rheometer (DHR) with starch pasting cell (DHR-2, TA instruments, New Castle, Delaware, USA). A mixture of 3.5 g starch (14 g/100 g moisture) in 25 ml of distilled water was stirred at 160 rpm. Samples were held at 50 °C for 1 min and then heated to 95 °C at 4 K/min and held at 95 °C for 5 min. Subsequently, samples were cooled to 50 °C at 4 K/min and held at 50 °C for 5 min. A plot of viscosity (Pa.s) vs. time (s) was used to determine pasting temperature, peak and final viscosity, holding strength, setback and breakdown.

2.8. Solubility and swelling power

Solubility and swelling power was determined using leach method (Leach, McCowen, & Schoch, 1959) modified by Balasubramanian, Sharma, Kaur, & Bhardwaj (2014); Kusumayanti, Handayani, & Santosa (2015) and Subramanian, Hosney, & Bramel-Cox (1994). Starch (0.1 g) was heated in 10 ml of water at 70, 80, and 90 °C for 30 min. Samples were stirred occasionally to avoid lump formation and then centrifuged at 3,000 rpm for 15 min. Supernatant was removed and starch sediment was weighed. Supernatant was dried for 2 h at 130 °C and then weighed.

$$Solubility(\%) = (W_{ss} * 100) / W_s \quad (1)$$

Where, W_{ss} is the weight of soluble starch (g) and W_s is the weight of the sample (g).

$$Swelling\ power(\%) = (W_{sp} * 100) / (W_s * (100 - \%solubility)) \quad (2)$$

Where, W_{sp} is the weight of sediment paste (g) and W_s is the weight of sample (g).

2.9. Water binding capacity

Water binding capacity (WBC) was determined using the method described by Yamazaki (1953). A mixture of 2.5 g (dry basis) starch in 25 mL distilled water was stirred for 30 min and centrifuged at 3,000 rpm for 10 min. Excess water was removed and then residue is weighed.

$$WBC(\%) = (W_{rs} * 100) / W_s \quad (3)$$

Where, W_{rs} is the weight of residual starch (g) and W_s is the weight of sample (g).

2.10. Paste clarity

Paste clarity was measured according to method described by Craig, Maningat, Seib, & Hosney (1989). Starch (2 g/100 g dry basis) aqueous mixture was heated and stirred in water bath for 30 min at 95 °C. Samples were cooled and stored for 4 d at 4 °C and percent transmittance was

measured everyday at 640 nm against water blank using UV–VIS Spectrophotometer (EVO 60 ThermoFisher scientific, Waltham, MA USA).

3. Results and discussion

3.1. Physico-chemical analysis

Proximate content of extracted proso millet starch is presented in Table1. Starch extraction yield was 54.1% and it had low residual protein (1.21 g/100 g), lipid (0.27 g/100 g) and ash (0.62 g/100 g). The amylose content and WBC properties of native, HTM and AM proso millet starches are presented in Table 2. The AM starch showed significant decline ($P<0.05$) in apparent amylose content from 28.51% in native starch to 25.78% in AM starch, which may be due to the attack of acid on amorphous section of starch. Hoover (2000) proposed that acid preferentially attacks the amorphous regions in the granules which leads to cleavage of amylose molecules causing reduction in amylose content. However, HTM starch with 29.08% amylose content showed no significant ($P<0.05$) change in amylose content. Rafiq, Singh, & Saxena (2016) and Sun, Han, Wang, & Xiong (2014) reported similar results for acid modified and hydrothermal modified horse chestnut and sorghum starch respectively.

3.2. Water binding capacity (WBC)

WBC of native starch showed significant ($P<0.05$) decline from 138.43 to 108.13% upon AM but HTM increased the WBC to 191.5%. WBC of HTM starch increased due to the increased hydrophilicity, which reduces the crystalline regions and improves the accessible binding sites in the amorphous region resulting improved WBC. Similar reports for increased WBC due to hydrophilic affinity have been reported in white sorghum and chestnut starch (Rafiq et al., 2016). The AM starch showed low WBC than native proso starch which may be due to the reduced

accessible binding sites caused by reduced amorphous region in starch granules. Balasubramanian et al. (2014) reported similar trend in AM and HTM pearl millet starch and Kaur, Oberoi, Sogi, & Gill (2011) has reported a decrease in WBC of various acid treated starches from different botanical sources

3.3.Solubility and swelling power

The solubility and swelling power of native and modified starches are presented in Table 3. The solubility of native starch increased from 2.62 to 34.88% whereas increase in AM starch is significantly large ($P<0.05$) from 18.97 to 86.17% with rise in temperature from 70 to 90 °C. However, HTM starch solubility increased from 1.71 to 12.45% but it is significantly ($P<0.05$) lower than native starch solubility. Swelling power also showed increase with increase in temperature. Native starch swelling power increased from 4.69 to 24.99%, AM starch solubility increased from 4.94 to 21.26% and HTM starch solubility also rises from 5.29 to 10.37% from 70 to 90 °C. According to Lawal & Adebawale (2005) and da Rosa Zavareze & Dias (2011) the decreased solubility and swelling power of HTM starch compared to native starch has been credited to starch granule's internal reordering providing higher interactions between starch functional groups, formation of more ordered double helical amylopectin clusters and the formation of amylose-lipid complexes within starch granules. In addition, the physical variations within the starch granules and unravelling of double helices of crystalline region, which reduced the granular stability might be accountable for the drop in swelling capacity and starch solubility at higher temperature (Leach et al., 1959; Olayinka, Adebawale, & Olu-Owolabi, 2008). Balasubramanian et al. (2014); Gunaratne & Hoover (2002) reported similar results of reduced swelling power and solubility with heat moisture treated pearl millet starch and cassava starch, respectively.

Solubility of AM starch was higher as compared to native proso starch and increased progressively with increase in temperature. AM leads to the structural weakening and depolymerization of starch granules and similar effect on pearl millet was observed by Balasubramanian et al. (2014). However, swelling power showed no significant ($P < 0.05$) change at 70 °C, increase at 80 °C but significant ($P < 0.05$) reduction at 90 °C was observed which may be due to amylose leaching, resulting in starch damage which limits swelling of starch (Jane, Wong, & McPherson, 1997). Acid hydrolysis causes an increase in percentage crystallinity as the crystalline region is not accessible to acid and the amorphous regions are being broken down. Rigidity of entangled amylopectin linkages in the crystalline area of the starch controls swelling and thus, increased crystallinity may cause reduction in swelling power of the AM (Kainuma & French, 1971).

3.4. Light transmittance

Fig. 1 shows the native and modified proso millet starch light transmittance over a 4 d period. It is used to assess the level of starch paste retrogradation during storage, which depends on the swollen and non-swollen granules during gelatinization process (Craig et al., 1989; Sandhu, Singh, & Lim, 2007). The transmittance of native, AM and HTM pastes showed a gradual decrease with storage but native and AM showed pronounced reduction over time, 42 to 2% and 85 to 47%, respectively, which is a result of retrogradation tendency. HTM improve flexibility of chains within amorphous area of granules and resulted in significant ($P < 0.05$) low transmittance, less than 6% (Hoover & Vasanthan, 1994). AM starch showed higher transmittance which may be due to leaching of amorphous region causing better interactive bond formation between amylopectin molecules thus resulting in clear paste (Lawal, 2004). Corn and pinhao starch showed similar results on acid modification (Thys, Aires, Marczak, & Noreña, 2013).

3.5. Thermal properties

Degree of gelatinization of native, HTM and AM starches are shown in Table 4. HTM increased the onset (T_O), peak (T_P) and conclusion (T_C) temperatures to 79.13, 87.17 and 99.35 °C, respectively compared to native starch T_O , T_P and T_C which were 72.93, 78.61 and 94.55 °C, respectively. HTM starch needs more heat to break the bonds formed between freely moving amylose molecules and amylopectin chains present in crystalline regions, hence higher T_O and T_P (Sun et al., 2014). Moreover, high T_C is due to the reduced destabilization effect of amorphous regions on crystalline melting which is result of reduced swelling power (Gunaratne & Hoover, 2002). Li, Ward, & Gao (2011 and Sun et al. (2014) reported similar results for hydrothermal treated sorghum starch and mung bean starch, respectively. The decrease in enthalpy of gelatinization (ΔH_G) for HTM may be due to fewer double helices available to untangle. During modification some helices present in crystalline and non-crystalline regions are disrupted, which leads to reduction in relative crystallinity, hence reduction in enthalpy (Cooke & Gidley, 1992). Non-significant ($P<0.05$) change in gelatinization range was observed for HTM starches.

The acid treatment showed significant ($P<0.05$) decrease in the T_O (69.71 °C), T_P (77.26 °C) and increase in the range (26.56 °C). Acid modification focus on the amorphous region of the starch granules which no longer destabilizes the crystallites causing crystallites to melt at increased temperature resulting in wider range of gelatinization (Hoover, 2000). In addition, decreased in amorphous region results in an increase in relative crystallinity causing higher ΔH_G than HTM. Increase in enthalpy compared to HTM may also be due interaction between amylose-amylose and amylose-amylopectin causing formation of double helices which require more energy to break during gelatinization (Thirathumthavorn & Charoenrein, 2005).

Retrogradation properties of native, HTM and AM starches after 10 d of storage were measured and shown in Table 5. T_O , T_P and T_C of retrograded native, HTM and AM starches are significantly lower ($P<0.05$) when compared to T_O , T_P and T_C of gelatinization. ΔH_R (2.66 J/g) of native is lower than its ΔH_G (3.83 J/g) but HTM starch's ΔH_R (2.99 J/g) increased which may be due to the interplay between crystallinity changes and amylose-amylopectin interactions. The increase in ΔH_R was attributed to increased crystallinity on modification, which reduces the level of separation among the outer branches of adjacent amylopectin chain groups. Thus, during retrogradation, the formation and lateral association of double helices involving amylopectin chains would be stronger and occur more rapidly in HTM than in native starches (Hoover, 2010). AM starch showed similar ΔH_R (3.83 J/g) as ΔH_G (3.97 J/g) but higher than native starch ΔH_R (2.66 J/g) which is the result of high mobility of short chains and reduction in amylopectin branch points, consequently leading to high rate of realignment of chains during storage (Palma- Rodriguez et al., 2012).

3.6.Pasting properties

The pasting properties of the native, AM, HTM proso millet starches presented in Table 6 and pasting profiles of native and modified starches are illustrated in Fig. 2. Both modifications showed significant changes ($P<0.05$) in pasting profile compared to native starch. HTM starch showed higher pasting temperature, 83.90 °C compared to native starch 79.23 °C, which may be due to additional heat requirements for degradation of starch granules and formation of paste caused by increased cross linking within starch granules (Singh, Bawa, Riar, & Saxena, 2009). Low swelling power of HTM starch restricts amylose leaching and does not let the viscosity to rise, resulting in low peak viscosity (2.29 Pa.s) (Hoover, 2010).

AM starches showed lower values for peak, breakdown, final viscosities and holding strength, which are 0.07, 0.03, 0.109 and 0.037 Pa.s respectively compared to native starch's 4.60, 2.60, 3.68 and 1.99 Pa.s respectively. AM disrupts the amorphous region and weakens the starch granule structure and limits the swelling during gelatinization as amorphous region is primarily associated with starch swelling. During pasting, starch is unable to achieve its maximum swelling power which results in reduced peak viscosity (Wang & Wang, 2001). The secondary rise during cooling in the pasting curve is known as setback, which is a measure of retrogradation was minimum for AM and no-significant ($P < 0.05$) change in HTM (Thirathumthavorn & Charoenrein, 2005).

4. Conclusion

Proso millet starch modification by hydrothermal and acid resulted in a significant change in the physicochemical, functional, pasting and thermal properties. AM reduced the amylose content and WBC of starch, and also improved the clarity of the paste, whereas HTM had no effect on amylose content but increased the WBC and reduced the paste clarity. Both modifications significantly changed the swelling power and solubility of the native starch. The decrease in swelling is a desirable property for some food preparation such as noodle production. Modifications influenced the thermal and pasting properties, with low breakdown implying that starches were more stable during continued shearing and heating after modifications. Low setback viscosity after modification could enable the starches to be used in canned and frozen foods. The increased knowledge on the effects of modified proso millet starch presented in this study will help broaden the applications of proso millet starch in food and non-food industries.

5. Acknowledgment

This is publication No. 16-05-088 of the Kentucky Agricultural Experiment Station and is published with the approval of Director. The work is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch-Multistate under 1007893.

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Figure 1. Effect of modifications on proso millet starch % light transmittance over 4-day period, -●- Native, -○- AM, -□- HTM.

Figure 2. Effect of modifications on proso millet starch pasting profile, -●- Native, -○- AM, -□- HTM, - temperature.

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Table 1. Proximate content of extracted proso millet starch

	Content (g/100 g)
Starch yield	54.1 ± 0.11
Moisture	9.86 ± 0.08
Protein	1.21 ± 0.07
Ash	0.62 ± 0.02
Lipid	0.27 ± 0.01
Carbohydrates *	88.04 ± 0.04
Amylose	28.51 ± 0.22

The values are means ± standard deviation of
three replicates. *Carbohydrates was calculated:
100 – (Moisture + Protein + Ash + Lipid)

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410 Table 2. Effect of modifications on native proso millet starch's WBC and amylose content.

Type	WBC (%)	Amylose Content (%)
N	138.43 ^c ± 1.93	28.51 ^a ± 0.22
AM	108.13 ^b ± 0.76	25.78 ^b ± 0.25
HTM	191.65 ^a ± 1.94	29.08 ^a ± 0.38

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly (P<0.05). N: Native starch; AM: Acid modified starch; HTM: Hydrothermal modified starch.

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Table 3. Effect of modifications on native proso millet starch's solubility and swelling power at 70, 80 and 90°C.

Type	70°C		80°C		90°C	
	Solubility (%)	Swelling (%)	Solubility (%)	Swelling (%)	Solubility (%)	Swelling (%)
N	2.62 ^b ± 0.17	4.69 ^b ± 0.26	6.59 ^b ± 1.07	11.65 ^b ± 1.12	34.88 ^b ± 0.77	24.99 ^a ± 0.22
AM	18.97 ^a ± 1.35	4.94 ^{a,b} ± 0.41	67.98 ^a ± 4.64	17.46 ^a ± 2.48	86.17 ^a ± 1.77	21.26 ^b ± 1.68
HTM	1.71 ^c ± 0.34	5.29 ^a ± 0.23	7.25 ^b ± 1.36	7.78 ^c ± 0.63	12.45 ^c ± 3.31	10.37 ^c ± 1.06

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly (P<0.05). N: Native starch; AM: Acid modified starch; HTM: Hydrothermal modified starch.

Table 4. Effect of modifications on native proso millet starch's degree of gelatinization.

Type	T _o Onset (°C)	T _p Peak (°C)	T _c Stop (°C)	ΔH _G (J/g)	Range (T _c -T _o) (°C)
N	72.93 ^b ± 0.62	78.61 ^b ± 0.83	94.55 ^b ± 1.34	3.83 ^a ± 0.28	21.62 ^b ± 1.54
AM	69.71 ^c ± 1.67	77.26 ^c ± 0.36	96.27 ^b ± 2.93	3.97 ^a ± 0.55	26.56 ^a ± 1.44
HTM	79.13 ^a ± 1.70	87.17 ^a ± 1.46	99.35 ^a ± 1.36	1.95 ^b ± 0.09	20.21 ^b ± 1.12

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly (p<0.05).
N: Native starch; AM: Acid modified starch; HTM: Hydrothermal modified starch.

Table 5. Effect of modifications on native proso millet starch's retrogradation.

Type	T _o Onset (°C)	T _p Peak (°C)	T _c Stop (°C)	ΔH _R (J/g)	Range(T _c -T _o) (°C)
N	41.38 ^a ± 0.88	55.05 ^{a,b} ± 0.74	74.73 ^b ± 1.49	2.66 ^b ± 0.33	33.35 ^b ± 1.26
AM	42.21 ^a ± 1.90	56.58 ^a ± 2.14	78.36 ^a ± 2.09	3.83 ^a ± 0.47	36.15 ^a ± 1.53
HTM	41.49 ^a ± 0.47	54.62 ^b ± 0.52	75.05 ^b ± 1.26	2.99 ^b ± 0.08	33.55 ^b ± 0.99

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly (p<0.05). N: Native starch; AM: Acid modified starch; HTM: Hydrothermal modified starch.

494 Table 6. Effect of modifications on native proso millet starch's pasting properties.

Type	Pasting temperature (°C)	Peak Viscosity (Pa.s)	Holding strength (Pa.s)	Final Viscosity (Pa.s)	Setback (Pa.s)	Breakdown (Pa.s)
N	79.23 ^b ± 0.38	4.60 ^a ± 0.15	1.99 ^a ± 0.48	3.68 ^a ± 0.37	1.69 ^a ± 0.48	2.60 ^a ± 0.64
AM	79.57 ^b ± 0.04	0.07 ^c ± 0.01	0.04 ^b ± 0.01	0.11 ^b ± 0.01	0.07 ^b ± 0.01	0.03 ^b ± 0.01
HTM	83.90 ^a ± 1.94	2.29 ^b ± 0.90	1.96 ^a ± 0.74	3.21 ^a ± 1.50	1.25 ^{a,b} ± 0.76	0.32 ^b ± 0.16

The values are means ± standard deviation of three replicates. Means with different letter in a column differ significantly (p<0.05). N: Native starch; AM: Acid modified starch; HTM: Hydrothermal modified starch.