

A study on the thermal stability of amylose-amylopectin and amylopectin-amylopectin in cross-linked starches through iodine binding capacity

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ABSTRACT

It has been proved that when normal maize starch granules were cross-linked, amylose did not cross-link to one another, but amylose-amylopectin (A-AP) and amylopectin-amylopectin (AP-AP) were carried out. The objective of this study was to study the thermal stability of the cross-linked starches (CLs), and correlate it to iodine binding capacity (IBC) of native starch and CLs with different water bath temperatures (20, 40, 60, 80, 100 °C, the starch slurry was prepared at a temperature of 20 °C). CLs was prepared through cross-linking of normal maize starch with 0.1, 1, and 10% sodium trimetaphosphate (STMP) and 0.1% phosphorous oxychloride (POCl₃). At different temperatures, no differences were observed for the IBC of native starch, however, the IBC of CLs decreased with the increase of the water bath temperature. At boiling temperature, the IBC of all the cross-linked starches was similar to the native maize, indicating that nearly all the amylose was leached out and had formed blue complexes with iodine. In addition, at 20 °C, the IBC of the cross-linked starch was negatively related to the cross-linking agent level added.

1. Introduction

Cross-linked starch (CLs) is one of the most important chemically modified starches being used diversely to meet technological needs due to its high efficiency and low cost (Bemiller & Whistler, 2009). Cross-linking reaction is of very high effective, as very little amount of cross-linking reagent can induce great change of pasting properties, an extensive research has been carried out on the properties of CLs using different cross-linking reagents at different concentrations for different purposes (Hirsch & Kokini, 2002; Marques et al., 2006; Woo & Seib, 1997).

Conventionally, native starches are cross-linked to improve the thermal resistance and thermal stability of the starch paste in order to improve its functionality for industrial applications, and thus, to expand its usefulness (H. Liu, Ramsden, & Corke, 1999b; J. Singh, Kaur, & McCarthy, 2007). The thermal stability of starch paste is usually measured by Rapid ViscoAnalyzer (RVA) (Kim et al., 2017; C.; Liu, Wang, Chang, & Wang, 2015), the viscosity differences between two characteristic points are defined as paste thermal stability. However, the thermal stability of the starch paste of the native starch and its cross-linked derivatives is not the properties we are going to discuss in this

paper. What we are going to discuss is the thermal stability of the chemical linkages constructed by the cross-linking reaction.

STMP and POCl₃ are the most widely applied cross-linking reagents in the preparation of cross-linked starches. Both cross-linking reagents, STMP and POCl₃, produce the di-starch phosphate in starch granules. These two kinds of reagents can introduce intra- and/or inter-molecular linkages randomly inside starch granules (Ayoub & Rizvi, 2009; Hong & Zhengbiao, 2010; Jobling, 2004; Wongsagonsup et al., 2014). The chemical stability of the cross-linking reaction is belonging to the molecular category. When granular starch is cross-linked, three possible mechanisms happened, i.e., amylose-amylose, amylose-amylopectin (A-AP), and amylopectin-amylopectin (AP-AP), but it has been proved that amylose do not cross-link to one another, only A-AP and AP-AP could be carried out during the cross-linking reaction (Boyer, Shannon, & Garwood, 1976; Imberty, Buléon, Tran, & Pérez, 2010; Kasemsuwan & Jane, 1994).

IBC has been adopted as the ISO method for the determination of the amylose content (BSI British Standards Rice. Determination of amylose content, n.d.), which is based on the intensity of the blue color developed by the amylose-iodine complexes, as the blue intensity could be used to indicate the concentration of amylose content. It has been

Abbreviations: A-AP, amylose-amylopectin; AP-AP, amylopectin-amylopectin; CLs, cross-linked starch; STMP, sodium trimetaphosphate; POCl₃, phosphorous oxychloride; CL%, cross-linking degree; IBC, iodine binding capacity; NM, normal maize

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proved that after the cross-linking of amylose to the amylopectin, less free amylose could be detected (Jane, Xu, Radosavljevic, & Seib, 1992; Sukhija, Singh, & Riar, 2016), because the reaction product A-AP can be considered as a new amylopectin. The capacity of A-AP to bind iodine is as weak as amylopectin, and the free amylose content decreases with the increase of the cross-linking degree (Kou & Gao, 2018). In this paper, IBC was directly adopted to characterize the amount of free amylose.

Although the mechanism of cross-linking reaction has been studied, there have been few comparative studies on the thermal stability of CLs at molecular level. From the molecular level, two chemical linkages were constructed during the cross-linking reaction. A survey of literature also revealed that there is a dearth of information on the thermal stability of cross-linking reaction. One purpose of this study was to investigate the thermal behavior of native and CLs under water bath of different temperatures. The other purpose was to evaluate the different thermal properties of cross-linking reaction with two kinds of cross-linking reagents: (i) STMP and (ii) POCl₃. To achieve the above two purposes, we devised an experiment of bathing the native starch and CLs of different cross-linking agents and different degrees at a series of temperatures (20, 40, 60, 80, 100° C) for 30 min. Afterwards, the IBC of the native starch and cross-linked starches of different bath temperatures was tested.

2. Materials and methods

2.1. Materials

Normal maize (NM) starch was provided by Qinquangdao Lihua Starch Ltd. (Hebei, China), POCl₃ (99%) was purchased from the Aldrich Chemical Co. (St. Louis, MO), and STMP (powder) from Monsanto (St. Louis, MO). Other chemicals were reagent-grade.

2.2. Preparation of the cross-linked starches

CLs of POCl₃ was prepared according to the method reported by Bates, French, and Rundle (1943). Starches were cross-linked at 25° C and pH 11.0 by slurrying the starch (20%, db) with POCl₃ (by micro injector) for 1 h at 0.1% agent level with sodium sulfate at 3% (starch basis). After 1 h, the slurry was adjusted to pH 6.5 with 1 M hydrochloric acid, and the starch was recovered by centrifuging (3,000g, 10min); washing with water (3 × 100 mL) and 95% ethanol (1 × 100 mL); then drying at 50° C overnight.

CLs of STMP were prepared using the another previous reported method (Shukri & Shi, 2015) with some modification. Starches were cross-linked at 45° C and pH 11.5 by slurrying the starch (20%, db) with STMP for 3 h at three reagent levels (0.1, 1, 10%) with sodium sulfate at 10% (starch basis). After 3 h, the slurry was adjusted to pH 6.5 with 1 M hydrochloric acid, and the starch was recovered by centrifuging (3,000g, 10min); washing with water (3 × 100 mL) and 95% ethanol (1 × 100 mL); then drying at 50° C overnight.

2.3. Degree of cross-linking

The CL% of cross-linked starches was calculated as described by Kou and Gao (2018). The CL% based on colorimetric procedures was calculated as defined:

$$\text{CL\%} = (A - \alpha) / A \times 100\% \quad (1)$$

Where CL% is the degree of cross-linking, A is the absorbance of the native starch, α is the absorbance of the CLs.

2.4. Pasting properties

The pasting profiles were analyzed using a Micro Visco-Amylo-

Table 1

Crosslinking degree of native starch and CLs of 0.1% POCl₃, 0.1, 1, and 10% STMP.

Samples	Native starch	0.1% POCl ₃	0.1% STMP	1% STMP	10% STMP
CL%	0 ± 0	50.2 ± 4.0	24.8 ± 0.6	42.8 ± 0.8	82.9 ± 2.6

Graph (Brabender, Germany). Starch (both native and CLs, 6.00 g, db) was weighed accurately into canisters and distilled water. Distilled water was added to suspension containing 94% water. With a 700cmg torque, the starch slurry was heated from 30 to 95° C at a rate of 7.5° C/min and maintained at 95° C for 5min, then cooled to 50° C at the same rate. From the pasting profiles, peak viscosity, final viscosity at 95° C, and final viscosity were calculated using the Viscograph software provided with the instrument.

2.5. Iodine binding capacity (IBC)

The process to determine IBC of native starch and CLs at different bath temperatures was similar to the ISO method (Rice—determination of amylose content) (BSI British Standards Rice. Determination of amylose content, n.d.), which mainly consists of four parts: chemical gelatinization (to gelatinize the starch and eliminate the influence of heat treatment), color reaction, absorbance measurement and evaluation of IBC. The native starch and its CLs were gelatinized by sodium hydroxide (1 M) as the reagent to gelatinize the native starch and CLs. Starches (100 ± 0.5 mg, db) were weighed into a 15 mL centrifuge tube, then 1 mL ethanol added for sufficient dispersion of starch, it was preferred to apply a vortex mixer while adding the NaOH (9 mL) to ensure a homogeneous solution. The above solution was kept at room temperature, after that, the solution was transferred into 100 mL volumetric flask and distilled water was added to make it 100 mL (making a starch suspension 1 mg/mL), and heated at different bath temperatures (20, 40, 60, 80, 100° C, the starch slurry was prepared at a temperature of 20° C). After the heating process, 5 mL of the above chemical gelatinized starch solution was transferred into another 100 mL volumetric flask (with 50 mL distilled water inside), 1 mL acetic acid solution (1 M) was added to neutralization the solution, then 2 mL I₂-KI (configured according to the description of ISO approach) added and made 100 mL for the color reaction. 30 min later, the absorbance of the native starch and the CLs were measured. The blank solution was also configured according to ISO.

2.6. Solubility

Solubility of starches were determined by the method reported by Balasubramanian, Sharma, Kaur, and Bhardwaj (2014) with some modifications. Starch samples (0.3 g db) were taken in pre-weighed centrifuge tubes and heated with water (20 mL) at 20, 40, 60, 80 and 100° C for 30 min with continuous stirring to prevent lump formation. After cooling, the dispersion was centrifuged at 1008g for 15 min. Then supernatant was transferred in the pre-weighed petri-dishes and dried for 2 h at 130° C and weighed. The solubility was determined as:

$$\text{Solubility (\%)} = (\text{Weight of soluble starch} / \text{Weight of sample}) \times 100$$

2.7. Differential scanning calorimeter (DSC)

The thermal characteristics of the native and cross-linked starches were measured using a Perkin-Elmer DSC (Norwalk, CT, USA) equipped with a refrigerated cooling system and Pyris™ operation software (Perkin-Elmer). Starches were weighed into a stainless-steel pans designed to withstand high pressure, waters were added from a micro-syringe, the pans were hermetically sealed and stored overnight for

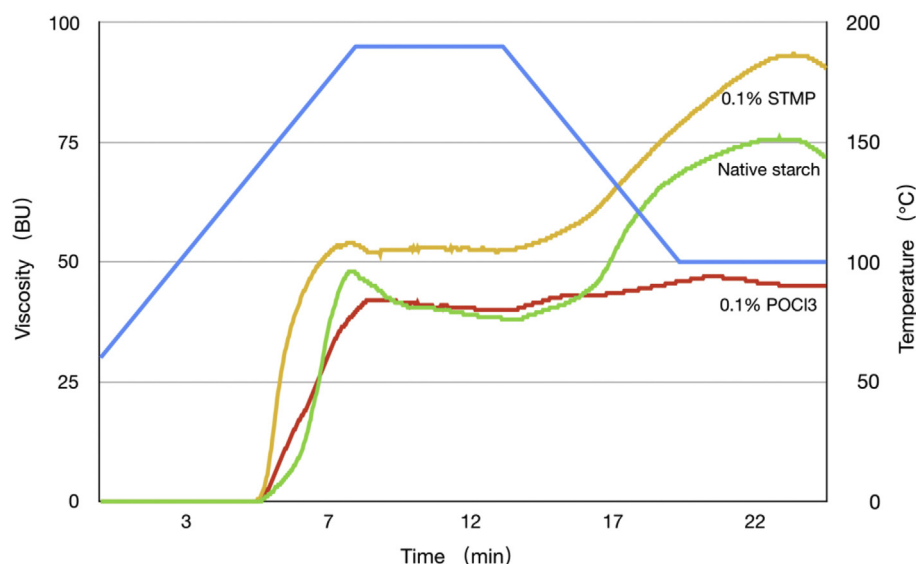


Fig. 1. Pasting properties of native starch and CLs of 0.1% POCl_3 and 0.1% STMP.

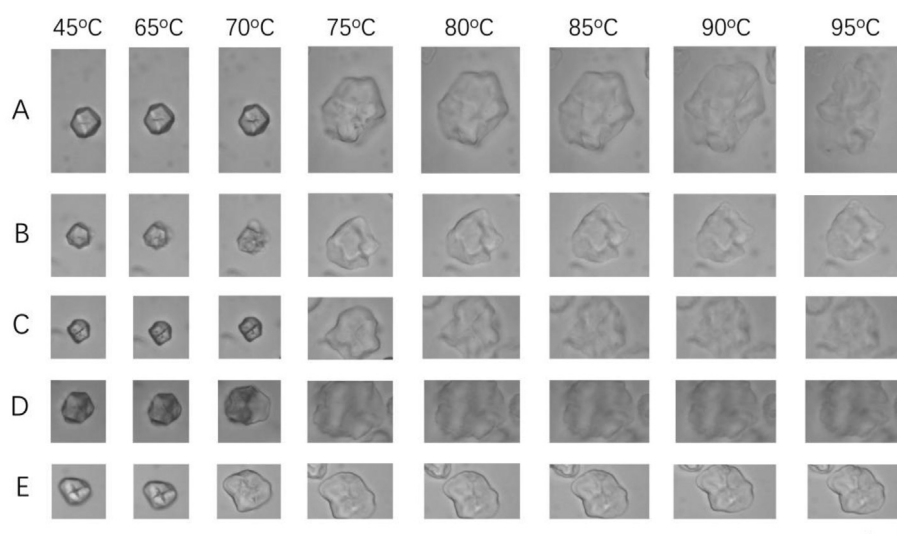


Fig. 2. The gelatinization process of starch granules. (A) Individual starch granule from native starch; (B) Individual starch granule from 0.1% POCl_3 cross-linked normal maize; (C), (D) and (E) Individual starch granules from 0.1, 1 and 10% STMP cross-linked normal maize. The columns showed the micro-morphologies of granules of native starch and CLs under normal light at different temperatures. Scale bar = 30 μm .

moisture equilibration. Samples were equilibrated at 20° C, and then heated to 120° C at a scanning rate of 10° C/min. An empty pan was used as inert reference.

2.8. Hot stage light-microscopy

Starch suspensions (1%, w/v) were prepared by suspending about 10 mg starch in 1.0 mL of distilled water. The dispersions were sealed between two glass coverslips using Dow Corning 732 sealant to prevent moisture loss during heating, and the sealed specimen was then mounted on the hot stage (model THMS600, Linkam, Surrey, U.K.) apparatus and observed under a polarization microscope (model BHS-2, Olympus Vanox, Japan) equipped with a digital camera, which can display live video of birefringence granules in real time. The hot stage was heated from 45° C to 95° C at a heating rate of 10° C/min. Each image (2048 × 1536 pixels) was saved as 12-bit TIFF image file, without compression (Li, Xie, Yu, & Gao, 2013).

2.9. Statistical analysis

Statistical analysis was performed using ORIGIN 9.0 (OriginLab Inc., Northampton, MA, USA). The data are expressed as means \pm standard

deviations of at least three independent determinations on one sample, and were analyzed by one-way analysis of variance (Lix, educational, 1996, 2016).

3. Results and discussion

3.1. Pasting properties of NM and lower crosslinked starch

The CL% of the CLs was presented in Table 1, at equal reagent level (0.1%), POCl_3 has the ability to impart higher CL% than STMP, because the reactivity of POCl_3 was much higher than that of STMP, many reports have confirmed it (Carmona-Garcia, Sanchez-Rivera, Méndez-Montealvo, Garza-Montoya, & Bello-Pérez, 2009; KNIGHT & OLSON, 1984). With the rise of the cross-linking reagent added, CL% increased (Kou & Gao, 2018). Although the two cross-linking reagent, POCl_3 and STMP, both produced the di-starch phosphate in the starch granules, the phosphate groups introduced by STMP mainly happened in the inner of the granule, while the POCl_3 is mainly in the surface of the granule (Carmona-Garcia et al., 2009). Irrespective of the reactive sites, the reactivity of the POCl_3 is at least 10 times higher than that of STMP, as the CL% of the CLs treated with 0.1% POCl_3 was higher than 1% STMP.

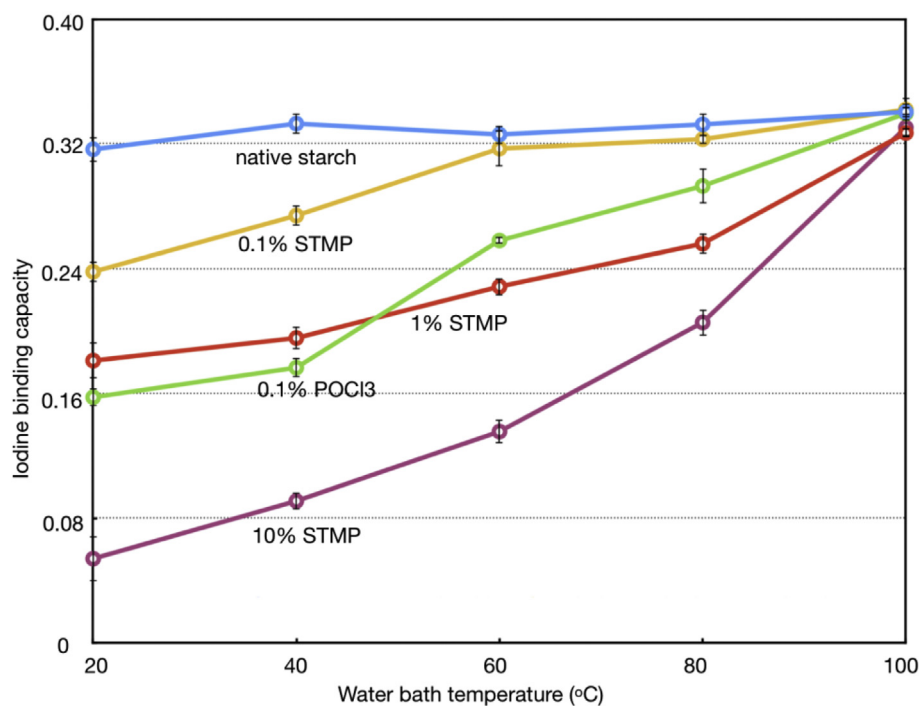


Fig. 3. Iodine binding capacity of native starch and CLs of 0.1% POCl₃, 0.1, 1, and 10% STMP.

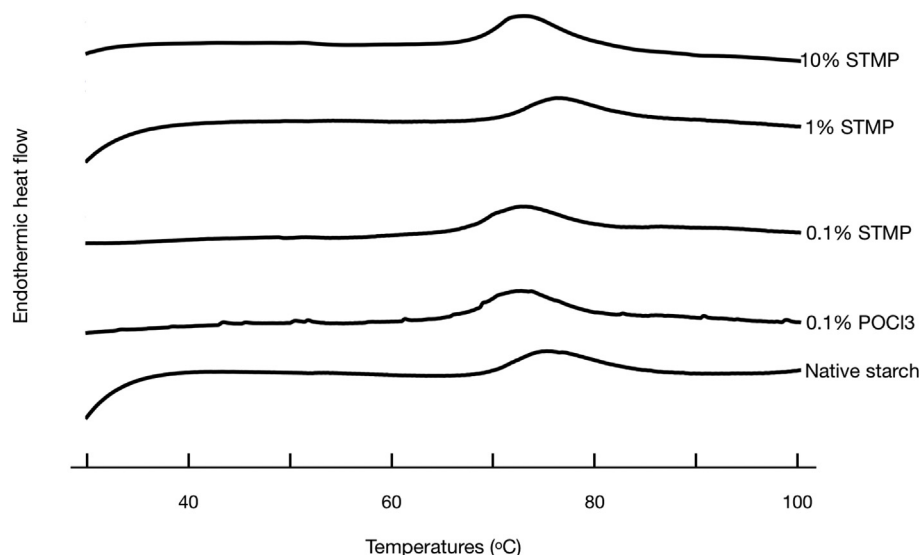


Fig. 4. DSC scanning thermogram of native starch and cross-linked starches.

Starch pasting properties were shown in Fig. 1. Remarkable differences in starch pasting properties were observed among NM, 0.1% POCl₃, and 0.1% STMP crosslinked starches. However, with higher cross-linking reagent levels added, the viscosity of 1% and 10% STMP cross-linked starch were not detectable and were not presented. The 0.1% STMP cross-linked starch showed significantly great peak viscosities (108 BU) than that of the 0.1% POCl₃ crosslinked starch and NM. The peak viscosities and final viscosities of the three starches followed the same order: 0.1% STMP > NM > 0.1% POCl₃. The NM was neither the lowest nor highest, this is because the cross linking of starches has two opposite effects on the paste of starch by two main mechanisms (Steeneken, 1989), on the one hand, crosslinking reduces the loss of solubles (amylose and small amylopectin) to improve paste viscosity, on the other hand, it can inhibit the swelling power of the granules, which can reduce the viscosity of the starch paste. At the same reagent

level, the former plays the dominant role in the 0.1% STMP CLs, the structure of the mass for building the paste is improved and the paste is stronger, while for the 0.1% POCl₃, the late mechanism is predominant and the past viscosity decreased, whereas the 1% and 10% STMP, the reagent levels were too high for the past to show viscosities detected by the appliance.

3.2. Gelatinization properties of NM and cross-linked starch granules

The gelatinization behavior of individual starch granules can be in situ viewed by observing the changes of granular morphology using light microscope with a hot stage (Cai, Zhao, Huang, Chen, & Wei, 2014; Li et al., 2013). During the process, the disruption and swelling of granules can be investigated simultaneously. The gelatinization processes of NM and CLs granules were presented in Fig. 2. Starch granules

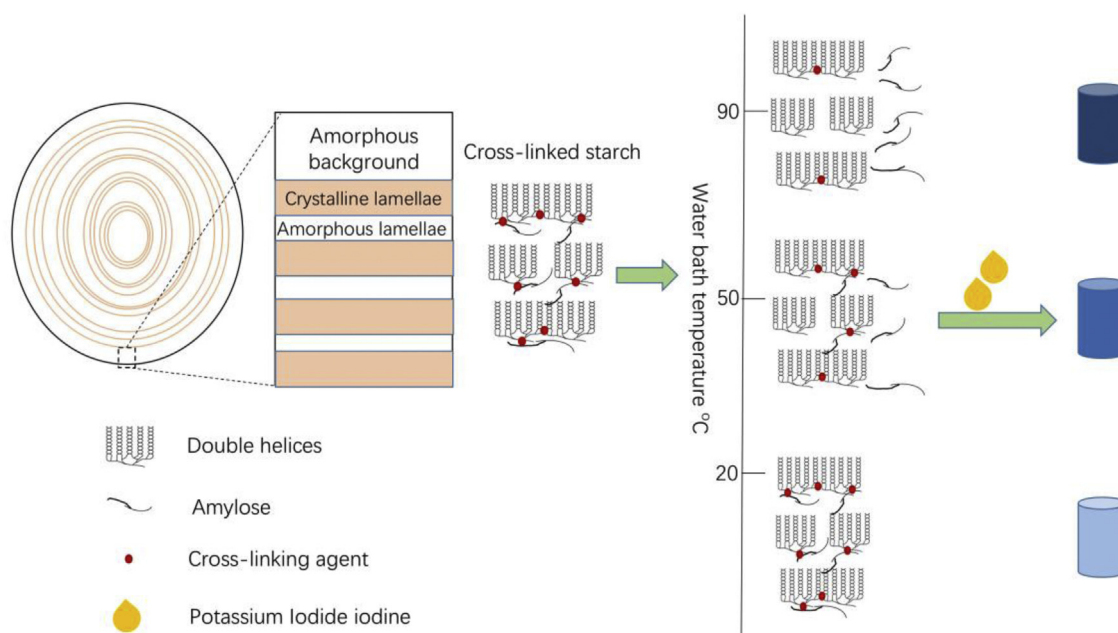


Fig. 5. Mechanism of IBC procedures for the determination of thermal stability of AP-AP and A-AP.

Table 2

Solubility of native starch and CLs of 0.1% POCl₃, 0.1, 1, and 10% STMP at different temperatures.

Water bath temperature/°C	Solubility (%)				
	Native starch	0.1% POCl ₃	0.1% STMP	1% STMP	10% STMP
20	nd ^a	nd	nd	nd	nd
40	1.55 ± 0.14	nd	nd	nd	nd
60	1.48 ± 0.04	nd	nd	nd	nd
80	6.9 ± 0.71	7.82 ± 0.32	10 ± 0.92	7.92 ± 0.81	2.8 ± 0.07
100	73.4 ± 4.74	13.45 ± 1.7	50.13 ± 2.58	12.85 ± 0.85	4.2 ± 0.14

^a nd, not detected.

showed their intact shapes before gelatinization as observed at 45° C and 65° C. At temperature 70° C and higher, the granules of NM and CLs all swelled and lost their intact granular shape, at 95° C, the NM lost the granular outlines while all the CLs still kept a deformed outline. This observation was assumed to be relevant to their gelatinization properties (since the swelling of the CLs are inhibited as the increase of CL%, and the outline of the 0.1% POCl₃ granule was clearer than the 0.1% STMP), and also suggesting the higher reactivity of the POCl₃.

3.3. Iodine binding capacity of NM and CLs under different bath temperatures

It has been proved that, after cross-linking, the amylose was cross-linked to the amylopectin, not just trapped by the un-gelatinized granules (Jane et al., 1992). The starch granules can be gelatinized by strong base at a concentration of sodium hydroxide of 1.2% (Ragheb, Abdel Thalouth, & Tawfik, 1995), in the ISO method for the determination of amylose content, 4% sodium hydroxide is applied to gelatinize the starch granules (BSI British Standards Rice. Determination of amylose content, n.d.), otherwise, the sodium hydroxide we used in this paper is 4%, which is sufficient to gelatinize all the starch granules, and a clear gelatinized pastes could be obtained at this concentration. Analysis of the IBC profiles (Fig. 3) showed that the all the CLs have the same IBC after a boiling water bath of 30 min, while at other bath temperatures, 0.1% STMP has the highest IBC and 10% STMP the lowest. At temperature 100° C, all the starches showed similar IBC to the NM, indicating that the thermal instability of A-AP in CLs of the two reagents was the same if given a boiling water bath. However, there was

an intersection between the IBC curves of 0.1% POCl₃ and 1% STMP, this observation is assumed to be the indication of the different cross-linking characteristics of the two cross-linking reagents. The 0.1% POCl₃ has a lower IBC at lower bath temperatures (20 and 40° C), while the 1% STMP has a higher IBC at higher bath temperature (60, 80° C), this suggests that starch cross-linked by the POCl₃ is more thermally stable at lower temperatures. The above observations (Fig. 3) was also assumed to be consistent with the different reactivity of the two reagents, as mentioned in literature (Bates et al., 1943; Carmona-Garcia et al., 2009; Woo & Seib, 1997), as the reaction duration of POCl₃ was usually shorter, the cross-linking of the POCl₃ is more happened on the outer layer of the starch granules, that is to say, relatively more A-AP are gathered in the outer layer of the starch granules, when the bath temperatures were high enough, the CLs cross-linked by POCl₃ was relatively less stable.

3.4. DSC analyses

Thermal properties of the native starch and CLs are shown in Fig. 4. On the one hand, it can be noticed that only one endothermic peak can be observed with 70% moisture content, the 0.1% STMP and 1% STMP both display a slightly higher gelatinization onset temperature, which is in accordance with previous studies (H. Liu, Corke, & Ramsden, 1999a; Woo & Seib, 2002), while the onset gelatinization temperatures of 10% STMP is in close with the native starch. On the other hand, the thermal differences of the A-AP and AP-AP cannot be detected. Although DSC is the most widely applied device to study the thermal properties of the starch and its derivations, it cannot be applied here to differentiate the

different thermal stability of the A-AP and AP-AP.

3.5. Thermal stability of A-AP and AP-AP

The mechanism of IBC procedures for the determination of thermal stability of AP-AP and A-AP has been demonstrated in Fig. 5. To begin with, the free amylose, which did not cross-link to the amylopectin, came out of the starch granules by the gelatinization effect of strong base without hot water bath (Jane et al., 1992; Kou & Gao, 2018). With the increase of the water bath temperature, more A-AP linkages were destroyed by the thermal treatment and more amylose came out of the starch granules, formed blue complexes with potassium iodide iodine, the blue intensity increased with the increase of the free amylose molecules, which makes the destruction of the A-AP linkages (thermal stability) detectable. Otherwise, this method cannot give statistical results of the thermal stability of the AP-AP linkages, to solve this problem, the solubility of NM and CLs at different water bath temperature was studied (Table 2). Below the gelatinization temperature of the NM starch, the solubility of the NM and all the CLs are not detectable, however, at above the gelatinization temperature, results of the solubility of CLs are in consistent with the CL% (Sukhija et al., 2016) and the IBC. Because, as vividly presented in Fig. 2, the outlines of highly CLs were still very clear at high temperatures, and its solubility was also much lower than that of the NM. It implied that the thermal stability of AP-AP was higher than that of A-AP.

4. Conclusion

The thermal stability of the two new linkages (A-AP and AP-AP) constructed by the cross-linking reaction of two different cross-linking reagents was analyzed through measuring IBC of the native and CLs. For the starches cross-linked by STMP, the IBC continuously increased with the increase of the bath temperature, until reaching a maximum at approximately 0.34 as the IBC of the native starch. In the case of 0.1% POCl₃, it also follows the similar trend as that of the starches cross-linked by STMP, however, the POCl₃ was found to be more inclined to be take place in the outer layer of the starch granules, because more amylose came out at a relative lower bath temperature.

For the CLs, with the increase of the cross-linking degree, less free amylose is left to form complexes with iodine, and can therefore have a reducing effect on the IBC. However, the A-AP was thermally unstable, with the increase of the water bath temperature, there is little evidence for differences in IBC between native and CLs. The results of the solubility study and the hot stage images shows that the CLs have a lower solubility and a more rigid structure than the native starches and also suggests that the AP-AP is thermally stable.

Based results of the thermal stability differentiation of A-AP and AP-AP, this paper firstly discussed the thermal stability of A-AP and AP-AP constructed by cross-linking reaction, respectively. Compared with DSC, the IBC method we applied in this paper to differentiate the thermal properties of A-AP and AP-AP gave some new insights into the thermal analysis of starch and its derivations. With increasing water bath temperature, more amylose molecules in the A-AP was destroyed and leaked out and make a higher IBC. The A-AP was thermally unstable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodhyd.2018.09.028>.

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