



## Preparation and characterisation of crosslinked waxy potato starch

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### ABSTRACT

Using waxy potato starch as the raw material, the preparation, and determination of physical and chemical properties of acetylated distarch adipate (ADA) were studied systematically. The experimental variables investigated include temperature, pH, and reaction time, which all have influence on the degree of substitution (DS) of crosslinked waxy potato starch. Results show that the optimal reaction was obtained at a pH range of 9.0–9.5, a temperature of 35 °C, and a reaction time of 2 h. The structures of ADA starch were characterised by FT-IR, and the results indicate a new absorption peak at 1732 cm<sup>-1</sup> which is assigned to the C=O stretching vibration. It was found through polarised light microscopy and scanning electron microscopy (SEM) that the starch granule structure did not substantially change after crosslinking. The modified waxy potato starch paste exhibited excellent viscosity stability, acid resistant, salt tolerance properties and good breakdown. The swelling of ADA starch granules was gradual over a wide range of temperatures.

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### 1. Introduction

Waxy potato starch has been developed from a potato mutant where amylose biosynthesis was inhibited resulting in tubers containing about 99% amylopectin (Visser, Suurs, Bruinenberg, Bleeker, & Jacobsen, 1997a).

Waxy potato starch combines the properties of normal potato starch with the advantage of lower viscosity and enhanced stability in pastes. Moreover, it shows a lower tendency to retrograde resulting in more stable and less turbid systems with lower viscosity (Kortstee et al., 1998; Nuessli, Handschin, Conde-Petit, & Zurich, 2000; Visser, Suurs, Bruinenberg, Bleeker, & Jacobsen, 1997b). However, it has some disadvantages which make it unsuitable for food systems and processing such as: narrow peak viscosity, undesirable texture, poor stability, and poor process tolerance (Light, 1990).

Cross-linking reagents may be any chemical containing two or more functional groups capable of reacting with at least two of the hydroxyl groups on the starch molecule, and thus causing a linkage of those groups. The most widely used cross-linking reagents for modifying food starches are mixes of adipic/acetic anhydride, and phosphorus oxychloride or sodium trimetaphosphate, which yield distarch adipates, distarch phosphates, respectively (Mali & Grossmann, 2001; Wurzburg, 1986). Adipic acid reacts with starch and produces both cross-linked starches and mono-derivatives (Wurzburg, 1960). Some advantages of cross-linked

starches over native starches are: increased stability towards heat, pH, shear, and freeze-thaw stability (Ellis, Cochrane, & Daley, 1998). When acetyl groups are introduced on starches, the tendency of sols of these starches to deteriorate in clarity, texture, and to syneresis when held at low temperatures, is greatly reduced. These constituent groups are stabilized starches by interrupting the linearity of amylose chains and segmenting of amylopectin branches. Cross-linked starches have been applied in soups, gravies, sauces, baby foods, fruit filling, pudding, and deep fried foods (Rutemberg & Solarek, 1984).

The present paper studies the chemical modification of waxy potato starch and its characterisation. The objectives of this study were to develop a greater understanding of how critical reaction parameters (temperature, pH and reaction time) in aqueous slurry affect the degree of substitution of acetylated distarch adipates (ADA) of waxy potato starch, and to study the structure of ADA starch and their physicochemical properties.

### 2. Materials and methods

#### 2.1. Materials

Waxy potato starch was obtained from AVEBE Company (Veenendam, Netherlands). Adipic acetic mixed anhydride was prepared by mixing adipic acid and acetic anhydride (mass ratio 1:30) (Wurzburg, 1960), which were purchased from Guang Zhou chemical reagent company (Guang Zhou, China). The other chemicals used in the study were all of analytical grade.

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## 2.2. Preparation of acetylated adipate crosslinked waxy potato starch

The general methodology for reactions of mixing adipic acid and acetic anhydride with waxy potato starch is given below. Starch slurry was prepared by adding 500 g waxy potato starch (dry basis, db) to 930 g deionized water at room temperature in a 2 L reaction vessel equipped with a mantle. The pH was maintained between 9.0 and 9.5 using a 3% (w/w) aqueous NaOH solution. The temperature was increased to 35 °C with a temperature controller and then 20 g mixed anhydride was added drop-wise to the slurry. The slurry was maintained at 35 °C for 2 h and then the pH was adjusted to 6.5 using diluted HCl (0.1 N). The resulting starch suspension was vacuum-filtered through filter paper (Whatman 110 mm, Whatman international Ltd. Maidstone, England) and washed twice with distilled water (used 1.25 L at a time) and once with alcohol. Afterwards the recovered starch was dried in a force-air oven (Model: CS101, Chong Qing experimental equipment factory, Chong Qing, China) at 40 °C for 24 h. The dried starch was ground gently in a mortar to pass through a standard 100-mesh sieve. The degree of substitution (DS) of ADA starch was determined according to the methods of Sanders and Brunt (1994). The preparation of ADA starch was duplicate following the same procedure.

## 2.3. Polarised light microscopy

Polarised light microscopy was performed using an OLYMPUS VANOX BHS-2 (Tokyo, Japan). ADA starch was suspended in distilled water (1% w/v). The starch slurry was then dropped on a glass slide; spread around then cover slip was placed. The sample was observed with a magnification of 400 $\times$ .

## 2.4. Scanning electron microscopy (SEM)

The appearance of native waxy potato starch and ADA starch granules were observed using a SEM according to the method of Hung and Morita (2005). Starch was suspended in 95% ethanol for a few minutes and sprinkled on double-sided adhesive tape mounted on an aluminium stub, and then coated with a thin film of Pt-Pd. Scanning was performed using a S-550 Scanning Electronic Microscope (Hitachi Ltd. Tokyo, Japan). The sample was observed with a magnification of 1000 $\times$ .

## 2.5. Fourier-transform infrared (FT-IR) spectroscopy

The IR spectra were obtained from samples in KBr pellets using a Vector 33 FT-IR spectrophotometer (Bruker Company, Ettlingen, Germany).

## 2.6. Swelling power of native waxy potato and ADA starches

Swelling power was determined using the method of Leach, McCowen, and Schoch (1959). Starch samples (0.25 g, db) were accurately weighed and transferred into clear dried test tubes and weighed with the test tubes ( $W_1$ ). Distilled water (50 mL) was added to the test tube and mixed thoroughly with a Variwhirl mixer (Model: A901, Salver Chem. Chicago, IL, USA) for 30 s. Because of the different swelling properties of native waxy potato starch and ADA starch, the resultant slurries were then heated in water bath at selected temperature; from 62 °C to 74 °C at two degree intervals for waxy potato starch and from 64 °C to 92 °C at four degree intervals for ADA starch. The suspension was then cooled rapidly to room temperature and centrifuged (5000g, 15 min) and sediment recovered. The weight of the residue (after decanting the supernatant) was obtained ( $W_2$ ).

Swelling of starch was then calculated to be  $W_2 - W_1$ /Weight of starch (dry matter basis).

## 2.7. Rheological measurements

Steady shear properties were studied at pH values of 3.0, 4.0, 5.0, 6.0 and 7.0 using a rheometer (Brookfield DV-I, Brookfield Engineering Laboratories, INC, Massachusetts, USA).

## 2.8. Pasting properties of starches

Native waxy potato and ADA starches were analysed using a Brabender viscograph "E" (C.W. Brabender instruments INC, Duisburg, Germany). Dry sample (13.8 g) was suspended in 446.2 mL distilled water or 1% (w/w) NaCl solution. Brabender cartridge (700 cm/g) was fitted with the rotation speed set to 75 rpm. The starch suspension was heated at 1.5 °C/min to 95 °C from 30 °C, then held for 30 min at 95 °C, cooled to 50 °C at a rate of 1.5 °C/min, and then held for 30 min at 50 °C.

## 2.9. Statistical analysis

Analyses of mean separation were performed performed by Tukey's HSD test using SigmaStat Version 2.0 (Jandel Scientific/SPSS Science, Chicago, IL, USA).

# 3. Results and discussion

## 3.1. Effect of pH of aqueous on the reaction

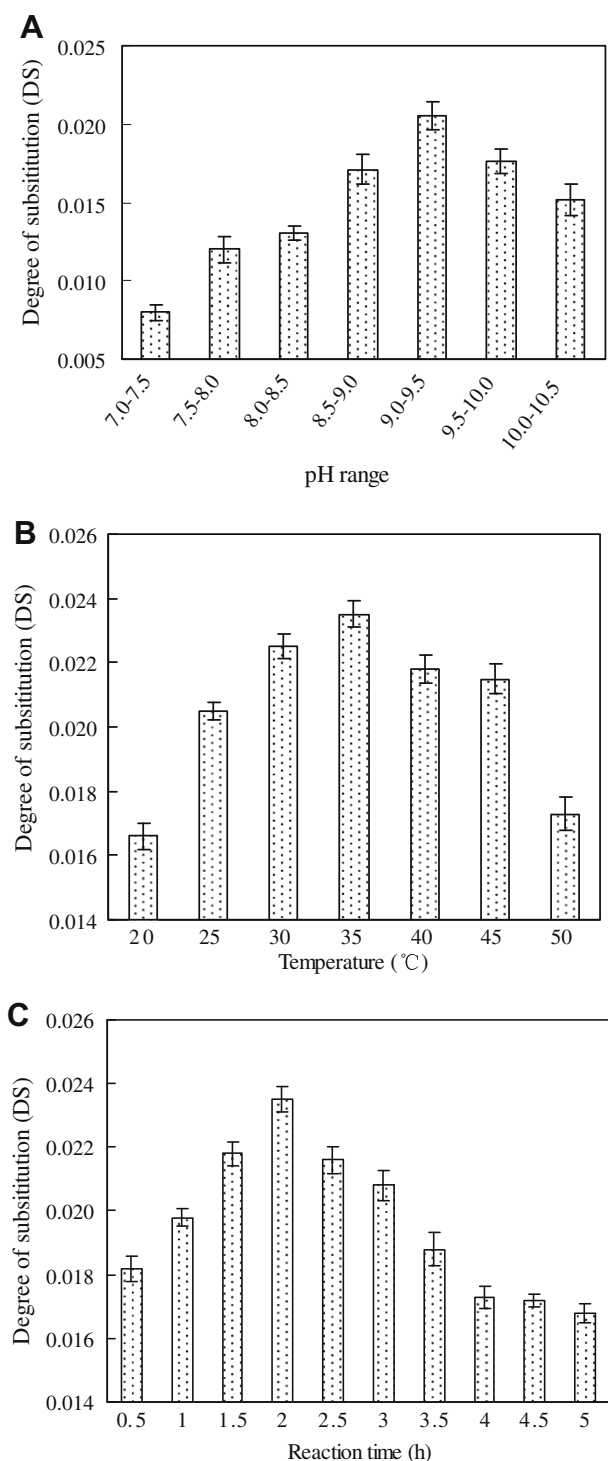
The effect of pH on the reaction was studied at the pH range of 7.0 to 10.5 whilst the other reaction parameters were kept constant. Inspection of Fig. 1 A shows that the highest degree of substitution was reached at the pH range of 9.0–9.5. However, pH above or below of this range had substantially detrimental effects on the reaction. These results may be explained by the fact that pH values >9.5 favour anhydride hydrolysis whereas pH values <9.0 do not satisfactorily activate the hydroxyl groups of starch molecules for the nucleophilic attack of the anhydride moieties. Hence, the pH range of 9.0–9.5 was used in subsequent experiments.

## 3.2. Effect of reaction temperature

Fig. 1B gives the results of variation in reaction temperature whilst holding the starch concentration at 35%, the anhydride at 4.0% (w/w) relative to dry starch, pH at 9.0–9.5 and reaction time of 2 h. Fig. 1B shows both positive and negative effects of temperature on the reaction efficiency. An increase in the reaction temperature from 20 °C to 35 °C resulted in a large increase in the degree of substitution. Further increase in the reaction temperature from 35 °C to 50 °C resulted in a decrease in the degree of substitution. A complex set of variables is expected to come into play when the reaction temperature is changed. For example, an increase in temperature will result in increased hydrolysis of anhydride. However, a higher temperature would be expected to also enhance the swelling of native starch granules which will increase the esterification. These results indicate that a better understanding how temperature affects hydrolysis of anhydride and starch granule swelling would help in explaining the above results.

## 3.3. Effect of reaction time

Variations in the reaction time and its effect on reaction efficiency were studied (Fig. 1C). Increasing the reaction time from 0.5 to 2 h resulted in higher reaction efficiency. However, prolonging the reaction time beyond 2 h resulted in decreased reaction efficiency. This could be due to that as the reaction progress, the



**Fig. 1.** Effects of different factors on the degree of substitution. A. Effects of varying reaction pH, starch concentration 35% (w/w), 25 °C, 2 h reaction time. B. Effects of varying reaction temperature, pH range 9.0–9.5, starch concentration 35% (w/w), reaction time 2 h. C. Effect of varying reaction time, starch concentration 35% (w/w), 35 °C, pH range 9.0–9.5.

anhydride is depleted due to esterification and hydrolysis reactions. As a result the reverse reaction of ester chain hydrolysis becomes dominant.

It has been reported that the esterification of starch with a hydrophobic reagent in aqueous slurry system could be reached to optimal reactive efficiency within 24 h reaction time (Jeon,

Viswanathan, & Gross, 1999), which is much longer than the reaction time of starch ester with water soluble mixed anhydride used in this study. For other reaction conditions are similar of both esterification, it indicates that the soluble reagent can react with starch in aqueous slurry more rapidly.

### 3.4. Granule morphology

Polarizing microscope is one of the most effective microscopic observations of native and modified starch granules (French, 1984). The polarised light micrograph of native waxy potato and ADA starches granules are shown in Fig. 2A (a,b). The native waxy potato starch granules (Fig. 2Aa) appear as distorted spherocystals, with a typical dark cross. The sign of birefringence is positive with respect to the spherocrystal radius, and the apparent intensity of birefringence is dependent on the thickness of the granule as well as on the degree of crystallinity and orientation of the crystallites (French, 1984). After crosslinking, the ADA starch granules (Fig. 2Ab) show no significant change in the birefringence, which means the acetylated adipate has little effect on the crystalline structure of native waxy potato starch.

The SEM image of native waxy potato and ADA starches granules are shown in Fig. 2B (a,b). Native waxy potato starches showed large, rounded or oval granular shapes with axial diameters of 12–72 and 14–44  $\mu\text{m}$ , respectively. In addition, the native waxy potato starch granules show some fissures on the surface. The results were consistent with those reported by McPherson and Jane (1999). Compared with Fig. 2Ba and Bb, it is readily visible that there is no significant change in the microstructure of the crosslinked starch, which indicates that the crosslinking by acetylated adipate had no negative effects on the morphology of waxy potato starch.

### 3.5. FTIR spectroscopy

The FTIR spectra of native waxy potato and ADA starches with different degree of substitution are shown in Fig. 3.

In curves Fig. 3(a), (b) and (c), an absorption peak at  $3357\text{ cm}^{-1}$  and  $2928\text{ cm}^{-1}$  should be resulting from  $-\text{OH}$  and  $-\text{CH}$  stretching vibration of the glucose unit. The absorptions at about  $1646\text{ cm}^{-1}$  and  $1300\text{ cm}^{-1}$  may be attributed to  $\text{H}_2\text{O}$  bending vibration (Zhang et al., 2007). Compared with the native waxy potato starch (a), a new absorption band at  $1732\text{ cm}^{-1}$  can be seen in curves (b, DS = 0.0173) and (c, DS = 0.0235), which is assigned to  $\text{C}=\text{O}$  stretching vibration, and enhanced absorption band at  $1732\text{ cm}^{-1}$  can be seen with an increasing degree of substitution in curve (c). The results indicate that native waxy potato starch was successfully reacted with mixing adipic acid and acetic anhydride.

### 3.6. Swelling power

Swelling power of the native waxy potato and ADA starches are shown in Fig. 4. The swelling of native waxy potato starch shows very steep swelling profile with a narrow temperature range ( $64\text{ }^\circ\text{C}$ – $74\text{ }^\circ\text{C}$ ). However, the swelling of ADA starch granules were more gradual over a wider range of temperatures ( $67\text{ }^\circ\text{C}$ – $90\text{ }^\circ\text{C}$ ), but the swelling power is significantly lower than that of their native counter parts.

In general, modified starches such as hydroxypropylated starches exhibited increased swelling power which is attributed to reduced interactions between glucan chains due to increased hydrophilicity of the starch from the introduction of hydroxypropyl groups. In addition inter- and intra-molecular hydrogen bonds in the glucan chains are disrupted. Thereby the granular structure of the starch is weakened and perhaps the free motion of the

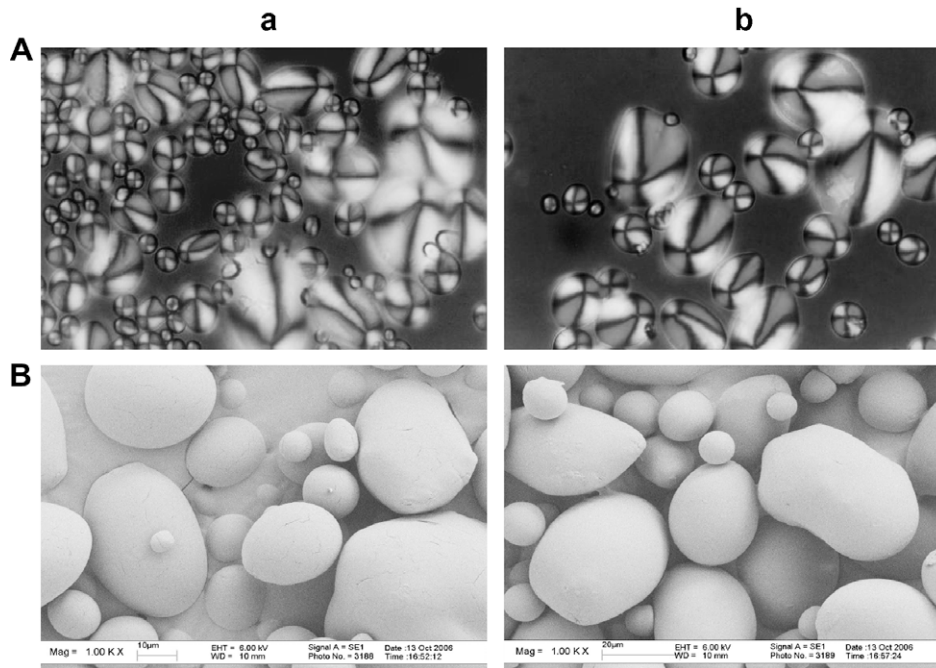


Fig. 2. Polarised light microscopy (A, 400 $\times$ ) and scanning electron microscopy (B, 1000 $\times$ ) of native waxy potato starch (a) and ADA starch (b, DS: 0.0235).

glucan chains in the amorphous regions increases, as described by Kaur, Singh and Singh (2004) and Choi and Kerr (2004). Whilst the crosslinked groups in the starch restrain the gelatinization of starch granules, it remains in its granular states in hot water with limited swelling.

### 3.7. The viscosity at different pH levels

Acids catalyse the hydrolysis of starch molecules. Hydrolysis is used to produce thin-boiling starches. Although food acids are weak acids, the starch granules is weakened when cooked in an acidic system, and an undesirable loss of viscosity can occur if the treatment is severe (Moore, Tuschhoff, Hastings, & Schanefelt, 1984).

The viscosity of native waxy potato and ADA starches at different pH levels are shown in Table 1. The viscosity of waxy potato starch decreased rapidly with a decreasing of pH, whilst the viscosity of ADA starch decreased slowly with the pH decreased. Moreover, with the DS increasing, the viscosity reduced indistinctively with good acid resistance. This is the result of crosslinking groups in the starch molecular chain. On the one hand, the crosslinking groups make the starch granules more difficult to swell, and on

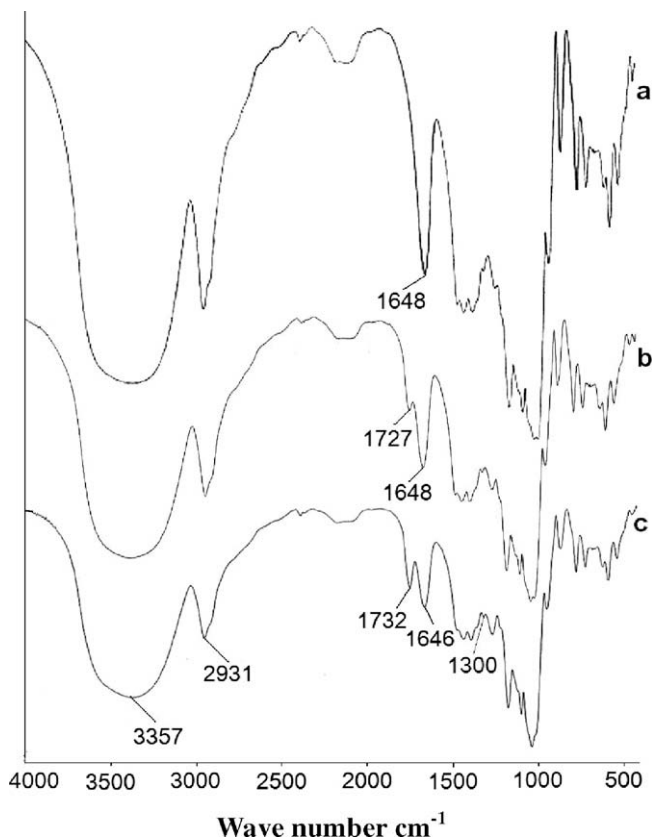


Fig. 3. FTIR Spectra of waxy potato and ADA starches (DS: a, 0, b, 0.0173, c, 0.0235).

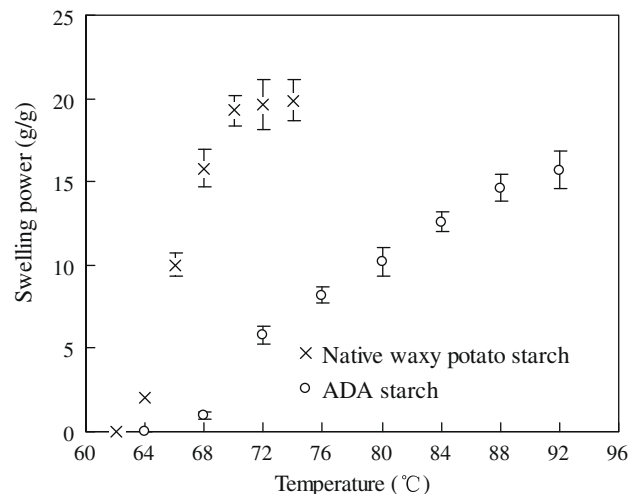


Fig. 4. Swelling powers of native waxy potato and ADA starches at different temperatures (DS of ADA starch: 0.0235).



**Table 1**  
Effects of pH on the viscosity of native waxy potato and ADA starches<sup>a</sup>.

Starch	DS	pH				
		3.0	4.0	5.0	6.0	7.0
Native waxy potato starch	0	1020±14	3030±16	4035±21	4645±24	4800±26
ADA starch	0.0115	1680±15	2160±18	2635±18	2830±24	3028±25
ADA starch	0.0235	987±6	1032±10	1124±11	1215±11	1435±14

<sup>a</sup> The data are averages of two measurements with standard deviation. Starch concentration: 1.5%, w/v, 25 °C, 30 rpm, m Pa.s. DS, Degree of substitution.

**Table 2**  
Pasting characteristics of native waxy potato and ADA starches<sup>a</sup>.

Starch	DS	$T_p$ (°C)	$P_v$ (BU)	$H_v$ (BU)	$H_{v30}$ (BU)	$C_v$ (BU)	BD(BU)	SB(BU)
Native waxy potato starch	0	64.7	919	300	217	299	702	82
Native waxy potato starch in 1% NaCl solution	0	69.3	342	299	135	172	207	37
ADA starch	0.0235	67.3	652	596	472	591	180	119
ADA starch in 1% NaCl solution	0.0235	69.3	398	396	351	439	47	88

<sup>a</sup> DS, Degree of substitution,  $T_p$ : pasting temperature,  $P_v$ : peak viscosity,  $H_v$ : hot paste viscosity,  $H_{v30}$ : holding 30 min of hot paste viscosity,  $C_v$ : cold paste viscosity, BD (Breakdown) =  $P_v - H_{v30}$ . SB (Setback) =  $C_v - H_{v30}$ . BU, Brabender units. Starch concentration 3% (w/v).

the other hand, the existing crosslinking groups make the starch molecular resistant to be degraded in an acid environment (Korimoto, 1994; Tamaki, Teranishi, & Yamaha, 1997).

### 3.8. Pasting properties

The Brabender viscosgrams of native and crosslinked waxy potato starches are presented in Table 2. A rise in pasting temperature and drop in peak viscosity was observed in crosslinked starch vs. their unreacted counterparts, whilst hot-paste viscosity,  $H_{v30}$  and cold paste viscosity were increased as a result of the crosslinking of waxy potato starch. These results agree with the crosslinking properties of starch. The breakdown value of a starch paste is defined as the difference between the peak viscosity and the viscosity after holding for 30 min at 95 °C (Huang, Li, & Fu, 2007; Muhammad, Hussin, Man, Ghazali, & Kennedy, 2000), is a measure of fragility of the granules, which was reduced after crosslinking. This indicates that the impact of crosslinking over their native counterparts. However, the setback (SB) value is increased after crosslinking. Since the SB value is an index that shows tendency to retrogradation of a starch paste (Ancona, Guerro, Matos, & Ortiz, 2001), this indicates that the ADA starch may more easily to be retrogradation.

Table 2 also presents the viscosity properties of starch in 1% NaCl solution which represents starches' salt tolerance. Compare with the starch paste without the addition of NaCl, the pasting temperature of both native waxy potato and ADA starches with 1% NaCl solution increased, and the peak viscosity, hot-paste viscosity,  $H_{v30}$  and cold paste viscosity decreased. But the viscosity of ADA starch in 1% NaCl solution decreased more slowly, moreover, the breakdown value are enhanced compared to native potato starch, indicating that the salt tolerance was improved after crosslinking. Salt can hydrate water and very effectively inhibit hydration of starch granules. As a consequence, the pasting temperature will increase and reduce the viscosity of the starches (Luallen, 2004; Moore et al., 1984), which is consistent with our results.

In general, cross-linked starches are used when a stable, high-viscosity starch paste is needed and particularly when the dispersion is to be subjected to high temperature, shear, low pH or salt solution. For instance, cross-linked starches are needed for salad dressings to provide the thickening without allowing viscosity breakdown by low pH and high shear of the homogenisation process. Whilst cross-linking may be the only modification, it is

usually employed in combination with other types of derivatization or modification (Rutenberg, 1984). As for ADA waxy potato starch, its tendency to be retrogradation (higher SB value compare with native counterparts) implies that other types of derivatization is needed to improve this property.

## 4. Conclusions

Reactions between waxy potato starch and a mixture of acetic anhydride and adipic acid under aqueous alkaline conditions were investigated. The preferred reaction conditions that lead to the highest anhydride conversion and degree of substitution were pH 9.0–9.5, 35 °C, 2 h reaction period, respectively. The substitution groups of ADA starch have new absorption peak at about 1732  $\text{cm}^{-1}$  in the FT-IR spectrum. The waxy potato starch granule structure morphology did not markedly change after modification according to the light polarised microscopy and SEM investigation. The crosslinked waxy potato starch exhibited improved viscosity stability, acid resistance, salt tolerance and good viscosity breakdown properties. The swelling ADA starch granules was gradual and over a wide range of temperatures. These outstanding properties of ADA starch will benefit the food industry. But the tendency of retrogradation should be improved, and the application of ADA starch in food needs further study.

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