Influence of Pretreatment on Cold Water Solubility and Esterification Activity of Starch

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Abstract: The aim of this study is to improve the Cold Water Solubility (CWS) and esterification activity of Native corn Starch (NS) by pretreatment NS using NaOH/urea aqueous solution. The influence of pretreatment on granule shape and crystal structure of corn starch was investigated by Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD). It has been found that the average particle size of Pretreatment corn Starch (PS) decreased to nanometer level, smaller than those of NS (4-15 μm). XRD revealed that crystalline pattern of PS was V_H-type, which was different from that of NS (A-type). The maximum CWS of PS was 96.77%, while the NS was only 0.3%. NS and PS were esterified with oleic acid catalyzed by lipase under the same conditions respectively. The effects of the pretreatment on esterification activity of the corn starches were investigated by analyzing the Degrees of Substitutions (DS) of the esterification products. The maximum DS of pretreatment starch oleate was 0.229, while the DS of native starch oleate was very low and even could not be detected.

Keywords: Cold water solubility, esterification activity, lipase, NaOH/urea aqueous solution, pretreatment starch

INTRODUCTION

Starch is one of the most abundant materials produced by nature which has already been found numerous industrial applications. It is one of the cheapest biopolymer and could be totally biodegraded, this leading to the growing interest in the nonfood usage of starch. Starch has been the choice materials for application in synthetic polymers. But the nature deficiencies of starch (such as un-solubility in cold water, easy to aging, retrogradation at low temperatures and low film-forming ability) limited its application. Therefore, starch is usually modified to obtain a particular property and to cater the requirements for its tailor-made (Wim et al., 2006). However, the intact starch granules inhibit chemical reagents from making contact with the molecules in the crystalline region, thus the chemical reactivity and reaction efficiency of native starch is usually low (LeCorre et al., 2012).

There are many methods to modify the structure in the crystalline region, or decrease the size of crystalline regions to increase reaction activity of starch and convert native starch into cold water-soluble starch (Wim et al., 2006). The Cold Water Solubility (CWS) is an important indicator for evaluation the physical properties of starch. It shows the water binding capacity of starch. The CWS is related with the molecular structure of starch, particle size and the content of amylase (Singh and Singh, 2003; Lin et al., 2003).

Cold-water soluble corn starch was first produced by Eastman and Moore (Eastman, 1984), by slurring corn starch in selected aqueous alcohols under the high temperature and pressure. A method for preparing granular cold water swelling/soluble starches by alcoholic-alkali treatments has been studied by Lim and Jane (1992). Subsequently, some different origin granular cold-water-soluble starch such as cold-water-soluble corn starch (Singh and Singh, 2003), cold-water-soluble banana starch (Arturo et al., 2000), cold water-soluble potato starch were prepared and studied using the alcoholic-alkaline method.

In this study, we reported an easy method to modify corn starch through hydrolyzing by using NaOH/urea solution as hydrolytic agent and re-precipitated out from the starch solution by adding ethanol drop-wise. The aim of this study is to improve the CWS of corn starch and to activate its reaction ability of esterification. Also, the mechanism of the method could improve the CWS and the esterification reaction activity of corn starch was investigated. To study the effect of process conditions on the CWS of PS, a number of experiments were performed at various ratio of NaOH to urea and the concentration of the aqueous solution of NaOH/Urea. Furthermore, the effect of the pre-cooling temperature and the amount of ethanol on the CWS were explored. The esterification reaction activity of PS with different CWS was compared with NS.

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MATERIALS AND METHODS

Chemicals and enzyme: Corn starch was purchased from Harbin Mei Wang Reagent Company, China. The water content was determined by drying the corn starch in a vacuum oven at 50°C until constant weight was achieved and was 16.2% (w/w). Oleic acid of analytical grade was purchased from Shanghai Chemical Co., China. Novozym 435 (Lipase B from Candida Antarctica immobilized on macroporous acrylic resin; specific activity: 10,000 U/g) was purchased from Novozymes, Denmark.; Dimethyl Sulfoxide (DMSO) of chromatography grade purchased from Shanghai Chemical Co., China. All the other chemicals are of analytical grade. This study was carried out in the Key Laboratory for Food Science and Engineering of Harbin University of Commerce since January 2012.

Starch pretreatment: According to method of Zhou and Zhang (2000), the aqueous solution containing NaOH/Urea at the desired ratio by weight was used as a solvent for starch. The mixture solution was pre-cooled to ideal temperature. Then the starch sample in the given amount was added immediately at ambient temperature of below 25°C. The NS was completely dissolved within 5 min by stirring at 3000 r/min and the resultant solution was transparent. The transparent starch solution was neutralized with HCl (15%) until it reached neutrality. Then, starch was precipitated out from the neutral starch solution by adding ethanol drop-wise. After various durations of dropping treatment, the precipitates were washed by successive centrifugations in 95% of ethanol until no HCl remained. There-after, they were washed with 100% of ethanol to remove water. The resulting precipitates were vacuum dried at 50°C for 24 h.

X-ray diffraction analysis: Native starch and pretreatment starch samples were put into a glass capillary and equilibrated overnight in a 98% RH (relative humidity) atmosphere. The capillary was sealed and X-rayed using a Philips PW3830 generator operating at the Ni-filtered Cu KR radiation wavelength (λ = 1.542 Å). Powder diffraction patterns were recorded on Fuji imaging plates read by a Fuji BAS 1800 II Phospho-Imager.

Determination of the relative degree of crystallinity: The relative degree of crystallinity of NS and PS were calculated as followed the method of Zheng (2002) (Fig. 1):

$$X_C = (A_1 + A_2 + A_3) / (A_1 + A_2 + A_3 + A_4) \quad (1)$$

X_C = Relative degree of crystallinity
A_1, A_2, A_3 = The area of crystalline region
A_4 = The area of non-crystalline region

Determination of the Cold Water Solubility (CWS): According to the method of Kurakake and Noguchi
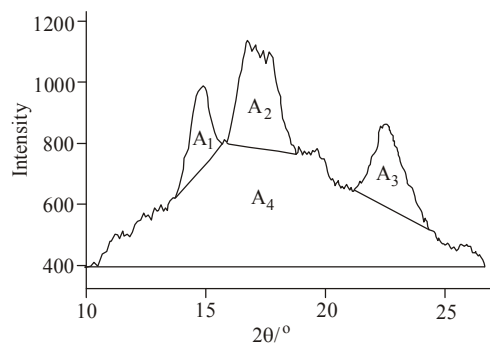

Fig. 1: Calculation of relative crystallinity

(1997), the NS and the PS were dispersed in distilled water at a concentration of 10 mg/mL at room temperature. Stir at 500 r/min for 15 s and at 1000 r/min for 2 min to prepare the starch solution. The solution was centrifuged at 6,000 r/min for 15 min and 25 mL supernatant was dried at 110°C until constant weight.

The solubility of NS and PS were calculated as followed:

$$CWS (\%) = (m_1 \times 100) / (m_0 \times 25) \times 100 \quad (2)$$

m_1 = The dry weight of 25 mL supernatant (g)
m_2 = The dry weight of sample (g)

Determination of the Degree of Substitution (DS) by methanolysis and GC analysis: The DS indicates the average number of substitutions per anhydroglucose unit. There are three free hydroxyl groups available for modification per anhydroglucose unit of starch, resulting in a maximum possible DS of 3 (Apostolos *et al.*, 2010).

Methanolysis of the starch oleate and GC analysis of the methyl oleate: A small sample 30 mg of starch oleate dissolved in 1 mL of DMSO was mixed with 1 mL of sodium methoxide (0.07 M) in methanol solution. This mixture was then heated (70°C) under reflux for 40 min, with constant shaking, then cooled and 1 mL of deionized water and 1 mL of n-heptane were added. The mixture was shaken for 1 min and left to settle. The top organic phase contained the methyl ester was removed and injected into the GC-FID (Perkin-Elmer Autosystem XL with a CP Simdist capillary column, oven set at 220°C, the injector at 250°C and the detector at 260°C).

Calculation DS of the starch oleate: Once the methyl oleate was quantified by GC chromatograph, the average mol of acyl groups per anhydroglucose unit was calculated to give the DS of the modified starch. DS was calculated according to the modified method of Kshirsagar and Singhal (2007) as followed:

$$DS = (n \times M_1) / (M_0 - n) \times (M_2 - M_{H2O}) \quad (3)$$

n   = Mol of esterifiable oleic acid (moL)
M_0 = Weight of sample (g)
M_1 = Molecular weight of anhydrous glucose unit, 162
M_2 = Molecular weight of oleic acid, 282.47
M_{H20} = Molecular weight of H_2O

Methods analysis: Scanning electron micrographs were obtained with a Quanta 400 scanning electron microscope (FEI Company, Holland) at an accelerating voltage of 20 kV.

X-ray diffraction analysis were carried out using XR3E2 apparatus (Vacuum Generators, U.K.) and operated at 36 kV under a current of 20 mA. X-ray diffraction data were recorded for dry starch powder at ambient temperature with a scattering angle step size of 0.02° between 4 and 30° on a Siemens D500 diffractometer equipped with a Cu-Kα anode with λ = 1.5406 Å.

FTIR spectra were recorded using a Vector 33 spectrometer (Brucker Company, Germany). Potassium bromide (KBr) disks were prepared from powdered samples mixed with dry KBr in the ratio of 1:100. The spectra were recorded in a transmittance mode from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}.

^{13}C NMR spectra were recorded at room temperature in DMSO-d6 by using Tetramethylsilane (TMS) as an internal reference on a Brucker DRX-400 NMR spectrometer (Germany) at 100 MHz.

RESULTS AND DISCUSSION

Effect of the concentration of NaOH/Urea aqueous solution on the CWS of PS. Alkalinity of the reaction mixture was an important factor for the CWS of PS. As shown in Fig. 2, the CWS of PS increased from 1.7 to 96.4% with the increase of the concentration of the NAOH/Urea aqueous solution (from 0 to 9%). However, the CWS of PS was still about 96% if further increased the concentration of the NaOH/Urea aqueous solution. The effect of the concentration of NaOH/Urea on the CWS of PS could be attributed to the extent of starch swelling during the treatment. Although NaOH and urea could promote starch swelling, the high concentration of NaOH/Urea aqueous solution made the decrease of water content. The shortage of water would result in the decrease of starch swelling extent.

Effect of the ratio of NaOH to urea on the CWS of PS: The reaction of starch with sodium hydroxide generated negative-charged alkali starch. The alkali starch combined with the strong hydrated sodium ions. Therefore a lot of water was brought into the starch macromolecules (Ruan *et al.*, 2005). That caused severe swelling of starch and break up the binding force between the amorphous region macromolecules of starch. But that could not overcome all the binding force between the starch macromolecules. As shown in
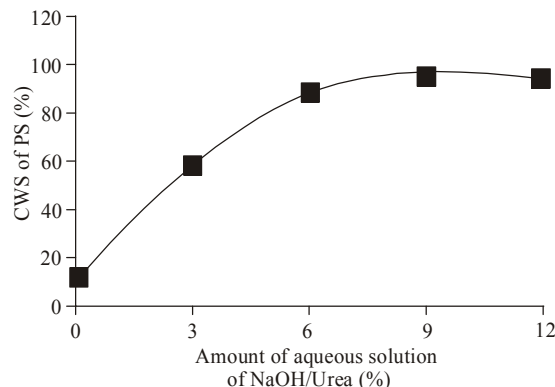

Fig. 2: Effect of the ratio of NaOH to urea
Pretreatment condition: The concentration of NaOH/Urea aqueous solution is 9%; The amount of ethanol is 50 mL; The pre-cooling temperature is -6°C; The concentration of starch is 5%; Pretreatment time is 30 min
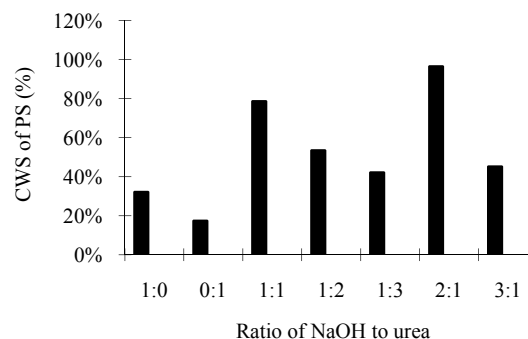

Fig. 3: Effect of the ratio of NaOH to urea
Pretreatment condition: The concentration of NaOH/Urea aqueous solution is 9%; The amount of ethanol is 50 mL; The pre-cooling temperature is -6°C; The concentration of starch is 5%; Pretreatment time is 30 min
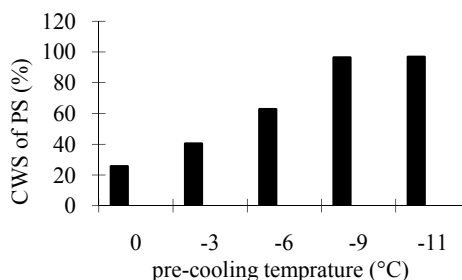

Fig. 4: Effect of the pre-cooling temperature on the CWS of PS
Pretreatment condition: The concentration of NaOH/Urea aqueous solution is 9%; NaOH: Urea = 2:1 (w/w); The amount of ethanol is 50 mL; The concentration of starch is 5%; pretreatment time is 30 min

Fig. 3, the CWS of the PS starch was only about 43.8%. But the CWS of PS was significantly improved by adding urea. This can be attributed to the polar CO and

NH_2 groups of urea molecule. These polar groups and starch macromolecules could form intermolecular hydrogen bonds (Qin and Gu, 2005) and break up the binding force between the crystalline region macromolecules of starch. When the ratio of NaOH to urea was 2:1, the maximum CWS of PS 96.4% was obtained.

Effect of the pre-cooling temperature on the CWS of PS: It has been found that pre-cooling temperature of the NaOH/urea aqueous solution played a crucial role on the CWS of PS. In this study, the effect of the pre-cooling temperature of the NaOH/urea aqueous solution on the CWS of PS was studied by changing the pre-cooling temperature from 5 to -12°C.

As shown in Fig. 4, the CWS of PS decreased with the increase of pre-cooling temperature of the NaOH/Urea aqueous solution. The formation of alkali starch is an exothermic reaction, so low temperature can promote the formation of alkali starch. Thus the higher temperature had the negative effect on the swelling and dissolution of the starch. The maximum CWS of PS (96.4%) was obtained at the temperature of -9°C. But the CWS of PS would not be higher than 97% at the further low temperature of below -9°C. Therefore, -9°C was selected as the optimum temperature.

Effect of the amount of ethanol on the CWS of PS: The role of ethanol is inhibition the starch particle swelling and maintaining the integrity of the starch particle. According to method of Geng *et al*. (2010), after neutralization, the starch molecules and ethanol formed a spiral compound (V complex). Once the ethanol was evaporated, the cavity was formed in the starch particles, thus the starch is in a metastable. That ensured the PS has excellent cold water solubility.

According to the results that showed in Fig. 5, the amount of ethanol indeed had a significantly effect on the CWS of PS. And the amount of ethanol was neither too less, nor too more. It must be a suitable amount. The CWS of PS increased from 27.7 to 96.4% with the increase of the amount of ethanol from 0 to 50 mL. But the CWS of PS would be less than 96.4% if further increase the amount of ethanol. And the CWS of PS was only 43.6% when the amount of ethanol increased to 100 mL. So the optimal amount of ethanol was 50 mL. The concentration of ethanol was related to the inhibiting ability of starch granule swelling. And the unduly inhibition of starch granule swelling would delay the dissociation of starch double helix structure. So the CWS of starch would decrease if the amount of ethanol was more than 50 mL.

The esterification activity of PS that with different CWS: In order to study the relationship between the esterification activity of PS with its CWS, a lot of experiments had been completed. The Degree of Substitution (DS) of the starch oleate was used to represent the esterification activity. The PS with
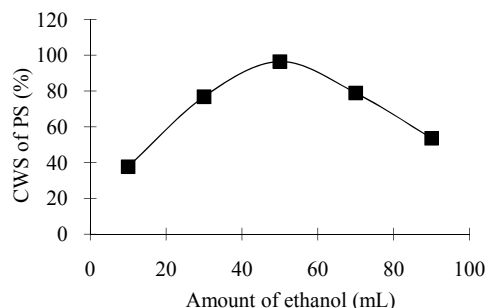

Fig. 5: Effect of the amount of ethanol on the CWS of PS Pretreatment condition: The concentration of NaOH/Urea aqueous solution is 9%; NaOH: Urea = 2:1 (w/w); The pre-cooling temperature is -9°C; The concentration of starch is 5% and pretreatment time is 30 min
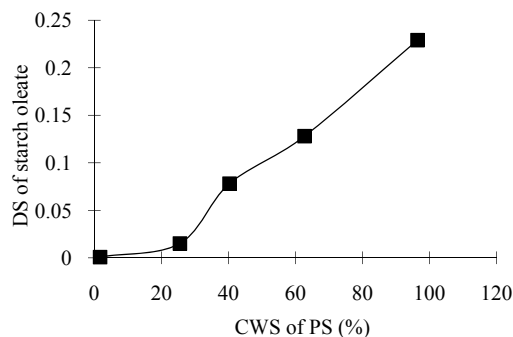

Fig. 6: Effect of the CWS of PS on the DS of starch oleate Reaction condition: PS/oleic acid = 1:4 (w/w); The amount of lipase is 5%, 65°C, 12 h)

different CWS (1.7, 25.5, 40.3, 62.7 and 96.4%, respectively) were chosen as the acyl acceptor to esterify with oleic acid. The results as shown in Fig. 6, the PS with high CWS must have the high esterification activity. These results proved that this pretreatment method indeed could improve the esterification activity of starch. And the esterification activity of PS with higher CWS was higher than that with lower CWS.

SEM micrographs of PS and NS. SEM micrographs in Fig. 7 showed the size and morphology of starch particles in the slurries. The NS (Fig. 6a) had a mean diameter of 10 μm which is within the granule range of 4-15 μm with a smooth surface. The average particle size decreased to nanometer level, with uneven size distribution and affinity to agglomerate after pretreatment using NaOH/Urea 2:1 (w/w) aqueous solution (Fig. 6b). Because of the existence of hydrogen bonds in native starch particles, the starch was insoluble in cold water. During the NaOH/Urea aqueous solution pretreatment process, the hydrogen bonds of starch were damaged. So the starch was gelatinized to the transparent homogeneous state. The interaction of urea with starch molecules inhibited the cross-linking between starch molecules. And that maintained the stability of the starch solution. The addition of anhydrous ethanol resulted in the formation of small

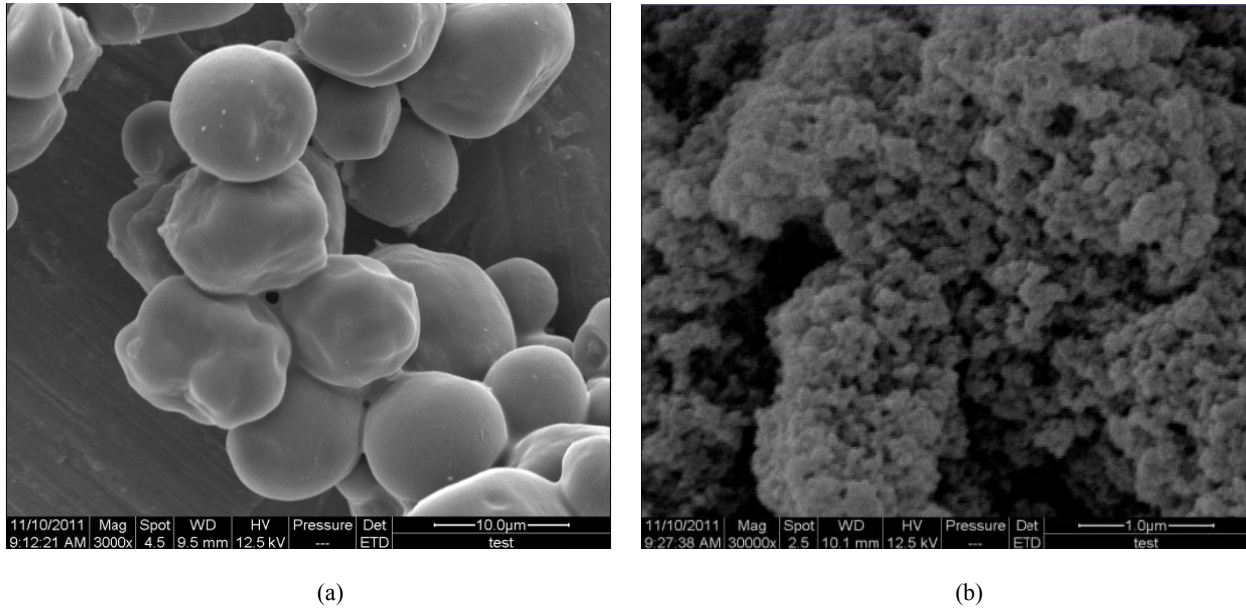(a)   (b)

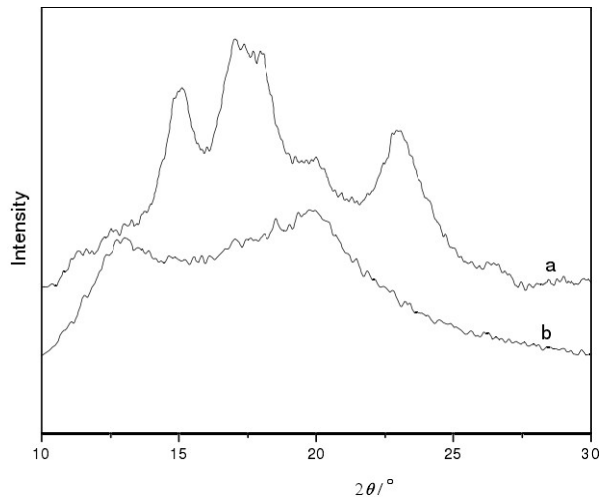Fig. 7: SEM images of NS (A) and PS (B)

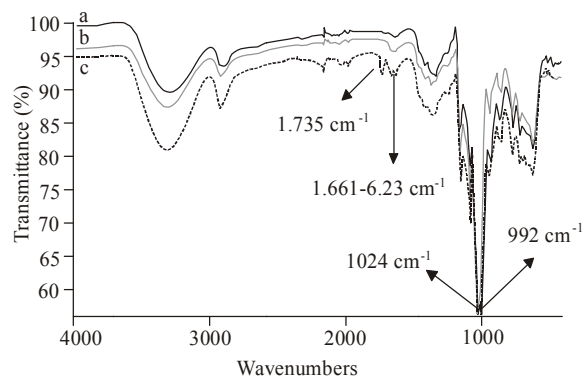Fig. 8: XRD spectra of NCS (a) and PCS (b)

Fig. 9: FT-IR spectra of NS (a), PS (b) and SO(c) of solvent-free system

size starch particles. The smaller size of the pretreatment starch improved the CWS of starch and the degree of substitution of starch ester, that maybe due to the increase of the surface area of starch. The increase of the surface area of starch creased more opportunities for other substrates to touch it.

X-ray diffraction analysis: The XRD patterns of NS and PS showed in Fig. 8. Strong diffraction peaks of X-ray diffraction patterns of NS appeared at $2\theta = 15.0°$, $17.0°$, $17.9°$, $22.9°$. It was shown that the crystal structure of NS is A-type. Strong diffraction peaks of X-ray diffraction patterns of PS appeared at $2\theta = 13.0°$ and $19.8°$, they are the characteristic diffraction peaks of the crystal structure of V_H-type. These results showed that the crystalline type of corn starch shift from A-type to V_H-type after pretreatment. That mainly because of the starch-ethanol single-helix inclusion compound formation. In strong alkaline solution, the hydroxyl of starch molecules are ionized that made the starch molecules take negative charges. The mutual exclusion between these negative charges of starch molecules promoted the swelling of starch particles until gelatinization. Eventually that made the expansion of the double helix structure of starch into the single helix structure (Chen and Jane, 1994). The crystalline structure was broken and the crystallization sequence was changed. After neutralization, the addition of ethanol made the starch single-helix inclusion compound formation with it (V_H-type complex).

According to the formula (1) calculated the relative degree of crystallinity, the relative degree of crystallinity of NS was 24.8% and PS was 10.32%. Compared with NS, the degree of crystallinity of PS decreased significantly. The decrease of the relative

degree of crystallinity made the amorphous region of corn starch increased, that will inevitably have an impact on its physical and chemical properties.

FT-IR analysis: In the NS spectrum, the characteristic peaks (954-1, 184/cm) are attributed to C-OH bond stretching. Another strong broad band due to hydroxyl bond stretching appears at 3,000-3,600/cm (Fig. 9a, b) which is reduced on Starch Oleate (SO) (Fig. 9c). A characteristic peak C = O bond stretching present in the starch occurred at 1,661-1,623/cm, which is intensified on the PS (Fig. 9a and b). That demonstrated the molecular chain of starch had fractured after pretreatment (Kiyoshi *et al*., 2012). A strong absorption band at 992/cm, which appears at 1,024/cm, probably due to the stretching of the C-O-C bond, was present in the spectra of the starch consistent with the earlier report by Zhao *et al*. (2007). The red shift of C-O-C bond stretching could weaken the hydrogen bond. An extremely broad band due to hydrogen bonded Hydroxyl groups (O-H) appeared at 3,400/cm which was attributed to the complex vibrational stretches associated with free, inter- and intramolecular bound hydroxyl groups which make up the gross structure of starch (Huang *et al*., 2004). The band at 2,919/cm is characteristic of C-H stretches. These spectra have similar profiles. The differences between NS and PS were the C = O bond stretching and the red shift of C-O-C bond stretching. There was no new peak present.

In comparison with the spectra of the PS, the major change of modified starch is the presence of a carbonyl C-O absorption frequency at 1,735/cm. The strong O-H stretching band at 3,400/cm in the SO decreased in intensity following esterification of starch with oleic acid.

CONCLUSION

Over the years, the starch is not liable to react with other chemical reagents was the crucial problem in the study field of starch chemical modification and how to resolve this problem was always the research emphasis. This present study solved the most difficult problem successfully and that is very important for the development of starch modification. Corn starch was successfully activated through treatment with NaOH/Urea aqueous solution at low temperature. The maximum CWS of PS (96.4%) had been obtained under the optimal conditions: the concentration of the NaOH/Urea is 9%, the ratio of NaOH to urea is 2:1, the pre-cooling temperature is -9C and the amount of ethanol is 50 mL. The average particle size decreased to nanometer level from 4-15 μm. The crystalline type of corn starch shift from A-type to V_H-type and the relative degree of crystallinity of corn starch had been decreased to 10.32%. The smaller particle size and the destruction of the crystal structure of starch after pretreatment endowed starch with higher cold-water solubility. The esterification activity of corn starch had been significantly improved after pretreatment. In comparison with the DS of native starch oleate, the high DS (0.229) of pretreatment starch oleate could be obtained by lipase-catalyzed esterification of starch with oleic acid in solvent-free system. The DS of SO and the CWS of PS was closely related. The DS of PS with higher CWS was greater than that with lower CWS. Obtained results indicated that the investigated method may be used for obtaining high CWS and high esterification activity starch.

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