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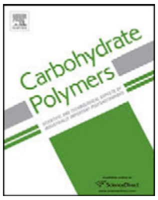

Lipase-coupling esterification of starch with octenyl succinic anhydride

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ABSTRACT

Enzymatic modification of starch was conducted by lipase-coupling esterification with octenyl succinic anhydride (OSA). Parameters affecting the esterification were systematically studied. Products were characterized by Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), X-ray diffraction, differential scanning calorimetry (DSC) and viscosity analysis (VA). Optimum condition for lipase-coupling OSA starch preparation was as follows: starch pretreatment at 65 °C for 15 min, starch concentration 35%, amount of lipase and OSA, 0.6% and 3%, reaction pH, temperature and time, 8.0, 40 °C and 30 min respectively, which resulted in 0.0195 of the degree of substitution and 84.05 ± 2.07% of the reaction efficiency. FT-IR spectroscopy confirmed the formation of OSA starch. SEM and X-ray diffraction showed apparent surface change, but no crystalline change. DSC and VA results indicated the synthesized OSA starch gelatinized rapidly with high viscosity. Attractively, reaction time drastically reduced to 30 min, showing vast potential for scale production of OSA starch.

1. Introduction

Starch is an abundant natural polysaccharide that is inexpensive, renewable, and fully biodegradable, thus can be widely used in various industries. However, limitations, such as low shear and thermal resistance and high tendency towards retrogradation restrict the application of native starch. To eliminate the restrictions, modified starches were developed to expand the usefulness of starch for industrial applications especially in foods (Jobling, 2004; Singh, Kaur, & McCarthy, 2007). Starch modification is generally achieved through physical methods as high temperature/pressure (Nilsson, Leeman, Wahlund, & Bergenstahl, 2006; Shogren, 2003) and chemical methods like etherification, esterification, cross-linking or oxidation (Tharanathan, 2005).

Many studies on the esterification of starches have been conducted, among which starch modified by esterification with octenyl succinic anhydride (OSA) was firstly patented by Caldwell and Wurzburg (1953), and widely studied (Bao, Xing, Phillips, & Corke, 2003; Bhosale & Singhal, 2006; Billmers & Mackewicz, 1997; Rivero, Balsamo, & Muller, 2009; Ruan, Chen, Fu, Xu, & He, 2009; Shogren, Viswanathan, Felker, & Gross, 2000; Song, He, Ruan, & Chen, 2006; Thirathumthavorn & Charoenrein, 2006). By OSA modification, starch can be used in many branches of industry as emulsifiers, glues, adhesives, degradable materials and auxiliaries with a wide range of rheological and functional properties (Nilsson et al., 2006; Tesch, Gerhards, & Schubert, 2002). OSA starch has been approved for food use by FDA in 1972, being used in food products such as sauces, puddings, and baby foods for over 30 years with maximum additive amount 3% and degree of substitution (DS) < 0.02. It has been further verified that OSA starch have special nutritional value, and it was considered a resistant starch, which could be used as a functional fiber for the treatment of certain human diseases (Wolf, Bauer, & Fahey, 1999).

OSA starch has been prepared by chemical method conducted at high temperature using harsh chemicals like NaOH, pyridine and anhydrides or acid chlorides of the acids. Different reactions occurred during the chemical process, resulted in undesirable by-products (Rutenberg & Solarek, 1984). However, enzyme method is environmentally friendly and occurs under milder conditions with improved product quality. Additionally, reaction rate may be greatly increased due to the high efficiency of enzymes. Enzymes like lipases and proteases have been used in esterification of starch with oils or long chain fatty acids (Qiao, Gu, & Cheng, 2006; Rajan, Prasad, & Abraham, 2006; Rajan, Sudha, & Abraham, 2008). But little work has been reported on the preparation of OSA starch through lipase-coupling esterification, which is expected to improve OSA Starch synthesis by speeding up esterification to avoid side reaction.

In this study, OSA starches were prepared from corn starch through lipase-coupling esterification. The major factors affecting the esterification process were systematically investigated. Fourier transform infrared (FT-IR) spectroscopy, scanning electron

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microscopy (SEM), X-ray diffraction and the visco analyser (RVA) were used for product characterization, with the aim to provide a unique pathway for OSA starch production, to make a complete insight into the factors affecting the pathway.

2. Materials and methods

2.1. Materials

Waxy corn starch was purchased from Shanxi Kangbao Starch Co. Ltd. (Taiyuan, China). OSA 99.9% was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). The lipase Novozym 435 was purchased from Novozymes Ltd., China with activity of 10 000 PLU/g determined by the synthesis of propyl laurate. Other chemicals and reagents used were of analytical grade and commercially available.

2.2. Preparation of OSA starch through lipase-coupling esterification

Waxy corn starch (25 g, dry weight) was suspended in distilled water (20–45%, w/w) with agitation and pretreated by heating. The pH of the suspension was adjusted with a pH meter by adding 1% NaOH solution. A weighed quantity of lipase was added at the beginning of reaction. A weighed quantity of OSA was added (diluted five times with absolute alcohol, v/v) slowly over 10 min. The reaction was continued at constant temperature and kept mixing for the required time. After reaction, the pH was adjusted to 6.5 with 1% HCl solution, the mixture was centrifuged, washed twice with distilled water and twice with 75% aqueous alcohol, and the solid was oven-dried at 45 °C for 24 h, and then passed through a 120 mesh nylon sieve (60 mm opening).

2.3. Determination of the degree of substitution

The degree of substitution (DS) is the average number of hydroxyl groups substituted per glucose unit. The DS of OSA starch was determined using the Kweon, Choi, Kim, & Lim (2001) method. 2 g OSA starch sample was accurately weighed and dispersed by stirring for 30 min in 15 ml of 2.5 M HCl isopropyl alcohol solution to form starch slurry. Then, 100 ml of 90% (v/v) aqueous isopropyl alcohol solution were added into the slurry with an additional stir for 10 min. The suspension was filtered through a glass filter and the residue was washed by 90% isopropyl alcohol solution until no detection of Cl^- (using 0.1 M $AgNO_3$ solution). The starch was re-dispersed in 300 ml of distilled water, and then the dispersion was heated up in a boiling water-bath for 20 min. The starch solution was titrated with 0.1 M standard NaOH solution, using phenolphthalein as an indicator. Native corn starch sample was simultaneously titrated as a control. The DS was calculated as follows: $[0.162 \times (V \times M)/W]/[1–0.210 \times (V \times M)/W]$, where V is the titration volume of NaOH solution (ml), M is the molarity of NaOH solution, and W is the dry weight (g) of the OSA starch. The reaction efficiency (RE) was calculated by the following equation: Actual DS/Theoretical DS × 100%. The theoretical DS was calculated by assuming that all of the added anhydride reacted with starch to form the ester derivative (Liu et al., 2008; Song et al., 2006).

2.4. Fourier transform infrared (FT-IR) spectroscopy

The change in chemical structure of the starch was qualitatively analyzed by using FT-IR (AVATAR 370, Thermo Nicolet, USA). The evidence of esterification will be verified by utilizing FT-IR showing the shift of the hydroxyl group to the carbonyl of ester group. For FT-IR spectroscopic analysis, the finely powdered starch samples (dried at 105 °C for 12 h to avoid moisture interference) were ground with KBr and then made into a film which was detected in the beam of the FT-IR spectrophotometer. The set spectrum was ranging from 400 to 4000 cm^{-1}.

2.5. X-ray diffraction

The X-ray patterns of starches were gained by an X-ray diffractometer (D/max-Ra, Rigaku, Tokyo, Japan) at 80 mA and 40 kV in operation. The scanning scope of the diffraction angle (2θ) was from 0° to 50° at 0.02° step size with a count time of 2 s. The starch samples were equilibrated at 40 °C for 24 h before scanning.

2.6. Scanning electron microscopy (SEM)

The surface topography of starch granules was tested by using SEM (XL-30-ESEM, Philips, Eindhoven, the Netherlands). Before testing, the samples were scattered onto metallic sample holders with conducting silver glue and sputtered with a layer of gold, and then photoed. The pictures were presented at magnifications of 2000× and 10 000×.

2.7. Differential scanning calorimetry (DSC)

DSC measurements were conducted with a Q100 DSC (TA Instrument, USA). Precise 2.5 mg of the dried samples were placed in closed stainless steel DSC pans and heated from 30 °C to 250 °C with a heating rate of 10 °C/min in a nitrogen atmosphere with N_2 filled at 50 ml/min. The glass transition temperature (T_g) is taken as the inflection point of the increment of special heat capacity and as the peak value of the endothermal process in the DSC curves.

2.8. Viscosity measurement

Viscosity was measured via a NDJ-1 rotating viscometer (Hengping scientific, Shanghai) at different stirring speed. Samples were prepared by dissolving 2.0 g starch in 25 ml distilled water and manually stirring the mixture for 1 min, achieving full dispersion before testing (Shih & Daigle, 2003). Constant readings (mPa s) were recorded, and the viscosity was calculated as follows: $\eta = K \times N$, where K is the parameter depending on the rotor and rate used, and N is the reading of test.

2.9. Statistical methods

All tests were performed in triplicate. Analysis of variance (ANOVA) was performed using the Duncan's multiple range test to compare treatment means. Significance was defined at $P < 0.05$.

3. Results and discussion

3.1. Preparation of OSA starch

The esterification rate and efficiency depend on the reagent type and reaction parameters. They also lied on the surface structure of the starch granules, which encompasses the outer and inner surface, depending on the pores and channels, and results in the formation of the so-called specific surface (Ruan et al., 2009). Thereby the preparation conditions were systematically investigated in this part. 95 °C for 15 min was used to inactivate lipase and thus stop the esterification process.

3.2. Effects on esterification

3.2.1. Effects of starch pretreatment on esterification

It is deduced that heating turns starch structure loose and gelatinized, facilitating water molecules entering into starch structure

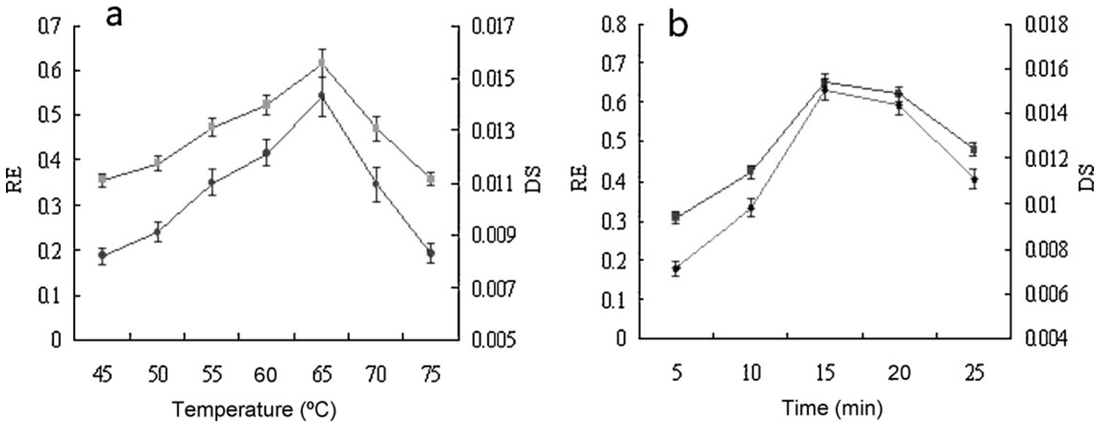

Fig. 1. Effects of starch pretreatment on esterification: (a) effect of pretreatment temperature, starch was pretreated at different temperatures for 30 min and (b) effect of pretreatment time, starch was pretreated at 65 °C for different time period. Esterification was conducted in the following conditions: starch concentration 30%, lipase 1% and OSA 3% (in proportion to water, w/w), reaction pH 8.5, temperature 35 °C and time 30 min (pooled data from three experiments are presented as means ± standard deviation of the mean ($n = 3$)).

and being constrained, thus the water activity on the surface of starch granules reducing, making lipase-coupling esterification feasible. As shown in Fig. 1(a), when starch pretreatment time was fixed at 30 min, the DS and RE of the lipase-coupling OSA modified corn starch reached a peak, 0.0143 and 61.64%, respectively at 65 °C. Maybe lower temperature is not enough for the starch granules loosing and free water reducing around the surface of starch granules. However, at temperature over 65 °C, the starch would excessively gelatinize, making stirring difficult and leading to drastic decreases on DS and RE. Since heating at 65 °C facilitates construction loosing of starch granule, how long should the pretreatment last? Fig. 1(b) demonstrated that both DS and RE significantly increased along with pretreatment time increasing from 5 min to 15 min, while decreased when over 15 min, indicating that neither insufficient nor excessive pretreatment could achieve satisfactory esterification. Consequently, optimized pretreatment condition for lipase-coupling OSA modified corn starch synthesis is heating at 65 °C for 15 min.

3.2.2. Effects of lipase addition on esterification

In Fig. 2, the DS and RE climbed up along with the increase on the lipase amount, while kept constant when the lipase quantity was over 0.6%. Lipase catalyzes either esterification or hydrolysis. The results of the present study indicated that the right point for substrates saturation and effective esterification is at 0.6% lipase addition, and 0.6% is the borderline amount for lipase catalysis direction towards esterification.

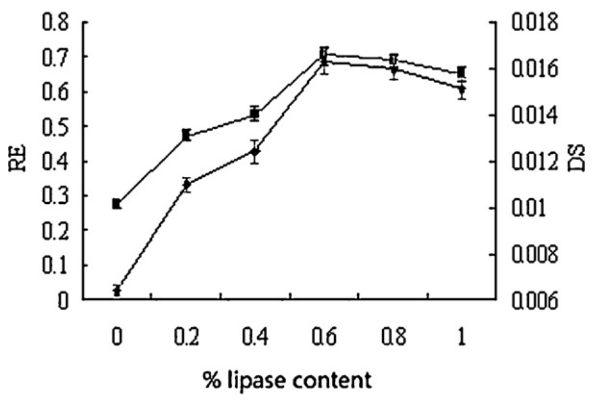

Fig. 2. Effect of lipase addition on esterification. Starch was pretreated at 65 °C for 15 min. Esterification was conducted in the following conditions: starch concentration 30%, and OSA 3% (in proportion to water, w/w), reaction pH 8.5, temperature 35 °C and time 30 min at various lipase additions (pooled data from three experiments are presented as means ± standard deviation of the mean ($n = 3$)).

3.2.3. Effects of pH and temperature of reaction system on esterification

The pH of the aqueous slurry is important for esterification of starch. The pH over 7.0 can enhance the nucleophilicity of the hydroxyl group and swell the starch particles as studied before (Rutenberg & Solarek, 1984). In order to study the influence of pH, the other reaction parameters were kept the same to Section 3.2.2, but the addition of lipase was fixed at 0.6%. Fig. 3(a) indicated that DS and RE attained the maximum values when pH of the reaction system reached 8.0, both DS and RE decreased as pH deviated from that point, one possible reason may be it is the optimum pH for enzyme catalysis at pH 8.0 in the given system. Also, it might be that pH value >8 favors side reactions. Whereas, when at pH <7.5, the hydroxyl groups of starch are not sufficiently activated for nucleophilic attack of the anhydride moieties. Thus, pH remained 8.0 in the following experiments. As for reaction temperature, Fig. 3(b) showed both the positive and negative effects of temperature on DS and RE. Increases on the reaction temperature from 30 °C to 40 °C resulted in increases on DS and RE. However, further increases on the reaction temperature from 40 °C to 70 °C make DS and RE decline. This may be because high temperature facilitates OSA diffusion into the starch granules as well as the swelling of the starch granules which will raise the esterification rate and efficiency, however, relatively high temperature may cause lipase inactivation and more OSA resolution in the aqueous phase which will increase hydrolysis.

3.2.4. Effects of starch and OSA concentration on esterification

The effects of different starch concentrations on esterification were shown in Fig. 4(a). It showed that an increase on the concentration of starch from 20% to 35% (in proportion to solvent, w/w) gave a steady increase in DS and RE, for the chances that starch contact with OSA increases accompanying the increase on starch concentration, resulting in DS and RE increases correspondingly, but DS and RE slightly decreased with further increase on the starch concentration up to 45%, it may be explained in that when the starch concentration becomes over 35%, it turns difficult to agitate the reaction system. Consequently, 35% was the best ($p < 0.01$) starch gel concentration for lipase-coupling OSA modification of corn starch. The effects of OSA concentration on esterification were

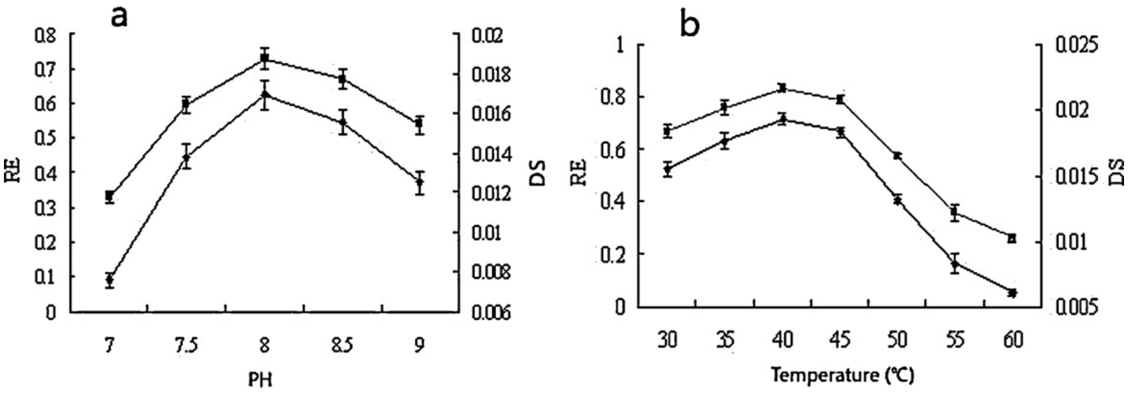

Fig. 3. Effects of pH and temperature of reaction system on esterification: (a) effect of reaction pH at 35 °C and (b) effect of reaction temperature at pH 8.0. Starch was pretreated at 65 °C for 15 min. Esterification was conducted in the following conditions: starch concentration 30%, lipase 0.6% and OSA 3% (in proportion to water, w/w), and reaction time 30 min (pooled data from three experiments are presented as means ± standard deviation of the mean ($n = 3$)).

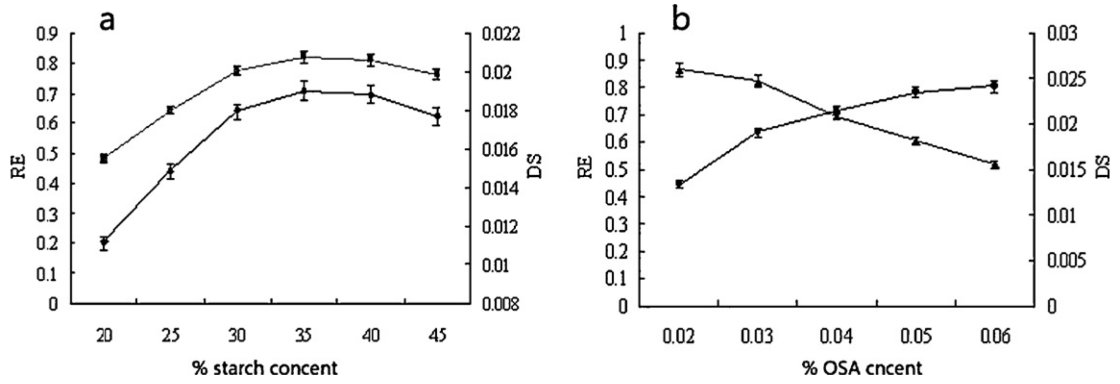

Fig. 4. Effects of starch and OSA concentrations on esterification: (a) effect of starch concentration with 3% OSA and (b) effect of OSA concentration with 35% starch. Starch was pretreated at 65 °C for 15 min. Esterification was conducted in the following conditions: lipase 0.6% (in proportion to water, w/w), reaction pH 8.0, temperature 40 °C, and time 30 min (pooled data from three experiments are presented as means ± standard deviation of the mean ($n = 3$)).

also displayed in Fig. 4(b). When the OSA concentration increased from 2% to 6%, DS increased but RE decreased, especially OSA concentration over 3% RE decreased sharply. Since the concentration of lipase was fixed, the reaction rate may reach the maximum with 3% OSA and kept constant when OSA is above 3%. Then there would be more unreacted OSA left. As a result, the RE decreased sharply.

3.2.5. Effects of reaction time on esterification

The reaction curves of esterification were presented in Fig. 5. With the reaction time increasing from 10 to 30 min, DS and RE values got rising. However, when the reaction lasted for more than 30 min, decreases on DS and RE showed up. The first reason for this phenomenon may be that the water activity on the surface of starch granules increases along with gradual separating of absorbed water from starch. The second reason is due to the OSA depletion along with the esterification process. Thus, the optimum reaction time was 30 min, beyond 30 min, adverse and side reactions may become dominant, hampering the esterification.

3.3. Properties of lipase-coupling synthesized OSA starch

3.3.1. General

The effects of lipase-coupling modification on thermal, morphological and pasting behaviours of corn starch may be characterized by instruments, such as Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction, scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and the visco analyser (RVA), respectively.

3.3.2. Fourier transform infrared (FT-IR) spectroscopy

Succinylation leads to the replacing of hydroxyl groups in the starch molecules with carbonyl groups of OSA. The shifts of hydroxyl groups into carbonyl groups could be ascertained by FT-IR spectroscopy (Marcazzan, Vianello, Scarpa, & Rigo, 1999). The FT-IR spectra of native and OSA modified starch were shown in Fig. 6. As shown in Fig. 6, native and OSA starches have similar profiles. For both starches, an extremely broad band, resulting from vibration

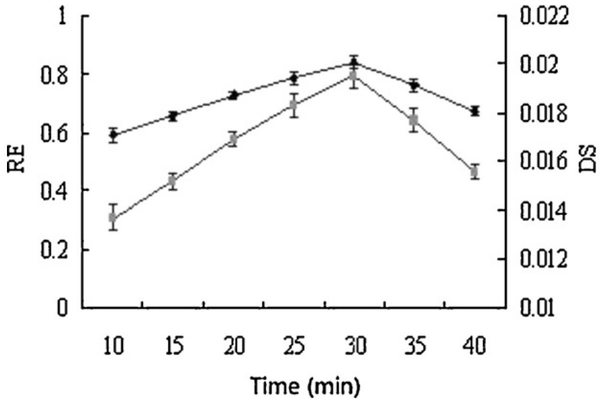

Fig. 5. Effects of reaction time on esterification. Starch was pretreated at 65 °C for 15 min. Esterification was conducted in the following conditions: starch concentration 35%, lipase 0.6% and OSA 3% (in proportion to water, w/w), reaction pH 8.0, temperature 40 °C at a various of reaction periods (pooled data from three experiments are presented as means ± standard deviation of the mean ($n = 3$)).

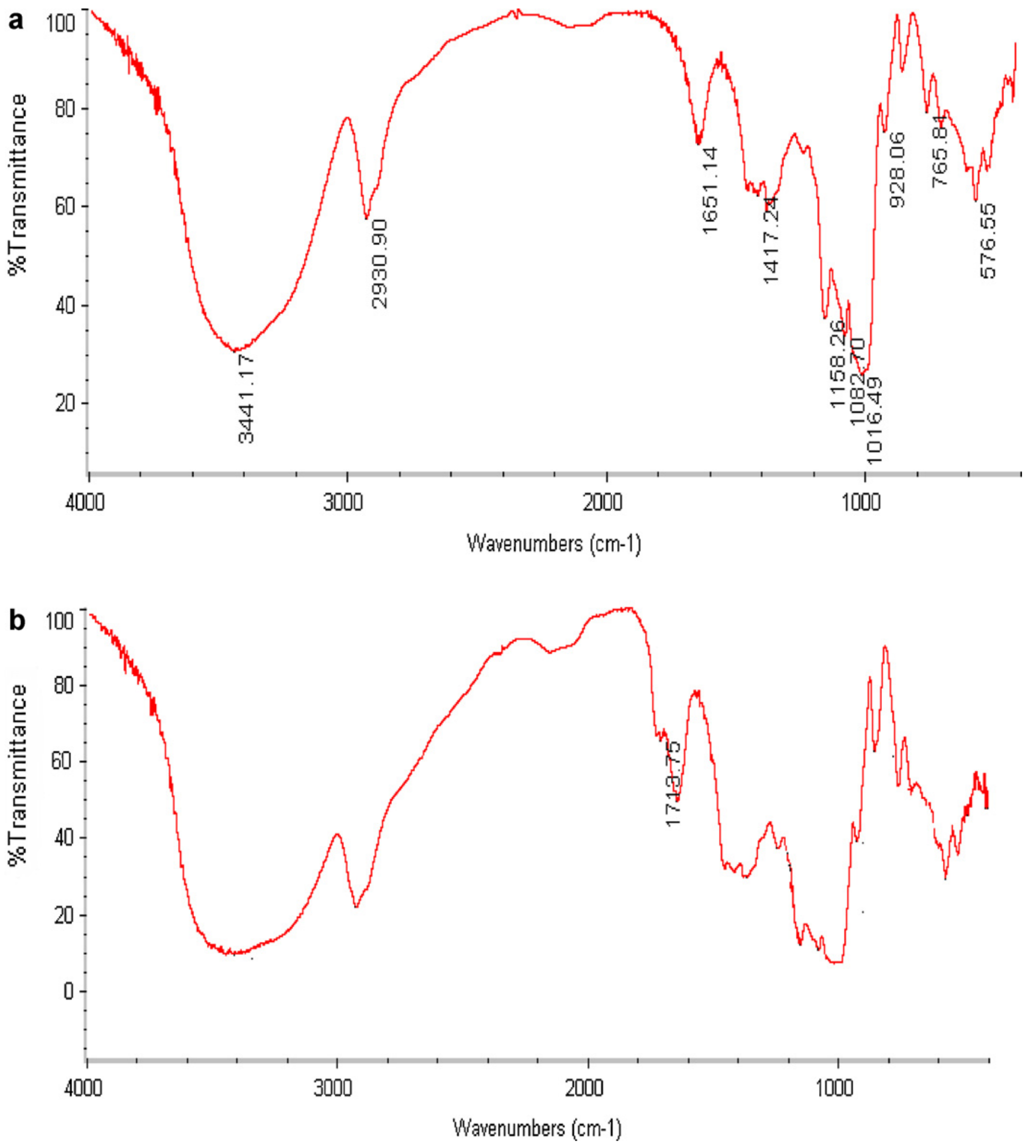

Fig. 6. The FT-IR spectroscopy of native corn starch (a) and OSA corn starch (DS 0.0195) (b).

of the hydroxyl groups (O–H), appeared at 3441 cm^{-1}; the band at 2930 cm^{-1} characterized the C–H stretching vibration; peak occurring at 1651 cm^{-1} referred to tightly bound water present in the starch granule; several discernible absorbancies at 1158 cm^{-1}, 1082 cm^{-1} and 1016 cm^{-1} were attributed to C–O bond stretching (Fang, Fowler, Sayers, & Williams, 2004; Mathew & Abraham, 2007). Compared with native corn starch, the spectrum of lipase-coupling synthesized OSA corn starch (DS 0.0195) shows a new peak at 1713 cm^{-1}, which indicates the formation of ester carbonyl groups (Fang et al., 2004; Marcazzan et al., 1999; Thygesen, Lokke, Micklander, & Engelsen, 2003).

3.3.3. *X-ray diffraction*

In order to illuminate the effect of OSA modification on the starch crystal structure, the native and OSA corn starches were measured by powder X-ray diffraction. Fig. 7 indicated that both native and lipase-coupling synthesized OSA corn starch (DS 0.0195) exhibited typical A-type diffraction pattern with strong reflection (Zobel, 1988), suggesting that lipase-coupling esterification did not change the crystalline pattern of OSA starch with DS around 0.0195, which was in accordance with the findings of Wang and Wang (2002), Song et al. (2006) and Ruan et al. (2009), who pointed out that the esterification occurred mainly in the amorphous regions and did not change the crystalline pattern of starches.

3.3.4. *Scanning electron microscopy (SEM)*

Starch modification includes physical, chemical and biochemical phenomena on the surface of contacting phases. Microscopy SEM has been widely utilized to test configuration changes caused by chemical modifications. Most of the structural changes upon hydroxypropylation take place at the relatively less organized central core region of the starch granule, i.e., where the hydroxypropyl groups are most densely deposited (Gallant, Bouchet, & Baldwin, 1997). SEM photomicrographs of native corn starch and lipase-coupling synthesized OSA starch were displayed in Fig. 8. The results showed that the lipase-coupling OSA treatment caused some changes in the structure of starch granules as compared with those of native starch. Though having channels that connect a central cavity, native starch granules were polygonal in shape with well-defined edges (Huber & BeMiller, 1997). However, OSA starch granules exhibited rough surfaces with apparent porous and cavities, and their edges lost some definition. The reason for this change probably is that OSA acted by first attacking the surface and

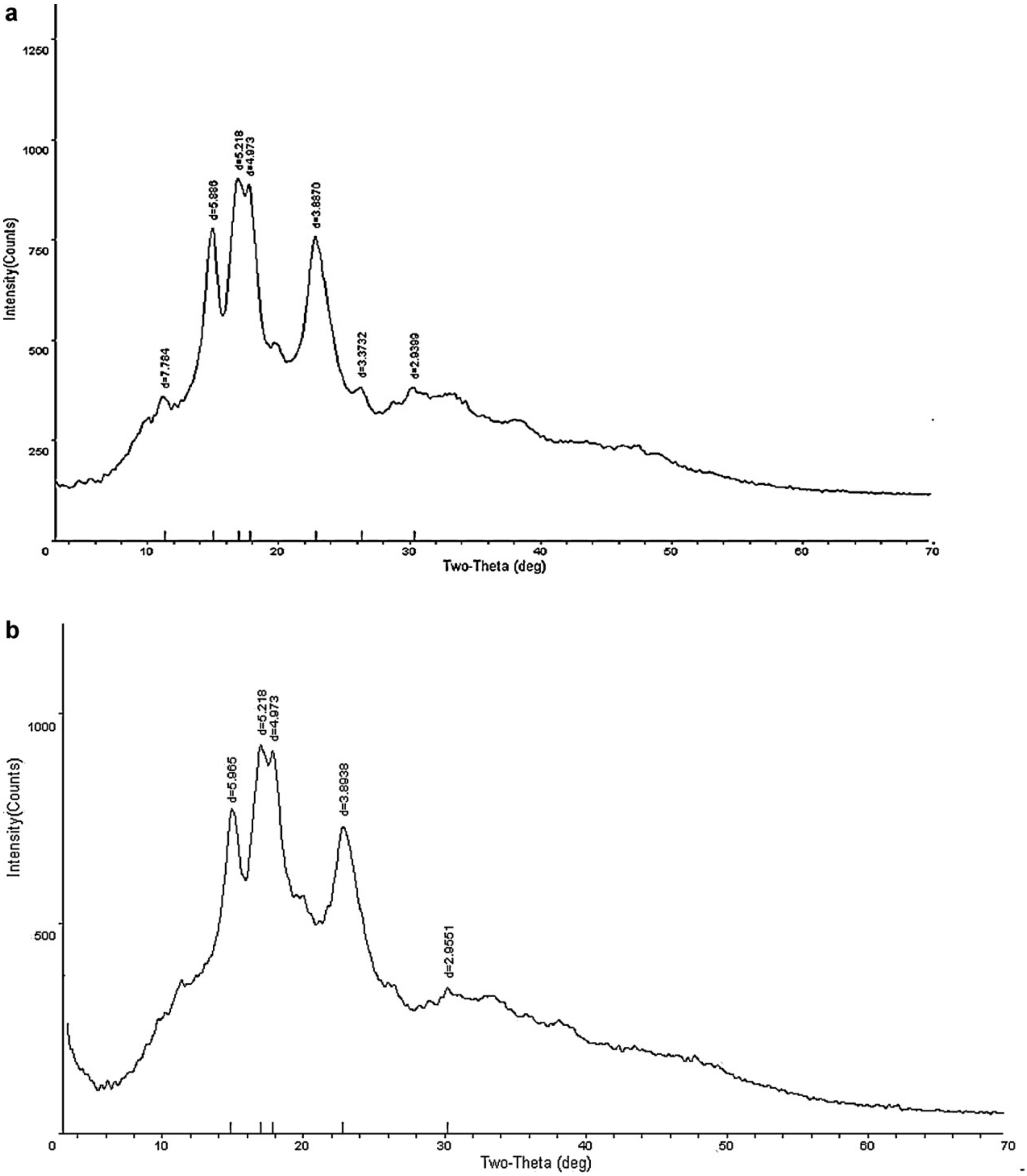

Fig. 7. X-diffraction of native corn starch (a) and OSA corn starch (DS 0.0195) (b).

forming pores on the surface. SEM photomicrographs indicated that lipase-coupling OSA modification moderately altered the granular structure of starch, although the crystal structure and type did not change.

3.3.5. Differential scanning calorimetry (DSC)

Gelatinization enthalpy reflects the energy required for granules rupture. Morikawa and Nishinari (2000) depicted that modification blocks gelatinization and some loss of structural order during the process of modification. The glass-transition temperature properties of native and lipase-coupling synthesized OSA corn starch (DS 0.0195) appeared similar to each other. But gelatinization peak for lipase-coupling modified starch was wider than that for native starch, and esterification increased the gelatinization temperature (T_{p}) yet decreased enthalpy (ΔH), which may be caused by molecular rearrangements of the amorphous regions and crystal perfections (Tester & Debon, 2000). Furthermore, a broad absorbance peak occurring in OSA starch over 80 °C. These changes are believed to result from granules damages after hydrating swell and recrystallization. The results indicated that though the modified starch kept its original crystal structure, its density reduced and that it was more likely to gelatinize owing to lower enthalpy in despite of higher gelatinization temperature, which is in agreement with Lawal report (2004).

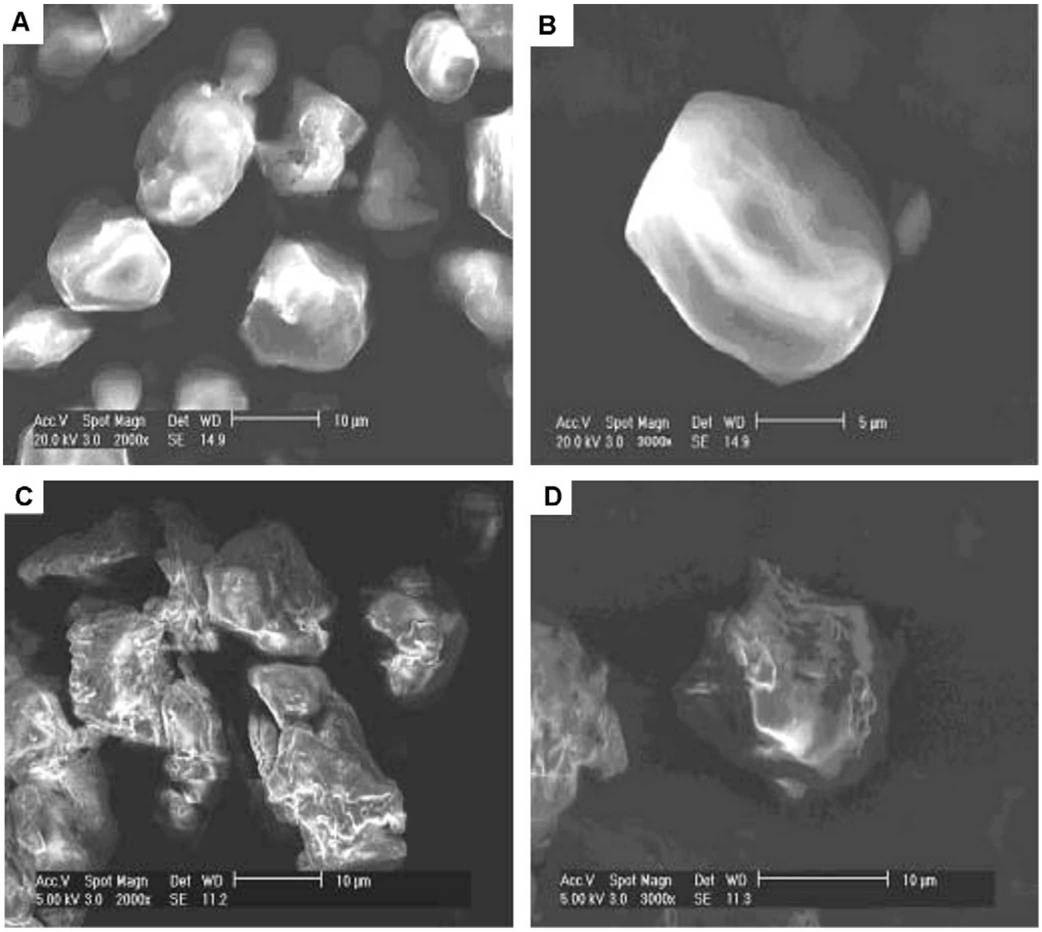

Fig. 8. SEM photomicrograph of native corn starch (a, b) and OSA corn starch (DS 0.0195) (c, d).

3.3.6. Viscosity

Starch apparent viscosity can be increased or reduced by applying a suitable modification (Agboola, Akingbala, & Oguntimein, 1991). Again, modification method, reaction conditions and starch source are the critical factors governing the pasting behaviour of starch pastes (Gonzalez & Perez, 2002). The pasting properties of the native and OSA modified corn starches at different shaking speeds and different starch concentrations demonstrated that the viscosity of OSA corn starches was higher than that of native starch in the range of designed concentrations. The peak apparent viscosities of the starches decreased as shaking speed increased for both the native and OSA corn starches in various concentrations. The incorporation of a bulky group, such as OSA, enhanced the overall pasting capacity of the starches. The intact starch had lower rise in the pasting curve and a longer peak time compared with OSA starch. This result reflected its relatively ordered structure and resistance to pasting of the native corn starch. Acetylation influences interactions between starch chains by steric hindrance, altering starch hydrophilicity and hydrogen bonding and resulting in a lower gelatinization temperature and a greater swelling of granules, the latter resulting in an increased peak viscosity (Liu, Ramsden, & Corke, 1997; Ruan et al., 2009; Singh & Kaur, 2004; Singh et al., 2007).

4. Conclusion

The lipase-coupling esterification strategy for OSA starch was developed and confirmed to be feasible and high efficient in a aqueous slurry system since the water activity surrounding starch granules decreases sharply due to its hydrating swell. The esterification conditions were optimized as follows: starch pretreatment temperature 65 °C, and pretreatment time 15 min, starch concentration 35%, amount of lipase and OSA 0.6%, 3%, reaction pH 8.0, reaction time 30 min, reaction temperature 40 °C. Under this condition, DS reached 0.0195 and RE was 84.05 ± 2.07%. FT-IR spectroscopy showed the characteristic vibration of the ester carbonyl groups in the OSA starch at 1713 cm^{-1}. SEM and X-ray diffraction showed that OSA groups acted by first attacking the surface and some pores formed, but OSA modification caused no change in the crystalline pattern of corn starch with DS 0.0195. After OSA modification, peak temperature (T_p) of gelatinization increases while enthalpy of gelatinization (ΔH) decreases, indicating that the modified starch kept its original crystal structure but its density reduced. Apparent viscosity results indicated that lipase-coupling synthesized starch gelatinized in a shorter time to achieve higher viscosities with increased concentration. Compared to previous studies, there is no property difference in OSA starch synthesized by lipase-coupling strategy, and the attained DS 0.0915 and RE 84.05% are satisfactory. The most attracting point is that the reaction time drastically declined from hours to 30 min, making the lipase-coupling esterification technically feasible for industrial scale-up production of OSA starch. In further studies, it is hopeful that lipase-coupling esterification be used in synthesis of various esters of starches or other polysaccharides from various sources to enhance product added values of farm products, or even wastes.

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