Preparation and Properties of Octenyl Succinic Anhydride Modified Early Indica Rice Starch

Octenyl succinic anhydride (OSA) modified early indica rice starch was prepared in aqueous slurry systems and the major factors affecting the esterification were investigated systematically. The physicochemical properties of the products were determined by means of Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), X-ray diffraction and Rapid Visco Analyser (RVA). The results indicated that the suitable parameters for the preparation of OSA starch from early indica rice starch in aqueous slurry systems were as follows: concentration of starch slurry 35% (in proportion to water, w/w), reaction period 4 h, pH of reaction system 8.5, reaction temperature 35°C, amount of OSA 3% (in proportion to starch, w/w). The degree of substitution (DS) was 0.018 and the reaction efficiency (RE) was 78%. FT-IR spectroscopy showed characteristic absorption of the ester carbonyl groups in the OSA starch at 1724 cm⁻¹. SEM and X-ray diffraction revealed that OSA groups acted by first attacking the surface and some pores formed, but OSA modification caused no change in the crystalline pattern of rice starch up to DS 0.046. RVA results indicated that the starch derivatives gelatinized at shorter time to achieve higher viscosities with increased OSA modification.

Keywords: Early indica rice; OSA starch; Esterification; Physicochemical properties

1 Introduction

Rice is one of the primary dietary sources of carbohydrates worldwide. Rice starch is one of the primary and major ingredients of various food products. It consists of tiny granules (< 5 μm) with a narrow size distribution [1]. The starch is white in color and has a neutral odor. In addition, rice starch causes minimal allergic reactions. However, the use of native rice starch is limited by its lack of stability under the conditions of temperature, shear, pH and refrigeration commonly applied to processed foods. For many applications, rice starch is modified to improve its functionality and to correct the shortcomings of native starch. This can be done physically or chemically. Chemically modified starches are widely used in food manufacturing and other industrial processes. The modification of starch with octenyl succinic anhydride (OSA) was patented by Caldwell and Wurzburg in 1953 [2]. In the United States, FDA has approved OSA starch for food use in 1972. The maximum level of OSA treatment allowed is 3% (degree of substitution (DS) ≈ 0.02).

OSA starch is synthesized in aqueous slurry systems. The different reactions occurring during the synthesis are shown in Fig. 1. Sodium hydroxide is used to enhance the nucleophilicity of the hydroxyl group and to swell the starch particles. But sodium hydroxide is also playing a role in undesired side reactions [3].

After OSA modification, starch is an effective emulsifier due to the addition of bifunctional groups that are both hydrophilic and hydrophobic [4]. OSA starch has been used in food products such as sauces, puddings, and baby foods for more than 30 years. Recently, OSA starch was reported to have special nutritional value. Heacock et
al. [5] found that esterification of starch with OSA might impair the binding of $\alpha$-amylase, thus decreasing the extent of starch digestion. Their study indicated that OSA starch appeared to be a resistant starch, which could be used as a functional fiber for the treatment of certain diseases.

Although many studies on the esterification of starches have been conducted [6–9], no work has been reported on the preparation conditions and properties of OSA starch from early indica rice starch. Early indica rice is largely planted in southern China, but its inferior eating and cooking quality results in a lower price in the national market [10]. So early indica rice could be a cheap alternative starch source for various starch production and starch modification companies in China.

In this study, OSA starches were prepared from early indica rice starch in aqueous slurry systems. The major factors affecting the esterification, including starch concentration, reaction time, pH of reaction system, reaction temperature and OSA concentration were investigated systematically. The physicochemical properties of the products were determined by means of Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), X-ray diffraction and the Rapid Visco Analyser (RVA). The results may give a deeper insight into the factors that affect the esterification process and allow determination of the optimum operating conditions to achieve OSA starch with a high degree of substitution. In addition, the results provide valuable information on the physicochemical properties of OSA modified early indica rice starch, which might be useful for the food industry.

2 Materials and Methods

2.1 Materials

The early indica rice starch used for this study was Jia-zao312, having the following approximate composition: amylose content 11.9 ± 0.2%, crude protein content 0.19 ± 0.02%, crude fat content 0.23 ± 0.03%, and moisture content 9.00 ± 0.16%. High-purity OSA was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The other chemicals used in the study were of analytical grade.

2.2 Preparation of OSA starch

Early indica rice starch (30 g, dry weight) was suspended in distilled water (20–40%, w/w) with agitation. The pH of the suspension was adjusted with a pH meter by adding 3% NaOH solution. A weighed quantity of OSA was added (diluted three times with absolute alcohol, v/v) slowly in 2 h. The reaction was continued for the required time. After reaction, the pH was adjusted to 6.5 with 3% HCl solution, the mixture was centrifuged, washed two times with distilled water and two times with 70% aqueous alcohol, and the solid oven-dried at 40°C for 24 h, then passed through a 180 mesh nylon sieve (90 μm 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 by titration [11]. To this end an OSA starch sample (5 g, dry weight) was accurately weighed and dispersed by stirring for 30 min in 25 mL 2.5 M HCl-isopropyl alcohol solution. 100 mL 90% (v/v) aqueous isopropyl alcohol solution, was added and the slurry stirred for additional 10 min. The suspension was filtered through a glass filter and the residue was washed with 90% isopropyl alcohol solution until no Cl could be detected any longer (using 0.1 M AgNO3 solution). The starch was re-dispersed in 300 mL distilled water, and then the dispersion was cooked 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. A blank was simultaneously titrated with native starch as a sample. The DS was calculated by the following equation:

$$DS = \frac{0.162 \times (A \times M) / W}{1 - [0.210 \times (A \times M) / W]}$$

Where: $A$ 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 as follows:

$$RE = \frac{Actual\ DS}{Theoretical\ DS} \times 100\%$$

The theoretical DS was calculated assuming that all of the added anhydride reacted with starch to form the ester derivative.

2.4 Fourier transform infrared (FT-IR) spectroscopy

The change in chemical structure of the starch was qualitatively analyzed by using FT-IR (Nexus 670, Nicolet, Madison, WI, USA). Samples were prepared by
grinding the finely powdered starch with KBr. The spectrum was recorded over the wavenumber range between 400 and 4000 cm\(^{-1}\). The starch samples were dried at 105°C for 12 h before analysis to avoid the interference of moisture.

2.5 Scanning electron microscopy (SEM)

The surface topography of starch granules was examined using SEM (XL-30-ESEM, Philips, Eindhoven, Netherlands). Before testing, the samples were fixed onto metallic sample holders with conducting silver glue and then sputtered with a layer of gold. Magnifications of 2,000 \(\times\) and 10,000 \(\times\) were used.

2.6 X-ray diffraction

The X-ray patterns of starches were obtained using an X-ray diffractometer (D/max-Ra, Rigaku, Tokyo, Japan) operated at 80 mA and 40 kV. The scanning region of the diffraction angle (2\(\theta\)) was from 5 to 50\(^\circ\) at 0.02\(^\circ\) step size with a count time of 2 s. The starch samples were equilibrated at 40°C for 24 h prior to the analysis.

2.7 Pasting properties

The pasting properties were determined using a Super 3D Rapid Visco Analyser (RVA) (Newport Scientific Instruments, Warriewood, Australia). The analysis was based on the AACC standard program for RVA analysis with some modification [12]. Samples were prepared by mixing starch (2.0 g) and 25 mL distilled water. The mixture was stirred manually for 1 min to facilitate dispersion before testing [13]. The initial speed of sample stirring in the analyzer was 960 rpm for 10 s, followed by 160 rpm for the remainder of the test. The heating and cooling cycles were programmed in the following manner: the samples were held at 50°C for 1 min, heated to 95°C in 3.75 min, held at 95°C for 2.5 min, cooled to 50°C in 3.75 min, and held at 50°C for 1.4 min. The peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown viscosity (BDV), setback viscosity (SBV) and peak time (PT) were recorded. The viscosity parameters were measured in Rapid Visco Units (RVU).

2.8 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

3.1.1 Effect of starch concentration on esterification

The first reaction parameter studied was the starch concentration in aqueous slurry systems. Fig. 2 showed that an increase in the concentration of starch from 20% to 35% (in proportion to water, w/w) gave a steady increase in DS and RE. Further increase in the starch concentration up to 40% decreased DS and RE. Starch and OSA are insoluble in water, so the esterification in aqueous slurry systems is a heterogeneous reaction. The chances that starch comes into contact with OSA increases with the increase in starch concentration, thus DS and RE increase correspondingly. However, when the starch concentration is higher than 40%, it becomes difficult to agitate the reaction system, thus DS and RE decrease. Compared with waxy corn starch, the optimum rice starch concentration is lower [6], it is thus dependent on the source of native starch.

3.1.2 Effect of reaction time on esterification

The effect of reaction time on DS and RE was studied (Fig. 3). Increasing the reaction time from 2 to 4 h resulted in higher DS and RE. Prolonging the reaction time beyond 4 h resulted in a decrease in DS and RE. An explanation was that as the reaction progressed, the concentration of anhydride was depleted due to esterification and hydrolysis reactions. As a result, the side reactions shown in Fig. 1 became dominant. Thus, the optimum reaction time was 4 h when OSA concentration was 3%.

Fig. 2. Effect of starch concentration on DS and RE. Reaction temperature was 35°C; pH 8.5; OSA concentration 3%; reaction time 3 h.
3.1.3 Effect of pH on esterification

The pH of the aqueous slurry is important for esterification. As a catalyst, NaOH starts the reaction by the formation of alcoholate ions along the starch polymer [3, 14]. In order to study the influence of pH, the other reaction parameters were identical to that above except that the reaction time was fixed at 3 h. Fig. 4 indicates that DS and RE decreased strongly when pH was \(< 7.5\) or \(> 9.5\). This may be explained by the fact that pH values \(> 9.5\) favor side reactions (Fig. 1), whereas at pH \(< 7.5\) the hydroxyl groups of starch are not sufficiently activated for nucleophilic attack of the anhydride moieties. Hence, pH 8.5 was used in subsequent experiments. This result was similar to that of Jeon et al. [6].

3.1.4 Effect of reaction temperature on esterification

Fig. 5 showed the positive and negative effects of temperature on DS and RE. Increase in the reaction temperature from 25°C to 35°C resulted in a large increase in DS and RE. Further increase in the reaction temperature from 35°C to 45°C decreased DS and RE. This is because an increase in temperature will enhance OSA diffusion into the starch granules as well as the swelling of the starch granules that will increase the esterification rate. However, a higher temperature will also result in enhanced solubility of OSA in the aqueous phase, which will increase hydrolysis. Thus, 35°C was concluded to be the most appropriate temperature for the OSA modification of early indica rice starch in aqueous slurry systems. The reaction temperature was higher than that used by Bao et al. [15], but the reaction time was shortened to obtain OSA starches with the similar DS.

3.1.5 Effect of OSA concentration on esterification

Fig. 6 shows the effect of OSA concentration on esterification. When the OSA concentration was increased from 3% to 15%, DS increased but RE decreased. This may result from insufficient mixing between the water-insoluble OSA and starch phases. In addition, the RE decreased by dilution when using higher amounts of anhydride and alkaline reagents. Hence, OSA concentrations of 3 to 5% (based on dry starch) are preferred for the preparation of low DS starch esters in water. If a higher conversion is needed, the dilution effect can be offset by
filtering unreacted starch from the reaction mixture and resuspending it in a smaller but defined amount of water. The esterification can be repeated using a second portion of anhydride [16].

3.2 Properties of OSA starch

3.2.1 Fourier transform infrared (FT-IR) spectroscopy

Succinylation leads to the substitution of hydroxyl groups in the starch molecules with carbonyl groups of OSA. The introduction of carbonyl groups could be confirmed by FT-IR spectroscopy [17]. The IR spectra of native and OSA modified starch are shown in Fig. 7. Native and OSA starch have similar profiles. In the fingerprint region, there are several discernible absorbencies at 1155, 1080, 1021 and 930 cm\(^{-1}\), which were attributed to C–O bond stretching [18]. Another characteristic peak occurred at 1638 cm\(^{-1}\) which presumably originates from tightly bound water present in the starch. The band at 2928 cm\(^{-1}\) is characteristic of the C–H stretching vibration. An extremely broad band resulting from vibration of the hydroxyl groups (O–H) appeared at 3390 cm\(^{-1}\). Compared with native starch, the spectrum of OSA starch shows a new peak at 1724 cm\(^{-1}\), which suggests the formation of ester carbonyl groups [17–19]. In the alkaline systems, OSA starch was synthesized in the form of starch sodium octenyl succinate (Fig. 1), thus a peak at 1572 cm\(^{-1}\) occurred, which is ascribed to the asymmetric stretching vibration of carboxylate RCOO\(^{-}\) [20, 21].
Fig. 8. SEM photomicrographs of native and OSA starch granules. (a) Native starch; (b) DS=0.018; (c) DS=0.025; (d) DS=0.046. (Micrographs of each starch sample were taken at 2000 x and 10000 x magnifications).
3.2.2 Scanning electron microscopy

Scanning electron photomicrographs of native and OSA starches from early indica rice are presented in Fig. 8. SEM investigations showed that the OSA treatment caused some changes in the structure of starch granules as compared with those of native starch. Native rice starch granules are polygonal in shape with well-defined edges, some authors reported a similar morphology of rice starch granules [22, 23]. OSA starch granules exhibited slightly rough surfaces and their edges lost some definition. The sides of these granules were porous and many cavities were observed, moreover, the damage increased with the increase of OSA treatment. This was probably because that OSA acted by first attacking the surface and forming pores on the surface. SEM photomicrographs indicated that OSA modification in aqueous slurry systems moderately altered the granular structure of rice starch.

3.2.3 X-ray diffraction

In order to study the effect of OSA modification on the crystal structure, the native and OSA starches were examined by powder X-ray diffraction. Fig. 9 indicated that both native and OSA rice starches showed typical A-type diffraction pattern with strong reflection at 15°, 17° and 23° [24, 25]. This result suggested that esterification did not change the crystalline pattern of OSA starches up to DS 0.046, although the granules of OSA starches have been damaged to some extent by the modification processes. This observation was in agreement with the finding of Wang et al. [26], who mentioned that the esterification occurred primarily in the amorphous regions and did not change the crystalline pattern of starches.

4 Conclusions

The preparation conditions of OSA starches from early indica rice starch under aqueous alkaline system were investigated. The suitable parameters were selected as follows: concentration of starch slurry 35% (in proportion to water, w/w), reaction time 4 h, pH of reaction system 8.5, reaction temperature 35°C, amount of OSA 3% (in proportion to starch, w/w). Under these conditions, DS was 0.018 and RE was 78%. FT-IR spectroscopy showed the characteristic vibration of the ester carbonyl groups in the OSA starch at 1724 cm⁻¹. SEM and X-ray diffraction showed that OSA groups acted by first attacking the sur-
face and some pores formed, but OSA modification caused no change in the crystalline pattern of rice starch up to DS 0.046. RVA results indicated that the starch derivatives gelatinized at shorter time to achieve higher viscosities with increased OSA modification. The information will be useful for promoting the use of the OSA starch in food industry.

After OSA modification, hydrophobic side chains are added to the originally merely hydrophilic starch molecule. By this means starch may adsorb to the interface of water and oil, thus stabilizing an emulsion. The stabilizing and digestibility properties of OSA modified early indica rice starches need further study.

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**References**


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