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Octenyl Succinic Anhydride Modified Early *Indica* Rice Starches Differing in Amylose Content

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Seven early *indica* rice starches with different amylose contents were modified by octenyl succinic anhydride (OSA) in aqueous suspension systems to evaluate the effect of amylose contents on starch esterification. The crystalline structure and pasting properties of starches were investigated using X-ray diffraction and a Rapid Visco Analyzer (RVA). The results indicated that the amylose content had a positive impact on the OSA modification. As the amylose content increased from 0 to 39.6%, the degree of substitution increased from 0.024 to 0.030 and the reaction efficiency increased from 62.8 to 77.5%. X-ray diffraction scans confirmed that the amylose was mainly present in the amorphous domain of the granule and was highly substituted after the OSA treatment. The RVA profiles demonstrated that the OSA starches had higher viscosities than their native counterparts. Moreover, negative correlations were observed between the amylose content and the major RVA parameters (e.g., peak viscosity, hot paste viscosity, cool paste viscosity, and breakdown viscosity).

KEYWORDS: Early *indica* rice; OSA starch; amylase content; relative crystallinity; pasting properties

INTRODUCTION

Rice is one of the major cereals worldwide and the staple food for people in Asia. Its starch is one of the primary ingredients of various food products. Like other starches, rice starch is composed of amylose and amylopectin that differ in both size and structure. Amylose is essentially a linear (1→4)-linked polymer of α-D-glucose with a few (1→6) branches. It is located mainly in the amorphous domains of the starch granule (*1*). In contrast, amylopectin is one of the largest naturally occurring macromolecules mainly composed of α-(1→6)-branched (1→4)-α-D-glucan. Amylopectin side chains make up the framework of the crystalline lamellae, whereas branching points are located in the disordered amorphous domains between the crystallites (*2*).

To meet different industrial applications, native starch is often modified to overcome its inherent shortcomings for a desirable and improved functionality. The modifications 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 (*3*). Because the OSA starch is considered to be an effective emulsifier due to the addition of dual functional hydrophilic and hydrophobic groups (*4*), it has been widely used in various food products such as sauces, puddings, and baby foods for more than 30 years. Recently, OSA starch was reported to have special nutritional values. Heacock et al. (*5*) found that esterification of starch with OSA could impair the binding of α-amylase, thus decreasing the extent of starch digestion. Their study indicated that the OSA starch appeared to be a resistant starch, which could be used as a functional fiber for the treatment of certain diseases.

Although OSA starches have been investigated regarding their preparative conditions, pasting and digestive properties, etc. (*6*−*10*), relatively little work has been done on the influence of amylose content (AC) on the OSA modification. The AC in starch affects many significant physical, chemical, and functional properties such as pasting temperature, viscosity, gel stability, water solubility, and the degree of resistance of the starch granules to in vitro digestion by amylases (*11*). These property changes in turn will affect the range of industrial applications of the starch. Such knowledge will be helpful in understanding the physical behavior of OSA starches as well as in the design of modified starches with new and improved properties.

In this study, the OSA starches were prepared from early *indica* rice starches with seven levels of AC. These cultivars are widely grown in southern China, but their inferior qualities lead them to lower prices on the national market (*12*). Therefore, these rice cultivars were selected in this study in an effort to improve their starch values through the OSA modification. The crystalline and pasting properties of the starches were investigated using X-ray diffraction and a Rapid Visco Analyzer (RVA). It is hoped that the results may give a deeper insight into the changes in esterification, crystalline, and pasting

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properties of OSA modified early *indica* rice starches related to varying amylose contents.

MATERIALS AND METHODS

Materials. The early *Indica* cultivars used for this study were G16, Jiazao312, M211, Hesheng No. 1, ZR9, R26, and R111, which were kindly provided by Dr. Dian-Xing Wu (Institute of Nuclear Agricultural Sciences, Zhejiang University, China). High-purity OSA was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). The other chemicals used in the study were of analytical grade.

Isolation of Starch. Rice starch was isolated by alkali extraction of proteins (*13*). Milled rice was steeped in a 0.1 M (0.4%, w/w) NaOH solution in a ratio of 1:2.5. The rice slurry was disintegrated for 3 min using a 50 μm milling slit of colloid mill (JM-L, Seven Stars Dairy Equipment Factory, Longwan, China). The slurry was agitated for 10 h at 35 °C and then centrifuged at 2000*g* for 10 min. The supernatant was discarded, and the sediment was washed twice with tap water (~3 times the sediment weight) and centrifuged. The residue was suspended again in tap water and adjusted to pH 7.0 by adding 3% HCl solution, and the slurry was centrifuged. The supernatant was discarded, and the dark tailing layer atop the starch was carefully scraped away and discarded. The starch was washed two times with tap water until the tailing fraction became negligible after centrifuging. The starch was dried in a convection oven at 40 °C for 24 h and then passed through a 180-mesh nylon sieve (90 μm opening).

Chemical Composition. Moisture (*14*), protein (*15*), and fat (*16*) contents of the isolated starches were determined according to American Association of Cereal Chemists (AACC) standard methods. The crude protein content was calculated by adopting 5.95 as a conversion factor (*17*). AC of the isolated starch was determined by using a standard iodine-binding method (*18*).

Preparation of Octenyl Succinylated Starches. Granular *indica* rice starch (30 g, dry weight) was suspended in distilled water (30%, w/w) with agitation. The pH of the suspension was maintained at 8.5 using a 3% NaOH solution. OSA (1.5 g) was added (diluted 3 parts with absolute alcohol) slowly in 2 h. The reaction was allowed to proceed for a total of 5 h at 35 °C. After the reaction, the pH was adjusted to 6.5 with a 3% HCl solution. The mixture was centrifuged and washed two times with distilled water and two times with 70% ethanol. Then the solid was oven-dried at 40 °C for 24 h before being passed through a 180-mesh nylon sieve (90 μm opening).

Determination of Degree of Substitution (DS). DS is the average number of hydroxyl groups substituted per glucose unit. The DS of OSA starch was determined using a titration method (*19*). The OSA starch (5.0 g) was accurately weighed and dispersed in 25 mL of 2.5 M HCl−isopropyl alcohol solution by stirring for 30 min. A total of 100 mL of 90% isopropyl alcohol solution (in proportion to water, v/v) was added and stirred for an additional 10 min. The suspension was filtered through a glass filter, and the residue was washed with 90% isopropyl alcohol solution until no Cl^- was detected (using 0.1 M $AgNO_3$ solution). The starch was redispersed in 300 mL of 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 control. The DS was calculated by using the equation (*19*)

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

where A is the titration volume of NaOH solution (mL), M is the molarity of the NaOH solution, and W is the dry weight (g) of the OSA starch.

The reaction efficiency (RE) was calculated as follows:

$$\mathrm{RE} = \frac{\text{actual DS}}{\text{theoretical DS}} \times 100\%$$

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

Table 1. Proximate Composition of *Indica* Rice Starches

cultivar	moisture content (%)	protein content[a] (%)	fat content[a] (%)	amylose content[a] (%)
G16	9.09	0.30	0.24	0
Jiazao312	9.00	0.19	0.23	11.9
M211	8.61	0.20	0.20	15.1
Hesheng No. 1	8.36	0.30	0.21	18.8
ZR9	9.70	0.38	0.22	24.9
R26	9.01	0.28	0.22	36.0
R111	8.72	0.26	0.20	39.6
LSD[b]	0.09	0.02	0.02	0.48

[a] On dry weight basis. [b] Least significant difference, $P < 0.05$.

X-ray Diffraction Measurements. The X-ray patterns of starches were obtained using an X-ray diffractometer (D/max-Ra, Rigaku Inc., Tokyo, Japan) operated at 80 mA and 40 kV. The scanning region of the diffraction angle (2θ) was from 5 to 50 at 0.02° step size. The relative crystallinity of starch samples was quantitatively estimated according to the method described by Komiya and Nara (*20*). The ratio of upper crystalline area to total diffraction area was calculated as the relative crystallinity. The starch samples were equilibrated at 40 °C for 24 h prior to the analysis.

Pasting Viscosity. The pasting properties were determined using a Rapid Visco Analyzer (RVA, model-3D, Newport Scientific Inc., Warriewood, Australia). The analysis was based on the AACC standard program for RVA analysis with some modifications (*21*). Samples were prepared by mixing 2.0 g of starch and 25 mL of distilled water. The mixture was stirred manually for 1 min to facilitate dispersion before testing (*22*). 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), and setback viscosity (SBV) were recorded. The viscosity parameters were measured in rapid viscosity units (RVU).

Statistical Methods. Analysis of variance (ANOVA) was performed using Duncan's multiple-range test to compare treatment means. Significance was defined at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical Composition of *Indica* Rice Starches. Amylose, moisture, crude protein, and crude fat contents of the isolated rice starches are shown in **Table 1**. The native rice starches had a wide range of AC (0−39.6%). Low total residual protein contents and crude fat contents in the isolated starches indicated the successful isolation of starches from whole kernels. These starch samples provided us a good platform for studying the relationship between AC and esterification.

Effect of AC on DS and RE. Previous studies found that the reactivities of amylose and amylopectin with methyl (*23*), propylene oxide (*24*), and acetyl (*25*) were different. Van der Burgt et al. (*23*) reported that methylation of starch granules within an aqueous suspension took place preferably in amylose compared with amylopectin. **Figure 1** shows that the AC has a positive impact on OSA modification. When the AC increased from 0 to 39.6%, the DS increased from 0.024 to 0.030 and the RE increased from 62.8 to 77.5%.

The preferential esterification of OSA to amylose versus amylopectin is in agreement with the model of the starch granule in which amylose is present in the amorphous domains (*1*, *2*). These domains are better accessible than crystalline lamellae that are constructed by amylopectin side chains. Therefore, structural difference between amylose and amylopectin is considered to be the causative factor for their different activities

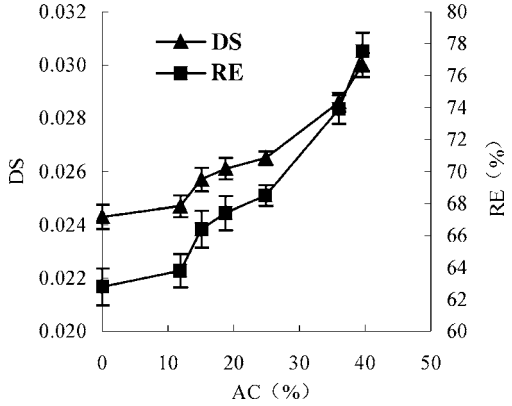

Figure 1. Effect of amylose content on DS and RE.

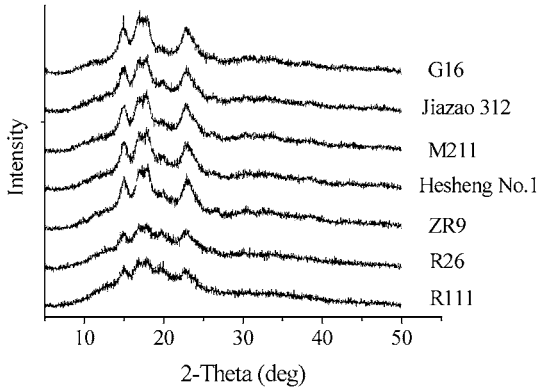

Figure 2. X-ray diffraction scans of native starches.

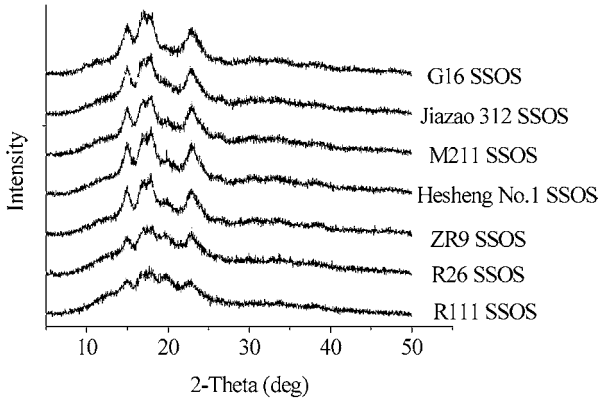

Figure 3. X-ray diffraction scans of OSA starches.

to OSA. This also helped us understand the apparent physical behavior of the OSA starches and facilitate the potential design of modified starches with new and improved properties.

X-ray Diffraction Patterns and Relative Crystallinity of Starches. The X-ray diffraction scans of native and OSA starches are presented in **Figures 2** and **3**, respectively. The corresponding relative crystallinity values calculated from the ratio of diffraction peak area and total diffraction area are given in **Table 2**. The X-ray diffraction patterns were comparable to those reported earlier for cereal starches. Both native rice starches and OSA rice starches showed typical A-type diffraction patterns with strong reflection at 15, 17, and 23° (*26*). Although no differences were noted between the native and OSA-modified rice starches in the X-ray diffraction pattern, the crystallinities of the native starches were consistently slightly higher than their esters. This result was in agreement with the report of Wang et al. (*27*), who indicated that the esterification occurred primarily

Table 2. Comparison of Relative Crystallinity of Native and OSA Starches

cultivar	AC (%)	native starches (%)	OSA starches (%)
G16	0	28.6	25.0
Jiazao312	11.9	24.5	22.5
M211	15.1	23.9	22.0
Hesheng No. 1	18.8	22.4	21.9
ZR9	24.9	21.2	21.0
R26	36.0	16.8	14.2
R111	39.6	15.0	13.3
LSD[a]	0.48	0.22	0.23

[a] Least significant difference, $P < 0.05$.

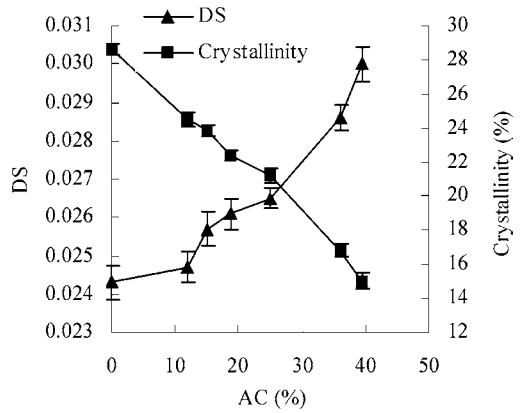

Figure 4. Effect of amylose content on crystallinity and DS.

in the amorphous regions and did not change the crystalline patterns of the starches.

Effect of AC on Crystallinity and DS. The degree of crystallinity of native rice starch was significantly influenced by AC. For instance, the relative crystallinity decreased from 28.6 to 15.0% as the AC increased from 0 to 39.6% (**Figure 4**). This agrees with the fact that the amylose is located in amorphous domains (*1*, *2*).

Figure 4 also displays a negative correlation between the crystallinity and DS. When the DS increased from 0.024 to 0.030, the corresponding crystallinity decreased from 28.6 to 15.0%. This observation corroborated the previous studies on differences in substitution behavior between amorphous and crystalline domains, wherein it was found that the former domains contained more substituents (*2*). Amylose is located in amorphous domains and is therefore better accessible to esterification.

RVA Profiles of Native and OSA Starches. RVA is a useful tool for the rapid evaluation of cooking and processing properties of cereal flours, starches, and their modified products. The measurement can be accomplished during a short testing time, and only a small amount of sample is required. The RVA profiles of native and OSA starches are displayed in **Figures 5** and **6**, respectively.

Viscosity curves reveal the effect of OSA treatment on the pasting properties of various *indica* rice starches. In general, OSA starches had higher viscosities than their native counterparts. The incorporation of a bulky group such as OSA enhanced the overall pasting capacity of the starches, and the modified starches tended to paste more extensively. The native starches had lower rises in the pasting curves, reflecting their relatively ordered structure and resistance to paste. This result was in agreement with our previous studies on crystallinity, wherein we reported that OSA starches had slightly lower degrees of crystallinity than their native starches (**Table 2**).

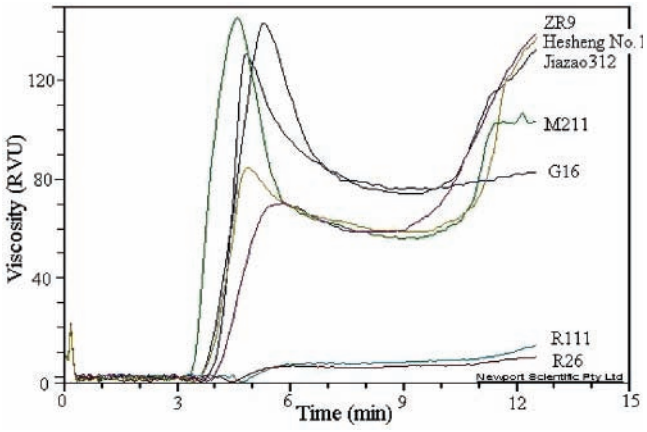

Figure 5. RVA pasting profiles of native starches.

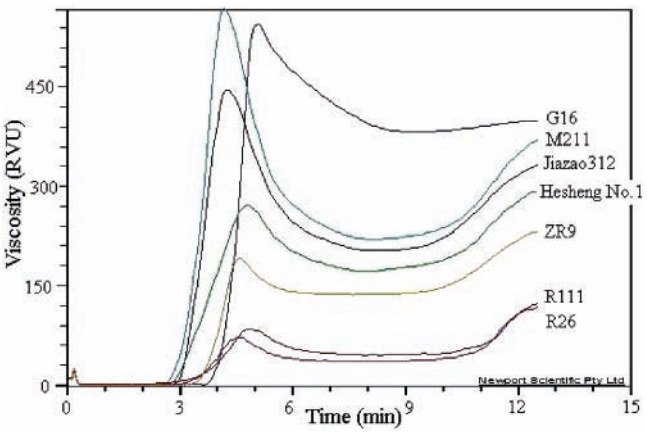

Figure 6. RVA pasting profiles of OSA starches.

Table 3 lists the major parameters of starch paste viscosity such as PKV, HPV, CPV, BDV, and SBV. The major RVA parameters indicated that the OSA starches had higher viscosities than the native starches. Moreover, negative correlations were observed between AC and the major RVA parameters. Four major parameters of the RVA profile, that is, PKV, HPV, CPV, and BDV, considerably decreased with the increase of AC. For example, when AC values increased from 0 to 39.6%, PKV decreased from 130.1 to 5.1 RVU for native starches and PKV decreased from 543.7 to 82.2 RVU for the OSA starches.

Correlation Coefficients between AC and RVA Parameters. The AC is important for structural and thermodynamic as well as functional and technological properties of starches from various botanical sources (*11*, *28*, *29*). The correlations between AC and RVA parameters for native and OSA modified rice starches are shown in **Table 4**.

For the native and OSA starches, AC was negatively correlated with PKV, HPV, BDV, and CPV, but positively correlated with SBV (**Table 4**). Compared with the native starches, the OSA starches had higher correlation coefficients. After the OSA modification, viscosities of rice starches increased greatly (**Figures 5** and **6**). Because the seven cultivars had significantly increased their viscosities after the OSA modification, they could be used to meet demands for different food products.

This study showed that the degree of changes in pasting properties of the OSA modified starches depended not only on their DS but also on the cultivars of the native starches.

Hu et al. (*30*) studied the digestibility of starches with different AC. They found the contents of resistant starch increased with the increasing amylose in the same type of rice. In our study, seven cultivars (i.e., G16, Jiazao312, M211, Hesheng No. 1, ZR9, R26, and R111) have different AC, so their contents of resistant starch may be different. After OSA modification, they will exhibit better paste clarity, increased resistance to retrogradation, and improved texture (*7*) and can be used in different food products.

Conclusions. The effect of varying AC on the OSA modification of early *indica* rice starch has been studied. The result revealed that the AC had a positive impact on the OSA modification in aqueous suspension systems. X-ray diffraction scans confirmed that amylose was mainly present in the amorphous domain of the granule and was therefore highly substituted. RVA profiles showed OSA starches had higher viscosities than their native counterparts. Moreover, negative correlations were observed between AC and the major RVA parameters (e.g., PKV, HPV, CPV, and BDV). However, the

Table 3. Major RVA Parameters of Native and OSA Starches

	native starches[a] (RVU[b])					OSA starches[a] (RVU)				
cultivar	PKV	HPV	CPV	BDV	SBV	PKV	HPV	CPV	BDV	SBV
G16	130.2	75.8	82.3	54.4	–47.8	543.8	380.3	397.5	163.5	–146.3
Jiazao312	142.9	73.6	132.5	69.3	–10.4	443.8	201.3	330.3	242.4	–113.4
M211	145.3	55.7	102.4	89.6	–42.8	566.8	217.8	369.3	349.0	–197.6
Hesheng No. 1	84.5	58.5	137.4	26.0	52.9	269.8	169.5	292.5	100.3	22.7
ZR9	69.8	58.3	138.7	11.5	68.9	188.8	134.9	231.2	53.9	42.3
R26	4.0	3.2	7.4	0.8	3.4	69.5	34.1	115.8	35.4	46.3
R111	5.2	4.8	12.3	0.4	7.2	82.3	43.3	122.1	38.9	39.8

[a] Mean of two determinations. [b] Rapid viscosity units.

Table 4. Pearson Correlation Coefficients (*r*) between AC and RVA Parameters for Native and OSA Starches

	native starches[a]					OSA starches[a]				
	PKV	HPV	BDV	CPV	SBV	PKV	HPV	BDV	CPV	SBV
AC	–0.91**	–0.92**	–0.77*	–0.61	0.46	–0.91**	–0.97**	–0.64	–0.96**	0.78*
PKV		0.91**	0.93**	0.72	–0.45		0.89**	0.89**	0.96**	–0.96**
HPV			0.70	0.87*	–0.14			0.57	0.93**	–0.77*
BDV				0.49	–0.65				0.78*	–0.93**
CPV					0.30					–0.85*

[a] *, significant at 0.05 level; **, significant at 0.01 level.

digestibility properties of the OSA-modified early *indica* rice starches with different AC need further study.

ABBREVIATIONS USED

OSA, octenyl succinic anhydride; AC, amylose content; RVA, Rapid Visco Analyzer; DS, degree of substitution; RE, reaction efficiency; PKV, peak viscosity; HPV, hot paste viscosity; CPV, cool paste viscosity; BDV, breakdown viscosity; SBV, setback viscosity; RVU, rapid viscosity units.

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