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# Hydrolysis process of normal rice starch by 1-butanol–hydrochloric acid

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ABSTRACT

The aim of this study was to examine 1-buatnol–hydrochloric acid (1-butanol–HCl) hydrolysis process of starch. Normal rice starch was hydrolyzed by 0.36% HCl in anhydrous 1-butanol at 40 °C from 4 h to 7 days. The changes in starch granule, crystalline, and molecular weight during the 1-butanol–HCl hydrolysis were determined using SEM, particle size analyzer, XRD, HPSEC–MALLS–RI, and iodine staining. SEM and particle size analyzer analysis showed that the granule morphology significantly changed and the granule size decreased from 5.47 μm to 5.07 μm after the hydrolysis by 1-butanol–HCl for 7 days. XRD results indicated that amorphous areas of normal rice starch were firstly hydrolyzed followed by the ordered areas during 1-butanol–HCl hydrolysis. The peak ($2\theta$) of V-type crystallinity was detected at around 20° in the XRD curves of 1-butanol–HCl-hydrolyzed starches. This peak confirmed the formation of amylose–1-butanol complex. HPSEC–MALLS–RI and iodine staining analysis demonstrated that the weight-average molecular weight of normal rice starch sharply decreased from $2.4 \times 10^7$ Da to $4.9 \times 10^5$ Da after 1-butanol–HCl hydrolysis for 4 h, then gradually decreased, and finally stabilized at about $2.6 \times 10^4$ Da after hydrolysis for 4 days. These results suggest that the attack of 1-butanol–HCl on normal rice starch preferentially occurred in the amylopectin molecules of amorphous areas.



## 1. Introduction

Acid hydrolysis has been widely investigated to modify native starches and create products for their application in food, paper, textile, and other industries (Li, Gao, Wang, Jiang, & Huang, 2010). It is also a useful tool to understand the inner structure of starch granules. Traditionally, acid hydrolysis of starch is achieved by degrading starch by acid in water. Recently, a new hydrolysis method, alcohol–acid hydrolysis, has attracted considerable interest. Compared with traditional acid hydrolysis, alcohol–acid hydrolysis shows altered properties, such as higher recovery of starch granules, less dextrin with low molecular weight, and narrower molecular weight distribution of the product (Chang, Lin, & Lii, 2004; Lin, Lee, & Chang, 2003; Ma & Robyt, 1987; Small, 1919).

Generally, the limiting average degree of polymerization (DP) of alcohol–acid-hydrolyzed starch progressively decreased in the sequence of methanol > ethanol > 2-propanol > 1-butanol (Ma & Robyt, 1987). Therefore, it was postulated that different alcohols induced different concentrations of acid inside starch granules. Moreover, the average DP of alcohol–acid-hydrolyzed starch depends upon its botanical source, acid concentration, concentration of alcohol, and treatment temperature (Chang, Lin, & Pan, 2010; Dutta, Paul, Kalita, & Mahanta, 2011; Lin, Pan, Hsu, Singh, & Chang, 2012).

The effects of alcohol–acid treatment on the physicochemical properties and molecule structures of different starches have been widely carried out (Chang, Lin, & Chang, 2006; Chung & Lai, 2006; Ferrini, Rocha, Demiate, & Franco, 2008; Jiang, Gao, Li, Zhang, & Huang, 2011; Luo, Fu, Gao, & Yu, 2011). For instance, most reports on alcohol–acid modification of starch concerned the effects of acid concentration, starch concentration, water content and alcohol types on these physicochemical properties. Nevertheless, the alcohol–acid hydrolysis process of rice starches has not been demonstrably investigated. Therefore, the objective of this study

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was further to systematically investigate the alcohol–acid hydrolysis process of normal rice starch from changes in starch granule (including morphology and size), crystallinity, and molecular weight during the hydrolysis by 1-buatnol–HCl for 7 d, which were detected by scanning electron microscopy (SEM), particle size analyzer, wide angle X-ray diffraction (XRD), high-performance size-exclusion chromatography coupled with a multi-angle laser light-scattering detector and a refractive index detector (HPSEC–MALLS–RI), and iodine staining.

## 2. Materials and methods

### 2.1. Materials

Normal rice starch was purchased from Shandong Mei-Jing Rice Inc. (Shandong, China). Normal rice starch was defatted using Soxhlet extraction with 85% aqueous methanol for 24 h (Chung, Jeong, & Lim, 2003). Methanol, 1-butanol, hydrochloric acid (HCl), and sodium nitrate ($NaNO_3$) were purchased from Sinopharm Chemical Reagent Co., Ltd. Dimethyl sulfoxide (DMSO) was HPLC-graded from Aladdin Reagents (Shanghai) Co., Ltd. All other chemicals and reagents were of analytical grade unless otherwise stated.

### 2.2. 1-Butanol–HCl hydrolysis of starch samples

The 1-butanol–HCl hydrolysis of normal rice starch was performed according to the procedure described previously (Chang et al., 2006) with minor modification. Normal rice starch with moisture content at 9.6% (25 g) was dispersed in 100 ml anhydrous 1-butanol. The reaction was started by adding 1 ml of concentrated (36% by weight) HCl and proceeded at 40 °C for 4 h, 8 h, 12 h, 16 h, 20 h, 24 h, 2 d, 3 d, 4 d, 5 d, 6 d, and 7 d with constantly stirring. The reaction was stopped by adding 14 ml of 1 M $NaHCO_3$. The solution was cooled in ice bath for 15 min and centrifuged at 3500 *g* for another 5 min. The precipitate was washed four times with 50% ethanol and dried in an air oven at 35 °C.

### 2.3. SEM analysis

Native and 1-butanol–HCl-hydrolyzed normal rice starches were adhered to a specimen holder using a silver plate and coated with a thin film of gold (10 nm) in a vacuum evaporator. The obtained specimens were observed in a scanning electron microscope (Quanta-200, FEI Company, Netherlands).

### 2.4. Particle size distribution

Native and 1-butanol–HCl-hydrolyzed normal rice starches (100 mg) were dispersed in 5 ml of water and stirred at 200 rpm for 10 min. The dispersion was then swiftly transferred into the sample cell of the particle size analyzer (Laser S3500, Microtrac, Montgomeryville, PA, USA). The flow rate of water was set at 60 ml/s and run for 30 s. Each sample was analyzed in triplicate.

### 2.5. XRD analysis

The crystalline type and the relative crystallinity of native and 1-buatnol–HCl-hydrolyzed normal rice starches were analyzed by X-ray diffractometer (D8 Advance, Bruker, Germany) at 40 kV and 40 mA with Cu Kα radiation ($\lambda = 0.154$ nm). The starch powder was packed tightly in a rectangular glass cell and scanned over the range of 4°–35° in the step of 4°/min and step size of 0.02° at 25 °C.

### 2.6. Molecular weight analysis

The weight-average molecular weight of native and 1-butanol–HCl-hydrolyzed normal rice starches was determined by HPSEC–MALLS–RI according to the procedure described previously (Yokoyama, Renner-Nantz, & Shoemaker, 1998) with little modification. Starch solution was prepared by dissolving 50 mg (db) of starch with 10 ml of 50 mM $NaNO_3$ in HPLC-graded dimethyl sulfoxide (50 mM $NaNO_3$-DMSO) solution in a boiling water bath for 1 h with constantly stirring and continuously stirred at 25 °C for 24 h. Each starch solution was then filtered through a 0.45 μm syringe filter (Millipore, Billerica, MA, USA). The resultant filtrate (0.1 ml) was injected into a HPSEC system. The system consisted of a pump (LC-20A, Shimadzu, Co., Kyoto, Japan), a MALLS detector (Dawn DSP, Wyatt Tech., Santa Barbara, CA, USA), and a RI detector (Waters 2414 differential refractometer). The columns used were Styragel HMW7 and Styragel HMW6 columns (Styragel, Waters, Milford, MA) connected in series and kept at 35 °C. The mobile phase was 50 mM $NaNO_3$-DMSO solution at a flow rate of 0.6 ml/min. The electronic outputs of the RI and MALLS detectors were collected by ASTRA software (version 5.4.3, Wyatt Tech.) and were used to determine the molecular weight of starch samples.

### 2.7. Iodine staining

Starch solution was prepared by dissolving 75 mg (db) of starch with 15 ml of 90% dimethyl sulfoxide (DMSO) solution in a boiling water bath for 1 h with constantly stirring, and then continuously stirred at room temperature for 24 h. The starch solution (0.1 ml) was diluted with deionized water (9.5 ml). The iodine solution (0.2 ml, 1 ml iodine solution containing 2 mg $I_2$ and 20 mg KI) was added. The mixture was brought to 10 ml with deionized water and immediately mixed. Afterwards it was left to stand for 15 min, the absorption spectra and the $\lambda_{max}$ were obtained by scanning in the range from 400 nm to 800 nm using a UV–Vis spectrophotometer (TU-1900, Rayleigh Analytical Instruments, Beijing, China).

### 2.8. Statistic analysis

Statistical analysis was performed using ORIGIN 8.0 program (OriginLab Inc., USA). Data were expressed as means ± standard deviations of at least three determinations on one sample for each time period and analyzed by a one-way analysis of variance (ANOVA). *P* value $\leq 0.05$ was regarded as significant throughout the study.

## 3. Results and discussion

### 3.1. SEM analysis

The granule morphology change of normal rice starch during 1-butanol–HCl hydrolysis was analyzed by SEM. Nature rice starch granules showed irregular polygonal shape with the diameter around 5 μm and smooth surfaces (Fig. 1a). After 1-butanol–HCl hydrolysis for 1 d, the great majority of the starch granules still maintained their original polygonal shapes but some starch granules presented slight fractures on the surface (Fig. 1b and c), suggesting that 1-butanol–HCl hydrolysis occurred to the surface of starch granules. Thereafter, several phenomena were observed for the longer hydrolysis time (Fig. 1d–f). First, deformations on the surfaces of 1-butanol–HCl-hydrolyzed starch granules occurred. Second, some starch granules were combined together, which was evidence of adhesion between 1-butanol–HCl-hydrolyzed starch granules. Third, most starch granules changed to pieces, indicating 1-butanol–HCl disruption of the granule interior. However, these