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# Effects of soy protein hydrolysates on maize starch retrogradation studied by IR spectra and ESI-MS analysis

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ABSTRACT

Starch retrogradation is the main cause of quality deterioration of starch-containing foods during storage. The purpose of this study is to find out whether certain soy protein polypeptide in hydrolysates will retard maize starch retrogradation. The results show that all soy protein hydrolysates retard maize starch retrogradation to a certain extent. The IR spectra of hydrolysates and the blends of hydrolysates and maize starch show that the polypeptides might act with reducing end of maize starch during retrogradation. The results of electrospray ionization-mass spectrometry [ESI-MS] show that the polypeptide ($m/z$ 863) is present in all three hydrolysates remarkedly retarding maize starch retrogradation and its relative abundence is also the highest. So the polypeptide containing seven amino acids probably is the key component to significantly inhibit maize starch retrogradation.



## 1. Introduction

Retrogradation is the main cause of quality deterioration of starch-containing foods during storage [1]. Soy proteins are often employed as ingredients in those foods [2–6], but they are seldom used to inhibit starch retrogradation, which may be due to the molecular aggregation of soy protein in food processing. Such aggregation hinders the interaction between soy protein and starch, so addition of soy protein cannot retard starch retrogradation. In the trial experiment, it is found that certain soy protein hydrolysates derived from acidic protease inhibit maize starch retrogradation significantly. The reason for such inhibition probably lies in the syneresis of the protein–corn starch gel [7], gelatinization temperature increasing [8], and forming of a more elastic than viscous behavior [9]. Soy protein is mainly composed of glutamic acid, arginine, aspartic acid, leucine, lysine and phenylalanine [10–13]. The main components of soy protein hydrolysates are polypeptides composed of those amino acids. In this paper, commercial acidic, neutral and alkaline proteases are used to hydrolyze soy protein into polypeptide and amino acids. The various water-soluble polypeptides in soy protein hydrolysates will interact with maize starch chain and have an influence on maize starch retrogradation in the food processing. IR spectra of maize starch and mixture of starch and hydrolysates are investigated with the aid of ESI-MS. Furthermore, the possible linkages between polypeptide and maize starch are also proposed.

## 2. Materials and methods

### 2.1. Materials

Maize starch (moisture content 35.6%, amylose content 27.8%) was offered by Shan Dong Jin-Cheng Co., Ltd. Butanol and ethanol were purchased from Tianjin Fuyu Fine Chemical Co., Ltd. Soy protein was offered by Ji Lin BuER Protein Limited Company. Microbial proteases (acidic, alkaline and neutral proteases), specifically catalyzing the hydrolysis of peptide bonds linked by amino acids such as Glu, Leu or Arg, were purchased from Tianjin Nuoao Sci & Tech Development Co., Ltd.

### 2.2. Methods

#### 2.2.1. Hydrolysis of soy protein by acidic, alkaline and neutral proteases

1 g soy protein was mixed with 100 mL water with stirring, and then 0.01 g proteases (20,000 U/g) were added to hydrolyze for 5 h, 7 h and 9 h at 25, 45, 55 °C. The pH of the mixtures

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**Table 1**
Effects of soybean protein hydrolysis by different proteases on retrogradation rate of maize starch.

| Hydrolysis temperature (°C) | pH of enzymatic solutions | Hydrolysis time (h) | Retrogradation rate (%) | | |
|---|---|---|---|---|---|
| | | | Acidic protease | Alkaline protease | Neutral protease |
| | | 5.0 | 5.5 ± 0.1 | 11.4 ± 0.1 | 6.8 ± 0.2 |
| 25 | 1.0 | 7.0 | 15.6 ± 0.2 | 4.9 ± 0.4 | 9.7 ± 0.1 |
| | | 9.0 | 7.1 ± 0.2 | 9.7 ± 0.3 | 5.3 ± 0.1 |
| | | 5.0 | 5.2 ± 0.3 | 14.2 ± 0.3 | 9.5 ± 0.2 |
| 45 | 1.0 | 7.0 | 16.2 ± 0.1 | 9.8 ± 0.2 | 12.5 ± 0.3 |
| | | 9.0 | 18.1 ± 0.1 | 12.0 ± 0.4 | 8.1 ± 0.4 |
| | | 5.0 | 7.4 ± 0.2 | 2.7 ± 0.1 | 8.3 ± 0.4 |
| 55 | 1.0 | 7.0 | 16.3 ± 0.3 | 15.2 ± 0.2 | 12.0 ± 0.1 |
| | | 9.0 | 8.8 ± 0.1 | 12.4 ± 0.2 | 8.3 ± 0.2 |
| | | 5.0 | 14.2 ± 0.3 | 13.5 ± 0.3 | 12.6 ± 0.3 |
| 25 | 3.0 | 7.0 | 13.4 ± 0.4 | 13.2 ± 0.2 | 10.7 ± 0.1 |
| | | 9.0 | 10.6 ± 0.4 | 14.7 ± 0.2 | 12.1 ± 0.2 |
| | | 5.0 | 9.9 ± 0.1 | 15.8 ± 0.3 | 8.7 ± 0.3 |
| 45 | 3.0 | 7.0 | 13.3 ± 0.3 | 15.1 ± 0.4 | 11.8 ± 0.2 |
| | | 9.0 | 12.7 ± 0.2 | 14.6 ± 0.2 | 12.8 ± 0.4 |
| | | 5.0 | 10.9 ± 0.1 | 14.8 ± 0.2 | 12.9 ± 0.2 |
| 55 | 3.0 | 7.0 | 11.4 ± 0.2 | 14.9 ± 0.3 | 12.7 ± 0.3 |
| | | 9.0 | 17.6 ± 0.1 | 13.3 ± 0.4 | 10.5 ± 0.1 |
| | | 5.0 | 10.7 ± 0.1 | 11.9 ± 0.4 | 8.2 ± 0.2 |
| 25 | 5.0 | 7.0 | 9.3 ± 0.2 | 9.7 ± 0.1 | 10.3 ± 0.1 |
| | | 9.0 | 11.3 ± 0.1 | 16.0 ± 0.3 | 9.1 ± 0.3 |
| | | 5.0 | 8.7 ± 0.1 | 9.8 ± 0.1 | 12.1 ± 0.1 |
| 45 | 5.0 | 7.0 | 7.8 ± 0.1 | 11.8 ± 0.1 | 14.2 ± 0.2 |
| | | 9.0 | 5.5 ± 0.2 | 12.4 ± 0.2 | 14.6 ± 0.3 |
| | | 5.0 | 12.8 ± 0.4 | 12.5 ± 0.2 | 15.8 ± 0.3 |
| 55 | 5.0 | 7.0 | 7.8 ± 0.1 | 14.6 ± 0.2 | 10.4 ± 0.1 |
| | | 9.0 | 12.4 ± 0.2 | 16.6 ± 0.4 | 10.6 ± 0.3 |
| | | 5.0 | 11.0 ± 0.2 | 8.5 ± 0.1 | 15.0 ± 0.4 |
| 25 | 7.0 | 7.0 | 11.0 ± 0.2 | 14.2 ± 0.4 | 9.0 ± 0.1 |
| | | 9.0 | 9.8 ± 0.1 | 14.7 ± 0.3 | 11.7 ± 0.2 |
| | | 5.0 | 10.0 ± 0.1 | 14.2 ± 0.2 | 13.7 ± 0.2 |
| 45 | 7.0 | 7.0 | 17.5 ± 0.1 | 14.3 ± 0.2 | 18.5 ± 0.3 |
| | | 9.0 | 13.5 ± 0.4 | 13.6 ± 0.3 | 19.1 ± 0.4 |
| | | 5.0 | 11.3 ± 0.2 | 12.0 ± 0.1 | 11.5 ± 0.3 |
| 55 | 7.0 | 7.0 | 18.7 ± 0.3 | 12.7 ± 0.3 | 8.3 ± 0.2 |
| | | 9.0 | 4.5 ± 0.1 | 13.1 ± 0.2 | 9.4 ± 0.3 |

The retrogradation rate of maize starch without soy protein hydrolysates is 20 ± 0.2%.

was adjusted to 1, 3, 5, 7 respectively by 6.0 M HCl or 2.0 M NaOH. The proteases were inactivated by being boiled for ten minutes. Then the enzymatic hydrolysates were obtained by centrifuge (3040 × *g* for 10 min) and water was supplemented to a constant volume 100 mL. Subsequently the pH of hydrolysates was adjusted to 6.0 by the slow addition of 3.0 M HCl or 3.0 M NaOH to stirred solutions. Such pH of hydrolysates solutions is favorable to maize starch retrogradation. Dried hydrolysates for IR spectra were obtained by removing water with hot air at the temperature of 55–60 °C in drying oven until constant weight.

### 2.2.2. Preparation and isolation of retrograded maize starches

The retrograded maize starches were prepared according to our previous method [14]. 10 g maize starches blended with 100 mL distilled water were gelatinized for 20 min at 95 °C by continuous stirring. Gelatinized starches were autoclaved at 120 °C for 30 min and retrograded at 4 °C for 24 h. Then 0.6 mL $\alpha$-amylase (12,000 U/mL) was added to hydrolyze not-retrograded starch into soluble carbohydrates for 6 h at 90 °C. Crude retrograded maize starch was precipitated by centrifuge (3040 × *g* for 5 min) and the supernatant fraction was discarded. The crude retrograded maize starch was washed with de-ionized water for three times to remove soluble impurities, and the purified retrograded starches were dried with hot air at the temperature of 55–60 °C in drying oven until constant weight.

The retrogradation rate (%) was calculated according to the following equation:

$$\text{retrogadation rate}\,(\%) = \left(\frac{m}{m_0}\right) \times 100,$$

where $m$ and $m_0$ were the weight of purified retrograded starch and native starch respectively.

The retrograded maize starch samples derived from maize starch blended with soy protein hydrolysates were prepared under the same treatment except that 100 mL distilled water was substituted by 100 mL soy protein hydrolysates.

All the samples duplicate five times and the average values of the samples were calculated, the significant deviation was calculated by subtracting values of the samples and of their averages.

### 2.2.3. Isolation of amylose and amylopectin in retrograded starches

The purified retrograded starches were dissolved in 4 M potassium hydroxide followed by neutralizing with 6 M hydrochloric acid. Then the amylose in retrograded maize starch was precipitated from the solution by adding 3 times the volume of 1-butanol, further isolation was done by centrifuge (3040 × *g* for 5 min). The supernatants, containing amylopectin, were collected, concentrated, and precipitated with excess ethanol. The amylopectin in supernatants was isolated by centrifuge (3040 × *g* for 5 min). The dry method of those amylose and amylopectin is the same as stated above.