Single Factor Experimental Study on The Preparation of Chitosan Oligosaccharide

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Abstract. Chitosan oligosaccharides were prepared by papain enzymatic hydrolysis. The single factor experiments were experimentalized to study the degradation of chitosan by papains. The concentration of reducing sugar and the yield of reducing sugar in the enzymatic hydrolysate were used as reference indicators. The effects of reaction time, pH, reaction temperature, amount of enzyme and substrate concentration were discussed. The results showed that the concentration of reducing sugar increased at first and then decreased with the increase of pH value and reaction temperature. With the prolonging of reaction time, the reaction slowed down and the enzymatic hydrolysis speed slowed down. The increase of the amount of enzyme promoted the reaction rate, and the concentration of reducing sugar also increased. The change of substrate concentration also led to the change of reducing sugar yield. The optimal conditions were obtained by the single factor experiment. When 50 °C of the reaction temperature, 5.0 of pH, 60 min of reaction time, substrate concentration reached 1.75% and the amount of enzyme reached 1:10. At the above condition, the concentration of chitosan oligosaccharide was 4.33 mg/mL and the chitosan oligosaccharide yield was up to 24.73%.

1. Introduction

Chitosan oligosaccharide is the degradation of chitosan after main chain rupture, degree of polymerization is approximately 2-20 oligosaccharides [1]. Compared with chitosan macromolecules, chitosan oligosaccharides have low molecular weight, good water solubility, moisture absorption and moisture retention, and are easy to be absorbed and utilized by the body. In addition, chitosan oligosaccharides have multiple physiological functions such as regulating immunity, regulating blood lipids, promoting the growth of beneficial bacteria, anti-oxidation, anti-tumor and bacteriostatic [2].

Currently, the main methods to obtain chitosan oligosaccharide by degrading chitosan include enzymatic hydrolysis, chemical degradation and physical degradation [3]. However, compared with chemical degradation method and physical degradation method, the enzymatic hydrolysis method overcomes the disadvantages of the product's relative molecular weight distribution is difficult to control and the uniformity is poor, which has the required reaction conditions are mild, energy saving, high efficiency, pollution-free and other advantages [4]. Therefore, enzymatic hydrolysis is the most ideal method for chitosan oligosaccharide degradation.

Enzymatic degradation can be divided into specific enzyme and non-specific enzyme degradation. Specific enzymes include chitosan, n-acetylglucosamine and chitinase, while non-specific enzymes...
include lysozyme, lipase, cellulase and papain. Compared with non-specific enzymes, specific enzymes cannot be industrialized and cost more [5].

In this study, chitosan oligosaccharide was prepared by degradation of chitosan by papain, and the effect of reaction time, pH, reaction temperature, enzyme base ratio and substrate concentration on the enzymatic hydrolysis efficiency of chitosan oligosaccharide was investigated by using reducing sugar concentration and reducing sugar yield of the degradation solution as indicators.

2. Factors influencing the degradation of chitosan by papain

2.1 Effect of reaction time on the degradation of chitosan enzyme

Table 1 and Figure 1 show the effect of reaction time on the degradation of chitosan. Before 30 min, the reducing sugar concentration increased rapidly. At 60 min, the reaction was basically completed, and the concentration of reducing sugar increased slowly. After enzymatic hydrolysis for 90 min, the reaction basically reached equilibrium. In the process of degradation, with the extension of reaction time, on the one hand, the amount of chitosan substrate becomes less and less, and the area in contact with the enzyme decreases, leading to the reaction slowing down. Therefore, the reaction time of 60 min was the best time for the enzymatic hydrolysis of chitosan.

| Number | 1 | 2 | 3 | 4 | 5 | 6 |
|--------|---|---|---|---|---|---|
| Time(min) | 15 | 30 | 45 | 60 | 75 | 90 |
| Absorbance | 0.108 | 0.282 | 0.327 | 0.356 | 0.363 | 0.375 |
| Reducing sugar concentration(mg/mL) | 0.102 | 0.509 | 0.614 | 0.682 | 0.698 | 0.726 |

Figure 1. Effect of reaction time on the degradation of the reaction.

2.2 Effect of pH on the degradation of chitosan enzyme

Table 2 and Figure 2 show the effect of pH on the degradation of chitosan. When the pH value is 2.5-3.5, the yield of enzymatic hydrolysis is lower, indicating that enzymatic hydrolysis is difficult to carry out in an over-acidic environment. When the pH value is 3.5-5.0, the concentration of reducing sugar increases rapidly with the increase of pH value. When the pH value was 5.0, the reducing sugar concentration in the enzymatic hydrolysate reached the highest point. When the pH value was greater than 5.0, chitosan in the enzymatic solution could not be completely dissolved, resulting in only partial
enzymatic hydrolysis of chitosan, and its reducing sugar concentration also showed a downward trend. It can be concluded from figure 3 that the most suitable pH for enzymatic hydrolysis is 5.0.

**Table 2.** Effect of pH on the enzymatic hydrolysis reaction.

| Number | 1   | 2   | 3   | 4   | 5   | 6   | 7   |
|--------|-----|-----|-----|-----|-----|-----|-----|
| pH     | 2.5 | 3.0 | 3.5 | 4.5 | 5.0 | 5.5 |
| Absorbance | 0.083 | 0.096 | 0.137 | 0.373 | 0.461 | 0.431 |
| Reducing sugar concentration (mg/mL) | 0.043 | 0.074 | 0.170 | 0.722 | 0.928 | 0.858 |

![Figure 2. Effect of pH on the enzymatic hydrolysis reaction.](image)

2.3 **Effect of reaction temperature on the degradation of chitosan enzyme**

Table 3 and Figure 3 show the effect of reaction temperature on the degradation of chitosan. Papain has a high sensitivity to reaction temperature. In the enzymatic hydrolysis of chitosan, the concentration of reducing sugar in the enzymatic hydrolysis solution increases with the increase of reaction temperature within the range of 30-45°C, until the peak value is reached at 45°C. As the temperature continued to rise, the reducing sugar concentration in the enzymatic hydrolysate decreased, indicating that papain activity decreased at reaction conditions above 45°C. Therefore, the enzymatic hydrolysis reaction is most suitable at 45°C.

**Table 3.** Effect of reaction temperature on the enzyme to enzymatic hydrolysis.

| Number | 1   | 2   | 3   | 4   | 5   | 6   |
|--------|-----|-----|-----|-----|-----|-----|
| Temperature (°C) | 30  | 35  | 40  | 45  | 50  | 55  |
| Absorbance | 0.271 | 0.348 | 0.412 | 0.500 | 0.378 | 0.262 |
| Reducing sugar concentration (mg/mL) | 0.483 | 0.663 | 0.813 | 1.019 | 0.733 | 0.462 |
Figure 3. Effect of reaction temperature on the enzymatic hydrolysis reaction.

2.4 Effect of substrate ratio (E:S) on the degradation of chitosan enzyme

Table 4 and Figure 4 show the effect of enzyme-base ratio on the degradation reaction of chitosan. From the results, it can be seen that in the range of enzyme base ratio 1:35 to 1:15, the increase of enzyme content has a promoting effect on the reaction rate, and the concentration of reducing sugar also increases accordingly. When the enzyme base ratio reached 1:15, the concentration of reducing sugar in the enzymatic hydrolysate was the highest, and the amount of reducing sugar produced by degradation decreased as the enzyme dosage continued to increase. Therefore, it can be concluded that the optimum enzymatic base ratio is 1:15.

Table 4. Effect of bottom on the reaction of the enzyme to the enzyme hydrolysis.

| Number | 1  | 2  | 3  | 4  | 5  | 6  |
|--------|----|----|----|----|----|----|
| E: S   | 1: 10 | 1: 15 | 1: 20 | 1: 25 | 1: 30 | 1: 35 |
| Amount of enzyme (g) | 0.2000 | 0.1333 | 0.0100 | 0.0080 | 0.0067 | 0.0057 |
| Absorbance | 0.704 | 0.723 | 0.362 | 0.313 | 0.236 | 0.198 |
| Reducing sugar concentration(mg/mL) | 1.496 | 1.540 | 0.969 | 0.918 | 0.801 | 0.401 |

Figure 4. Effect of enzyme bottom on enzymatic hydrolysis reaction.

2.5 Effect of substrate concentration C (%) on the degradation of chitosan enzyme

Table 5 and Figure 5 show the effect of substrate concentration on the degradation reaction of chitosan. Figure 6 shows that the change of substrate concentration also leads to the change of reducing sugar yield. It can be seen from the figure that the increase of substrate concentration does not necessarily improve the yield of enzymatic hydrolysis. When the substrate concentration was more than 1%, the
yield of chitooligosaccharide began to decline, which was due to the increase of the reaction substrate and the relatively small amount of enzyme compared with the substrate, leading to the decline of yield. When the substrate concentration reached 1.75%, the substrate and enzyme dosage were relatively appropriate, and the reducing sugar yield reached the peak. It can be concluded that the optimum substrate concentration in this enzymatic hydrolysis reaction is 1.75%.

Table 5. Effect of substrate concentration on the enzymatic hydrolysis reaction.

| Number | C(%)   | Substrate(g) | Amount of enzyme(g) | Absorbance | Reducing sugar yield(%) |
|--------|--------|--------------|---------------------|------------|-------------------------|
| 1      | 0.75   | 0.15         | 0.0060              | 0.223      | 0.951                   |
| 2      | 1.00   | 0.20         | 0.0080              | 0.346      | 1.540                   |
| 3      | 1.25   | 0.25         | 0.0100              | 0.384      | 0.696                   |
| 4      | 1.50   | 0.30         | 0.0120              | 0.492      | 0.581                   |
| 5      | 1.75   | 0.35         | 0.0140              | 0.588      | 0.401                   |
| 6      | 2.00   | 0.40         | 0.0160              | 0.628      | 0.312                   |

Figure 5. Effect of substrate concentration on the enzymatic hydrolysis reaction.

3. Conclusions
As the pH value and reaction temperature increase, the concentration of reducing sugar first increases and then decreases. When the pH value is 5.0, the reaction temperature is 45 ℃, the reaction time is 1.0 h, the substrate concentration is 1.75% and the enzyme substrate ratio is 1:10, the reducing sugar concentration reaches the maximum of 4.33 mg/mL and the yield reaches 24.73%. The optimum substrate concentration in this enzymatic hydrolysis reaction is 1.75%. The reaction rate promoted with the increase of the amount of enzyme, and the concentration of reducing sugar also increased. The reducing sugar yield changed because of the change of substrate concentration.

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