Influence factors of the chitosan degradation by pawpaw protease

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Abstract. The pawpaw protease is used to degrade chitosan to prepare a low molecular weight chitosan-reducing sugar. The effect of temperature, pH value, reaction time and enzyme addition on the degradation of chitosan was investigated by single factor experiment with the reducing sugar concentration of the degradation solution as the index. The experimental results show that with the increase of the amount of enzyme added and the prolongation of the reaction time, and the degradation rate of chitosan increased and the concentration of reducing sugar increased. As the increase of pH and reaction temperature, the concentration of reducing sugar first increases and then decreases. The optimal conditions for chitosan degradation is 4.1 of the pH value, 42 °C of the reaction temperature, 1.4 h of the reaction time, and enzyme addition amount is 30 U/mg. Under the above conditions, the reducing sugar concentration reaches 1.57 g/L, and the rate is 15.7%.

1. Introduction

Chitosan is a product formed by the hydrolysis and deacetylation of chitin by concentrated alkali. It is also called chitosan, soluble chitin, polyglucagon, and the chemical name is poly(1,4 glycoside)-2-Amino-2-deoxy-BD-glucose. The chemical name of chitin is (1,4)-2-acetamido-2-deoxy-BD-glucose, and the chemical structure is composed of 2-acetamido-2-deoxy-D-glucose through B-1,4 glycoside. The polysaccharide formed by the bond form has a molecular weight of more than 1 million. It is the only amino acid-containing homogeneous polysaccharide, and its structure is very similar to that of cellulose. It is a product in which the hydroxyl group at the second position of cellulose is replaced by an amide group. It is a natural product re-extracted by biosynthesis, has good biocompatibility and can be biodegraded [1].

Low molecular weight chitosan is a kind of low-polymerization degree (n generally 2-20) water-soluble amino sugar compound produced by hydrolysis of chitosan. Its chemical structure is...
homomeric or heteropolyoligosaccharide linked by N-acetyl-D-glucosamine (GLcNAc) and D-glucosamine (GLcN) via B-1,4 glycosidic bonds. Some of its physiological activities and functional properties are very significant, including the good water solubility of chitosan and moisturizing, antibacterial effects, anti-tumor and immune function [2].

Compared with chitosan macromolecules, low molecular weight chitosan has unique and superior physiological and biochemical properties, such as moisturizing, hygroscopic and water-soluble. The macromolecular chain structure of chitosan has a large number of -NH₂ and -OH groups and is an active substance of natural organisms containing nitrogen-containing polysaccharides. When chitosan degrades, a large number of strong polar groups of -OH and -NH₂ increase rapidly, which greatly improves the water solubility of low molecular weight chitosan and enhances its moisture absorption and moisturizing function. The low molecular weight chitosan with a certain molecular weight has better moisture absorption and moisturizing function than hyaluronic acid and glycerin, and its moisturizing performance will gradually increase with the decrease of the average molecular weight within a certain molecular weight range, and vice versa [3].

Under the action of enzymes such as papaya protease, the chitosan was degraded into low molecular weight reducing sugar. In this paper, the factors affecting the degradation of high molecular chitosan into low molecular chitosan were studied.

2. Influence factors of the chitosan degradation by papaya protease

2.1 Effect of enzyme dosage on degradation of chitosan

When the amount of enzyme added is 5, 10, 20, 30, 40, 50, 60 U/mg, in the chitosan degradation reaction, the reducing sugar release value is shown in Table 1, and at the same time according to Table 1, the relationship between the amount of enzyme added and reducing sugar can be made - Fig.1. When the concentration of chitosan in the reaction solution is constant, the amount of reducing sugar which starts to degrade will increase with the increase of papaya protease enzyme amount, but when the amount of papaya protease added reaches 30 U/mg, the amount of enzyme added continues to increase, and the amount of reducing sugar produced by the degradation remains substantially constant. Therefore, the amount of enzyme added is 30 U/mg, which is the optimal amount of enzyme for the degradation reaction.

| Experiment number | 1   | 2   | 3   | 4   | 5   | 6   | 7   |
|-------------------|-----|-----|-----|-----|-----|-----|-----|
| Enzyme amount (U/mg) | 5   | 10  | 20  | 30  | 40  | 50  | 60  |
| Absorbance         | 0.583 | 1.290 | 1.706 | 1.933 | 1.983 | 2.002 | 2.032 |
| Reducing sugar concentration (g/L) | 0.485 | 1.011 | 1.320 | 1.489 | 1.526 | 1.540 | 1.563 |
Figure 1. Effect of enzyme amount on degradation of chitosan

2.2 Effect of reaction time on degradation of chitosan

In the case of different reaction times, the concentration of reducing sugar in the degradation reaction is shown in Table 2, and according to Table 2, the relationship between the reaction time and reducing sugar is shown in Fig.2. Before the reaction proceeded to 1.5h, the concentration of reducing sugar increased rapidly; when the reaction was 1.5h, the reaction was almost completed, and the concentration of reducing sugar increased slowly; after 1.5h, the reaction basically reached equilibrium. The reason may be that as the degradation reaction progresses, the amount of chitosan substrate decreases and the accumulation of degradation products inhibits the degradation reaction, which makes the degradation rate of the enzyme slower, but it is also possible that the enzyme is gradually inactivated during the reaction. Therefore, the reaction time of 1.5 h was chosen as the best time to determine the degradation activity of chitosanase in pawpaw protease.

Table 2. Effect of reaction time on chitosan degradation

| Experiment number | 1   | 2   | 3   | 4   | 5   | 6   | 7   |
|-------------------|-----|-----|-----|-----|-----|-----|-----|
| Reaction time (h) | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 |
| Absorbance        | 1.456 | 1.724 | 1.996 | 1.933 | 1.956 | 1.935 | 1.915 |
| Reducing sugar    | 1.134 | 1.333 | 1.535 | 1.489 | 1.506 | 1.490 | 1.475 |
Reducing sugar concentration (g/L)

**Figure 2.** Effect of reaction time on chitosan degradation

2.3 *Effect of pH on the degradation of chitosan*

The concentration of reducing sugars degraded by chitosan under different pH values and enzymes is shown in Table 3. According to Table 3, the relationship between reaction time and reducing sugar is shown in Fig.3. Fig.3 reflects the change of chitosan degradation reaction with pH value. When the pH value is in the range of 3.0~4.3, the concentration of reducing sugar produced by degradation increases with the increase of pH value; when the pH is 4.3, the reducing sugar content in the degradation liquid is the highest; when the pH value is greater than 4.3, the reducing sugar concentration in the degradation liquid begins to decrease. Therefore, the degradation reaction is optimal at pH = 4.3.

**Table 3.** Effect of pH on the degradation of chitosan

| Experiment number | 1    | 2    | 3    | 4    | 5    | 6    | 7    |
|-------------------|------|------|------|------|------|------|------|
| pH                | 3.00 | 3.40 | 3.70 | 4.00 | 4.30 | 4.60 | 5.00 |
| Absorbance        | 1.056| 1.288| 1.616| 1.921| 1.987| 1.813| 1.653|
| Reducing sugar concentration (g/L) | 0.837 | 1.009 | 1.253 | 1.480 | 1.529 | 1.400 | 1.281 |
2.4 Effect of reaction temperature on degradation of chitosan

The concentration of reducing sugar in the degradation reaction at different reaction temperatures is shown in Table 4. According to Table 4, the relationship between reaction time and reducing sugar is shown in Fig.4. Fig.4 reaction degradation reaction with the change of reaction temperature, the enzyme is highly sensitive to temperature, when the reaction temperature is lower than the optimal temperature, the reaction rate increases with temperature; when the reaction temperature is higher than the optimal temperature, the enzyme will gradually deactivate and the reaction rate will decrease. As shown in Fig.4, in the reaction of pawpaw protease-degrading chitosan to prepare low-molecular-weight chitosan, in the range of 30-45 °C, the concentration of reducing sugar gradually increases with the increase of reaction temperature, reaching the highest value at 45 °C. Continue to increase the temperature, the reducing sugar concentration is reduced, which indicates that when papamel is degraded by pawpaw protease, the pawpaw protease deformation is inactivated under high temperature conditions. Therefore, the optimum temperature for pawpaw protease degradation of chitosan is 45 °C.

| Experiment number | 1   | 2   | 3   | 4   | 5   | 6   | 7   |
|-------------------|-----|-----|-----|-----|-----|-----|-----|
| Reaction temperature (°C) | 30  | 35  | 40  | 45  | 50  | 55  | 60  |
| Absorbance        | 1.693 | 1.750 | 1.835 | 1.934 | 1.900 | 1.818 | 1.765 |
| Reducing sugar concentration (g/L) | 1.310 | 1.353 | 1.416 | 1.490 | 1.464 | 1.403 | 1.364 |

Figure 3. Effect of pH on the degradation of chitosan
Reducing sugar concentration (mg/ml)

Temperature (℃)

Figure 4. Effect of reaction temperature on degradation of chitosan

3. Conclusion

(1) With the increase of the amount of enzyme added and the prolongation of the reaction time, the degradation rate of chitosan increased and the concentration of reducing sugar increased. As the pH and reaction temperature increase, the concentration of reducing sugar first increases and then decreases.

(2) The optimum conditions for the degradation of chitosan by pawpaw protease is 4.1 of the pH value, 42 °C of the reaction temperature, 1.4 h of the reaction time, and 30 U/mg of enzyme addition amount.

(3) Under the reaction conditions, the molecular weight of the low molecular weight chitosan produced by the degradation is 2766.34, and the degradation yield is 15.7%. The degradation efficiency and economic benefit of chitosan are both the best.

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