Production of Citric Acid from Apple Pomace Enzymolyzed by Cellulase

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Abstract：Cellulase can evidently increase the content of glucose and has a significant effect on the production of citric acid from apple pomace by *Aspergillus niger*. Based on experiments, a cellulolytic enzyme named cellulase A_6 was found able to produce about 170 g glucose from 1 kg dried apple pomace after 12 h reaction, with cellulase concentration of 20 U/g in the medium at 50°C, natural pH without pretreatment of alkali. Using the treated apple pomace as a liquid state substrate, *Aspergillus niger*-C selected out was able to produce about 256 g citric acid from 1 kg dried apple pomace at 35°C in 3 d or 30°C in 5 d with flask rotation speed of 210 r/min, and the conversion of citric acid could reach 80% based on the amount of sugar consumed.

Key words：citric acid; apple pomace; cellulase; fermentation
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1 INTRODUCTION

Apple pomace, the residue left from juice extraction, consists of about 25% of the weight of fresh fruit. Approximately several million tons of apple pomace are produced annually in China, and the quantity is expected to increase year after year. Because of high content of organic acids and very little protein(about 6% in dry apple pomace), apple pomace cannot be used as feedstuff directly. At present, almost all apple pomace is discarded. When fermented by natural microorganism, it emits bad niff and causes a serious environmental problem. This restricts the development of apple juice production. However, apple pomace is rich in carbohydrate, and it consists of 19.2% reducing sugar and 24.5% crude fibre. In recent years, much more research efforts have been put into using apple pomace as an energy source to alleviate the waste disposal problem[1–4].

Citric acid is a commercially important product that has been produced by submerged fermentation of sugar with *Aspergillus niger*. Thus, severing apple pomace as substrate to produce citric acid is an alternative way of comprehensive utilization. Lu[5] and Shojaosadati et al.[6] have reported their studies respectively; even some pilot production has also been conducted. But, the fact that reducing sugar in apple pomece is less and unsuitable for the citric acid production directly should be taken consideration, which may be another important limiting factor in practical

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production. So adding cellulase to increase the content of reducing sugar seems necessary and effective to get higher yield of citric acid. The objective of this study is to further optimize the fermention process and make it available in industry.

2 MATERIAL AND METHODS

2.1 Raw Material

Apple pomace used in this study was obtained from Shaanxi Fruit Processing Plant, Xi′an. It was dried in a hot-air dehydrator to moisture content of approximately 7% and stored at room temperature till needed. Prior to its use, the dried pomace was porphyrized by mill and sifted through the 0.25 mm screen.

Celluslase-A_6, a kind of ready commodity was obtained from Shaanxi Institute of Enzyme Science, its activity is 120 U/g.

Three strains of *Aspergillus niger* were obtained from the Department of Biology of Northwest University, which were stored in glycerol at –5^{o}C. Each strain grew and was maintained on a sterilized apple juice agar slant at 30^{o}C for 7 d. A spore inoculum was prepared by adding 3 ml of sterilized distilled water to each slant and shaking for one minute, then the spore solution was adjusted to certain concentration for use with sterilized water.

2.2 Method of Enzymolysis

20 g of apple pomace was introduced into a 300 ml triangular flask. Proper amoumt of distilled water (about 50 ml) and crude cellulase A_6 were added to it. Adjusting pH with NaOH solution, putting it into a constant temperature incubator motionlessly for several hours at certain temperature after the triangular flask was wrapt by 4 layers of general gauze to avoid contamination. Then transfer apple pomace enzymolyzed by cellulase into hot water of 80^{o}C and measure the content of sugar.

2.3 Liquid State Fermentation

The treated apple pomace by cellulase in the 300 ml triangular flask was added in 80 ml distilled water, sterilized at 121^{o}C for 20 min after the triangular flask wrapt by gauze, cooled, inoculated, fermented in a table concentrator for some days. Then the fermented apple pomace was transferred into hot water, and the content of citric acid and total acid were measured.

2.4 Analytical Methods

Reducing sugar was determined as glucose by the colorimetric method of 3.5– nitryl salicylic acid (DNS). Citric acid was measured by the colorimetric method of pyridine-acetic anhydride. The content of total acid was determined by titration with NaOH solution[2].

3 RESULTS AND DISCUSSION

3.1 Optimizing the Production of Reducing Sugar

3.1.1 Effect of alkali on enzymolysis

Six parallel samples were treated under the same conditions. In three of them, apple pomace was pretreated by marinating in 40^{o}C, 3% NaOH solution for 24 h before adding cellulase[7]. The

result showed that 31.08% reducing sugar was obtained (reducing sugar in original apple pomace was 19.22%) for the apple pomace without pretreatment of alkali. In contrast, the content of reducing sugar in pretreated apple pomace reduced to 13.59%. The reason for this phenomenon has two facts: (1) Apple pomace itself is more abundant in reducing sugar than corn stalk and rice hull; (2) Excessive alkali must be removed for the next step of experiment, so some reducing sugar is lost in washing[8,9].

3.1.2 Effect of time on enzymolysis

Figure 1 indicates the effect of enzymolysis time on the production of reducing sugar. The content of reducing sugar increases rapidly between 1~12 h and reaches a higher level after 12 h. Continuing the process, the reducing sugar production increases slowly and almost no significant change appears beyond 24 h. Enzymolysis of 12 h seems suitable in industrial production.

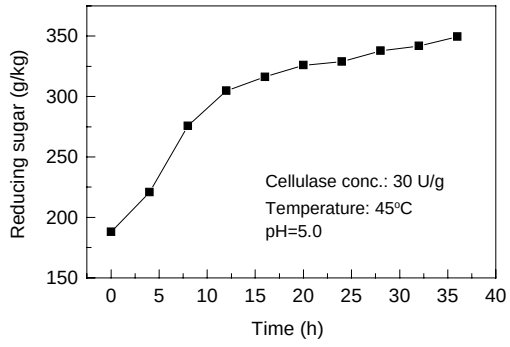

Fig.1 Effect of time on enzymolysis

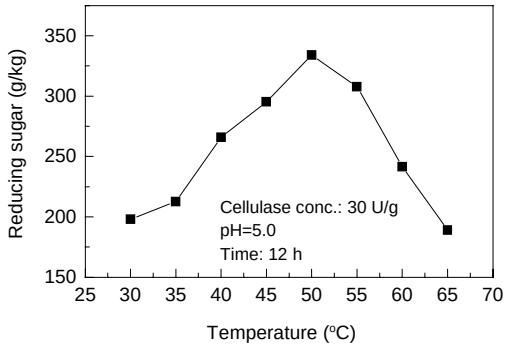

Fig.2 Effect of temperature on enzymolysis

3.1.3 Effect of temperature on enzymolysis

Temperature has profound influence on the activity of cellulase and is a key factor to the yield of reducing sugar. As shown in Fig.2, cellulase-A_6 can gave the highest yield of reducing sugar at 50°C.

3.1.4 Effect of cellulase-A_6 concentration on enzymolysis

The effect of the concentration of cellulase-A_6 on the production of reducing sugar is shown in Fig.3. Because the production becomes much slower as the concentration is increased from 20 U/g

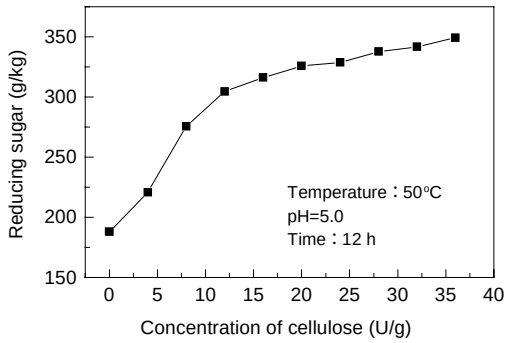

Fig.3 Effect of the cellulose concentration on enzymolysis

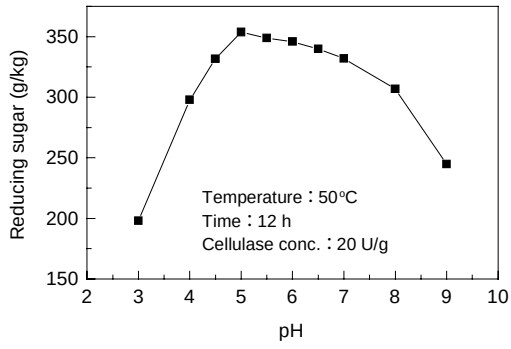

Fig.4 Effect of pH on enzymolysis

to 35 U/g (enzyme activity per gram substrate), the concentration of 20 U/g is proper. Moreover, the less consumption of costly cellulase predicates lower manufacturing cost in industry.

3.1.5 Effect of pH on enzymolysis

The production of reducing sugar from apple pomace is not significantly affected by pH in the range of 4.7~6.5 and it can reach the maximum at pH 5.0 as shown in Fig.4. In fact, apple pomace has a pH just in the range of 4.7~6.5. At the same time, the use of cellulase is less and don't affect pH of the whole system. So pH can be ignored in enzymolysis process.

3.2 Optimizing Citric Acid Production from Enzymolyzed Apple Pomace by Cellulase

The yield of citric acid varied considerably and depended on (1) strain of *Aspergillus niger* used, (2) inoculum size, (3) rotation rate of shake culture, and (4) temperature and time of fermentation[10–12]. From Figs.5, 6 and Tables 1, 2, the optimal conditions of production are as follows: using *Aspergillus niger*-C, inoculum of 4×10^4 g^{-1}, higher rotation speed, and 35°C for 3 d or 30°C for 5 d. In addition, different nitrogen sources and their additional quantity had been studied. 0.3% of NH_4Cl is better to increase the production of citric acid, but its effect on the yield of citric acid is far less than that of the above factors.

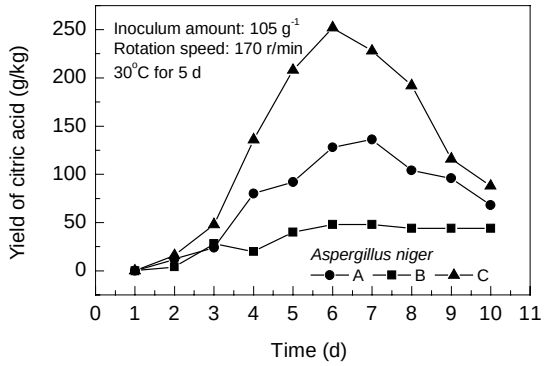

Fig.5 Effect of different strains of *Aspergillus niger* on the yield of citric acid

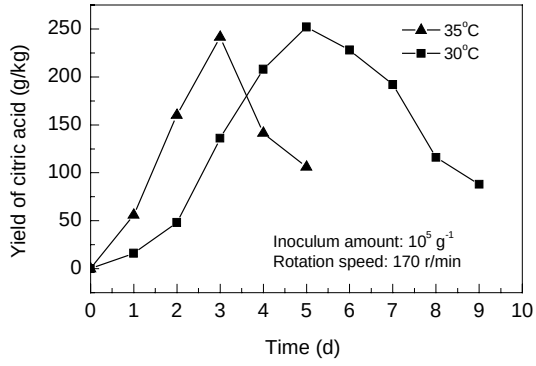

Fig.6 Effect of time on the yield of citric acid at different temperatures

Table 1 Effect of inoculum on the yield of citric acid

Amount of inoculum ($\times10^4$ g^{-1})	0.2	1	2.5	4	5	10	25
Citric acid (g/kg)	128.1	176.2	220.0	240.1	241.7	236.8	252.0
Total acid (g/kg)	200.2	208.0	236.6	260.1	252.9	246.0	265.1

Note: Rotation speed 170 r/min, 30°C for 5 d.

Table 2 Effect of the rotation speed of flask on the yield of citric acid

Rotation speed (r/min)	80	160	210
Citric acid (g/kg)	153.9	191.5	220.5
Total acid (g/kg)	170.1	201.6	227.3

Note: Inoculum amount 4×10^4 g^{-1}, 30°C for 5 d.

However, three important problems should be taken consideration in the following work: (1) *Aspergillus niger*-C selected in this work needs to be bred better so as to suit for production of citric

acid from apple pomace; (2) The yield of citric acid decreases obviously when production is scaled up, and the reason is to be explored; (3) The extraction rate of citric acid is low, so it is necessary to use advanced separation methods in further work.

4 CONCLUSION

The optimum conditions of enzymolysis and fermentation of apple pomace have been obtained. Under the optimal conditions, the content of reducing sugar of apple pomace can reach 36.3% and the yield of citric acid is 256 g per kg dried apple pomace that is about 80% based on the amount of consumed sugar.

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