Study on the technology of compound enzymatic hydrolysis of whole passion fruit

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Abstract. Fresh Whole Passion Fruit was used as raw material, The enzymatic hydrolysis technology of Passion Fruit by Complex enzyme were studied, The effects of enzyme dosage, Enzyme ratio(cellulose: pectinase), pH, temperature and time on the hydrolysis were investigated by single-tests and orthogonal tests, the hydrolysis indicators of single-factor tests and orthogonal tests were juice yield. The optimal hydrolysis conditions of Passion Fruit by Complex enzyme were enzyme dosage 0.12%, Enzyme ratio 5:1, hydrolysis temperature 50℃, pH4.0 and time 3.5 h. Under such conditions, juice yield of Passion Fruit was 92.91%.

1. Introduction
Passion fruit belongs to the herbaceous vines of Passifloraceae family, known as passionflower, egg fruit, rattan fruit, etc. [1]. It is tropical fruit mainly distributed in Australia, South Africa, Kenya, Brazil, the United States Hawaii, Florida and other tropical or subtropical regions [2]. According to the shape and quality of passion fruit ,it can be divided into purple passion fruit, yellow fruit and purple-yellow fruit hybrids [3]. The Passion fruit was unique flavor and nutrient-rich, containing 17 kinds of amino acids, 21 kinds of trace elements [4] and medicinal value of alkaloids and flavonoids [5], with quench thirst , eliminating fatigue, keeping beauty, Antibacterial anti-cancer, cholesterol-lowering and other health effects [6]. Passion fruit is known as the most aromatic fruit in the world, containing guava, mango, apple, pineapple and many other kinds of fruit aroma ingredients, shown that passion fruit juice contained more than 60 kinds of esters and other fragrant ingredients, with strong Unique fragrance [7].

Passion fruit consists of rich juice, about 30%-40%. In the processing of the passion fruit, Peeled pulp was processed fruit juice [8-9], and Separated peel made into preserved fruit [10]. In recent years, there are researches of passion fruit vinegar [11-12], passion fruit wine [13], but passion fruit juice, especially the peel contains a lot of pectin, cellulose, hemicellulose and other substances, which reduced the juice rate [14]and greatly affected the use of processing, also affected the quality of passion fruit juice. Therefore, the use of biological enzymes thoroughly hydrolyzed cellulose and pectin of passion fruit pulp, peel solved the production of fruit juice in the low rate, that can take full advantage of passion fruit, to solve the problem of passion fruit dried fruit. In this paper, we use cellulose and pectinase to digest the passion fruit, and the juice yield as an index to evaluate enzymatic levels. The research of enzymatic hydrolysis is intended to provide a theoretical and application basis.
for the utilization of passion fruit.

2. Materials and methods

Materials

Passion fruit was provided by Taixing Supermarket (China), picked no pests and diseases, no mildew, skin formation of ripe fruit; Pectinase (10000U/ml) was purchased from Novozymes (China) Biotechnology Co., Ltd.; Cellulase (100FBG/g) was purchased from Novozymes (China) Biotechnology Co., Ltd.; Juicer (Jd-322) was purchased from Jinda Electric Co., Ltd.; Digital Thermostatic Water Bath (HH-6) was bought from Guohua Electric Co., Ltd.; pH meter (FE20) was purchased from METTLER TOLEDO Co., Ltd.; Electronic balance (YT1201N) was bought from Shanghai Instrument Factory Co., Ltd.; Vacuum Pump (DP-02) was purchased from Wuxi Jiuping Co., Ltd.

Experimental design

The single factor and orthogonal test were used to optimize the enzymolysis conditions, and the juice yield was used as the index. Designed that enzyme ratio (cellulase: pectinase) was 1:1, 1:2, 1:3, 1:4, 1:5, 5:1, 4:1, 3:1, 2:1; enzyme dosage was 0, 0.02%, 0.04%, 0.06%, 0.08%, 0.10%, 0.12%, 0.14%, 0.16%, 0.18%, 0.2%; the enzymolysis temperature was 30℃, 35℃, 50℃, 55℃, 60℃; the action time was 1h, 2h, 3h, 4h, 5h; pH was 3.0, 3.5, 4.0, 4.5, 5.0, 5.5. Based on the single factor test, the L9 (34) orthogonal test was used to optimize the enzymatic hydrolysis of passion fruit.

Determination of juice yield

Weigh a certain amount of passion fruit pulp (m₁) for enzymatic hydrolysis, after the end of the enzyme, collected fruit pomace drying by Suction filtering, and weigh the fruit pomace (m₂) [15], calculate the juice rate, the formula is as follows:

\[
\text{juice yield \%} = \frac{m_1 - m_2}{m_1} \times 100\%
\]

Where \(m_1\) is the weight of passion fruit pulp; \(m_2\) is the weight of fruit pomace after Suction filtering.

3. Results and Analysis

Determination of enzyme ratio.

Under the conditions of enzyme dosage 0.2%, temperature 50℃, pH 4.5, enzymolysis time 4h, the effect of different enzyme ratio (cellulase: pectinase) on change trend of juice yield was showed in Fig.1.

![Fig.1 Effect of enzyme ratio on juice yield](image1)

![Fig.2 Effect of enzyme dosage on juice yield](image2)

It reflected in Fig.1 when the proportion of pectinase is more than cellulase in the range of 1:2 ~ 1:5, juice yield was increased first and then decreased, but the trend was not obvious, which revealed when the pectinase is the leading role in composite enzymatic hydrolysis, the impact on the juice yield is not obvious. On the other hand, the juice yield was gradually increase with the increase of proportion of cellulose in the range of 2:1~5:1, it appeared the highest juice yield 91.80% at enzyme ratio of 5:1. Overall, juice yield is higher when the proportion of cellulase more than pectinase,
indicating that the cellulase in the compound enzyme played a more significant role. Therefore, the enzyme ratio of 5:1 is appropriate.

**Determination of enzyme dosage.** When enzyme hydrolysis conditions of enzyme ratio, temperature, time, pH were fixed at 5:1, 50°C, 4h, 4.5, respectively, the effect of different enzyme dosage on trend of juice yield was showed in Fig.2.

From the Fig.2, it reflected that it is a significant effect on juice yield to add compound enzyme, and it showed a trend of rapid rise when enzyme dosage was lower than 0.1%, this is because that with the increase of enzyme dosage, enzyme increasingly digested of the substrate; But enzyme dosage exceed 0.1%, the juice yield tends to be stable, and it is due to enzyme added has been saturated with the substrate. So we made sure the enzyme dosage is 0.1%.

**Determination of temperature.** Temperature is an important factor for enzyme activity, the enzyme activity for too high-temperature will decline or even was inactivation, too low-temperature was not conducive to the role of enzymes [16]. When enzyme hydrolysis conditions of enzyme dosage, enzyme ratio, time, pH were fixed at 0.1%, 5:1, 4h, 4.5, respectively, the effect of different temperature on trend of juice yield was showed in Fig.3.

From the Fig.3, it can be seen that with the rise of temperature, the juice yield increases first and then decreases, this is because that the ability of the enzyme increased with the temperature changed from 30°C to 50°C. When the temperature exceed 50°C, the activity of the complex enzyme is limited [17], resulting in a decrease in juice yield. So we made sure the enzymolysis of temperature is 50°C.

**Determination of pH.** When enzyme hydrolysis conditions of enzyme dosage, enzyme ratio, temperature, time were fixed at 0.1%, 5:1, 50°C, 4h, respectively, the effect of different pH on trend of juice yield was showed in Fig.4.

From the Fig.4, It reflected that juice yield were significantly increased in the range of pH3.0~pH4.0, and reached the highest juice yield 92.03% at pH4.0, but the juice yield declined rapidly when the pH was higher than 4.0. This is due to each enzyme has the optimum range of pH, at different pH conditions, the dissociation state of the polar group and charges on enzyme activity center is different. Only in a certain dissociation state can the enzyme protein form the intermediate with the substrate [18], so we made sure the pH of enzymolysis is 4.0.

**Determination of time.** When enzyme hydrolysis conditions of enzyme dosage, enzyme ratio, temperature, pH were fixed at 0.1%, 5:1, 50°C, 4.0, respectively, the effect of different time on trend of juice yield was showed in Fig.5.
Fig. 5 Effect of hydrolysis time on juice yield

It reflected in Fig. 5 that juice yield was slowly increased in the early 1h~2h of enzymolysis, significantly increased in 2h~3h, and had a trend of steady after 3h. This indicated that increase of time would lead to enzymatic fully, thereby increasing the juice yield, and enzyme reach to saturation when the time was higher than 3h, so we made sure the enzymolysis of time is 3h.

**Orthogonal test results** On the basis of single factor test, chosen enzyme dosage (A), enzyme ratio (B), temperature (C) and time (D) as the main factors of the orthogonal design. Table 1 was the orthogonal test of L9 (3^4), orthogonal analysis and the results was in table 2.

| Experiment number | A   | B   | C   | D   | Juice yield (%) |
|-------------------|-----|-----|-----|-----|-----------------|
| 1                 | 1   | 1   | 1   | 1   | 90.76           |
| 2                 | 1   | 2   | 2   | 2   | 91.22           |
| 3                 | 1   | 3   | 3   | 3   | 89.40           |
| 4                 | 2   | 1   | 2   | 3   | 92.41           |
| 5                 | 2   | 2   | 3   | 1   | 90.55           |
| 6                 | 2   | 3   | 1   | 2   | 91.63           |
| 7                 | 3   | 1   | 3   | 2   | 90.49           |
| 8                 | 3   | 2   | 1   | 3   | 92.75           |
| 9                 | 3   | 3   | 2   | 1   | 92.86           |

| K1                 | 271.38 | 273.65 | 275.1 | 274.2 |
| K2                 | 274.59 | 274.53 | 276.5 | 273.3 |
| K3                 | 276.09 | 273.88 | 270.4 | 274.6 |
| k1                 | 90.46  | 91.217 | 91.71 | 91.39 |
| k2                 | 91.528 | 91.508 | 92.16 | 91.11 |
| k3                 | 92.03  | 91.293 | 90.15 | 91.52 |
| R                  | 1.57   | 0.29167 | 2.0185 | 0.4083 |

It can be seen from table 2 that enzyme dosage and temperature have a greater impact on the juice yield, in which the order of influence of each factor on juice yield is: C > A > D > B, as well as temperature > enzyme dosage> time> enzyme ratio, the optimum hydrolysis condition is A3B2C2D3 from R, exactly enzyme dosage 0.12%, enzyme ratio 5:1, temperature 50℃, time 3.5h.

**Verification test** the best factor combination A3B2C2D3 is not in the combination of orthogonal tables, so it need for verification. verification Results showed when the optimum conditions were A3B2C2D3, the yield was 92.91%, which was higher than combination of A2B3C2D1 that was the
highest in the orthogonal table. This indicated that orthogonal optimization tests was feasible, so the optimum conditions were as follows: enzyme dosage 0.12%, enzyme ratio 5:1, temperature 50℃, time 3.5h.

4. Conclusion
Whole passion fruit was used as raw materials, the enzymatic hydrolysis technology of passion fruit was studied by single factor and orthogonal optimization. Results showed that hydrolysis conditions of passion fruit were: enzyme dosage 0.12%, enzyme ratio 5:1, temperature 50℃, time 3.5h. Under this condition, the juice yield was 92.91%.

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