Study the Effect of Pectase on Juice Yield of *Rubus corchorifolius*

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Abstract. The experiment of effect of pectase on juice yield of raspberry were studied in quantity, reaction temperature and time of enzymolysis through single factor and orthogonal experiments in this paper. The optimum conditions were as followed: pectase content 0.07%, temperature 45°C, enzymatic enzymolysis time 3.5h and under these conditions the juice of pectase contain its active ingredient greatly.

1. Introduction

*Rubus corchorifolius*, also known as raspberries, pallets, marin, etc., belong to Rosaceal, Rubus plants, for perennially small shrubs. In 1993, the United Nations food and agriculture organization (FAO) recommended raspberries as the international "third generation fruit" [1]. The fruits are rich in vitamin C and E, superoxide dismutase (SOD) and natural pigment [2.3].

At present, the pectinase enzymolysis technology has been widely used in juice processing, it can effectively improve the fruit juice yield, improve the juice [4.5], the filtering efficiency of reducing the amount of chemical clarifying agent, improve the quality of fruit juice, preserved fruit juice of nutrients, simplifying the juice processing technology, shorten processing time, increase production efficiency [6.7].

In this paper, we study the pectinase influence on raspberry juice yield, aimed at the preparation of pure juice yield, high clarity good nutrition raspberry drink, at the same time keep the active ingredient, as far as possible in order to provide theoretical basis for the industrialization of raspberry juice production.

2. Materials and Methods

2.1. Material raspberry fruit, provided by Beijing huiyuan juice & beverage group co., LTD

2.2. Main reagent pectinase, purchased from novozymes tianjin biotechnology co., LTD

2.3. Main instruments and equipment
Digital display thermostatic water bath pot, electronic balance, juicer, hand-held refractometer, 752C ultraviolet visible spectrophotometer, low-speed table centrifuge, etc.
2.4. **Method**

2.4.1. **Process flow.** Raw materials, cleaning, crushing, enzyme treatment, juicing, filtration, clarification, mixing, filling and sealing, sterilization, cooling, storage

2.4.2. **Operating points.**
1. Select ripe and intact raspberry fruits from raw materials, and remove impurities and the fruit with mildew and pests.
2. Wash, broken and rinse thoroughly, remove sand, soil, etc., slowly and gently, so as to prevent damage to the flesh; Crushing should be appropriate, too fine, too thick are not appropriate.
3. After the enzyme treatment, a certain amount of pectinase was added into the broken raspberry, which was put into a constant temperature water bath pot for a period of time to accelerate the decomposition of pectin in the raspberry and facilitate the extraction of juice.
4. After enzyme treatment, the apple pulp of raspberry was poured into the press for pulping and deseeding, and the pulp after seed removal was set aside.
5. Filter the pulp with two layers of gauze and four layers of gauze.
6. Sterilization 10-15 min with 100 °C hot water.

2.4.3. **Enzyme treatment test**

2.4.3.1. **Effect of pectinase addition on juice yield**
Weigh the raspberry five copies, each 200.0 g, broken after adding pectinase, adding quantity were 0.02%, 0.04%, 0.06%, 0.08%, 0.10%, enzymolysis temperature 45 °C, enzymolysis time was 3.5 h, then take out the juice, calculate the juice yield under different enzymatic quantity.

2.4.3.2. **Effect of enzymatic hydrolysis temperature on juice yield**
Weigh the raspberry five copies, each 200.0 g, broken after add 0.06% pectinase, enzymolysis time was 3.5 h, enzymolysis temperature 30 °C, 35 °C, 40 °C, 45 °C, and 50 °C respectively, and then take out the juice, the juice yield of different enzyme solution temperature calculation.

2.4.3.3. **Effect of enzymatic hydrolysis time on juice yield**
Weigh an raspberry is of five copies, each 200.0 g, broken after add 0.06% pectinase, enzymolysis temperature 45 °C, enzymolysis time were 2.5 h, 4.5 h, 4.0 h, 3.5 h, 3.0 h, then take out the juice, calculate the juice yield of different digestion time.

2.4.3.4. **Orthogonal optimization test of enzymatic hydrolysis conditions**
Adding in the pectinase quantity, digestion temperature, digestion time, such as single factor affecting raspberry juice yield test, on the basis of according to the orthogonal table L9 (34) orthogonal experiment was carried out, determine the optimum process conditions of enzymatic treatment, test indicators have juice yield, soluble solids, total sugar, total acid, vitamin C and light transmittance. The test design is shown in table 1.

| Factors | Amount of pectinase added/% | Enzyme solution temperature/°C | Enzymatic hydrolysis time/h |
|---------|-----------------------------|-------------------------------|-----------------------------|
| A       | B                           | C                             |
| 1       | 0.05                        | 40                            | 3.0                         |
| 2       | 0.06                        | 45                            | 3.5                         |
| 3       | 0.07                        | 50                            | 4.0                         |
2.5. Determination methods [8,9]

Juice extraction rate = raspberry juice quality/raspberry fruit quality × 100%; Determination of soluble solids content: hand-held refractometer; Total sugar content determination: direct titration method; Determination of total acid content: titration method; Determination of vitamin C content: 2, 6-dichlorindophenol titration; Transmittance: after enzymatic hydrolysis, the juice was centrifuged for 100ml and 2000r/min for 10min, and the supernatant was taken to measure the transmittance at 660nm.

3. Results and discussion

3.1. Effects of pectinase addition on juice yield

In enzyme solution temperature 45 ℃, enzymolysis time was 3.5 h, pectinase adding quantity were 0.02%, 0.04%, 0.06%, 0.08% and 0.1% under the conditions of enzymatic hydrolysis, the filtered juice of weighing tree enzyme juice quality, calculate the juice yield different enzymatic quantity. The result was shown in figure 1. Adding pectinase can significantly increase the juice output rate of raspberry. As the amount of enzyme added increases, the juice output rate of raspberry also increases, showing a trend of first rapid and then slow down. However, when the amount of added enzyme reached 0.06%, the juice production rate of the enzyme decreased. The enzymatic hydrolysis effect did not increase significantly. Therefore, increasing the amount of enzyme will increase the production cost of raspberry juice and affect the taste of raspberry juice. Therefore, it is more appropriate to determine the amount of added enzyme at 0.06%.

![Figure 1. Effect of adding pectinase on the content of juice yield](image1)

3.2. Effect of enzymatic hydrolysis temperature on juice production rate

In pectinase adding amount was 0.06%, the enzymatic hydrolysis time of 3.5 h, enzymolysis temperature 30 ℃, 35 ℃ and 40 ℃ respectively, 45 ℃, 50 ℃ , after the filtering juice , weighing the quality of the juice.

![Figure 2. Effect of temperature on the content of juice yield](image2)
highest. Continue to raise the temperature, raspberry juice rate decline instead. Because when the temperature exceeds a certain temperature pectinase enzyme solution will be passivation inactivation, resulting in a decline in enzyme activity and thus juice rate lower. Moreover, when the temperature is lower, the aroma substances of raspberry juice can be retained to the maximum extent. IN addition, high temperature increases energy consumption and costs. As a result, the optimum enzymolysis temperature 45 ℃.

3.3. Effect of enzymatic hydrolysis time on juice production rate
In the pectinase adding amount was 0.06%, the enzymatic hydrolysis temperature 45℃, enzymolysis time were 2.5 h, 3.0h, 3.5h, 4.0h, 4.5h, respectively under the conditions of enzymatic hydrolysis, after filtering and weighing the juice quality, calculate the yield of different digestion time. As can be seen from figure 3, the longer the enzymatic hydrolysis time is, the more sufficient the enzymatic hydrolysis action is, and the higher the juice output rate is. After the enzymatic hydrolysis time reaches 3.5h, the juice output rate increases gradually. When the time was 3.5h, the juice output rate reached 82.0%.If the enzymatic hydrolysis time is prolonged, the production cost will be increased, and the loss of nutrients will also be caused if the time is too long. The optimal enzymatic hydrolysis time was 3.5h, which was considered from the aspects of obtaining better juice yield, improving production efficiency and reducing production cost.

![Figure 3. Effect of time on the content of juice yield](image)

3.4. Effects of optimal enzymatic hydrolysis conditions on juice extraction rate and active component content of raspberry
According to the above single-factor test, the amount of pectin enzyme added, the enzymatic hydrolysis temperature and the enzymatic hydrolysis time were selected for orthogonal optimization test according to orthogonal table L9 (34). The results were shown in table 2 and table 3.

| Test Number | A       | B     | C     | Juice Yield | Soluble Solid | Contents of Total Sugar | Total Acid Content | Vitamin C (mg/100ml) | Light Transmittance e% |
|-------------|---------|-------|-------|-------------|---------------|------------------------|--------------------|-----------------------|------------------------|
| 1           | 1(0.05) | 1(40) | 1(3.0) | 67.5        | 10.5          | 10.4                   | 1.9                | 26                    | 50.3                   |
| 2           | 1       | 2(45) | 2(3.5) | 82.6        | 11.0          | 10.6                   | 2.3                | 35                    | 63.7                   |
| 3           | 1       | 3(50) | 3(4.0) | 72.1        | 10.4          | 10.7                   | 2.5                | 28                    | 52.6                   |
| 4           | 2(0.06) | 1     | 2     | 75.9        | 10.8          | 10.4                   | 2.0                | 39                    | 54.6                   |
| 5           | 2       | 2     | 3     | 83.5        | 10.6          | 10.3                   | 2.7                | 27                    | 59.3                   |
| 6           | 2       | 3     | 1     | 80.5        | 10.6          | 10.5                   | 2.4                | 35                    | 53.9                   |
| 7           | 3(0.07) | 1     | 3     | 78.6        | 10.5          | 10.8                   | 2.8                | 33                    | 64.8                   |
| 8           | 3       | 2     | 1     | 84.6        | 11.2          | 10.3                   | 2.1                | 30                    | 59.2                   |
| 9           | 3       | 3     | 2     | 80.9        | 10.4          | 10.2                   | 1.9                | 28                    | 57.4                   |

K1: 74.1  74.0  77.5

K2: 80.0  83.6  79.8

K3: 81.4  77.8  78.1

Table 2. The orthogonal experiment result of the pectase condition
From table 2, the enzymatic hydrolysis conditions on the influence of raspberry juice yield the best conditions for A3B2C2. the best addition amount of 0.07% of pectinase enzymolysis, best temperature 45℃, enzyme solution is the best time of 3.5 h. R value analysis results show that the impact sequence on the raspberry juice yield sequence for B>A>C, enzyme solution temperature is the main factor of raspberry juice yield, followed by the addition ratio of pectinase, minimal impact for digestion time.

In addition, the contents of soluble solids, total sugar, total acid and vitamin C in raspberry juice under various enzymatic hydrolysis conditions varied little. It can be seen they have little effect on the amount of pectinase added, the temperature and the time of enzymatic hydrolysis. However, the light transmittance of raspberry juice under the condition of variations from 50.3% to 64.8%, poor R value analysis result shows that the factors impact on the raspberry juice transmittance order for B > A > C, the main factor was the enzyme solution temperature, followed by the addition ratio of pectinase, finally for digestion time, the results and the impact on the juice yield is nearly the same.

Table 3. Analysis of variance of orthogonal test L9(3^4)

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|--------|----|----------|-------------|---------|--------|
| A      | 2  | 112.2744 | 56.1372     | 5.29    | 0.0001 |
| B      | 2  | 166.1211 | 83.0605     | 7.42    | 0.0001 |
| C      | 2  | 33.5811  | 16.7905     | 2.79    | 0.0001 |
| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
| Model  | 6  | 311.9766 | 51.9961     | 5.14    | 0.0001 |
| Error  | 11 | 2154.3011| 195.8455    |         |        |
| Corrected Total | 17 | 2466.2777 |             |         |        |

It can be seen from table 3 that the main order of influence on juice production rate and light transmittance is: enzymatic hydrolysis temperature > pectinase addition amount > enzymatic hydrolysis time, which is consistent with the orthogonal test results. According to the anova table, the P values of enzyme addition, enzymatic hydrolysis temperature and enzymatic hydrolysis time were all less than 0.01, with significant difference.

4. Conclusion
Pectinase can improve raspberry juice yield. The best conditions for enzyme solution, pectinase adding amount of 0.07%, temperature 45℃, time of 3.5 h. Under these conditions, the content of soluble solids, total sugar, total acid and vitamin C in raspberry juice had little effect, and the light transmittance of raspberry juice had great influence.

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