Optimization of Blanching Process of hawthorn using central composite design and response surface method

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Abstract. Response surface method (RSM) was used to optimize hawthorn blanching process based on single-factor experiments. There were two numerical (time and temperature) and one categorical variable (POD enzyme activity) that was used for the evaluation of the hawthorn blanching quality. The changes in POD enzyme activity, procyanidin content, and total flavonoid content were also analyzed. The regression model (P<0.05) was obtained through Central-Composite experimental design, indicating that the model could evaluate the residual POD enzyme activity in the blanching process under different parameters. The results show that time 103s and temperature 92.5℃ were the best blanching conditions. Under these optimized conditions, the residual POD enzyme activity of hawthorn was within a reasonable range, which was 0.7731 U·g⁻¹·min⁻¹. The calculated results were basically consistent with the model predicted values (0.7989 U·g⁻¹·min⁻¹).

1. Introduction

Crataegus sp., commonly known as hawthorn, or hawberry, is a fresh fruit which belong to Rosaceae family and is found widely distributed in North China. Hawthorn has many unique characteristics like excellent sour-sweat flavor and precious nutritional value. Medicinal properties could be found in the fruits, leaves, and flowers of various hawthorn species. Besides being a nutritious fruit, hawthorn is also a famous Chinese medicinal herb that has been utilized for a variety of therapeutic purposes with a long history of use [1, 4]. Many shreds of evidence suggest that hawthorn fruits are rich in polyphenols, which play an essential role in antioxidant, anti-inflammatory, and lipid-lowering [12, 14, 15]. The extraction of active components from fruits, leaves, and flowers of hawthorn and antioxidant effects have been studied extensively [5, 15]. Some pieces of evidence showed that procyanidin (PC) content in hawthorn is noteworthy, which makes it good source of edible PC [2, 6, 8, 11]. These indicate that hawthorn may be an important potential source of dietary antioxidants.

Blanching is an essential heat treatment of fruit and vegetable processing before freezing or extracting, which influences the quality of the product significantly [20, 22]. The physiochemical and nutritional properties of fruits changed after blanching treatment, which affected the bioactive compounds and antioxidant activity of fruit [21]. The primary purpose of blanching is to inactivate the enzymes (such as peroxidase, POD, and polyphenol oxidase, PPO), prevents the reduction of polyphenols (such as PC and total flavonoids) [7].
Timing and temperature are the most critical factors during the process of phenol extraction from plant tissue [16]. Compared with other temperature-time combinations, showed that higher temperatures (100°C) and a shorter blanching time (10min) are much more effective for vegetables, prevent the loss of chlorophyll and active components [17]. On the contrary, if the processing time is prolonged, more pigment and nutrients will be lost. Reducing the blanching temperature, especially below the boiling point, can maintain more than 80% of the active ingredients in some fruits [13].

To sum up, the efficacy and nutritional value of hawthorn fruit are related to active antioxidant substances. However, antioxidant compounds are unstable and perishable with long blanching time. The decomposition of these active components should be considered in the optimization of process parameters. Nevertheless, little is known about the effect of different blanching conditions on the content of active antioxidant ingredients in hawthorn. In addition, the effect of blanching treatment on antioxidant components such as total flavonoids and proanthocyanins in hawthorn fruit has not been reported. At present, there is no research on the optimization of blanching technology to improve the antioxidant activity of hawthorn products.

In this study, the effects of blanching time and blanching temperature on POD enzyme activity, total flavonoids, and proanthocyanin content of hawthorn fruit were studied. The project team optimized the blanching process of hawthorn by evaluating the changes of POD enzyme activity, total flavonoids and proanthocyanin content of hawthorn. Finally, the best blanching conditions were predicted by using the CCD model to obtain ideal hawthorn products with high antioxidant activity in the actual production.

2. Materials and methods

2.1. Hawthorn samples
This study takes a typical variety of hawthorn "Waibahong" as the research object, which was a typical cultivation variety of hawthorn in north China. The sample of hawthorn fruits was harvested from Tianjin Jizhou in November 2019. They were selected with similar size and ripening situation, with no infection and physical damage. Samples were cleaned and stored in polyethylene boxes at 4°C.

2.2. Single factor test of blanching time
Hawthorn samples in a ratio of 1:10 (w/v) were blanched at 98°C with time duration of 50, 60, 70, 80, 90, 100, 110, and 120 s, respectively. After blanching, surface of the sample was dried. 50 g of sample was transferred into a self-sealing bag and soaked in ice water for 10 mins at room temperature. Thereafter, activity of residual POD enzyme was measured.

2.3. Single factor test of blanching temperature
Hawthorn fruit in a ratio of 1:25 (w/v) was blanched at 78, 83, 88, 93, and 98 °C for time duration of 120s. After blanching, surface of the samples was dried. 50 g of sample was transferred into a self-sealing bag and soaked in ice water for 10 mins at room temperature. Thereafter, activity of residual POD enzyme was measured.

2.4. Effect of blanching time on the content of PC and total flavonoids content in hawthorn
Hawthorn samples in a ratio of 1:10 (w/v) were blanched at 98 °C for 1, 2, 3, 4, 5, 6, and 9 min respectively. After blanching, surface of the samples was dried, 50 g of sample was transferred into a self-sealing bag and soaked in ice water for 10 mins at room temperature. Thereafter, activity of residual POD enzyme was measured.

2.5. Central Composite Design (CCD) and statistical analysis
The Central-Composite design under the response surface methodology (RSM) developed by the Design Expert software (Version 8.0.6) was used further to optimize the process of hawthorn blanching of single factor. The two significant factors, blanching time (A), blanching temperature(B) were the two in
dependent variables, which were studied at five different levels (-1.414, -1, 0, 1, 1.414). The actual factors levels shown in Table 1.

| Variable       | Factor, % | -1.414 | -1  | 0  | 1  | 1.414 |
|----------------|-----------|--------|-----|----|----|-------|
| A  blanching time | 95.86     | 100    | 110 | 120| 124.14 |
| B  blanching temperature | 88.76 | 90    | 93  | 96 | 97.24 |

2.6. Determination of residual POD enzyme activity
The residual POD enzyme activity of hawthorn samples was determined according to the standard methods of the Experiment Guidance of Postharvest Physiology and Biochemistry of Fruits and Vegetables [3].

2.7. Determination of residual PC content
The residual PC content of hawthorn samples was determined according to the standard methods of the Technical Standards for Testing & Assessment of Health Food [9].

2.8. Determination of residual total flavonoids content
The residual total flavonoids content of hawthorn samples was determined according to the standard methods of the Determination of Total flavonoids in Foods Spectrophotometry [18].

3. Results and discussion

3.1. Effect of blanching time on POD enzyme activity of hawthorn
Figure 1 shows that the POD enzyme activity of hawthorn decreased gradually with the extension of blanching time. When the blanching time reached 70s and above, POD enzyme activity decreased sharply and then leveled off. There was no significant difference between the POD enzyme activity of 110s and 120s. This indicated that the POD enzyme in hawthorn was inactivated when the blanching time reaches 110s, and 110s could be the appropriate time for enzyme inactivation.

![Figure 1. Effect of blanching time on POD enzyme activity of hawthorn.](image-url)
3.2. Effect of blanching temperature on POD enzyme activity of hawthorn

With the increase of blanching temperature, POD enzyme activity in hawthorn decreased gradually. At 93°C, POD enzyme activity decreased to 0.426 U·g⁻¹·min⁻¹, and its activity was far less than 10% of that of fresh sample POD enzyme activity (9.4 U·g⁻¹·min⁻¹). POD enzyme activity almost down to zero at 98°C, indicating that 93°C can be used as an appropriate blanching temperature.

![Figure 2. Effect of blanching temperature on POD enzyme activity of hawthorn.](image)

3.3. Effect of blanching time on the content of PC in hawthorn

According to figure 3, with the extension of blanching time, the content of PC in hawthorn showed a decreasing trend. However, this decreasing trend was not significant within 6 minutes, indicating that the PC content was not effected within the 120 seconds blanching time set by a single factor.

![Figure 3. Effect of blanching time on PC content of hawthorn.](image)
3.4. Effect of blanching time on the content of total flavonoids in hawthorn

As can be seen from figure 4, with the extension of blanching time, the content of total flavonoids in hawthorn showed a decreasing trend. However, the decreasing trend of total flavonoids was not significant within 120 seconds, indicating that the total flavonoids content was not affected within the 120 seconds blanching time set by a single factor.

In summary, oxidation decomposition of hawthorn PC and total flavonoids will not be caused by hot blanching temperature and time set by the test, so it is unnecessary to consider the effect of hot blanching on PC and total flavonoids of hawthorn.

Figure 4. Effect of blanching time on total flavonoids content in hawthorn.

3.5. Blanching process optimization

Experimental design using CCD of two independent variables, including five replicates at the central point that is given in Table 2, which shows the experimental response.

| Table 2. The experimental design and results of CCD. |
|----------------|-------|----------------|
| Run | A     | B     | Y/(U·g⁻¹·min⁻¹) |
|-----|-------|-------|-----------------|
| 1   | 0     | 0     | 0.632           |
| 2   | 0     | 0     | 0.479           |
| 3   | -1.414| 0     | 0.814           |
| 4   | 0     | -1.414| 1.539           |
| 5   | 0     | 0     | 0.421           |
| 6   | 1     | -1    | 0.976           |
| 7   | 1     | 1     | 0.0589          |
| 8   | 0     | 0     | 0.478           |
| 9   | 0     | 0     | 0.425           |
| 10  | -1    | -1    | 1.491           |
| 11  | 0     | 1.414 | 0.0695          |
| 12  | -1    | 1     | 0.0834          |
| 13  | 1.414 | 0     | 0.182           |
3.6. Regression analysis of the data
Design-Expert software was applied for regression analysis and model fitting of experimental data based on the experimental results of Table 2. The experiment results analysis was performed by Design-Expert software to fit out equations:

\[ Y = 0.59 - 0.18A - 0.55B \]  \hspace{1cm} (1)

Where \( Y \) represents the POD enzyme activity, while \( A \) and \( B \) represents blanching time and blanching temperature, respectively.

3.7. Variance analysis
The ANOVA test verified the applicability of the model, which calculates statistical analysis showing the results in Table 3. F-test determined the effects of variables and the more noticeable effect on the variables with the lower p-value. The R-squared value provides a method for the variability of response values that can be explained by experimental factors and interactions.

The p-value could test the significance of each coefficient and reveal the interaction pattern between independent variables [10]; if the p-value less than 0.05, the corresponding variables would be more significant.

As shown in Table 3, the p-value of POD enzyme activity (Y) demonstrates a high significance for regression equation (p < 0.0001 < 0.001), and the p-value of lack of fit was 0.0535 (p > 0.05), which indicates that the model adequately fitted the experimental data, which proves the adequacy of the regression model. What is more, the results showed that two independent variables, the blanching time (A), blanching temperature (B), which both had significant effects (p<0.05). The order of variables affecting POD enzyme activity was as follows: blanching temperature (B) > blanching time (A). The value of the determination coefficient R-Squared, which was 90.23%, means the fitting degree of the equation is promising. The value of the pred coefficient (PredR-Squared= 81.25%) was in reasonable agreement with the adjustment coefficient (Adj R-Squared= 88.28%) value, which indicated a preferable correlation between the predictive and the measured value of POD enzyme activity.

**Table 3.** The ANOVA of POD enzyme activity (Y) as response values.

| Source       | DF | MS  | F    | Pr>F     |
|--------------|----|-----|------|----------|
| Model        | 2  | 1.34| 46.19| <0.0001  |
| A            | 1  | 0.26| 8.85 | 0.0139   |
| B            | 1  | 2.42| 83.53| <0.0001  |
| Residual     | 10 | 0.029|     |          |
| Lack of Fit  | 6  | 0.043| 5.92 | 0.0535   |
| Pure Error   | 4  | 7.34E-003| |          |
| Cor Total    | 12 |     |      |          |

***p<0.001, **p<0.01, *p<0.05.

Therefore, this model was practical and feasible for predicting the POD enzyme activity of hawthorn under various combinations of variables.

Two-dimensional contour plot could describe the interactions between independent variables (blanching time and blanching temperature) that were significant or not. And the three-dimensional response surface plots were plotted to assess the effect of independent variables on the response value [23]. The blanching technology of hawthorn was investigated when two varieties kept in the experimental range (Figure 5).
The response surface and contour lines of the regression model fitted with the Design-expert software that shown in figure 5. Previous studies have shown that the best quality of all kinds of fruits and vegetables was that the residual activity of POD enzyme does not exceed 10% [19]. Therefore, with the response value of POD enzyme activity in hawthorn at 0.0589~1.539 U·g⁻¹·min⁻¹ (POD enzyme activity residual rate of 0.6%~16%) as the optimization target, the optimal blanching time of hawthorn was 103.14s, the blanching temperature was 92.52℃, the POD enzyme activity was 0.7989 U·g⁻¹·min⁻¹, the POD enzyme activity residual rate was 8.32%, fully meeting the requirements of hawthorn blanching quality.

Figure 5. Response surface and contour plots of the influence of blanching temperature and blanching time on POD enzyme activity of hawthorn.
4. Verification test
In this work, the POD enzyme activity was calculated by blanching tests with the experimental parameter at 92.5°C for 103s, then sampling count. The experiment was carried out in three parallel groups, then average was calculated. Finally, after blanching treatment, POD enzyme activity in the experimental group was 0.7731 U·g⁻¹·min⁻¹, which was close to the predicted value (0.7989 U·g⁻¹·min⁻¹).

5. Conclusions
The Central-Composite experiment design optimized the blanching process for hawthorn. The blanching process had the optimal value when the blanching parameters of time and temperature were 103s and 92.5°C, respectively. Meanwhile, the POD enzyme activity of hawthorn was within a reasonable range, which was 0.7989 U·g⁻¹·min⁻¹.

The experimental results verify the prediction model and showed that the optimized conditions were reliable and effective, providing a reference for the research of the hawthorn blanching process.

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