EXPERIMENTAL STUDY

Optimization of vinegar-steaming process for Wuweizi (Fructus Schisandrae Chinensis) with response surface method

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Abstract

OBJECTIVE: To optimize the vinegar-steaming process of Wuweizi (Fructus Schisandrae Chinensis) using the response surface method (RSM) based on the Box-Behnken design.

METHODS: A regression model was constructed with the response variables, the content of Deoxyschizandrin, and the three explanatory factors: length of steaming time, the quantity of vinegar and length of moistening time to evaluate the effects on the processing of Wuweizi (Fructus Schisandrae Chinensis).

RESULTS: There was a linear relationship between the content of Deoxyschizandrin and the three explanatory factors. When the steaming time was 5.49 h, with 2.365 g of vinegar added and a moistening time of 4.13 h, the content of Deoxyschizandrin reached the maximum predicted value of 0.1076%, and under the conditions the average content of Deoxyschizandrin was 0.1058%.

CONCLUSION: The correlation coefficient of the nonlinear mathematical model was relatively high and the model matched the data well, potentially providing a method for the study of the steaming process.

INTRODUCTION

Response surface methodology (RSM) is a valuable tool to design biological processes and functions by the regression model. RSM is also called regression design. It is a method that collects data actively to construct a regression model, most often, a multiple linear regression. The model involves multiple factors, and it is used in many areas such as food, chemical engineering and so on. Wuweizi (Fructus Schisandrae Chinensis) can be processed by a number of methods including vinegar-steaming, wine-steaming and honey-steaming. Vinegar-steaming and alcoholic-steaming are the most common methods. Each method has its own advantages. This study is intended to optimize the vinegar-steaming process of Wuweizi (Fructus Schisandrae Chinensis) with RSM based on the Box-Behnken design proposal.

MATERIALS, REAGENTS AND INSTRUMENTS

Materials: Wuweizi (Fructus Schisandrae Chinensis) were identified as the dry fruits of Schisandra Sphenanthera Rehd et wils' by Feng Yonghui (Xi’an Medical University).
Reagents: Deoxyschizandrin standard was provided by the National Institute for The Control of Pharmaceutical and Biological Products. All reagents were chromatographically pure.
Instruments: 2690 HPLC (Waters Co., Milford, MA, USA), diode array detector.

**METHODS**

**Single factor experiments**

Steaming time: Ten-gram samples of dried Wuweizi (*Fructus Schisandrae Chinensis*) were treated with 2 g vinegar for 4 h after even mixing by stirring, and then each sample was steamed in a water bath for 2, 3, 4, 5 or 6 h. The fruits were then dried at 50°C for 4 h, and the Deoxyschizandrin content was determined by HPLC. Chromatographic conditions as follows: chromatography column: Symmetry C18 column (4.6 mm × 150 mm, 5 μm). Mobile phase: methanol-acetonitrile and water (1:1:1). Flow rate: 1 mL/min. Column temperature: 25°C. Detection wavelength: 250 nm. Sample size: 10 μL

From Figure 1, the Deoxyschizandrin content in steamed Wuweizi (*Fructus Schisandrae Chinensis*) shows a marked increase with steaming time, reaching a maximum at 5 h. After this time point, the Deoxyschizandrin content decreases with additional steaming time.

**Quantity of vinegar**

Ten-gram samples of dried Wuweizi (*Fructus Schisandrae Chinensis*) were each treated with 0.5, 1, 1.5, 2 or 2.5 g vinegar for 4 h after even mixing by stirring, and then placed into a water bath and steamed for 5 h. The samples were then dried at 50°C for 4 h. They were then steamed in a water bath for 4 h, and then dried at 50°C for 4 h. The Deoxyschizandrin content was determined by HPLC using the same chromatography conditions as described above.

From Figure 2, the Deoxyschizandrin content in steamed Wuweizi (*Fructus Schisandrae Chinensis*) shows a marked increase with the addition of vinegar, with a maximum at 2 g added vinegar.

**Moistening time**

Ten-gram samples of dried Wuweizi (*Fructus Schisandrae Chinensis*) were each treated with 2 g vinegar, and then placed into a water bath and steamed for 5 h, and then dried at 50°C for 4 h. The Deoxyschizandrin content was then determined by HPLC using the same chromatography conditions as described above.

From Figure 3, the Deoxyschizandrin content in steamed Wuweizi (*Fructus Schisandrae Chinensis*) shows a marked increase with moistening time with a maximum effect at 4 h.

**RESULTS**

**Encoding and resetting results to fit the Box-Behnken design**

A response surface was designed to measure the effects of the three explanatory factors: steaming time, the quantity of vinegar, and moistening time on the response variable, the content of Deoxyschizandrin in steamed Wuweizi (*Fructus Schisandrae Chinensis*) in order to establish a regression model. The factors and levels in the response surface design of Deoxyschizandrin in Schisandrea Sphenantherae Fructus are listed in Table 1.

The response surface method (RSM) was used, based on the Box-Behnken design, to estimate the best conditions for the content of Deoxyschizandrin. Response surface design matrix and experimental results of Wuweizi (*Fructus Schisandrae Chinensis*) were shown in Table 2.
A multivariate fitted regression model was constructed from the data to give:

\[ Y = 0.1059 + 0.0085A + 0.0055B + 0.0026C + 0.0048AB + 0.0019AC + 0.0030BC - 0.0126A^2 - 0.0105B^2 - 0.0213C^2 + \epsilon \]

From Table 3, the model was highly significant (\( P_{\text{model}} < 0.0001 \)), and the lack of fit analysis had less significance (\( R^2 = 0.9917 \)).

Response surface plot for the best condition and the interactive effects of the condition

According to the regression model and the RSM model by Design Expert, the response surface of multiple factors was constructed. As shown in Figures 4-6, when one of the three factors is set to 0, the other two factors influence the Deoxyschizandrin content. It was found that within the experimental range, the Deoxyschizandrin content, initially increased with respect to two factors and then decreased. Both the steaming time and the quantity of added vinegar affected the Deoxyschizandrin content whilst influence of the moistening time was relatively small.

To further verify the value of the best point, the first order partial derivative of the regression equation was calculated:

\[ 0.0085 - 0.0252A + 0.0048B + 0.0019C = 0; \]
\[ 0.0055 + 0.0048A - 0.0210B + 0.0030C = 0; \]
\[ 0.0026 + 0.0019A + 0.0030B - 0.0426C = 0. \]

This results in \( A = 0.49, B = 0.73, C = 0.13 \). These numbers were applied to the formula and the following optimized conditions obtained: steaming time 5.49 h, added vinegar 2.365 g and moistening time 4.13 h, maximizing the Deoxyschizandrin content up to 0.1076%.

DISCUSSION

The processing conditions in our study were generally consistent with those reported in the literature with the exception that the amount of vinegar added was slightly larger. Owing to the limitation of objective conditions, our study was only able to investigate one response variable. Additional studies would be able to take into account more variables such as Schizandrin B, other lignans, polysaccharides and volatile oils, in order to further optimize the conditions for processing of Wuweizi (Fructus Schisandrae Chinensis).

By using the response surface method, a nonlinear regression model possessing a high \( R^2 \) value was developed with an efficiently small number of experiments. With this model, an optimized processing condition was estimated that approximated real world conditions. This strongly suggests that the application of this research method is valid in the field of TCM processing.

Using the design expert to estimate best conditions for processing, which was then tested by a regression model, the average content of Deoxyschizandrin obtained by testing in triplicate was 0.1058%. The difference between the experimental value and the theoretical value obtained was only 1.67%, which suggests that this model accurately reflects the relationship between Deoxyschizandrin content and the three explanatory factors.
REFERENCES


13. Tian HH, Ping NS, Yong JS. Preparative separation and purification of deoxyschizandrin and γ-schisandrin from Schisandra chinensis (Turcz.) Baill by high-speed counter-current chromatography. JCA 2005; 1066: 239.


