

Polyphenol Content and Antioxidant Capacity of Herbal Tea from Vietnamese Water Hyssop (*Bacopa monnieri*)

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Abstract— A sustainable process to produce herbal tea from Vietnamese water hyssop (*Bacopa monnieri*) has been conducted. The product can be used as traditional Ayurvedic medicinal herb. In this study, factors affecting drying process have been investigated including temperature ranging from 60 to 80°C, material thickness from 0.18 to 0.54 g/cm² and air velocity from 0.5 to 1.5 m/s. Total polyphenols and soluble solid contents were observed during the process. Antioxidant capacity of raw and dried water hyssop have been also evaluated. The optimum result showed that at temperature of 60°C, material thickness of 0.18 g/cm² and air velocity of 1.5 m/s, polyphenols content and total soluble solids in final herbal tea were found at 15.13 mg/g gallic acid equivalent and 4.00%. With saponins concentrations of 0.53% and 0.50%, the IC50 values of 3.94 µg/ml and 4.45 µg/ml were found in the raw and dried water hyssop tea, respectively. It's required to dry water hyssop at optimum condition in 180 minutes and chop materials into 1mm lengths before being used as herbal tea.

Keywords— Polyphenols; saponin; IC50; *Bacopa monnieri*.

I. INTRODUCTION

Water hyssop (*Bacopa monnieri*) is widely grown in the tropics and subtropics regions including India, Sri Lanka, South China, Taiwan and Southeast Asia. Besides used as a vegetable, with many special features water hyssop can be used as a traditional medicine in countries like India, Southeast Asia and China [1], [2]. This herb is also used in the pharmaceutical industry because of its valuable bioactive compounds such as alkaloids, saponins [3] - [8].

In Vietnam, Water hyssop grows almost nationwide, especially in the midland plains and the northern and southern Vietnam. However, it has been mainly used as fresh vegetables. Nowadays there is a trend to used natural herb as functional food. The purpose of this study focused on drying process of Vietnamese water hyssop and its antioxidant properties.

II. MATERIALS AND METHODS

A. Materials

Water hyssop has been obtained from the local market at Trang Dai district, Bien Hoa city, Dong Nai province, Vietnam.

Following procedure has been used to dry the water hyssop tea: Water hyssop => cleaning => cool drying =>

blanching => hot air drying => grinding => packaging => Herbal tea.

B. Chemicals

Folin-Ciocalteu agent, DPPH and galic acid were supplied by Merck (Germany). Oleanolic acid was from Sigma (USA).

C. Analysis methods

Saponins determined by the spectrophotometric method at 538 nm using oleanolic acid as standard [9]. Content of total polyphenols was assessed by colorimetric method using Folin-Ciocalteu reagents. Antioxidant properties were determined by colorimetric method described in Fu and Shieh [10].

D. Experimental Design

Response surface methodology was used to determine optimal process parameters to dry the water hyssop. The general form of a response surface of response variable Y as a function of n independent process variables from X₁ to X_n is presented as Equation (1).

$$Y = B_0 + \sum_{i=1}^n B_i X_i + \sum_{i=1}^n B_{ii} X_i^2 + \sum_{\substack{i,j=1 \\ i \neq j}}^n B_{ij} X_i X_j \quad (1)$$

where B_0 is the constant, B_i is the linear coefficient; B_{ii} is the quadratic coefficient; and B_{ij} is the cross-product coefficient. A central composite design (CCD) was employed to the experimental drying data. In this study three independent process variables are temperature (X_1), material thickness (X_2) and air velocity (X_3). Stat Ease® Design expert version 6.0.7 was used to fit the quadratic response surface model to the experimental data. Table 1 shows treatment levels and coded values for the independent process variables used in developing the experimental data. The response variables selected were polyphenols content Y_1 (mg/g) and total soluble solids (TSS) Y_2 (°Brix).

TABLE I
TREATMENT LEVELS AND CODED VALUES OF THE INDEPENDENT VARIABLES.

| Independent variables | Symbols | Levels | |
|---|---------|---------|-------|
| | | Uncoded | Coded |
| Temperature (°C) | X_1 | 60 | -1 |
| | | 70 | 0 |
| | | 80 | 1 |
| Material thickness (g/cm ²) | X_2 | 0.18 | -1 |
| | | 0.36 | 0 |
| | | 0.54 | 1 |
| Air velocity (m/s) | X_3 | 0.5 | -1 |
| | | 1.0 | 0 |
| | | 1.5 | 1 |

III. RESULTS AND DISCUSSIONS

A. Response surface analysis of drying process

Experimental results of drying water hyssop are shown in Table 2. The responses of polyphenols content and TSS are fitted in the coded models, in which all the coefficients of Equation 1 are obtained.

Effects of linear, quadratic and cross-product coefficients of second-order models on polyphenols content and TSS are shown in Table 3.

Omitted terms which are insignificant at $p \geq 0.05$, the linear regression equations for polyphenols content Y_1 (mg/g) and total soluble solids Y_2 (%) are presented as follow:

$$Y_1 = 11.36 - 2.09 X_1 - 2.60 X_2 + 0.99 X_3 - 1.29 X_2^2 + 0.65 X_2 X_3 \quad (2)$$

$$Y_2 = 3.25 - 0.57 X_1 - 0.1 X_2 + 0.09 X_3 + 0.22 X_1^2 - 0.18 X_3^2 \quad (3)$$

Using these optimized models for water hyssop drying, the correlations between the experimental and predicted values of polyphenols contents and TSS are presented in Figure 1. Results show that actual and predicted values are closely distributed. Moreover, measure of goodness-of-fit of linear regression of polyphenols contents ($R^2 = 0.98$) and TSS ($R^2 = 0.97$) indicate a high degree of correlation between investigated processing parameters and response variables.

TABLE II
EXPERIMENTAL CCD MATRIX DESIGN AND RESULTS OBTAINED OF DIFFERENT RESPONSE VARIABLES.

| Exp. | Coded | | | Experimental values | |
|------|-------|-------|-------|-----------------------------------|--------------------|
| | X_1 | X_2 | X_3 | Polyphenols content, Y_1 (mg/g) | TSS, Y_2 (°Brix) |
| 1 | -1 | -1 | -1 | 14.36 | 4.0 |
| 2 | -1 | -1 | 1 | 15.13 | 4.0 |
| 3 | -1 | 0 | 0 | 13.88 | 4.0 |
| 4 | -1 | 1 | -1 | 7.699 | 3.8 |
| 5 | -1 | 1 | 1 | 11.91 | 4.0 |
| 6 | 0 | -1 | 0 | 11.92 | 3.5 |
| 7 | 0 | 0 | -1 | 10.14 | 3.0 |
| 8 | 0 | 0 | 0 | 11.74 | 3.3 |
| 9 | 0 | 0 | 0 | 11.72 | 3.3 |
| 10 | 0 | 0 | 0 | 11.45 | 3.3 |
| 11 | 0 | 0 | 0 | 11.45 | 3.3 |
| 12 | 0 | 0 | 0 | 11.58 | 3.3 |
| 13 | 0 | 0 | 0 | 11.25 | 3.3 |
| 14 | 0 | 0 | 1 | 12.05 | 3.0 |
| 15 | 0 | 1 | 0 | 7.699 | 3.0 |
| 16 | 1 | -1 | -1 | 11.04 | 2.8 |
| 17 | 1 | -1 | 1 | 11.63 | 3.0 |
| 18 | 1 | 0 | 0 | 8.697 | 2.8 |
| 19 | 1 | 1 | -1 | 4.147 | 2.5 |
| 20 | 1 | 1 | 1 | 6.562 | 3.0 |

TABLE III
ESTIMATED REGRESSION COEFFICIENTS OF SECOND-ORDER MODELS OF DIFFERENT RESPONSE VARIABLES.

| Term | Polyphenols content | | TSS | |
|----------------|------------------------|---------|------------------------|---------|
| | Regression coefficient | p value | Regression coefficient | p value |
| Constant B_0 | 11.36 | | 3.25 | |
| Linear | | | | |
| B_1 | -2.09 | <.0001 | -0.57 | <.0001 |
| B_2 | -2.61 | <.0001 | -0.1 | 0.02 |
| B_3 | 0.99 | <.0001 | 0.09 | 0.03 |
| Quadratic | | | | |
| B_{11} | 0.19 | 0.54 | 0.22 | 0.009 |
| B_{22} | -1.29 | 0.001 | 0.07 | 0.31 |
| B_{33} | -0.01 | 0.97 | -0.18 | 0.03 |
| Cross-product | | | | |
| B_{12} | -0.26 | 0.16 | -0.01 | 0.76 |
| B_{13} | -0.26 | 0.18 | 0.06 | 0.15 |
| B_{23} | 0.66 | 0.003 | 0.06 | 0.15 |
| R^2 | 0.98 | | 0.97 | |

It can be observed from Table 2 and 3 that all investigated factors significantly affects both polyphenols content and TSS of the dried herbal tea. Increasing the drying temperature and air velocity or reduce material thickness can accelerate the drying process of water hyssop. However polyphenols compounds are easily oxidized during the drying under hot air because they contain the pirogalic or

pirocatesic groups which are vulnerable to redox reaction. The experimental result are similar to those reported on lowering polyphenols content during drying process of tomatoes [11], asparagus [12], sweet potatoes [13], corn [14], and chili [15].

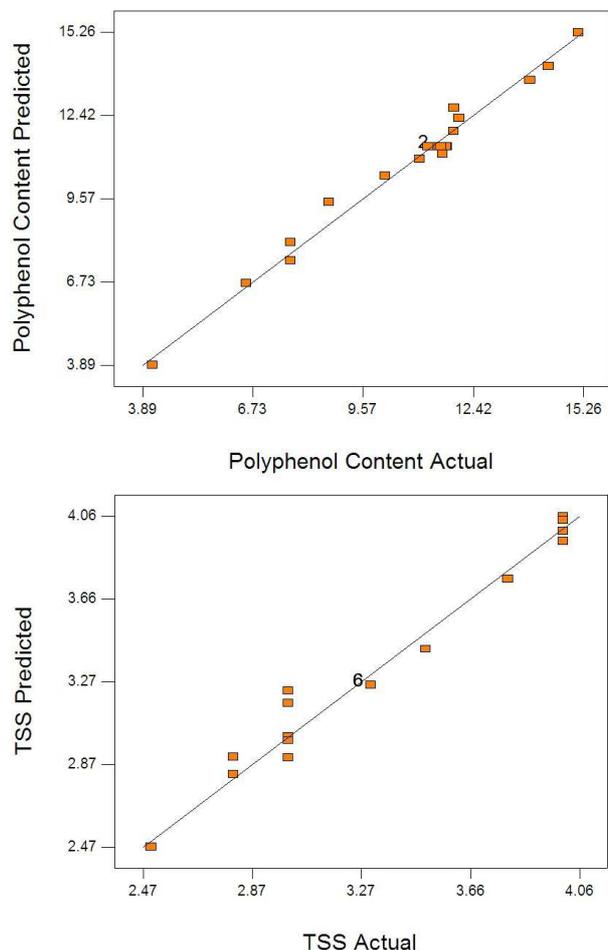


Fig. 1 Predicted vs. experimental values of different response variables: (a) polyphenols content (mg/g); (b) Total soluble solid (°Brix).

The contour plots of response surfaces can be used to explore the changes of response variables. Figures 2 - 3 show the dependence of chosen response values on the changes of process parameters around the center values developed in the CCD design. For example the effect of drying temperature on response variables at a central air velocity of 1.0 m/s can be observed. With material thickness of 0.36 g/cm² increasing drying temperature from 60°C to 80°C will decrease polyphenols content from 13.88% to 8.697% and TSS from 4.0 to 2.8 Brix.

Predicted optimal processing parameters are developed by JMP software. The results show that drying temperature of 60°C, material thickness of 0.18 g/cm² and air velocity of 1.5 m/s are selected as the appropriate conditions to dry the water hyssop. To be confirmed, the drying process is performed at predicted optimal drying temperature, material thickness and air velocity. Predicted and experimental data at optimal conditions are shown in Table 4. Experimental results show that predicted and actual values of polyphenols content and TSS are quite similar. At optimal conditions,

drying performance can be achieved at polyphenols content of 15.13% and TSS of 4.00 °Brix.

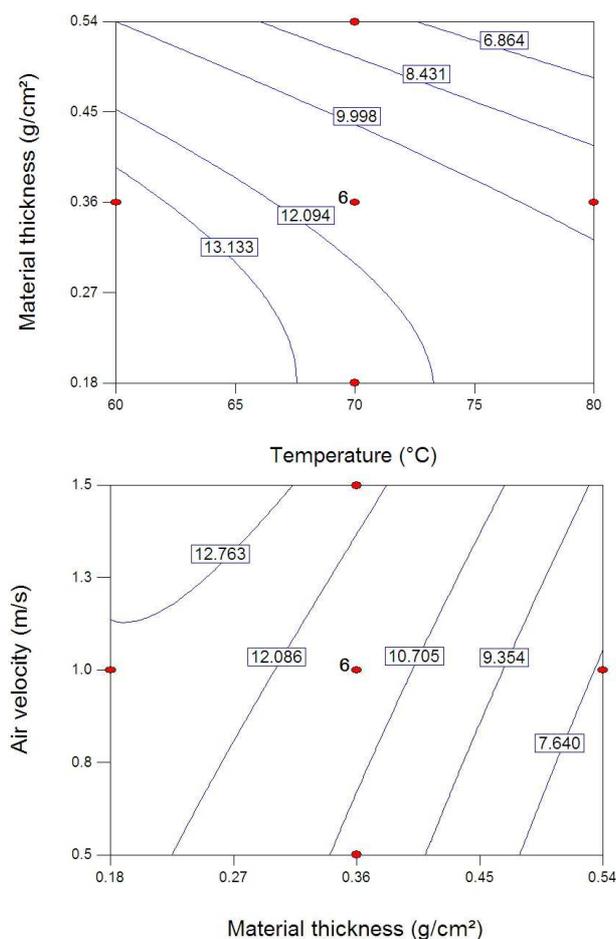


Fig. 2 Effect of process parameters on the polyphenols content (mg/g).

TABLE IV
PREDICTED AND ACTUAL DATA AT OPTIMAL POINTS.

| Response variables | Predicted values | Experimental values |
|--------------------------------------|------------------|---------------------|
| Polyphenols content (%) | 15.26 | 15.13 |
| Total soluble solids content (°Brix) | 3.99 | 4.00 |

B. Drying curve of water hyssop tea

Figure 4 shows moisture content at 30-minutes intervals when water hyssop is dried at the optimum condition of drying temperature, material thickness and air velocity density. Because the initial moisture content of water hyssop is rather high, it is required to chop the raw material into 1mm lengths and dry in 180 minutes to obtain a reduction from 93.98% to the the final moisture content of 5.94% as fulfilled the requirement by Vietnamese standard for herbal tea, TCVN 7975:2008.

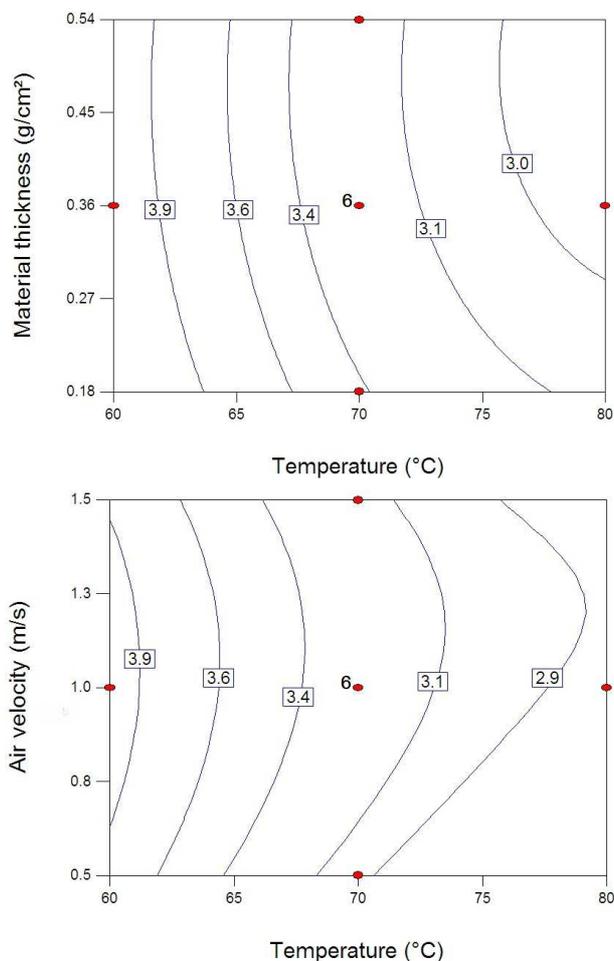


Fig. 3 Effect of process parameters on the total soluble solid content (°Brix).

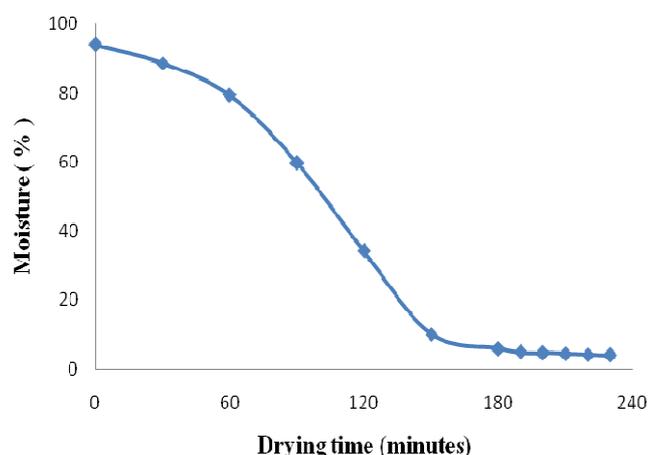


Fig. 4 Drying curve of water hyssop.

C. Polyphenols content and antioxidant capacity

The dried water hyssop has a great potential to be used as herbal tea because of its valuable bioactive compounds. Table 5 shows the contents of saponins and polyphenols in the raw material and finished product. Their antioxidant activity (IC₅₀) and ability to capture free radical DPPH compared to vitamin C has also reported.

Concentrations of saponins respectively of raw materials and finished tea products are 0.529% and 0.499% respectively.

TABLE V
COMPARISON OF SAPONINS, POLYPHENOLS, ANTIOXIDANT ACTIVITY IN RAW MATERIAL AND DRIED WATER HYSSOP.

| | Saponins | Poly-phenols | Anti-oxidant activity (IC 50) | Free radical scavenging activity (compared to Vit C) |
|------------------|----------|--------------|-------------------------------|--|
| Raw water hyssop | 0.53 (%) | 16.03 (%) | 3.937 (µg/ml) | 56.0 (%) |
| Water hyssop tea | 0.50 (%) | 14.72 (%) | 4.446 (µg/ml) | 49.6 (%) |

It can be observed that amount of saponins in the finished product decreased compared with the raw material. The polyphenols content in finished products is also lower than the raw material because of oxidation during the drying process. As a result the ability to capture free radicals in raw material is also lightly higher than the processed water hyssop tea.

IV. CONCLUSIONS

Drying of water hyssop to obtain herbal tea has been successfully studied. Response surface methodology using central composite design can be employed to predict polyphenols content and total soluble solids based on processing parameters of drying temperature, material thickness and air velocity. The optimal drying conditions are chosen at 60 °C, material density of 0.18 g/cm² and air velocity at 1.5 m/s. After 180 minutes of drying, water hyssop tea obtained moisture of 5.94% and its saponin and polyphenols content can be achieved at 0.499% and 14.717% of the total soluble solids respectively.

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