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Optimization of Temperature and Drying Time on Flavonoids Levels of Anjasmoro Variety Soybean (*Glycine Max*) Extract Using Response Surface Methodology

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ABSTRACT

Soybeans (*Glycine max*) contain a number of nutritional and health-supporting compounds, such as protein, fibers, phytosterols, and isoflavones. The pharmacological activities of soybeans include improving the immune system, acting as an antioxidant, inhibiting carcinogenesis, and reducing cholesterol levels. Anjasmoro variety is one of the superior varieties of large-seeded soybeans and is in great demand by farmers. The temperature and drying time of soybeans can have an effect on the level of compounds extracted. The aim of this research was to optimize flavonoid extraction from soybeans based on temperature and drying time using the response surface methodology (RSM). The results show that the optimal temperature and drying time of soybeans was obtained at 60.027 °C and 6.036 hours, with optimal flavonoid levels of 21.9707 mg QE/g. The verification results based on laboratory test confirmed that the level of flavonoids at optimal condition was 20.6461 mg QE/g, with a deviation of 6.03 % from prediction value and accuracy results of 93.97 %. The optimal drying temperature and drying time of soybeans can be used to obtain the highest flavonoid levels.

Keywords: *Glycine max*; *flavonoids; optimization; response surface methodology*

INTRODUCTION

Soybeans (*Glycine max*) are one of the largest food crop commodities in Indonesia after rice and corn. People use soybeans as a source of vegetable protein and as a health modulator. Soybeans are generally consumed in the form of processed products, including tofu, tempeh, soy sauce, tauco, soy milk, soy chips, soy nuggets, soy sticks, and various forms of snacks (Afiani & Aprilla, 2023; Aryanta, 2023). Soybeans can improve the body's immune system, act as antioxidants, inhibit cancer, reduce cholesterol levels, prevent diabetes, and manage problems related to postmenopause (Ahmad et al., 2014). Processed tofu, tempeh, and soybean flour products can reduce glucose levels in hyperglycemic mice (Hosea & Kartodarsono, 2022). Soybeans contain high amounts of protein, fibers, phytosterols, and flavonoid compounds in the form of isoflavones, genistein, and daidzein (Ahmad et al., 2014). According to Ahmad et al. (2014), isoflavones prevent degenerative illnesses by acting as an antioxidants. The isoflavones blocking the disfiguration of β-carotene, a vitamin precursor, preventing lipid oxidation, and boosting the

activity of glutathione peroxidase in the liver. Soybeans have several varieties, one of which is the Anjasmoro variety. Anjasmoro variety is known as a superior variety, with large seed size and in great demand by Indonesian farmers (Ramadhani et al., 2018).

The drying process of soybeans affects the levels of polyphenols and isoflavones in the dry seed hence affect their quality (Ferreira et al., 2019). Therefore, it is necessary to optimize the temperature and duration of soybeans drying process. The method used is the response surface methodology (RSM). RSM is an experimental strategy that is useful to find the optimal response (optimal condition) by determining midpoint and star arm runs (Yusof et al., 2019) when the response is influenced by several factors. The parameter to be optimized were temperature and drying time of soybean and the flavonoid levels were analyzed using colorimetry method.

METHODS

Equipment

The main equipment used in this research was the Jasco V-730 UV-Vis double beam spectrophotometer and other supporting devices.

Materials

Soybeans (*Glycine max*) were obtained from the Bogor Center for Agricultural Biotechnology and Genetic Resources Research. The variety of soybeans was Anjasmoro (Leguminosae). Aluminum chloride (Merck), quercetin (Sigma-Aldrich), sodium acetate (Merck), hydrochloric acid (Merck), sulfuric acid (Merck), and other pro analysis grade reagents were also obtained.

Sample Preparation

Fresh soybeans were dried using oven temperatures of 50 °C, 60 °C, and 70 °C, with drying times of 5, 6, and 7 hours, respectively. The dried soybeans samples were sieved using a 40-mesh sieve. The research design method for the drying process was the central composite design (CCD); it can be seen in Table 1.

Table 1. Variation of Temperature and Drying Time of Soybean

No.	Temperature (°C)	Time (Hours)
1	74	6
2	60	7.4
3	60	6
4	60	6
5	70	5
6	60	6
7	50	5
8	60	4.6
9	60	6
10	70	7
11	50	7
12	46	6
13	60	6

Extract Preparation

A total of 50 grams soybean powder were macerated using 250 mL of 70 % ethanol. After 24 hours, the extract was filtered, and the residue was remacerated with ethanol for 24 hours. All filtrate obtained was concentrated using rotary evaporator at a temperature of 60 °C until a thick extract was obtained.

Qualitative Tests of Phytochemical Compounds

Qualitative tests were carried out to detect the presence of polyphenols and flavonoids compounds in the extracts (Shaikh & Patil, 2020).

Analysis of Flavonoid Levels in Extracts

The sample solution was made by weighing 50.0 mg of extract from all treatments, and diluting it with 70 % ethanol in a 50-mL volumetric flask, respectively. The sample solution of 0.5 mL was pipetted, adding 1.5 mL of 96 % ethanol, 0.1 mL of 10 % aluminum

chloride, 0.1 mL of 1M sodium acetate, and 2.8 mL of aquadest and homogenizing. The solution was shaken until homogeneous, then left at room temperature for 30 minutes. The absorbance was measured using UV-Vis spectrophotometry at 430 nm. A calibration curve was created by measuring the absorbance of the quercetin standard series (Shraim et al., 2021).

RESULTS AND DISCUSSION

Flavonoids from soybean powder were extracted with 70 % ethanol solvent. Ethanol solvent commonly used in extraction of natural compounds able to dissolve almost all types of compounds, both polar and non-polaris groups (Joshi & Adhikari, 2019). The disadvantages of using ethanol in high concentration from an economic perspective are the expensive and inefficient extraction costs (Alam et al., 2013; Shi et al., 2003). Flavonoids from soybeans also can be extracted using 70 % methanol as well as ethanol at a concentration of 60–99.5 % (Chen et al., 2021; Malenčić, et al., 2021; Le et al., 2019; Woumbo et al., 2021; Hsieh et al., 2022). However, methanol is rarely used due to its toxicity properties (Joshi & Adhikari, 2019).

During the extraction process, the solvent will diffuse into the solid part of the plant, and the active compound will dissolve according to the polarity of the solvent (Pandey & Tripathi, 2019). The extract obtained varies in quality and quantity depending on the solvent and other parameters such as temperature, time and extraction method. Differences in extraction parameters also affect the compounds, especially flavonoids contained in the extract (Oreopoulou et al., 2019; Yeop et al., 2019). The soybean extract generally contains amounts of protein, carbohydrates, fats, and minerals contained (Abd-all et al., 2019). The Soybean extract generally contained polyphenols, tannins, flavonoids, proanthocyanidins, alkaloids, steroids, glycosides, and saponins (Malenčić, et al., 2007; Jaya & Das, 2019).

Qualitative analysis as a preliminary test in this research shows that the presence of flavonoid content detected the extract, so that further research can be carried out to determine the flavonoid content of soybean extract obtained from process of extraction at different temperatures and times.

Table 2. Level of Flavonoid in Soybeans

Runs	Temperature (°C)	Time (Hour)	Level (mg QE/g extract)
1	74	6	10.5384
2	60	7.4	11.4281
3	60	6	21.0375
4	60	6	20.0487
5	70	5	6.8936
6	60	6	22.1393
7	50	5	5.9089
8	60	4.6	8.9735
9	60	6	22.2013
10	70	7	6.3353
11	50	7	6.9101
12	46	6	10.5197
13	60	6	24.2819

The data show that drying process using a high temperature oven shortens the time but can reduce food quality. High drying temperatures will cause damage and decrease to the active substances contained in a food ingredient. High temperature is not suitable to dry substances with flavonoids and other thermolabile compounds (Tzanova et al., 2020).

The optimal condition to obtain flavonoid levels in soybean extracts were analyzed using RSM with the central composite design (CCD). The models displayed in Design Expert 13 software include linear models, linear models with interaction between to factors quadratic models, and cubic models. The selection of an appropriate model is based on the sequential model sum of squares, the lack of fit test, and model summary statistics. Optimization limit factor and response can be seen in Tables 3.

Table 3. Optimization Limit Factor and Response

Variable	Lower Limit	Upper Limit
A: Drying temperature (°C)	50	70
B: Drying time (hour)	5	7
Flavonoid levels (mg QE/g)	5.9089	24.2729

The response of flavonoid levels measured with ANOVA can be seen in Table 4. The model can be stated to be significant with $p < 0.05$. This means the model is valid to determine the optimal condition because the response can be precisely explained by the selected model. The p value for the temperature factor (A) was $0.948 > 0.05$. The value of the time factor (B) was $0.5666 > 0.05$, and the interaction of the two factors (AB) was $0.7437 > 0.05$. This means that temperature (A), time (B), and the interaction between the two factors (AB) have no significant effect on flavonoid levels, while temperature (A^2) and time (B^2) factors have a significant effect on flavonoid levels because the p value < 0.05, with the value being 0.0001 and 0.0001, respectively. The p value for lack of fit was $0.1249 > 0.05$, which means that it is not significant. Therefore, the selected model is appropriate for describing data on flavonoid levels. Based on the results of the RSM analysis, the following equation to determine the optimal condition was derived from the fitting (corresponding) equation 1.

Table 4. Response of Flavonoid Levels

Source	Sum of Square	Degree of Freedom	Mean Square	F value	P value	Significance
Model	567.74	5	113.55	21.6	0.0004	Significant
A-A	0.024	1	0.024	0.0046	0.948	
B-B	1.9	1	1.9	0.3615	0.5666	
AB	0.608	1	0.608	0.1157	0.7437	
A^2	308.85	1	308.85	58.76	0.0001	
B^2	324.34	1	324.34	61.71	0.0001	
Residual	36.79	7	5.26			
Lack of fit	26.81	3	8.94	3.58	0.1249	Not significant
Pure error	9.98	4	2.5			
Cor total	604.54	12				

$$Y = -488.0719 + 8.3124\ A + 85.95688\ B - 0.038988\ A*B - 0.067598\ A^2 - 6.92732\ B^2 \quad (1)$$

Y = Flavonoid levels (mg QE/g)
A = Drying temperature (°C)
B = Drying time (hour)
A*B = Interaction temperature to time
A^2 = Quadratic temperature (°C)
B^2 = Quadratic time (hour)

Figure 1 was a 3D graph of the model surface that shows interaction of extraction variables with drying time and drying. The graph is from a quadratic model in the form of a parabola. Flavonoid levels are higher if they are in the reddish-orange area. This research was carried out to obtain an optimal solution for temperature and drying time factors of soybeans so as to obtain the highest flavonoid levels. Experimental values and predicted values of response variables under optimal extraction conditions can be seen in Table 5.

Verification of flavonoid levels was carried out to determine actual flavonoid levels based on wet laboratory analysis and to determine the accuracy of the model prediction. Estimated optimal flavonoid levels using the fitting (corresponding) model were obtained by drying soybeans at a temperature of 60.027 °C for 6.036 hours, which produced flavonoid levels up to 21.9707 mg QE/g. The verification results for flavonoid levels (20.6461 mg QE/g) were lower than predicted, with a deviation of 6.03 %. Their accuracy was 93.97 %.

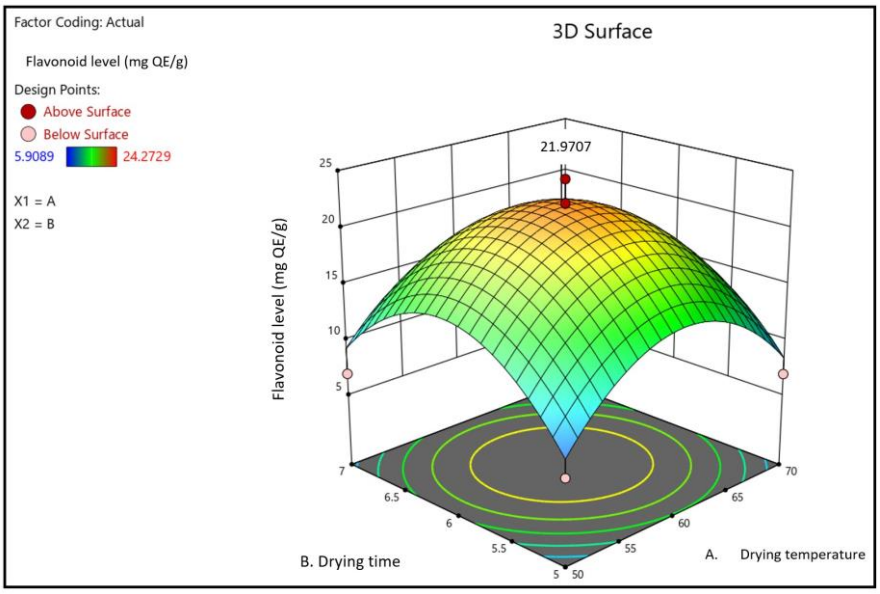

Figure 1. Interaction of drying time and drying temperature in extraction process

Table 5. Prediction Values and Verification Values of Optimal Parameters in Soybean Extraction Process

Parameters	Value
Drying temperature (°C)	60.027
Drying time (hour)	6.036
Prediction of flavonoid levels (mg QE/g)	21.9707
Desirability	0.873
Verification of flavonoid levels (mg QE/g)	20.6461
Accuracy of flavonoid levels (%)	93.97
Deviation (%)	6.03

CONCLUSION

The study results showed the optimal drying temperature and drying time for Anjasmoro variety soybeans using RSM were obtained at 60.027 °C for 6.036 hours, with optimal flavonoid levels of 21.9707 mg QE/g with 95 % degree of accuracy. The optimal verification of flavonoid levels based on wet laboratory analysis was 20.6461 mg QE/g, with a deviation of 6.03 % and 93.97 % degree of accuracy.

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