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Extraction Process of Polyphenols from Soybean (*Glycine max* L.) Sprouts: Optimization and Evaluation of Antioxidant Activity

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Abstract: This research aimed to optimize the total polyphenol content (TPC) extracted from soybean sprout powder under different experimental parameters, including ethanol concentration (60–100% *v/v*), extraction temperature (40–80 °C), extraction time (15–150 min), material:solvent ratio (1:4–1:10 g/mL), the number extraction cycles (1, 2 and 3 times), the age of sprout (0–7 days), and the used part of the sprout (cotyledon, hypocotyl, or radicle). The obtained results were used in response surface methodology, in combination with a central composite design, to model the total polyphenol content (TPC) with respect to three variables, including ethanol concentration, extraction temperature, and material:solvent ratio. The experimental conditions for optimal recovery of TPC consisted of ethanol concentration of 88% (*v/v*), extraction temperature of 59 °C, material:solvent ratio of 1:6.5 g/mL, extraction time of 60 min, and 2 cycles of maceration. In addition, for maximal TPC, the sprout should undergo the germination of 5 days and the radicle fraction should be used. Based on the suggested optimum conditions, the obtained and verified TPC was 19.801 mg genistein (GE)/g dry weight (d.w.). The obtained dried extract also exhibited low antioxidant activity.

Keywords: soybean sprout; isoflavone; total polyphenol content; optimization; antioxidant activity

1. Introduction

Due to the favorable geographic and climatic conditions, Vietnam possesses a rich source of medicinal materials and agricultural crops. One of the Far East's oldest crops is soybean (*Glycine max* (L.) Merrill), a native species of China. The crop has been used in various forms as one of the most important sources of dietary protein and oil thanks to its excellent protein-producing efficiency per unit of land, profitability, and potential use as a defense crop against famine. Soybean protein is also among plant proteins of best quality because it contains essential amino acids and holds the potential to replace proteins of animal origin [1]. Recently, the soybean has been claimed as a potential weapon against chronic diseases [2–7].

The soybean seed and sprout have been shown to possess phytochemicals, such as phenolic acids, saponins, phytosterols, isoflavones, phytates, and trypsin inhibitors, which are valuable in prevention of degenerative conditions [8]. Among them, isoflavones, phytoestrogen compounds belonging to polyphenols, have mechanism of action and function almost identical to those of female hormones. Structurally, isoflavones are oxygen heterocycles containing a 3-phenylchroman skeleton that is hydroxylated at 4 and 7 positions [9,10]. Since the structure is similar to that of estrogen, isoflavones are able to resist factors causing hormone-related cancers [11]. In vitro investigations and animal models have shown that isoflavones are a good candidate for inhibition of hormone-related cancers, such as breast, endometrial (uterine), and prostatic. Isoflavone also figures prominently due to its role as a classic dietary antioxidant. Other health benefits of isoflavone may include immunomodulation and risk reduction of chronic diseases, including cardiovascular diseases, diabetes, cancer, osteoporosis, and obesity [12–16].

Isoflavone content in the soybean sprout is generally higher than that in the soybean seed and the discrepancy can range from 20% to 50% after 24 h of germination [17]. In terms of isoflavone composition, the soybean sprout has been found to accumulate glycitein in large quantities in the hypocotyl, whereas the leaf fraction contains mostly daidzein and genistein [18]. In addition, coumestrol, an estrogen-like isoflavone, and its glucosides were also found in the soybean seed coats. These results were also confirmed in another comparative study where, among 46 different beans and bean sprout samples, coumestrol was found in soybean, black mappe, and green gram sprouts [19].

The sprouting process of the soybean is also suggested to cause total phenolic and flavonoid content to rise. To be specific, in a previous germination investigation involving six legume sprouts, the total phenolic content in the soybean was found to double after 120 h of germination, averaging 28.27 mg gallic acid (GAE).g^{-1} dry weight [20]. The same study also suggested that soybean sprout was ranked second only after peanut sprouts in terms of antioxidant activity and phenolic content. Another extensive review indicated that both total phenolic and flavonoid content of the soybean sprout methanolic extract far exceeded those of the cowpea and mungbean sprouts. Composition-wise, the major phenolic acid in the soybean sprout was salicylic acid, followed by ferulic acid and 3-hydroxycinnamic acid [21].

Attempts to achieve efficient recovery of biologically active compounds from plant materials could contribute to the development of food products in many ways. First, through optimization, improvements in existing manufacturing systems could be made, possibly leading to reduced material use, and in turn economic advantages. Second, successful surveys on plants that potentially produce useful compounds could propose new uses for existing species. For soybean sprout, despite its widespread culinary applications and established nutritional value, to date, industrial use of the sprout is limited and detailed investigation regarding isolation processes of valuable compounds from the material has been uncomprehensive. To our knowledge, only the isoflavone content of the soybean sprout cotyledon extract has been investigated and optimized, leaving total phenolic content unexplored [22]. Therefore, the aim of this research is to apply a centered-central composite design (CCD) subjected to response surface methodology (RSM) [23–26] to optimize the recoveries of total polyphenol (TPC). Considered optimization parameters include ethanol concentration, temperature of extraction, time of extraction, material:solvent ratio, and the number of extraction cycles. The results of this study are expected to contribute to the development of a new approach for large-scale production of phenolic compounds from soybean sprouts.

2. Materials and Methods

2.1. Plant Sample Preparation

The soybean seeds (DT2010 variety) [27] were harvested at Cu Chi Commune, Ho Chi Minh City, Vietnam, in February, 2019. Soybean seeds were preserved in zip bags, placed in a dry, ventilated place, and kept away from moisture during the study.

Soybean seeds were then germinated with the following procedure. First, 2 kg of soybean seeds were soaked into 20,000 mL of warm water at 35–40 °C for 3–5 h. Afterwards, the seeds were spread in baskets in a dark place and covered with dark colored towels to avoid light. The seeds were kept at room temperature (25–30 °C) and allowed to germinate for zero to 7 days. Water was sprayed twice daily to provide moisture during sprouting.

At day 0, after soaking in warm water, the sprouts had a length of 1.4 cm. On the second and the third day, the length of the sprouts was 3.3 cm and 7.4 cm, respectively. On the third day, the primary roots appeared and the length reached 10.3 cm. From the fourth to the seventh day, the epicotyl appeared, developed, and the first leaf appeared at the sixth day. The length of the sprouts at the fourth, fifth, sixth, and seventh days was 12.5 cm, 16.1 cm, 18.3 cm, and 22.4 cm, respectively (Figure 1). Soybean sprouts after being germinated for a suitable time were dried until the humidity was less than 12% and then crushed. The color of dried hypocotyl and roots were dark brown and that of dried cotyledon was golden brown. Crushed soybean germ powder had dark brown color.

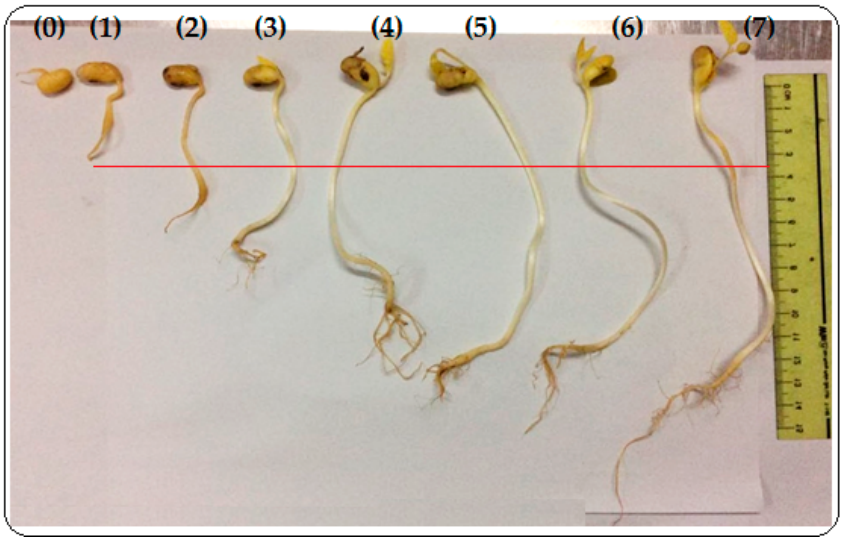


Figure 1. Germination progress of soybean seeds over 7 days.

2.2. Extraction Method

Maceration of plant material was employed in this study. In brief, twenty grams of powdered soybean sprouts were extracted under different extraction parameters, such as ethanol concentration (60–99.5% *v/v*), extraction temperature (40–80 °C), extraction time (30–150 min), material-solvent ratio (1:4–1:10 g/mL), and the number of extraction cycles (1, 2, and 3 times). The mixture of powder and solvent was agitated at 120 rpm. After extraction, the solvent was evaporated from the extract liquid at 50 °C under vacuum to obtain the dried extract.

2.3. Optimization of Extraction Process by Response Surface Methodology (RSM)

Response Surface Methodology (RSM), in combination with central composite design (CCD), was adopted to investigate the interaction of experimental variables and to produce a statistical model for the extraction process [28]. The factor levels for the RSM optimization process were selected based on single factor optimization method, as shown in Table 1. The three selected variables were ethanol concentration (X_1), temperature (X_2), and the ratio of material/ solvent (X_3). Each variable consisted of 3 different levels ranging from low (−1), to medium (0), and to high (+1). The number of experiments performed was $N = 2^k + 2k + 3$ ($N = 20$ with $k = 3$), in which k is the number of independent variables and 2k of additional experiments at the α. Distance from center to point $\alpha = 2^{k/4}$ ($\alpha = 1.68$ with $k = 3$). All studies were conducted at five levels ($-\alpha$, −1, 0, +1, $+\alpha$). Thus, in this study, 20 experiments were carried out with 2^3 experiments of total planning, 3 replicated experiments at the center to evaluate

errors, and 6 additional experiments (Table 2). The model that describe the contents of isoflavone as the response variable (Y) with respect to experimental parameters is established as follows:

$$Y(TPC) = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} {x_i}^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{3} \beta_{ij} x_i \beta x_j$$

where Y shows the response; β_0, β_i, β_{ii}, and β_{ij} indicate the regression coefficient for the intercept, liner, square, and interaction, respectively; x_i and x_j represent the independent factors; and n shows the number of factor involved.

The empirical model was evaluated using analysis of variance (ANOVA) at 5% significance level. The significant terms of the model were also obtained by ANOVA. The adequacy of the model was identified based on R-squared, adj-R-squared, predicted-R-squared, F-value, and the lack-of-fitness statistic.

The adequacy of the model was validated by reproducing the experiment in triplicate with obtained optimal parameters. The obtained results were then verified by comparing the predicted value with the actual values obtained from the experimental work.

Table 1. Experimental factors with their levels used in the experiment.

Factor	Symbol	Level		
		−1	**0**	**+1**
EtOH concentration (%)	X_1	85	90	95
Temperature (°C)	X_2	55	60	65
Solvent-material ratio	X_3	4	6	8

Table 2. Central composite design of coded factors.

Run	X_1	X_2	X_3
1	(−1)	(−1)	(−1)
2	1	(−1)	(−1)
3	(−1)	1	(−1)
4	1	1	(−1)
5	(−1)	(−1)	1
6	1	(−1)	1
7	(−1)	1	1
8	1	1	1
9	(−α)	0	0
10	(+α)	0	0
11	0	(−α)	0
12	0	(+α)	0
13	0	0	(−α)
14	0	0	(+α)
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

2.4. Quantification of Total Polyphenol Content (TPC) Based on Folin-Ciocalteu (FC) Methodology

The determination of TPC in the dried extract was done using the technique described by Singleton with modification [29,30]. First, the calibration curve was established by mixing 1 mL of aliquots with concentrations of 125, 250, 500, 750, 1000, 1250, and 1500 µg/mL genistein (GE, Sigma-Aldrich, St. Louis, MO, USA) solutions with 200 µL of Folin Ciocalteu (FC, Sigma-Aldrich, St. Louis, MO, USA) reagent,

3160 µL of water, and 600 µL sodium carbonate (Sigma-Aldrich, St. Louis, MO, USA) solution (20%). The absorbance was measured with a spectrophotometer (Shimadzu, Kyoto, Japan) after 30 min at 760 nm.

Testing sample for quantification of TPC was prepared as follows. First, 42 g of dried extract was dissolved in 6 mL of dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) to produce the stock solution. Then, 40 µL of the stock solution was mixed with 200 µL of FC. The solution was sonicated for 5 min at room temperature, followed by addition of 3160 µL of water and 600 µL sodium carbonate solution (20%). The final solution was sonicated for 30 min at room temperature. The blank sample was prepared identically, except for the stock solution, which was replaced by 40 µL of DMSO.

Testing sample was measured 3 times to obtain the average value. A UV-Vis Spectrophotometer (Shimadzu, Kyoto, Japan) was used to take the absorbance of the mixture at 760 nm wavelength against DMSO blank. The standard curve (100–1000 mg/ L) was prepared from genistein to calculate the concentration of the sample. The obtained result was explicated as mg of genistein per mg of the dried extract (mg genistein/ mg). The amount of TPC was calculated using the following equation:

$$\text{TPC}\left(\frac{mg\ Genistein}{g\ d.w}\right) = \frac{C_1\ m_1}{C_2 m}$$

where C_1 is the concentration obtained from Genistein standard curve (mg GE/L DMSO), C_2 is the concentration of the dried extract (mg dried extract/L DMSO), m_1 is the weight of total dried extract (mg), and m is the weight of dried material (soybean sprouts powder) (g).

2.5. Antioxidation Evaluation with 1,1-Diphenyl-2-Picrylhydrazyl (DPPH)

DPPH measurements were used based on the method of Brand-Williams and co-workers and Lee and co-workers [31,32], in which the determination of antioxidant potency is based on the scavenging activity of the stable DPPH free radical.

DPPH was first mixed with methanol (Sigma-Aldrich, St. Louis, MO, USA) 80% (with OD 517 nm = 0.80 ± 0.02) to form the solution with the concentration of 40 µg/mL. Vitamin C (Sigma-Aldrich, St. Louis, MO, USA), mixed in methanol 80% with concentrations of 0–100 µg/mL, was used as the positive control.

To commence the experiment, the sample was first dissolved in methanol 80%. Then, 180 µL of the prepared DPPH solution was added into 120 µL of the sample solution. The resultant solution was shaken, stored in darkness at 30 °C for 30 min, and then measured at a wavelength at 517 nm. Each experiment was repeated 3 times to calculate the average value. Besides, the color solution was prepared by adding 180 µL of 80% MeOH (Sigma-Aldrich, St. Louis, MO, USA) solution to 120 µL of the sample solution (120 µL). The absorbance of the color solution was measured at 517 nm. Each experience was repeated 3 times to calculate the average. The blank solution was prepared by adding 180 µL of the prepared DPPH solution into 120 µL of 80% MeOH solution.

The percentage of DPPH radical scavenging is calculated according to the following formula:

$$\text{DPPH}(\%) = \frac{A_b - (A_S - A_C)}{A_b}$$

where A_b is the optical density of the blank sample; A_s is the optical density of sample; A_c is the optical density of pigment; and IC_{50} value is calculated by the graph of % inhibition.

2.6. Instruments

The following instruments were used: moisture meter (MA35, Sartorius, Göttingen, Germany); UV-Vis spectrophotometer (Thermo Genesys 10S UV-Vis, Waltham, MA, USA); ultrasonic bath (Elma S 100 H, Elma, Singen, Germany); Elisa microplate reader (2100-C Optic Ivymen System, Biotech S.L., Madrid, Spain); rotary evaporator Buchi-R210 (Marshall Scientific, Hampton, NH, USA).

3. Results and Discussion

3.1. Single-Factor Investigations with Regard to TPC

3.1.1. Influence of Ethanol Concentration

Ethanol has been extensively used for extraction of biologically active compounds from plants due to its low toxicity. To enhance the extraction efficiency of polyphenol in soybean sprouts, ethanol is usually used with water at different concentrations due to its high dielectric property. Figure 2 indicates the effect of different ethanol concentrations ranging from 60% to 99.5% on recoveries of TPC. The TPC recovered from soybean sprout at 60% ethanol was 3.4 mg GE/g dry weight (d.w.). Further increase of ethanol concentration to 80% enhanced the yield of recovery to 7.1 (mg GE/g d.w.) and the yield peaked at about 9.0 mg GE/g d.w. at 90% concentration. However, raising the concentration past 90% to 99.5% lowered the yield to 7.4 mg GE/g d.w.

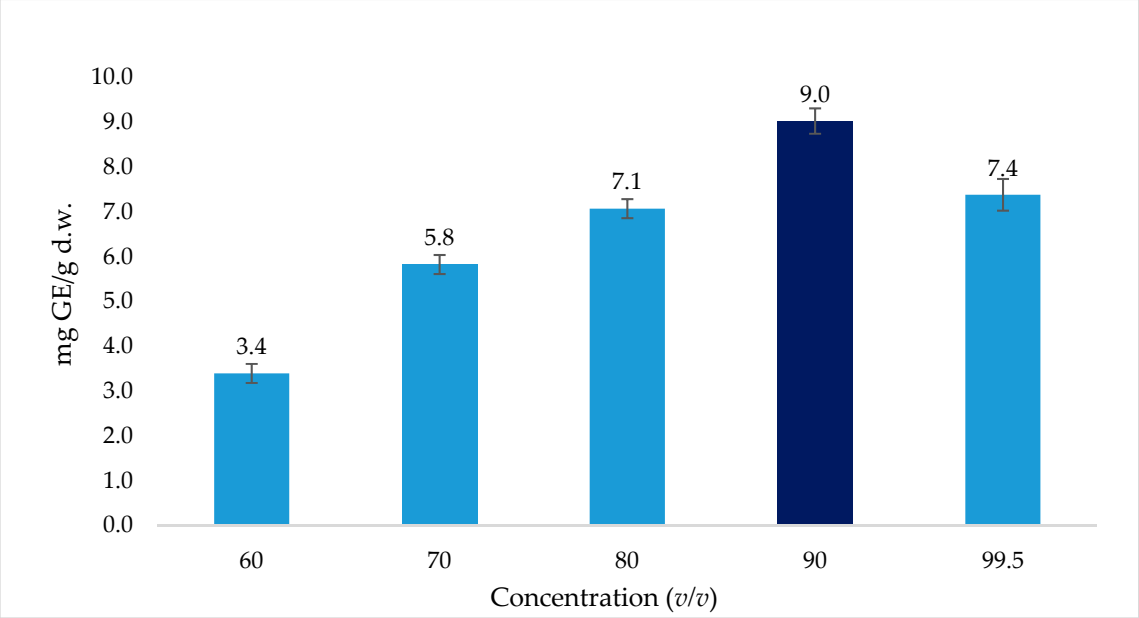


Figure 2. Effect of ethanol concentration.

Since most of the bioactive components, such as saponins, phenolics, and flavonoids, are highly polar, pure ethanol is not capable of fully extracting bioactive compounds. Therefore, water should be added to enhance the recovery yield. However, excess addition of water to the extraction solvent may generate water-soluble gums existing in soybean sprouts, rather than TPC, which might complicate the filtration process and reduces the isoflavone yield through absorption by the impurities, such as protein and fat. From the above observation, the ethanol concentration of 90% was selected for subsequent experiments.

3.1.2. Influence of Extraction Temperature

Figure 3 illustrates the TPC in relation to extraction temperature. As the temperature was elevated from 40 °C to 60 °C, the TPC increased gradually from 6.6 to the peak of 11.1 mg GE/g d.w. The mechanism through which temperature elevation improves recovery of extractant could be explained by enhanced diffusivity of solvent and improved solubility of phenolic compounds in solvents. In addition, increased mass transfer of phenolic compounds, caused by reduction in the viscosity and surface tension, might contribute to improvement of extracted phenolic compounds. However, TPC decreased dramatically to the minimum point of 6.0 mg GE/g when the temperature increased from 60 °C to 80 °C. This decrease may be due to the thermal destruction of polyphenols at high temperature.

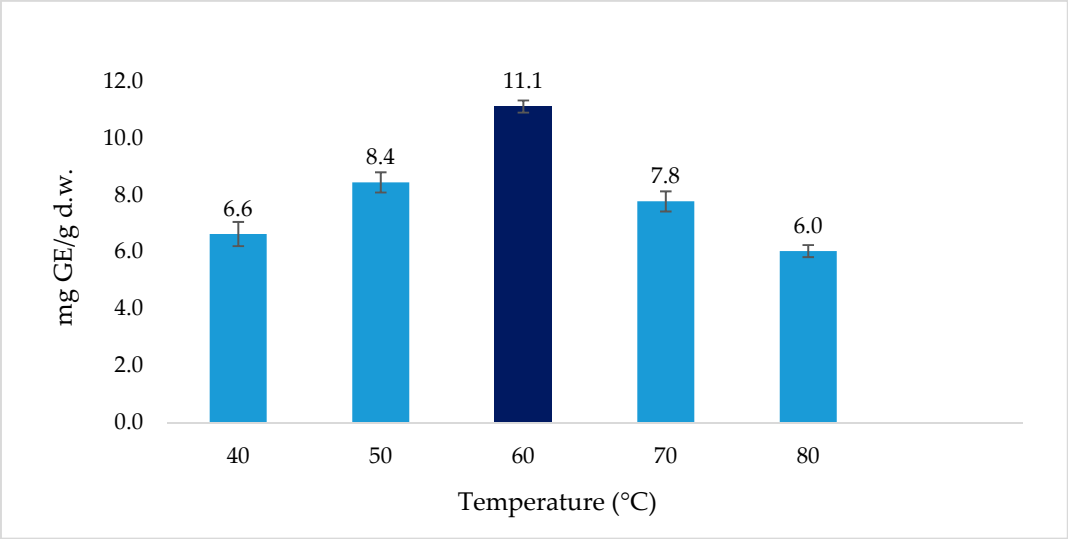


Figure 3. Effect of extraction temperature.

3.1.3. Influence of Material:Solvent Ratio

Material-to-solvent ratio is an important parameter in the extraction process, since the diffusion mechanism relies on the difference in concentrations between material and the solution. In liquid–solid extraction, the amount of substances collected depends on the amount of solvent. The ratio of material and solvent was allowed to vary from 1:04 to 1:10.

Figure 4 shows that when the material to solvent ratio increased from 1:04 to 1:06, TPC increased from 6.7 to 12.7 mg GE/g d.w. and reached the peak of 13.0 mg GE/g d.w. as the ratio reached 1:08. This is because the addition of solvent elevates the difference of concentration gradient, thus maintaining the diffusion until equilibrium is reached. At the equilibrium state where the amount of polyphenols in the material is exhausted, the addition of ethanol will not promote the TPC yield any longer. Evidently, differences of yields that were achieved at ratios of 1:06, 1:08, and 1:10 were subtle and not statistically significant. As a result, the raw material:solvent ratio of 1:06 was more economical and selected for subsequent experiments.

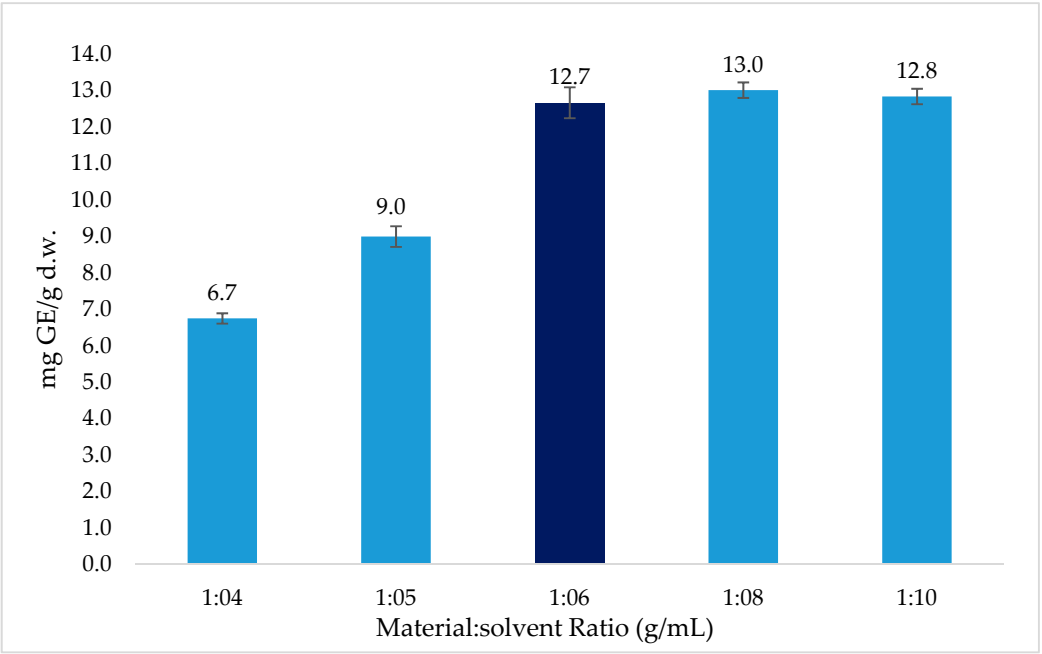


Figure 4. Effect of material:solvent ratio.

3.1.4. Influence of Extraction Time

Figure 5 presents the effect of extraction time on the TPC. It was observed that the extraction time was positively correlated with the TPC in the first 60 min, as evidenced by a steady rise of TPC from 5.4 to 12.8 when prolonging the time from 15 to 60 min. From 60 to 150 min, the TPC appeared to be unchanged, at around 12.7 mg GE/g d.w. This could be explained by the Fick's second law of diffusion, which articulates that an eventual equilibrium between material solute and the solvent will be achieved [33]. Therefore, prolonging the time period past a certain point had no effect on the extraction of phenolic compounds. Therefore, 60 min was selected as the suitable extraction time.

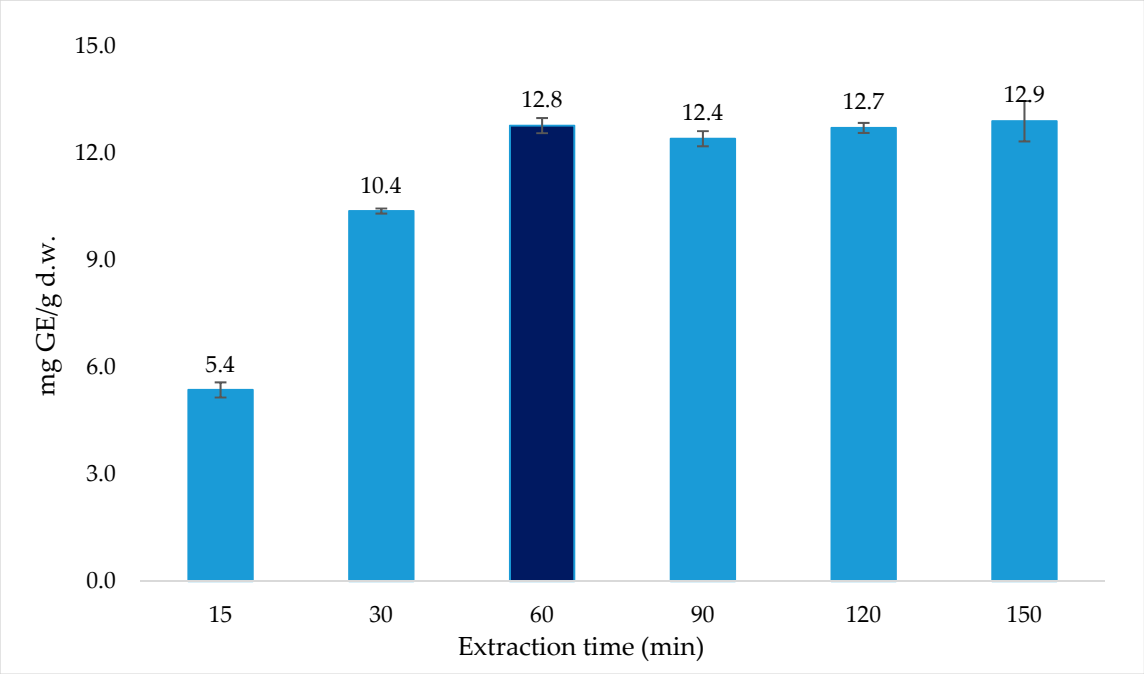


Figure 5. Effect of extraction time.

3.1.5. Influence of Number of Extraction Cycles

From Figure 6, it was indicated that TPC was not completely extracted after the first cycle. In fact, the yield of the second cycle was higher than that of the first cycle and TPC after two cycles, totaling 12.8 mg GE/g d.w. After the third extraction cycle, total TPC rose from 12.8 to 14.0 mg GE/g d.w., suggesting that most of TPC has been exhausted after the third cycle. Considering the minor yield enhancement after the third cycle, extraction through two cycles was selected as the suitable procedure for subsequent experiments.

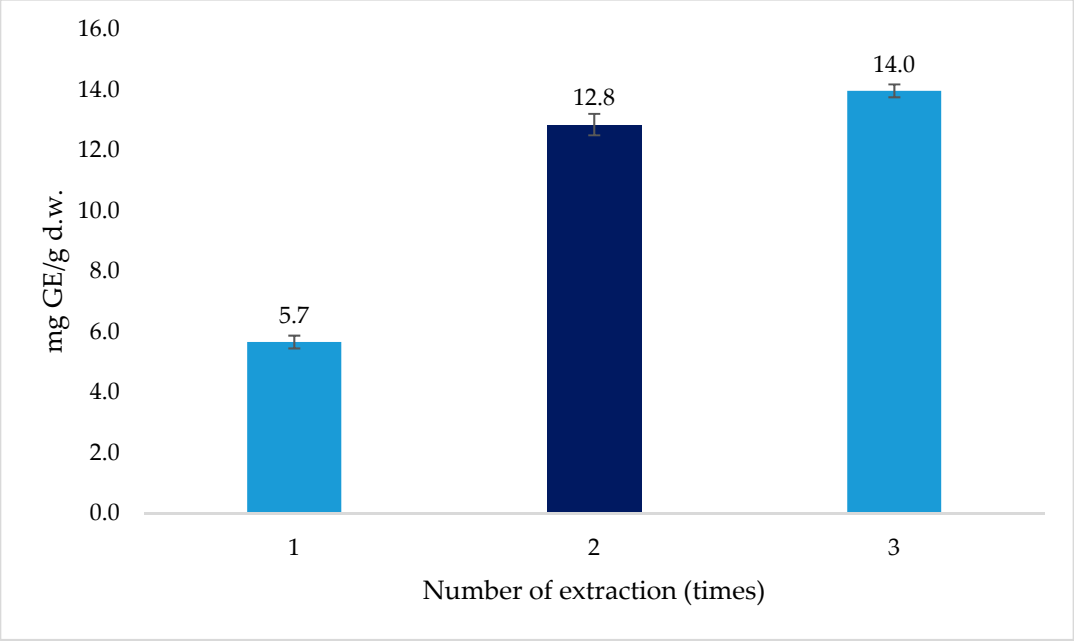


Figure 6. Effect of the number of extraction.

3.1.6. Influence of Germination Time

Figure 7 illustrates changes in TPC with regard to germination day. Within the first five days, it is clear that the TPC correlated positively with germination time and exhibited a turning point at the fifth day. Without germination, the TPC in the soybean seeds was initially 5.5 mg GE/g d.w. After the first day of germination, the TPC of soybean sprouts expanded around 1.5 times, reaching 9.4 mg GE/g d.w. Afterwards, TPC kept continuously increasing from 12.8 to 16.8 mg GE/g d.w. and reached a peak of 19.6 mg GE/g d.w. at the fifth day. However, the TPC declined from 19.6 to 14.5 mg GE/g d.w. when the germination period increased from 5 days to 7 days. The results followed a similar trend as reported by Phommalth, who observed the highest isoflavone content in the whole sprout (Pungsannamulkong and Aga3 varieties) on the seventh day of germination [17]. After 7 days, there was a decreasing trend in isoflavone content as the germination period increased.

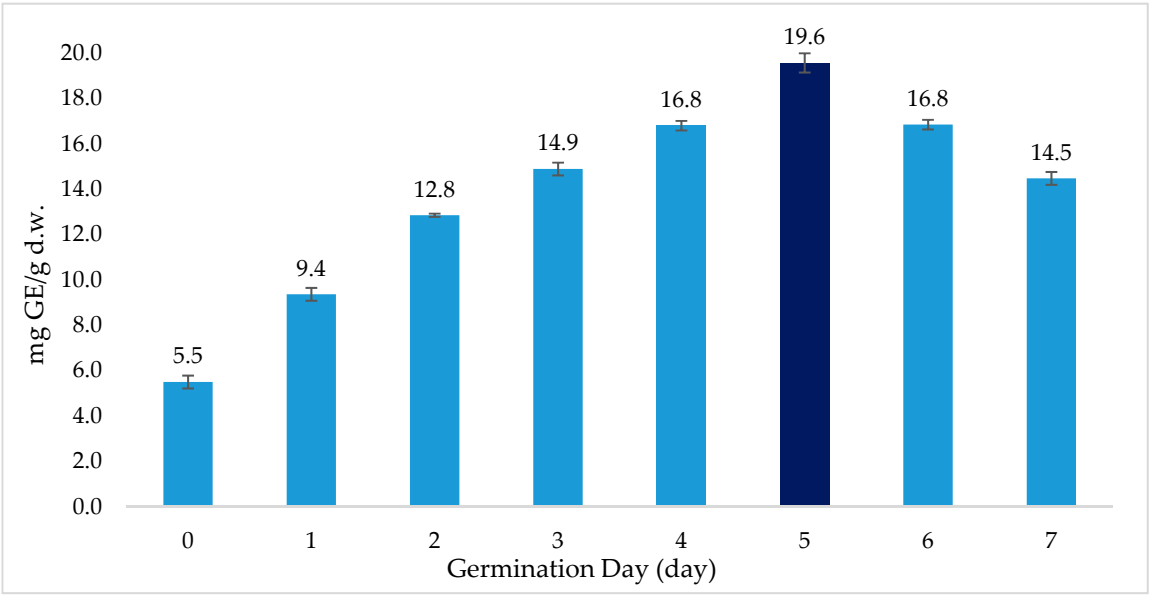


Figure 7. Effect of germination day.

3.1.7. Influence of the Structural Parts of the Soybean Sprouts

From Figure 8, it was observed that the TPC content in the radicle fraction was the highest at 24.4 mg GE/g d.w. The figure is 1.82-times higher than that in hypocotyl (13.4 mg GE/g d.w.) and 3-times higher than that in cotyledon (8.0 mg GE/g d.w.). This is consistent with a previous study, where the isoflavone content in the hypocotyl was found at a higher level than that in the cotyledon of non-sprouted soy-bean seeds, while the radicle of germinated seeds showed the highest isoflavone content [34].

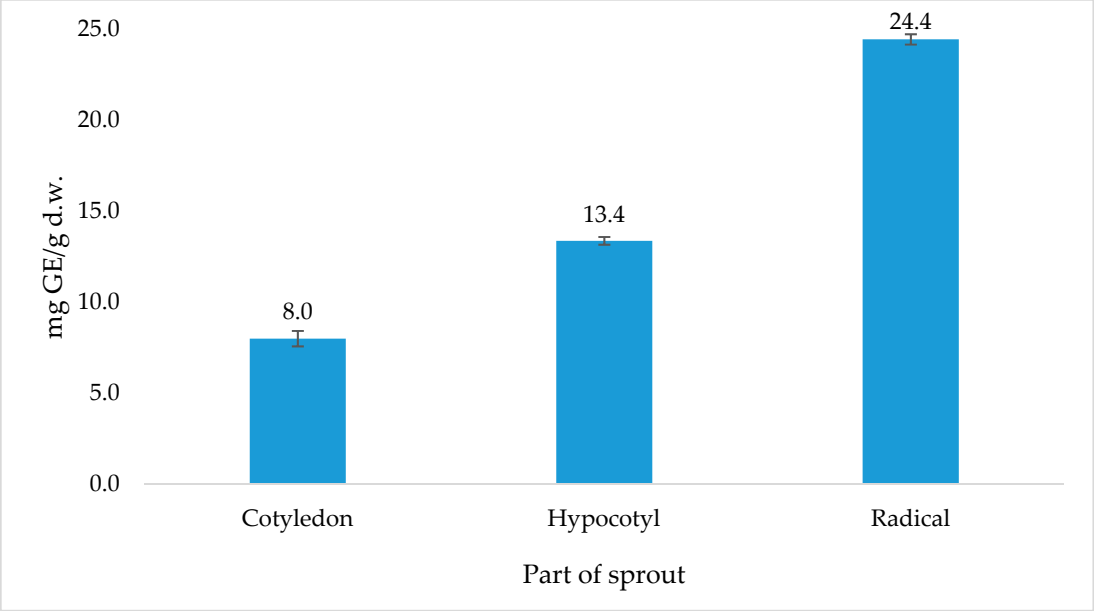


Figure 8. The polyphenol content in different parts of soybean sprouts.

3.2. Optimization Process with RSM

3.2.1. Model Fitting Using RSM

Variations of factors for optimization of processes were selected based on single factor experiments. To be specific, the factor levels for optimization of TPC included ethanol concentration X_1 (85–95% *v/v*), extraction temperature X_2 (55–65 °C), and material:solvent ratio X_3 (1:4–1:8 g/mL). Extraction time and germination day were fixed at 60 min and 5 days, respectively. The obtained optimum response was 19.621 (mg GE/g d.w.), based on the average of center points (optimum condition). The result of experimental design based on CCD and corresponding responses are shown in Table 3. The second-order polynomial equation of optimized condition for TPC is as follows:

$$Y_{TPC} = 19.62 - 0.7572X_1 - 0.3258X_2 + 0.5415X_3 - 1.25{X_1}^2 - 1.32{X_2}^2 - 0.8648{X_3}^2$$

where Y is the responses, X_1, X_2, and X_3 are the factors, including ethanol concentration, extraction temperature, and material-solvent ratio, respectively. Table 4 shows the result of analysis of variance. Overall, most of model terms and interaction factors were statistically significant ($p < 0.05$). The coefficient of determination (R^2) was 0.9902, which indicates a good correlation between predicted and actual (experimental) values. The suitability of the model also relates to a good agreement between predicted R^2 and adjusted R^2. Furthermore, for a fitted model, a non-significant lack-of-fit and Adequate precision value greater than 4 are desirable. Thus, the terms of experimental design obtained in this study suggested that models are reliable and have good predictability. This could be manually confirmed by examining the actual and predicted response in Table 3.

Table 3. Central composite design of actual factors and responses based on actual and predicted values.

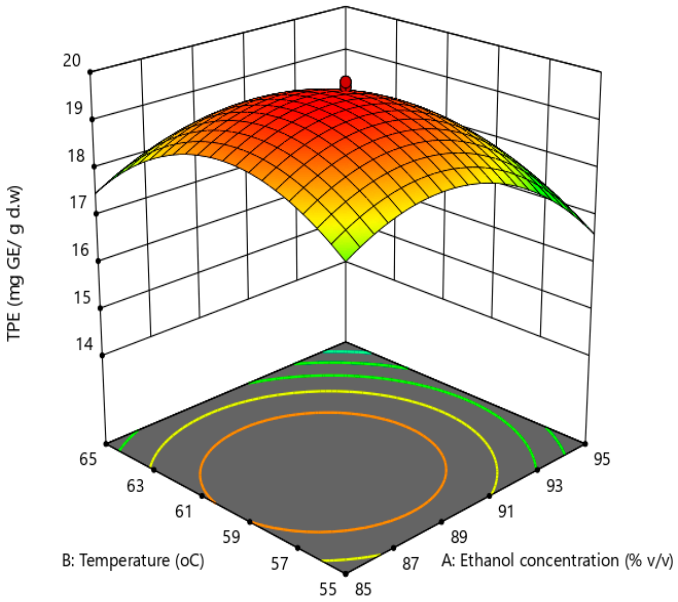
Run	Factors			Response	
	X_1	X_2	X_3	Y_{TPC} (Actual) (mg GE/g d.w.)	Y_{TPC} (Predict) (mg GE/g d.w.)
1	85	55	4	16.7	16.54
2	95	55	4	15.1	15.33
3	85	65	4	15.7	15.99
4	95	65	4	14.9	14.68
5	85	55	8	17.6	17.92
6	95	55	8	16.4	16.61
7	85	65	8	17.4	17.27
8	95	65	8	15.2	15.46
9	81.6	60	6	17.5	17.35
10	98.4	60	6	14.8	14.81
11	90	51.6	6	16.5	16.43
12	90	68.4	6	15.4	15.33
13	90	60	2.6	16.3	16.20
14	90	60	9.4	18.2	18.04
15	90	60	6	19.7	19.621
16	90	60	6	19.5	19.621
17	90	60	6	19.8	19.621
18	90	60	6	19.5	19.621
19	90	60	6	19.4	19.621
20	90	60	6	19.8	19.621

Table 4. Analysis of Variance (ANOVA) results for the quadratic model for optimization of bioactive compounds from soybean sprouts.

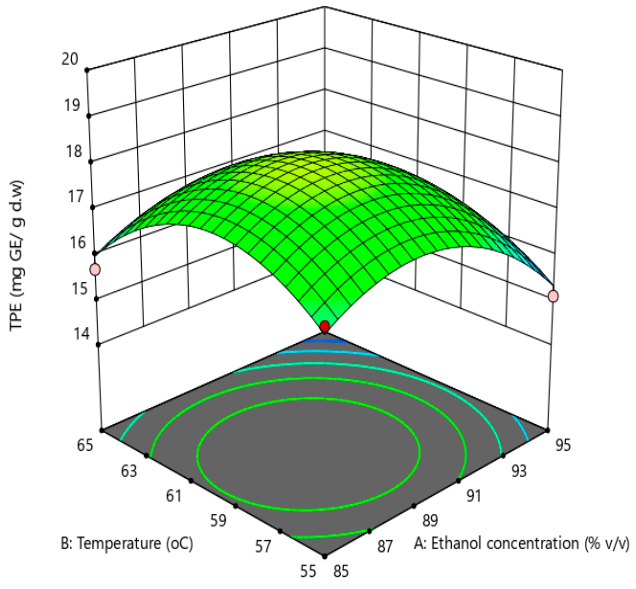
Factors	Total Polyphenol Content (TPC)			
Source	Sum of squares	*F*-value	*p*-value	-
Model	62.89	110.03	<0.0001	Significant
X_1	7.83	123.3	<0.0001	-
X_2	1.45	22.83	0.0007	-
X_3	4	63.06	<0.0001	-
X_1X_2	0.005	0.0787	0.7847	Not significant
X_1X_3	0.125	1.97	0.1909	-
X_2X_3	0.005	0.0787	0.7847	-
X_1^2	22.65	356.68	<0.0001	Significant
X_2^2	25.28	398.71	<0.0001	-
X_3^2	10.78	169.71	<0.0001	-
Residual	0.6350	-	-	-
Lack of Fit	0.4867	3.28	0.1091	Not significant
Pure Error	0.1483	-	-	-
Cor Total	63.52	-	-	-
Coefficient of Variation	1.46	-	-	-
PRESS	4.55	-	-	-
R^2	0.9900	-	-	-
R^2 Adjusted	0.9810	-	-	-
R^2 Predicted	0.9284	-	-	-
Adequate Precision	277.367	-	-	-

3.2.2. Analysis of Response Surface

Figure 9 depicts interaction impact of ethanol concentration and temperature on the TPC at different material-solvent ratios. Apparently, both factors exerted positive impacts on the TPC production. The change in the ratio also seemed to shift the response surface upward without altering the surface shape. The shape of the response also indicated that the yield of TPC was rapidly enhanced with elevating temperature extraction and ethanol concentration. At ratios of 1:4, 1:6, and 1:8 g/mL, further calculations showed that maximum yield was 16.7, 19.7, and 17.6 mg GE/g d.w., respectively.

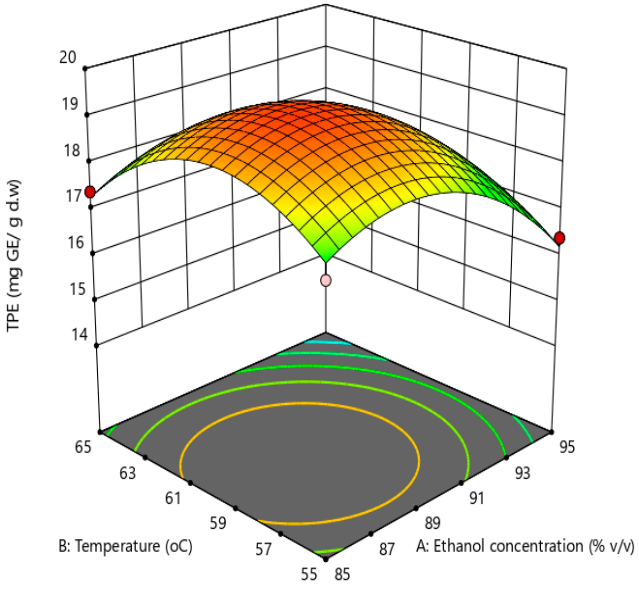


(**a**)



(**b**)

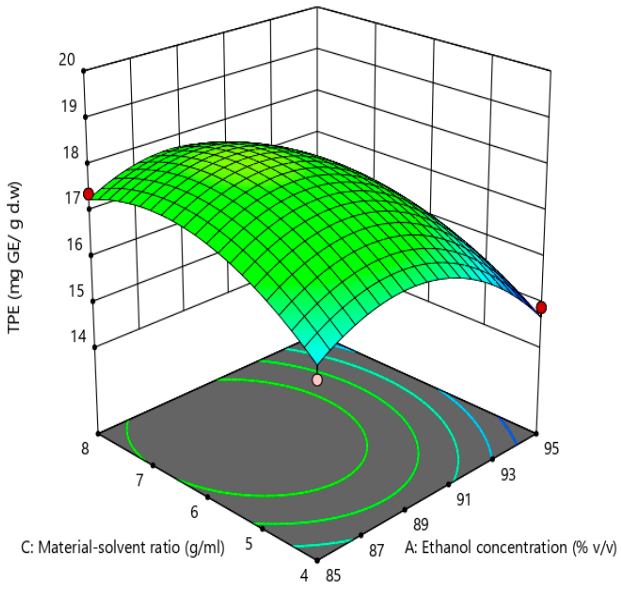
Figure 9. *Cont.*



(**c**)

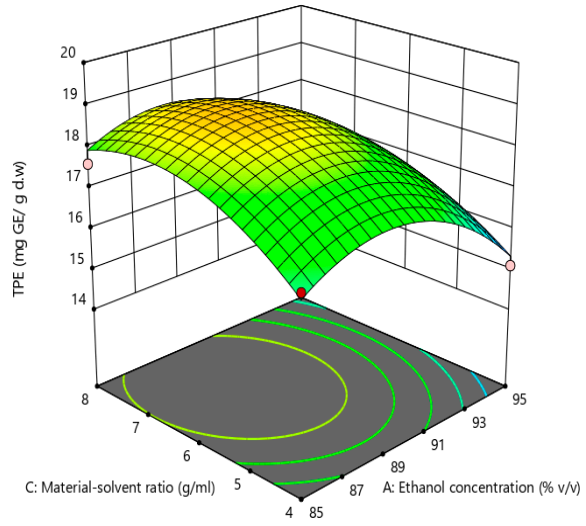
Figure 9. Response surface plot showing effects of extraction temperature and ethanol concentration on yield of TPC. (**a**) Material-solvent ratio of 1:4; (**b**) Material-solvent ratio of 1:6; (**c**) Material-solvent ratio of 1:8.

Figure 10 presents the response surface plot which gave the extraction yield of TPC as a function of material:solvent ratio and ethanol concentration. Different plots according to fixed temperatures (55 °C, 60 °C, and 65 °C) are illustrated. It was indicated that the extraction yield of TPC increased analogously with the material:solvent ratio and ethanol concentration. However, as the ethanol concentration began surpassing 90% (*v*/*v*), TPC started to decline. Of the three subplots, the plot of extraction temperature of 60 °C had a calculated TPC peak at 19.7 mg GE/g d.w.

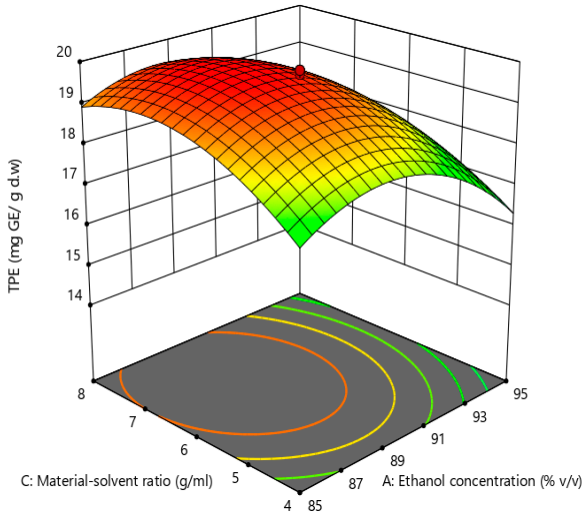


(**a**)

Figure 10. *Cont.*



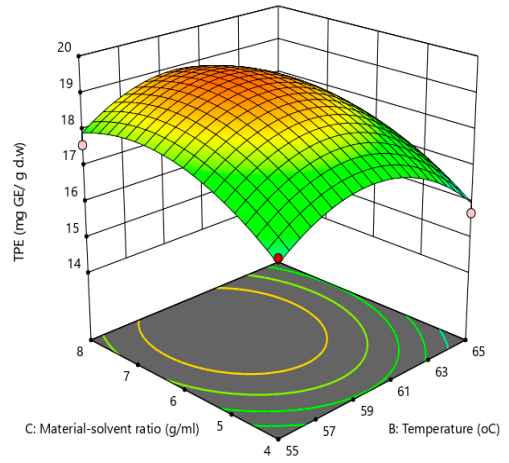
(**b**)



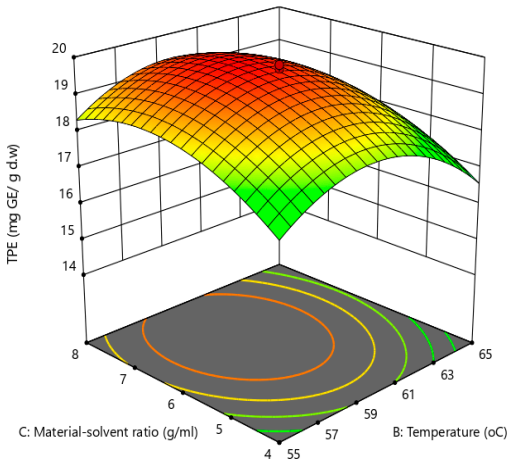
(**c**)

Figure 10. Response surface plot showing effects of material:solvent ratio and ethanol concentration on yield of TPC. (**a**) Extraction temperature of 55 °C; (**b**) Extraction temperature of 60 °C; (**c**) Extraction temperature of 65 °C.

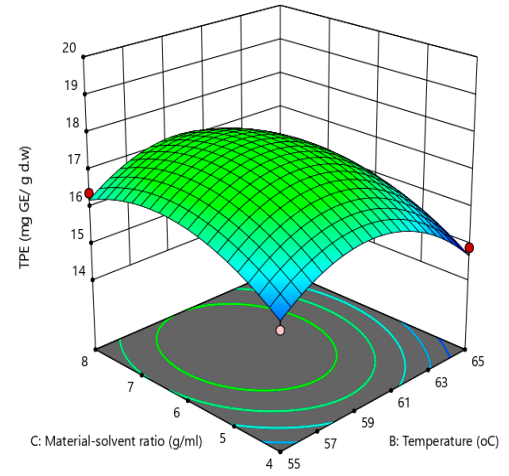
The extraction yield of TPC was investigated at different ethanol concentrations (85%, 90%, and 95% *v*/*v*) with respect to two variables, namely material:solvent ratio and extraction temperature, as shown in Figure 11. Similar to Figure 10, the extraction yield increased with increase of ratio of solid–liquid and extraction temperature from 55 to 60 °C. However, elevating the temperature past 60 °C caused extraction yield of TPC to diminish. Maximum TPC was calculated to be 17.6, 19.7, and 16.4 mg GE/g d.w., in which the peak was recognized at a temperature of 60 °C.



(**a**)



(**b**)



(**c**)

Figure 11. Response surface plot showing effects of material:solvent ratio and extraction temperature on yield of TPC. (**a**) EtOH concentration of 85% (*v*/*v*); (**b**) EtOH concentration of 90% (*v*/*v*); (**c**) EtOH concentration of 95% (*v*/*v*).

3.2.3. Validation of the Model

Validations of the models were carried out based on optimum extraction conditions obtained from RSM analysis. Table 5 shows the parameters and the results of validation experiments. Triplicate attempts of this experiment gave a TPC result of 19.11 ± 0.25 mg GE/g d.w. This indicated that there is a desirable agreement between predicted and experimental (actual) values. By applying the paired t-test, no significant variance between actual and predicted values ($p < 0.05$) was observed. Therefore, the generated response model was adequate in predicting the TPC yield.

Table 5. The result of optimum point experiment.

EtOH Concentration (% *v*/*v*)	Extraction Temperature (°C)	Material-Solvent (g/mL)	TPC Model (mg GE/g d.w.)	TPC Experiment (mg GE/g d.w.)	Error with Model (%)
88	59	1:6.5	19.801	19.32 18.88 19.14	2.42 4.65 3.34
Average TPC ± Standard Deviation				19.11 ± 0.25	

3.3. Evaluating the Antioxidant Ability of Dried Extract at Optimum Conditions

Compared with the positive control of ascorbic acid, the results showed that soybean sprouts extracts possessed a concentration-dependent relationship with DPPH scavenging assay activity (Figure 12). When the test sample was prepared at a concentration 1000 µg/mL, the free radical inhibition percentage of soybean sprouts dried extract in this study was 28.6%. The IC_{50} of soybean sprouts was 1350 µg/mL, lower than that of the vitamin C by approximately 193 times. This results was similar with the study by Khang et al. (2016), which reported the antioxidation activity of soybean at a concentration almost identical to our study at 26.64% [20], and indicated that the soybean sprouts dried extract used in this study possessed low antioxidant capacity.

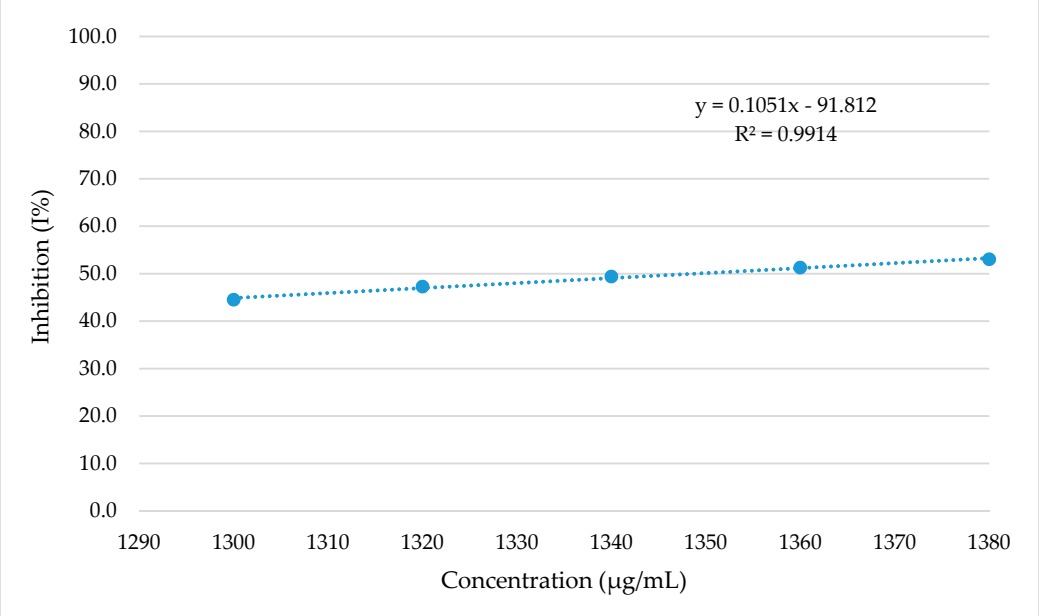


Figure 12. The antioxidant ability of dried extract obtained at optimum conditions.

4. Conclusions

The present study has successfully performed the extraction of polyphenols from soybean sprouts via maceration technique and investigated the relationship between TPC and various experimental

parameters, including ethanol concentration, extraction temperature, extraction time, material:solvent ratio, extraction cycle, number of germination days, and the used structural part of the soybean sprout. To achieve this, multiple single-factor experiments were carried out and the resultant conditions were used to further optimize the TPC via RSM. The optimization with RSM procedure proceeded with respect to three selected parameters, including material:solvent ratio, extraction temperature, and ethanol concentration. The final optimum parameters that gave maximum TPC are as follows: extraction time of 60 min, extraction temperature of 59 °C, ethanol concentration of 88% (*v/v*), material-solvent ratio of 1:6.5 g/mL, maceration through 2 cycles, and germination period of 5 days, with the plant material being the radicle part of the sprouts. Validation experiments showed that these conditions yielded a TPC of 19.11 ± 0.25 mg GE/g d.w. The obtained dried extract exhibited low antioxidant activity. Further studies should focus on scalability and feasibility assessment of the maceration production of biologically active compounds from soybean sprouts. In addition, compositional determination of the extracted products should be contemplated.

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Conflicts of Interest: The authors declare no conflict of interest.

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