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Multi-response optimization of cellulase-assisted hydrolysis for enhanced protein solubilization and bioactive compound release in defatted black sesame meal

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ABSTRACT

In this study, a byproduct of oil extraction, defatted black sesame meal, was utilized to obtain proteins and bioactive compounds using enzyme-assisted extraction. The study investigated the influence of three key factors (Enzyme concentration, temperature, and hydrolysis time). The response surface methodology was used to optimize the responses simultaneously. With high R^2 determination coefficients (0.9483 to 0.9956) and P values of lack-of-fit higher than 0.0005 (0.0785 to 0.5435), the obtained models showed high accuracy and could be used to predict changes in responses. The optimal values for the three investigated variables were found to be 1245 U/kg substrate, 49°C, and 7.25 hours, respectively. The extract obtained then contained 2.23% soluble dry solids, 1.886 g/100 mL soluble protein, 16.14 mg GAE/100 mL total phenolics, and 40.99 mg/100 mL lignans. Antioxidant activity was demonstrated by the IC_{50} value of DPPH free radical scavenging ability, which was 35.52 mg DW/mL. Simultaneously, through Pearson correlation analysis and principal component analysis, the study proved that this antioxidant activity was mainly contributed to by the phenolics, especially the lignans. The extract obtained from defatted black sesame meal can be used to enhance the nutritional value of food products or, at a higher level, to be processed into functional foods.

Contribution/Originality: This is one of the very few studies establishing an effective approach for employing cellulase to turn defatted black sesame meal, an agricultural byproduct, into functional components. This study contributes to the existing literature by showing that the obtained antioxidant activity is mainly due to the bioactive chemicals rather than soluble protein.

1. INTRODUCTION

The vegetable oil industry is growing rapidly to satisfy increasing consumer demand. The market for sesame oil, in particular, is increasing significantly, and this trend is estimated to continue from 2022 to 2032 due to its health benefits. And Asia appears to be the largest producer of sesame oil, with an output of approximately 777,000 tons in 2019. Thanks to its high omega-6 and omega-9 content, sesame oil helps lower bad cholesterol and increase good cholesterol, thereby preventing atherosclerosis and blood pressure-related diseases (Oboulbiga et al., 2023).

However, after the pressing and extraction, in addition to the main product, cooking oil, the factory also receives the byproduct, sesame meal. Sesame meal retains almost all of the protein in the sesame seed and also water-soluble

bioactive substances, especially phenolic and lignan (Bodoira, Velez, Andreatta, Martínez, & Maestri, 2017; Wan, Zhou, Zhao, & Hou, 2023). Although it has high value, the sesame meal is currently often discarded or used as animal feed (Wei et al., 2022), which causes waste and economic losses for the factory. Therefore, recovering the nutritional and functional components in sesame meal is a necessary approach to move towards the current circular economy trend.

On the other hand, protein and bioactive substances in sesame meal often exist in a bound form or are trapped in the plant cell wall matrix, making extraction by conventional methods very difficult (Bodoira et al., 2017). Therefore, it is necessary to break down the cell wall structure to facilitate the release of the internal components. And one of the methods that has been preferred recently is the application of enzymes during the hydrolysis due to its high efficiency and environmental friendliness compared to chemical methods (Qian et al., 2023; Sari, Bruins, & Sanders, 2013). However, similar to other biochemical processes, the efficiency of enzyme-induced hydrolysis also depends on many factors, and if these conditions are not optimal, the extraction process will not obtain high efficiency or, conversely, may cause protein denaturation or destruction of sensitive substances (Qian et al., 2023). Therefore, many studies have been conducted to determine the optimal conditions for the hydrolysis process, but most current studies on sesame meal or by-products from oilseeds mainly focus on optimizing the influence of factors on a single response (Bouloumpasi, Skendi, Christaki, Biliaderis, & Irakli, 2024; Sarkis, Michel, Tessaro, & Marczak, 2014).

Therefore, this study investigated the influence of cellulase concentration, hydrolysis temperature, and reaction time on the efficiency of simultaneous extraction of protein, lignan, phenolic, and antioxidant activity from defatted black sesame meal. Multi-response optimization helps to find the optimal parameters for the desired overall effect. In addition, the study also identifies which specific components significantly contribute to antioxidant efficacy, thereby providing a scientific basis for selecting quality indicators in the production process.

This paper is divided into four sections in a sequential manner: *(i)* A summary of relevant research; *(ii)* Information about materials, experimental design, analytical methods, and data processing; *(iii)* Optimization results using the response surface method based on central composite design; and *(iv)* A summary of the main conclusions, practical application directions, and some issues that need to be resolved.

2. LITERATURE REVIEW

After pressing and extracting the oil, the remaining black sesame meal contains about 50% plant protein and is particularly outstanding compared to other oilseeds because it is rich in methionine and tryptophan, essential amino acids for the human body (Wan et al., 2023). The main phenolic components in sesame are lignans, especially rich in sesamin, sesamolin, and sesaminol derivatives such as sesaminol triglucoside and sesaminol diglucoside (Wan et al., 2023). These bioactive compounds have been shown to exhibit strong antibacterial and antioxidant activity. They are also associated with many health benefits, including reducing cholesterol levels, lowering the risk of hypertension and cardiovascular disease, and reducing cancer mortality (Rodríguez-García, Sánchez-Quesada, Toledo, Delgado-Rodríguez, & Gaforio, 2019).

Sesame seeds vary in color from black, brown, and yellow to white. A study by Cui et al. (2021) found that the protein content of sesame seeds increased with darker seed coat color. Indeed, black sesame seeds had a higher protein content than white sesame seeds (Cui et al., 2021). Studies have also shown that the lignan content of sesame seeds is closely related to seed coat color, with black sesame seeds having the highest content of sesamin, sesamol, and total lignans. The total lignan content in yellow, black, brown, and white sesame seeds was 2.52-5.94, 3.56-12.76, 2.66-6.68, and 2.83-5.66 mg/g, respectively (Shi, Liu, Jin, & Wang, 2017).

Enzyme-assisted extraction has emerged as an effective method (Qian et al., 2023; Sari et al., 2013). In fact, research showed that adding enzymes increased protein extraction efficiency from 80% (without enzymes) to 90% for soybean meal and from 15-30% (without enzymes) to 50-80% for rapeseed and microalgae meals (Sari et al., 2013). For flaxseed meal, optimized enzymatic hydrolysis increased protein extraction efficiency 14-fold compared to

conventional methods (Ribeiro, Barreto, & Coelho, 2013). A recent study on mulberry leaves also demonstrated that ultrasonic-assisted cellulase hydrolysis achieved a protein content of 62.69%, which is 21.94% higher than that of the traditional alkaline extraction method (Fan et al., 2023).

Similarly, the sequential enzymatic hydrolysis process in rice bran has been shown to increase total phenolic content by 46.24%, flavonoid content by 79.13%, and ferric reducing antioxidant power (FRAP) by 159.14% (Liu et al., 2017). In flaxseed, phenolic extraction increased 10-fold through EAE (Ribeiro et al., 2013). Furthermore, a study showed that combining cellulase with autoclave pretreatment increased the release of bound phenolics from rice bran by 1.74 to 5.56 times (Zhao et al., 2023). In date syrup production, the combination of pectinase and cellulase increased the total soluble solids to 72.37 g/100g (Abbès et al., 2011).

The efficiency of enzyme-assisted extraction depends on many parameters such as the solids-to-solvent ratio, enzyme concentration, temperature, and pH (Qian et al., 2023). However, the optimal conditions for protein dissolution may differ from those for the release of bioactive substances, so multi-response optimization is needed to maximize overall efficiency. Response surface methodology (RSM) is a powerful statistical tool widely used to determine the interactions between these variables (Bouloumpasi et al., 2024; Sarkis et al., 2014).

In summary, to utilize this valuable byproduct, the study simultaneously applied cellulase-assisted hydrolysis combined with an optimized experimental design to obtain the highest yield of protein and bioactive compounds.

3. MATERIALS AND METHODS

3.1. Raw Materials

Dried black sesame seeds (*Sesamum indicum* L.) were obtained from a local commercial supplier (Ngan Anh, Vietnam). Oil was mechanically extracted using a laboratory-scale screw press (NNF 807A, NaniFood, Thailand). The resulting defatted sesame meal was ground and passed through a 1 mm sieve to obtain a uniform powder, which was stored in airtight containers at room temperature until use. The cellulase enzyme (Celluclast 1.5 L, Novozymes, Denmark) has an activity of 779 EGU/g (EGU = endoglucanase units).

3.2. Experimental Design and Enzymatic Hydrolysis

The cellulase-assisted hydrolysis experiment was designed using the Portable Statgraphics Centurion software by the Response Surface Methodology (RSM) with a model of Central Composite Design (CCD). Three independent variables were enzyme concentration (X_1), hydrolysis temperature (X_2), and hydrolysis time (X_3). Before designing the optimization experiment, three single-factor experiments were carried out based on the manufacturer's recommended operating conditions for a wide range of enzyme concentration (0-1400 U/kg), hydrolysis temperature (40-70°C), hydrolysis time (0-9 h to 0-8). Consequently, the smaller research ranges of 1031.82-1368.18 U/kg, 42-58°C, and 6.16- 7.84 h were selected. Table 2 presents the coded and actual values for each variable. Five levels were used to encode each variable: -1.68179, -1 (low), 0 (center), +1 (high), and +1.68179. Six replications of the central point were included in the total of 20 runs.

For each experimental run, defatted sesame meal was dispersed in distilled water at a solid-to-liquid ratio of 1:10 (w/v). The pH of the suspension was adjusted to 5.0 (within the optimal range according to the manufacturer's recommendation of 4.5-6.0) using dilute hydrochloric acid. The required amount of cellulase was then added according to the experimental design matrix. The hydrolysis process was carried out by placing conical flasks containing the mixture in a thermostatically controlled water bath with convection. After hydrolysis, the enzyme was inactivated by heating the mixture at 95°C for 10 min. The hydrolysate was then centrifuged at 5000 rpm for 15 min, and the supernatant was collected for analysis.

3.3. Analytical Methods

The total soluble solids were measured using a refractometer (Atago PAL-1, Japan). Soluble protein content (g/100mL) in the hydrolysate supernatant was determined by the Lowry method using bovine serum albumin (0-0.4 mg/mL) as the calibration standard (Satpathy, Dash, Sahoo, Anwar, & Parida, 2020) and a Spectrophotometer UV-VIS (752N, Inesa, China). The Folin-Ciocalteu assay was used to measure total phenolic content. A calibration curve was constructed using gallic acid standard over a concentration range of 0-0.1 mg/mL and reported as mg gallic acid equivalent per 100 mL (mgGAE/100mL) (Torres, Osaki, Silveira, dos Santos, & Chow, 2024). Lignan content (mg/100 mL) was quantified by the spectrophotometric method after extraction with hexane-chloroform solvent and calculated using the specific absorption coefficient of sesamin ($E^{1\%}_{1cm}$ = 231.1) (Bhatnagar, Hemavathy, & Gopala Krishna, 2015). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test was used to assess antioxidant activity. IC_{50} values, or the concentration of the extract needed for 50% inhibition, are used to express the results of this experiment (Gulcin & Alwasel, 2023; Martinez-Morales, Alonso-Castro, Zapata-Morales, Carranza-Álvarez, & Aragon-Martinez, 2020). By drying a known volume at 105°C to constant weight, the dry matter concentration of the supernatant was calculated, and the IC_{50} values were expressed as mg dry weight (DW) per mL (mg DW/mL).

3.4. Statistical and Multivariate Analysis

Experimental data were analyzed by the Portable Statgraphics Centurion software. Standardized Pareto charts were used to illustrate the statistically significant impact of three independent variables on responses at the 95% confidence level (the P-value less than 0.05). To predict the optimal conditions, a second-order polynomial equation was created (Equation 1), in which X_i and X_j were independent variables, Y is the expected response, β_o is the constant, and β_i, β_{ii}, and β_{ij} are the variable coefficients for the linear, quadratic, and interaction terms, respectively. The coefficient of determination (R^2) and the P-value of lack-of-fit were used to measure the quality of polynomial models. Three-dimensional plots and the corresponding contour plots were used to show the relationship between two variables and answers.

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad (1)$$

Pearson correlation analysis was conducted to evaluate the relationships among soluble protein, total phenolic content, lignan content, and IC_{50} values using the mean results of the 20 experimental runs. Principal component analysis (PCA) was performed on standardized response variables to explore multivariate relationships and identify the main factors governing functional properties.

4. RESULTS AND DISCUSSION

4.1. Composition of Defatted Black Sesame Meal

The composition of defatted black sesame meal is presented in Table 1. The relatively low moisture content, only 5.43%, creates an unfavorable environment for microorganisms and enzymes, thereby extending the shelf life of sesame meal and facilitating subsequent production processes. After oil pressing, the protein content increased significantly (38.15%) compared to that of the sesame seed due to the removal of a large amount of lipid. However, this protein content is a little lower than the value (42.21%) achieved in the study of Jimoh, Fagbenro, and Adeparusi (2011), possibly due to differences in sesame variety and extraction method.

Total phenolic content reached 207.50 mg GAE/100 g, indicating that a sizable quantity of phenolic compounds was still present in the meal after oil extraction. Sesame contains phenolic compounds in both free and bound forms. These chemicals are frequently linked to the structure of the cell wall, which may restrict their extraction without additional processing (Bodoira et al., 2017).

Interestingly, the lignan concentration was 723.88 mg/100 g, which is in line with findings that sesame is one of the dietary sources of lignans, including sesamin and sesamolin (Wei et al., 2022). These substances are well known

for their health-promoting and antioxidant capacity, which includes their capacity to scavenge free radicals and possible anti-inflammatory actions (Pathak, Rai, Kumari, & Bhat, 2014).

In general, even after oil extraction, defatted black sesame meal maintained its high nutritional potential and bioactivity. Consequently, value-enhancing techniques like enzyme-assisted hydrolysis could improve functional and antioxidant qualities by increasing the release of compounds into soluble fractions.

Table 1. Proximate composition and bioactive contents of defatted black sesame meal.

Component	Value
Moisture (%)	5.43 ± 0.12
Crude protein (%)	38.15 ± 0.84
Total phenolic content (mg GAE/100g)	207.50 ± 6.21
Lignan content (mg/100g)	723.88 ± 18.47

4.2. Effects of Enzymatic Hydrolysis on Responses

The effect of enzyme concentration, hydrolysis temperature, and hydrolysis time on the responses was illustrated in Table 2. Total soluble solids, an indicator of the release of soluble substances, and four functional responses associated with protein solubility, the release of bioactive substances (phenolics and lignans), and antioxidant activity (IC_{50}) are among these responses.

Table 2. Actual and coded values of the variables and the corresponding responses.

Run	Enzyme concentration (U/kg)	Hydrolysis temperature (°C)	Hydrolysis time (h)	Total soluble solids (%)	Protein content (g/100mL)	Phenolic content (mgGAE/100mL)	Lignan (mg/100mL)	IC_{50} (mgDW/mL)
1	1200	50	7	2.18	1.819	13.40	40.53	38.39
2	1100	45	7.5	1.88	1.400	8.93	38.73	48.38
3	1300	45	6.5	1.80	1.702	6.95	31.55	54.68
4	1200	50	7	2.24	1.809	15.19	38.92	39.34
5	1300	45	7.5	2.06	1.722	12.81	38.92	43.63
6	1100	55	7.5	1.69	1.459	8.55	39.24	49.33
7	1031.82	50	7	1.81	1.152	8.23	38.10	51.89
8	1200	41.591	7	1.79	1.505	8.23	38.40	50.56
9	1368.18	50	7	2.10	1.804	13.40	39.25	38.97
10	1300	55	7.5	1.82	1.825	12.98	38.57	44.36
11	1200	50	7	2.21	1.805	15.03	40.17	39.18
12	1100	55	6.5	1.66	1.260	6.48	29.61	55.47
13	1200	50	7	2.16	1.832	15.63	39.72	35.10
14	1100	45	6.5	1.60	1.183	6.33	27.38	57.11
15	1200	58.409	7	1.79	1.648	8.36	39.00	50.15
16	1300	55	6.5	1.80	1.727	7.51	33.97	52.10
17	1200	50	7	2.23	1.817	15.26	39.50	35.75
18	1200	50	7	2.16	1.854	15.61	39.99	38.85
19	1200	50	7.8409	2.05	1.683	13.19	37.76	37.47
20	1200	50	6.1591	1.64	1.499	6.15	23.12	59.69

The standardized Pareto charts from Figure 1 compared the statistical significance levels of linear, quadratic, and interaction effects of three independent variables on each response. The effect levels were arranged from greatest to weakest in the same order as the bars on the charts, which were displayed from top to bottom. The statistical significance limit at 95% confidence (P = 0.05) was shown on charts by a blue vertical line. When this vertical line was crossed by the variable horizontal bar, the effect was deemed substantial. While total phenolic content and the IC_{50} value were not significantly impacted by temperature, it was evident that all examined variables (enzyme

concentration, temperature, and hydrolysis time) had a notable linear effect on total soluble solids, soluble protein content, and lignan content.

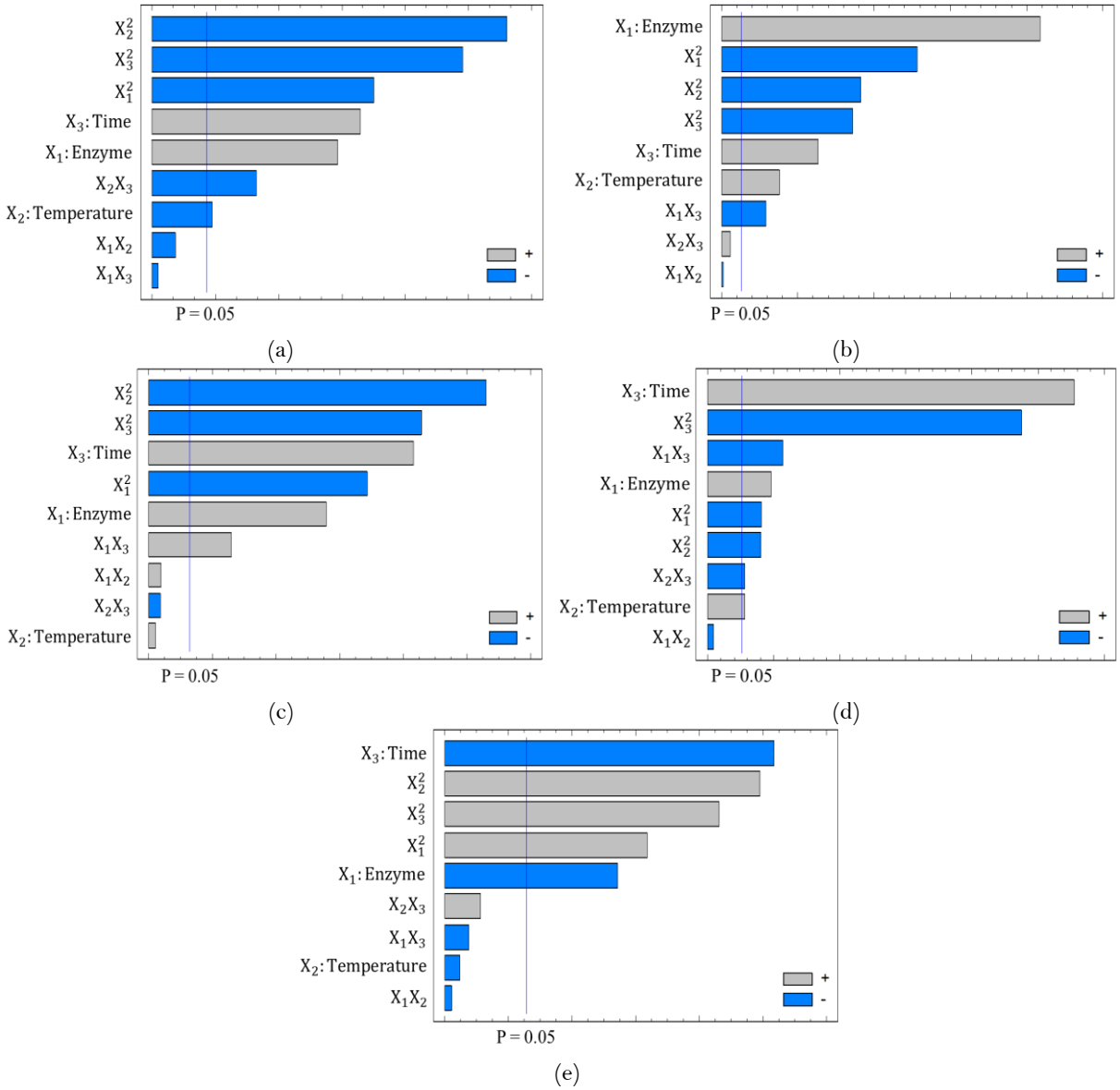

Figure 1. Standardized Pareto charts illustrating the effects of enzyme concentration (X_1), hydrolysis temperature (X_2), and hydrolysis time (X_3), including interaction and quadratic terms, on (a) total soluble solids, (b) soluble protein content, (c) total phenolic content, (d) lignan content, and (e) IC_{50}.

4.3. Model Fitting and Adequacy of Regression Equations

The coded quadratic regression equations showing the relationship between the responses (Y) and three independent variables (X), along with their statistical parameters, are summarized in Table 3. The goodness of fit of models is assessed through the coefficient of determination (R^2). A near-perfect match between the model and the data is indicated by an R^2 close to 1, but a calibrated model is comparable to simply taking the mean, as indicated by an R^2 close to 0. The calibrated R^2 value is more suitable for comparing models with different numbers of independent variables (Alexander, Tropsha, & Winkler, 2015). The fitted second-order models adequately represented the experimental data, as evidenced by the high coefficients of determination (R^2 = 0.9483-0.9956) displayed by all models. Strong predictive reliability was confirmed by adjusted R^2 values (0.9018-0.9917) that closely matched the R^2. Furthermore, the test for "lack-of-fit" was carried out by comparing the variability of the model residuals to the variability between observations in order to assess if the chosen model was enough to describe the experimental data.

The P-values for lack-of-fit were higher than 0.05, indicating that random error rather than inadequate models was responsible for the residual variation (DiRenzo, Hanks, & Miller, 2023). These results corroborated that the obtained models fit the experimental data at the 5% significance level and could be used to accurately forecast response changes depending on three variables. The estimated versus experimental value scatter plots in Figure 2 provided more compelling evidence of this. The close clustering of data points along the regression line confirms the adequacy and robustness of the quadratic models for describing the enzymatic hydrolysis system.

Table 3. Quadratic regression models (coded variables) and statistical adequacy parameters for the responses.

Response	Quadratic regression model (coded variables)	R^2	Adjusted R^2	Lack-of-fit (p-value)
Total soluble solids (Y_1, %)	$Y_1 = -66.7952 + 0.0259924X_1 + 0.82008X_2 + 9.10743X_3 - 0.00000969138X_1^2 - 0.0000275X_1X_2 - 0.000075X_1X_3 - 0.00620998X_2^2 - 0.0245X_2X_3 - 0.543217X_3^2$	0.9669	0.9370	0.0785
Soluble protein (Y_2, g/100 mL)	$Y_2 = -49.2108 + 0.0364436X_1 + 0.334488X_2 + 5.42023X_3 - 0.000012125X_1^2 - 0.000002X_1X_2 - 0.000745X_1X_3 - 0.003457X_2^2 + 0.003X_2X_3 - 0.325194X_3^2$	0.9956	0.9917	0.2868
Total phenolic content (Y_3, mg GAE/100 mL)	$Y_3 = -781.138 + 0.278166X_1 + 10.1592X_2 + 100.78X_3 - 0.000163773X_1^2 + 0.00024X_1X_2 + 0.01665X_1X_3 - 0.101147X_2^2 - 0.046X_2X_3 - 8.1702X_3^2$	0.9922	0.9852	0.5435
Lignan content (Y_4, mg/100 mL)	$Y_4 = -1144.51 + 0.317739X_1 + 4.22868X_2 + 243.418X_3 - 0.0000601579X_1^2 - 0.0001675X_1X_2 - 0.022525X_1X_3 - 0.0237096X_2^2 - 0.2245X_2X_3 - 14.0523X_3^2$	0.9848	0.9711	0.1046
Antioxidant activity (IC_{50}, Y_5, mg DW/mL)	$Y_5 = 1908.24 - 0.689642X_1 - 21.1537X_2 - 249.701X_3 + 0.000310612X_1^2 - 0.00029X_1X_2 - 0.0098X1X_3 + 0.193894X_2^2 + 0.295X2X_3 + 16.8792X_3^2$	0.9483	0.9018	0.1716

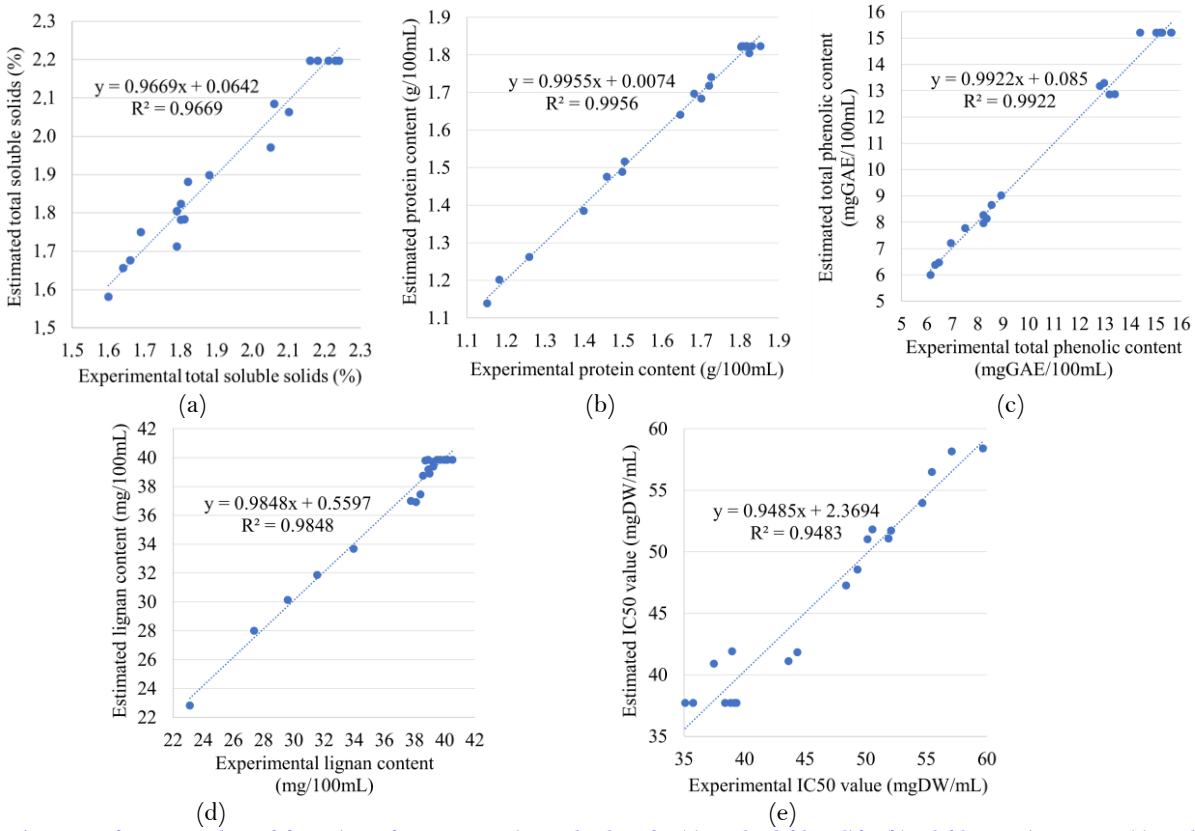

Figure 2. The scatter plots of the estimated versus experimental values for (a) total soluble solids, (b) soluble protein content, (c) total phenolic content, (d) lignan content, and (e) IC_{50}.

4.4. Response Surfaces and Contour Plots for Responses

4.4.1. Total Soluble Solids

The initial total soluble solids were only 0.59%, then gradually increased during hydrolysis and reached 1.60% to 2.24% (an increase of approximately 2.7 to 3.8 times), demonstrating the gradual release of soluble components into the aqueous phase during cellulase-assisted extraction. The response surface plot (Figure 3) showed that enzyme concentration and hydrolysis time positively affect the total dissolved solids. This is because cellulase cleaves β-1,4-glycosidic bonds in the polysaccharide, leading to weakening of the cell wall and allowing low molecular weight compounds to diffuse into the aqueous phase (Sarkis et al., 2014). Meanwhile, excessively high or low temperatures are unfavorable for cellulase enzyme activity, reducing hydrolysis efficiency, thereby limiting the diffusion and dissolution of components. In a similar study, Abbès et al. (2011) also indicated that the soluble solids content in the date powder and water mixture was significantly improved when cellulase enzyme was applied under suitable conditions compared to the control sample (Abbès et al., 2011). However, the increase became less noticeable and sometimes slightly decreased as the hydrolysis time was extended due to the decrease in substrate concentration and the partial decomposition of hydrolysis products (Hadidi, Tan, Assadpour, & Jafari, 2024).

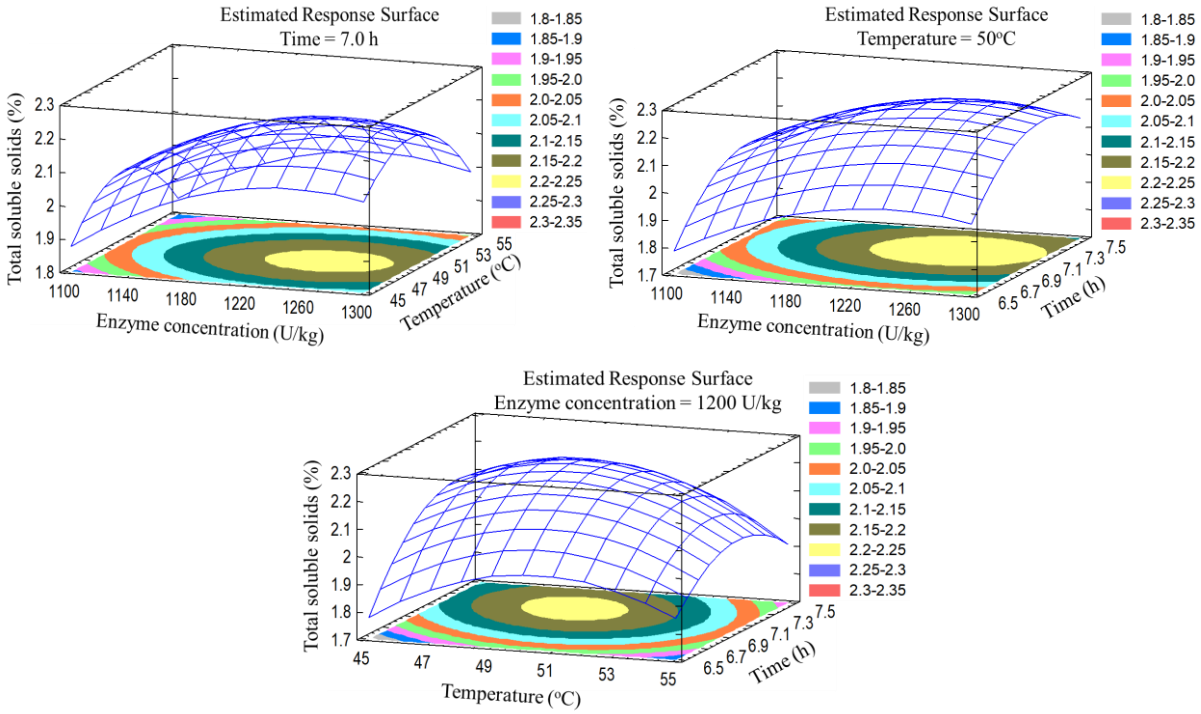

Figure 3. Response surface and contour plots for the effect of hydrolysis variables on the total soluble solids.

4.4.2. Soluble Protein Content

The soluble protein content increased from an initial 0.489 g/100 mL to 1.152–1.854 g/100 mL after hydrolysis. The enzyme concentration showed a significant positive effect (Figure 4). Although cellulase does not directly hydrolyze proteins, disrupting the cellulose and hemicellulose network improves mass transfer (Bouloumpasi et al., 2024; Sarkis et al., 2014) and thus facilitates the release of proteins into the aqueous phase. However, the effect of temperature and hydrolysis time on soluble protein content was not linear. When the temperature and time were increased to moderate levels, the amount of protein escaping from the matrix into the hydrolysate also rose. But at high hydrolysis temperatures or long reaction times, thermal denaturation (Van de Vondel, Lambrecht, & Delcour, 2022) or the formation of protein-phenol complexes (Ozdal, Capanoglu, & Altay, 2013) can occur, reducing the solubility of proteins in the hydrolysate. Similarly, in another study, Fan et al. (2023) found that the amount of soluble

protein in mulberry leaf extract was significantly improved when cellulase was applied with ultrasound assistance at optimal values of enzyme concentration, temperature, and pH (Fan et al., 2023).

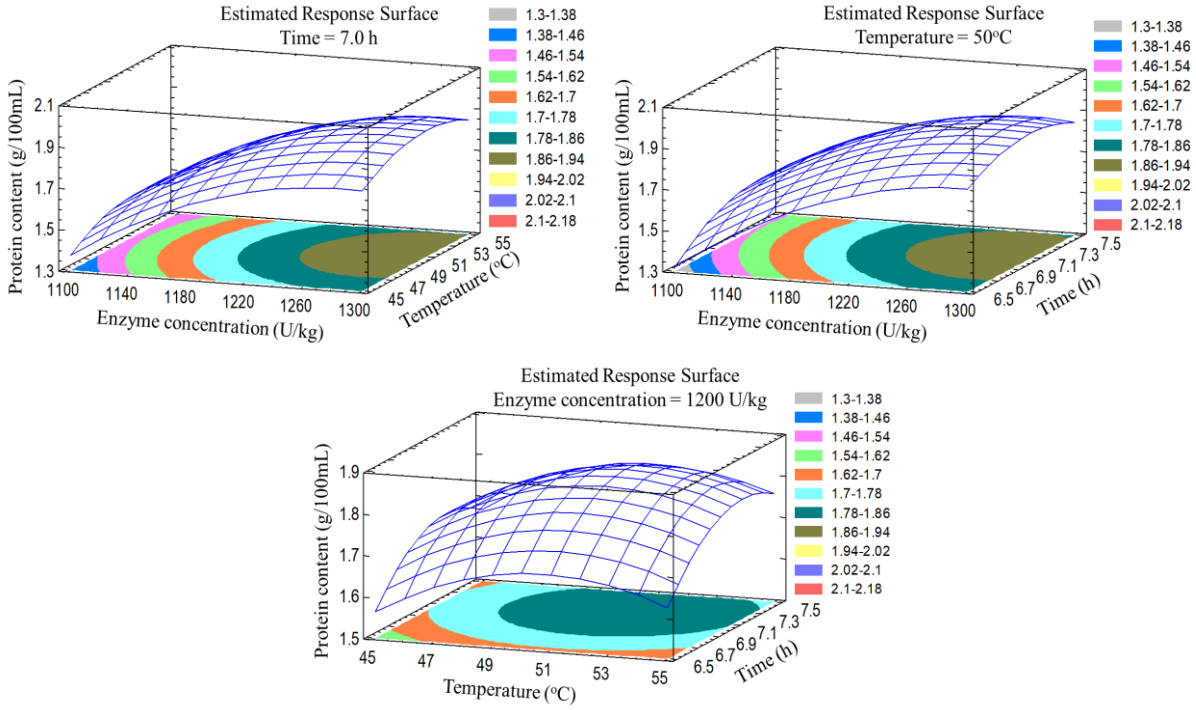

Figure 4. Response surface and contour plots for the effect of hydrolysis variables on the soluble protein content.

4.4.3. Total Phenolic Content

The application of cellulase enzyme significantly improved the total phenolic content. The results showed that this value increased by about 1.5-3.8 times compared to the initial content of 4.08 mg GAE/100 mL (Figure 5). Phenolic compounds present in seeds often form strong covalent bonds with components of plant cell walls through ester, ether, and C-C bonds (Jiang et al., 2023). Some phenolics exist in a free state but are trapped in these matrices, making extraction difficult. Therefore, it is necessary to use the cellulase enzyme to break the covalent bonds as well as to decompose polysaccharides in the cell wall to release them easily. The findings of Zhao et al. (2023) proved a significant improvement in the total phenolic content in rice bran with rising cellulase concentration (Zhao et al., 2023). In addition, the release of phenolics also depended on the hydrolysis temperature. Within a moderate range, increasing temperature enhanced diffusion, thereby promoting faster enzyme kinetics. However, if the temperature exceeded the optimal value, the total phenolic content tended to decrease due to reduced enzyme activity and poor stability of phenolic compounds at higher temperatures (Antony & Farid, 2022; Daniel, Danson, Eisenthal, Lee, & Peterson, 2008). Similarly, although matrix breakdown improves extraction efficiency, excessively long reaction times can promote oxidation, structural changes, or polymerization of phenolic compounds (Durmus, Gulsunoglu-Konuskan, & Kilic-Akyilmaz, 2024). Similar trends have been observed in onions and other plants, where increasing extraction temperature increases total phenolic content, but excessively high temperatures lead to reduced recovery or stability of phenolics (Akowuah, Mariam, & Chin, 2009; Sharma et al., 2015).

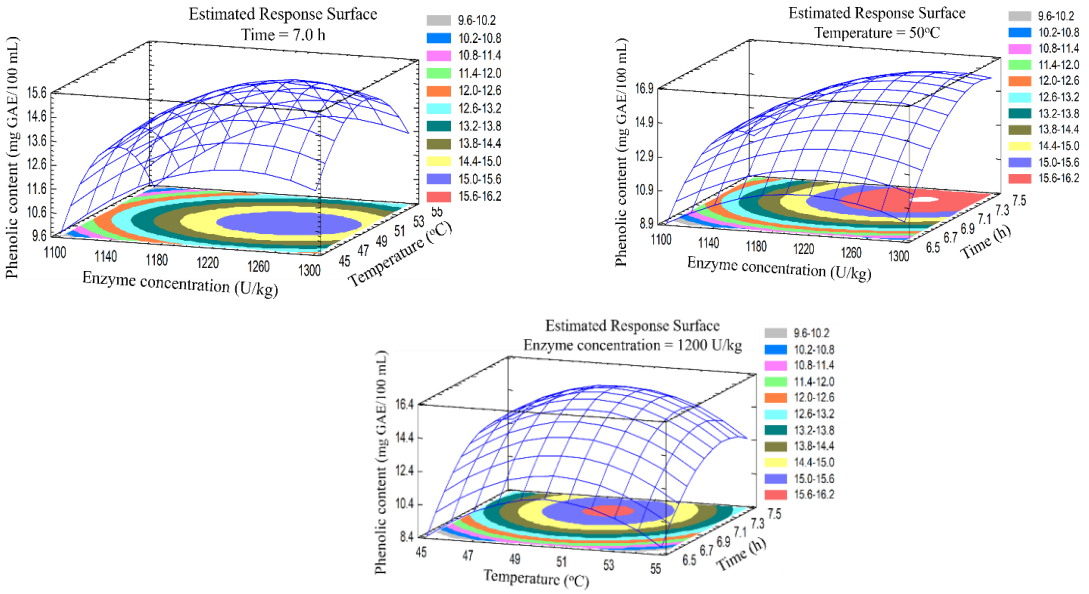

Figure 5. Response surface and contour plots for the effect of hydrolysis variables on the total phenolic content.

4.4.4. Lignan Content

The lignan content increased significantly after hydrolysis and ranged from 23.12 to 40.53 mg/100 mL compared to the initial value of 10.68 mg/100 mL (Figure 6). Since lignan is the main phenolic component in black sesame meal (Wan et al., 2023), the changing trend in this component appeared to be similar to that of phenolics. Specifically, lignan release in the hydrolysate was enhanced at higher cellulase concentrations and longer hydrolysis times. This was also demonstrated in previous studies on other materials such as rice bran, flaxseed meal, and lignocellulose biomass, where the results showed that the release of phenolics and their derivatives was significantly improved with increasing enzyme concentration and extending the reaction time (Liu et al., 2017; Ribeiro et al., 2013; Santos et al., 2026). However, prolonged processing led to a minor decrease in lignan content. This decrease could be caused by the heat sensitivity and oxidation of sesame lignans, especially sesamolin, which transforms into sesamol and other lignan derivatives (Andargie, Vinas, Rathgeb, Möller, & Karlovsky, 2021; Berenshtein, Okun, & Shpigelman, 2024).

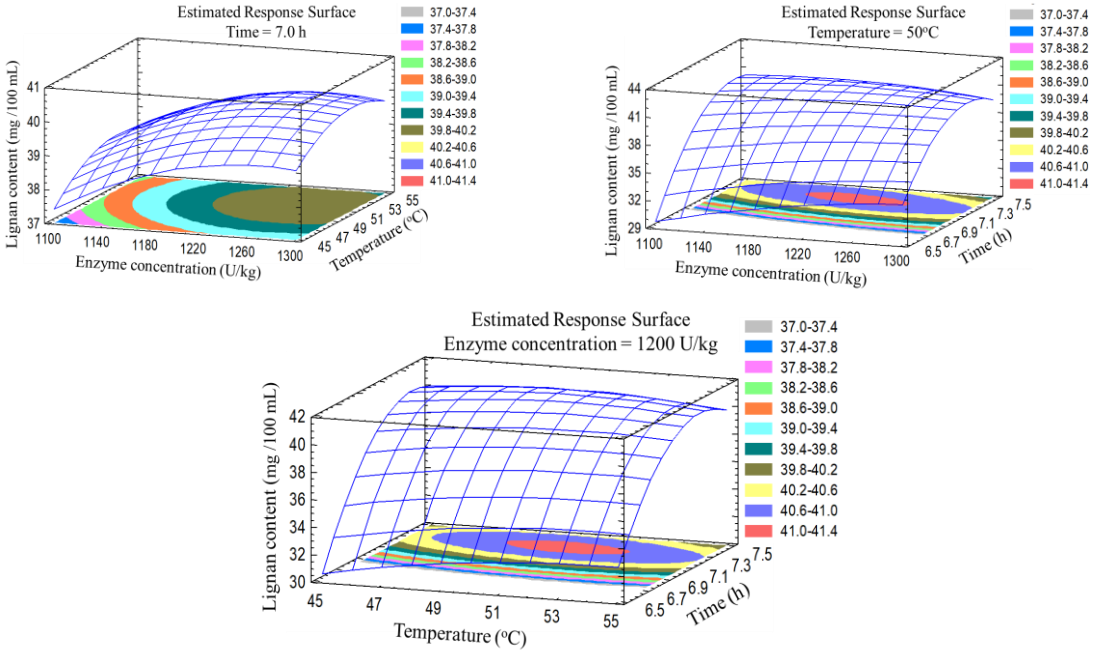

Figure 6. Response surface and contour plots for the effect of hydrolysis variables on the lignan content.

4.4.5. Antioxidant Activity

The pre-hydrolysis mixture had an IC_{50} value of 140.58 mg DW/mL. This value decreased significantly after hydrolysis with cellulase under different conditions (35.10 to 59.69 mg DW/mL), demonstrating that the free radical scavenging ability was significantly improved. The results in Figure 7 showed that the IC_{50} value decreased significantly with increasing enzyme concentration and prolonged hydrolysis time due to the release of bioactive compounds having strong antioxidant properties (Gulcin, 2025) in black sesame meal. Meanwhile, antioxidant activity was only improved within the optimal temperature range for the enzyme and tended to increase slightly under excessive conditions. This changing trend was entirely consistent with the results of the protein, total phenolic, and lignan content during hydrolysis. In another study, Paié-Ribeiro et al. (2024) also found that olive cake samples with higher total flavonoid and phenolic content had improved antioxidant capacity through FRAP, DPPH, and ABTS assays (Paié-Ribeiro et al., 2024).

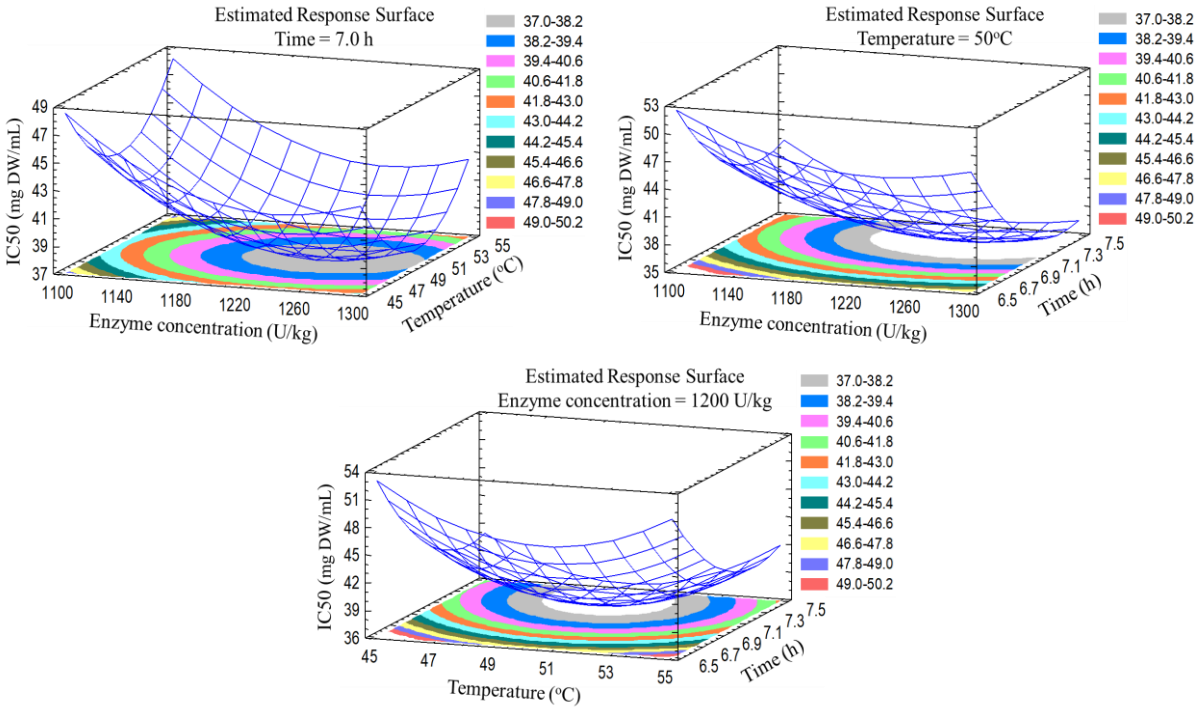

Figure 7. Response surface and contour plots for the effect of hydrolysis variables on IC_{50} value.

4.5. Multi-Response Optimization and Empirical Validation

After five responses were modeled, simultaneous optimization of all responses was conducted using a desired function approach to maximize total soluble solids, soluble protein content, total phenolic content, and lignan content while minimizing IC_{50}. The overlaid contour plots (Figure 8) delineated an optimal operating region in which all responses were simultaneously satisfied. This method enables the identification of operating circumstances that result in overall optimal efficiency by merging several response variables into a single composite desirability value (Gamero-Salinas & López-Fidalgo, 2024; Hernández, Seid Ahmed, Curra, & Pérez, 2025). The model predicted that the hydrolysate would contain 1.886 g/100 mL soluble protein, 16.14 mg GAE/100 mL total phenolics, and 40.99 mg/100 mL lignans, with a total soluble solid of 2.23% and an IC_{50} value of 35.52 mg DW/mL when the enzyme was added at a concentration of 1245 U/kg substrate and the hydrolysis was performed at 49°C for 7.25 hours. The predicted optimum fell within the experimental design space, indicating that responses were obtained by interpolation rather than extrapolation of the regression model.

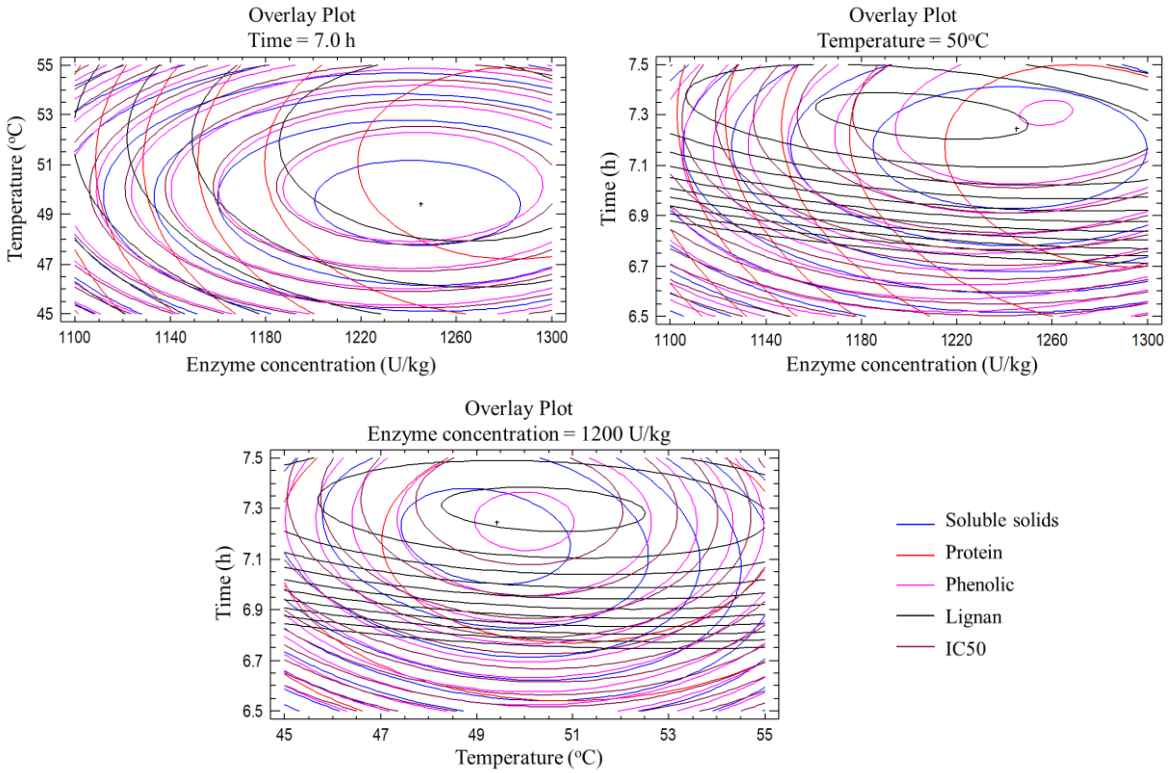

Figure 8. Overlaid contour plots for the optimal region.

These results were further verified by experimenting with the obtained optimal conditions. The experimental values for total soluble solids (2.25%), soluble protein content (1.877 g/100mL), total phenolic content (16.89 mgGAE/100mL), lignan content (40.61 mg/100mL), and IC_{50} (34.89 mgDW/mL) were close to the predicted values (the difference was less than 5%), confirming the reliability and accuracy of the established models.

4.6. Correlation Analysis

Pearson correlation analysis (Figure 9) showed a significant relationship among the responses. Even though five responses were modeled, the four functional responses (Y_2–Y_1) were the focus of further Pearson correlation analysis, and total soluble solids (Y_1) were kept as an additional matrix disintegration indication. Total phenolic content and lignan content indicated a very strong positive connection ($r = 0.93$), suggesting that lignans make up a significant portion of the phenolic group in sesame hydrolysates. This was in line with the fact that lignan-type phenolics are known to predominate in sesame seeds (Bekele et al., 2025). There was a significant negative connection ($r = -0.89$) between lignan content and IC_{50} and also between total phenolic content and IC_{50} ($r = -0.71$). These results confirmed that increased liberation of phenolic and lignan compounds considerably improved radical-scavenging capacity, as lower IC_{50} values indicated stronger antioxidant activity (Falodun, Okafor, Erharuyi, & Okugbo, 2025). The stronger correlation observed for lignans suggested that they may be the principal contributors to antioxidant activity under the investigated conditions. Soluble protein exhibited a moderate negative correlation with IC_{50} ($r = -0.60$). The smaller correlation coefficient indicates that protein-derived contributions are secondary to phenolic and lignan-related processes, even if bioactive peptides produced during hydrolysis may contribute to radical-scavenging action in plant-based systems (Zhu et al., 2024). These findings demonstrated that antioxidant activity in cellulase-assisted sesame hydrolysates was primarily governed by the release of phenolic and lignan compounds rather than protein solubilization alone.

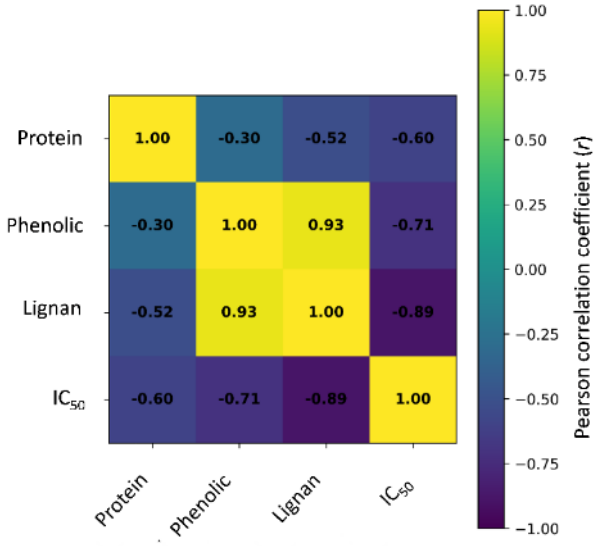

Figure 9. Pearson correlation heatmap of responses.

4.7. Principal Component Analysis

Principal component analysis (PCA) was performed to better understand the multivariate relationship between the responses (Figure 10). Only 12.37% of the total variance is explained by the second principal component (PC2), compared to 81.45% by the first principal component (PC1). Thus, the cumulative total variance is 93.82%. This suggests that the two major components provide a relatively sufficient explanation for the dataset, with a proportion above 90% (Greenacre et al., 2022).

From the PCA plot, a strong inverse relationship can be seen between protein, total phenolic, and lignan content and antioxidant activity (IC_{50}), meaning that the higher the content of these components, the lower the IC_{50} value, indicating stronger DPPH free radical scavenging ability. However, the total phenolic and lignan content were mainly arranged along PC1, while the soluble protein content was mainly arranged along PC2. This result demonstrated that the release of phenolic compounds, especially lignans, was the main factor contributing to the antioxidant effect of the hydrolysate from sesame meal.

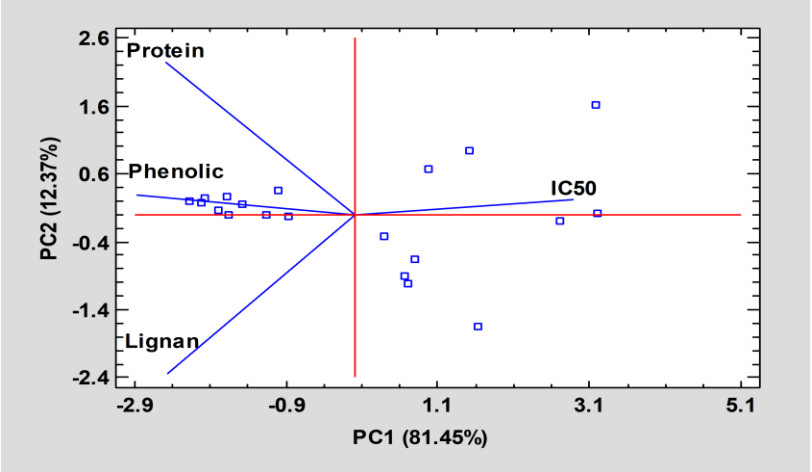

Figure 10. PCA biplot of the first two principal components showing the distribution of responses.

5. CONCLUSIONS

Protein solubility and the release of bioactive compounds from defatted black sesame meal were both improved by cellulase-assisted hydrolysis. The hydrolysate produced 2.23% soluble solids, 1.886 g/100 mL soluble protein, 16.14 mg GAE/100 mL total phenolics, and 40.99 mg/100 mL lignans and had improved antioxidant potential with

an IC_{50} value of 35.52 mg DW/mL under ideal conditions (enzyme concentration of 1245 U/kg substrate, hydrolysis temperature of 49°C, and exposure duration of 7.25 hours). Pearson correlation and principal component analysis revealed that antioxidant activity was primarily governed by phenolic and lignan release rather than protein solubilization alone. These findings have shown the potential application of sesame meal in foods and functional foods to enhance nutritional value and biological activity. Even though this study found an ideal procedure in the lab, there are still issues with mass transfer efficiency and enzyme costs when applying it to the industrial scale. The analysis of the technological and economic efficiency should be the main focus of future study. In addition, the content of phytic acid, an anti-nutritional component present in black sesame, should also be monitored during hydrolysis and application of this hydrolysate in functional food products.

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