

OPTIMIZATION OF CELLULASE-ASSISTED EXTRACTION PARAMETERS TO ENHANCE THE YIELD OF NUTRITIONAL COMPOUNDS FROM OYSTER MUSHROOMS (*PLEUROTUS SAJOR-CAJU*)

Giang N.T.N.^{1,6}, Tan N.D.^{2,6}, Ha, H.T.N.^{2,6}, Thanh, D.V.^{2,6}, Diem, L.T.T.^{1,6}, Khai T.V.^{3,6}, Duy, Q.T.K.^{4,6}, Luan, H.V.^{5,6} and Quyen, D.K.^{2,6*}

Address(es):

¹ Experimental-practical Area, An Giang University, Ung Van Khiem, 18, 90000, Long Xuyen, An Giang, Vietnam.

² Food Technology Department, Agriculture and Natural Resource Faculty, An Giang University, Ung Van Khiem, 18, 90000, Long Xuyen, An Giang, Vietnam.

³ Crop Science Department, Agriculture and Natural Resource Faculty, An Giang University, Ung Van Khiem, 18, 90000, Long Xuyen, An Giang, Vietnam.

⁴ Student of DH22TP, Food Technology Department, Agriculture and Natural Resource Faculty, An Giang University, Ung Van Khiem, 18, 90000, Long Xuyen, An Giang, Vietnam.

⁵ Student of DH21TP, Food Technology Department, Agriculture and Natural Resource Faculty, An Giang University, Ung Van Khiem, 18, 90000, Long Xuyen, An Giang, Vietnam.

⁶ Vietnam National University Ho Chi Minh city, Vietnam.

*Corresponding author: dkquyen@agu.edu.vn

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ABSTRACT

Oyster mushroom (*Pleurotus sajor-caju*) has substantial potential as a raw material widely utilized in food production. This study aimed to optimize the extraction conditions of dried oyster mushroom material with the assistance of cellulase enzyme. A central composite design was employed, incorporating varying dilution ratios of water to dried mushroom powder (15:1, 20:1, 25:1), enzyme concentrations (0.3, 0.4, 0.5 w/v), and pH levels of 4.5, 5.0, and 5.5. The results showed excellent agreement with quadratic regression models, with $R^2 > 0.88$. Based on the response surface plots, the optimal parameters were determined to be a dilution ratio of 1:19.6, a pH of 5, and a cellulase concentration of 0.44% (w/v). Under these optimal conditions, the nutrient content (saccharose, reducing sugar, protein, amino protein) and the levels of bioactive compounds (lysine, β -glucan, phenolics, flavonoids) per 100 grams of dry matter were quantified as 8.24 g, 3.60 g, 3.95 g, 3.10 g, 0.08 g, 2.17 mg, 4.93 g TAE, and 0.65 g QE, respectively.

Keywords: enzyme-assisted concentration, optimal conditions, mushroom, nutrient, bioactive compounds

INTRODUCTION

Pleurotus sajor-caju stands out as a preferable edible mushroom, which is highly cultivated and commercialized (Tran, 2022). This crop possesses its nutritional and medicinal characteristics. Particularly, this mushroom is a source of carbohydrates and proteins, offering approximately 70 to 90% of its dry weight, while the fat content is low in most species, ranging from about 1-2% (Galappthi et al., 2021). Its protein varies in water-soluble and essential amino acids consist of lysine, tryptophan, and sulfur-containing amino acids. Additionally, it contains a significant number of vital vitamins and minerals (Galappthi et al., 2021), which are identified as bioactive polymers that support the maintenance of human gut microbiota (Jayachandran et al., 2017). Due to these properties, *P. sajor-caju* is acknowledged as a significant raw material utilized in the food processing sector. The extraction of bioactive compounds from *P. sajor-caju* is a crucial step in maximizing its nutritional and functional potential. Traditional extraction methods often rely on solvents and heat, which may lead to degradation of heat-sensitive compounds and reduced bioactivity. Enzyme-assisted extraction (EAE) has emerged as an efficient and environmentally friendly alternative to conventional methods (Adithya et al., 2021). Enzymes act as biocatalysts that break down complex cell wall structures, enhancing the release of intracellular compounds with minimal loss of bioactivity (Gardossi et al., 2010). Cellulase, in particular, plays a pivotal role in EAE by hydrolyzing cellulose, the primary structural component of plant cell walls, into smaller sugar units. This enzymatic degradation weakens the cell wall integrity, facilitating the release of intracellular nutrients such as proteins, amino acids, and phenolic compounds. Studies have shown that using cellulase can significantly enhance the yield of bioactive compounds, as it selectively targets cellulose without affecting other valuable macromolecules (Zhao et al., 2016). Increasing the solvent-to-substrate ratio and enzyme concentration further improves extraction efficiency by creating a favorable concentration gradient that promotes diffusion (Zhao et al., 2016). Commercial cellulase preparations often contain a mixture of enzymes that can target multiple structural components, including hemicellulose and pectin, contributing to more effective extraction outcomes (Zhang et al., 2018). Several

previous studies have investigated the effects of enzyme-assisted extraction on mushrooms and other plant materials. For instance, research by Guadalupe et al. (2007) demonstrated that enzymatic treatment enhances the recovery of phenolic compounds and improves antioxidant activity in plant extracts. However, limited studies have specifically focused on optimizing cellulase-assisted extraction for *P. sajor-caju*, leaving a research gap in understanding the precise conditions required to maximize nutrient and bioactive compound yield. Furthermore, preprocessing methods such as dehydration can significantly influence extraction efficiency. Dehydration removes bound water from raw materials, increasing the diffusion rate and improving enzyme interaction with the substrate (Jolita et al., 2022). This preprocessing step can potentially enhance the enzymatic hydrolysis process, further increasing the efficiency of bioactive compound extraction.

The present study aims to address this research gap by optimizing the extraction conditions for dried *P. sajor-caju* powder using cellulase enzyme. A central composite design was employed to evaluate the effects of key parameters, including the ratio of water to dried mushroom powder, enzyme concentration, and pH levels. The objective is to determine the optimal conditions that maximize the extraction yield of essential nutrients and bioactive compounds, thereby providing valuable insights for the food and pharmaceutical industries.

MATERIAL AND METHODS

Pleurotus sajor-caju mushrooms were freshly harvested and transported to An Giang University, Vietnam, and Vietnam National University, Ho Chi Minh City (Vietnam). The mushrooms were sorted based on the following parameters: length (12.5±2.5 cm), cap diameter (6.6±3.5 cm), and weight (10.48±4.32 g) (Giang et al., 2021).

Experimental design

Fresh mushrooms were trimmed, cleaned, drained, and spread out on a stainless-steel tray (40x60 cm²), and dried at 60°C in oven dryer (ESCO, OFA-10-8, Indonesia) until the moisture content was less than 10% (Giang and Thuy, 2020).

Subsequently, the dried oyster mushroom specimens were grounded and passed through a 1 mm mesh sieve to obtain a fine powder. For each experimental treatment, 50 g of the resulting mushroom powder was employed. The extraction conditions included pH, which was adjusted using 10% (w/v) citric acid, the water-to-dried mushroom powder ratio, and the cellulase concentration. These parameters are presented in Table 1. The extraction of mushroom powder was conducted at 50°C for 8 hours (Nguyen and Nguyen, 2021). The cellulase enzyme after hydrolysis was deactivated at 90°C for 15 minutes. The obtain extract was filtered and analyzed for mentioned nutritional criteria below.

The optimization of extraction parameters was set up utilizing Response Surface Methodology (RSM) through central composite design (CCD) 2³+star, in order to evaluate the influence of the factors and reflected by the multivariate equation (Tuyen et al., 2022). The experiment involved 3 factors: the water to material ratio (A) (15:1, 20:1, and 25:1 v/w), pH (B) (4.5, 5.0, and 5.5), and cellulase concentration (C) (0.3, 0.4, and 0.5 w/v). The design comprised 6 central points and 20 runs (8 factorial, 6 axial, and 6 central), each factor was investigated at 5 levels, denoted from -α to +α (where α = ±1.682), employing the STAGRAPHICS centurion software version 16.1. The coded variables are outlined in Table 1.

Table 1 Variable coding and investigated levels of water:material ratio, pH and cellulase concentration.

Variables	Codes	Levels				
		-α	-1	0	+1	+α
Water:material ratio (v/w)	A	11.60:1	15:1	20:1	25:1	28.41:1
pH	B	4.16	4.5	5.0	5.5	5.84
Cellulase concentration (w/v)	C	0.23	0.3	0.4	0.5	0.57

Determination of chemical components in the extract solution

Saccharose and reducing sugar content (g/100 g of dry matter) was analysed based on the DNS method ((Nielsen, 2017b)). **Protein content** (g/100 g dry matter)

was quantified employed the Lowry theory (Nielsen, 2010). **Amino acid content** (g/100 g of dry matter) was assessed followed the Ninhydrin method (Friedman, 2004). **β-glucan content** (mg/100 g dry matter) was performed using the phenol-sulfuric acid assay (Fournier, 2001). **Lysine content** (g/100 g of dry matter) was determined following the method of Hasani et al. (2007). **The concentration of phenolic** (g TAE/100 g of dry matter) and **flavonoid** (g QE/100 g of dry matter) were detected using a colorimetric reagent (Folin-Ciocalteu) based on a described method of Sumaiyah et al. (2015). These assays were applied with several modifications and the standard equations followed Nguyen and Nguyen (2021).

Data analysis methods

Data were processed using STATGRAPHICS Centurion 16.1 to perform analysis of variance (ANOVA) and LSD tests for mean comparison at the 5% significance level (p = 0.05). Microsoft Excel was employed for calculations and graphical representations. Model adequacy during optimization was assessed through the coefficient of determination (R²), and the response surface optimization equation was formulated from the general experimental model, as presented in Equation 1 below.

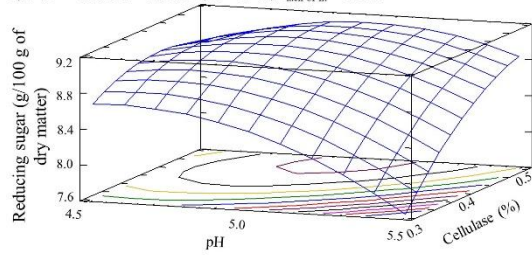
$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^k \sum_{j=1}^k \beta_{ij} X_i X_j \quad (1)$$

In the equation, Y is the objective function, β₀ is the constant term, β_i is the linear coefficient, β_{ii} is the squared coefficient, β_{ij} is the interaction coefficient, and X_i and X_j are the survey variables.

RESULTS AND DISCUSSION

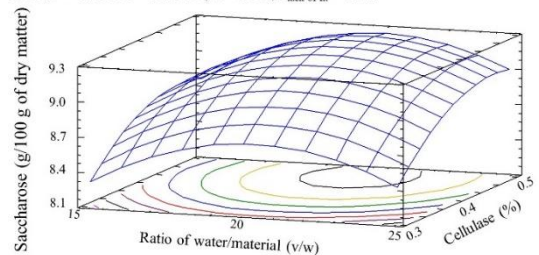
The influence of the water/material ratio, pH and cellulase concentration on the chemical composition of the oyster mushrooms extract is depicted in Figure 1.

$$Y_1 = -26.410 + 0.753A + 11.721B - 6.632C - 0.016A^2 - 0.032AB + 0.190AC - 1.294B^2 + 3.641BC - 15.076C^2; R^2=0.944; P_{lack-of-fit} = 0.080$$



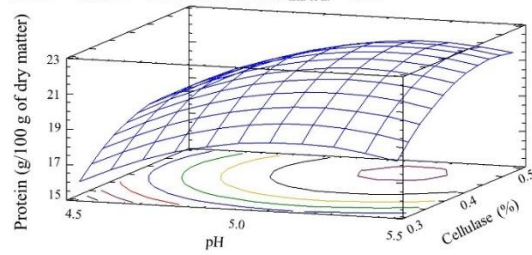
(a) Reducing sugar

$$Y_2 = -6.834 + 0.467A + 3.020B + 11.500C - 0.014A^2 + 0.019AB - 0.003AC - 0.324B^2 + 0.283BC - 13.157C^2; R^2=0.911; P_{lack-of-fit} = 0.147$$



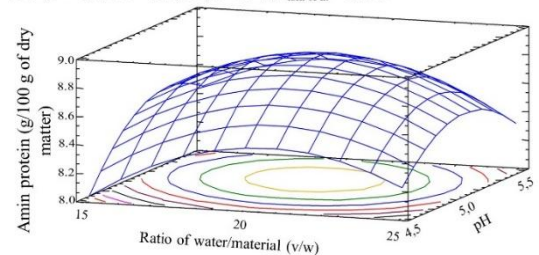
(b) Saccharose

$$Y_3 = -200.956 + 4.142A + 63.349B + 77.690C - 0.109A^2 + 0.032AB - 0.506AC - 6.302B^2 + 4.124BC - 92.993C^2; R^2=0.996; P_{lack-of-fit} = 0.182$$



(c) Amino acid

$$Y_4 = -26.410 + 0.753A + 11.721B - 6.632C - 0.016A^2 - 0.032AB + 0.190AC - 1.294B^2 + 3.641BC - 15.076C^2; R^2=0.944; P_{lack-of-fit} = 0.2925$$



(d) Protein

Figure 1 The correlation between pH and the supplemental water ratio at a cellulase concentration of 0.4 to the chemical composition of the extracted solution (a) reducing sugar, (b) saccharose, (c) amino protein, (d) protein

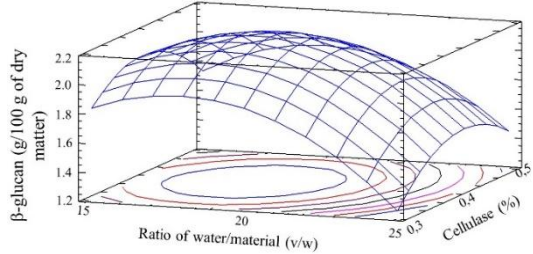
When optimizing three independent variables, specifically water/material ratio, also known as the dilution ratio (A), pH (B), and cellulase concentration (C), using Response Surface Methodology (RSM), the results indicate that the coefficient of influence of individual variables (A, B and C) is positive, meaning that an increase in the variable value will increase the value of the objective function and vice versa (Nguyen et al., 2019). Specifically, the sucrose content (per 100 g dry matter) of the extract solution increased, reaching the highest level of 8.94 g at pH 5.0. Additionally, the sucrose content of the extract solution reached its highest value of 9.05 g at a water ratio of 20:1. Furthermore, the sucrose amount increased from 8.34 g and reached a maximum of 9.05 g when the concentration of supplemental

cellulase enzyme increased from 0.3 to 0.4. However, the sucrose content tends to remain stable as the pH and concentration of supplemental enzyme increase to 5.5 and 5.0, respectively. Conversely, the sucrose content decreases, reaching a value of 8.63 g if the dilution ratio continues to increase to 25:1 (v/w). Similarly, the reducing sugar content (per 100 g dry matter) increases and reaches a high value when the concentration of supplemental enzyme is increased to 0.4 and stabilizes at 3.58 g. As the solvent extraction ratio increases to 20:1 (v/w), the reducing sugar level peaks at 3.48 g; however, if the dilution ratio continues to increase, the reducing sugar content tends to decrease. Similar results occur when

increasing the pH of the solution. Specifically, the reducing sugar level increases to a maximum of 3.54 at pH 5.0 and decreases to 3.16 g at pH 5.5. Furthermore, the protein and amino acid content (per 100 g dry matter) of the extract solution increase as the investigated values increase, reaching maximum values of 3.91 g and 3.09 g respectively at pH 5, cellulase concentration of 0.4, and a water:mushroom substrate ratio of 20:1. However, if these parameters continue to increase, the protein and amino acid content tend to decrease, especially when diluted to a ratio of 25:1, the protein content decreases rapidly from 3.33 g to 2.54 g, and the amino acid content decreases from 3.07 g to 2.10 g. The water/material ratio, greatly influences the levels of soluble compounds. A higher solvent ratio increases the contact of the material with the solvent; the increasing concentration gradient enhances diffusion rates and extraction efficiency (Cacace and Mazza, 2003; Sulyman et al., 2020; Ngo and Hoang, 2023). An appropriate dilution ratio improves solubility and mass transfer rates; however, excessively high dilution ratios can affect the efficiency of the mass transfer process (Wang et al., 2021). Excessive solvent volumes during liquid-liquid phase partitioning may dilute proteins and amino acids into the aqueous phase, reducing their concentrations in the obtained extract (Rudakov et al., 2022). Moreover, using excessive solvent leads to solvent wastage; additional time and energy are required to remove the solvent after extraction (Cacace and Mazza, 2003). In this study, increasing the solvent volume to a ratio of 25:1 results in a significant decrease in the levels of chemical compounds and bioactive components compared to the remaining dilution ratios. Enzymes are highly sensitive to the environmental pH, and pH plays a crucial role in the reaction rate of enzymes. Additionally, each enzyme operates optimally

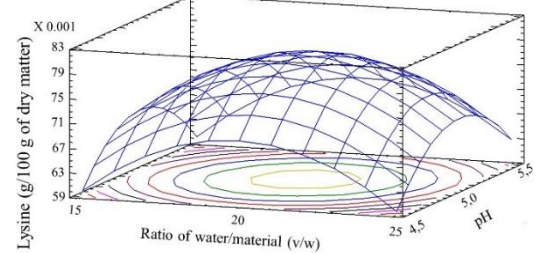
within a specific pH range, and for cellulase enzymes, the optimal pH conditions in this experiment were observed to be in the range of 4.5-5.0 (Sulyman et al., 2020; Nguyen and Nguyen, 2021). Optimal pH can maximize the activity of cellulase, facilitating cell wall breakdown, allowing for the efficient release and extraction of nutrients and bioactive compounds (Pinelo et al., 2006; Puri et al., 2013). The activity of cellulase and its ability to hydrolyze cell walls weaken due to the loss of enzyme ionization balance as the solvent pH continues to increase towards neutral and alkaline regions. This may be attributed to structural alterations of the enzyme or the destruction of the enzyme's active center, reducing its ability to interact with substrates (Li et al., 2020; Sulyman et al., 2020; Wei et al., 2021). Furthermore, due to the ionization nature of reducing sugars, as pH increases, the ionization ability of reducing sugar molecules changes, becoming less soluble in the solvent or unable to form the necessary complexes for extraction (Kunz et al., 2011). Additionally, pH variations can alter the hydrophobic (water-attracting) and hydrophilic (water-repelling) properties of compounds in the extraction solvent. This can reduce the solubility of reducing agents in the solvent, decreasing extraction efficiency (Kudirka et al., 2023). The correlation and influence of the investigated conditions on the composition of bioactive components in the extract solution are shown in Figure 2. For bioactive compounds, the levels of β-glucan, lysine, phenolic, and flavonoid (per 100 g dry matter) increase to suitable values and then gradually decrease with increasing pH, reaching their highest values at pH 5.0 with respective values of 2.16 g, 0.08 g, 0.492 g TAE and 0.646 g QE.

$$Y_{\beta} = -100.504 + 2.378A + 26.668B + 63.103C - 0.034A^2 - 0.186AB - 0.334AC - 2.075B^2 - 3.195BC - 47.265C^2; R^2=0.999; P_{\text{lack-of-fit}} = 0.099$$



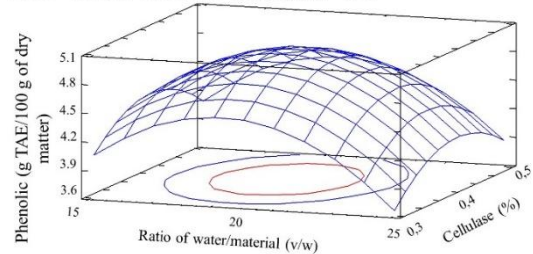
(a) β-glucan

$$Y_L = -0.909 + 0.012A + 0.332B + 0.116C - 0.109A^2 - 0.001AB + 0.001AC + 0.006B^2 - 0.035BC + 0.033C^2; R^2=0.896; P_{\text{lack-of-fit}} = 0.068$$



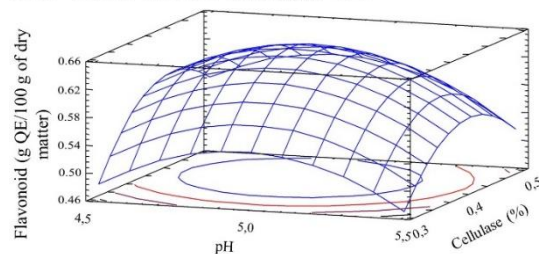
(b) Lysine

$$Y_P = -44.509 + 1.084A + 0.332B + 12.538C + 33.959A^2 - 0.026AB - 0.019AC - 1.075B^2 - 2.964BC - 24.384C^2; R^2=0.974; P_{\text{lack-of-fit}} = 0.074$$



(c) Phenolic

$$Y_F = -10.417 + 0.226A + 3.027B + 6.821C - 0.003A^2 - 0.017AB - 0.093AC - 0.266B^2 - 0.153BC - 4.766C^2; R^2=0.979; P_{\text{lack-of-fit}} = 0.945$$



(d) Flavonoid

Figure 2 The correlation and influence of the surveyed factors on the bioactive components in the extracted solution (a) β-glucan, (b) lysine, (c) phenolic, and (d) flavonoid

Enzymes are employed to facilitate the extraction of bioactive compounds from plant-based materials (Ngo and Hoang, 2023). The presence of cellulase aids in cellulose hydrolysis within plant cell walls, breaking polyphenol and polysaccharide bonds, and releasing nutrients from plant materials into the solvent (Ngo and Hoang, 2023; Yin et al., 2023). Results indicate that the appropriate supplementation of cellulase enhances the efficiency of extracting chemical compounds and bioactive substances from oyster mushroom substrate as cellulase positively influences cellulose degradation, thereby enhancing nutrient dissolution into the solvent (Rostami and Gharibzahedi, 2017; Ngo and Hoang, 2023; Yin et al., 2023). However, when cellulase supplementation increases to 0.5%, the interaction between cellulase and substrates reaches saturation levels, leading to competitive molecular interactions and a decrease in extraction efficiency (Huynh

et al., 2014). According to Ngo and Hoang (2023), excessively high enzyme concentrations do not enhance extraction efficiency, as cellulase is inhibited by products generated during hydrolysis, such as glucose and xylobiose, resulting in decreased activity.

The regression equations (Figures 1 and 2) describe the relationship between the independent variables and their interactions with the chemical components and bioactive compounds of the extract from mushroom substrates, showing $R^2 > 0.88$ and $P_{\text{Lack-of-fit}}$ values > 0.05 . This indicates the high suitability of the model for predicting the objective function. Additionally, the Pareto chart (Figure 3) also demonstrates that the sucrose, reducing sugar, phenolic, flavonoid, glutamic and lysine contents are most influenced by the dilution ratio.

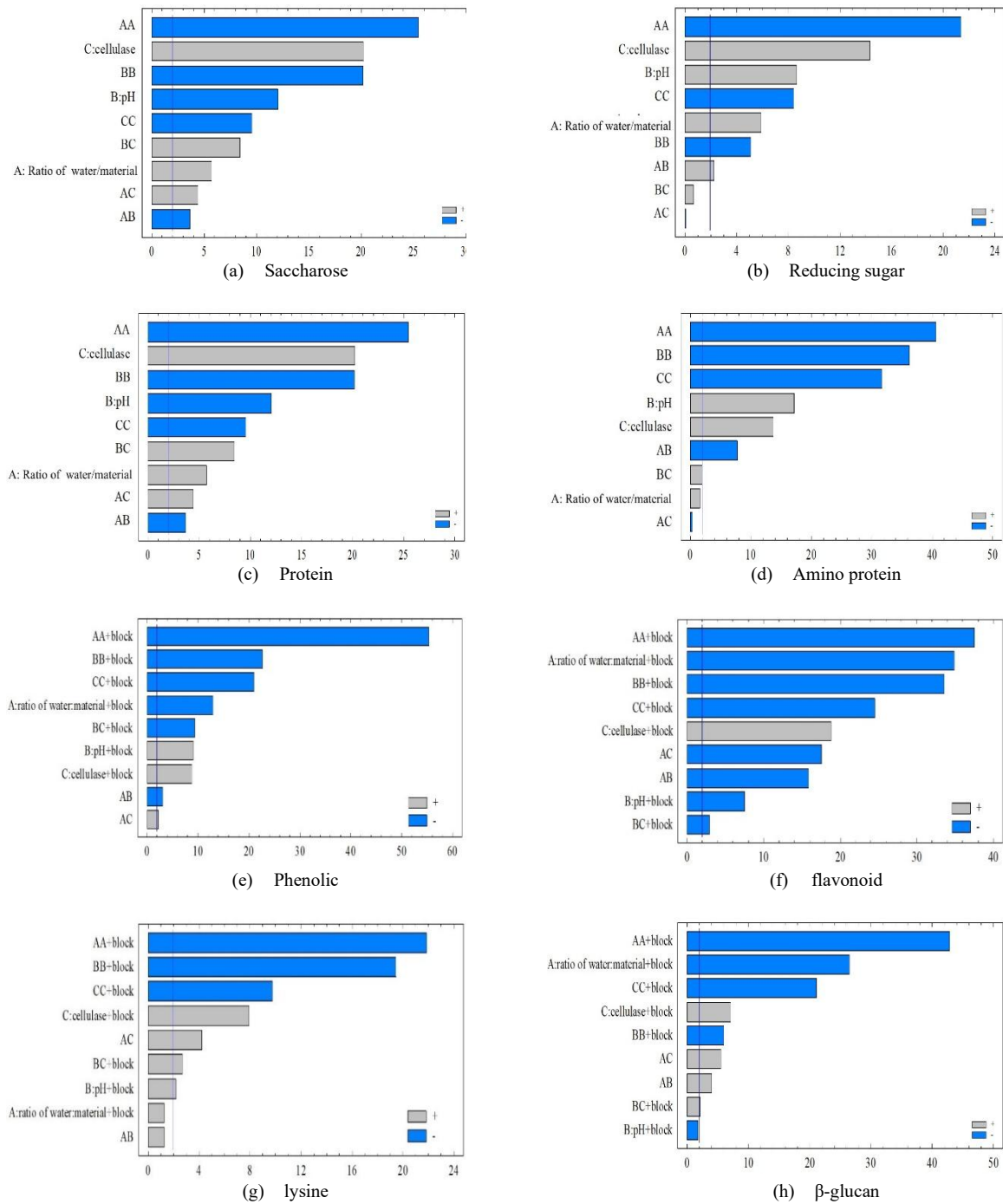


Figure 3 The Pareto chart illustrates the influence of the factors: water:material ratio (A), pH (B), and cellulase concentration (C) on (a) reducing sugar, (b) total sugar, (c) protein, (d) amino acid, (e) phenolic, (f) flavonoid, (g) lysine, (h) β -glucan

The simultaneous optimization of multiple response surfaces has found the optimal parameters for the extraction process of nutrients from mushroom substrates with the assistance of cellulase enzyme: the water/material ratio is 19.56, pH is 5.04, and supplemental cellulase is 0.44% (Figure 4). The experimental extraction process was conducted with a water/material ratio of 19.6, pH of 5.00, and cellulase concentration of 0.44%. The results show that the nutrient levels are equivalent to

the values predicted by the model, with differences ranging from 0.00-0.10% (Table 2). These findings are consistent with previous observations on nutrient extraction supported by cellulase enzyme from plant materials by **Nguyen and Nguyen (2021)**; **Le et al. (2022)**; **Ngo and Hoang (2023)**; **Yin et al. (2023)**.

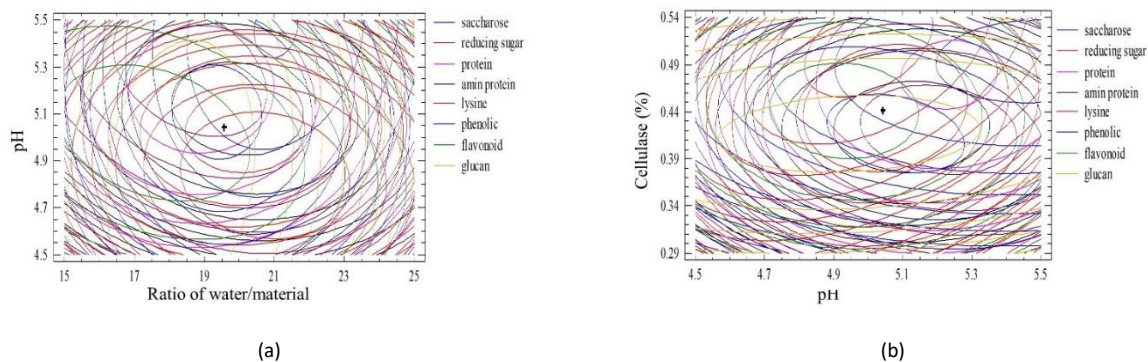


Figure 4 The contour plot illustrates the simultaneous optimization of multiple response surfaces (saccharose, reducing sugar, protein, amino acid, β-glucan, lysine, phenolic and flavonoid) based on (a) the ratio of water/material and pH, (b) pH and cellulase (with one factor fixed at the centre point)

Table 2 Experimental and the optimization models results

Target functions	Unit	Values of experiment	Values of optimization models
Saccharose	g/100 g of dry matter	8.24±0.00	8.24
Reducing sugar	g/100 g of dry matter	3.60±0.01	3.61
Protein	g/100 g of dry matter	3.95±0.01	3.95
Amino protein	g/100 g of dry matter	3.10±0.01	3.11
Phenolic	g TAE/100 g of dry matter	4.94±0.00	4.93
Flavonoid	g QE/100 g of dry matter	0.65±0.01	0.65
Lysine	g/100 g of dry matter	0.08±0.00	0.08
β-glucan	mg/100 g of dry matter	2.18±0.01	2.17

CONCLUSION

The statistical evaluation of the ultimate parameters chosen for the experiment, comprising the ratio of mushroom powder to water at 1:19.6, pH level of 5.0, and cellulase-assisted concentration set at 0.44% (v/w). The response surface experiment results demonstrated outcomes that closely aligned with the predicted values. These findings offer valuable insights for the large-scale extraction of nutrients and bioactive compounds from *Pleurotus sajor-caju* in the food processing sector.

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