

Article

Optimization of Ultrasonic-Assisted Enzymatic Extraction, Purification, and Antioxidant Activity of Polyphenols from Almond Hull

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Abstract

Almond processing byproducts are rich in bioactive polyphenols but severely underutilized due to inefficient conventional extraction methods. This study presents the first systematic optimization of an integrated ultrasound-assisted enzymatic extraction and AB-8 macroporous resin purification process for almond hull polyphenols, addressing the limitations of low yield, high impurity content, and bioactivity loss in traditional approaches. Extraction parameters were optimized via single-factor experiments combined with Box–Behnken response surface methodology, while purification conditions were refined through static and dynamic adsorption–desorption tests. Structural characterization and antioxidant evaluation were performed using Ultraviolet-Visible Spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays. Under optimal conditions, the polyphenol yield reached 23.67 mg/g. After purification, polyphenol purity increased 5.88-fold, flavonoid purity improved 4.62-fold, and DPPH/FRAP antioxidant activities were enhanced 5.0-fold and 6.5-fold, respectively. Purified polyphenols retained intact phenolic structures and exhibited a loose porous microstructure. This green process provides a technical basis for high-value utilization of almond hulls. Limitations include lack of polyphenol monomer identification, in vivo efficacy validation and industrial economic feasibility assessment.

Keywords: almond hull; polyphenols; ultrasonic-assisted enzymatic extraction; macroporous resin purification; antioxidant activity

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1. Introduction

Almond (*Prunus dulcis*) is one of the most widely cultivated nut crops worldwide, with a global production exceeding 3 million tons annually. Almond hull, the fleshy outer layer of the almond fruit, accounts for about 50% of the fruit [1], and is a major by-product of the almond processing industry. At present, most almond hulls are directly discarded or used as low-value animal feed, which not only causes serious waste of biomass resources, but also brings environmental pollution risks [2]. Therefore, exploring the high-value utilization of almond hulls has important economic and environmental significance.

Previous studies have confirmed that almond hulls are rich in bioactive substances, especially polyphenols [1]. It is noteworthy that the content and composition of bioactive compounds in *Amygdalus communis* L. green hulls vary depending on cultivar origin and harvest batch. Plant polyphenols have been widely reported to have excellent antioxidant, anti-inflammatory, antibacterial, and anti-tumor activities [3–6], and are widely used in food, cosmetics, and pharmaceutical industries. However, the extraction efficiency and purity of polyphenols from plant by-products are the key factors restricting their application. Traditional solvent extraction methods have the disadvantages of long extraction time, high organic solvent consumption, low extraction efficiency, and easy degradation of active ingredients. Ultrasonic-assisted enzymatic extraction (UAEE) is a novel green extraction technology, which combines the cavitation effect of ultrasound with the specific degradation effect of enzymes on plant cell walls. It can effectively break the cell wall structure, promote the release of intracellular active ingredients, and has the advantages of high extraction efficiency, mild reaction conditions, and environmental friendliness [7].

In addition, crude plant polyphenol extracts commonly contain non-polyphenolic impurities including polysaccharides, proteins and pigments, which not only interfere with the accurate measurement of the true biological activity of target polyphenols but also cause ambiguity in activity attribution, thus limiting the further research and application of polyphenols. Macroporous resin adsorption is a commonly used purification technology for plant polyphenols, which has the advantages of high adsorption selectivity, easy regeneration, low cost and simple operation and is suitable for large-scale industrial production [8].

This study hypothesizes that UAEE can significantly improve the extraction efficiency and polyphenol yield from almond hulls compared with conventional extraction methods. In addition, the optimized macroporous resin purification process is expected to enhance the purity, physicochemical properties, and antioxidant activity of the extracted almond hull polyphenols. Therefore, this study aims to: (1) optimize the UAEE process parameters of almond hull polyphenols using single-factor experiments and response surface methodology; (2) optimize the static and dynamic adsorption and desorption conditions of macroporous resin for almond hull polyphenols; (3) characterize the physicochemical properties of the purified polyphenols and evaluate their antioxidant activity. This study will provide an efficient and green technical system for the extraction and purification of almond hull polyphenols, and lay a theoretical foundation for the high-value utilization of almond processing by-products.

2. Materials and Methods

2.1. Materials

The almond hulls used in this study were collected from California, USA, which were derived from *Prunus dulcis* (Mill.) D.A. Webb cv. Monterey (Rosaceae). The samples were thoroughly dried in the United States before being shipped to China. After arrival, intact and mold-free hulls were selected and sequentially washed and re-dried, then stored in an ultra-low temperature freezer at -80°C .

Sodium hydroxide and sodium carbonate were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd.; Shanghai, China; hydrochloric acid was purchased from Shanghai Macklin Biochemical Technology Co., Ltd.; Shanghai, China; anhydrous ethanol was purchased from Tianjin Zhiyuan Chemical Reagent Co., Ltd.; Tianjin, China; Folin-phenol was purchased from Sinopharm Chemical Reagent Co., Ltd.; Beijing, China; cellulase (50 U/mg) was purchased from Zhejiang Yinuo Biotechnology Co., Ltd.; Jinhua, China; pectinase (100 U/mg) was purchased from Henan Wanbang Chemical Technology Co., Ltd. Zhengzhou, China.

2.2. Methods

2.2.1. Single-Factor Experiment of Polyphenol Extraction from Almond Hulls

Pretreated almond hulls were soaked in deionized water at 50 °C for 3 h and ground with a colloid mill for 3.5 h to achieve adequate swelling and tissue breakdown, ultimately reducing their particle size to less than 100 µm and creating a necessary foundation for subsequent polyphenol extraction.

Polyphenol extraction yield was selected as the evaluation index, and a single-factor experimental design was adopted in this study. Throughout the experiments, the ultrasonic power (180 W), ultrasonic frequency (40 kHz) and initial pH value (4.5) for enzymatic hydrolysis of almond hull were kept constant. The initial pH was adjusted using 1% citric acid–sodium citrate buffer solution. The effects of liquid–solid ratio (10–50 mL/g), enzymolysis temperature (30–70 °C), enzymolysis time (0–60 min), compound enzyme ratio (pectinase to cellulase, 1–17), and total enzyme dosage (0.05–10.00%, based on the dry weight of raw materials) on polyphenol extraction were investigated sequentially. Strict single-variable control was implemented, wherein only one factor was altered in each test while all other experimental conditions remained unchanged.

2.2.2. Response Surface Methodology for Polyphenol Extraction from Almond Hulls

A three-factor three-level Box–Behnken design (Design-Expert 13.0) was employed to optimize the enzymolysis process, with compound enzyme ratio, enzymolysis temperature and liquid–solid ratio as independent variables and polyphenol content as the response value. Factor levels were determined by single-factor experiments (Table 1). All experiments were performed in triplicate, with statistical significance set at $p < 0.05$.

Table 1. Experimental design of the response surface method for polyphenol extraction from almond hulls.

Factor	Level		
	−1	0	1
Compound Enzyme Ratio (A)/(g:g)	11	13	15
Enzymolysis temperature (B)/°C	35	40	45
Liquid-Solid ratio (C)/(mL/g)	35	40	45

2.2.3. Static Adsorption and Desorption Experiments

AB-8 Pre-Treatment

An appropriate amount of AB-8 macroporous adsorption resin was weighed and soaked in 95% (*v/v*) ethanol for 24 h for activation, so as to remove residual monomers from resin synthesis and impurities trapped in the pores. The resin was then repeatedly rinsed with deionized water until no obvious ethanol odor remained. Afterwards, the resin was immersed in 4% (*v/v*) hydrochloric acid (HCl) solution for 6 h to eliminate cationic impurities, and then rinsed with deionized water until the eluate showed a neutral pH. Next, the resin was soaked in 4% (*v/v*) sodium hydroxide (NaOH) solution for another 6 h to remove anionic impurities. Finally, deionized water was used to wash the resin until the outflow was neutral. Excess water was removed by vacuum filtration, and the treated resin was set aside for subsequent use.

Static Adsorption and Desorption Curves

Previous studies have confirmed that AB-8 macroporous resin is a commonly used and optimal resin for polyphenol purification [9,10]. A total of 2 g of pretreated AB-8 macroporous resin was placed in a conical flask, followed by adding 30 mL of 2 mg/mL almond hull crude polyphenol extract. The mixture was oscillated for adsorption at 25 °C and 130 rpm in a TY-70B constant-temperature oscillator (Tianjin Test Instrument Co., Ltd., Tianjin China) for 5 h, with samples collected hourly for polyphenol concentration analysis. After adsorption, the resin was suction-filtered, rinsed with distilled water, and then desorbed with 30 mL of 50% (*v/v*) ethanol solution under identical temperature and shaking conditions for 5 h; samples were also taken hourly to determine polyphenol content. The adsorption (1) and desorption (2) performances of the resin were calculated via established formulas. All experiments were independently conducted in triplicate [11].

$$\text{Adsorption rate (\%)} = \frac{C_0 - C_1}{C_0} \times 100 \quad (1)$$

$$\text{Desorption rate (\%)} = \frac{C_2 - C_1}{C_0 - C_1} \times 100 \quad (2)$$

where C_0 , C_1 , and C_2 (in mg/mL) represent the concentration of total polyphenols in the initial solution, the solution after adsorption, and the solution after desorption, respectively.

Optimization of Static Adsorption and Desorption Conditions

A total of 2 g of pretreated AB-8 macroporous resin was accurately weighed, and 45 samples were prepared and randomly divided into three groups. Group 1 was added with almond hull polyphenol extracts at pH 4, 5, 6, 7, and 8; Group 2 was added with the extracts at mass concentrations of 0.25, 0.5, 1.0, 2.0, and 3.0 mg/mL; Group 3 was added with the extract at pH 4 and 0.5 mg/mL. All samples were subjected to adsorption oscillation at 25 °C and 130 r/min for 3 h. After adsorption, the supernatant was collected for polyphenol determination, and the adsorption rate was calculated to screen the optimal adsorption pH and extract concentration.

The supernatant of Group 3 was discarded, and the resin was gently rinsed three times with ultrapure water to remove residual unadsorbed polyphenols. Then, 30 mL of ethanol solutions at volume fractions of 10%, 30%, 50%, 70%, and 90% were added separately, followed by desorption oscillation at 25 °C and 130 r/min for 2 h. The supernatant was sampled for polyphenol measurement, and the desorption rate was calculated to determine the optimal ethanol elution concentration.

2.2.4. Dynamic Adsorption and Elution Condition Optimization

Determination of Breakthrough Point

Wet packing was employed: 30.0 g of pretreated AB-8 macroporous resin was packed into a 2.6 × 40 cm chromatography column. The column was equilibrated with ultrapure water for 12 h to stabilize the resin. The crude almond hull polyphenol extract (0.5 mg/mL) was loaded at 2.0 mL/min, and effluents were collected every 20 mL for absorbance determination. A curve was plotted with loading volume as the abscissa and effluent concentration as the ordinate.

Optimization of Flow Rate Conditions for Purification of Almond Hull Polyphenols by AB-8 Macroporous Resin

Forty-five portions (30.0 g each) of pretreated AB-8 macroporous resin were weighed and randomly divided into three groups (15 portions per group). Group 1 was loaded at 1.0, 1.5, 2.0, 2.5, and 3.0 mL/min. Groups 2 and 3 were loaded at the optimal flow rate

determined from Group 1. Effluents were collected to determine polyphenol contents from almond hulls, and adsorption curves were plotted as adsorption rate versus loading volume. Group 2 was eluted at 1.0, 1.5, 2.0, 2.5, and 3.0 mL/min. Eluates were collected for absorbance measurement, and desorption curves were plotted as desorption rate versus elution volume. Group 3 was eluted at the optimal elution flow rate obtained from Group 2, with eluates collected in 20 mL fractions. Fractional desorption curves were plotted as desorption rate versus elution volume.

Determination of Total Polyphenols

Total polyphenol content was determined by the Folin–Ciocalteu colorimetric method. Using gallic acid as the standard, a series of standard solutions (10–60 µg/mL) were prepared. After mixing with Folin–Ciocalteu reagent and sodium carbonate solution, the mixture was incubated in the dark for 40 min, and absorbance was measured at 765 nm. The regression equation was $y = 0.0145x + 0.0359$ ($R^2 = 0.9979$). Samples were appropriately diluted and analyzed under the same conditions. The total polyphenol content of the samples was calculated according to Equation (3):

$$\text{Total polyphenols (mg/g)} = \frac{C_1 \times V_1 \times N}{M \times 1000} \quad (3)$$

where C_1 , total polyphenol concentration in the sample solution (µg/mL); V_1 , volume of test solution (mL); N , dilution factor; M , mass of almond hull sample (g).

Determination of Total Flavonoids

The total flavonoids were determined using an assay kit, and the detailed procedures are as follows.

Rutin standard stock solutions were prepared with concentrations of 10, 20, 30, 40, 50 and 60 µg/mL. Six stoppered test tubes were taken, and 1 mL of each standard solution was added into the corresponding tube. Subsequently, 1.2 mL of extract was added, followed by 0.2 mL of sodium nitrite solution. The mixture was fully blended and allowed to stand for 6 min. Next, 0.2 mL of aluminum nitrate solution was added. After shaking and standing for another 6 min, 1 mL of sodium hydroxide solution and 1.4 mL of deionized water were added sequentially, and the solution was left to stand for 15 min. The absorbance of each tube was measured at a wavelength of 510 nm. The standard curve was plotted with rutin concentration (µg/mL) as the abscissa and absorbance as the ordinate. The fitted regression equation was $y = 0.0127x - 0.0036$ ($R^2 = 0.9989$).

The test samples were diluted appropriately to ensure the measured absorbance values fell within the linear range of the standard curve. The subsequent experimental operations for sample detection were consistent with those for standard solutions. The total flavonoid content of the samples was calculated according to Equation (4):

$$\text{Total Flavonoids (mg/g)} = \frac{C_2 \times V_2 \times D}{M \times 1000} \quad (4)$$

where C_2 , rutin concentration obtained from the standard curve, µg/mL; V_2 , total volume of the sample extract, mL; D , sample dilution factor; M , mass of almond hull sample, g.

Determination of Antioxidant Activity

The total antioxidant capacity of the samples was comprehensively characterized by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant power (FRAP) assay.

DPPH Radical Scavenging Activity Assay

Determined using a DPPH assay kit (BIOISCO, AO043, Iseju (Lianyungang, China) Biotechnology Co., Ltd., Lianyungang, China).

Almond hull polyphenol extract samples were centrifuged at 4000 rpm and 4 °C for 10 min, and the supernatant was collected. The supernatant was appropriately diluted with pre-chilled kit extraction solution to prepare test samples, with three parallel replicates for each sample. The test sample was mixed with the DPPH solution at a volume ratio of 1:18, and the mixture was incubated in the dark at room temperature for 20 min. Absorbance at 515 nm was measured to record the blank absorbance (A_2) and the sample absorbance (A_1). The absorbance difference was calculated as $\Delta A = A_1 - A_2$.

Total antioxidant capacity was quantified as Trolox equivalents. The regression equation was $y = 0.7072x - 0.0081$ ($R^2 = 0.9977$), where x is the Trolox concentration ($\mu\text{mol/mL}$) and y is ΔA . The volume-based total antioxidant capacity of the almond hull polyphenol extract samples was calculated according to Equation (5):

$$\text{Total antioxidant capacity } (\mu\text{mol}\cdot\text{Trolox/mL}) = 1.414 \times (\Delta A + 0.0081) \quad (5)$$

Ferric Reducing Antioxidant Power (FRAP) Assay

Determined using a FRAP assay kit (BIOISCO, AO037, Iseju (Lianyungang, China) Biotechnology Co., Ltd., Lianyungang, China).

Prior to the assay, fresh working reagent was prepared by mixing FRAP buffer, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution, and ferric chloride solution at a volume ratio of 10:1:1. The reagent was pre-warmed in a 37 °C water bath before use. Almond hull polyphenol extract samples were prepared in the same manner as described in Section Determination of DPPH Radical-Scavenging Activity. Each sample was analyzed in three parallel replicates. The diluted sample was mixed with the working reagent at a volume ratio of 1:19. After thorough mixing, the mixture was incubated at room temperature for 20 min. Absorbance was measured at 593 nm to record the blank absorbance (A_4) and test absorbance (A_3). The absorbance difference was calculated as $\Delta A = A_3 - A_4$. The regression equation was $y = 2.4832x + 0.0134$ ($R^2 = 0.9996$), where x represents Trolox concentration ($\mu\text{mol/mL}$) and y represents ΔA . The volume-based total antioxidant capacity of the almond hull polyphenol extract samples was calculated according to Equation (6):

$$\text{Total antioxidant capacity } (\mu\text{mol}\cdot\text{Trolox/mL}) = 0.4027 \times (\Delta A - 0.0134) \quad (6)$$

Determination of Purity of Total Polyphenols and Flavonoids

The purity of almond hull polyphenols was determined by the method of Haiyun Jiang et al. Accurately weighed constant-weight purified polyphenols were dissolved in methanol to a known mass concentration. Total phenolic content was measured by the Folin–Ciocalteu assay. Purity was calculated by Equation (7) and expressed as percentage on a dry basis [12].

$$\text{Purity (\%)} = \frac{C_3 \times V_3}{M} \times 100 \quad (7)$$

where C_3 , polyphenol concentration (mg/mL); V_3 , volume of prepared almond hull polyphenol solution (mL); M , mass of freeze-dried powder (mg).

The determination procedure for the purity of total flavonoids was analogous to that for total polyphenols, and thus no further details are provided herein.

2.2.5. Characterization of Almond Hull Polyphenols

UV-Vis Spectral Analysis

Purified polyphenols from almond hulls were dissolved to prepare solutions of appropriate concentrations. Ultraviolet-visible (UV-Vis) absorption spectra were recorded

in the wavelength range of 200–600 nm using a model 7600-1 UV-Vis spectrophotometer (Shanghai Jinghua Technology Instrument Co., Ltd., Shanghai, China) [13].

FT-IR Spectral Analysis

Under dry conditions, polyphenols were mixed with potassium bromide (KBr) at a mass ratio of 1:100, pressed into pellets using a pellet press, and scanned with a Fourier transform infrared (FTIR) spectrometer over the wavenumber range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} with 32 accumulations [14].

SEM Analysis

The morphology of the samples was characterized by a JEOL JSM-IT500HR scanning electron microscope (JEOL Ltd., Akishima, Tokyo, Japan). Freeze-dried powders were mounted on stubs with carbon conductive tape, sputter-coated with gold, and imaged at 3.0 kV to obtain micrographs at various magnifications [15].

2.2.6. Statistical Analysis

Origin 2021 was used to draw line graphs, column graphs and standard calibration curves. Design-Expert 13.0 was applied to create 3D response surface plots and contour plots for response surface methodology experiments.

All experiments were performed with three biological replicates and three technical replicates. All data are expressed as the mean $\pm$ standard deviation ($n = 3$). The Shapiro–Wilk test was used to verify the normality of all datasets. One-way analysis of variance (ANOVA) was applied to evaluate the overall differences among groups, and the Tukey HSD post hoc test was adopted for pairwise comparisons.

3. Results

3.1. Results of Single-Factor Experiments of Polyphenol Extraction from Almond Hulls

Figure 1A illustrates that polyphenol extraction yield from almond hulls rose initially and then leveled off as the liquid–solid ratio increased. Distinct extraction behaviors were observed at different ratio intervals. In the range of 10–40 mL/g, sufficient solvent completely permeated almond hull tissues, promoted enzyme diffusion and enzyme–substrate interaction, and effectively enhanced polyphenol liberation. The yield peaked at 19.60 mg/g at 40 mL/g, where the system approached thermodynamic equilibrium; thus, additional solvent could not further boost the extraction performance. A minor decline in yield occurred when the ratio increased to 40–50 mL/g, owing to reduced enzymatic activity caused by enzyme dilution and the co-elution of impurities that suppressed enzymatic hydrolysis [16,17]. Balancing extraction efficiency, solvent usage and downstream processing costs, 40 mL/g was determined as the optimal liquid–solid ratio.

As shown in Figure 1B, the extraction yield of polyphenols from almond hulls increased first and then decreased with the rise in temperature. In the range of 20–40 °C, temperature elevation synergized with ultrasound to improve extraction performance. Heat softened lignin and cellulose in cell walls and loosened the dense tissue structure, facilitating the penetration of enzymes; meanwhile, extreme physical forces generated by ultrasound cavitation disrupted cell walls and released bound polyphenols. Elevated temperature also accelerated enzymatic reactions, and 40 °C was identified as the optimum temperature for cellulase and pectinase, where the maximum polyphenol yield of 19.61 mg/g was achieved along with optimal enzyme conformation and catalytic activity. When the temperature exceeded 40 °C, the yield declined continuously and dropped to 18.45 mg/g at 60 °C. Excessive temperature combined with ultrasound induced irreversible thermal denaturation of enzymes and reduced their activity. Additionally, endogenous

polyphenol oxidase (PPO) was activated to accelerate the oxidative degradation of polyphenols. These two adverse effects jointly caused the gradual reduction in extraction yield.

As shown in Figure 1C, the polyphenol extraction yield from almond hulls exhibited a unimodal trend with ultrasound-assisted enzymolysis time. The yield increased significantly from 14.52 mg/g at 0 min to a maximum of 19.83 mg/g at 40 min, then declined to 17.52 mg/g at 60 min. From 0 to 40 min, cellulase and pectinase synergistically degraded cell wall components (including crystalline cellulose) over time, gradually loosening the dense tissue structure and promoting continuous polyphenol release. Complete disruption of cell wall integrity at 40 min maximized polyphenol dissolution, yielding the peak value. Beyond 40 min, two inhibitory effects drove yield reduction: accumulated reducing sugars (e.g., glucose, galacturonic acid) competitively inhibited enzyme activity by binding to active sites, while dissolved oxygen and ultrasound-induced reactive oxygen species accelerated polyphenol oxidation, with degradation rates eventually exceeding release rates [18]. Considering enzymatic efficiency and polyphenol stability, 40 min was selected as the optimal hydrolysis time.

As shown in Figure 1D, the extraction yield of polyphenols from almond hulls exhibited an initial significant increase followed by a gradual decline as the compound enzyme ratio (pectinase:cellulase) increased. At low ratios, insufficient pectinase failed to fully degrade the pectin components in the cell wall, leaving cellulose trapped within the pectin matrix and inaccessible to cellulase, thereby limiting the degradation efficiency of the cell wall skeleton. With the increase in pectinase proportion, pectin was gradually degraded, exposing the cellulose substrates and allowing cellulase to efficiently break down the cellulose skeleton, which promoted the continuous release of intracellular polyphenols. When the compound enzyme ratio reached 13 g/g, the catalytic rates and substrate matching of the two enzymes were optimized, resulting in the highest cell wall degradation efficiency and the maximum polyphenol yield of 21.33 mg/g. However, when the ratio exceeded 13 g/g, excess pectinase disrupted the balance of the enzyme system, leading to a reduction in polyphenol yield to 18.98 mg/g at 17 g/g. Collectively, the optimal compound enzyme ratio for polyphenol extraction from almond hulls was determined to be 13 g/g.

As shown in Figure 1E, the polyphenol content increased significantly to a peak and then decreased progressively with rising total enzyme dosage. When the dosage increased from 0.05% to 0.1%, more contact sites formed between enzymes and cell walls, which facilitated cell wall disruption. Accordingly, the polyphenol content rose markedly from 20.85 mg/g to 22.38 mg/g. At 0.1% enzyme dosage, all accessible catalytic sites on substrates were fully saturated, and the enzymatic degradation reached its maximum rate, yielding the highest polyphenol content. Further elevation of enzyme dosage led to a significant reduction, with the value dropping to 20.02 mg/g at 10%. This decline is mainly attributed to three factors: excess enzymes increase the viscosity of the extraction system and reduce polyphenol yield; high concentrations of free enzymes bind non-specifically to polyphenols via hydrogen bonding and hydrophobic interactions to form insoluble complexes, lowering the level of detectable free polyphenols; excessive enzymatic degradation releases non-polyphenolic compounds, which complicate subsequent separation and purification [19]. Thus, the optimal total enzyme dosage for almond hull polyphenol extraction under these conditions is 0.1%.

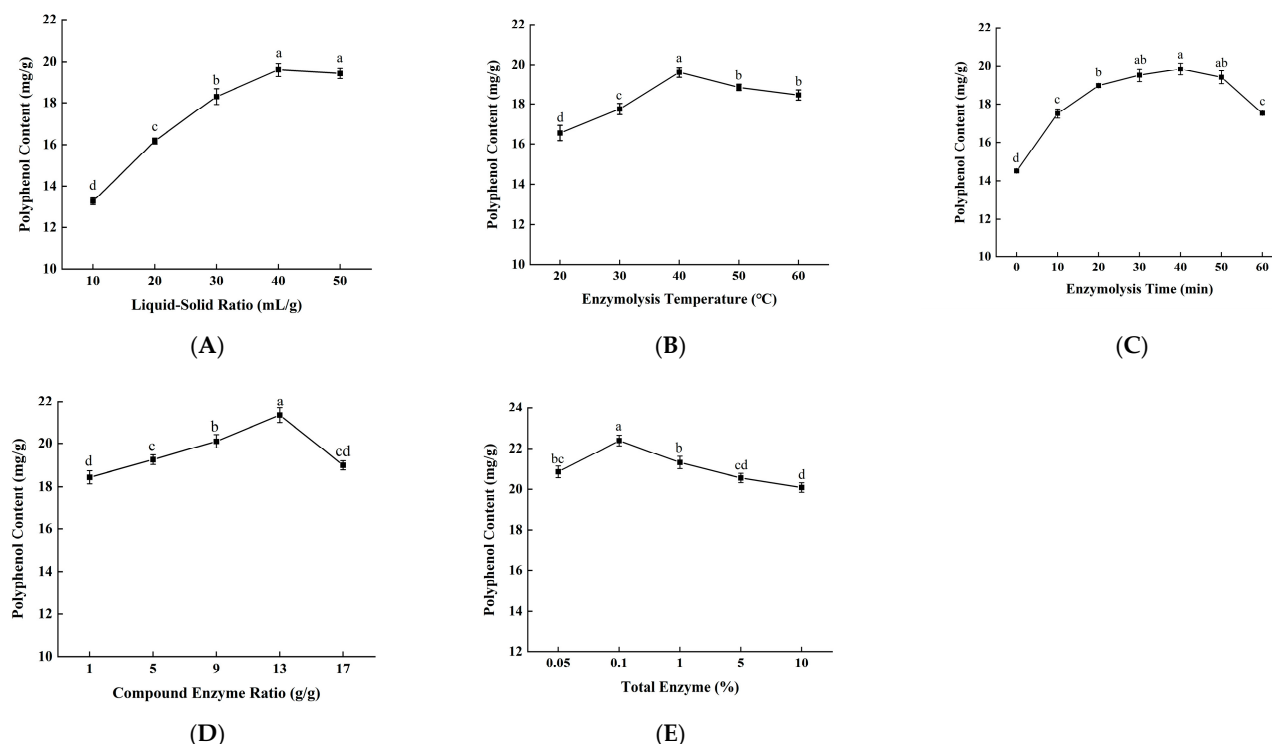


Figure 1. Effects of Liquid-Solid Ratio (A), Enzymolysis Temperature (B), Enzymolysis Time (C), Compound Enzyme Ratio (D), and Total Enzyme Dosage (E) on the Extraction of Almond Hull Polyphenols. Different lowercase letters in the figure indicate statistically significant differences among groups ($p < 0.05$, Tukey's HSD test).

3.2. Results of Response-Surface Methodology of Polyphenol Extraction from Almond Hulls

Based on single-factor experimental results, a three-factor, three-level Box–Behnken design was employed using Design-Expert 13.0 software to optimize the ultrasonic-assisted enzymatic extraction of polyphenols from almond hulls. The central values for the compound enzyme ratio (A), enzymolysis temperature (B), and liquid–solid ratio (C) were set at 13, 40 °C, and 40 mL/g, respectively. The experimental data were fitted to a second-order polynomial regression model: $Y = 22.43 + 0.19A + 0.335B + 0.470C - 0.30AB - 0.445AC - 0.545BC - 0.9070A^2 - 0.9570B^2 - 1.09C^2$. Analysis of variance (ANOVA) confirmed the model's statistical significance ($F = 27.20$, $p < 0.01$) with a non-significant lack-of-fit ($p = 0.8540$), indicating its adequacy for predicting polyphenol extraction yields. The coefficient of determination ($R^2 = 0.9722$) demonstrated excellent agreement between predicted and experimental values (Table 2.). The relative influence of factors on extraction yield followed the order: $C > B > A$.

Table 2. Analysis of variance (ANOVA) for the regression model of polyphenol extraction from almond hull.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value	Significance
Model	19.02	9	2.11	27.20	0.0001	**
A	0.2888	1	0.2888	3.72	0.0952	ns
B	0.8978	1	0.8978	11.55	0.0115	*
C	1.77	1	1.77	22.74	0.0020	**
AB	0.3600	1	0.3600	4.63	0.0684	ns
AC	0.7921	1	0.7921	10.19	0.0152	*
BC	1.19	1	1.19	15.29	0.0058	**
A ²	3.46	1	3.46	44.58	0.0003	**

B ²	3.86	1	3.86	49.63	0.0002	**
C ²	4.98	1	4.98	64.03	<0.0001	**
Lack of Fit	0.0876	3	0.0292	0.2560	0.8540	ns
Pure Error	0.4563	4	0.1141	-	-	
Cor Total	19.57	16	-	-	-	
R ²		0.9722				
Adjusted R ²		0.9365				
Predicted R ²		0.8919				

Note: * $p < 0.05$, statistically significant difference; ** $p < 0.01$, highly statistically significant difference.

Three-dimensional (3D) response surface plots and two-dimensional (2D) contour plots (Figures 2–4) were generated from the fitted quadratic regression model to elucidate the pairwise interactive effects of the three independent variables on polyphenol extraction yield. The 3D surfaces provide a visual representation of how combinations of two factors influence the response, while the morphology of the contour plots reflects the intensity of these interactions: elliptical contours indicate strong and statistically significant interactions, whereas near-circular contours correspond to weak or negligible interactions.

Figure 2 presents the contour plot and three-dimensional (3D) response surface plot illustrating the effects of compound enzyme ratio (A) and enzymolysis temperature (B) on polyphenol yield from almond hulls, with the liquid–solid ratio fixed at 40 mL/g. The 3D response surface exhibits a typical unimodal parabolic shape, where polyphenol yield first increases and then decreases with increasing levels of both factors. The relatively gentle surface curvature and approximately circular contour lines indicate no statistically significant interaction between the two factors ($p = 0.0684$). However, this p -value is close to the critical threshold of 0.05, suggesting a potential trend of interaction. The maximum polyphenol yield was obtained at a compound enzyme ratio of 13 and an enzymolysis temperature of 40 °C, which is in perfect agreement with the central levels of the experimental design.

Figure 3 presents the two-dimensional (2D) contour plot and three-dimensional (3D) response surface plot illustrating the interactive effects of compound enzyme ratio (A) and liquid–solid ratio (C) on polyphenol yield from almond hulls, with the enzymolysis temperature fixed at 40 °C. The 3D response surface exhibits a characteristic unimodal parabolic profile, where polyphenol yield first increases and then decreases with increasing levels of both factors, consistent with the results of the single-factor experiments. The relatively steep surface curvature and distinctly elliptical contour lines are hallmark features of a statistically significant interaction between the two factors ($p = 0.0152$). The minor axis of the ellipses is oriented along the liquid–solid ratio axis, indicating that the liquid–solid ratio is the dominant factor influencing this interaction and exerts a greater influence on polyphenol yield than the compound enzyme ratio. The maximum polyphenol yield was achieved at a compound enzyme ratio of 13 and a liquid–solid ratio of 40 mL/g, which coincides exactly with the central levels of the experimental design.

Figure 4 presents the two-dimensional (2D) contour plot and three-dimensional (3D) response surface plot illustrating the interactive effects of enzymolysis temperature (B) and liquid–solid ratio (C) on polyphenol yield from almond hulls, with the compound enzyme ratio fixed at 13. The 3D response surface exhibits a characteristic unimodal parabolic profile, where polyphenol yield first increases and then decreases with increasing levels of both factors, in excellent agreement with the conclusions obtained from the single-factor experiments. Comparison of the three pairwise interaction plots reveals that this response surface has the steepest surface curvature and distinctly elliptical contour lines, which are hallmark features of a highly statistically significant interaction between the two factors ($p = 0.0058 < 0.01$), representing the strongest interaction observed in this study.

The minor axis of the elliptical contours is oriented along the liquid–solid ratio axis, further confirming that the liquid–solid ratio is the most dominant factor influencing polyphenol yield. The maximum polyphenol yield was achieved at an enzymolysis temperature of 40 °C and a liquid–solid ratio of 40 mL/g, which coincides exactly with the central levels of the Box–Behnken experimental design.

Based on the validated second-order polynomial regression model, the numerical optimization module of Design-Expert 13.0 software was employed to optimize the ultrasonic-assisted enzymatic extraction process of polyphenols from almond hulls. The optimal process parameters were determined as follows: a compound enzyme ratio of 13.1, an enzymolysis temperature of 41 °C, a liquid–solid ratio of 41 mL/g, an enzymolysis time of 40 min, a total enzyme dosage of 0.1% (dry basis), an ultrasonic power of 180 W, an ultrasonic frequency of 40 kHz, and an initial pH of almond hull homogenate of 4.5. Under these conditions, the predicted polyphenol yield was 23.89 mg/g. Three independent parallel validation experiments were conducted under the optimal conditions, resulting in an average actual polyphenol yield of 23.67 mg/g, with a relative error of only 0.92%. The results demonstrate that the established regression model has high prediction accuracy, and the optimized extraction process is stable and reliable.

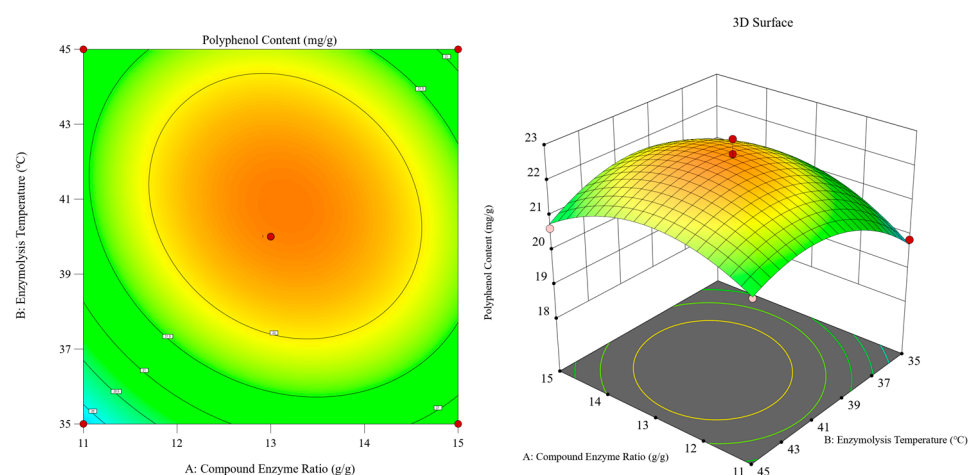


Figure 2. Interaction of A (compound enzyme ratio) and B (enzymolysis temperature) on polyphenol yield.

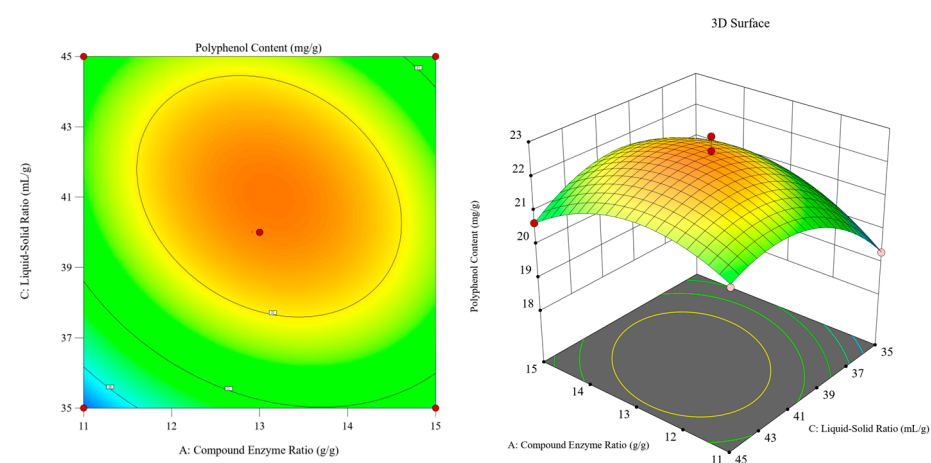


Figure 3. Interaction of A (compound enzyme ratio) and C (liquid–solid ratio) on polyphenol yield.

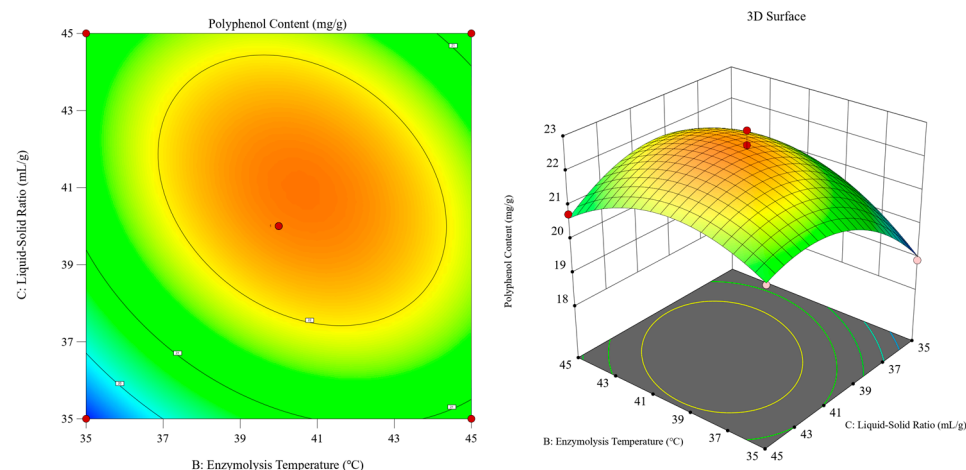


Figure 4. Interaction of B (enzymolysis temperature) and C (liquid–solid ratio) on polyphenol yield.

3.3. Results of Static Adsorption and Desorption Experiments

3.3.1. Static Adsorption and Desorption Curves

The static adsorption profile of almond hull polyphenols onto AB-8 macroporous resin (Figure 5, left) showed rapid adsorption within the first 2 h, driven by abundant available surface active sites, leading to a steep drop in solution polyphenol concentration. The adsorption rate slowed between 2 and 5 h as surface sites were occupied and polyphenols diffused into internal resin pores. Equilibrium was attained at 3 h with no further significant polyphenol uptake, confirming active site saturation, and the equilibrium adsorption efficiency was 79.41%.

The corresponding static desorption profile (Figure 5, right) exhibited a fast initial desorption phase within 1 h, attributed to easy elution of surface-adsorbed polyphenols by ethanol. The desorption rate decreased from 1 to 2 h as pore-entrapped polyphenols required overcoming adsorption forces. Desorption equilibrium was reached at 2 h with stable polyphenol concentration in the eluent, yielding an equilibrium desorption efficiency of 89.16%.

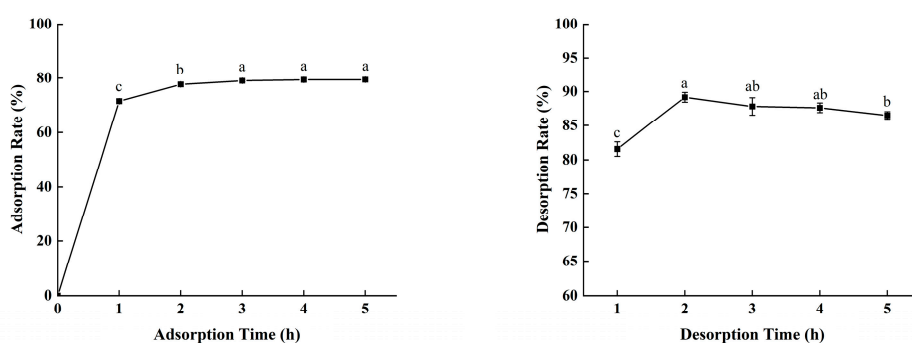


Figure 5. Static adsorption curve (left) and Static desorption curve (right). Different lowercase letters in the figure indicate statistically significant differences among groups ($p < 0.05$, Tukey's HSD test).

3.3.2. Optimization Results of Static Adsorption and Desorption Conditions

The adsorption behavior of AB-8 macroporous resin toward polyphenols varies with the pH of the sample solution. Essentially, pH modulates the affinity between polyphen-

nols and the resin by altering the ionization degree of polyphenols [20]. As shown in Figure 6A, the adsorption rate of AB-8 resin decreased markedly as the pH of the extract increased from 4 to 8, reaching a maximum of 86.56% at pH 4. Under acidic conditions, polyphenols mainly exist in an undissociated molecular form with appropriate polarity, thereby enabling effective adsorption onto the resin through hydrogen bonds and hydrophobic interactions [21]. Under alkaline conditions, polyphenols ionize to form water-soluble anions, which reduces their affinity for the resin and consequently leads to a decrease in adsorption efficiency [22]. Accordingly, pH 4 is determined as the optimal loading pH for the purification of almond hull polyphenols using AB-8 macroporous resin.

The static adsorption performance of the resin is closely related to the sample loading concentration. An appropriate concentration can fully exploit the adsorption potential, shorten the adsorption time, and improve adsorption efficiency. As shown in Figure 6B, within the range of 0.25–0.5 mg/mL, the adsorption rate of polyphenols on AB-8 macroporous resin increased with rising sample concentration, reaching a maximum of 93.21% at 0.5 mg/mL. However, further increasing the concentration led to a decline in adsorption rate. This may be attributed to the approaching saturation of adsorption sites for polyphenols on the resin. In addition, a higher sample concentration was accompanied by increased impurities, which competed with polyphenols for active sites on the macroporous resin, thus reducing the adsorption capacity [23]. Therefore, 0.5 mg/mL was selected as the optimal loading concentration for subsequent optimization experiments.

As shown in Figure 6C, the desorption rate of polyphenols from almond hull first increased and then decreased with the rise in ethanol volume fraction, reaching a maximum of 81.74% at 50% ethanol. This is because increasing ethanol concentration strengthens the interaction between the eluent and polyphenols, facilitating their desorption. The polarity of 50% ethanol matches well with that of the adsorbed polyphenols, leading to the strongest desorption effect. Excessively high ethanol content, however, co-elutes other strongly polar impurities, resulting in a lower desorption rate of polyphenols. Therefore, 50% ethanol was chosen as the eluent for AB-8 macroporous resin.

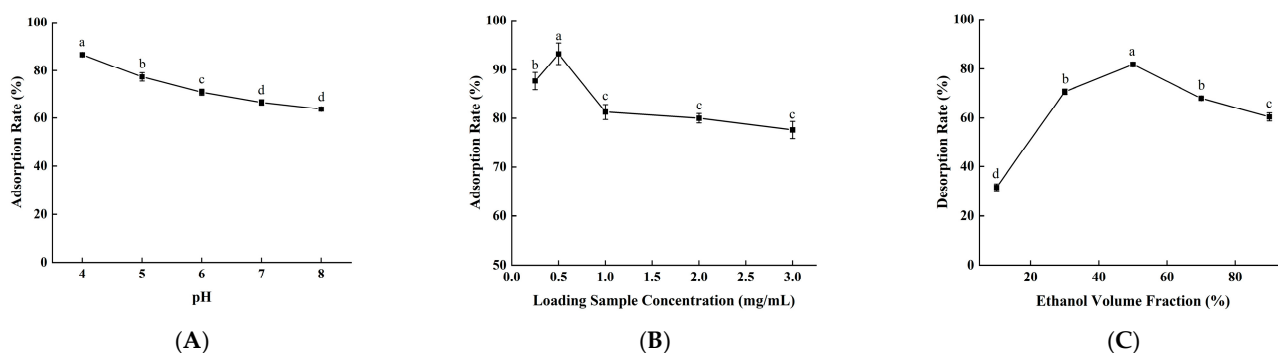


Figure 6. Effect of sample pH (A), loading sample concentration (B) and ethanol volume fraction (C) on static adsorption rate of AB-8 macroporous resin for almond hull polyphenols. Different lower-case letters in the figure indicate statistically significant differences among groups ($p < 0.05$, Tukey's HSD test).

3.4. Optimization Results of Dynamic Adsorption and Elution Conditions

3.4.1. Determination of Breakthrough Point

As shown in Figure 7, the polyphenol concentration in the effluent was positively correlated with the sample loading volume. Within 0–80 mL, the resin adsorption capacity for polyphenols was not saturated, and no polyphenols were detected in the effluent. In

the range of 80–140 mL, the adsorption rate of the resin gradually lagged behind the loading rate, resulting in accelerated breakthrough of polyphenols. When the loading volume reached 200 mL, the effluent polyphenol concentration was 1.6 $\mu\text{g/mL}$, approximately one-tenth of the initial concentration (16.2 $\mu\text{g/mL}$), which was defined as the breakthrough point of the macroporous resin [24]. Therefore, a sample loading volume of 200 mL was selected for subsequent experiments.

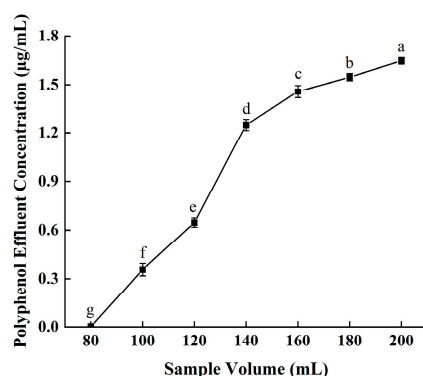


Figure 7. Breakthrough curve of AB-8 macroporous resin for the adsorption of polyphenols. Different lowercase letters in the figure indicate statistically significant differences among groups ($p < 0.05$, Tukey's HSD test).

3.4.2. Optimization of Dynamic Purification Conditions for Almond Hull Polyphenols

As depicted in Figure 8A, the loading flow rate exerted a remarkable effect on the adsorption rate of polyphenols from almond hulls onto AB-8 macroporous resin. The adsorption rate increased continuously with the flow rate ranging from 1.0 to 2.0 mL/min and reached a maximum of 97.48% at 2.0 mL/min, followed by a slight decrease yet remaining above 90% as the flow rate further rose to 3.0 mL/min. This could be attributed to insufficient utilization of adsorption sites at low flow rates, improved mass transfer at moderate flow rates, and inadequate residence time for full adsorption at excessively high flow rates [25]. Accordingly, 2.0 mL/min was determined as the optimal loading flow rate, balancing adsorption performance and mass transfer efficiency, which provides a key basis for the large-scale purification process.

As shown in Figure 8B, the desorption rate of the macroporous resin first increased and then decreased with increasing elution flow rate. At low flow rates of 1.0–2.0 mL/min, long contact time was accompanied by slow desorption product removal, forming a local high-concentration layer and concentration-difference resistance that inhibited competitive binding and thus lowered desorption efficiency [26]. At 2.0 mL/min, desorbed products were promptly removed, concentration polarization was alleviated, and enhanced turbulence improved contact between the eluent and adsorption sites, yielding the highest desorption rate of 90.70%. Further increasing the flow rate to 2.0–3.0 mL/min drastically shortened residence time, preventing sufficient interaction between the eluent and resin pores and resulting in reduced desorption due to incomplete polyphenol release [27]. Accordingly, 2.0 mL/min was selected as the optimal elution flow rate for efficient purification.

As shown in Figure 8C, stepwise elution behavior was observed using 50% ethanol as the eluent at a flow rate of 2 mL/min. The concentration of polyphenols from almond hulls rose sharply within 40–80 mL of elution volume, peaking at 88.57 $\mu\text{g/mL}$ at 80 mL, then gradually declined until nearly all adsorbed polyphenols were eluted at 160 mL, after which the eluate concentration stabilized with no obvious changes. Thus, 160 mL was chosen as the optimal elution volume for subsequent experiments.

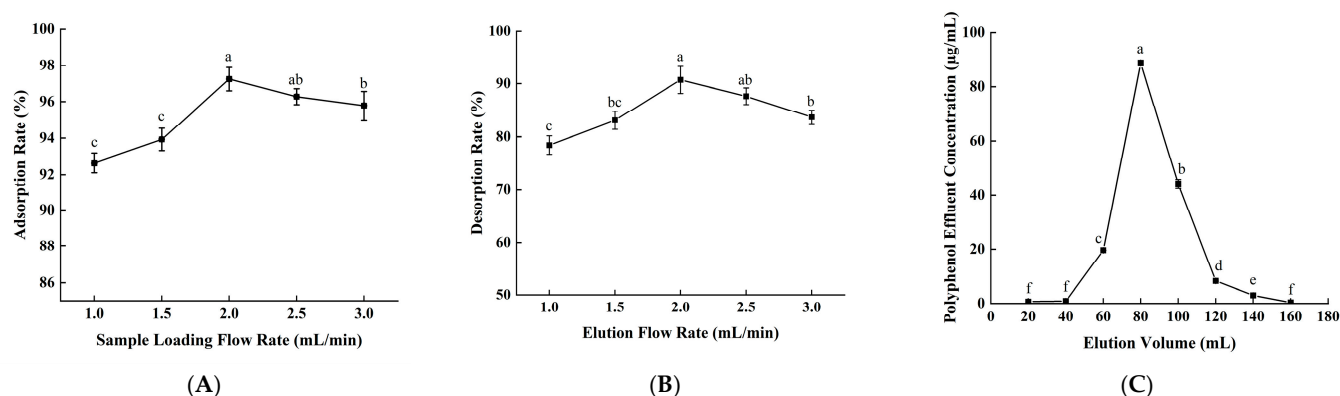


Figure 8. Effects of loading flow rate (A) and elution flow rate (B) on the adsorption rate of AB-8 macroporous resin, as well as the effect of elution volume (C) on its desorption rate. Different lowercase letters in the figure indicate statistically significant differences among groups ($p < 0.05$, Tukey's HSD test).

3.5. Results of Polyphenol Purity Determination

Three verification experiments were conducted under the optimized purification process: AB-8 macroporous resin was used as the adsorbent, the sample concentration was 0.5 mg/mL, the sample pH was 4.0, the loading flow rate was 2.0 mL/min, the loading volume was 200 mL, the eluent was 50% (v/v) ethanol, the elution flow rate was 2.0 mL/min, and the elution volume was 160 mL. The results demonstrated a 5.88-fold increase in total polyphenol purity and a 4.62-fold increase in total flavonoid purity from almond hulls.

3.6. Results of Antioxidant Activity Determination

Both DPPH and FRAP are widely used in vitro antioxidant capacity assays. The DPPH method, fully known as the 2,2-diphenyl-1-picrylhydrazyl method, was first proposed by Blois in 1958. Its core principle is based on the utilization of DPPH, a stable nitrogen-centered free radical. Due to the delocalization of the unpaired electron across the entire molecule, DPPH does not undergo dimerization. It exhibits a deep purple color in ethanol or methanol solutions and has a characteristic maximum absorption peak at wavelengths of 515–520 nm. When antioxidants are present in the system, they react with DPPH radicals primarily through two mechanisms: hydrogen atom transfer [28] and single electron transfer [29]. These two mechanisms usually occur simultaneously, and the dominant one depends on the structure and solubility of the antioxidant as well as the reaction conditions (e.g., solvent, pH value). After the reaction, DPPH radicals are reduced, causing the solution to gradually change from deep purple to pale yellow. The absorbance value measured at 515–520 nm decreases accordingly, and the extent of this decrease is positively correlated with the free radical scavenging capacity of the antioxidant. The FRAP assay, short for Ferric Reducing Antioxidant Power assay, was developed by Benzie and Strain in 1996. This method relies on electron transfer reactions under acidic conditions. At pH 3.6, an environment that maintains the solubility of ferric ions and facilitates electron transfer, ferric ions (Fe^{3+}) bind with TPTZ to form the colorless complex $[\text{Fe}^{3+}(\text{TPTZ})_2]^{3+}$. In the presence of antioxidants, electrons are donated to the above complex, reducing Fe^{3+} to ferrous ions (Fe^{2+}) and generating the intensely blue-colored $[\text{Fe}^{2+}(\text{TPTZ})_2]^{2+}$ complex [30].

The antioxidant activity was evaluated by the DPPH radical scavenging assay and the FRAP ferric reducing antioxidant power assay, as shown in Figure 9. The DPPH radical scavenging capacity of the unpurified crude extract was only approximately 0.4 μmol Trolox/mL, and the FRAP value was about 0.2 μmol Trolox/mL. After purification, the

DPPH radical scavenging capacity of almond hull polyphenols increased significantly to approximately 2.0 $\mu\text{mol Trolox/mL}$, and the FRAP value also increased significantly to about 1.3 $\mu\text{mol Trolox/mL}$. This indicated that the purification process could effectively remove inactive impurities in the extract, enrich antioxidant active components such as polyphenols, and significantly enhance the free radical scavenging capacity and ferric reducing antioxidant activity of the sample.

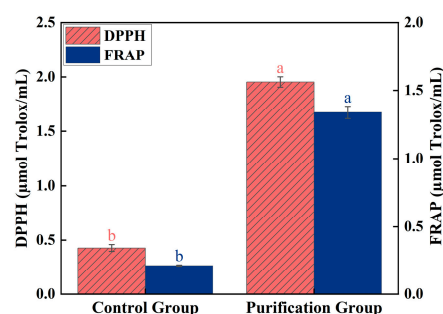


Figure 9. Antioxidant Activity of Almond Hull Polyphenol Extracts Before and After Purification. Different lowercase letters in the figure indicate statistically significant differences among groups ($p < 0.05$, Tukey's HSD test).

3.7. Results of Polyphenol Characterization

3.7.1. UV-Vis Spectral Analysis of Almond Hull Polyphenols

As shown in Figure 10, the UV spectrum indicated that the characteristic absorption peaks of plant polyphenols were mainly concentrated in the range of 280–400 nm, which was attributed to the $\pi \rightarrow \pi^*$ electron transition of the aromatic ring conjugated system in the molecule [31]. Compared with the unpurified crude sample, the absorbance of the characteristic absorption peak of polyphenols in the range of 280–400 nm of the purified sample was significantly enhanced, and the overall spectral trend was highly consistent with the classic UV spectral characteristics of phenols. This result indicated that the purification process established in this study could effectively enrich the polyphenol components in almond hulls.

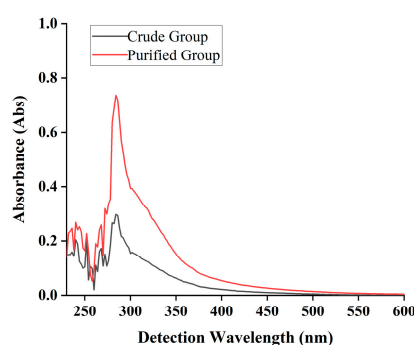


Figure 10. UV-Vis Spectral Analysis of Almond Hull Polyphenols Before and After Purification.

3.7.2. FT-IR Spectral Analysis of Almond Hull Polyphenols

Figure 11 shows the FT-IR spectra of the crude almond hull polyphenol extract and the purified polyphenols. Both samples before and after purification exhibited typical characteristic absorptions of plant polyphenols: the peak at 3000–3600 cm^{-1} was assigned to the stretching vibration of hydroxyl O–H, the peak at 1600–1650 cm^{-1} corresponded to the skeleton stretching vibration of aromatic ring C=C, the peak at 1000–1300 cm^{-1} was the stretching vibration of phenolic hydroxyl C–O, and the peak at 2900–3000 cm^{-1} was the

stretching vibration of alkyl saturated C–H [32]. Compared with the crude sample, the hydroxyl absorption peak of the purified sample was sharper and more symmetrical, and the characteristic peaks of phenols were clearer. In conclusion, both extracts displayed typical FT-IR characteristic peaks of plant polyphenols. After purification, the hydroxyl peak became sharper and more symmetric, and the characteristic peaks of phenolic substances were obviously enhanced.

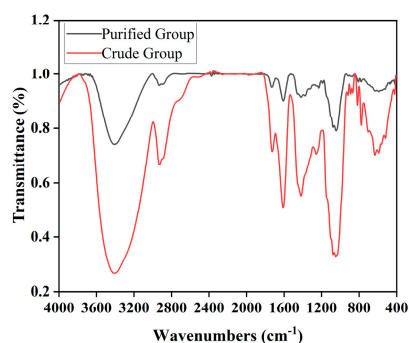


Figure 11. FT-IR Spectral Analysis of Almond Hull Polyphenols Before and After Purification.

3.7.3. SEM Analysis of Almond Hull Polyphenols

Scanning electron microscopy (SEM) characterization revealed that, at 1100× magnification, the purified almond hull polyphenols (Figure 12, left) exhibited the unpurified sample (a heterogeneous particle size distribution, featuring both rod-like structures and a loose network morphology formed by the aggregation of fine particles. In contrast, Figure 12, right) presented smooth, compact particles. These differences in aggregation state and surface characteristics may be attributed to the removal of macromolecular impurities (e.g., polysaccharides and proteins) during purification: the elimination of such impurities facilitated the formation of the loose structure in the purified fraction, while the unpurified sample displayed a compact appearance due to the encapsulation effect of residual impurities [33]. Collectively, these morphological differences provided preliminary structural evidence for elucidating the regulatory effects of purification on the physicochemical properties and functional activities of almond hull polyphenols, although the direct correlation between these morphological variations and functional improvements remains to be further corroborated with subsequent experimental data.

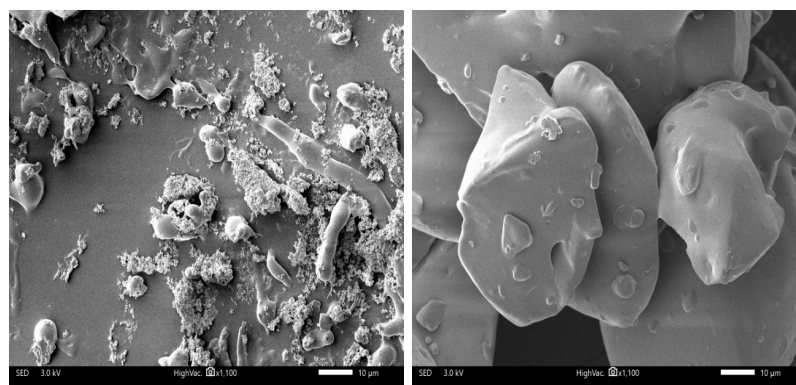


Figure 12. Comparative SEM micrographs of almond hull polyphenols before (left) and after (right) purification.

4. Discussion

Polyphenols are among the most valuable bioactive components in almond hulls. Conventional water and ethanol extraction suffers from low efficiency, high impurity levels, and potential polyphenol degradation, which has long restricted the utilization of almond hull resources. This study employed an ultrasound-assisted complex enzymatic hydrolysis for polyphenol extraction. The cavitation effect of ultrasound enhances mass transfer and shortens extraction time, while complex enzymatic hydrolysis (pectinase + cellulase) gently degrades plant cell walls, avoiding structural damage caused by high temperature, strong acid or alkali, thus maximally preserving the natural bioactivity of polyphenols. This integrated approach exhibits the advantages of green and efficient extraction, consistent with the current trend of green extraction technologies for plant bioactive ingredients. The optimal conditions were determined as follows: ultrasonic power of 180 W, ultrasonic frequency of 40 kHz, complex enzyme dosage of 0.1% (*w/w*), pectinase-to-cellulase ratio of 13 (g/g), enzymolysis time of 40 min, temperature of 41 °C, and liquid–solid ratio of 41 mL/g.

During purification, AB-8 macroporous resin was employed for efficient separation based on the polarity differences between polyphenols and impurities. Macroporous resin has been widely used in the purification of plant polyphenols owing to its high selectivity, low cost and simple operation. Static and dynamic adsorption experiments were performed to determine the optimal purification parameters: sample loading volume 200 mL, elution volume 160 mL, sample flow rate 2.0 mL/min, and elution flow rate 2.0 mL/min. Results from three replicate validation tests revealed that the present purification process enhanced the purity of total polyphenols and total flavonoids by 5.88- and 4.62-fold, respectively, enabling efficient polyphenol enrichment and impurity removal. The purification efficiency obtained in this study was comparable to that reported by Yaxin Zhu et al., who achieved a flavonoid purity of 61.35% from walnut peeling wastewater using AB-8 resin [34]. Similarly, Jingyi Chen et al. obtained polyphenols with a purity of 57.72% from oil-tea camellia fruit shells via ultrasonic-assisted extraction followed by AB-8 purification, representing an approximately 2.32-fold purity improvement [26]. Notably, our result was significantly superior to the 17.43% polyphenol yield reported by Li-Feng Yang et al. using DM-301 macroporous resin for polyphenol purification from sweet potato leaves [35]. The pronounced discrepancy in purification efficiency can be attributed to two key factors. First, the ultrasonic-assisted complex enzymatic hydrolysis (UA-CEH) synergistic extraction employed herein released more bound polyphenols and reduced co-extracted macromolecular impurities (e.g., polysaccharides, proteins) compared with conventional solvent extraction, providing a superior feedstock for subsequent purification. Second, the selective use of AB-8 resin endowed this study with high adsorption capacity and specificity toward almond hull polyphenols, as Wang et al. verified its excellent adsorption–desorption performance for procyanidins and chlorogenic acid from almond skins [36]. In contrast, the DM-301 resin utilized by Li-Feng Yang et al. exhibited lower selectivity for polyphenols from walnut seed coats. Furthermore, the purified fraction exhibited 5-fold and 6.5-fold enhancements in DPPH radical scavenging capacity and FRAP total reducing power, respectively, confirming that AB-8 resin effectively removes polysaccharides, proteins, and other macromolecular impurities while concentrating functional polyphenols. Characterization via UV-Vis spectroscopy, FT-IR, and SEM revealed that purified polyphenols retained intact functional groups and displayed a loose, porous microstructure with increased specific surface area, which facilitates enhanced biological activity.

Compared with conventional purification processes, this preparation route obviates the requirement for large-scale complex auxiliary equipment, and is characterized by straightforward operation and remarkable cost-effectiveness. The polyphenolic compounds derived from almond green husks, enriched via this process, provide essential

material support for the subsequent optimization of microencapsulation technologies and the development of active components for functional foods. Furthermore, this work furnishes critical research evidence and technical reference for exploring high-value conversion pathways of almond green husk processing by-products.

5. Conclusions

In this work, we optimized the ultrasonic-assisted enzymatic extraction and AB-8 macroporous resin purification of polyphenols from almond hulls. The optimal conditions achieved a higher extraction yield and polyphenol purity, accompanied by greatly strengthened antioxidant activity and efficient impurity removal. This green and efficient technical route provides solid technical support for the high-value utilization of almond processing by-products and the development of natural antioxidant products.

6. Limitations

1. This study only completed the extraction and content determination of total polyphenols from almond hulls. Polyphenol monomers were not analyzed by Liquid Chromatography–Mass Spectrometry (LC-MS), so the categories, molecular structures and relative proportions of core active polyphenols could not be clarified. This limits further research on their action mechanisms.
2. The antioxidant capacity of the polyphenols was evaluated solely by in vitro chemical assays, while cellular tests and in vivo animal experiments were not carried out. Since in vitro conditions differ greatly from the complex metabolic environment in living organisms, the in vivo antioxidant efficacy, action pathways and biosafety of the polyphenols remain unvalidated. The applicability and reliability of the research conclusions need to be further enhanced.
3. This study was limited to laboratory-scale experiments with only the extraction process optimized. Economic indicators such as production costs, energy consumption and processing losses were not assessed for industrial application, and industrial feasibility was not analyzed. Furthermore, AB-8 macroporous adsorption resin was adopted based on existing literature without screening alternative adsorption resins. The resin's reusability, regeneration stability and system material balance were not investigated, so the overall economic benefits and industrial application costs of the process were not comprehensively evaluated.

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Data Availability Statement: The original data presented in the study are included in the article; further inquiries can be directed to the author.

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