Optimization of Supercritical Fluid Extraction of Essential Oil and Vitamin E from Discarded Tobacco Leaves Using Response Surface Methodology

Xiaowei Tantai,[a,+] Chuntao Zhang,[b,+] Ruoxin Wu,[b] Dou Ma,[c,*] and Xiaoming Xiao [a,*]

** Corresponding authors: 271435867@qq.com (D.M.); xmxiao@tju.edu.cn; (X.X.)*

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GRAPHICAL ABSTRACT

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Xiaowei Tantai,[a,+] Chuntao Zhang,[b,+] Ruoxin Wu,[b] Dou Ma,[c,*] and Xiaoming Xiao [a,*]

Discarded tobacco leaves, a substantial agricultural by-product, represent an underutilized reservoir of bioactive phytochemicals. This study investigated the application of Supercritical Fluid Extraction (SFE) using CO_2 as a green technology for the recovery of tobacco oleoresin and α-tocopherol (Vitamin E). A Box-Behnken Design (BBD) integrated with Response Surface Methodology (RSM) was employed to optimize critical process variables: extraction pressure, temperature, and time. Statistical analysis identified the model-predicted optimum conditions as 21.3 MPa, 45.8 °C, and 63.4 min, yielding a maximum oleoresin recovery of 1.36%. This empirical result exhibited high concordance with the model prediction, validating the robustness of the optimization. Gas Chromatography-Mass Spectrometry (GC-MS) analysis demonstrated the tunable selectivity of the SFE process. The relative content of the high-value antioxidant Vitamin E was preferentially enriched to 19.1% under density-controlled conditions (25 MPa and 40 °C), minimizing the co-extraction of impurities. These findings established SFE as a superior, eco-friendly strategy for the valorization of tobacco residues, offering a sustainable pathway for the production of functional ingredients for pharmaceutical and cosmetic applications.

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Keywords: Supercritical fluid extraction; Tobacco waste valorization; Response surface methodology; Vitamin E; Essential oil

*Contact information: a: School of Chemical Engineering and Technology, Tianjin University, Tianjin, China; b: Tianjin Work Station, Technical Center, Shanghai Tobacco Group Co., Ltd., Tianjin, China; c: Hefei Design Institute of China National Tobacco Corporation, Hefei, China; +These authors contributed equally to this work.*Corresponding authors: 271435867@qq.com (D.M.); xmxiao@tju.edu.cn; (X.X.)*

INTRODUCTION

Global tobacco cultivation generates substantial agricultural by-products, predominantly discarded tobacco leaves, *i.e.*, tobacco leaf byproducts or non-compliant leaves generated during processing and sorting, which are traditionally treated as environmental pollutants (Tan *et al.* 2024; Wang *et al.* 2025). Yet, circular economy frameworks increasingly reclassify this biomass as an underutilized reservoir of high-value compounds rather than waste (Banožić *et al.* 2020; Yao *et al.* 2025). These leaves encapsulate a complex matrix of bioactive phytochemicals, notably nicotine, solanesol, and distinct aroma constituents (Shen *et al.* 2024). Specifically, tobacco oleoresin functions as a pivotal feedstock for fragrance formulation and product quality modulation.

Concurrently, the presence of α-tocopherol (Vitamin E)—an antioxidant critical to pharmaceutical and cosmetic applications—positions tobacco waste as a viable source to mitigate global supply deficits (Ferreira *et al.* 2022). Consequently, the efficient extraction of these metabolites provides a synergistic solution for environmental remediation and resource valorization.

Despite this economic potential, the efficient recovery of these bioactive components presents substantial technical hurdles. Conventional isolation protocols for lipophilic ingredients predominantly employ hydrodistillation and organic solvent extraction (*e.g.*, Soxhlet) (Uddin *et al.* 2018; Nazlić *et al.* 2023). Although well established, these methods face increasing scrutiny due to inherent inefficiencies, including prolonged extraction durations, high energy demands, and reliance on hazardous solvents such as n-hexane (Jayawardena *et al.* 2025; Kopp and Lauritano 2025; Krivošija *et al.* 2025). The associated emission of volatile organic compounds (VOCs) and the potential for toxic residue retention compromise safety, restricting applicability in pharmaceutical and food sectors (Nadaf and Kalburgi 2022). Crucially, the elevated temperatures intrinsic to these processes prove detrimental to thermolabile analytes. Notably, Vitamin E exhibits significant susceptibility to oxidative and thermal degradation, resulting in diminished yields and bioactivity under the sustained thermal stress of distillation or refluxing (Mohamad *et al.* 2017). Consequently, the development of alternative "green" extraction technologies operating under mild conditions is imperative to preserve the structural integrity of these sensitive constituents.

To address these limitations, Supercritical Fluid Extraction (SFE) utilizing carbon dioxide (CO_2) represents a robust green alternative for agricultural residue valorization (Prado *et al.* 2025; Soni *et al.* 2025). Distinct from organic solvents, supercritical CO_2 is non-toxic, non-flammable, and chemically inert, facilitating the production of extracts devoid of hazardous residues. Significantly, its near-ambient critical temperature (31.1 °C) enables processing under mild conditions, effectively mitigating the thermal degradation of labile constituents such as Vitamin E (Grosso *et al.* 2015; Fomo *et al.* 2020; Khalati *et al.* 2023). Moreover, the solvation power of supercritical CO_2 is tunable *via* pressure and temperature modulation, permitting the selective co-extraction of target lipophilic compounds, including essential oils and tocopherols (Davarnejad *et al.* 2010; Liu *et al.* 2025). Nevertheless, SFE efficiency depends on multiple interacting variables—specifically pressure, temperature, and extraction time—which influence yields in a non-linear manner (Herrero *et al.* 2010; Vafaei *et al.* 2022). To resolve this complexity, Response Surface Methodology (RSM) has been employed as a rigorous statistical framework. In contrast to single-factor experimentation, RSM permits simultaneous evaluation of variable interactions and the derivation of mathematical models to identify optimum conditions for extraction efficiency (Sarfarazi *et al.* 2015; Kumar and Reji 2023).

Consequently, the primary objective of this study is to establish an optimized Supercritical Fluid Extraction protocol for the simultaneous recovery of tobacco essential oil and Vitamin E from discarded leaves. A Box-Behnken Design (BBD) integrated with RSM was employed to rigorously evaluate the individual and synergistic effects of extraction pressure, temperature, and time on yield. Furthermore, the chemical composition of the extracted oleoresin was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) to validate the enrichment of target bioactives. Ultimately, this research delineates a sustainable, solvent-free strategy for the effective valorization of tobacco agricultural by-products.

EXPERIMENTAL

Materials

Discarded tobacco leaves (*Nicotiana tabacum* L.), representing non-compliant leaves generated during industrial sorting and processing, were procured from Luoyang Tobacco Company (Luoyang, China). Analytical grade ethanol was sourced from Tianjin Jiangtian Chemical Technology Co., Ltd. (Tianjin, China). High-purity carbon dioxide (CO_2, 99.999%) was supplied by Tianjin Baisida Gas Co., Ltd. (Tianjin, China).

Sample Preparation

The discarded tobacco leaves were collected fresh after industrial sorting and were dried under vacuum at 35 °C until constant weight (approximately 4 h). The dried biomass was pulverized using a laboratory blender and sieved through a 100-mesh screen to ensure particle size homogeneity. Processed samples were stored in vacuum-sealed polyethylene bags under dark, ambient conditions to mitigate moisture re-absorption and oxidative degradation. Before extraction, the discarded tobacco leaves were not subjected to any pretreatment intended to remove specific constituents.

Supercritical Fluid Extraction (SFE) Procedure

Extractions were conducted using a laboratory-scale SFE system (Model SFT-105-XW, Supercritical Fluids Technology Inc., Newark, DE, USA). For each experimental run, 15.0 g of prepared tobacco powder was loaded into the extraction vessel. Ethanol served as the collection solvent. Extractions proceeded under dynamic conditions for specified durations. The extraction yield (Y) was calculated according to Eq. 1,

$$Y=\frac{m_{extract}}{m_{\mathrm{feed}}}\times 100\% \tag{1}$$

where m_{extract} is the mass of the obtained oleoresin (g) and m_{feed} is the mass of the dried tobacco powder loaded (g). All extraction yields reported in this study were calculated on a dry-weight basis.

Experimental Design

To define the operational boundaries, single-factor experiments initially evaluated the influence of four independent variables—CO_2 flow rate (1.32 to 5.47 mL/min), extraction time (45 to 105 min), temperature (40 to 60 °C), and pressure (15 to 25 MPa)—on extraction yield (Table 1). Based on these trials, the CO_2 flow rate was fixed at its optimal level to simplify the experimental matrix.

Table 1. Experimental Design for Single-Factor Analysis

Extraction Parameters	Units	Levels of Parameters				
Flow of CO_2	mL/min	1.32	3.57	4.32	5.47	-
Extraction time	min	45	60	75	90	105
Extraction temperature	°C	40	45	50	55	60
Extraction pressure	MPa	15	17.5	20	22.5	25

Subsequently, a three-factor, three-level Box-Behnken Design (BBD) was employed to optimize the remaining critical variables: extraction time (X_1), temperature (X_2), and pressure (X_3). The experimental range and coded levels are presented in Table 2.

Table 2. Independent Variables and Coded Levels for Box-Behnken Design

Independent Variables	Symbol	Coded Levels		
		-1	0	+1
Extraction Time (min)	X_1	45	60	75
Temperature (°C)	X_2	40	45	50
Pressure (MPa)	X_3	17.5	20	22.5

Data analysis was performed using Design-Expert software (Version 8.0.6, State-Ease Inc., Minneapolis, USA). A second-order polynomial model was fitted to correlate the independent variables with the response (Y), as described in Eq. (2),

$$Y=\beta_0+\sum_{k=1}^{3}\beta_k X_k+\sum_{k=1}^{3}\beta_{kk}X_k^{2}+\sum_{k=1}^{2}\sum_{j=2}^{3}\beta_{kj}X_k X_j \qquad (2)$$

where Y is the extraction yield of the oleoresin, β_0 represents the model intercept, and β_k, β_j, and β_{kj} are regression coefficients of variables representing the linear, quadratic and cross products of X_1, X_2, X_3 on response, respectively.

Analytical Methods

The chemical profile of the extracted oleoresin was characterized using Gas Chromatography-Mass Spectrometry (GC-MS; Agilent 7890B-7000D). Separation utilized an HP-5MS capillary column with helium as the carrier gas at a constant flow rate. The thermal program was initiated at 50 °C (held for 2 min), ramped to 230 °C at 4 °C/min, and finally to 255 °C at 1 °C/min. Quantitative analysis was conducted using a Gas Chromatograph equipped with a Flame Ionization Detector (GC-FID) and an HP-5 capillary column under identical thermal conditions. Selected constituents were tentatively identified based on GC-MS mass spectral matching. Because retention indices were not determined in the present study, only the constituents most relevant to the present discussion are listed in Table S1.

RESULTS AND DISCUSSION

The Optimal Levels Selection of Different Extraction Parameters

To delineate the operational boundaries for the subsequent RSM optimization, the influence of four independent variables—CO_2 flow rate, extraction time, pressure, and temperature—on tobacco oleoresin yield was systematically evaluated.

The mass transfer efficiency in SFE is critically governed by solvent flow dynamics. As depicted in Fig. 1(a), the extraction yield exhibited a marked increase as the flow rate was raised from 1.32 to 4.32 mL/min. This positive correlation is ascribed to the reduction of external mass transfer resistance surrounding the tobacco matrix; higher superficial velocities diminish the boundary film thickness, thereby facilitating solute diffusion into the bulk phase. Conversely, elevating the flow rate to 5.47 mL/min induced a minor yield reduction. This inflection suggests that excessive flow velocities curtail the solvent-solute residence time, preventing equilibrium establishment and causing solvent channeling. Balancing solvent economy with extraction efficiency, a constant flow rate of 4.32 mL/min was adopted for subsequent trials.

The extraction kinetics are presented in Fig. 1(b). A rapid yield increment was observed during the initial 45 to 60 min, corresponding to the solubility-controlled phase where surface-accessible solutes are readily desorbed. Beyond 60 min, the process transitioned into a diffusion-controlled regime, characterized by a plateau in extraction rate as solutes migrated from the internal porous matrix. Extended extraction durations (>60 min) provided negligible marginal gains and posed risks of volatile compound loss or thermal degradation of sensitive components. Consequently, 60 min was designated as the center point, establishing an experimental range of 45 to 75 min for RSM optimization.

Extraction pressure modulates supercritical CO_2 density, thereby dictating solvation power. As shown in Fig. 1(c), yield was positively correlated with pressure between 15 and 20 MPa, consistent with the enhanced solubility of lipophilic constituents (essential oils, tocopherols) in denser fluids. However, pressures exceeding 20 MPa resulted in a yield decline. While higher density theoretically augments solubility, the concomitant increase in fluid viscosity and reduction in solute diffusivity impede mass transfer. Furthermore, excessive pressure can induce mechanical compaction of the tobacco powder bed, restricting solvent percolation and increasing internal resistance. Therefore, 20 MPa was selected as the optimum center point (range: 17.5 to 22.5 MPa).

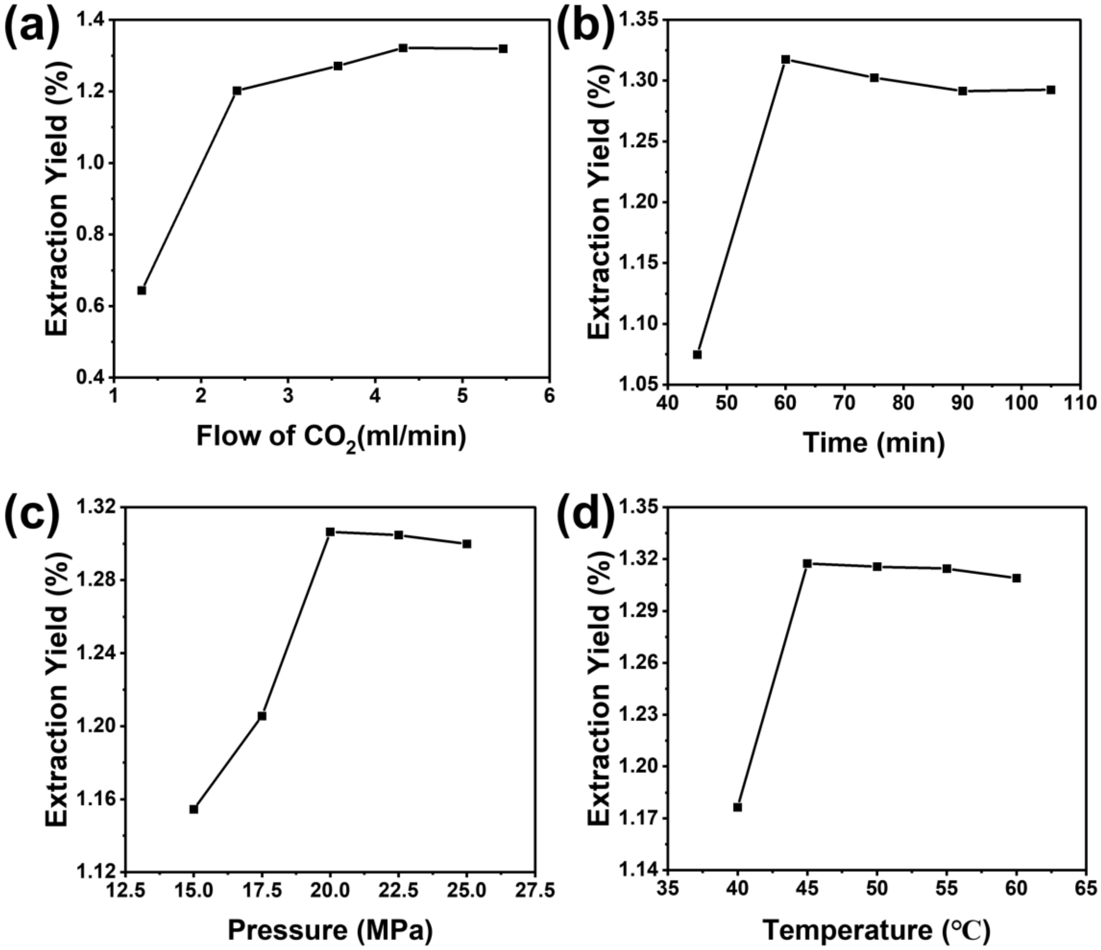

Fig. 1. Effects of independent variables on the extraction yield of tobacco oleoresin: (a) CO_2 flow rate; (b) extraction time; (c) extraction pressure; and (d) extraction temperature

Temperature exerts a non-monotonic influence on SFE yield due to the retrograde condensation phenomenon (crossover effect). While elevated temperatures enhance solute vapor pressure and desorption kinetics, they simultaneously reduce solvent density and solvation power. As illustrated in Fig. 1(d), the yield was maximized at 45 °C. Below this threshold, solute desorption energy was insufficient; above 45 °C, the density reduction effect dominated over the vapor pressure enhancement, diminishing yield. Crucially, given the thermolability of Vitamin E, operating at elevated temperatures risks oxidative degradation. Accordingly, 45 °C was identified as the optimal center point, defining a range of 40 to 50 °C for optimization.

Model Fitting and Statistical Analysis

The experimental design matrix and corresponding extraction yields for the 17 runs of the BBD are detailed in Table 3. Multiple regression analysis was applied to these data to quantify the correlation between the extraction yield and the independent variables. The resulting second-order polynomial model, expressed in terms of coded factors, is presented in Eq. 3,

$$Y = 1.340 + 0.041X_1 + 0.017X_2 + 0.033X_3 - 0.011X_1X_2 - 0.023X_1X_3 + 0.040X_2X_3 - 0.061X_1^2 - 0.110X_2^2 - 0.033X_3^2 \quad (3)$$

where Y represents the predicted extraction yield; and X_1, X_2, and X_3 correspond to the coded values of extraction time, temperature, and pressure, respectively.

Table 3. Box-Behnken Design Matrix and Observed Response Values

Standard	Run	X_1 Time (min)	X_2 Temperature (°C)	X_3 Pressure (MPa)	Extraction Yield (%)
5	14	45 (-1)	45 (0)	17.5 (-1)	1.167
7	3	45 (-1)	45 (0)	22.5 (1)	1.261
1	4	45 (-1)	40 (-1)	20.0(0)	1.090
3	6	45 (-1)	50 (1)	20.0(0)	1.165
9	11	60 (0)	40 (-1)	17.5 (-1)	1.191
10	13	60 (0)	50 (1)	17.5 (-1)	1.126
12	2	60 (0)	50 (1)	22.5 (1)	1.291
11	8	60 (0)	40 (-1)	22.5(1)	1.195
17	12	60 (0)	45 (0)	20.0 (0)	1.319
13	15	60 (0)	45 (0)	20.0 (0)	1.345
16	10	60 (0)	45 (0)	20.0 (0)	1.351
15	1	60 (0)	45 (0)	20.0(0)	1.345
14	9	60 (0)	45 (0)	20.0(0)	1.363
6	17	75 (1)	45 (0)	17.5(-1)	1.286
2	5	75 (1)	40 (-1)	20.0(0)	1.203
8	7	75 (1)	45 (0)	22.5 (1)	1.288
4	16	75 (1)	50 (1)	20.0 (0)	1.235

The statistical adequacy of the proposed model was evaluated *via* Analysis of Variance (ANOVA), as summarized in Table 4. The model exhibited a high F-value of 33.49 and a very low p-value ($p<0.0001$), confirming that the regression is statistically significant. The probability that an F-value of this magnitude could occur due to noise was merely 0.01%. Furthermore, the "Lack of Fit" F-value of 0.26 relative to the pure error was insignificant ($p=0.2552>0.05$). A non-significant lack of fit corroborates the model's validity, indicating it satisfactorily approximates the experimental data within the studied domain.

Table 4. ANOVA for the Response Surface Quadratic Model

Source	Coefficient	Sum of Squares	df	Mean Square	F-Value	p-value (Prob > F)	Significance
Model	1.34	0.11	9	0.012	33.49	0.0001	Yes
X_1-Time	0.041	0.014	1	0.014	36.51	0.0005	Yes
X_2-Temp	0.017	2.38×10^{-3}	1	2.38×10^{-3}	6.42	0.0390	Yes
X_3-Pressure	0.033	8.78×10^{-3}	1	8.78×10^{-3}	23.68	0.0018	Yes
X_1X_2	-0.011	4.62×10^{-4}	1	4.62×10^{-4}	1.25	0.3010	No
X_1X_3	-0.023	2.12×10^{-3}	1	2.12×10^{-3}	5.71	0.0482	Yes
X_2X_3	0.040	6.48×10^{-3}	1	6.48×10^{-3}	17.48	0.0041	Yes
X_1^2	-0.061	0.016	1	0.016	41.99	0.0003	Yes
X_2^2	-0.110	0.051	1	0.051	138.84	< 0.0001	Yes
X_3^2	-0.033	4.67×10^{-3}	1	4.67×10^{-3}	12.60	0.0094	Yes
Residual	—	2.59×10^{-3}	7	3.71×10^{-4}	—	—	—
Lack of Fit	—	1.56×10^{-3}	3	5.20×10^{-4}	2.01	0.2552	No
Pure Error	—	1.04×10^{-3}	4	2.59×10^{-4}	—	—	—
Cor Total	—	0.11	16	—	—	—	—

Note: $R^2 = 0.9773$; Adj $R^2 = 0.9481$; Std. Dev. = 0.019.

Model accuracy was further verified by the coefficient of determination (R^2). The calculated R^2 of 0.9773 indicates that 97.73% of the response variability was explained by the model. The adjusted coefficient of determination (Adj $R^2 = 0.9481$) was in close agreement with the R^2 value (difference < 0.2), suggesting the model did not suffer from overfitting and provides a robust representation of the system.

The significance of individual model terms was assessed based on p-values. As detailed in Table 4, the linear terms for extraction time (X_1, $p < 0.0005$) and pressure (X_3, $p < 0.0018$) were highly significant contributors to yield, whereas temperature (X_2, $p = 0.0390$) exhibited a lesser but statistically relevant impact. Notably, all quadratic terms (X_1^2, X_2^2, X_3^2) were significant ($p < 0.01$), confirming that the relationship between variables and yield was curvilinear rather than linear, characterized by a distinct optimum peak. Regarding interactions, the interplay between temperature and pressure (X_2X_3) was highly significant ($p = 0.0041$), while the time-pressure interaction (X_1X_3) was also significant ($p = 0.0482$). This implies that the optimal extraction pressure was dynamically dependent on the selected temperature and duration.

Analysis of Interactive Effects

The three-dimensional (3D) response surface plots and corresponding two-dimensional (2D) contour plots were generated from the regression equation to visualize the reciprocal interactions between independent variables and identify optimal extraction conditions (Fig. 2). In each visualization, the extraction yield is plotted as a function of two variables, while the third is held constant at its center point (0).

The interaction between extraction temperature (X_2) and pressure (X_3) exerted the most pronounced impact on oleoresin yield ($p < 0.01$), as illustrated in Fig. 2a and 2d. The response surface exhibited a distinct convex geometry, confirming that the optimum lies well within the experimental domain. Notably, the effect of temperature on yield was pressure-dependent, a phenomenon characteristic of the “crossover effect” in supercritical fluids. At lower pressures, increasing temperature resulted in negligible variation or a slight yield reduction due to the dominant decrease in fluid density. Conversely, at higher pressures, elevated temperatures markedly enhanced the yield. In this high-pressure regime, the solvent density remained sufficient to maintain solvation power, allowing the increased solute vapor pressure—driven by thermal energy—to dominate mass transfer. Consequently, a synergistic combination of elevated pressure and moderate temperature is critical for maximizing recovery.

Figures 2b and 2e depict the interactive effect of extraction time (X_1) and pressure (X_3). ANOVA results confirmed this interaction as statistically significant ($p < 0.05$), as evidenced by the elliptical distortion of the contour plot. At lower pressures, the extraction yield increased linearly with time, indicating a slower mass transfer rate. However, at higher pressures, the yield plateaued more rapidly. This behavior suggests that elevated pressure significantly augments solvent density and solvating power, thereby accelerating mass transfer kinetics and abbreviating the time required to reach equilibrium. Thus, prolonging extraction at high pressures offers diminishing returns and may compromise process economics without improving yield.

The interaction between extraction time (X_1) and temperature (X_2) is shown in Fig. 2c and 2f. Consistent with the ANOVA table, this interaction was not statistically significant ($p > 0.05$). The response surface exhibited a comparatively planar profile relative to other interactions, implying that the influence of temperature is consistent across the tested time durations. Nevertheless, the significance of the quadratic terms (X_1^2, X_2^2) resulted in a curvilinear profile where yield initially increased before declining. This confirms the existence of discrete optima for both time and temperature, balancing the kinetics of extraction against the risk of thermal degradation for sensitive bioactive compounds like Vitamin E.

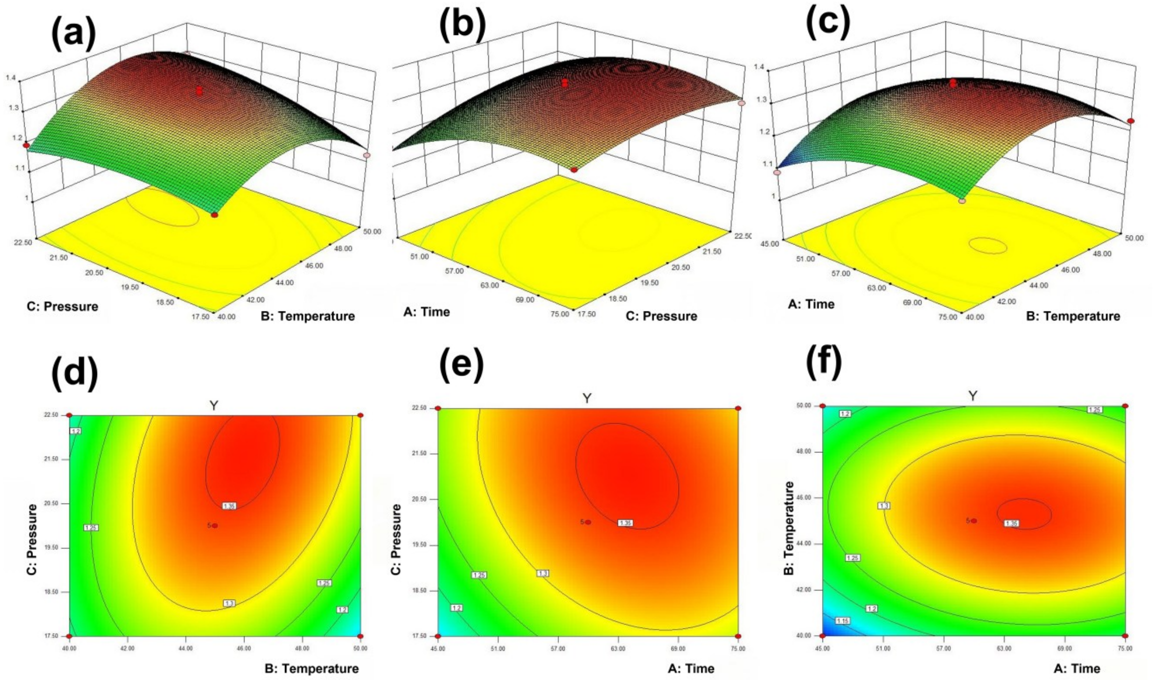

Fig. 2. Response surface plots (3D) (a-c) and contour plots (2D) (d-f) showing the interactive effects of variables on the extraction yield: (a,d) temperature and pressure; (b,e) time and pressure; and (c,f) time and temperature

Optimization of Extraction Conditions and Model Verification

Canonical analysis of the second-order polynomial model (Eq. 3) was performed to determine the predicted optimum within the investigated domain for oleoresin recovery. The theoretical optima for the independent variables were identified as: extraction time (X_1) = 63.4 min, temperature (X_2) = 45.8 °C, and pressure (X_3) = 21.3 MPa. Under these conditions, the model predicted a maximum extraction yield of 1.36%.

To corroborate the predictive accuracy of the regression model, verification trials were conducted in triplicate under these specified conditions. The resulting empirical yield was 1.36 ± 0.03%. The high concordance between the predicted value (1.356%) and the experimental mean (1.36%) indicates a negligible relative error. This validation confirms the robustness of the Box-Behnken Design in optimizing SFE parameters and demonstrates that the established model accurately describes the extraction kinetics of discarded tobacco leaves within the investigated domain.

Chemical Profile and Selectivity Analysis

Beyond maximizing total extraction yield, elucidating variations in chemical composition is imperative for evaluating the selectivity of the SFE process. Chromatographic profiles of tobacco oleoresin obtained under varying conditions were characterized by GC-MS. The specific influence of extraction pressure and temperature on the relative abundance of key bioactive constituents—namely Vitamin E, nicotine, pinane, and 4,8,13-cyclotetradecatriene-1,3-diol—is illustrated in Fig. 3.

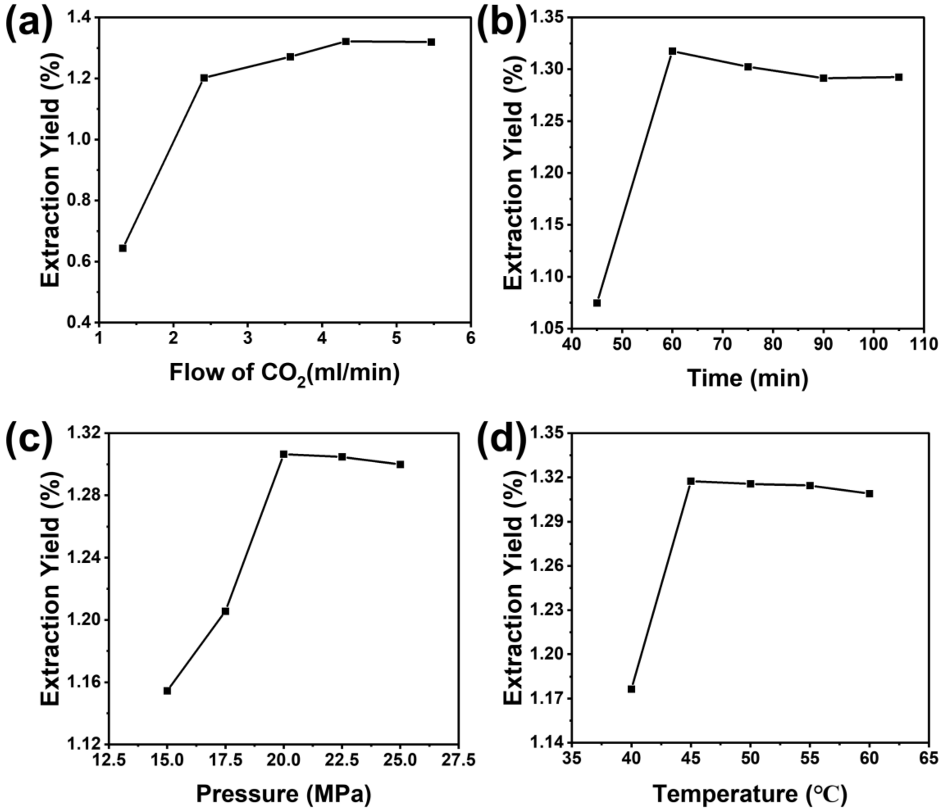

Fig. 3. Effects of SFE parameters on the relative content of major constituents in tobacco oleoresin: (a) effect of extraction pressure; and (b) effect of extraction temperature

As depicted in Fig. 3a, extraction pressure significantly modulated the oleoresin composition. The relative content of nicotine exhibited an inverse correlation with pressure from 15 to 20 MPa, stabilizing thereafter. Conversely, the concentration of the target bioactive, Vitamin E, remained stable at lower pressures (15 to 22.5 MPa) but surged at 25 MPa. This behavior is governed by the tunable density of supercritical CO_2. At elevated pressures (*e.g.*, 25 MPa), the increased fluid density enhances solvation power and carrying capacity for high-molecular-weight lipophiles such as Vitamin E. Consequently, higher pressures appear favorable for the enrichment of Vitamin E while simultaneously reducing the relative proportion of nicotine.

The impact of extraction temperature on the chemical profile followed a distinct parabolic trajectory (Fig. 3b). The relative content of Vitamin E increased gradually from 40 °C, peaked at 55 °C, and subsequently declined at 60 °C. The initial ascent is attributed to the temperature-induced increase in solute vapor pressure, facilitating desorption from the matrix. However, the decline observed at 60 °C implies thermal degradation. Given that Vitamin E is a thermolabile antioxidant, excessive thermal stress risks oxidation or structural breakdown. In contrast, pinane fluctuated but maintained relatively high stability across the range. These results suggest that an optimal thermal window (50 to 55 °C) exists, balancing enhanced mass transfer kinetics with the preservation of structural integrity.

The relative peak areas of selected constituents under representative conditions are summarized in Table S1. Consistent with the trends observed in Fig. 3, the SFE process exhibited compositional selectivity. Notably, at 40 °C and 25 MPa, the relative content of the target bioactive, Vitamin E, reached its maximum value of 19.1%, compared with 6.97% at 40 °C and 20 MPa. Under the representative conditions summarized in Table S1, nicotine remained a notable constituent of the extract, although it was not the dominant constituent on a relative peak area basis. These results indicate that tuning the solvent density altered the relative composition of the extract rather than reflecting any prior removal of specific constituents from the raw material.

CONCLUSIONS

1. This study demonstrated the potential of Supercritical Fluid Extraction (SFE) as a sustainable and efficient strategy for the valorization of discarded tobacco leaves. Through the application of Box-Behnken Design (BBD) and response surface methodology (RSM), the complex synergistic interactions among extraction pressure, temperature, and time were rigorously elucidated. The optimal processing parameters for maximizing oleoresin recovery were identified as a pressure of 21.3 MPa, a temperature of 45.8 °C, and a duration of 63.4 min. Under these conditions, an experimental yield of 1.36% was achieved, exhibiting high concordance with model predictions and confirming the robustness of the statistical optimization.
2. Beyond the maximization of crude yield, a pivotal outcome of this research is the demonstration of SFE's tunable selectivity. Chromatographic characterization revealed that specific high-pressure conditions (25 MPa at 40 °C) preferentially enriched the extract with high-value bioactive constituents. Most notably, the relative content of α-tocopherol (Vitamin E) reached 19.1%, significantly outperforming extracts obtained at lower pressures.

3. These findings suggest that SFE is a promising approach for fractionating complex plant matrices and preserving thermolabile compounds under the investigated conditions. Ultimately, this protocol provides a way to avoid the usage of petroleum-based solvents for the conversion of agricultural tobacco by-products into functional ingredients, although further pilot-scale validation and process-economic assessment are still needed.

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Author Contributions

X.T. and C.Z. contributed equally to this work. X.T.: Conceptualization, Methodology, Formal analysis, and Writing—Original Draft. C.Z.: Methodology, Formal analysis, Data curation, and Writing—Original Draft. R.W.: Investigation, Data curation, and Visualization. D.M.: Resources, Methodology, and Supervision. X.X.: Conceptualization, Writing—Review & Editing, Supervision, and Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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APPENDIX

Table S1. Relative Peak Areas of Selected Constituents in Tobacco Oleoresin under Representative SFE conditions

Constituent	A 40 °C 20 MPa	B 40 °C 25 MPa	C 50 °C 20 MPa
Nicotine	14.6	13.8	12.1
α-tocopherol (Vitamin E)	7.00	19.1	10.2
Pinane	31.0	24.7	25.0
4,8,13-Cyclotetradecatriene-1,3-diol	24.4	20.7	20.3
Other constituents	23.0	21.7	32.4
Total	100	100	100

Note: Values are relative peak areas (%) obtained by GC-MS under representative SFE conditions. Only selected constituents directly relevant to the discussion are shown here. All remaining detected peaks are grouped as “Other constituents”. Compound assignments should be regarded as tentative because retention indices were not determined in the present study.