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RESEARCH

Microwave-Assisted Extraction for Valorization of Black Mustard Seed By-Products: Optimization of Phenolic Compounds Recovery and Antioxidant Properties

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Abstract

The present investigation is part of the valorization of interstitial by-products, focusing on the extraction of phenolic compounds using green extraction methods. Microwave-assisted extraction (MAE) was used as an advanced processing technique and systematically optimized using Response Surface Methodology (RSM) to investigate the impact of key parameters, including ethanol concentration, microwave power, and irradiation time on total phenolic content (TPC) and antioxidant activity (AA) of black mustard seed by-product (BMSBP). The central composite design (CCD) was used to study the effects of three independent variables, microwave power (100–700 Watt), irradiation time (2–12 min), and Ethanol concentration (40–80%). Statistical optimization revealed that the following extraction parameters represented the optimal combination for maximizing the bioactive compounds recovery: Ethanol concentration of 45% (v/v), an irradiation time of 9.9 min, and a microwave power of 500 W. Under these optimal conditions, corresponding experimental values for TPC and AA were 859.54 ± 2.43 mg GAE/100 g DW and 68.85 ± 1.23%, respectively. Overall, this study highlights the potential of MAE as an effective processing technique, with RSM playing a key role in modeling the extraction process and identifying optimal conditions by evaluating the influence of key parameters. Moreover, it underscores the feasibility of converting mustard seed waste into a valuable by-product as a source of natural antioxidants.

Keywords Mustard seed by-products · Microwave-assisted extraction · Optimization · Response surface methodology · Total phenolic content · Antioxidant activity

Introduction

Mustard belongs to the Brassicaceae family (formerly Cruciferae). The most commonly cultivated species within this family include black mustard (*Brassica nigra*), brown mustard (*Brassica juncea*), and white mustard (*Sinapis alba*). Mustard seed is produced in all continents except Australia and Antarctica, with Asia being the largest producer, particularly Nepal which contributes 210 thousand tons, while Africa records the lowest production worldwide (Aslan 2023). Its seeds are highly valued for their pungent flavor and high oil content, making them a key raw material in the food-processing and condiment industries (Dua et al 2014; Nath et al 2019).

A recent study by (Sharif et al. 2017) on the complete profile of three mustard seed genotypes revealed an oil content of up to 41.98%, with erucic acid being the dominant fatty acid (51.35%), followed by protein (28%) and carbohydrates (18.85%). The mineral composition was characterized by the presence of calcium (Ca), zinc (Zn), copper (Cu), and iron (Fe). In addition to this composition, mustard seeds contain unique nitrogen- and sulfur-rich secondary metabolites known as glucosinolates, which are responsible for their characteristic flavor and pungency (Singh and Singh 2025). Furthermore, the phytochemical

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profile of mustard seeds also includes phenolic compounds such as flavonoids particularly flavanols, benzoic acids and mainly p-hydroxycinnamic derivative (Cartea et al. 2010) with sinapic acid as the predominant one (over 73%), which may occur in free or bound forms or be esterified with choline to form sinapine which accounts for 70–90% of the total phenolics, with small amounts of free sinapic acid (Thiyam et al. 2006). For instance, total phenolic and total flavonoid contents in the black mustard seeds were estimated as 20–32 mg of gallic acid equivalent per 1 g and 4–7 mg of quercetin equivalent per 1 g respectively (Đorđević et al. 2021).

Furthermore, several research studies have highlighted the significant role of *Brassica* species in the pharmaceutical domain and in traditional medicine (Ayadi et al. 2022; Singh and Singh 2025), due to their ability to exert a wide range of biological activities, including antidiabetic effects, anti-inflammatory activity, regulation of metabolic syndrome, and neuroprotective properties (Mattosinhos et al. 2022; Ayadi et al. 2022; Grover et al. 2002). Bioactive compounds are often directly linked to therapeutic and pharmaceutical effects. These compounds are part of secondary metabolites found not only in plant material but also in the by-products. Consequently, recent research has increasingly focused on valorising industrial by-products by extracting bioactive compounds, providing a sustainable approach to obtain valuable compounds while minimizing large-scale harvesting of plants, as a way to manage agri-food waste and preserve the environment (Sharma et al. 2021; Amrane-Abider et al. 2023).

The industrial processing of mustard seeds generates a substantial oleaginous biomass, representing up to 60% of the seed's total weight (Sehwag and Das 2015). This by-product, commonly referred to as mustard bran or meal, is often considered waste despite its notable bioactive potential. It contains approximately (1–5 w% by weight) of phenolic compounds (Thiyam et al. 2006). Phytochemical analyses of mustard meal extracts have revealed a profile rich in sinapine (approximately 62%) and sinapic acid (around 16%) (Das et al 2009), highlighting its potential as a valuable source of phenolic antioxidants. These phenolic molecules exhibit a broad spectrum of bioactivities, including potent antioxidant, antimicrobial, anti-inflammatory, anticancer, and anxiolytic effects (Nićiforović & Abramovič, 2014; Siger et al 2024). Owing to their diverse therapeutic properties, significant scientific efforts have been directed toward optimizing extraction and purification techniques, with the aim of valorising agro-industrial by-products as alternative and sustainable sources of high-value bioactive compounds (Achinivu et al 2021; Kumar & Goel 2019).

Process intensification (PI) offers a strategic approach to enhance extraction performance while minimizing environmental and health-related impacts. Among emerging technologies, MAE has demonstrated significant potential as a PI tool, owing to its capacity for rapid internal heating and efficient disruption of plant cell structures (DiNardo et al 2020). This mechanism facilitates the selective recovery of target compounds in significantly shorter timeframes, with reduced solvent consumption and lower energy demands, thereby offering a sustainable and economically viable alternative to conventional extraction methods (Amrane-Abider et al. 2023). However, the efficiency of the extraction process is intrinsically dependent on the operational parameters employed during the procedure (Boscariol et al. 2019). Consequently, the rigorous optimization of experimental conditions is paramount to achieving maximal performance (Reungoat et al 2021).

Although phenolic extraction from mustard residues has been previously explored, including one study that applied MAE by Anghel 2023, the study relied solely on a one-factor-at-a-time (OFAT) approach. This prevented the evaluation of interactions between key variables and resulted in non-optimized conditions. To date, no study has used Design of Experiments (DOE) or RSM to model and optimize MAE for phenolic recovery from black mustard by-products. This gap underscores the need for a green, intensified, and statistically optimized extraction strategy to support the sustainable valorisation of mustard bran.

DOE is a statistical approach that enables the systematic evaluation of multiple factors and their effects on one or more responses. It provides an efficient way to plan experiments, analyze variable influences, and identify significant interactions. Compared with the traditional OFAT approach, DOE offers several advantages, including fewer experimental runs, lower costs, and reduced time and resource consumption. Moreover, DOE can generate a mathematical model describing factor response relationships, which serves as the foundation for RSM. The latter is built upon DOE by applying advanced statistical and mathematical techniques to develop predictive models, assess interactions, and determine optimal conditions that yield the most desirable outcomes (Bezerra et al. 2008; Luiz Caleffo Piva Bigão et al. 2024).

In light of this gap, this study investigates the combined effects of ethanol concentration, microwave power, and irradiation time on the extraction of phenolic antioxidants from BMSBP using MAE coupled with RSM. This plant-based matrix remains underexplored in the context of MAE optimization, and its extraction behavior cannot be directly extrapolated from other plant materials studied in the literature. Unlike previous studies that focused solely on phenolic recovery, this work simultaneously assesses antioxidant activity to confirm the functional relevance of the recovered compounds. Collectively, this work represents, to the best of our knowledge, the first statistically optimized microwave-assisted extraction strategy for BMSBP, contributing to the identification of optimal conditions for maximizing

their functional potential and offering a solid foundation for future scale-up and industrial application in the food, health, and cosmetic sectors, thereby delivering both scientific and practical significance beyond incremental methodological application.

Materials and Methods

Sample Preparation

Industrial mustard residue was kindly furnished by CEVETAL SPA (Bejaia, Algeria); it is the waste of the mechanical cold pressing of black mustard seeds (*Brassica nigra*) grown in India. The mustard residue consists of seed hulls, residual cotyledons, and other minor seed fractions. The raw mustard waste was lyophilized using a Christ Alpha 1–2 LD Plus freeze dryer (Germany), then ground using a basic grinder and sieved into particles of 250 µm; the obtained powder was stored in the dark at 4 °C prior to analysis.

Chemical Reagents

Gallic acid was purchased from Bio-chem-chemopharma (UK). Folin–Ciocâlteu, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and solvents (ethanol, methanol, acetone) were from Sigma Chemical (Sigma Aldrich GmbH, Germany). All reagents and chemicals were of analytical quality; distilled water was used for solution preparation.

Microwave-Assisted Extraction (MAE)

Bioactive compounds were extracted from black mustard seed by-product (BMSBP) using a microwave oven (MAX-MO23S, MAXIPOWER, China) (Haddadi-Guemghar et al. 2014). Briefly, a mass of 0.1 g of (BMSBP) powder was mixed with 10 mL of extraction solvent in a round-bottom flask of 50 mL. The flask was placed in a microwave oven and connected to a condenser. Subsequent to irradiation, the flask was removed and cooled in an ice water bath. The BMSBP extract was then centrifuged at 5000 rpm for 15 min using a Digicen 21R centrifuge (Orto Alresa, Madrid, Spain). The supernatant was collected and stored in a refrigerator at 4 °C prior to analysis.

Analytical Methods

Determination of Total Phenolics Content

TPC was quantified spectrophotometrically using the Folin–Ciocâlteu reagent following the method originally described by Singleton and Rossi (1965), as reported by Velioglu et al. (Velioglu et al. 1998) with slight modifications. Briefly, 0.2 mL of the extract was mixed with 0.75 mL of Folin–Ciocâlteu reagent (previously diluted tenfold with distilled water) and incubated at room temperature for 5 min. Subsequently, 0.75 mL of a 7% (w/v) aqueous sodium carbonate solution was added. The reaction mixture was then kept in the dark at room temperature for 60 min. Absorbance was measured at 750 nm against a reagent blank consisting of the extraction solvent without sample, using a Shimadzu UV-1800 spectrophotometer. The TPC was expressed as milligrams of gallic acid equivalents per 100 g of dry weight of BMSBP (mg GAE/100 g DW).

Antioxidant Activity

The antioxidant activity of BMSBP extracts was evaluated using the in vitro DPPH radical scavenging assay, following the protocol described by Blois and adapted by Park et al. (2017) (Park et al. 2017). The DPPH working solution was prepared by dissolving 2,2-di(4-tert-octylphenyl)−1-picrylhydrazyl in 96% ethanol to obtain a final concentration of 0.1 mM. An aliquot of 0.1 mL of the phenolic extract was added to 0.9 mL of the DPPH solution. The mixture was vigorously vortexed and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a Shimadzu UV-1800 spectrophotometer, with the extraction solvent used as the blank and a DPPH solution with solvent replacing the sample serving as the control. The DPPH radical scavenging activity was determined by the reduction in absorbance and calculated using the following equation:

$$\text{Percentage of inhibition } (I\,\%) = ((Ac - As)/Ac \times 100)$$

where (Ac) and (As) are the absorbances at 517 nm of the control and sample, respectively.

Experimental Design and Statistical Analysis

The key parameters influencing the extraction of TPC and AA during MAE were optimized using DOE and (RSM), implemented via JMP® Pro 13.0.0 (SAS Institute) statistical software. CCD was applied to assess the individual and interactive effects of three independent variables: ethanol concentration (X_1, 40–80%), irradiation time (X_2, 2–12 min), and microwave power (X_3, 100–700 Watt), each tested at three equidistant levels $(-1, 0, +1)$. The experimental matrix consisted of 16 runs, including two replicates at the center point (Table 1), and all experiments were performed in triplicate to ensure statistical robustness and reproducibility. The selection of factor levels was guided by preliminary experiments designed to define the optimal range for maximizing TPC and AA.

The experimental data were fitted using a second-order polynomial equation as follows:

$$Y_n = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (1)$$

where Y_n is one of the responses, β_0 represents the constant coefficient, β_i, β_{ii}, β_{ij} are the regression coefficients for the linear, quadratic, and interaction terms, respectively, and X_i and X_j are the independent variables, was then used by the software to generate the response surfaces. The adequacy of the model was determined by evaluating the lack of fit, coefficient of determination (R^2), and the Fisher's test value (F-value) obtained from the analysis of variance (ANOVA). Statistical significance of model parameters was determined at the 5% probability level ($p = 0.05$).

Results and Discussion

Model Fitting Analysis

To assess the adequacy and predictive power of the developed models, a model-fitting analysis was conducted using RSM. Statistical parameters such as ANOVA, coefficients of determination (R^2) and adjusted R^2 (Adj.R^2), and coefficient of variation (CV) were evaluated, providing insights into the significance and reliability of the regression models describing the extraction responses.

ANOVA as shown in Table 2, the high F-values and low p-values indicate that both TPC and AA models were highly significant (F-value = 56.34; $p < 0.0001$) and (F-value = 18.65; $p < 0.0010$) respectively, suggesting that the variations in the measured responses were predominantly attributed to the tested extraction factors rather than random error. Additionally, the ANOVA results for both responses showed a non-significant lack of fit ($p > 0.05$), indicating that the models are accurate and have good predictive capability within the range of variables tested.

As indicated by the fit statistics in Table 2, the degree of agreement between experimental and predicted values for TPC and AA is reflected by the coefficient of determination (R^2), which was 0.99 for TPC and 0.97 for AA, these high R^2 values indicate that 99% and 97% of the variability in TPC and AA, respectively, can be explained by the selected variables in the design matrix. The adjusted coefficient of determination (Adj.R^2), which accounts for the number of predictors in the model, was also high (0.97 for TPC and 0.91 for AA), further confirming the relevance of the included variables. Adj.R^2 increases when significant variables are incorporated and decreases when non-significant ones are added, thus providing a more accurate measure of model fit. Moreover, the low coefficients of variation (CV), which were below 10% (1.11% for TPC and 2.07% for AA), indicate excellent precision, reproducibility, and reliability of the model predictions (Chen 2016; Wong and Nillian 2023). The measured and predicted values for TPC and AA across all CCD runs are presented in Table 3, showing close agreement between experimental and model-predicted responses, further confirming the reliability and accuracy of the developed models. Based on these findings, the quadratic polynomial model showed high effectiveness in simulating the extraction process, demonstrating strong predictive accuracy for both total phenolic content and antioxidant activity.

Table 3 shows the p-values of all evaluated parameters. TPC was strongly influenced by irradiation time and microwave power ($p < 0.0001$ for both), and by the quadratic term of microwave power ($p = 0.0006$). The interaction between irradiation time and microwave power had a smaller effect ($p = 0.0150$) along with slight effect of the quadratic effect of irradiation time ($p = 0.0255$). The AA response was strongly affected by irradiation time ($p = 0.0004$) and slightly by microwave power ($p = 0.0136$). Interaction terms, namely (Ethanol concentration; microwave power) and (irradiation time; microwave power), were also significant ($p = 0.0065$ and 0.0414, respectively). Additionally, the quadratic terms of irradiation time and microwave power were significant ($p = 0.0040$ and 0.0062, respectively).

Least-squares regression analysis was applied to estimate the coefficients of the quadratic models. Based on this, simplified regression equations (Eqs. (2) and (3)) were developed to effectively fit the experimental data and identify optimal extraction conditions. These reduced models include only the significant terms while maintaining the overall predictive quality (Simić et al. 2016). The coded equations also allow for assessing the relative influence of each factor, where the sign of the coefficient indicates the direction of the effect.

Table 1 Central composite design matrix for MAE on BMSBP

Run	Independent variables		
	X_1 (%)	X_2 (min)	X_3 (W)
1	40	2	100
2	80	2	100
3	60	7	100
4	40	12	100
5	80	12	100
6	60	2	400
7	40	7	400
8	60	7	400
9	60	7	400
10	80	7	400
11	60	12	400
12	40	2	700
13	80	2	700
14	60	7	700
15	40	12	700
16	80	12	700

Table 2 Analysis of variance (ANOVA) and parameter estimates for the experimental results obtained using MAE for total phenolic compounds (TPC) and antioxidant activity (AA)

Studied factors (sorted parameter estimates)		*P*-value	
		TPC	AA
Linear terms	Ethanol concentration	0.1382	0.0493*
	Irradiation time	<0.0001***	0.0004***
	Microwave power	<0.0001***	0.0136*
Interaction terms	Ethanol concentration, Irradiation time	0.5951	0.6106
	Ethanol concentration, Microwave power	0.2130	0.0065**
	Irradiation time, microwave power	0.0150*	0.0414*
Quadratic terms	Ethanol concentration, ethanol concentration	0.1168	0.9004
	Irradiation time, irradiation time	0.0255*	0.0040**
	Microwave power, microwave power	0.0006***	0.0062**
Analyses of variance		TPC	AA
DF	Model	9	8
	Lack of fit	5	5
	Pure error	1	1
Sum of squares	Model	40,770.23	285.24
	Lack of fit	447.09	7.15
	Pure error	35.36	3.05
F-value	Model	56.34	18.65
	Lack of fit	2.53	0.47
p-value	Model	<0.0001***	0.0010***
	Lack of fit	0.4430	0.7961
	R^2	0.99	0.97
	Adj.R^2	0.97	0.91
	CV (%)	1.11	2.07

DF degree of freedom, R^2 coefficient of determination, *Adj.*R^2 adjusted coefficient of determination, *CV* coefficient of variation

***$p<0.001$; **$p<0.01$; *$p<0.05$

$$Y_{TPC} = 848.11 + 36.76X_2 + 39.41X_3 - 10.68X_2X_3 - 16.31X_2^2 - 36.68X_3^2 \quad (2)$$

$$Y_{AA} = 67.23 - 1.03X_1 + 2.9X_2 - 1.42X_3 - 1.88X_1X_3 - 1.19X_2X_3 - 3.64X_2^2 - 3.31X_3^2 \quad (3)$$

The regression coefficients in Eqs. (2) and (3) provide both qualitative and quantitative insight into the influence of each variable on TPC and AA. Positive coefficients indicate a promoting effect on the response, whereas negative coefficients reflect either an inhibitory influence or a decline beyond an optimal threshold. The magnitude of each coefficient reflects the relative contribution of the corresponding factor.

Equation (2) shows that the TPC increased with irradiation time (X_2) and microwave power (X_3), indicating a positive relationship between these factors and TPC, however, their interaction (X_2X_3) and quadratic effects (X_2^2 and X_3^2) negatively influenced TPC, revealing a decline beyond certain levels, consistent with Simic et al. (2016), who found that irradiation time and microwave power individually promoted TPC, yet exerted a negative effect when combined in interaction, as explained by (Zhang et al. 2011), MAE is inherently a rapid extraction technique, exceeding the optimal irradiation time particularly at high power levels causes accumulated thermal energy to drive oxidative degradation and structural breakdown of phenolic compounds, reversing the initial extraction gains. Ethanol concentration (X_1) and its associated terms (X_1^2, X_1X_2, X_1X_3) were not significant, suggesting that other variables had a more dominant impact on extraction efficiency, This finding aligns with (Reungoat et al. 2020; Zhang et al. 2024), who reported that ethanol concentration had no significant linear effect on phenolic compound extraction from mustard seed and meal, Among all parameters, microwave power exhibited the strongest influence on TPC, as evidenced by its highest coefficient (39.41) and lowest p-value (<0.0001).

Table 3 predicted and experimental results of TPC and AA responses for BMSBP obtained from CCD runs

Run	TPC (mg GAE/100 g DW)		AA (%)	
	Measured	Predicted	Measured	Predicted
1	698.07	699.50	56.23	56.60
2	693.94	696.81	58.37	58.83
3	770.55	772.02	65.79	65.34
4	796.54	790.83	65.93	65.28
5	795.32	795.25	66.24	66.52
6	803.12	795.05	61.87	60.69
7	824.07	833.15	67.3	68.35
8	847.92	848.11	68.5	67.23
9	856.33	848.11	66.03	67.23
10	847.92	842.85	67.33	66.32
11	856.48	868.57	65.27	66.49
12	791.8	790.86	60.19	59.90
13	801.13	805.83	53.96	54.61
14	848.3	850.84	62.01	62.50
15	843.33	839.46	64.28	63.81
16	863.98	861.54	57.9	57.53

As indicated in Eq. (3), AA increased with irradiation time (X_2), while ethanol concentration (X_1), microwave power (X_3), their interactions (X_1X_3, X_2X_3), and the quadratic terms (X_2^2, X_3^2) had negative effects. The similar trend observed between irradiation time and microwave power on both TPC and AA is consistent with the well-established relationship between phenolic compounds and antioxidant activity; indeed, a high correlation between TPC and DPPH radical-scavenging activity ($r = 0.93$) has been reported in different mustard seed fraction extracts, confirming that phenolic compounds are the primary contributors to antioxidant activity measured by DPPH radical scavenging assay in mustard-based matrices (Mayengbam et al. 2014). Furthermore, since phenolic compounds are heat-sensitive, prolonged exposure to intense microwave irradiation promotes their thermal degradation, thereby reducing both TPC and AA simultaneously.

Unlike TPC, where ethanol concentration showed no significant effect, AA was negatively influenced by X_1 and its interaction with microwave power (X_1X_3). This discrepancy may be attributed to the fact that DPPH radical-scavenging activity reflects not only the quantity but also the specific profile of extracted antioxidants, which is more sensitive to ethanol concentration. At higher ethanol concentrations, a shift in the polarity of the extraction medium may favor certain compound classes while limiting the recovery of more polar antioxidants with stronger radical-scavenging capacity (Li et al. 2012). The interaction between ethanol concentration and irradiation time (X_1X_2) and the quadratic term of ethanol concentration (X_1^2) were not significant. The most pronounced effect was the quadratic term of irradiation time, with a coefficient of 3.64; however, this was counterbalanced by the strong positive linear effect of irradiation time, indicating that AA initially increased before declining at higher exposure levels.

Response Surface Plots

Three-dimensional response surface plots were built using two significant interaction terms, keeping the third factor at its zero (central) level, to evaluate the effect of these interactions on the studied responses TPC and AA.

A notable interaction between microwave power and irradiation time was evident, as shown in the three-dimensional response surface plot (Fig. 1), The TPC yield increased significantly when irradiation time was extended from 2 to 10 min, and then tended to stabilize around 12 min. in parallel, raising the microwave power from 100 to 600 W enhanced the extraction efficiency, resulting in a higher TPC (874 mg GAE/g DW). However, beyond 600 W a decline in TPC was observed. This trend reflects the dual role of microwave-induced heating, where moderate power and irradiation time optimize mass transfer and extraction efficiency, while excessive conditions lead to compound degradation. This dual nature is characteristic of MAE, a PI technology that boosts extraction yields through rapid and uniform volumetric heating. Nonetheless, the effectiveness of MAE is strongly influenced by the interplay between microwave power, irradiation duration, and the characteristics of the sample matrix (DiNardo et al. 2020). During MAE, ionic conduction and dipole rotation are the key mechanisms through which microwave energy interacts with molecules, generating friction that heats the solution (Zhang et al. 2011; Wong and Nillian 2023).While longer irradiation times can improve yields by facilitating mass transfer and promoting solvent penetration and solute diffusion until equilibrium is reached, prolonged exposure to high temperatures can degrade heat-sensitive phenolic compounds, thereby reducing the total phenolic content (Tomasi et al. 2023; Gavrila et al. 2025).

As shown in Fig. 2 A, the AA of black mustard seed by-products increase significantly with long extraction times (from 2 to 12 min) and high microwave power levels (from 100 to 700 W). The maximum AA value (68%) was observed at an optimal combination of 300 W microwave power and 9 min of irradiation time. The elliptical shape of the contour plot suggests an antagonistic interaction between microwave power and irradiation time, whereby exceeding the optimal levels of both factors results in a reduction of AA. Moreover, the elliptical shape observed in the contour plot illustrates this interaction, indicating that both factors exert a combined negative effect when surpassing certain thresholds, resulting in a peak response followed by a decline.

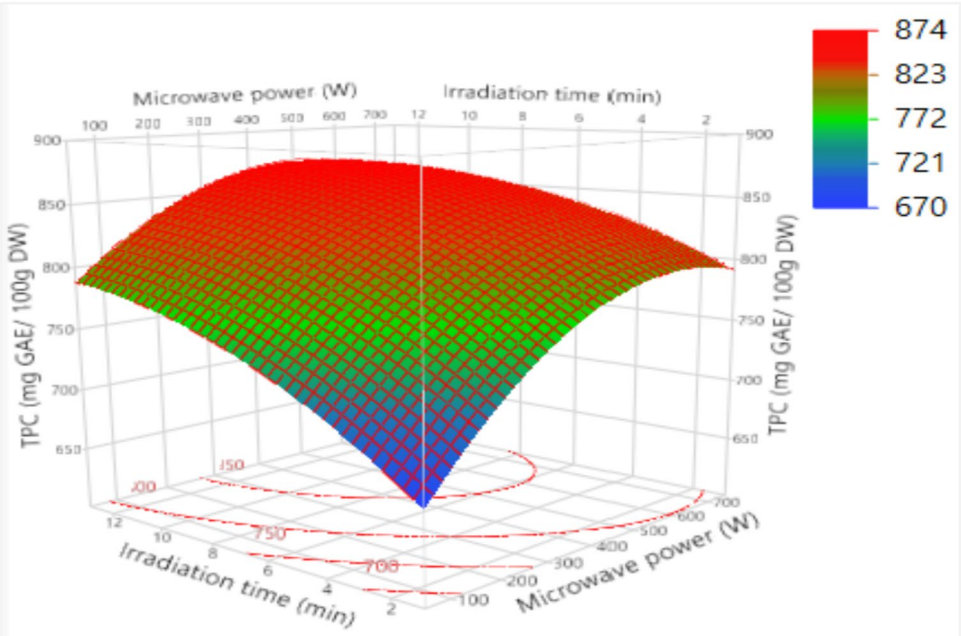


Fig. 1 Response surface plots of interaction effect between microwave power and irradiation time on TPC

This decline is likely due to the degradation of antioxidant compounds, suggesting that both factors negatively impacted DPPH radical inhibition when interacting at higher levels.

Whether considering the extraction of phenolic compounds Fig. 1 or the DPPH antioxidant activity Fig. 2 A, the observed results are consistent with several previous studies that examined the influence of microwave power–time interactions. Indeed, prolonged irradiation combined with elevated power levels has been suggested to negatively impact extraction efficiency, likely due to the degradation or oxidation of thermolabile bioactive compounds during the process (Amrane-Abider et al. 2023; Gavrila et al. 2025).

Figure 2B illustrates the interaction effect between microwave power and ethanol concentration on DPPH inhibition. The response surface reveals that Microwave power had a strong effect on the extraction process, shown by the changes in DPPH radical scavenging activity of BMSBP extracts (Fig. 2B). AA declined notably at ethanol concentrations between 60 and 80% and microwave powers above 600 W. The optimal extraction conditions were observed with an Ethanol concentration of 40% and microwave power ranging from 300 to just below 600 W at 400 W, under which DPPH inhibition reached approximately 69%, at these conditions, the combination of moderate ethanol concentration and sufficient microwave power enables optimal extraction. This efficiency may be attributed to the ability of microwave energy to rapidly heat the solvent–matrix system, as reported by (Gavrila et al. 2025), Extraction temperature varies with microwave power, leading to enhanced cell wall disruption and the release of polyphenols.

Besides, it is well established that AA efficiency is significantly affected by both the solvent's properties and the specific chemical structure and –OH positioning of the antioxidant compounds being extracted (Paré 2003). Previous studies have shown that ethanol–water mixtures around 50% are particularly effective due to their balanced polarity, which favors the solubilization of phenolic constituents. The dielectric properties of the solvent, particularly the dielectric constant and loss factor of ethanol–water mixtures, govern microwave energy absorption efficiency; as water content increases, the dielectric loss of the mixture increases, enhancing volumetric heating and improving extraction efficiency (Yilmaz and Toledo 2006; Vinatoru et al. 2017). Additionally, the presence of water in the solvent mixture, due to its high dipole moment, enhances microwave energy absorption, which promotes uniform heating and improves extraction efficiency (Yilmaz and Toledo 2006; Vinatoru et al. 2017). Furthermore, microwave energy preferentially heats moisture within plant cells, generating internal steam pressure that disrupts cell walls and facilitates the release of intracellular phenolic compounds into the solvent, a process strongly influenced by the structural characteristics of the matrix, including cell wall composition and moisture content (Zhang et al. 2011; DiNardo et al. 2020). An optimal ethanol concentration of 40% is in accordance with Das et al. (2009), describing the phenolic content in mustard meals as the major water-soluble antioxidant since they possess one hydroxyl group.

Assessment of Optimal Process Parameters and Model Validation

The model developed using CCD predicted the optimal conditions for the extraction of TPC and AA. Specifically,

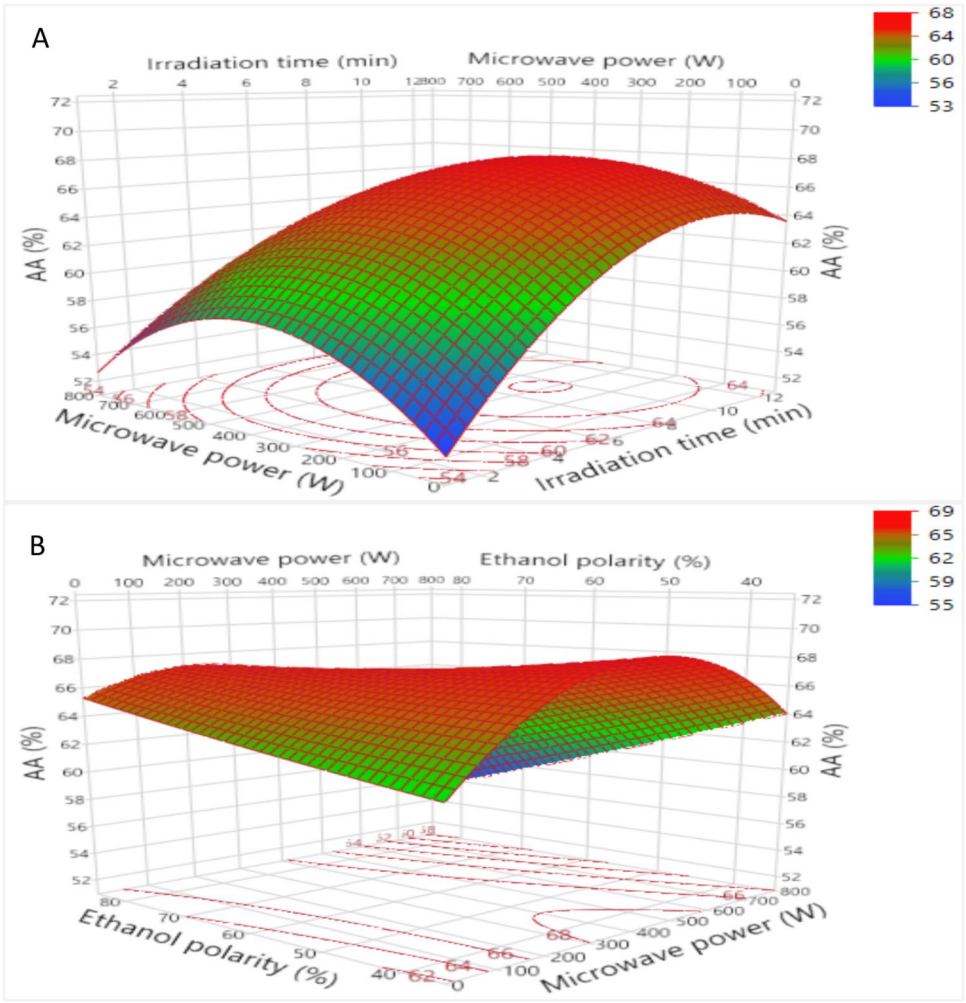


Fig. 2 Response surface plots for the interaction effect of microwave power and irradiation time (**A**) and the interaction effect of microwave power and ethanol concentration (**B**) on AA

it indicated that the ideal ethanol concentration, irradiation time, and microwave power were 68%, 12 min, and 500 W for TPC, and 40%, 8.99 min, and 400 W for AA. Under these conditions, the theoretical TPC and AA values predicted by RSM were 875.94 mg GAE/100 g DW and 69.03%, respectively.

Recognizing the need for a balance between the quantity of extracted phenolics and their bioactivity, a simultaneous optimization using the Desirability function was performed (Fig. 3). This approach identified the optimal extraction conditions as follows: 45% ethanol in water (X_1), 9.9 min (X_2), and 500 W (X_3), achieving a desirability value of 0.90, which is expected to result in a TPC of 858.7 mg GAE/100 g DW and an AA of 68.31%.

To validate the adequacy of the model in predicting optimal values and the simultaneous optimization using the Desirability function, a statistical comparison between the experimental and predicted responses was conducted using a one-sample *t*-test. MAE was applied under both simultaneous and individual optimal conditions to ensure the accuracy and acceptability of the extraction process. Triplicate experiments were performed under these optimized conditions, and the results were compared with the predicted optimal values (Table 4). Based on the results, no significant differences were observed between the experimental and predicted responses, confirming the validity of the models.

The optimal conditions obtained in the present study for MAE were 45% ethanol, 500-W microwave power, and 9.9 min of irradiation time, resulting in a TPC of 859.54 mg GAE/100 g DW and an AA of 68.85%. These results demonstrate the high efficiency of MAE in recovering phenolic compounds while maintaining strong antioxidant activity from BMSBP under relatively mild extraction conditions.

In the context of RSM optimization applied to mustard residues using other extraction techniques, previous studies have mainly focused on sinapine yield, as sinapine represents more than 80% of the phenolic compounds present in mustard seed (Achinivu et al. 2021; Nguyen et al. 2021; Zhang et al. 2024). Direct quantitative comparison between studies is limited, as TPC was determined using the Folin–Ciocâlteu method and expressed as gallic acid equivalents, whereas

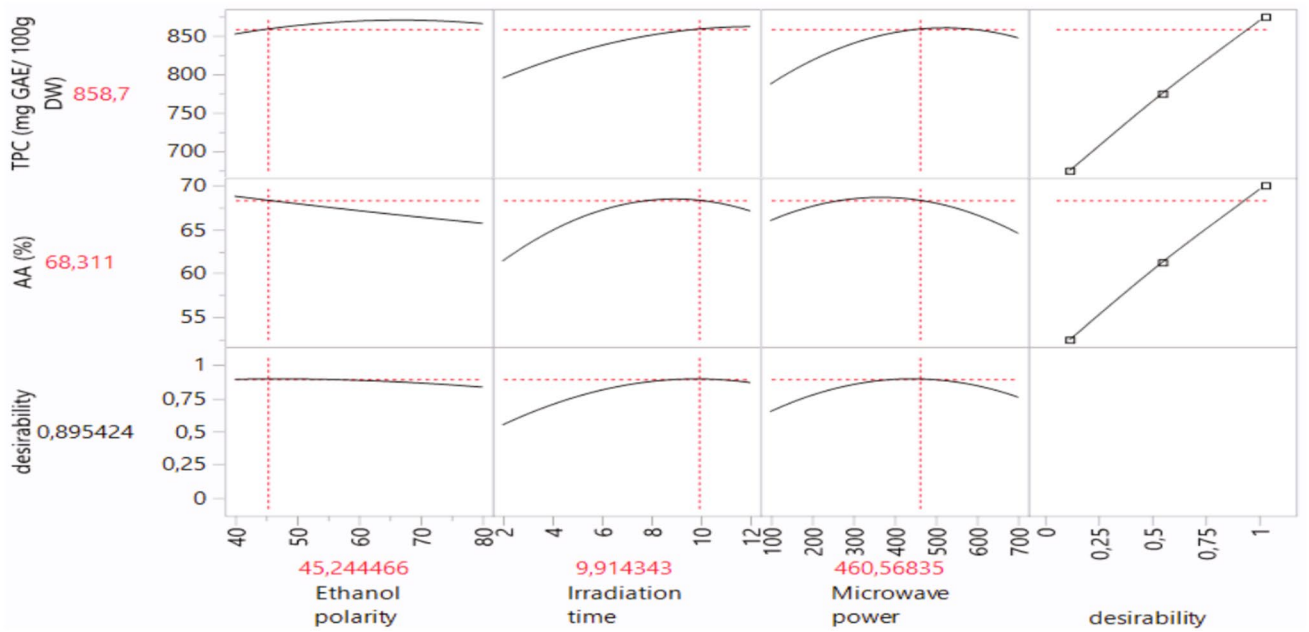


Fig. 3 Simultaneous optimization of TPC and AA values using desirability function analysis

other studies quantified individual phenolic compounds by HPLC and sometimes used spectrophotometric assays with standards similar to sinapine.

Reungoat et al 2020, they applied a central composite face-centered design and found that the extraction should be carried out at 75 °C with 70% ethanol and a ratio of 10 mL/g mustard bran dry matter (MBDM) for 30 min to reach a sinapine concentration of 8.8 ± 0,1 mg/g (MBDM) using conventional maceration. Similarly Flourat et al 2019 optimized maceration extraction for TPC, reporting longer extraction times (approximately 4 h) and lower temperatures (55 °C), yielding up to 10 mg ethyl sinapate equivalents/g of defatted and dried matter under conditions of 55 °C, 4 h, and 47% ethanol, and up to 13 mg ethyl sinapate equivalents/g of non-pretreated matter when applying 55 °C, 3.98 h, and 64.83% ethanol. Regarding unconventional extraction techniques, Chadni et al. (2023) optimized ultrasound-assisted extraction using a Box–Behnken design and identified optimal conditions of 75 °C, 70% ethanol, and 100% ultrasound amplitude, resulting in a sinapine yield of 6.90 ± 0.03 mg/g dry matter.

These results highlight how extraction conditions strongly influence the yield of target compounds and demonstrate the value of experimental design and RSM in improving process efficiency. The optimized conditions identified in this study provided efficient extraction performance, as reflected by both the measured responses and the overall process outcomes. Building on these results, MAE enabled effective extraction of phenolic compounds with high antioxidant activity while markedly reducing processing time, solvent usage, and energy consumption relative to conventional and ultrasound-assisted methods.

To further evaluate the outcomes of the optimization and emphasize the value of the by-product fraction, whole mustard seeds were extracted under the same conditions applied to the BMSBP matrix. The TPC and AA of the whole seeds were 1083.61 mg GAE/100 g DW and 66.31%, respectively. While the whole seeds contained higher TPC, the BMSBP extract maintained strong antioxidant activity, highlighting that the by-product still contains a substantial fraction of bioactive compounds and can serve as a valuable source for functional ingredient recovery.

Table 4 Predicted and experimental values of the responses at optimum extraction conditions

Outcomes	TPC (mg GAE/100 g DW)	AA (%)	Simultaneous optimum	
			TPC (mg GAE/100 g DW)	AA (%)
Predicted	875.94	69.03	858.7	68.31
Experimental	877.58 ^{ns} ± 14.04	69.72 ^{ns} ± 0.62	859.54 ^{ns} ± 2.43	68.85 ^{ns} ± 1.23

Values are represented as mean ± SD ($n = 3$)

ns not significant difference ($p \geq 0.05$)

Supporting this, Mayengbam et al. (2014) similarly evaluated the TPC in different mustard seed parts: whole seeds, hulls, and cotyledons, using ultrasonic-assisted extraction with 70% aqueous methanol, reporting 1031 ± 32; 624 ± 40 and 1060 ± 24 mg sinapic acid equivalent per 100 g, respectively. These findings demonstrate that significant phenolic compounds remain in residual seed parts, reinforcing that by-products are a promising and sustainable source of phenolic compounds.

Conclusion

This study on the MAE of phenolic compounds from BMSBP was successfully carried out by combining RSM with CCD as the experimental design. Ethanol concentration, microwave power, and irradiation time were identified as critical variables, while TPC and AA were the responses. A validated model was developed for each response, and the optimal conditions for MAE were determined as 45% ethanol concentration, 500 W microwave power and 9.9 min of irradiation time, yielding 859.54 mg GAE/100 g DW for TPC and an AA of 68.85%.

This finding provides inspiration for using green technologies, such as MAE, to valorise waste biomass and recover sensitive bioactive compounds. The application of RSM proved to be a powerful statistical tool, enabling rapid and reliable optimization of the extraction process.

Since the ultimate goal of biomass valorization is to support its integration into industrial processes as natural sources of innovative, value-added products, future work should focus on detailed compositional analysis of the extracts, deeper evaluation of their biological activities through realistic food or medical simulative systems, and investigation of additional process variables. Scale-up studies will also be crucial for enhancing the industrial applicability of this approach. Overall, these perspectives align well with the principles of building a bio-circular economy.

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Data Availability The authors confirm that the data supporting the findings of this study are available within the article and further data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics Approval No humans or animals were used in this research.

Competing interests The authors declare no competing interests.

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