Scientific paper

Microwave-Assisted Extraction of Capsaicin and Dihydrocapsaicin from Green Chili with Process Optimization as per Response Surface Methodology

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Abstract

Capsaicin and dihydrocapsaicin are highly valuable natural compounds. In this study, microwave-assisted extraction (MAE) was optimized for the extraction of these compounds from *Capsicum annuum* L. using two solvents: ethyl acetate and acetone. The optimization was conducted using response surface methodology (RSM), and high-performance liquid chromatography (HPLC) was used for quantification of the compounds. The independent variables considered were microwave power (W), irradiation time (s), and solvent-to-solid ratio (SSR). For MAE-acetone, the optimal yields were 19.3 mg/g dry matter (DM) for capsaicin and 10.2 mg/g DM for dihydrocapsaicin. MAE-ethyl acetate yielded higher amounts, with 22.1 mg/g DM for capsaicin and 10.6 mg/g DM for dihydrocapsaicin. The optimal conditions for capsaicin in both solvents were 60 s, 220 W, and 30 mL/g SSR, while for dihydrocapsaicin, the conditions were 40 s, 220 W, and 40 mL/g SSR. Thus, MAE-ethyl acetate proved to be more effective than MAE-acetone for the extraction of both compounds. It is, therefore, preferable due to its efficiency and environmental safety and, thus, is a promising technique for industrial applications.

Keywords: *Capsicum annuum* L.; Capsaicin; Dihydrocapsaicin; Microwave-assisted extraction; RSM optimization

1. Introduction

Capsaicin and dihydrocapsaicin are two highly important bioactive compounds. They belong to a broader class of compounds called capsaicinoids and are abundantly found in chili peppers, genus Capsicum. The common species of the genus Capsicum include *C. annuum* L., *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*. In general, they make around 90% of the capsaicinoids present in hot peppers, capsaicin around 69% and dihydrocapsaicin 22%.[1]

Capsaicin and dihydrocapsaicin (Fig. 1) are recognized for their wide-ranging therapeutic applications. Capsaicin is well-established for its analgesic properties, particularly in the management of chronic pain conditions such as arthritis, neuropathy, and post-herpetic neuralgia.[2] Dihydrocapsaicin shares similar pharmacological properties and contributes to the overall potency of capsaicin-containing preparations.[3] Beyond pain relief, these compounds have shown potential in weight management by enhancing thermogenesis and lipid metabolism.[4,5]

Additionally, emerging research also suggests their anti-cancer properties, with studies indicating the ability of these compounds to induce apoptosis in various cancer cell lines.[6] The antimicrobial and anti-inflammatory activities of capsaicin and dihydrocapsaicin further highlight their significance in developing novel therapeutic agents.[7] Herbal products based on chili constituents are already on the market. For instance, capsaicin-based topical creams are available in many brands for pain management, particularly in conditions like arthritis and neuropathic pain.[8]

Capsaicin and dihydrocapsaicin are not only highly valuable but also expensive compounds. Their molecular structures make them difficult to synthesize chemically, making their extraction from natural sources, such as chili peppers, crucial.[9] The ability to efficiently extract these compounds is essential for advancing therapeutic and pharmaceutical applications and ensuring a sustainable supply of these bioactives. This research focuses on the efficient extraction of capsaicin and dihydrocapsaicin using green and sustainable methods.

Given the therapeutic importance of these compounds, coupled with the challenges associated with their

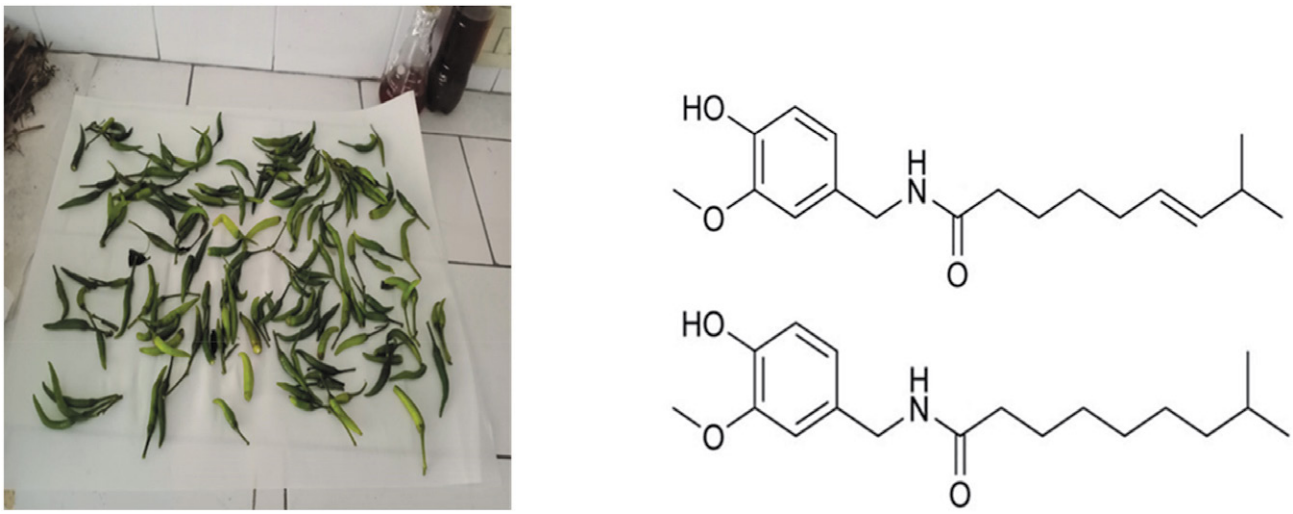

Fig. 1. *Capsicum annuum* peppers and chemical structure of capsaicin (upper) and dihydrocapsaicin (lower).

synthetic production, developing efficient and sustainable extraction methods from natural sources is crucial.

Capsicum species, the natural source of capsaicin and other capsaicinoids, are abundantly available almost globally. *Capsicum annuum* L. is cultivated as a profitable crop in many regions of the world.[10]

Chili pepper cultivation is widespread across the world, constituting a sustainable source of capsaicinoids. Thus, the global production of *Capsicum* was approximately 38 million tons in 2019. Asia leads as the largest producer, accounting for approximately 65% of the global output. The Americas, Europe, Africa, and Oceania contribute 13.3%, 11.9%, 10.1%, and 0.2%, respectively.[11,12,13,14] For fresh peppers, Asia is responsible for 67.3% of the world's production, and Africa accounts for 10%.[15,16]

Thus, from the abundantly available chili peppers, capsaicin and other capsaicinoids can be extracted on a large scale. A host of methods are available to extract these bioactive compounds from chili peppers. However, the efficiency of these methods, as well as their health and environmental safety, are crucial issues that must be kept in mind while selecting and developing a strategy for this purpose.[17] Literature shows several valuable studies on the extraction of capsaicinoids from chili using various strategies. Each technique presents a unique balance of advantages and disadvantages concerning efficiency, health and environmental safety, and economic viability. Conventional approaches, such as Soxhlet extraction and maceration, typically involve substantial volumes of organic solvents and prolonged extraction times, consequently raising significant health and environmental concerns.[18] More advanced techniques have also been studied for the extraction of chili bioactives, including ultrasound-assisted extraction (UAE),[19,20] enzyme-assisted extraction (EAE),[21] pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE),[22,23] and surfactant-assisted extraction.[24] Among these evolving methodologies, microwave-assisted extraction (MAE) stands out as a promising and rapidly developing technique. MAE is characterized by its comparatively rapid extraction times, reduced solvent requirements, and high yields of capsaicinoids.[18,25] Early investigations by Williams et al. (2004) demonstrated MAE's superior efficiency over conventional reflux and shaken flask methods, specifically noting acetone's effectiveness among the tested solvents (hexane, methanol, and methylene chloride).[26] More recently, Hussain et al. (2022) reported MAE to be more efficient than UAE when employing deep eutectic solvents.[27] Furthermore, Waqas et al. (2022) identified ethyl acetate as a highly efficient solvent in the surfactant-assisted extraction of capsaicin.[24] While these studies collectively affirm MAE's considerable potential and highlight the individual efficacy of both acetone and ethyl acetate as solvents, a comprehensive optimization of capsaicinoid extraction utilizing MAE with these solvents, particularly with the focus on discovering an industrially viable method, remains to be thoroughly investigated. The current research directly addresses this gap by employing response surface methodology (RSM) to optimize the microwave-assisted extraction of capsaicinoids from chili using ethyl acetate and acetone, thereby advancing towards an industrially viable process for obtaining these valuable compounds.

From this perspective, microwave-assisted extraction (MAE) is a technique of choice. It is not only efficient but also eco-friendly and time- and energy-saving.[28] Its flexibility in working with heat-sensitive chemicals without causing noticeable deterioration also increases its usefulness. Thus, adaptability, speed, selectivity, and efficiency of MAE make it a potent tool in bioactive compound extraction.[29]

Apart from the techniques, the choice of solvents used as extractants is also crucial. A solvent selected for this purpose should meet the requirements of efficiency in terms of extraction yield from chili dry matter, health and

environmental safety, and cost-effectiveness. For this study, two solvents were selected, which were ethyl acetate and acetone. Previous studies have shown them to be highly efficient in extracting capsaicinoids from chili.[30] The physical characteristics of these solvents make them reasonably suitable for capsaicinoid extraction.[31,32] Both ethyl acetate and acetone are moderately polar; however, acetone is more suitable for comparatively more polar substances as it has a much higher dielectric constant and a higher dipole moment. In addition, ethyl acetate is considered more environmentally friendly than acetone as it has a higher boiling point, lower vapor pressure, and a higher flash point.[33] Capsaicinoids, with their combination of polar (vanillyl and amide) and nonpolar (alkyl chain) functionalities, exhibit good solubility in moderately polar solvents like ethyl acetate. Their logK_{ow} (octanol-water partition coefficient) values of ~3.0–4.5 indicate they are moderately lipophilic but neither extremely nonpolar (like hexane) nor extremely polar (like water).[34] Ethyl acetate and acetone fall nicely into this range. Comparatively low boiling points of ethyl acetate and acetone (77 °C and 56 °C, respectively) make them easy to evaporate off after extraction to isolate the capsaicinoid extracts without high temperatures that could degrade the target compounds.

While excessive exposure to ethyl acetate can cause health issues like skin irritation and unconsciousness, it is generally considered a low-toxicity substance due to its rapid breakdown into ethanol and acetic acid in the body.[35] It is approved for use in food products up to 25 mg/kg and is even used as a flavoring agent because of its fruity aroma.[36] In addition, it can be produced from bio-based ethanol and is biodegradable in air and water.

To understand and manipulate the effects of various factors on the extraction yield of a desired phytochemical, discerning optimal conditions are important. To this end, response surface methodology, abbreviated as RSM, is a commonly used method. The method is applied through its designs, of which the Box-Behnken design (BBD) is commonly employed.[37,38] If Y is a response that depends on several factors, X, their relationship can be shown in the form of a second-order polynomial equation. A common form of this equation is given below[39,40] (Eq. 1):

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

The intercept coefficient is shown by β_0 and regression coefficients for linear, interactive, and quadratic terms are shown by β_i, β_{ij}, and β_{ii}. The data acquired according to the design are fitted in this equation and solved for various responses. Analysis of variance (ANOVA) is carried out to determine the significance of the suggested model and its terms.

Based on the gaps identified in existing literature, this study was designed to evaluate the potential of MAE for the efficient recovery of capsaicin and dihydrocapsaicin from green chilies. Although various extraction techniques such as Soxhlet, ultrasound-assisted extraction, and pressurized liquid extraction have been employed previously, limitations in extraction time, energy consumption, and solvent usage persist. MAE presents a promising green alternative, yet there remains limited comparative data on solvent efficiency using MAE specifically for capsaicinoids. Furthermore, few studies have utilized response surface methodology (RSM) to optimize the conditions for MAE in this context.

To address these gaps, the current research investigated and compared the extraction efficiency of two solvents, ethyl acetate and acetone, under MAE. A three-factor Box-Behnken design of RSM was applied to optimize critical parameters influencing extraction efficiency.[41] The novelty of this study lies in its integrated approach combining MAE with process optimization and solvent comparison, offering insights into a potentially more sustainable and effective extraction strategy for capsaicinoids. This work aims to provide a scalable and environmentally conscious alternative for the recovery of bioactive chili compounds.

The bioactive compound extraction method based on microwave heating in combination with green solvents like ethyl acetate and acetone, and RSM-based process optimization are the main elements of the novelty of this research. It was hypothesized that using microwave technology coupled with ethyl acetate or acetone would result in a viable method for obtaining these valuable compounds from chili.

2. Materials and Methods

1. 1. Chemicals

Acetone (≥99.5%), acetonitrile (≥99.9%), and ethyl acetate (≥99.5%) were purchased from Sigma-Aldrich (Steinheim, Germany) and used without further purification. Standard compounds of capsaicin (≥98%) and dihydrocapsaicin (≥98%) were obtained from Chem-Impex International Inc. (Wood Dale, USA).

1. 2. Collection and Preparation of Plant Material

A sample of green chili pepper (cultivar: Hybrid 7864 Red Hard) was obtained from Shujabad (29.8717° N, 71.3231° E), Pakistan. Two kilograms of green chili peppers were carefully selected as the source material. The pedicles were removed from the peppers, resulting in a reduced weight of 1154 g. After being washed and allowed to air dry for two weeks, the peppers were processed with a grinder into a fine powder. The ground pepper powder was sieved to ensure uniformity in size (80 mesh size, 77 μm). The finely ground powder was placed into a sealed bag to prevent moisture absorption or contamination.

1. 3. Design of Experiment (DoE) as Per RSM

The pivotal stage of RSM is the design of the experiment. A software (Design-Expert version 13, Stat-Ease, Inc., MN, USA) was used to produce a 3-factor-3-level Box-Behnken design. The design gave 17 orders, including five central points. The design is shown in Table 1.[42] The MAE factors were time (20 min, 40 min, 60 min), power (220 W, 330 W, 440 W), and SSR (20 mL/g, 30 mL/g, 40 mL/g).

1. 4. Extract Preparation

A domestic microwave oven (model DW-142 HZP, Dawlance, Pakistan) was used for the study, operating at a fixed frequency of 2450 MHz and allowing for a manual adjustment of power levels. The extraction was conducted in open vessels, specifically 250-mL borosilicate glass conical flasks, to prevent pressure build-up and allow for safe heating of the solvent-sample mixtures.

A total of 17 conical flasks, labelled according to the Box-Behnken design matrix, were each filled with a fixed amount of finely ground green chili pepper and 30 mL of ethyl acetate or acetone. After microwave exposure under specified conditions, the mixtures were allowed to cool to room temperature.

The resulting extracts were filtered under vacuum using a Buchner funnel fitted with Whatman No. 1 filter paper to separate the solid residue from the liquid phase. The filtrates were transferred to clean conical flasks and covered with perforated aluminum foil to allow controlled evaporation of the solvents. The flasks were placed in a fume hood at ambient conditions to facilitate solvent removal, leaving behind the dried extracts for further analysis.

1. 5. High-performance Liquid Chromatography (HPLC)

The primary aim of this study was to optimize MAE conditions for capsaicin and dihydrocapsaicin. An HPLC method was employed to quantify these compounds in the extracts. The method used was based on standard reversed-phase chromatography.[43] The HPLC analysis was performed using a Shimadzu LC-10AS series system equipped with an SPD-6AV UV detector and a C18 column (250 × 4.6 mm). The mobile phase consisted of acetonitrile and water (70:30, *v/v*), delivered at 1.0 mL/min in isocratic mode. The detection wavelength was set to 280 nm.[44]

Calibration curves were constructed using standard solutions of capsaicin and dihydrocapsaicin in acetonitrile: water (70:30) over the concentration range of 5-100 µg/mL. Each standard was analyzed in triplicate. The calibration equations were:

Capsaicin: $y = 1.89 \cdot 10^5 x + 1.37 \cdot 10^5$, $R^2 = 0.9977$, %RSD of peak area = 1.02

Dihydrocapsaicin: $y = 1.1 \cdot 10^5 x + 6.23 \cdot 10^3$, $R^2 = 0.9979$, %RSD = 1.21

Extracts were analyzed by dissolving 10 mg of dried MAE sample in 10 mL of the mobile phase, followed by filtration through a 0.45 µm membrane filter. A 20 µL aliquot was injected for analysis. All determinations were performed in triplicate. To ensure the reproducibility and precision of the method, all quantitative analyses were performed using at least three parallel determinations. The consistency of these replicates is demonstrated by the low relative standard deviation (%RSD) of the peak areas.

The straight-line equations derived from these calibration curves were used to estimate capsaicinoid contents in each extract. The linearity of each curve was high, as indicated by a high coefficient of determination (R^2). This demonstrates a strong linear relationship between the concentration of the standard and the instrument's response, confirming the reliability of the quantitative measurements. The results are shown in Table 1. These results were subjected to data analysis and modeling according to RSM.

2. Results and Discussion

2. 1. Capsaicin and Dihydrocapsaicin Extraction Yield

The Box-Behnken design and the experimental yields of capsaicin and dihydrocapsaicin in acetone and ethyl acetate are shown in Table 1.

For MAE-acetone and MAE-ethyl acetate, the highest capsaicin extraction yields from dry matter were in standard order 2 (time 60 s, power 220 W, SSR 30 mL/g). The highest capsaicin contents in MAE-acetone and MAE-ethyl acetate were 19.3 mg/g and 21.8 mg/g of dry matter (DM), respectively. The corresponding predicted values predicted by optimization models were 19.3 mg/g DM and 22.1 mg/g of DM, respectively. The second highest capsaicin yields were in standard order 3 (time 20 s, power 440 W, SSR 30 mL/g), which for MAE-acetone and MAE-ethyl acetate were 18.4 mg/g DM and 20.9 mg/g of DM, respectively, and the corresponding predicted values were 18.6 mg/g DM and 21.2 mg/g of DM, respectively. Thus, standard orders 2 and 3 provide two alternative pathways for efficient extraction of capsaicin. Standard order 2, however, produced slightly better results. It also requires lower microwave power than standard order 3, which makes it industrially more favorable.

The highest dihydrocapsaicin contents in MAE-acetone and MAE-ethyl acetate were 10.1 mg/g DM and 10.5 mg/g DM, respectively, which were in standard order 11 (time 40 s, power 220 W, SSR 40 mL/g). The corre-

Table 1. Experimental design and the results of microwave-assisted extraction of capsaicin and dihydrocapsaicin from green chili peppers using acetone and ethyl acetate as solvents.

					MAE-acetone		MAE-ethyl acetate	
		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 1	Response 2
Standard order	Run order	A: Time	B: Power	C: SSR	Capsaicin	Dihydrocapsaicin	Capsaicin	Dihydrocapsaicin
		s	W	mL/g	mg/g DM	mg/g DM	mg/g DM	mg/g DM
5	1	20	330	20	3.85	2.83	5.55	7.29
6	2	60	330	20	3.68	4.34	5.38	1.99
14	3	40	330	30	1.68	3.99	4.23	2.07
16	4	40	330	30	0.864	4.38	3.41	1.97
12	5	40	440	40	3.30	7.63	6.70	6.78
7	6	20	330	40	12.4	7.56	15.8	7.29
10	7	40	440	20	0.518	4.03	2.22	4.59
2	8	60	220	30	19.3	4.26	21.8	4.21
1	9	20	220	30	12.1	7.66	14.6	7.68
17	10	40	330	30	2.16	3.36	4.71	2.26
9	11	40	220	20	1.75	4.58	3.45	4.32
8	12	60	330	40	8.01	8.73	11.4	9.23
4	13	60	440	30	6.31	6.45	8.87	3.12
13	14	40	330	30	1.74	4.98	4.29	4.06
15	15	40	330	30	2.02	5.14	4.57	2.72
3	16	20	440	30	18.4	2.96	20.9	3.44
11	17	40	220	40	6.26	10.1	9.66	10.6

Note: Each result value is the mean of three determinations.

sponding values predicted by optimization models were 10.1 mg/g DM and 10.6 mg/g DM, respectively.

Thus, MAE-ethyl acetate proved to be more powerful than MAE-acetone in extracting both compounds; however, the yields of dihydrocapsaicin in both solvents were almost comparable. On the other hand, as highlighted above, there was an appreciable difference in the capsaicin extraction in the two solvents.

2. 2. Optimization of MAE-acetone and MAE-ethyl Acetate

For method optimization for capsaicin and dihydrocapsaicin in both solvents, the data set in Table 1 was analyzed. The analysis predicted quadratic models for both compounds in MAE-acetone as well as in MAE-ethyl acetate. Analysis of variance (ANOVA) was conducted to determine the significance of the predicted models and their terms. The models and terms having p-values < 0.05 were considered significant. The ANOVA results of MAE-acetone and MAE-ethyl acetate are shown in Tables 2 and 3.

As Tables 2 and 3 show, predicted models for capsaicin and dihydrocapsaicin in both solvents were highly significant, as is witnessed by their large F-values and very small p-values. Most terms were also highly significant except for those whose p-values (the probability that the observed results could occur by chance) were > 0.05. A non-significant lack of fit (p > 0.05) implied that the given model fit the data well without substantial error. Residual indicates unexplained variability in the data; thus, the lower values of all the suggested models suggest a better model fit.

Based on ANOVA, model regression equations for MAE-acetone and MAE-ethyl acetate for capsaicin (mg/g) and dihydrocapsaicin in the coded form are displayed in Table 4.

As the fit statistics show, the R-square, adjusted R-square, and predicted R-square values of all the models were high (86% or higher). These high values indicate that the predicted models are statistically robust and reliable. These values suggest that a significant proportion of the variability in the response variables (capsaicin and dihydrocapsaicin yields) is explained by the factors considered in the models. This implies that the optimization process was effective and that the identified conditions can be confidently used to achieve high yields of these valuable compounds. The low standard deviation (Std. Dev.) values indicate that the predictions of the model are consistently close to the mean, suggesting reliable and precise predictions.

Similarly, the moderate C.V.% (% coefficient of variation) values also indicate the good fit of the models. In a process, a low C.V. % is desirable as it is an indicator of the precision and consistency of the model. In the current study, it has moderate values, which indicate acceptable precision and accuracy.

Table 2. ANOVA table for MAE-acetone.

Capsaicin by MAE-acetone					
Source of variation	**Sum of Squares**	**df**	**Mean Square**	**F-value**	***p*-value**
Model	563.83	9	62.65	77.43	< 0.0001
A: Time	10.9	1	10.9	13.47	0.008
B: Power	14.73	1	14.73	18.21	0.0037
C: SSR	50.63	1	50.63	62.58	< 0.0001
AB	93.05	1	93.05	115.01	< 0.0001
AC	4.43	1	4.43	5.47	0.0519
BC	0.7373	1	0.7373	0.9113	0.3716
A^2	280.62	1	280.62	346.85	< 0.0001
B^2	72.24	1	72.24	89.29	< 0.0001
C^2	34.98	1	34.98	43.24	0.0003
Residual	5.66	7	0.8091		
Lack of Fit	4.65	3	1.55	6.13	0.0561
Pure Error	1.01	4	0.2528		
Cor Total	569.49	16			
Dihydrocapsaicin by MAE-acetone					
Source of variation	**Sum of Squares**	**df**	**Mean Square**	**F-value**	***p*-value**
Model	563.83	9	62.65	77.43	< 0.0001
A: Time	10.9	1	10.9	13.47	0.008
B: Power	14.73	1	14.73	18.21	0.0037
C: SSR	50.63	1	50.63	62.58	< 0.0001
AB	93.05	1	93.05	115.01	< 0.0001
AC	4.43	1	4.43	5.47	0.0519
BC	0.7373	1	0.7373	0.9113	0.3716
A^2	280.62	1	280.62	346.85	< 0.0001
B^2	72.24	1	72.24	89.29	< 0.0001
C^2	34.98	1	34.98	43.24	0.0003
Residual	5.66	7	0.8091		
Lack of Fit	4.65	3	1.55	6.13	0.0561
Pure Error	1.01	4	0.2528		
Cor Total	569.49	16			

Note: The terms with *p*-values < 0.050 were significant and *p*-values < 0.0001 were highly significant.

Table 4. Model regression equations for MAE-acetone and MAE-ethyl acetate.

MAE-acetone	
$Y(\text{Capsaicin}) = 1.69 - 1.17A - 1.36B + 2.52C - 4.82AB + 8.16A^2 + 4.41B^2 - 2.88C^2 + \epsilon$	Eq. 2
$Y(\text{Dihydrocapsaicin}) = 4.3709 + 0.3498A - 0.6948B + 2.29C + 1.72AB + 0.1138A^2 + 0.8484B^2 + 1.37C^2 + \epsilon$	Eq. 3
MAE-ethyl acetate	
$Y(\text{Capsaicin}) = 4.24 - 1.17A - 1.36B + 3.36C - 4.82AB + 8.16A^2 + 4.14B^2 - 2.88C^2 + \epsilon$	Eq. 4
$Y(\text{Dihydrocapsaicin}) = 2.61537 - 0.8897A - 1.10B + 1.96C + 1.82AC - 1.01BC + 0.9482A^2 + 1.05B^2 + 2.90C^2 + \epsilon$	Eq. 5

The equations contained only the significant terms (*p*-value < 0.05). The adequacy of the models was demonstrated by highly supporting fit statistics, which are displayed in Table 5.

Tables 2 and 3 present the ANOVA results for capsaicin and dihydrocapsaicin extracted using MAE with acetone and ethyl acetate. All models were statistically significant, as shown by high F-values and very low *p*-values (< 0.0001), indicating that the variables (microwave time, power, and solvent-to-solid ratio) had a strong effect on extraction yield. Most terms, especially quadratic and some interaction terms, were also significant ($p < 0.05$), confirming both linear and nonlinear effects. The lack-of-fit tests were non-significant, and residual errors were low, suggesting that the models fit the experimental data well.

The regression equations in Table 4 include only the significant terms and are used to predict extraction yields under different conditions. These "predicted values" are

Table 3. ANOVA table for MAE-ethyl acetate.

Capsaicin by MAE-ethyl acetate					
Source of variation	**Sum of Squares**	**df**	**Mean Square**	**F-value**	***p*-value**
Model	603.74	9	67.08	82.91	< 0.0001
A: Time	10.9	1	10.9	13.47	0.008
B: Power	14.73	1	14.73	18.21	0.0037
C: SFR	90.54	1	90.54	111.91	< 0.0001
AB	93.05	1	93.05	115.01	< 0.0001
AC	4.43	1	4.43	5.47	0.0519
BC	0.7373	1	0.7373	0.9113	0.3716
A^2	280.62	1	280.62	346.85	< 0.0001
B^2	72.24	1	72.24	89.29	< 0.0001
C^2	34.98	1	34.98	43.24	0.0003
Residual	5.66	7	0.8091		
Lack of Fit	4.65	3	1.55	6.13	0.0561
Pure Error	1.01	4	0.2528		
Cor Total	609.4	16			
Dihydrocapsaicin by MAE-ethyl acetate					
Source of variation	**Sum of Squares**	**df**	**Mean Square**	**F-value**	***p*-value**
Model	113.8	9	12.64	25.05	0.0002
A: Time	6.33	1	6.33	12.54	0.0094
B: Power	9.74	1	9.74	19.29	0.0032
C: SFR	30.72	1	30.72	60.84	0.0001
AB	2.47	1	2.47	4.89	0.0626
AC	13.23	1	13.23	26.21	0.0014
BC	4.08	1	4.08	8.09	0.0249
A^2	3.79	1	3.79	7.5	0.029
B^2	4.62	1	4.62	9.15	0.0193
C^2	35.37	1	35.37	70.06	< 0.0001
Residual	3.53	7	0.5049		
Lack of Fit	0.6071	3	0.2024	0.2766	0.8403
Pure Error	2.93	4	0.7317		
Cor Total	117.34	16			

Note: The terms with *p*-values < 0.050 were significant and *p*-values < 0.0001 were highly significant.

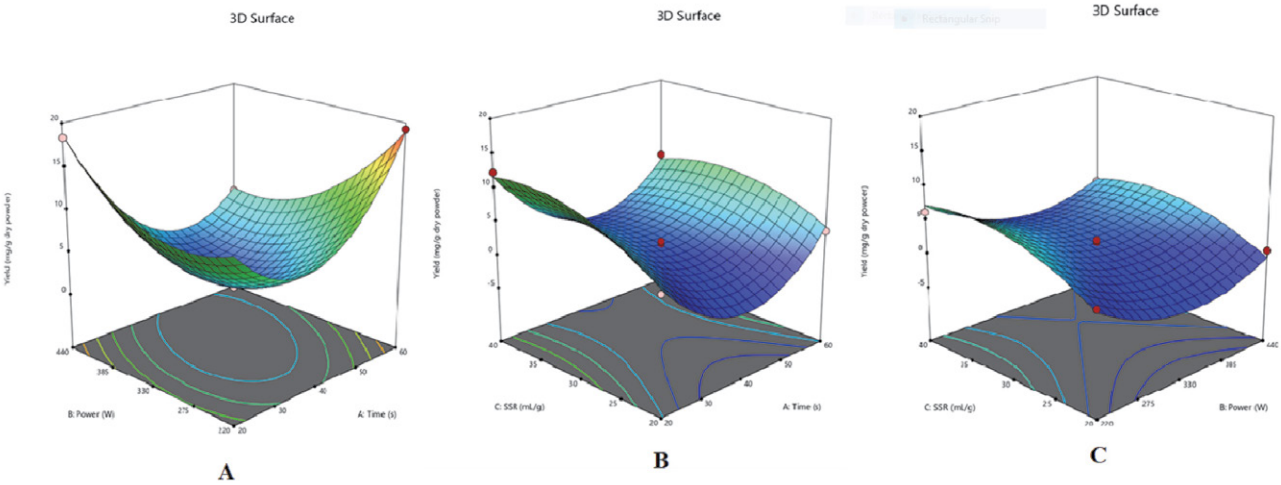

Fig. 2. 3D Surface plots for MAE-acetone displaying interactive effect of (A) power and time, (B) SSR and time, and (C) SSR and power on capsaicin extraction. Red color shows high value, and blue color shows low value.

Table 5. Fit statistics for MAE-acetone and MAE-ethyl acetate models.

Capsaicin in MAE-acetone			
Std. Dev.	0.8995	R^2	0.9901
Mean	6.13	Adjusted R^2	0.9773
C.V. %	14.68	Predicted R^2	0.8665
		Adeq Precision	28.2704
Dihydrocapsaicin in MAE-acetone			
Std. Dev.	0.6566	R^2	0.9595
Mean	5.47	Adjusted R^2	0.9073
C.V. %	12.00	Predicted R^2	0.7654
		Adeq Precision	14.9016
Capsaicin in MAE-ethyl acetate			
Std. Dev.	0.8995	R^2	0.9907
Mean	8.67	Adjusted R^2	0.9788
C.V. %	10.37	Predicted R^2	0.8753
		Adeq Precision	29.5002
Dihydrocapsaicin in MAE-ethyl acetate			
Std. Dev.	0.7105	R^2	0.9699
Mean	4.92	Adjusted R^2	0.9312
C.V. %	14.45	Predicted R^2	0.8782
		Adeq Precision	16.2227

calculated from the model equations and help identify optimal settings without further experimentation. Table 5 confirms the reliability of these models with high R^2, adjusted R^2, and predicted R^2 values (all above 0.75), low coefficients of variation (C.V. < 15%), and high adequate precision values. Overall, the models are robust, predictive, and suitable for optimizing MAE conditions for both target compounds.

2. 3. MAE-acetone 3D Surface Plots

MAE-acetone 3D surface plots for capsaicin are shown in Fig. 2 and for dihydrocapsaicin in Fig. 3.

2. 3. 1. Interactive Effect of Time and Power

MAE-acetone capsaicin 3D surface plot (Fig. 2A) illustrates the interactive effect of microwave power and microwave irradiation time on the extraction yield of capsaicin using acetone as the solvent, with a fixed solvent-to-solid ratio of 30 mL/g. The plot shows a saddle-like shape (dipping in the middle and rising on two sides, with a minimum in the center), indicating that both microwave power and extraction time significantly influence the capsaicin extraction. The curved nature of the surface and the changing slopes indicate a significant interaction between time and power. The effect of one factor depends on the level of the other.

At a lower power level (around 220 W), the yield of capsaicin is relatively low, regardless of the extraction time. The yield improves significantly as the microwave power increases to a moderate level (around 330 W). This suggests that an optimal power level is crucial for efficient extraction, as it likely facilitates better solvent penetration and effective disruption of plant cell walls, releasing more capsaicin. However, further increasing the power beyond 330 W seems to reduce the yield, possibly due to excessive heating, which could degrade capsaicin or reduce its solubility in acetone.

At shorter extraction times (20–30 s), the yield is low, likely because the solvent does not have enough time to fully extract capsaicin from the plant matrix. The yield increases with time, reaching a peak at around 50–55 s. Beyond this optimal extraction time, the yield plateaus or even decreases slightly, suggesting that prolonged exposure might lead to capsaicin degradation or the loss of solvent efficiency.

It can, thus, be concluded that the optimal capsaicin yield, around 20 mg/g DM, is achieved at a moderate microwave power level (~330 W) and an extraction time of approximately 55 s. Operating under these conditions likely balances efficient energy input and sufficient extraction time without causing significant thermal degradation or solvent evaporation. Over- or under-shooting these parameters can lead to suboptimal yields due to factors such as incomplete extraction, solvent evaporation, or compound degradation.

Low time and high power: This might lead to incomplete extraction because the solvent doesn't have enough time to fully penetrate the plant matrix and extract capsaicin. High power might also cause overheating, which can degrade capsaicin or reduce its solubility, leading to lower yields.

High time and low power: While this might prevent overheating, the low power may not provide enough energy to efficiently disrupt the plant cells and enhance the extraction. Prolonged extraction time can sometimes cause the solvent to become saturated, after which additional extraction time does not contribute much to increasing the yield.

Thus, the plot of time and power suggests that a moderate combination of time and power (around 330 W for 55 s) is optimal for maximizing capsaicin extraction yield. This balanced approach ensures efficient energy input for proper cell disruption and sufficient extraction time without causing degradation or inefficiency

2. 3. 2. Interactive Effect of Time and SSR

The 3D surface plot (Fig. 2B) illustrates the interactive effect of microwave irradiation time and solvent-to-solid ratio (SSR) on the yield of capsaicin when using a microwave power fixed at 330 W. The yield of capsaicin increases significantly with time initially, but shows a decreasing trend at prolonged extraction times. This suggests that longer extraction times may lead to the degrada-

tion of capsaicin or cause it to remain trapped within the solid matrix.

A higher solvent-to-solid ratio generally improves the yield up to an optimal point. Beyond this optimal SSR, the yield either remains constant or decreases, likely due to the dilution effect (where the solvent's capacity to extract capsaicin becomes less effective over extended periods) or insufficient solvent interaction with the solid matrix.

At a low extraction time, an increase in SSR leads to a high yield, as the solvent can effectively penetrate the solid matrix and extract capsaicin. As time increases with a low SSR, the yield decreases, indicating that longer exposure at low SSR does not benefit the extraction process.

Thus, for optimal capsaicin yield at 330 W microwave power, a balance between time and SSR is crucial. Medium SSR with medium to high extraction time seems to offer the best results. However, extremely high or low SSR combined with long extraction times can negatively affect the capsaicin yield. This indicates that while sufficient solvent is necessary for efficient extraction, too much solvent over extended time periods can counteract the benefits, leading to lower extraction yield from dry matter.

2. 3. 3. Interactive Effect of Power and SSR

This 3D surface plot (Fig. 2C) illustrates the interactive effect of microwave power (B) and solvent-to-solid ratio (SSR) (C) at a fixed extraction time of 40 s on the extraction yield of capsaicin. The plot demonstrates a noticeable interaction between microwave power and SSR. The highest yield of capsaicin is observed at high microwave power combined with an optimal SSR. However, at very high SSR values, even increasing power does not significantly improve the yield, suggesting that there is an optimal balance between the amount of solvent and the microwave energy applied. Thus, for maximizing capsaicin extraction yield from chili dry matter under these conditions, a moderate SSR around 25-30 mL/g combined with high microwave power near 330 W is the most effective. Going beyond this SSR or power does not yield substantial improvements and may even reduce efficiency due to over-dilution or excessive energy input, leading to potential degradation of capsaicin.

Considering all the interactive effects, the following conclusions can be drawn: a combination of low power (e.g., 220 W) and long time (e.g., 60 s), or, alternatively, short time (e.g., 20 s) and high power (e.g., 440 W), keeping a moderate SSR (e.g., 30 mL/g) is the most effective. These conditions should provide the most efficient extraction of capsaicin while minimizing energy usage. As the results (Table 1) show, a comparable capsaicin yield of around 20 mg/g DM was obtained under each of these two sets of conditions. However, the low-power strategy produced slightly better results.

3D Surface plots (MAE-acetone for dihydrocapsaicin (Fig. 3) demonstrating the interactive effect of (A) power and time, (B) SSR and time, and (C) SSR and power on dihydrocapsaicin extraction have important implications for extraction process optimization. Like those of capsaicin, these 3D plots of dihydrocapsaicin also have curvatures and saddle-like shapes. This is not unexpected because they show a quadratic model, which has been suggested by optimization and ANOVA operations. In 3D plot (Fig. 3A) (interactive effect of power and time), there is a prominent maximum increase at high power-high time. It may mean that dihydrocapsaicin requires harsher power-time conditions for maximum extraction. In 3D surface plot (Fig. 3B) (interactive effect of SSR and time), the maximum lies at high SSR and time, and SSR having a more drastic effect on the dihydrocapsaicin extraction as compared to time.

The interactive effect of SSR and power (at a fixed time of 40 s) on dihydrocapsaicin extraction (Fig. 3C) shows an interesting trend. The optimal extraction occurs at low power levels coupled with high SSR. This suggests

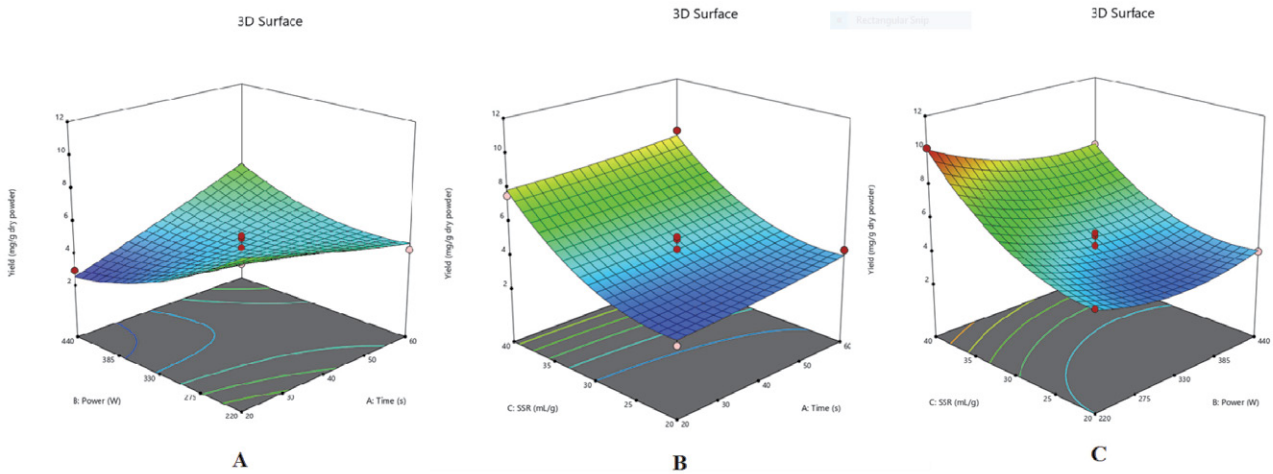

Fig. 3. 3D Surface plots for MAE-acetone displaying interactive impact of (A) power and time, (B) SSR and time, and (C) SSR and power on dihydrocapsaicin extraction. Red color shows high value and blue color shows low value.

that when a greater volume of solvent is present, the dihydrocapsaicin is more efficiently extracted, and further increasing the microwave power may slightly reduce the extraction efficiency. This could be due to the potential for excessive power to cause thermal degradation of the compound. Therefore, maintaining a low power while ensuring a high SSR appears to be the most effective approach for maximizing dihydrocapsaicin extraction outcomes under these specific conditions. In MAE, the amount of solvent plays a crucial role in how much heat is generated. A large volume of a polar solvent like acetone can lead to more heat generation by microwaves and result in a higher overall temperature in the extraction system, which may cause molecular degradation.

2. 4. MAE-ethyl Acetate 3D Surface Plots

MAE-ethyl acetate 3D surface plots for capsaicin are shown in Fig. 4 and for dihydrocapsaicin in Fig. 5. They closely resemble the 3D surface plots of MAE-acetone for capsaicin and dihydrocapsaicin, indicating similar conclusions. It means the overall impact of MAE-acetone and MAE-ethyl acetate on the extraction of these two phytochemicals is similar; that is, both solvents under the MAE framework show similar extraction behavior toward capsaicin and dihydrocapsaicin. As for exploring optimal conditions, both methods have similar practical implications. The 3D surface plots provided valuable insights into the optimal conditions for obtaining capsaicin and dihydrocapsaicin using MAE-acetone or MAE-ethyl acetate methods.

2. 5. Validation Study for MAE-acetone and MAE-ethyl Acetate

The numerical optimization method was conducted by keeping input factors in range and response at the 'maximize' option.[44] This suggested the following conditions for capsaicin: 60 s time, 220 W power, and 30 mL/g SSR,

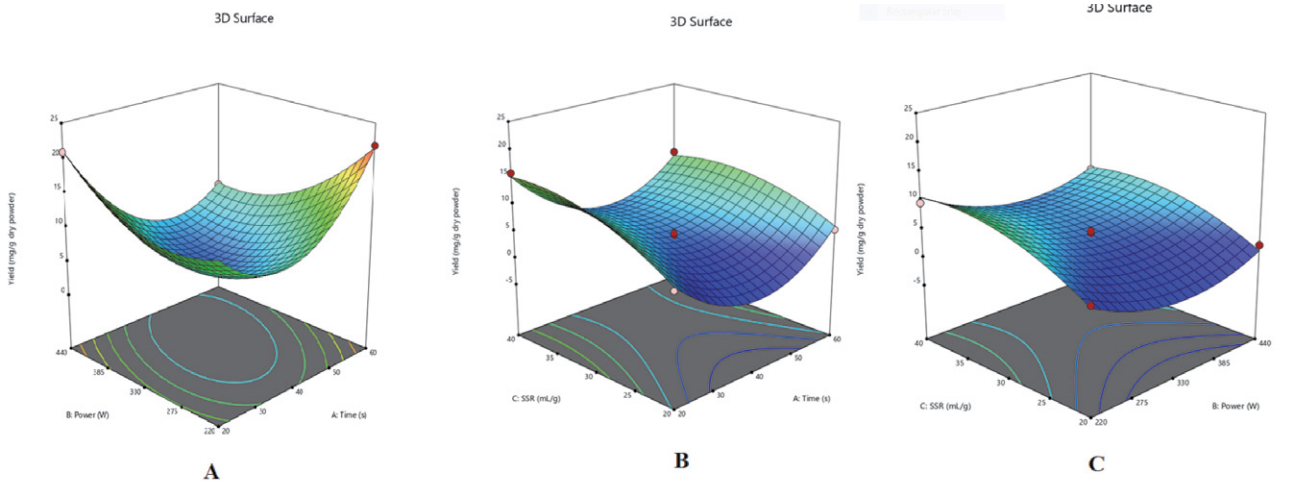

Fig. 4. 3D Surface plots for MAE-ethyl acetate displaying interactive impact of (A) power and time, (B) SSR and time, and (C) SSR and power on capsaicin extraction. Red color shows high value, and blue color shows low value.

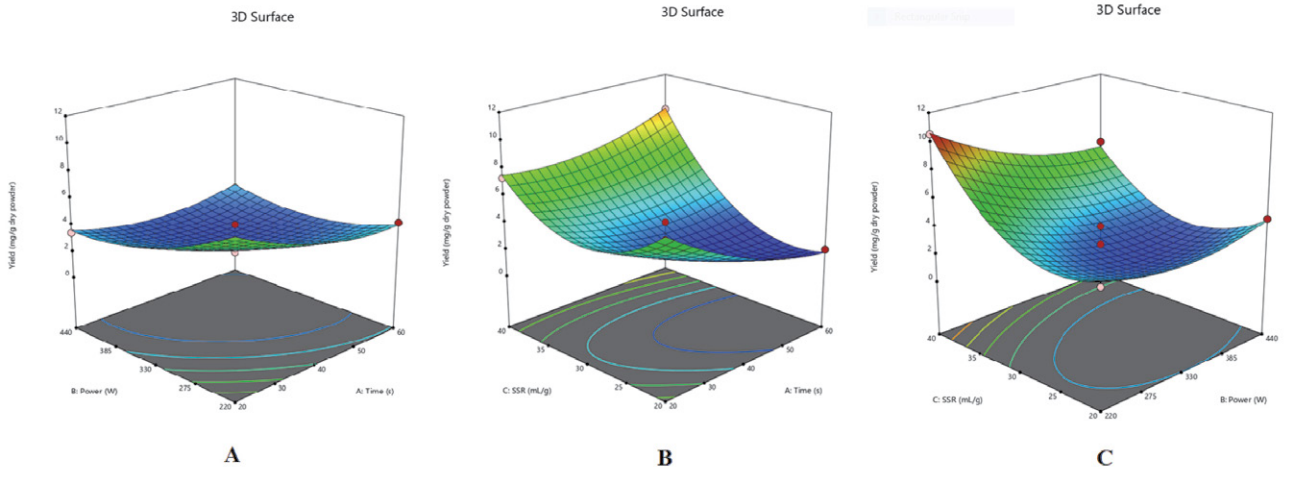

Fig. 5. 3D Surface plots for MAE-ethyl acetate displaying interactive impact of (A) power and time, (B) SSR and time, and (C) SSR and power on dihydrocapsaicin extraction. Red color shows high value, and blue color shows low value.

and for dihydrocapsaicin: 40 s time, 220 W power, and 40 mL/g SSR. The extraction yield of capsaicin and dihydrocapsaicin predicted for the optimal conditions and corresponding experimental values are shown in Table 6, along with %RSD (% relative standard deviations). It is notable that in each case, the %RSD value is very small, which is a strong indicator of the reliability of the predicted model.

Table 6. Validation study for MAE-acetone and MAE-ethyl acetate*.

MAE-acetone			
Responses	Predicted values mg/g DM	Experimental values mg/g DM	% RSD
Capsaicin	19.3	19.2	0.367
Dihydrocapsaicin	10.2	10.1	0.697
MAE-ethyl acetate			
Capsaicin	22.1	21.8	0.966
Dihydrocapsaicin	10.6	10.5	0.670

*Conditions for capsaicin: 60 s time, 220 W power, and 30 mL/g SSR; conditions for dihydrocapsaicin: 40 s time, 220 W power, and 40 mL/g SSR.

In MAE-ethyl acetate and MAE-acetone optimization, two different sets of conditions (power and time) can be identified that gave almost the same capsaicin yield (Table 1). The first set of conditions comprised a microwave irradiation time of 60 s, microwave power of 220 W, and SSR (solvent-to-solid ratio) of 30 mL/g, and the second set of conditions consisted of a microwave irradiation time of 20 s, microwave power of 440 W, and SSR of 30 mL/g.

Under the first set of conditions (60 s, 220 W), the extraction yield of capsaicin both in MAE-ethyl acetate and MAE-acetone was slightly higher than in the second set of conditions (20 s, 440 W).

In evaluating which set of conditions to prefer for industrial applications, several factors should be considered, including energy efficiency, consistency, extraction yield, and equipment wear and tear. The first set uses lower microwave power and a longer time, which generally results in a more gradual heating. This method is more energy-efficient as it uses less power, which can be crucial in large-scale operations to reduce operational costs and energy consumption. Similarly, operating at high power (440 W) could put more strain on the microwave equipment, potentially leading to more frequent maintenance or reduced equipment lifespan. Low power settings (220 W) are generally less harsh on equipment.

2. 6. Comparison of MAE-acetone and MAE-ethyl Acetate

Based on the comparison between MAE using acetone and MAE using ethyl acetate, it is evident that ethyl acetate is more efficient in extracting both capsaicin and dihydrocapsaicin.

Consequently, MAE-ethyl acetate may be preferred for industrial or research applications where maximizing the extraction yields of capsaicin and dihydrocapsaicin is critical. The differences in the efficiency of capsaicin and dihydrocapsaicin extraction between acetone and ethyl acetate can be attributed to several chemical factors related to the properties of these solvents, such as polarity, solubilizing ability, boiling point, affinity for lipophilic compounds, and interaction with microwave energy. Ethyl acetate is moderately polar, while acetone is slightly more polar. Capsaicin and dihydrocapsaicin are relatively less polar compounds. The moderate polarity of ethyl acetate may strike an optimal balance, allowing it to better dissolve these hydrophobic compounds compared to acetone. This increased solubility can lead to more efficient extraction. Ethyl acetate has a lower dielectric constant (6.02) compared to acetone (20.7). This lower dielectric constant of ethyl acetate means it interacts less strongly with polar solutes like water, making it more effective in extracting non-polar compounds such as capsaicin and dihydrocapsaicin. Ethyl acetate has a higher boiling point (77 °C) compared to acetone (56 °C). During microwave-assisted extraction, the slightly higher boiling point of ethyl acetate allows for a more controlled extraction process at elevated temperatures, reducing the risk of solvent evaporation and degradation of the target compounds. This can result in higher yields. Finally, the higher vapor pressure and lower boiling point of acetone make it more prone to rapid evaporation under microwave conditions. This can lead to lower extraction efficiencies as the solvent may evaporate before fully extracting the target compounds. Ethyl acetate, with its slightly higher boiling point and lower vapor pressure, can maintain its liquid state more effectively during microwave heating, leading to more efficient extraction.

Thus, MAE-ethyl acetate is preferable not only based on its safety but also efficiency.[45] Ethyl acetate has a lower polarity index compared to acetone, which makes it more effective in extracting slightly less polar compounds. This might explain why, under similar MAE conditions, ethyl acetate results in a slightly higher yield.

2. 7. Comparison with Previous Studies

The extraction of capsaicin and related capsaicinoids from chili peppers has been the focus of numerous studies, each employing a variety of solvents and extraction techniques. Waqas et al. (2022) conducted a comparative analysis of different solvents for capsaicin extraction, revealing that ethyl acetate was notably effective, yielding 7.4 mg/g of capsaicin, while acetone yielded 2.9 mg/g.[24] These results underscore the variability in extraction efficiency based on solvent choice. In contrast, our study achieved significantly higher extraction yields using the same solvents—highlighting the superior efficiency of our opti-

mized extraction strategy. Williams et al. (2004) used MAE with ethanol to extract capsaicinoids from fresh pepper samples, achieving a yield of around 86.36%.[26] This study aligns with the growing body of research demonstrating the effectiveness of MAE in maximizing bioactive compound yield. Furthermore, Hussain et al. (2022) explored the use of deep eutectic solvents (DESs), choline chloride-urea, and choline chloride-glycerol in MAE, reporting capsaicin yields of 18.88 mg/g and 14.49 mg/g, respectively.[27] Notably, the yield obtained with choline chloride-urea in their study is comparable to the results of our current research, further validating the efficacy of MAE in capsaicin extraction.

The observed variations in extraction yield across different studies can be attributed to several factors, including the nature of the sample, the specific chili pepper varieties or cultivars used, geographical and environmental influences, and the extraction techniques and conditions employed. For instance, the maturity of the chili peppers, the presence of other bioactive compounds, and the solvent's polarity all play critical roles in determining extraction efficiency.[47,48]

In addition, differences in microwave power, irradiation time, and solvent-to-solid ratios significantly influence the outcomes of MAE processes. These parameters, when optimized, can lead to higher yield and greater extraction efficiency. The ability of the current study to achieve a higher extraction yield with both ethyl acetate and acetone suggests that careful optimization of MAE parameters, coupled with the selection of appropriate solvents, can significantly enhance the extraction of capsaicinoids from chili peppers.

To conclude, the findings of this research not only demonstrate the effectiveness of MAE using ethyl acetate and acetone but also contribute to the growing body of knowledge on the optimal conditions for capsaicin and dihydrocapsaicin extraction, with potential applications in both the food and pharmaceutical industries. This work highlights the importance of continued research and method optimization in the field of natural product extraction.

3. Conclusions

Capsaicin and dihydrocapsaicin are significant bioactive compounds found across various species of the Capsicum genus, known for their wide-ranging health benefits and industrial applications. This study focused on evaluating and comparing the efficiency of two solvent systems—acetone and ethyl acetate—in the microwave-assisted extraction (MAE) of these compounds from green peppers (*Capsicum annuum* L.). Using response surface methodology (RSM), the extraction conditions were meticulously optimized, with the resulting models validated through statistical analysis and experimental data. Ethyl acetate under MAE proved to be a more efficient solvent for capsaicin extraction from chili peppers than acetone, with an extraction yield of 22.1 mg/g dry matter (DM) as compared to 19.3 mg/g DM in acetone, while these solvents showed comparable efficiency for dihydrocapsaicin, with a yield of around 10 mg/g DM in both. The minimal %RSD between observed and predicted values demonstrated RSM as a viable optimization technique for the extraction systems investigated in this study.

Thus, under the MAE setup, ethyl acetate has been demonstrated to be a viable extraction medium for capsaicin and dihydrocapsaicin. This makes it an attractive option for large-scale industrial applications focused on the extraction of these valuable bioactive compounds.

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4. References

1. M. Kaiser, I. Higuera, F. M. Goycoolea, *Fruit Veg. Phytochem. Chem. Hum. Health.* **2017**, 499–514. **DOI:**10.1002/9781119158042.ch23
2. E. M. Petran, A. Periferakis, L. Troumpata, A. T. Periferakis, A. E. Scheau, I. A. Badarau, K. Periferakis, A. Caruntu, I. Savulescu-Fiedler, R. M. Sima, et al., *Curr. Issues Mol. Biol.* **2024**, *46*, 7895–7943. **DOI:**10.3390/cimb46080468
3. E. S. Fernandes, A. R. A. Cerqueira, A. G. Soares, S. K. Costa, *Drug Discov. Mother Nat.* **2016**, 91–125. **DOI:**10.1007/978-3-319-41342-6_5
4. J. Zheng, S. Zheng, Q. Feng, Q. Zhang, X. Xiao, *Biosci. Rep.* **2017**, *37(3)*, BSR20170286. **DOI:**10.1042/BSR20170286
5. W. Zhang, Y. Zhang, J. Fan, Z. Feng, X. Song, *Mol. Med. Rep.* **2024**, *29(3)*, 1–8. **DOI:**10.3892/mmr.2024.13162
6. W. Zhang, Q. Zhang, L. Wang, Q. Zhou, P. Wang, Y. Qing, C. Sun, *Br. J. Nutr.* **2023**, *130(9)*, 1645–1656. **DOI:**10.1017/S0007114523000697
7. G. Sethi, M. K. Shanmugam, S. Warrier, M. Merarchi, F. Arfuso, A. P. Kumar, A. Bishayee, *Nutrients* **2018**, *10(5)*, 645. **DOI:**10.3390/nu10050645
8. M. Yasin, L. Li, M. Donovan-Mak, Z. H. Chen, S. K. Panchal, *Foods* **2023**, *12(4)*, 907. **DOI:**10.3390/foods12040907
9. Y. Xiang, X. Xu, T. Zhang, X. Wu, D. Fan, Y. Hu, R. Xie, *Exp. Cell Res.* **2022**, *417(2)*, 113227. **DOI:**10.1016/j.yexcr.2022.113227
10. S. Kumar, M. Sarpras, F. Mushtaq, S. Singh, A. Thattantavide, A. Kumar, *Capsaicinoids: From Nat. Sources to Biosynth. and Clin. Appl.* **2024**, 1–24, Springer Nature, Singapore. **DOI:**10.1007/978-981-99-7779-6_1

11. L.G. Po, M. Siddiq, T. Shahzad, *Handb. Veg. Veg. Process*. **2018**, 633–660. **DOI:**10.1002/9781119098935.ch27
12. S.C. Hespeler, H. Nemati, E. Dehghan-Niri, *Artif. Intell. Agric*. **2021**, *5*, 102–117. **DOI:**10.1016/j.aiia.2021.05.003
13. T. Hernández-Pérez, M. D. R. Gómez-García, M. E. Valverde, O. Paredes-López, *Compreh. Rev. Food Sci. Food Saf.* **2020**, *19(6)*, 2972–2993. **DOI:**10.1111/1541-4337.12634
14. K. Bhutia, V. Khanna, T. Meetei, N. Bhutia, *Agrotechnology* **2018**, *7(2)*, 1–4. **DOI:**10.4172/2168-9881.1000180
15. Q. Ishfaqe, A. Sami, M. Zeshan Haider, A. Ahmad, M. Shafiq, Q. Ali, M. A. Manzoor, *Front. Microbiol.* **2024**, *15*, 1437553. **DOI:**10.3389/fmicb.2024.1437553
16. K. R. Karim, M. Y. Rafii, A. B. Misran, M. F. B. Ismail, A. R. Harun, M. M. H. Khan, M. F. N. Chowdhury, *Agronomy* **2021**, *11(11)*, 2217. **DOI:**10.3390/agronomy11112217
17. H. E. Cortes-Ferre, M. Antunes-Ricardo, J. A. Gutiérrez-Uribe*, Front. Nutr.* **2022**, *9*, 942805. **DOI:**10.3389/fnut.2022.942805
18. M. Lu, C. T. Ho, Q. Huang, *J. Food Drug Anal*. **2017**, *25(1)*, 27–36. **DOI:**10.1016/j.jfda.2016.10.023
19. P. F. Pradana, M. D. D. Khoirul, E. A. Septian, *J. Eng*. **2017**, *3*, 6–10. **DOI:**10.12962/joe.v3i1.2265
20. Q. Yang, A. M. Mariga, L. Wen, Q. Hu, W. Yang, M. Xie, J. Liu, F. Pei, *Food Chem*. **2025**, *464(Pt 2)*, 141773. **DOI:**10.1016/j.foodchem.2024.141773
21. H. E. Cortés-Ferré, D. Guajardo-Flores, G. Romero-De La Vega, J. A. Gutierrez-Uribe, *Front. Sustain. Food. Syst.* **2021**, *4*, 588534. **DOI:**10.3389/fsufs.2020.588534
22. K. A. Avilés-Betanzos, M. Scampicchio, G. Ferrentino, M. O. Ramírez-Sucre, I. M. Rodríguez-Buenfil, *Processes* **2023**, *11(8)*, 2272. **DOI:**10.3390/pr11082272
23. A. C. de Aguiar, P. dos Santos, J. P. Coutinho, G. F. Barbero, H. T. Godoy, J. Martínez, *LWT-Food Sci. Tech*. **2014**, *59(2)*, 1239–1246. **DOI:**10.1016/j.lwt.2014.06.014
24. M. Waqas, D. Ahmed, M. T. Qamar, *Heliyon* **2022**, *8(8)*, e10273. **DOI:**10.1016/j.heliyon.2022.e10273
25. S. Chuichulcherm, S. Prommakort, P. Srinophakun, A. Thanapimmetha, *Ind. Crop. Prod.* **2013**, *44*, 473–479. **DOI:**10.1016/j.indcrop.2012.10.007
26. O. J. Williams, G. V. Raghavan, V. Orsat, J. Dai, *J. Food Biochem.* **2004**, *28(2)*, 113–122. **DOI:**10.1111/j.1745-4514.2004.tb00059.x
27. M. Hussain, M. T. Qamar, D. Ahmed, *Int. J. Veg. Sci.* **2022**, *28(4)*, 312–319. **DOI:**10.1080/19315260.2021.1960459
28. A. M. Sousa, V. D. Alves, S. Morais, C. Delerue-Matos, M. P. Gonçalves, *Bioresour. Technol.* **2010**, *101(9)*, 3258–3267. **DOI:**10.1016/j.biortech.2009.12.061
29. H. López-Salazar, B. H. Camacho-Díaz, M. A. Ocampo, A. R. Jiménez-Aparicio, *Bioresources* **2023**, *18(3)*, 6614. **DOI:**10.15376/biores.18.3.Lopez-Salazar
30. R. F. Remler, *Ind. Eng. Chem.* **1923**, *15(7)*, 717–720. **DOI:**10.1021/ie50163a023
31. B. González, N. Calvar, E. Gómez, Á. Domínguez, *J. Chem. Thermodyn*. **2007**, *39(12)*, 1578–1588. **DOI:**10.1016/j.jct.2007.05.004
32. J. T. Timothy, S. K. Purdy, J. Shen, F. B. Nelson, R. Mustafa, D. J. Wiens, M. J. Reaney, *Toxicol. Rep.* **2021**, *8*, 785–792. **DOI:**10.1016/j.toxrep.2021.03.034
33. F. Chemat, M. Abert Vian, H. K. Ravi, B. Khadhraoui, S. Hilali, S. Perino, A. S. Fabiano Tixier, *Molecules* **2019**, *24(16)*, 3007. **DOI:**10.3390/molecules24163007
34. S. S. Efimova, O. S. Ostroumova, *Membranes* **2023**, *13(4)*, 453. **DOI:**10.3390/membranes13040453
35. K. R. Reshna, S. Gopi, P. Balakrishnan, *Am. Chem. Soc.* **2022**, 1–19. **DOI:**10.1021/bk-2022-1433.ch001
36. Y. P. Silva, T. A. Ferreira, G. B. Celli, M. S. Brooks, *Waste Biomass Valori.* **2019**, *10*, 2851–2861. **DOI:**10.1007/s12649-018-0317-7
37. A. Nooraziah, V. J. Tiagrajah, *Appl. Mech. Mater.* **2014**, *666*, 235–239. **DOI:**10.4028/www.scientific.net/AMM.666.235
38. Y. Lu, B. Cui, *Molecules* **2019**, *24(21)*, 3956. **DOI:**10.3390/molecules24213956
39. R. H. Myers, D. C. Montgomery, C. M. Anderson-Cook, *Response Surf. Methodol.* **2002**, *2*, 328–333. **DOI:**10.1080/00224065.2017.11917988
40. M. Anis, D. Ahmed, N. Anis, *Folia Hortic.* **2022**, *34(2)*, 249–262. **DOI:**10.2478/fhort-2022-0019
41. M. M. A. Iqbal, W. A. W. A. Bakar, S. Toemen, F. I. A. Razak, N. I. W. Azelee, *Arab. J. Chem.* **2020**, *13(2)*, 4170–4179. **DOI:**10.1016/j.arabjc.2019.06.010
42. K. R. Bryenton, A. R. Cameron, K. L. Kirk, N. Saad, P. Strongman, N. Volodin, *Algorithms* **2020**, *13(11)*, 286. **DOI:**10.3390/a13110286
43. C. L. Guo, H. Y. Chen, B. L. Cui, Y. H. Chen, Y. F. Zhou, X. S. Peng, Q. Wang, *Int. J. Anal. Chem.* **2015**, *2015(1)*, 912631. **DOI:**10.1155/2015/912631
44. N. K. Alruwaili, *Int. J. Anal. Chem.* **2021**, *2021(1)*, 8833900. **DOI:**10.1155/2021/8833900
45. F. Sohail, D. Ahmed, *Acta Chim. Slov.* **2023**, *70(4)*, 533–544. **DOI:**10.17344/acsi.2023.8419
46. I. Atique, D. Ahmed, M. Maqsood, W. Malik, *Int. J. Veg. Sci.* **2018**, *24(3)*, 212–226. **DOI:**10.1080/19315260.2017.1409304
47. M. J., Akhter, S. Akhter, S. Islam, M. S. H. Sarker, S. K. Hasan, *Heliyon* **2024**, *10(17)*, e37406. **DOI:**10.1016/j.heliyon.2024.e37406
48. E. Herrera-Pool, A. L. Ramos-Díaz, J. D. Padilla de la Rosa, U. García-Cruz, M. A. Lizardi-Jiménez, T. Ayora-Talavera, J. C. Cuevas-Bernardino, N. Pacheco, *Food Sci.* **2025**, *90(1)*, e17630. **DOI:**10.1111/1750-3841.17630

Povzetek

Kapsaicin in dihidrokapsaicin sta naravni spojini z visoko vrednostjo. V tej študiji smo optimizirali ekstrakcijo z mikrovalovi (MAE) za ekstrakcijo teh spojin iz *Capsicum annuum* L. z uporabo dveh topil: etil acetat in aceton. Optimizacijo smo izvedli z metodo odzivne površine (RSM), za kvantifikacijo spojin pa smo uporabili visokoločljivostno tekočinsko kromatografijo (HPLC). Upoštevane neodvisne spremenljivke so bile moč mikrovalov (W), čas obsevanja (s) in razmerje topilo-trdna snov (SSR). Za MAE z acetonom sta bila optimalna izkoristka 19,3 mg/g suhe snovi (s.s.) za kapsaicin in 10,2 mg/g s.s. za dihidrokapsaicin. MAE z etil acetatom je dala višje količine: 22,1 mg/g s.s. za kapsaicin in 10,6 mg/g s.s. za dihidrokapsaicin. Optimalni pogoji za kapsaicin v obeh topilih so bili 60 s, 220 W in 30 mL/g SSR, za dihidrokapsaicin pa 40 s, 220 W in 40 mL/g SSR. Tako se je MAE z etil acetatom izkazala kot bolj učinkovita kot MAE z acetonom za ekstrakcijo obeh spojin. Torej je bila izbrana zaradi svoje učinkovitosti in okoljske varnosti ter je obetavna tehnika za industrijske aplikacije.