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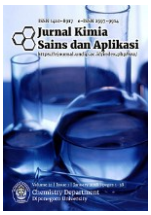

Optimizing Maceration Extraction of Myristicin and Methyl Eugenol from Nutmeg (*Myristica fragrans* Houtt.): An RSM Box–Behnken Approach

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Abstract

Myristicin is the key marker of nutmeg (*Myristica fragrans*) quality, while methyl eugenol is an undesirable compound requiring strict monitoring. This study aimed to optimize the extraction conditions of these compounds from nutmeg seeds and mace as a pretreatment step prior to electrochemical quantification. Optimization was performed using Response Surface Methodology (RSM) with a Box–Behnken Design (BBD), considering extraction time (h), ethanol concentration (%), and the sample-to-solvent ratio (g/mL) as independent variables. The analytes were quantified by Gas Chromatography–Mass Spectrometry (GC-MS), and the experimental data were modeled using Design Expert software. Regression models indicated that ethanol concentration and solvent volume significantly influenced extraction yields, while extraction time had a statistically minimal effect (p-values > 0.05, small effect sizes). Nevertheless, practical differences were observed between matrices: nutmeg seeds reached optimal extraction within 2 hours, whereas mace required 17 hours, likely due to its higher essential oil content and fibrous structure, which slows diffusion kinetics. Thus, although time effects were not statistically significant, extended extraction was practically preferred for mace to ensure adequate recovery. Response definitions were specified as follows: Y1 = myristicin concentration (mg/mL), Y2 = methyl eugenol concentration (mg/mL), and % extraction yield. The optimal extraction conditions were achieved with 90% ethanol and 50 mL solvent, maximizing analyte detectability while maintaining reproducibility across seed and mace extracts. These optimized ethanolic matrices are compatible with subsequent electrochemical quantification, given their aqueous/ethanolic composition and anticipated sensitivity of electrochemical detection.

1. Introduction

Nutmeg (*Myristica fragrans*), a native Indonesian plant, is a strategically valuable export commodity. *Myristica fragrans* Houtt., commonly known as nutmeg, produces two valuable spices: nutmeg and mace. Both have been widely used not only in culinary traditions but also in traditional medicine due to their broad spectrum

of pharmacological activities. Among the many pharmacological activities found in nutmeg and mace, myristicin is considered the main bioactive component. These compounds are primarily responsible for nutmeg's distinctive aroma, as well as various biological properties such as antibacterial and anti-inflammatory effects [1], antiplaque [2], anticancer [3, 4], antiangiogenic [5], and antirheumatic [6].

The quality of Indonesian nutmeg as an export commodity remains a challenge. One of these is the presence of methyl eugenol, a compound reported to have carcinogenic potential [7, 8]. High methyl eugenol content can degrade nutmeg quality and limit compliance with international standards. Therefore, efforts are needed to determine the levels of hazardous substances and active ingredients in nutmeg. One method for determining these levels is using electrochemistry. Electrochemical methods have several advantages over Gas Chromatography–Mass Spectrometry, particularly in terms of efficiency and ease of operation. This technique requires lower equipment and operational costs, faster analysis times, and relatively simple sample preparation without the need for complex separation or derivatization processes. Furthermore, electrochemical methods can be developed into portable sensors for field applications and real-time monitoring, with lower solvent consumption, making them more environmentally friendly. The flexibility of electrode surface modification also allows for increased sensitivity and selectivity, making them ideal for rapid screening of redox-active compounds. Before determining the levels electrochemically, all compounds to be determined must first be extracted.

Extraction is an important step prior to quantitative analysis, as extraction conditions greatly affect the results. One of the extraction methods is maceration. Maceration is widely used due to its simplicity, relatively low cost, and ability to maintain the stability of thermolabile compounds such as myristicin. However, this method has limitations, including long extraction times, large solvent requirements, and lower efficiency compared to modern techniques. Nevertheless, maceration remains an important basic method in phytochemical studies and can serve as a starting point for developing more efficient and cost-effective methods, as well as being easy to apply, since no specific apparatus is required [9].

Several studies have reported the development of extraction methods by optimizing maceration parameters [10, 11]. Determining efficient maceration parameters can be developed using experimental designs [9], including using Response Surface Methodology (RSM) [11]. RSM, using Expert Design software based on Box–Behnken Design (BBD), allows for efficient evaluation of variable interactions with minimal trials and has been successfully applied to optimize natural product extraction [12, 13, 14]. This approach allows for evaluation of interactions between variables with a minimal number of trials, while simultaneously increasing the efficiency and accuracy of extraction optimization [15, 16].

Based on this background, the study aims to determine optimal maceration conditions for extracting myristicin and methyl eugenol from nutmeg. The solvents to be used are water and a water-ethanol mixture, as analyte measurements by electrochemistry require aqueous solutions. The optimization objectives are threefold: (a) maximizing the extraction of myristicin and methyl eugenol, (b) enabling accurate monitoring of both analytes, and (c) minimizing solvent consumption. Ethanol was chosen due to its polarity and effectiveness in extracting semi-polar compounds such as myristicin and methyl eugenol. To evaluate its performance, ethanol concentration was varied within the range of 60–90%. The macerated nutmeg seed extract was evaporated using a rotary evaporator until a concentrated extract was obtained. This evaporation was expected to completely remove the ethanol solvent from the extract.

The maceration extract with various ethanol concentrations was then evaporated using a rotary evaporator to obtain a concentrated extract. The concentrated extract obtained after rotary evaporation was free of ethanol. This concentrated extract will be used for actual sample measurement by dissolving it in Britton Robinson buffer pH 5 using the electrochemical sensor principle of a Glassy Carbon Electrode (GCE) modified with Fe_3O_4-graphene [17]. The results are expected to contribute to the detection of bioactive compounds, support quality standards for Indonesian nutmeg products, and open up opportunities for its use in the pharmaceutical sector and the development of natural product-based industries.

2. Experimental

2.1. Materials

The nutmeg seed and mace samples used for extraction were nutmeg (*Myristica fragrans* Houtt.) purchased from farmers in Sitaro Regency, North Sulawesi, Indonesia. Myristicin (Sigma-Aldrich) and methyl eugenol standards (CV. WINGJA). The solvents used for extraction were analytical grade ethanol and hexane (Sigma-Aldrich).

2.2. Experimental Design

The study began with the preparation of nutmeg seeds and mace, which were dried and ground into powder. The resulting powder was used for extraction optimization by varying ethanol concentration (A, 60–90%), sample-to-solvent ratio (B, 10 g sample to 50–150 mL solvent), and extraction time (C, 2–24 hours). The experimental design was established using Response Surface Methodology (RSM) with Box-Behnken Design (BBD) [17], comprising 12 experimental runs as presented in Table 1. All experiments were performed at room temperature, with manual stirring for about 3 minutes at the beginning and every 30 minutes thereafter. The maceration process was sealed using aluminium foil to limit the evaporation. The success of myristicin and methyl eugenol extraction from nutmeg seeds and mace was evaluated based on the amount of both analytes extracted, as determined by GC-MS. Optimal conditions were identified using Design-Expert 13.0.5.0.

Table 1. Experimental design for extraction conditions in the seeds and mace of nutmeg

Standard order	Run	Independent Variable		
		A. Concentration of ethanol (%)	B. Sample-to-solvent ratio (10 g sample mass to mL solvent volume, w/v)	C. Time of maceration (hours)
9	1	75	50	2
7	2	60	100	24
2	3	90	50	13
10	4	75	150	2
5	5	60	100	2
11	6	75	50	24
6	7	90	100	2
4	8	90	150	13
8	9	90	100	24
3	10	60	150	13
12	11	75	150	24
1	12	60	50	13

2.2.1. Extraction Yield

The extract of all runs was then evaporated using a rotary evaporator to get a dried extract. The extraction yield was determined as the weight of the dried extract divided by the weight of the dried samples. The extraction yield was reported as % yield (dry extract mass or dry sample mass) [18]. The reported data are the means from three replicates.

2.2.2. Determination of the Amount of Analyte in the Extract

The quantitative amounts of analytes (myristicin and methyl eugenol) in the extract were determined using an Agilent Technologies 7890B GC-MS with a capillary column HP–5MS UI (length 30 m × diameter 0.25 mm × film thickness 0.25 µm). The oven temperature was initially set to 50°C for 5 minutes, then raised at 5°C/minute to 280°C, and held for 20 minutes. Injection port temperature 280°C with splitless mode and injection volume of 1 µL. Helium with a flow rate of 1 mL/minute was used as the carrier gas. MS Parameters were a m/z range of 35–600 with an Electron Impact (EI) system [19].

The standards of myristicin and methyl eugenol at serial concentrations were injected into the GC-MS system. The correlation between analyte concentration and the peak area was used to construct a standard curve. The amount of analyte in the dried extracts was determined from the peak area of the corresponding analyte in the extract, with extraction yields and solvent volumes used for correction. Each sample dried extract (10 mg) was dissolved in 2 mL of n-hexane by ultrasonication for 1 hour, then filtered through a 0.22 µm syringe filter. About 1 µL of the sample was injected into the GCMS.

2.2.3. Statistical Analysis

A statistical analysis of this optimization design was carried out following Juliana *et al.* [17], with slight modifications. Data were analyzed using Design Expert 13.0.5.0 statistical software. Analysis of variance (ANOVA) was used to determine the coefficients for linear, quadratic, and interaction terms in the regression. Response surface plots were obtained for each interaction. Furthermore, the model's suitability was confirmed by comparing the predicted and experimental values with the R^2 value. The selected optimum condition is the one that yields the highest combined yield of the two analytes.

3. Results and Discussion

3.1. Extraction Yield

The extraction yield was calculated, and the results for all experimental conditions are presented in Table 2. The extraction yield of mace was consistently higher than that of the seeds [20]. The extraction yield also depends on factors such as the solvent used, the sample-to-solvent ratio, and the extraction time [21]. However, a high extraction yield does not always correspond to a higher number of analytes extracted. Therefore, to confirm the actual number of analytes obtained, their concentrations must be quantified.

3.2. Analyte Concentration on Extract

The myristicin and methyl eugenol belong to the phenylpropanoid group [22, 23], which can be determined by GC-MS. The retention times of myristicin and methyl eugenol were 24.4 and 27.3 minutes, respectively. Using the standard curve, the amount of the analytes was determined. The chromatogram of the seed and mace extract is shown in Figure 1. Based on the chromatogram, the myristicin peak is the dominant, while the methyl eugenol peak is minor.

The extraction yields and concentrations of myristicin and methyl eugenol in nutmeg seed and mace extracts showed considerable variation across the different experimental runs (Table 2). The amount of myristicin in the mace extract is about twice that compared to the seed extract, indicating that mace possesses a higher extractive capacity under the applied conditions. This agrees with the previous report that myristicin, a marker compound of nutmeg quality, is predominantly concentrated in the mace [24, 25].

Table 2. Responses from experimental design and responses of the dependent variables to the extraction condition in the seeds and mace of nutmeg

Run	Seeds			Mace		
	% extraction yield	mg myristicin in mL extract (Y1)	mg methyl Eugenol in mL extract (Y2)	% extraction yield	mg myristicin in mL extract (Y1)	mg methyl Eugenol in mL extract (Y2)
1	7.836	0.164	0.004	12.201	0.303	0.029
2	11.028	0.042	0.002	14.507	0.119	0.010
3	8.014	0.110	0.003	17.330	0.377	0.040
4	11.985	0.030	0.001	22.521	0.126	0.010
5	10.956	0.062	0.002	13.996	0.143	0.014
6	7.930	0.113	0.003	12.176	0.285	0.034
7	9.877	0.120	0.003	20.784	0.276	0.032
8	11.908	0.081	0.002	21.273	0.187	0.022
9	11.215	0.097	0.002	20.131	0.251	0.029
10	11.026	0.029	0.001	15.576	0.128	0.012
11	13.451	0.069	0.002	21.361	0.184	0.023
12	7.725	0.062	0.003	12.978	0.284	0.033

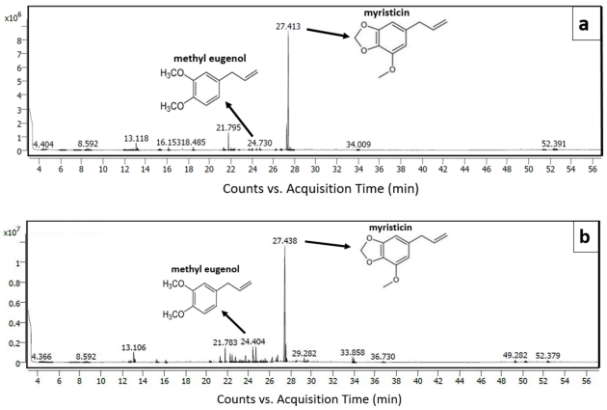

Figure 1. GCMS chromatogram of (a) seed extract and (b) mace extract

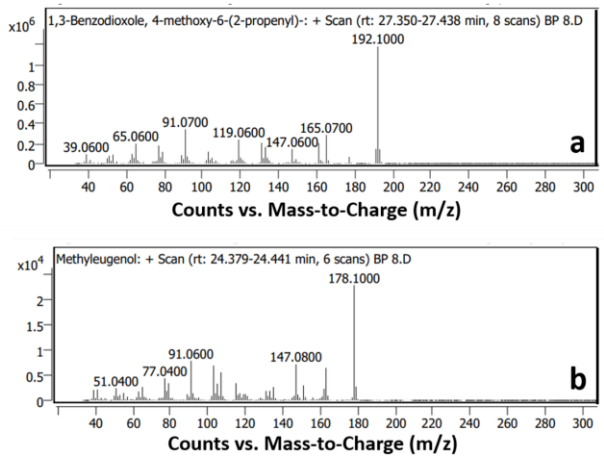

Figure 2. Mass spectrometry spectrum of (a) myristicin and (b) methyl eugenol

Methyl eugenol extracted from mace is also higher than from seed extract. Although methyl eugenol is considered an undesirable compound due to safety concerns, its higher presence in mace indicates the need for careful monitoring and selective extraction methods when targeting mace-derived products. Interestingly, the runs with the highest mace extraction yields (Runs 4, 8, and 11) also showed higher concentrations of both myristicin and methyl eugenol, suggesting a positive correlation between extraction yield and analyte recovery. However, the ratio between desirable (myristicin) and undesirable (methyl eugenol) compounds varied.

Mass spectrum data confirmed the identity of both target compounds (Figure 2). Peaks at m/z 178 and 147 identified methyl eugenol, where fragment 147 was formed due to the removal of the methoxy group from the main structure. Meanwhile, peaks at m/z 193 and 165 confirmed the presence of myristicin, with fragment 165 showing a characteristic side chain or dioxol ring cleavage pattern. These results are consistent with the literature and support the quantitative data obtained from RSM.

3.3. Model and Analysis of Variance (ANOVA)

Based on the myristicin and methyl eugenol concentrations in the nutmeg seed extract, the suggested models for both are linear. ANOVA for both myristicin and methyl eugenol concentrations showed significant results ($p < 0.05$). The residuals for each extract were nearly zero, indicating that the three-factor design influenced all responses. The following ANOVA results for each response are shown in Table 3. The same pattern was found in the nutmeg mace extract. The suggested model is linear for myristicin and methyl eugenol ($p < 0.05$).

Table 3a. ANOVA data of the extraction condition with a linear model for response 1: concentration of myristicin in seeds

Source	Sum of Square	df	Mean Square	F-value	p-value	
Model	67.17	3	22.39	4.02	0.0514	Not significant
A-concentration of ethanol	57.20	1	57.20	10.26	0.0126	
B-volume of ethanol	9.84	1	9.84	1.77	0.2206	
C-time of maceration	0.1260	1	0.1260	0.0226	0.8842	
Residual	44.60	8	5.57			
Cor total	111.77	11				

Table 3b. ANOVA data of the extraction condition with a linear model for response 2: concentration of methyl eugenol in seeds

Source	Sum of Square	df	Mean Square	F-value	p-value	
Model	0.0202	3	0.0067	4.97	0.0310	significant
A-concentration of ethanol	0.0117	1	0.0117	8.62	0.0188	
B-volume of ethanol	0.0081	1	0.0081	6.00	0.0399	
C-time of maceration	0.0004	1	0.0004	0.3038	0.5966	
Residual	0.0108	8	0.0014			
Cor total	0.0310	11				

Table 3c. ANOVA data of extraction condition with a linear model for response 1: concentration of myristicin in mace

Source	Sum of Square	df	Mean Square	F-value	p-value	
Model	323.25	3	107.75	12.01	0.0025	significant
A-concentration of ethanol	200.12	1	200.12	22.31	0.0015	
B-volume of ethanol	122.08	1	122.08	13.61	0.0061	
C-time of maceration	1.04	1	1.04	0.1158	0.7423	
Residual	71.76	8	8.97			
Cor total	395.00	11				

Table 3d. ANOVA data of the extraction condition with a linear model for response 2: concentration of methyl eugenol in mace

Source	Sum of Square	df	Mean Square	F-value	p-value	
Model	5.98	3	1.99	6.20	0.0175	significant
A-concentration of ethanol	4.31	1	4.31	13.42	0.0064	
B-volume of ethanol	1.37	1	1.37	4.28	0.0724	
C-time of maceration	0.2923	1	0.2923	0.9103	0.3680	
Residual	2.57	8	0.3211			
Cor total	8.55	11				

The model F-value of 4.02 implies a 5.14% chance that an F-value this large could occur by chance. p-values less than 0.0500 indicate model terms are significant. In this case, A is a significant model term (Table 3a). Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The model F- value of 4.97 implies the model is significant. There is only a 3.10% chance that an F-value this large could occur due to noise. p-values less than 0.0500 indicate model terms are significant (Table 3b). In this case, A and B are significant model terms. The model F-value of 12.01 implies the model is significant. There is only a 0.25% chance that an F-value this large could occur due to noise. p-values less than 0.0500 indicate model terms are significant (Table 3c). In this case, A and B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The model F-value of 6.20 implies the model is significant. There is only a 1.75% chance that an F-value this large could occur due to noise. p-values less than 0.0500 indicate model terms are significant (Table 3d). In this case, A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

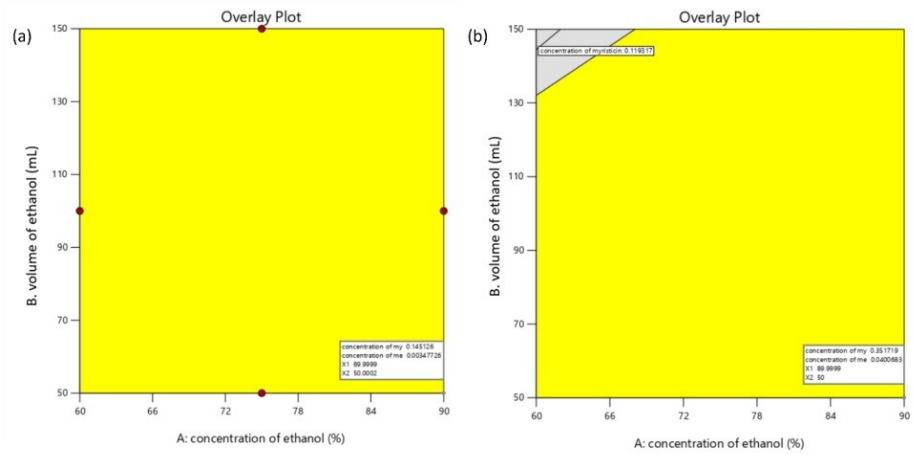

Figure 3. Graphical overlay plot: concentration of myristicin at (a) 2 hours in seeds, (b) 17 hours in mace

Table 4. The linear equations for myristicin concentration and methyl eugenol concentration in nutmeg seeds and mace

Samples	Y	Linear equations
Seeds	mg myristicin in mL extract	= +0.017691 + 0.001764 A − 0.000601 B − 0.000629 C
	mg methyl eugenol in ml extract	= +0.002209 + 0.000023 A − 0.000016 B − 0.000012 C
Mace	mg myristicin in mL extract	= +0.118308 + 0.003479 A − 0.001560 B − 0.000099 C
	mg methyl eugenol in ml extract	= +0.003584 + 0.000476 A − 0.000170 B + 0.000126 C

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor, and the intercept is not at the center of the design space (Table 4).

Based on the results and the kinetics analysis of the process, increasing the solvent volume in the maceration method does not always directly correlate with an increase in analyte concentration in the extract. Although the addition of solvent can increase the total amount of dissolved compounds through increasing the diffusion gradient in the initial stage, once the system reaches distribution equilibrium between the matrix and the liquid phase, the additional solvent tends to only increase the volume of the extract phase without increasing the analyte mass proportionally. As a result, the analyte concentration (mg myristicin/mg extract) decreases due to the dilution effect. In addition, the use of excess solvent has the potential to increase the co-extraction of unwanted matrix compounds, thereby reducing the selectivity and relative concentration of the target analyte. Therefore, optimizing the material-to-solvent ratio is a critical parameter in maceration to achieve a balance between extraction efficiency and optimal analyte concentration, rather than simply maximizing the volume of solvent used [26, 27].

The equation in terms of actual factors can be used to predict the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor, and the intercept is not at the center of the design space. In mace containing a higher concentration of methyl eugenol than seeds, a shorter time is beneficial for optimum extraction of myristicin, but limits the extraction of methyl eugenol.

The overlay plot shows the optimal operational region (yellow) that meets the maximum concentration criteria for both analytes (Figure 3). Given that the time variable (C) was not statistically significant, the optimum point was chosen at the shortest extraction duration within the design range of seeds. This aimed to increase process efficiency without reducing the yield of extracted myristicin.

To visualize the relationship between the three factors as dependent variables and the response of myristicin concentration in nutmeg seeds as an independent variable, the linear model polynomial equation is shown in a three-dimensional (3D) surface plot, with a minimum myristicin concentration of 0.0288 mg/mL to 0.16448 mg/mL. By increasing the ethanol concentration and volume of ethanol as the solvent, the myristicin amount in the extract is increase linear at 2-hour maceration time (Figure 4a for mace and Figure 4g for seeds), at 17 hours (Figure 4b for mace and Figure 4h for seed), at 24 hours (Figure 4c for mace and Figure 4i for seed). The myristicine content from nutmeg seed extraction decreases by increasing the extraction time, as shown by the negative coefficient for time (C) in Table 4.

In the maceration method, the yields of volatile and non-volatile compounds are influenced by diffusion mechanisms, partition equilibrium, and chemical stability. In nutmeg, volatile compounds are extracted rapidly because the high concentration gradient at the beginning allows fast diffusion, and the permeable cell walls enable compounds stored in oil glands or vacuoles to escape quickly into the solvent. Most compounds are extracted within the first few hours, and equilibrium is reached soon after, since nutmeg is semi-nonpolar and soluble in organic solvents such as ethanol. Extending

maceration beyond this point does not improve yield and may even reduce it due to oxidation, re-adsorption, or enzymatic degradation. Thus, nutmeg extraction follows a fast extraction, early equilibrium, and eventual degradation pattern [28].

In contrast, mace extraction is governed more by solvent penetration and the gradual softening of the cellular matrix. The compounds in mace are located deeper in the tissue or bound to cell walls, forming complexes with proteins or polysaccharides, which slows their release. As maceration time increases, the solvent penetrates further, causing cell wall swelling and releasing bound compounds, leading to a progressive increase in yield. Moreover, mace constituents are relatively stable toward oxidation and evaporation, allowing prolonged extraction without significant loss. Consequently, myristicin extraction from mace follows a slow diffusion-controlled process with progressive yield enhancement, explaining the higher yields observed at longer maceration times [29].

The same trend was also found on methyl eugenol as an analyte. The increasing of ethanol concentration and volume will increase the amount of methyl eugenol in the extract (Figure 4c-4e for mace and Figure 4j-4l for seed). The longer the maceration time, is not significantly increase the amount of myristicin and methyl eugenol in the mace and seeds extract.

The linear increase in myristicin and methyl eugenol in the extract is largely due to ethanol concentration (A). Myristicin and methyl eugenol are nonpolar compounds that will be easier to extract by nonpolar solvent. Myristicin is a nonpolar to slightly polar compound. This is because its structure is dominated by an aromatic ring and a methylenedioxy group, which make it more lipophilic, while its methoxy and methylenedioxy groups confer a slight polar character. Practically, myristicin is poorly soluble in water but soluble in nonpolar organic solvents such as ether and benzene. Log P of myristicin is 2.6 [30]. On the other hand, methyl eugenol is a semi-polar compound with predominantly lipophilic (nonpolar) properties. It is insoluble in water but easily dissolves in organic solvents such as ethanol, ether, and benzene. Log P of methyl eugenol is 2.69 [26]. Since the target of this research is to detect the amounts of the two analytes using an electrochemical instrument, the solvent should be polar, such as water or ethanol. The optimal ethanol concentration for extracting both analytes is 90%.

At a fixed sample mass of 10 g, increasing the solvent volume primarily led to dilution of the extract, outweighing any improvement in mass transfer. Consequently, smaller solvent volumes yielded extracts with higher analyte concentrations, thereby facilitating more reliable detection.

The optimal ethanol volume for extraction was 50 mL, corresponding to a 1:5 sample-to-solvent ratio. This ratio represents the lowest solvent-to-sample proportion that still ensures effective recovery of the target compound, myristicin, as supported by previous studies [31, 32]. Excessive solvent volumes, while potentially enhancing diffusion, reduce analyte detectability due to dilution. Thus, maintaining this minimal yet sufficient solvent volume balances extraction efficiency with analytical sensitivity.

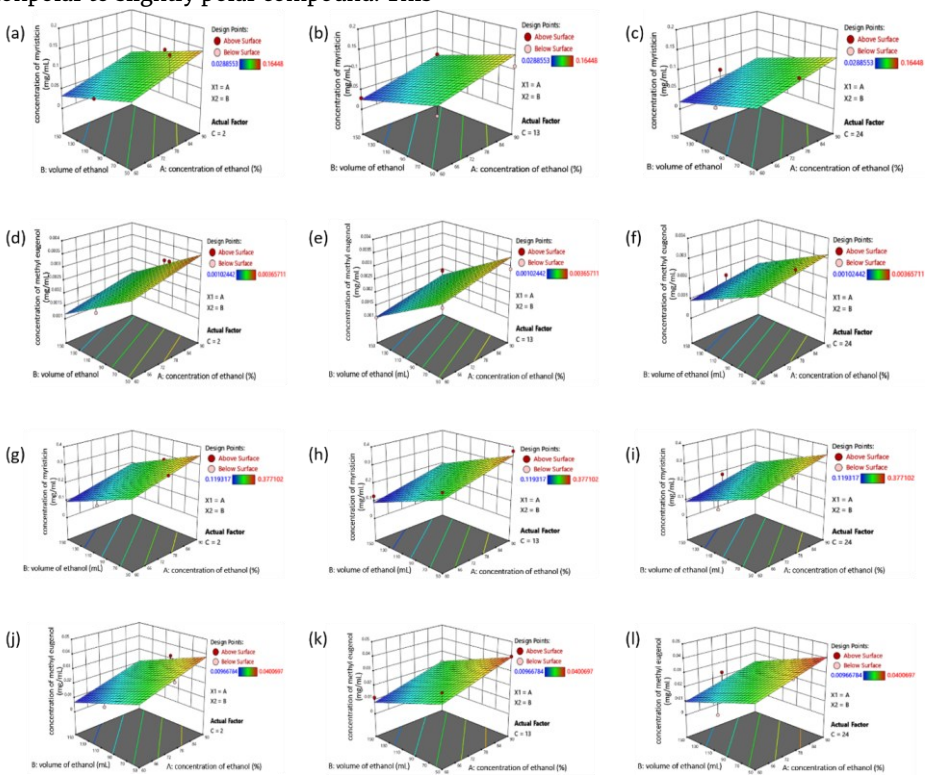

Figure 4. 3D contour plots of analyte content in the extract as a function of independent variables (ethanol concentration and ethanol volume). Panels (a–f) represent mace extract, while (g–l) represent seed extract. Panels (a–c) and (g–i) correspond to myristicin, whereas (d–f) and (j–l) correspond to methyl eugenol. Extraction times are 2 h for (a), (d), (g), and (j); 12 h for (b), (e), (h), and (k); and 24 h for (c), (f), (i), and (l)

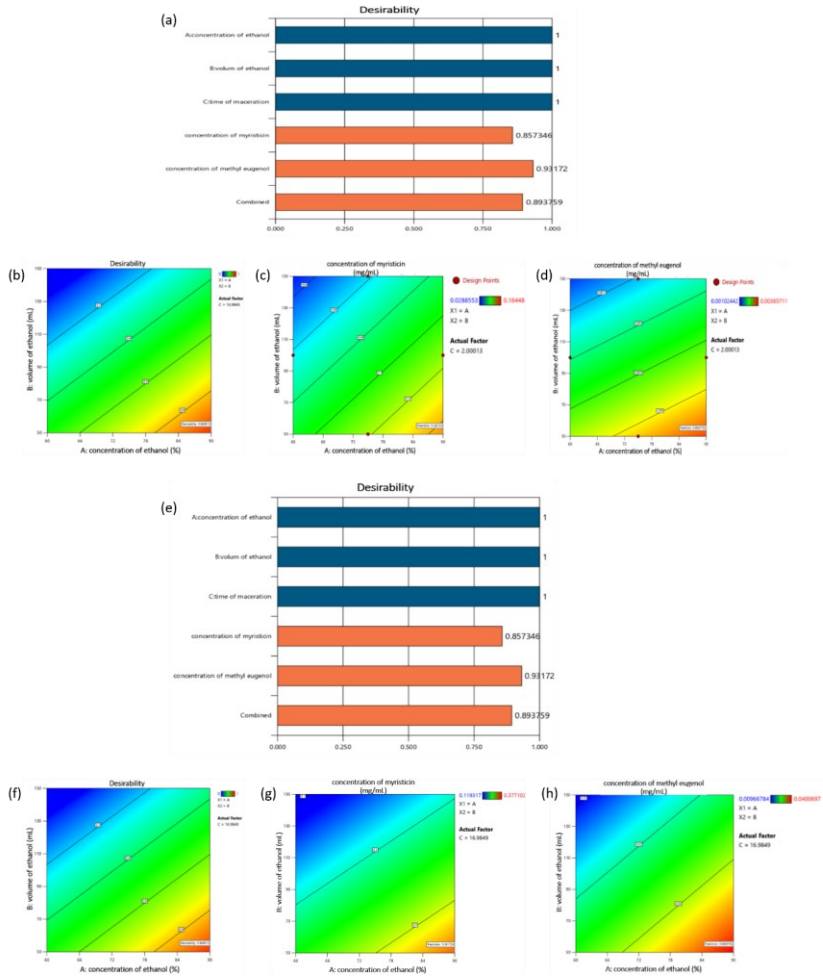

Figure 5. Optimal conditions for maximizing myristicin and methyl eugenol in seed extract (a–d) and mace extract (e– h). Desirability plots are shown in (a, b) and (e, f), while the amounts of myristicin are presented in (c) and (g), and methyl eugenol in (d) and (h)

The desirability function approach was applied to identify the optimum extraction conditions that simultaneously maximize the amount of analytes in the extract while minimizing the volume of ethanol used as solvent. Under the optimum conditions of 90% ethanol concentration, 50 mL ethanol volume, and 2 hours of maceration time, the desirability of myristicin concentration was 0.857346, and the desirability of methyl eugenol concentration was 0.93172, which is close to the combined desirability of 0.893759 for seed extract (Figure 5a-5d). Both values are close to 1, indicating that the desirability function successfully aligns both responses toward a common optimum. Methyl eugenol extracted under the same experimental parameters also needs to be monitored, as low concentrations indicate good nutmeg quality. However, desirability is determined based on the extraction objective, which is to optimize myristicin as the primary target compound in the nutmeg sample. Higher myristicin concentrations indicate better nutmeg quality. Overall, the high desirability values obtained confirm that RSM, combined with the desirability function, is a reliable and powerful tool for optimizing multiple response variables in extraction.

The overall desirability value of mace extract also showed very good results, namely 0.901573 for myristicin concentration and 1 for methyl eugenol concentration desirability, and a combined desirability value of 0.949512, which indicated that the conditions were very satisfactory between the targeted responses so that the designed optimization model was effective in balancing the yield and purity of myristicin and methyl eugenol analytes (Figure 5e-5h). These conditions were found to be 90% ethanol concentration, 50 mL ethanol volume, and 17 hours of maceration time.

The optimal extraction conditions for analytes from nutmeg seed and mace were identical in terms of solvent composition and volume, namely 90% ethanol and 50 mL, with the only difference being maceration time. The optimal extraction time was 2 hours for nutmeg seed and 17 hours for mace. This difference can be attributed to extraction kinetics and matrix-dependent diffusion.

Nutmeg seed contains higher levels of total (34.47% vs. 22.55%) and fixed oils (26.43% vs. 23.73%), whereas mace has a higher proportion of essential oils (8.79% vs. 5.95%) [31]. The dense, starchy matrix of the seed facilitates solvent penetration and accelerates analyte diffusion, leading to faster equilibrium. In contrast, the fibrous and essential oil-rich structure of mace creates more viscous microenvironments and stronger analyte–matrix interactions, which reduce the effective diffusion coefficient and slow mass transfer. Consequently, longer extraction times are required for mace to reach equilibrium.

The concentration of myristicin obtained from the RSM design further serves as a reference for validating the response of the Fe_3O_4/graphene-modified GCE electrochemical sensor in detecting myristicin in nutmeg samples [17].

4. Conclusion

The optimum maceration conditions for extracting myristicin and methyl eugenol from nutmeg seed is 90% ethanol concentration, 50 mL ethanol volume, and 2 hours of maceration time, while for mace are ethanol concentration of 89.9%, ethanol volume of 50 mL, and maceration time of 17 hours. Future studies should include method validation for routine quality control, with emphasis on recovery, sensitivity metrics (LOD/LOQ), and reproducibility. Additionally, alignment of optimized extraction conditions with the electrochemical method performance will be important to ensure compatibility and robustness for practical applications in nutmeg quality monitoring.

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References

[1] Chia-Jung Lee, Chun-Wei Huang, Lih-Geeng Chen, Ching-Chiung Wang, (+)-*Erythro*-$\Delta^{8'}$-7*S*,8*R*-Dihydroxy-3,30,50-Trimethoxy-8-O-40-Neolignan, an Anti-Acne Component in Degreasing *Myristica fragrans* Houtt, *Molecules*, 25, 19, (2020), 4563 https://doi.org/10.3390/molecules25194563

[2] Monika Vasantrao Padol, Prashanth Vishwakarma, Arun Suresh Dodamani, Anup Wamanrao Gore, Karina Shyamlal Chachlani, Shradha Pramod Kharkar, Comparative evaluation of nutmeg mouthwash and 0.2% chlorhexidine gluconate mouthwash on halitosis and plaque control: A randomized clinical trial, *Journal of Indian Society of Periodontology*, 26, 4, (2020), 384-389 https://doi.org/10.4103/jisp.jisp_868_20

[3] Hailan Bao, Qi Muge, Anticancer effect of myristicin on hepatic carcinoma and related molecular mechanism, *Pharmaceutical Biology*, 59, 1, (2021), 1126-1132 https://doi.org/10.1080/13880209.2021.1961825

[4] Elisa Frederico Seneme, Daiane Carla Dos Santos, Carolina Afonso de Lima, Ícaro Augusto Maccari Zelioli, Juliana Mozer Sciani, Giovanna Barbarini Longato, Effects of Myristicin in Association with Chemotherapies on the Reversal of the Multidrug Resistance (MDR) Mechanism in Cancer, *Pharmaceuticals*, 15, 10, (2022), 1233 https://doi.org/10.3390/ph15101233

[5] Sawsan S. Al-Rawi, Ahmad Hamdy Ibrahim, Heshu Jalal Ahmed, Zhikal Omar Khudhur, Therapeutic, and pharmacological prospects of nutmeg seed: A comprehensive review for novel drug potential insights, *Saudi Pharmaceutical Journal*, 32, 6, (2024), 102067 https://doi.org/10.1016/j.jsps.2024.102067

[6] Muhammad Imran, Abdul Haleem Shah, Niamat Ullah, Suliman Yousef Alomar, Abdur Rehman, Naeem Ur Rehman, Asif Nawaz, Rabia Baloch, Ali Zaman, Hafiz Abdul Rafey, Khizar Abbas, Adnan Amin, Integrated computational analysis, *in vitro*, *in vivo* investigation on *Myristica fragrans* Houtt. essential oils for potential anti rheumatic activities, *Journal of King Saud University - Science*, 36, 5, (2024), 103177 https://doi.org/10.1016/j.jksus.2024.103177

[7] Fuat Karakuş, Zübeyde Tanrıverdi, Burak Kuzu, In Silico Network Toxicology, Molecular Docking, and Multi-Level Bioinformatics Reveal Methyl Eugenol-Induced Hepatocellular Carcinoma Mechanisms in Humans, *Cancer Medicine*, 14, 10, (2025), 1-11 https://doi.org/10.1002/cam4.70768

[8] Xiaoling Zhang, Susan P. Felter, Anne Marie Api, Kaushal Joshi, Dan Selechnik, A Cautionary tale for using read-across for cancer hazard classification: Case study of isoeugenol and methyl eugenol, *Regulatory Toxicology and Pharmacology*, 136, (2022), 105280 https://doi.org/10.1016/j.yrtph.2022.105280

[9] Rudi Heryanto, Siti Sa'diah, Waras Nurcholis, Application of two-level factorial design in optimization of maceration extraction of *Curcuma longa* curcuminoids extracts, *Journal of Applied Biology & Biotechnology*, 12, 5, (2024), 143-148 https://doi.org/10.7324/JABB.2024.179132

[10] Anthonin Gori, Benjamin Boucherle, Aurélien Rey, Maxime Rome, Nicola Fuzzati, Marine Peuchmaur, Development of an innovative maceration technique to optimize extraction and phase partition of natural products, *Fitoterapia*, 148, (2021), 104798 https://doi.org/10.1016/j.fitote.2020.104798

[11] Manoj Madanahalli Ramesh, Nischith Sirawase Shankar, Annegowda Hardur Venkatappa, Driving/Critical Factors Considered During Extraction to Obtain Bioactive Enriched Extracts, *Pharmacognosy Reviews*, 18, 35, (2024), 68-81 https://doi.org/10.5530/phrev.2024.18.7

[12] Alexander Weremfo, Samuel Abassah-Oppong, Felix Adulley, Kwabena Dabie, Salifu Seidu-Larry, Response surface methodology as a tool to optimize the extraction of bioactive compounds from plant sources, *Journal of the Science of Food and Agriculture*, 103, 1, (2023), 26-36 https://doi.org/10.1002/jsfa.12121

[13] Ivana Karabegoić, Sandra Stamenković Stojanović, B. Danilović, Optimization of food-grade bioactive compound extraction from plant sources by response surface methodology approach: A comprehensive review, *Maso International – Journal of Food Science and Technology*, 13, 1, (2023), 31-47 https://doi.org/10.2478/mjfst-2023-0004

[14] Syeda Nashvia Adin, Isha Gupta, Mohd Aqil, Mohd Mujeeb, Abdul Ahad, BBD Driven Optimization of Extraction of Therapeutically Active Xanthanoid Mangiferin from *Mangifera indica* L. Leaves and its Antioxidant Activity, *Pharmacognosy Research*, 15, 1, (2022), 84-93 https://doi.org/10.5530/097484900279

[15] Vicente Amirpasha Tirado-Kulieva, Manuel Sánchez-Chero, Mario Villegas Yarlequé, Gretell Fiorela Villegas Aguilar, Gilberto Carrión-Barco, Abraham Guillermo Ygnacio Santa Cruz, José Sánchez-Chero, An Overview on the Use of Response Surface Methodology to Model and Optimize Extraction Processes in the Food Industry, *Current Research in Nutrition and Food Science*, 9, 3, (2021), 745-754 https://doi.org/10.12944/CRNFSJ.9.3.03

[16] Filipa Paulo, Loleny Tavares, Lúcia Santos, Response Surface Modeling and Optimization of the Extraction of Phenolic Antioxidants from Olive Mill Pomace, *Molecules*, 27, 23, (2022), 8620 https://doi.org/10.3390/molecules27238620

[17] Dara Juliana, Bambang Pontjo Priosoeryanto, Waras Nurcholis, Optimization of Cardamom Fruit (*Amomum compactum*) Extraction Focused on Total Terpenoid Extraction and Cytotoxic Activity Using Response Surface Methodology, *Jurnal Jamu Indonesia*, 8, 2, (2023), 57-69 https://doi.org/10.29244/jji.v8i2.328

[18] Irmanida Batubara, Yanico Hadi Prayogo, Stéphane Dumarcay, Christine Gerardin, Philippe Gerardin, Wayan Darmawan, Ika Resmeiliana, Maria Celeste Ruiz, Auliya Ilmiawati, Harlinda Kuspradini, Mohamad Rafi, Phytochemical Profile and In Vitro – In Silico Antibacterial Activity of *Melia azedarach* Leaf and Twig Extracts Obtained Using Solvents of Different Polarities, *Sci*, 7, 4, (2025), 167 https://doi.org/10.3390/sci7040167

[19] Irmanida Batubara, Indah Fajarwati, Y. W. Sari, Gilles J. Guillemin, I. Maulidya, Dinda Iryawati, W. Wahyuni, Evaluation of acute and sub-acute toxicity of *Curcuma aeruginosa* Roxb. essential oil in Sprague Dawley rats, *Research in Pharmaceutical Sciences*, 20, 3, (2025), 456-468 https://doi.org/10.4103/RPS.RPS__9__24

[20] Saengnapa Champasuri, Arunporn Itharat, Bioactivities of Ethanolic Extracts of Three Parts (Wood, Nutmeg and Mace) from *Myristica fragrans* Houtt., *Journal of the Medical Association of Thailand*, 99, (2016), S124-S130

[21] Halid Makić, Samira Hotić, Elvisa Hodžić, Nenad Stojanović, Jasmina Ibrahimpašić, Samira Dedić, Emina Ćehajić Gradinović, Influence of Maceration, Solvent Types, and Extraction Durations on the Yield of Milk Thistle Seeds (*Silybum Marianum*) Extraction, *Technologica Acta*, 17, 1, (2024), 67-76 https://doi.org/10.51558/2232-7568.2023.17.1.67

[22] Manh Tuan Ha, Ngoc Khanh Vu, Thu Huong Tran, Jeong Ah Kim, Mi Hee Woo, Byung Sun Min, Phytochemical and pharmacological properties of *Myristica fragrans* Houtt.: an updated review, *Archives of Pharmacal Research*, 43, 11, (2020), 1067-1092 https://doi.org/10.1007/s12272-020-01285-4

[23] Mega Ferdina Warsito, A Review on Chemical Composition, Bioactivity, and Toxicity of *Myristica fragrans* Houtt. Essential Oil, *Indonesian Journal of Pharmacy*, 32, 3, (2021), 304-313 https://doi.org/10.22146/ijp.1271

[24] Khairan Khairan, Meutia Faradilla, Binawati Ginting, Elly Sufriadi, Adinda Gusti Vonna, Muslim Akmal, Amalia, Ernawati, Indra, Hizir Sofyan, Muhammad Diah, Syaifullah Muhammad, Isolation of Nutmeg Essential Oil (*Myristica fragrans* houtt) from Aceh Indonesia and Their Antioxidant and Antibacterial Activities, *Indonesian Journal of Pharmacy*, 35, 3, (2024), 425-436 https://doi.org/10.22146/ijp.8399

[25] Ismiyarto, Ngadiwiyana, Rani Mustika, Isolasi, Identifikasi Minyak Atsiri Fuli Pala (*Myristica fragrans*) dan Uji Aktivitas Sebagai Larvasida, *Jurnal Kimia Sains dan Aplikasi*, 12, 1, (2009), 23-30 https://doi.org/10.14710/jksa.12.1.23-30

[26] Nikolaos Nenadis, Maria Papapostolou, Maria Z. Tsimidou, Suggestions on the Contribution of Methyl Eugenol and Eugenol to Bay Laurel (*Laurus nobilis* L.) Essential Oil Preservative Activity through Radical Scavenging, *Molecules*, 26, 8, (2021), 2342 https://doi.org/10.3390/molecules26082342

[27] Lidwina Angelica Soetantijo, Hendrik O. Lintang, Heriyanto, Mitha Ayu Pratama Handojo, Tatas Hardo Panintingjati Brotosudarmo, Optimization of Adsorption and Desorption Time in the Extraction of Volatile Compounds in Brewed Java Arabica Coffee Using the HS-SPME/GC-MS Technique, *Jurnal Kimia Sains dan Aplikasi*, 25, 2, (2022), 49-55 https://doi.org/10.14710/jksa.25.2.49-55

[28] Erik V. Petersson, Jiayin Liu, Per J. R. Sjöberg, Rolf Danielsson, Charlotta Turner, Pressurized Hot Water Extraction of anthocyanins from red onion: A study on extraction and degradation rates, *Analytica Chimica Acta*, 663, 1, (2010), 27-32 https://doi.org/10.1016/j.aca.2010.01.023

[29] Nashwa F. S. Morsy, A comparative study of nutmeg (*Myristica fragrans* Houtt.) oleoresins obtained by conventional and green extraction techniques, *Journal of Food Science and Technology*, 53, 10, (2016), 3770-3777 https://doi.org/10.1007/s13197-016-2363-0

[30] Muliadi, Mudzuna Quraisyah Basimin, Ahmad Muchsin Jayali, Density Functional Theory for QSAR Antioxidant Compound Myristicin Derivatives, *Indonesian Journal of Chemical Research*, 9, 1, (2021), 49-56 https://doi.org/10.30598//ijcr.2021.9-mul

[31] Salony Azam Sheikh, Vallamkondu Manasa, Palak Daga, Ajay W. Tumaney, Does fixed oil impart more antioxidant potential to spices than essential oils? A comparative study with seed (nutmeg) and aril (mace) oil from the spice *myristica fragrans*, *Journal of Food Science and Technology*, 62, 11, (2025), 2173-2182 https://doi.org/10.1007/s13197-024-06164-0

[32] Nicky Rahmana Putra, Ahmad Hazim Abdul Aziz, Hasmadi Mamat, Dwila Nur Rizkiyah, Mohd Azizi Che Yunus, Irianto Irianto, Lailatul Qomariyah, Green extraction of nutmeg (*Myristica fragrans*) phytochemicals: Prospective strategies and roadblocks, *Open Agriculture*, 9, 1, (2024), 20220285 https://doi.org/10.1515/opag-2022-0285