Microwave-assisted Natural Deep Eutectic Solvents Pretreatment Followed by Hydrodistillation Coupled with GC-MS for Analysis of Essential Oil from Turmeric (*Curcuma longa* L.)

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Abstract: In the past decade, natural deep eutectic solvents (NADESs) as green and sustainable extraction solvents with great potential for the efficient extraction of bioactive compounds from the plants are emerging. In this study, a microwave-assisted technology is used to prepare natural deep eutectic solvents. And natural deep eutectic solvents as pretreatment solvents coupled with microwave-assisted hydrodistillation (MAHD) for isolating essential oil (EO) derived from turmeric (*Curcuma longa* L.) is investigated. To improve the essential oil yield of turmeric (*Curcuma longa* L.) as a target, various factors affecting extraction efficiency including the type and amount of natural deep eutectic solvents, pretreatment time, pretreatment temperature and hydrodistillation (HD) time are discussed and optimized through central composite design (CCD) of the response surface methodology (RSM). The optimal conditions are as follows: natural deep eutectic solvent composed of choline chloride and oxalic acid (molar ratio with 1:1) as a pretreatment solvent, an amount of 60 g, a pretreatment time of 5 min, a pretreatment temperature of 84 °C, a hydrodistillation time of 76 min. Under the optimum conditions, the highest essential oil yield of 0.85% is achieved. Additionally, the essential oil is analyzed by using gas chromatography-mass spectrometry (GC-MS), with a total of 49 compounds being identified. Through combining natural deep eutectic solvents with a microwave-assisted hydrodistillation technique, this work provides an eco-friendly extraction way of isolating essential oil, which boosts development in the monitoring other spice quality field.

Key words: natural deep eutectic solvent, microwave-assisted technology, microwave-assisted hydrodistillation, essential oil, turmeric

1 Introduction

Essential oils (EO) or volatile oils, also known as essences, originate from the secondary metabolism of plants and have complex chemical components¹⁾. Turmeric (*Curcuma longa* L.) belongs to the family Zingiberaceae, is a perennial rhizomatous shrub native to Southern Asia. At present, the cultivation of this species is also widely distributed in some African countries²⁾. The turmeric essential oil is widely used in traditional and modern system of medicine due to the presence of several medicinal properties like antibacterial, antifungal, antiviral, antiworm, antiallergic, anti-parasitic, antinociceptive, anti-platelet, antimutagenic and anti-arthritic properties etc³⁻⁶⁾.

Extraction method is one of prime factors that determine the quality of essential oil. Inappropriate extraction procedure can lead to the damage or alter action of chemical signature of essential oil⁷⁾. EO is generally extracted by physical methods, steam distillation, hydrodistillation (HD)

Abbreviations: EO; essential oil, DES; deep eutectic solvents, NADESs; natural deep eutectic solvents, CCD; central composite design, RSM; response surface methodology, GC-MS; gas chromatography-mass spectrometry, MAHD; microwave-assisted hydrodistillation, HD; hydrodistillation, HBD; hydrogen bond donors, HBA; hydrogen bond acceptors, FT-IR; Fourier Transform Infrared, ANOVA; Analysis of variance

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or solvent extraction. On commercial scale, steam distillation or hydrodistillation is the most preferred method for the extraction of EO⁸⁾. Technologies of traditional extraction are often inconvenient, energy-consuming or require a large amount of hazardous and expensive organic solvents and can alter the quality of the produced EOs due to the damage or losses of specific compounds under high temperature conditions or the presence of solvent residues⁹⁾. This encourages researchers to find substitutes and techniques based on their cost-effective criteria. Microwave dielectric heating is common in various processes in the food industry all over the world¹⁰⁾. Microwave-assisted hydrodistillation (MAHD) is more and more applied in EO extraction¹¹⁾. Compared to HD, MAHD is an extraction process that requires a short extraction period, low energy source, and is environmentally friendly¹²⁾.

Additionally, pretreatment is indispensable to overcome the natural recalcitrance of plant cell walls^{13, 14)}, and enhance the release of intracellular compounds. Recently, deep eutectic solvents (DESs) have emerged as a class of novel green fluids, which are composed of hydrogen bond donors (HBD) and acceptors (HBA)^{15, 16)} to form a new solvent mixture with lower melting point than those of the individual components¹⁷⁾. They show many favorable properties including negligible vapor pressure, nonflammability, low toxicity, biocompatibility and the possibility of being made from renewable materials; at the same time, their physico-chemical properties can be tuned by modifying the HBA/HBD structures or by changing the molar ratio of these components¹⁸⁾. Besides, DESs are proved to be good pretreatment solvents^{19, 20)} which could possess the outstanding ability due of its ability in cellulose, hemicellulose and lignin disruption²¹⁾. Natural deep eutectic solvents (NADESs), which are directly composed of natural products, such as organic acids, amino acids, sugars, choline or urea, have received increasing attentions because they are composed of primary metabolites in living cells. NADESs have advantages of being composed of naturally-occurring compounds, being much more sustainable and posing practically no environmental hazards^{22–24)}.

So far, there are three main methods available for preparing NADESs consisting of heating and stirring method²⁵⁾, evaporating method²⁶⁾ and freeze drying method²⁷⁾. Nevertheless the time and energy that these processes demand could be controversial with regard to the greenness of these solvents. The use of microwave radiation is one of the most promising techniques because of its advantages such as higher yields, milder reaction conditions and shorter reaction times²⁸⁾. As a consequence, microwave-assisted technology provides great potential of preparing natural deep eutectic solvents.

In this study, a novel extraction method that natural deep eutectic solvents of microwave-assisted preparation pretreatment followed by MAHD for extraction of essential

oil from turmeric (*Curcuma longa* L.) was proposed. To the best of our knowledge, this type of pretreatment approach in terms of NADESs through microwave irradiation synthesis for isolating EO from turmeric has not been reported elsewhere. The NADESs based on microwave-assisted preparation were characterized by Fourier Transform Infrared (FT-IR) and a comparison of extraction efficiency between two different preparation methods was also included. The effects of this extraction method on the EO yield were systematically investigated by response surface methodology. The compositions of the EO are analyzed by using gas chromatography-mass spectrometry (GC-MS) and compared with the EO obtained from other extraction methods.

2 Materials and Methods

2.1 Reagents, materials and samples

Dry turmeric samples of *Curcuma longa* L. were purchased from traditional Chinese medicine shops (Taizhou, China). All the dry samples were pulverized into powder before using for experiments. Choline chloride, fructose, lactic acid, glucose, malic acid, oxalic acid and *n*-hexane of analytical grade were purchased from Aladdin Co., Ltd. (Shanghai, China). C₈–C₂₀ *n*-alkanes of chromatographic grade were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). Helium gas of high purity (99.999%) was purchased from Hangzhou Special Gas Production Co. Ltd. (Hangzhou, China).

2.2 Preparation of natural deep eutectic solvents

Microwave-assisted technology is used to prepare NADESs. A two component mixture, HBA and HBD, with a calculated amount of deionized water was added to a glass flask and exposed to microwave (400 W) at 80°C until a homogeneous liquid was formed (15–30 min). After the preliminary synthesis tests, five types of NADESs were satisfactorily synthesized. It revealed that a significant reduction in energy and time consumption was observed comparing with the traditional preparing methods. **Table 1** showed the composition, molar ratios, and symbols of NADESs used in this study.

As a comparative study, both of the HBDs and HBAs species were mixed together to synthesize NADESs at proper molar ratios with magnetic agitation at 80°C, until a transparent and homogeneous solution was formed (2–4 h). Then a calculated amount of deionized water was added into NADESs for experiment.

2.3 Characterization of natural deep eutectic solvents

FT-IR spectroscopy could provide the useful information for identifying the presence of functional groups or chemical bonds in a molecule or an interaction system. The syn-

Table 1 The composition, molar ratios, and symbols of NADESs were used in this study.

NADES name	HBA	HBD	Molar ratio (mol/mol)
NADES-1	Choline chloride	Fructose	3:2
NADES-2	Choline chloride	Lactic acid	1:2
NADES-3	Choline chloride	Malic acid	1:1
NADES-4	Choline chloride	Glucose	2:1
NADES-5	Choline chloride	Oxalic acid	1:1

Fig. 1 The device for the NADES-based MAHD process.

thesis of NADES by microwave-assisted technology was confirmed by FT-IR Spectroscopy (T27, Bruker, Germany). The chemical characterization of these solvents was accomplished by identification of bonds present through infra-red induced absorption at a frequency related to bond vibration²⁹⁾.

2.4 NADES-based MAHD

The NADES-based MAHD extraction of the target compounds procedure consisted of three successive processes: microwave-assisted NADES pretreatment, fast heating, and MAHD. A microwave reactor (XH-300UA, Xianghu Science and Technology Development Co. Ltd, Beijing, China) was used in experiments. The device was represented in Fig. 1.

In the first stage, 30 g of the sample with an accurately

weighed amount of various NADESs was mixed in a 500 mL three-necked flask. Then the three-necked flask was placed into the microwave reactor where the mixture was pretreated by microwave irradiation with a specifically set microwave power (600 W), temperature (84°C), and reaction time (5 min).

For the second part, 250 mL of water was added into the flask. The experimental apparatus sequentially constructed with a water-knockout vessel and a condenser pipe which was outside the microwave reactor was connected on the flask to achieve the purpose of collecting EO and the reflux of condensed water. The reaction conditions were microwave power (600 W), reaction temperature (110°C), reaction time (5 min). This process was designed to give a momentary increase in the temperature of the reaction mixture to its boiling point.

Finally, the purpose of the third procedure was to maintain HD. The microwave power was done with a designated value (300 W), while the temperature remained to be 110°C. According to the experimental requirements, the reaction time was also set to a specified value (76 min). After HD, the EO was separated from aqueous phase and dried over anhydrous Na₂SO₄. Then, the samples were weighed and stored in dark tubes at 4°C until analysis.

The following formula was used to calculate the EO yield of the sample.

$$\text{EO yield (\%)} = \frac{\text{weight of EO}}{\text{weight of powder}} \times 100$$

2.5 Experimental design and statistical analysis

In order to obtain the maximum EO yield of turmeric by means of NADES-based MAHD method. The pretreatment time (X_1 : 5–9 min), the pretreatment temperature (X_2 : 60–100°C), and the HD time (X_3 : 40–80 min) were selected as the three main independent variables while the response variables were the extraction yield and a central composite design (CCD) combined with response surface methodology (RSM) was employed for optimizing the operating conditions. The CCD includes a three-level full factorial design (+1, −1), overlying by the center points (coded 0), and the star points (+ α , − α). The star points establish new extremes for the low and high settings for all parameters and provide estimation of the curvature in the model³⁰⁾. The range of these three factors and central point were chosen in terms of the preliminary experiments. Table 2 showed the range of independent variables and their coded levels obtained after preliminary experiments and were used in the experimental design. In this CCD, these 20 randomized experiments together including six replicates at center point were employed for fitting the full quadratic equation model. The general full second-order polynomial equation is:

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i < j} \beta_{ij} X_i X_j$$

Design-Expert software (Version 8.0) was used to carry out the design of experiments, analysis of the results and prediction of the responses. Analysis of variance (ANOVA) was used for calculating and modeling of the optimum conditions for NADES-based MAHD of EO from turmeric.

2.6 GC–MS analysis

Each EO sample was diluted with *n*-hexane and then added an appropriate amount of anhydrous sodium sulfate to purify the moisture in the solution. Then EO was analyzed by gas chromatography coupled to mass spectrometry (GC–MS) comprising a GC 2000 Series (Focused Photonics, Hangzhou, China) instrument coupled with a Mars 6100 ion trap mass detector using a DB-5MS capillary column (30 m × 0.25 mm id × 0.25 μm thickness). The GC–MS was performed using the following operating conditions: carrier gas helium; flow velocity 0.8 mL/min; split ratio 5:1; injection volume 1 μL; injection temperature 250°C; oven temperature progress holding 45°C for 3 min, from 60°C to 90°C at the rate of 5°C min^{−1} and holding for 1 min, from 90°C to 250°C at the rate of 3°C min^{−1} and holding 250°C for 5 min; ionization mode used at electronic impact 70 eV; transfer line temperature 250°C; ion trap temperature 180°C; manifold temperature 50°C; and mass range *m/z* 45–550.

Identification of the constituents was based on comparison of the obtained mass spectra with those in the NIST Mass Spectral Search Program (NIST 2011 version mass spectral database; National Institute of Standards and Technology, Washington, DC, USA) connected to a Mars 6100 spectrometer and homemade library mass spectra built from pure substances and components of known oils and MS literature. The constituents were confirmed by comparison of their Kovats retention indices relative to C₈–C₂₀ *n*-alkanes (Sigma Chemical, St. Louis, MO, USA) with those of authentic standards or in the published literature. Identification was assumed when a good match of the mass spectrum and Retention Index (RI) were achieved. Quantitative analysis in percent was performed by peak area normalization measurements³¹⁾.

2.7 Comparison of different extraction methods

In order to prove the method feasibility, some different methods such as MAHD and HD were applied to extract EO from the turmeric for comparison. The conditions of MAHD could be divided into two stages: (1) microwave power, temperature and reaction time were set to 600 W, 110°C and 5 min, respectively, for fast heating; (2) microwave power, temperature and reaction time were set to 300 W, 110°C and 76 min, respectively, for HD. HD was traditional HD where conditions were conventional heating at

Table 2 Experimental levels of independent variables.

Variables	Units	Symbol	Variable levels				
			− α (−1.6)	−1	0	+1	+ α (+1.6)
pretreatment time	min	X_1	3.64	5	7	9	10.36
pretreatment temperature	°C	X_2	46.36	60	80	100	113.64
hydrodistillation time	min	X_3	26.36	40	60	80	93.64

110°C for 4 h.

3 Results and Discussion

3.1 Characterization of natural deep eutectic solvents

The synthetic route of NADES was shown in Fig. 2A. As an example, FT-IR was utilized for the chemical characterization of the prepared NADES composed of choline chloride and oxalic acid to confirm the existence of bonds in the individual components and between the HBA and HBD molecules. The hydrogen bond formation between the halide anion of choline chloride and oxalic acid is the major force for NADES formation. As seen in Fig. 2B, a strong, broad peak at approximate 3400 cm^{-1} was obtained for absorption bands of -OH, proving that there were lots of hydrogen bonds in NADES. Furthermore, the sharp peaks at around $800\text{--}1100\text{ cm}^{-1}$ were also characteristic peaks indicating formation of hydrogen bonding between the individual components³²⁾. On the other hand, NADESs were characterized by bands in the $3150\text{--}3400\text{ cm}^{-1}$ range, which correspond to the N-H stretching vibrations characteristic of choline chloride, which overlap with the O-H stretching

vibrations, thus demonstrating formation of a hydrogen bond between HBD and HBA³³⁾. The peaks at approximate 1680 cm^{-1} belonged to the absorption bands of $\text{C}=\text{O}$, maintaining the characteristic bands of the precursors. These bands were associated with the features of NADES comprised of choline chloride and oxalic acid. The results presented similar FT-IR spectra to those obtained in a literature search³⁴⁾ when using the heating and stirring method.

3.2 The comparison of synthesis methods of NADES

To demonstrate the effectiveness of synthetic techniques, NADESs synthesized by microwave-assisted preparation method and the classical heating and stirring method were compared under the same extraction conditions. The EO yield for NADES composed of choline chloride and oxalic acid (molar ratio with 1:1) obtained by microwave-assisted preparation method and the classical heating and stirring method were 0.73%, 0.66%, respectively. The result showed that both two methods have similar values of EO yield. Microwave technology was better than heating and stirring method in terms of energy saving and reduced waste time. Therefore, NADESs synthesized by microwave-assisted preparation method were used for the following experiment.

3.3 Screening of natural deep eutectic solvents

In this work, NADESs acted as a driving force for destroying the membrane structure of plant cells, allowing EO to diffuse out. Therefore, correct selection of NADESs type and extraction method for target compounds extracted from the sample was especially important. Five NADESs at a certain molar ratio were tested in this study by EO yield and the result was shown in Fig. 3A. The chart demonstrated that NADES-5 composed of choline chloride and oxalic acid at a molar ratio of 1:1 possessed the higher EO yields than others. The reason for this phenomenon was that DES might provide a mild acid-base catalysis mechanism which would instigate the controlled cleavage of labile ether linkages among phenylpropane units and thus led to lignin depolymerization and/or separation from the biomass³⁵⁾. In addition, oxalic acid was the most acidic among the ingredients used as HBD resulting in the best dissolving effect on the plant cell wall. Therefore, NADES-5 was chosen as the pretreatment solvent for the following experiment. Each sample was prepared and investigated in triplicate prior to analysis in this work.

3.4 The effect of the amount of natural deep eutectic solvents

The amount of NADES could affect the EO yield, which depended on the solubilizing capacity of different the amount of NADES for plant cell wall. To evaluate the influence of the amount of NADES, different amount of NADES

Fig. 2 The synthetic route and FT-IR spectra of natural deep eutectic solvents.

(A) The synthetic route of natural deep eutectic solvents.

(B) FT-IR spectra of natural deep eutectic solvent composed of choline chloride and oxalic acid.

Fig. 3 Effect of NADES types, amount and extraction methods for EO yield.

(A) Effect of NADES types for EO yield. Extraction conditions: first stage: microwave power, 600 W; temperature, 80°C; time, 5 min; second stage: microwave power, 600 W; temperature, 110°C; time, 5 min; third stage: microwave power, 300 W; temperature, 110°C; time, 40 min.

(B) Effect of NADES amount for EO yield. Extraction conditions: first stage: microwave power, 600 W; temperature, 80°C; time, 5 min; second stage: microwave power, 600 W; temperature, 110°C; time, 5 min; third stage: microwave power, 300 W; temperature, 110°C; time, 40 min.

(C) Effect of extraction methods for EO yield. Extraction conditions: (HD): conventional heating at 110°C for 4 h. (MAHD): first stage: microwave power, 600 W; temperature, 110°C; time, 5 min; second stage: microwave power, 300 W; temperature, 110°C; time, 76 min. (NADES-based MAHD): first stage: microwave power, 600 W; temperature, 84°C; time, 5 min; second stage: microwave power, 600 W; temperature, 110°C; time, 5 min; third stage: microwave power, 300 W; temperature, 110°C; time, 76 min.

(20, 40, 60, 80, 100 g) were investigated in the same experimental conditions. As can be seen in **Fig. 3B**, the EO yield of the turmeric increased with the amount of NADES from 20 up to 60 g because the amount of NADES was too small to be in full contact with the powder when the NADES amount was less than 60 g. However, when the amount of NADES was over 60 g, the yield kept declining steadily. This was due to the fact that NADES liquids had limited solvent capacity for the plant cell wall to some extent. Besides, adding NADES a lot would affect the mass transfer and reduce the yield of EO on account of the viscosity of NADES. Thus, 60 g was selected as the optimal amount of NADES for further experiments.

3.5 Optimization of the extraction conditions by RSM

3.5.1 Fitting the model

RSM was a fast and economical mathematical tool which reasonably investigated the influences of independent factors and their interaction on the response variable. Re-

sponse surface optimization was more advantageous than the traditional single parameter optimization in that it saved time, space and raw material³⁶⁾. Microwave-assisted NADES pretreatment followed by HD extraction of EO from turmeric was optimized using RSM. To examine the combined effect of three different independent variables (pretreatment time, pretreatment temperature, and HD time) on the EO yield, 20 experiments were performed. The experimental design, together with experimental data and predicted responses, was provided in **Table 3**. According to **Table 3**, the EO yields ranged from 0.44 to 0.83%, and the maximum yield was obtained at pretreatment time: 5 min, pretreatment temperature: 100°C, and HD time of 80 min.

The model established by second-order polynomial regression equation could replace the experimental real point to explain response results. This mathematical regression model was shown below in terms of coded levels³⁷⁾:

Table 3 Central composite design (CCD) matrix with the actual responses and predicted values for yield of essential oil from turmeric.

Run	Independent variable			Response (yield; %)	
	X ₁ (min)	X ₂ (°C)	X ₃ (min)	Experimental	Predicted
1	5.00	60.00	40.00	0.44	0.43
2	9.00	60.00	40.00	0.51	0.50
3	5.00	100.00	40.00	0.49	0.48
4	9.00	100.00	40.00	0.41	0.41
5	5.00	60.00	80.00	0.79	0.77
6	9.00	60.00	80.00	0.67	0.67
7	5.00	100.00	80.00	0.83	0.82
8	9.00	100.00	80.00	0.58	0.58
9	3.64	80.00	60.00	0.71	0.73
10	10.36	80.00	60.00	0.58	0.58
11	7.00	46.36	60.00	0.63	0.65
12	7.00	113.64	60.00	0.61	0.61
13	7.00	80.00	26.36	0.27	0.28
14	7.00	80.00	93.64	0.7	0.71
15	7.00	80.00	60.00	0.8	0.79
16	7.00	80.00	60.00	0.81	0.79
17	7.00	80.00	60.00	0.77	0.79
18	7.00	80.00	60.00	0.8	0.79
19	7.00	80.00	60.00	0.77	0.79
20	7.00	80.00	60.00	0.79	0.79

$$Y = +0.79 - 0.044 * X_1 - 9.785E - 0.03 * X_2 + 0.13 * X_3 - 0.035 * X_1 * X_2 - 0.045 * X_1 * X_3 - 0.047 * X_1^2 - 0.056 * X_2^2 - 0.10 * X_3^2$$

where Y was the EO extraction yield (% w/w), X₁ was the pretreatment time (min), X₂ was pretreatment temperature (°C), and X₃ was the HD time (min).

Table 4 showed analysis of variance (ANOVA) for the model. The *p*-value less than 0.05 had significant effects on the yield of EO, while values greater than 0.05 indicated that the model terms were not significant³⁸⁾. The coefficient of determination (R-Squared) of the predicted model was 0.9937, along with non-significant lack-of-fit (*p* > 0.05), suggesting a good fit, the predicted model seemed to reasonably represent the observed values. Therefore, the response was sufficiently accounted for by the model. The significance of each coefficient was determined using the F-test and *p*-value. On the basis of ANOVA results, X₁, X₃, X₁X₂, and X₁X₃ had significant effects on the EO yield (*p*-value less than 0.05).

3.5.2 Analysis of response surface

The RSM was taken as an optimization tool to visualize the effect of the independent variables. The three-dimen-

sional response surface showed these effects of the mutual interaction of independent variables. According to **Fig. 4**, the regression model was used to depict the interactive effects of three operational parameters for extraction yield of EO³⁷⁾.

The 3D response surface plot of the combined effects of pretreatment time and pretreatment temperature on the yield of EO was depicted in **Fig. 4A** indicating that lower pretreatment time led to higher yield of EO. On the one hand, the yield of EO decreased with decrease in pretreatment time. The reason for this phenomenon was the formation of esters (monoester or cross-linked diester) between hydroxyl groups of cellulose with oxalic acid, which can prevent the dissolution and hydrolysis of cellulose³⁹⁾ after the long pretreatment time. On the other hand, the yield was increased when the pretreatment temperature increased from 70°C to 84°C. However, further increase in pretreatment temperature could have a negative impact on the yield of EO. Too high temperature had many disadvantages involving the high consumption of energy and the loss of the highly volatile low-boiling-point compounds. Taking into account the above results, it could be inferred that the maximum EO yield occurred at low pretreatment

Table 4 Analysis of variance (ANOVA) for the experiments.

Source	Sum of squares	df	Mean squares	F-value	P-value	Significance
Model	0.48	9	0.053	175.10	< 0.0001	significant
X ₁	0.026	1	0.026	86.60	< 0.0001	
X ₂	1.308E-003	1	1.308E-003	4.32	0.0645	
X ₃	0.22	1	0.22	734.32	< 0.0001	
X ₁ X ₂	9.800E-003	1	9.800E-003	32.34	0.0002	
X ₁ X ₃	0.016	1	0.016	53.46	< 0.0001	
X ₂ X ₃	0.000	1	0.000	0.000	1.0000	
X ₁ ²	0.032	1	0.032	106.02	< 0.0001	
X ₂ ²	0.045	1	0.045	149.43	< 0.0001	
X ₃ ²	0.16	1	0.16	512.27	< 0.0001	
Residual	3.030E-003	10	3.030E-004			not significant
Lack of Fit	1.630E-003	5	3.260E-004	1.16	0.4357	
Pure Error	1.400E-003	5	2.800E-004			
Cor Total	0.48	19				
R-Squared	0.99					
Adj R-Squared	0.99					
Pred R-Squared	0.97

Fig. 4 Response surface plot showing the (A) the interactive effect of pretreatment time and pretreatment temperature on EO yield; (B) the interactive effect of pretreatment time and hydrodistillation time on EO yield; (C) the interactive effect of hydrodistillation time and pretreatment temperature on EO yield.

time and higher pretreatment temperature.

The combined effect of the pretreatment time and the HD time on the EO yield is indicated by **Fig. 4B**. The HD time exhibited a significant effect on the EO yield, whereas the effect of the pretreatment time was weaker. The EO yield increased linearly when the temperature ranged from 60 to 74 min, but the yield decreased when the time was higher than 74 min. Thus, the maximum EO yield of tur-

meric was obtained at high level of HD time and low level of the pretreatment time.

Figure 4C showed the interaction effects of HD time and pretreatment temperature on the extraction yield of essential oil. Overall, under all the extraction time, the extraction yields increased with the increase in HD time. The increase in extraction time didn't have a significant effect on the EO yields at low temperatures (60–70°C). However, at

high temperature (70–84°C), increasing the extraction time would lead to an increase in the yield of EO. The highly viscous DESs showed very large viscosity decreases with increasing temperature⁴⁰⁾. The temperature had a large effect on NADES viscosity, and thus, highly viscous NADESs, which cannot be used for practical purposes at close to ambient conditions, could be considered for technical applications at higher temperature. In order to speed up the mass transfer rate and improve the extraction yield, the higher extraction yields were observed at high temperature and long HD time.

3.5.3 Validation of the models

In view of the above results, the selected variables were further optimized by using Design-Expert. The software identified the following optimum conditions: 5 (min) pretreatment time (X_1), pretreatment temperature of 84 (°C) (X_2), and 76 min of HD time (X_3). Furthermore, the validity of the model was checked and the experimental value (0.85%) was closed with the predicted values (0.86%). Therefore, the extraction conditions from RSM models could be considered to be reliable and reasonable.

To ensure the repeatability of the results in extraction of EO from turmeric using NADESs, the critical experiments were replicated three times. The uncertainty of the results was found to be within $\pm 2\%$.

3.6 The comparison of different methods

According to Fig. 3C, NADES-based MAHD had the highest EO yield in comparison with other two methods, which indicated that NADESs for the pretreatment of turmeric had a remarkable impact on the increase of EO

yields.

Quantitative analyses of turmeric oil were carried out using GC-MS and the total ion chromatograms were shown in Fig. 5. The relative level of each component was calculated by comparing its peak area with the total area that was calculated from the detected peaks⁴¹⁾. The EO compounds obtained by different methods and the relative peak area of each identified compound were presented in Table 5. A total number of 49 compounds were identified by the three extraction methods. Simultaneously, 39, 24 and 21 compounds were identified by NADES-based MAHD, MAHD, HD methods, which accounted for 98.22%, 98.17%, and 98.56%, respectively, of the essential oil extracted from the turmeric. There were more compounds present in EOs obtained by NADES-based MAHD compared with those obtained by MAHD and HD suggesting the effectiveness of NADESs pretreatment stage.

In the light of Table 5, the major components were ar-turmerone (25.78%), α -turmerone (16.16%) in the essential oils obtained by NADES-based MAHD which are principle compounds of turmeric oil in line with previous reports⁴²⁾ contributing to the strong fragrance. In the same time, the contents of ar-turmerone, α -turmerone are 31.35%, 21.38% by MAHD and 34.69%, 18.66% by HD of turmeric oil, respectively.

The remaining major components found in the EO were α -himachalene (7.49%), alloaromadendrene (5.87%), γ -muurolene (4.74%), δ -selinene (4.53%), by way of NADES-based MAHD. The EO of turmeric had α -curcumene (3.78%), α -zingiberene (4.66%), α -selinene (6.98%), germacrone (13.32%) as other major constituents

Fig. 5 The total ion chromatograms of EO obtained from three extraction methods. (A) NADES-based MAHD; (B) MAHD; (C) HD.

Table 5 Chemical compositions of EO from the turmeric.

NO.	Retention time (min)	Compound name	Formula	CAS#	MW	RI ^a (RI ^b)	ID method	Relative peak area (%)		
								NADES-based MAHD	MAHD	HD
1	21.48	Carvotanacetone	C ₁₀ H ₁₆ O	499-71-8	152	1247(1247)	MS, RI	0.45	ND	ND
2	23.37	Thymol	C ₁₀ H ₁₄ O	89-83-8	150	1292(1292)	MS, RI	0.11	ND	ND
3	25.86	Eugenol	C ₁₀ H ₁₂ O ₂	97-53-0	164	1351(1351)	MS, RI	ND	ND	1.49
4	26.83	Ylangene	C ₁₅ H ₂₄	14912-44-8	204	1374(1373)	MS, RI	1.04	ND	ND
5	26.91	β-Cubebene	C ₁₅ H ₂₄	17699-14-8	204	1376(1381)	MS, RI	0.55	ND	ND
6	28.45	α-Cedrene	C ₁₅ H ₂₄	469-61-4	204	1401(1404)	MS, RI	0.32	ND	0.29
7	28.61	Caryophyllene	C ₁₅ H ₂₄	87-44-5	204	1418(1417)	MS, RI	0.42	0.65	1.37
8	28.82	α-Gurjunene	C ₁₅ H ₂₄	489-40-7	204	1422(1419)	MS, RI	0.16	ND	ND
9	29.17	Aromadendrene	C ₁₅ H ₂₄	109119-91-7	204	1431(1434)	MS, RI	0.23	ND	ND
10	29.21	<i>trans</i> -α-Bergamotene	C ₁₅ H ₂₄	13474-59-4	204	1432(1433)	MS, RI	ND	0.18	ND
11	29.77	α-Himachalene	C ₁₅ H ₂₄	3853-83-6	204	1445(1448)	MS, RI	7.49	ND	ND
12	30.08	(<i>E</i>)-β-Farnesene	C ₁₅ H ₂₄	28973-97-9	204	1453(1454)	MS, RI	ND	0.83	ND
13	30.25	Alloaromadendrene	C ₁₅ H ₂₄	25246-27-9	204	1455(1458)	MS, RI	5.87	0.76	ND
14	30.47	β-Selinene	C ₁₅ H ₂₄	17066-67-0	204	1463(1465)	MS, RI	1.40	ND	ND
15	30.83	γ-Gurjunene	C ₁₅ H ₂₄	22567-17-5	204	1472(1473)	MS, RI	1.24	ND	ND
16	30.96	γ-Murolene	C ₁₅ H ₂₄	30021-74-0	204	1474(1473)	MS, RI	4.74	1.02	0.48
17	31.00	δ-Selinene	C ₁₅ H ₂₄	28624-23-9	204	1476(1475)	MS, RI	4.53	ND	ND
18	31.09	Acoradiene	C ₁₅ H ₂₄	24048-44-0	204	1478(1471)	MS, RI	3.99	ND	ND
19	31.19	α-Curcumene	C ₁₅ H ₂₂	644-30-4	202	1481(1476)	MS, RI	0.66	3.78	3.71
20	31.40	α-Murolene	C ₁₅ H ₂₄	31983-22-9	204	1485(1488)	MS, RI	0.21	ND	ND
21	31.61	α-Selinene	C ₁₅ H ₂₄	5951-67-7	204	1491(1494)	MS, RI	0.21	ND	ND
22	31.75	β-Himachalene	C ₁₅ H ₂₄	1461-03-6	204	1494(1499)	MS, RI	3.07	ND	ND
23	31.77	α-Zingiberene	C ₁₅ H ₂₄	495-60-3	204	1495(1496)	MS, RI	ND	4.66	4.96
24	31.89	Curzerene	C ₁₅ H ₂₄	15423-57-1	204	1497(1498)	MS, RI	0.51	ND	ND
25	32.25	Valencene	C ₁₅ H ₂₄	4630-7-3	204	1507(1501)	MS, RI	1.45	1.62	1.66
26	32.63	δ-Cadinene	C ₁₅ H ₂₄	483-76-1	204	1517(1515)	MS, RI	0.21	0.30	ND
27	32.92	β-Sesquiphellandrene	C ₁₅ H ₂₄	20307-83-9	204	1524(1523)	MS, RI	ND	ND	7.14
28	32.96	α-Selinene	C ₁₅ H ₂₄	473-13-2	204	1525(1517)	MS, RI	0.54	6.98	ND
29	33.32	β-Bisabolene	C ₁₅ H ₂₄	495-61-4	204	1534(1525)	MS, RI	ND	0.25	ND
30	33.49	Eudesma-3,7(11)-diene	C ₁₅ H ₂₄	6813-21-4	204	1539(1540)	MS, RI	0.23	ND	ND
31	33.91	Calamenene	C ₁₅ H ₂₂	483-77-2	202	1550(1542)	MS, RI	0.38	ND	ND
32	34.79	Cedrol	C ₁₅ H ₂₆ O	77-53-2	222	1572(1573)	MS	0.88	0.74	0.88
33	34.82	Spathulenol	C ₁₅ H ₂₄ O	6750-60-3	220	1573(1570)	MS, RI	0.41	ND	ND
34	35.41	Longiborneol	C ₁₅ H ₂₆ O	465-24-7	222	1588(1592)	MS, RI	2.22	0.84	0.78
35	35.70	<i>cis</i> -β-Elemenone	C ₁₅ H ₂₂ O	32663-57-3	218	1595(1592)	MS, RI	ND	0.66	0.75
36	35.94	β-Santalol	C ₁₅ H ₂₄ O	37172-32-0	220	1602	MS	2.25	0.53	0.66
37	36.35	Cubenol	C ₁₅ H ₂₆ O	21284-22-0	222	1613(1613)	MS, RI	0.18	0.79	0.76
38	36.54	(<i>Z</i>)-α-Santalol	C ₁₅ H ₂₄ O	115-71-9	220	1618	MS	0.28	ND	0.52
39	37.38	Torreyol	C ₁₅ H ₂₆ O	19435-97-3	222	1639(1640)	MS, RI	0.28	0.45	0.51
40	37.40	β-Eudesmol	C ₁₅ H ₂₆ O	473-15-4	222	1640(1648)	MS, RI	1.45	ND	ND
41	37.96	α-Cadinol	C ₁₅ H ₂₆ O	481-34-5	222	1654(1654)	MS, RI	ND	3.11	ND
42	38.19	ar-Tumerone	C ₁₅ H ₂₀ O	532-65-0	216	1660(1664)	MS, RI	25.78	31.35	34.69
43	38.48	α-Tumerone	C ₁₅ H ₂₂ O	180315-67-7	218	1668	MS	16.16	21.38	18.66
44	39.08	α-Bisabolol	C ₁₅ H ₂₆ O	515-69-5	222	1683(1685)	MS, RI	ND	ND	0.65
45	39.30	<i>Z</i> -α- <i>trans</i> -Bergamotol	C ₁₅ H ₂₄ O	88034-74-6	220	1689(1697)	MS, RI	4.03	1.56	1.38
46	39.59	Germacrone	C ₁₅ H ₂₂ O	6902-91-6	218	1700(1697)	MS, RI	2.02	13.32	15.01

Table 5 Continued.

NO.	Retention time (min)	Compound name	Formula	CAS#	MW	RI ^a (RI ^b)	ID method	Relative peak area (%)		
								NADES-based MAHD	MAHD	HD
47	40.76	Zerumbone	C ₁₅ H ₂₂ O	471-05-6	218	1729(1733)	MS, RI	0.60	ND	ND
48	41.41	(-)-Drimenol	C ₁₅ H ₂₆ O	19078-37-6	222	1747(1750)	MS, RI	ND	0.26	ND
49	41.99	Aristol-9-en-8-one	C ₁₅ H ₂₂ O	6831-17-0	218	1765(1758)	MS, RI	1.67	2.15	2.21
The number of certified essential oil components						39		24		21
Total identified peak area (%)						98.22		98.17		98.56
The number of non-oxygenated compounds						23		11		7
Total non-oxygenated compounds (%)						39.45		21.03		19.61
The number of oxygenated compounds						16		13		14
Total oxygenated compounds (%)						58.77		77.14		78.95

CAS# Chemical Abstracts Service Registry Number;

MW molecular weight;

RI^a Calculation retention index based on the DB-5MS column;RI^b Retention index in the literature;

ID identification;

ND not detected.

by means of MAHD. In HD, other major compounds of EO were α -curcumen (3.71%), α -zingiberene (4.96%), β -sesquiphellandrene (7.14%) and germacrone (15.01%). The difference in the major components of turmeric EO by different ways could be observed. Only 14 chemical compositions of EO such as caryophyllene, α -curcumen, valencene and germacrone were common in three ways. Many of compounds (e.g., ylangene, α -himachalene, α -gurjunene and β -eudesmol) were only detected after the pretreatment of NADESs. It proved that NADESs with the dissolution ability to biomaterials could lead to a better release of the active and valuable ingredients embedded in plant matrices. In addition, the EOs of isolated either by MAHD or HD were rather similar in their composition with a few differences in their quantity (relative peak area). The chemical compositions of EOs obtained by HD and MAHD had some differences as well as similarities with the cases reported in other regions^{43–45}. Hence, geographical origin of turmeric appeared to have great influence on the EO quality⁴⁶. Wide variations were found in the EO obtained in NADES-based MAHD method by comparison with those reported in other literatures^{47–49}. Including extraction and analysis methods, the huge differences in the oil chemical profile might be also due to genotype, variety, differential geography, climate, season, cultivation practices, fertilizer application, stress during growth or maturity, harvesting time, stage of maturity and storage^{50–52}. It was worth noting that the number of oxygenated compounds in NADES-based MAHD remained at a relatively high level in comparison with other two methods. Oxygenated compounds in terms of their contribution to the fragrance of the plant are more valuable than monoterpenes hydrocarbons⁵³. Therefore, this novel technology had great potential for a much better production of natural EO that were

rich in the valuable oxygenated compounds from aromatic plant materials.

4 Conclusions

From the experiment, synthesis of NADESs by microwave-assisted method could save the synthesis time and energy. Comparing with the traditional synthesis methods, both two methods had similar values of EO yield. It showed that microwave irradiation was a promising technique to synthesize NADESs. To get more EO yields from turmeric, NADESs by microwave-assisted preparation were as pretreatment solvents coupled with microwave-assisted HD for the extraction of EO from turmeric. The main extraction parameters such as pretreatment time, pretreatment temperature and HD time were optimized through CCD of the RSM. Under the optimal conditions, NADES composed of choline chloride and oxalic acid with a component molar ratio of 1:1 and an amount of 60 g in NADES obtained the highest EO yield of 0.85%. By GC-MS analysis, a total of 49 compounds were identified. The number of total compounds including valuable oxygenated compounds obtained by NADES-based MAHD were higher than other two methods. In brief, the proposed method brought about more identified compounds and EO yields than MAHD and HD methods.

Such a powerful approach can provide an energy efficient and environment friendly process and a more detailed insight on the volatile fraction of turmeric to satisfy the needs of the food and fragrance industries.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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