Fine Chemical Engineering
https://ojs.wiserpub.com/index.php/FCE/

Research Article

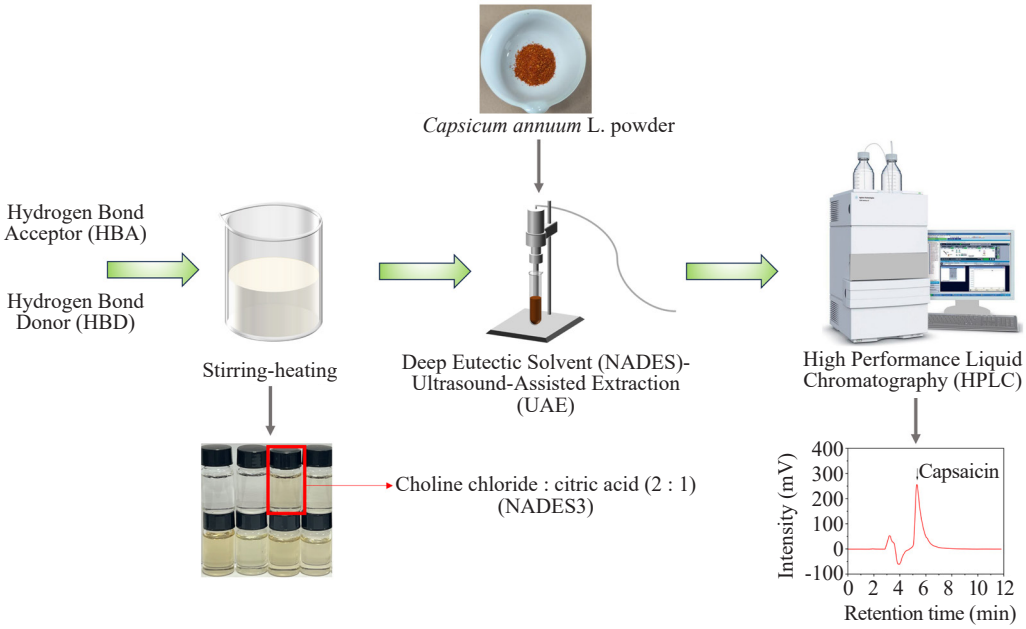
Extraction of Capsaicin from the Millet Pepper by Natural Deep Eutectic Solvent

Beibei Fang, Maoan Huang, Qing Liu, Xuchun Zhai, Chengli Yang, Dali Li*

Department of Bioengineering, School of Environmental & Biological Engineering, Nanjing University of Science & Technology, Nanjing, China
E-mail: lidali@njust.edu.cn

Received: 6 March 2025; **Revised:** 29 April 2025; **Accepted:** 22 May 2025

Graphical Abstract:



Abstract: This study aimed to design a Natural Deep Eutectic Solvent (NADES) for extracting capsaicin from the millet pepper (*Capsicum annuum* L.). Results demonstrated that NADES3 composed of choline chloride and citric acid (2 : 1 molar ratio) yielded 4.23 mg capsaicin per gram of pepper under optimized conditions (30% water content, 20 mL/g liquid-solid ratio, 20 min ultrasound). Post-enrichment with AB-8 macroporous resin, capsaicin recovery efficiency reached 87.66%. Molecular dynamics simulation revealed a stronger NADES3/capsaicin interaction compared to ethanol/capsaicin, indicating that capsaicin exhibits better homogeneous dispersion in NADES3 than in ethanol. Moreover, the recyclable NADES3 could be reused at least 4 times, showing that the proposed extraction method was eco-friendly. Thus, this study will serve as a reference for the future application of NADES in extracting other alkaloids, both in

DOI: https://doi.org/10.37256/fce.6220256694

laboratory and industrial settings.

***Keywords*:** natural deep eutectic solvent, ultrasound-assisted extraction, capsaicin

1. Introduction

Capsaicin (CAP) is a occurring bioactive alkaloid that confers the characteristic pungency to certain *Capsicum* (pepper) species.[1] Beyond its culinary applications, CAP and its derivatives are widely utilized in therapeutic contexts, including anti-inflammatory, antioxidant, antitumor and anti-obesity, etc.[2-4]

There exist multiple potential avenues for the acquisition of CAP. The most straightforward method for extracting CAP involves the utilization of an appropriate organic solvent or alternative ionic liquids,[5] which may threaten the environment. To tackle this challenge, environment-friendly and sustainable solvents have attracted considerable attention. Natural Deep Eutectic Solvent (NADES) is recognized as a novel type of ionic liquid characterized by the following properties: low cost, easy accessibility, non-volatility, biodegradability, and recyclability.[6,7] It is composed of natural components, such as sugars, and natural organic acids, all of which are considered safer and more environment-friendly alternatives to conventional solvents.[8] The NADES is a mixture of two or more components, comprising Hydrogen Bond Donors (HBD) and Hydrogen Bond Acceptors (HBA).[9] Different combinations of components of NADES can produce adjustable affinity of solvent for the extraction of different compounds from plants, which can assist in customizing a more suitable extraction solvent system.[10]

Among many extraction techniques to employ NADES as an extractant, the cavitation triggered by ultrasound can enhance the mass transfer efficiency of the extractant, which is ideal for NADES that possess higher viscosity.[11] On the one hand, the mechanical effects generated by ultrasound promote the collision between plants and extractants, which is beneficial to the partition of active substances from plants to extractants.[12,13] On the other hand, Ultrasound-Assisted Extraction (UAE) takes advantage of allows for scaling up to meet industrial requirements.[14]

In this study, the use of NADES-UAE was demonstrated as an efficient method for extracting CAP from the millet pepper, with the extraction conditions optimized. To elucidate the mechanism behind the disparate performance of extractants during extraction, the intermolecular interaction between NADES and CAP was investigated using Molecular Dynamics (MD) simulation.

2. Material and methods

2.1 *Chemicals and material*

The millet pepper (*Capsicum annuum* L.) was purchased from the Market of Nanjing Farmers. The plant was identified by a botanist, Assoc. Prof. Dr. Ruofu Shi, School of Environmental and Biological Engineering, Nanjing University of Science and Technology (NJUST). The voucher specimen has been kept at the Department of Bioengineering, School of Environmental and Biological Engineering, NJUST. N-hexane, ethyl acetate, choline chloride (ChCl), iodine and acetonitrile were purchased from Aladdin Co., Ltd. (Shanghai, China). D-(+)-Glucose, lactic acid, urea, ethylene glycol, glycerol, HCl and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ferric chloride, potassium ferricyanide, CAP (97% purity), Citric Acid (CA) and betaine were purchased from Macklin Co., Ltd. (Shanghai, China). AB-8 macroporous resin was purchased from Samsung Resin Co., Ltd. (Anhui, China).

2.2 *Preparation of NADES*

A stirring-heating method was used to prepare NADES. The HBA and HBD were mixed in sealed flasks in corresponding molar ratios, and components of NADES were shown in Table 1. Then, the mixture was heated at 80 °C with a magnetic stirrer until uniform and transparent liquid was obtained. The prepared NADES was added with calculated amounts (20%, w/w) of water and stored at room temperature.

Table 1. Screening of NADESs

Name	Composite of NADES	Chemical formulas	Molar ratio	Y_{CAP} (mg/g)
NADES1	Choline chloride : Glycerol	$C_5H_{14}ClNO : C_3H_8O_3$	1 : 2	0.08
NADES2	Choline chloride : Ethylene glycol	$C_5H_{14}ClNO : (CH_2OH)_2$	1 : 2	1.27
NADES3	Choline chloride : Citric acid	$C_5H_{14}ClNO : C_6H_8O_7$	2 : 1	2.42
NADES4	Choline chloride : Lactic acid	$C_5H_{14}ClNO : C_3H_6O_3$	1 : 2	0.37
NADES5	Choline chloride : Glucose	$C_5H_{14}ClNO : C_6H_{12}O_6$	2 : 1	0.84
NADES6	Choline chloride : Urea	$C_5H_{14}ClNO : CO(NH_2)_2$	1 : 2	1.16
NADES7	Betaine : Citric acid	$C_5H_{11}NO_2 : C_6H_8O_7$	2 : 1	1.48
NADES8	Betaine : Urea	$C_5H_{11}NO_2 : CO(NH_2)_2$	1 : 2	1.13

2.3 *NADES-UAE procedure*

The millet peppers were oven-dried at 50 °C, then homogenized into powder using a pulverizer (Joyoung, JYZ-D02V, Hangzhou, China) and sieved to a uniform particle size of 60-80 mesh. Before extracting, the powder was sealed and stored in a desiccator at room temperature.

The powder of millet pepper was mixed with NADES at a liquid-solid ratio of 5 mL/g. Subsequently, a probe-type cell crusher (Vibra cell vc130PB, Sonic and Materials, USA) was performed for extracting at an ultrasound power of 97.5 W. The sample was sonicated at room temperature for 10 min. The mixture was filtered through 4 layers of gauze and the filtrate was collected. A 50 μL of NADES extraction was diluted 5 times with ethanol, then centrifuged at 8,000 ×g for 10 min and the supernatant was collected for analysis by Thin-Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) analysis.

The extracted yield of CAP (Y_{CAP}) was calculated by formulae (1):

$$Y_{CAP}(\text{mg/g}) = \frac{X_{CAP} \times V_{NADES}}{M} \quad (1)$$

Where X_{CAP} (mg/mL) represents the concentrations of CAP in NADES extraction, which can be obtained using HPLC, V_{NADES} (mL) represents the volume of NADES extraction, M (g) represents the mass of the powder.

2.4 *MD simulation*

The MD simulation analysis was performed using the Materials Studio (MS) 2020 simulation package. A Condensed-phase Optimized Molecular Potentials for Atomistic Simulation Studies (COMPASSII) force field and Ewald summation techniques with an accuracy of 0.0001 kcal/mol were used to model the intermolecular interaction in the system at 298 K and 1 atm.[15]

The structures of molecules or ions, such as ethanol, Ch^+, Cl^-, CA, water and CAP were downloaded from the PubChem web server and optimized using geometry optimization in the Forcite module. The Amorphous Cell computational module was utilized to construct simulation boxes for different systems of NADES3-CAP and ethanol-CAP. The number of molecules in the simulation boxes was based on the molar ratios. The NADES3-CAP box consisted of 20 Ch^+, 20 Cl^-, 10 CA, 5 molecules of CAP and 33 molecules of water, the ethanol-CAP box consisted of 63 molecules of ethanol and 5 molecules of CAP. The simulation boxes were minimized using geometry optimization.[16]

The simulation was initiated by equilibrating the system under a constant Number of molecules, Pressure and Temperature (NPT) ensemble for 50 ps.[17] Finally the simulation was continued by dynamic mode with constant Number

of molecules, Volume and Temperature (NVT) ensemble using a Nose thermostat for 1,000 ps. The time step was set at 1.0 fs.[15]

2.5 *Optimization of the extraction conditions*

Based on the results of NADES-UAE, the NADES3 with optimal extraction efficacy was selected for further solvent screening.

For improving the efficiency of the mass transfer in extraction process and facilitating the recovery of the NADES3, the liquid-solid ratio was established at a value of 10 mL/g during the optimisation of the extraction conditions. The effects of water content (20, 30, 40, 50%), liquid-solid ratio (10, 20, 30, 40, 50 mL/g) and ultrasound time (5, 10, 15, 20 min) at room temperature on the extraction efficacy of the NADES3 were evaluated.

2.6 *TLC*

The samples were spotted on silica gel thin-layer plates (HSGF 254, Yantai Jiangyou Silica Gel Development, China). The mixture of N-hexane: ethyl acetate (1 : 1 (v/v)) was selected for TLC.

2.7 *HPLC*

The HPLC was performed on a Shimadzu HPLC system equipped with a detector (Shimadzu, SPD-10Avp, Japan), using a C18 column (4.6 × 250 mm, 5 μm, Alltech, USA).

The sample was filtered through a 0.45 μm microfiltration membrane. The mobile phase was acetonitrile: water (1 : 1 (v/v)) at a flow rate of 1.5 mL/min. The 222 nm wavelength was used for detecting CAP.

2.8 *Enrichment of CAP*

During adsorption, 0.5 g of AB-8 macroporous resin was taken and mixed with 1 mL of NADES extraction for 6 h at room temperature. For desorption, 1 mL of ethanol was added to 0.1 g of the adsorped AB-8 macroporous resin, then incubated at 150 r/min, 37 °C for 2 h.

The Adsorption ratio (A), Desorption ratio (D), and Recovery efficiency (R) were calculated using formulae (2), (3) and (4):

$$\mathrm{A}(\%) = \frac{C_0 - C_e}{C_0} \times 100\% \tag{2}$$

$$\mathrm{D}(\%) = \frac{C_d \times V_d}{(C_0 - C_e) \times V_0} \times 100\% \tag{3}$$

$$\mathrm{R}(\%) = \frac{C_d \times V_d}{C_0 \times V_0} \times 100\% \tag{4}$$

Where C_0 is the concentration of CAP in NADES extraction (mg/mL); C_e is the equilibrium concentration of CAP in NADES extraction after adsorption (mg/mL); C_d is the concentration of CAP in the desorption solution (mg/mL); V_0 is the volume of NADES extraction (mL); and V_d is the volume of desorption solution (mL).

2.9 *Reuse of NADES*

The recovered NADES3 was used to extract a new sample of millet pepper. Four cycles of the NADES3 recovery and new extraction were conducted using the same method to further determine the yield of CAP in each NADES3 extraction.

2.10 *Statistical analysis*

All the experiments were carried out in triplicate if not otherwise specified, and the results were expressed as mean ± Standard Deviation (SD). Statistical analysis and data visualization were performed using the Origin software (version 2023bSR1).

3. Results and discussion

3.1 *Screening of NADESs*

Eight kinds of NADES were prepared for screening their performance in extracting CAP from the millet pepper. As shown in Table 1, the NADES3 was the best with Y_{CAP} of 2.42 mg/g.

The extraction efficiency observed in the current study was comparatively lower than that reported by Qin et al. for Capsaicin (CAP) extraction. This discrepancy may be attributed to variations in experimental parameters, particularly extraction time and ultrasonic power intensity.[18]

3.2 *MD simulation analysis*

The NADES3 was selected for Molecular Dynamics (MD) simulation and compared with ethanol phases. Apparently, the average noncovalent interaction energy of CAP with NADES3 was -278.62 kcal/mol, which was lower than that with ethanol (-258.45 kcal/mol) from 400 to 1,000 ps (Figure 1A). It could be observed that the average hydrogen bond number of the CAP formed with NADES3 (39.62) in the range of 0 ~ 1,000 ps was much higher than that with ethanol (13.03) under the condition that there was no significant difference in the quality of hydrogen bonds (Figure 1B), indicating that CAP dissolved in NADES3 exhibited greater stability and maintained a more homogeneous dispersion compared to that in ethanol.[10] This may be because NADES containing both HBA and HBD can form more hydrogen bonds with the extracted phenolic compounds, thereby enhancing the dissolution capacity of the extract.[19]

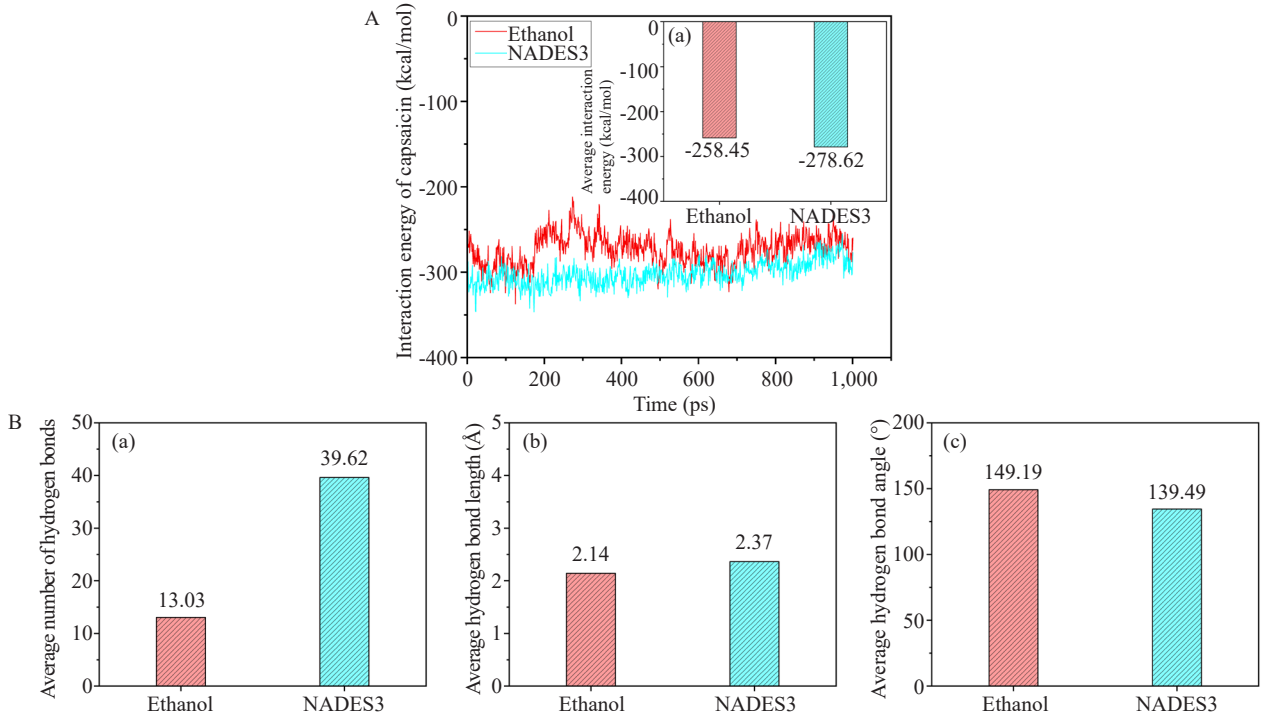


Figure 1. (A) Noncovalent intermolecular interaction energy of CAP with different solvents. (a: the average energy from 400 to 1,000 ps); (B) The a-c correspond to the average hydrogen bonds number (a), length (b) and angle (c) between CAP and different solvents from 0 ~ 1,000 ps, respectively

3.3 *Effect of water content, liquid-solid ratio and ultrasound time*

As shown in Figure 2a, the NADES3 with a water content of 30% showed a higher Y_{CAP} (3.01 mg/g). This might be because high viscosity was detrimental to the mass transfer of the target compounds from plant matrices to the extractant.[20] However, CAP was extremely difficult to dissolve in water and a further increase in water content might reduce the extraction yield by weakening the interaction between NADES3 and CAP.[21,22]

As shown in Figure 2b, the highest Y_{CAP} was 3.39 mg/g at a liquid-solid ratio of 20 mL/g. This might indicate that an excessive amount of solvent had an adverse impact on the extraction process.[23]

The ultrasound time was an important parameter of the extraction process. The Y_{CAP} reached a value of 4.23 mg/g at an ultrasound time of 20 min (Figure 2c). The cavitation effect of the ultrasound enhanced the swelling and hydration, which probably increased the exposure of the solute and the extraction medium and facilitated its release into the solvent.[7]

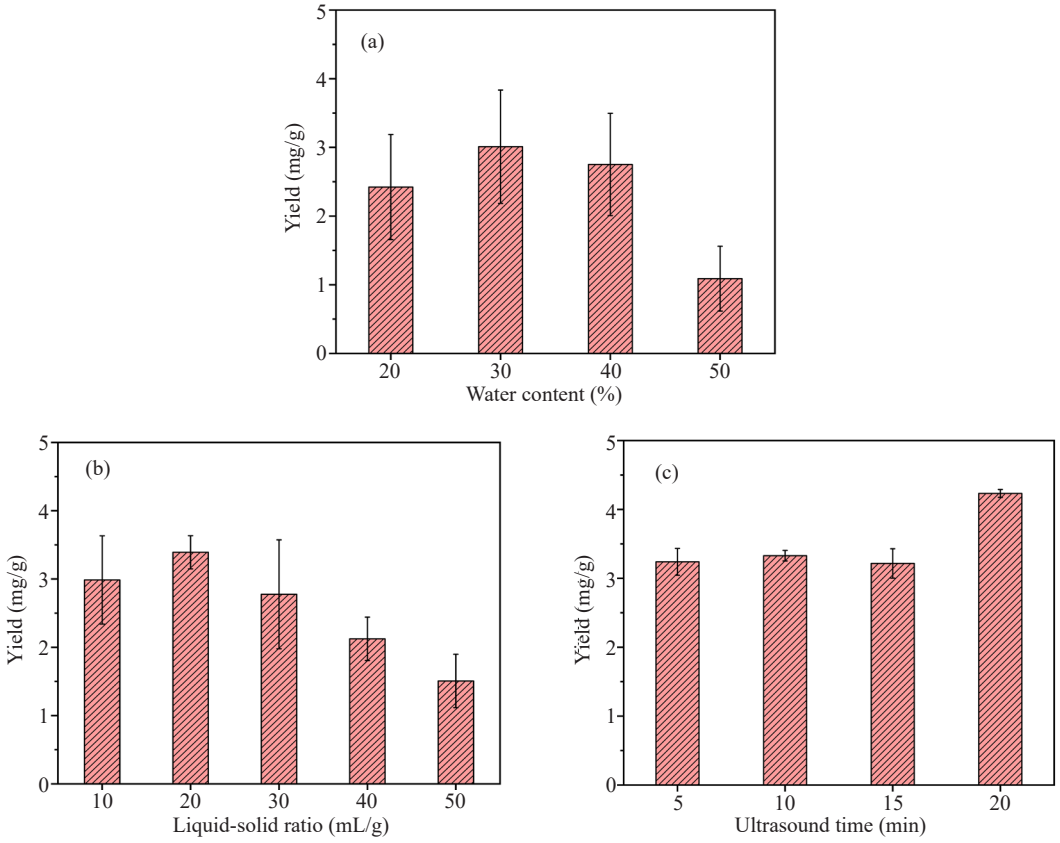


Figure 2. Effects of extraction conditions, (a) water content, (b) liquid-solid ratio, and (c) ultrasound time

3.4 *Reuse capacity of NADES3*

The highest recovery of CAP was 87.7% proving that the AB-8 resin could effectively enrich CAP from the NADES3.

To evaluate the reuse capacity of NADES3, the powder of millet pepper was extracted using the reclaimed NADES3. It could be observed that the well-designed NADES3 had good recycling ability in the extraction of CAP and could be reused at least 4 times (Figure 3), aligning with the precept of green and sustainable development.

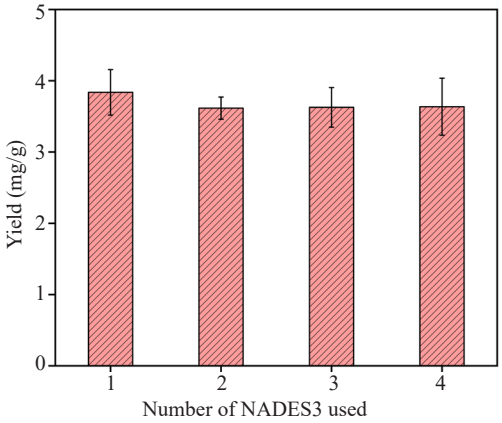


Figure 3. Reuse capacity of NADES3

4. Conclusion

In conclusion, the extraction of CAP from the powder of millet pepper was accomplished using a new method of NADES-UAE. Simulation indicated that NADES-UAE has more potential than the conventional extraction method. Furthermore, MD analysis was used to investigate the extraction mechanism caused by different solvents and enabled the theoretical substantiation of this research through the simulation of the interaction between different solvents and capsaicin, which may facilitate the customization of NADES with higher extraction performance. The method of MD will be useful for developing solvents for extracting natural products.

Specifically, the extraction process disrupted the cellular walls, promoting capsaicin release. The liberated capsaicin formed more stable hydrogen bonds with NADES3, significantly improving the solubility of capsaicin in NADES. Given this, NADES-UAE represents an efficient and industrially viable method for capsaicin extraction. This green chemistry approach facilitates sustainable valorization of natural materials while demonstrating significant potential for expanding capsaicin applications in food technology and biotechnological sectors.

Conflict of interest

The authors declare that there is no conflict of interest.

References

[1] Orosz, M. J.; Rávai, B.; Mátravölgyi, B.; Bálint, E. Flow synthesis of capsaicin and capsaicinoid analogues. *ACS Sustainable Chem. Eng.* **2024,** *12*, 7913-7923.

[2] Basith, S.; Cui, M.; Hong, S.; Choi, S. Harnessing the therapeutic potential of capsaicin and its analogues in pain and other diseases. *Molecules* **2016,** *21*, 966.

[3] Zhang, S.; Wang, D.; Huang, J.; Hu, Y.; Xu, Y. Application of capsaicin as a potential new therapeutic drug in human cancers. *J. Clin. Pharm. Ther.* **2020,** *45*, 16-28.

[4] Bogusz, S., Jr.; Libardi, S. H.; Dias, F. F. G.; Coutinho, J. P.; Bochi, V. C.; Rodrigues, D.; Melo, A. M. T.; Godoy, H. T. Brazilian *Capsicum* peppers: Capsaicinoid content and antioxidant activity. *J. Sci. Food Agric.* **2018,** *98*(1), 217-224.

[5] Chinn, M. S.; Sharma-Shivappa, R. R.; Cotter, J. L. Solvent extraction and quantification of capsaicinoids from *Capsicum chinense*. *Food Bioprod. Process.* **2011,** *89*(4), 340-345.

[6] Azzouz, A.; Hayyan, M. Are deep eutectic solvents biodegradable? *Process Saf. Environ. Prot.* **2023,** *176*, 1021-1025.

[7] Wang, H.; Qin, Z.; Bian, R.; Stubbings, W. A.; Liu, L.-Y.; Li, F.; Zhao, X.; Wu, F.; Wang, S. Single injection by LC-ESI-MS/MS for simultaneous determination of organophosphate tri-and di-esters in plant tissue based on

ultrasonic-assisted sequential extraction and single-step purification. *Food Chem.* **2024,** *437*, 137917.
[8] Cao, C.; Nian, B.; Li, Y.; Wu, S.; Liu, Y. Multiple hydrogen-bonding interactions enhance the solubility of starch in natural deep eutectic solvents: Molecule and macroscopic scale insights. *J. Agric. Food Chem.* **2019,** *67*(45), 12366-12373.
[9] Li, X.; Row, K. H. Development of deep eutectic solvents applied in extraction and separation. *J. Sep. Sci.* **2016,** *39*(18), 3505-3520.
[10] Fu, X.; Belwal, T.; He, Y.; Xu, Y.; Li, L.; Luo, Z. UPLC-Triple-TOF/MS characterization of phenolic constituents and the influence of natural deep eutectic solvents on extraction of *Carya cathayensis* Sarg. peels: Composition, extraction mechanism and *in vitro* biological activities. *Food Chem.* **2022,** *370*, 131042.
[11] Zhao, Y.; Wan, H.; Yang, J.; Huang, Y.; He, Y.; Wan, H.; Li, C. Ultrasound-assisted preparation of 'ready-to-use' extracts from *Radix Paeoniae Rubra* with natural deep eutectic solvents and neuroprotectivity evaluation of the extracts against cerebral ischemic/reperfusion injury. *Ultrason. Sonochem.* **2022,** *84*, 105968.
[12] Medlej, M. K.; Cherri, B.; Nasser, G.; Zaviska, F.; Hijazi, A.; Li, S.; Pochat-Bohatier, C. Optimization of polysaccharides extraction from a wild species of *Ornithogalum* combining ultrasound and maceration and their antioxidant properties. *Int. J. Biol. Macromol.* **2020,** *161*, 958-968.
[13] Tong, X.; Yang, J.; Zhao, Y.; Wan, H.; He, Y.; Zhang, L.; Wan, H.; Li, C. Greener extraction process and enhanced *in vivo* bioavailability of bioactive components from *Carthamus tinctorius* L. by natural deep eutectic solvents. *Food Chem.* **2021,** *348*, 129090.
[14] Belwal, T.; Chemat, F.; Venskutonis, P. R.; Cravotto, G.; Jaiswal, D. K.; Bhatt, I. D.; Devkota, H. P.; Luo, Z. Recent advances in scaling-up of non-conventional extraction techniques: Learning from successes and failures. *TrAC Trends Anal. Chem.* **2020,** *127*, 115895.
[15] Hashim, N. A.; Abdul Mudalip, S. K.; Harun, N.; Che Man, R.; Sulaiman, S. Z.; Arshad, Z. I. M.; Shaarani, S. M. Molecular dynamics simulation of *Mahkota Dewa* (*Phaleria macrocarpa*) extract in subcritical water extraction process. *IOP Conf. Ser.: Mater. Sci. Eng.* **2018,** *358*, 012033.
[16] Ismail, M.; Bakaruddin, B. R.; Lethesh, K. C.; Mutalib, M. I. A.; Shah, S. N. Experimental and theoretical study on extraction and recovery of naphthenic acid using dicyanamide-based ionic liquids. *Sep. Purif. Technol.* **2019,** *213*, 199-212.
[17] Yao, J.; Xiao, L.; Li, C.; Wang, B.; Chen, Y.; Yan, X.; Cui, Z. Exploration of the multiscale interaction mechanism between natural deep eutectic solvents and silybin by QC calculation and MD simulation. *J. Mol. Liq.* **2022,** *363*, 119768.
[18] Yang, Q.; Mariga, A. M.; Wen, L.; Hu, Q.; Xie, M.; Liu, J.; Pei, F. Extraction mechanism and bio-activities of capsaicinoids from lantern peppers placenta using deep eutectic solvents. *Food Chem.* **2025,** *464*, 141773.
[19] Cunha, S. C.; Fernandes, J. O. Extraction techniques with deep eutectic solvents. *TrAC Trends Anal. Chem.* **2018,** *105*, 225-239.
[20] Xing, C.; Cui, W.-Q.; Zhang, Y.; Zou, X.-S.; Hao, J.-Y.; Zheng, S.-D.; Wang, T.-T.; Wang, X.-Z.; Wu, T.; Liu, Y.-Y.; Chen, X.-Y.; Yuan, S.-G.; Zhang, Z.-Y.; Li, Y.-H. Ultrasound-assisted deep eutectic solvents extraction of glabridin and isoliquiritigenin from *Glycyrrhiza glabra*: Optimization, extraction mechanism and *in vitro* bioactivities. *Ultrason. Sonochem.* **2022,** *83*, 105946.
[21] Pan, C.; Zhao, L.; Zhao, D. Microwave-assisted green extraction of antioxidant components from *Osmanthus fragrans* (Lour) flower using natural deep eutectic solvents. *J. Appl. Res. Med. Aromat. Plants* **2021,** *20*, 100285.
[22] Aslan Türker, D.; Doğan, M. Application of deep eutectic solvents as a green and biodegradable media for extraction of anthocyanin from black carrots. *LWT* **2021,** *138*, 110775.
[23] Kumar, K.; Srivastav, S.; Sharanagat, V. S. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. *Ultrason. Sonochem.* **2021,** *70*, 105325.