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Process optimization of deep eutectic solvent-based microwave-assisted extraction of flavonoids from Ziziphi Spinosae Semen using response surface methodology

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Abstract

Total flavonoids were extracted from Ziziphi Spinosae Semen (ZSS) by applying microwave-assisted extraction with low eutectic solvent (DES) as the extractant. This study investigated the effect of 21 different components of DES on the yield of total flavonoids in ZSS. The results revealed that the DES system, which consists of choline chloride and lactic acid with a molar ratio of 2:1, achieved the best flavonoid extraction efficiency. Based on the single-factor test, response surface methodology was used to optimize and determine the optimal process conditions for the extraction of total flavonoids from ZSS with deep eutectic solvent. The maximum flavonoids yields were achieved at 36.24 mg/g within the 50% DES water content, a microwave power of 600 W, a microwave time of 60 s, a liquid-solid ratio of 1:28 mg/mL, an extraction time of 66 min, and an extraction temperature of 93 °C. The macroporous resin was used to recover flavonoids from extracts. The DM130 resin showed higher adsorption/desorption performance. The total flavonoids of ZSS were extracted using the recovered DES with a yield of 32.58 mg/g and a high reusability of 89.9%.

Keywords: Ziziphi Spinosae Semen; deep eutectic solvents; total flavonoids; microwave-assisted extraction.

Practical Application: The total flavonoids extraction process of sour date kernel optimized in this study can be used as a theoretical basis for developing and utilizing sour date kernel resources. DES can be an excellent alternative to traditional organic solvents to extract effective bioactive substances in Chinese medicine.

1 Introduction

Ziziphi Spinosae Semen (ZSS), the seed of *Ziziphus jujuba Mill*. var. *spinosa* (Bunge) Hu ex H. F. Chou, is one of the most famous traditional Chinese medicines (State Pharmacopoeia Committee, 2000). ZSS is mainly produced in Australia, Europe, Asia, and especially in the inland areas of northern China, such as Hebei, Shaanxi, Liaoning, and Henan (He et al., 2020). As a precious traditional Chinese medicine, modern pharmacological research has shown that the extract of ZSS possesses a wide range of biological activities, such as ameliorating effect of learning and memory, sedation and hypnosis, antibacterial, anti-inflammatory, antioxidant, antidepressant, antianxiety, and hypoglycemic (Liu et al., 2018; Zheng et al., 2022). ZSS has a wide range of biological activities mainly attributed to the rich bioactive components of the ZSS, including flavonoids, saponins, alkaloids, and fatty acids (He et al., 2020). Therefore, it is necessary to explore an efficient process to extract flavonoids from ZSS to explore the value of this traditional Chinese medicine fully.

At present, various efficient methods for extracting flavonoids from plant material have been reported, such as reflux, decoction, maceration, percolation, and soxhlet (Blicharski & Oniszczuk, 2017). Traditional extraction techniques do not solve all problems in the field of extraction due to their time consumption, energy dissipation, low selectivity, and require large amounts of solvents, which are commonly toxic (Farzaneh & Carvalho, 2017). Therefore, the use of traditional extraction methods in the food, cosmetics, and pharmaceutical industries is limited (Briones-Labarca et al., 2019). Flavonoids are mainly extracted with various organic solvents due to their low solubility in water. The resulting environmental pollution and harmful residues have received considerable critical attention (Xia et al., 2021). Advanced extraction methods are being developed to overcome the problems of traditional extraction methods (Chaves et al., 2020). Modern extraction techniques include ultrasound-assisted extraction, microwave-assisted extraction (MAE), solid phase extraction, supercritical fluid extraction, enzyme-assisted extraction, pressurized liquid extraction, and a combination of different techniques (Al-Hatim et al., 2022). Compared with the traditional methods, MAE has many merits, such as shorter extraction time, less solvent use, higher extraction rate, better productivity, and higher quality products. Tran et al. (2022) evaluated the effect of extraction conditions of three extraction methods on the yield of polyphenols and flavonoids in Pomelo peels and found that among the three methods, microwave-assisted extraction was considered the most efficient method to obtain the high yield of polyphenols. More importantly, MAE can also replace organic solvents with alternative "green" solvents, thus reducing the use of organic solvents (Qu et al., 2021). In addition, MAE allows for higher yields in shorter extraction times, making the extraction of total flavonoids more environmentally friendly and efficient (Chaves et al., 2020).

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DES was first reported by Abbott et al. (2003) and typically consists of a mixture of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) in appropriate proportions, held together by hydrogen bonding. Compared with organic solvents, DES not only possesses high solubility but also possesses some merits of low toxicity or nontoxicity, low cost, environmental friendliness, low volatility, and designable structure. Currently, DES has been widely used to extract active ingredients of traditional Chinese medicine, such as flavonoids, phenolic acids, and alkaloids. When using DES to extract flavonoids, higher yields were always obtained than conventional solvents (Wu et al., 2022). However, most DESs exhibit viscosities higher than many conventional organic solvents, which is not conducive to the rapid diffusion of target components. MAE can heat the extraction solvent in a short time, reducing the viscosity and surface tension of DES and contributing to the dissolution of the active ingredients of Chinese medicine (Yang, 2018). Therefore, combining microwave assistance with DES may be an efficient and environmentally friendly method for extracting flavonoids in traditional Chinese medicine.

A large number of studies have been published on the extraction of total flavonoids from ZSS using various methods and solvents. Nevertheless, no studies have been found about applying DES in MAE of ZSS total flavonoids. Furthermore, there is still a lack of reports on the recovery of total ZSS flavonoids from DES extracts. Various DES combinations were evaluated in this study for their efficiency in extracting flavonoids from ZSS. After preliminary screening, the best DES system was selected. Subsequently, the main extraction conditions parameters related to the yield of total flavonoids from ZSS were optimized by single-factor and response surface methods. Finally, a macroporous resin adsorption method was used to recover DES and the total flavonoids of ZSS, and the sustainable utilization of the method was evaluated. This study provides a solid theoretical basis for the development and utilization of ZSS.

2 Materials and methods

2.1 Materials and chemicals

ZSS was purchased in Zanhuang County, Hebe Province, China. The ZSS was degreased by the microwave-assisted method. The stir-fried ZSS materials are ground with a high-speed grinder (BF-02, Hebei Benchen Technology Co Ltd, Hebei, China) and passed through a # 100 mesh. The sample was weighed with an analytical electron balance (± 0.01 g accuracy, BSA124S-CW, Shanghai Balance Instrument Plant, Shanghai, China). The powders (100 g) were radiated with 400 mL of petroleum ether under a microwave power of 600 W for 100 s. The solution after radiation was filtered, and the obtained residue was dried in an electric heating constant temperature blast drying oven (DHG-9145B, Shanghai Jinghong Experi- mental Facilities Corporation Ltd, Shanghai, China) at 80 °C for 30 min to remove the residual petroleum ether. Finally, the obtained defatted ZSS powder was placed in a desiccator for storage.

Choline chloride (≥ 98% mass fraction purity) was purchased from Beijing FeiMo Biotechnology Co., Ltd. (Beijing, China). Rutin (≥ 98% mass fraction purity) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Urea, glucose, lactic acid, sorbitol, glycerol, ethylene glycol, 1,4-butanediol, sodium nitrite, aluminum nitrate, and sodium hydroxide (all ≥ 99% mass fraction purity) were purchased from Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Macroporous resins (AB-8, DM130, HPD100, DM301, and D101) were purchased from Cangzhou Bon Adsorber Technology Co., Ltd. (Heibei, China).

2.2 Preparation of DESs

DES was prepared by the previously reported heating method (Dai et al., 2013). Choline chloride (ChCl) was used as HBA, and urea, glucose, lactic acid, sorbitol, glycerol, ethylene glycol, and 1,4-butanediol were used as HBD. The HBA and the HBD are mixed in a specific molar ratio at 80 °C and stirred for 30~90 min until a stable homogeneous liquid is formed. In the synthesis of DES, a certain amount of water was added to the reaction system. Twenty-one DES systems composed of two components were obtained (Table 1).

2.3 Extraction of total flavonoids from ZSS

ZSS powder (1 g) was mixed separately with 21 different DES containing 50% water (25 mL). Subsequently, the samples were placed in a microwave oven and irradiated with 600 W power for 60 s (M1-211A, Guangdong Midea Microwave and Electrical Appliances Manufacturing Co Ltd, Guangdong, China). The beaker was then placed in a 90 °C water bath and stirred continuously for 70 min. After the reaction, the mixture was centrifuged at 10,000 G for 15 min to remove the solids (3-18k, Sigma Laborzentrifugen Co Ltd, Germany). The supernatant was taken and stored by filtration through a 0.45 μm nylon membrane.

Table 1. Different compositions of DESs applied in this work.

NO.	Type of HBD	Abbreviation	ChCl/HBD Ratio
DES-1	Urea	U	2:1
DES-2			1:1
DES-3			1:2
DES-4	Glucose	Glu	2:1
DES-5			1:1
DES-6			1:2
DES-7	Lactic acid	LA	2:1
DES-8			1:1
DES-9			1:2
DES-10	Sorbitol	SO	2:1
DES-11			1:1
DES-12			1:2
DES-13	Glycerol	GL	2:1
DES-14			1:1
DES-15			1:2
DES-16	Ethylene glycol	EG	2:1
DES-17			1:1
DES-18			1:2
DES-19	1,4-Butanediol	BDO	2:1
DES-20			1:1
DES-21			1:2

2.4 Drawing standard curve and determining flavonoid content

The previously reported method was used with slight modifications (Pan et al., 2021). First, 0.3 mL 5% (w/v) sodium nitrite solution was added to 1 mL extract, shaken well, and allowed to stand for 6 min. Then 0.3 mL of 10% aluminum nitrate solution was added, shaken well, and left to react for 6 min. Finally, 4 mL of 4% sodium hydroxide solution was added. The mixture was made up to 10 mL with distilled water, shaken well, and left to stand for 15 min. Total flavonoid concentration was evaluated at 510 nm (UV2100B, Shanghai Metash Instruments Co Ltd, Shanghai, China). In the range of 0.2 to 0.8 mg/mL with a calibration curve regression equation of y = 12.26x - 0.0014 (R^2 = 0.9993). The total flavonoid yield was calculated according to the following Equation 1:

$$W=\frac{X\times V_0\times V_2}{m\times V_1} \tag{1}$$

Which W is the yield of the total flavonoids of ZSS; X is the concentration of the total flavonoids of ZSS; m is the mass of the sample (defatted ZSS powder); V_0 is the volume of the extract solution after fixing; V_1 is the volume of the extract solution used in measuring the absorbance value, and V_2 is the volume of the extract solution to be measured.

2.5 DES type and optimal ratio

The effect of 21 DESs on the total flavonoid yield of ZSS was investigated to determine the optimal composition of DESs. The water content of each DES was fixed at 50%, and the extraction of total flavonoids from ZSS was carried out at three HBA: HBD ratios (2:1, 1:1, 1:2). The total flavonoid yield was measured, and the ratio corresponding to the highest yield was used as the best HBA: HBD ratio (Shang et al., 2021).

2.6 Single-factor investigation

The single-factor experimental design was carried out to evaluate the effects of the water content of DES (30, 40, 50, 60, 70%), microwave power (400, 500, 600, 700, 800 W), microwave radiation time (30, 60, 90, 120, 150 s), material-to-liquid ratio (1:15, 1:20, 1:25, 1:30, 1:35 mg/g), extraction temperature (60, 70, 80, 90, 100 °C) and extraction time (40, 50, 60, 70, 80 min) on the yield of total flavonoids in ZSS, respectively. In determining these specific ranges of values, we have benefited from the existing knowledge on the extraction of flavonoids from previous studies (Pal & Jadeja, 2019; Wu et al., 2022).

2.7 RSM experiments for extracting ZSS total flavonoids with DES

Based on the results of the above experiment, we used the response surface methodology to optimize the extraction process parameters using Design-Expert Ver. 13 (Stat-Ease Inc., Minneapolis, MN, USA). The Box-Behnken design (BBD) was applied to obtain the optimal values for the three leading independent variables in this extraction: solid-liquid ratio (A, 1:20, 1:25, 1:30 g/mL), extraction time (B, 60, 70, 80 min) and extraction temperature (C, 80, 90, 100 °C) (Table 2).

2.8 Validation of RSM models

The optimized parameters from the RSM models were validated by performing the MAE accordingly. The experimental results obtained were then compared to those predicted by the models.

2.9 Recovery of total flavonoids and reuse of DESs

Five macroporous resins (AB-8, DM130, HPD100, DM301, and D101) were used to recover total flavonoids from ZSS. The DES-extract flavonoid solution (10 mL) was loaded in a beaker containing 5 g of the pretreated macroporous resins. The mixture was shaken at room temperature for 12 hours at 150 r/min for adsorption. After the macroporous resin entirely adsorbed the flavonoids, the supernatant was collected to determine the adsorption rate. In the meantime, the saturated macroporous resin was filtered and washed, and then acetone equivalent to the volume of DES was added. After shaking at 150 r/min for 12 hours, the macroporous resin was filtered to obtain the acetone desorbate. In the meantime, DES was recovered and stored for the subsequent extraction.

2.10 Statistical analysis

Each test was performed three times in parallel, and the results are expressed as mean ± standard deviation. The regression equation and the significance statistics of the RSM model were analyzed by Design-Expert 13 (Stat-Ease Inc., USA). In BBD experiments, significance analysis of the factors and their interactions was based on analysis of variance (ANOVA), and significant differences ($p < 0.05$) were considered significant.

3 Results and discussion

3.1 Screening of optimal DESs

The type of DES determines its properties. The appropriate type of DES with different properties (solubility, polarity, physicochemical interactions, viscosity, and surface tension) plays a crucial role in extracting target components with solvents (Wang et al., 2019). Therefore, a rational selection of DES is essential in this experiment. In this study, the effect of 21 types of DES on the total flavonoid yield was investigated using the microwave-assisted method under the same extraction conditions using defatted ZSS powders as raw material. As shown in Figure 1, DES-7 showed the best extraction effect with a total flavonoid yield of 31.21 mg/g. This result may be explained by

Table 2. Design of RSM experiments.

Levels	A. Solid-liquid ratio (g/mL)	B. Extraction time (min)	C. Extraction temperature (°C)
-1	1:20	60	80
0	1:25	70	90
1	1:30	80	100

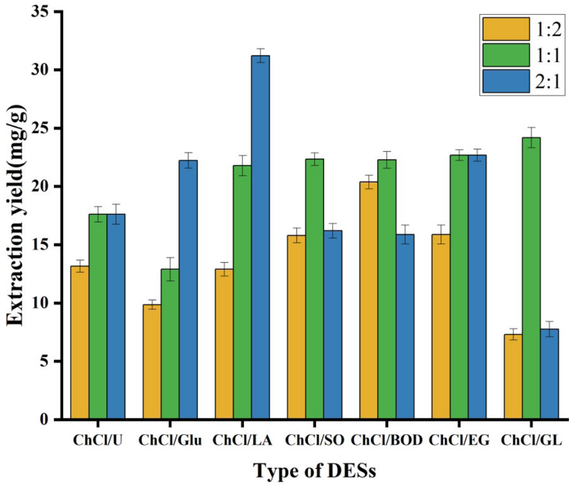

Figure 1. Effect of different types of deep eutectic solvents on the yield of total flavonoid from Ziziphi Spinosae Semen.

the stronger hydrogen bonding of the organic acid-based DES to the flavonoids. Furthermore, DES-7 has a lower viscosity and better flowability, facilitating the diffusion of the target components (Wang et al., 2019). Xia et al. (2021) extracted total flavonoids from Polygonatum odoratum rhizomes and found the highest yield when ChCl/lactic acid DES was used as an extractant. Wang et al. (2018c) found similar results when extracting Baicalin from Scutellaria baicalensis Georgi using the microwave-assisted method. Baicalin had the highest equilibrium solubility and yield in DES, consisting of ChCl and lactic acid.

Polarity is another significant factor affecting solubility. Theoretically, bioactive components can be easily extracted with similar polarity solvents (Wang et al., 2018b). As seen in Figure 1, the maximum yield of total ZSS flavonoids was reached when the molar ratio of ChCl and lactic acid was 2:1, which may be due to the similar polarity of DES-7 and the total ZSS flavonoids (Wang et al., 2018c). Meanwhile, the increase in the lactic acid ratio effectively reduced the viscosity of the DES system, which promoted the diffusion of total ZSS flavonoids and improved extraction efficiency. However, since the water has been added to the DES system to adjust the viscosity, the continued increase of the lactic acid ratio would weaken the interaction between the target substance and DES. On the contrary, Xia et al. (2021) showed different results when using DES consisting of ChCl and lactic acid to extract total flavonoids from the rhizomes of Polygonatum odoratum. Specifically, the total flavonoids yield increased and then decreased as the ChCl ratio decreased. The highest total flavonoid yield was achieved when the molar ratio of ChCl and lactic acid was 1:2. This discrepancy could be attributed to the different water content of DES used in the experiments.

Based on these results, DES-7 consisting of ChCl and lactic acid (molar ratio of 2:1), was considered the most suitable solvent for extracting total flavonoids from ZSS and was applied in subsequent experiments.

3.2 Optimization of microwave-assisted DES extraction of total flavonoids from ZSS

Effect of DES water content on the yield of total flavonoids from ZSS

A significant obstacle to DES as an extractant is its generally high viscosity, which can be improved by adding water or increasing the temperature (Yang, 2018). Furthermore, the high costs associated with using pure DES in laboratory-scale extractions can be addressed by adding the appropriate amount of water (Skarpalezos & Detsi, 2019). From Figure 2A, we can see that the total flavonoid yield increased gradually with an increase in water content from 30% to 50%. The yield of ZSS total flavonoids peaked when the water content arrived at 50%. Nevertheless, when the water content increased above 50%, the extraction yields had a clear trend of decreasing. This is likely attributed to the high water content effectively reducing the viscosity and surface tension of the DES, which is more conducive to mass transfer action (Shang et al., 2021). However, with water content greater than 50%, the hydrogen bonding backbone of the DES fraction is disrupted, resulting in a reduction in the solubility of total flavonoids in DES (Meng et al., 2018). Dai et al. (2013) found that adding 50% water caused a break in the hydrogen bond between the two components, resulting in a significant change in the polarity of both DESs. Meng et al. (2018) showed a similar trend of increasing and then decreasing flavonoid yield with increasing water content when using DES to extract flavonoids from Pollen Typhae. Therefore, a 50% DES water content was optimal and used for further experiments.

Effect of microwave power on the yield of total flavonoids from ZSS

The microwave power provides localized heating in the sample, which acts as the driving force for MAE by disrupting the plant matrix and allowing the target components to diffuse and dissolve in the solvent (Hiew et al., 2022). Increasing the microwave power will generally shorten the extraction time and thus improve the extraction efficiency (Pan et al., 2021). The effect of microwave power on the yield of total flavonoids from ZSS is depicted in Figure 2B. As the microwave power increased from 400 to 600 W, the total flavonoid yield increased significantly. However, the total flavonoid yield decreased when power increased above 600 W. This may be due to the increase in microwave power leading to a rapid increase in temperature, which accelerates the diffusion of the target components. However, when the temperature is too high, the activity of flavonoids is lost, and the solubility of impurities increases (Wang et al., 2018c). This trend was similar to that of Xu et al. (2021) when they used a microwave-assisted method to extract flavonoids from Phyllostachys heterocycla leaves. Specifically, the total flavonoid yield showed a trend from increase to decrease with increasing microwave power. Similar results were obtained when Le et al. (2019) used a microwave-assisted method to extract total flavonoids from Fruits of Docynia indica. Therefore, a microwave power of 600 W was selected for the subsequent study.

Effect of microwave time on the yield of total flavonoids from ZSS

The microwave time is a crucial parameter for obtaining high-quality products. In general, the yield of the target product

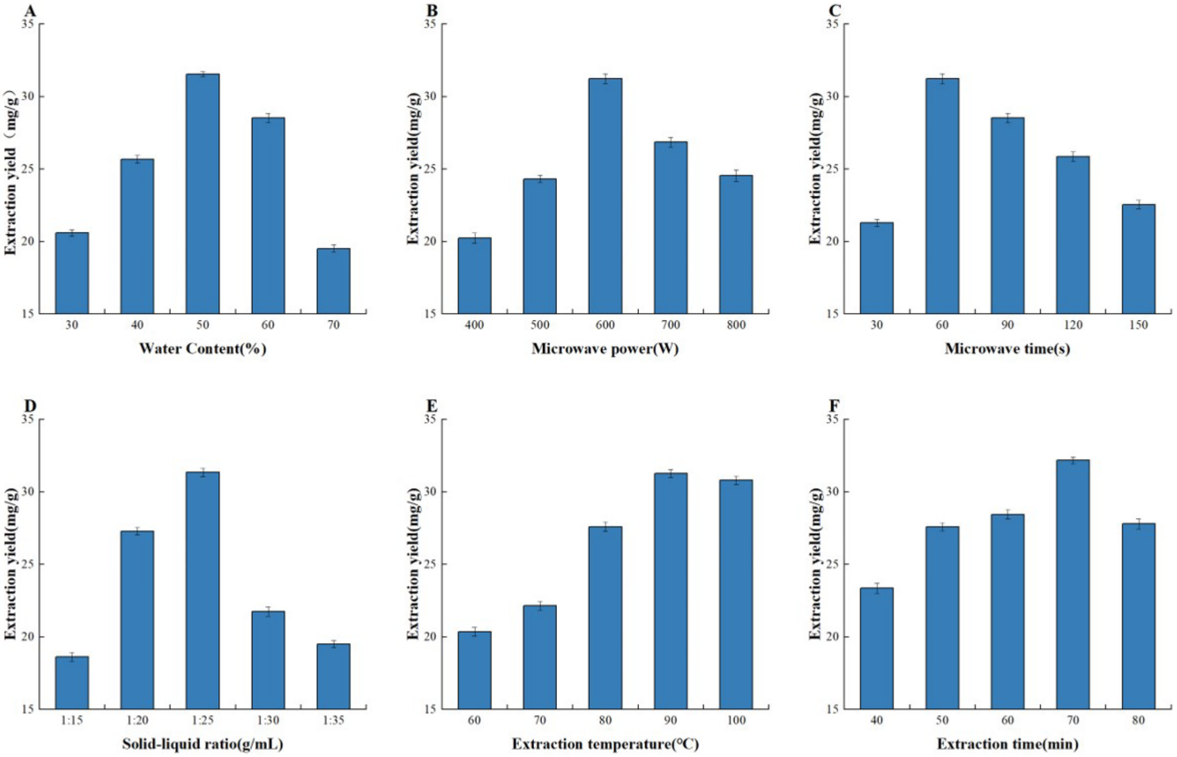

Figure 2. Water content (A), microwave power (B), microwave time (C), solid-liquid ratio (D), extraction temperature (E), and extraction time (F) on the yield of total flavonoids from ZSS.

can be improved by increasing the extraction time, despite the risk of degradation (Akhtar et al., 2019). As shown in Figure 2C, the total flavonoids yield increased significantly with increasing microwave time from 30 to 60 seconds. However, when the microwave time exceeded 60 s, the total flavonoids yield decreased gradually. This is likely due to the faster dissolution rate of the target component with increasing time until a dynamic equilibrium is reached between the target component and the microwave radiation time (Yu et al., 2021). Nevertheless, the microwave time is too long, which can cause flavonoids to become unstable and lead to a lower yield of total flavonoids (Wang et al., 2020). A similar trend was observed when Wang et al. (2012) used the MAE extract of total flavonoids in Toona Sinensis leaves. Furthermore, Alara et al. (2018), who used MAE of total flavonoids from Vernonia amygdalina leaf, also pointed out that excessive irradiation time may lead to thermal degradation of flavonoids, resulting in a lower total flavonoid yield. Therefore, a microwave time of 60 s was chosen for the follow-up study.

Effect of solid-liquid ratio on the yield of total flavonoids from ZSS

By choosing a suitable solid-liquid ratio, the diffusion of the solvent into the material can be accelerated, thus increasing the extraction efficiency of the target component and reducing the amount of extractant (Yu et al., 2021). The effect of the solid-liquid ratio on the yield of total flavonoids from ZSS is shown in Figure 2D. Specifically, when the solid-liquid ratio increased from 1:15 g/mL to 1:25 g/mL, the total flavonoid yield also increased. However, the yield of total flavonoids was significantly reduced when the solid-liquid ratio was greater than 1:25 g/mL. This may be due to the fact that, as the volume of DES increases, the total flavonoids can be dissolved more fully. Furthermore, larger volumes of solvent require more microwave energy to be absorbed, resulting in insufficient energy to promote cell wall rupture for the effective leaching of flavonoids (Alara et al., 2018). This is consistent with the phenomenon Cui et al. (2018) produced when DES was used to extract the main flavonoids from sea buckthorn leaves, increasing flavonoid yield when the solid-liquid ratio increased. However, too large a volume of DES decreased the flavonoid yield. In a study by Li et al. (2015) on the extraction of catechins from tea leaves using microwave-assisted methods, it was also found that the yield increased and subsequently decreased with an increasing solid-liquid ratio. Therefore, a solid-liquid ratio of 1:25 g/mL was considered optimal and used for further experiments.

Effect of extraction temperature on the yield of total flavonoids from ZSS

The rise in extraction temperature increases the mobility of the DES while the solubility of the target components in DES increases. Furthermore, the rise in solution temperature can weaken hydrogen bonding within the cell wall, thus promoting the dissolution and disruption of the cell wall by DES and improving extraction efficiency (Wang et al., 2020).

The effect of the extraction temperature on the yield of total flavonoids from ZSS is shown in Figure 2E. Total flavonoids from ZSS increased significantly with an increase in extraction temperature from 60 to 90 °C. However, the total flavonoid yield decreased after the temperature exceeded 90 °C. This may be due to increasing the extraction temperature and decreasing the viscosity of DES, leading to faster molecular movement. Additionally, higher temperatures can lead to the degradation of sensitive flavonoids and the leaching of more impurities (Wang et al., 2018a). Yeong et al. (2022) and Alara et al. (2018) showed a similar trend. That is, as the extraction temperature increased, the yield of flavonoids gradually increased and subsequently decreased. Therefore, 90 °C was considered the optimal extraction temperature and was used for further experiments.

Effect of extraction time on the yield of total flavonoids from ZSS

Extraction time is an essential factor that affects extraction efficiency. A longer extraction time increases the cost, while a shorter extraction time may leave a large amount of the target in the sample, resulting in an inefficient extraction process (Skarpalezos & Detsi, 2019). The effect of the extraction time on the yield of total flavonoids from ZSS is shown in Figure 2E. The extract yield was increased by extending the extraction time when the time ranged from 40 to 70 min. Nevertheless, the total flavonoid yield decreased when the extraction time exceeded 70 min. This phenomenon may be due to the target component dissolving faster over time, but prolonged extraction can lead to changes in the molecular structure of flavonoids (Akther et al., 2021). Lee et al. (2022) and Xia et al. (2021) showed a similar trend of increasing and then decreasing flavonoid yield with increasing extraction time when using microwave-assisted DES to extract flavonoids. Therefore, 70 min was considered the optimal extraction time and used for further experiments.

3.3 Results of RSM experiments

According to the principles of Box-Behnken combinatorial design, 17 experiments, each replicated three times, were randomized to investigate the effects of different variables on the total flavonoid yield of ZSS. Table 3 shows the factors affecting the total flavonoid yield of ZSS, their levels, and the experimental results.

Using Design-Expert 13, the regression model of the solid-liquid ratio (A), extraction time (B), and extraction temperature (C) were established as follows (Equation 2):

$$Y = -66.75 + 1.67A + 0.78B + 1.24C - 0.0017AB + 0.0010AC + 0.00018BC - 0.034A^2 - 0.0055^2 - 0.0070C^2 \tag{2}$$

As shown in Table 4, the regression model coefficients were $R^2 = 0.9512$ and $p = 0.0008$, indicating that the experimental model reached a significant level and was suitable for the experimental design of DESs for the extraction of total flavonoids from ZSS.

According to the significance criteria, the primary terms (A and C) had significant effects on the extraction rate of total flavonoids from ZSS ($p < 0.05$).

Furthermore, $AdjR^2 = 0.8884$ may explain the variation in response values in the regression model. The slight difference between $AdjR^2 = 0.8884$ and $PredR^2 = 0.7083$ suggests that our model and the data obtained are sufficient to accurately reflect the correlation between the three factors and the total flavonoid yield. From the F value, it can be seen that the order of effect on the yield of total flavonoids from ZSS is the material-liquid ratio (A) > heating temperature (C) > heating time (B).

Based on 3D response surface plots, the effects of the three variables on the total flavonoid yield and interaction effects between the variables were visualized. Figure 3 intuitively shows

Table 3. The results of RSM experiments.

Run	A: Solid-liquid ratio/(g/mL)	B: Extraction time/min	C: Extraction temperature/°C	Y: Total flavonoids yield/(mg/g)
1	0	0	0	37.81
2	1	0	1	35.75
3	-1	0	-1	36.11
4	0	1	1	36.53
5	1	0	-1	35.38
6	-1	0	1	36.28
7	1	-1	0	35.95
8	0	1	-1	35.73
9	0	0	0	37.35
10	-1	-1	0	36.35
11	1	1	0	35.54
12	0	0	0	37.43
13	0	-1	1	36.61
14	-1	1	0	36.28
15	0	0	0	37.05
16	0	-1	-1	35.88
17	0	0	0	37.50

Table 4. Results of significance test and variance analysis of the regression model.

Source	Sum of squares	df	Mean squares	F-value	*p*-value	
Model	8.42	9	0.9357	15.15	0.0008	Significant
A	0.7200	1	0.7200	11.66	0.0112	
B	0.0630	1	0.0630	1.02	0.3460	
C	0.5356	1	0.5356	8.67	0.0216	
AB	0.0289	1	0.0289	0.4681	0.5159	
AC	0.0100	1	0.0100	0.1620	0.6994	
BC	0.0012	1	0.0012	0.0198	0.8920	
A^2	3.06	1	3.06	49.59	0.0002	
B^2	1.25	1	1.25	20.27	0.0028	
C^2	2.04	1	2.04	32.96	0.0007	
Residual	0.4322	7	0.0026			
Lack of fit	0.1321	3	0.0031	0.5871	0.6550	Not significant
Pure error	0.3001	4	0.0023			
Cor total	8.85	16				
R^2=0.9512	AdjR^2 = 0.8884	PredR^2 = 0.7083				

how each of the three factors (A-C) affects the total flavonoid yield in ZSS. It can be seen that the response surfaces of all three factors have a large slope (the slope of the surface is positively correlated with the interaction between the two factors), indicating that each factor has a significant effect on the total flavonoid yield. This result is consistent with the ANOVA results in Table 4.

3.4 Validation of RSM models

The optimization analysis of the prediction model showed that the optimal extraction process parameters are as follows: solid-liquid ratio, 1:27.54/mL; extraction time, 65.92 min; and extraction temperature, 93.31 °C. The process was adjusted to the following parameters for the actual extraction: solid-liquid ratio, 1:28 g/mL, extraction time, 66 min, and extraction temperature, 93.00 °C. Three parallel validation tests were conducted under these conditions, and the total flavonoid yield was 36.24 mg/g, similar to the predicted value (37.03 mg/g). Based on these results, the model proved to be reliable.

3.5 ZSS total flavonoids collection and DES reuse

DES is a synthetic solvent with a relatively low vapor pressure that cannot be effectively recovered by conventional reduced pressure concentration. It is necessary to provide a suitable recycling method for DES. Therefore, this paper compares five macroporous resins' adsorption and desorption capacities (AB-8, DM130, HPD100, DM301, and D101) for total ZSS flavonoids. As Table 5 shows, the adsorption rate of DM301 was up to 88.85%. The adsorption rates of D101 and HPD100 were slightly lower than those of DM301, with 85.68% and 84.31%, respectively. Furthermore, a high desorption rate of 85.87% was obtained for DM301, slightly lower than that of HPD100 (89.08%). Based on the above results, DM301 proved to have excellent adsorption and desorption properties. Taken together, the recovery of DES and collection of flavonoids with microporous resin DM301 is simple and effective. After the flavonoids were adsorbed with macroporous resin, the DESs could be recovered for the next extraction cycle. After the macroporous resin adsorbed the flavonoids, the recovered DES was used again to extract total flavonoids from ZSS with a yield of 30.28 mg/g and a recovery of 65.0%. The above results can indicate that the DES recovered by the macroporous resin can be reused.

Table 5. Comparison of adsorption and desorption performance.

Resin type	Dosage (g)	Adsorption rate (%)	Desorption rate (%)
AB-8	5	81.16	82.22
DM130	5	75.65	83.41
HPD100	5	84.31	89.08
DM301	5	88.58	85.87
D101	5	85.68	66.25

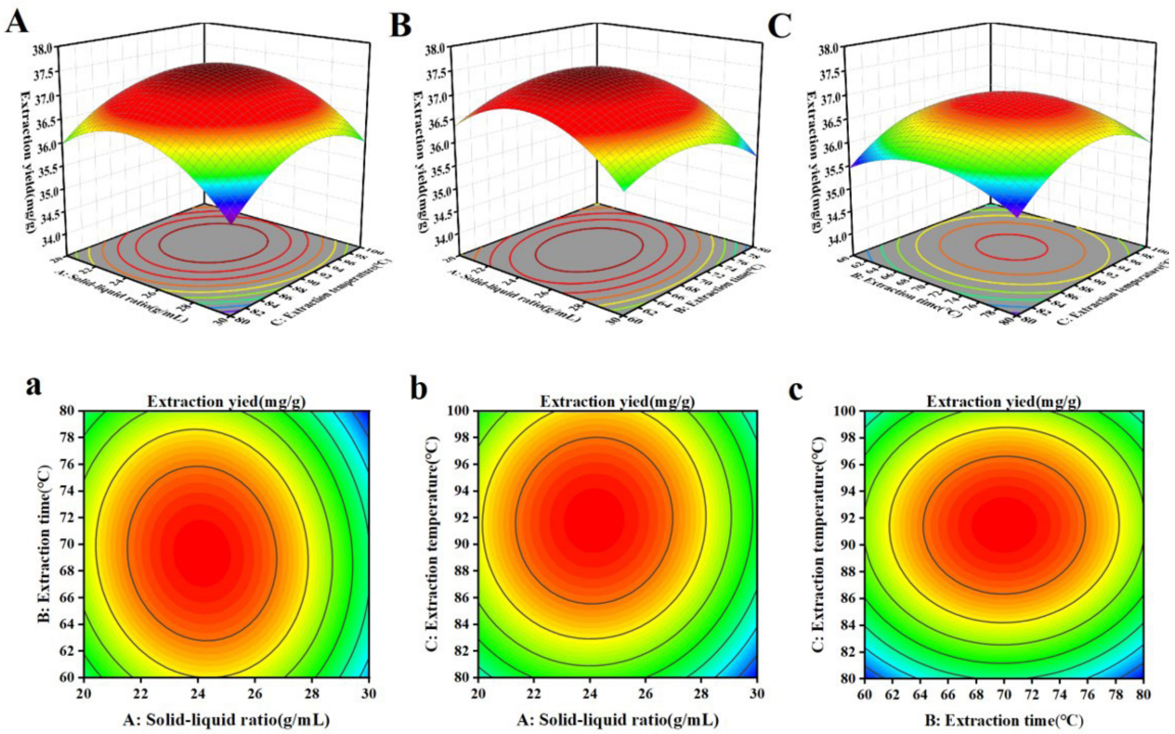

Figure 3. Three-dimensional response surface and two-dimensional contour plots of microwave-assisted deep eutectic solvent extraction. A-C and a-c, interactions among solid-liquid ratio, extraction time, and extraction temperature, respectively.

4 Conclusion

In this paper, a new green extraction solvent DES was used to extract the total flavonoids of ZSS, and DES composed of ChCl and lactic acid synthesized in a 2:1 molar ratio was found to be the best extraction solvent. After that, the extraction conditions of total flavonoids were optimized by single-factor investigation and RSM experiments. The optimal water content of DES, microwave power, microwave time, solid-liquid ratio, extraction time, and extraction temperature was found to be 50%, 600 W, 60 s, 1:28 g/mL, 66 min, and 93 °C, respectively. The yield of total flavonoids was determined to be 36.24 mg/g under these optimal conditions. In addition, the recovery of total flavonoids and DES was performed by adsorption on macroporous resin. The results showed that DM301 macroporous resin was the most effective for the recovery of total flavonoids, and the recovered DES had excellent reusability. This study shows that the excellent properties of DES indicate it is promising and has the potential as a green solvent for the extraction of bioactive compounds from traditional Chinese medicine.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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