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From medicinal and food homologous biomass to total glycosides in *Rehmannia glutinosa* leaves extracts: Microemulsion-based negative pressure cavitation-assisted extraction

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A B S T R A C T

Food-grade and sustainable peppermint oil microemulsion was prepared through gradual screening of components for extraction of total glycosides in *Rehmannia glutinosa* leaves. The optimal microemulsion was: 6:3:1:25.71 (Tween 80: ethanol: peppermint oil: 0.02 mol/L KCl, w-w:w:w). 1 mol/L KCl solution was used for back-extraction to achieve sustainability of microemulsion. Microemulsion could be used at least 4 cycles. To optimize sustainability of microemulsion, negative pressure cavitation-assisted extraction technology was used as an auxiliary method. Which also makes extraction more suitable for large-scale industrial production. Consequently, microemulsion-based negative pressure cavitation-assisted extraction technology was developed. The optimal conditions for technology were as follows: 5.5 of pH, 37 min of time, and 10 mL/g of liquid-solid ratio. The yield of total glycosides was 45.13 mg/g. This newly established technology, as a cleaner alternative approach, can therefore be used to extract total glycosides from *Rehmannia glutinosa* leaves.

Abbreviations: R,, *glutinosa*: *Rehmannia glutinosa* Libosch; RGL, *R. glutinosa* leaves; TGRGL, Total glucosides from RGL; MAE, Microwave-assisted extraction; UAE, Ultrasonic-assisted extraction; NPCAE, Negative pressure cavitation assisted extraction; MB-NPCAE, Microemulsion-based NPCAE; Span 80, Sorbitan monooleate; AOT, Sodium bis-(2-ethylhexyl) sulfosuccinate; P204, Di (2-ethylhexyl) phosphoric acid; TEM, Transmission electron microscopy; NPC, negative pressure cavitation; BBD, Box-Behnken design; W/O, Water in oil

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1. Introduction

Rehmannia glutinosa Libosch. (*R. glutinosa*), an herbal, belongs to Scrophulariaceae family is famous for its medicine and food homology (Zong et al., 2014; Liu et al., 2017). According to *The Grand Compendium of Materia Medica* (Bencao Gangmu), a classical Chinese medical work written in the 16th century, the roots, tender shoots and young leaves of *R. glutinosa* can be used as food (Jia et al., 2020). Porridge, tea, soup and cold dishes are forms in which *R. glutinosa* is often consumed (Jia et al., 2020; Zhang, 2021; Yu, 2013). Additional, main components of *R. glutinosa* leaves (RGL), catalpol, ajugol and acteoside, have obvious antibacterial and antioxidant activities (Wang et al., 2022; Nogueira and Lopes, 2011; Liu et al., 2017; Zhou et al., 2017; Piatczak et al., 2020). The main reasons for food spoilage are oxygen exposure and microbial contamination (Aihaiti et al., 2022). Therefore, total glucosides from RGL (TGRGL), catalpol, ajugol and acteoside, has the potential to be used as a natural food preservative (Xu et al., 2020). This could reduce the risk of liver damage and carcinogenicity to consumers from synthetic preservatives (Kulawik et al., 2013). Meanwhile, according to the reports that TGRGL also has pharmacological activities such as antitumor, liver-protecting, and hypoglycemic et al. (Khan et al., 2022; Zhang et al., 2021; Yan et al., 2018). Consequently, extraction of TGRGL is particularly important from the perspective of food and medicine.

The current extraction solvents employed for natural active ingredients are mostly traditional organic solvents (methanol, ethyl acetate) (Belandria et al., 2016), ionic liquids (Hou et al., 2019), eutectic solvents (Bertolo et al., 2021) and supercritical fluids (Ferrentino et al., 2020). Although these solvents are widely used, defects are inevitable. For example, high toxicity, risk of environmental pollution in operation process, low extraction yield, high cost, complicated preparation and use procedures, non-food grade, non-recyclability, and detection obstacles et al. (Chemat et al., 2010). Considering the drawbacks of existing solvents, development of a green, low-cost, sustainable and easy-to-operate new alternative solvent has become a hot topic.

Microemulsion is a new type of emulsion system with great potential developed on the basis of traditional nanoemulsion in recent years. It not only has the characteristics of traditional nanoemulsion kinetic stability, but also makes up for the defect of traditional nanoemulsion thermodynamic instability (Klossek et al., 2014). As a low-viscosity, thermodynamically stable, isotropic transparent or translucent oil-water mixture system, microemulsions are composed of a water phase, surfactant, co-surfactant, and oil phase (Hayes et al., 2017; Wang et al., 2020). Compared with other solvents, greater interface area, higher selectivity, and higher solubility of compounds are advantages of microemulsion (Abbasi and Radi, 2016; Guo et al., 2020; Zhu et al., 2020). Nanoscale particle size of microemulsions yield a larger specific surface area than traditional solvents, which enables the target components to contact the microemulsion more fully (Qin et al., 2021).

The application of microemulsion involves many fields such as cosmetics, chemical synthesis, wastewater treatment, new energy and pharmaceuticals (Garcia et al., 2021; Asgari et al., 2019; Galiano et al., 2015; Leng et al., 2019; Wang et al., 2022). Nevertheless, the application of microemulsions to extract active ingredients is still relatively scarce. Some studies have directly applied it as an extraction solvent for extraction of lutein (Jivan and Abbasi, 2020), juglone (Li et al., 2021), and naphthoquinone (Qin et al., 2021). Among the methods described above, the microemulsion used to extract lutein was not prepared through gradual screening of components. Although the microemulsion used for extraction of juglone has been optimized, it does not belong to category of food-grade. The microemulsion used for extraction of naphthoquinone compounds is neither food-grade nor prepared through gradual screening of components. Vitally important, none of systems mentioned above achieve sustainable use of microemulsions. In this study, the extraction yield of TGRGL was used as an index to initially screen components used in preparation of microemulsions and optimize the obtained system. Furthermore, a cleaner microemulsion for TGRGL extraction was established. Sustainability of microemulsion extraction solvents was achieved by back-extraction.

In addition to extraction solvent, choice of auxiliary means is another important factor that affects extraction yields of target compounds. Traditional extraction methods such as Soxhlet extraction and heating reflux extraction are time-consuming, laborious, and consume large amounts of organic solvents (Qi et al., 2015; Mohamed et al., 2015). To overcome the above shortcomings, extraction methods such as microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), supercritical fluid extraction (SFE), and pulsed power-assisted extraction (PPAE) have been successively developed for the extraction of active ingredients (Kwiatkowski et al., 2020; Ullah et al., 2019; Soldan et al., 2021; Khursheed et al., 2021). Although these techniques improve extraction efficiency and reduce the volume of organic solvents required, expensive equipment, complicated operation, and loss of solvents, have become new challenges. In this situation, negative pressure cavitation assisted extraction (NPCAE) is proposed, which can make extraction solvent and extracted material more intimately contact each other, such that target compounds can be released into extraction solvent more efficiently (Kou et al., 2021; Roohinejad et al., 2016; Cui et al., 2020). Consequently, as a new technology involving high efficiency, simple device, simple operation, and low solvent loss (Wang et al., 2018; Roohinejad et al., 2016), NPCAE has been increasingly widely used in the extraction of active ingredients in recent years (Jiao et al., 2014; Roohinejad et al., 2016; Cui et al., 2020).

In this study, a food grade and sustainable peppermint oil microemulsion was prepared through gradual screening of components. To optimize sustainability of microemulsion, NPCAE was used as an auxiliary method. Additionally, adjustability of NPCAE tank capacity, ease of operation and high efficiency of extraction make microemulsion extraction suitable for large-scale production. Consequently, a novel and effective microemulsion-based NPCAE (MB-NPCAE) technology for the extraction of TGRGL was developed.

2. Materials and methods

2.1. Plant materials and reagents

RGL was obtained from Kanghui Huaiyao Planting Professional Cooperative (Jiaozuo City, Henan Province, China) and identified by Prof. Chunjian Zhao. After drying at 40 °C, RGL was crushed through 40-mesh sieve for use.

Acteoside, catalpol, and ajugol standards (≥98 %) were purchased from Shanghai Maclean Biochemical Technology

Co., Ltd. (Shanghai City, China). Sorbitan monooleate (Span 80), sodium bis-(2-ethylhexyl) sulfosuccinate (AOT), di (2-ethylhexyl) phosphoric acid (P_{204}), and other chemicals were purchased from Shanghai Dibai Biotechnology Co., Ltd. (Shanghai City, China).

2.2. *Determination of TGRGL*

A Kromasil 100–5 C_{18} column (250 mm × 4.6 mm, 5 µm), with acetonitrile (A) and formic acid aqueous solution (B) was used for the determination of acteoside, ajugol, and catalpol using High performance liquid chromatography (Agilent 1200, Agilent TechnologiesInc., USA). Gradient elution of acteoside, ajugol, and catalpol was performed over 0–15 min, 1–18 % A (v/v); 15–30 min, 18–31 % A (v/v); 30–35 min, 31–1 % A (v/v); 35–40 min, 1 % A (v/v). The detection conditions were 203 nm of wavelength, 35 °C of column temperature, and an injection volume of 20 µL.

2.3. *Establishment of microemulsion for TGRGL*

0.02 mol/L KCl solution was added drop by drop to the mixture of peppermint oil and ethanol while maintaining magnetic stirring at 500 r/min to prepare microemulsion for TGRGL. The mass ratio of Tween 80, ethanol, peppermint oil and 0.02 mol/L KCl solution was 6:3:1:25.71. The establishment process and specific optimization of microemulsion system were shown in Section S1 of Supplementary material.

2.4. *Extraction of TGRGL by MB-NPCAE*

2.4.1. *Extraction procedure for TGRGL by MB-NPCAE*

RGL powder and microemulsion were added from inlet of negative pressure cavitation (NPC) device. Intake valve was opened to form environment of negative pressure. After extraction was completed, intake valve and power supply were turned off in sequence (Qi et al., 2015)

2.4.2. *Optimization of MB-NPCAE by single factors*

The effects of various factors on TGRGL extraction yields were explored, and the operation process is described in Section 2.4.1. Single-factor parameters of each group are shown in Table 1.

2.4.3. *Response surface analysis of MB-NPCAE*

Interactions between various factors on TGRGL extraction yields were explored using response surface method and Box-Behnken design (BBD) based on single-factor experiments. The parameter levels of BBD are shown in Table 2.

2.4.4. *Comparison of TGRGL yields obtained by microemulsion and individual component in microemulsion*

RGL powder was mixed with microemulsion and individual components (ethanol, Tween 80, peppermint oil, and 0.02 mol/L KCl solution) at a liquid-solid ratio of 10 mL/g. Under the optimal extraction conditions identified in Sections 2.4.2 and 2.4.3, TGRGL was extracted by NPC based on Section 2.4.1 operation method.

2.4.5. *Comparison of different extraction methods for TGRGL*

TGRGL yields obtained using different extraction methods were compared. The types of extraction methods and their respective extraction conditions are listed in Table 3.

Table 1 – Parameters of single factor experiment.

Single factors	Extraction conditions					
	Liquid-solid ratio (mL/g)	Water content of pool (%)	pH of microemulsion	Extraction time (min)	Concentration of salt solution (mol/L)	Negative pressure (MPa)
Liquid-solid ratio (mL/g)	5, 10, 20, 30, 40	70	7	35	0.04	-0.06
Water content of pool (%)	10	50, 60, 70, 80, 90	7	35	0.04	-0.06
pH of microemulsion	10	70	4, 5, 6, 7, 8	35	0.04	-0.06
Extraction time (min)	10	70	7	15, 25, 35, 45, 55	0.04	-0.06
Concentration of salt solution (mol/L)	10	70	7	35	0, 0.02, 0.04, 0.06, 0.08	-0.06
Negative pressure (MPa)	10	70	7	35	0.04	-0.02, – 0.04, – 0.06, – 0.08, – 0.1

Table 2 – Level design of various factors in BBD experiment.

Factor	Parameter level		
	-1	0	1
KCl concentration (mol/L)	0	0.02	0.04
Water content of pool (%)	60	70	80
pH of microemulsion	4	5	6
Extraction time (min)	25	35	45

2.5. *Reusability of microemulsion*

Microemulsions used for extraction were recovered by back-extraction and reconstituted in 0.02 mol/L KCl solution. After diluting microemulsion extract solution which dissolves TGRGL to four times the original concentration, the pH was adjusted to 7.5. It was then mixed with 7 times the volume of the KCl solution and shaken for 30 min at a speed of 180 rpm. Subsequently, the mixed solution was transferred to a separation funnel and allowed to stand for. Upper liquid phase was removed after separation, then 0.02 mol/L KCl solution was added dropwise while maintaining magnetic stirring at 500 r/min to restore microemulsion (Tween 80: peppermint oil: ethanol: 0.02 mol/L KCl, 6:3:1:25.71, w-w:w:w).

The reconstituted microemulsion was purified. After the reconstituted microemulsion was shaken in shaker at speed of 180 rpm for 30 min, it was allowed to stand in separation funnel for 24 h and the excess liquid from lower layer was released. This process was repeated twice to complete purification of the reconstituted microemulsion. Reusability of the reconstituted microemulsion based on extraction yields of the reconstituted microemulsion for TGRGL was evaluated.

3. Results and discussion

3.1. *Mechanism of microemulsion extraction*

RGL powder and the prepared peppermint oil microemulsion were added to the extraction tank of NPC to carry out MB-NPCAE for TGRGL. Operational flowchart of MB-NPCAE and diagram of TGRGL extraction mechanism are shown in Fig. 1 and Fig. 2, respectively.

In the process of NPCAE, the high energy in microemulsion bubbles was released while cavitation bubbles were disrupted or contracted. This caused the pressure inside cell to be greater than that outside cell. The pressure difference between the interior and exterior of cell enlarged the mesh of

Table 3 – Extraction conditions of each extraction method.

	Liquid-solid ratio (mg/g)	Temperature (℃)	Time (min)	pH	Powder (W)	Negative pressure (MPa)
Ethanol-HRE	10	75	150	5.5	—	—
Methanol-HRE	10	75	150	5.5	—	—
Ethanol-UAE	10	40	37	5.5	200	—
Methanol-UAE	10	40	37	5.5	200	—
MB-UAE	10	40	37	5.5	200	—
Ethanol-NPCAE	10	25	37	5.5	—	-0.06
Methanol-NPCAE	10	25	37	5.5	—	-0.06
MB-NPCAE	10	25	37	5.5	—	-0.06

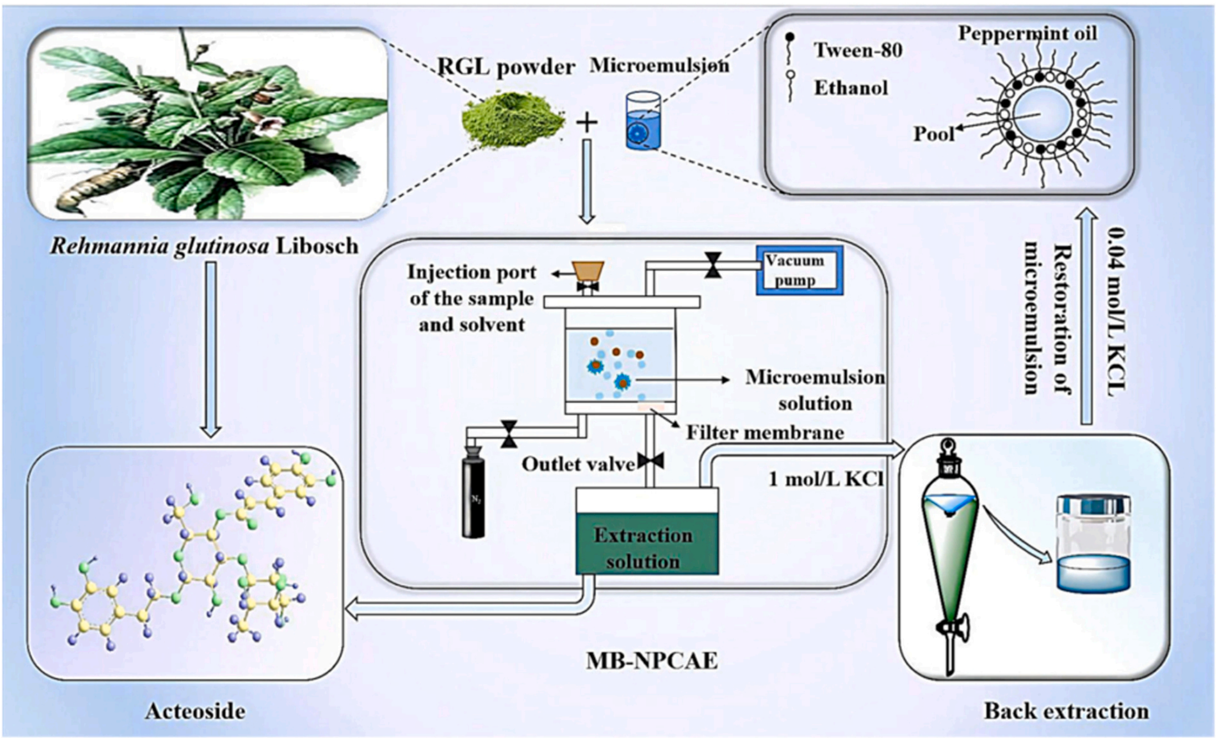

Fig. 1 – Operational flowchart for MB-NPCAE.

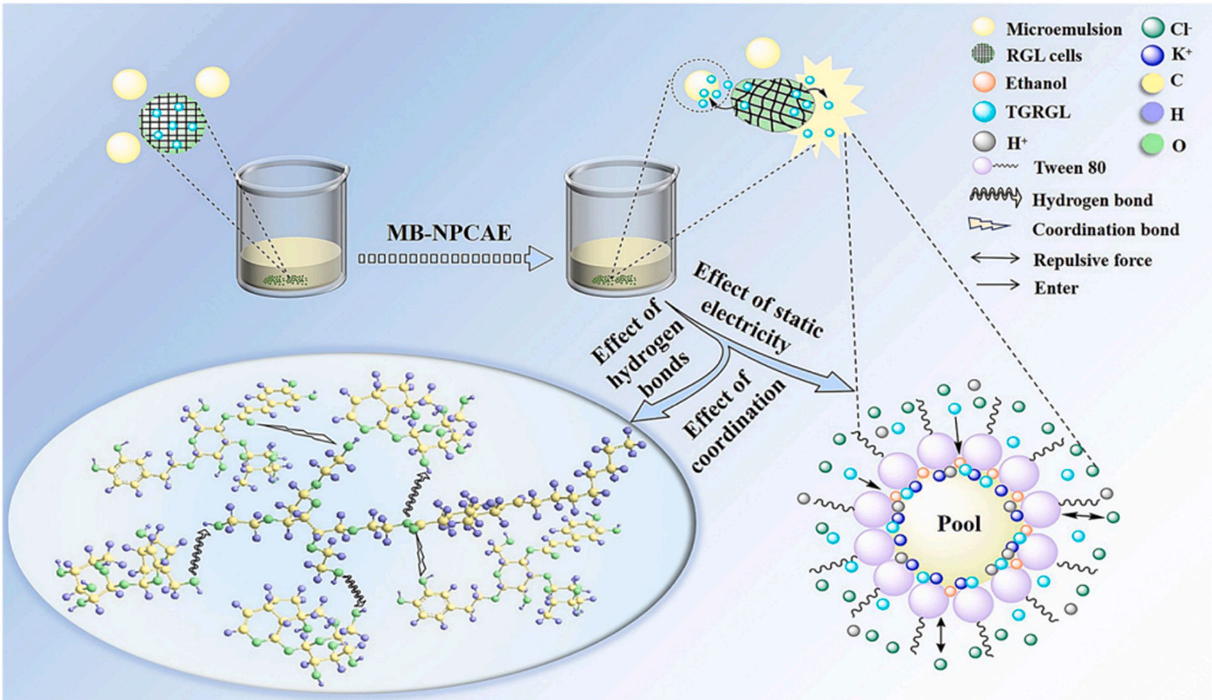

Fig. 2 – Mechanism of TGRGL extraction.

three-dimensional network structure of cell wall. The enlarged cell wall pores and the higher pressure inside the cell compared to the outside increased the release of TGRGL from the inside of the cell to the extracellular microemulsion. The microemulsion used as TGRGL carrier outside of RGL cells is composed of a co-surfactant, surfactant, oil phase, and water phase. Alcohols are often used as co-surfactants in microemulsions. The type and concentration of alcohol have significant impact on type and "pool" capacity of microemulsions. Ethanol, as the co-surfactant in peppermint oil microemulsion, acts as a regulator between interfacial membrane and surfactant. Ethanol was distributed at oil-water interface or embedded in Tween 80 molecules when the concentration of ethanol in microemulsion was low. This avoided the shortcomings of tight oil-water interface arrangements and increased microemulsion particle size caused by the large amount of high-concentration co-surfactant aggregated and adsorbed on the surfactant. Strong fluidity of interface film can improve efficiency of TGRGL entering the microemulsion solubilization "pool". The smaller particle size of microemulsion resulted in greater specific surface area, which increased the probability of microemulsion contacting TGRGL. It has been reported that short-chain alcohols are used as co-surfactants and are more likely to be distributed on hydrophilic side to form W/O-type microemulsions (Wan et al., 2015). TGRGL is water-soluble, which is consistent with the characteristics of W/O, which is more suitable for solubilizing water-soluble components. Therefore, TGRGL released into extracellular microemulsion can be solubilized into "pool" through interactions with Tween 80. When the pH of microemulsion was 5.5, the liquid environment was weakly acidic. The diffusion electric double layer formed near the polar head of surfactant was adjusted by KCl aqueous solution, and free H^+ in microemulsion accumulated near negatively charged polar tip of microemulsion core. This made it easier for acteoside, catalpol, and ajugol to be solubilized to the "pool" under the action of static electricity. The oxygen on carbonyl in acteoside molecule coordinated with the oxygen on ether group or the hydroxyl oxygen in Tween 80. The oxygen on carbonyl in Tween 80 can coordinate with the oxygen on ether and hydroxyl in catalpol and ajugol. In addition, the hydrogen on hydroxyl in TGRGL and the oxygen on Tween 80, as well as the oxygen on TGRGL and the hydrogen on hydroxyl in Tween 80 also form intermolecular hydrogen bonds, which further increased the solubilization of TGRGL to "pool".

3.2. *Extraction of TGRGL by MB-NPCAE*

3.2.1. *Single factor optimization for TGRGL yield*

3.2.1.1. *Effect of KCl concentration on extraction yield*. In addition to the type of electrolyte, the concentration of electrolytes is a parameter that affects ionic strength which plays a crucial role in the extraction performance of microemulsions (Wan et al., 2015). Thus, KCl concentration in the microemulsion was optimized. As shown in Fig. 3A, the yield of ajugol, catalpol, acteoside, and TGRGL increases with the increase of KCl concentration when KCl concentration is 0–0.02 mol/L. However, the yield of ajugol, catalpol, acteoside, and TGRGL decreases with the increase of KCl concentration when KCl concentration is 0.02–0.08 mol/L. This is consistent with the reports that the a little amount of salt ions added can promote the dissolution of the target component, while, excessive salt ions will promote the salt-out effect and reduce the solubility of target components (Zhang et al., 2019; Falade et al., 2003). This is because the low concentration of salt ions can increase the ionic activity of the target component, while the high concentration of salt ions neutralize the surface charge of the target component to make the target component gather, resulting in salt-out effect (Falade et al., 2003; Zhang et al., 2019). Therefore, 0.02 mol/L is the optimal KCl concentration.

3.2.1.2. *Effect of liquid-solid ratio on extraction yield*. The relationship between the liquid-solid ratio and extraction

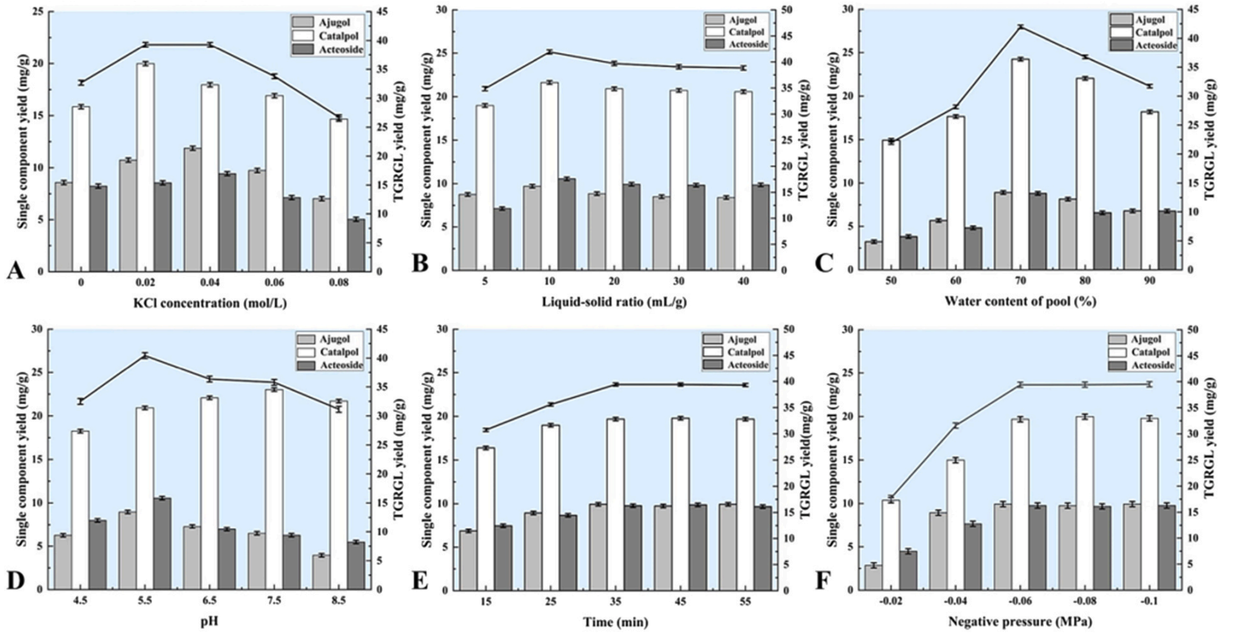

Fig. 3 – The effect of various factors on TGRGL extraction yields.

yield of TGRGL was explored. Extraction of TGRGL with microemulsions is a process of leaching equilibrium. The greater the liquid-solid ratio, the lower the concentration of TGRGL in the microemulsion, which was more conducive to the equilibrium shift in the direction of TGRGL leaching. Therefore, the TGRGL yield obtained at a liquid-solid ratio of 10 mL/g was higher than that obtained at 5 mL/g. However, as the liquid-solid ratio continued to increase, the extraction yield of TGRGL declined. This may be because the large amount of microemulsion increased the resistance of NPCAE to the mass transfer process, which weakened the cell wall-rupturing ability of NPC. In this case, the microemulsion could not penetrate the cell wall to combine with TGRGL, which caused the extraction yield of TGRGL to drop slightly when the liquid-solid ratio was > 10 mL/g (Fig. 3B). Therefore, 10 mL/g was selected as the optimal liquid-solid ratio for TGRGL extraction.

3.2.1.3. *Effect of water content on extraction yield*. The higher the water content of microemulsion, the larger the “pool.” The larger the “pool”, the greater its ability to solubilize TGRGL. Therefore, the extraction yield of TGRGL increased with increasing water content during the initial stages (Fig. 3C). However, the higher the water content, the better the performance of the microemulsion. The particle size and surface tension of the microemulsion increased with increasing water content (Wan et al., 2015). As the particle size of the microemulsion increases, it becomes more difficult for the microemulsion to enter the cell interior. Meanwhile, an increase in surface tension also reduces the stability of the microemulsion (Wan et al., 2015). The increase in particle size and surface tension of the microemulsion had a gradually stronger negative effect on the extraction yield of TGRGL than the positive effect of increasing the water content of the microemulsion on the solubilization ability of TGRGL. Thus, when the water content of the microemulsion was > 70%, the extraction yield of TGRGL decreased. Therefore, 70% was determined to be the optimal microemulsion water content for obtaining TGRGL.

3.2.1.4. *Effect of pH on extraction yield*. When the solution was acidic, H^+ in the microemulsion was coordinated with oxygen atoms on the hydroxyl and ether groups of ajugol, catalpol, and acteoside, which made ajugol, catalpol, and acteoside present positive charge. The positively charged ajugol, catalpol, and acteoside were easily attracted to polar heads negatively charged on the surface of the microemulsion reverse colloidal nucleus. This made it easier for ajugol, catalpol, and acteoside to enter the microemulsion "pool" and be extracted by the microemulsion. When the pH was > 5.5, the yield of TGRGL decreased. When the pH was < 5.5, H^+ levels in the microemulsion were excessive. Except for H^+ involved in coordination, the free H^+ still in the microemulsion competes with ajugol, catalpol, and acteoside, which are positively charged due to coordination. TGRGL and free H^+ can be attracted to the polar head with a negative point in the reverse colloidal nucleus. Therefore, the capacity of the microemulsion "pool" to accommodate ajugol, catalpol, and acteoside was reduced. For this reason, TGRGL yields also declined when the pH was < 5.5. It could be seen that pH 5.5 was the most suitable for obtaining TGRGL from microemulsions (Fig. 3D).

3.2.1.5. *Effect of time on extraction yield*. The relationship curve between extraction time and TGRGL yield showed that the yield of TGRGL first increased and then tended to be stable (Fig. 3E). The mass transfer was limited because the extraction time was too short, which was insufficient to completely release TGRGL from RGL. The extraction yield of TGRGL gradually increased with the extension of extraction time, and the yield of TGRGL reached a maximum at 45 min. When the extraction time was further extended, the extraction yield of TGRGL did not increase. From the perspective of extraction yield and energy saving, 45 min was used as the best extraction time for subsequent experiments.

3.2.1.6. *Effect of negative pressure on extraction yield*. With increases in the negative pressure from − 0.02 to − 0.06 Mpa,

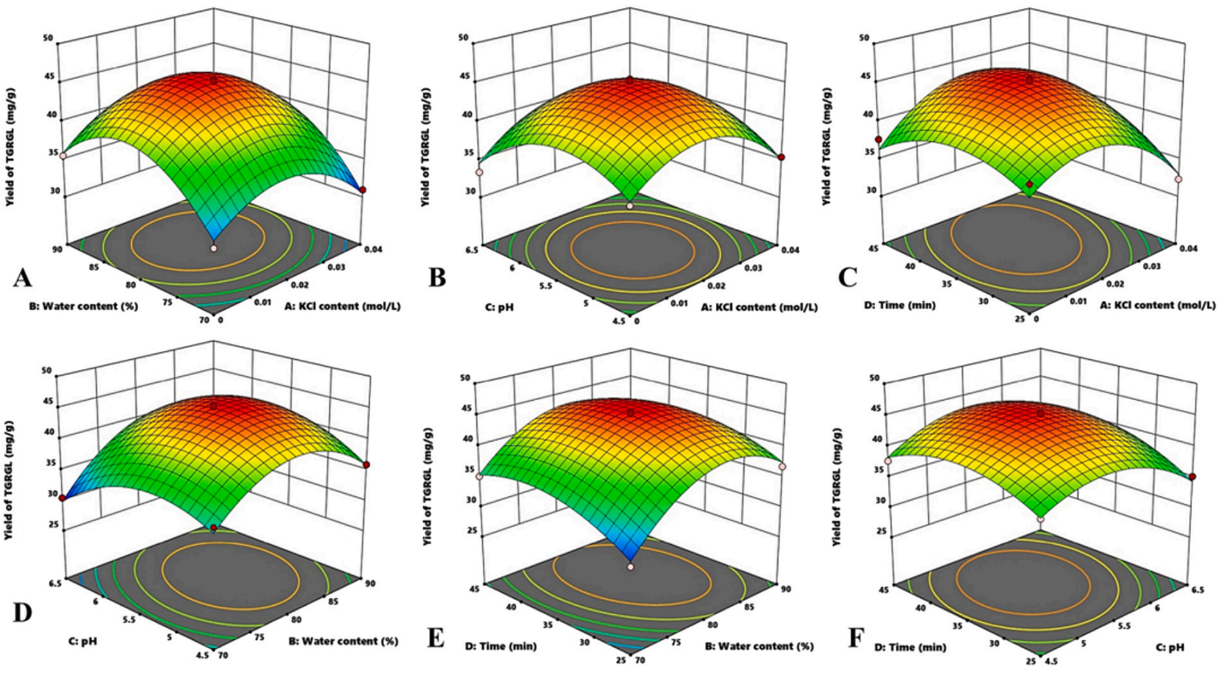

Fig. 4 – The interaction influence of various factors on the extraction yield of TGRG.

the extraction yield of TGRGL increased gradually (Fig. 3F). The extraction yield of TGRGL then no longer increased significantly with further increased in negative pressure until it reached a maximum at − 0.06 Mpa. When the negative pressure was < −0.06 Mpa, RGL powder was not well dispersed in the microemulsion because the low negative pressure resulted in insufficient contact between RGL powder and microemulsion, and incomplete extraction. When negative pressure was > −0.06 Mpa, the RGL powder was more vigorously dispersed. The turbulence generated made the extraction of TGRGL reach equilibrium at − 0.06 Mpa of negative pressure. Consequently, − 0.06Mpa was selected as the optimum negative pressure for subsequent tests.

3.2.2. *Response surface analysis of MB-NPCAE*

Based on single-factor experimental results, response surface optimization of the liquid-solid ratio, KCl concentration, water content, and time was carried out using the TGRGL extraction yield as the response value. The quadratic regression equation between the variable and the response value established through the BBD experiment is shown in Eq. (1).

$$Y = 45.01 - 0.3150A + 2.39B - 0.605C + 1.35D + 0.7125AB + 0.7775AC + 2.01AD + 1.61BC - 0.7987BD + 0.9725CD - 5.03A^2 - 6.31B^2 - 4.39C^2 - 3.33D^2 \quad (1)$$

where A is the KCl concentration (mol/L), B is the water content (%), C is the pH, D is the time (min), and Y is the yield of TGRGL (mg/g).

The relationship between TGRGL yield and each variable was investigated using analysis of variance. The R^2 value of the model coefficient was 0.9742, the p-value of the model was < 0.05, and the p-value for the lack of fit was > 0.05. The model is true and effective and accurately reflects the relationship between variables and response values.

A 3D model diagram of the interactive influence of various factors on TGRGL yield is shown in Fig. 4. The optimal MB-NPCAE conditions for obtaining TGRGL were 0.020 mol/L KCl, 71.83 % water content, pH 5.49, and 36.83 min. Considering the convenience of actual operation, the conditions were adjusted as follows: KCl concentration 0.02 mol/L, water content, 72%; pH 5.5, and time, 37 min. After adjusting the conditions, the actual yield of TGRGL was 45.13 mg/g, which was consistent with the TGRGL yield of 45.36 mg/g predicted by the model.

3.2.3. *Comparison of TGRGL yields obtained by microemulsion and individual component in microemulsion*

To verify the performance of the microemulsion solvent, the TGRGL yield obtained using microemulsion and that using individual component in the microemulsion was compared. Under the same extraction conditions, the TGRGL yield obtained by microemulsion was 45.13 mg/g, which was 34.72-, 125.36-, 7.71- and 1.82-fold of the TGRGL yields obtained by peppermint oil, Tween 80, 0.02 mol/L KCl and ethanol, respectively (Fig. 5A). Consequently, peppermint oil, ethanol, Tween 80 and 0.02 mol/L KCl were combined to prepare a microemulsion, the yield of TGRGL increased. This implies that compared to the individual components in microemulsion, the preparation of microemulsion is meaningful for the extraction of TGRGL.

3.2.4. *Comparison of TGRGL yield by different extraction methods*

As shown in Fig. 5B, MB-UAE increased the yield of TGRGL by 1.97- and 1.89-fold compared to ethanol-UAE and methanol-UAE, respectively. The extraction yields of TGRGL and MB-NPCAE were 1.82- and 1.72-fold higher than those of ethanol-NPCAE and methanol-NPCAE, respectively. It can be seen that microemulsions can achieve higher yields of TGRGL than traditional extraction solvents (ethanol and methanol).

Ethanol-NPCAE yields were 1.08- and 3.31-fold higher than those of ethanol-UAE and ethanol-HRE, respectively.

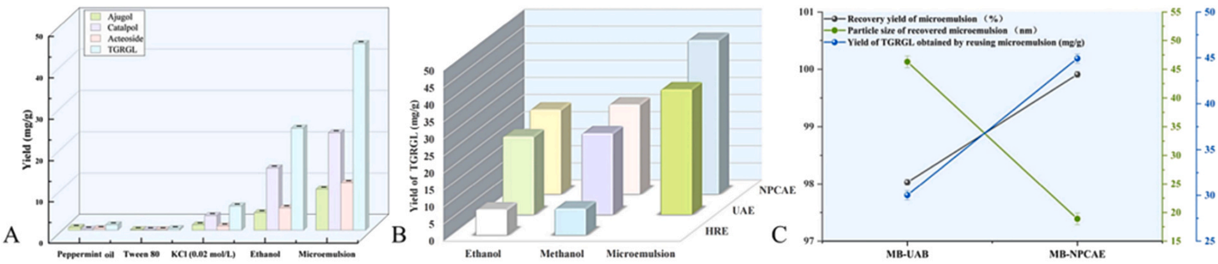

Fig. 5 – Results of comparative experiments (A. Effects of microemulsion and individual components in microemulsion on TGRGL yields; B. Effects of different extraction methods on TGRGL yields; C. Comparison of recovery of microemulsion by different methods).

Compared with methanol-UAE and methanol-HRE, the TGRGL yield was increased by methanol-NPCAE by 1.10- and 3.37-fold, respectively. MB-NPCAE increased the yield of TGRGL by 1.23-fold compared to MB-UAE.

After MB-NPCAE and MB-UAE were first used, the recovery yield of the microemulsion, particle size, and TGRGL yield of the reconstituted microemulsion were explored. From the perspective of the recovery yield of the microemulsion, there was almost no loss of microemulsion after extraction by MB-NPCAE (99.91 % of recovery yield). Compared with MB-NPCAE, MB-UAE exhibited a lower recovery yield of the microemulsion (98.03 % of recovery yield) (Fig. 5C). This may be due to the volatilization of peppermint oil and ethanol resulting from the non-sealed environment and heat released during the ultrasonication process. The particle size of the purified microemulsion used in MB-NPCAE (18.9 nm) was almost unchanged, whereas the particle size of the microemulsion used in MB-UAE (46.3 nm) was enlarged. This may be related to the loss of peppermint oil during ultrasound treatment. The loss of peppermint oil caused the microemulsion to be restored to the original ratio, resulting in a higher water ratio. The interfacial tension increased, which led to an increase in the microemulsion particle size (Wan et al., 2015). This increase in microemulsion particle size led to a decrease in the specific surface area of the microemulsion, which decreased the yield of TGRGL. This was also consistent with the results obtained using reconstituted microemulsion to extract TGRGL (Fig. 5C). The TGRGL yield obtained by MB-NPCAE (44.92 mg/g) was much greater than that obtained by MB-UAE (30.02 mg/g). Therefore, from a sustainability perspective, MB-NPCAE has greater potential than MB-UAE.

When MB-NPCAE, ethanol-HRE, and methanol-HRE were used to extract TGRGL, it was found that the TGRGL yield obtained by MB-NPCAE was 6.03- and 5.80-fold that by ethanol-HRE and methanol-HRE, respectively (Fig. 5B).

3.3. *Reusability of microemulsion*

The recyclability of solvents is one of the criteria for sustainable solvents. The microemulsion can be recovered and reused via back-extraction. The recovered microemulsion was purified according to the method described in Section 2.5, and then used as a solvent to extract TGRGL again. It was found that TGRGL yield obtained using five times regenerated microemulsion was 45.13, 44.02, 40.96, 36.31 and 21.24 mg/g, respectively. The TGRGL yield obtained from the fifth regenerated microemulsion was significantly lower than that obtained from the first used microemulsion. Therefore, the microemulsion solvent can be used as sustainable solvent at least 4 cycles to ensure TGRGL yield.

4. Conclusion

After screening and optimization, a food-grade and sustainable peppermint oil microemulsion was established for the first time and used as a novel and efficient solvent for the extraction of TGRGL. To overcome the volatility of peppermint oil in microemulsion, optimize the sustainability of microemulsion, and make the extraction more suitable for large-scale production, NPCAE as an auxiliary means, MB-NPCAE technology was proposed. Under the optimized MB-NPCAE conditions, TGRGL was obtained with a high yield of 45.13 mg/g. By back-extraction, the microemulsion solvent could be used at least 4 cycles. As a food-grade, sustainable and efficient solvent, peppermint oil microemulsion opens up a new field of microemulsion applications for the extraction of natural products. Furthermore, MB-NPCAE technology not only further develops the utilization value of RGL, but also is expected to be used as a guideline for biomass utilization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cherd.2023.02.039.

References

Abbasi, S., Radi, M., 2016. Food grade microemulsion systems: canola oil/lecithin: *n*-propanol/water. Food Chem. 194, 972–979. https://doi.org/10.1016/j.foodchem.2015.08.078

Aihaiti, K., Li, J., Yaermaimaiti, S., Liu, L., Xin, X.L., Aisa, H.A., 2022. Non-volatile compounds of *Hyssopus cuspidatus* Boriss and their antioxidant and antimicrobial activities. Food Chem. 374, 131638. https://doi.org/10.1016/j.foodchem.2021.131638

Asgari, S., Saberi, A.H., McClements, D.J., Lin, M., 2019. Microemulsions as nanoreactors for synthesis of biopolymer

nanoparticles. Trends Food Sci. Technol. 86 (42), 118–130. https://doi.org/10.1016/j.tifs.2019.02.008
Belandria, V., Oliveira, P.M.A.D., Chartier, A., Rabi, J.A., Oliveira, A.L.D., Bostyn, S., 2016. Pressurized-fluid extraction of cafestol and kahweol diterpenes from green coffee. Innov. Food Sci. Emerg. Technol. 37, 145–152. https://doi.org/10.1016/j.ifset.2016.07.022
Bertolo, M.R.V., Martins, V.C.A., Plepis, A.M.G., Junior, S.B., 2021. Utilization of pomegranate peel waste: natural deep eutectic solvents as a green strategy to recover valuable phenolic compounds. J. Clean. Prod. 327, 129471. https://doi.org/10.1016/j.jclepro.2021.129471
Chemat, F., Huma, Z.E., Khan, M.K., 2010. Applications of ultrasound in food technology: processing, preservation and extraction. Ultrason. Sonochem. 18 (4), 813–835. https://doi.org/10.1016/j.ultsonch.2010.11.023
Cui, Q., Liu, J.Z., Yu, L., Gao, M.Z., Wang, L.T., Wang, W., Zhao, X.H., Fu, Y.J., Jiang, J.C., 2020. Experimental and simulative studies on the implications of natural and green surfactant for extracting flavonoids. J. Clean. Prod. 274, 122652. https://doi.org/10.1016/j.jclepro.2020.122652
Falade, K.O., Adedeji, A.A., Akingbala, J.O., 2003. Effect of soybean substitution for cowpea on physical, compositional, sensory and sorption properties of *akara* Ogbomoso. Eur. Food Res. Technol. 217 (6), 492–497. https://doi.org/10.1007/s00217-003-0776-2
Ferrentino, G., Giampiccolo, S., Morozova, K., Haman, N., Spilimbergo, S., Scampicchio, M., 2020. Supercritical fluid extraction of oils from apple seeds: process optimization, chemical characterization and comparison with a conventional solvent extraction. Innov. Food Sci. Emerg. Technol. 64, 102428. https://doi.org/10.1016/j.ifset.2020.102428
Galiano, F., Figoli, A., Deowan, S.A., Johnson, D., Altinkaya, S.A., Veltri, L., Luca, G.D., Mancuso, R., Hilal, N., Gabriele, B., Hoinkis, J., 2015. A step forward to a more efficient wastewater treatment by membrane surface modification *via* polymerizable bicontinuous microemulsion. J. Membr. Sci. 15, 103–114. https://doi.org/10.1016/j.memsci.2015.02.019
Garcia, L.B., Pires, G.A., Oliveira, D.A.J., Silva, L.A.O., Gomes, A.F., Amaral, J.G., Pereira, G.R., Ruela, A.L.M., 2021. Incorporation of glycolic extract of cocoa beans (*Theobroma cacao* L.) into microemulsions and emulgels for skincare. Ind. Crops Prod. 161, 113181.https://doi.org/10.1016/j.indcrop.2020.113181.
Guo, Y., Li, H.Y., Yuan, Y.H., Huang, J., Diao, J., Xie, B., 2020. Microemulsion extraction: an efficient way for simultaneous detoxification and resource recovery of hazardous wastewater containing V(V) and Cr(VI). J. Hazard. Mater. 386, 121948. https://doi.org/10.1016/j.jhazmat.2019.121948
Hayes, D.G., Ye, R., Dunlap, R.N., Cuneo, M.J., Pingali, S.V., O'Neill, H.M., Urban, V.S., 2017. Protein extraction into the bicontinuous microemulsion phase of a Water/SDS/pentanol/dodecane winsor-III system: effect on nanostructure and protein conformation. Colloids Surf. B: Biointerfaces 160, 144–153. https://doi.org/10.1016/j.colsurfb.2017.09.005
Hou, K.X., Bao, M.L., Wang, L., Zhang, H., Yang, L., Zhao, H.T., Wang, Z.Y., 2019. Aqueous enzymatic pretreatment ionic liquid-lithium salt based microwave-assisted extraction of essential oil and procyanidins from pinecones of *Pinus koraiensis*. J. Clean. Prod. 236, 117581. https://doi.org/10.1016/j.jclepro.2019.07.056
Jia, C.L., Wang, J.Y., Zhao, K.J., Peng, H.S., Gui, S.Y., 2020. Records of medicinal and edible plants in the herb section of compendium of materia medica and their revelation. Mod. Chin. Med. 22 (11), 1769–1777. https://doi.org/10.13313/j.issn.1673-4890.20200811005
Jiao, J., Wei, F.Y., Gai, Q.Y., Wang, W., Luo, M., Fu, Y.J., Ma, W., 2014. A pilot-scale homogenization-assisted negative pressure cavitation extraction of *Astragalus polysaccharides*. Int. J. Biol. Macromol. 67, 189–194. https://doi.org/10.1016/j.ijbiomac.2014.03.018
Jivan, M.J., Abbasi, S., 2020. Novel approach for lutein extraction: food grade microemulsion containing soy lecithin & sunflower oil. Innov. Food Sci. Emerg. Technol. 66, 102505. https://doi.org/10.1016/j.ifset.2020.102505
Khan, R.A., Hossain, R., Roy, P., Jain, D., Mohammad Saikat, A.S., Roy Shuvo, A.P., Akram, M., Elbossaty, W.F., Khan, I.N., Painuli, S., Semwal, P., Rauf, A., Islam, M.T., Khan, H., 2022. Anticancer effects of acteoside: mechanistic insights and therapeutic status. Eur. J. Pharmacol. 916, 174699. https://doi.org/10.1016/j.ejphar.2021.174699
Khursheed, A.S., Oladipupo, O.O., Zhang, B., Nurul, H., Soottawat, B., 2021. Pulsed electric field assisted process for extraction of bioactive compounds from custard apple (*Annona squamosa*) leaves. Food Chem. 359 (11), 129976. https://doi.org/10.1016/j.foodchem.2021.129976
Klossek, M.L., Marcus, J., Touraud, D., Kunz, W., 2014. Highly water dilutable green microemulsions. Colloids Surf. A Physicochem. Eng. Asp. 442, 105–110. https://doi.org/10.1016/j.colsurfa.2012.12.061
Kou, P., Wang, S.X., Pan, H.Y., Wan, N., Wang, X.Q., Liu, Z.G., Zhao, C.J., Jiang, S.G., Fu, Y.J., 2021. Preparative separation of specific triterpenoids from Inonotus obliquus based on negative-pressure cavitation extraction and high-speed countercurrent chromatography. J. Taiwan Inst. Chem. Eng. 125, 69–77. https://doi.org/10.1016/j.jtice.2021.06.024
Kulawik, P., Özogul, F., Glew, R., Özogul, Y., 2013. Significance of antioxidants for seafood safety and human health. J. Agric. Food Chem. 61 (3), 475–491. https://doi.org/10.1021/jf304266s
Kwiatkowski, M., Kravchuk, O., Skouroumounis, G.K., Taylor, D.K., 2020. Response surface parallel optimization of extraction of total phenolics from separate white and red grape skin mixtures with microwave-assisted and conventional thermal methods. J. Clean. Prod. 251, 119563. https://doi.org/10.1016/j.jclepro.2019.119563
Leng, L.J., Chen, J., Leng, S.Q., Li, W.Y., Huang, H.J., Li, H., Yuan, X.Z., Li, J., Zhou, W.G., 2019. Surfactant assisted upgrading fuel properties of waste cooking oil biodiesel. J. Clean. Prod. 210, 1376–1384. https://doi.org/10.1016/j.jclepro.2018.11.027
Li, C.Y., Meng, X.M., Tian, M.F., Li, S., Tian, Y., Wang, T.T., Zhao, C.J., 2021. A novel method to extract juglone from *Juglans mandshurica* waste branches using a water-in-oil microemulsion. Waste Biomass Valoriz. 13 (3), 1547–1563. https://doi.org/10.1007/s12649-021-01611-x
Liu, C.Y., Ma, R.F., Wang, L.L., Zhu, R.Y., Liu, H.X., Guo, Y.B., Zhao, B.S., Zhao, S.G., Tang, J.F., Li, Y., Niu, J.Z., Fu, M., Zhang, D.W., Gao, S.H., 2017. Rehmanniae radix in osteoporosis: a review of traditional Chinese medicinal uses, phytochemistry, pharmacokinetics and pharmacology. J. Ethnopharmacol. 198, 351–362. https://doi.org/10.1016/j.jep.2017.01.021
Mohamed, K., Elena, R.S., Jana, Š.Ž., Anet, R.J., Mladen, B., Nabil, G., Nadia, B., Barba, F.J., 2015. Current and new insights in the sustainable and green recovery of nutritionally valuable compounds from *Stevia rebaudiana* Bertoni. J. Agric. Food Chem. 63, 6835–6846. https://doi.org/10.1021/acs.jafc.5b01994
Nogueira, C.R., Lopes, L.M.X., 2011. Antiplasmodial natural products. Molecules 16 (3), 2146–2190. https://doi.org/10.3390/molecules16032146
Piatczak, E., Kuzma, L., Koztowska, W., Lisiecki, P., Szemraj, M., Plachno, B.J., Gonciarz, W., Chmiela, M., Matkowski, A., Zielinska, S., 2020. Phenylethanoid and iridoid glycosides production in Rehmannia elata N.E.Brown ex Prein. In vitro shoot cultures and their biological activity. Ind. Crops Prod. 158, 113050. https://doi.org/10.1016/j.indcrop.2020.113050
Qi, X.L., Peng, X., Huang, Y.Y., Li, L., Wei, Z.F., Zu, Y.G., Fu, Y.J., 2015. Green and efficient extraction of bioactive flavonoids from *Equisetum palustre* L. by deep eutectic solvents-based negative pressure cavitation method combined with macroporous resin enrichment. Ind. Crops Prod. 70, 142–148.https://doi.org/10.1016/j.indcrop.2015.03.026.
Qin, D.Y., Xiang, B., Zhou, X., Qiu, S.Y., Xi, J., 2021. Microemulsion as solvent for naphthoquinones extraction from walnut (*Juglans mandshurica* Maxim) green husk using high voltage electrical discharge. Sep. Purif. Technol. 281, 119983. https://doi.org/10.1016/j.seppur.2021.119983

Roohinejad, S., Koubaa, M., Barba, F.J., Greiner, R., Orlien, V., Lebovka, N.I., 2016. Negative pressure cavitation extraction: a novel method for extraction of food bioactive compounds from plant materials. Trends Food Sci. Technol. 52, 98–108. https://doi.org/10.1016/j.tifs.2016.04.005

Soldan, A.C.F., Arvelos, S., Watanabe, E.O., Hori, C.E., 2021. Supercritical fluid extraction of oleoresin from *Capsicum annuum* industrial waste. J. Clean. Prod. 297, 126593. https://doi.org/10.1016/j.jclepro.2021.126593

Ullah, Z., Man, Z., Khan, A.S., Muhammad, N., Mahmood, H., Ghanem, O.B., Ahmad, P., Shah, M.U.H., Rashid, M.U., Raheel, M., 2019. Extraction of valuable chemicals from sustainable rice husk waste using ultrasonic assisted ionic liquids technology. J. Clean. Prod. 220, 620–629. https://doi.org/10.1016/j.jclepro.2019.02.041

Wan, Z.Y., Wu, F., Li, Y., Xie, X.A., Li, L., 2015. Mesoscopic simulation of Tween 80/n-Alcohol Microemulsion system. Mod. Food Sci. Technol. 31 (8), 177–183. https://doi.org/10.13982/j.mfst.1673-9078.2015.8.029

Wang, N.N., Wang, J.R., Li, Y., Li, L., Xie, X.A., 2020. Reverse microemulsion prepared by AOT/CTAB/SDS/Tween80 for extraction of tea residues protein. J. Mol. Liq. 320, 114474. https://doi.org/10.1016/j.molliq.2020.114474

Wang, R.M., Wang, L., Zhang, L., Wan, S.T., Li, C.F., Liu, S.X., 2022. Solvents effect on phenolics, iridoids, antioxidant activity, antibacterial activity, and pancreatic lipase inhibition activity of noni (*Morinda citrifolia* L.) fruit extract. Food Chem. 377, 131989. https://doi.org/10.1016/j.foodchem.2021.131989

Wang, T., Guo, N., Wang, S.X., Kou, P., Zhao, C.J., Fu, Y.J., 2018. Ultrasound-negative pressure cavitation extraction of phenolic compounds from blueberry leaves and evaluation of its DPPH radical scavenging activity. Food Bioprod. Process. 108, 69–80. https://doi.org/10.1016/j.fbp.2018.01.003

Xu, Z., Dai, X.X., Zhang, Q.Y., Su, S.L., Yan, H., Zhu, Y., Shang, E.X., Qian, D.W., Duan, J.A., 2020. Protective effects and mechanisms of *Rehmannia glutinosa* leaves total glycoside on early kidney injury in db/db mice. Biomed. Pharmacother. 125, 109926. https://doi.org/10.1016/j.biopha.2020.109926

Yan, J., Wang, C., Jin, Y., Meng, Q., Liu, Q., Liu, Z., Liu, K., Sun, H., 2018. Catalpol ameliorates hepatic insulin resistance in type 2 diabetes through acting on AMPK/NOX4/PI3K/AKT pathway. Pharmacol. Res. 130, 466–480. https://doi.org/10.1016/j.phrs.2017.12.026

Yu, C.M., 2013. Three healthy porridges in spring. Openings 18, 114. https://doi.org/10.3969/j.issn.1005-393X.2013.18.028

Zhang, B., 2021. On the beauty of Su Dongpo's food and culture. J. Huanggang Polytech. 23 (4), 6–10. https://doi.org/10.3969/j.issn.1672-1047.2021.04.02

Zhang, F., Wang, M., Xue, J., Liang, Y., Chen, Z., 2019. Effects of different pH and ionic strength on functional properties of grape seed protein isolate. Food Sci. Technol. 44 (9), 277–282. https://doi.org/10.13684/j.cnki.spkj.2019.09.048

Zhang, H., Lu, J., Liu, H., Guan, L., Xu, S., Wang, Z., Qiu, Y., Liu, H., Peng, L., Men, X., 2021. Ajugol enhances TFEB-mediated lysosome biogenesis and lipophagy to alleviate non-alcoholic fatty liver disease. Pharmacol. Res. 174, 105964. https://doi.org/10.1016/j.phrs.2021.105964

Zhou, F., Xu, T., Zhao, Y.J., Song, H.X., Zhang, L.Q., Wu, X.D., Lu, B.Y., 2017. Chitosan-coated liposomes as delivery systems for improving the stability and oral bioavailability of acteoside. Food Hydrocoll. 83, 17–24. https://doi.org/10.1016/j.foodhyd.2018.04.040

Zhu, X.C., He, M., Zhang, W., Wei, H.B., Lyu, X.J., Wang, Q.B., You, X.F., Li, L., 2020. Formulation design of microemulsion collector based on gemini surfactant in coal flotation. J. Clean. Prod. 257, 120496. https://doi.org/10.1016/j.jclepro.2020.120496

Zong, W.R., Cheong, K.L., Wu, D.T., Li, J., Zhao, J., Li, S.P., 2014. Preparation and purification of raffinose family oligosaccharides from *Rehmannia glutinosa* Libosch. by fast protein liquid chromatography coupled with refractive index detection. Sep. Purif. Technol. 138, 98–103. https://doi.org/10.1016/j.seppur.2014.10.001