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Optimization of green deep eutectic solvent (DES) extraction of *Chenopodium quinoa* Willd. husks saponins by response surface methodology and their antioxidant activities†

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Quinoa saponins have outstanding activity, and there are an increasing number of extraction methods, but there are few research programs on green preparation technology. The extraction conditions of quinoa saponins with deep eutectic solvents (DESs) were optimized by single-factor experiments combined with response surface methodology. The antioxidant capacity of saponins extracted by DESs and traditional methods was evaluated by the DPPH clearance rate, iron ion chelation rate and potassium ferricyanide reducing power. The results show that the optimal DES is choline chloride: 1,2-propylene glycol (1 : 1), and its water content is 40%. The optimal extraction conditions were as follows: the solid-to-solvent ratio was 0.05 g mL^{-1}, the extraction time was 89 min, and the extraction temperature was 75 °C. Under these conditions, the extraction of quinoa saponins by DES was more effective than the traditional extraction methods. The saponins extracted by DES and traditional methods were analyzed by UPLC-MS, and five main saponins were identified. Quantitative analysis by HPLC-UV showed that Q1 (m/z = 971) and Q2 (m/z = 809) had higher contents of saponins. *In vitro* antioxidant experiments showed that all DES saponin extracts showed good antioxidant capacity. This study provides new insight into the development and utilization of quinoa saponins.

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1. Introduction

Quinoa, an annual dicotyledonous plant of the amaranthus family, grows at high altitudes, has strong tolerance and adaptability to cold and arid environments, and has received extensive attention from the scientific community because of its high nutritional value.[1,2] Quinoa is rich in proteins, amino acids, starch, fiber, and minerals, especially amino acids, which are rich and balanced, and its seeds have a high content of saponins and flavonol glycosides. In traditional studies, mechanical grinding is often used to remove husks. Due to the high content of saponins in the husks of quinoa seeds (approximately 3–8% of the whole plant), thus wasting a lot of saponin resources. Current *in vitro* and *in vivo* bioactivity studies have shown that quinoa saponins have a wide range of beneficial properties, including antioxidant, antidiabetic, anti-inflammatory, antimicrobial, antidiabetic, anti-inflammatory, antimicrobial and anticancer functions.[3] Although quinoa saponins have many activities, the practical application of quinoa saponins has not received attention; a few of them are included in feed, and most of them are discarded, which not only results in the loss of active substances and the waste of resources but also poses a threat to the environment.[4,5]

Deep eutectic solvents (DESs) were first proposed by Abbott in 2003 and are considered green solvents because of their advantages, such as simple synthesis, designable structure and environmental friendliness.[6,7] In recent years, the use of deep eutectic solvents has been reported for the extraction of phenolic compounds in *Carthamus tinctorius* L., flavonoids from Pollen Typhae and polysaccharides from *Camellia oleifera* Abel.[8] Quinoa saponin is one of the main active substances in quinoa husks, and the common extraction solvents used to extract saponin in the current study are methanol, ethanol, and water. Among the traditional extraction methods, the ultrasonic extraction method of 70% methanol and 70% ethanol had a better extraction effect. Espinoza *et al.* used ethanol solution as the extraction solvent to optimize the extraction conditions of quinoa saponin, and the extraction rate was greatly improved, but this method still required many organic solvents.[9] These organic solvents have disadvantages such as environmental

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pollution, volatilization and toxicity.[10] To improve the environment and reduce the pollution of organic solvents, deep eutectic solvents (DESs) have emerged as a new type of green nonpolluting extraction solvent. DESs are formed by the interaction of two or three hydrogen bond donors and hydrogen bond acceptors through hydrogen bonding or electrostatic forces and are mixtures with a lower melting point than the individual components, with a melting point of less than 100 °C.[11–13] Hydrogen bond acceptors (HBAs) are mostly quaternary ammonium salts, quaternary phosphates, betaines, imidazolyl salts, *etc.*; hydrogen bond donors (HBDs) are mostly amides, carboxylic acids, polyols, *etc.* As shown in Fig. S1,† the most common DESs are based on choline chloride mixed with other ingredients, such as glycerol.[14] Previously, Taco used choline chloride with glycerol to extract saponins from quinoa seeds, and the extraction rate was superior to that of conventional organic solvents. Saponins containing C=O are more conducive to the formation of hydrogen bonds, so the efficiency is increased. DES is too viscous and can lead to low extraction rate, because part of the saponin are soluble in water, the addition of water can improve the extraction rate.[15] However, previous studies prepared a single DES and lacked studies on quinoa husks saponins, so the DESs prepared in this study were based on four types, acid-based, amino-based, sugar-based, and alcohol-based, with a wide variety of species, which broadened the idea of the study of plant compounds extracted by DESs. The main criteria for selecting the components of DESs in this study are that they are all easy to purchase in the market, low cost, high safety, good biodegradability, and recyclable, and the use of DESs to extract saponins from quinoa husks in this study provides a new way of thinking in the study of saponins.

2. Materials and methods

2.1 Chemicals

Chenopodium quinoa Willd. husks were obtained from Ulanqab (Inner Mongolia, China). Q1 (3-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabino-pyranosyl-phytolaccagenic acid 28-*O*-β-D-glucopyranosyl) and Q2 (3-*O*-α-L-arab-inopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester) (NMR, purity ≥ 98%) saponin controls were pre-prepared by laboratory. Choline chloride, betaine, citric acid, urea, glucose, 1,2-propylene glycol, glycerol, vanillin, perchloric acid, ethanol, DPPH, ferrous chloride and ferric chloride were purchased from China National Pharmaceutical Industry Co., Ltd. (Beijing, China). All chemicals were of analytical grade unless they were specially mentioned. Methanol and acetonitrile were of chromatographic purity and purchased from China National Pharmaceutical Industry Co., Ltd. (Beijing, China).

2.2 Apparatus and instruments

The HPLC LC-20AT was obtained from Shimadzu Corp. (Japan). The UPLC-Q-Exactive was purchased from Thermo Fisher (Waltham, MA, USA). Freeze-drying system FDU-1200 was from Shanghai Airon Co. (Shanghai, China). The enzyme labeling instrument 1510 was from Thermo Fisher (Waltham, MA, USA). Solid phase extractor LC-CQ-24Y was purchased from Shanghai Bangxi Instrument Technology (Shanghai, China).

2.3 Qualitative determination of saponins

The electrospray ionization (ESI) mass spectrometry (MS) data were recorded on an Thermo Fisher Scientific UPLC-Q-Exactiveinstrument with ACQUITY UPLC BEH C18 column (3.0 × 100 mm, 1.7 μm). The UPLC conditions for the UPLC-MS analysis were as follows: column temperature: 40 °C; injection volume: 4 μL; mobile phase A, acetonitrile; mobile phase B, 0.1% ammonium acetate solution; gradient elution (mobile phase A concentration): 0–5 min: 10% B, 5–10 min: 10–28% B, 10–15 min: 28–35% B, 15–20 min: 35–40% B, 20–25 min: 40–45% B, 25–30 min: 45–85% B, 30–32 min: 85–10% B, 32–37 min: 10% B; flow rate was 0.3 mL min^{-1}.[16]

The ESI parameters were as follows: isolation window was 4.0 *m*/*z*, AGC target was 3×10^6, the carrier gas (N_2), sheath gas flow rate: 35 arb, the column oven was 30 °C, data were collected in negative ion mode $[M-H]^-$, scans were conducted over 100–1500 *m*/*z*, the spray voltage was 3.5 kV, the capillary voltage was 50 V, and the capillary temperature was 320 °C.[17]

2.4 Quantitative determination of saponins

2.4.1 Vanillin glacial acetic acid-perchloric acid colorimetric method. Q1 was selected to draw the standard curve. 1 mg Q1 was dissolved with methanol in 10 mL, standard solution 0, 0.2, 0.4, 0.6, 0.8 and 1 mL were marked by 0–5 respectively, then dried in water bath with 60 °C. Followed by 0.2 mL 5% vanillin glacial acetic acid solution and 0.8 mL perchloric acid added after the standard solution cooled to room temperature, shooked well and bathed in 60 °C for 15 min. 4 mL glacial acetic acid was added in each cooled tubes, shooked well and left at room temperature for half an hour. Finally, the absorbance was measured at 560 nm wavelength by using the method of enzyme labeling instrument. The standard curve was established with concentration as abscissa and absorbance as ordinate.[18]

2.4.2 High performance liquid chromatography (HPLC). HPLC data were recorded on a Shimadzu LC-20AT HPLC instrument equipped with YMC ODS-Pack C18 column (4.6 mm × 250 mm, 5 μm). The HPLC conditions were as follows: mobile phase A was ultrapure water and mobile phase B was acetonitrile; binary high-pressure gradient elution was 0–5 min: 10% B, 5–10 min: 10–15% B, 10–15 min: 15–20% B, 15–25 min: 20–22% B, 25–35 min: 22–28% B, 35–45 min: 28–35% B, 45–50 min: 35–40% B, 50–55 min: 40–45% B, 55–60 min: 45–60% B, 60–65 min: 60–85% B, 65–70 min: 85–10% B, 70–75 min: 10% B;[19] the detection wavelength was 202 nm; the column temperature was 30 °C; the flow rate was 1 mL min^{-1}; and the injection volume was 20 μL.

Q1 and Q2 were selected to draw the standard curve. Configure the working solutions of Q1 and Q2 standards 10 μg mL^{-1}, 50 μg mL^{-1}, 300 μg mL^{-1}, 500 μg mL^{-1}, 750 μg mL^{-1} and 1500 μg mL^{-1} respectively. Pipette 1 mL of the above working solution separately and pass through 0.45 μm microporous filter membrane. Elution was carried out according to the above

chromatographic conditions and the peak area integral values were recorded. The standard curve is established with concentration as abscissa and peak area integral value as ordinate.

2.5 DES preparation

DESs were prepared by combining hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs) in different molar ratios using the heating and stirring method as described in the literature.[10] The heating method is the most commonly used synthetic method because it does not require purification and has a simple operation procedure. DESs were prepared by the heating and stirring method by weighing a certain molar ratio of HBD and HBA, mixing them in a beaker, adding a magnetic stirrer, adding an appropriate amount of water, heating in a water bath at 70 °C and stirring magnetically until a clear and transparent liquid was formed. A total of 42 DESs were prepared in this study. Choline chloride and betaine were used for HBD, and citric acid, urea, glucose, 1,2-propylene glycol, and glycerol were used for HBA. Information on the prepared DESs is shown in Table 1.

2.6 Screening of the water content of DESs

The water content of DESs is an important factor affecting the extraction effect of target components, therefore, the effect of different water content of DESs was examined. Different water contents (20, 30, 40, 50, 60, 70 and 80%) were examined using uniform solid-to-solvent ratios, extraction temperature and extraction time to finalize the optimum water content.

2.7 HLB solid-phase extraction column treatment

In the traditional method, the separation and purification of saponins are generally carried out using macroporous resins. But the manual filling of the column is cumbersome and has low accuracy and a long operation time. HLB column was used in this study as a solid-phase extraction method for the purification of saponins. According to Jeong's method,[12] the HLB column was processed as follows:

The HLB column was activated with 12 mL of deionized water and 12 mL of methanol. The diluted samples were kept in the HLB column for 10 min and then passed through the HLB at a flow rate of 1 drop per s. The DES was recovered by freeze-drying effluent liquid. Then the HLB column was rinsed with methanol, the eluate was collected, and the saponins were obtained by evaporation.

2.8 Experimental design

2.8.1 One-way experimental design. According to the above saponin quantification method, the optimal DES and its water content were determined. The effects of different solid-to-solvent ratios, extraction temperatures, and extraction times on the saponin extraction rate during ultrasonic extraction of quinoa saponins were further examined.

2.8.2 Response surface optimization test. Design-expert was used for experimental design and statistical analysis. Three variables of ultrasonic extraction time (A, min), extraction temperature (B, °C) and solid-to-solvent ratios (C, g mL^{-1}) were selected as independent variables, and the Box–Behnken response surface optimization experimental design was carried out with the response value of saponin extraction rate D. A total of 17 experiments were performed, and the experimental protocols are shown in Table 2.

2.9 Determination of antioxidant activity

DES extract, 70% methanol extract, 70% ethanol extract, 100% ethanol extract, and 1 mmol per L VC solution using the same conditions for saponin extraction were configured into 0.20, 0.40, 0.60, 0.80, and 1.00 mL per mL solutions. VC solution was used as a positive control.

2.9.1 Measurement of DPPH free radical scavenging rate. This experiment was carried out according to the literature with slight modifications.[17] DPPH (0.788 mg) was accurately weighed and fixed with 95% ethanol into a 20 mL brown volumetric flask, kept away from light, and shaken well to obtain a 0.1 mmol per L DPPH solution. One hundred microliters of

Table 2 Analytical factors and levels for BBD

	Levels		
Factors	−1	0	1
Extraction time A (min)	50	70	90
Extraction temperature B (°C)	45	60	75
Solid-to-solvent ratios C (g mL^{-1})	0.05	0.1	0.15

Table 1 Composition of different DESs

Type	No.	DESs	Mole ratio
Acid-based DESs	DES1	Choline chloride : citric acid	1 : 1; 1 : 2; 1 : 3; 1 : 4
	DES2	Betaine : citric acid	1 : 1; 1 : 2; 1 : 3; 1 : 4
Amamine-based DESs	DES3	Choline chloride : urea	1 : 1; 1 : 2; 1 : 3; 1 : 4
	DES4	Betaine : urea	1 : 1; 1 : 2; 1 : 3; 1 : 4
Sugar-based DESs	DES5	Choline chloride : glucose	1 : 1; 1 : 2; 1 : 3; 1 : 4; 2 : 1
	DES6	Betaine : glucose	1 : 1; 1 : 2; 1 : 3; 1 : 4; 2 : 1
Alcohol-based DESs	DES7	Choline chloride : 1,2-propylene glycol	1 : 1; 1 : 2; 1 : 3; 1 : 4
	DES8	Betaine : 1,2-propylene glycol	1 : 1; 1 : 2; 1 : 3; 1 : 4
	DES9	Choline chloride : glycerol	1 : 1; 1 : 2; 1 : 3; 1 : 4
	DES10	Betaine : glycerol	1 : 1; 1 : 2; 1 : 3; 1 : 4

the solution to be tested (0.20, 0.40, 0.60, 0.80, 1.00 mL mL^{-1} DES extract, 70% methanol extract, 70% ethanol extract, 100% ethanol extract, and 1 mmol per L VC solution) was mixed with 100 μL of DPPH solution and reacted for 30 min in darkness, and the absorbance A_i was determined at 571 nm. The absorbance A_i was measured at 571 nm. One hundred microliters of the test solution was mixed with 100 μL of 95% ethanol solution and reacted in the dark for 30 min, and the absorbance A_j was measured at 571 nm. One hundred microliters of DPPH solution was mixed with 100 μL of 95% ethanol solution and reacted in the dark for 30 min, and the absorbance A_c was measured at 571 nm. The formula was calculated as the clearance rate:

$$\text{Clearance rate}(\%) = 1 - \frac{(A_i - A_j)}{A_c} \times 100 \quad (1)$$

2.9.2 Determination of iron ion chelation. This experiment was performed according to the method of Llorent-Martínez with slight modifications.[20] A total of 100 μL of the solution to be tested (0.20, 0.40, 0.60, 0.80, 1.00 mL mL^{-1} DES extract, 70% methanol extract, 70% ethanol extract, 100% ethanol extract, 1 mmol per L VC solution) was placed in a test tube, and 5 μL of 2 mmol per L ferrous chloride solution and 185 μL of ultrapure water were mixed well. Then, 10 μL of 5 mmol per L phenanthroline solution was added, mixed with vigorous shaking, and left to stand for 10 min at room temperature. The absorbance A_1 was measured at a wavelength of 562 nm. The magnitude of the absorbance A_2 of the blank control group was determined by replacing the sample with ultrapure water, and then the Fe^{2+} sequestration rate:

$$\text{Sequestration rate}(\%) = \left(1 - \frac{A_1}{A_2}\right) \times 100 \quad (2)$$

2.9.3 Determination of reducing power by the potassium ferricyanide method. The experiment was carried out according to the method of Wang with slight modifications.[21] One hundred microliters of different concentrations of the solution to be tested (0.20, 0.40, 0.60, 0.80, 1.00 mL mL^{-1} DES extract, 70% methanol extract, 70% ethanol extract, 100% ethanol extract, and 1 mmol per L VC solution) were placed in a test tube, and 0.1 mL of 0.2 mol per L phosphate buffer (pH = 6.6) and 0.1 mL of 1% $K_3Fe(CN)_6$ were mixed well and reacted at 50 °C for 20 min. Then, 0.1 mL of 10% trichloroacetic acid solution was added, mixed and centrifuged for 10 min. Then, 0.1 mL of supernatant was taken, 0.5 mL of ultrapure water was added, and 100 μL of $FeCl_3$ solution was added. After mixing and standing for 10 min, 200 μL was placed in a 96-well plate, the absorbance A_i was measured at 700 nm, the absorbance A_0 was measured by replacing the sample with ultrapure water, and the difference in absorbance was used to express the reducing power, which was calculated by the formula:

$$\text{Absorbance increment} = A_i - A_0 \quad (3)$$

2.10 Statistical analysis

All experiments were conducted in triplicate. Data analysis was performed using SPSS Statistics version 25. The experiment data were analyzed statistically with Design-expert 13. Analysis of variance (ANOVA) was performed for calculations and modeling of optimal conditions. Values of $p < 0.05$ were regarded as significant.

3. Results and analysis

3.1 Qualitative determination of saponins

The extracted saponins were characterized by ultrahigh-performance liquid chromatography-mass spectrometry (UPLC-MS) analysis, and UPLC-MS has higher sensitivity and a lower detection limit, and more saponins can be detected in the same sample.[22] As shown in Fig. 1, although the peak times of the main saponins extracted from DES and 70% methanol and 70% ethanol were slightly different, the types of saponins were similar. The 100% ethanol extract had fewer saponin types, which was related to the low extraction rate.

According to UPLC-MS analysis, the saponins extracted by the four extraction methods were hederagenin or phytolaccagenic acid saponins. According to the determined mass-charge ratio, the ion fragment information of the main saponins is shown in Fig. S2.† [23] The mass-charge ratio is different from that of Dini because the negative ion mode $[M-H]^-$ was adopted in this study.[23] The substance names are shown in Table S1,† which is consistent with the study.[24] The retention time is different from the study of Colson, possibly because the elution procedure of UPLC-MS is different.[25] In a previous study by Taco, a choline chlorine–glycerin–water system was used to extract quinoa saponins, and compared with traditional solvents, the main types of saponins identified were consistent with those in this study.[15] The study of Taco on the thermal stability of DESs showed that green solvents were more stable than traditional solvent extracts of quinoa saponins, so DESs could replace traditional organic solvents to extract quinoa husks saponins.

3.2 Quantitative determination of saponins

According to the results of HPLC analysis in Fig. S3,† Q1 and Q2 were the major saponin elements in all tested quinoa husks samples regardless of whether the quinoa saponins were extracted using conventional or green solvents, so the extracted saponins were quantified using Q1 and Q2 saponin controls. According to the analysis of the literature, the mass-charge ratio $m/z = 971$ for Q1 and $m/z = 809$ for Q2 and the ionic peak of the saponin fragment are shown in Fig. S2(c).† [23,24]

3.3 Deep eutectic solvent screening

3.3.1 Vanillin glacial acetic acid-perchloric acid colorimetric method. The standard curve was made with absorbance as the vertical coordinate and saponin concentration as the horizontal coordinate. In this study, the standard curve $Y_1 = 7.251X_1 + 0.0622$, $R^2 = 0.9937$ was obtained after measurement with Q1 as the standard. Saponins were extracted ultrasonically

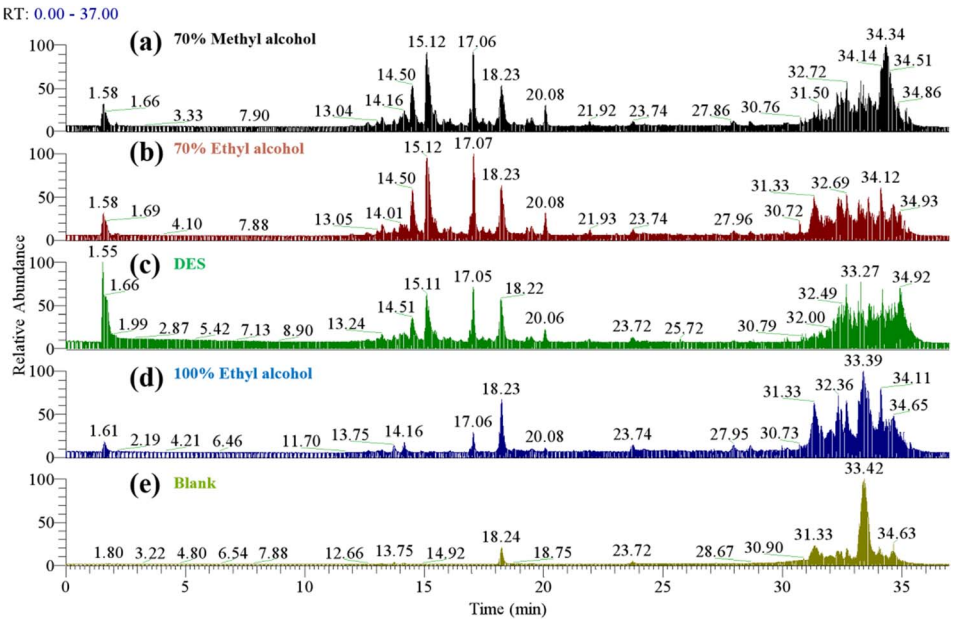

Fig. 1 The total ion chromatogram of 70% methanol extract (a), 70% ethanol extract (b), DES extract (c) and 100% methanol extract (d), blank control (e).

using the same solid-to-solvent ratio, extraction time and extraction temperature and then centrifuged at 5000 r min^{-1} for 10 min. The supernatant was taken, diluted, and the content of the saponins was determined. The contents of the saponins extracted from DES1, DES2, DES3, DES4, DES9 and DES10 were determined by the colorimetric method of vanillin-glacial acetic acid–acetic acid–perchloric acid and the results showed that DES4 (1 : 4) had the highest saponin extraction rate. Because DES5, DES6, DES7, and DES8, these DESs themselves will react with the saponin chromogenic reaction, and the vanillin glacial acetic acid-perchloric acid colorimetric method will produce errors. Therefore, these are determined using the 3.3.2 method for saponin content.

3.3.2 Determination of saponin content by high-performance liquid chromatography. The peak area integral value was taken as the vertical coordinate, and the solubility of the standard was taken as the horizontal coordinate to make the standard curve. In this study, the Q1 standard curve $Y_2 = 12208X_2 + 734\,719$, $R^2 = 0.989$ was obtained after measurement, and the Q2 standard curve $Y_3 = 329063X_3 + 65\,472$, $R^2 = 0.9962$ was obtained after measurement. DES extracts were treated as in section 3.3.1. We determined the contents of the saponins extracted from DES4 (1 : 4), DES5, DES6, DES7, and DES8 using liquid chromatography and the results showed that DES7 (1 : 1) had the highest saponin extraction rate.

3.4 Determination of DES water content

DES has a strong viscosity. To make DES easy to handle, water is usually added to reduce the viscosity of DES but also changes the polarity of DES.[26] Different polarities of the solvent on quinoa saponin solubility are different, and adding the appropriate amount of water can improve the saponin extraction rate. For example, ethanol aqueous solution with higher polarity is more effective than pure ethanol. In the study of Taco, two DESs, choline–glycerin chloride or choline–glycerin–water, were used to extract quinoa saponins.[15] Comparing the two extraction methods, it was found that the extraction rate of saponins in the choline–glycerin–water system was higher. This indicates that the saponin extraction rate can be improved mainly by increasing the solvent polarity.[14] However, adding too much water will destroy the supramolecular structure consisting of hydrogen bonds between DES, so according to the experimental results shown in Fig. 2(a), we finally determined DES7 (1 : 1) with a water content of 40% for subsequent experimental studies.

3.5 Determination of one-way tests

3.5.1 Determination of extraction time. The effects of different extraction times (10, 30, 50, 70, 90 and 110 min) on the saponin extraction rate were investigated when all other factors, such as the extraction temperature and solid-to-solvent ratio, were consistent. From Fig. 2(b), it can be seen that with increasing extraction time, the extraction rate of saponin also gradually increased but increased to a certain time after the extraction rate began to decline, so 70 min was selected as the optimal extraction time.

3.5.2 Determination of extraction temperature. The effect of different extraction temperatures (15, 30, 45, 60 and 75 °C) on the saponin extraction rate was examined when all other factors, such as extraction time and solid-to-solvent ratio, were consistent. As shown in Fig. 2(c), the saponin extraction rate increased with increasing extraction temperature. Appropriately increasing the temperature can reduce the viscosity of DESs, increase the diffusion coefficient, and destroy the

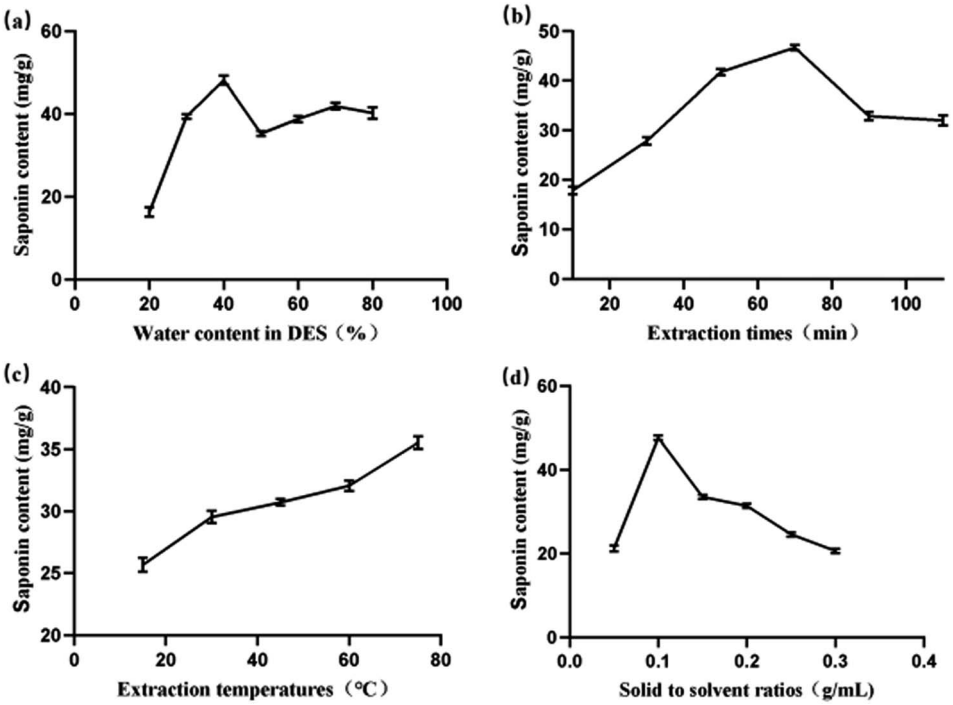

Fig. 2 (a) The effect of water content of DESs on the yield of saponins from quinoa husks. (b) The effect of extraction time on the yield of saponins from quinoa husks. (c) The effect of extraction temperature on the yield of saponins from quinoa husks. (d) The effect of solid-to-solvent ratios on the yield of saponins from quinoa husks.

intermolecular interactions, thus improving the solubilization of the active substances because the high temperature can accelerate the permeation of DESs and promote the transfer of saponins from quinoa to DESs, therefore increasing the extraction rate. Considering that higher temperatures can also cause the cavitation effect of ultrasound, too high a temperature cannot be used, so according to the results shown in Fig. 2(c), we chose 75 °C as the optimal extraction temperature.

3.5.3 Determination of solid-to-solvent ratios. The solid-to-solvent ratios affect the diffusion of solutes into the solvent, and to maximize the extraction efficiency and minimize solvent wastage, we investigated the effect of solid-to-solvent ratios (0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 g mL^{-1}) on the saponin extraction rate when the extraction time and extraction temperature were consistent. In this study, the effect of solid-to-solvent ratios was examined by fixing the amount of DES and increasing the amount of quinoa husks ratio by ratio, and the results are shown in Fig. 2(d). With the increase of quinoa husks, the saponin extraction rate increased, but when the quinoa husks content was too high, the solid particles in the solution increased, the sample and the solvent could not be mixed sufficiently, and the saponin extraction rate gradually decreased.[27] Therefore, 0.1 g mL^{-1} was finally selected as the optimal solid-to-solvent ratio.

3.6 Response surface optimization test

3.6.1 Response surface optimization test results and goodness-of-fit analysis. Based on the results of the one-way test, DES with an optimal saponin extraction rate from quinoa husks and its proportional composition and water content were selected for subsequent tests. Extraction time A (min), extraction temperature B (°C), and solid-to-solvent ratios C (g mL^{-1}) were selected as independent variables, and saponin extraction rate D was used as the response value to carry out a three-factor, three-level Box–Behnken response surface optimization experimental design with a total of 17 experimental scenarios, and the results of the experiments are shown in Tables 3 and 4.[9] Based on the results of the experiments, multivariate regression fitting analyses were carried out, and a quadratic multinomial regression equation was obtained with the response value of the saponin extraction rate. The quadratic multinomial regression equation was:

$$D = 31.24 + 0.8031A + 1.83B - 3.45C + 0.4935AB - 1.24AC - 3.8BC + 1.11A^2 + 12.17B^2 + 2.51C^2$$

Analysis of variance (ANOVA) was used to assess the relationship between independent and response variables and the optimal conditions for the extraction method.[28] In the response surface model for this saponin extraction rate, $R^2 = 0.9124$, $p < 0.01$, the overall model reached the significance level. The lack of fit term indicates that the probability of the model predicted value not fitting the actual value is not significant, and its $p = 0.4907$, $p > 0.05$, which indicates that the lack of fit term is not significant, and this model is chosen appropriately.[29] The coefficient of variation C.V. = 8.95% indicates that the model has good repeatability. According to the results in Table 4, it can be seen that the primary term C solid-to-solvent ratio reached

Table 3 Response surface experimental design and results

Run	Extraction time A (min)	Extraction temperature B (°C)	Solid-to-solvent ratios C (g mL^{-1})	Saponin content D (mg g^{-1})
1	70	45	0.15	43.252
2	70	45	0.05	41.190
3	90	45	0.1	45.541
4	70	60	0.1	27.758
5	90	60	0.15	29.597
6	90	60	0.05	40.361
7	50	60	0.05	37.631
8	70	60	0.1	28.939
9	70	60	0.1	29.534
10	50	75	0.1	42.513
11	70	60	0.1	34.832
12	70	75	0.05	56.179
13	90	75	0.1	46.472
14	50	60	0.15	31.846
15	70	75	0.15	43.046
16	70	60	0.1	35.159
17	50	45	0.1	43.556

Table 4 Analysis of mean square deviation of regression equation[a]

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value	Significant
Model	873.08	9	97.01	8.10	0.0058	$p < 0.05$*
A	5.16	1	5.16	0.4309	0.5325	
B	26.90	1	26.90	2.25	0.1776	
C	95.35	1	95.35	7.96	0.0257	$p < 0.05$*
AB	0.9741	1	0.9741	0.0814	0.7837	
AC	6.20	1	6.20	0.5176	0.4952	
BC	57.72	1	57.72	4.82	0.0641	
A^2	5.18	1	5.18	0.4325	0.5318	
B^2	623.32	1	623.32	52.06	0.0002	$p < 0.05$*
C^2	26.43	1	26.43	2.21	0.1810	
Lack of fit	35.22	3	11.74	0.9664	0.4907	Not significant

[a] Note: *means significant.

a significant level ($p < 0.05$), and the effect of the secondary term B^2 on the saponin extraction rate reached a highly significant level ($p < 0.01$). The order of the magnitude of the effect of the three factors on the saponin extraction rate was solid-to-solvent ratio (C) > extraction temperature (B) > extraction time (A).

Hu used ethanol to extract saponins from *Eclipta prostrasta* for response surface optimization experiments.[30] The extraction time was 3 h, which was longer than that of this study and consumed a large amount of organic solvents. Guo reported that the extraction time of notoginseng saponins from notoginseng leaves was 1.5 hours,[31] and the reflux extraction time with Soxhlet by Medina-Meza was approximately 3 hours.[32] Compared with other quinoa saponin extraction methods, this study used a shorter time to avoid the deterioration of metabolites and the rise in medium temperature caused by long-term extraction. The solid-to-solvent ratios in the study of Taco were consistent with those in this study, but the extraction time was shorter and the extraction rate was lower, probably because the interaction between temperature and time was not considered in the extraction rate.[15]

3.6.2 Analysis of factor interactions. Contour lines can show the relationship between response values and variables and the interaction effects among variables in a more visual way.[33] The shape of the contour lines can reflect the strength of the interaction effect, with oval shapes indicating a significant interaction between two factors, while circles are the opposite, *i.e.*, the flatter the contour plots are, the greater the effect of the interacting two factors on the saponin extraction rate.[28]

As shown in Fig. 3, extraction temperature and solid-to-solvent ratio had the largest interaction effect on the extraction of quinoa saponins by DESs, and extraction time and extraction temperature and extraction time and solid-to-solvent ratio had the least significant interactions, which is consistent with the results of the analysis of variance (ANOVA).

3.6.3 Determination of the optimal extraction process and validation tests. The regression model predicted the optimal extraction process of quinoa saponins as follows: an extraction time of 89.14 min, an extraction temperature of 74.89 °C, a solid-to-solvent ratio of 0.053 g mL^{-1}, and a theoretically predicted saponin extraction rate of 56 mg g^{-1}. Considering the

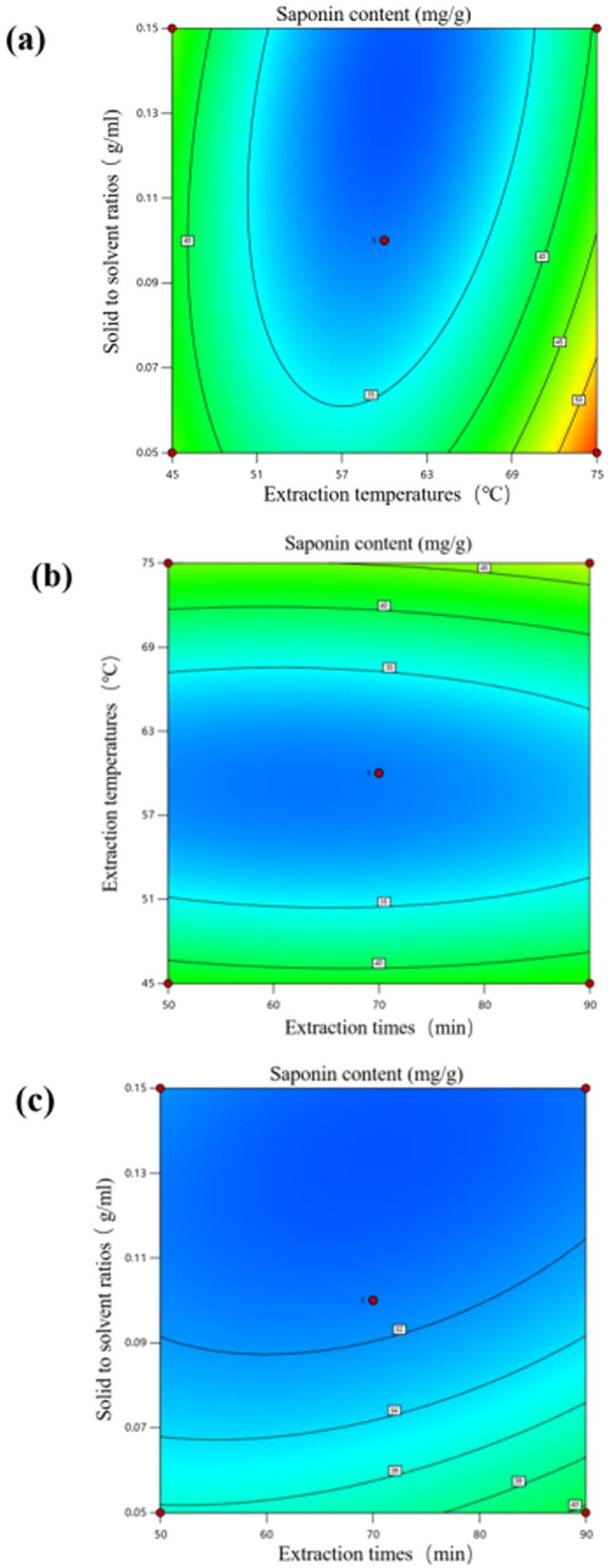

Fig. 3 Contour plots for the effects of the interactions of extraction temperature and solid-to-solvent ratios (a), extraction time and extraction temperature (b), extraction time and solid-to-solvent ratios (c) on the yield of saponins from quinoa husks.

practical practicability, we set the extraction time to 89 min, the extraction temperature to 75 °C, and the solid-to-solvent ratio to 0.05 g mL^{-1}. The saponin yield was 53.72 ± 0.1 mg g^{-1}. The relative error with the predicted value was 4.07%, which was less than 5%, indicating that the established model was more consistent with the actual situation. Therefore, the optimal extraction conditions for quinoa saponins using response surface methodology for DESs are desirable.

3.7 Comparison of saponin extraction methods

Studies have shown that DES is an effective green medium for stabilizing bioactive saponins in quinoa and has the potential to replace organic solvents.[34] According to the traditional saponin extraction method, the optimized extraction conditions were used to extract quinoa saponins using 70% methanol, 70% ethanol, and 100% ethanol, and the saponin extraction rates were 37.05 ± 0.16%, 32.75 ± 0.1%, and 8.64 ± 0.1%, respectively, and the saponin extraction rate of DESs was 53.72 ± 0.1%.[35] As shown in Fig. 4, compared with conventional organic solvents, the extraction effect of DESs was significantly better than that of conventional organic solvents which is consistent with Tu's study.[10] This indicates that the method proposed in this study is more efficient, simple, and has better prospects for application.

3.8 DES recovery

In this study, hydrophilic–lipophilic balanced adsorbents (HLB columns) were used to selectively enrich quinoa saponins with a lipophilic dammarane fraction and hydrophilic sugars.[12] Solid-phase extraction was used to separate quinoa saponins and extraction solvents, and freeze-drying of the separated extraction solvents allowed the recovery of deep-eutectic solvents.

3.9 Antioxidant activity results and analysis

3.9.1 Analysis of results for DPPH clearance. The trend of the scavenging rate of DPPH radicals by different

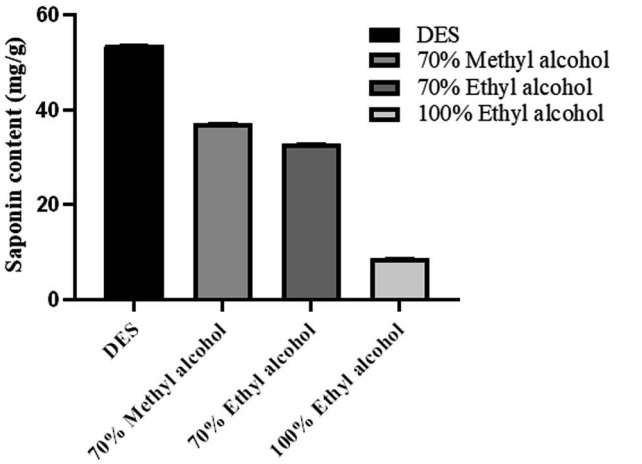

Fig. 4 Comparison of extraction rates of different extraction solvents.

concentrations of DES saponin extract, 70% methanol saponin extract, 70% ethanol saponin extract, and 100% ethanol saponin extract is shown in Fig. 5(a). All of the saponin extracts had some ability to scavenge DPPH radicals, and the scavenging rate gradually increased with increasing concentration. The comparative study showed that the scavenging of DPPH by VC in the positive control group changed only slightly with increasing sample concentration, and the scavenging of DPPH radicals by VC had a high and stable trend. For the samples, the DES saponin extract scavenged DPPH radicals slightly more than the organic solvent saponin extract at low concentrations; however, the DES saponin extract and 70% methanol saponin extract were similar when the concentration was greater than 0.6 mL mL^{-1}, and the DES saponin extract and 70% ethanol saponin extract were similar when the concentration was greater than 0.8 mL mL^{-1}. The DES extract scavenging rate was higher than the other three extracts, and experiments by Dong showed that this showed a dose relationship with its saponin-containing concentration and indicated that it has some

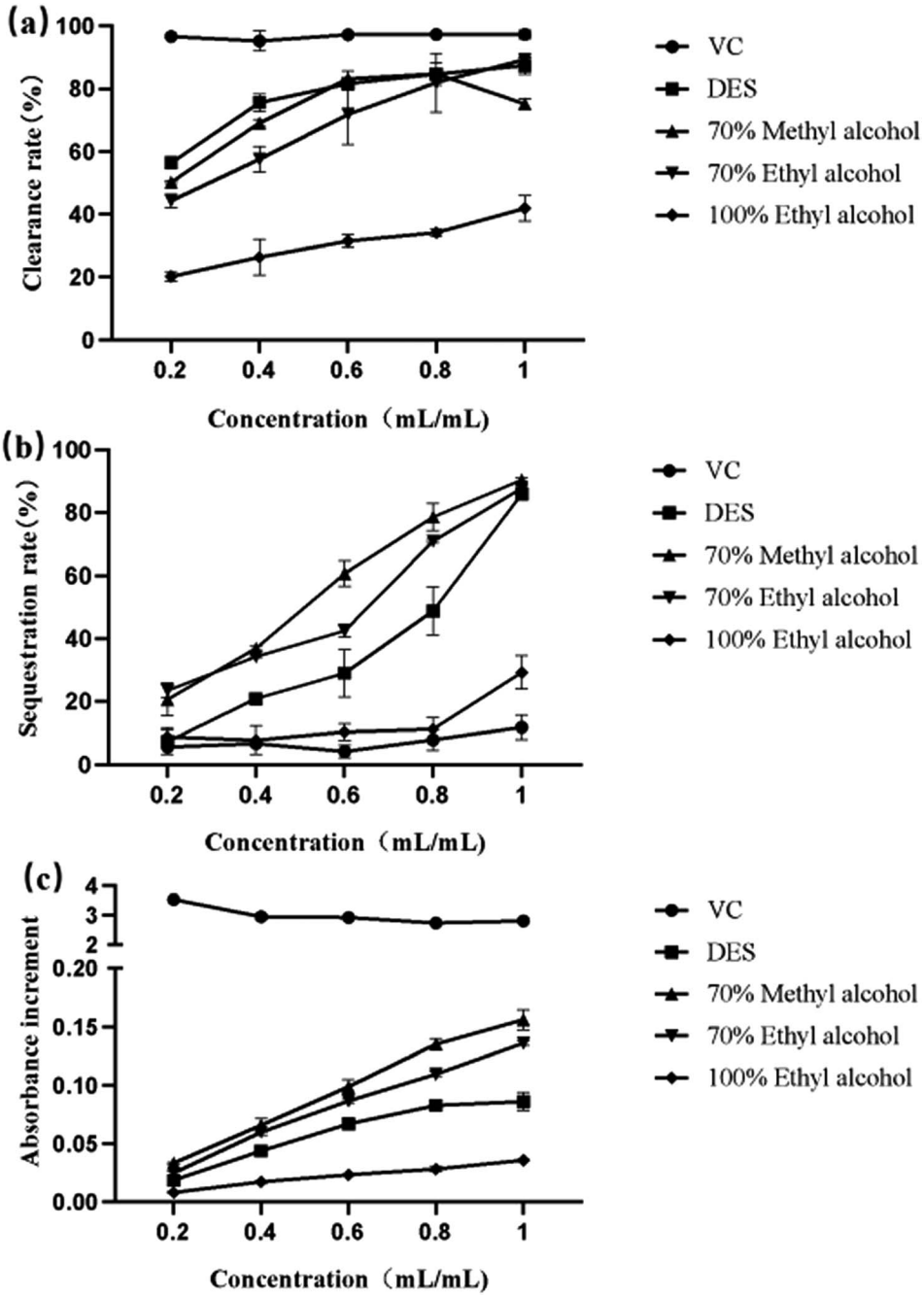

Fig. 5 (a) Comparison of DPPH scavenging rate of four saponin extracts and positive control VC. (b) Comparison of chelation rate of four saponin extracts and positive control VC. (c) Comparison of reducing power of four saponin extracts and positive control VC.

antioxidant capacity and can be used to delay or limit lipid oxidation.[6]

3.9.2 Analysis of the results of the chelating rate of iron ions. The ability to chelate ferrous ions is one of the commonly used methods to assess the antioxidant activity of a subject. The trend of the ferrous ion chelating rate for different concentrations of DES saponin extract, 70% methanol saponin extract, 70% ethanol saponin extract, and 100% ethanol saponin extract is shown in Fig. 5(b). The quinoa saponins extracted by all four extraction methods showed better ferrous ion chelating ability and showed a linear relationship with concentration. At low concentrations, the 70% methanol saponin extract and 70% ethanol saponin extract had higher chelating ability for ferrous ions than the DES saponin extract, but when the concentration was 1 mL mL^{-1}, the DES saponin extract, 70% methanol saponin extract, and 70% ethanol saponin extract had similar chelation rates of ferrous ions. It was inferred that the ferrous ion chelating ability increased with increasing saponin concentration.

3.9.3 Analysis of the reducing power results of the potassium ferricyanide method. The trend of the scavenging rate of DPPH radicals by different concentrations of DES saponin extract, 70% methanol saponin extract, 70% ethanol saponin extract, and 100% ethanol saponin extract is shown in Fig. 5(c). Among them, the reducing power of the potassium ferricyanide method of the VC positive control was the best. According to Fig. 5(c), the reducing power of the DES saponin extract was not the highest, and the reducing power of the 70% methanol saponin extract and 70% ethanol saponin extract was always better than that of the DES saponin extract, which may be related to the difference in the type of saponin it contains. Plant polyphenols also have good reducing power, and 70% methanol extract and 70% ethanol extract may contain plant polyphenols, which may be another reason for the high reducing power.[17] In conclusion, the antioxidant capacity was evaluated in terms of reducing power using the potassium ferricyanide method, and all four extracts had reducing power attributed to the presence of saponins and showed a linear relationship with concentration.

4. Conclusion

A total of 42 green DESs were prepared in this study, and the effects of extraction conditions on the extraction rate of quinoa saponins from DESs were investigated using a one-way test combined with a response surface test. The optimum extraction process conditions obtained were an extraction time of 89 min, an extraction temperature of 75 °C, and a material-to-liquid ratio of 0.05 g mL^{-1}. Under these conditions, the predicted saponin extraction rate was 56 mg g^{-1}, and the actual saponin yield was 53.72 ± 0.1 mg g^{-1}, which was not much different from the predicted value. This process will provide a theoretical basis for subsequent further optimization. Moreover, under these optimized conditions, the extraction of quinoa saponins by the DES was less time consuming and greener than that of conventional organic solvents. The new green extraction solvent prepared in this study breaks the concept that traditional organic solvents have been used to extract plant compounds, making DESs an attractive green solvent.[36]

The results of *in vitro* antioxidant experiments showed that all DES saponin extracts exhibited good antioxidant capacity and showed a linear relationship with the concentration, and this study provides a reference for quinoa saponin treatment of diseases caused by oxidative stress. As shown in Table S2,† this study provides a new method for the further development and utilization of quinoa saponin.

Author contributions

Yuqing Cai: methodology, software, writing – original draft. Hui Gao: resources, writing – review & editing. Linmeng Song: methodology, data curation. Feiyan Tao: writing – review & editing, supervision, data curation. Xueying Ji: methodology. Yuan Yu: supervision. Yuqing Cao: writing – review & editing. Shaojian Tang: supervision, writing – review & editing. Peng Xue: conceptualization, funding acquisition, resources, supervision, project administration, writing – review & editing.

Conflicts of interest

None.

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