Heliyon 10 (2024) e33066

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Ultrasound-assisted natural deep eutectic solvent extraction of anthocyanin from *Vitis davidii* Foex. pomace: Optimization, identification, antioxidant activity and stability

Shushu Zhang [a], Shuhua Lin [b], Juhua Zhang [a,c,**], Wei Liu [b,d,*]

[a] *Longping Branch, College of Biology, Hunan University, Changsha, 410125, China*
[b] *Hunan Agricultural Product Processing Institute, Changsha, 410125, China*
[c] *Hunan Academy of Agricultural Sciences, Changsha, 410125, China*
[d] *Hunan Provincial Key Laboratory for Fruits and Vegetables Storage Processing and Quality Safety, Changsha, 410125, China*

ARTICLE INFO

Keywords:
Vitis davidii foex. pomace
Anthocyanins
Ultrasonic-assisted natural deep eutectic solvent
Composition identification
Antioxidant capacity

ABSTRACT

An efficient and environmentally friendly extraction method utilizing an ultrasonic-assisted natural deep eutectic solvent (UAE-NADES) was developed for the extraction of anthocyanins from *Vitis davidii* Foex. A screening process was conducted to evaluate seven different NADESs, resulting in the selection of a high-efficiency NADES (choline chloride-glycerol (ChGly)). To analyze the influence of significant factors and their interactive effects on the total anthocyanin content (TAC), response surface methodology (RSM) was employed. Furthermore, the conditions of extraction were optimized to attain the most productive yield of total anthocyanin content. The theoretical optimal conditions were determined to be a liquid−solid ratio of 34.46 mL/g, an extraction temperature of 322.79 K and an ultrasonic power of 431.67 W, under which the verification TAC value (3.682 ± 0.051 mg/g) was highly consistent with the theoretical value (3.690 mg/g). Seventeen anthocyanins were identified by UPLC−MS/MS. The contents of the main anthocyanins peonidin-3,5-*O*-diglucoside, malvidin-3,5-*O*-diglucoside, malvidin-3-*O*-5-*O*-(6-*O*-coumaroyl)-diglucoside, and malvidin-3-*O*-(6-*O*-*p*-coumaroyl)-glucoside in the ChGly extracts were significantly higher than those in the acid−alcohol extract. Stability assays showed that the stability of anthocyanins in ChGly is higher than that in acidified alcohol at higher temperature, pH and stronger illumination. In vitro antioxidant results showed that the antioxidant capacities of the compounds extracted through the use of UAE-NADES were higher than those extracted using acidified alcohol. Additionally, the thermal behavior of anthocyanin extracts was further characterized through DSC analysis, highlighting the influence of ChGly or acidic ethanol. The results indicate that UAE-NADES exhibits a significant effect on the extraction

Abbreviations: **UAE-NADES**, ultrasonic-assisted natural deep eutectic solvent; **ChCl**, Choline chloride; **ChGly**, Choline chloride-glycerol; **TAC**, the total anthocyanin content; **UPLC-MS/MS**, ultra-performance liquid chromatography-tandem mass spectrometry; **FAO**, the United Nation's Food and Agriculture Organization; **HBA**, hydrogen bond acceptor; **HBD**, hydrogen bond donor; **BBD**, Box-Behnken design; **ANOVA**, analysis of variance; **DPPH**, 2,2-diphenyl-1-picrylhydrazylradical; **ABTS**, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); **RSA**, radical scavenging rates; **DSC**, differential scanning calorimetry analysis; **RSM**, response surface methodology; $\mathbf{T_d}$, thermal denaturation temperature.

* Corresponding author.
** Corresponding author.
E-mail addresses: 150405653@qq.com (S. Lin), 498282528@qq.com (J. Zhang), liuwei0505@126.com (W. Liu).

https://doi.org/10.1016/j.heliyon.2024.e33066
Received 1 August 2023; Received in revised form 7 June 2024; Accepted 13 June 2024
Available online 14 June 2024

of anthocyanins from plant byproducts, suggesting that its potential for use in the food sector is considerable.

1. Introduction

Vitis davidii Foex. Originated in China and belongs to the wild species of Vitis widely distributed in southern China. *Vitis davidii* Foex. is regarded as a valuable fruit that is appreciated for its unique flavor, excellent taste, and nutritional properties. Nevertheless, it is seldom eaten as fresh fruit due to its small grain, thick peel and high number of seeds. Therefore, *Vitis davidii* Foex. is suitable for processing into wine and juice, and the processing generates a considerable amount of waste, including its peel, stem, seed, and kernel, which are often discarded as byproducts [1]. Byproducts from juice production not only cause environmental pollution but also seriously waste valuable resources. As a report from the Food and Agriculture Organization (FAO) of the United Nations stated, more than 30 % of the resources that go into food production are lost as byproducts [2]. *Vitis davidii* Foex. pomace is rich in anthocyanins. It has been reported that anthocyanins possess multiple pharmacological activities, such as antioxidation, apoptosis inhibition, anti-inflammation, cardiovascular protection [3], myopia prevention and intestinal flora diversity properties [4,5]. Because of their attractive colors and desirable biological activities, anthocyanins have appeared in food, medicine and other industries. Nevertheless, it is commonly observed that anthocyanins exhibit heightened susceptibility to sunlight, heat, and pH inactivation. Furthermore, conventional solvent extraction methods frequently result in deterioration, diminished color intensity, and inadequate processability, thereby restricting their commercial application. Therefore, the effective extraction of anthocyanins from *Vitis davidii* Foex. pomace is urgently needed to improve their yield and stabilization for environmental protection and economic value.

Extraction is a significant and crucial step in the comprehensive utilization of byproducts, that is, extracting the required active compound at the optimal yield without affecting their physiological functions. Anthocyanins are intracellular secondary metabolites, and the efficient extraction of anthocyanins from *Vitis davidii* Foex. requires the breaking down of the cell wall. Conventionally, organic solvent extraction is typically the means by which anthocyanins are extracted. Nevertheless, organic solvent waste liquid is not only expensive to treat [6] but also harmful to human health and easily causes environmental pollution. Recently, NADESs have attracted the interest of scholars due to their remarkable advantages, such as high thermal stability, low cost and environmental friendliness [7]. NADES is a eutectic mixture formed by hydrogen bond acceptors (HBAs, such as choline chloride) and hydrogen bond donors (HBDs, such as glucose, 1,2-propanediol and glycerol) [8], which are natural components existing in cells and organisms. NADES extraction can directly interact with the target compound or indirectly interact with the cell wall cellulose molecular chain to destroy the cell wall and release the target compound from the plant matrix, thus improving the extraction effect [9,10]. In addition, it was found that NADESs produced by a wide range of natural ingredients exhibit high solubilization and stabilizing abilities and have been successfully used to extract bioactive compounds including phlorotannins [11], curcumins [12], anthocyanins [13], alkaloids [14], steroid glycosides [15], sesquiterpene [16], essential oil [17], saponines [18], triterpene saponins [19], glycosides [9] and aglicons of phenyletanes and phenylpropanoids [20]. Among them, the extraction, purification, and preconcentration procedures mediated by NADES have been proven to be superior to traditional solvents. For example, Santos-Martín et al. [21] developed for the environmentally friendly extraction of phenolic compounds from blueberry leaves using NADES. Their research found that the lactic-based NADES enabled the extraction of a wide range of hydroxycinnamic acids and flavonol derivatives, whereas the choline-based NADES was selective towards the extraction of anthocyanins. Zannou et al. [22] used 16 kinds of NADES to extract anthocyanins from blackberry, and found that NADESs were the most prominent solvents for the recovery of the targeted anthocyanins. Soukaina et al. [23] screened out 2 kinds of choline chloride NADES for extracting antioxidant polyphenols from mint. Their results supported that the proposed NADES are able to provide a higher selectivity toward flavonoids such as apigenin derivative, kaempferol coumaroyl hexoside, epi gallocatechin and quercetin o glucopyranoside gallate. Ultrasound-assisted extraction has been considered a "green" extraction technology due to its advantages, such as short processing time, high extraction capacity, low cost, easy operation, and environmental friendliness, and has been widely studied in the extraction of anthocyanins from plant materials. Ultrasonic-assisted extraction mainly uses ultrasonic cavitation, mechanical effects and thermal effects to efficiently destroy plant cell and vacuole walls, which greatly improves the penetration ability of NADESs and promotes the release, diffusion and dissolution of intracellular products. The combination of UAE-NADES methods allows the benefits of both methods to be utilized. Therefore, it provides an effective and green alternative to traditional extraction techniques. Most recently, UAE and NADES were used to extract active anthocyanin substances from raspberry powder, and the maximum extracted amount of anthocyanins reached 1.378 ± 0.009 mg/g [24]. In another study, it was found that the extraction rate of mulberry anthocyanins by six kinds of NADESs was higher than that by acidified ethanol [25]. Therefore, it is necessary to extract anthocyanins from *Vitis davidii* Foex. pomace by developing a separation technique using green solvent using NADES solvent. Analysis of the physicochemical properties of NADES, species, and NADES-specific optimization on anthocyanin extraction is critical to achieve maximum yield for further expansion of applications. In addition, the difference between anthocyanins extracted from UAE-ChGly and conventional extraction solvents needs to be clarified for possible future commercial applications. Furthermore, to the best of our knowledge, compared to most existing studies on the impact of NADES on the yield and purity of extracted compounds, the multiple stability protective effects of NADES (ChGly) on extracted anthocyanins have not been reported. Hence, the UAE-NADES method is used to extract anthocyanins from Vitis davidii Foex. to achieve the maximum yield of anthocyanins in this study. The extraction conditions were optimized by RSM, and the influences of the liquid–solid ratio, ultrasonic power and temperature on the extraction amount of anthocyanins were studied. Compared with the acidified ethanol extraction method, the extraction amount, anthocyanin composition, stability and antioxidant activity in vitro were investigated. This study

provides an effective and green alternative extraction method for the food sector.

2. Materials and methods

2.1. *Vitis davidii* Foex. pomace

The squeezed *Vitis davidii* Foex. pomace (35 kg) was kindly donated by the juice producer Hunan Veichu Juice &Wine Co., Ltd. (Huaihua, Hunan Province, China), placed into a plastic bag, and sent to the laboratory in October 2022. The pomace was exposed to hot air drying for 24 h at 318.15 K in an oven (XMTD-8222, Shanghai Jinghong Laboratory Instrument Co. Ltd., Shanghai, China) to a water content of <5 %. The dried pomaces were ground into fine powder with a mill (DE-500 g, Zhejiang Hongjingtian Industry & Trade Co., Ltd., Zhejiang, China), sieved with a 40 mesh sieve, sealed in an aluminum foil self-sealing bag to avoid the influence of light, and deposited at 233.15 K for future use.

2.2. Chemical and materials

Choline chloride (>98.0 %), malic acid (>98.0 %), oxalic acid (>98.0 %), lactic acid (>98.0 %), malonic acid (>99.0 %), 1,2-propanediol (>99.0 %), glycerol (>98.0 %), 1,4-butanediol (>99.0 %), the analytical grade absolute EtOH, hydrochloric acid, methyl alcohol and formic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). AB-8 macroporous resin was obtained from Tianjin Guangfu Science and Technology Development Co., Ltd. (Tianjin, China). The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay kit, ABTS (2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonate)) assay kit and OH assay kit were purchased from Suzhou Keming Biology Technology Co., Ltd. (Suzhou, China).

2.3. Preparation of NADES

Based on and literature review and preliminary experiments, we select 7 kinds of NADES commonly used in extracting anthocyanins from *Vitis davidii* Foex. pomace as shown in Table 1, among which hydrogen bond donors include organic acids and alcohols. Seven different NADESs were prepared by the heat and stirring method as depicted in the literature with slight modifications [26]. Choline chloride was weighed for HBA and HBD, mixed at a proper ratio, and then 35 % (mass ratio, %) distilled water was added. Then, the mixture was stirred continuously at 353.15 K for 2–4 h until a clear, transparent and uniform liquid was created. The composition and mole ratios of the NADES are shown in Table 1.

2.4. Extraction and optimization

Traditional heating and stirring-assisted extraction was used for the most efficient NADES screening within the tested range. Water and 70 % (v/v) ethanol acidified with 0.1 % HCl were used as controls. Simply put, the powder sample was mixed with a solvent with a liquid–solid ratio of 40 mL/g in a beaker, heated in a constant-temperature water bath of 313.15 K, and magnetically stirred at the same speed for 40 min. The extract was centrifuged at 5000 g (Avanti J-26 XP, Beckman Coulter, Inc., USA) for 10 min at 277.15 K, and the supernatant was kept at 253.15 K for further study, which was performed within 2 weeks. Subsequently, the water content and molar ratio of ChGly were optimized.

Ultrasound-assisted extraction of *Vitis davidii* Foex. pomace was performed to improve the anthocyanin extraction effect using the NADES. The influence of each factor on anthocyanin extraction and the best level range of each factor were determined by a single-factor experiment. The optimal conditions for each factor were determined by the highest total anthocyanin content, expressed as mg cyanidin-3-*O*-glucoside equivalents/g of dried *Vitis davidii* Foex. Pomace weight and were used in all subsequent optimization experiments. Each factor was tested at 5–6 levels, and a total of 23 experiments were conducted by varying one factor level each time while remaining other factor terms at a steady level. Anthocyanins were extracted at different liquid–solid ratios (10–60, mL/g), ultrasonic times (20–60 min), temperatures (303.15–353.15 K), and ultrasonic powers (280–630 W) (KQ-700DE, Kunshan Ultrasonic Instruments Co., Ltd., Suzhou, China). The single-factor experiment was repeated three times for each group, and then the average value was calculated. Based on the single-factor test, a three-level, three-factor Box–Behnken design (BBD) was used to investigate the relationship between extraction conditions (liquid–solid ratio (A), temperature (B), and ultrasonic power (C)) and total anthocyanin content (Y) and to determine the interactive effects of each factor for optimization. Table 2 shows each independent variable and level.

Table 1
Composition, abbreviations and mole ratios of NADESs.

Serial number	HBA	HBD	Abbreviation	Molar ratio
NADES 1	Choline chloride	Malic acid	ChMa	1.5 : 1
NADES 2		Oxalic acid	ChOx	1 : 1
NADES 3		lactic acid	ChLa	1 : 1
NADES 4		malonic acid	ChMal	1 : 2
NADES 5		1,2-Propanediol	ChPg	1 : 2
NADES 6		Glycerol	ChGly	1 : 2
NADES 7		1,4- butanediol	ChBu	1 : 2

The response factor is the total anthocyanin content (TAC), and the following formula is a second-order polynomial equation. :

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k-1} \beta_{ij} X_i X_j \quad (1)$$

where Y is the response term for Cy3-gal yield, β_0 is the interception coefficient, and β_i, β_{ii} and β_{ij} are the linear, quadratic and interactive terms, respectively. Independent variables were coded as X_i and X_j. ANOVA ($p < 0.05$) was used to determine the significant linear, quadratic, and interactive effects of factors on the response. Design Expert version 11 software (Stat-Ease Inc., USA) was used to plot three-dimensional response surface graphs. All experiments in the model were done in triplicate.

2.5. Determination of TAC by the pH-differential method

The TAC was determined by the pH-difference method with slight modification [14,15,27].

The sample is the supernatant of all the extracts after centrifugation at 5000 r/min and 277.15 k (DL-2018HR, Anhui Zhongke Duling Commercial Electrical Appliance Co., Ltd., Anhui, China).

Preparation of buffer solution with pH 1.0: accurately weigh 3.725 g KCl, dissolve it in distilled water and fix the volume to 250 mL; accurately measure 4.25 mL of concentrated hydrochloric acid to a constant volume of 250 mL with distilled water, and mix KCl solution with HCl solution at a ratio of 25:67. Adjust the pH to 1.0 ± 0.1 with KCl solution.

Preparation of pH 4.5 buffer solution: accurately weigh 6.8 g $CH_3COONa{\cdot}3H_2O$, dissolve it in distilled water, and fix the volume to 250 mL; measure 2.9 mL of acetic acid to 250 mL, and mix them according to the ratio of 1: 1 (v: v, 100 mL:100 mL). Adjust the pH to 4.5 ± 0.1 with HCl solution.

The sample (2.5 mL) was adjusted to 25 mL with buffers of pH 1.0 and pH 4.5 and incubated at 313.15 K in the dark for approximately 70 min. In accordance with Eq. (1), the TAC was calculated as cyanidin-3-*O*-glucoside from the absorbance values at λ_{max} and 700 nm (UV-1800, Shimadzu Co., Ltd., Japan).

$$\mathrm{TAC(mg/g)} = \frac{A \times MW \times DF \times V}{\varepsilon \times M \times L} \quad (2)$$

where $A = (A_{\lambda max} - A_{700nm})_{pH1.0} - (A_{\lambda max} - A_{700nm})_{pH4.5}$, MW and ε are the molecular weight (449.2 g/mol) and molar extinction coefficient (26900 L mol^{-1} cm^{-1}) of the standard (cyanidin-3-*O*-glucoside), respectively; DF is the dilution factor, V is the solution volume (mL); M is the quantity of dried sample (g); and L is the optical path diameter of the cuvette (1 cm). The results were expressed in mg/g.

2.6. Purification procedure by AB-8 macroporous resins

Anthocyanin crude extract was purified by AB-8 macroporous resin as described by Sixu Lin [28]. Briefly, an AB-8 macroporous resin-loaded chromatographic column with a 1/2 loading capacity was used to purify the supernatant. After loading, the sample was allowed to stand for 12 h so that it could adsorb onto the column, and then ultrapure water was used to wash away glycerol, protein, sugar and polar compounds. The anthocyanin was eluted with 70 % acidic methyl alcohol (methyl alcohol 70 mL/100 mL, formic acid 0.1 mL/100 mL) to obtain an anthocyanin-rich solution, and then rotary evaporation was carried out at 313.15 K to remove ethanol. The obtained concentrated solution was made into powder by a vacuum freeze-drying machine and reposited at 193.15 K.

2.7. Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) analysis

The powder (2 mg) prepared in Section 2.6 was dissolved in 1.0 mL of 70 % aqueous methanol (0.1 % hydrochloric acid) and then filtered with a 0.22 μm syringe filter (Millipore Co., Shanghai, China). Then, anthocyanin extracts were analyzed using a UPLC–ESI–MS/MS system (UPLC, ExionLC™ AD, https://sciex.com.cn/; MS, Applied Biosystems 6500 Triple Quadrupole, https://sciex.com.cn/). The analytical conditions were as follows: UPLC: column, Waters ACQUITY BEH C18 (1.7 μm, 2.1 mm × 100 mm); a gradient elution (mobile phase A: water, 0.1 % formic acid; mobile phase B: methanol, 0.1 % formic acid) process was performed as follows: 5 % B at 0 min, 50 % B at 6 min, 95 % B at 12 min, hold for 2 min, 5 % B at 14 min, hold for 2 min; flow rate, 0.35 mL/min; temperature, 40 °C; injection volume, 2 μL. The MS analysis was performed on the QTRAP® 6500 LC–MS/MS System equipped with an ESI Turbo Ion-Spray interface operating in positive ion mode and controlled by Analyst 1.6.3 software (Sciex). The ESI source operation

Table 2
Factors and levels of response surface experiment.

Levels	Factors		
	A. Liquid-solid ratio (mL/g)	B. Extraction temperature (K)	C. Ultrasonic power (W)
−1	20	313.15	350
0	30	323.15	420
1	40	333.15	490

parameters were as follows: ion source, turbo spray, source temperature 823.15 K, ion spray voltage (IS) 5500 V (positive ion mode), and curtain gas (CUR) 35 psi. The decluttering potential (DP) and collision energy (CE) for individual MRM transitions were determined with further DP and CE optimization. A specific set of multiple reaction monitoring (MRM) transitions was monitored for each period according to the anthocyanins eluted within this period. A local metabolic database (http://www.metware.cn/) was used for MRM analysis. The quantities of anthocyanin compounds were determined by employing an external calibration curve consisting of 17 standards of anthocyanin compounds.

2.8. Stability evaluation of the anthocyanin extracts

The purpose of our research was to assess the stability of anthocyanins extracted through the utilization of UAE-NADES (ChGly) or conventional acidic ethanol while subjecting them to different temperature, pH, and illumination conditions to make a comparative analysis. The results are expressed as the anthocyanin retention rate. For the stability determination under different temperature conditions, including 293.15 K, 323.15 K and 353.15 K, the remaining TAC was measured every hour. To assess stability under diverse pH conditions, 0.1 % hydrochloric acid (v/v) and 0.1 % sodium hydroxide solution (v/v) were utilized to adjust the pH within the range of 1.0–6.0. The anthocyanin extracts were kept at a temperature of 313.15 K in a light-proof environment, and the remaining TAC was measured every hour. For the stability measurement under different illumination environments, darkness, indoor natural light and light with an illumination intensity of 3500 lx were selected. The temperature was room temperature, and the experiment was conducted in spring in Changsha (China). The remaining TAC was measured every hour.

2.9. Antioxidant activity assay

The in vitro antioxidant capacity of anthocyanins extracted by UAE-ChGly or traditional acidic ethanol was determined by three approaches with several test kits (Suzhou Keming Biotechnology Co., Ltd., www.cominbio.com), including 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and OH kits. The freeze-dried anthocyanin powder was dissolved in absolute ethanol to prepare the test solution with the same concentration gradient. The working solutions of DPPH, ABTS and OH were prepared according to the instructions.

DPPH reagent (100 μL) was mixed with 100 μL of sample and incubated in the dark for 20 min at room temperature, and the absorbance was determined at 515 nm. Similarly, the DPPH working solution (100 μL) and 100 μL of absolute ethanol were mixed thoroughly as blank controls, and the absorbance was measured. The DPPH free radical scavenging rates (RSAs) of the samples were calculated as follows:

$$\text{DPPH RSA}\ (\%) = \left(A_{\text{control}} - A_{\text{anthocyanin}}\right) / A_{\text{control}} \times 100\% \tag{3}$$

The ABTS reagent (100 μL) and 100 μL of the sample were mixed thoroughly, and the absorbance at 734 nm was measured within 10 min. Similarly, the ABTS reagent (100 μL) and 100 μL of absolute ethanol were mixed thoroughly as blank controls, and the absorbance was measured. The ABTS RSA of the samples was calculated as follows:

$$\text{ABTS RSA}\ (\%) = \left(A_{\text{control}} - A_{\text{anthocyanin}}\right) / A_{\text{control}} \times 100\% \tag{4}$$

Following the manufacturer's instructions, 100 μL of reagent I, 100 μL of reagent II, 500 μL of distilled water and 200 μL of reagent III were added to the control tube; 100 μL of reagent I, 600 μL of distilled water and 200 μL of reagent III were added to the blank tube; and 100 μL of reagent I, 100 μL of reagent II, 200 μL of reagent III and 500 μL of sample were added to the measuring tube. The samples were mixed thoroughly and incubated at 310.15 K in the dark for 20 min. Then, 200 μL was absorbed, and the absorbance was measured at 510 nm in a 96-well plate. The absorbances of the control tube, blank tube and measuring tube were recorded as A_1, A_2 and A_3, respectively. The OH RSA of the samples was calculated according to the following formula:

$$\text{OH RSA}\ (\%) = (A_1 - A_3) / (A_1 - A_2) \times 100\% \tag{5}$$

2.10. Differential scanning calorimetry analysis (DSC)

The thermal behavior of anthocyanins extracted using UAE-NADES (ChGly) or conventional acidic ethanol was evaluated using differential scanning calorimetry (DSC25, TA Instruments Co., Ltd., USA). By using these two methods, freeze-dried anthocyanin powder was loaded into aluminum crucibles with covers and heated from 288.15 K to 523.15 K with N_2 flow (50 mL/min) at the rate of increasing by 10 K per minute.

2.11. Statistical analysis

Assays were performed in triplicate, and data are presented as the mean ± standard deviation. Analysis of variance (ANOVA) was performed using IBM SPSS 26, and Origin Pro 2021 software was used to arrange the data. Statistical analysis was performed using response surface methodology (RSM) by the software Design-Expert 13. When $P < 0.05$, we considered it statistically significant.

3. Results and discussion

3.1. Screening of NADESs for Vitis davidii Foex. pomace anthocyanin extraction

3.1.1. Effect of different types of NADES

The composition of NADESs has a significant effect on their inherent physicochemical properties (including density, viscosity, polarity, pH and solubility) and inevitably affects extraction capacity. We used ChCl as the HBA and acids (malic, oxalic, lactic, malonic acid) and alcohol substances (1,2-propanediol, glycerol, and 1,4-butanediol) as the HBD in this study. As shown in Fig. 1(A), most of the NADESs showed better extraction effects than water. If 70 % acidified ethanol was taken as a reference, four different NADESs, namely, ChGly, ChBu, ChOx and ChPg, possessed significantly higher efficiencies than acidified ethanol. Among the seven NADESs, the TAC extracted using ChGly was the highest, followed by ChBu, ChOx, ChPg, ChLa, ChMa and ChMal, which was approximately 40 % and 13 % higher than that of water and acidified ethanol, respectively. A similar result was reported for *Aronia melanocarpa* anthocyanin extraction using NADES [28]. It is possible to explain these results by the correlation between the viscosity of the NADESs and the TAC extracted from the NADESs [29]. The viscosity of NADESs mainly depends on the magnitude of hydrogen bonds and intermolecular interactions. The high viscosity of the NADES was mainly due to the formation of a hydrogen bond network between the HBA and HBD [30]. The more hydrogen bonds there are, the greater the viscosity of the NADES [28]. The number of functional groups, such as hydroxyl, carboxyl, carbonyl and amino groups, is closely correlated with the magnitude of hydrogen bonds and intermolecular interactions, such as van der Waals and electrostatic forces. The main drawbacks of these high viscosity solvents are causing mass transfer problems and limiting the extraction process [31]. In our study, the number of hydroxyl groups in glycerol was

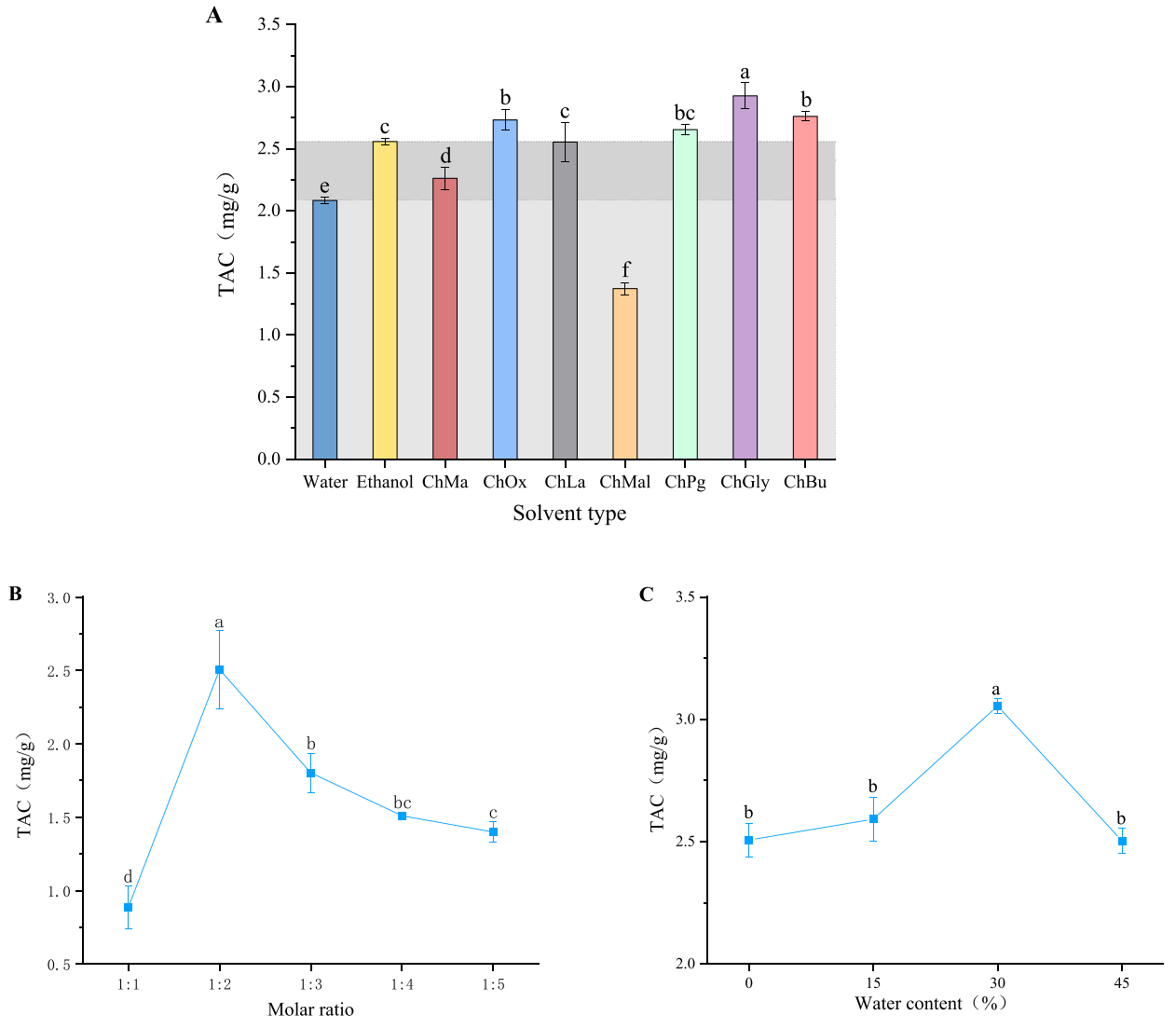

Fig. 1. Effect of (A) different types of NADES, (B) molar ratio of ChGly and (C) water content in ChGly on TAC.

the lowest among all HBDs used. Furthermore, NADESs prepared by ChCl and glycerol had the least viscosity and the highest TAC; moreover, the extraction efficiency of total anthocyanin by ChGly was obviously higher than that of acidic ethanol or distilled water. Thus, ChGly was chosen for *Vitis davidii* Foex. pomace anthocyanin extraction in the subsequent tests.

3.1.2. Effect of molar ratio and water content of NADESs

It is well known that the molar ratio of HBA and HBD has a remarkable impact on their inherent properties, which directly leads to different extraction rates [25]. In our study, anthocyanins were extracted from *Vitis davidii* Foex. pomace by NADES with different molar ratios of ChCl/Gly. The results shown in Fig. 1(B) indicate that with increasing molar ratio, the yield of anthocyanins in *Vitis davidii* Foex. increased first and then decreased. It was also found that under the extraction conditions of a liquid–solid ratio of 40 mL/g, a temperature of 313.15 K, and heating and stirring for 40 min, the ChGly formed by ChCl and Gly in a molar ratio of 1:2 had the maximum TAC, which was 2.507 ± 0.268 mg/g. The main reason is that the ChGly system with this molar ratio has better stability, which promotes the improvement of extraction yield [32]. Different molar ratios could affect NADES stability. When the molar ratio is between 1:1 and 1:2, the ChGly mixture is stable. It may be that the hydroxyl group (-OH) in Gly needs to combine with the chloride ion (Cl^-) or the hydroxyl group (-OH) in ChCl to form a stable hydrogen bond structure. When the molar ratio was 1:4 and 1:5, the extraction rate was obviously reduced. First, due to the presence of too many hydroxyl groups, Gly cannot bind fully to ChCl groups, resulting in reduced stability. Second, the increase in the glycerol ratio leads to an increase in solvent viscosity and a decrease in anthocyanin diffusion. Therefore, to obtain satisfactory solvent stability and the highest efficiency, a molar ratio of 1:2 should be selected. Ali et al. [33] also suggested that different composition ratios of DES may lead to changes in the properties of DES (there are many factors to consider, such as diffusion and mass transfer, pH value, and polarity) and the interaction of intramolecular or intermolecular hydrogen bonds between DES and compounds, thus affecting the extraction effect of compounds.

The water content (mass ratio, %) of the ChCl/Gly system could affect the viscosity of the solvent and the mass transfer rate of

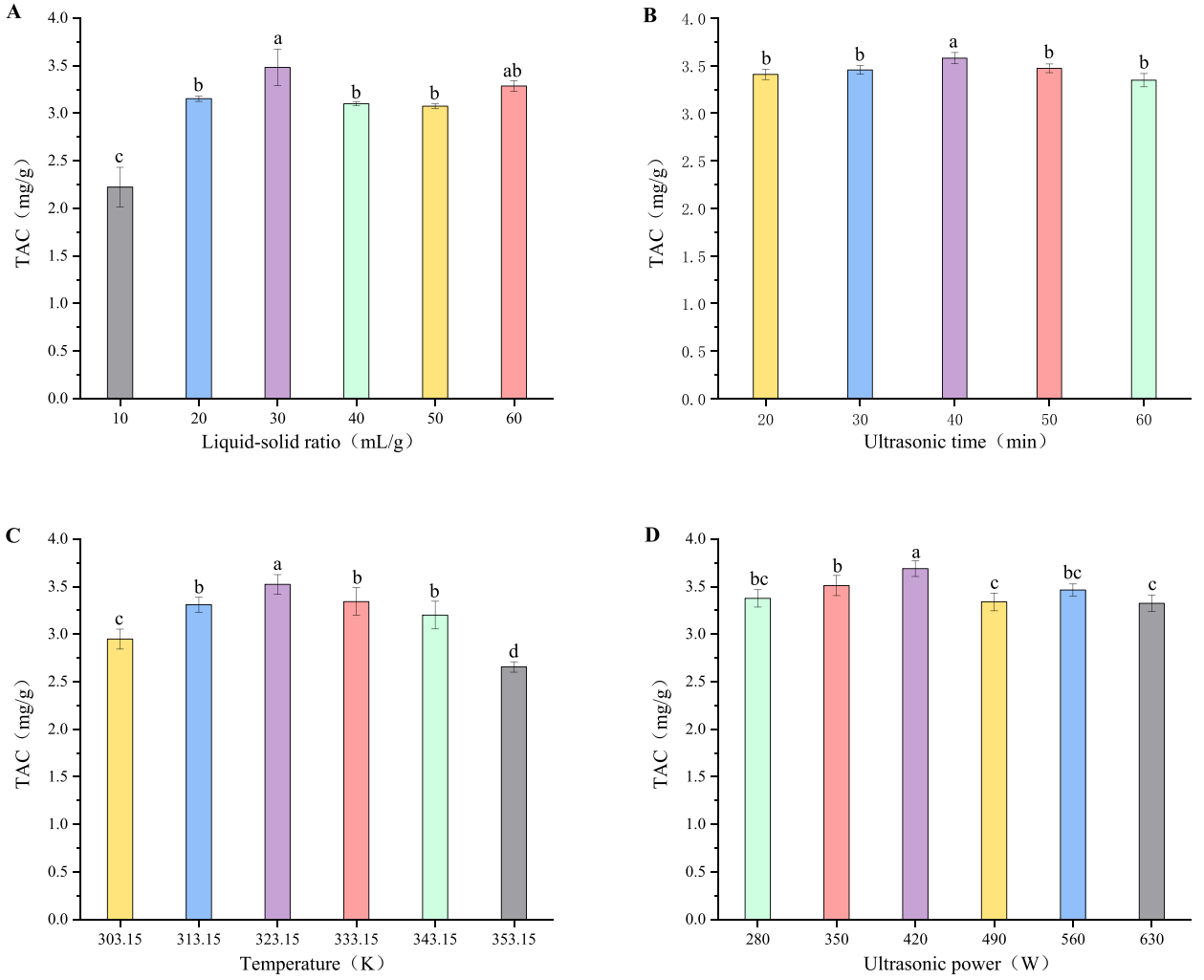

Fig. 2. Effect of (A) liquid-solid ratio, (B) extraction time, (C) temperature and (D) ultrasonic power on TAC during extraction.

anthocyanins; thus, the TAC extracted was basically dependent on the water content [34]. In our study, anthocyanin extraction was carried out at different water contents (0–45 %). According to Fig. 1(C), with increasing water content to 30 %, the extraction amount of anthocyanins increased gradually. This is because the existing water reduces the viscosity, thus increasing the diffusion speed of anthocyanins. When the water content increased to 30 %, the maximum TAC was 3.055 ± 0.030 mg/g. Adding a proper amount of water to NADESs can not only reduce the viscosity of the solvent but also promote the formation of hydrogen bonds by the reaction between choline chloride and water because water can also be used as an HBD in NADESs [35], which further increases the ability of NADESs to extract anthocyanins. Nevertheless, if the water content exceeds 30 %, the extraction amount of anthocyanins gradually decreases with increasing water content. This is attributed to the fact that adding surplus water will destroy the hydrogen bond formed between the HBA and the HBD [36], and the interaction between the solvent and the target compounds will be reduced [37], resulting in a decline in extraction capacity. Hence, ChGly (the molar ratio of ChCl to Gly was 1:2, and the water content was 30 %) was selected as the extraction solvent of anthocyanins from *Vitis davidii* Foex. pomace.

3.2. Single factors effect on TAC

To evaluate the impact of the liquid–solid ratio on the amount of anthocyanin extracted from *Vitis davidii* Foex. by ChGly, the liquid–solid ratio was varied from 10:1 to 60:1 (mL/g) (other parameters: 20 min, 323.15 K, 350 W). As shown in Fig. 2(A), with increasing solvent dosage, the TAC from *Vitis davidii* Foex. gradually increased and reached the highest value of 3.482 ± 0.191 mg/g when the liquid–solid ratio was 30 mL/g, which was 1.67 times and 1.34 times that of water extraction (2.084 ± 0.024 mg/g) and acidified ethanol (2.592 ± 0.064 mg/g) extraction respectively. Then, the TAC decreased significantly and stabilized. This explains that with the increase in solvent dosage, the active substance would completely contact the solvent, and the as the liquid–solid ratio increased, more anthocyanidin was dissolved, and more anthocyanins could be extracted in a certain time. However, when the liquid–solid ratio is higher than 30 mL/g, the effective influence on a larger extraction amount is finite, which is similar to the result reported for anthocyanin extraction from blueberry pomace with NADESs [38]. Anthocyanins can also be considered hydrogen bond donors, which in turn compete with the hydrogen bond donors (HBDs) used to prepare NADES. If the HBD used for preparing NADES has sufficient hydrogen bonding donor groups and branches, it will block interactions and may lead to steric hindrance between chloride anions and anthocyanins.

To study the effect of extraction time on the anthocyanin yield of *Vitis davidii* Foex., the ultrasonic extraction time was set at 20–60 min (other parameters: 30:1 mL/g, 323.15 K, 350 W). As shown in Fig. 2(B)–as the ultrasonic time increased from 20 min to 40 min, the extraction amount of anthocyanins showed a slight increasing trend up to 3.579 ± 0.060 mg/g, and then no significant change occurred with the extension of ultrasonic time. At 40min, the TAC extracted by ChGly was 1.71 times and 1.38 times that of water extraction and acidified ethanol extraction, respectively.The sample to extraction time had no significant effect on a number of the experimental levels. For example, differences between 20, 30, 50, and 60 min were nonsignificant. At all testing times, the extraction amount at 40 min was significantly greater than that at other times. Similar results were also reported during anthocyanin extraction from *Lycium ruthenicum* Murr. by ultrasonic extraction with ethanol [39].

To explore the impact of temperature on the amount of anthocyanin extracted from *Vitis davidii* Foex, a range from 303.15 to 353.15 K (other parameters: 30:1 mL/g, 40 min, 350 W) was studied. As shown in Fig. 2(C), with the temperature increasing from 303.15 K to 323.15 K, the anthocyanin yield of *Vitis davidii* Foex. increased steadily, reaching a maximum of 3.523 ± 0.103 mg/g, after which a sharp downward trend was observed with higher temperature. When the extraction temperature was 323.15 K, the TAC extracted by ChGly was 1.69 times and 1.36 times that extracted by water and acidified ethanol, respectively.The temperature during ultrasonication was conducive to generating stronger cavitation effects that loosened the sample cell structure, thereby facilitating solvent permeation [40]. In this experiment, the rise in temperature up to a maximum of 323.15 K will accelerate the mass transfer in

Table 3
Optimal design of anthocyanin extraction and response value results.

Run	A. Liquid-solid ratio (mL/g)	B. Extraction temperature (K)	C. Ultrasonic power (W)	TAC (mg/g)
1	30	323.15	420	3.664
2	30	323.15	420	3.665
3	40	333.15	420	3.498
4	20	323.15	490	3.407
5	30	333.15	350	3.472
6	40	323.15	350	3.454
7	30	323.15	420	3.670
8	30	323.15	420	3.714
9	20	313.15	420	3.363
10	30	313.15	490	3.476
11	20	323.15	350	3.370
12	30	333.15	490	3.419
13	40	323.15	490	3.576
14	40	313.15	420	3.581
15	30	313.15	350	3.253
16	30	323.15	420	3.666
17	20	333.15	420	3.560

the environment and reduce the viscosity and surface tension of the solvent, which will help to enhance the cavitation effect and increase the anthocyanin yield of *Vitis davidii* Foex [41]. However, anthocyanins are heat-sensitive substances, and high temperatures over 323.15 K might lead to their thermal degradation.

To study the effect of ultrasonic power on the anthocyanin yield of *Vitis davidii* Foex., the ultrasonic power was set at 280–630 W (other parameters: 30:1 mL/g, 40 min, 323.15 K). As shown in Fig. 2(D), when the ultrasonic power was increased from 280 W to 420 W, the anthocyanin yield gradually increased, with the highest value of 3.689 ± 0.084 mg/g, and then the yield decreased significantly with increasing ultrasonic power. Ultrasonic power can adjust the cavitation effect, thus affecting the extraction components. Under the experimental conditions, a moderate ultrasonic power of 420 W can cause the optimal cavitation effect and obtain the maximum anthocyanin extraction. Excessive ultrasonic power can cause instantaneous ultrahigh temperature and ultrahigh pressure, which will cause the regression of bioactive substances, thus reducing the anthocyanin yield [38].

When determining the influence of various factors at different levels on the extraction yield, the following order was obtained: extraction temperature, liquid–solid ratio, ultrasound power, and extraction time. Compared with other factors, it was found that the ultrasound time had the lowest impact, so a constant time of 40 min was maintained in the response surface test.

3.3. Optimization of the extraction process of anthocyanins using BBD

3.3.1. Response surface test results and variance analysis

The results of the single-factor experiment showed that three factors had important effects on the extraction of anthocyanins from grapes. Namely liquid–solid ratio, extraction temperature and ultrasonic power. Three-factor and three-level experiments were carried out by BBD. See Table 3 for the independent variable values and response variable values of each experimental item. BBD is used to clarify the variance in the experimental results. The ANOVA results are interpreted in Table 4. This model reached a remarkably significant level ($P < 0.0001$), and the lack of fit was not significant ($p = 0.4515 > 0.05$), which indicated that the established model was reliable. The R^2 is 0.9882, and the Adj R^2 is 0.9729, showing that the model can explain the change of 97.29 % of the response variable values and independent variable values have a significant linear relationship with the response variable values, and the model has a high goodness of fit. The coefficient of variation (CV) value of 0.6194 % indicates low deviations between the experimental and predicted values, confirming the reliability and precision of the experiment. The Adeq Precision value is 24.7876, which indicates that the signal of the model is sufficient for simulation and prediction of the extraction process of anthocyanins from *Vitis davidii* Foex. pomace [42].

According to the data of the variance analysis in Table 4, the significance level and importance of each influencing factor can also be obtained. The liquid–solid ratio (A), the extraction temperature (B) and the ultrasonic power (C), which affect the extraction of anthocyanins, reached a highly significant level ($p < 0.01$). The order in which each factor influences the value of the response variable was as follows: the liquid–solid ratio (A) > the ultrasonic power (C) > the extraction temperature (B). Among the partial cross items of the model, only the interaction of the liquid–solid ratio and ultrasonic power is not significant for the extraction of anthocyanins, and the other partial cross items are highly significant. The quadratic term of the model reached a highly significant level. The regression equation of the ternary second-order polynomial obtained by the model calculation is:

$$TAC(\mathrm{mg/g}) = 3.6758 + 0.05117625A + 0.03458B + 0.04109875C - 0.07AB + 0.0213525AC - 0.069025BC - 0.06433375A^2 - 0.11096625B^2 - 0.15981875C^2 \quad (6)$$

Table 4
Analysis of variance of each test factor in RSM.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	significance
Model	0.2789	9	0.0310	64.88	<0.0001	***
A. Liquid-solid ratio	0.0210	1	0.0210	43.87	0.0003	***
B. Extraction temperature	0.0096	1	0.0096	20.03	0.0029	**
C. Ultrasonic power	0.0135	1	0.0135	28.29	0.0011	**
AB	0.0196	1	0.0196	41.04	0.0004	***
AC	0.0018	1	0.0018	3.82	0.0916	
BC	0.0191	1	0.0191	39.90	0.0004	***
A^2	0.0174	1	0.0174	36.49	0.0005	***
B^2	0.0518	1	0.0518	108.55	<0.0001	***
C^2	0.1075	1	0.1075	225.16	<0.0001	***
Residual	0.0033	7	0.0005			
Lack of Fit	0.0015	3	0.0005	1.08	0.4515	
Pure Error	0.0018	4	0.0005			
Cor Total	0.2823	16				
R^2	0.9882					
Adjusted R^2	0.9731					
Predicted R^2	0.9059					
Adeq Precision	24.8561					
CV(%)	0.6194					

Level of significance: **p < 0.01, ***p < 0.001.

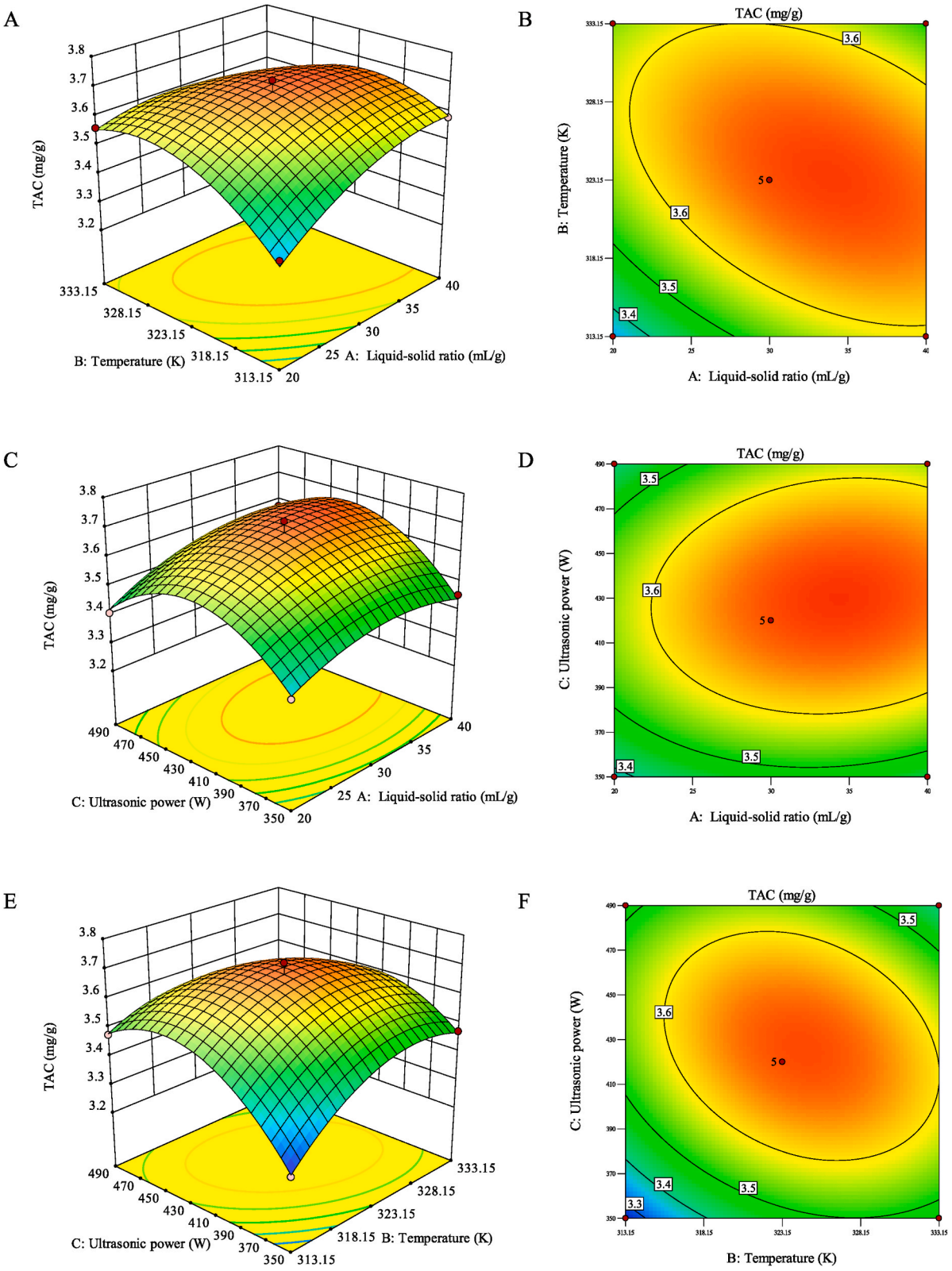

Fig. 3. Three-dimensional and two-dimensional response surface diagrams of anthocyanins extracted by UAE-ChGly affected by (A) liquid-solid ratio and (B) temperature, (C) liquid-solid ratio and (D) ultrasonic power, (E) temperature and (F) ultrasonic power.

3.3.2. Optimization of interactions between factors and model verification

The 3D graphs and contour plots of the response surface can directly show the influence of the interaction between the two independent variables on TAC. In the 3D graphs, the steeper the surface, the more obvious the interaction between the two independent variables, while the gentler the surface suggested the opposite. In contour plots, the shape of the contour represents the intensity of interaction, the elliptical shape indicates that the interaction between two independent variables is significant, while the circle suggests the exact opposite result [42].

Fig. 3(A-B) shows the response surface 3D graphs and contour plots of the mutual effect between the liquid–solid ratio and temperature on the TAC. As shown in Fig. 3(A), the response surface is steep, reflecting that the liquid–solid ratio and extraction temperature have a significant interaction on the TAC. It can be observed from Fig. 3(B) that the contour lines of the two independent variables are evenly distributed along the axis, and the contour lines are oval, indicating that the interaction between the two independent variables is strong, which significantly affects the extraction amount of anthocyanins. It can also be perceived from Fig. 3. A-B that with the enhancement of the liquid–solid ratio and extraction temperature, the TAC first increases and then decreases. When the liquid–solid ratio is 30 mL/g and the ultrasound temperature is 323.15 K, the TAC reaches the highest value.

Fig. 3(C-D) shows the response surface 3D graphs and contour plots of the mutual effect between the liquid–solid ratio and power on the TAC. It can be observed from Fig. 3(C) that the response surface is relatively flat, indicating that the interaction between the liquid–solid ratio and power on the TAC is not significant. As seen from the contour plots in Fig. 3(D), the contour along the ultrasonic power axis is denser than the liquid–solid ratio, indicating that the ultrasonic power has a more significant impact than the liquid–solid ratio. The contour line is round ($P > 0.05$), which indicates that the interaction of the two independent variables has no significant effect on the TAC. It can also be perceived from Fig. 3(C-D) that with the growth of the liquid–solid ratio and ultrasonic power, the TAC first increases and then decreases. When the liquid–solid ratio is 30 mL/g and the power is 420 W, the TAC reaches the highest value.

Fig. 3(E-F) shows the responses of surface 3D graphs and contour plots to the interaction between extraction temperature and ultrasonic power on the TAC. It can be perceived from Fig. 3(E) that the response surface is steep, showing that the interaction between temperature and power on the TAC is significant. The change in contour along the axis of power is denser than that of extraction temperature, which indicates that the effects of power are more significant than the effects of extraction temperature. It can also be seen from Fig. 3(E-F) that with the enhancement of extraction temperature and power, the TAC first increases and then decreases. When the temperature is 323.15 K and the power is 420 W, the extraction amount reaches the highest value.

In addition, it can be seen that a very high temperature and power lead to a decrease in the TAC, which involves a very high temperature, and ultrasonication will lead to the amassing of free radicals [43]. The more free radicals there are, the more anthocyanins will be oxidized and degraded. Therefore, an appropriate value should be selected for the parameters to take full advantage of the ultrasonic cavitation effect, the mechanical action and the thermal action to promote the delivery of intracellular anthocyanins to obtain the maximum extraction efficiency.

On the basis of the results of the BBD experiment and regression equation, the optimum extraction conditions for anthocyanins from *Vitis davidii* Foex. pomace by UAE-ChGly was determined as follows: liquid–solid ratio of 34.46 mL/g, extraction temperature of 322.79 K and ultrasonic power of 431.67 W. Under these factor values, TAC was 3.68997 mg/g. Considering the operability and cost of the experiment, the optimum technological conditions were revised as follows: liquid–solid ratio of 34 mL/g, extraction temperature of 323.15 K, and ultrasonic power of 430 W. Under these circumstances, the experimental verification value of anthocyanin extraction was 3.682 ± 0.051 mg/g, which was close to the predicted value, indicating that the optimized extraction parameters of anthocyanin from *Vitis davidii* Foex. pomace in this study was reliable and could fit the actual extraction.

Table 5
Mass spectrometry data and identification of anthocyanins extracted by UAE-ChGly or acidic ethanol.

Peak	RT (min)	Compounds	Q1 (Da)	Q3 (Da)	Molecular Weight	UAE-ChGly(mg/g)	acidic ethanol (mg/g)
1	11.34	Delphinidin-3-*O*-(6-*O*-*p*-coumaroyl)-glucoside	611.14	303.1	611.14	0.38 ± 0.09	0.14 ± 0.01
2	4.64	Delphinidin-3,5-*O*-diglucoside	627	303.1	627.16	0.13 ± 0.00	0.02 ± 0.00
3	11.26	Delphinidin-3-*O*-5-*O*-(6-*O*-coumaroyl)-diglucoside	773.19	303.1	773.19	0.18 ± 0.02	0.03 ± 0.00
4	12.85	Malvidin-3-*O*-(6-*O*-*p*-coumaroyl)-glucoside	639.17	331.1	639.17	21.67 ± 0.23	8.95 ± 0.06
5	12.40	Malvidin-3-*O*-5-*O*-(6-*O*-coumaroyl)-diglucoside	801.22	331.1	801.22	52.22 ± 3.51	22.09 ± 1.37
6	7.84	Malvidin-3-*O*-sambubioside-5-*O*-glucoside	787.4	331.3	787.23	2.15 ± 0.17	3.10 ± 0.36
7	9.59	Malvidin-3-*O*-sophoroside	655.2	331.3	655.19	0.46 ± 0.06	0.14 ± 0.01
8	10.22	Malvidin-3-*O*-rutinoside	639.06	331.1	639.19	0.52 ± 0.10	0.27 ± 0.09
9	9.98	Malvidin-3-*O*-glucoside	493.1	331.1	493.13	17.39 ± 0.94	8.52 ± 0.74
10	7.19	Malvidin-3,5-*O*-diglucoside	655.4	331.1	655.19	465.44 ± 2.37	228.61 ± 0.96
11	6.45	Pelargonidin-3,5-*O*-diglucoside	595.1	271.1	595.17	0.27 ± 0.08	0.09 ± 0.02
12	12.96	Peonidin-3-*O*-(6-*O*-*p*-coumaroyl)-glucoside	609.16	301.1	609.16	0.43 ± 0.08	0.21 ± 0.00
13	6.95	Peonidin-3,5-*O*-diglucoside	625.2	301.1	625.18	20.50 ± 1.02	8.69 ± 0.41
14	12.46	Peonidin-3-*O*-5-*O*-(6-*O*-coumaroyl)-diglucoside	771.21	301.1	771.21	1.41 ± 0.08	0.67 ± 0.11
15	9.23	Peonidin-3-*O*-glucoside	463.3	301.1	463.12	0.50 ± 0.01	0.29 ± 0.01
16	8.32	Petunidin-3-*O*-glucoside	479.1	317.1	479.12	0.13 ± 0.05	0.01 ± 0.00
17	12.74	Petunidin-3-*O*-(6-*O*-*p*-coumaroyl)-glucoside	625.18	317.1	625.16	0.97 ± 0.07	0.15 ± 0.00

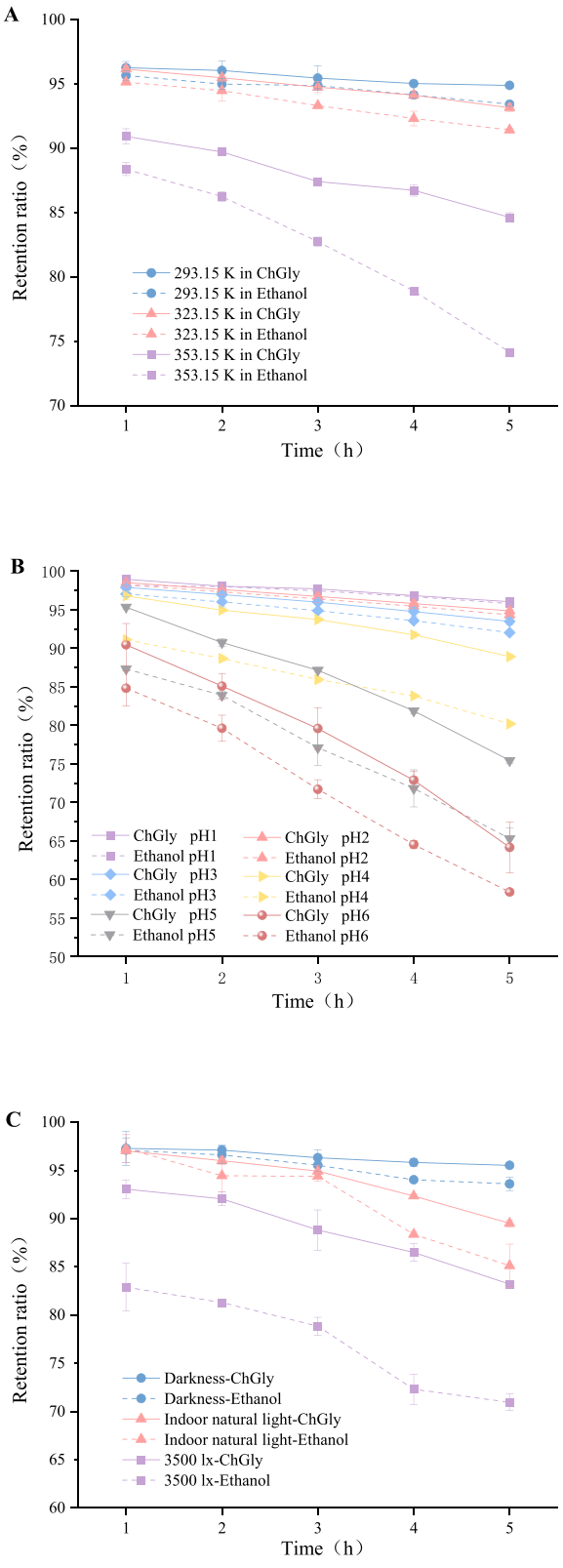

Fig. 4. Retention rates of anthocyanins in ChGly and acidified ethanol extracts at (A) different temperatures, (B) pH values and (C) light conditions.

3.4. Anthocyanin identification

Pure anthocyanins were recovered from acidic ethanol or ChGly by the purification method shown in 2.6.

UPLC−MS/MS was used to identify the anthocyanins extracted from *Vitis davidii* Foex. pomace under acidic ethanol or the optimum extraction conditions of UAE-ChGly. The composition and content differences of anthocyanins extracted by the two methods were further compared.

Based on the metabolite database of 108 anthocyanin components, including cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin and their glycosylated or methylated derivatives, in this study, the components of anthocyanins were qualitatively and quantitatively analyzed. As shown in Table 5, a total of 17 anthocyanin compounds were detected, including 7 malvidin derivatives, 3 delphinidin derivatives, 1 pelargonidin derivative, 4 peonidin derivatives, and 2 petunidin derivatives. Additionally, the contents of 17 anthocyanins were higher in UAE-ChGly than in acidic ethanol extracts. Among these 17 anthocyanins, malvidin-3,5-*O*-diglucoside, malvidin-3-*O*-5-*O*-(6-*O*-coumaroyl)-diglucoside, malvidin-3-*O*-(6-*O*-*p*-coumaroyl)-glucoside, and peonidin-3,5-*O*-diglucoside were 2.04-, 2.36-, 2.42-, and 2.36-fold higher in UAE-ChGly, respectively, than in acidic ethanol extracts. Compared with acidic ethanol, UAE-ChGly increased the contents of all anthocyanins except malvidin-3-*O*-sambubioside-5-*O*-glucoside, which indicates that ChGly has more particular appeal and selectivity for extracting active compounds from *Vitis davidii* Foex. pomace [38]. In addition, we found that malvidin-3,5-*O*-diglucoside displayed higher levels (465.44 mg/kg) in UAE-ChGly extracts. Derivatives of malvidin were dominant in the anthocyanin composition of *Vitis davidii* Foex. pomace. This is slightly different from red-fleshed grapes [44,45]. This result is similar to the composition of anthocyanins in *V. davidii* and wine made by *Vitis davidii* Foex [31,32,46]. This shows that the anthocyanin composition of different grape varieties will be different, and the anthocyanin composition and proportion of the same grape variety are similar. UAE-ChGly is more efficient than acidified ethanol in extracting anthocyanins from *Vitis davidii* Foex. pomace.

3.5. Characterization of anthocyanins extracted by UAE-ChGly and acidified ethanol method

3.5.1. Multistability analysis

Anthocyanins have poor stability during extraction and storage and are easily affected by temperature, pH and light, which leads to a decrease in their content and the loss of biological activity [47]. Hence, it is essential to strengthen the stability of anthocyanins for their extraction and storage. In this study, the stability of anthocyanins in the extract of the UAE-ChGly method and traditional acidification ethanol method were compared.

The thermal stability of anthocyanins extracted by UAE-ChGly or acidified ethanol in a certain temperature range was compared, as represented in Fig. 4(A). The anthocyanins were steady for each extractive solution when stored at 293.15 K and 323.15 K, with over 90 % remaining anthocyanins in the extractive solutions at the last hour of the experiment. Notably, it was found that the degradation rate of anthocyanins in ChGly was lower than that of acidified ethanol during the whole experiment. Furthermore, the anthocyanins in the two extracts were obviously degraded at 353.15 K, and the preservation rate of anthocyanins in ChGly was 10.49 % more than that in acidified ethanol at 5 h, which revealed that ChGly had a protective effect on anthocyanins in a high-temperature environment. Fu et al. [38] recently obtained similar results when extracting anthocyanins from blueberry pomace with NADES (ChOa) as an extractant.

As shown in Fig. 4(B), pH significantly affected the stability of anthocyanins in the two solvents. With increasing pH, the stability of anthocyanins in both extracts decreased. When the pH is 1–2, anthocyanins mainly exist in the form of stable red flavylium. With increasing pH, anthocyanins will exist in the form of colorless methanol pseudobases (pH 4–5) or be converted into blue quinone bases (pH 6), which will have a negative impact on the stability of anthocyanins [48]. Within the experimental range, under acidic conditions ($pH \leq 3$), the anthocyanins in the two extraction solvents were relatively stable, and the retention rate was higher than 90 % after 5 h. However, at higher pH, compared with acidified ethanol, the degradation rate of anthocyanins in ChGly was obviously slower. This corresponds with the previous experimental results. Oancea et al. [49] showed that red onion anthocyanins were more stable at low pH values (pH 1) and extremely unstable in alkaline environments (pH 9). Amit B. Das et al. [50] explored the degradation of anthocyanins in purple rice bran at pH 2–8. Similarly, they found that anthocyanins had the best stability and the lowest degradation rate at a low pH value (pH 2).

In the test of illumination stability, anthocyanin was steady in the dark in the two solvents, as shown in Fig. 4(C). In contrast, indoor natural light and light irradiation with 3500 lx illumination significantly reduced the anthocyanin retention rate. In the study of the light stability of corn anthocyanins by Bharat et al. [51], similar results were achieved. When exposed to light, anthocyanins will be photodegraded, which may be due to more nonacylated anthocyanins. Because strong light can interact with anthocyanin molecules and water molecules, its ground state changes to a singlet or triplet state, and then a series of free radical reactions occur, which leads to a decrease in the anthocyanin retention rate [38]. The final retention rates of anthocyanins in ChGly and acidified ethanol were 89.52 % ± 0.35 % and 85.13 % ± 2.23 %, respectively, under indoor natural light irradiation and 83.22 % ± 0.09 % and 70.96 ± 0.88 %, respectively, under 3500 lx light irradiation, which indicated that the photostability of anthocyanins in ChGly was higher than the photostability of acidified ethanol.

The multiple-stability of anthocyanins in ChGly is higher at higher temperatures, pH, and stronger illumination, which may be attributed to the fact that the hydrogen bond interaction between NADES(ChGly) HBD and HBA can maintain the spatial structure of bioactive compounds and inhibit their degradation [52]. Similar result was recently recorded by Obluchinskaya et al. [53] After 360 days of storage, about 70 % of phloretin was degraded in ethanol, while only about 20 % was degraded in NADES. Dai et al. [54] observed the hydrogen bond interaction between NADES (choline chloride/xylitol) and quercetin by using a high-resolution magic

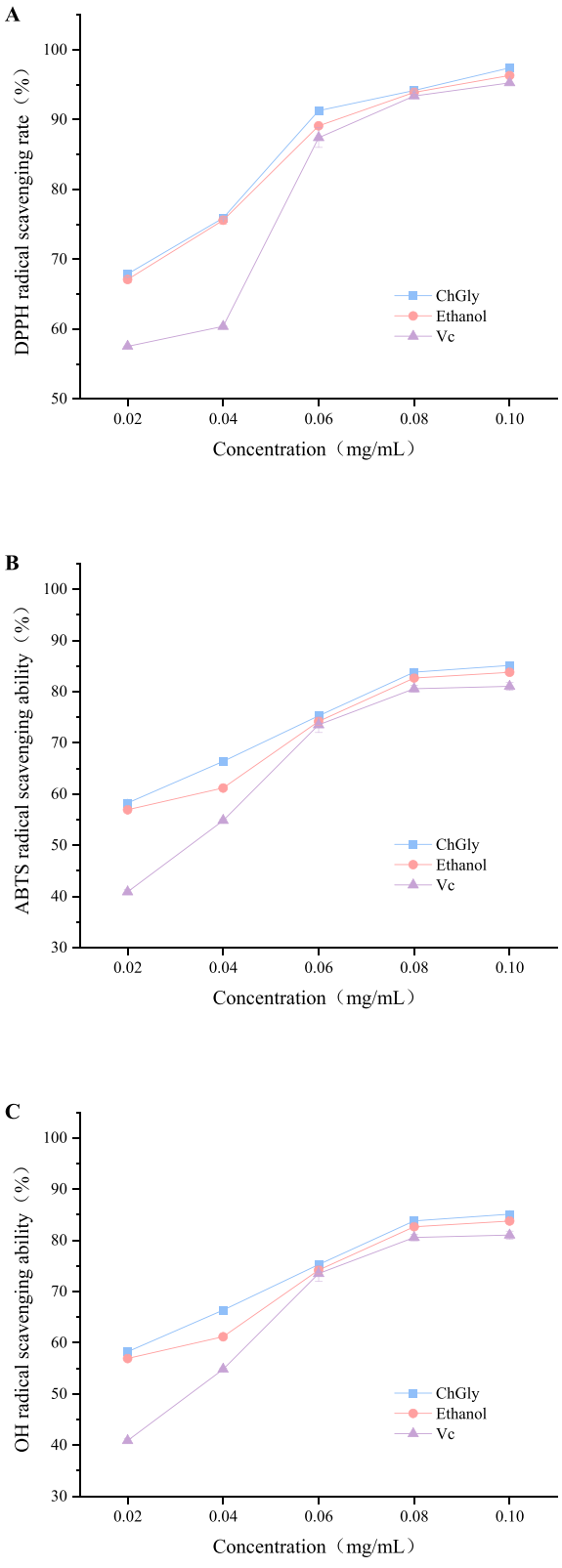

Fig. 5. Determination of (A) DPPH, (B) ABTS and (C) OH RSA.

angle spinning nuclear magnetic resonance spectrum. They thought that this interaction would significantly reduce the fluidity of the solute, thus reducing the contact between the solute and oxygen and inhibiting oxidative degradation. Through a comparative study on the multiple stabilities of anthocyanins in acidified ethanol and ChGly, it was found that the anthocyanins extracted by ChGly had higher temperature, pH, and light stability, which indicated that the protective effect of ChGly on anthocyanins extracted was higher than that of acidic ethanol, indicating that ChGly was a good substitute for traditional organic solvents.

3.5.2. Oxidation resistance

The oxidation resistance of anthocyanins extracted by UAE-ChGly and acidic ethanol were compared in vitro. We studied the scavenging abilities of DPPH· radicals, ABTS radicals, and OH· radicals. DPPH· is a stable free radical that is soluble in polar solvents such as ethanol and has a maximum absorption at a wavelength of 515 nm. Adding antioxidants to DPPH solution will cause a decoloration reaction, so the DPPH RSA can be calculated by comparing the changes in absorbance before and after adding the sample to quantify the oxidation resistance of the sample. As shown in Fig. 5(A), the DPPH RSA of anthocyanins extracted by the two methods and Vc increased with increasing concentration. In the experimental concentration range, the DPPH RSA of anthocyanin extracted by UAE-ChGly is always higher than that extracted by acidic ethanol, and both of them are more than that of the Vc matched group. This phenomenon may be due to the different structures and contents of anthocyanins extracted by different methods, which is similar to the results reported by extracting anthocyanins from blue honeysuckle fruit with NADESs [55].

The ABTS method can be used to evaluate hydrophilic and lipophilic oxidation resistance. ABTS is oxidized to produce the stable blue–green cationic radical $ABTS^{+}$, which can be dissolved in water or acidic ethanol and has a maximum absorption at 734 nm. As soon as the sample is added to the $ABTS^{+}$ solution, the antioxidant components in it react with $ABTS^{+}$ and fade the reaction system. To quantify the antioxidant capacity of the sample, the change in absorbance was measured at 734 nm, and the RSA of ABTS was calculated. As shown in Fig. 5(B), the ABTS RSA of anthocyanins extracted by UAE-ChGly or acidic ethanol and Vc increased with increasing concentration. In the experimental concentration range, the ABTS RSA of anthocyanin extracted by UAE-ChGly was always higher than that extracted by acidic ethanol, and both were higher than that of the Vc control group. Oscar Zannou et al. [56] also found that the phenolic compounds of borage (Echium amoenum) flowers extracted by NADES have higher oxidation resistance than those extracted by ethanol.

In the Fenton reaction, hydrogen peroxide combines with ferrous ions to produce hydroxyl radicals. Salicylic acid can efficiently capture the generated hydroxyl radicals and react with them to generate the colored Compound 2,3-dihydroxy benzoic acid, which has a maximum absorption peak at 510 nm. After adding substances with scavenging ability, the colored compounds will decrease, so the ability to scavenge hydroxyl radicals of samples can be judged according to the absorbance value. As shown in Fig. 5(C), within the experimental concentration range, the hydroxyl radical scavenging ability of UAE-ChGly extracted anthocyanins was always slightly higher than that of acid ethanol-extracted anthocyanins and Vc. Consistent with the results reported by Sixu Lin [28], the antioxidant capacity of anthocyanins extracted by NADES combined with the physical field is better than that extracted by traditional ethanol solvent. The two extraction methods lead to different extraction efficiencies of anthocyanins, and the antioxidant activity increases significantly with increasing anthocyanin content. This study shows that NADESs have the potential to replace traditional organic extraction solvents, which are safer, greener and more efficient.

3.5.3. Thermal behavior analysis

DSC is a crucial method for determining the thermal properties of extracted active substances, which can reflect the structural properties and composition of bioactive compounds. The thermal stability of *Vitis* davidii Foex. anthocyanins is influenced by the hydrogen bond size, free volume and degree of structural hydration in NADES [57]. The DSC thermograms of anthocyanin extracted by

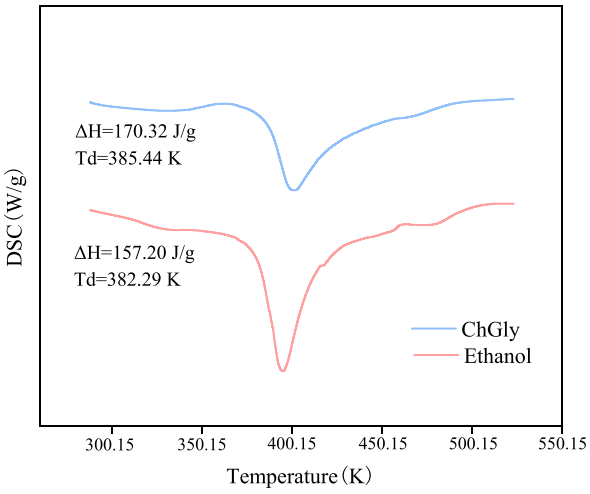

Fig. 6. DSC plots of anthocyanin extracted by UAE-ChGly or acidified ethanol.

UAE-ChGly or acidified ethanol are shown in Fig. 6. According to Fig. 6, both extracts obtained from UAE-ChGly and acidified ethanol showed two very similar curves, both of which had a strong peak, indicating a similar composition of anthocyanins. It is worth noting that the UAE-ChGly extraction method will also have an influence on the anthocyanins. Anthocyanins extracted by UAE-ChGly have higher enthalpy (Δ H) and thermal denaturation temperature (T_d) than anthocyanins extracted by acidified ethanol, indicating that anthocyanins extracted by UAE-ChGly need higher energy for denaturation and have higher thermal stability [58]. In brief, UAE-ChGly indeed improved the thermal stability of anthocyanins. The observed outcome can be attributed to the alteration in the composition and structure of anthocyanins extracted through UAE-ChGly in comparison to those extracted using acidic ethanol. This finding further corroborates the results obtained from flight mass spectrometry and antioxidant activity determination.

4. Conclusions

In this study, anthocyanins were extracted from *Vitis davidii* Foex. pomace using UAE-ChGly and optimization of extraction conditions by response surface design with three factors and three levels. The theoretical optimum conditions with the highest TAC yield were a liquid−solid ratio of 34 mL/g, an extraction temperature of 323.15 K and an ultrasonic power of 430 W (after revision). Under these conditions, the TAC reached 3.682 ± 0.051 mg/g. Compared with traditional acidified ethanol, UAE-ChGly has a higher extraction efficiency and is more environmentally friendly. Seventeen anthocyanins were identified from *Vitis davidii* Foex. pomace by UPLC−MS/MS analysis, and UAE-NADES significantly increased the content of the main anthocyanins. The experiments used to determine the stability differences of anthocyanins showed that compared with acidified ethanol, ChGly had a protective effect on the stability of anthocyanins under high temperature, different pH and light conditions. The antioxidant capacity of the two extraction methods was compared by in vitro antioxidant experiments. The results indicated that UAE-ChGly and acidified ethanol extraction resulted in different TACs. It was found that antioxidant activity increased significantly with increasing TAC. The UAE-NADES technology employs efficient, selective, and environmentally friendly NADES instead of traditional organic solvents, combined with the lower solvent consumption, energy, and processing time of UAE, which significantly improve the extraction rate of *Vitis davidii* Foex. anthocyanins. In addition, due to the non-toxic properties of NADES, the anthocyanin exhibit good potential for direct application in food, cosmetics, and pharmaceutical formulations. However, further research is needed in the future on the separation of NADES/target compounds and the recovery of NADES, which are crucial for the green industrial application of technology. In addition, there are challenges in the industrial application of ultrasonic extraction technology, such as ultrasonic blank area in large-diameter extraction tank, which affects the extraction effect. The combination of ultrasonic technology and other emerging technologies is a good research direction. With the deepening of research and improvement of equipment, ultrasonic extraction will be more widely used in food, medicine, chemical industry and other fields. Overall, UAE-ChGly provides a green and high-efficiency method for anthocyanin extraction from *Vitis davidii* Foex. pomace and has the potential for use in numerous industries, including food, dietary supplements, cosmetics, and pharmaceuticals.

Funding

This research was funded by Key Research and Development Program of Hunan Province of China (2023NK2033), and the Hunan agricultural science and technology innovation fund project (2022CX45, 2022CX133).

5. Institutional review board statement: not applicable

Informed Consent Statement: Not applicable.

Data availability statement

The data presented in this study are available in this article.

CRediT authorship contribution statement

Shushu Zhang: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Shuhua Lin:** Software, Formal analysis, Data curation. **Juhua Zhang:** Writing – review & editing, Methodology, Formal analysis. **Wei Liu:** Writing – review & editing, Project administration, Methodology, Funding acquisition.

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.

Acknowledgments

Thanks to the juice producer Hunan Veichu Juice &Wine Co., Ltd. (Huaihua, Hunan Province, China) for donating the squeezed *Vitis davidii* Foex. pomace.

References

[1] H.Q. Ma, A.X. Hou, J.J. Tang, A.A. Zhong, K. Li, Y. Xiao, Z.J. Li, Antioxidant activity of *Vitis davidii* Foex seed and its efssfects on gut microbiota during colonic fermentation after in vitro simulated digestion, Foods 1117 (2022) 17.

[2] S. Koutsoukos, T. Tsiaka, A. Tzani, P. Zoumpoulakis, A. Detsi, Choline chloride and tartaric acid, a natural deep eutectic solvent for the efficient extraction of phenolic and carotenoid compounds, J. Clean. Prod. 241 (2019) 11.

[3] S. Silva, E.M. Costa, C. Calhau, R.M. Morais, M.E. Pintado, Anthocyanin extraction from plant tissues: a review, Crit. Rev. Food Sci. Nutr. 5714 (2017) 3072–3083.

[4] L.H. Chen, B. Hu, Y.H. Qin, G.B. Hu, J.T. Zhao, Advance of the negative regulation of anthocyanin biosynthesis by MYB transcription factors, Plant Physiol. Biochem. 136 (2019) 178–187.

[5] P. Ongkowijoyo, D.A. Luna-Vital, E.G. de Mejia, Extraction techniques and analysis of anthocyanins from food sources by mass spectrometry: an update, Food Chem. 250 (2018) 113–126.

[6] B.J. Allison, C.W. Simmons, Obtaining multiple coproducts from red grape pomace via anthocyanin extraction and biogas production, J. Agric. Food Chem. 6630 (2018) 8045–8053.

[7] Y. Wang, H. Cheng, J.R. He, Q.X. Yao, L.L. Li, Z.H. Liang, X. Li, Enzymes-catalyzed knoevenagel condensation promoted by Ionic liquid and deep eutectic solvent, Catal. Lett. 1524 (2022) 1215–1223.

[8] L.B. Santos, R.S. Assis, J.A. Barreto, M.A. Bezerra, C.G. Novaes, V.A. Lemos, Deep eutectic solvents in liquid-phase microextraction: contribution to green chemistry, Trac-Trends Anal. Chem. 146 (2022) 12.

[9] N.P.E. Hikmawanti, D. Ramadon, I. Jantan, A. Mun'im, Natural deep eutectic solvents (NADES): phytochemical extraction performance enhancer for pharmaceutical and nutraceutical product development, Plants-Basel 1010 (2021) 18.

[10] B. Ozturk, C. Parkinson, M. Gonzalez-Miquel, Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents, Sep. Purif. Technol. 206 (2018) 1–13.

[11] E.D. Obluchinskaya, A.V. Daurtseva, O.N. Pozharitskaya, E.V. Flisyuk, A.N. Shikov, Natural deep eutectic solvents as alternatives for extracting phlorotannins from brown algae, Pharm. Chem. J. 53 (2019) 243–247.

[12] V. Huber, L. Muller, P. Degot, D. Touraud, W. Kunz, NADES-based surfactant-free microemulsions for solubilization and extraction of curcumin from *Curcuma Longa*, Food Chem. 355 (2021) 129624.

[13] N. Vannuchi, S. dos Passos Ramos, T.M. Mazzo, E. Longo, F.P. Bonsanto, A.R.C. Braga, V.V. de Rosso, Natural deep eutectic solvents (NADES)-Extracted Anthocyanins: bioaccessibility in electrospun PEO microfibers, Food Res. Int. 177 (2024) 113898.

[14] J. Torres-Vega, S. Gómez-Alonso, J. Pérez-Navarro, E. Pastene-Navarrete, Green extraction of alkaloids and polyphenols from *Peumus boldus* leaves with natural deep eutectic solvents and profiling by HPLC-PDA-IT-MS/MS and HPLC-QTOF-MS/MS, Plants 9 (2) (2020) 242.

[15] S. Saffarionpour, Deep eutectic solvents for sustainable extraction of polyphenols and saponins from plant sources: assessment of the impact of influencing factors, Sep. Sci. Technol. (2024) 1–42.

[16] J. Vladić, S. Kovačević, K. Aladić, S. Rebocho, S. Jokić, S. Podunavac-Kuzmanović, A.R.C. Duarte, I. Jerković, Novel insights into the recovery and stabilization of *Rosmarinus officinalis* volatile aroma compounds using green solvents, Food Bioprocess Technol. (2023) 1–16.

[17] Y. Zhao, P. Wang, W. Zheng, G. Yu, Z. Li, Y. She, M. Lee, Three-stage microwave extraction of cumin (*Cuminum cyminum* L.) Seed essential oil with natural deep eutectic solvents, Ind. Crops Prod. 140 (2019) 111660.

[18] P.S. Suresh, P.P. Singh, S. Kapoor, Y.S. Padwad, U. Sharma, Lactic acid-based deep eutectic solvent: an efficient green media for the selective extraction of steroidal saponins from *Trillium govanianum*, Sep. Purif. Technol. 294 (2022) 121105.

[19] A.A. Petrochenko, A. Orlova, N. Frolova, E.B. Serebryakov, A. Soboleva, E.V. Flisyuk, A. Frolov, A.N. Shikov, Natural deep eutectic solvents for the extraction of triterpene saponins from *Aralia elata* var. *mandshurica* (Rupr. & Maxim.) J. Wen, Molecules 28 (8) (2023) 3614.

[20] A.N. Shikov, V.M. Kosman, E.V. Flissyuk, I.E. Smekhova, A. Elameen, O.N. Pozharitskaya, Natural deep eutectic solvents for the extraction of phenyletanes and phenylpropanoids of *Rhodiola rosea* L, Molecules 25 (8) (2020) 1826.

[21] M. Santos-Martín, J. Cubero-Cardoso, R. González-Domínguez, E. Cortés-Trivino, A. Sayago, J. Urbano, Á. Fernández-Recamales, Ultrasound-assisted extraction of phenolic compounds from blueberry leaves using natural deep eutectic solvents (NADES) for the valorization of agrifood wastes, Biomass Bioenergy 175 (2023) 106882.

[22] O. Zannou, I. Koca, Greener extraction of anthocyanins and antioxidant activity from blackberry (*Rubus* spp) using natural deep eutectic solvents, LWT 158 (2022) 113184.

[23] K. Soukaina, Z. Safaa, H. Soukaina, C. Hicham, C. Bouchra, Choline chloride-based deep eutectic solvents (NADES): potential use as green extraction media for polyphenols from *Mentha pulegium*, antioxidant activity, and antifungal activity, Microchem. J. (2024) 110174.

[24] H.K. Xue, J.Q. Tan, Q. Li, J.T. Tang, X. Cai, Optimization ultrasound-assisted deep eutectic solvent extraction of anthocyanins from raspberry using response surface methodology coupled with genetic algorithm, Foods 910 (2020) 20.

[25] Y.H. Bi, X.W. Chi, R. Zhang, Y.H. Lu, Z.Y. Wang, Q. Dong, C.X. Ding, R.L. Yang, L. Jiang, Highly efficient extraction of mulberry anthocyanins in deep eutectic solvents: insights of degradation kinetics and stability evaluation, Innov. Food Sci. Emerg. Technol. 66 (2020) 7.

[26] A.P. Abbott, G. Capper, D.L. Davies, R.K. Rasheed, V. Tambyrajah, Novel solvent properties of choline chloride/urea mixtures, Chem. Commun. 1 (2003) 70–71.

[27] J. Lee, R.W. Durst, R.E. Wrolstad, Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study, J. AOAC Int. 885 (2005) 1269–1278.

[28] S.X. Lin, X.J. Meng, C. Tan, Y.Q. Tong, M.Z. Wan, M.Y. Wang, Y. Zhao, H.T. Deng, Y.W. Kong, Y. Ma, Composition and antioxidant activity of anthocyanins from *Aronia melanocarpa* extracted using an ultrasonic-microwave-assisted natural deep eutectic solvent extraction method, Ultrason. Sonochem. 89 (2022) 15.

[29] Y.T. Dai, E. Rozema, R. Verpoorte, Y.H. Choi, Application of natural deep eutectic solvents to the extraction of anthocyanins from *Catharanthus roseus* with high extractability and stability replacing conventional organic solvents, J. Chromatogr. A 1434 (2016) 50–56.

[30] J.L. Zuo, P.R. Ma, S.Q. Geng, Y.Z. Kong, X. Li, Z.S. Fan, Y.L. Zhang, A. Dong, Q. Zhou, Optimization of the extraction process of flavonoids from *Trollius ledebouri* with natural deep eutectic solvents, J. Separ. Sci. 453 (2022) 717–727.

[31] P. Gullon, B. Gullon, A. Romani, G. Rocchetti, J.M. Lorenzo, Smart advanced solvents for bioactive compounds recovery from agri-food by-products: a review, Trends Food Sci. Technol. 101 (2020) 182–197.

[32] Y.Y. Si, S.W. Sun, K. Liu, Y. Liu, H.L. Shi, K. Zhao, J. Wang, W. Wang, Novel deep eutectic solvent based on levulinic acid and 1,4-Butanediol as an extraction media for bioactive alkaloid rutaecarpine, Processes 73 (2019) 17.

[33] M.C. Ali, J. Chen, H.J. Zhang, Z. Li, L. Zhao, H.D. Qiu, Effective extraction of flavonoids from *Lycium barbarum* L. fruits by deep eutectic solvents-based ultrasound-assisted extraction, Talanta 203 (2019) 16–22.

[34] M.C. Bubalo, N. Curko, M. Tomasevic, K.K. Ganic, I.R. Redovnikovic, Green extraction of grape skin phenolics by using deep eutectic solvents, Food Chem. 200 (2016) 159–166.

[35] Y.C. Hou, C.F. Yao, W.Z. Wu, Deep eutectic solvents: green solvents for separation applications, Acta Phys.-Chim. Sin. 348 (2018) 873–885.

[36] Y.T. Dai, G.J. Witkamp, R. Verpoorte, Y.H. Choi, Tailoring properties of natural deep eutectic solvents with water to facilitate their applications, Food Chem. 187 (2015) 14–19.

[37] J.G. Nie, D.T. Chen, Y.B. Lu, Deep eutectic solvents based ultrasonic extraction of polysaccharides from edible brown seaweed *Sargassum horneri*, J. Mar. Sci. Eng. 86 (2020) 10.

[38] X.Z. Fu, D. Wang, T. Belwal, J. Xie, Y.Q. Xu, L. Li, L.G. Zou, L.X. Zhang, Z.S. Luo, Natural deep eutectic solvent enhanced pulse-ultrasonication assisted extraction as a multi-stability protective and efficient green strategy to extract anthocyanin from blueberry pomace, LWT-Food Sci. Technol. 144 (2021) 12.

[39] P. Liu, W.R. Li, Z.Z. Hu, X.G. Qin, G. Liu, Isolation, purification, identification, and stability of anthocyanins from *Lycium ruthenicum* Murr, LWT-Food Sci. Technol. 126 (2020) 9.

[40] T. Belwal, H. Huang, L. Li, Z.H. Duan, X.B. Zhang, H. Aalim, Z.S. Luo, Optimization model for ultrasonic-assisted and scale-up extraction of anthocyanins from *Pyrus communis* 'Starkrimson' fruit peel, Food Chem. 297 (2019) 12.
[41] Y. Tao, D.W. Sun, Enhancement of food processes by ultrasound: a review, Crit. Rev. Food Sci. Nutr. 554 (2015) 570–594.
[42] X.H. Fan, X.Y. Zhang, Q.A. Zhang, W.Q. Zhao, F.F. Shi, Optimization of ultrasound parameters and its effect on the properties of the activity of beta-glucosidase in apricot kernels, Ultrason. Sonochem. 52 (2019) 468–476.
[43] Q.A. Zhang, H. Shen, X.H. Fan, Y. Shen, X. Wang, Y. Song, Changes of gallic acid mediated by ultrasound in a model extraction solution, Ultrason. Sonochem. 22 (2015) 149–154.
[44] L.Z. Lu, Y.Z. Yang, G.Y. Zhong, Z.C. Liang, L.L. Cheng, Phytochemical composition and content of red-fleshed grape accessions, Horticulturae 95 (2023) 14.
[45] S.C. Li, L. Sun, X.C. Fan, Y. Zhang, J.F. Jiang, C.H. Liu, Polymorphism of anthocyanin concentration and composition in Chinese wild grapes, Aust. J. Grape Wine Res. 271 (2021) 34–41.
[46] F.L. Han, Y.L. Ju, X.R. Ruan, X.F. Zhao, X.F. Yue, X.F. Zhuang, M.Y. Qin, Y.L. Fang, Color, anthocyanin, and antioxidant characteristics of young wines produced from spine grapes (*Vitis davidii* Foex) in China, Food Nutr. Res. 61 (2017) 11.
[47] Z.Q. Chen, C. Wang, X.D. Gao, Y. Chen, R.K. Santhanam, C.L. Wang, L.L. Xu, H.X. Chen, Interaction characterization of preheated soy protein isolate with cyanidin-3-O-glucoside and their effects on the stability of black soybean seed coat anthocyanins extracts, Food Chem. 271 (2019) 266–273.
[48] D.A. Turker, M. Dogan, Ultrasound-assisted natural deep eutectic solvent extraction of anthocyanin from black carrots: optimization, cytotoxicity, in-vitro bioavailability and stability, Food Bioprod. Process. 132 (2022) 99–113.
[49] S. Oancea, O. Draghici, pH and thermal stability of anthocyanin-based optimised extracts of Romanian red onion cultivars, Czech J. Food Sci. 313 (2013) 283–291.
[50] A.B. Das, V.V. Goud, C. Das, Degradation kinetics of anthocyanins from purple rice bran and effect of hydrocolloids on its stability, J. Food Process. Eng. 434 (2020) 10.
[51] B. Bharat, B. Bhushan, P. Ajay, M.M. Kumar, D.M. Chander, K. Yathish, J.B. Singh, K. Pardeep, A.S. Kumar, S. Alla, FTIR spectra, antioxidant capacity and degradation kinetics of maize anthocyanin extract under variable process conditions, Applied Food Research 31 (2023).
[52] N. Guo, K. Ping, Y.W. Jiang, L.T. Wang, L.J. Niu, Z.M. Liu, Y.J. Fu, Natural deep eutectic solvents couple with integrative extraction technique as an effective approach for mulberry anthocyanin extraction, Food Chem. 296 (2019) 78–85.
[53] E.D. Obluchinskaya, O.N. Pozharitskaya, L.V. Zakharova, A.V. Daurtseva, E.V. Flisyuk, A.N. Shikov, Efficacy of natural deep eutectic solvents for extraction of hydrophilic and lipophilic compounds from *Fucus vesiculosus*, Molecules 26 (14) (2021) 4198.
[54] Y.T. Dai, J. van Spronsen, G.J. Witkamp, R. Verpoorte, Y.H. Choi, Natural deep eutectic solvents as new potential media for green technology, Anal. Chim. Acta 766 (2013) 61–68.
[55] P. Kou, N. Wan, L.T. Wang, H.Y. Pan, J. Jiao, C.J. Zhao, Z.G. Liu, X.Q. Wang, Y.J. Fu, A sustainable and efficient preparation process of anthocyanins from blue honeysuckle fruit and comprehensive bioactivity assessment, J. Taiwan Inst. Chem. Eng. 116 (2020) 3–10.
[56] O. Zannou, H. Pashazadeh, M. Ghellam, I. Koca, A.A. Kaddour, F. Leriche, Appraisal of phenolic compounds, antioxidant activity and in vitro gastrointestinal digestion of borage (*Echium amoenum*) flowers using natural deep eutectic solvent (NADES), Biomass Convers. Biorefinery 13 (2022).
[57] J.S. Farah, M.C. Silva, A.G. Cruz, V. Calado, Differential calorimetry scanning: current background and application in authenticity of dairy products, Curr. Opin. Food Sci. 22 (2018) 88–94.
[58] M.D. Moura, B.D.G. da Costa, M.A. Giaconia, R.R. de Andrade, A.R.C. Braga, M.B. Braga, Jaboticaba powders production by freeze-drying: influence of octenyl succinic anhydride-modified starch concentrations over anthocyanins and physical properties, J. Food Process. Eng. 463 (2023) 14.