Deep eutectic solvent-based ultrasonic assisted extraction of polysaccharides and antioxidants from *Astragalus hamosus* L. seedpod

Reza Tabaraki[1*], Fariba Fatahi[1]

https://orcid.org/0000-0002-3422-9423,
https://orcid.org/0009-0003-0179-7648
[1]*Department of Chemistry, Faculty of Science, Ilam University, Ilam, Iran.*
Corresponding author: Tel: ++98 843 2227022; Fax: ++98 843 2227022.
E-mail address: rezatabaraki@yahoo.com; r.tabaraki@ilam.ac.ir

Abstract
In this study, the efficiency of various solvents and techniques was compared for the extraction of polysaccharides and antioxidants from *Astragalus hamosus*. Among twenty solvents and four extraction techniques, ultrasonic-assisted extraction using choline chloride-urea (a deep eutectic solvent, DES) demonstrated the best performance. Extraction variables were optimized by the response surface methodology (RSM), and Box-Behnken design (BBD). Extraction yield and total carbohydrate content (TCC) were 44.5% and 2.90 mgGlu/gdw, respectively. Rhamnose, mannose and galactose (molar ratio: 0.34:1.18:1.0) were major monosaccharides in the extracted polysaccharides. Six antioxidant assays—total phenolic content (TPC), total flavonoid content (TFC), DPPH assay, ferric-reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC), and reducing power assay (RPA)—were used to evaluate antioxidant capacity. Additionally, different extraction methods, including ultrasonic-assisted, microwave-assisted, maceration, and Soxhlet extractions were compared.
Keywords: *Astragalus hamosus* L., Antioxidants, Deep eutectic solvent, Leguminosae, Polysaccharides, Ultrasonic assisted extraction, Response surface methodology.

1. Introduction

Plants have a unique place in traditional medicine (El Jabboury, et al., 2023; Mohammadhosseini et al., 2019; Sharif and Jabeen, 2024). *Astragalus* L. (the Leguminosae family) grows widely in the temperate regions of Europe, Africa, and Asia. One of the species of this family, *Astragalus hamosus* L. (seedpod, Fig. 1) is known as "Iklil-ul-Malik", "Milk Vetch", and "Nakhonak" (Zoghlami and Zouaghi, 2003). It is an herbaceous perennial plant that grows up to 60 cm. This plant has anti-bronchitis, anti-diarrheal, anti-migraine, antispasmodic, anti-inflammatory, and anti-influenza properties. It is also used as general strengthening of the body, pain reliever and soft swelling softener, an eye enhancer, and a blood thinner (Al-Snafi and Esmail, 2015).
Different flavanols and flavonoids, such as the glycosides 7-*O*-methyl-kaempferol 4'-β-D-galactopyranoside, hyperoside, isoquercitrin, rutin, and astragalin, identified in *A. hamosus* (Hamedi et al., 2016; Krasteva et al., 2007; Shkondrov et al., 2021; Shkondrov and Krasteva, 2021). Hamedi et al. (2016) analyzed several phytochemicals in *A. hamosus* seedpods, including total ash (75.00 mg/g), acid-insoluble ash (8.33 mg/g), water-soluble ash (40.00 mg/g), free amino acid content (3.33% *w/w*), soluble sugar content (8.83% *w/w*), and identified nineteen fatty acids in the pod oil, such as linoleic acid (48.64%), linolenic acid (25.35%), lauric acid (8.12%), and stearic acid (6.38%). Triterpenoid saponin contents (Nafti et al., 2022) were also determined. Anti-inflammatory and analgesic activity (Hakim et al., 2010; Shojaii et al., 2015), anti-proliferative effects on breast cancer cells (Mahmoodi et al., 2022) and oral toxicity (Hassanzadeh-Taheri et al., 2018) of *A. hamosus* extract were also evaluated.
Today, organic solvents have largely been replaced by deep eutectic solvents (DES). DES are a new class of solvents characterized by a melting point lower than that of their individual components: a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD). They can be liquid at room temperature or below 70 °C. Compared to ionic liquids (ILs), DES offer several advantages, including simple and low-cost synthesis from tunable starting materials, environmental friendliness, low vapor pressure, water miscibility, non-flammability, biodegradability, and low toxicity. Various methods such as heating and stirring, evaporation, and freeze-drying can be employed for DES preparation (Dinis and Coutinho, 2022).

In recent years, DESs have been primarily used as solvents in sustainable chemistry applications (Liu et al., 2022), including material synthesis and exfoliation, metal processing, biodiesel production, contaminant removal, and water treatment (Zaib et al., 2023). Natural compounds in plants, such as proteins and lignin (Kaoui et al., 2023), carbohydrates (Chen and Lahaye, 2021), flavonoids (Xinyu et al., 2022), isoflavones (Duru et al., 2022), and phenolics (Wu et al., 2020; Zannou et al., 2022), have been successfully extracted by DESs. DESs were also used in a variety of extraction techniques, including microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), mechanochemical extraction (MCE), liquid phase microextraction (LPME), and solid phase microextraction (SPME) (Li and Row, 2016; Jafari et al., 2023). Polysaccharides extractions by DESs were also performed by different methods such as UAE (Adelia and Samavati, 2015), MAE (Shang et al., 2021), supercritical fluid extraction (Benvenutti et al., 2022), enzyme-assisted extraction (EAE) (Liang et al., 2019), and IR-assisted extraction (Guo et al., 2021). Ultrasound waves produce cavitation in solvent and cause the cell wall disruption and materials release from sample to solvent. This accelerated mass transfer increases the extraction yield and decreases the extraction time.

Aim of this work was selection the best solvent and extraction methods for antioxidants and polysaccharides extraction from *A. hamosus*. Twenty solvents and four techniques (UAE, MAE, maceration extraction (ME), and Soxhlet extraction (SE)) were compared. Total carbohydrate and total phenolic contents were responses, and extraction variables were optimized by RSM. Antioxidant capacity of the extract was evaluated by six assays.

2. Experimental

2.1 Reagents and materials

Choline chloride, citric acid, lactic acid, malic acid, gallic acid, ethylene glycol, urea, L-proline, glycerol, thiourea, fructose, xylitol, acetone, methanol, ethanol, phenol, glucose, sulfuric acid, Folin-Ciocalteu reagent, 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), butanol, chloroform and sodium carbonate were analytical grade and obtained from Merck. 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), quercetin, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma–Aldrich. *A. hamosus* seedpods were purchased from a local herbal market in Ilam, Iran, in September 2020. The seedpods were cleaned and milled using a laboratory electric mill.

2.2 Apparatus

An Elmasonic ultrasonic bath (S30H, Germany; 2.75 L; 37 kHz; 280 W) was used for ultrasonic-assisted extractions. Absorbance measurements were performed using a Varian UV/Vis spectrophotometer. A microwave oven (Samsung CE3280EB, 500 W) was employed for microwave-assisted extractions.

2.3 Extraction

Table 1 shows the prepared DES solvents, HBA, and HBD molecules and their molar ratios. HBD and HBA were mixed, heated at 80 °C for 60 min (Wu et al., 2020). Finally, water (10% *v/v*) was added to transparent liquids.

A. hamosus (20 g, powder particle size: 177-250 µm) were defatted with petroleum ether (250 mL, 70 °C, 6 h). The degreased sample (1.0 g) was extracted with DES. Then, proteins were removed from the extract with Sevag solvent (chloroform and butanol with 1:4 ratio). Polysaccharides can be crystallized by storing the supernatant overnight at 4 °C in ethanol. Extraction yield was calculated from the weight of dried polysaccharides (Fatahi and Tabaraki, 2023). Each extraction was repeated three times.

2.4 Optimization of extraction variables

Optimization of extraction variables was done by response surface methodology (RSM). Second order polynomial equation was fitted to experimental data and then, optimum conditions and the relationship between operating parameters and the response were determined. The statistical significance of the fitted model was investigated

by the analysis of variance (ANOVA). The Box–Behnken design (BBD) is one of good, rotatable and three level experimental designs for RSM. It was shown that the BBD design is more efficient than the central composite design and the three-level full factorial designs (Ferreira et al., 2007). Table 2 shows the BBD design for antioxidant and polysaccharides extractions. Variable levels were determined by preliminary experiments.

2.5 Software

All calculations were done by the Minitab 16 (Minitab Inc., State College, PA, USA) software. A second-order polynominal regression model containing the coefficient of linear, quadratic and interaction terms was fitted to experimental data. An analysis of variance (ANOVA) with 95% confidence level was then carried out for each response variable in order to test the significances of all terms in the polynomial model and model suitability by computing the *p*-value.

2.6 Carbohydrate and antioxidant assays

TCC was measured by the phenol-sulfuric acid assay (Singleton et al., 1999) based on the conversion of the polysaccharides to monosaccharides and furfural. Linear calibration curve was: y = 0.0809x + 0.011 (R^2 = 0.999; 0.25-1.25 mg L^{-1}, standard: glucose).
Total phenolic content (TPC) was determined by the Folin-Ciocalteu reagent (Pierre Brat et al., 2005). Linear calibration curve was y = 499.41x + 0.0227 (R^2 = 0.998; 0.0003-0.0017 M; standard: Gallic acid).
The $AlCl_3$ colorimetry (Wang et al., 2017) was done for total flavonoid content (TFC) evaluation. The procedure involves mixing 250 µL of the extract, 1.25 mL of water, and 75 µL of 5% $NaNO_2$, followed by a 6-minute incubation. Next, 150 µL of 10% aluminum chloride is added, and the mixture is allowed to stand for 5 minutes. Then, 0.5 mL of NaOH (1.0 M) is added, and the total volume is adjusted to 4 mL with distilled water. Finally, the absorbance is measured at a wavelength of 510 nm. Linear calibration curve was: y = 22.21x + 0.125 (R^2 = 0.996; 0.001-0.05 mg mL^{-1}; standard: Quercetin).
In the $DPPH^{\bullet}$ assay (Wu et al., 2019), 0.5 mL of extract was added to DPPH solution (2.5 mL, 100 µM) and stored in dark for 30 min and then, solution absorbance was measured at 517 nm.
In the ferric-reducing antioxidant power (FRAP) assay (Benzie method, 1996), absorbance was measured at 593 nm. Calibration curve equation was y = 661.5x + 0.026 (R^2 = 0.998; 0.0005-0.0029 M).
In the reducing power assay (RPA) method (Oyaizu, 1986), antioxidants reduce $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$. Absorbance was measured at 700 nm. The linear calibration curve was: y = 2.915x + 0.066 (R^2 = 0.986; 0.05-0.5 mg mL^{-1}; standard: Trolox).
In the Trolox equivalent antioxidant capacity (TEAC) assay (Re et al., 1999), antioxidants decolorize the $ABTS^{\bullet+}$ radical. Absorbance was measured at 700 nm. The linear calibration curve was y = -1.7957x + 0.9021 (R^2 = 0.996; 0.05-0.5 mM; standard: Trolox).

2.7 Comparison of the UAE with other extraction methods

In all experiments, the V/m ratio was 100 (0.6 g *A. hamosus* + 60 mL water or DES). Water was used in microwave-assisted extraction (MAE, 500 W, 6 min), maceration (ME), and Soxhlet (SE) extractions (70 °C, 60 min). Finally, the extracts were cooled and centrifuged (10000 g, 10 min) and used for further analysis.

2.8 Monosaccharide analysis

GC-FID (Varian 3400, USA, capillary column DM-2330 (30 m × 0.32 mm × 0.2 µm) was used for identification the monosaccharides composition of polysaccharides and uronic acid units of polysaccharides regarding the previously reported method (Taylor and Conrad, 1972; Sahragard and Jahanbin, 2017). Standards were *D*-galactose, D-glucose, D-mannose, L-rhamnose, D-xylose, and L-arabinose. Myo-inositol was the internal standard. GC experimental variables were N_2 flow rate = 1.2 mL min^{-1}, $T_{injector}$ = 250 °C, and $T_{detector}$ = 300 °C, T_{column} = 20 °C (2 min), 20 to 250 °C (rate = 8 °C/min) and 250 °C (3 min).

3. Results and discussion

3.1 Solvent selection

As shown in Fig. 2, the best solvents for polysaccharides extraction were choline chloride-urea (TCC = 0.584 mg Glu/gdw), choline chloride-lactic acid (0.577 mg Glu/gdw), and water (0.517 mg Glu/gdw). The best solvents for polyphenols extraction were choline chloride-citric acid (TPC = 0.391 mg GAE/gdw), choline chloride-urea (0.372 mg GAE/gdw), and glycerol-urea (0.323 mg GAE/gdw). Therefore, choline chloride-urea deep eutectic solvent was select as extraction solvent. Some of the reported physical properties of this DES (choline chloride-urea, mole ratio 1:2) are melting point (134 °C), freezing point (12 °C), viscosity (750 cP at 25 °C, and 169 cP at 40 ºC), density (1.25 g/cm^3), and ionic conductivity (0.199 mS/cm at 40 °C) (Zhang et al., 2012).

3.2 Optimization of polysaccharides extraction

RSM model based on 29 Box-Behnken experiments (Table 2) for time (t), temperature (T), solvent-to-solid ratio (V/m) and water content in DES (WC) (Eq. 1) was:

$$TCC = 1.38 - 4.87\times10^{-2}\times\mathbf{T} - 2.75\times10^{-3}\times WC + 2.82\times10^{-2}\times\mathbf{V/m} - 4.72\times10^{-3}\times t + 3.30\times10^{-4}\times\mathbf{T^2} + 2.10\times10^{-4}\times(WC)^2 - 1.00\times10^{-4}\times\mathbf{(V/m)^2} - 3.40\times10^{-4}\times\mathbf{t^2} - 1.70\times10^{-4}\times T\times WC - 1.00\times10^{-6}\times T\times V/m + 4.20\times10^{-4}\times\mathbf{T\times t} - 1.30\times10^{-4}\times WC\times V/m + 6.00\times10^{-5}\times WC\times t + 2.40\times10^{-4}\times\mathbf{V/m\times t} \quad (1)$$

R^2 = 0.981; Lack of fit = 0.317 (p > 0.05);

Bold terms are significant (confidence level = 95%; ANOVA). As shown, linear (T and V/m), quadratic (T^2, $(V/m)^2$, and t^2) and interaction (T×t and V/m ×t) terms were significant. An insignificant lack of fit (0.317> 0.05) means the model fits data well (goodness-of-fit). Variables effects on TCC are shown in Figs. 3 and 4 (response surface and contour plots). In each plot, TCC was drawn by keeping two variables constant (at their middle values) while varying the other two.

The increase in temperature led to a rise in TCC. This effect is attributed to enhanced solubility of polysaccharides and an increased interaction rate between solvent molecules and the plant cell wall, facilitating diffusion and mass transfer. However, the activity and structure of the extracted polysaccharides may be altered at high temperatures due to hydrolysis and decomposition (Heydarian et al., 2017). Additionally, TCC increased with a higher volume-to-mass (V/m) ratio, likely because of improved solvent penetration into cells and more efficient desorption of polysaccharides from the cell matrix (Rostami and Gharibzahedi, 2016). The ultrasonic extraction method generates microbubbles in the extraction medium; the collapse of these bubbles produces intense mechanical shocks that disrupt the cell wall, releasing cellular compounds. Prolonged sonication time further enhances mass transfer, cavitation, and extraction efficiency (Jooyandeh et al., 2018).

The predicted and experimental TCC and extraction yield at optimal conditions (T = 70 °C, t = 60 min, WC = 10% v/v, and V/m = 100 mL/g) were 2.86, 2.90 mg Glu/gdw and 44.5%, respectively.

3.3 Structural analysis of extracted polysaccharides

FT-IR spectrum of *A. hamosus* polysaccharides is shown in Fig. 5A. O−H and C−H stretching absorption bands appeared at 3433 cm^{-1} and 2881 cm^{-1}, respectively. Two sharp bands (1623 and 1666 cm^{-1}) are attributed to the C=O vibrations in the polysaccharide backbone because of uronic acid (Jooyandeh et al., 2018). Peak at 1456 cm^{-1} indicates the stretching vibrations of C-H in the monosaccharide ring (Sahragard and Jahanbin 2017). The C–O–C glycosidic band vibration, along with the C–C and C–O deformation vibrations in the pyranose form of sugars, appeared at 1082 cm^{-1} (Tadayoni et al., 2015). The bands at 871 and 956 cm^{-1} are attributed to the α- and β-glycosidic linkages, as well as to the mannopyranose and galactopyranose units (Ustyuzhanina et al., 2016).

Monosaccharide composition analysis was done by gas chromatography (Fig. 5B). The extracted polysaccharides were hydrolyzed by trifluoroacetic acid, followed by reduction and acetylation. Molar ratio of monosaccharides in extracted polysaccharides was rhamnose (0.34): mannose (1.18): galactose (1.0).

Antioxidant activity of the extracted polysaccharides were evaluated by TEAC, RPA, FRAP and DPPH assays. Results were 670 µmol Trolox per gram of solid polysaccharides, 276.5 µmol Trolox per gram of dry polysaccharides, 81.1 mM Fe(II) per gram of dry polysaccharides and 57.7%, respectively.

3.4 Optimization of antioxidants extraction

Optimization of total phenolic extraction variables was done by BBD and RSM (Table 2). Second-order polynomial equation for TPC (Eq. 2) was:

$$TPC = -1.37 + 2.53\times10^{-2}\times T + 4.26\times10^{-2}\times \mathbf{WC} + 7.75\times10^{-3}\times V/m + 3.06\times10^{-2}\times t - 2.30\times10^{-4}\times T^2 - 3.60\times10^{-4}\times \mathbf{(WC)^2} - 9.00\times10^{-5}\times \mathbf{(V/m)^2} - 4.70\times10^{-4}\times \mathbf{t^2} - 3.90\times10^{-4} \times T\times WC + 8.00\times10^{-5}\times T\times V/m + 2.00\times10^{-5}\times T\times t + 1.20\times10^{-4}\times WC\times V/m - 1.20\times10^{-4}\times WC\times t + 1.40\times10^{-4}\times V/m\times t \quad (2)$$

R^2=0.900; Lack of fit=0.161 (p > 0.05)

Bold terms are significant (confidence level = 95%; ANOVA). As shown, significant terms were linear (WC), and quadratic ($(WC)^2$, $(V/m)^2$, and t^2) terms. Three dimensional RSM and contour plots are shown in Figs. 6 and 7. Water content in DES (WC) appeared in the all of significant terms in TPC equation. Increasing water content in DES (10 to 50%) improved the extraction of the phenolic compounds. The presence of the water in DES modifies the viscosity and polarity of the DES. Increase in the water content in DES reduces its viscosity, increase its polarity, increase in solubility of polar compounds such as polyphenols and improves penetration in plant matrices and mass transport from it to solution (Bubalo et al., 2016). Liquid-to-solid ratio (V/m) is another important process variable. At high V/m values, concentration gradient between the inside of the plant cells and the outside solvent is high that results an increase in the diffusion rate, mass transfer and extraction yield. As shown in Fig. 5, total phenolic content was increased by the time but after 50 min, it had continuous decrease which is probably due to the degradation of polyphenols. Although, temperature helps to the better penetration of the solvent into the plant matrix by decreasing the density and viscosity of solvent, but high temperatures could induce polyphenols degradation. At optimal conditions (t = 51 min, T = 30 °C, V/m = 100 mL/g, WC = 50% *v/v*), predicted and experimental TPC were 1.30 and 1.22 mg GAE/gdw, respectively.

3.5 Comparison of extraction techniques

Plant extracts are complex mixtures; therefore, their antioxidant capacity must be evaluated using various chemical assays such as FRAP, DPPH, and ABTS with different mechanisms. Under optimal conditions, ultrasonic-assisted extraction (UAE) was performed, followed by six antioxidant assays. The DES-based UAE extraction was also compared with other extraction methods (Table 3). UAE demonstrated higher efficiency compared to microwave-assisted extraction, maceration, and Soxhlet extraction. The efficiency of choline chloride-urea (the selected DES) was superior to that of water (Fig. 2), with an approximately 11.5% improvement in TCC (0.584 vs. 0.517 mg Glu/gdw), and a 21% increase in TPC (0.372 *vs*. 0.295 mg GAE/gdw). The results of TCC for choline chloride-urea (DES) and water in UAE method were statistically compared by the *t*-test. The difference was statistically significant (TCC_{DES} = 0.584 mg Glu/gdw; TCC_{water} = 0.517 mg Glu/gdw, s = 0.02; t_{exp} = 5.8; $t_{critical}$ = 4.3; p = 0.05; $t_{exp} > t_{critical}$). The difference for TPC was also statistically significant (TPC_{DES} = 0.372 mg GAE/gdw; TPC_{water} = 0.295 mg GAE/gdw, s = 0.02; t_{exp} = 6.7; $t_{critical}$ = 4.3; p = 0.05; $t_{exp} > t_{critical}$).

4. Concluding remarks

The genus *Astragalus* comprises approximately 3,494 species (Sadeghi et al., 2023). Although there are scientific studies on the pharmaceutical effects of *A. hamosus* seedpods, to the best of our knowledge, there are no reports on polysaccharides content and TCC, TPC, TFC, FRAP, DPPH, TEAC, or RPA assays for *A. hamosus* seedpods. However, limited data are available for other species within this genus. Development of ecofriendly and sustainable techniques and green solvents for extracting phytochemicals from medicinal plant is becoming increasingly important. Therefore, this study was designed to develop a green extraction technique for polysaccharides and antioxidants from *A. hamosus* for the first time. In this study, twenty solvents and four extraction methods—ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), maceration

extraction (ME), and Soxhlet extraction (SE)—were compared for their efficiency in extracting carbohydrates and phenolics from *A. hamosus*. Ultimately, choline chloride-urea (a DES) combined with ultrasonic-assisted extraction was selected. Experimental variables were optimized by the experimental design and response surface methodology. The DES-UAE method was also applied for polyphenolic extraction, and six antioxidant assays were employed to evaluate the antioxidant capacity of the extract. TCC and TPC for choline chloride-urea and water in UAE-assisted extraction were statistically compared using a t-test, revealing significant differences. These results showed that deep eutectic solvent as a green solvent, has greater extraction capability than water, methanol, ethanol and acetone.

Structural characterization of DES-extracted polysaccharides and antioxidants by HPLC-MS could be a promising topic for future research. These results expand our understanding of phytochemical extraction using DES. Exploring the relationship between the separated polysaccharides and antioxidants of *Astragalus hamosus* pod and their anti-bronchitis, anti-diarrheal, anti-migraine, antispasmodic, anti-inflammatory, and anti-influenza properties is a real need for future studies. Additionally, pharmacokinetic and pharmacodynamic studies of these substances must be considered.

List of abbreviations

ABTS: 2,2′-Azino-Bis-(3-Ethylbenzthiazoline-6-Sulfonic Acid; **ANOVA:** Analysis of Variance; **BBD:** Box-Behnken Design; **DES:** Deep Eutectic Solvents; **DPPH:** 1,1-Diphenyl-2-Picrylhydrazyl; **EAE:** Enzyme-Assisted Extraction; **FRAP:** Ferric-Reducing Antioxidant Power; **HBA:** Hydrogen Bond Acceptor; **HBD:** Hydrogen Bond Donor; **IL:** Ionic Liquid; **LPME:** Liquid Phase Microextraction; **MAE:** Microwave-Assisted Extraction; **MCE:** Mechanochemical Extraction; **ME:** Maceration Extraction; **mg GAE/gdw:** mg of Gallic Acid Equivalent /g of Dry Weight; **mg Glu/gdw:** mg Glucose Per g of Dry Weight; mg QE/gdw: mg of Quercetin /g of Dry Weight; **RPA:** Reducing Power Assay; **RSM:** Response Surface Methodology; **SE:** Soxhlet Extraction; **SPME:** Solid Phase Microextraction; **T:** Temperature; **t:** time; **TCC:** Total Carbohydrate Content; **TEAC:** Trolox Equivalent Antioxidant Capacity; **TFC:** Total Flavonoid Content; **TFC:** Total Flavonoid Content; **TPC:** Total Phenolic Content; **TPTZ:** 2,4,6-Tris (2-Pyridyl)-S-Triazine; **Trolox:** 6-Hydroxy-2,5,7,8-Tetramethylchroman-2-Carboxylic Acid; **UAE:** Ultrasound-Assisted Extraction; **V/m:** Solvent-To-Solid Ratio; **WC:** Water Content in DES; **µmol Trolox/gdw:** µmol of Trolox Equivalent/g of Dry Weight.

Author contribution statement

Conceptualization and literature search were performed by Reza Tabaraki and Fariba Fatahi. The first draft of the manuscript was prepared by Fariba Fatahi. Reza Tabaraki and Fariba Fatahi critically analyzed and gave suggestions to finalize the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Table 1. Composition of deep eutectic solvents.

No.	Abbreviation	Hydrogen bond acceptor	Hydrogen bond donor	Molar ratio
1	ChCl-Lac	Choline chloride	Lactic acid	1:1
2	ChCl-MA	Choline chloride	Malic acid	1:1
3	ChCl-Xyl	Choline chloride	Xylitol	1:1
4	ChCl-EGly	Choline chloride	Ethylene glycol	1:2
5	ChCl-Urea	Choline chloride	Urea	1:2
6	ChCl-Cit	Choline chloride	Citric acid	1:1
7	ChCl-Gly	Choline chloride	Glycerol	1:1
8	ChCl-Glu	Choline chloride	Glucose	1:1
9	ChCl-TUrea	Choline chloride	Thiourea	2:1
10	ChCl-Fru	Choline chloride	Fructose	1:1
11	Gly-Urea	Glycerol	Urea	1:1
12	LA-Glu	Lactic acid	Glucose	1:1
13	Pro-LA	Proline	Lactic acid	1:2
14	Pro-Gly	Proline	Glycerol	2:5
15	Cit-Gly	Citric acid	Glycerol	1:2
16	Glu-Cit	Glucose	Citric acid	1:1

Table 2. Box-Behnken experimental design.

No.	X_1	X_2	X_3	X_4	T (C)	WC%*	V/m**	t (min)	TPC	TCC
1	+1	0	-1	0	70	30	20	40	0.23	0.54
2	+1	0	+1	0	70	30	100	40	1.08	2.19
3	0	0	0	0	50	30	60	40	1.07	1.57
4	0	0	0	0	50	30	60	40	1.03	1.47
5	0	-1	-1	0	50	10	20	40	0.26	0.59
6	-1	-1	0	0	30	10	60	40	0.59	1.73
7	+1	0	0	-1	70	30	60	20	0.82	1.17
8	-1	0	0	-1	30	30	60	20	1.03	1.77
9	0	+1	0	-1	50	50	60	20	0.84	1.14
10	0	0	0	0	50	30	60	40	1.10	1.44
11	-1	0	0	+1	30	30	60	60	0.70	1.59
12	0	0	-1	-1	50	30	20	20	0.22	0.27
13	+1	0	0	+1	70	٣٠	60	60	0.53	1.66
14	0	-1	+1	0	50	10	100	40	1.02	2.59
15	0	0	+1	-1	50	30	100	20	0.80	1.8
16	+1	-1	0	0	70	10	60	40	0.87	1.63
17	0	-1	0	+1	50	10	60	60	0.61	1.53
18	-1	0	+1	0	30	30	100	40	1.17	2.25
19	0	+1	0	+1	50	50	60	60	0.75	1.4
20	-1	+1	0	0	30	50	60	40	0.91	1.8
21	0	+1	-1	0	50	50	20	40	0.31	0.58
22	0	-1	0	-1	50	10	60	20	0.51	1.37
23	0	0	+1	+1	50	30	100	60	1.29	2.4
24	0	+1	+1	0	50	50	100	40	1.45	2.18
25	+1	+1	0	0	70	50	60	40	0.57	1.43
26	0	0	0	0	50	30	60	40	0.85	1.32
27	0	0	-1	+1	50	30	20	60	0.25	0.11
28	0	0	0	0	50	30	60	40	1.00	1.54
29	-1	0	-1	0	30	30	20	40	0.59	0.59

*Water content in DES (WC), v/v%.
**Liquid to solid ratio (V/m), mL/g.

Table 3. Comparison of different extraction methods.

Method	DES-UAE	ME	SE	MAE
TCC	2.90± 0.01	2.04±0.01	1.99±0.01	2.1±0.01
TPC	1.22± 0.02	1.03±0.01	0.97±0.01	1.11±0.01
TFC	37.91±0.02	14.43±0.04	10.84±0.01	24.25±0.02
DPPH	56.25±1.58	37.22±1.09	31.35±1.21	41.25±1.42
TEAC	1300±0.06	1047±0.06	1021±0.08	1124±0.07
FRAP	56.97±1.02	21.25±1.29	18.34±1.78	35.81±1.46
RPA	279.9±0.04	163.66±0.04	112.23±0.04	187.35±0.05

Conditions: 0.6 g of the *Astragalus hamosus*, 60 mL of solvent, 70 °C, 60 min.
TCC: mg Glu/gdw (mg glucose per g of dry weight).
TPC: mg GAE/gdw (mg of gallic acid equivalent /g of dry weight).
TFC: mg QE/gdw (mg of quercetin /g of dry weight).
DDPH: percentage of radical scavenging activity.
TEAC: µmol Trolox/gdw (µmol of Trolox equivalent/g of dry weight).
FRAP: mM Fe(II)/ gdw.
RPA: µmol Trolox/ gdw (µmol of Trolox equivalent/g of dry weight).

Figure 1. The photograph of *Astragalus hamosus* seedpods.

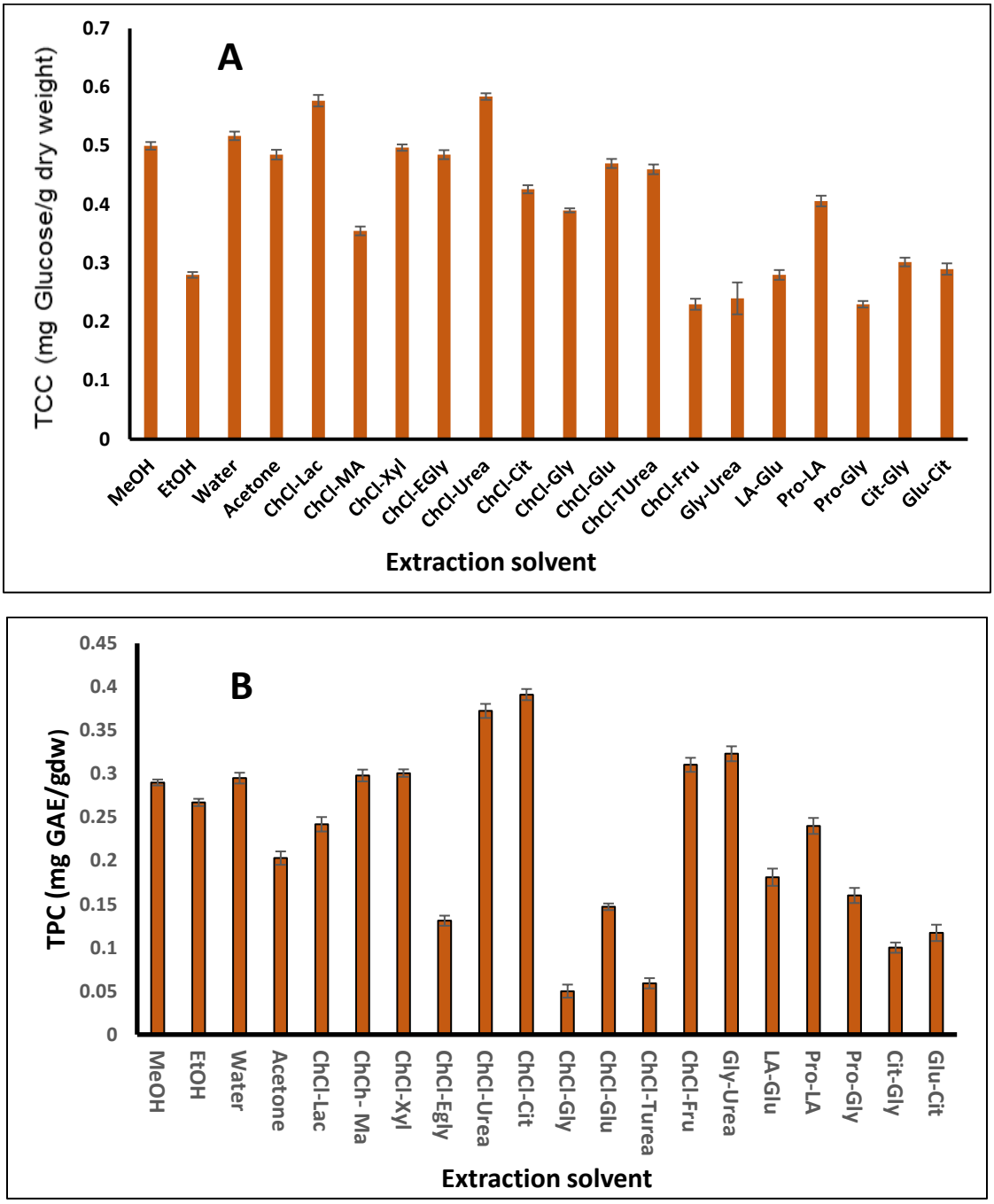

Figure 2. Solvent selection for polysaccharides (A) and antioxidants (B) extraction (experimental conditions: 0.1 g of the *Astragalus hamosus*, 2 mL of DES or solvent, 1 mL water, 50 °C, 50 min, ultrasonic-assisted extraction).

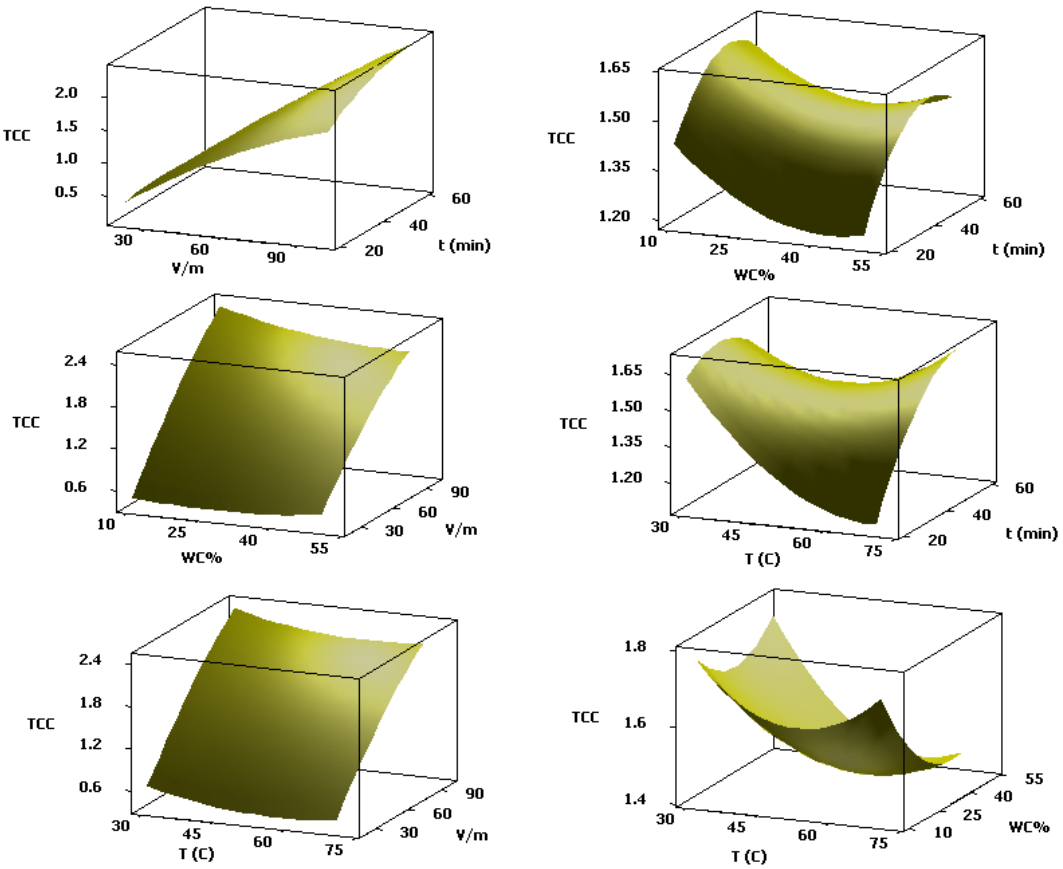

Figure 3. Response surface plots of TCC vs. extraction temperature (T), the water content in DES (WC), liquid/solid ratio (V/m), and extraction time (t).

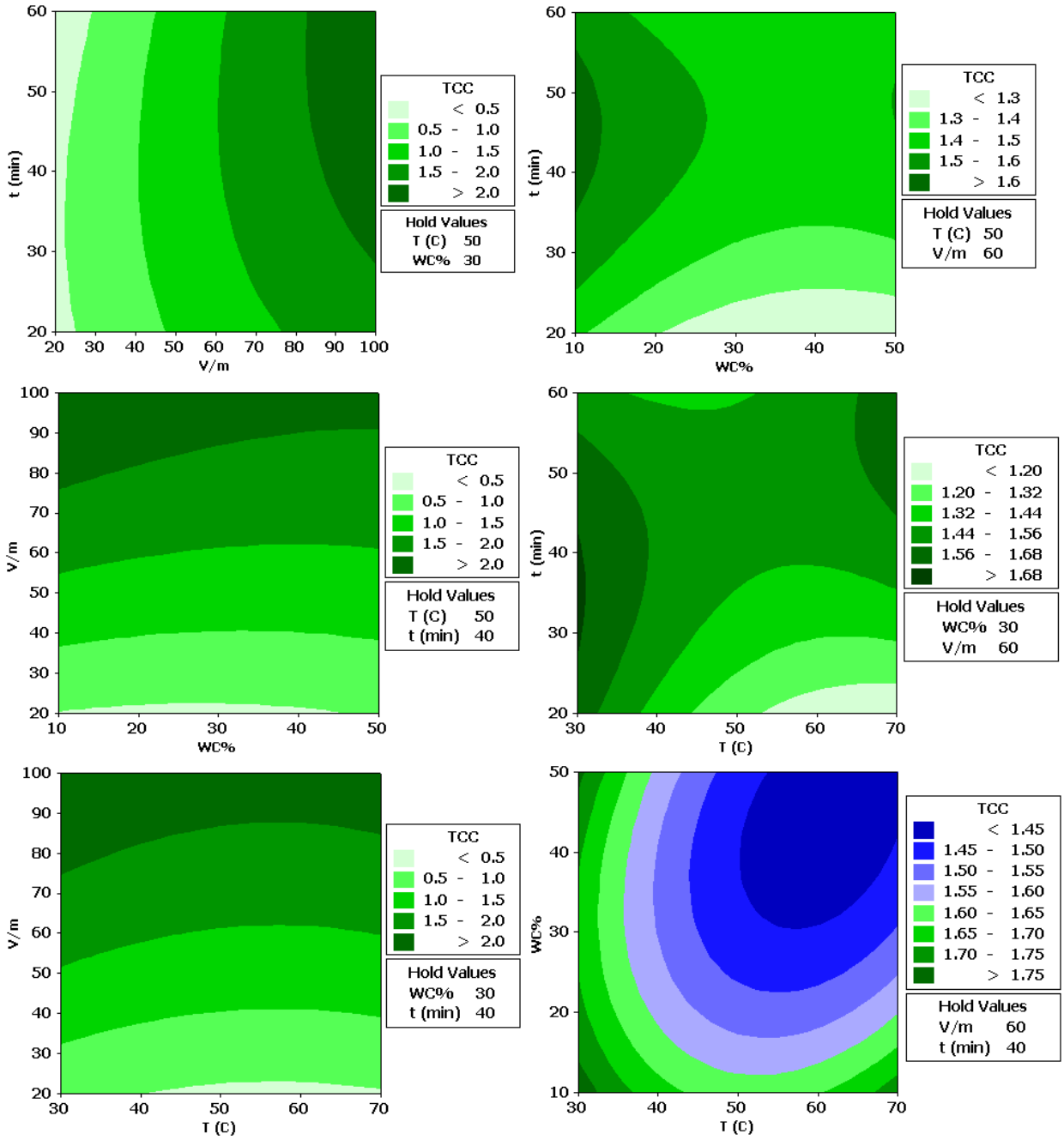

Figure 4. Contour plots of TCC *vs.* extraction variables.

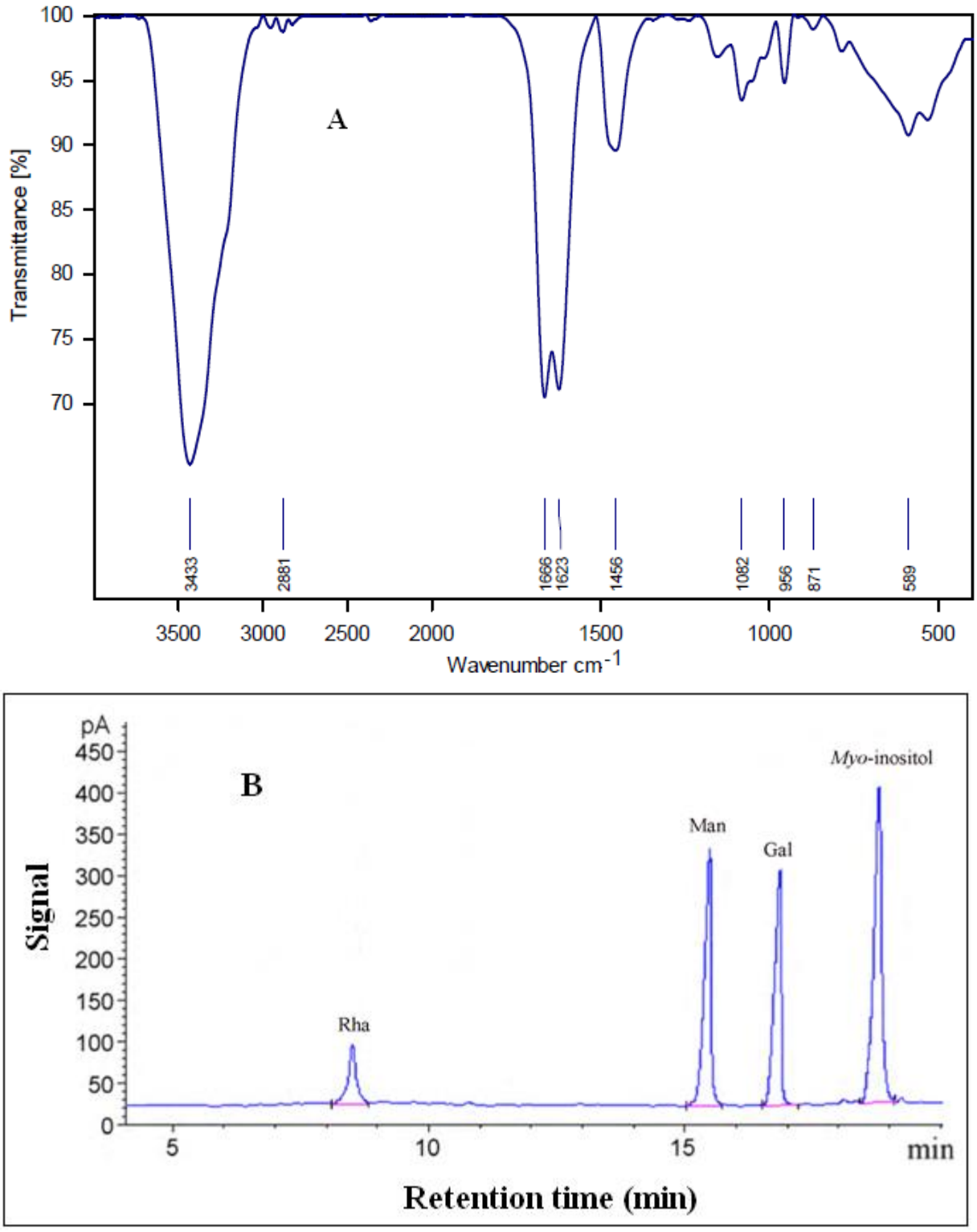

Figure 5. A) FT-IR spectrum and B) Gas chromatogram analysis of monosaccharide composition.

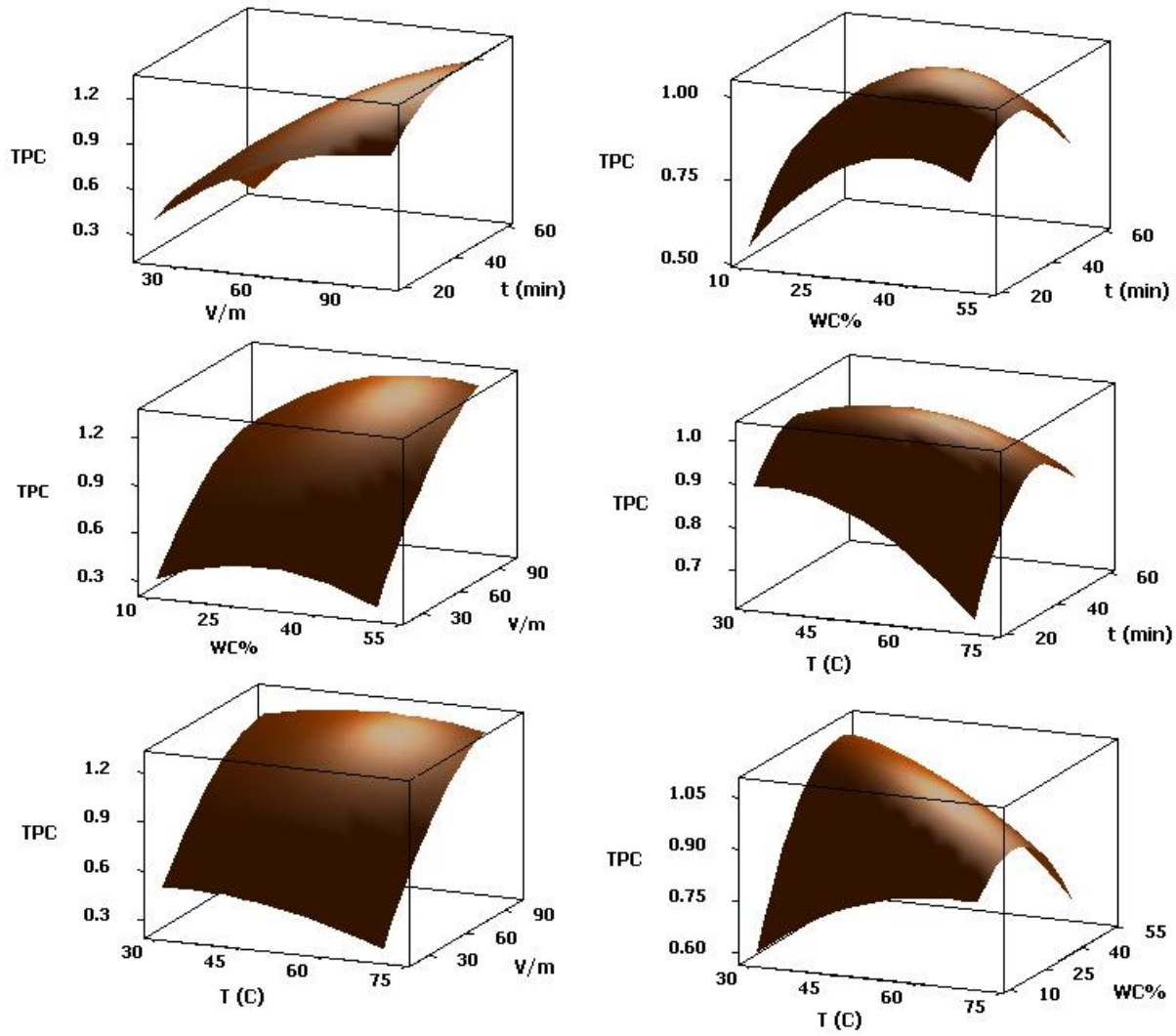

Figure 6. Response surface plots of TPC vs. extraction temperature (T), the water content in DES (WC), liquid/solid ratio (V/m), and extraction time (t).

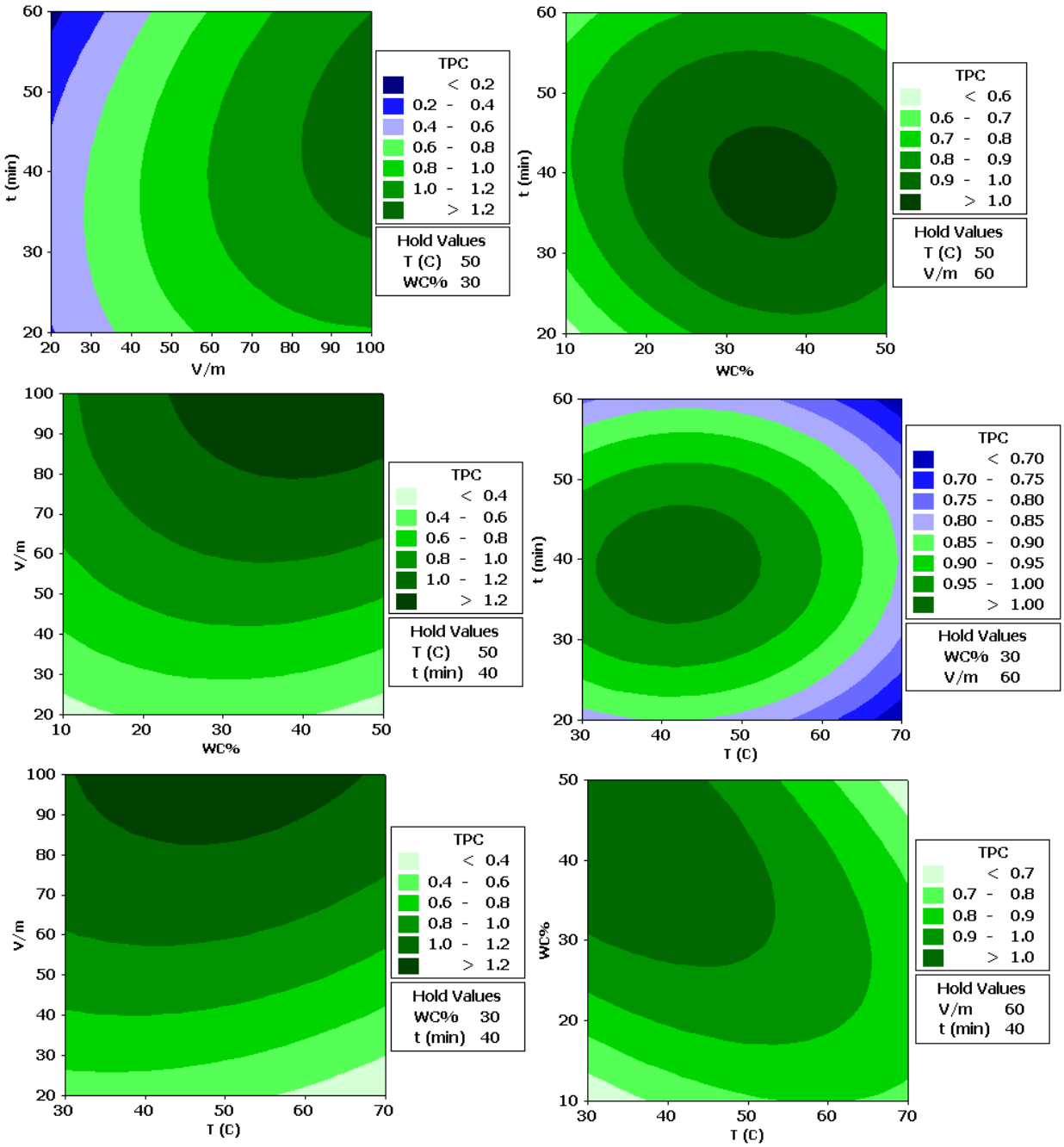

Figure 7. Contour plots of TPC *vs.* extraction variables.