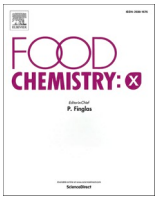
Food Chemistry: X 21 (2024) 101083

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Efficient extraction of pectic polysaccharides from thinned unripe kiwifruits by deep eutectic solvent-based methods: Chemical structures and bioactivities

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ARTICLE INFO

Keywords:
Thinned young kiwifruit
Deep eutectic solvent
Pectic polysaccharide
Structural feature
Biological function

ABSTRACT

To promote the potentially industrial applications of thinned unripe kiwifruits, two deep eutectic solvent-based methods, including deep eutectic solvent-assisted extraction (DAE) and microwave-assisted deep eutectic solvent extraction (MDE), were optimized for the extraction of polysaccharides from thinned unripe kiwifruits (YKP). Results showed that the yields of YKP-D prepared by DAE and YKP-DM prepared by MDE were extremely higher than YKP-H prepared by hot water extraction. Furthermore, YKP-H, YKP-D, and YKP-DM were mainly composed of pectic polysaccharides, including homogalacturonan (HG) and rhamnogalacturonan I (RG I) domains. Besides, both YKP-D and YKP-DM exhibited stronger antioxidant, anti-glycosylation, and immunomodulatory effects than those of YKP-H, and their higher contents of uronic acids and bound polyphenols as well as lower molecular weights could partially contribute to their bioactivities. Overall, these results revealed that the developed MDE method could be utilized as a promising method for highly efficient extraction of YKP with superior beneficial effects.

1. Introduction

Kiwifruit (*Actinidia* spp.) is a commercially important fruit crop, which is very popular world widely because of its attractive flavor and distinctively nutritional value (Li et al., 2023; Wang et al., 2021). It is widely planted around the world, especially in China, and China accounts for more than 50 % of the world's production and ranks first in terms of the planted area (Li et al., 2023). Indeed, the species with highly

Abbreviations: ABTS, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); AG, aminoguanidine; AGEs, advanced glycation end-products; BBD, Box-Behnken design; BHT, butylated hydroxytoluene; DAE, deep eutectic solvent-assisted extraction; DES, deep eutectic solvent; DMEM, Dulbecco's modified eagle medium; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric-reducing antioxidant power; FT-IR, fourier transform infrared spectroscopy; HG, homogalacturonan; HPLC, high performance liquid chromatography; HWE, hot water extraction; IL-6, interleukin-6; MAE, microwave-assisted extraction; MDE, microwave-assisted deep eutectic solvent extraction; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide; NMR, nuclear magnetic resonance spectroscopy; NO, nitric oxide; PMP, 1-phenyl-3-methyl-5-pyrazolone; RG I, rhamnogalacturonan I; *SEC*, size exclusion chromatography; *SEC*-MALLS-RID, size exclusion chromatography coupled with a multi angle laser light scattering and a refractive index detector; SFD, single-factor experimental design; TNF-α, tumor necrosis factor-alpha; UAE, ultrasound-assisted extraction; UDE, ultrasound-assisted deep eutectic solvent extraction; YKP, polysaccharides from thinned unripe kiwifruits; YKP-H, polysaccharides extracted from thinned unripe kiwifruits by hot water extraction; YKP-D, polysaccharides extracted from thinned unripe kiwifruits by deep eutectic solvent-assisted extraction; YKP-DM, polysaccharides extracted from thinned unripe kiwifruits by microwave-assisted deep eutectic solvent extraction.

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https://doi.org/10.1016/j.fochx.2023.101083
Received 16 October 2023; Received in revised form 13 December 2023; Accepted 15 December 2023
Available online 17 December 2023

commercial value, including *A. chinensis* and *A. deliciosa*, are widely planted in China (Li et al., 2023; Wang et al., 2021). It is widely believed that the dietary intake of kiwifruits can be a good choice for the prevention and management of oxidative damages and metabolic disorders (Li et al., 2023; Wang et al., 2021). In fact, kiwifruit exhibits multiple beneficial effects, e.g., antioxidant, immunomodulatory, hypoglycemic, and anti-obesity effects, which are closely associated with its abundant nutrients and bioactive components (e.g., polyphenols and pectic polysaccharides) (Li et al., 2023; Wang et al., 2021). Generally, pectic polysaccharides have been considered one of the most important bioactive components in kiwifruits (Li et al., 2023), which are primarily consisted of galacturonic acid, galactose, arabinose, and rhamnose (Wu et al., 2019; Yuliarti et al., 2015; Yuliarti et al., 2012). These pectic polysaccharides possess diverse beneficial effects, such as antioxidant, antiglycation, immunomodulatory, and prebiotic effects, which can be utilized as functional food ingredients or fortified ingredients (Li et al., 2023).

Generally, to promote the quality and yield of kiwifruit, fruit thinning is commonly carried out by fruit farmers during its growing period (Jiao et al., 2019), and approximate thirty percentages or even one half of unripe kiwifruits will be removed. Thinned unripe kiwifruits usually possess a low maturity, and are not suitable for direct consumption. Therefore, they are usually discarded in the orchard (Jiao et al., 2019), causing a severe pollution of environment and a waste of resource. In fact, like mature kiwifruits, thinned unripe kiwifruits also contain abundant nutrients and bioactive components (e.g., polyphenols and polysaccharides). A previous study has demonstrated that thinned unripe kiwifruits contain more abundant polyphenols than mature kiwifruits (Jiao et al., 2019). Nevertheless, studies on polysaccharides from thinned unripe kiwifruits and their potentially industrial applications in the food science are limited. Hence, to improve their utilization as functional food ingredients and functional foods in the food industry, the studies on the extraction, characterization, and beneficial effects of polysaccharides from thinned unripe kiwifruits are urgent required.

Conventional hot water extraction (HWE) is widely applied as a traditional method for the preparation of natural polysaccharides from fruits and vegetables. However, HWE always possesses a low extraction efficiency (Han et al., 2019). In recent years, the deep eutectic solvent (DES) has gained much attention to be utilized as a new and green solvent for the extraction of polysaccharides because of its distinctive extraction efficiency (Shafie et al., 2019; Wu et al., 2021; Zhang & Wang, 2017). Indeed, studies have revealed that natural polysaccharides prepared by deep eutectic solvent-assisted extraction (DAE) exhibit higher yields and stronger biological activities than those of traditional extraction methods (Wu et al., 2021; Zhang & Wang, 2017; Zou et al., 2022). For instance, the extraction yield of *Zizyphus jujube* polysaccharide extracted by the DAE method (6.36 %) was about 2.04 times than that of *Zizyphus jujube* polysaccharide extracted by the HWE method (3.12 %) (Zou et al., 2022). In addition to the extraction solvent, some physical auxiliary extraction techniques, such as microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), have also been utilized to enhance the extraction efficiency of natural polysaccharides (Han et al., 2019). Recently, both microwave-assisted deep eutectic solvent extraction (MDE) and ultrasound-assisted deep eutectic solvent extraction (UDE) have been developed for the efficient extraction of natural polysaccharides. Compared with conventional extraction methods, both MDE and UDE possess shorter extraction time, higher extraction efficiency, and lower energy consumption (Shang et al., 2021; Wu et al., 2022a; Zhang & Wang, 2017). Therefore, we hypothesized that DES-based extraction methods could be utilized as highly efficient techniques for the preparation of polysaccharides from fruits and their by-products with superior biological activities.

To date, both DAE and MDE methods have never been performed for extracting polysaccharides from thinned unripe kiwifruits (YKP). In addition, the impacts of different extraction techniques (e.g., HWE, DAE, and MDE) on the chemical structures and beneficial effects of YKP were still unclear. Therefore, the extraction conditions of DAE and MDE were first optimized. Afterward, the structural features and biological activities of YKP prepared by different techniques were studied. The findings could afford some new insights into the development of thinned unripe kiwifruits and YKP as value-added functional products.

2. Materials and methods

2.1. Materials and chemical reagents

Unripe fruits of *A. chinensis* cv 'HY' were thinned 40 days after fruit-set, which were cultivated in Deyang Kiwifruit Planting Base, Sichuan, China (GPS coordinate 104°9′17″E, 31°23′47″N). After washing and removing the branches and leaves, thinned unripe kiwifruits were freeze-dried at – 40 ℃ for 48 h.

2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), potassium ferricyanide, trichloroacetic acid, and aminoguanidine (AG), 1-phenyl-3-methyl-5-pyrazolone (PMP), and Griess reagent were obtained from Sigma-Aldrich (St. Louis, MO, USA). Thermo-stable α-amylase, glucoamylase, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT), Dulbecco's modified eagle (DMEM) medium, tumor necrosis factor-alpha (TNF-α) ELISA Kit, and interleukin-6 (IL-6) ELISA Kit were obtained from Solarbio Life Sciences (Beijing, China) and Elabscience (Wuhan, China).

2.2. Preparation of YKP by HWE

Preparation of YKP by HWE was conducted as previously described (Han et al., 2019). The sample powder (10.0 g) was merged with 80 % of ethanol (1: 10, *w/v*) to remove ethanol-soluble components by using an ultrasonic cleaning tank at 480 W and 25 ℃ for 20 min (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China). Subsequently, polysaccharides in the residues were extracted with deionized water (1: 30, *w/v*) at 95 ℃ for 4 h. Afterward, the supernatant was evaporated to about 100 mL by a rotary evaporator (Shanghai YaRong Biochemistry Instrument Co., Ltd., Shanghai, China). Furthermore, the thermo-stable α-amylase (5 U/mL, 85 ℃, and 8 h) and glucoamylase (5 U/mL, 59 ℃, and 10 h) were sequentially added to de-starch. After the removal of starch, both fractional ethanol-precipitation and membrane isolation techniques were carried out for the partial separation of polysaccharides. The final concentration of ethanol in the extracted supernatant was set as 70 % (*v/v*), and then the precipitates were redissolved in deionized water (50 mL), and the supernatant was ultra-filtered by using an ultrafiltration device with a molar mass cutoff of 3.5 kDa for three times (Amicon ultra-15 centrifugal filter, Merck KGaA, Darmstadt, Germany). Finally, polysaccharides extracted from thinned unripe kiwifruits by HWE were collected by freeze-drying at –40 ℃ for 48 h, and named YKP-H.

2.3. Preparation of YKP by DAE

DES solution was consisted of choline chloride (ChCl) and ethylene glycol (EG) in a molar ratio of 1: 3, which was prepared as previously described (Wu et al., 2021). A single-factor experimental design (SFD) was firstly carried out for the optimization of the DAE conditions. The extraction parameters, including water content (15, 30, 45, 60, and 75 %) in DES solution, extraction time (120, 150, 180, 210, and 240 min), and liquid–solid ratio (20, 30, 40, 50, and 60 mL/g), were optimized. The extraction temperature was set as 95 ℃ based on a previous study (Wu et al., 2021). After the extraction, the following steps for removing starch and partial isolation of polysaccharides were carried out in accordance with Section 2.2, and polysaccharides extracted from thinned unripe kiwifruits by DAE was named YKP-D. Furthermore, a three-factor Box-Behnken design (BBD) with three levels was employed to further optimize the DAE conditions. The independent parameters contained extraction time (X_{A1}, 180, 210, and 240 min), liquid–solid

ratio (X_{A2}, 40, 50, and 60 mL/g), and water content (X_{A3}, 45, 60, and 75 %) in DES solution. A total of 17 experiments were designed in this study, and the response surface factors were shown in Table S1. The data were analyzed by using Design-Expert software. The data were fitted to a second-order polynomial model as follows:

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_i X_j$$

where Y is the yield of YKP; X_i and X_j are different variables; A_0, A_i, A_{ii}, and A_{ij} are the regression coefficients.

2.4. Preparation of YKP by MDE

The DES solution was the same as mentioned in Section 2.3. The MDE was carried out by using a laboratory microwave oven (MKX-H1C1A, MAKEWAVE, Qingdao, China). The SFD was also firstly applied. The extraction parameters, including water content (15, 30, 45, 6, and 75 %) in DES solution, extraction power (400, 480, 560, 640, and 720 W), and extraction time (6, 12, 18, 24, and 30 min), were optimized. The extraction temperature and liquid–solid ratio were set as 95 ℃ and 50 mL/g based on the optimal extraction conditions of the DAE method. After the extraction, the following steps for removing starch and partial isolation of polysaccharides were also carried out following Section 2.2, and polysaccharides extracted from thinned unripe kiwifruits by the MDE method was named YKP-DM. Furthermore, a BBD was also conducted to further optimize the extraction conditions of MDE. The independent variables included extraction time (X_{B1}, 12, 18, and 24 min), extraction power (X_{B2}, 560, 640, and 720 W), and water content (X_{B3}, 15, 30, and 45 %) in DES solution. The experimental design was also displayed in Table S1. The experimental results were also analyzed, and fitted to a second-order polynomial model as follows:

$$Y = B_0 + \sum_{i=1}^{3} B_i X_i + \sum_{i=1}^{3} B_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} B_{ij} X_i X_j$$

where Y is the yield of YKP; X_i and X_j are different parameters; B_0, B_i, B_{ii}, and B_{ij} are the regression coefficients.

2.5. Characterization of polysaccharides extracted from thinned unripe kiwifruits by different techniques

To study physicochemical properties of polysaccharides extracted from thinned unripe kiwifruits by different techniques (YKP-H, YKP-D, and YKP-DM), the contents of polysaccharides, uronic acids, proteins, and bound polyphenols in each sample were detected by colorimetric approaches according to previous studies (Hu et al., 2023; Wu et al., 2023). Furthermore, to compare structural features of polysaccharides extracted from thinned young kiwifruits by different techniques, the molecular weights, apparent viscosities, monosaccharide units, functional groups, and glycosidic linkages were measured in the present study. Briefly, their molecular weights were measured by size exclusion chromatography coupled with a multi angle laser light scattering and a refractive index detector (*SEC*-MALLS-RID, Wyatt Technology Co., Santa Barbara, CA, USA) as previously described (Wu et al., 2022b). Their apparent viscosities were determined by using a modular compact rheometer (MCR 302e, Anton Paar GmbH, Graz, Austria) as previously described (Wu et al., 2022b), and the rheological curves were generated over a shear rate range of 0.01 to 100 s^{-1}. Their monosaccharide units were determined by high performance liquid chromatography (HPLC, L-20A, Shimadzu, Japan) coupled with pre-column derivatization using PMP as previously reported (Hu et al., 2023). Their functional groups and esterification degrees were analyzed by fourier transform infrared spectroscopy (FT-IR, PerkinElmer, Waltham, MA, USA) according to a previous study (Hu et al., 2023). Their glycosidic linkages were analyzed by one-dimensional nuclear magnetic resonance spectroscopy (1D NMR (Bruker, Rheinstetten, Germany) as previously reported (Wu et al., 2023).

2.6. Evaluation of biological activities of polysaccharides extracted from thinned unripe kiwifruits by different techniques

Various beneficial effects (e.g., antioxidant, anti-glycosylation, and immunomodulatory effects) of polysaccharides extracted from thinned unripe kiwifruits by different techniques (YKP-H, YKP-D, and YKP-DM) were evaluated and compared by *in vitro* models as previously reported (Hu et al., 2023; Wu et al., 2022c; Wu et al., 2023). In brief, to compare their antioxidant effects, the ABTS and DPPH radical scavenging abilities as well as ferric-reducing antioxidant power (FRAP) were determined according to previous methods (Hu et al., 2023; Wu et al., 2023). To compare their anti-glycosylation effects, a BSA/Glc model was carried out for the measurement of their inhibitory effects against the formation of advanced glycation end-products (AGEs) (Wu et al., 2023). To compare their immunomodulatory effects, a RAW 264.7 cell model was applied for the determination of their effects on the cell viability and the release of nitric oxide (NO), IL-6, and TNF-α as previously reported (Hu et al., 2023; Wu et al., 2022c).

2.7. Statistical analysis

Experimental design and data analysis were performed by using Design Expert 11, IBM SPSS Statistics, and Origin 2022. One-way analysis of variance (one-way ANOVA) and student's *t*-test were applied to evaluate statistical significances ($p < 0.05$).

3. Results and discussion

3.1. Optimal extraction conditions of DAE and MDE methods

3.1.1. Optimal extraction conditions of the DAE method

Recent studies have revealed that DES can be utilized as a new and green solvent for the highly efficient extraction of natural polysaccharides (Shafie et al., 2019; Wu et al., 2021; Zhang & Wang, 2017). Usually, the water content in DES solution has been considered one of the most important extraction parameters (Wu et al., 2021), which can change the viscosity and polarity of DES, thereby resulting in different extraction efficiencies of natural polysaccharides. As shown in Fig. 1A, the water content in DES solution significantly influenced the extraction yields of YKP. As the increase in water contents ranged from 15 % to 60 %, the yields of YKP increased. Nevertheless, as the further increase in water content, the yields of YKP declined. This result was similar to previous studies that the low water content could make DES difficult to penetrate into the internal structure of the sample due to its high viscosity, while an excessive water content could hinder the interactions between polysaccharides and DES (Shang et al., 2021; Wu et al., 2021; Zhang & Wang, 2017). Therefore, an appropriate water content in DES solution could facilitate the dissolution of natural polysaccharides. The optimal water content was determined to be 60 %. Besides, according to the SFD analysis, the optimal extraction time and liquid–solid ratio were measured to be 210 min and 50 mL/g, respectively.

Furthermore, according to the BBD matrix and experimental results (Table S1), the corresponding quadratic polynomial equation was obtained, and the equation was as follows:

$$\begin{aligned} Y = & -50.38 + 0.32X_{A1} + 0.58X_{A2} + 0.34X_{A3} + 0.00015X_{A1}X_{A2} \\ & - 0.000561X_{A1}X_{A3} - 0.0007X_{A2}X_{A3} - 0.000667X_{A1}^2 - 0.005583X_{A2}^2 \\ & - 0.001559X_{A3}^2 \end{aligned}$$

where Y is the predicted value of YKP, X_{A1}, X_{A2}, and X_{A3} are extraction time (min), liquid–solid ratio (mL/g), and water content in DES solution (%), respectively.

As displayed in Table 1, results revealed that the fitted model was

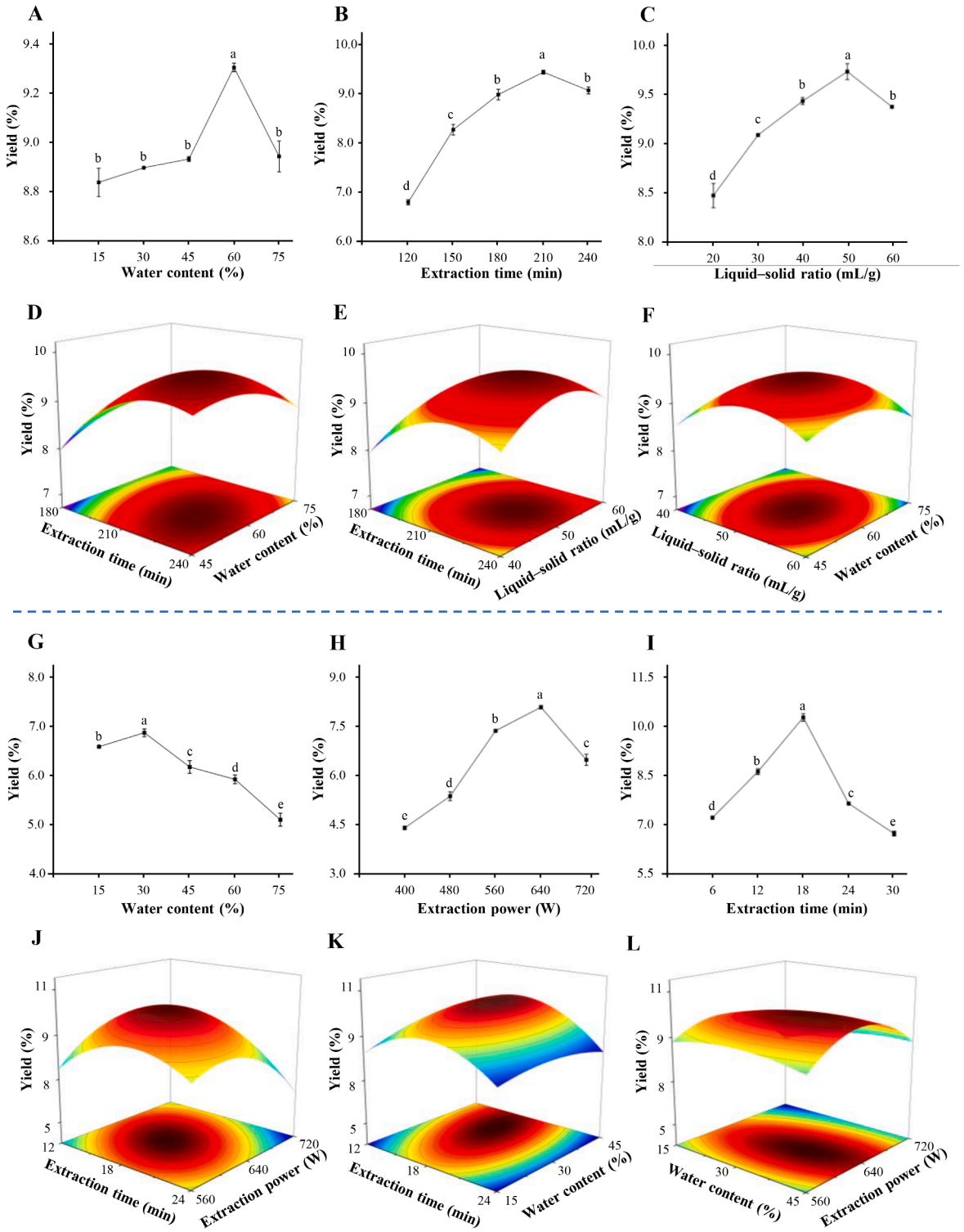


Fig. 1. Effects of extraction parameters on the yields of polysaccharides extracted from thinned unripe kiwifruits. A, B, and C indicate the effects of water content in DES solution, extraction time, and liquid–solid ratio on the yields of polysaccharides extracted by deep eutectic solvent-assisted extraction, respectively; D, E, and F indicate interactions among water content in DES solution, extraction time, and liquid–solid ratio on the yields of polysaccharides extracted by deep eutectic solvent-assisted extraction. G, H, and I indicate the effects of water content in DES solution, extraction power, and extraction time on the yields of polysaccharides extracted by microwave-assisted deep eutectic solvent extraction; J, K, and L indicate interactions among water content in DES solution, extraction power, and extraction time on the yields of polysaccharides extracted by microwave-assisted deep eutectic solvent extraction.

highly significant ($p < 0.0001$), indicating that it could effectively explain the variation in the yields of YKP (Wu et al., 2021; Wu et al., 2022a). Additionally, the lack of fit was not statistically significant, indicating that the fitted model could adequately predict the yields of YKP (Wu et al., 2021; Wu et al., 2022a). The values of R^2 (0.9989) and R^2_{adj} (0.9976) were extremely similar, suggesting that the predicted value was extremely close to the detected value. Furthermore, it could confirm that the fitted model was repeatable and reliable according to

Table 1
Variance analysis of regression models for deep eutectic solvent-assisted extraction (DAE) and microwave-assisted deep eutectic solvent extraction (MDE).

	Deep eutectic solvent-assisted extraction					Microwave-assisted deep eutectic solvent extraction				
	Sum of squares	**df**	**Mean square**	***F*-value**	***p*-value**	**Sum of squares**	**df**	**Mean square**	***F*-value**	***p*-value**
Model	5.51	9	0.6122	739.55	< 0.0001**	21.48	9	2.39	39.24	< 0.0001**
X_{A1} (X_{B1})	1.4	1	1.4	1694.51	<0.0001**	0.8269	1	0.8269	13.75	0.0076**
X_{A2} (X_{B2})	0.0761	1	0.0761	91.86	<0.0001**	1.3	1	1.3	21.62	0.0023**
X_{A3} (X_{B3})	0.0055	1	0.055	6.66	0.0364*	0.5207	1	0.5207	8.66	0.0216*
$X_{A1}X_{A2}$ ($X_{B1}X_{B2}$)	0.0081	1	0.0081	9.78	0.0167*	2.31	1	2.31	38.47	0.0004**
$X_{A1}X_{A3}$ ($X_{B1}X_{B3}$)	0.255	1	0.255	308.05	<0.0001**	0.4583	1	0.4583	7.62	0.0281*
$X_{A2}X_{A3}$ ($X_{B2}X_{B3}$)	0.441	1	0.441	53.27	0.0002**	0.4206	1	0.4206	6.99	0.0332*
X_{A2}^2 (X_{B2}^2)	1.52	1	1.52	1835.56	< 0.0001**	6.17	1	6.17	102.62	< 0.0001**
X_{A2}^2 (X_{B2}^2)	1.31	1	1.31	1585.03	< 0.0001**	7.49	1	7.49	124.48	< 0.0001**
X_{A2}^2 (X_{B2}^2)	0.518	1	0.518	625.71	<0.0001**	0.6681	1	0.6681	11.11	0.0125*
Residual	0.0058	7	0.0008			0.4209	7	0.0601		
Lack of fit	0.0047	3	0.0016	5.57	0.0653	0.1969	3	0.0656	1.17	0.4247
Pure error	0.0011	4	0.0003			0.224	4	0.056		
Correlation total	5.52	16				21.91	16			

X_{A1}, extraction time (min); X_{A2}, liquid–solid ratio (mL/g); X_{A3}: water content in DES solution (%, *v/v*);
X_{B1}, extraction time (min); X_{B2}, extraction power (W); X_{B3}: water content in DES solution (%, *v/v*);
DAE, $R^2 = 0.9989$, $R^2_{adj} = 0.9976$, coefficient of variation (*CV*) = 0.3261 %, and adeq. precision = 73.8756;
MDE, $R^2 = 0.9808$, $R^2_{adj} = 0.9561$, coefficient of variation (*CV*) = 2.88 %, and adeq. precision = 21.4226.
Significant differences are shown by * $p < 0.05$, ** $p < 0.01$.

the values of the coefficient variation and the adequate precision (Wu et al., 2021; Wu et al., 2022a). As shown in Table 1, the linear coefficients of X_{A1}, X_{A2}, and X_{A3}, interaction coefficients of $X_{A1}X_{A2}$, $X_{A1}X_{A3}$, and $X_{A2}X_{A3}$, and quadratic term coefficients of X_{A1}^2, X_{A2}^2, and X_{A3}^2 of the fitted model were significant with *p*-values lower than 0.05, suggesting that these extraction parameters had notably impacts on the yields of YKP. Indeed, the three-dimensional response surface plots could also obviously reveal that the interactions between extraction time and water content, extraction time and liquid–solid ratio, and liquid–solid ratio and water content were significant (Fig. 1D, E, and F) (Wu et al., 2021; Wu et al., 2022a).

Furthermore, the predicted optimal extraction conditions of DAE were determined to be as follows: water content of 57.05 %, liquid–solid ratio of 51.22 mL/g, and extraction time of 221.83 min. Under the validation conditions (water content of 57 %, liquid–solid ratio of 51 mL/g, and extraction time of 222 min), the actual yield of YKP was measured to be 9.65 % ± 0.05 %, which was very close to the predicted value of 9.625 %. Collectively, these results suggested that the fitted model of the DAE method was accurate and adequate in this study. Moreover, the extraction yield (9.65 % ± 0.05 %) of YKP prepared by the DAE method was more than two times higher than that of the HWE method (4.22 % ± 0.15 %). Nevertheless, although the extraction yield of the DAE method was very high, its extraction time was too long (222 min), which could limit its industrial applications. Thus, to reduce the extraction time and maintain the high extraction yield, the MDE method was further conducted in this study.

3.1.2. Optimal extraction conditions of the MDE method

Usually, compared with conventional extraction methods, the MAE method always has the strengths of shorter time and higher extraction efficiency (Han et al., 2019; Shang et al., 2021; Wu et al., 2022a). Generally, the extraction parameters of MDE, e.g., extraction time, extraction power, and water content in DES solution, can obviously affect the extraction yield (Shang et al., 2021; Wu et al., 2022a). Fig. 1 also shows the effects of water content in DES solution, extraction power, and extraction time on the yield of YKP. As shown in Fig. 1G, the optimal water content of MDE was determined to be 30 %, which was quite different from that of DAE. Besides, as displayed in Fig. 1H, the extraction yields of YKP increased as the microwave power increased from 400 W to 640 W. However, excessive microwave power may cause the degradation of YKP, resulting in a low yield of YKP at 720 W (Han et al., 2019; Wu et al., 2022a). Besides, the optimal extraction time was determined to be 18 min. Moreover, the BBD data were also presented in Table S1, and the corresponding quadratic polynomial equation was as follows:

$$Y = -99.33 + 2.28X_{B1} + 0.28X_{B2} + 0.018X_{B3} - 0.001584X_{B1}X_{B2} - 0.003761X_{B1}X_{B3} + 0.00027X_{B2}X_{B3} - 0.03363X_{B1}^2 - 0.000208X_{B2}^2 - 0.00177X_{B3}^2$$

The predicted yield of YKP (Y, %) was modeled by using factors including microwave time (X_{B1}, min), microwave power (X_{B2}, W), and water content in DES solution (X_{B3}, %, *v/v*).

Furthermore, according to the *P* value (<0.0001) and *F* value (39.24), the fitted model could well clarify the variation in the yields of YKP (Wu et al., 2021; Wu et al., 2022a). Besides, based on the values of the lack of fit, the R^2 (0.9808) and the R^2_{adj} (0.9561) (Table 1), the fitted model could precisely predict the yields of YKP (Wu et al., 2021; Wu et al., 2022a). In addition, based on the values of the coefficient variation and the adequate precision, the fitted model possessed good repeatability and reliability (Wu et al., 2021; Wu et al., 2022a). Furthermore, the linear, interaction, and quadratic term coefficients of all extraction factors were also significant (Table 1), indicating that all independent parameters could significantly influence the extraction rates of YKP. Indeed, the three-dimensional response surface plots also indicated that the interactions between microwave time and microwave power, microwave time and water content, and microwave power and water content were significant (Fig. 1J, K, and L) (Wu et al., 2021; Wu et al., 2022a). All data showed that the microwave time, microwave power, and water content were the decisive parameters for the YKP extraction.

Based on the analysis of the experimental results, the predicted optimal extraction conditions were determined as follows: microwave time of 17.01 min, microwave power of 635.23 W, and water content of 35.51 %. Under the validation conditions (extraction time of 17 min, extraction power of 635 W, and water content of 36 %), the actual extraction yield of YKP was measured to be 10.04 % ± 0.16 %, which was very close to the predicted value of 9.991 %. Besides, compared with the DAE method, the developed MDE method not only had a higher yield of YKP, but also possessed an extremely shorter extraction time (17 min). In addition, compared with different mature kiwifruits (e.g., *A. deliciosa* and *A. chinensis*, about 2.6 % – 5.4 %) (Carnachan et al., 2012; Han et al., 2019; Wu et al., 2019; Yuliarti et al., 2012), the thinned unripe kiwifruits contained more abundant non-starch polysaccharides (about 10.04 %), suggesting that the thinned unripe kiwifruits could be good resources of polysaccharides. Overall, all results suggested that the

developed MDE method could be utilized as a highly efficient method to prepare polysaccharides from thinned unripe kiwifruits.

3.2. Physicochemical properties and chemical structures of polysaccharides extracted from thinned unripe kiwifruits by different techniques

3.2.1. Comparison of chemical compositions of YKP-H, YKP-D, and YKP-DM

To well understand the impacts of different techniques on the physicochemical properties of YKP, the chemical compositions of YKP-H, YKP-D, and YKP-DM were determined and compared. Results revealed that different techniques could not only significantly affect the yields of YKP, but also notably influence its physicochemical properties (Table 2). In detail, it was shown that total polysaccharides in YKP-H, YKP-D, and YKP-DM ranged from 88.49 mg/100 mg (YKP-D) to 94.28 mg/100 mg (YKP-H), with the uronic acids in the range of 23.51 mg/100 mg (YKP-H) – 30.50 mg/100 mg (YKP-DM), suggesting that YKP-H, YKP-D, and YKP-DM were pectic polysaccharides, similar to those of mature kiwifruits (Han et al., 2019; Wu et al., 2019; Yuliarti et al., 2015; Yuliarti et al., 2012). Generally, the content of uronic acids can reflect the structural characteristics and functional properties of pectic polysaccharides, and a higher content of uronic acids can contribute to better bioactivities (Yang et al., 2022). Besides, only minor proteins were observed in YKP-H, YKP-D, and YKP-DM, with the contents in the range of 2.38 – 3.42 mg/100 mg, suggesting that the contribution of proteins to the functional properties of YKP could be ignored. Furthermore, few bound polyphenols were found in YKP-H, YKP-D, and YKP-DM, with the contents in the range of 1.01 (YKP-H) – 8.62 mg GAE/100 mg (YKP-D). Compared with the YKP-H, the higher contents of polyphenols were found in both YKP-D and YKP-DM, which could be attributed to the high solubility of polyphenols in the DES solution (Ruesgas-Ramón et al., 2017). Generally, the bound polyphenols are considered the partial chemical composition of plant dietary fibers (e.g., pectin and hemicellulose), which can partially contribute to their beneficial effects, such as antioxidant, antiglycation, prebiotic, and hypoglycemic effects (Huang et al., 2022; Liu et al., 2019).

Table 2
Chemical compositions, molecular weights, and monosaccharide units of polysaccharides extracted from thinned unripe kiwifruits by different techniques.

	YKP-H	YKP-D	YKP-DM
Extraction yields (%)	4.22 ± 0.15^{c}	9.65 ± 0.05^{b}	10.04 ± 0.16 ^{a}
Total polysaccharides (mg/100 mg)	94.28 ± 0.72 ^{a}	88.49 ± 1.30^{b}	92.53 ± 1.29 ^{ab}
Total uronic acids (mg/100 mg)	23.51 ± 0.81^{c}	26.52 ± 0.23^{b}	30.50 ± 1.63 ^{a}
Total proteins (mg/100 mg)	3.31 ± 0.41 ^{a}	2.38 ± 0.17^{b}	3.42 ± 0.43 ^{a}
Total polyphenols (mg GAE/100 mg)	1.01 ± 0.01^{c}	8.62 ± 0.22 ^{a}	6.64 ± 0.26^{b}
Degree of esterification (%)	46.29 ± 0.35 ^{a}	39.07 ± 0.47^{b}	32.68 ± 0.32^{c}
$M_w \times 10^5$ (Da)	6.081 ± 0.035 ^{a}	1.656 ± 0.016^{b}	1.265 ± 0.015^{c}
M_w/M_n	2.312	2.636	2.686
Monosaccharide units			
Galactose (Gal)	3.33	2.39	2.26
Arabinose (Ara)	1.99	1.23	0.94
Galacturonic acid (GalA)	1.00	1.00	1.00
Rhamnose (Rha)	0.29	0.24	0.30
Glucuronic acid (GlcA)	0.38	0.18	0.12
Mannose (Man)	0.49	0.23	0.16
Glucose (Glc)	0.21	0.15	0.26

YKP-H, YKP-D, and YKP-DM indicate polysaccharides extracted from thinned unripe kiwifruits by hot water extraction, deep eutectic solvent-assisted extraction, and microwave-assisted deep eutectic solvent extraction, respectively; Superscripts (a–c) differ significantly ($p < 0.05$) among YKP-H, YKP-D, and YKP-DM.

3.2.2. Comparison of physical properties of YKP-H, YKP-D, and YKP-DM

Physical properties, e.g., particle size, molecular weight, and rheological property, can determine the biological functions and processing characteristics of natural polysaccharides. As shown in Fig. 2, molecular masses and apparent viscosities of YKP-H, YKP-D, and YKP-DM were determined. It could be observed that the size exclusion chromatography (*SEC*) profile of YKP-H was different from those of YKP-D and YKP-DM (Fig. 2A). Obviously, the proportion of high molecular weight fraction (eluted from about 15 min to 17.5 min) in YKP-H was higher than those in YKP-D and YKP-DM. In fact, the molecular weight of YKP-H (6.081×10^5 Da) was also significantly higher than those of YKP-D (1.656×10^5 Da) and YKP-DM (1.265×10^5 Da), indicating that different extraction techniques could impact their molecular weights (Han et al., 2019; Wu et al., 2022a; Yuliarti et al., 2015). Furthermore, the polydispersity (M_w/M_n) of YKP-H, YKP-D, and YKP-DM was in the range of 2.312 – 2.686, suggesting that they exhibited a relatively broad distribution.

The difference found in the molecular weights of YKP-H, YKP-D, and YKP-DM may result in the difference in their rheological properties. So, their apparent viscosities were further studied. As displayed in Fig. 2B, the apparent viscosity of YKP-H was much higher than those of YKP-D and YKP-DM, similar to the order of their molecular weights, indicating that the molecular weight could affect their apparent viscosities (Wu et al., 2022a). In addition, both YKP-H and YKP-D exhibited non-Newtonian and Newtonian shear-thinning behaviors at low shear rates ($0 – 50\ s^{-1}$) and high shear rates ($50 – 100\ s^{-1}$), respectively. However, YKP-DM only exhibited a Newtonian fluid behavior, which might be due to the microwave-induced degradation of YKP-DM.

3.2.3. Comparison of chemical structures of YKP-H, YKP-D, and YKP-DM

As known to all, the chemical structures of natural polysaccharides play a crucial role in their biological functions and processing properties (Ferreira et al., 2015). To well understand the chemical structures of YKP-H, YKP-D, and YKP-DM, their monosaccharide units, functional groups, and glycosidic linkages were studied by HPLC, FT-IR, and 1D NMR analysis, respectively. Fig. 2C displayed the HPLC profiles of monosaccharide units of YKP-H, YKP-D, and YKP-DM. Their monosaccharide units were the same, suggesting that the primary chemical structure of YKP was overall stable under different extraction conditions. Seven monosaccharide units, including galactose (Gal), arabinose (Ara), galacturonic acid (GalA), mannose (Man), glucuronic acid (GlcA), rhamnose (Rha), and glucose (Glc), were found in YKP-H, YKP-D, and YKP-DM, and Gal, Ara, and GalA were determined as the major monosaccharide units (Table 2). Obviously, these polysaccharides extracted from thinned young kiwifruits were rich in pectic polysaccharides based on their monosaccharide units (Wu et al., 2021; Wu et al., 2019). Additionally, the major monosaccharide units in polysaccharides extracted from unripe kiwifruits were very similar to those in polysaccharides obtained from different mature kiwifruits (e.g., *A. deliciosa* and *A. chinensis*) (Wu et al., 2019). Generally, the basic monosaccharide units of homogalacturonan (HG) and rhamnogalacturonan I (RG I) pectic domains include GalA, Rha, GlcA, Gal, and Ara (Hu et al., 2023; Wu et al., 2022a). Hence, according to the monosaccharide units in YKP-H, YKP-D, and YKP-DM, it was suggested that both HG and RG I might exist in pectic polysaccharides extracted from thinned young kiwifruits (Carnachan et al., 2012; Wu et al., 2019). In fact, the major pectic polysaccharides in mature kiwifruits contain HG and RG I substituted with arabinan and arabinogalactan side-chains (Carnachan et al., 2012), suggesting that polysaccharides extracted from unripe kiwifruits possessed similar chemical structures to those of mature kiwifruits. In addition, the value of GalA/Rha (MR1 ratio) could reflect the ratio of HG and RG I in the complex pectic polysaccharides. The value of MR1 ratio of YKP-H, YKP-D, and YKP-DM was in the range of 3.33 – 4.17, suggesting that YKP were rich in both HG and RG I pectic domains. In

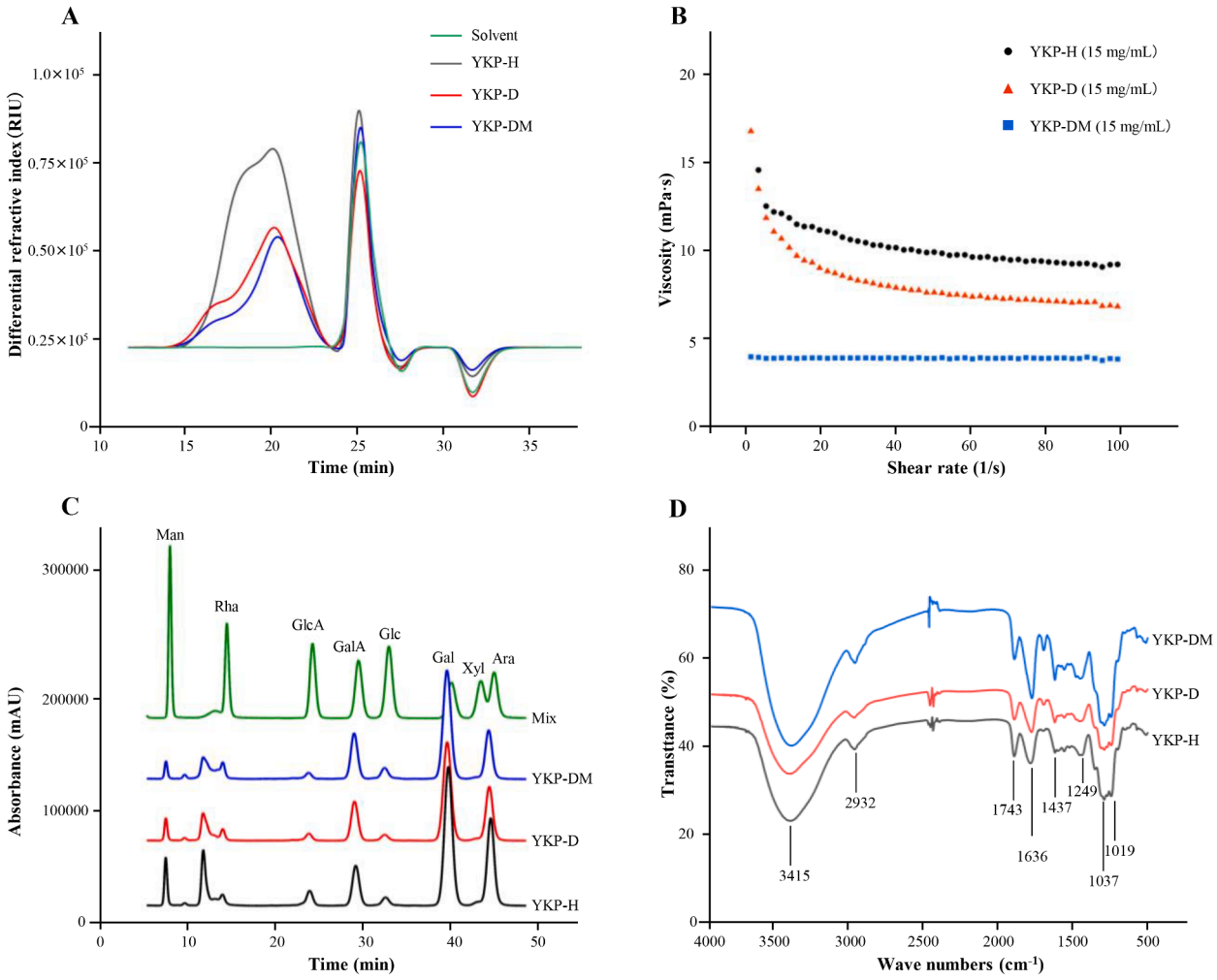


Fig. 2. Size exclusion profiles (A), apparent viscosities (B), monosaccharide units (C), and FT-IR spectra (D) of YKP-H, YKP-D, and YKP-DM. YKP-H, YKP-D, and YKP-DM indicate polysaccharides extracted from thinned unripe kiwifruits by hot water extraction, deep eutectic solvent-assisted extraction, and microwave-assisted deep eutectic solvent extraction, respectively.

addition, Man and Glc are basic monosaccharide units of hemicelluloses, e.g., xyloglucan and galactomannan (Wu et al., 2023). Thus, a small amount of galactomannan might also exist in YKP (Carnachan et al., 2012).

Fig. 2D shows the FT-IR spectra of YKP-H, YKP-D, and YKP-DM. Their FT-IR spectra were similar, with the typical signals of pectic polysaccharides, including 3415, 2932, 1743, 1636, 1437, 1249, 1037, and 1019 cm^{-1}, which indicated that their functional groups were similar. Especially, the signal at 1743 cm^{-1} corresponded to the asymmetric stretching vibration of carbonyl double bonds (C═O) of esterified carboxyl groups (Wu et al., 2019), while the signal at 1636 cm^{-1} is attributed to the asymmetric stretching vibration of carbonyl double bonds (C═O) of free carboxyl groups. Collectively, these observations indicated the existence of uronic acids in all samples, confirming that thinned young kiwifruits contain pectic polysaccharides (Wu et al., 2019). In addition, according to the peak areas of signals at 1743 cm^{-1} and 1636 cm^{-1} (Wu et al., 2022a), the esterification degrees of YKP-H, YKP-D, and YKP-DM were calculated to be 46.29 %, 39.07 %, and 32.68 %, respectively, indicating that the esterification degree of YKP could be obviously reduced by DES-based extraction methods.

Furthermore, Fig. 3 shows the ^{1}H and ^{13}C NMR spectra of YKP-H, YKP-D and YKP-DM. Results revealed that their 1D NMR spectra were also similar, further confirming that their primary chemical structures were overall stable under different extraction conditions. The typical ^{1}H NMR signals of pectic polysaccharides, including HG, RG I, and arabinogalactan, were observed, e.g., the ^{1}H NMR signals ranged from 5.10 to 5.44 ppm suggested the existence of the residues of α-L-Ara*f* and α-L-Rha*p*, the ^{1}H NMR signals ranged from 4.40 to 4.53 ppm indicated the existence of the residue of β-D-Gal*p*, and the ^{1}H NMR signals of 3.81 ppm and 4.97 ppm suggested the existence of the residue of GalAM*ep* (Guo et al., 2021; Redgwell et al., 2011; Wu et al., 2021; Wu et al., 2022a; Yao et al., 2021). In detail, the 1,4-α-D-GalAM*ep* residue was observed at 4.97 ppm, 170.59 ppm, and 100.35 ppm, and the GalA-OCH3 was also measured at 3.81 ppm and 52.78 ppm (Wu et al., 2021; Wu et al., 2023; Yao et al., 2021). Besides, the O-acetyl group was observed at 2.08 ppm and 19.87 ppm (Hu et al., 2023; Wu et al., 2023). The 1,2-α-L-Rha*p* and 1,2,4-α-L-Rha*p* residues were found at 5.32/1.25 ppm and 5.27/1.31 ppm, respectively (Yao et al., 2021). Therefore, results confirmed that HG and RG I pectic domains existed in YKP. Furthermore, the T-α-L-Ara*f* residue was found at 5.15 ppm and 107.38 ppm, and the 1,5-α-L-Ara*f*, 1,3-α-L-Ara*f*, and 1,3,5-α-L-Ara*f* residues were observed at 5.10 ppm, 5.44 ppm, and 5.24 ppm, respectively (Guo et al., 2022; Wu et al., 2021; Yao et al., 2021). Indeed, the C-1 signal of both 1,3-α-L-Ara*f* and 1,5-α-L-Ara*f* residues was observed at 109.30 ppm (Guo et al., 2021; Redgwell et al., 2011). Signals at 4.53 ppm and 103.10 ppm indicated the existence of 1,3-β-D-Gal*p*, and signals at 4.46 ppm and 103.22 ppm suggested the presence of 1,3,6-β-D-Gal*p* (Guo et al., 2021; Redgwell et al., 2011), and the signal at 4.40 ppm indicated the existence of T-β-D-Gal*p* (Yao et al., 2021). Therefore, these results indicated that both arabinan and arabinogalactan might exist as the side chains of

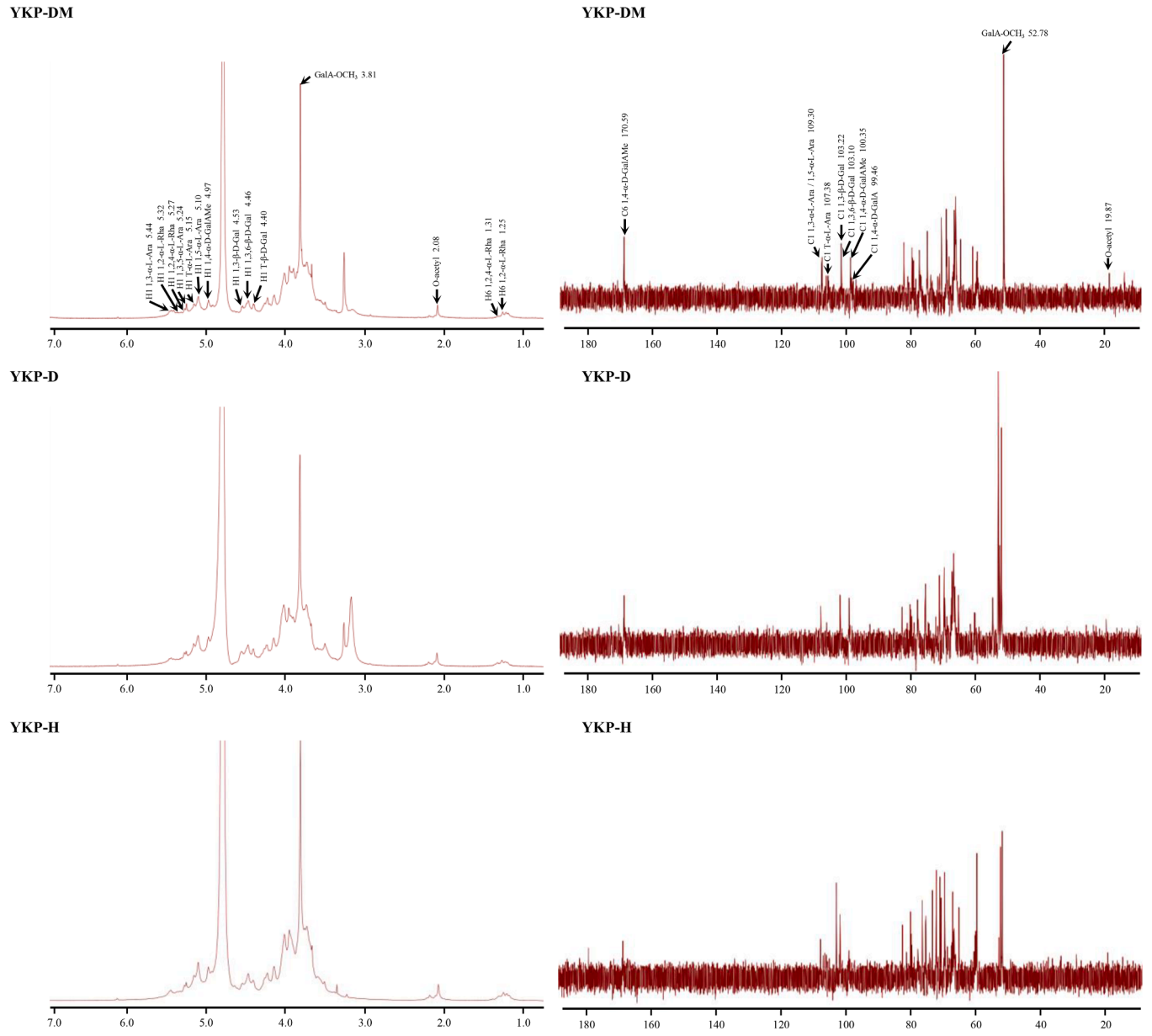


Fig. 3. ^{1}H NMR (left) and ^{13}C NMR (right) spectra of YKP-H, YKP-D, and YKP-DM. The codes (YKP-H, YKP-D, and YKP-DM) were the same as in Fig. 2.

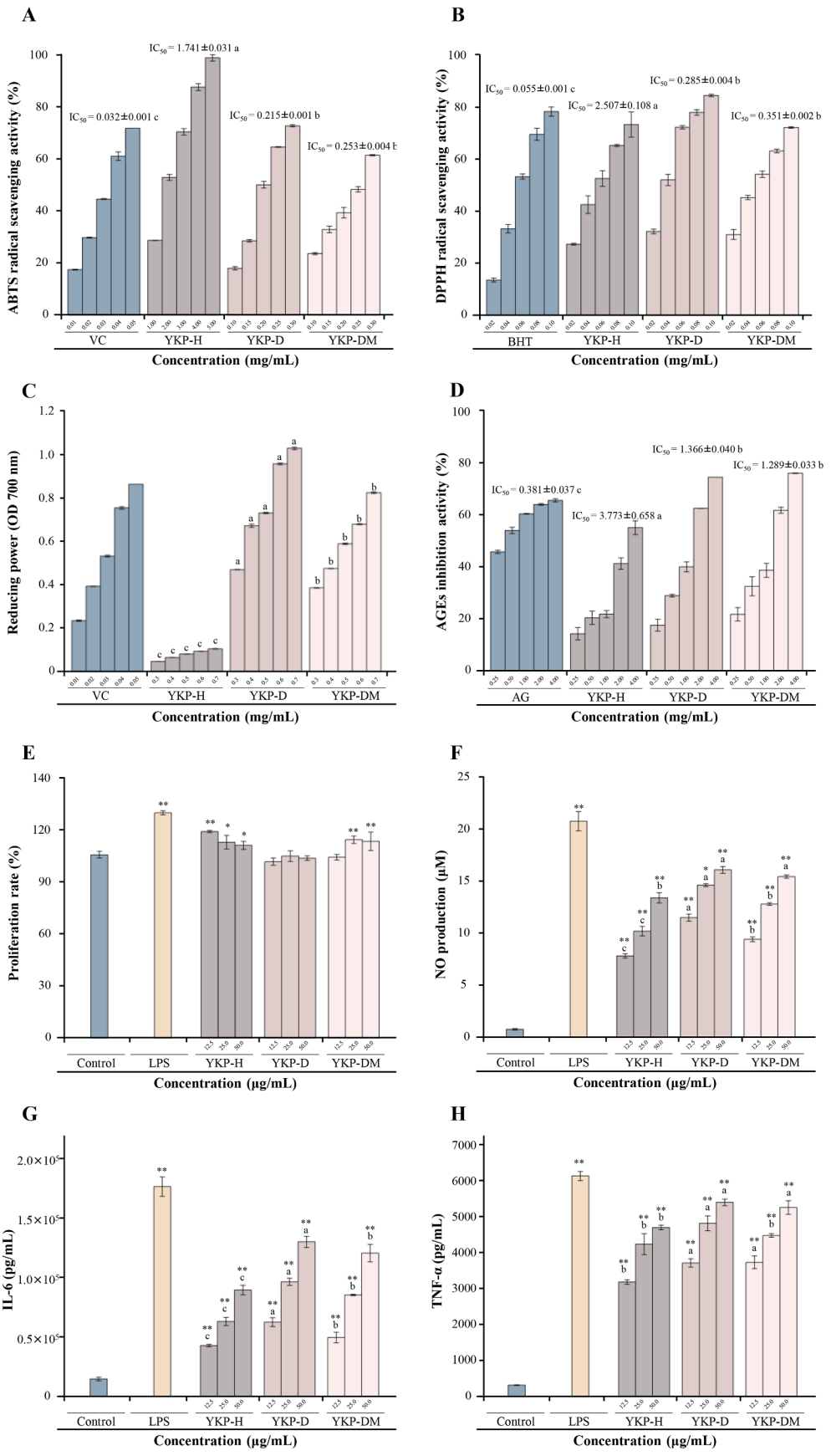
RG I pectic domain. Collectively, based on the results from monosaccharide analysis and NMR analysis, both HG and RG I with arabinan and arabinogalactan as side chains might exist as major pectic polysaccharides in YKP-H, YKP-D and YKP-DM. Previous studies have also demonstrated that the cell wall polysaccharide extracted from mature kiwifruits (e.g., *A. deliciosa* and *A. chinensis*) also contain HG and RG I pectic domains (Carnachan et al., 2012; Li et al., 2023).

3.3. Comparison of biological functions of YKP-H, YKP-D, and YKP-DM

3.3.1. In vitro antioxidant activity

Kiwifruits have been considered natural antioxidants due to the existence of a high content of vitamin C and polyphenolics (Li et al., 2023; Wang et al., 2021). Actually, apart from vitamin C and polyphenolics, kiwifruit polysaccharides have also been reported to exhibit strong antioxidant activity, which can partially contribute to their antioxidant capacities (Han et al., 2019; Li et al., 2023; Wu et al., 2019). However, the information about the antioxidant activity of YKP is still limited. In this study, the antioxidant activity of YKP-H, YKP-D, and YKP-DM was determined by evaluating their scavenging abilities against ABTS and DPPH free radicals as well as their ferric-reducing antioxidant powers.

Based on the results shown in Fig. 4, all tested samples exhibited notable antioxidant activities. Indeed, YKP-D and YKP-DM exhibited much higher antioxidant activity than that of YKP-H. In detail, the IC_{50} values of YKP-D and YKP-DM against ABTS and DPPH radicals were determined as 0.215 mg/mL and 0.253 mg/mL (Fig. 4A), and 0.285 mg/mL and 0.351 mg/mL (Fig. 4B), respectively, which were much lower than those of YKP-H (ABTS, 1.741 mg/mL; DPPH, 2.507 mg/mL). Besides, both YKP-D and YKP-DM exhibited much higher values of FRAP than that of YKP-H. In addition, the antioxidant activity of YKP-D was slightly higher than that of YKP-DM. Interestingly, the IC_{50} values of YKP-D and YKP-DM against ABTS and DPPH radicals were significantly lower than those of pectic polysaccharides extracted from different mature kiwifruits (IC_{50} values for ABTS, 1.26 mg/mL – 22.72 mg/mL; IC_{50} values for DPPH, about 2.33 mg/mL – 3.38 mg/mL) (Han et al., 2019; Wu et al., 2019), suggesting that pectic polysaccharides extracted from thinned unripe kiwifruits by DES-based extraction methods possessed good potentials to be utilized as natural antioxidants.



(caption on next page)

Fig. 4. *In vitro* antioxidant (A, B, and C), anti-glycosylation (D), and immunomodulatory effects (E, F, G, and H) of YKP-H, YKP-D, and YKP-DM. A, B, and C indicate ABTS and DPPH radical scavenging abilities as well as reducing powers of YKP-H, YKP-D and YKP-DM, respectively; D indicates the inhibitory effects of YKP-H, YKP-D and YKP-DM on the formation of AGEs; E indicates effects of YKP-H, YKP-D, and YKP-DM on the proliferation of RAW 264.7 cells; F, G, and H indicate effects of YKP-H, YKP-D, and YKP-DM on the NO production and the release of cytokines (IL-6 and TNF-α) from RAW 264.7 cells. The codes (YKP-H, YKP-D, and YKP-DM) were the same as in Fig. 2; Significant differences in antioxidant and anti-glycosylation effects ($p < 0.05$) among the positive control, YKP-H, YKP-D, and YKP-DM are shown by data bearing different letters (a–c); Significant differences in cell proliferation and productions of NO, IL-6, and TNF-α in LPS, YKP-H, YKP-D, and YKP-DM vs. control are shown by * $p < 0.05$, ** $p < 0.01$. Significant differences ($p < 0.05$) in productions of NO, IL-6, and TNF-α among YKP-H, YKP-D, and YKP-DM are also shown by data bearing different letters (a–c).

Furthermore, the physicochemical and structural properties, e.g., high content of uronic acids and bound polyphenols, low molecular mass, and low methylation degree, are always associated with a high antioxidant activity of complex pectic polysaccharides (Chen et al., 2021; Naqash et al., 2021; Wu et al., 2022c; Wu et al., 2023). Therefore, compared with YKP-H, the lower molecular weights, lower degrees of methylation, higher contents of uronic acids and bound polyphenolics observed in YKP-D and YKP-DM could contribute to their higher antioxidant activities.

3.4. *In vitro anti-glycosylation effect of YKP-H, YKP-D, and YKP-DM*

Advanced glycosylation end products (AGEs) are always associated with the development of oxidative stress response. Usually, natural pectic polysaccharides possess remarkable antioxidant activity, which can inhibit the generation of AGEs induced by the Maillard reaction (Jia et al., 2023). Previous studies have revealed that pectic polysaccharides from *Actinidia arguta* possess remarkable antioxidant activity and obvious anti-glycosylation effect (Zhu, Wang, et al., 2019; Zhu, Zhang, et al., 2019). In the present study, pectic polysaccharides from thinned unripe kiwifruits possessed obviously *in vitro* antioxidant capacities. So, the anti-glycosylation effects of YKP-H, YKP-D, and YKP-DM were investigated.

As displayed in Fig. 4D, compared with the positive control (AG, IC_{50} = 0.381 mg/mL), all tested samples displayed moderate inhibitory effects against the formation of AGEs induced by the Maillard reaction. In addition, YKP-D and YKP-DM displayed much stronger inhibitory effects against the formation of AGEs than that of YKP-H, similar to their antioxidant activities. More specifically, the IC_{50} values of YKP-D and YKP-DM against the formation of AGEs were calculated to be 1.366 mg/mL and 1.289 mg/mL (Fig. 4D), respectively, which were much lower than those of YKP-H (3.773 mg/mL). In fact, the complex pectic polysaccharides possess notable antioxidant activities, which can scavenge free radicals and active dicarbonyl compounds during the generation of AGEs induced by the Maillard reaction (Jia et al., 2023). Therefore, the higher inhibitory effects of YKP-D and YKP-DM on the formation of AGEs could be partially owing to their higher antioxidant activities. Furthermore, the inhibitory effects of YKP-D and YKP-DM on the formation of AGEs were similar to those of polysaccharides from *A. arguta* fruits (Zhu, Wang, et al., 2019; Zhu, Zhang, et al., 2019), blackberry fruits (Dou et al., 2021), and raspberry fruits (Yu et al., 2015). Overall, these results also suggest that the developed MDE method is a promising approach for highly efficient preparation of YKP with a remarkable anti-glycosylation effect.

3.5. *In vitro immunomodulatory effect of YKP-H, YKP-D, and YKP-DM*

Immune cell acts a critical role in the immune system, and macrophage acts a key role in innate and adaptive immune responses (Ferreira et al., 2015). When the body is invaded by pathogenic organisms, macrophages can regulate the immunity system via modulating the production of NO, IL-6, and TNF-α (Ferreira et al., 2015). Many studies have shown that polysaccharides can maintain human health by regulating the immune system (Ferreira et al., 2015; Zhao et al., 2020). Therefore, the immunomodulatory effects of YKP-H, YKP-D, and YKP-DM were investigated.

As displayed in Fig. 4E, YKP-H, YKP-D, and YKP-DM exhibited no cytotoxic effects on RAW 264.7 macrophages at concentrations ranging from 12.5 μg/mL to 50.0 μg/mL. Besides, all samples could stimulate the release of NO, IL-6, and TNF-α from immune cells at the tested concentrations (Fig. 4F, G, and H). Notably, different extraction techniques also significantly influenced the immunomodulatory effect of YKP. In detail, polysaccharides extracted from thinned unripe kiwifruits by DES-based methods also displayed significantly stronger immunomodulatory effects than that of traditional hot water extraction, similar to previous studies that polysaccharides prepared by DES-based methods possess higher bioactivities than those of conventional extraction methods (Wang et al., 2022; Wu et al., 2021). More specifically, at a concentration of 50.0 μg/mL, the levels of NO, IL-6, and TNF-α produced from macrophages induced by YKP-H were 13.57 μM, 8.29×10^4 pg/mL, and 0.49×10^4 pg/mL, respectively, which were obviously lower than those of YKP-D (16.29 μM, 12.04×10^4 pg/mL, and 0.57×10^4 pg/mL, respectively) and YKP-DM (15.64 μM, 11.16×10^4 pg/mL, and 0.55×10^4 pg/mL, respectively). In fact, many experimental results demonstrated that the physicochemical characteristics of pectic polysaccharides could influence their immunomodulatory effects (Ferreira et al., 2015; Zhao et al., 2020). Therefore, the differences observed in the immunomodulatory effects of YKP-H, YKP-D, and YKP-DM could be attributed to their diverse structures. Several studies have revealed that the immunomodulatory effects of pectic polysaccharides exhibit a positive correlation with their contents of uronic acids and a negative correlation with their molecular weights (Ketha & Gudipati, 2018; Wang et al., 2021; Yuan et al., 2020; Zhang et al., 2019). Therefore, the higher contents of uronic acids and lower molecular weights of YKP-D and YKP-DM might partially contribute to their stronger immunomodulatory effects. Nevertheless, more studies should be conducted to unveil their precise structure-independent relationship. Overall, these results indicate that the DES-based extraction methods are promising approaches for the preparation of YKP with high immunomodulatory effects, and YKP can be developed into natural immunomodulators for improving human health.

4. Conclusions

In this study, to improve the industrial applications of thinned unripe kiwifruits, two deep eutectic solvent-based extraction methods, including DAE and MDE, were optimized for extracting YKP. According to our results, both DAE and MDE methods achieved extremely high extraction yields of YKP (YKP-D, 9.65 %; YKP-DM, 10.04 %), which were more than two times higher than that of traditional HWE (YKP-H, 4.22 %). Especially, the MDE method possessed an extremely shorter extraction time (17 min) than the DAE method (222 min). Furthermore, different extraction methods obviously affected the chemical composition (e.g., uronic acids and bound polyphenols), molecular weight, and apparent viscosity of YKP, while the primary chemical structure of YKP was overall stable. YKP-H, YKP-D, and YKP-DM were mainly composed of complex pectic polysaccharides, including HG and RG I with arabinan and arabinogalactan as side chains. In addition, both YKP-D and YKP-DM exhibited stronger antioxidant, anti-glycosylation, and immunomodulatory effects than those of YKP-H, which might be partially attributed to the higher contents of uronic acids and bound polyphenols and lower molecular weights. Overall, these results suggest that the developed MDE method is a promising approach for the efficient extraction of bioactive YKP, and YKP can potentially serve as functional

food ingredients or health products.

CRediT authorship contribution statement

Ding-Tao Wu: Data curation, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – original draft. **Jin-Lei Geng:** Formal analysis, Investigation, Methodology, Writing – original draft, Validation. **Jie Li:** Formal analysis, Investigation, Validation. **Wen Deng:** Investigation, Validation. **Yao Zhang:** Resources, Validation. **Yi-Chen Hu:** Formal analysis, Resources. **Liang Zou:** Formal analysis, Resources. **Yu Xia:** Resources. **Qi-Guo Zhuang:** Resources, Writing – review & editing. **Hong-Yan Liu:** Formal analysis, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Ren-You Gan:** Formal analysis, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the Scientific Research Fund Projects of Science and Technology Department of Sichuan Province (No. 2022JDJQ0063 and No. 2023YFN0011), the Local Financial Funds of National Agricultural Science and Technology Center, Chengdu, China (No. NASC2021TD01), and the Agricultural Science and Technology Innovation Program of Chinese Academy of Agricultural Sciences, Chengdu, China (No. ASTIP-IUA-2023003).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101083.

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