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Effects of different extraction methods on the structural properties and bioactivities of polysaccharides extracted from Qingke (Tibetan hulless barley)

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ABSTRACT

In this study, four extraction methods, including ultrasonic assisted extraction (UAE), hot water extraction (HWE), microwave assisted extraction (MAE), and pressurized water extraction (PWE), were used to extract Qingke polysaccharides (THBs), and their physicochemical structures, *in vitro* antioxidant activities, and *in vitro* hypolipidemic activities were investigated and compared. Results demonstrated that the yields, the chemical compositions, the apparent viscosities, the molecular weights, and the molar ratios of constituent monosaccharides of THBs varied by different extraction methods. THB-P extracted by PWE possessed the highest molecular weight and apparent viscosity among all tested THBs. In addition, all THBs extracted by different methods exerted obvious *in vitro* antioxidant activities, *in vitro* binding capacities, and inhibitory effects on the pancreatic lipase. Indeed, both antioxidant activities and hypolipidemic activities of THB-P were higher than that of THB-H, THB-U, and THB-M obtained by HWE, UAE, and MAE, respectively, which might be due to the high molecular weight and apparent viscosity of THB-P. The findings indicated that the pressurized water extraction could be a convenient method for the extraction of polysaccharides from Qingke with high biological activities for applications in the functional food fields.

1. Introduction

Hordeum vulgare Linn. var. *nudum* Hook.f. (Qingke, Tibetan hulless barley) is an important cereal crop and a feed crop in the Tibetan Plateau (Lin et al., 2018a,b; Zhu et al., 2015). It has also been explored as functional food ingredients due to its beneficial effects in recent years. Numerous researches have indicated that the regular intake of barley flour can decrease the risks of diabetes, hyperlipidemia, and hypertension (Idehen et al., 2017; Lin et al., 2018a,b). Generally, β-glucans are regarded as one of the most crucial bioactive ingredients in Qingke polysaccharides (Tong et al., 2015; Zhang et al., 2018). β-glucans exert significant physiological effects in decreasing glycemic response, serum lipids, and plasma cholesterol (Bae et al., 2010; Drozdowski et al., 2010; Tong et al., 2015). β-glucans also have various important biological activities, including anti-hyperlipidemic, anti-hyperglycemia, anti-oxidant, anti-proliferative, and anti-inflammatory effects (Liu et al., 2018; Shah et al., 2017; Suchecka et al., 2017; Zhao et al., 2014), which are always connected with their chemical compositions, rheological properties, and molecular weights (Lin et al., 2018a,b; Shah et al., 2017; Suchecka et al., 2016). Therefore, Qingke polysaccharides, particularly Qingke β-glucans, have great potential applications in the functional food fields.

Extraction processes play important roles in the application of Qingke polysaccharides in the functional food industry. Different extraction methods can significantly affect the yields, structural properties, and bioactivities of natural polysaccharides (Guo et al., 2019; Yuan et al., 2019). Generally, hot water extraction (HWE) is widely known as a traditional way to obtain polysaccharides from natural

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plants (He et al., 2018). Nevertheless, HWE has the shortcomings of high extraction temperature, long extraction time, and relatively low extraction efficiency. Therefore, several physical extraction methods, such as microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), and pressurized water extraction (PWE), are taken into considerations, which can facilitate the extraction procedure with high extraction efficiency. Both UAE and MAE are usually adopted to raise the dissolution of polysaccharides because of their efficient performances on mechanical destruction or biodegradation of plant cell walls (Guo et al., 2019; Shang et al., 2018; Silva et al., 2018). In addition, the PWE can enhance the water solubility of polysaccharides, and lower the solvent surface tension and viscosity, allowing easier penetration into the plant cell walls (Xu et al., 2016). As far as we know, effects of different extraction methods (HWE, UAE, MAE, and PWE) on the structural properties and biological activities of Qingke polysaccharides (THBs) have never been investigated. Whether the structural properties and bioactivities of THBs are influenced by different extraction methods remains unknown.

Hence, in the present research, to explore the effects of different extraction methods on the structural characteristics and biological activities of THBs, and to further understand the relationship between the structural characteristics and biological activities of Qingke polysaccharides, four extraction methods, including HWE, MAE, UAE, and PWE, were utilized to extract THBs, and their physicochemical structures, *in vitro* antioxidant activities, and *in vitro* hypolipidemic activities were investigated and compared. Results from this research will present a scientific basis to select excellent potential extraction method for the preparation of THBs with high-activity for the application in the functional food fields.

2. Material and methods

2.1. Material and chemicals

The blue Qingke was obtained from Changdu, Tibet, China. The samples were dried, smashed, and screened through mesh size of 60, and stored at −20 °C.

Carboxymethyl cellulose, sodium cholate, sodium taurocholate, oleic acid, sodium deoxycholate, sodium glycocholate, cholesterol, PMP, mannose, glucose, galactose, rhamnose, glucuronic acid, xylose, galacturonic acid, arabinose, and ABTS were purchased from Sigma-Aldrich (St. Louis, MO, USA). Amyloglucosidase (1000 U/g), heat-stable α-amylase (40000 U/g), pancreatic lipase (4000 U/g), pancreatin (4000 U/g), and a free cholesterol assay kit were purchased from Solarbio (Beijing, China). The mixed linkage β-glucan assay kit was purchased from Megazyme (Wicklow, Ireland). All chemicals were of analytical grade.

2.2. Extraction of Qingke polysaccharides (THBs)

2.2.1. Hot water extraction

Hot water extraction (HWE) was conducted by the previously reported method (Guo et al., 2018). Briefly, Qingke polysaccharides were extracted twice with deionized water (1: 20, *w/v*) at 50 °C for 2 h. After extraction, Qingke polysaccharides were obtained by freeze drying, and the content of total polysaccharides was estimated by phenol-H_2SO_4 colorimetry. Furthermore, the content of β-glucans was estimated by the mixed linkage β-glucan assay kit, and the content of proteins was measured by Bradford's method. The contents of β-glucans, total polysaccharides, and proteins of THB-H were presented as gram of β-glucans, total polysaccharides, and proteins per 100 g of crude polysaccharides (THB-H) extracted from Qingke powder (g/100 g THB-H), respectively.

2.2.2. Ultrasonic assisted extraction

Ultrasonic assisted extraction (UAE) was also performed by the previously reported method with slight adjustments (Guo et al., 2019). Briefly, polysaccharides were extracted with deionized water (1: 40, *w/v*) by an Ultrasonic Processor (650 W, 24 kHz, Scientz Company, China) for 20 min at the ultrasonic amplitude of 70% and room temperature. Finally, the content of total polysaccharides, and contents of proteins and β-glucans of Qingke polysaccharides extracted by UAE (THB-U) were also determined as the methods described in section 2.2.1.

2.2.3. Pressurized water extraction

Pressurized water extraction (PWE) was also conducted by the previously reported method with slight adjustments (Yuan et al., 2019). Briefly, Qingke polysaccharides were extracted with deionized water (1: 30, *w/v*) by the laboratory-scale high pressure reactor (LEC-300, Shanghai Laibei Scientific Instruments Co., Ltd., Shanghai, China) for 40 min at 1.6 MPa and 55 °C. Finally, the content of total polysaccharides, and contents of proteins and β-glucans of Qingke polysaccharides extracted by PWE (THB-P) were also determined as the methods described in section 2.2.1.

2.2.4. Microwave assisted extraction

Microwave assisted extraction (MAE) was also conducted by the previously reported method with slight adjustments (Guo et al., 2019). Briefly, Briefly, Qingke polysaccharides were extracted with deionized water (1: 30, *w/v*) by the MKJ-J1-3 microwave extractor (Makewave Company, Qingdao, China) for 10 min at 480 W and 85 °C. During MAE extraction, the heat-stable α-amylase (20 U/mL) was added to prevent the influence of starch gelatinization. Afterwards, the content of total polysaccharides, and contents of proteins and β-glucans of Qingke polysaccharides extracted by MAE (THB-M) were also determined as the methods described in section 2.2.1.

2.3. Physicochemical characterization of polysaccharides extracted by different methods

2.3.1. Determination of molecular weights (M_w) and constituent monosaccharides

The weight-average M_w and M_w/M_n (polydispersities) of THB-H, THB-U, THB-P, and THB-M were estimated by high performance size exclusion chromatography coupled with multi angle laser light scattering and refractive index detector (Wyatt Technology Co., Santa Barbara, CA, USA) (Guo et al., 2018). The Shodex OHpak SB-806M HQ column was used at 30 °C. In addition, constituent monosaccharides of THB-H, THB-U, THB-P, and THB-M were also measured by HPLC (high performance liquid chromatography, Agilent Technologies, Santa Clara, CA, USA) analysis based on the previously reported methods (Yuan et al., 2019).

2.3.2. Determination of apparent viscosities

The apparent viscosities of THB-H, THB-U, THB-P, and THB-M were determined by a DHR-1 (Discovery Hybrid Rheometer-1, TA instruments, New Castle DE, USA) equipped with a parallel steel plate (40 mm diameter, 1.0 mm gap) by previously reported methods with slight adjustments (Nie et al., 2019; Zhu et al., 2018). The apparent viscosities of samples were measured at 15.0 mg/mL and 10.0 mg/mL. The samples were determined over the range of 0.1–100 s^{-1} at 25 °C.

2.3.3. FT-IR spectroscopy analysis

The FT-IR spectroscopy analysis of THB-H, THB-U, THB-P, and THB-M was conducted by a Nicolet iS10 FT-IR (ThermoFisher scientific, Waltham, MA, USA) at a frequency range of 400–4000 cm^{-1} (Guo et al., 2019).

2.4. Evaluation of antioxidant activities and hypolipidemic activities of polysaccharides extracted by different methods

2.4.1. In vitro antioxidant activities

The reducing powers, NO (nitric oxide) radical scavenging activities,

Table 1
Chemical compositions, molecular weights (M_w), polydispersities (M_w/M_n), and molar ratios of compositional monosaccharides of Qingke polysaccharides (THBs).

Samples	THB-M	THB-U	THB-H	THB-P
Extraction yields (%)	3.62 ± 0.08 ^{a}	3.28 ± 0.12 ^{b}	2.93 ± 0.09 ^{c}	3.56 ± 0.08 ^{a}
β-glucans contents (g/100g THBs)	80.25 ± 1.34 ^{b}	79.25 ± 1.61 ^{b}	86.42 ± 1.56 ^{a}	84.27 ± 1.70 ^{a}
Total polysaccharides contents (g/100g THBs)	86.77 ± 1.53 ^{b}	82.02 ± 1.98 ^{c}	91.03 ± 1.65 ^{a}	90.61 ± 1.82 ^{a}
Protein contents (g/100g THBs)	1.24 ± 0.06 ^{c}	1.84 ± 0.12 ^{a}	1.53 ± 0.11 ^{b}	1.32 ± 0.07 ^{c}
$M_w \times 10^5$ (Da)	1.10 (±0.28%) ^{d}	1.26 (±0.85%) ^{c}	1.78 (±0.53%) ^{b}	1.86 (±0.45%) ^{a}
M_w/M_n	1.93 (±0.44%)	2.09 (±0.71%)	1.63 (±0.84%)	1.64 (±0.70%)
Monosaccharides and molar ratios				
Glucose	11.07	10.06	13.83	12.56
Galactose	0.51	0.51	0.53	0.54
Xylose	1.00	1.00	1.00	1.00
Arabinose	1.54	1.50	1.51	1.27

THB-M, THB-U, THB-H, and **THB-P,** Qingke polysaccharides extracted by microwave assisted extraction, ultrasonic assisted extraction, hot water extraction, and pressurized water extraction, respectively; The error bars are standard deviations; Each experiment was repeated for three times; Values represent mean ± standard deviation, and superscripts a-d differ significantly ($p < 0.05$) column wise between different extraction methods; Statistical significances were carried out by ANOVA and Ducan's test.

and ABTS radical scavenging activities of THB-H, THB-U, THB-P, and THB-M were measured by previously reported methods (Guo et al., 2019), respectively.

2.4.2. In vitro hypolipidemic activities

The *in vitro* binding properties (bile acid binding capacity, cholesterol binding capacity, and fat binding capacity) and inhibitory effects on pancreatic lipase of THB-H, THB-U, THB-P, and THB-M were also determined by previously reported methods (Guo et al., 2018), respectively. The fat binding capacities of THB-H, THB-U, THB-P, and THB-M were presented as gram of binding oil per gram of Qingke polysaccharides (g/g). In addition, the cholesterol binding capacities of THB-H, THB-U, THB-P, and THB-M were presented as milligram of binding cholesterol per gram of Qingke polysaccharides (mg/g). The bile acid binding capacities of THB-H, THB-U, THB-P, and THB-M were represented as percent of blank control (%). Furthermore, the inhibitory effects on the pancreatic lipase of THB-H, THB-U, THB-P, and THB-M were presented as IC_{50} values (mg/mL) by a logarithmic regression curve.

2.5. Statistical analysis

All results were repeated for three times, and data were represented in means ± standard deviations. Origin 9.0 software was applied for statistical analysis. Statistical significances were carried out by one-way ANOVA (analysis of variance), taking a level of $p < 0.05$ as significant to Duncan's multiple range test.

3. Results and discussions

3.1. Effects of extraction methods on the structural properties of THBs

3.1.1. Chemical compositions of THBs

The contents of total β-glucans and soluble β-glucans in blue Qingke are determined to be 5.94% (*w/w*) and 2.29% (*w/w*) in the previous study (Lin et al., 2018a,b). Table 1 summarized the chemical compositions and yields of THBs prepared by HWE, PWE, UAE, and MAE. As shown in Table 1, the extraction yields of THBs ranged from 2.93% to 3.62%, which were higher than that of soluble β-glucans extracted from Qingke in the previous study (Lin et al., 2018a,b). The extraction yields of THB-P and THB-M were similar, which were significantly ($p < 0.05$)

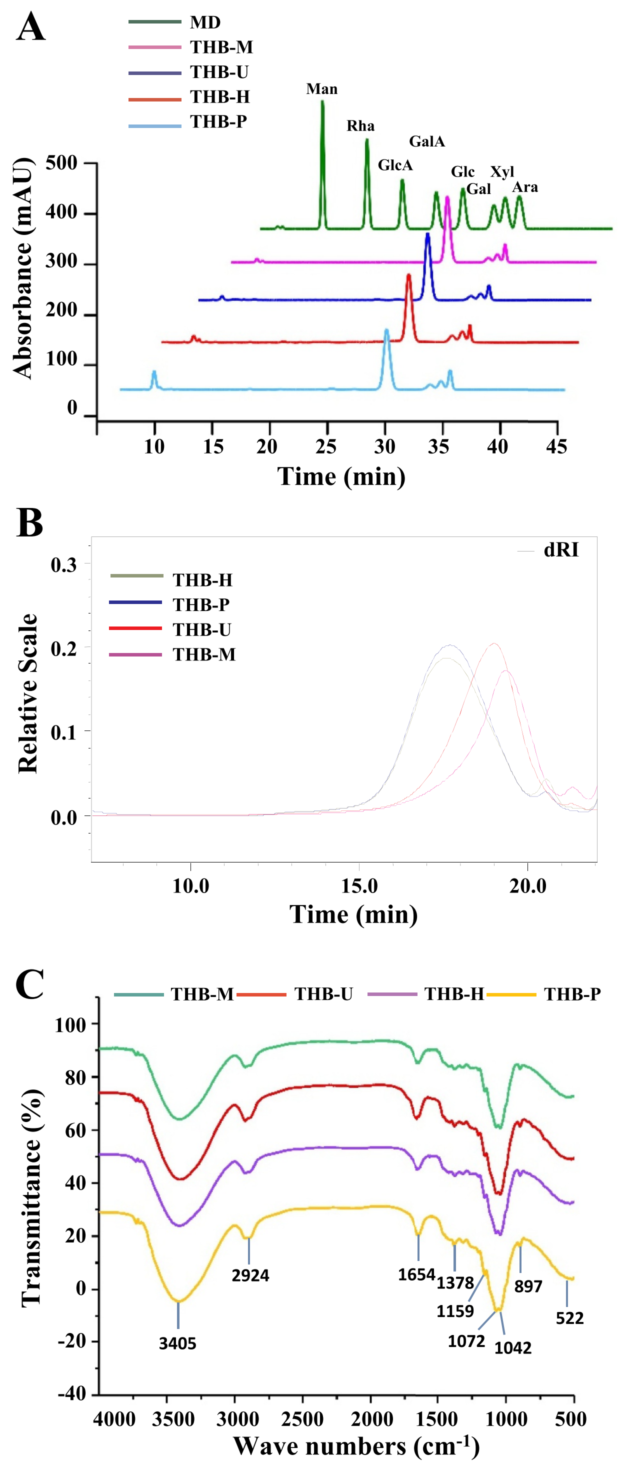

Fig. 1. HPLC profiles (A), HPSEC chromatograms (B), and FT-IR spectra (C) of THB-M, THB-U, THB-H, and THB-P.
THB-M, THB-U, THB-H, and **THB-P,** Qingke polysaccharides extracted by microwave assisted extraction, ultrasonic assisted extraction, hot water extraction, and pressurized water extraction, respectively; **MD,** mixed standard of monosaccharides; **Man,** mannose; **Rha,** rhamnose; **GlcA,** glucuronic acid; **GalA,** galacturonic acid; **Glc,** glucose; **Gal,** galactose; **Xyl,** xylose; **Ara,** arabinose.

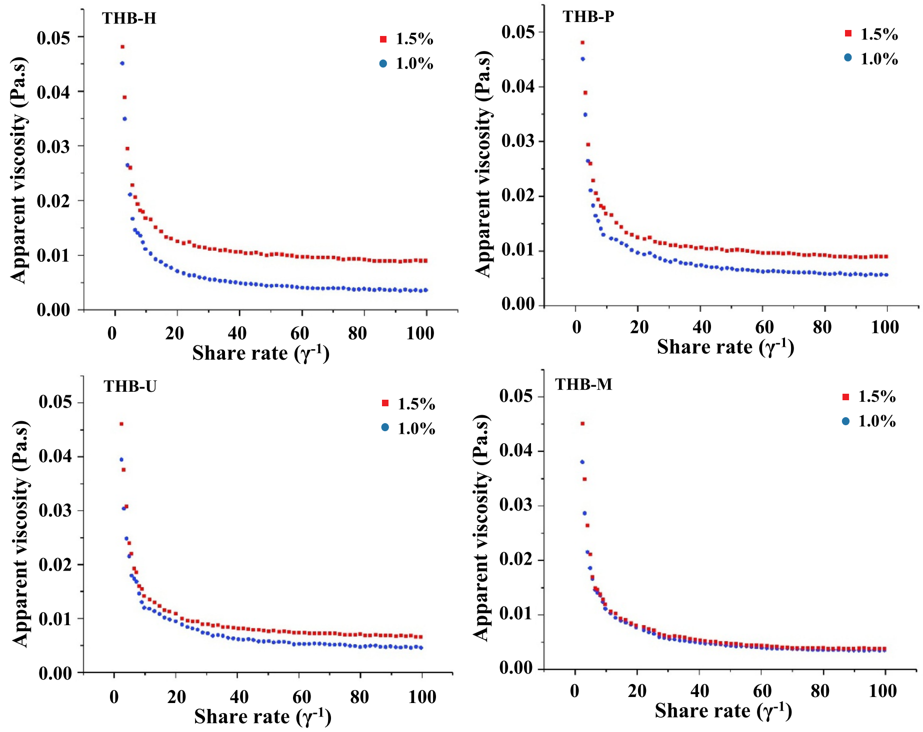

Fig. 2. Effects of shear rate on the apparent viscosities of THB-M, THB-U, THB-H, and THB-P
THB-M, THB-U, THB-H, and **THB-P,** Qingke polysaccharides extracted by microwave assisted extraction, ultrasonic assisted extraction, hot water extraction, and pressurized water extraction, respectively.

higher than that of THB-H and THB-U. Indeed, regarding the extraction time and energy consumption of four extraction methods, MAE, UAE, and PWE could be much better than HWE for the extraction of Qingke polysaccharides. In addition, different extraction methods could also affect the purities of polysaccharides, which were similar with previous studies (Yuan et al., 2019). The contents of total polysaccharides in THB-H, THB-U, THB-P, and THB-M were measured to be 91.03 ± 1.65 g/100 g THB-H, 82.02 ± 1.98 g/100 g THB-U, 90.61 ± 1.82 g/100 g THB-P, and 86.77 ± 1.53 g/100 g THB-M, respectively, which suggested that all THBs extracted by different extraction methods exhibited relatively high contents of polysaccharides. Similarly, the contents of β-glucans in THB-H, THB-U, THB-P, and THB-M were measured to be 86.42 ± 1.56 g/100 g THB-H, 79.25 ± 1.61 g/100 g THB-U, 84.27 ± 1.70 g/100 g THB-P, and 80.25 ± 1.34 g/100 g THB-M, respectively, which showed that β-glucans were the main components in Qingke polysaccharides (THBs). The contents of β-glucans in both THB-H and THB-P were significantly ($p < 0.05$) higher than that of THB-M and THB-U. Furthermore, the contents of proteins in THB-H, THB-U, THB-P, and THB-M ranged from 1.24 to 1.84 g/100 g THBs. The significantly ($p < 0.05$) highest content of proteins was determined in THB-U among all tested THBs, while the lowest contents of proteins were found in both THB-M and THB-P.

3.1.2. Constituent monosaccharides, molecular weights, and apparent viscosities of THBs

It is generally assumed that bioactivities of natural polysaccharides are closely associated with their constituent monosaccharides, molecular weights, and viscosities (Lin et al., 2018a,b; Shah et al., 2017; Suchecka et al., 2017; Zhu et al., 2018). Thus, effects of four extraction procedures on the constituent monosaccharides, M_w, and apparent viscosities of THBs were studied. Fig. 1A showed that HPLC-UV profiles of THB-H, THB-U, THB-P, and THB-M were similar. The compositional monosaccharides of THB-H, THB-U, THB-P, and THB-M were determined as Glc, Ara, Xyl, and Gal, which was consistent with the previous study (Guo et al., 2018). Molar ratios of Glc, Ara, Xyl, and Gal in THB-H, THB-U, THB-P and THB-M were summarized Table 1, which indicated that Glc was the dominant monosaccharide in THBs. Results suggested that β-glucans and arabinoxylans existed in Qingke polysaccharides according to their constituent monosaccharides. Furthermore, results showed that different extraction methods had no effects on the types of constituent monosaccharides in THBs, but significantly affected their molar ratios. Similar studies have also indicated that extraction methods can influence the constituent monosaccharides of natural polysaccharides (Dong et al., 2016; He et al., 2018).

Furthermore, molecular weights of THB-H, THB-U, THB-P, and THB-M were also summarized in Table 1, which ranged from 1.10×10^5 Da to 1.86×10^5 Da. Results showed that different extraction methods significantly affected the molecular weights of THBs. The molecular weights of THB-P extracted by PWE and THB-H extracted by HWE were significantly ($p < 0.05$) higher than that of THB-M extracted by MAE and THB-U extracted by UAE, which demonstrated that both MAE and UAE could significantly degraded the molecular weights of Qingke polysaccharides. Indeed, the sharp degradations of polysaccharides were observed in HPSEC chromatograms of both THB-U and THB-M (Fig. 1B). Results further suggested that THBs were degraded during UAE and MAE, which were similar with the results from previous studies (Hu et al., 2018; Yuan et al., 2019). Furthermore, the polydispersities of THB-H, THB-U, THB-P, and THB-M ranged from 1.63 to 2.09, which were consistent with their HPSEC chromatograms.

Moreover, Fig. 2 showed effects of shear rate on the apparent viscosities of THBs extracted by four extraction methods. Results showed that the apparent viscosities of THB-H, THB-U, THB-P, and THB-M decreased with the increasing shear rate (0.1–100 s^{-1}), respectively. The apparent viscosities of THB-H, THB-U, and THB-P exhibited a dose-dependent manner, but the apparent viscosity of THB-M was not significant. Furthermore, the order of the apparent viscosities of THBs was as follows: THB-P > THB-H > THB-U > THB-M. Results indicated that different extraction methods significantly affected the apparent viscosities of polysaccharides, which were similar with the results of polysaccharides from onion (Zhu et al., 2018), okra (Yuan et al., 2019), and

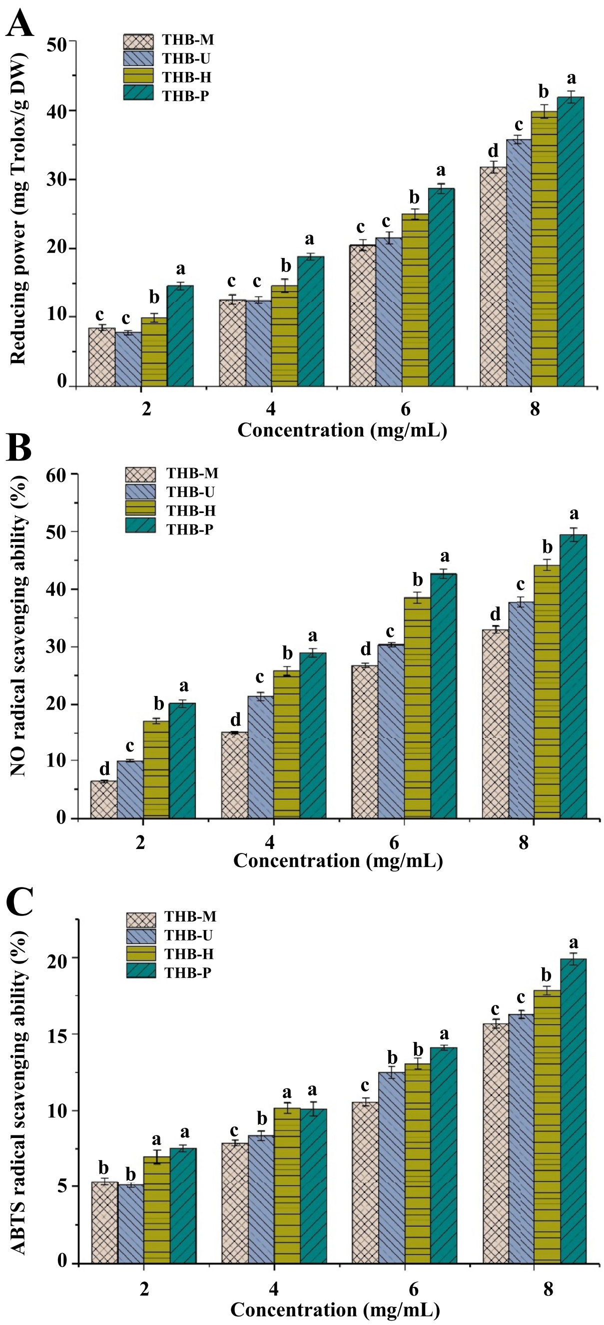

Fig. 3. Reducing power (A), nitric oxide radical scavenging activity (B), and ABTS radical scavenging activity (C) of THB-M, THB-U, THB-H, and THB-P

THB-M, THB-U, THB-H, and THB-P, Qingke polysaccharides extracted by microwave assisted extraction, ultrasonic assisted extraction, hot water extraction, and pressurized water extraction, respectively; Each experiment was repeated for three times; The error bars are standard deviations; Significant ($p < 0.05$) differences are shown by data bearing different letters (a–d); Statistical significances were carried out by ANOVA and Ducan's test.

wheat bran (Yan et al., 2019). Indeed, results indicated that the pressure assisted extraction had no effect on the apparent viscosities of THBs. However, both UAE and MAE significantly reduced the apparent viscosities of THBs, which might be related to the decrease of their molecular weights. These results indicated that the apparent viscosities of Qingke polysaccharides had close relationship with their molecular weights.

3.1.3. FT-IR spectra of THBs

The FT-IR spectra were used to determine the structural characteristics of THBs. Fig. 1C showed the FT-IR spectra of THB-H, THB-U, THB-P, and THB-M. The FT-IR spectra of THBs were similar, which showed that THBs extracted by four extraction methods exhibited similar absorption peaks. Briefly, 3405 cm^{-1} and 2924 cm^{-1} are the broad peaks caused by the stretching vibration of hydroxyl group and the C–H asymmetric stretching vibration (Wang et al., 2012). The broad band at 1654 cm^{-1} is due to the presence of water molecules in sample (Hematian Sourki et al., 2017), and the absorption peak at 1378 cm^{-1} is assigned to the C–H variable angle vibration (Guo et al., 2019), respectively. The absorption peak at 897 cm^{-1} is the characteristic absorption peak of the C–H in β-linkage, and the absorption peak at 1042 cm^{-1} is due to the C–O absorption peak of the pyran ring structure (Qian et al., 2015).

3.2. Effects of extraction methods on the in vitro antioxidant activities of THBs

Polysaccharides extracted from Qingke exert moderate antioxidant activities, and different extraction methods can change the antioxidant activities of polysaccharides (Dong et al., 2016). Therefore, effects of different extraction methods on the antioxidant activities of THBs were measured and compared. The reducing powers, NO and ABTS radical scavenging activities of THB-H, THB-U, THB-P, and THB-M were shown in Fig. 3. As shown in Fig. 3, THBs extracted by different extraction methods exhibited obvious reducing powers, NO and ABTS radical scavenging activities with a dose-dependent manner, respectively. Different extraction methods significantly influenced the antioxidant activities of THBs, which were similar with previous studies that antioxidant activities of polysaccharides varied by different extraction procedures (Dong et al., 2016; Guo et al., 2019; Shang et al., 2018; Yan et al., 2019; Yuan et al., 2019). At the concentration of 8.0 mg/mL, the reducing powers of THB-P, THB-H, THB-U, and THB-M were measured to be 41.83 ± 0.86 μg Trolox/mg, 39.74 ± 0.98 μg Trolox/mg, 35.72 ± 0.58 μg Trolox/mg, and 31.78 ± 0.83 μg Trolox/mg, respectively; Furthermore, the NO radical scavenging activities of THB-P, THB-H, THB-U, and THB-M were measured to be 49.35 ± 1.16%, 44.10 ± 0.98%, 37.71 ± 0.87%, and 32.96 ± 0.65%, respectively; Moreover, the ABTS radical scavenging activities of THB-P, THB-H, THB-U, and THB-M were measured to be 19.88 ± 0.39%, 17.83 ± 0.28%, 16.28 ± 0.25%, and 15.67 ± 0.31%, respectively. All results indicated that the antioxidant activities of THB-P extracted by PWE were significantly ($p < 0.05$) higher than that of THB-H, THB-U, and THB-M, which demonstrated that the PWE could be a potential method for the extraction of THBs with relatively high antioxidant activities. Generally, antioxidant activities of polysaccharides extracted from natural resources are related to their chemical compositions, M_w, constituent monosaccharides, and functional groups (Nie et al., 2019; Wu et al., 2019; Yuan et al., 2019).

3.3. Effects of extraction methods on the in vitro hypolipidemic activities of THBs

3.3.1. Binding capacities of THBs

Excessive intake of bile acids and fat can lead to obesity and some other diseases (cardiovascular disease, diabetes, and cancer) (Guo et al., 2018; Liu et al., 2018). In addition, previous studies showed that β-glucan can reduce the intestinal excess intake of lipids (Bae et al., 2010; Drozdowski et al., 2010). The *in vitro* binding capacities of THBs extracted by different extraction methods were evaluated, and results were summarized in Fig. 4. The fat (Fig. 4A), cholesterol (Fig. 4B), and bile acid (Fig. 4C) binding abilities of THB-P, THB-H, THB-U, and THB-M ranged from 1.87 ± 0.02 to 2.49 ± 0.04 g/g, from 33.59 ± 0.69 to 38.42 ± 0.61 mg/g, and from (25.98 ± 0.34)% to (33.20 ± 0.55)%, respectively. Results showed that the *in vitro* binding capacities of THBs varied by different extraction methods. Indeed, the significantly highest fat,

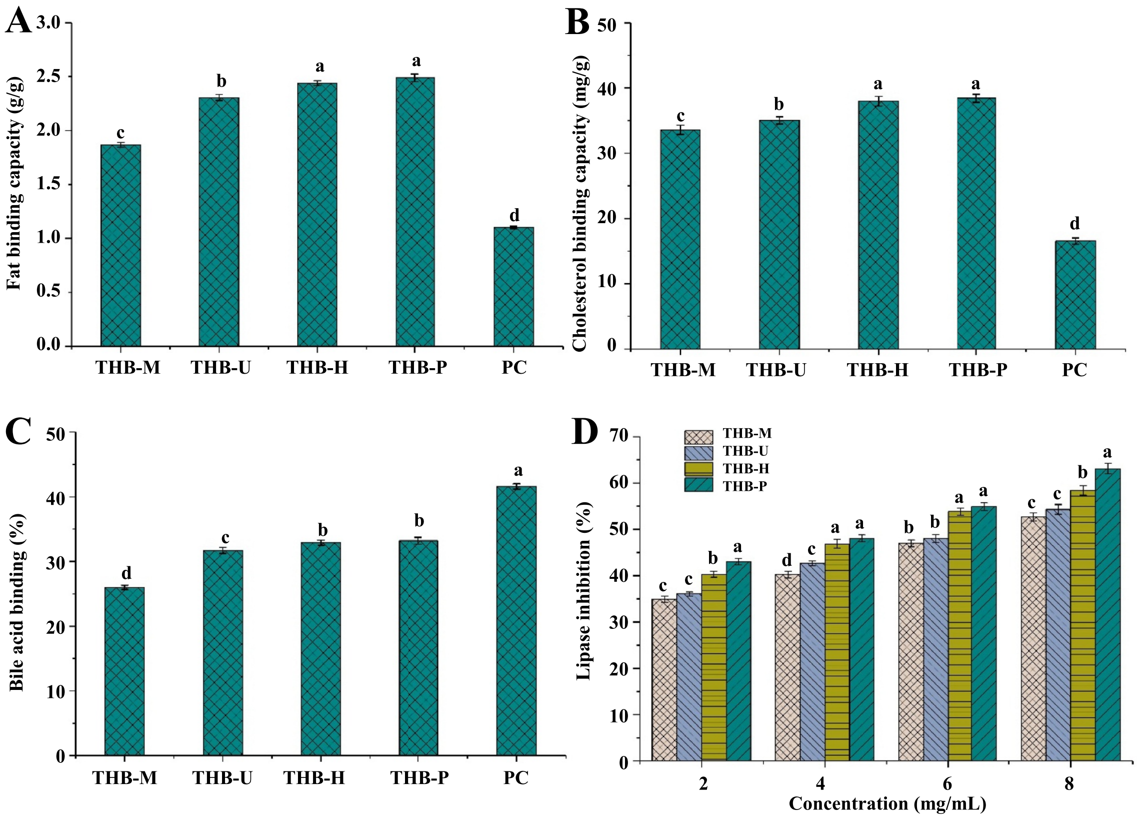

Fig. 4. The fat (A), cholesterol (B), bile acid (C) binding capacities, and *in vitro* inhibitory effects on the pancreatic lipase (D) of THB-M, THB-U, THB-H, and THB-P

THB-M, THB-U, THB-H, and **THB-P,** Qingke polysaccharides extracted by microwave assisted extraction, ultrasonic assisted extraction, hot water extraction, and pressurized water extraction, respectively; **PC,** positive control; Carboxymethyl cellulose was used as positive control in fat binding and cholesterol binding abilities, and cholestyramine was used as positive control in bile acid binding ability; Each experiment was repeated for three times; Values represent mean ± standard deviation, and superscripts a-c differ significantly ($p < 0.05$) column wise between different extraction methods. Statistical significances were carried out by ANOVA and Ducan's test.

cholesterol, and bile acid binding abilities were observed in both THB-P and THB-H extracted by PWE and HWE, respectively, followed by lower in THB-U extracted by UAE, and the lowest in THB-M extracted by MAE (Fig. 4). These findings further indicated that the PWE could be a potential method for the extraction of THBs with relatively high binding capacities. The relatively high binding capacities of THB-P extracted by PWE might be attributed to its relatively high molecular weight and apparent viscosity (Nie et al., 2019; Zhang et al., 2018). Furthermore, although the bile acid binding abilities of THB-H, THB-U, THB-P, and THB-M were lower than that of cholestyramine (the positive control), THBs extracted by different extraction methods also exhibited obvious bile acid binding abilities. In addition, considering β-glucans were the main components in Qingke polysaccharides, which suggested that Qingke polysaccharides have significant *in vitro* binding abilities may be correlated to β-glucans. These findings suggested that THBs, especially THB-P extracted by PWE, possessed potential to be explored as functional foods or medicine for the prevention of hyperlipidemia.

3.3.2. *In vitro inhibitory effects of THBs on the pancreatic lipase*

Pancreatic lipase belongs to the most essential enzymes that involve in the digestion of triglyceride. Generally, the inhibition of lipase activity is an important way for the regulation of the obesity and hyperlipidemia (Jin et al., 2017). Thus, effects of different extraction methods on the inhibitory effects of THBs on the pancreatic lipase were studied. Fig. 4D showed the inhibitory activities of THBs extracted by different extraction methods on the pancreatic lipase. All THBs exhibited obvious inhibition effects against pancreatic lipase with a dose-dependent manner. Results showed that different extraction methods also affected the inhibitory effects of THBs on the pancreatic lipase. The significantly highest inhibition effect on the pancreatic lipase was measured in THB-P (63.20 ± 1.11%) at the concentration of 8.0 mg/mL, followed by lower in THB-H (58.39 ± 0.98%), and the lowest in both THB-U (54.67 ± 1.03%) and THB-M (52.65 ± 0.83%). According to the apparent viscosities and molecular weights of THBs as mentioned above, results suggested that the inhibitory effects of THBs on the pancreatic lipase might be also associated with their apparent viscosities and molecular weights (Lin et al., 2018a,b; Zhang et al., 2018). Furthermore, the IC_{50} values of inhibitory effects of THB-P, THB-H, THB-U, and THB-M on the pancreatic lipase were measured to be 3.761 mg/mL, 4.487 mg/mL, 6.423 mg/mL, and 7.276 mg/mL, respectively, which further confirmed that the inhibitory effect of THB-P extracted by PWE on the pancreatic lipase was higher than that of THB-H, THB-U, and THB-M. These findings indicated that the PWE could be an efficient method for the extraction of THBs with relatively high bioactivities for applications in the functional food fields.

4. Conclusions

Different extraction methods significantly affected the extraction yields, chemical compositions, M_w, apparent viscosities, and molar ratios of constituent monosaccharides of THBs. Especially, THB-P extracted by PWE possessed the highest molecular weight and apparent viscosity among all tested THBs. Indeed, antioxidant activities, *in vitro* binding capacities, and inhibitory effects on pancreatic lipase of THBs also varied by different extraction methods. The highest antioxidant activities, *in vitro* binding capacities, and pancreatic lipase inhibition effects were also observed in THB-P among all tested samples. The higher biological activities of THB-P may correlate to its higher molecular weight, higher apparent viscosity, and higher β-glucan content. These findings showed that pressurized water extraction could be an excellent potential extraction method for the extraction of polysaccharides from Qingke with high bioactivities for industrial applications.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

CRediT authorship contribution statement

Jing-Liu He: Formal analysis, Investigation, Resources, Software, Writing - original draft. **Huan Guo:** Data curation, Formal analysis, Investigation, Validation, Resources, Software, Writing - original draft. **Si-Yu Wei:** Formal analysis, Investigation. **Jia Zhou:** Formal analysis, Investigation. **Pan-Ying Xiang:** Formal analysis, Investigation. **Lu Liu:** Formal analysis, Investigation. **Li Zhao:** Formal analysis, Investigation, Software. **Wen Qin:** Resources, Software. **Ren-You Gan:** Formal analysis, Funding acquisition, Methodology, Supervision. **Ding-Tao Wu:** Data curation, Formal analysis, Funding acquisition, Methodology, Supervision, Project administration, Writing - review & editing.

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Appendix A. Supplementary data

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