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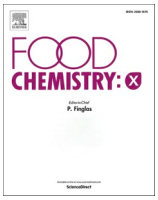

Effects of different chemical modifications on physicochemical and antioxidation properties of *Lycium barbarum* seed dreg polysaccharides

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ABSTRACT

Recent studies have witnessed that chemical modification can improve the physicochemical and functional properties of plants' polysaccharides. Herein, we modified the natural *Lycium barbarum* seed dreg polysaccharides (LBSDPs) by sulfation (S-LBSDPs), phosphorylation (P-LBSDPs), and carboxymethylation (C-LBSDPs), and evaluated the chemical composition and antioxidant activity of their derivatives. Natural polysaccharides and their derivatives exhibited typical polysaccharide absorption peaks and characteristic group absorption peaks in FT-IR spectra along with maximum UV absorption. After modification, the total sugar and protein contents of the derivatives were decreased, whereas the uronic acid content was increased. Among the three derivatives, sulfated polysaccharides displayed excellent thermal stability. S-LBSDP and P-LBSDP showed the highest ABTS radical scavenging and reducing power while S-LBSDPs and C-LBSDPs showed better DPPH radical scavenging effect, and P-LBSDPs showed considerable Fe^{2+} chelating ability. Our data indicate that chemical modifications can impart a positive effect on the antioxidant potential of plant-derived polysaccharides.

1. Introduction

Polysaccharides, as natural ingredients, possess such as easy degradability, nontoxicity (Yoon et al., 2003), cost-effectiveness (Wang et al., 2023), making them extensively widely used in the food, medicine, agriculture, and cosmetics industries (Eghbaljoo et al., 2022). Goji (*Lycium barbarum*), a medicinal and food homologous woody shrub, constitutes polysaccharide as its main component. The multiple functional properties of Goji polysaccharides have been reported, including antioxidant (Zeng et al., 2023), antitumor (Ma et al., 2022), antiviral (Li et al., 2023), and anti-inflammatory effects (Lian, Liu, Chang, Nochi, & Chao, 2023). So far, studies have primarily focused on water extraction methods to obtain polysaccharides from Goji berries (Wang et al., 2023). However, limited attention has been given about the by-product of the Goji berry processing industry. Typically, a small amount of Goji seed dreg is often used for animal feed while the rest is directly discarded (Bora, Ragaee, & Abdel-Aal, 2019).

It has been known that the structure and function of polysaccharides could be selectively altered by physical, chemical, and biological modifications (Tang et al., 2019). Among the three widely used modifications, chemical modification is more effective, which can enhance the structure and function of resulting polysaccharides. On the basis of safety, common chemical modifications mainly include carboxymethylation, sulfation, and phosphorylation (Mukherjee et al., 2022). After chemical modification of polysaccharides, some functional groups may be replaced or a new one can be introduced, which leads to alterations in their structural composition, molecular weight, connection mode, and ionic properties of polysaccharides, thereby influencing their physical, chemical, and functional potential (Maciel et al., 2019).

So far, different methods or solvents have been used to extract, separate, and purify polysaccharides from various plants (Khvostov, Tolstikova, Borisov, & Dushkin, 2019). Our research group has extensively studied various methods for polysaccharide modification, mainly emphasizing the four different solutions (hot buffer, chelating agent,

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dilute alkali, and concentrated alkali) from wide range of plant materials such as pony seed dreg (Shi et al., 2016), onion (Zhu et al., 2018), potato peel (Xu et al., 2019), and *Amana edulis* (Ji et al., 2019) by continuous fractionation extraction. Our previous studies have also reported the effect of altered chemical composition and molecular weight on the free radical scavenging, anticancer, and bacteriostatic abilities of the modified polysaccharides. The physicochemical and functional properties of plant polysaccharides from different sources or extracted by different solvents were found different to some extent. The chemical modification of the polysaccharides from peony seed dreg could improve the scavenging capacity for some free radicals (Li et al., 2018).

With the original intention of improving the utilization of resources and reducing environmental pressure, we selected the *L. barbarum* seed dreg (LBSD), the by-product of *L. barbarum* seed oil, as the raw material and prepared LBSD polysaccharides (LBSDPs) using four different solutions, and found that the obtained LBSDPs displayed notable antioxidant capacity (Zhang et al., 2022). However, there are no data available regarding the chemically modified L. *barbarum* polysaccharides and their related physicochemical and functional changes.

In this study, four types of LBSDPs were chemically modified by sulfation, phosphorylation, and carboxymethylation methods. The UV–Vis absorption spectroscopy and Fourier-transform infrared spectroscopy (FT-IR) were used to evaluate whether natural polysaccharides were successfully modified. The scanning electron microscopy (SEM) and differential scanning calorimetry (DSC) were used to investigate the differences in the physicochemical properties of the derivatives. The effects of modification on the antioxidant capacity of natural polysaccharides were also investigated. This study provides a suitable modification approach to improve the antioxidant capacity of plant polysaccharide derivatives.

2. Materials and methods

2.1. Materials and reagents

The L. *barbarum* seed dreg, a by-product of seed oil extraction, was obtained from Ningxia Wolfberry Goji Industry Co., Ltd. (Yinchuan, China). The obtained dreg was dried and screened (60 meshes) before extraction. Uronic acid and glucose were both purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). DPPH and ABTS were purchased from Sigma Aldrich Trading Co., Ltd. (New Jersey, USA) and Aladdin (Shanghai, China), respectively. Phenanthrazine was procured from McLean Biotechnology Co., Ltd. (Chengdu, China). All the other chemical reagents used in this study were of analytical grade.

2.2. Continuous extraction of LBSDPs

As described in the previous study (Zhang et al., 2022), different solvents were used for the crude extraction from the LBSD in a continuous and graded way. The crude extract was deproteinized, dialyzed, freeze-dried, and subsequently purified to obtain four different polysaccharide fractions such as hot buffer (HBSS), chelating agent (CHSS), dilute alkaline (DASS), and concentrated alkaline (CASS).

2.3. Preparation of sulfated LBSDPs

In an ice water bath, a certain amount of concentrated H_2SO_4 (98.3%, 7.5 mL), *n*-butanol (2.5 mL), and $(NH_4)_2SO_4$ (0.13 g) were placed in a 100-mL beaker and allowed to react in a magnetic stirrer under 0 °C for 30 min. Then, 0.5-g LBSDPs was added to the beaker and allowed to react for 2 h in the ice water bath. The resulting reaction solution was adjusted to neutral with 70% NaOH solution by volume fraction and then transferred to the dialysis bag. After 48 h of dialysis with flowing tap water, distilled water was used for 24 h of dialysis with a regular change during the dialysis period. Following dialysis, three times the volume of 95% ethanol was added to the dialysate, fully mixed, and allowed to stand overnight. The S-LBSDPs were obtained by dissolving the precipitate with an appropriate amount of distilled water and then freeze-dried (Li et al., 2018).

According to a previous study (Li et al., 2018), the degree of substitution (DS) of S-LBSDPs was determined by $BaSO_4$ turbidimetry with K_2SO_4 as the standard and calculated as follows:

$$DS = 1.62 \times S\%32 - 1.02 \times S\% \quad (1)$$

2.4. Preparation of phosphorylated LBSDPs

Phosphoric reagents (sodium tripolyphosphate and sodium trimetaphosphate) were weighed at a ratio of 6:1 and dissolved in 100-mL distilled water to prepare phosphoric reagents. LBSDPs (1 g) and Na_2SO_4 (5 g) were sequentially added to the phosphorylation reagent. After they were dissolved, the pH was changed to 8.6 and phosphorylation was performed at 75 °C for 4 h. Following the reaction, three volumes of 95% ethanol solution were added into the solution and allowed to stand for 24 h. The precipitation was dissolved, dialyzed, and freeze-dried to obtain P-LBSDPs (Cao et al., 2020).

The DS of P-LBSDPs were determined by the molybdenum blue photometric method and calculated as below:

$$\mathrm{DS} = \frac{1.62 \times \mathrm{P}\%}{31 - 0.84 \times \mathrm{P}\%} \quad (2)$$

2.5. Preparation of Carboxymethylated LBSDPs

A total of 500 mg of LBSDPs were accurately weighed and mixed with 2.5-mol/L NaOH solution in a 50-mL beaker and stirred at 80 °C for 1 h until completely mixed, and then 3% chloroacetic acid was gradually added. After reacting in a water bath of 80 °C for 5 h, the pH was changed to neutral after cooling to room temperature, dialyzed for 2 days, and vacuum dried to obtain C-LBSDPs (Teng, Wang, Zeb, & Xiu, 2022).

The DS of C-LBSDPs was determined by acid–base titration. A total of 10 mg of C-LBSDPs were added to 10 mL of 0.01-mol/L NaOH standard solution, stirred at 40 °C for 30 min, and cooled to room temperature. Then, 0.01-mol/L HCl standard solution was used to add excessive NaOH in reverse direction until the color of the phenolphthalein indicator faded (Li et al., 2018).

The DS was calculated as follows:

$$\mathrm{DS} = \frac{0.132\mathrm{A}}{1 - 0.058\mathrm{A}} \quad (3)$$

$$\mathrm{A} = \frac{\mathrm{V_0C_0} - \mathrm{V_1C_1}}{\mathrm{W}} \quad (4)$$

A: he carboxymethyl content (%); V_0: the volume of NaOH (mL); C_0: the concentration of NaOH (mol/L); V_1: the consumed volume of HCl (mL); C_1: the concentration of HCl (mol/L); W: the mass of C-LBSDP samples (g).

2.6. Fourier-transform infrared spectroscopy

The structure of sulfated, phosphorylated, and carboxymethyl derivatives of LBSDPs was analyzed using an infrared spectrophotometer (Spectrum Two, Perkin Elmer, Waltham, USA) within a certain wavelength range. Briefly, ATR mode was selected for measurement, and then a small amount of dry sample was placed in the center of the test bench. The pressure bar was rotated and the sample was pressed, and scanned (4000–400 cm^{-1}).

2.7. UV absorption peak test

LBSDPs and their chemically modified derivatives were dissolved into a mass fraction of 1 mg/mL for UV–Vis spectrophotometric analysis

(UV-1800 spectrophotometer, Shimadzu, Japan) under a scanning range of 200–900 nm.

2.8. Determination of total sugar, uronic acid, and protein content

With D-glucose as the standard, the total sugar content of LBSDPs and modified LBSDPs was determined by phenol sulfuric acid method (Angkawisittpan & Manasri, 2012); with galacturonic acid as the standard, the content of uronic acid in LBSDPs and their derivatives was determined by the *m*-hydroxybiphenyl method (Cesaretti, Luppi, Maccari, & Volpi, 2003). The protein content of LBSDPs and their derivatives was determined with Lowry methods (Lowry, Rosebrough, Farr, & Randall, 1951) using a BCA protein quantitative kit purchased from White Shark Technology Co., Ltd. (Guangzhou, China).

2.9. SEM

SEM can reveal the surface microstructure of natural polysaccharides and their derivatives. The fully dried LBSDPs and their modified derivatives were scattered on a tape attached to the aluminum plate. After carefully removing the excess polysaccharide with an ear-washing ball, the samples were gold coated to make the sample conductive and placed in the sample room of the scanning electron microscope, followed by adjusting the accelerating voltage of 10 kV, and amplification by 200 and 1000 times.

2.10. Differential scanning calorimeter (DSC) analysis

The thermal properties of LBSDP powder and its chemically modified derivatives were determined by Q200 (TA Instruments, Newcastle, USA). Briefly, with the blank crucible as the control, the dried samples were added to the crucible and sealed. Under the N_2 50-mL/min gas flow, the temperature was increased from 20 °C to 250 °C at the rate of 10 °C/min.

2.11. Antioxidant abilities assay

2.11.1. ABTS radical scavenging ability

ABTS radical scavenging capacity is achieved through the reaction of antioxidant and stable cationic radical ABTS. For the ABTS working solution, pure water was used to dilute the stock solution to 734 nm, and the absorption wavelength reached about 0.70 ± 0.02. A total of 4-mL ABTS working solution was added into 0.2-mL 0.2 mg/mL of the sample to be tested and allowed to react at room temperature in the dark for 9 min followed by absorbance determination (A_{734} nm) as A_1. The ABTS potassium persulfate mixed solution and samples were replaced with pure water, and the above operation was repeated, and the measured absorbance was A_0 and A_2. A certain concentration of V_C was also treated as a positive control (Zhang et al., 2022). At the same time, the ABTS free radical scavenging capacity in the sample was calculated according to the standard curve of V_C on ABTS free radical scavenging capacity. The results were expressed in two ways: V_C equivalent (mg/mg) and free radical scavenging rate (%). All the measurements were repeated three times and finally expressed as an average value. The calculation formula was as follows:

$$\text{ABTS radical scavenging rate}\ (\%) = \frac{1-(A_0-A_1)+A_2}{A_0}\times 100 \quad (5)$$

2.11.2. DPPH radical scavenging ability

The determination of the DPPH radical scavenging rate of LBSDPs and modified products was conducted following a previous study with few modifications (Zhang et al., 2022). First, 0.1-mM DPPH solution was prepared and stored away from direct exposure to light. A total of 2 mL (0.2 mg/mL) of LBSDPs and their chemically modified products were accurately weighed, and then 2.5 mL of DPPH solution was poured. After mixing, the solution was left to stand for 30 min under dark conditions, and the absorbance A_1 was measured at 517 nm. The sample to be tested and the DPPH were replaced by pure water, respectively, the above operation was repeated, and the measured absorbances were A_0 and A_2. A certain concentration of V_C was considered as a positive control (Zhang et al., 2022). At the same time, the free radical scavenging capacity of DPPH in the sample was calculated according to the standard curve of V_C scavenging capacity for DPPH free radicals. The results were expressed in two ways: V_C equivalent (mg/mg) and free radical clearance (%). All the measurements were repeated three times, with the average value as the final result. The DPPH radical scavenging rate of LBSDPs and their chemically modified products can be calculated according to the following formula:

$$\text{DPPH radical scavenging rate}\ (\%) = \frac{1-(A_0-A_1)+A_2}{A_0}\times 100 \quad (6)$$

2.11.3. Fe^{2+} chelating ability

In this experiment, 0.2-mg/mL LBSDPs and their chemically modified products were used to determine the chelating capacity of Fe^{2+}. A total of 1.0 mL of the sample to be tested, 0.1 mL of 2-mM $FeCl_2$, 0.2 mL of 5-mM phenanthroline solution, and 2.7-mL H_2O were added in turn, mixed well, and stored under dark conditions at room temperature for 10 min. After the reaction, the absorbance at 562 nm was A_1. Pure water was used to replace the samples and the above reaction solution, the above operation was repeated, and the measured absorbances were A_0 and A_2. In this experiment, EDTA-2Na of a certain concentration was used as the positive control group, and the Fe^{2+} chelating capacity of the sample to be tested was calculated according to the standard curve drawn by the corresponding relationship between EDTA-2Na and Fe^{2+} chelating capacity. The results were expressed in two ways: EDTA-2Na equivalent (mg/mg) and Fe^{2+} chelating capacity (%). The average value shall be taken as the final result after three parallel determinations. The determination formula of the Fe^{2+} chelating capacity of the sample to be tested was as follows:

$$Fe^{2+}\text{chelating rate}\ (\%) = \frac{1-(A_0-A_1)+A_2}{A_0}\times 100 \quad (7)$$

2.11.4. Reducing power ability

A total of 1.0 mL of 0.2-mg/mL aqueous solution of natural LBDPs and chemically modified LBDPs were weighed, and then 2.5 mL of 0.2-mol/L phosphate buffer and 1% $K_3Fe(CN)_6$ were successively poured. After 20 min, in a constant temperature of water bath at 50 °C, 2.5 mL of 10% TCA was poured. After 10 min, 2.5 mL of the reaction solution was diluted with equal volume and allowed to react with 1.0 mL of 0.1% $FeCl_3$. The absorbance determined at 700 nm was recorded as A_1. The sample to be tested was replaced by deionized water. After the same treatment conditions, the absorbance at 700 nm was recorded as A_0 (Zhang et al., 2022). V_C of a certain concentration was selected as the positive control, and the reduction capacity of the sample was tested according to the standard curve drawn by the corresponding relationship between V_C and reduction capacity. The results were expressed in two ways: V_C equivalent (mg/mg) and reducing power (A_1–A_0). The average value was taken as the final result after three parallel determinations.

2.12. Statistical analysis

To ensure the reliability of the experimental data, all the experiments were repeated three times in parallel. The data were presented as the mean ± SD of three replications. Statistical analysis ($P < 0.05$) was conducted by one way analysis of variance and Pearson multivariate test using SPSS 17.0 software (SPSS, Chicago, USA).

3. Results and discussions

3.1. UV analysis of LBSDPs and their derivatives

In the UV spectra of LBSDPs range of 200–800 nm, the four natural polysaccharides had relatively concentrated maximum absorption peaks, namely 214 nm, 210 nm, 208 nm, and 216 nm (Fig. 1A–D), respectively, which was consistent with the previously reported experimental results (Cao et al., 2020; Li et al., 2018). The UV–Vis spectra of modified LBSDPs were not significantly different from those of natural polysaccharides, and their maximum absorption peaks were similar, which indicated that the composition of natural polysaccharides and their derivatives was relatively single and uniform (Cao et al., 2020). In the range of 250–280 nm, all the samples exhibited weak absorption peaks. Previous studies showed that the bands in this wavelength range correspond to –CO–NH–, which indicated that there were some trace proteins in detected samples (Xie et al., 2020). The results were consistent with the protein content determined by the BCA protein quantitative kit. At the same time, the absorption peak of S-LBSDPs at 260 nm indicated the existence of S═O, which may be due to the transformation of the n–π^* sulfate group (Li, Wang, Xiong, Yu, & Peng, 2020).

3.2. FT-IR analysis of LBSDPs and their derivatives

The FT-IR spectra of LBSDPs and modified products (S-LBSDPs, P-LBSDPs, and C-LBSDPs) were revealed in Fig. 1E–H. It could be seen that the spectra of LBSDPs and their derivatives shared similar profiles in general and exhibited typical absorption peaks of polysaccharides at around 3428 cm^{-1}, 2932 cm^{-1}, 1646 cm^{-1}, 1460 cm^{-1}, 1249 cm^{-1}, and 1028 cm^{-1} in particular, which were designated as tensile vibration of O—H groups, tensile vibration of C—H bonds and total sugar content index, bound water, deformation vibration of C—H bonds, asymmetric tensile vibration, and symmetric tensile vibration of C–O–C bond, respectively (Xiao et al., 2019). These typical polysaccharide absorption peak signals indicated that the absorbing major body of the carbohydrate chain did not alter even after the chemical modifications.

Specific signal peaks of sulfate derivatives were observed in S-LBSDPs. There were two characteristic absorption peaks observed at 1250 cm^{-1} and 826 cm^{-1}, which were designated as the asymmetric tensile vibration of S═O and the symmetric tensile vibration of C–O–S (Chen et al., 2022). Therefore, it confirmed the successful inclusion of the sulfate group into LBSDPs, indicating the synthesis of sulfated derivatives.

In phosphorylated LBSDPs, the absorptions were obtained at 1268 cm^{-1}, 1025 cm^{-1}, and 836 cm^{-1} as a result of the existence of three absorption bonds P═O, P–OH, and P–O–C, respectively (Hu et al., 2020). The above absorption peaks demonstrated the successful incorporation of phosphate groups into the LBSDP structure.

Compared with natural polysaccharides, the peaks at 1596 cm^{-1}, 1407 cm^{-1}, and 1322 cm^{-1} of carboxymethyl-modified polysaccharides were due to the asymmetric stretching vibration of C═O in –COO–, the vibration of methylene (–CH_2–) connected to the carboxyl group, and the symmetric stretching vibration of –CO–, respectively (An et al., 2022). The existence of these three characteristic absorption peaks confirmed carboxymethylated LBSDPs.

The FT-IR data (Fig. 1) revealed that chemical modification had successfully introduced sulfation, phosphorylation, and carboxymethylation groups into natural polysaccharides without changing the absorption main body of the carbohydrate chain. Interestingly, in a specific spectral range, slight differences could be found in the absorption peaks of the four natural polysaccharides under the same modification method, which might be attributed to the structural differences of polysaccharides or different degrees of substitution.

3.3. Chemical compositions and DS of LBSDPs and their derivatives

The DS, total sugar content, uronic acid, and protein content of LBSDPs and their derivatives are shown in Tables 1 and 2. The results showed that the chemical composition of LBSDPs changed significantly after sulfation, phosphorylation, and carboxymethylation.

DS result usually indicates the degree to which hydroxyl groups in the molecular structure of polysaccharides are replaced by various groups (sulfate, phosphate, carboxymethyl, acetyl, etc.), and DS is one of the key points affecting the structure and biological activity of chemically modified polysaccharides (Li et al., 2022). The types of polysaccharides and different modification methods are known to affect the DS of derivatives. The order of DS of S-LBSDPs was shown as follows: S-

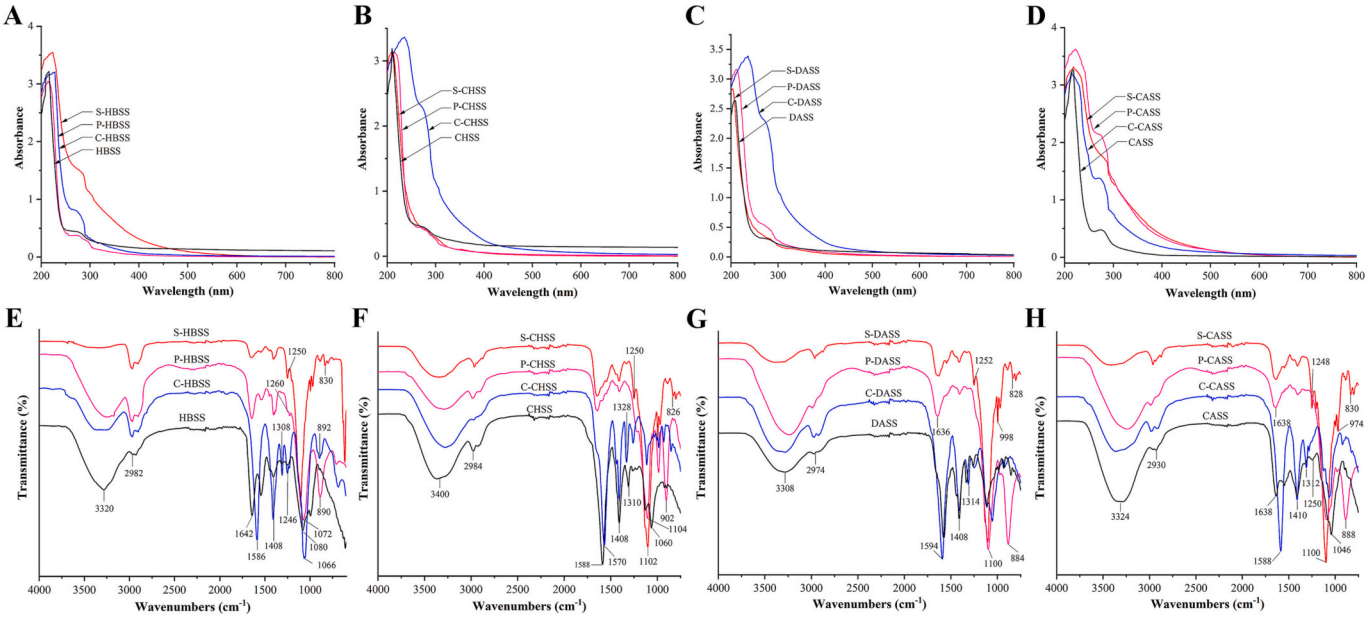

Fig. 1. The UV and FT-IR spectra of *Lycium barbarum* seed dreg polysaccharides and their modified derivatives. UV spectra (A–D); FT-IR spectra (E–H). LBSDP, *Lycium barbarum* seed dreg polysaccharide; HBSS, hot buffer soluble solution; CHSS, chelating agent soluble solution; DASS, dilute alkaline soluble solution; CASS, concentrated alkaline soluble solution; S-LBSDP, sulfated LBSDP; P-LBSDP, phosphorylated LBSDP; C-LBSDP, carboxymethylated LBSDP.

Table 1
The degree of substitution of *Lycium barbarum* seed dreg polysaccharides and their modified derivatives.

Samples	Degree of substitution		
	Sulfated LBSDPs	Phosphorylated LBSDPs	Carboxymethylated LBSDPs
HBSS	0.606 ± 0.049^{bc}	0.540 ± 0.001^{a}	0.676 ± 0.016^{b}
CHSS	0.567 ± 0.022^{c}	0.542 ± 0.002^{a}	0.519 ± 0.018^{d}
DASS	0.649 ± 0.031^{b}	0.381 ± 0.003^{c}	0.714 ± 0.014^{a}
CASS	0.804 ± 0.039^{a}	0.487 ± 0.002^{b}	0.585 ± 0.014^{c}

Note: Values were shown as mean ± *SD* of three replicates. Lowercase letters represent significant differences ($P < 0.05$) in values within columns. LBSDP, *Lycium barbarum* seed dreg polysaccharide; HBSS, hot buffer soluble solution; CHSS, chelating agent soluble solution; DASS, dilute alkaline soluble solution; CASS, concentrated alkaline soluble solution; S-LBSDP, sulfated LBSDP; P-LBSDP, phosphorylated LBSDP; C-LBSDP, carboxymethylated LBSDP.

CHSS > S-HBSS > S-DASS > S-CASS. Among the phosphorylated polysaccharides, the DS of P-HBSS was the highest, which was consistent with the results obtained for *A. edulis* polysaccharides (Cao et al., 2020). After derivatization of LBSDPs by carboxymethylation, the DS of P-DASS was the highest, whereas that of P-CHSS was the lowest. Some studies showed that the DS affects the biological activity of polysaccharides, with both too high and too low values leading to a reduction in their biological activity (Li et al., 2022).

The total sugar and protein content of different modified LBSDPs decreased to varying degrees, whereas the uronic acid content increased (Table 2). However, under the same chemical modification, the changes in the content of different polysaccharides in the chemical composition were different. After sulfate modification, LBSDPs showed comparatively low total sugar content. The order of total sugar content was as follows: S-DASS > S-HBSS > S-CASS > S-CHSS. The total sugar content of P-HBSS was the highest (65.13% ± 1.06%), while that of P-DASS was the lowest (57.43% ± 1.58%). Among the derivatives, the total sugar content of C-LBSDPs derivatives decreased most significantly, which might be due to the maximum DS of carboxymethylation compared with sulfation and phosphorylation. Among them, C-DASS had the highest total sugar content (68.70% ± 1.04%), followed by C-CASS (66.84% ± 4.75%), C-CHSS (64.23% ± 1.91%), and C-HBSS (52.42% ± 1.13%). The total sugar content of the four polysaccharides under different chemical modification methods was as follows: LBSBDPs > S-LBSDPs > P-LBSDPs > C-LBSDPs. The content of uronic acid in the modified polysaccharides was remarkably higher than that in the natural polysaccharide components ($P < 0.05$), as reported by Li et al. (2018), which might be due to the hydrolysis of natural polysaccharides in the modified preparation of polysaccharides, and ultimately led to the increase of the uronic acid content (Rizkyana et al., 2022). Compared with natural polysaccharides, the protein content of S-LBSDPs, P-LBSDPs, and C-LBSDPs decreased, and the decrease was the most significant for S-LBSDPs.

3.4. Thermal properties of LBSDPs and modified products

DSC curve illustrated the thermal transformation of LBSDPs and their derivatives, which was supportive of understanding the thermal changes of food during processing or storage. Fig. 2 shows that natural LBSDPs and modified products had a single endothermic valley within the temperature range of 20 °C–250 °C, which indicated the endothermic enthalpy change required for the complete melting of 1.0 g polysaccharides (ΔH). After HBSS was modified by different methods, its peak temperature increased, which revealed that the thermal stability of LBDSPs after sulfation, phosphorylation, and carboxymethylation was improved to some extent. However, the peak temperature of derivatives of CHSS and CASS decreased. The peak temperature of DASS varied with different modification methods. For example, the peak temperature of S-DASS and C-DASS decreased, whereas that of P-DASS increased. The above phenomenon might be caused by different modification methods that have different changes to the structure of polysaccharides (Cao et al., 2020). The enthalpy change of polysaccharide was different after different modifications, for example, the enthalpy changes of HBSS increased after phosphorylation, while the enthalpy change of HBSS decreased after sulfation and carboxymethyl treatments. The sulfation and phosphorylation of CHSS and DASS could increase their enthalpy values, while the products after carboxymethylation showed a decreasing trend. The enthalpy changes of P-CASS and C-CASS increased, whereas that of S-CASS decreased. The increase in enthalpy indicated the increase of reactive active substances produced during thermal decomposition. The reason for the difference in the above results might be that the hydroxyl group in the polysaccharide molecule had different action sites or the number of substituted groups, which changed the structure of the polysaccharide (Li et al., 2020). In addition, the peak temperature and enthalpy change of different polysaccharides under the same modification method were also different, which might be due to the different carbon water interaction intensity or structure of the four polysaccharides (Cao et al., 2020; Li et al., 2018).

The above results showed that sulfated polysaccharides had excellent thermal stability. At the same time, the enthalpy changes of most LBSDP derivatives increased and more reactive active substances were produced. The thermal properties of polysaccharides represent an important feature of food industry applications, providing a basis for their changes in the process of food heat treatment.

3.5. Surface micromorphology of LBSDPs and modified products

The surface micromorphology of polymers could be observed using SEM, which could help researchers comprehend the physical and functional properties of samples. Fig. 3 revealed that LBSDPs and their chemically modified products had somewhat different

Table 2
The total sugar content, uronic acid content, and protein content of *Lycium barbarum* seed dreg polysaccharides (LBSDPs) and their modified derivatives.

Samples	Total sugar (%)				Uronic acid (%)				Protein (%)			
	LBSDPs	S-LBSDPs	P-LBSDPs	C-LBSDPs	LBSDPs	S-LBSDPs	P-LBSDPs	C-LBSDPs	LBSDPs	S-LBSDPs	P-LBSDPs	C-LBSDPs
HBSS	83.79 ± 0.83^{a}	74.36 ± 1.46^{ab}	65.13 ± 1.06^{a}	52.42 ± 1.13^{b}	0.69 ± 0.04^{ab}	7.03 ± 0.50^{a}	5.19 ± 0.39^{a}	4.80 ± 0.69^{a}	0.42 ± 0.02^{c}	0.23 ± 0.15^{b}	0.38 ± 0.04^{c}	0.41 ± 0.20^{d}
CHSS	80.22 ± 2.16^{b}	71.85 ± 2.27^{b}	64.85 ± 1.30^{a}	64.23 ± 1.91^{a}	0.79 ± 0.03^{a}	1.69 ± 0.25^{b}	1.04 ± 0.54^{b}	1.07 ± 0.35^{b}	0.80 ± 0.08^{c}	0.70 ± 0.20^{b}	0.73 ± 0.30^{c}	0.78 ± 0.13^{c}
DASS	85.69 ± 0.83^{a}	75.50 ± 1.74^{a}	57.43 ± 1.58^{b}	68.70 ± 1.04^{a}	0.10 ± 0.04^{b}	0.91 ± 0.34^{b}	0.42 ± 0.25^{b}	0.84 ± 0.54^{b}	3.43 ± 0.08^{a}	3.14 ± 1.50^{a}	3.29 ± 0.34^{a}	3.42 ± 0.15^{a}
CASS	84.44 ± 1.37^{a}	72.92 ± 1.63^{ab}	63.00 ± 1.27^{a}	66.84 ± 4.75^{a}	0.41 ± 0.06^{ab}	1.14 ± 0.50^{b}	0.91 ± 0.34^{b}	1.10 ± 0.54^{b}	2.46 ± 0.06^{b}	1.76 ± 1.62^{ab}	2.28 ± 0.19^{b}	2.45 ± 0.19^{b}

Note: Values were shown as mean ± *SD* of three replicates. Lowercase letters represent significant differences ($P < 0.05$) in values within columns. LBSDP, *Lycium barbarum* seed dreg polysaccharide; HBSS, hot buffer soluble solution; CHSS, chelating agent soluble solution; DASS, dilute alkaline soluble solution; CASS, concentrated alkaline soluble solution; S-LBSDP, sulfated LBSDP; P-LBSDP, phosphorylated LBSDP; C-LBSDP, carboxymethylated LBSDP.

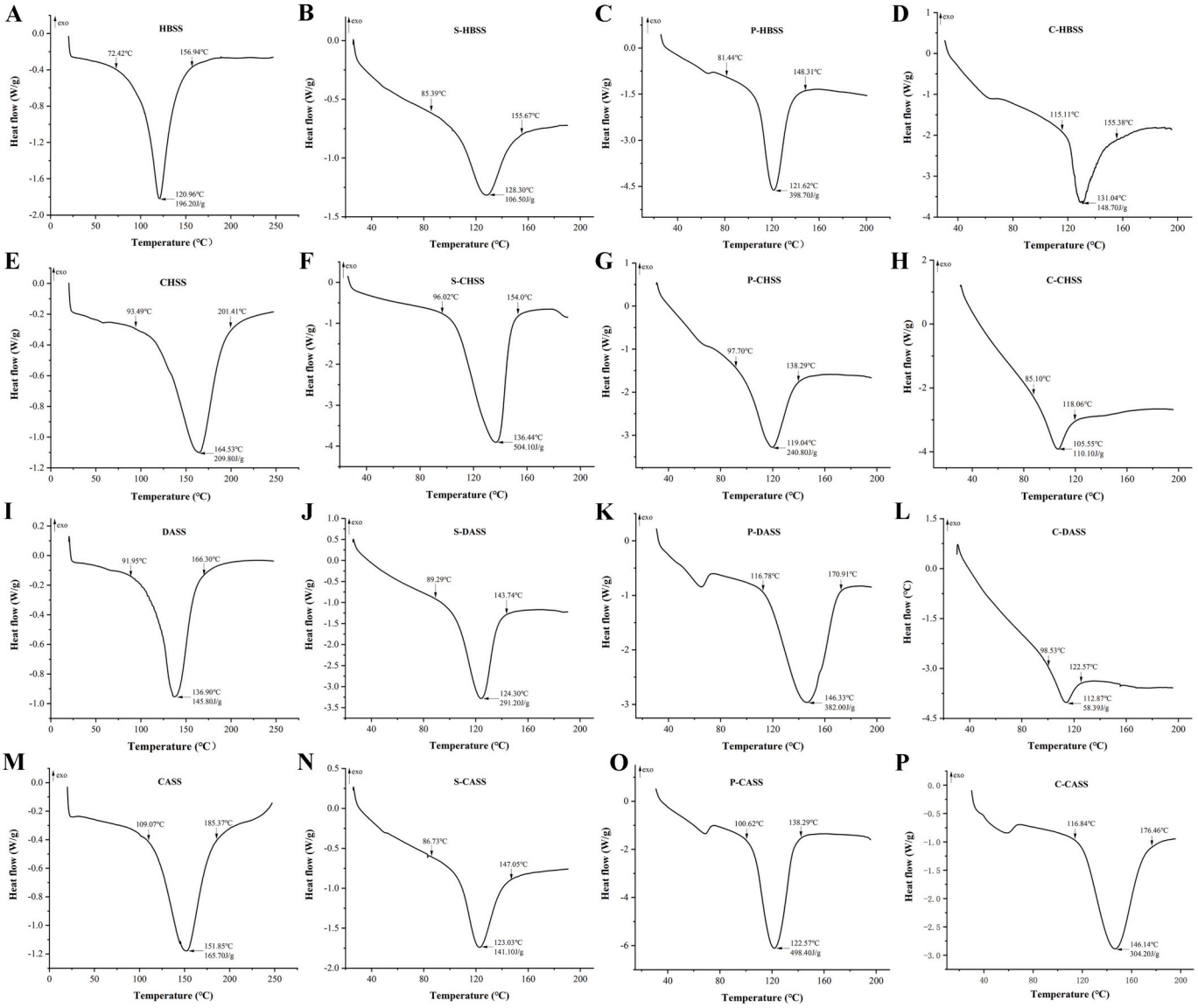

Fig. 2. The DSC properties of *Lycium barbarum* seed dreg polysaccharides and their modified derivatives. LBSDP, *Lycium barbarum* seed dreg polysaccharide; HBSS, hot buffer soluble solution; CHSS, chelating agent soluble solution; DASS, dilute alkaline soluble solution; CASS, concentrated alkaline soluble solution; S-LBSDP, sulfated LBSDP; P-LBSDP, phosphorylated LBSDP; C-LBSDP, carboxymethylated LBSDP.

micromorphology and structure. It was observed that three chemical modification methods had significant effects on the size and morphology of polysaccharides. Under the same chemical modification, the morphological changes of LBSDPs were different, which might be attributed to the structural differences of LBSDPs (Zhang et al., 2022). The natural polysaccharides showed the appearance of sheets, balls, or sticks with different sizes and uneven distribution. For sulfation modification, HBSS and CASS after sulfation modification showed rough surfaces clustered into balls. However, S-CHSS and S-DASS were more uniform and broken, with rough surface shapes. This difference might be due to the fact that CHSS and DASS were relatively simple in appearance compared with HBSS and CASS, showing a single sheet shape, which was easier to break (Zhang et al., 2022). After phosphorylation, four polysaccharides appeared flake and globular. Among them, the surface of the lamellar polysaccharide was rough, with a pit-like structure, and the mesh size was different. This phenomenon might be attributed to the fact that the glycosidic bond was replaced by the phosphate radical group during the modification, which could increase the water solubility of polysaccharide, thus affecting its biological activity (Xie et al., 2020). The spherical surface was smooth and fine, which might be the result of the strong interaction of glycosidic bonds between monosaccharides (Ming et al., 2017). After carboxymethyl modification, it showed irregular sheet, rod, and ball shapes, and rough and uneven surfaces, which might be the result of the interaction of glycosidic bonds with the addition of carboxymethyl groups (Ren et al., 2018). The results showed that different chemical modification methods could introduce chemical groups that distorted and transformed the configuration and orientation of the sugar ring and cause changes in the size and shape of the physical structure of polysaccharides.

3.6. ABTS radical scavenging ability of LBSDPs and modified products

When the substance has the ability to remove ABTS groups, it can combine with the blue–green ABTS groups, thus making the solution lighter or even colorless (Li et al., 2018). The scavenging ability of LBSDPs and their derivatives to ABTS radicals are shown in Fig. 4A–D. Compared with natural members, the sulfated, phosphorylated, and carboxymethyl-modified products had an enhanced ability to scavenge ABTS free radicals, which might be caused by the promotion of ABTS free radical scavenging ability by the addition of chemical groups,

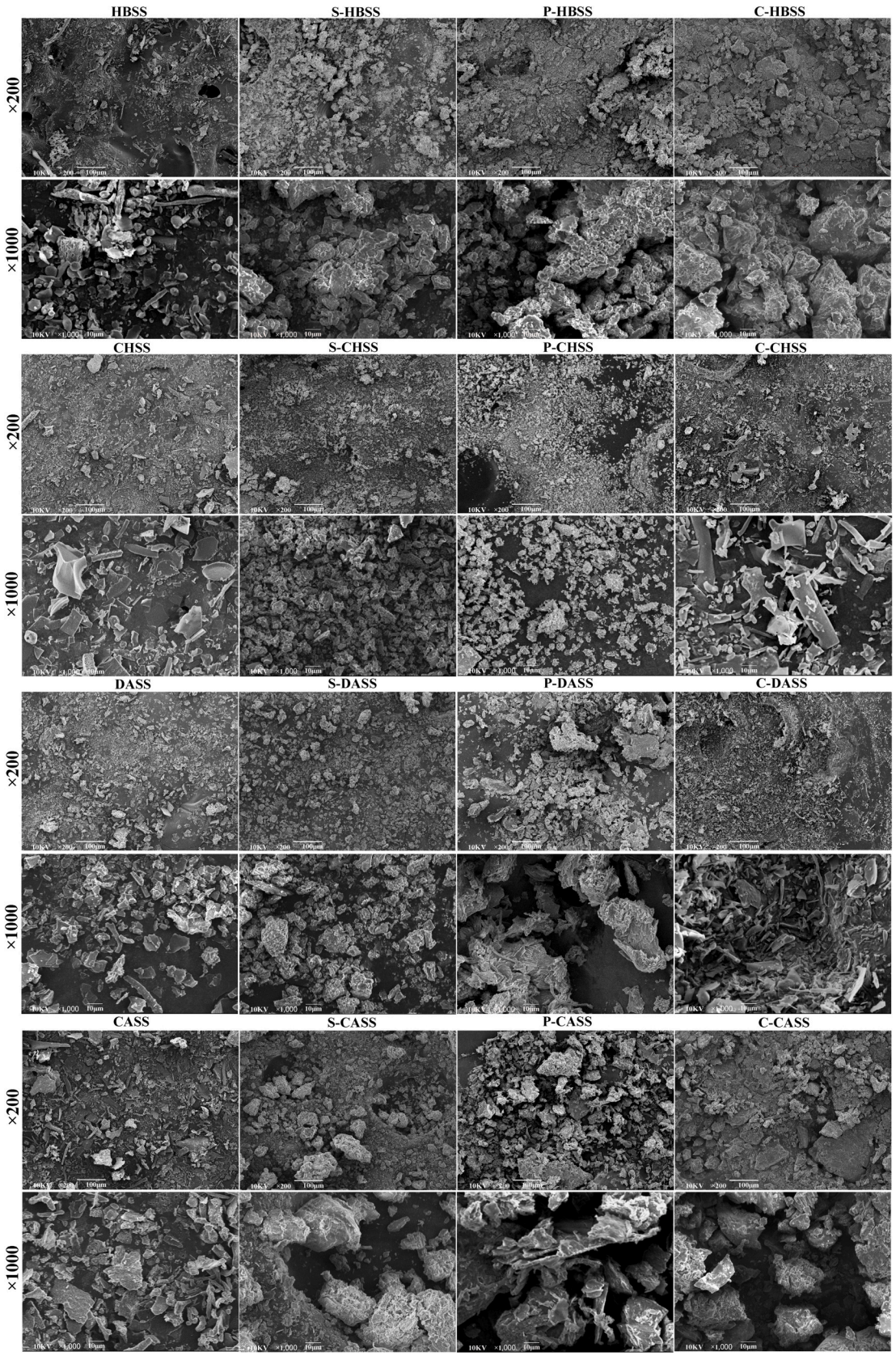

Fig. 3. The microstructure on surface morphology of *Lycium barbarum* seed dreg polysaccharides and their modified derivatives. LBSDP, *Lycium barbarum* seed dreg polysaccharide; HBSS, hot buffer soluble solution; CHSS, chelating agent soluble solution; DASS, dilute alkaline soluble solution; CASS, concentrated alkaline soluble solution; S-LBSDP, sulfated LBSDP; P-LBSDP, phosphorylated LBSDP; C-LBSDP, carboxymethylated LBSDP.

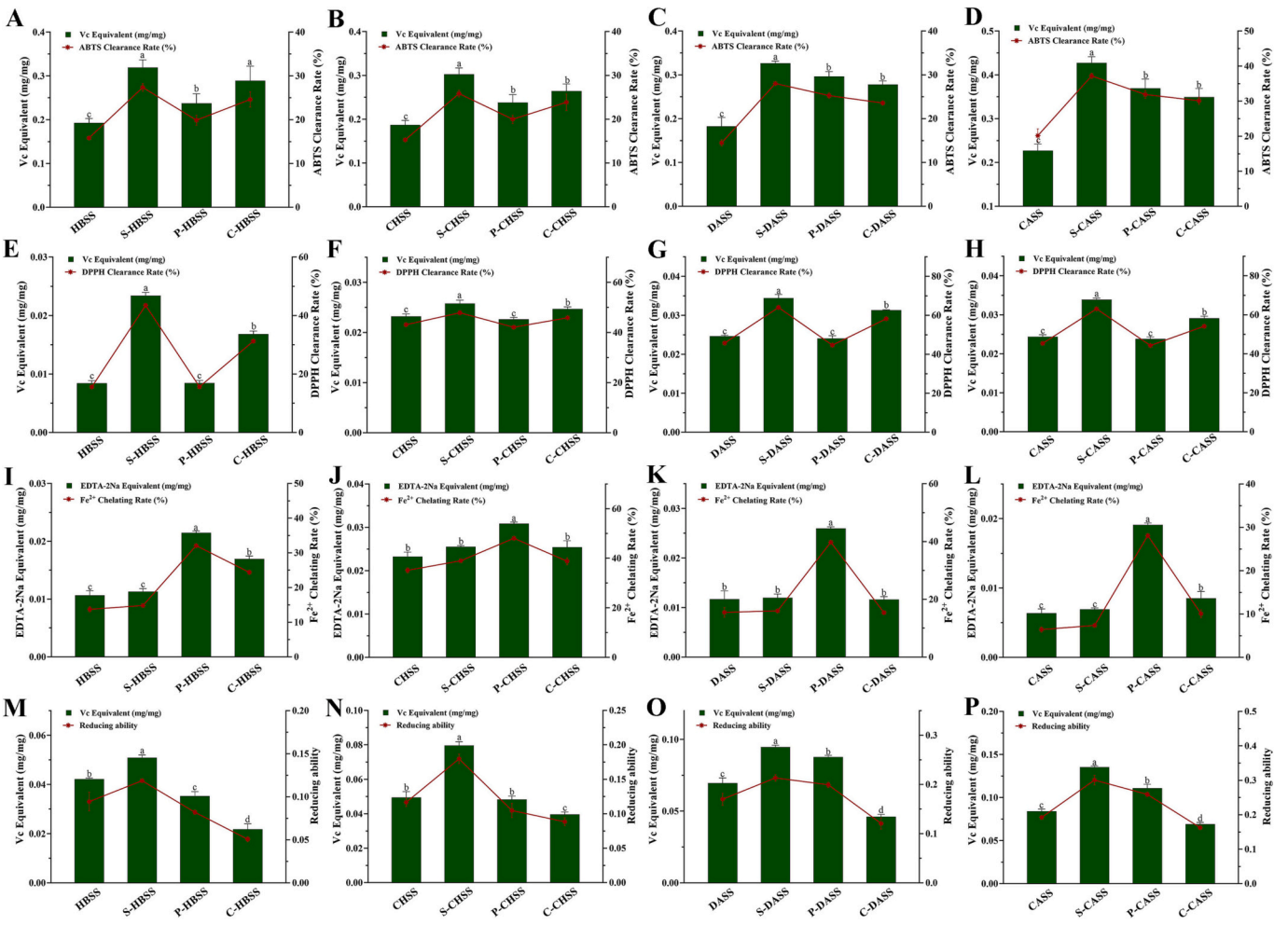

Fig. 4. Antioxidant abilities of *Lycium barbarum* seed dreg polysaccharides and their modified derivatives. ABTS radical scavenging ability of LBSDPs and their modified derivatives (A–D); DPPH radical scavenging ability of LBSDPs and their modified derivatives (E–H); the chelating ability on Fe^{2+} of LBSDPs and their modified derivatives (I–L); reducing power ability of LBSDPs and their modified derivatives (M–P). Values were shown as mean ± *SD* of three replicates. Lowercase letters represent significant differences ($P < 0.05$). LBSD, *Lycium barbarum* seed dreg; LBSDP, *Lycium barbarum* seed dreg polysaccharide; HBSS, hot buffer soluble solution; CHSS, chelating agent soluble solution; DASS, dilute alkaline soluble solution; CASS, concentrated alkaline soluble solution; S-LBSDP, sulfated LBSDP; DS, degree of substitution; P-LBSDP, phosphorylated LBSDP; C-LBSDP, carboxymethylated LBSDP.

thereby enhancing the antioxidant activity of derivatives. Compared with phosphorylated and carboxymethyl-modified products, sulfated derivatives displayed the largest scavenging capacities, which were S-HBSS (27.37%), S-CHSS (25.82%), S-DASS (28.03%), and S-CASS (37.22%), respectively. This result might be caused by the activation of hydrogen atoms in ectopic carbon by sulfate groups, which improved the hydrogen supply ability of polysaccharides. Among them, the scavenging ability of S-CASS increased most significantly, from 20.17% to 37.22%, and the V_C equivalent reached 0.43 ± 0.01 mg/mg. This outcome could be attributed to the higher DS of S-CASS compared with the other three sulfuric acid derivatives. The above results indicated that sulfation treatment could be a promising approach to enhance the antioxidant capacity of natural polysaccharides, which was consistent with the experimental results of Li et al. (2018).

3.7. DPPH radical scavenging ability of LBSDPs and modified products

The hydroxyl group in the polysaccharide molecule could provide pairing for the lone pair of unstable electrons in the DPPH molecule, making it form a stable DPPH-H conjugate, which was characterized by color weakening or disappearance (Li, Lin, et al., 2022). In this study, the results of DPPH removal by LBSDPs and chemically modified LBSDPs were demonstrated in Fig. 4E–H. After the sulfation and carboxymethylation of LBSDPs, their scavenging capacity for DPPH radicals increased, which might be caused by the strong hydrogen supply ability generated by hydrogen activation on heterotopic carbon (Hu et al., 2020). In previous reports, after sulfation and carboxymethylation, the antioxidant capacity of DPPH of *Cyclocarya paliurus* polysaccharides increased significantly (Han et al., 2021; Xiong, Huang, & Huang, 2019). However, the scavenging capacity of phosphorylated products was not significantly different from those of natural polysaccharides. One possible reason was that the introduced phosphate group did not have a great impact on the conformation, charge density, or polarity of LBSDPs, which led to little change in their scavenging ability (Han et al., 2021). In addition, under the same modification method, different polysaccharides had slightly different scavenging activities for DPPH free radicals, which might be due to the influence of the content of uronic acid and glycosidic bonds in polysaccharides on the introduction of different substituents (Cao et al., 2020; Li et al., 2018). The differences in antioxidant capacity of DPPH scavenging ability of LBSDPs and their derivatives might be related to the changes of molecular size, esterification degree, and chemical composition due to modifications.

3.8. Fe^{2+} chelating ability of LBSDPs and modified products

Transition metal ions, like Fe^{2+}, can cause lipid oxidation and cell damage, mainly because they have the ability to decompose hydrogen and lipid peroxidase, thus promoting the production of free radicals. To reduce the damage of these metal ions, previous studies introduced materials with the ability to chelate these ions and oxidation protection (Li et al., 2018). Therefore, the chelating capacity of substances to Fe^{2+} was a significant index to estimate the antioxidant capacity of antioxidants. As shown in Fig. 4I–L, the chemical modification of LBSDPs had a certain impact on Fe^{2+} chelating capacity with a different trend. Compared with sulfation and carboxymethyl modifications, the chelating ability of phosphorylated derivatives to Fe^{2+} was significantly stronger than that of natural LBSDPs. The chelating ability of P-LBDSDPs to Fe^{2+} was 32.07%, 48.08%, 39.76%, and 28.10%, respectively. The above differences might be attributed to the various contents of –OH, $–PO_3H_2$ in samples (Li et al., 2018). Due to the derivatization of the polysaccharide molecular structure after phosphorylation modification, the introduction of the above-mentioned functional groups led to corresponding changes in its biological activity and better Fe^{2+} chelating ability. Sulfation and carboxymethyl derivatives had little effect on enhancing the chelating ability of Fe^{2+}.

3.9. Reducing the power ability of LBSDPs and modified products

Antioxidants scavenge free radicals by giving electrons through their own reduction. The reducing power of the substance is positively correlated with the antioxidant ability, that is, the greater the reducing ability, the stronger the antioxidant capacity. The reducing power of LBSDPs and their modified products is shown in Fig. 4M–4P. Three chemical modification methods had different effects on the reducing power of natural LBSDPs, whereas sulfated derivatives had the largest reducing power. The total reducing power of S-LBSDPs was 0.12, 0.18, 0.27, and 0.30, respectively, and the V_C equivalent was 0.05 mg/mg, 0.08 mg/mg, 0.09 mg/mg, and 0.13 mg/mg, respectively. This might be due to the introduction of some $–OSO_3H$ groups into the molecules of LBSDPs after sulfation modification, and the hydrolysis of some branch chains, resulting in the change of spatial structure (Anjali et al., 2022). In addition, compared with natural members, there was no significant change in the reduction ability of phosphorylation and carboxymethyl derivatives, and their effects were even weaker. It might be due to the low DS that the introduced phosphate and carboxymethyl groups had insufficient ability to activate hydrogen atoms on the heteroterminal carbon chain of polysaccharides, resulting in a small amount of hydrogen atom donors. The above results were consistent with the results obtained by Li et al. (2018) and Cao et al. (2020). Compared with natural polysaccharides, the sulfated products showed stronger reducing power, whereas phosphorylation and carboxymethylation exerted little or even weak effect (Cao et al., 2020; Li et al., 2018).

4. Conclusion

Four polysaccharides prepared from LBSD were modified using three different chemical modification methods: sulfation, phosphorylation, and carboxymethylation. UV and infrared spectra confirmed the successful chemical modification. The total sugar and protein content of the derivatives decreased to varying degrees, whereas the uronic acid content increased. Sulfated polysaccharides exhibited excellent thermal stability. At the same time, the enthalpy changes of chemically modified LBSDPs increased. S-LBSDP and P-LBSDP showed the highest antioxidant capacity. Our research results speculated that chemical modifications could exert a significant effect on the improvement of the antioxidant activity of polysaccharide derivatives, and it can further lead to novel research avenues for developing natural antioxidants. Our data lay the foundation for the application of polysaccharides and their derivatives as antioxidants in the food and health industry.

CRediT authorship contribution statement

Xiu-Xiu Zhang: Writing – original draft, Methodology, Investigation. **Wang-Wei Zhang:** Methodology, Investigation. **Zhi-Jing Ni:** Resources, Project administration, Formal analysis, Data curation. **Kiran Thakur:** Writing – review & editing, Software, Resources. **Jian-Guo Zhang:** Validation, Software, Resources. **Mohammad Rizwan Khan:** Writing – review & editing, Funding acquisition. **Wen-Di Xu:** Formal analysis, Data curation. **Zhao-Jun Wei:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

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

No data was used for the research described in the article.

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Further reading

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