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Effects of ultra-high-pressure on the quality of polysaccharides from the fresh stems of *Dendrobium officinale*

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Abstract

Fresh medicine is the use of fresh Chinese herbs after mining. The aim of the present work was to investigate the feasibility of ultra-high-pressure processing in the fresh medicine field. The typical fresh medicine *Dendrobium officinale* was used as the research material. The effects of pressure level (100 - 500 MPa), processing duration (0.5 - 2.5 min), number of processing cycle (1 - 5), and temperature (20 - 100°C) on the polysaccharide loss (in pressure transmitting medium) and dissolution efficiency (in boiling water) were studied. The results show that all parameters not only caused a dramatic loss of polysaccharides but also significantly increased the dissolution quantity, with the largest loss resulting from the number of repetitive processing cycles, which reached 47.11% after five processing steps. Analysis of the infrared spectroscopy and antioxidant activity results showed that the structure and antioxidant activity of the polysaccharides extracted from the stems processed by the parameters with the maximum value were markedly different, and the antioxidant activity of the polysaccharides extracted from the stems processed five times was the weakest. Ultra-high-pressure processing has good prospects in fresh medicine, while further research on process optimisation is necessary.

Introduction

Dendrobium officinale Kimura et Migo is an orchid that is known as a valuable Chinese herbal medicine. It has been recorded in the "Sheng Nong's Herbal Classic" and "Compendium of Materia Medica" that *D. officinale* can improve immunity and nourish the body (Lv *et al.,* 2013). Modern pharmacological research and clinical applications have shown that *D. officinale* has the pharmacological actions of improving immunity, regulating blood sugar, lowering blood pressure, resisting fatigue, oxidative damage and tumours, protecting the liver, clearing heat, engendering fluid and liquid, nourishing yin, relieving cough, and reducing sputum (Ng *et al.,* 2012). It is also recorded in the "Compendium of Materia Medica" that dried *D. officinale* is more suitable for deficiency syndromes, while fresh *D. officinale* is more suitable for excess syndromes. In clinical practice, the dried stems of *D. officinale* are mainly used to treat the so-called heat deficiency, and the fresh stems are mainly used to treat excessive heat (Levy *et al.,* 2015). This shows that fresh *D. officinale* has special effects. The food culture of *Dendrobium* is drinking the water extracted from its stems. However, the stems of *D. officinale* are rich in mucus, which blocks the dissolution of the active substances such as the polysaccharides, and weakens the nutritional value and medicinal effects, thus reducing the efficacy of fresh *D. officinale* (He *et al.,* 2018). This is the technical bottleneck for the development of the *Dendrobium* industry. There are two key points to improve the pharmacological activities of fresh *D. officinale*: (i) to reduce the loss of active substances during processing, and (ii) to improve the dissolution efficiency of active substances during hot water extraction for drinking (Li *et al.,* 2013).

Ultra-high-pressure processing is a conventional non-thermal processing technology for food products. This technology has the advantages of sterilising, inactivating enzymes, retaining nutrients in food, and maintaining food colour and flavour (Daryaei *et al.,* 2016). The principle of this technology is that ultra-high-pressure promotes solvent penetration into material tissues and cells, thus quickly destroying the tissue and cell structures (Palaniyandi *et al.,* 2017), changing the conformation of macromolecules, destroying the binding force between molecules (Błaszczak *et al.,* 2017), and achieving sterilization, enzyme elimination, macromolecular modification, and solubilisation of active substances (Cheok *et al.,* 2014). Therefore, ultra-high-pressure technology has broad application prospects in the field of *Dendrobium*.

The nutritional values and medicinal effects

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of *D. officinale* primarily come from the polysaccharides, which make up approximately 30 - 45% of the content, and are used as the quality standard of *Dendrobium* products (Chinese Pharmacopoeia Commission, 2015). The polysaccharides of *D. officinale* have some health functions, including protecting the liver, improving eyesight, and benefiting the stomach (Wei *et al.,* 2016). However, the polysaccharides of *D. officinale* have a high viscosity which leads to their low solubility, and physical and chemical instability (Xu *et al.,* 2013). However, studies have shown that ultra-high-pressure treatment can increase the content of water-soluble polysaccharides in the fresh juice of *D. officinale*, and increase the bioavailability of the polysaccharides (Zhu *et al.,* 2018). However, to the best of our knowledge, studies on the solubilisation and quality of polysaccharides extracted from the fresh stems of *D. officinale* by ultra-high-pressure processing are presently rather limited.

Based on the above, it is necessary to investigate the effects of processing level, processing duration, number of processing cycles, and temperature on the extraction efficiency of polysaccharides by hot water and the polysaccharide loss rate in water, in the pressure-transfer medium from polypropylene bags. The antioxidant activities *in vitro* and *in vivo* were used to evaluate the quality of polysaccharides. The present work is theoretically significant for the development of *Dendrobium* as a fresh medicine, and has important practical application value for breaking the bottleneck of the *Dendrobium* industry.

Materials and methods

Materials and reagents

Fresh stems of *D. officinale* were provided by the School of Biology and Food Engineering of Chuzhou University. Concentrated sulphuric acid, re-steamed distilled phenol (99.5% phenol), absolute ethanol, and glucose standard D-galactosamine (D-Gal, Sigma-Aldrich) (Sinopharm Group Chemical Reagent Co., Ltd., Shanghai, China) were used, and were of analytical grade. A hydroxyl radical determination kit, a 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) determination kit, an anti-superoxide anion determination kit, and a Fe^{2+}-chelating determination kit were purchased from Hefei Jingke Biotechnology Co., Ltd. (China).

Experimental instruments

Ultra-high-pressure equipment (HPP.LI-600/3, Tianjin Huatai Senmiao Bioengineering Technology Co., Ltd., Tianjing, China), a UV-visible spectrophotometer (T-6, Beijing Persee General Instrument Co., Ltd., Beijing, China), and a Fourier transform infrared spectrometer (FTIR-650, Tianjin Port East Technology Co., Ltd., Tianjin, China) were used.

Ultra-high-pressure processing

A certain weight of *D. officinale* fresh stems was placed in high-pressure polypropylene bags, and then deionised water (10-fold) was added, followed by heat sealing after air extraction with a vacuum packing machine. The sealed bags were placed in the kettle of the high-pressure equipment, and ultra-high-pressure treatment was conducted according to the set conditions. The bags were removed, and the stems and transfer medium were separated. Water from the pressure-transfer medium in the polypropylene bag was used to detect the polysaccharide content. The stems were used to determine the extraction efficiency of polysaccharides. The stems were put into a Soxhlet extractor and extracted with boiling water. Then, the content of polysaccharides was determined every 2 h until no more polysaccharides dissolved.

Experimental design

First, the effect of pressure level (100, 200, 300, 400, and 500 MPa) on polysaccharide loss and dissolution quantity was explored, under the conditions of the desired pressure once for 1.5 min at 60°C. Then, the effect of pressure holding time (0.5, 1, 1.5, 2, and 2.5 min) on polysaccharide loss and dissolution quantity was tested at 300 MPa at 60°C. Third, the effects of different numbers of repetitive processing steps (1, 2, 3, 4, and 5) on polysaccharide loss and dissolution quantity were measured at 300 MPa for 1.5 min at 60°C. Finally, the effects of temperature (20, 40, 60, 80, and 100°C) on the loss and dissolution quantity were determined at 300 MPa for 1.5 min.

Polysaccharide determination

The polysaccharide standard curve was prepared according to Luo *et al.* (2016), and the standard curve was determined to be y = 10.885x - 0.0551, with R^2 = 0.9988, x = concentration of the glucose standard in mg/L, and y = absorbance.

The total polysaccharides in *D. officinale* were measured as follows: the fresh stems of *D. officinale* were homogenised into liquid by a 20-fold excess of water, and then extracted for 30 min at 100°C. After filtering, the filtrate was removed, and the residue was extracted two times under the same

conditions. The filtrates were combined, and 2 mL of the extract was removed to determine the absorbance. The standard curve was used to calculate the polysaccharide content from the measured absorbance value.

The polysaccharide content in the extracts was determined as follows: fresh stems were placed into a Soxhlet extractor, a 10-fold excess of deionised water was added, and the polysaccharides were extracted at 100°C. Then, 2 mL of the extracts was removed every 2 h to determine the absorbance. The standard curve was used to calculate the polysaccharide content from the measured absorbance value.
The polysaccharide content in the pressure-transmitting medium was measured as follows: 2 mL of pressure-transmitting medium was removed to measure the absorbance. The standard curve was used to calculate the polysaccharide content from the measured absorbance value.

Calculation of polysaccharide content

The polysaccharide content in the sample was expressed as the mass fraction ω, and the calculation was carried out using Eq. 1, with the unit of milligrams per millilitre (mg/mL):

$$\omega = (m_1 \times V_1 / m_2 \times V_2) \times 0.9 \times 10^{-4} \quad \text{(Eq. 1)}$$

where, m_1 = content of polysaccharides in the sample liquid measured from the standard curve, m_2 = quantity of the sample, both with units of mg, V_1 = constant volume sample, V_2 = volume of the sample liquid removed during colorimetric determination, both with units of mL; and 0.9 = correction factor for the conversion of glucose to dextran.

Calculation of loss rate

The polysaccharide loss rate was expressed as the mass fraction L, and the calculation was carried out using Eq. 2 with the unit of the percentage (%):

$$L = (m_3 / m_4) \times \% \quad \text{(Eq. 2)}$$

where, m^3 = content of polysaccharide in the pressure transmitting medium, and m^4 = total polysaccharides in *D. officinale*, both with units of mg.

Extraction of polysaccharides

According to the results of single-factor experiments, the levels of the factors that yielded the greatest effect on the dissolution efficiency of polysaccharides in boiling water were selected to process the fresh stems of *D. officinale*. After ultra-high-pressure processing, the polysaccharides in the extraction solution were mixed with anhydrous ethanol (1:4), and incubated overnight at 4°C. Then, the precipitate was centrifuged to obtain the crude *D. officinale* polysaccharides, after which the pigment was eliminated, giving the *D. officinale* polysaccharides (DOPs), denoted as D-I, D-II, D-III, and D-IV.

Spectral analysis

Samples of polysaccharide (0.05 mg/mL) were scanned in the wavelength range of 190 - 380 nm in an UV-Vis spectrophotometer. A small amount of potassium bromide crystals was added to a small amount of DOP. This mixture was ground into impalpable particles in an agate mortar, pressed into transparent sheets with a tablet press, and scanned in the infrared region of 400 - 4000 cm^{-1} with a micro-infrared spectrometer.

Determination of the antioxidant capacity in vitro

The hydroxyl radical and DPPH scavenging activity as well as the Fe^{2+}-chelating activity of the polysaccharides extracted by different ultra-high-pressure processing parameters was determined as instructed by the manufacturer's application manual (Hefei Jingke Biological Technology Co., Ltd., China). The hydroxyl radical, DPPH scavenging activity, and Fe2+-chelating capacity ϕ were calculated using Eq. 3:

$$\phi = [(B-S)/B] \times \% \quad \text{Eq. 3)}$$

where, B = blank absorbance, and S = sample absorbance.

Determination of the antioxidant capacity in vivo
Animal experimental design

Eight-week-old healthy male Kunming mice with body weights of 20 ± 2 g were used to evaluate the antioxidant capacity *in vivo*. All mice were reared at 50 - 60% relative humidity with a temperature of 22 ± 1°C, and a 12 h light/12 h dark cycle. All mice had free access to water and food throughout the experiments. All treatments were approved by the Academic Ethics Committee of Hefei University of Technology.

After one week of acclimation to the environment, the experimental mice were randomly assigned into twenty-six groups (six per group). The model mice were induced by D-Gal (100 mg/kg body weight) via hypodermic injection, and then fed the same volume of physiological saline by gastric gavage once per day. D-I groups were given a gavage

of D-I at doses of 40 mg/kg body weight daily, and hypodermically injected with D-Gal (100 mg/kg body weight daily). The D-II, D-III, and D-IV groups were all fed in the same way as the D-I groups. All mice were managed once a day for thirty days.

Biochemical assay

Twelve hours after the last dose, mouse blood was collected from the posterior orbital sinus, which was then centrifuged to obtain the erythrocytes. Then, the mice were sacrificed via decapitation, and sterilised with 57% alcohol. The livers and hearts were aseptically excised, immediately homogenised in ice-cold physiological saline (10%), centrifuged, and the obtained supernatant was temporarily saved for further analysis. All processing was performed at 4°C.

The inhibitory effects on MDA generation, GSH-Px, and SOD were measured according to the instructions of commercially available kits (Biotech Shanghai Engineering Co., Ltd., China).

Data processing and analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences program (SPSS version 13.0). Differences between groups were assessed by one-way analysis of variance (ANOVA) and least significant difference (LSD) to judge whether the results were statistically significant. The critical p value of both tests was set to 0.05, and Origin 8.0.6 software was used for mapping.

Results

Effect of ultra-high-pressure processing on the loss rate of polysaccharides

Figure 1 shows the effects of processing duration, temperature, and the number of applied pressure cycles on the loss rate of polysaccharides in the pressure-transmitting medium. These four ultra-high-pressure processing parameters all caused significant loss ($p < 0.05$), and the loss rate increased with increases in the parameters ($p < 0.05$). Among them, the number of processing cycles had the greatest effect on the polysaccharide loss rate; the loss rate reached 21.84% when the pressure was applied twice, and the loss rate was nearly half

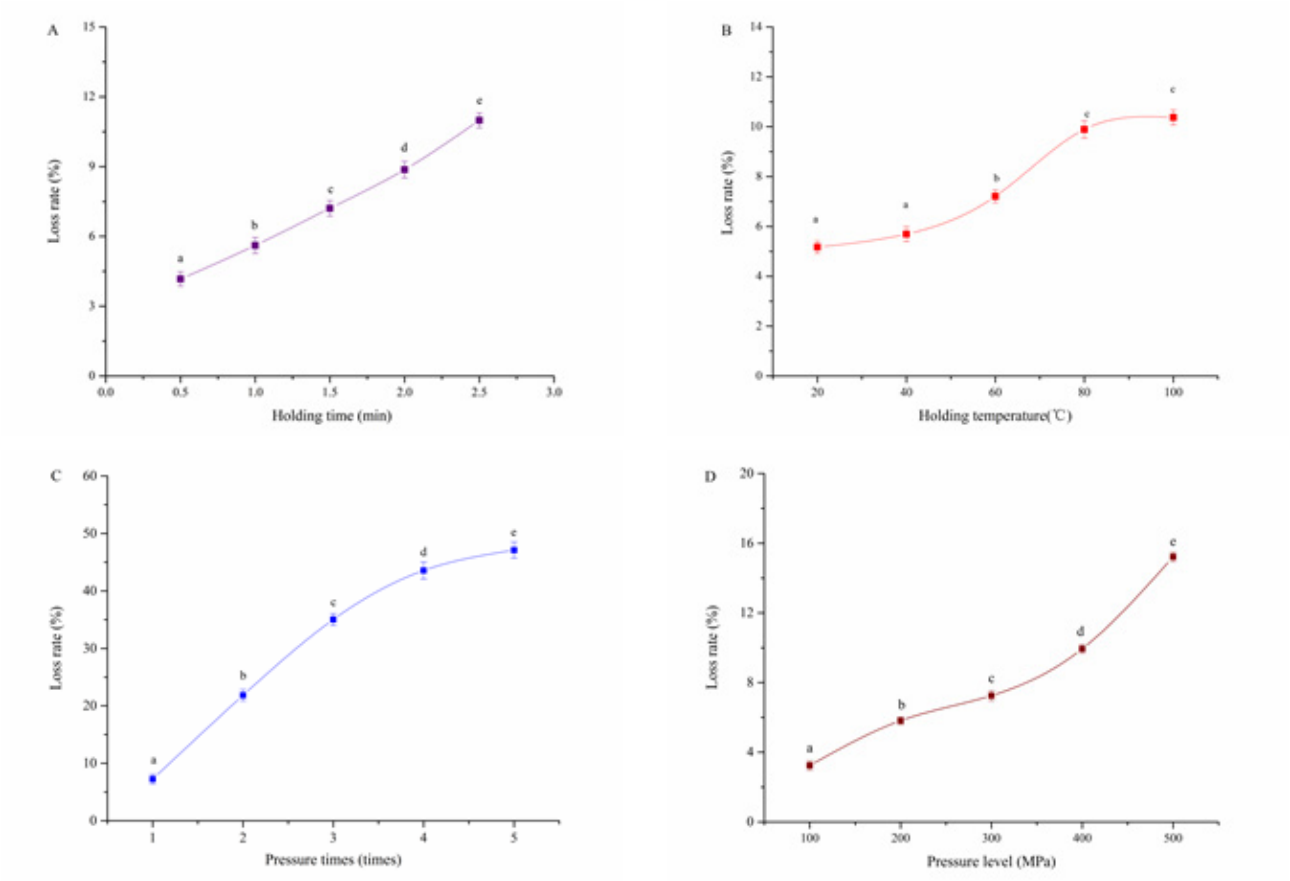

Figure 1. The loss rate of polysaccharides in the pressure transfer medium. (A) processing duration; (B) pressure temperature; (C) number of processing cycles; and (D) pressure level. The data are presented as mean ± SD of triplicates ($n = 3$), and evaluated by one-way ANOVA followed by LSD to detect inter-group differences. Different superscript lowercase (a-e) in groups denote significant difference ($p < 0.05$).

(47.11%) after five pressure cycles (Figure 1C). The second factor was the pressure level, and the loss rate reached 7.25, 9.94, and 15.22% at 300, 400, and 500 MPa, respectively (Figure 1D). The polysaccharide loss rate and duration showed a linear correlation and reached 10.99% after 2.5 min (Figure 1A). When the temperature reached 60°C, the loss of polysaccharide was obvious (7.21%), and the loss rates were 9.89 and 10.37% at 80 and 100°C, respectively (Figure 1B).

Effects of ultra-high-pressure processing on the dissolution efficiency of polysaccharides

The dissolution efficiency of polysaccharides from *D. officinale* processed by ultra-high-pressure in boiling water is shown in Figure 2. Ultra-high-pressure processing had a significant effect on the dissolution efficiency of the polysaccharides. As compared to the control (without ultra-high-pressure processing), the greater the pressure level and temperature, the longer the processing duration; and the larger the number of applied pressure cycles, the higher the dissolution efficiency ($p < 0.05$). However, Figure 2C shows that as the number of repetitive processing cycles increased, the increasing trend of dissolution efficiency decreased, and the dissolution trend of polysaccharides in boiling water was not obvious after five processing cycles. This may be related to the loss of polysaccharides.

Infrared spectral scan results

The infrared spectra of four polysaccharides from the maximum processing level, temperature, cycles, and time are shown in Figure 3. Figure 3 shows that the characteristic absorption peaks of polysaccharides were 3390, 1640, and 1400 cm^{-1}. The broad peak at 3380 cm^{-1} was the characteristic of the stretching vibration of intermolecular and intramolecular hydrogen bonds from the O-H groups. The absorption peak at 2972 - 2880 cm^{-1} was the C-H stretching vibration. The characteristic peak of the C=O stretching vibration was at 1680 cm-*1*, indicating that the polysaccharide was a glycoprotein conjugate. The peak at 1625 cm^{-1} was the O-H absorption peak. The 1400 cm^{-1} peak was the C-H angular vibration absorption peak. The stretching vibration peak at 1100 - 900 cm^{-1} was from C-O-C (Khemakhem *et al.,* 2018). Four polysaccharides had

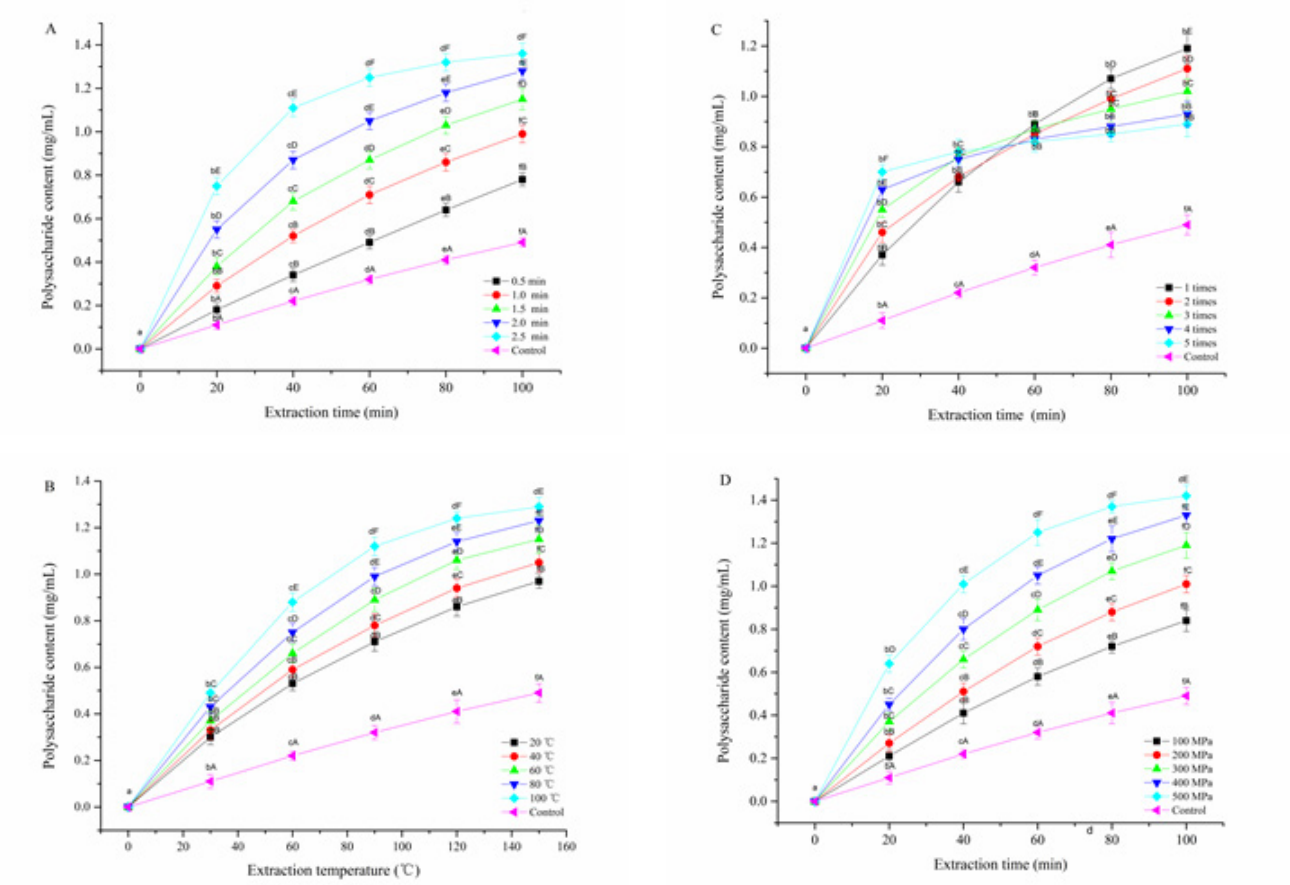

Figure 2. The dissolution efficiency of polysaccharides in boiling water. (A) processing duration; (B) pressure temperature; (C) the number of processing cycles; and (D) pressure level. The data are presented as mean ± SD of triplicates (n = 3), and evaluated by one-way ANOVA. Different superscript lowercase ($^{a-e}$) in groups were considered to be statistically significant ($p < 0.05$). Different superscript uppercase ($^{A-F}$) among groups denote significant difference ($p < 0.05$).

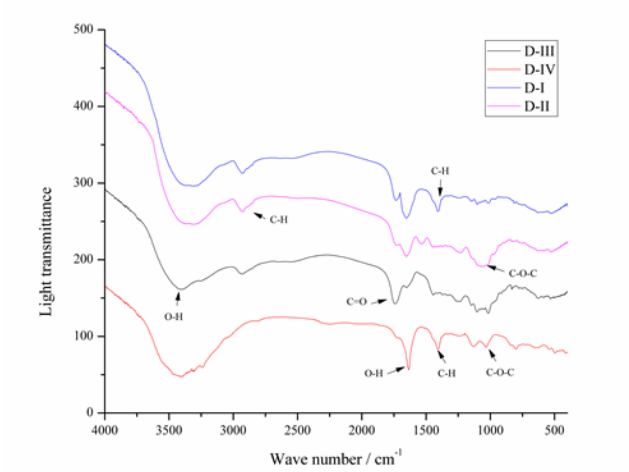

Figure 3. Infrared spectra of DOPs from the fresh stem extracted under different conditions. (D-I) DOP by pressure duration of 2.5 min at 300 MPa at 60°C; (D-II) DOP by pressure temperature of 100°C at 300 MPa for 1.5 min; (D-III) DOP by five processing cycles at 300 MPa for 1.5 min at 60°C; and (D-IV) DOP by pressure level of 500 MPa for 1.5 min at 60°C.

O-H groups, and the O-H amplitude of DOP-1 was the highest. DOP-2, DOP-3, and DOP-4 had C-H and C=O groups, and the C=O amplitude of DOP-2 was the highest. DOP-1, DOP-2, and DOP-3 had a C-O-C group, and the C-O-C amplitude of DOP-3 was the greatest. This indicates that the effect of pressure level, time, temperature, and cycles is different for different polysaccharides dissolution characteristics.

Antioxidant activity of polysaccharides in vitro

The OH scavenging activities of the polysaccharides extracted from the different ultra-high-pressure treatments were 50.64, 59.48, 30.66, and 62.15%; the Fe^{2+}-chelating abilities were 65.27, 60.54, 23.49, and 80.44%; and the DPPH scavenging activities were 29.59, 30.13, 15.53, and 37.22% at 3.0 mg/mL for D-I, D-II, D-III, and D-IV, respectively (Table 1). It was obvious that polysaccharides of D-IV exhibited the highest antioxidant activities as compared to the other polysaccharides ($p < 0.05$), and D-III exhibited the lowest antioxidant activities.

Antioxidant activity of polysaccharides in vivo

The SOD activities of the polysaccharides extracted from different ultra-high-pressure treatments were 92.04, 87.26, 64.55, and 119.08 U/mg protein, and the GSH-Px activities were 224.08, 249.33, 202.52, and 281.55 in the livers of the model mice at 3.0 mg/mL for D-I, D-II, D-III, and D-IV, respectively (Table 2). It was obvious that SOD and GSH-Px activities of all DOPs were significantly higher than that of the control

Table 1. DOPs from different ultra-high-pressure treatments, and their antioxidant activities (*in vitro*).

DOPs	·OH scavenging activity (%)	DPPH scavenging activity (%)	Fe^{2+}-chelating ability (%)
D-I	50.64 ± 0.70^{b}	29.59 ± 0.17^{b}	65.27 ± 0.52^{b}
D-II	59.48 ± 0.65^{b}	30.13 ± 0.09^{b}	60.54 ± 0.33^{b}
D-III	30.66 ± 0.32^{a}	15.53 ± 0.61^{a}	23.49 ± 0.07^{a}
D-IV	62.15 ± 1.17^{c}	37.22 ± 0.25^{c}	80.44 ± 0.21^{c}

•OH, DPPH scavenging activity, and Fe^{2+}-chelating ability were tested at 3.0 mg/mL. (D-I) DOP by pressure time of 2.5 min; (D-II) DOP by pressure temperature of 100°C; (D-III) DOP by pressure times of five times; (D-IV) DOP by pressure level of 500 MPa. The data are presented as mean ± SD of triplicates ($n = 3$), and different superscript lowercase ($^{a-c}$) denote significant difference ($p < 0.05$).

($p < 0.05$), the SOD and GSH-Px activities of D-IV exhibited the highest antioxidant capacity as compared to the other polysaccharides ($p < 0.05$), and D-III exhibited the lowest SOD and GSH-Px activities ($p < 0.05$). The MDA levels were 27.27, 28.14, 30.25, and 22.05 for D-I, D-II, D-III, and D-IV, respectively (Table 2). It was obvious that all MDA levels of experimental group were significantly lower than that of the control ($p < 0.05$), D-IV had the lowest MDA level among the polysaccharides ($p < 0.05$), and D-III exhibited the highest MDA level among the polysaccharides ($p < 0.05$).

Table 2. DOPs from different ultra-high-pressure treatments, and their antioxidant activities (in vivo).

DOPs	SOD (U/mg protein)	GSH-Px (U/mg protein)	MDA (nmol/mg protein)
D-Gal	43.14 ± 4.24^{a}	183.45 ± 6.09^{a}	34.55 ± 1.23^{a}
D-I	92.04 ± 5.88^{c}	244.07 ± 8.22^{c}	27.27 ± 1.17^{c}
D-II	87.26 ± 4.76^{c}	249.33 ± 7.45^{c}	28.14 ± 1.09^{c}
D-III	64.55 ± 4.87^{b}	202.52 ± 9.03^{b}	30.52 ± 1.21^{b}
D-IV	119.08 ± 6.09^{d}	281.55 ± 11.21^{d}	22.05 ± 1.02^{d}

SOD, GSH-Px activity, and MDA level were tested using model mice induced by D-Gal at 40 mg/kg. (D-I) DOP by pressure time of 2.5 min; (D-II) DOP by pressure temperature of 100°C; (D-III) DOP by pressure times of five times; (D-IV) DOP by pressure level of 500 MPa. The data are presented as mean ± SD of six replicates ($n = 6$), different superscript lowercase ($^{a-d}$) denote significant difference ($p < 0.05$).

Discussion

Traditional Chinese medicine and Western medicine have their own characteristics for clinical treatment (Li *et al.,* 2019). For example, traditional Chinese medicine plays a key role in fighting the new coronavirus and SARS (Yang *et al.,* 2020). Fresh medicine is an important part of traditional Chinese

medicine; however, drinking the extracted solution of fresh medicine is the diet culture of traditional Chinese medicine (Wu and Liang, 2018). Therefore, an increasing number of researchers are interested in the study of fresh Chinese medicine, including improving biological activity and developing new products (Huo *et al.,* 2017).

Ultra-high-pressure processing technology is a food processing technology at low temperature, and research has shown that this processing technology can kill bacteria, keep the food fresh, and promote the dissolution of active substances (Balasubramaniam, 2015). Therefore, it has been applied in the study of fresh medicine in recent years, including sterilisation, enzyme denaturation, and the extraction of active substances (Jun *et al.,* 2011). However, research on the effects of ultra-high-pressure processing on fresh medicine as a whole is very limited. In the present work, *D. officinale* was used as the research material because it is a typical fresh medicine (He *et al.,* 2018), and the pressure level, temperature, processing duration, and number of processing cycles all improved the dissolution efficiency of polysaccharides (Figure 2). However, all these caused some degree of polysaccharide loss, particularly after processing more than twice (Figure 1). Therefore, although ultra-high-pressure technology is a very good technique for fresh medicine processing, further research on processing technology is needed.

There are several reasons for the increase in the dissolution efficiency of polysaccharides from *D. officinale* treated by ultra-high-pressure processing. One is that ultra-high-pressure processing has destructive effects on tissues, cells, and molecular binding forces which result in the release of active substances from cells (Palaniyandi *et al.,* 2017). Matser and Timmermans (2016) found that the degree of destruction of cell structure and intermolecular forces plays a major role in the polysaccharide dissolution amount. Moreover, according to Barba *et al.* (2015), when the pressure parameters increase, the destruction of the physical properties of viscous polysaccharides is aggravated, the viscosity weakens, and the dissolution resistance of polysaccharides is reduced, thus resulting in a significant increase in dissolution. In addition, Yan and Xi (2017) found that boiling water reduced the viscosity of mucopolysaccharides, and the viscosity coefficient weakened when the extraction time increased, thus resulting in a decrease in the dissolution resistance of polysaccharides from *D. officinale.*

Different structures of polysaccharides have different physical properties (Duan *et al.,* 2018). The difference in the dissolution efficiency of polysaccharides from *D. officinale* illustrated the different compositions of the polysaccharides. The infrared scanning images can distinguish the groups in the polysaccharide structure. The infrared scanning images of the four polysaccharides from *D. officinale* confirmed that the structure of the polysaccharide extracts from different pressure processing conditions were different to some extent. Pandey *et al.* (2012) noted that IR peak height can also be used to determine the content of specific groups. Fei *et al.* (2017) quantitatively evaluated the degree of substitution (DS) of highly acetylated cellulose acetate (CA) using FTIR, and found that the specific peak parameter ratio can effectively evaluate and monitor the acetylation process. In the present work, the peak parameters of the hydroxyl groups in the polysaccharides extracted by 500 MPa were the largest, followed by 100°C and 2.5 min, and five cycles showed the minimum amount of polysaccharides. These results suggested that the structure of the polysaccharides from *D. officinale* treated by different ultra-high-pressure parameters is different, which may be because small molecular weight polysaccharides are dissolved first (Zhan *et al.,* 2019). Moreover, Li *et al.* (2020) reported that high-pressure processing can change the structural and rheological properties of polysaccharides.

Based on the research findings such as those described above, it is reasonable to deduce that the antioxidant activity of the polysaccharides extracted by different ultra-high-pressure parameters should be different. Therefore, the present work further explored the antioxidant activities of the polysaccharides extracted by different ultra-high-pressure parameters *in vivo* and *in vitro*, and found that there was a difference in the antioxidant activities among the polysaccharides (Tables 1 and 2). The results further confirmed that the ultra-high-pressure processing parameters have a significant effect on the extraction rate and antioxidant activity of the polysaccharides (Gao *et al.,* 2020). Many researchers have also optimised the extraction process of polysaccharides, e.g., extraction of polysaccharides from *Umbilicaria* from yellow mountains (Sun and Wei, 2020), and there is synergy among the ultra-high-pressure processing parameters, thus resulting in stronger disruption of the cell structure and physical properties of the mucous in fresh stems as compared to individual parameters (Balasubramaniam, 2015). Optimising the extraction process, increasing the extraction rate of polysaccharides, and enhancing the biological activity of fresh medicine

are therefore necessary (Huang *et al.,* 2019).

Conclusions

In the present work, the fresh stems *of D. officinale* were used as material for fresh medicine research, and the effects of ultra-high-pressure technology on the dissolution efficiency of polysaccharides from the stems of *D. officinale* were investigated. The results showed that all the pressure parameters of pressure level, duration, number of processing cycles, and temperature caused an obvious loss of polysaccharides, and increased the dissolution efficiency of polysaccharides from *D. officinale*. In particular, the composition and antioxidant activity of polysaccharides treated with different parameters were different. These findings showed that ultra-high-pressure technology is a very good technique in fresh medicine processing, while further investigations on the optimisation of polysaccharide extraction from the fresh stems of *D. officinale* are necessary.

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