

Study on physicochemical properties of polysaccharides and glycosides from *Rehmannia glutinosa* under different processing conditions

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Abstract: AIM: The physicochemical properties of polysaccharides and changes in the glycoside composition under different processing conditions of *Rehmannia glutinosa* were studied. METHODS: High-pressure processing temperature, processing time, processing frequency, and thickness of fresh *Rehmannia glutinosa* slices were taken as factors, and the contents of polysaccharides, catalpol, and rehmannioside D were taken as evaluation indexes. The physicochemical properties and the inhibition of α -glucosidase were characterized via ion chromatography, Fourier transform infrared spectroscopy, and environmental scanning electron microscopy. RESULTS: The parameter values for the highest polysaccharide content were as follows: processing temperature of 110 °C, processing time of 4 h, processing frequency of 2 times, and slice thickness of 1.0–1.5 cm. The parameter values for the highest α -glucosidase inhibition rate were as follows: processing temperature of 120 °C and processing time of 2 h. The surface morphology of polysaccharides from *Rehmannia glutinosa* showed irregular fragments, and glucose and galactose were dominant. The α -glucosidase inhibitory activity of polysaccharides from processed *Rehmannia glutinosa* reached 60% at a low concentration of 0.05 mg/mL.

Keywords: *Rehmannia glutinosa*; high-pressure processing process; polysaccharides; glycosides

1. Introduction

Rehmannia glutinosa is the fresh or dried root of *Rehmannia glutinosa* Libosch. According to different processing methods, *Rehmannia glutinosa* can be divided into three types: fresh *Rehmannia glutinosa*, raw *Rehmannia glutinosa*, and cooked *Rehmannia glutinosa*. Chemical composition is the basis of the efficacy of traditional Chinese medicine. After *Rehmannia glutinosa* is processed, changes in the chemical composition will cause its medicinal properties to change (from cooling to warming). Among the chemical composition, sugars (such as oligosaccharides and polysaccharides) and glycosides are the main active ingredients in *Rehmannia glutinosa* [1]. *Rehmannia glutinosa* polysaccharides have the effects of improving the body's antioxidant capacity and immunity, anti-anxiety, and anti-fatigue, and lowering blood sugar [2]. Preliminary research performed in this study found that the polysaccharide extraction rates of fresh *Rehmannia glutinosa*, raw *Rehmannia glutinosa*, high-pressure steamed raw *Rehmannia glutinosa*, and “nine steaming and nine sun-drying” cooked *Rehmannia glutinosa* were 0.61%, 0.64%, 3.45%, and 7.29%, respectively. The polysaccharide content in cooked *Rehmannia glutinosa* increased after processing, and the polysaccharide immune activity of cooked *Rehmannia glutinosa* was higher than those of raw and fresh *Rehmannia glutinosa* [3,4]. High-pressure steaming, as a modern *Rehmannia glutinosa* processing method,

has the advantages of high efficiency, short time, and environmental friendliness and is widely used in industrial production [5]. However, there are no specific standards to refer to for the high-pressure steaming method. Therefore, investigating changes in polysaccharide and glycoside contents and compositions, as well as polysaccharide activity under high-pressure steaming conditions, is particularly important for improving high-pressure steaming standards.

2. Materials and methods

2.1. Instrument

The instruments used were a vertical pressure steam sterilizer (Shanghai Shen'an Medical Equipment Factory, model LDZF-50KB), a rotary evaporator (Shanghai Yarong Biochemical Instrument Factory, model RE-52AA) a full-wavelength microplate reader (Botten Instrument Co., Ltd., USA, model Epoch), a high-performance liquid chromatograph (Agilent Technologies, USA, model Agilent1100), and a part-per-million balance (Swiss Mettler Toledo Co., Switzerland, model XP204).

2.2. Reagents

The reagents used were a catalpol standard (Chengdu Ruifanshi Biotechnology Co., Ltd, Lot No.: L04J12Y136474, purity $\geq 98\%$), a rehmannonoside D standard (Dubiside Biotechnology Co., Ltd. and Chengdu Bioinstrumentation, Chinese Academy of Sciences, Lot No. MUST-21052010, purity $\geq 99.19\%$), a verbasoside standard (Shanghai Yuanye Biotechnology Co., Ltd, Lot No. W14010C100217, purity $\geq 98\%$), anisoacteoside standard (Chengdu Aifa Bio-Tech Co., Ltd, Lot No. AF20020206, purity $\geq 98\%$), α -glucosidase (Shanghai Yuanye Biotechnology Co., Ltd, Lot No. R31D10Y107277, concentration $\geq 700,000$ U/mL), and acetonitrile (Merck, Germany, Lot No. I1133829105). Other chemical reagents were analytically pure.

2.3. Analysis of samples

Fresh *Rehmannia glutinosa* was purchased from Wuzhi County, Jiaozuo, Henan Province, China. The fresh *Rehmannia glutinosa* was of the Jinjiu variety. It was identified as *Rehmannia glutinosa* by Dr. Wen Chunnan of the College of Agriculture, Henan Agricultural University.

2.4. Experimental methods

Single-factor experiments

The experiments were based on the initial steaming condition of fresh *Rehmannia glutinosa* at slice thickness of 1.0 cm (with 40% rice wine added and soaked for 2 h), steaming temperature of 126 °C, steaming time of 2.0 h, and steaming frequency of 1 time. **Table 1** shows the values of the varied parameters of high-pressure steaming temperature, steaming time, steaming frequency, and slice thickness for investigating their effects on the *Rehmannia glutinosa* product yield, polysaccharide extraction rate,

and glycoside content of cooked *Rehmannia glutinosa*. Each group of the single-factor experiments was repeated three times and the average value was taken.

Table 1. Level table of factors in single-factor experiments.

Level	Factors			
	Temperature (°C)	Time (h)	Frequency (time)	Thickness (cm)
1	105	1.5	1	0.1
2	110	2.0	2	0.5
3	120	2.5	3	1.0
4	130	4.0	4	1.5
5	134	6.0	6	2.0

2.5. Extraction of crude polysaccharides

Polysaccharides were extracted using the method in Qian et al. [3]. *Rehmannia glutinosa* was cut into uniformly sized pieces and 100 g was weighed accurately, followed by the addition of 2 L of distilled water. The mixture was boiled and then extracted for 3.0 h twice. The extract was mixed and concentrated to 100 mL using a rotary evaporator at 45 °C. Absolute ethanol was added until the ethanol volume fraction was 75% and the mixture was placed in a refrigerator at 4 °C overnight before being centrifuged at 3500 rpm for 10 min. Dissolve the precipitate with an appropriate amount of distilled water and dialyze to obtain a polysaccharide solution. Place in an electric blast drying oven, dry and weigh at 40 °C, and calculate the polysaccharide extraction rate.

2.6. Determination of glycosides

2.6.1. Chromatography process

Waters SunFire™ C18 column (4.6×250 mm, 5 μm) with column temperature of 30 °C. The detection wavelengths were as follows: 203 nm for 0–30 min (catalpol and rehmannioside D) and 334 nm for 30–50 min (mullein and isomullein). The flow rate was 1 mL/min, the injection volume was 10 μL, and the mobile phase was acetonitrile (A)-0.1% phosphoric acid solution (B). The gradient is shown in **Table 2**.

Table 2. Mobile phase gradient.

Time (min)	Phase A (%)	Phase B (%)
0–12	1	99
12–20	1–15	99–85
20–30	15–19	85–81
30–50	19–20	81–80
50–52	20–100	80–0
52–60	100	0

2.6.2. Preparation of reference solutions

Appropriate amounts of catalpol, rehmannioside D, verbascoside, and isoacteoside reference standards were precisely weighed, where each was placed in a beaker to be dissolved in an appropriate amount of the mobile phase (0.1% phosphoric acid, 99:1 v/v acetonitrile). The mixed solution was placed in a 20mL volumetric flask, where the mobile phase was added to adjust the volume to the mark, and the mixed solution was mixed well. In the end, 0.25 mg, 0.125 mg, 0.125 mg, and 0.125 mg of reference solutions of catalpol, rehmannioside D, verbascoside, and isoacteoside, respectively, were obtained for later use.

2.6.3. Preparation of test solution

The steamed *Rehmannia glutinosa* was cut into uniformly sized pieces, accurately weighed at 1.0 g, and placed in an Erlenmeyer flask, where 50 mL of methanol was added to extract glycosides under an ultrasonic power of 200 W. The extraction was paused for 10 minutes after 30 min, for a total of 60 min of extraction time. The mixture was brought to room temperature, and methanol was used to make up for the lost weight [6]. A pipette was used to accurately draw an additional 20 mL of the filtrate and a rotary evaporator was used to concentrate the additional filtrate at 40 °C. After dissolving in the mobile phase, the mixture was transferred to a 5mL volumetric flask, with the volume adjusted to the mark, and the mixture was shaken well and filtered with a 0.45µm microporous membrane filter for later use.

2.6.4. Specificity experiments

The reference solutions and test solutions were analyzed according to the chromatography process described in Subsection 2.6.1. The result showed that the peak shape of each component was good and there were no interfering components.

2.6.5. Examination of linear relationships

The mobile phase was used to dilute the reference solution to obtain a series of concentration gradients (catalpol: 0.0078–1.0 mg/mL; rehmannioside D, verbascoside, and isoacteoside: 0.0039–0.5 mg/mL) to produce standard curves, where the concentration was the abscissa and the integrated value of the peak area was the ordinate. The calculated regression equations of the four components were as follows: $Y_{\text{catalpol}} = 7480.8X - 52.869$ ($R^2 = 0.9993$), $Y_{\text{rehmannioside D}} = 9843.1X + 29.667$ ($R^2 = 0.9997$), $Y_{\text{verbascoside}} = 29101X + 26.293$ ($R^2 = 1$), $Y_{\text{isoacteoside}} = 22744X + 18.752$ ($R^2 = 1$). It can be seen from the R^2 values of the regression equations that each component had a good linear relationship within its corresponding range.

2.6.6. Intermediate precision experiment

The control sample and the test sample solution were measured six times according to the chromatography process described in Subsection 2.6.1. The result showed the relative standard deviation (RSD) percentages of the peak areas of catalpol, rehmannioside D, verbascoside, and isoacteoside of 0.07%, 0.90%, 1.00%, and 0.15%, respectively, indicating that the instrument had good precision.

2.6.7. Stability test of test solution

The control sample and the test sample solution were tested for content stability. The result showed that the RSD percentages of the peak areas of catalpol,

rehmannioside D, verbascoside, and isoacteoside were 0.20%, 0.33%, 0.77%, and 0.06%, respectively. This showed that the test sample solution had good stability within 10 h.

2.6.8. Reproducible experiments

The same batch of control sample and test sample solution were taken, and detection and analysis were performed according to the chromatography process described in Subsection 2.6.1. The RSD percentages of the peak areas of catalpol, rehmmanioside D, verbascoside, and isoacteoside were 1.06%, 0.51%, 0.44%, and 0.63%, respectively, indicating that the method had good repeatability.

2.7. Physicochemical properties of crude polysaccharide

The polysaccharide content was determined via the phenol–sulfuric acid method, the soluble protein content was determined via the Bradford method [7], the polyphenol content was detected via the Folin-Ciocalteu method, and the amino acid content was determined using an amino acid detection kit.

The determination of the sugar components of monosaccharides followed the method in Ruan et al. [8] with slight modifications, where 10.0 mg of CV-1 was accurately weighed and then mixed with 3 mL of trifluoroacetic acid (TFA) with a concentration of 4 mol/L. After hydrolysis at 121 °C for 6 h, 10 mL of methanol was added. A rotary evaporator was used under reduced pressure to remove excess TFA, and a 5mL NaOH aqueous solution with a concentration of 50 mmol/L was added to obtain a CV-1 solution. The solution was filtered with a 0.45µm water-based membrane filter to be tested. Measurements were made using Fourier transform infrared spectroscopy (Nicolet iS10, USA) with a scanning range of 4000–500cm⁻¹. X-ray diffraction (XRD) spectra were collected using an X-ray diffractometer (Miniflex 600, Japan) with a scanning angle range of 5°–80° and a step size of 0.02°. Environmental scanning electron microscopy (ESEM) (FEI Q45, USA) with an accelerating voltage of 5 kV was used for detection.

2.8. α-glucosidase inhibition

Referring to the method in Wang et al. [9], acarbose was used as a positive control to study the inhibitory effect of *Rehmannia glutinosa* polysaccharides on α-glucosidase at different steaming temperatures and steaming times.

3. Result

3.1. Analysis of polysaccharide yields

Modern research shows that during the processing of *Rehmannia glutinosa*, the oligosaccharide content decreases sharply, while the polysaccharide content, which is the highest among the active ingredients of *Rehmannia glutinosa*, increases significantly. Therefore, it is crucial to investigate the impact of high-pressure steaming conditions on polysaccharide yield [10]. It can be seen from **Figure 1** that with the increase in steaming temperature, steaming time, steaming frequency, and slice thickness, the crude polysaccharide yield in cooked *Rehmannia glutinosa* showed a trend of first increasing and then decreasing, which was similar to the changing trend

of the yield of steamed finished product. Hence, the recommended condition for the high-pressure steaming of *Rehmannia glutinosa* based on the yield of crude polysaccharides was as follows: steaming temperature of 105–110 °C, steaming time of 4.0–5.0 h, steaming frequency of 2–3 times, and fresh *Rehmannia glutinosa* slice thickness of 1.0–1.5 cm.

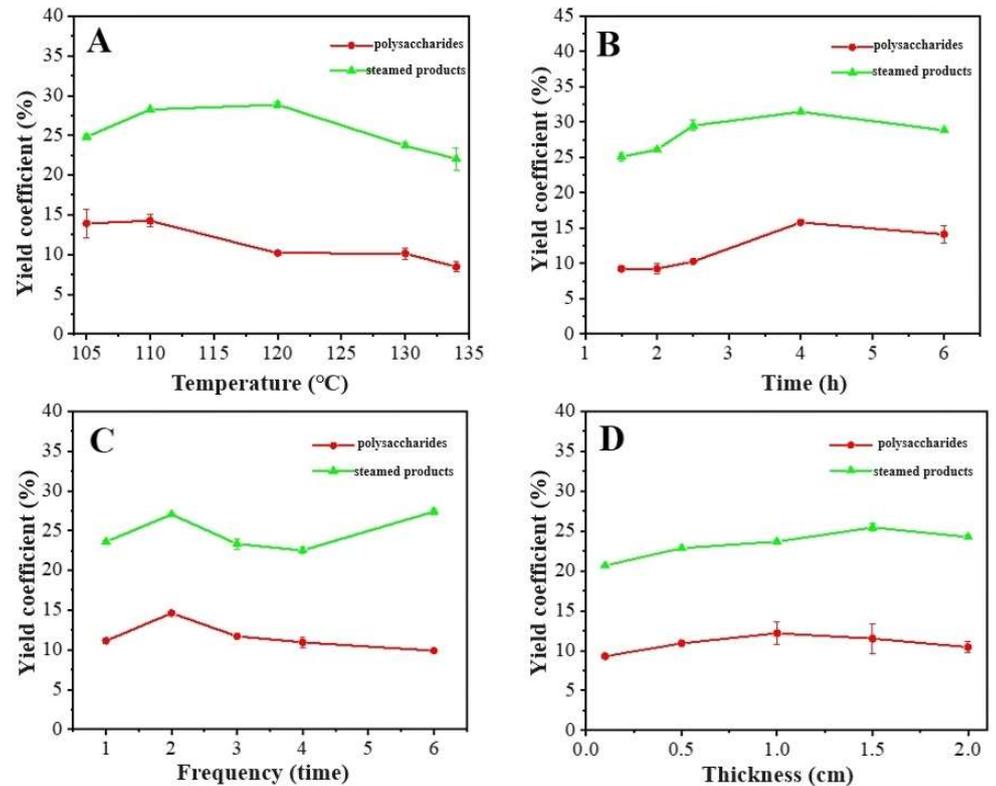


Figure 1. *Rehmannia glutinosa* yields and polysaccharide extraction yields: (A) steaming temperature, (B) steaming time, (C) steaming frequency, and (D) slice thickness.

Note: Crude polysaccharide yield (%) = mass of alcohol precipitate/mass of cooked *Rehmannia glutinosa* × 100

Steamed product yield (%) = mass of cooked *Rehmannia glutinosa*/mass of fresh *Rehmannia glutinosa* × 100

3.2. Glycoside content analysis

The result of the effects of different steaming temperatures and steaming frequencies on the main glycosides in *Rehmannia glutinosa* is shown in **Figure 2**. The contents of catalpol, rehmannioside D, and verbascoside were $2.34 \pm 0.06\%$, $0.06 \pm 0.50\%$, and $0.005 \pm 0.00\%$, respectively. Isomurascoside was beyond the detection range and was not detected. Catalpol is the main bitter component in *Rehmannia glutinosa*, and the decrease in catalpol content after steaming may be related to the poor stability of the chemical structure of this component and the rupture of ester bonds caused by heating [11]. This is an important reason why the bitter and cooling nature of *Rehmannia glutinosa* turns into sweet and warming. As the steaming temperature, steaming time, and the number of steaming times of cooked *Rehmannia* increased, the catalpol content decreased significantly, which is consistent with the research results

in Yan et al. [12]. The catalpol content remained basically stable after steaming for 4.0 h and after steaming twice. Research has shown that during the baking process of *Rehmannia glutinosa*, catalpol undergoes the enzymatic degradation of β -glucosidase and acid hydrolysis [13]. Among the different thicknesses of fresh *Rehmannia* slices, the catalpol content in cooked *Rehmannia glutinosa* obtained by steaming 1.0cm *Rehmannia glutinosa* slices was the lowest (0.63%). The reason may be that during the cutting process, the nuclear membrane and vacuolar membrane of the fresh *Rehmannia glutinosa* ruptured. As a result, β -glucosidase came into contact with catalpol and promoted its degradation. Since fresh *Rehmannia glutinosa* cut into smaller pieces was more severely damaged, catalpol degraded faster [14]. The catalpol content in the cooked *Rehmannia glutinosa* slices obtained by steaming the thinnest (0.1 cm) fresh *Rehmannia glutinosa* slices was higher. The reason may be that during processing, the internal temperature of the thin fresh *Rehmannia glutinosa* slices rose faster and promoted the inactivation of β -glucosidase. Under different steaming conditions, the content of *rehmannioside D* did not change much and complied with the regulations in the *Chinese Pharmacopoeia*.

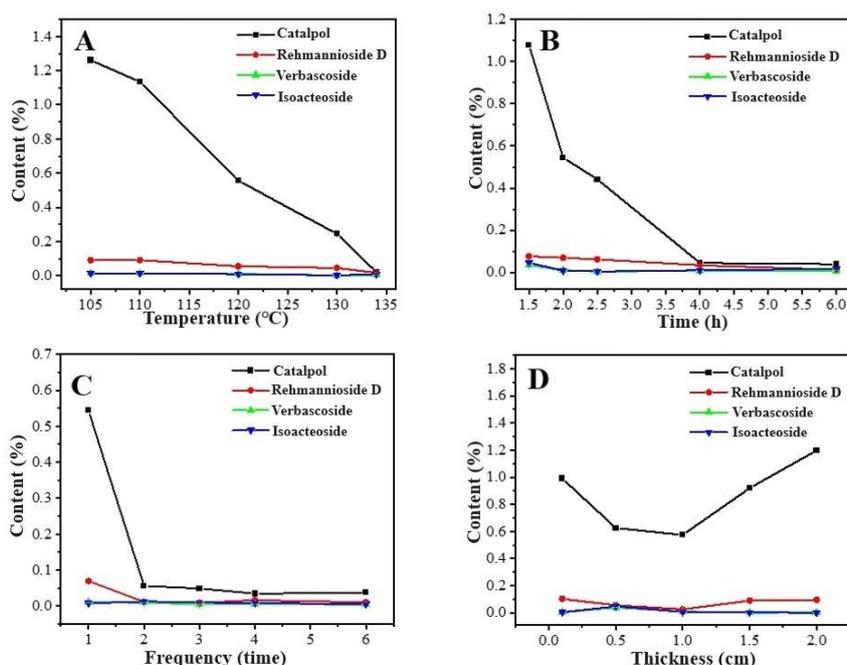


Figure 2. Glycoside contents in *Rehmannia glutinosa* at different (A) steaming temperatures, (B) steaming times, (C) steaming frequencies and (D) slice thicknesses.

3.3. Analysis of physical and chemical properties of crude polysaccharides

3.3.1. Chemical components

The main component of the cell walls of polysaccharides is high-molecular-weight polysaccharides, which are generally insoluble in water. Therefore, high-temperature and high-pressure processing have been used to break up the cell walls to promote the dissolution of polysaccharides [15,16]. Crude polysaccharides extracted from *Rehmannia glutinosa* have been obtained by using different processing

techniques, where the sugar content of polysaccharides ranges from 60% to 90%, the protein content ranges from 10% to 20%, and the polyphenol and amino acid contents are less than 10%.

3.3.2. Monosaccharide component analysis

The result of the components of monosaccharides in polysaccharides is shown in **Table 3**. The polysaccharides in cooked *Rehmannia glutinosa* obtained at different steaming temperatures and steaming times were mainly composed of glucose and galactose and contained small amounts of fructose, glucuronic acid, and galacturonic acid. This is consistent with the research results in Lu et al. [17]. As the steaming time increased, the glucose content gradually decreased and the galactose content gradually increased. Under different steaming conditions, the sum of glucose and galactose contents of each polysaccharide component ranged from 79% to 92%. As the steaming temperature and steaming time increased, the sum of glucose and galactose contents basically increased first and then showed a downward trend. The total percentage of glucose and galactose (91.27%) in polysaccharides after steaming *Rehmannia glutinosa* at 110 °C was higher than those at other steaming temperatures, and the total percentage of glucose and galactose (89.56%) in polysaccharides after 2.5 h of steaming was higher than those of other steaming times. The reason may be that glucose and galactose are isomers and these two components were isomerized during the process of concoction. The fructose content increased with the increase in steaming temperature, which may be related to the increase in the sweetness of *Rehmannia glutinosa* after concoction [18].

Table 3. Composition of monosaccharides in polysaccharide components.

Molar ratio	Fuc	Rha	Ara	Gal	Glc	Xyl	Man	Fru	GalA	GlcA
105 °C	-	-	0.19	20.22	68.76	-	-	9.61	0.06	1.16
110 °C	-	-	-	38.69	52.58	-	0.04	5.25	0.91	2.54
120 °C	-	-	-	22.80	67.46	-	-	7.93	0.73	1.08
130 °C	6.42	-	0.03	31.35	57.32	-	-	2.87	0.33	1.68
134 °C	5.15	-	-	32.76	53.70	-	0.37	4.32	1.92	1.77
1.5 h	6.93	-	0.09	25.95	61.16	-	2.35	1.83	0.48	1.20
2.0 h	10.88	-	-	24.89	60.71	-	-	1.76	1.38	0.39
2.5 h	5.00	1.75	0.00	36.69	52.87	-	-	3.20	0.16	0.33
4.0 h	4.67	-	0.44	35.90	45.83	-	-	6.54	5.91	0.72
6.0 h	6.56	0.07	0.32	38.13	41.84	-	3.34	8.29	0.59	0.86

Note: “-” means not detected.

3.3.3. Infrared spectral analysis

Infrared spectroscopy has been used for the identification of different sugars, determination of sugar configuration, identification of substituents, etc. [19]. As shown in **Figure 3**, the infrared spectra had a strong absorption peak caused by -OH stretching vibration near 3400 cm^{-1} , indicating that there were intramolecular or intermolecular hydrogen bonds in the sample, and there was a strong -OH bond at 2930 cm^{-1} . The C-H stretching vibration absorption peaks of -CH₃, -CH₂, -CH, O-H, and C-H are the characteristic groups of polysaccharides. The absorption peak at 1734

cm^{-1} represented esterified uronic acid, and 1618 cm^{-1} represented the characteristic absorption peak of the carbonyl vibration of the free carboxyl group of uronic acid, indicating the presence of carboxyl groups in the polysaccharide component [20]. The C-H deformation vibration peak of the functional group methyl ($-\text{CH}_3$) and methine ($-\text{CH}_2-$) appeared at 1420 cm^{-1} . Around 1020 cm^{-1} and 1235 cm^{-1} were two C-O stretching vibration absorption peaks of C-O-C and C-O-H on the pyranose ring. This result is consistent with the result of monosaccharide component analysis [21].

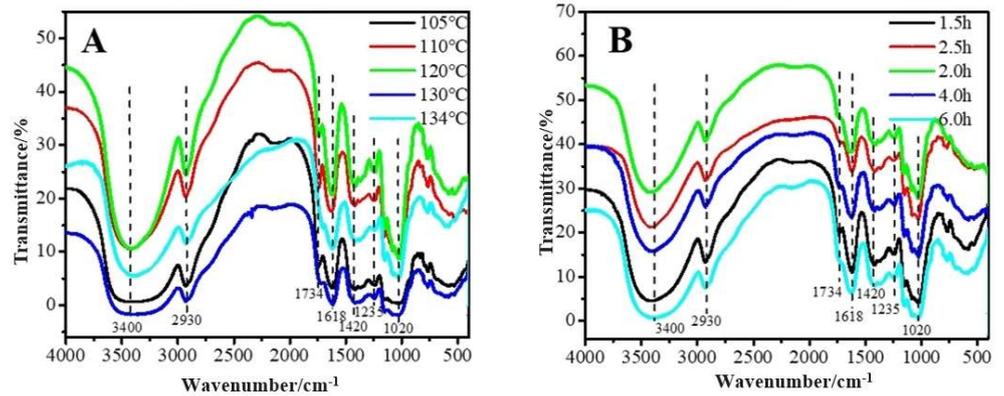


Figure 3. Infrared spectra of *Rehmannia glutinosa* polysaccharides at different (A) steaming temperatures and (B) steaming time.

3.3.4. X-ray diffraction (XRD) analysis

The polysaccharides in *Rehmannia glutinosa* obtained at different steaming temperatures and steaming times were detected via XRD and analyzed using Jade 6.5 software. The result is shown in **Figure 4**. The crystallinity of fresh *Rehmannia glutinosa* steamed at $105 \text{ }^\circ\text{C}$, $110 \text{ }^\circ\text{C}$, $120 \text{ }^\circ\text{C}$, $130 \text{ }^\circ\text{C}$, and $134 \text{ }^\circ\text{C}$ under high pressure was 16.18%, 16.54%, 16.42%, 18.91%, and 17.09%, respectively. The crystallinity values of polysaccharides obtained by steaming at $130 \text{ }^\circ\text{C}$ and above were higher than those at below $130 \text{ }^\circ\text{C}$, indicating that the samples were treated at high temperatures, in which the relative proportions of ash and carbon contents increased. The crystallinity values of fresh *Rehmannia glutinosa* autoclaved for 1.5 h, 2 h, 2.5 h, 4.0 h, and 6.0 h were 20.61% and 16.49%. The crystallinity of the substance was related to the stability of the substance.

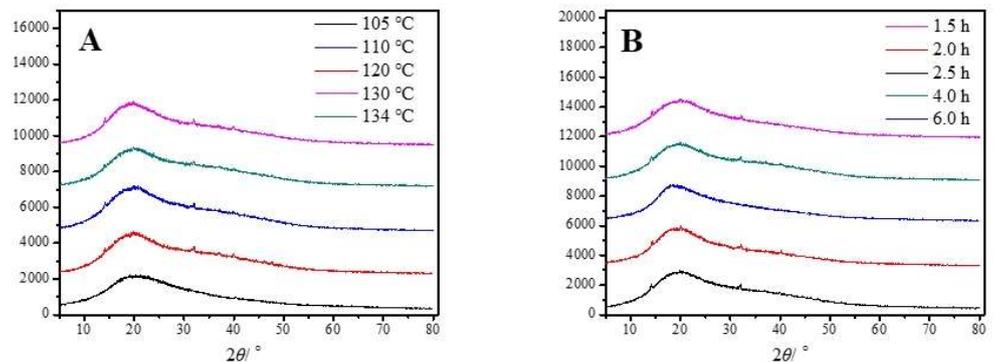


Figure 4. X-ray diffraction patterns of *Rehmannia glutinosa* polysaccharides at different (A) steaming temperatures and (B) steaming times.

3.3.5. ESEM analysis

The ESEM result of polysaccharides from *Rehmannia glutinosa* obtained at different steaming temperatures and steaming times is shown in **Figure 5**. Each polysaccharide sample showed a flaky structure. The polysaccharides from *Rehmannia glutinosa* obtained at different steaming temperatures were fragmented, as clearly observed under 500× magnification. The polysaccharide fragments at steaming temperatures of 130 °C and 134 °C were large in size but there were obvious holes on the surface. Under 1500× magnification, the polysaccharides' surface was observed as relatively smooth. Under 1500× magnification, there were obvious cracks seen on the polysaccharides' surface after steaming for 2.0 h, and there were holes on the polysaccharides' surface after steaming for 2.5 h. After steaming for 4.0 h, there were many obvious rough dents on the polysaccharides' surface, indicating that the polysaccharides in the cooked *Rehmannia glutinosa* were damaged to varying degrees as steaming time increased. The flaky structure of *Rehmannia glutinosa* after steaming for 6.0 h was thicker, indicating a higher degree of molecular aggregation [22].

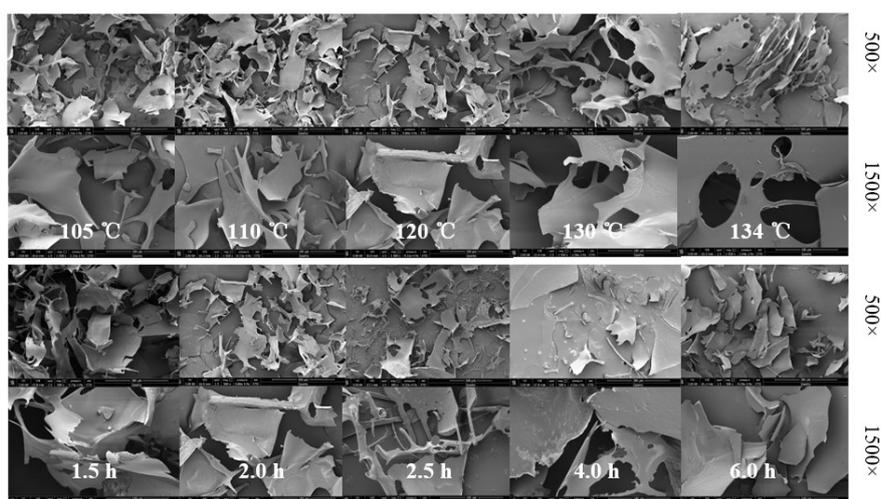


Figure 5. Scanning electron microscopy images of *Rehmannia glutinosa* polysaccharides at different steaming temperatures and steaming times.

3.3.6. Inhibition of α -glucosidase

The result of the inhibitory effects of *Rehmannia glutinosa* polysaccharides on α -glucosidase is shown in **Figure 6**. The polysaccharides from *Rehmannia glutinosa* obtained at different steaming temperatures and steaming times showed a strong inhibitory effect on α -glucosidase at a lower concentration (0.05 mg/mL) (the inhibition rate reached 60%) and showed concentration correlation. Yu and Li [23] studied pumpkin polysaccharides and showed that pumpkin polysaccharides with higher galactose and glucose contents had a significant inhibitory effect on α -glucosidase, which was similar to the results of this study. At concentrations of 0.05 mg/mL and 2.00 mg/mL, the inhibitory activities of *Rehmannia glutinosa* polysaccharides steamed at 130 °C on α -glucosidase were better than those of *Rehmannia glutinosa* polysaccharides steamed at other steaming temperatures. When the sample concentration increased to 5.00 mg/mL, the α -glucosidase inhibition rates

of *Rehmannia glutinosa* polysaccharides obtained by steaming at 120 °C, 130 °C, and 134 °C reached more than 90%.

At the concentration of 0.05 mg·mL⁻¹, the inhibitory activity of *Rehmannia glutinosa* polysaccharides steamed for 1.5 h on α -glucosidase was better than those at other steaming times. At a concentration of 2.00 mg/mL, the inhibition rates of *Rehmannia glutinosa* polysaccharides on α -glucosidase after steaming for 1.5 h, 2.0 h and 2.5 h were all above 70%, which were superior to those of other *Rehmannia glutinosa* polysaccharides steamed for 1.5 h, 2.0 h, and 2.5 h. At the concentration of 5.00 mg/mL, *Rehmannia glutinosa* after steaming for 2.0 h had a better inhibitory activity on α -glucosidase, with an inhibition rate of 95%.

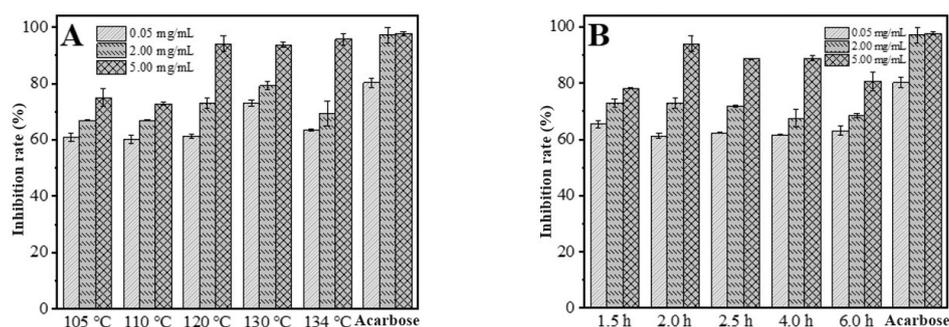


Figure 6. Result of α -glucosidase inhibition activities of *Rehmannia glutinosa* polysaccharides at different (A) steaming temperatures and (B) steaming times.

4. Discussion

Polysaccharides and glycosides are the main active ingredients in *Rehmannia glutinosa*. This study used a modern high-pressure steaming method to steam fresh *Rehmannia glutinosa*. Crude polysaccharides and glycosides of the steamed products were used as evaluation indicators to investigate the effects of high-pressure steaming under different conditions. Dynamic changes in polysaccharide components and in-vitro hypoglycemic activities of polysaccharides from *Rehmannia glutinosa* were obtained with different processing parameters. The result showed that there were certain processing parameter values of steaming temperature, steaming time, steaming frequency, and slice thickness where the yields of crude polysaccharides and steamed products were better. The result also showed better α -glucosidase inhibitory activities at a certain steaming temperature and steaming time.

Cutting and processing Chinese medicinal materials while they are fresh is the general trend for the development of authentic medicinal materials. In addition, from ancient times to the era of the Qing Dynasty, there were many records of steaming and drying cooked *Rehmannia glutinosa* as a raw material, but all previous editions of the Chinese Pharmacopoeia stipulated that cooked *Rehmannia glutinosa* is a processed product of raw *Rehmannia glutinosa*, not a processed product of fresh *Rehmannia glutinosa* [24]. *Rehmannia glutinosa* is considered an effective drug in the treatment of diabetes in traditional Chinese medicine. Among them, catalpol, *rehmannioside D*, oligosaccharides and polysaccharides play an important role [25–27]. This study used fresh *Rehmannia glutinosa* as raw material and used a high-pressure steaming method to process *Rehmannia glutinosa*, which was in line with the “Guidance on

Standardizing the Fresh Cutting and Processing of Chinese Medicinal Materials from Origin in Henan Province” issued by the Henan Provincial Food and Drug Administration [28]. The high-pressure steaming and processing technology was explored using polysaccharide yield, glycoside content, and polysaccharide inhibition of α -glucosidase activity as evaluation indicators. During the extraction process, high temperature and high pressure in the early stage promoted the lysis of part of the cell walls of *Rehmannia glutinosa* and released a large number of polysaccharides. At steaming temperature exceeding 110 °C, steaming time exceeding 4.0 h, and steaming frequency exceeding 2 times, the yields of crude polysaccharides decreased. The reason may be that as the steaming intensity increased, the crude polysaccharides were hydrolyzed into oligosaccharides and monosaccharides [3]. From the result, the amounts of verbascoside were low and relatively stable under all steaming conditions. Ren et al. [29] showed that verbascoside decreased to varying degrees during the processing of fresh *Rehmannia glutinosa*, and it was inferred that verbascoside was not thermally stable or was converted with iso-verbascoside. Research by Yu et al. [30] showed that fresh *Rehmannia glutinosa* will produce isomurabasin after being processed at a certain temperature and time, and the content of isomurabasin in *Rehmannia glutinosa* will change with different processing times. The production and changes of glycosides are greatly affected by time and temperature.

Preliminary research performed in the present study found that the immune effect of cooked *Rehmannia* polysaccharides obtained via high-pressure steaming was better than those of fresh *Rehmannia glutinosa* polysaccharides and raw *Rehmannia glutinosa* polysaccharides. Since the high-pressure steaming method is green, efficient, and environmentally friendly, it has gradually been adopted by modern enterprises. However, there is no perfect standard for the high-pressure steaming of *Rehmannia glutinosa*. Optimizing the multi-factor condition of high-pressure steaming based on the steaming yield of cooked *Rehmannia glutinosa* and the structure and activity of the active ingredients of cooked *Rehmannia glutinosa* is crucial to improving the quality of high-pressure steamed *Rehmannia glutinosa*, and hence more research needs to be carried out. Compared with the traditional cooking process, the high-pressure steaming method saves time and resources to a certain extent and requires relatively simple equipment. It essentially realizes the parametric processing of decoction pieces, which has a certain production-guiding significance. This study responded to the call of the Henan Provincial Food and Drug Administration and provided theoretical support for the development of fresh-cut and processed Chinese medicinal materials originating in Henan Province.

5. Conclusion

The study showed that the trend of crude polysaccharide yield from *Rehmannia glutinosa* processed under various conditions was basically the same as that of the steamed product yield, and the purity of polysaccharides extracted from the products ranged from 60% to 90%. The catalpol content in *Rehmannia glutinosa* significantly reduced after high-pressure steaming, while the content of rehmannioside D was in accordance with the standard of the Chinese Pharmacopoeia and the contents of mauritioside and isomauritioside were lower. The polysaccharides of

Rehmanniae glutinosa under different high-pressure steaming conditions showed a potential application value in hypoglycemia treatment.

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