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Article

Difference analysis of oligosaccharides in different varieties of *Rehmannia glutinosa*

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Abstract: The oligosaccharide and monosaccharide contents of different varieties of *Rehmannia glutinosa* were compared to explore the difference in the oligosaccharide and monosaccharide contents in different varieties and to provide a scientific basis for formulation of product quality standards of *Rehmannia glutinosa.* The contents of fructose, glucose, sucrose, raffinose, and stachyose were determined via high-performance liquid chromatography. The total sugar content in the fresh *Rehmannia glutinosa* varieties in descending order was as follows: Beijing Tuodu 3 > Huaifeng > Jinjiu > Huaizhong 1 > Beijing 3. The total sugar content in the raw *Rehmannia glutinosa* varieties in descending order was as follows: Jinjiu > Huaifeng > Beijing Tuodu 3 > Beijing 3 > Huaizhong 1. The stachyose content was the highest among the five oligosaccharides. The total sugar content in the Beijing Tuodu 3 variety was the highest among the fresh *Rehmannia glutinosa* varieties, and the total sugar content in the Jinjiu variety was the highest among the raw *Rehmannia glutinosa* varieties. There were significant differences in the oligosaccharide and monosaccharide contents among the different varieties.

Keywords: varieties; *Rehmannia glutinosa*; oligosaccharides; differences; high-performance liquid chromatography

1. Introduction

Rehmannia glutinosa is the dried root of the Scrophulariaceae plant *Rehmannia glutinosa* Libosch. It is one of the traditional Chinese medicines in China. *Rehmannia glutinosa* is cultivated in Henan, Shanxi, Hebei, Shandong, Inner Mongolia, and other places in China. Since the quality of *Rehmannia glutinosa* from Jiaozuo, Henan, is the best, Henan "Huai Rehmannia" has been used as an authentic medicinal material [1]. There are three medicinal types of *Rehmannia glutinosa*: fresh *Rehmannia glutinosa*, raw *Rehmannia glutinosa*, and cooked *Rehmannia glutinosa* [2]. Fresh *Rehmannia glutinosa* is obtained by washing fresh *Rehmannia glutinosa* materials to remove fibrous roots and impurities. Raw *Rehmannia glutinosa* is obtained by moistening fresh *Rehmannia glutinosa* materials and cutting them into thick slices before drying. Cooked *Rehmannia glutinosa* is obtained by steaming and drying the raw *Rehmannia glutinosa* materials until 80% dry or stewing in wine and then drying until the mucus on the skin is slightly dry, followed by cutting it into pieces or thick slices and drying. In traditional Chinese medicine, *Rehmannia glutinosa* is sweet, bitter, and cold in nature. It has the effects of clearing heat and cooling blood, replenishing the essence

and marrow, lowering blood sugar, enhancing immunity, and improving hematopoietic activity [3,4]. It is widely used to treat diseases, such as hypertension, autoimmune meningitis, osteoporosis, blood deficiency, constipation, and diabetes [5–8]. The main components of *Rehmannia glutinosa* are oligosaccharides, polysaccharides, and iridoid glycosides. In addition, it also contains amino acids and trace elements [9,10].

The main monosaccharide components of polysaccharides include rhamnose, arabinose, glucose, galactose, xylose, mannose, fructose, ribose, galacturonic acid, glucuronic acid, and mannuronic acid [11]. Oligosaccharides are formed by the shrinkage polymerization of 2 to 9 monosaccharide molecules. Oligosaccharides in *Rehmannia glutinosa* mainly include sucrose, azadiragibiose, raffinose, mannotriose, stachyose, verbascose, etc. [12]. Studies have found that *Rehmannia glutinosa* oligosaccharides have a variety of activities and play an important role in regulating blood sugar [13], regulating immune responses [14], enhancing the hematopoietic function [15], and improving memory [16]. The sugar composition and content in *Rehmannia glutinosa* are affected by various factors, such as the place of origin, variety, and environment [17], and so it is necessary to measure and analyze its sugar content. Preliminary research results have shown [18] that raw *Rehmannia glutinosa* and fresh *Rehmannia glutinosa* contain high levels of small-molecule sugars. Different varieties of *Rehmannia glutinosa* show differences in the content and type of sugars, which in turn gives them unique biological activities [19]. Therefore, this study used the water extraction method [20] to extract sugar substances from five different varieties of fresh *Rehmannia glutinosa* and raw *Rehmannia glutinosa*. High-performance liquid chromatography–refractive index detector (HPLC-RID) was used to determine the oligosaccharide and monosaccharide contents [21–24]. The differences in the oligosaccharide and monosaccharide contents between the five different varieties of *Rehmannia glutinosa* were analyzed and compared.

2. Instruments and materials

2.1. Instruments

The instruments used were an ME204 electronic balance, a KQ-500E ultrasonic cleaner, L420 desktop low-speed centrifuge, an Agilent Infinity 1260 high-performance liquid chromatograph, and Agilent Zorbox NH_2 (4.6 × 250 mm, 5 μm) chromatography column.

2.2. Reagents

The reagents used were distilled water, Wahaha purified water, chromatographically pure acetonitrile, and the standards for fructose, raffinose, glucose, stachyose, and sucrose.

2.3. Medicinal materials

The raw *Rehmannia glutinosa* and fresh *Rehmannia glutinosa* medicinal materials used in the experiment were collected from Beijing Tuodu 3, Huaizhong 1, and Jinjiu in Wuzhi County of Henan's Jiaozuo City, and from Huaifeng and Beijing

3 in Wen County of Jiaozuo City. Three batches of each of the five varieties of fresh *Rehmannia glutinosa* and three batches of each of the five varieties of raw *Rehmannia glutinosa* were collected in late October during the study, for a total of 30 batches of medicinal materials.

3. Experimental method

3.1. Sample preparation

Fresh *Rehmannia glutinosa* materials were washed with soil, cut into thin slices, and freeze-dried using a vacuum freeze dryer, before being ground into powder with a small powder grinder and the powder was then passed through a No. 2 sieve to obtain fresh *Rehmannia glutinosa* powder samples. A separate batch of fresh *Rehmannia glutinosa* materials were cleaned, baked directly at the place of production until 80% dry, and then ground into powder with a small powder grinder. The powder was then passed through a No. 2 sieve to obtain raw *Rehmannia glutinosa* powder samples.

3.2. Determination of oligosaccharide and monosaccharide contents

Since sugars lack chromophores, sugar components do not absorb in the normal ultraviolet and visible light regions [21,22], and so differential refractive index detectors are often used to detect sugar components [20].

3.2.1. Chromatography process

The chromatography column was an Agilent Zorbox NH2 (4.6 × 250 mm, 5 μm) column. The detector was a RID detector, and the mobile phase was 72% acetonitrile. The detector flow rate was 1 mL/min, the column oven temperature was 35 ℃, and the injection volume was 10 μL.

3.2.2. Preparation of reference solutions

Appropriate amounts of reference fructose, glucose, sucrose, raffinose, and stachyose substances were precisely weighed and placed in a 5mL volumetric flask. Then, water was added to prepare mixed reference solutions with concentrations of 0.925 mg/mL, 0.94 mg/mL, 0.745 mg/mL, 1.115 mg/mL, and 1.32 mg/mL.

3.2.3. Preparation of test solution

Each *Rehmannia glutinosa* powder sample obtained through pre-treatment was accurately weighed at 0.5 g and added to 25 mL of distilled water, where the mixture was sealed and weighed, followed by ultrasonic extraction for 1 h. Next, the mixture was let cool and weighed, with the weight replenished with water, and then the mixture was shaken well. The mixture was subsequently centrifuged at 4000 r/min for 10 min and let stand. After the suspended powder precipitated, it was filtered with a 0.22μm water-based membrane microfilter into a sample vial for later use.

3.3. Methodological review

3.3.1. Examination of linear relationships

Each of the reference solution prepared as described in Subsection 3.2.2 was injected at 1, 2, 4, 8, and 10 μL into the chromatography column according to the chromatography process described in Subsection 3.2.1. By drawing the standard

curve, the regression equations of the oligosaccharides and monosaccharides were obtained, as shown in **Table 1**, which shows that the five sugar substances had a good linear relationship within the linear range.

Table 1. Regression equations for five sugar substances.

Name	Regression equation	R^2	Linear range
Fructose	$y = 77703x + 333.33$	0.9994	0.034–0.503 mg/mL
Glucose	$y = 66928x + 4234.8$	0.9990	0.178–3.560 mg/mL
Sucrose	$y = 125242x + 1067.8$	0.9993	0.0225–0.4500 mg/mL
Raffinose	$y = 69150x + 344.75$	0.9991	0.027–0.548 mg/mL
Stachyose	$y = 76938x + 107.59$	0.9992	0.031–0.612 g/mL

3.3.2. Repeatability test

Six accurately weighed batches of the *Rehmannia glutinosa* powder were made, and test solutions were prepared according to the method described in Subsection "3.2.3", where 10 μL of a sample was injected into the chromatography column according to the chromatography process described in Subsection "3.2.1". The content of each sugar component of each sample was calculated based on the peak area. The result is shown in **Table 2**, where the percentages of the relative standard deviation (RSD) were all less than 2%, indicating that this method had good repeatability.

Table 2. Repeatability test result for five sugar substances.

Component	Batch	Content (%)	RSD (%)
Fructose	1	2.14	0.91
	2	2.18	
	3	2.13	
	4	2.12	
	5	2.14	
	6	2.14	
Glucose	1	0.86	1.27
	2	0.86	
	3	0.85	
	4	0.84	
	5	0.84	
	6	0.84	
Sucrose	1	4.37	0.97
	2	4.43	
	3	4.39	
	4	4.44	
	5	4.33	
	6	4.37	
Raffinose	1	2.82	1.00
	2	2.78	
	3	2.77	
	4	2.84	
	5	2.81	
	6	2.77	
Stachyose	1	46.93	0.35
	2	47.40	
	3	47.08	
	4	47.07	
	5	47.14	
	6	47.00	

3.3.3. Stability test

The same *Rehmannia glutinosa* test solution was drawn at 10 μL according to the chromatography process described in Subsection "3.2.1" at 0, 2, 4, 8, 12, and 24 h. The retention time of each measured peak was basically the same, and the RSD percentage values of the five sugar components were calculated based on the peak area. The result is shown in **Table 3**. The RSD percentages were all less than 2%, indicating that the test solution was stable for at least 24 h.

Table 3. Stability experimental result of five sugar substances.

Component	Period (h)	Peak area	RSD (%)
Fructose	0	34,713.1	1.96
	2	35,433.8	
	4	34,685.6	
	8	34,330.1	
	12	35,989.7	
	24	34,218.7	
Glucose	0	12,149.4	1.13
	2	12,035.4	
	4	11,800.5	
	8	11,859.7	
	12	11,826.4	
	24	11,943.8	
Sucrose	0	137,716.0	1.41
	2	135,308.4	
	4	137,762.8	
	8	140,128.8	
	12	135,972.6	
	24	135,047.9	
Raffinose	0	42,244.0	1.17
	2	41,342.7	
	4	41,546.2	
	8	42,540.7	
	12	41,409.5	
	24	41,619.8	
Stachyose	0	793,162.8	0.83

3.3.4. Precision test

The reference solution at 10 μL was accurately drawn and injected into the chromatography column to measure the peak area of each sugar component, and the process was repeated for six times. The result is shown in **Table 4**. The RSD percentages were all less than 2%, indicating good precision.

Table 4. Precision test result for five sugar substances.

Component	Frequency	Peak area	RSD (%)
Fructose	1	20,540.4	1.90
	2	20,718.5	
	3	21,173.0	
	4	21,380.3	
	5	20,807.5	
	6	20,278.7	
Glucose	1	131,524.1	1.20
	2	132,642.1	

Table 4. (*Continued*).

Component	Frequency	Peak area	RSD (%)
Glucose	3	133,529.7	1.20
	4	131,460.6	
	5	131,693.2	
	6	128,953.0	
Sucrose	1	31,402.3	1.40
	2	31,352.7	
	3	30,623.8	
	4	31,901.6	
	5	31,316.7	
	6	30,843.2	
Raffinose	1	20,054.6	0.30
	2	20,000.0	
	3	19,925.7	
	4	19,876.1	
	5	20,041.1	
	6	20,000.24	
Stachyose	1	26,239.1	1.20

3.3.5. Sample recovery test

Six batches of the *Rehmannia glutinosa* powder were accurately weighed at 0.5 g each, followed by accurately adding standard fructose at 0.13 mg, glucose at 0.33 g, sucrose at 0.03 mg, raffinose at 0.04 mg, and stachyose at 0.05 mg. A test solution was prepared according to the method described in Subsection "3.2.3" and 10 μL of each sample was injected. Detection was performed according to the chromatography process described in Subsection "3.2.1" and the peak area was recorded. According to the calculation result of the standard curve method, the average recovery rates of fructose, glucose, sucrose, raffinose, and stachyose were 103.52%, 103.48%, 102.37%, 99.46%, and 98.23%, respectively, and the RSD percentages were 0.94%, 1.38%, 0.94%, 1.40%, and 0.93%, respectively. The result showed that this method can be used to simultaneously determine the above five components in *Rehmannia glutinosa* and that the recovery rate was good.

4. Results and analysis

4.1. Comparison of sugar content in five varieties of fresh *Rehmannia glutinosa*

The experimental data were analyzed using Excel 2019 statistical software. As shown in **Figure 1**, among the five varieties of fresh *Rehmannia glutinosa*, the content of stachyose was the highest, which was significantly higher than the content of other sugar compounds. The content of each sugar compound was different in different varieties. In terms of fructose, the Beijing Tuodu 3 variety had the highest content and the Huaifeng variety had the lowest. In terms of glucose, the Beijing 3 variety had the

highest content and the Beijing Tuodu 3 variety had the lowest content. In terms of sucrose, the Huaifeng variety had the highest content, followed by the Huaizhong 1 variety, while the Jinjiu variety had the lowest sucrose content. In terms of raffinose, the Huaizhong 1 variety had the highest content, followed by the raffinose content in the Huaifeng 1 variety, which was not much different than that of the Huaizhong 1 variety, while the Beijing Tuodu 3 variety had the lowest content. In terms of stachyose, the Beijing Tuodu 3 variety had the highest content, followed by the Huaifeng variety, while the Beijing 3 variety had the lowest content.

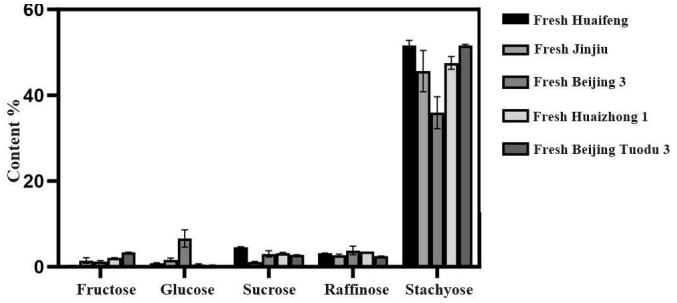

Figure 1. Comparison of sugar content among five varieties of fresh *Rehmannia glutinosa*.

4.2. Comparison of sugar content in five varieties of raw *Rehmannia glutinosa*

As shown in **Figure 2**, in the comparison of the contents of oligosaccharides and monosaccharides, stachyose still accounted for a relatively high proportion in raw *Rehmannia glutinosa*, but compared with that of fresh *Rehmannia glutinosa*, the stachyose content in the five varieties of raw *Rehmannia glutinosa* decreased. In terms of fructose, the Huaifeng variety had the highest content and the Beijing 3 variety had the lowest. In terms of glucose, the Huaizhong 1 variety had the highest content and the Huaifeng variety had the lowest content. In terms of sucrose, the Huaifeng variety had the highest content, followed by the sucrose content in the Huaizhong 1 variety, which was not much different from that of the Huaifeng variety, while the Jinjiu variety had the lowest content. In terms of raffinose, the Huaizhong 1 variety had the highest content, the Beijing Tuodu 3 variety had the second-highest content, and the Jinjiu variety had the lowest content. In terms of stachyose, the Jinjiu variety had the highest content, followed by the Huaifeng variety, and the Huaizhong 1 variety had the lowest.

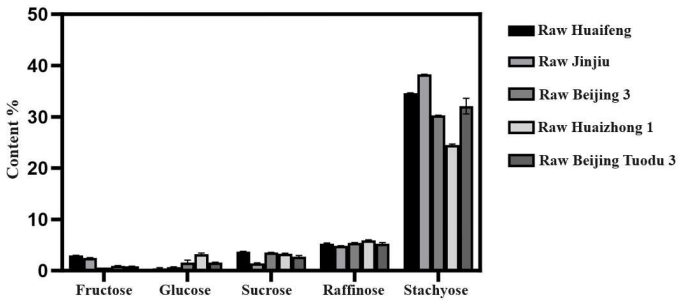

Figure 2. Comparison of sugar content among five varieties of raw *Rehmannia glutinosa*.

4.3. Comparison and difference analysis of total sugar content in five varieties of *Rehmannia glutinosa*

The comparison of the total sugar content in the different varieties of *Rehmannia glutinosa* is shown in **Figure 3**. The total sugar content in the five varieties of fresh *Rehmannia glutinosa* was higher than that of raw *Rehmannia glutinosa*. Among the fresh *Rehmannia glutinosa* varieties, the Beijing Tuodu 3 variety had the highest total sugar content, followed by the Huaifeng variety, while the Beijing 3 variety had the lowest total sugar content. Among the raw *Rehmannia glutinosa* varieties, the Jinjiu variety had the highest total sugar content, followed by the Huaifeng variety, while the Huaizhong 1 variety had the lowest total sugar content.

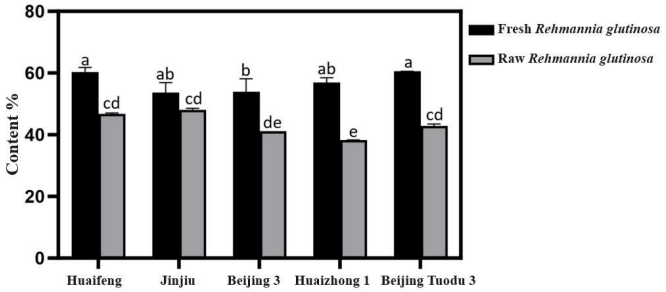

Figure 3. Total sugar content in five varieties of *Rehmannia glutinosa*. When $P < 0.05$, there is a significant difference between varieties.

A statistical difference analysis was conducted on the total sugar content in the five varieties of *Rehmannia glutinosa*. The result is shown in **Figure 3**. Among the fresh *Rehmannia glutinosa* varieties, there was a significant difference between the Huaifeng and Beijing 3 varieties and between the Beijing Tuodu 3 and Beijing 3 varieties, while the differences between the other varieties were not significant. Among the raw *Rehmannia glutinosa* varieties, the difference between the Beijing 3 and Beijing Tuodu 3 varieties was not significant, but both varieties were significantly different from the other three varieties. The difference between the Huaifeng and Jinjiu varieties was not significant, but both are significantly different from the other three varieties. There were significant differences between the Huaizhong 1 variety and the other four varieties.

5. Discussion and conclusion

It can be seen from the measurement results that the contents of the five measured sugar components, which were fructose, glucose, sucrose, raffinose, and stachyose, in the different varieties of *Rehmannia glutinosa* were significantly different. This reflected that the variety factor had a significant impact on the sugar content in *Rehmannia glutinosa*. Among the fresh *Rehmannia glutinosa* varieties, the Beijing Tuodu 3 variety had the highest total sugar content, while the Beijing 3 variety had the lowest total sugar content. Among the raw *Rehmannia glutinosa* varieties, the Jinjiu variety had the highest total sugar content, while the Huaizhong 1 variety had the lowest total sugar content. There were also obvious differences in the overall sugar content in the five varieties of *Rehmannia glutinosa*. The content of stachyose was the highest, which was significantly higher than the content of other sugar compounds.

Comparing the sugar content in the five varieties of fresh *Rehmannia glutinosa* and raw *Rehmannia glutinosa*, it was found that the stachyose content in the raw *Rehmannia glutinosa* varieties decreased, which was consistent with the experimental results in Xue et al. [25], while the raffinose content increased. Qiu et al. [23] found that stachyose is less stable at higher temperatures and longer times and that some stachyose may decompose during the processing of *Rehmannia glutinosa*. Lei and Jia [26] found that during the processing of fresh *Rehmannia glutinosa* into raw *Rehmannia glutinosa*, stachyose mainly decomposed by galactose removal, resulting in an increase in the raffinose content. In addition, the test results showed that the measurement results of this experiment were accurate, highly precise, and reproducible, and the testing was simple to perform. The method can be applied to the content determination of the main sugar components in *Rehmannia glutinosa* and can provide scientific product quality control for authentic medicinal *Rehmannia glutinosa*.

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References

1. Cai SJ. Quality Evaluation of in Different Commercial Grades of *Rehmannia glutinosa* Libosch [Master's thesis]. Henan University of Chinese Medicine; 2016.
2. Zhang FF. Effects of Differently Processed *Rehmannia* Roots on Yin Deficiency Animals [Master's thesis]. Shandong University; 2018.
3. Yi W, Liu Y, Li N, et al. Comparison of the content oligosaccharides in different varieties of *Rehmannia glutinosa.* Chinese Wild Plant Resources. 2011; 30(6): 66-69.
4. Li M, Jiang H, Hao Y, et al. A systematic review on botany, processing, application, phytochemistry and pharmacological action of *Radix Rehmnniae*. Journal of Ethnopharmacology. 2022; 285: 114820. doi: 10.1016/j.jep.2021.114820
5. Liu C, Ma R, Wang L, et al. *Rehmanniae Radix* in osteoporosis: A review of traditional Chinese medicinal uses, phytochemistry, pharmacokinetics and pharmacology. Journal of Ethnopharmacology. 2017; 198: 351-362. doi: 10.1016/j.jep.2017.01.021
6. Rahmat E, Okello D, Kim H, et al. Scale-up production of *Rehmannia glutinosa* adventitious root biomass in bioreactors and improvement of its acteoside content by elicitation. Industrial Crops and Products. 2021; 172: 114059. doi: 10.1016/j.indcrop.2021.114059
7. Gu M, Yuan Y, Qin Z, et al. A combined quality evaluation method that integrates chemical constituents, appearance traits and origins of raw *Rehmanniae Radix* pieces. Chinese Journal of Natural Medicines. 2021; 19(7): 551-560. doi: 10.1016/S1875-5364(21)60056-0
8. Lu M, Chang C, Chao C, et al. Structural changes, and anti-inflammatory, anti-cancer potential of polysaccharides from multiple processing of *Rehmannia glutinosa*. International Journal of Biological Macromolecules. 2022; 206: 621-632. doi:

10.1016/j.ijbiomac.2022.02.112
9. Yu L, Li X, Zhang H, et al. Simultaneous determination of monosaccharide and oligosaccharides by RP-HPLC-RID in Huai-*Radix Rehmanniae*. Chinese Journal of Pharmaceutical Analysis. 2013; 33(6): 977-982.
10. Xue S, Wang L, Chen S, et al. Study on commercial specification and quality characteristics of *Radix Rehmanni*. World Science and Technology-Modernization of Traditional Chinese Medicine. 2017; 19(3): 497-507.
11. Qian Y. Study on Physicochemical Properties of Saccharides from Different Processed Products of *Rehmannia Glutinosa* and Optimization of High Pressure Steaming Conditions [Master's thesis]. Henan Agricultural University; 2022.
12. Qiu J, Zhang R, Jia Z, et al. Change of oligosaccharide and catalpol contents in *Rehmanniae Radix* at various processing phases. Chinese Traditional and Herbal Drugs. 2011; 42(12): 2434-2437.
13. Zhang R, Zhou J, Li M, et al. Ameliorating effect and potential mechanism of *Rehmannia glutinosa* oligosaccharides on the impaired glucose metabolism in chronic stress rats fed with high-fat diet. Phytomedicine. 2014; 21(5): 607-614. doi: 10.1016/j.phymed.2013.11.008
14. Chen H, Lu L, Guo H, et al. Effect of ROS on blood lipid metabolism and VEGF level in kidney tissue of diabetic rats. Laboratory Medicine and Clinic. 2021; 18(7): 919-922, 928.
15. Wu W, Wen X, Zhao Y. Research progress of *Rehmannia glutinosa* oligosaccharides and its pharmacological activity. Journal of Chinese Medicinal Materials. 2006; 5: 507-510.
16. Jiang Q. *Rehmannia Glutinosa* Oligosaccharide on the Treatment of Vascular Dementia and Mechanism. Jiangsu University; 2013.
17. Qiu J, Wang C, Li M, et al. Changes of the content of oligosaccharide and catalpol in *Rehmannia glutinosa* at various phases of processing with wine. Medical Journal of National Defending Forces in Northwest China. 2021; 42(7): 610-614.
18. Qian Y, Wang L, Zhang Z. Physical-chemical properties of heteropolysaccharides from different processed forms of *Radix Rehmannia*. Process Biochem. 2022; 121: 481-492. doi: 10.1016/j.procbio.2022.07.032
19. Wang Q, Song J, Li Y, et al. Research progress on extraction, purification and pharmacological action of *Rehmannia glutinosa* polysaccharides. Chinese Traditional and Herbal Drugs. 2023; 54(11): 3734-3744.
20. Zhang H, Zhang R, Jia Z, et al. Optimization of extraction technology of effective parts of *Rehmannia glutinosa* oligosaccharide by orthogonal experiment. Chinese Journal of Experimental Traditional Medical Formulae. 2010; 16(5): 27-29.
21. Qiu J, Zhang R, Jia Z, et al. Determination of oligosaccharides in *Rehmannia glutinosa* by HPLC. Chinese Journal of Experimental Traditional Medical Formulae. 2009; 15(8): 8-9.
22. Song L, Xue S, Wang L, et al. Analysis of chemical constituents from *Rehmannia glutinosa* with different storage years. Chinese Traditional Patent Medicine. 2020; 42(10): 2661-2669.
23. Qiu J, Zhang R, Jia Z, et al. Determination of content of oligosaccharide and catalpol in *Rehmannia* root by HPLC. Chinese Journal of Experimental Traditional Medical Formulae. 2010; 16(17): 110-113.
24. Li X, Dong C, Qi D, et al. Quality evaluation of fresh *Rehmannia glutinosa* from different habitats by HPLC multicomponent determination and principal component analysis. Jiangsu Agricultural Sciences. 2020; 48(19): 225-229.
25. Xue S, Chen Y, Chen S, et al. Simultaneous determination of eight saccharides contents in *Radia Rehmanniae* and its different processed products by HPLC-ELSD. Chinese Journal of Pharmaceutical Analysis. 2023; 43(6): 939-949.
26. Lei J, Jia J. Research progress in the stachyose in *Rehmannia glutinosa*. Medical Recapitulate. 2010; 16(6): 935-937. doi: 10.3969/j.issn.1006-2084.2010.06.046