Optimization of Solid-State Fermentation Conditions of *Astragali Radix* Residues and Changes of Its Nine Constituents Content Before and After Fermentation

Cuiyan Liu
Anhui Agricultural University https://orcid.org/0000-0002-5159-0712
Yun Zhu
Anhui Agricultural University
Guangtai Fan
Anhui Agricultural University
Jingang Gu
Chinese Academy of Agricultural Sciences
Chunyang Han (luckyhcy@ahau.edu.cn)
Anhui Agricultural University

Research article

Keywords: Solid-state fermentation, Astragali Radix residues, Aspergillus niger, Constituent, Optimization

Posted Date: September 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-80025/v1

Abstract

Background: For the last few years huge quantity of herb residues are discharged in China, and so far there is not a good solution. The current methods of disposal and utilization of herb residues (stacking, incineration, and landfill) were not only great harmful to the ecological environment, but also cause the waste of medicinal herb resources. The fermentation can further decompose and transform the active ingredients and nutrients in herb residue, so the fermentation technology has good application prospect in the treatment of herb residues. In this study, we investigated the applicability of fermentation processes with the *Aspergillus niger* (ACCC 30583) strain for the reuse of *Astragali Radix* residues (ARR) by comparing the content changes of its nine constituents before and after fermentation. The contents of total protein and crude fat in the ARR were determined via near-infrared scanning; the iron, copper, zinc, and manganese contents were assayed with atomic absorption spectroscopy, the calycosin-7-glucoside and astragaloside Ⅳ content were determined by HPLC, and the polysaccharide content was determined via phenol sulfuric acid spectrophotometry.

Results: The optimum fermentation conditions for ARR with ACCC 30583 was culture medium content 60%, fermentation time 5 days, and fermentation temperature 28 ℃. Compared with the residues before fermentation, the contents of total protein, calycosin-7-glucoside, astragaloside Ⅳ, *Astragali Radix* polysaccharides (ARP), and manganese increased significantly, the iron content decreased significantly, and the crude fat, zinc, and copper contents exhibited no significant changes.

Conclusions: The solid fermentation of ARR with the *Aspergillus niger* (ACCC 30583) strain effectively promoted the separation of astragaloside Ⅳ and ARP from ARR, which provided methodological basis for the effective reuse of herb residues.

Background

As the knowledge of natural botanical medicine grows and the demand for raw plant extracts (i.e., Chinese herbal medicine) continues to rise, the extract manufacturing industry has continued to expand accordingly in China. However, the disposal of residues derived from the growing number of herbal medicine products has become a key challenge. Current data suggest that the present annual output of Chinese herbal medicines is approximately 70 million tons [1]. Moreover, during the processing and industrialization of these products, 35 million tons of solid waste (e.g. medicinal plant residues) are produced, which are typically sent to a landfill or incinerated [2]. Notably, due to the limitations of current extraction procedures, Chinese herbal medicine residues still contain many bioactive substances such as flavonoids and polysaccharides [3, 4]. Therefore, disposing of these solid wastes through landfills or incineration is not only an enormous environmental burden but also a waste of valuable resources with therapeutic potential [5-7]. Therefore, there is a growing interest to repurpose these Chinese herbal medicine waste products by recycling these biomass resources in an effective environmentally-friendly manner [8]. Nonetheless, an optimal recycling procedure is yet to be developed.

Fermentation entails the use of microorganisms or enzymes to transform raw materials into useful products through specific metabolic pathways under appropriate conditions, or the preparation of microbial biomass itself, direct metabolites, or secondary metabolites via microbial activity [9]. *Astragali Radix* residues (ARR) is the remaining insoluble residue derived from *Astragali Radix* extraction. In this study, ARR were used as raw fermentation material, and *Aspergillus niger* (ACCC 30583) was used as a cellulose-degrading fermentation strain. By optimizing the fermentation conditions, the contents of astragaloside Ⅳ, calycosin-7-glucoside, ARP, crude protein, crude fat, iron, manganese, zinc, and copper in ARR were determined. Afterward, the changes in the content of each component of ARR before and after fermentation were compared. Therefore, this study provides insights into useful technical approaches for the reuse of ARR.

Materials And Methods

Experimental materials

The *Aspergillus niger* (ACCC 30583) strain was purchased from the Agricultural Culture Collection of China. The following reagents were used for experimental purposes: astragaloside IV (National Institutes for Food and Drug Control); calycosin-7-glucoside standard (Shanghai Yuanye Biotechnology Co., Ltd.); formic acid, methanol, hydrochloric acid (Sinopharm Chemical Reagent Beijing Co., Ltd.); 1000 μg mL^{-1} copper, iron, zinc, manganese single-element standard solutions (National Nonferrous Metals and Electronic Materials Analysis and Testing Center).

Preparation ofARR and culture medium

ARR preparation. After the *Astragali Radix* decoction pieces were extracted twice with water, the residue was obtained and dried at 60 ℃.

Potato Dextrose Agar (PDA) liquid medium preparation. The PDA liquid medium was composed of potato (200 g), glucose (20 g), and water (1000 mL).

Aspergillus niger culture medium preparation. The optimum enzyme-producing medium composition for *Aspergillus niger* (ACCC 30583) determined in previous experiments was: corn stalk powder, 22.5 g; wheat bran, 7.5 g; $(NH_4)_2SO_4$, 2 g; water, 1000 mL; pH=5.0 [10].

Optimization of ARR solid fermentation conditions

Aspergillus niger cellulase culture medium preparation

Aspergillus niger (30583) spores were collected and a $1×10^8$ spore mL^{-1} suspension was prepared with sterile deionized water. This suspension (1 mL) was then added to the PDA liquid medium. After 24 h of shock culture at 28 ℃, the bacteria-containing PDA medium was added to an *Aspergillus niger* (ACCC 30583) optimal cellulase-producing medium at a 5% inoculation volume and cultured at 28 ℃ and 160 r min^{-1} for 72 h.

Effect of cellulase culture medium amount on *Aspergillus niger*-mediated fermentation

Six portions of ARR were placed in different triangular flasks, and different amounts of cellulase culture solution produced by *Aspergillus niger* (30583) were added. The contents of cellulase culture medium were 30%, 40%, 50%, 60%, 70%, and 80%. The mixtures were fermented in a constant temperature incubator at 28 ℃ for 5 d. All experiments were conducted in triplicate. Astragaloside IV precipitation was measured after fermentation.

Effect of temperature on fermentation

The six portions of ARR were mixed with cellulase culture solution produced by *Aspergillus niger* (30583) in a ratio of 1 : 1, and then those mixtures were respectively fermented at 24, 26, 28, 30 and 32 ℃ for 5 d. All experiments were conducted in triplicate. Astragaloside IV precipitation was measured after fermentation.

Effect of time on fermentation

Six portions of ARR were placed in different triangular flasks, and different amounts of cellulase culture solution produced by *Aspergillus niger* (30583) were added. The mixtures containing 50% culture medium were prepared and fermented for 3、4、5、6、7、8 d at 28 ℃. All experiments were conducted in triplicate. Astragaloside IV precipitation was measured after fermentation.

Orthogonal experiment

According to single-factor analyses of the effect of culture medium content, fermentation time, or temperature on the precipitation of astragaloside IV during the solid fermentation of ARR, the experimental culture medium contents were selected to be 50%, 60%, and 70%, the fermentation times were 4, 5, and 6 d, and the fermentation temperatures were 26, 28, and 30 ℃. A three-factor and three-level L9 (3^3) orthogonal experiment was then conducted to determine the best conditions for solid fermentation of ARR.

Determination of nine components in ARR before and after fermentation

Astragaloside IV content determination via HPLC

HPLC was performed with the following chromatographic conditions (C18 column): mobile phase, acetonitrile : water = 30 : 70; flow rate = 1 mL min^{-1}; detection wavelength, 210 nm; column temperature, 30 °C; injection volume, 10 μL. Here, we investigated whether the linear relationship met the quantitative requirements and also if the experimental evaluation parameters of precision, stability, and recovery were reliable.

Preparation of reference and test solutions

Calycosin-7-glucoside was accurately weighed and mixed with methanol to obtain a standard solution (500 g mL^{-1}). Samples were then obtained from the *Aspergillus niger* culture medium and ARR mixtures, after which they were centrifuged at 4000 r min^{-1} for 10 min. The supernatant was recovered and concentrated to 10 mL with a rotary evaporator. The extraction procedure was carried out four times with saturated n-butanol (40 mL per extraction). The n-butanol solution was then mixed and thoroughly washed with ammonia water twice (40 mL per wash). Afterward, the ammonia solution was discarded, the n-butanol solution was allowed to evaporate, and the resulting residue was mixed with 5 mL of deionized water. D101 type macroporous adsorption resin columns (inner diameter 1.5 cm, column height 12 cm) were used to elute the samples with 50 mL of water. The eluent was then discarded and eluted with 70% ethanol. This second eluent was collected, evaporated, and dried, and the residue was dissolved with methanol, with a constant methanol capacity of 10 mL.

Calycosin-7-glucoside content determination via HPLC

HPLC was performed with a C18 chromatographic column; gradient elution was conducted with acetonitrile as mobile phase A and 0.2% formic acid solution as mobile phase B. The elution conditions were: 0–20 min from 20% A to 40% A; 20–30 min 40% A; 30–40 min from 40% A to 20% A; flow rate, 1.0 mL min^{-1}; detection wavelength, 260 nm; column temperature, 30°C; injection volume, 10 μL. As described above, we investigated whether the linear relationship met the quantitative requirements and also if the experimental evaluation parameters of precision, stability, and recovery were reliable.

Reference and test solution preparation: Calycosin-7-glucoside was accurately weighed and mixed with methanol to make a standard solution (500 g mL^{-1}). A 1 g sample of ARR was accurately weighed before and after fermentation and placed in an appropriately labeled round-bottom flask, after which 50 mL of methanol were added; the mixture was weighed once again. After reflux heating for 4 h and cooling to room temperature, the weight was measured. Methanol was used to compensate for lost weight, after which the mixture was thoroughly shaken and filtered. The filtrate was discarded and evaporated in a water bath. The residue was dissolved in methanol and transferred to a 5 mL volumetric bottle; the volume was adjusted with methanol. The resulting preparation was used as a test solution for all downstream experiments.

Determination of *AstragaliRadix* polysaccharide content via the phenol-sulfuric acid method

An anhydrous glucose reference was accurately weighed and mixed with ultrapure water to make a 1 mg mL^{-1} glucose reference solution; a standard curve was then constructed.

ARR powder samples (5 g) were taken before and after fermentation. These samples were then mixed with pure water (50 mL), heated to boil for 1 h, and filtered. The residue was extracted twice again as above, and then all the filtrates were combined and dried at 100 ℃ water bath. The samples were then precipitated with 60% and 80% ethanol for 5 h in sequence and centrifuged at 4000 r min^{-1} for 5 min. The deposit was diluted to 50 mL with ultrapure water; when mixed fully, a 2-mL sample of solution was taken

and diluted to 100 mL with ultrapure water, which was ARR test solution, and its concentration was 2 mg mL^{-1} (2 mg of ARR powder per mL of test solution).

The reference and test solutions (2 mL) were measured and transferred to appropriately labeled test tubes with stoppers. A phenol solution (3%, 2 mL) and a sulfuric acid solution (98%, 9 mL) were then added successively; the mixture was then shaken vigorously. Immediately after cooling to room temperature, the absorbance was measured at a 490 nm wavelength using a TU-1901 dual-beam UV-visible spectrophotometer. The *Astragali Radix* polysaccharide content was then calculated.

Total protein and crude fat determination via near-infrared scanning

ARR powder with an 80-mesh particle size was transferred into a glass dish and measured with a near-infrared scanner. The ARR powder samples before and after fermentation (i.e., sampled in triplicate) were analyzed in duplicate, after which the results were averaged.

Metal element content determination via atomic absorption spectrometry

Metal elemental composition was measured in accordance with the GB/T13885-2003 national standard "Determination of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc contents in feed." A series of standard working solutions were prepared with 1 g L^{-1} iron, copper, zinc, and manganese stock solutions.

The ARR powder (1 g) was weighed before and after fermentation, after which it was mixed with a hydrochloric acid solution (1 : 10; 100 mL), fully dissolved, and left undisturbed prior to filtration. The filtrate was then analyzed with an atomic absorption spectrometer to determine copper, iron, zinc, and manganese contents in the sample.

Statistical analysis

The SPSS 19.0 software was used for data analysis. All measurement data were expressed as mean ± standard deviation (mean ± SD). Comparisons before and after fermentation were performed via the paired t-test. A $p < 0.05$ indicated a significant difference and $p < 0.01$ indicated an extremely significant difference.

Results

Optimization of ARR solid fermentation conditions

Effect of cellulase culture medium amount on *Aspergillus niger*-mediated fermentation

Fig. 1 illustrates the relationship between the amount of astragaloside IV in the ARR and the content of the cellulase culture solution produced by Aspergillus niger. Fig. 1 demonstrates that, with the continuous increase of culture solution content, the amount of astragaloside IV increases first and then decreases,

and the culture solution accounts for 60% of the total volume of the fermentation product (i.e., ARR + Aspergillus niger culture solution), reaching a maximum at 0.4% (0.44 ± 0.0015 mg g^{-1}). This demonstrates that the optimal moisture content for Aspergillus niger solid fermentation ARR was 60%.

Effect of temperature on fermentation

Fig. 2 illustrates the relationship between the amount of astragaloside IV and fermentation temperature during ARR solid fermentation. It can be seen from Fig. 2 that when the fermentation temperature is between 24 and 28 °C, the amount of astragaloside IV significantly increases with increased temperature, reaching a maximum at 0.4 °C (0.46 ± 0.020 mg g^{-1}). Moreover, when the fermentation temperature is 28–32 °C, the amount of astragaloside IV decreases as the temperature increases. Therefore, the optimal temperature for solid fermentation of ARR was found to be 28 °C.

Effect of time on fermentation

It can be seen from Fig. 3 that the amount of astragaloside IV increases gradually as fermentation time increases. Particularly, astragaloside IV concentrations changed substantially during the first 3–5 d of fermentation, with less pronounced changes occurring thereafter. The optimal duration for solid fermentation of ARR was thus found to be 5 d.

Orthogonalexperiment results

Tables 1 and 2 summarized the results of the orthogonal experiment, whereby three factors and three levels (culture medium content, fermentation time, and fermentation temperature) were evaluated.

According to Table 1, the R values of the culture broth content, fermentation time, and fermentation temperature were 0.108, 0.024, and 0.057, respectively. Notably, the order of the three factors affecting the solid fermentation of ARR was: moisture content > temperature > fermentation time. Moreover, based on the Average value in the Table 1, the best fermentation conditions for solid fermentation of *Aspergillus niger* with ARR were the following: a water content of 60%, fermentation time 4 d, and fermentation temperature 28 °C.

Contents of nine components inARRbefore and after fermentation

Astragaloside Ⅳ and calycosin-7-glucoside content

According to the astragaloside IV and calycosin-7-glucoside peaks measured by liquid chromatography, the standard curves of the two substances were calculated as follows: Y = 28.85x - 1.825 (R^2 = 0.999), with a linear range of 0.12–0.54 μg, and Y = 475.64x + 12.039 (R^2 = 0.997), with a linear range of 0.45–4.05 μg, respectively. The contents of astragaloside IV and calycosin-7-glucoside in ARR before and after fermentation (Table 3) were calculated according to Fig. 4 and Fig. 5.

Astragali Radix polysaccharide content

According to our tests, the standard curve equation for ARP was Y = 0.008x + 0.010, R^2 = 0.999, with a linear range of 0–100 μg mL^{-1}. The polysaccharide contents in ARR before fermentation was 36.34 ± 3.12 mg g^{-1}, which increased significantly to 49.90 ± 3.48 mg g^{-1} after fermentation ($p < 0.01$). Fig. 6 illustrates the differences between polysaccharide contents in ARR before and after fermentation.

Total protein and crude fatcontents

Table 4 summarizes the total protein and crude fat contents of ARR before and after fermentation. Notably, we observed that under the same humidity conditions, the total protein and crude ash contents in the ARR after fermentation was significantly higher than before fermentation ($p < 0.05$), whereas the crude fat content remained largely constant before and after fermentation ($p > 0.05$).

Contents of four metallic elements

Linear regression was performed for each element, with the concentration as the abscissa and the absorbance as the ordinate. It can be seen from Table 5 that the concentration of each element exhibited a good linear relationship with the absorbance.

The results demonstrated that the iron content in ARR after fermentation was significantly lower than before fermentation ($p < 0.01$), and the manganese content was significantly higher than before fermentation ($p < 0.01$) (Table 6).

Conclusions

Selection of fermentation strain ACCC 30583

Aspergillus niger is a common species of *Aspergillus*, which is widely distributed in food, plant products, and soil around the world. Notably, some *Aspergillus* strains are utilized in industrial fermentation processes [11-13]. The industrial use of *Aspergillus niger* is generally regarded as being highly safe. According to the FDA, *Aspergillus niger* can be directly used in food production or feed [14]. *Aspergillus niger* hyphae are relatively developed, with fast asexual growth and propagation speed, as well as strong adaptability, and thus are often used for fermentation feed in the breeding industry [15]. The overarching purpose of this study was to use the fermented ARR as an animal feed additive. Before that, our research team had screened 11 strains of cellulase-producing fungi and bacteria. An enzyme activity test determined that among the 11 strains, *Aspergillus niger* ACCC 30583 and Bacillus 01784 had the highest enzyme production activity. We then investigated the influence of the *Aspergillus niger* ACCC 30583 and Bacillus 01784 culture media on the precipitation of Astragaloside Ⅳ, the active component of ARR. Our results showed that Astragaloside Ⅳ yields were higher in *Aspergillus niger* ACCC 30583 fermentation, and was therefore deemed the optimal strain for ARR fermentation. Our team then investigated the effects of fermentation temperature, time, carbon source composition, carbon source concentration, inoculum amount, and nitrogen source concentration on the cellulase production activity of *Aspergillus niger* ACCC 30583, and determined the optimal cellulase production conditions through a four-factor (nitrogen source

concentration, carbon source concentration, inoculum amount, and fermentation time) orthogonal experiment [10]. In this study, a three-level orthogonal experiment was carried out to determine the optimal conditions for ARR solid fermentation. We concluded that the optimal culture medium content, fermentation time, and fermentation temperature were 60%, 4 days, and 28 ℃, respectively. Under these conditions, the fermentation of ARR by the *Aspergillus niger* ACCC 30583 strain effectively promoted the separation of astragaloside Ⅳ from the residues. Additionally, another potential advantage of using ARR fermented by *Aspergillus niger* as a feed additive is that *Aspergillus niger* produces many kinds of enzymes during the fermentation process, among which proteases can decompose the protein in feed and facilitate animal digestion (i.e., by compensating endogenous enzyme deficiencies), stimulate the secretion of endogenous enzymes, accelerate the digestion and absorption of nutrients, and improve feed utilization efficiency [16].

Importance of ARR as a test substance

Astragali Radix (Huangqi), which is widely used in medicine or food, is thought to reinforce qi and strengthen the spleen, and is therefore suitable to treat diseases associated with qi failure and blood deficiencies [17]. Pharmacological studies have demonstrated that *Astragali Radix* has a wide range of clinical applications, including anti-tumor, antioxidative, anti-diabetes, antibacterial, and antiviral and immunostimulant properties [18-21]. However, due to extraction technology limitations, the extraction efficiency of *Astragali Radix* is low, resulting in ARR with a large amount of bioactive substances. Huang et al. used HPLC to detect the astragaloside Ⅳ contents in *Astragali Radix* and ARR, and demonstrated that ARR contained 72.08% of the total astragaloside Ⅳ content in *Astragali Radix* [22]. Moreover, Zhou et al. used HPLC to detect the astragaloside Ⅳ content in ARR discarded by Dali Pharmaceutical Co. Ltd. and found that the astragaloside Ⅳ content was as high as 0.74 μg g^{-1} [23]. The results of this study showed that the astragaloside Ⅳ content in residues extracted twice via the regular water extraction method was 0.13 mg g^{-1}. In addition to functional components such as astragaloside Ⅳ, ARR also contain an abundance of nutrients such as crude fat, crude protein, amino acids, and minerals, which could be used as growth enhancers in animal feed [24]. Previous studies have shown that ARR is a natural and safe feed additive, which can accelerate animal growth and improve immunity and meat quality [25-26]. Thus, the potential of ARR as feed additives for animal production should be studied further.

Astragaloside IV as the basis for judging the effectiveness of ARR fermentation

Saponins are among the most representative active components of *Astragali Radix*. Currently, more than 40 saponins, such as astragaloside Ⅳ and isoastragaloside, have been isolated from ARR, among which astragaloside Ⅳ has the best biological activity [27] and is thus often referred to as a "super *Astragali Radix* polysaccharide." Astragaloside Ⅳ has broad pharmacological applications, such as neuroprotection and liver protection, as well as anti-cancer and anti-diabetes effects [28]. Zang et al. demonstrated that astragaloside IV is a commonly used Chinese patent medicine for patients with chronic heart failure [29]. Moreover, Leng et al. reported that astragaloside IV has protective effects against endothelial dysfunction in diabetic rats [30]. Astragaloside IV has also been reportedly used as a quality-control marker of

Astragali Radix in the Chinese Pharmacopoeia (2005 version) [31]. Therefore, astragaloside IV is arguably the most representative component of *Astragali Radix*.

Analysis of test results

Our results demonstrated that the contents of bioactive substances such as astragaloside IV, calycosin-7-glucoside, and ARP in ARR increased significantly after *Aspergillus niger*-mediated fermentation. This may be because *Astragali Radix* contains more lignin and cellulose, which provides nutrition for the growth of *Aspergillus niger*, thereby allowing these microorganisms to produce lignin-degrading enzymes and cellulase, which can decompose the cell walls of *Astragali Radix*. Thus, astragaloside Ⅱ, isoflavones, and polysaccharides in *Astragali Radix* can be separated from cell walls or protein complexes. The total protein content in ARR was notably increased by fermentation, which was directly related to the many enzymes produced by *Aspergillus niger* (i.e., enzymes are proteins themselves). The increase in crude ash may be mainly attributed to metabolic waste after fermentation. The mechanisms by which elemental metal contents in ARR were modified by fermentation remain unclear. It has been speculated that *Aspergillus niger* can use iron and other minerals contained in ARR to participate in the synthesis of its own organic compounds during its growth process, and the hydrochloric acid used in the analytical digestion process cannot disrupt the bonds between the iron and the aforementioned compounds, resulting in a decrease in the amount of iron detected [32-33]. Similarly, the increase of manganese content may also be due to chemical changes or associations before and after fermentation. However, this theory needs to be further confirmed.

In conclusion, *Aspergillus niger*-mediated fermentation greatly improved the separation of active substances and nutrients in ARR, thereby facilitating the repurposing of these residues as animal feed additives. Moreover, our proposed method has important implications for the treatment of other Chinese herbal medicine residues after extraction.

Abbreviations

ARR: *Astragali Radix* residues; ARP: *Astragali Radix* polysaccharides; PDA: Potato Dextrose Agar

Declarations

Acknowledgements

Not applicable.

Authors' contributions

CYL, YZ and GTF conceived and designed the experiments. YZ, GTF performed experiments. CYL and YZ analyzed the data and prepared the Figures and Tables and wrote the manuscript. JGG and CYH corrected and proofread the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

Funding

This work was supported by the National Key Research and Development Program of China (2018YFD0502003) and the Natural Science Foundation of the Anhui Higher Education Institutions of China (Grant No. KJ2017A129).

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Wang H, Feng F. Identification of components in Zhi-Zi-Da-Huang decoction by HPLC coupled with electrospray ionization tandem mass spectrometry, photodiode array and fluorescence detectors. J Pharmaceut Biomed. 2009;49:1157–65.
2. Liu WW, Liu YX, Zhao Y, Guo DL, Zhang BL. Research progress on comprehensive utilization of Chinese medicinal residue. J Pharm Res. 2013;32:49–50.
3. Zhou Y, Selvam A, Wong JW. Chinese medicinal herbal residues as a bulking agent for food waste composting. Bioresource Technol. 2018;249:182–8.
4. Hashemi SR, Davoodi H. Herbal plant sand their derivatives as growth and health promoters in animal nutrition. Vet Res Commun. 2011;35:169–80.
5. Wu DL, Xiao XZ, Li YB. The utilizing situation of Chinese medicine residue. Guangdong Chem Ind. 2015;42:103–5.
6. Abdallah A, Zhang P, Abubakari AH, Elemba E, Zhong QZ, Sun ZW. Reclamation of *Astragalus* by-product through dietary inclusion in ruminant diets: Effects on growth performance, nutrient

digestibility, rumen fermentation, blood biochemical parameters, and humoral immune response in sheep. Evid-Based Compl Alt. 2019a;2019:8530961.

7. Zhao ZC, Tumenbayaer. Research progress on comprehensive utilization of extracting residues from traditional Chinese Medicine. Anim Husbandry Feed Sci. 2019;40:47–52.
8. Ni KK, Wang XK, Lu Y, Guo LN, Li XM, Yang FY. Exploring the silage quality of alfalfa ensiled with the residues of astragalus and hawthorn. Bioresource Technol. 2020;297:122249.
9. Sanchez S, Demain AL. Metabolic regulation of fermentation processes. Enzyme Microb Tech. 2002;31:895–906.
10. Liu F. Optimization of the solid fermentation conditions of the Astragalus slag. Anhui Agr Univ. 2015.
11. Park HS, Jun SC, Han KH, Hong SB, Yu JH. Diversity, application, and synthetic biology of industrially important *Aspergillus* fungi. Adv Appl Microbiol. 2017;100:161–202.
12. Baker SE. *Aspergillus niger* genomics: Past, present and into the future. Med Mycol. 2006;44:17–21.
13. Ma XG, Zhang ZZ, Liu Y, Zhao GC. Breeding of high citric acid-producing *Aspergillus niger* strain by aerospace mutagenesis and its optimization of ferm entation conditions. China Brewing. 2012;5:40–4.
14. Wang JH, Cao FL, Zhu ZL, Zhang XH, Sheng QQ, Qin WS. Improvement of quality and digestibility of Moringa oleifera leaves feed via solid-state fermentation by *Aspergillus niger*. Int J Chem React Eng. 2018;16:1–14.
15. Shi CY, He J, Wang JP, Yu J, Yu B, Mao XB, Zhang P, Huang ZQ, Cheng DW. Effects of *Aspergillus niger* fermented rapeseed meal on nutrient digestibility, growth performance and serum parameters in growing pigs. Anim Sci J. 2016;87:557–63.
16. Zhuang TL, Li H, Guo FX, Sun ZT. Production of bio-feed enriched of enzymes from apple pomace in solid state fermentation by *Aspergillus niger*. Sci Tech Food Ind. 2010;12:171–5.
17. Li YS, Han MY, Chen BX, Zhang MX, Zhang JS, Jin CX, Qi YW. Regulatory effects of Astragali radix on lymphocytes and their surface molecules in mouse. Immunol J. 2020;36:52–7.
18. Zhang L, Shergis JL, Yang LH, Zhang AL, Xue CC, Guo XF, Zhang L, Zhou SZ, Zeng L, Mao W. *Astragalus membranaceus* (HuangQi) as adjunctive therapy for diabetic kidney disease: An updated systematic review and meta-analysis. J Ethnopharmacol. 2019;239:111921.
19. Park YJ, Thwe AA, Li X, Kim YJ, Kim JK, Arasu MV, Al-Dhabi NA, Park SU. Triterpene and flavonoid biosynthesis and metabolic profiling of hairy roots, adventitious roots, and seedling roots of *Astragalus membranaceus*. J Agr Food Chem. 2015;63:8862–9.
20. Liu Y, Liu J, Wu KX, Guo XR, Tang ZH. A rapid method for sensitive profiling of bioactive triterpene and flavonoid from Astragalus mongholicus and *Astragalus membranaceus* by ultra-pressure liquid chromatography with tandem mass spectrometry. J Chromatogr B. 2018;1085:110–8.
21. Chen W, Ju J, Yang YH, Wang H, Chen WJ, Zhao XL, Ye HY, Zhang Y. *Astragalus* polysaccharides protect cardiac stem and progenitor cells by the inhibition of oxidative stress-mediated apoptosis in diabetic hearts. Drug Des Dev Ther. 2018;12:943–54.

22. Huang YF, Liu J, Huang JW, Zhang YM. Determination of astragaloside IV in dreg of Radix astragali by high-performance liquid chromatography. Acta Scientiarum Nat U Sunyatseni. 2009;48:146–8.
23. Zhou P, Wang CJ, Deng X. Determination the content of astragaloside Ⅳ in dreg of Radix astragali. J Anhui Agr Sci. 2011;39:7773–4.
24. Sun HQ, Zhu ZY, Yang XY, Meng M, Dai LC, Zhang YM. Preliminary characterization and immunostimulatory activity of a novel functional polysaccharides from *Astragalus* residue fermented by *Paecilomyces sinensis*. RSC Adv. 2017;7:23875–81.
25. Abdallah A, Zhang P, Elemba E, Zhong Q, Sun Z. Carcass characteristics, meat quality, and functional compound deposition in sheep fed diets supplemented with *Astragalus membranaceus* by-product. Anim Feed Sci Tech. 2020;259:114346.
26. Abdallah A, Zhang P, Zhong Q, Sun, Z. Application of traditional Chinese herbal medicine by-products as dietary feed supplements and antibiotic replacements in animal production. Curr Drug Metab. 2019b;20:54–64.
27. Ren S, Zhang H, Mu Y, Sun M, Liu P. Pharmacological effects of Astragaloside IV: a literature review. J Tradit Chin Med. 2013;33:413–6.
28. Zhang JQ, Wu CX, Gao L, Du GH, Qin XM. Chapter Four - Astragaloside IV derived from *Astragalus membranaceus*: A research review on the pharmacological effects. Adv Pharmacol. 2020;87:89–112.
29. Zang YB, Wan JJ, Zhang Z, Huang S, Liu X, Zhang WD. An updated role of astragaloside IV in heart failure. Biomed Pharmacother. 2020;126:110012.
30. Leng B, Tang F, Lu M, Zhang Z, Wang H, Zhang Y. Astragaloside IV improves vascular endothelial dysfunction by inhibiting the TLR4/NF-kappaB signaling pathway. Life Sci. 2018;209:111–21.
31. Monschein M, Ardjomand-Woelkart K, Rieder J, Wolf I, Heydel B, Kunert O, Heuberger H, Bauer R. Accelerated sample preparation and formation of astragaloside IV in Astragali Radix. Pharm Biol. 2013;52:403–9.
32. Odoni DI, Van Gaal MP, Schonewille T, Tamayoramos JA, Martins Dos Santos VA, Suarezdiez M, Schaap PJ. *Aspergillus niger* secretes citrate to increase iron bioavailability. Front microbiol. 2017;8:1424.
33. Chaerun SK, Sulistyo RS, Minwal WP, Mubarok MZ. Indirect bioleaching of low-grade nickel limonite and saprolite ores using fungal metabolic organic acids generated by *Aspergillus niger*. Hydrometallurgy. 2017;174:29–37.

Tables

Table 1

Orthogonal *experiment* results (3 factors and 3 levels; culture medium content, fermentation time, and fermentation temperature)

Number	Moisture content	Time (d)	Temperature (°C)	Astragaloside IV precipitation (mg g^{-1})
1	50%	4	26	0.20975
2	50%	5	28	0.26641
3	50%	6	30	0.30095
4	60%	4	30	0.46345
5	60%	5	26	0.34130
6	60%	6	28	0.29543
7	70%	4	28	0.26530
8	70%	5	30	0.32638
9	70%	6	26	0.27193
Average value 1	0.259	0.313	0.277	
Average value 2	0.367	0.311	0.334	
Average value 3	0.288	0.289	0.303	
Range	0.108	0.024	0.057	

Table 2

Analysis of variance of culture medium content, fermentation time, and fermentation temperature via a 3-factor and 3-level orthogonal test

Factor	Sum of Squares	df	F ratio	F value
Moisture content	0.019	2	1.854	4.46
Fermentation time	0.001	2	0.098	4.46
Fermentation temperature	0.005	2	0.488	4.46
Deviation	0.04	8		

Table 3
Determination of astragaloside IV and calycosin-7-glucoside in *Astragali Radix* residues before and after fermentation

Group	Astragaloside IV (μg g^{-1})	Calycosin-7-glucoside (μg g^{-1})
Before fermentation	0.13 ± 0.011	6.1 ± 0.77
After fermentation	0.63 ± 0.090*	8.15 ± 1.12*

Note: * indicates a significant difference between pre- and post-fermentation ($p < 0.05$)

Table 4
Near-infrared scanning results of total protein and crude fat contents in *Astragali Radix* residues before and after fermentation (%)

Sample	Total protein	Crude fat	Crude ash
Before fermentation	9.85 ± 0.40	4.35 ± 0.30	6.93 ± 0.31
After fermentation	19.84 ± 0.46*	5.16 ± 1.06	12.20 ± 0.38*

Note: * indicates a significant difference between pre- and post-fermentation ($p < 0$ .05)

Table 5
Standard curve equations for the four metallic elements analyzed herein

Metallic elements	Standard curve	R^2	Linear range (mg L^{-1})
Iron	Y = 0.0415x + 0.2395	0.999	4-16
Copper	Y=0.1088x + 0.0373	0.9994	0.4-6
Manganese	Y=0.1665x + 0.0497	0.9993	0.4-6
Zinc	Y = 0.4037x + 0.0519	0.9983	0.2-2

Table 6
Determination results of iron, copper, manganese, and zinc in *Astragali Radix* residues before and after fermentation (mg kg^{-1})

Sample	Iron	Copper	Manganese	Zinc
Before fermentation	211.06 ± 17.88	31.16 ± 6.22	25.22 ± 5.98	149.54 ± 11.29
After fermentation	137.70 ± 11.54*	28.48 ± 4.26	96.05 ± 7.92*	151.76 ± 10.34

Note: * indicates a significant difference between pre- and post-fermentation ($p < 0.05$)

Figures

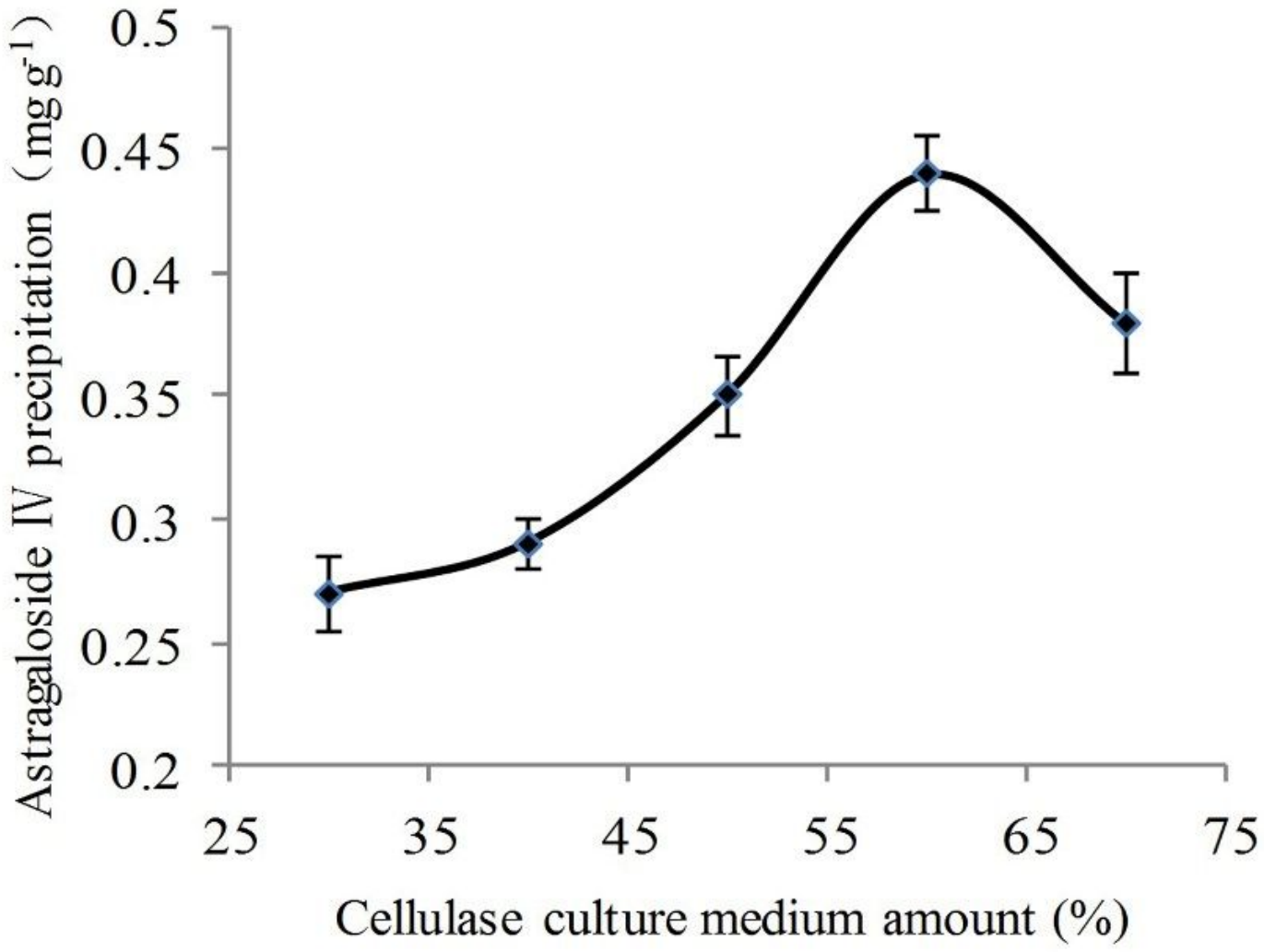

Figure 1

Effect of Aspergillus niger cellulase culture medium on astragaloside Ⅳ precipitation

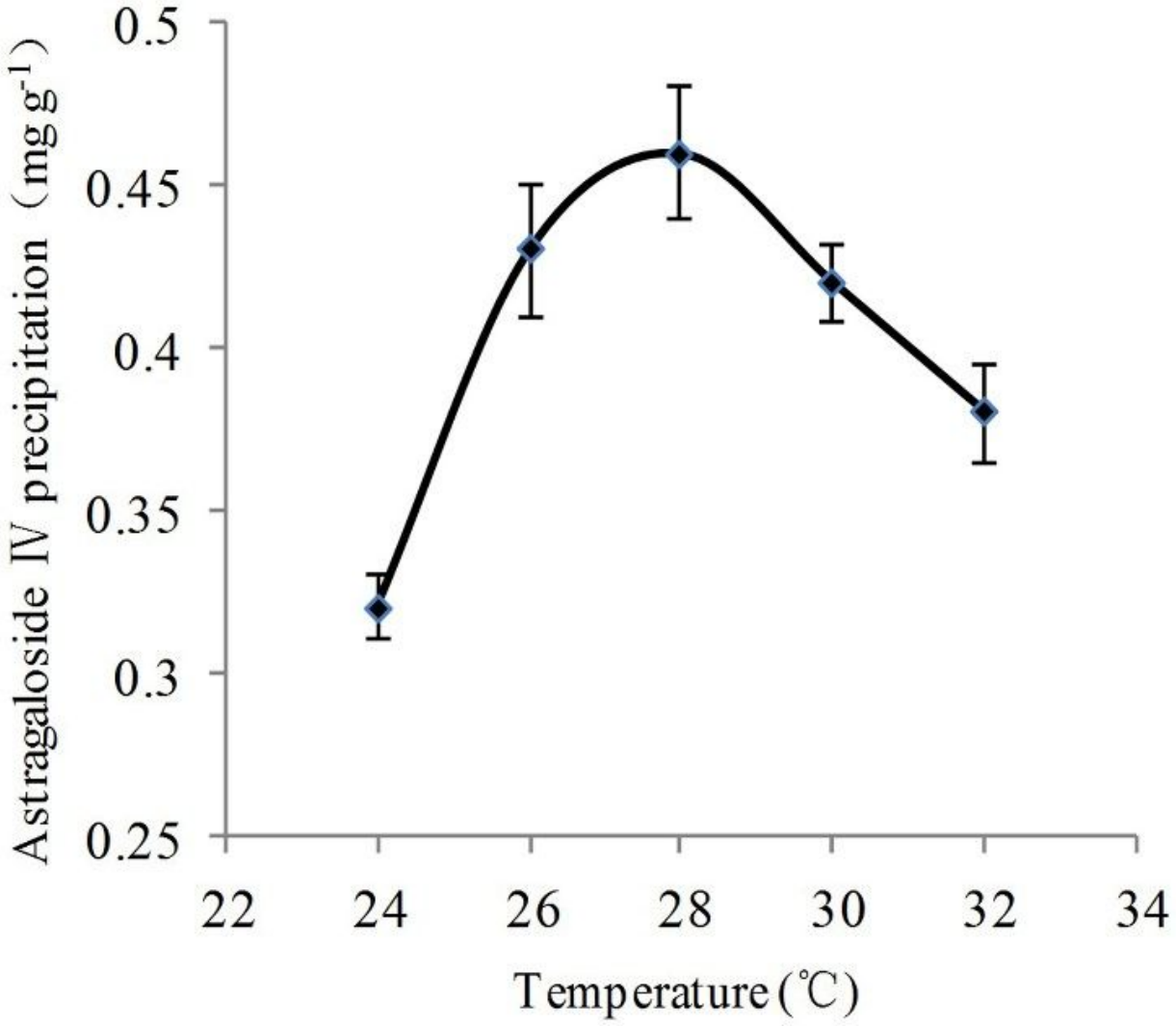

Figure 2

Effect of temperature on astragaloside Ⅳ precipitation

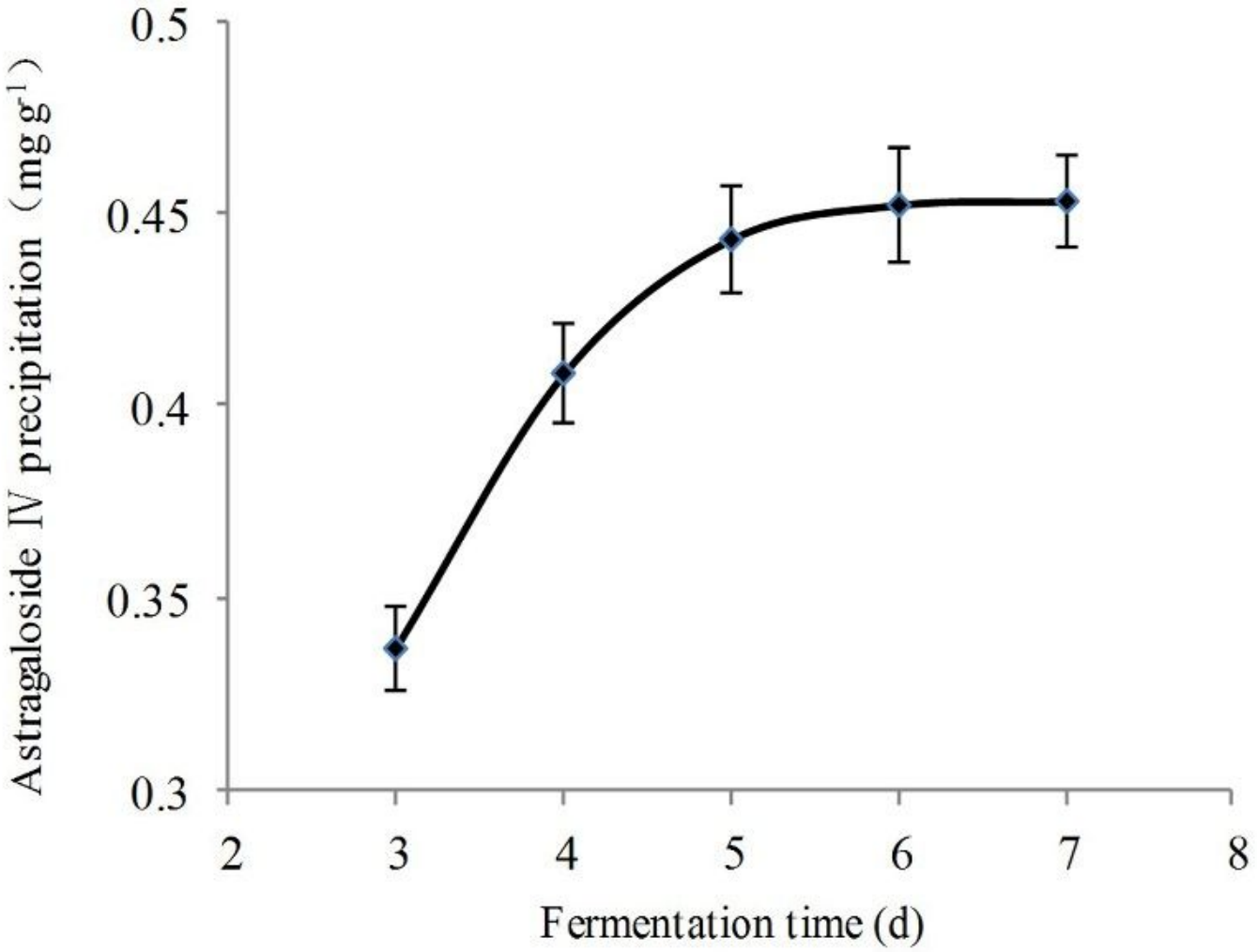

Figure 3

Effect of time on astragaloside Ⅳ precipitation

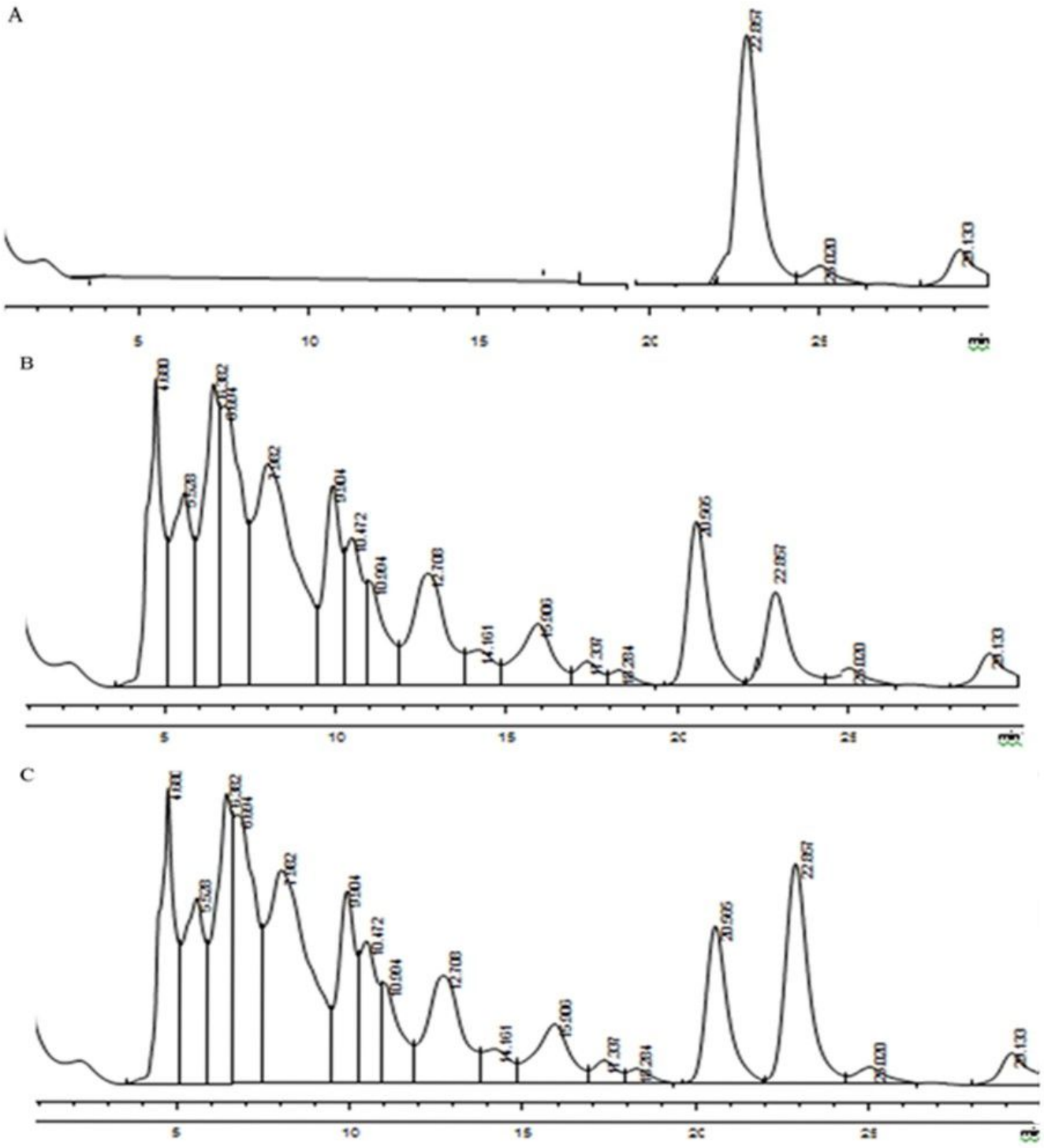

Figure 4

Chromatogram of astragaloside IV before and after fermentation (A: astragaloside IV standard; B: before fermentation; C: after fermentation)

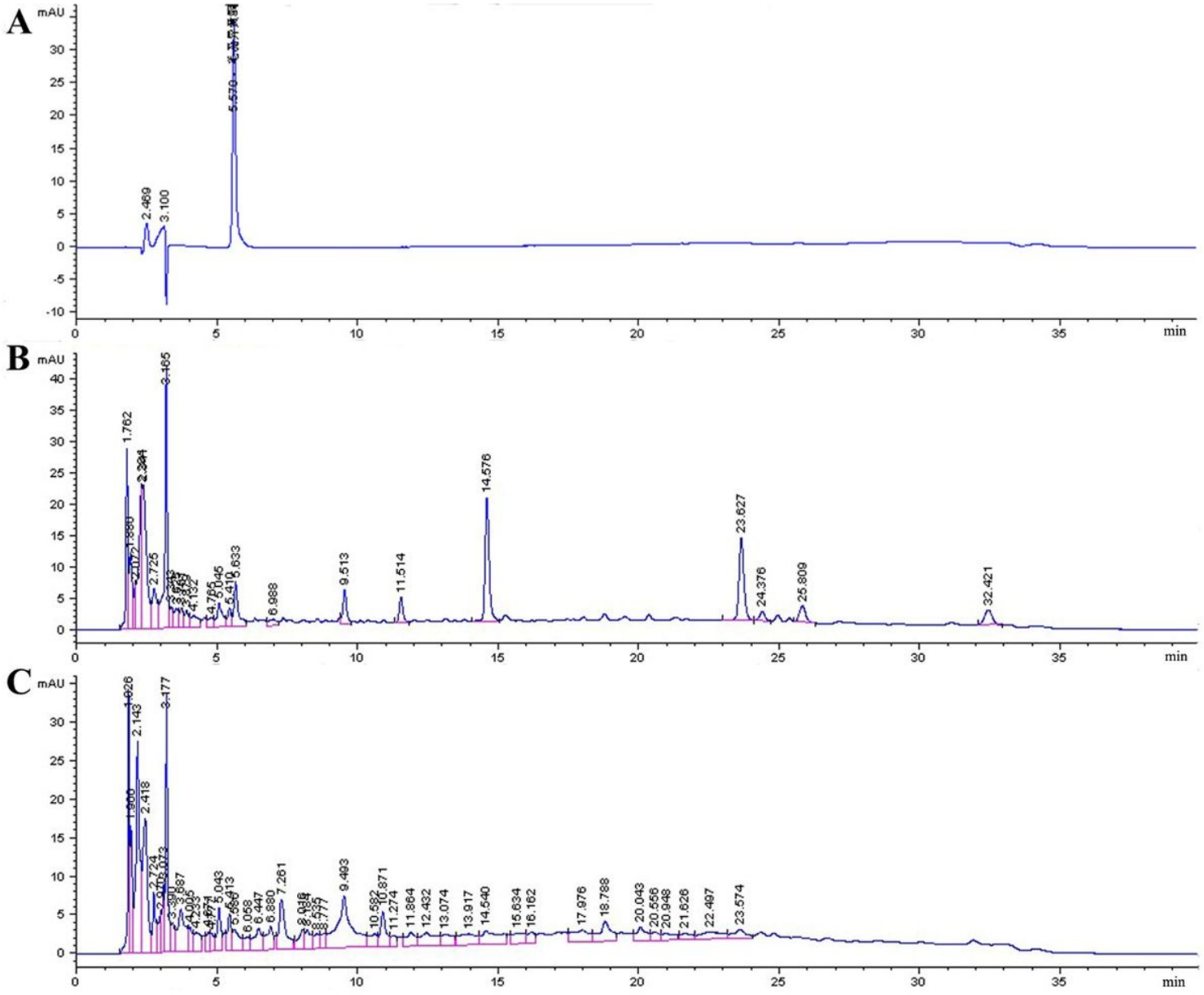

Figure 5

Chromatogram of calycosin-7-glucoside before and after fermentation (A: calycosin-7-glucoside standard; B: before fermentation; C: after fermentation)

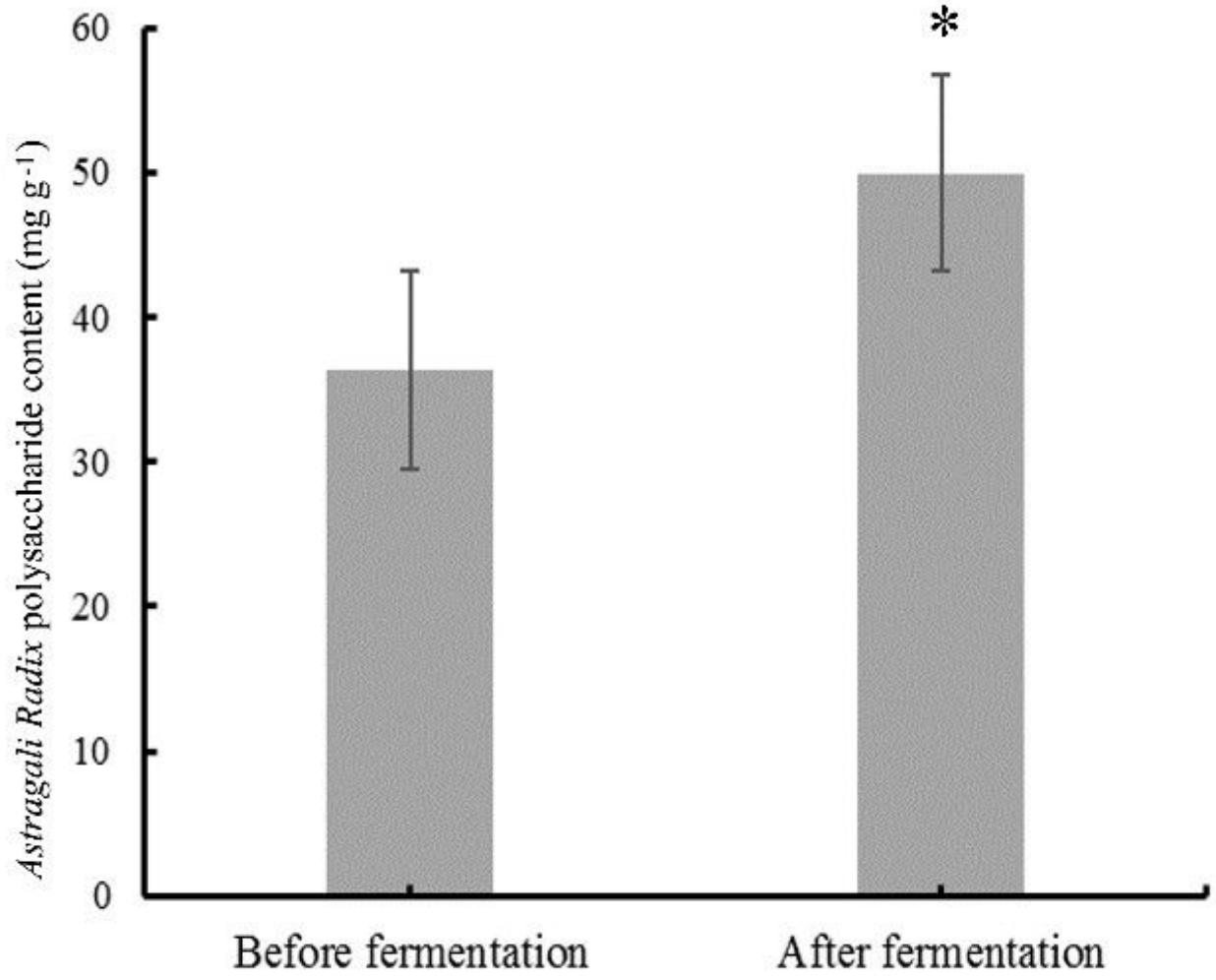

Figure 6

Astragali Radix polysaccharide content in Astragali Radix residues before and after fermentation