



Original Article

Quantitative and HPLC fingerprint analysis combined with chemometrics for quality evaluation of *Codonopsis Radix* processed with different methods

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ABSTRACT

Objective: To observe the effect of different processing methods on the quality of *Codonopsis Radix* and provide data reference for the reasonable processing in the producing areas.

Methods: An analytical strategy that combined quantitative and HPLC fingerprint analysis with chemometrics was developed for the quality evaluation of *Codonopsis Radix*. Lobetyolin, polysaccharide, sucrose, glucose, and fructose were detected simultaneously in *Codonopsis Radix* samples treated with different processing methods including dryer-drying, sun-drying, shade-drying, sulfur fumigation, and kneading, etc.

Results: The results showed that different processing methods had an obvious impact on the quality of *Codonopsis Radix*. Sun-drying or shade-drying was recommended, and sulfur fumigation should be avoided. And dryer-drying at 40 °C–50 °C was suggested to meet the requirement for large scale processing. In addition, based on the content of polysaccharide, glucose, and fructose, it was advised to knead for three times. As for lobetyolin and sucrose, kneading should be avoided.

Conclusions: Our findings demonstrated that processing method had a big impact on the quality of *Codonopsis Radix*. Based on the results, the appropriate processing methods directed at different requirements were recommended. The study could lay a foundation for the reasonable processing of *Codonopsis Radix* in producing areas.

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1. Introduction

Codonopsis Radix (CR) is the dried root of *Codonopsis pilosula* (Franch.) Nannf., *C. pilosula* Nannf. var. *modesta* (Nannf.) L. T. Shen, and *C. tangshen* Oliv. It has the efficacy of strengthening the spleen and tonifying the lung, as well as nourishing blood and engendering liquid, and is used to treat hypofunction of the spleen and the lung manifested by shortness of breath, cough, palpitation, anorexia, loose stools, and wasting-thirst caused by internal heat, etc. (China Pharmacopoeia Committee, 2015). Modern research shows that CR contains a variety of chemical constituents, such as polyacetlenes, polyenes, flavonoids, lignans, alkaloids, coumarins, terpenoids, steroids, organic acids, and saccharides, etc., which has the bioactivities of promoting blood hematopoiesis, strengthening

immunity, resisting fatigue, and delaying ageing, etc. (He, Zhu, Wang, Xu, & Hu, 2004; Huang, Zhang, Kang, Yu, & Guo, 2018; Wang, Deng, Shi, & Deng, 2011; Zhu, He, Wang, Xu, & Xu, 2001). Among them, lobetyolin is one of the active constituents displaying protective effect on gastric mucosal lesion induced by ethanol, which is consistent with the traditional efficacy of strengthening the spleen and the stomach (Song, Wang, Li, & Zhong, 2008). Lobetyolin is also the reference substance of TLC identification of CR in Chinese Pharmacopoeia (2015 edition) and is often used as the index component for the quality evaluation of CR in literatures. Polysaccharide has the effect of strengthening immunity, eliminating free radical, and promoting blood hematopoiesis (Chen & Wang, 2007). Oligosaccharide and monosaccharide such as sucrose, glucose, and fructose, etc. are important for the property, structure, and structure-activity relationship study of polysaccharide (Fan et al., 2009; Xia et al., 2011; Xu, Xia, Wang, & Wang, 2008) and have significant bioactivities (Anand, Chhina, Sharma, Dua, & Singh, 1964; Rani & Gupta, 2012; Roglans et al., 2007). Although several researches have focused on the detection of lobetyolin

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(Huang et al., 2017; Lv et al., 2017; Wang, Liu, Guo, Pang, & Zhao, 2007), polysaccharides (Wang, Chen, Zou, Qin, & Zhu, 2016; Zhen, Gao, & Cao, 2014), and monosaccharides (Wang & Shi, 2001) to explicate the quality of CR, few studies have been done to detect these components concurrently and analyze the quality of CR comprehensively. Therefore, lobetyolin, polysaccharide, sucrose, glucose, and fructose are chosen as the index components to assess the quality of CR roundly.

Primary process in producing areas is an important step for the production of Chinese materia medica (CMM) and affects the quality as well as clinical efficacy of CMM directly. Sun-drying and shade-drying are traditional processing methods of CR, and sun-drying is stipulated as the drying method of CR in Chinese Pharmacopoeia (2015 edition). Sun-drying and shade-drying are easy and simple, but they occupy large areas and consume vast manpower. In addition, the drying period is long and is affected by weather changes, thus the medicinal materials get mildew and rot easily (Chen, Liu, Zhang, Gao, & Zhang, 2010). Dryer-drying is drying under specific temperature in a drying oven or room. It is time-saving and not subject to natural conditions, but it needs particular heating or drainage facilities and the temperature should not be too high. Sulfur fumigation is a drying method of continuous smoking by the import of sulfur burning fume through specially-made furnace and pipe in the stack. It can prevent worm-eaten and improve the appearance of medicinal materials. However, it can lead to excessive level of sulfur dioxide residue and research shows that the content of lobetyolin in CR declines sharply after sulfur fumigation (Li et al., 2010). Kneading is a traditional processing method of CR by rubbing repeatedly from cover to cover after combing the medicinal materials into small bundles. It can remove impurities and keep the skin and flesh clingy and firm. Thus, the medicinal materials become uniform and smooth, and are suitable for preservation. In addition, research shows that kneading can increase the content of polysaccharide in CR (He, Li, Pan, & Li, 2006). In our previous resource investigation on CR, it is found that the processing methods in different producing areas are different. For instance, the processing method of sun-drying combined with shade-drying is generally adopted in Weiyuan County, Gansu Province. In Lingchuan County, Shanxi Province, the processing method of sun-drying is mainly employed, and traditional processing method of kneading is still reserved. In addition, the processing method of sulfur fumigation is occasionally used in individual producing areas. Nevertheless, study on the effect of processing method on the quality of CR is limited at present. In order to find out how different processing methods have an impact on the quality of CR and recommend appropriate processing method in producing areas, relevant research is urgently needed.

In the present study, to observe the influence of different processing methods on the quality of CR, an analytical strategy that combined quantitative analysis of multi-compounds and qualitative analysis of HPLC fingerprint with chemometrics was developed. Lobetyolin, polysaccharide, sucrose, glucose, and fructose were detected simultaneously in CR samples treated with 12 kinds of processing methods including dryer-drying, sun-drying,

shade-drying, sulfur fumigation, and kneading, etc., which covered all the processing methods in main producing areas. The results could provide data reference for the reasonable processing of CR in producing areas.

2. Materials and methods

2.1. Materials

CR samples were collected from the standardized production base in Shili Village, Shili Town, Min County, Dingxi City, Gansu Province (34°29' N, 103°57' E, 2340 m) in October 2016. All samples were authenticated by Prof. Bengang Zhang as the dried root of *Codonopsis pilosula* (Franch.) Nannf., and the voucher specimen with the reference voucher number DS-20161001-01 was deposited in our laboratory for future reference. The processing method information was listed in Table 1.

2.2. Chemicals and reagents

Waters 2695 HPLC system (USA) coupled with 2996 DAD was used for the determination of lobetyolin and the establishment of HPLC fingerprint, and the data were processed by Empower 2 data station. Agilent 1260 HPLC system (USA) coupled with 380 ELSD was used for the determination of sucrose, glucose and fructose, and the data were handled by ChemStation. Absorbance measurement of polysaccharide was carried out on a UV2550 spectrophotometer (Shimadzu, Japan). AL204 electronic analytical balance (Mettler Toledo), KQ-300DE ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.), and XMTD-6000 water bath kettle (Beijing Changfeng Instrument Co.) were used for sample pretreatment. Chromatographic grade acetonitrile was purchased from Honeywell Burdick & Jackson (Muskegon, USA). Deionized water (18.2 MU) was obtained from a Milli-Q System (Millipore, Billerica, MA, USA). Analytical grade methanol, ethanol, and sulfuric acid were purchased from Beijing Chemical Works (Beijing, China). Analytical grade phenol was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Lobetyolin was purchased from Tianjin Mark Biotechnology Co., Ltd. (Tianjin, China). The purity was more than 98% and the batch number was 20151202. Sucrose, D-anhydrous glucose, and fructose were purchased from National Institutes for Food and Drug Control. Their purity was more than 99% and the batch numbers were 111507-201303, 110833-201506, and 100231-201606, respectively.

2.3. Samples and standard solutions preparation

The preparation of standard solution and sample solution of lobetyolin and polysaccharide were based on the methods in literature (Gao et al., 2016). And the preparation of standard solution and sample solution of sucrose, glucose, and fructose were based on the methods in literature (Cao et al., 2017).

Table 1
Processing method information of *Codonopsis Radix* samples.

No.	Processing methods	Logogram	No.	Processing methods	Logogram
S1-3	Dryer-drying for 5 times at 30 °C without kneading	T30-DD5-NK	S19-21	Sun-drying and kneading for once	SND-K1
S4-6	Dryer-drying for 5 times at 40 °C without kneading	T40-DD5-NK	S22-24	Sun-drying and kneading for 3 times	SND-K3
S7-9	Dryer-drying for 5 times at 50 °C without kneading	T50-DD5-NK	S25-27	Sun-drying and kneading for 5 times	SND-K5
S10-12	Dryer-drying for once at 40 °C without kneading	T40-DD1-NK	S28-30	Shade-drying without kneading	SDD-NK
S13-15	Dryer-drying for 5 times at 40 °C and kneading for once	T40-DD5-K1	S31-33	Shade-drying and kneading for once	SDD-K1
S16-18	Sun-drying without kneading	SND-NK	S34-36	Sulfur fumigation after sun-drying without kneading	SND-SF-NK

Annotation: For drying time of dryer-drying, drying once was conducted by drying 44 h in succession, and drying five times was conducted within 5 d, that was drying 10 h each day in first 2 d, and 8 h each day in latter 3 d.

Briefly, for the preparation of standard solution of lobetyolin, a moderate amount of lobetyolin was dissolved in chromatographic methanol and diluted to make a solution with 40 µg lobetyolin per 1 mL for analysis. For the preparation of sample solution of lobetyolin, 1 g powdered sample was weighed and suspended with 30 mL 70% methanol, and extracted by ultrasonication for twice for 30 min each time, after compensating the loss of weight with extraction solvent, the sample solution was filtered through a 0.22 µm membrane filter prior to further analysis.

For the preparation of standard solution of polysaccharide, a moderate amount of glucose was dissolved in distilled water and diluted to prepare a solution containing 0.11 mg glucose per 1 mL for analysis. For the preparation of sample solution of polysaccharide, 0.25 g powdered sample was weighed and added with 150 mL 80% ethanol, and extracted by refluxing for 1 h in water bath. After filtration, the residue was washed by 80% hot ethanol. Residue and filter paper were suspended with 150 mL distilled water and extracted by refluxing for 1 h in boiling water bath. After filtration, the residue was washed by hot water. The filtrate and lotion were transferred to a volumetric flask to make 250 mL. Then 2 mL was precisely measured and diluted with distilled water to 10 mL and used as sample solution.

For the preparation of standard solution of sucrose, glucose, and fructose, moderate amount of the corresponding reference substances were dissolved in distilled water and diluted to prepare a solution containing a mixture of 3.7, 2.4, and 3.2 mg of sucrose, glucose, and fructose respectively each 1 mL for further analysis. For the preparation of sample solution of these saccharides, 1 g powdered sample was weighed and suspended with 50 mL distilled water, and extracted by refluxing for 1 h in boiling water bath, after compensating the loss of weight with extraction solvent, the sample solution was filtered through a 0.22 µm membrane filter prior to further analysis.

2.4. Lobetyolin determination and HPLC fingerprint establishment

The content of lobetyolin and the HPLC fingerprint of CR samples were obtained as previously described (Gao et al., 2016). Briefly, chromatographic separation was performed on a Waters XBridge C₁₈ column (4.6 mm × 250 mm, 5 µm). A linear gradient elution of solvent A (Water) and solvent B (Acetonitrile) were applied with the following program: 0–8 min, 5% B, 8–10 min, 5%–8% B, 10–25 min, 8%–15% B, 25–26 min, 15%–20% B, 26–36 min, 20% B, 36–48 min, 20%–37% B, 48–55 min, 37%–60% B, 55–70 min, 60% B, 70–80 min, 60%–90% B, 80–90 min, 90% B. The flow rate was set at 0.8 mL/min and the injection volume was 10 µL. The wavelength was set at 267 nm. And the column was maintained at 30 °C. The quantification of lobetyolin in samples and the establishment of HPLC fingerprint were accomplished under the above conditions.

2.5. Determination of polysaccharide

The content detection of polysaccharide of CR samples was based on the methods in literature (Gao et al., 2016). Briefly, 1 mL sample solution was added with 1 mL 5% phenol solution, and further added with 5 mL sulfuric acid. After being heated for 20 min in boiling water bath and then cooled for 5 min in ice bath, the absorbance of the solution was measured at the wavelength of 490 nm compared with the corresponding blank reagent. The detection of polysaccharide in samples was achieved under the above conditions.

2.6. Determination of sucrose, glucose, and fructose

The content of sucrose, glucose, and fructose of CR samples were got as previously described in literature (Cao et al., 2017). Briefly, chromatographic separation was performed on an Agilent Hi-Plex Ca column (7.7 mm × 300 mm). The mobile phase was deionized water. The flow rate was set at 0.5 mL/min and the injection volume was 5 µL. The column was maintained at 80 °C. The drift tube temperature and the evaporating tube temperature were set at 30 °C and 60 °C, respectively. The flow rate of carrier gas was set at 1.4 L/min. The signal gain was 1. The determination of sucrose, glucose, and fructose in samples was completed under the above conditions.

2.7. Chemometrics analysis

HPLC fingerprint and similarity analysis was performed by the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A). Hierarchical clustering analysis and data analysis of lobetyolin, polysaccharide, sucrose, glucose, and fructose were completed with SPSS Software (Version 21.0). Comparisons were performed by Student's *t* test or one-way ANOVA followed by Student-Newman-Keuls (S-N-K). *P* < 0.05 was considered statistically significant. All data were the result of at least three independent experiments.

3. Results and discussion

3.1. Content of lobetyolin

The content of lobetyolin of CR samples processed with different methods was obtained and the results were listed in Table 2. As it could be seen, the content of lobetyolin in CR samples was in the range of 0.136%–0.271%, with the RSD less than 1.45%.

3.2. Content of polysaccharide

The content of polysaccharide of CR samples processed with different methods was obtained and the results were listed in

Table 2
Content of lobetyolin in *Codonopsis Radix* samples (*n* = 3).

No.	Lobetyolin/%	RSD/%	No.	Lobetyolin/%	RSD/%	No.	Lobetyolin/%	RSD/%
S1	0.160	0.94	S13	0.187	0.94	S25	0.145	0.19
S2	0.164	1.25	S14	0.184	0.41	S26	0.136	1.14
S3	0.170	0.61	S15	0.185	0.17	S27	0.154	1.45
S4	0.203	0.39	S16	0.249	0.51	S28	0.269	0.39
S5	0.197	1.05	S17	0.261	0.48	S29	0.270	0.17
S6	0.205	0.60	S18	0.259	0.35	S30	0.271	0.26
S7	0.206	0.45	S19	0.186	0.47	S31	0.175	0.32
S8	0.209	0.37	S20	0.188	0.73	S32	0.174	0.25
S9	0.200	0.61	S21	0.187	0.18	S33	0.175	0.16
S10	0.159	0.85	S22	0.242	0.24	S34	0.170	0.44
S11	0.170	0.39	S23	0.246	0.13	S35	0.172	0.27
S12	0.163	0.42	S24	0.249	0.39	S36	0.172	0.36

Table 3
Content of polysaccharide in *Codonopsis Radix* samples (n = 3).

No.	Polysaccharide/%	RSD/%	No.	Polysaccharide/%	RSD/%	No.	Polysaccharide/%	RSD/%
S1	13.72	1.31	S13	14.61	1.45	S25	15.60	1.45
S2	13.31	1.75	S14	13.80	1.38	S26	16.31	0.74
S3	12.91	1.03	S15	13.66	0.76	S27	16.46	1.75
S4	13.51	1.41	S16	16.09	0.86	S28	10.54	1.32
S5	13.23	1.63	S17	15.63	1.22	S29	10.01	1.67
S6	13.04	1.35	S18	15.64	0.29	S30	9.73	1.70
S7	14.23	0.98	S19	19.21	1.95	S31	13.45	0.41
S8	13.78	0.86	S20	19.70	1.63	S32	14.07	1.60
S9	13.58	1.03	S21	19.97	1.66	S33	14.50	0.50
S10	14.55	1.80	S22	14.83	1.22	S34	13.21	0.91
S11	15.36	0.86	S23	14.64	1.64	S35	12.91	1.86
S12	15.72	1.21	S24	15.47	1.39	S36	13.05	0.94

Table 4
Content of sucrose, glucose, and fructose in *Codonopsis Radix* samples (n = 3).

No.	Sucrose/%	RSD/%	Glucose/%	RSD/%	Fructose/%	RSD/%
S1	16.18	0.09	1.99	0.80	18.60	0.24
S2	16.19	0.03	1.88	1.09	18.78	0.10
S3	16.17	0.01	1.94	0.23	18.66	0.11
S4	17.05	0.14	2.41	0.72	21.20	0.14
S5	17.40	0.06	2.29	0.47	20.22	0.08
S6	16.59	0.01	2.33	0.48	21.00	0.08
S7	17.04	0.05	2.26	0.27	20.32	0.15
S8	16.51	0.08	2.40	0.45	21.37	0.08
S9	17.30	0.01	2.32	0.33	20.74	0.15
S10	15.59	0.09	3.00	0.26	22.00	0.15
S11	16.55	0.09	3.06	0.47	22.46	0.11
S12	16.60	0.12	3.07	0.30	23.02	0.10
S13	13.46	0.11	3.08	0.42	24.04	0.08
S14	13.53	0.03	3.15	0.57	24.88	0.65
S15	13.49	0.05	3.10	0.22	24.33	0.09
S16	13.71	0.08	3.20	0.33	24.01	0.09
S17	13.38	0.22	3.17	0.31	24.39	0.11
S18	13.40	0.10	3.19	0.34	24.68	0.07
S19	8.26	0.21	4.44	0.61	25.78	0.06
S20	8.37	0.12	4.51	0.53	25.59	0.13
S21	8.69	0.06	4.55	0.23	25.73	0.07
S22	12.07	0.06	2.64	0.94	18.96	0.10
S23	12.05	0.06	2.63	0.93	19.09	0.17
S24	12.02	0.04	2.63	1.07	19.36	0.11
S25	11.42	0.02	3.45	0.53	23.74	0.10
S26	11.38	0.11	3.44	0.80	24.30	0.06
S27	11.40	0.15	3.45	0.55	24.12	0.12
S28	17.51	0.04	3.02	0.73	20.59	0.12
S29	17.46	0.08	3.02	0.60	20.68	0.14
S30	17.41	0.08	3.03	0.56	20.80	0.08
S31	12.43	0.30	5.15	0.22	25.91	0.10
S32	12.77	0.14	5.18	0.41	26.61	0.08
S33	12.51	0.11	5.16	0.03	26.49	0.09
S34	3.61	0.47	6.96	0.15	36.04	0.40
S35	3.69	0.32	7.09	0.16	36.40	0.70
S36	3.89	0.40	7.27	0.14	36.80	0.69

Table 3. The results showed that the content of polysaccharide in CR samples was in the range of 9.73%–19.97%, with the RSD less than 1.95%.

3.3. Content of sucrose, glucose and fructose

The content of sucrose, glucose, and fructose of CR samples treated with different processing methods was obtained and the results were listed in Table 4. It was observed that the content of sucrose in CR samples was in the range of 3.61%–17.51%, with the RSD less than 0.47%. The content of glucose in CR samples was in the range of 1.88%–7.27%, with the RSD less than 1.09%. The content of fructose in CR samples was in the range of 18.60%–36.80%, with the RSD less than 0.70%.

3.4. HPLC fingerprint analysis and similarity analysis

HPLC fingerprint analysis of CR samples was conducted and the similarity values were calculated. HPLC fingerprint of reference (R) and that of CR samples were shown in Fig. 1 and the similarity analysis results were listed in Table 5. HPLC fingerprint analysis showed that there were 23 common peaks in CR samples, among them, peak 20 was identified as lobetyolin by comparison with reference substance and set as reference peak S. The similarity analysis indicated that the similarity values were in the range of 0.948–0.996 compared with the reference fingerprint, which manifested that there were different degrees of difference on the chemical constituents of CR samples. Thus, different processing methods had an influence on the chemical constituents of CR samples. It was necessary to further analyze the detail influence of processing methods on the quality of CR.

3.5. Hierarchical clustering analysis and process condition analysis

Hierarchical clustering analysis combined with data analysis of lobetyolin, polysaccharide, sucrose, glucose, and fructose were conducted for the quality evaluation of CR treated with different processing methods. Moreover, different drying method, dryer-drying temperature, dryer-drying times, kneading and kneading times were analyzed to provide data reference for the reasonable processing in producing areas.

3.5.1. Drying method

To compare the influence on the chemical constituents of CR by four kinds of drying methods, including dryer-drying, sun-drying, shade-drying, and sulfur fumigation, the corresponding samples were selected. According to the traditional processing methods in Min County, dryer-drying was processed for five times at 40 °C, sun-drying was processed under direct sunlight, shade-drying was processed without direct sunlight, and sulfur fumigation was processed after sun-drying. And all these processing methods were processed without kneading. Thus, samples T40-DD5-NK (S4-6), SND-NK (S16-18), SDD-NK (S28-30), and SND-SF-NK (S34-36) were selected to represent the samples of four kinds of drying methods, respectively.

The relative peak areas of the common peaks from HPLC fingerprints were used to conduct hierarchical cluster analysis and the results were shown in Fig. 2A. The samples could be divided into two groups. Samples SND-SF-NK (S34-36) were classified as one group, and the other samples were classified as another group.

The content of various components of the samples was analyzed and the results were shown in Fig. 3A–Fig. 7A, respectively. The content of lobetyolin was as follow: SDD-NK > SND-NK > T40-DD5-NK > SND-SF-NK ($P < 0.05$), the content of polysaccharide was as follow: SND-NK > T40-DD5-NK and SND-SF-NK > SDD-NK

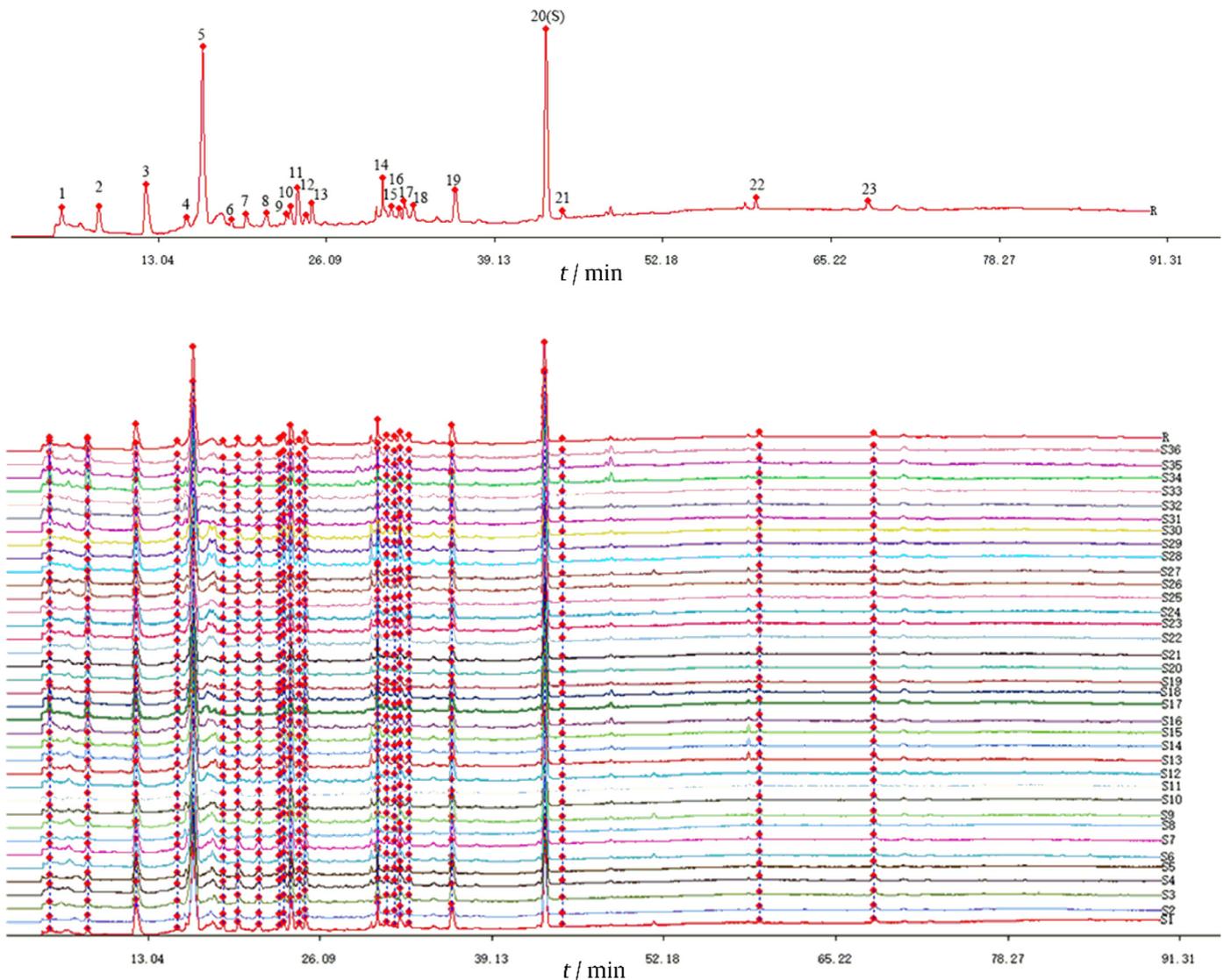


Fig. 1. HPLC fingerprints of reference (R) and *Codonopsis Radix* samples.

Table 5
Similarity analysis based on HPLC fingerprints of *Codonopsis Radix* samples.

No.	S reference	No.	S reference	No.	S reference
S1	0.978	S13	0.996	S25	0.970
S2	0.983	S14	0.995	S26	0.990
S3	0.983	S15	0.995	S27	0.987
S4	0.979	S16	0.992	S28	0.991
S5	0.983	S17	0.992	S29	0.991
S6	0.952	S18	0.992	S30	0.991
S7	0.985	S19	0.996	S31	0.989
S8	0.986	S20	0.996	S32	0.992
S9	0.984	S21	0.996	S33	0.948
S10	0.992	S22	0.972	S34	0.983
S11	0.994	S23	0.978	S35	0.971
S12	0.992	S24	0.983	S36	0.988

($P < 0.05$), the content of sucrose was as follow: SDD-NK > T40-DD5-NK > SND-NK > SND-SF-NK ($P < 0.05$), the content of glucose was as follow: SND-SF-NK > SND-NK > SDD-NK > T40-DD5-NK ($P < 0.05$), and the content of fructose was as follow: SND-SF-NK > SND-NK > T40-DD5-NK > SDD-NK ($P < 0.05$).

The results indicated that samples processed with sulfur fumigation were different from those processed with the other three

drying methods. Although the content of glucose and fructose was higher in samples treated with sulfur fumigation, the content of lobetyolin and sucrose was decreased significantly. Thus, sulfur fumigation should be avoided in the actual process except for the special requirement for monosaccharide content. For the other three drying methods, the content of lobetyolin and sucrose in samples treat with shade-drying was higher. And the content of polysaccharide, glucose, and fructose in samples treated with sun-drying was higher. Therefore, shade-drying or sun-drying were recommended for the processing of CR according to the requirement of different components, which was in accordance with the traditional processing method of CR.

3.5.2. Dryer-drying temperature

To compare the influence on the chemical constituents of CR by three kinds of dryer-drying temperatures, including 30 °C, 40 °C, and 50 °C, the corresponding samples were selected. All the samples were processed by dryer-drying for five times without kneading. Thus, samples T30-DD5-NK (S1-3), T40-DD5-NK (S4-6), and T50-DD5-NK (S7-9) were selected to compare the difference of their chemical constituents.

The relative peak areas of the common peaks from HPLC fingerprints were used to conduct hierarchical cluster analysis and

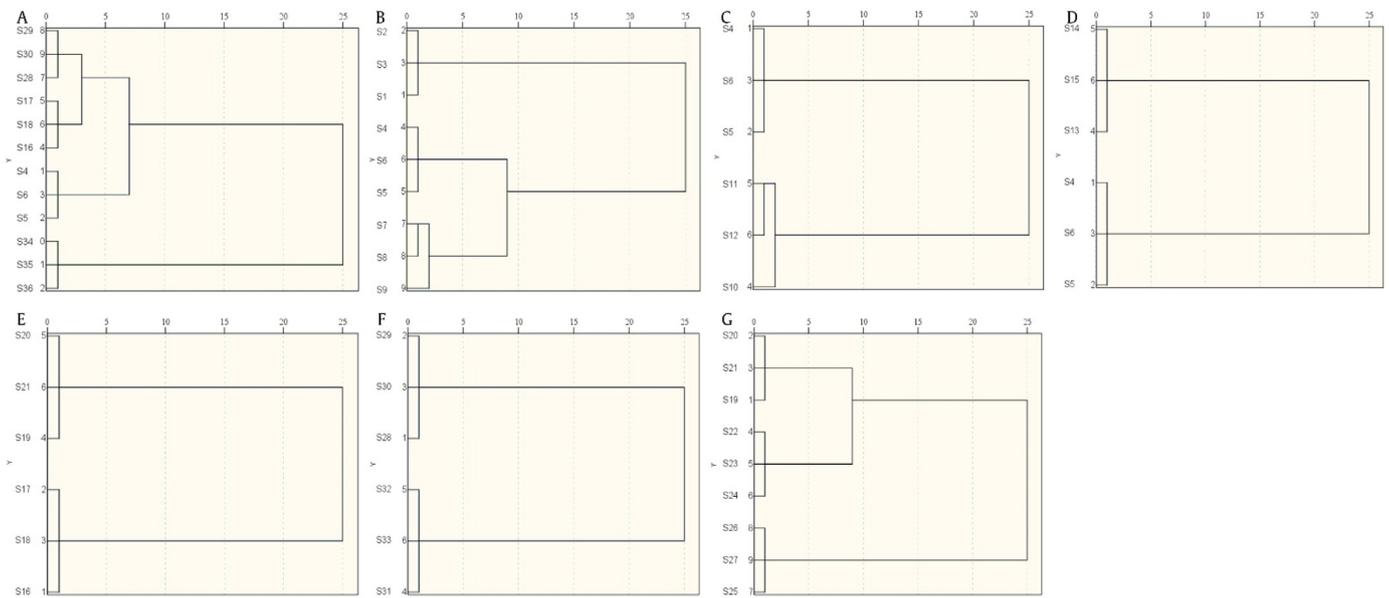


Fig. 2. Hierarchical cluster analysis of *Codonopsis Radix* samples treated with different processing methods.

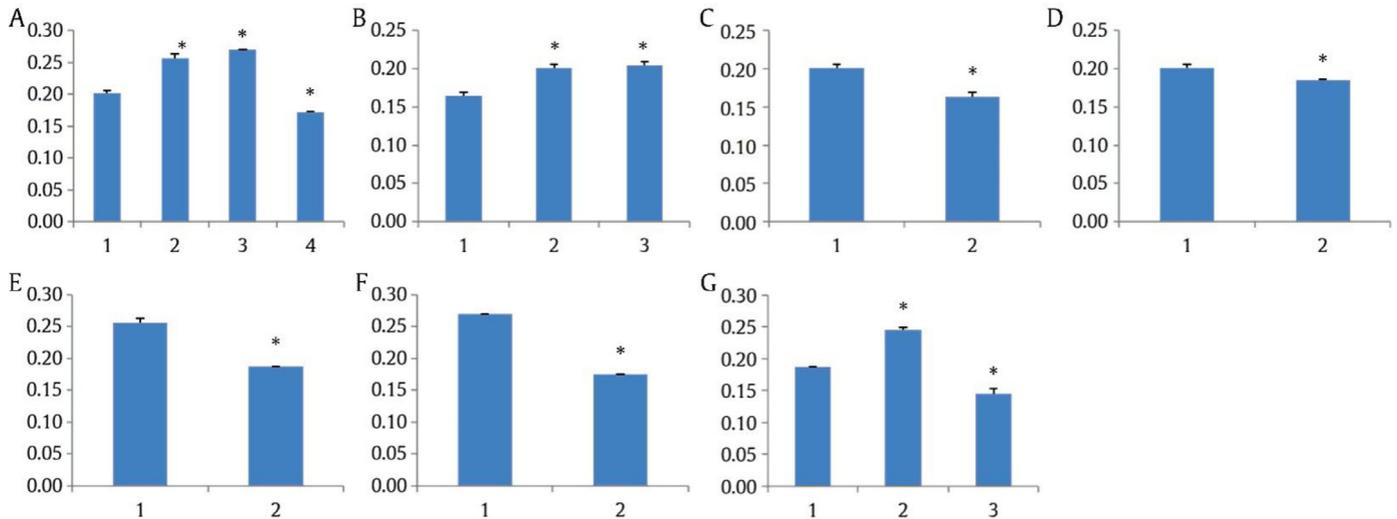


Fig. 3. Content of lobetyolin in *Codonopsis Radix* samples treated with different processing methods (mean \pm SD, $n = 3$) (* $P < 0.05$ vs group 1, same as below).

Note: (A) 1: T40-DD5-NK; 2: SND-NK; 3: SDD-NK; 4: SND-SF-NK; (B) 1: T30-DD5-NK; 2: T40-DD5-NK; 3: T50-DD5-NK; (C) 1: T40-DD5-NK; 2: T40-DD1-NK; (D) 1: T40-DD5-NK; 2: T40-DD5-K1; (E) 1: SND-NK; 2: SND-K1; (F) 1: SDD-NK; 2: SDD-K1; (G) 1: SND-K1; 2: SND-K3; 3: SND-K5, same as below.

the results were shown in Fig. 2B. The samples could be divided into two groups. Samples T30-DD5-NK (S1-3) were classified as one group, and the other samples were classified as another group.

The content of various components of the samples was analyzed and the results were shown in Fig. 3B–Fig. 7B, respectively. For the content of lobetyolin, T40-DD5-NK and T50-DD5-NK $>$ T30-DD5-NK ($P < 0.05$). For the content of polysaccharide, the difference is insignificant ($P > 0.05$). For the content of sucrose, T40-DD5-NK and T50-DD5-NK $>$ T30-DD5-NK ($P < 0.05$). For the content of glucose, T40-DD5-NK and T50-DD5-NK $>$ T30-DD5-NK ($P < 0.05$). For the content of fructose, T40-DD5-NK and T50-DD5-NK $>$ T30-DD5-NK ($P < 0.05$).

The results illustrated that samples treated with dryer-drying at 30 °C were different from those treated with dryer-drying at 40 °C and 50 °C. And the content of lobetyolin, polysaccharide, sucrose, glucose, and fructose was all higher in samples processed with dryer-drying at 40 °C and 50 °C. Therefore, it was recommended to choose 40 °C–50 °C as the appropriate drying temperature in actual process.

3.5.3. Dryer-drying times

To compare the influence of dryer-drying times on the chemical constituents of CR, samples for drying once and five times were selected. All the samples were processed by dryer-drying at 40 °C without kneading. Thus, samples T40-DD5-NK (S4-6) and T40-DD1-NK (S10-12) were selected to compare the difference of their chemical constituents.

The relative peak areas of the common peaks from HPLC fingerprints were used to conduct hierarchical cluster analysis and the results were shown in Fig. 2C. The samples could be divided into two groups. Samples T40-DD5-NK (S4-6) were classified as one group. And samples T40-DD1-NK (S10-12) were classified as another group.

The content of various components of the samples were analyzed and the results were shown in Fig. 3C–Fig. 7C, respectively. For the content of lobetyolin, T40-DD5-NK $>$ T40-DD1-NK ($P < 0.05$). For the content of polysaccharide, glucose, and fructose, T40-DD1-NK $>$ T40-DD5-NK ($P < 0.05$). The difference for content of sucrose was insignificant ($P > 0.05$).

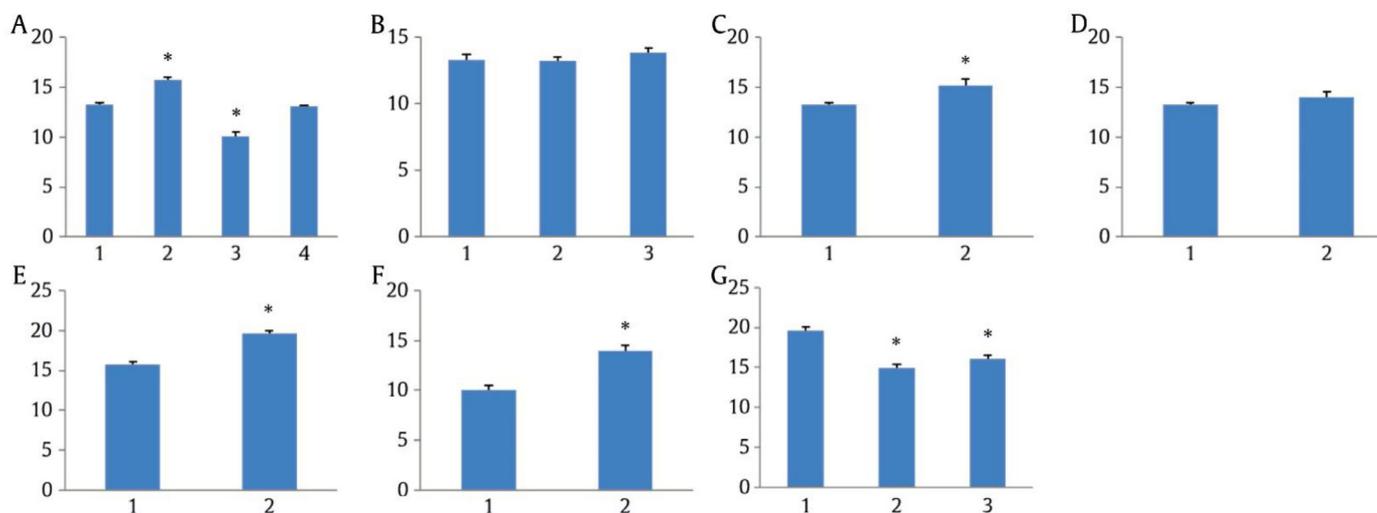


Fig. 4. Content of polysaccharide in *Codonopsis Radix* samples treated with different processing methods (mean ± SD, n = 3).

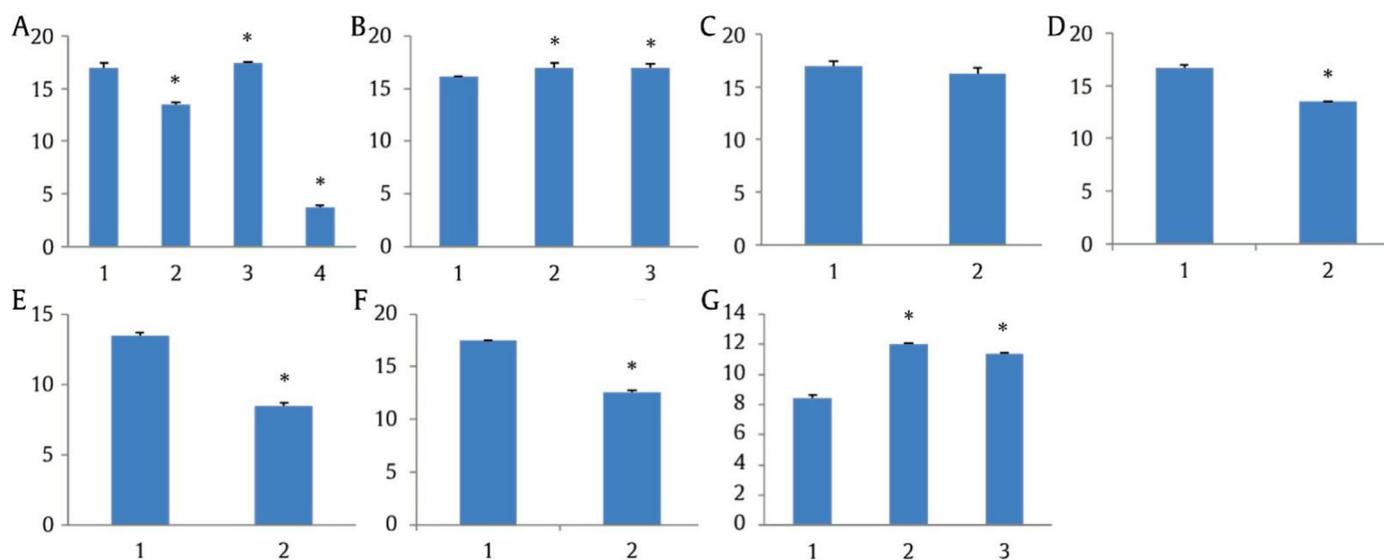


Fig. 5. Content of sucrose in *Codonopsis Radix* samples treated with different processing methods (mean ± SD, n = 3).

The results revealed that samples treated with different drying times could be distinguished well. The content of lobetyolin and sucrose was higher in samples treated with dryer-drying for five times, while the content of polysaccharide, glucose, and fructose was higher in samples treated with dryer-drying for once. Therefore, it was recommended to choose appropriate drying times according to the requirement of different components.

3.5.4. Kneading

To compare the influence of kneading on the chemical constituents of CR, the corresponding samples were selected. Samples processed with dryer-drying, sun-drying, and shade-drying were all designed to be processed with or without kneading. Thus, for dryer-drying, samples T40-DD5-NK (S4-6) and T40-DD5-K1 (S13-15) were selected. For sun-drying, samples SND-NK (S16-18) and SND-K1 (S19-21) were selected. For shade-drying, samples SDD-NK (S28-30) and SDD-K1 (S31-33) were selected.

The relative peak areas of the common peaks from HPLC fingerprints were used to conduct hierarchical cluster analysis and the results were shown in Fig. 2D–2F, respectively. The dryer-drying samples could be divided into two groups: Samples T40-DD5-NK (S4-6) were classified as one group, and samples T40-DD5-K1 (S13-

15) were classified as another group. The sun-drying samples could be divided into two groups: Samples SND-NK (S16-18) were classified as one group, and samples SND-K1 (S19-21) were classified as another group. The shade-drying samples were also divided into two groups. Samples SDD-NK (S28-30) were classified as one group, and samples SDD-K1 (S31-33) were classified as another group.

The content of various components of the dryer-drying samples, sun-drying samples, and shade-drying samples were analyzed and the results were shown in Fig. 3D–3F, Fig. 4D–4F, Fig. 5D–5F, Fig. 6D–6F, and Fig. 7D–7F, respectively. In regard to the dryer-drying samples, for the content of lobetyolin and sucrose, T40-DD5-NK > T40-DD5-K1 ($P < 0.05$). For the content of polysaccharide, T40-DD5-K1 > T40-DD5-NK ($P > 0.05$). For the content of glucose and fructose, T40-DD5-K1 > T40-DD5-NK ($P < 0.05$). In regard to the sun-drying samples, for the content of lobetyolin and sucrose, SND-NK > SND-K1 ($P < 0.05$). For the content of polysaccharide, glucose and fructose, SND-K1 > SND-NK ($P < 0.05$). In regard to the shade-drying samples, for the content of lobetyolin and sucrose, SDD-NK > SDD-K1 ($P < 0.05$). For the content of polysaccharide, glucose, and fructose, SDD-K1 > SDD-NK ($P < 0.05$).

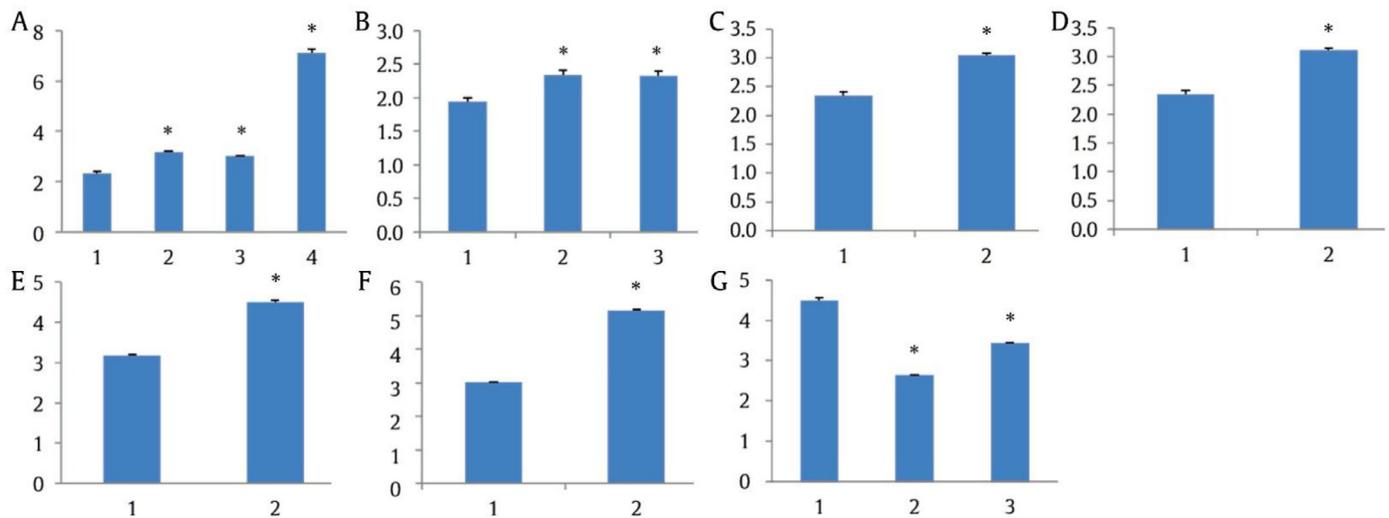


Fig. 6. Content of glucose in *Codonopsis Radix* samples treated with different processing methods (mean ± SD, n = 3).

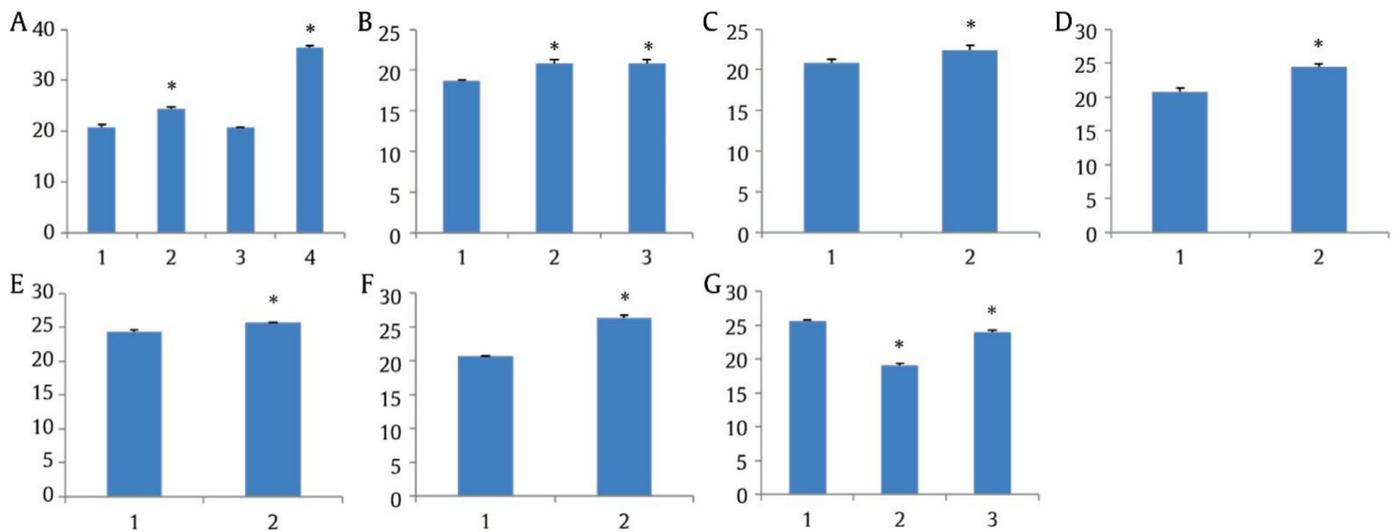


Fig. 7. Content of fructose in *Codonopsis Radix* samples treated with different processing methods (mean ± SD, n = 3).

Based on the above analysis, samples processed with or without kneading could be distinguished well. Big differences in samples treated with or without kneading were observed. On the whole, for different samples treated with dryer-drying, sun-drying or shade-drying, the content of lobetyolin and sucrose in samples treated without kneading was higher. The content of polysaccharide, glucose, and fructose in samples treated with kneading was also higher. Therefore, during the process of kneading, the content of polysaccharide, glucose, and fructose was increased significantly, which was consistent with the results in literature (He et al., 2006).

3.5.5. Kneading times

To compare the influence of kneading times on the chemical constituents of CR, samples with kneading once, three times, and five times were selected. All the samples were processed by sun-drying. Thus, samples SND-K1 (S19-21), SND-K3 (S22-24), and SND-K5 (S25-27) were selected to compare the difference of their chemical constituents.

The relative peak areas of the common peaks from HPLC fingerprints were used to conduct hierarchical cluster analysis and the results were shown in Fig. 2G. The samples could be divided

into two groups. Samples SND-K5 (S25-27) were classified as one group, and the other samples were classified as another group.

The content of various components of the samples was analyzed and the results were shown in Figs. 3G-7G, respectively. For the content of lobetyolin, SND-K3 > SND-K1 > SND-K5 ($P < 0.05$). For the content of polysaccharide, SND-K1 > SND-K5 > SND-K3 ($P < 0.05$). For the content of sucrose, SND-K3 > SND-K5 > SND-K1 ($P < 0.05$). For the content of glucose, SND-K1 > SND-K5 > SND-K3 ($P < 0.05$). For the content of fructose, SND-K1 > SND-K5 > SND-K3 ($P < 0.05$).

The results manifested that samples treated with different kneading times could be distinguished well. The content of lobetyolin and sucrose in samples treated with kneading for three times was higher. And the content of polysaccharide, glucose, and fructose in samples treated with kneading for once was higher. Thus, it was appropriate to choose the corresponding kneading times based on different chemical components.

3.6. Discussion

It could be concluded from the results that the effects of different processing methods on the content of lobetyolin and sucrose tended to be consistent, while those of polysaccharide,

glucose, and fructose tended to be consistent. Lobetyolin was composed of an aglycone and a glucose (Gao et al., 2018). If it was damaged and decomposed during processing, the content of dissociative glucose would increase and vice versa. Thus, the change in the content of lobetyolin was opposite to that of glucose. Polysaccharide was polymerized with several monosaccharides and their derivatives. After processed with different methods, it could be hydrolyzed to oligosaccharides such as sucrose, and could be further hydrolyzed to monosaccharides (Gao et al., 2018). In the present study, the change in content of polysaccharide was opposite to that of sucrose. The possible cause was that most polysaccharide was hydrolyzed to disaccharides, and had not been hydrolyzed to monosaccharide during processing. Sucrose was a disaccharide and could be hydrolyzed to glucose and fructose. Therefore, the change in the content of sucrose was opposite to those of glucose and fructose. Actually, there was a complicated process in the change of different components of CR, the result was a dynamic balance of various transformation with the influence of different processing methods. The mechanism of transformation would be exploited in further studies.

4. Conclusions

An analytical strategy that combined quantitative analysis and HPLC fingerprint with chemometrics was developed for the quality evaluation of CR treated with 12 kinds of different processing methods, including dryer-drying, sun-drying, shade-drying, sulfur fumigation, and kneading, etc. The results illustrated that the holistic chemical constituents of CR samples processed with different methods varied greatly, which demonstrated that different processing methods had a big impact on the quality of CR. According to the results, sun-drying or shade-drying was recommended for the processing of CR, and sulfur fumigation should be avoided except for special circumstances. And dryer-drying was recommended to meet the requirement of enterprises for large scale processing of CR. According to the content of lobetyolin and sucrose, shade-drying was advised. If dryer-drying was needed, it was advised to dry at 40 °C–50 °C for five times, and kneading should be avoided. In producing areas used to kneading, it was suggested to knead once. On the other hand, based on the content of polysaccharide, glucose, and fructose, sun-drying was recommended. If dryer-drying was required, it was suggested to dry at 40 °C–50 °C for once. Kneading was suggested in this case, and it was suggested to knead for three times. All these results had considerable value for the reasonable processing of CR and could lay a foundation for the rational utilization of CR.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

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