

Analysis of Gingerol Content Difference in Dried Ginger, Processed Ginger and Ginger Based on RP-HPLC

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Abstract: Objective: To establish a method for determination of 6- gingerol, 8- gingerol and 10- gingerol in dried ginger, processed ginger and ginger. Methods: Gingerol, 6-gingerol, 10-gingerol, 6-gingerol and 8-gingerol were determined by RP-HPLC. Results: the contents of 6-gingerol, 8-gingerol and 10-gingerol were 7.06%, 1.88% and 2.52% respectively. The linear correlation coefficient r of the standard curve was 0.9997, and the RSD of peak area was 0.43% after 5 consecutive injections; The maximum relative deviation of three detection results of the same sample is 4.7%. Conclusion: RP-HPLC method for the determination of gingerol in dried ginger, ginger and processed ginger is simple and accurate.

1. Introduction

Dried ginger is the dry rhizome of Zingiberaceae plant Ginger, which has the effects of warming the middle-warmer, dispelling cold, restoring yang, dredging the pulse, warming the lung and resolving drinks. Researchers believe that gingerols content in gingerbread is very low. After processing ginger, its composition changes in different degrees, which can lead to changes in drug properties and functional indications. Ginger is warm and main, and is good at relieving exterior syndrome and dispelling cold, and stopping vomiting; Dried in the sun to obtain dried ginger, which is pungent and hot in nature, is the main guard and is good at warming and rejuvenating; Among them, 6- gingerol is the main component of gingerols, which has a wide range of pharmacological effects such as inhibiting platelet aggregation, scavenging free radicals, resisting inflammation, relieving pain, enhancing myocardial contractility and increasing heart rate [1]. In addition, the chemical constituents of ginger oil with skin, ginger oil without skin and ginger skin oil extracted by CO₂ supercritical fluid extraction were determined and analyzed by GC-MS, and a total of compounds were identified, with different contents in different parts. Modern pharmacological experiments show that gingerol has many functions such as antioxidation, anti-inflammation, protecting liver and promoting bile flow, inhibiting central nervous system, resisting tumor, etc. Therefore, it is of great significance to determine the contents of gingerols in dried ginger.

Different processed products can be made under different processing conditions, and after processing, the components in ginger will also change to some extent, thus changing its therapeutic function [3]. Accurate determination of the components is an important means to test the quality of ginger and processed products. At present, the detection of gingerol is mostly by RP-HPLC [4-5]. In this study, the content of 6- gingerol in dried ginger, ginger and processed ginger can be quickly determined by RP-HPLC. The results are accurate and reliable, and can be used as a useful standard for quality control and evaluation of medicinal ginger together with the determination of volatile oil.

2. Materials and Methods

2.1 Instruments and reagents

In this study, Agilent1100 high performance liquid chromatograph, matched chromatographic workstation, diode array monitor, electronic balance and ultrasonic cleaner were used. Dried ginger

and processed ginger are purchased from commercial pharmacies, while ginger is purchased from supermarkets. 6- gingerol reference substance (self-made, managed test and spectral analysis, the content purity measured by RP-HPLC area normalization method is 99.0%); Methanol is chromatographically pure, water is distilled water, and other reagents are analytically pure.

2.2 Chromatographic conditions

Chromatographic column: Park Jung Su Hypersil ODS 2(250 mm×4.6 mm, 5μ m); Mobile phase: acetonitrile-water (74: 38); The flow rate was 1 .5mL·min⁻¹; Detection wavelength: 240nm; Column temperature: 23°C; Sample size: 15 μL. Under this chromatographic condition, the chromatographic peaks of 6- gingerol, 8- gingerol and 10- gingerol were completely separated from other components in the sample.

2.3 Preparation of samples

Grinding dried ginger (water content 15.2%), and sieving with No.3 sieve; Weigh 1.5 g ginger powder into a 100 m L volumetric flask and add methanol to the scale; Ultrasonic treatment at 45°C (frequency 10 kHz, power 100 W) for 35 min; Concentrate into sample No.1 with mass concentration of 25 mg/m L; Samples No.2 and No.3 were prepared in the same way.

2.4 Peel dried ginger

Take appropriate amount of peeled dried ginger and dried ginger powder with skin (sieve No.2), soak overnight, distill to obtain volatile oil according to volatile oil determination method A in Chinese Pharmacopoeia, read and record the results, collect the volatile oil and store it at low temperature for later use. Among them, the determination results of total volatile oil of peeled dried ginger and peeled dried ginger are shown in Table 1 below:

Table 1. Determination results of total volatile oil from peeled dried ginger and dried ginger with skin

Sample	Peel dried ginger (m L/50)	Dried ginger with skin (m L/50)
1	1.71	1.82
2	1.62	1.80
3	0.77	1.83

2.5 Selection of determination conditions

Taking absolute ethyl alcohol solvent as blank, the absolute ethyl alcohol solution of vanillin and the absolute ethyl alcohol extract of *Zingiber officinale* with skin were spectrally scanned in the wavelength range of 100-400nm, and the maximum absorption wavelength was investigated. The results show that both the test solution and the reference solution have the maximum absorption wavelength at 258nm, so 258nm is determined as the determination wavelength. The absorption curve is shown in fig. 1.

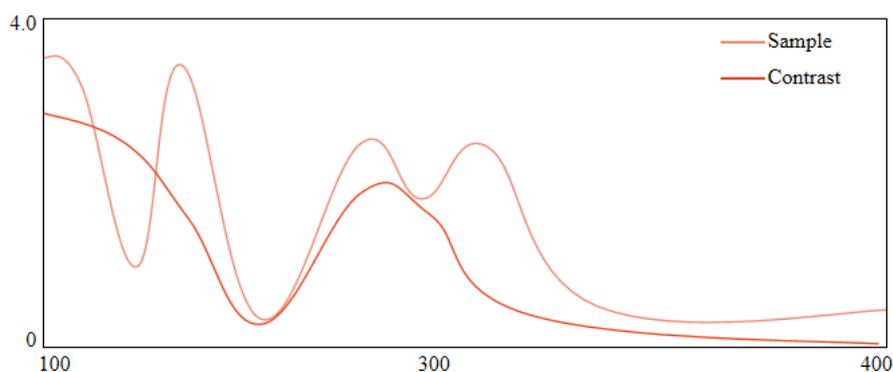


Figure 1. Absorption spectrum of absolute ethyl alcohol

3. Result

3.1 Investigation of linear relationship

Serial reference solutions with different concentrations were continuously injected for determination ($n = 3$), and the standard curve was drawn with the concentration of reference solutions as abscissa (X) and the peak area value as ordinate (Y), and the regression equation was calculated (Table 2). The results showed that the linear range of 6- gingerol was 0.7021-3.7216/ μ g, 8-gingerol was 0.3077-1.2965 μ g, and 10-gingerol was 0.3628-1.8857 μ g.

Table 2. Linear relationship results of three components

Component	linearity range/ μ g	Regression equation	r
6-gingerol	0.7021-3.7216	$Y=8.0217X+7.82$	0.9993
8-gingerol	0.3077-1.2965	$Y=14.2401X+5.07$	0.9992
10-gingerol	0.3628-1.8857	$Y=13.7962X+9.17$	0.9997

3.2 Precision test

Three kinds of gingerol standard solutions with low, medium and high concentrations were taken respectively, and the intra-day precision of the method was investigated by continuous sampling for three times. Results: The RSD of peak areas of 6- gingerol, 8- gingerol and 10- gingerol were 0.15%, 0.22% and 0.32% respectively ($n = 10$). Then, samples were injected continuously for 5 days, and the inter-day precision of the method was investigated. Results: The RSD of peak areas of 6- gingerol, 8- gingerol and 10- gingerol were 0.26%, 0.33% and 0.43% respectively ($n = 10$), which indicated that the precision of the method was good.

3.3 Stability investigation

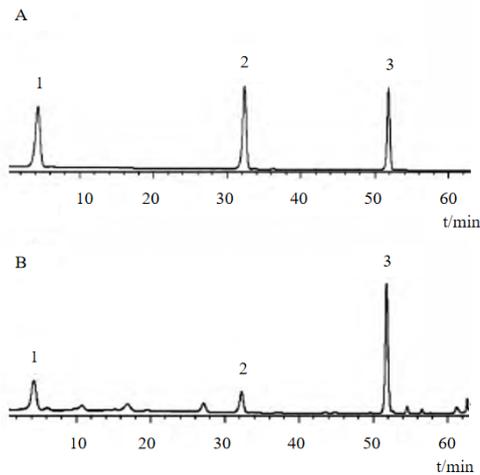
Take about 0.15g of dried ginger powder, weigh it accurately, prepare sample solution, and determine the content of 6- gingerol at 0, 0.05, 1.5, 2.5, 4.5, 8.5, 10.5 and 24 hours respectively. The calculated result of stability RSD is 1.71%, which shows that the sample solution is stable within 24 hours.

3.4 Recovery rate test

Accurately weigh 6 samples of the same batch with known content, accurately add 6- gingerol reference substance respectively, and prepare them according to the preparation method of test solution. The average recovery rate is 96.32%, and the SRD is 0.94% ($n=8$).

3.5 Sample content determination

Samples of ginger, dried ginger and processed ginger were taken respectively, and the test solution was prepared according to the above method. Accurately suck 5 μ l of mixed reference solution and test solution, inject them into the high performance liquid chromatograph, measure them according to the above chromatographic conditions, and record the chromatogram (Figure 2). At the same time, the contents of each component in the three samples were calculated, and the results are shown in Table 3.



A, mixing reference substances; B, sample; 1,6-gingerol; 2,8-gingerol; 3,10-gingerol

Figure 2. RP-HPLC diagram of mixed reference substance and sample

Table 3. Determination results of five gingerols in ginger and its processed products ($\text{mg} \cdot \text{g}^{-1}$, $n=5$)

Sample	Zingerone	6-Gingerol	8-Gingerol	6-Shogaol	10-Gingerol
Ginger	—	0.46	0.17	0.02	0.21
Rhizoma Zingiberis	—	7.06	1.88	1.05	2.52
Baked ginger	0.11	7.32	1.36	1.43	2.31

3.6 Drawing of standard curve

Take 5 standard substances with mass concentrations of 0.04, 0.06, 0.08, 0.10 and 0.12 $\text{mg} \cdot \text{mL}^{-1}$, each with a sample volume of 10 μl , repeat for 3 times, and take the average peak area. See table 4 for concentration of standard substance and average area of chromatographic peak, and fig. 3 for NVA standard curve.

Table 4. Concentration of standard substance and average area of chromatographic peak

Serial number	Mass concentration of standard substance ($\text{mg} \cdot \text{mL}^{-1}$)	Average chromatographic peak area
1	0.04	305
2	0.06	555
3	0.08	715
4	0.10	850
5	0.12	694

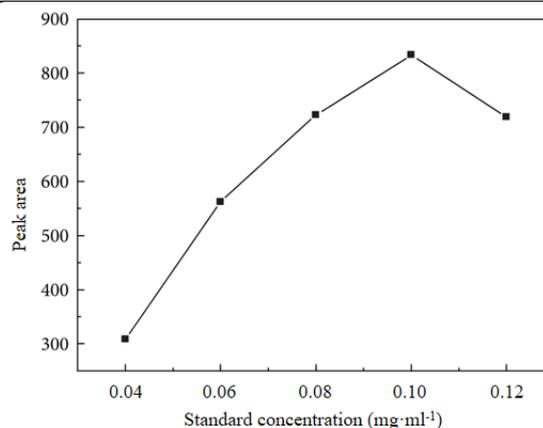


Figure 3. NVA standard curve

4. Discussion

In this paper, the peeling technology of dried ginger was studied by TLC identification, GC-MS detection and total phenol content. It can be seen that thin layer qualitative analysis shows that there is no obvious difference between the chemical components of alcohol-soluble parts and water-soluble parts. We designed RP-HPLC internal standard method to determine its content, but methanol-water as mobile phase can not obtain baseline separation, while acetonitrile-water isocratic elution can achieve better separation effect, but the analysis time is too long and there is tailing phenomenon, so acetonitrile-water gradient elution is adopted, and a proper amount of acetic acid is added in water to improve the peak shape [6]. In addition, by comparing the separation conditions of methanol-water system and acetonitrile-water system as mobile phase, it is found that the separation degree of acetonitrile-water system is satisfactory. At the same time, the choice of mobile phase was optimized when establishing the detection method of high performance liquid chromatography. From the determination results, gingerol appears when processing ginger, and the content of gingerol is relatively small, and the content of gingerol increases in ginger charcoal, which indicates that gingerol can only be obtained by processing ginger or processed products of ginger charcoal.

Ultrasonic extraction with methanol can avoid the influence of high temperature on 6- gingerol, and methanol has good solubility. The established RP-HPLC-DAD method for determination of 6- gingerol in dried ginger, ginger and processed ginger is rapid, simple, economical and accurate. In addition, 50%, 75% and pure methanol were used as extraction solvents respectively, and it was found that pure methanol had higher extraction efficiency for these components. The method has the advantages of simple sample pretreatment, simple operation, good reproducibility, and good repeatability, which overcomes the disadvantages of poor specificity and low accuracy of colorimetry, and also solves the disadvantages of RP-HPLC external standard reference substance 6- gingerol, which is difficult to store and unfavorable to operation [7]. Compared with processed ginger, dried ginger does not contain gingerol, but other chemical components are slightly higher than processed ginger. However, except for the higher contents of gingerol and 6- gingerol, the contents of other compounds in ginger charcoal decreased obviously. At the same time, it can be found from the research results that the content of 6- gingerol does not decrease but increases with the deepening of processing degree, and its reaction mechanism needs further study.

At present, the chemical constituents and pharmacological effects of dried ginger, processed ginger and ginger charcoal have made some progress. However, there is no in-depth report on the change trend of chemical components in the processing of the three traditional Chinese medicines, and the correlation between the effective components in the medicinal materials and the efficacy [8]. In this experiment, 6-gingerol, 8-gingerol and 10-gingerol were used as indexes to study the dried ginger slices prepared by different processing methods of the same batch of ginger, and the processed ginger and ginger charcoal for the first time. The results showed that the contents of three gingerols in dried ginger tablets prepared by different processing methods from the same batch of ginger were different. At the same time, this experiment compared methanol-water and acetonitrile-water mobile phase systems, and analyzed the retention time and separation degree. The results showed that the separation effect of acetonitrile-water system was better, and the chromatographic peaks of three gingerols and their adjacent chromatographic peaks could achieve baseline separation. It can be used for the quality control of other preparations and products containing 6- gingerol, and provides a beneficial supplement for the quality evaluation of related products in Chinese Pharmacopoeia. The average content of 6- gingerol in processed ginger is higher than that in dried ginger. Whether this difference is directly related to the origin, harvest season and processing of medicinal materials needs further study.

Comparing the characteristic wavelengths of 240 nm and 258nm, it is found that 240 nm is at the shoulder of the absorption curve and is vulnerable to interference; Choosing 258 nm as the detection wavelength, the chromatogram has good peak shape and high sensitivity. High-purity synthetic vanillin nonanoamide has stable properties and low price, which is similar to 6- gingerol in molecular weight and has the same chromophore. As an external standard, qualitative and

quantitative analysis of gingerol can solve the problem that the standard reference substance of gingerol is difficult to obtain. Compared with dried ginger, there are five components in the volatile oil of processed ginger. The contents of three gingerols in dried ginger slices processed while fresh were higher than those in traditional processing. Further investigation of processed products showed that the contents of three gingerols were lower than those in processed ginger slices processed by traditional processing. According to the determination results, there are great differences in chemical composition between ginger and different processed products, which determines that their pharmaceutical effects are different. When using ginger as raw material for pharmaceutical materials, it is necessary to consider the influence and changes of different processing methods on the components.

5. Summary

The drying process of dried ginger slices is different between the two processing methods. The traditional processing method needs to repeat the drying process twice, and at the same time, one more water treatment process, which may be the reason why the content of three gingerols in dried ginger slices processed by fresh-cut processing method is higher than that of dried ginger slices processed by traditional processing method. RP-HPLC can accurately determine the gingerol content in ginger and processed products, which provides a corresponding analysis method for the determination of ginger and processed products, and the composition content of gingerol also changes during processing. The changes of the contents of five gingerols before and after processing ginger were preliminarily discussed. Meanwhile, the method is simple, rapid, high in precision and good in reproducibility. This method can provide a basis for the quality control and evaluation of medicinal ginger and its related products, and provide a useful supplement for the content determination method in Pharmacopoeia.

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