Effects of different processing methods on the quality of Polygonatum cyrtonema Hua

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Abstract

Background: In this study, we explored the effects of different processing methods on the quality of Polygonatum cyrtonema Hua (PCP), and the role of Huangjiu in the processing procedure. Methods: The sensory characteristics of the crude product, steamed product, and wine-processed product of PCP were described. The colorimeter was used to analyze the chromatic values of three different processed products on PCP. At the same time, the contents of the water extract and alcohol extract were measured separately. The content of three different processing Polygonatum Polysaccharide (PCP) was determined using 0.2% anthrone-sulfuric acid. The correlation difference between the chromatic values and chemical composition of different PCP products was analyzed using various analytical methods. Results: The surface colors gradually deepened, the sweetness increased, the viscosity strengthened, and the tongue-numbing sensation disappeared after PCP processing. The contents of extract and L* gradually decreased from the crude to the steamed to the wine-processed product, consistent with the pattern of surface color alteration. While, E*a* gradually increased. The content of PCP was crude product > wine-processed product > steamed product. The results of multivariate statistical analysis showed that the samples processed for crude, steamed, and wine-processed products were clustered into three classes. The correlation analysis showed that L* and E*a* were highly significant positively correlated with the content of PCP, and a was significantly negatively correlated with the content of PCP. Conclusion: The results showed that the wine-processed product had the best quality. The internal quality of the PCP was correlated with its characteristics and chromatic value. In this study, we investigated the internal and external quality of three different products of PCP in order to provide a reference for further research on the impact of different processing methods on PCP quality, the standardization of PCP processing, and the role of Huangjiu in the processing of PCP.

Keywords: Polygonatum cyrtonema Hua; different products; chemical composition; chromatic value; characteristics
Introduction

PC has the effect of tonifying qi (Qi is the fundamental substance of the human body with the ability to convert and transmit), nourishing yin (Yin nourishes the internal organs by supporting the essence, blood, and fluids, thereby promoting the full development and quality of the human body), strengthening the spleen, moistening the lungs, and benefiting the kidneys. It is a traditional medicinal and dietary tonic in Chinese medicine [1]. According to the "Shi Liao Ben Cao" and other ancient literature, the crude product of PC is pungent and can cause throat irritation [2]. The purpose of processing PC is to eliminate the irritation caused by crude product to the throat. The second reason is to make it easy to store. And the third reason is to enhance its tonic effect and improve its clinical efficacy [3-5]. So the processed PC is primarily used in clinical practice. The processing methods of PC have a long history. According to ancient literature, there are more than 20 different processing methods, including single steaming, repeat steaming, nine-steam-nine-bask processing with Huangjiu, processing with black bean juice, etc [6, 7]. The Pharmacopoeia of the People’s Republic of China (ChP) 2020 Edition only includes the wine-processed product, and the clinical application primarily focuses on the wine-processed product [8].

The chemical composition of PC will change to different degrees after steaming or wine-steaming, which will have a certain impact on the clinical pharmacological effect of PC [9-11]. PCP is one of the main biologically active ingredients with various pharmacological effects, such as lowering blood lipid and blood glucose, anti-tumor, treatment of senile dementia, antioxidant effects, enhancement of the immune system [12-14]. In the ChP of 2020, PCP is also specified as the sole chemical component to be assessed in the quality inspection of PC [8].

The theory of 'Identify the shape and discuss the quality' emphasizes that color is crucial for recognizing the characteristics of the physical properties of different chemical compositions in Chinese medicine. 'If the color changes, the taste will change, and if the taste changes, the quality will change’ [15]. The variations in color to a certain extent reflect changes in endogenous substances of the medicine. This indicates that the appearance characteristics is an external manifestation of the intrinsic quality of Chinese medicine. The previous studies have shown that the apparent color characteristics are closely related to internal quality of PC. The chromatic value is used to quantitatively analyze the apparent color of Chinese medicine, which objectively quantifies the subjective color. So its application in the field of Chinese medicine connotations is expanding.

At present, most research is focused on the differences in intrinsic and extrinsic quality among crude, steamed, wine-processed products. Zhuahu Y et al. measured the chromatic value and contents of relevant chemical composition of wine-processed PC at different time points, then analyzed the correlation of both [16]. Finding a significant relationship between the $E_0^a$ value and the contents of relevant chemical composition. Tianmei W et al. determined the contents of extracts, PCP and other substances, then overall rating were made by subordinate function method [17]. During the processing with decoction pieces as the test material, the ranking of the overall score is processing with wine > nine-steam-nine-bask processing > steamed. Lin C et al. measured the taste value and PCP content of the raw products, one-steam-one-bask to nine-steam-nine-bask of PC [18]. They found that the taste value and the content of PCP gradually decreased with an increase in processing times. Lack of systematic comparative studies on the quality differences among crude products, steamed products and wine-processed products.

Therefore, this study explores the quality differences in processing PC by using appearance characteristics, chromatic value, water extract, alcohol extract, PCP as indicators. The study aimed to compare the effects of different processing methods on the quality of PC and to preliminarily investigate the role of Huangjiu in processing. To provide a reference for standardizing the processing on a PC.

Materials and methods

Reagents

Polygonatum cyronema Hua (Lot. 2020042401) was purchased from Xinshao Nanmo Biotechnology Co. Ltd., China. Huangjiu (Lot. 20180812) was obtained from Huishan Shaoxing wine Co. Ltd., China. Asphodele (Lot. 4290021) was purchased from Beijing Suolaibao Biotechnology Co. Ltd., China. D (+) anhydrous glucose (Lot. S22J12H137237) was obtained from Shanghai Yuanye Biotechnology Co. Ltd., China. Anhydrous ethanol (Lot. 20230301003) was purchased from Tianjin Zhiyuan Chemical Reagent Co. Ltd., China.

Instruments

756PC UV-Visible Spectrophotometer (Shanghai Sunyu Hengfeng Scientific Instrument Co.); HH-56A Electrothermal constant temperature water bath (Beijing Kewei Yongxing Instrument Co.); U-3010 Colorimeter (Hitachi High-Technologies Corporation); ZN-02 Small pulverizer (Beijing Xingshi Lihe Technology Development Co.); YX-280D-TL Portable Pressure Steam Sterilizer (Hefei Huatui Medical Equipment Co.).

Preparation of different processing of PC

Preparation of crude decoction pieces. According to the requirements of ChP and the previous study, the crude product was collected, washed with water, slightly moistened, sliced, and dried at 60 °C.

Preparation of steamed decoction pieces. The crude product was purified by removing impurities and washed. Then, it was steamed under high pressure of 0.06 MPa for 1 h, followed by steaming under normal pressure for 7 h. Finally, it was sliced and dried at 60 °C.

Preparation of wine-processed decoction pieces. The crude PC was purified by removing impurities and washed. Then, it was steamed with Huangjiu under high pressure of 0.06 MPa for 1 h, followed by steaming under normal pressure for 7 h. Finally, it was sliced and dried at 60 °C.

The characteristics

Characteristics were traditionally the most common method for identifying quality, offering the advantages of intuition and effectiveness. The Bencao Tu Jing records: "The juice should be fully extracted, resulting in a black, lacquer-like color. The taste is as sweet as caramel." Took samples of different products and observed their characteristics. The sensory indicators evaluation primarily included assessing the color, texture, taste characteristics, odor [19-22].

Determination of the chromatic value

The colorimeter was initially calibrated using a standard white plate before the measurement began. After calibration, the samples of different products were taken (sieved through the No. 3 sieve) and pressed onto slides. The pressed samples had a thickness of approximately 1–3 mm. The samples were placed on the white bottom plate of the colorimeter, and the chromatic values of the sample powders were measured. Chromatic value $L^*$ indicates brightness, the brightness of the sample to be tested increases with the increase of the $L^*$ value [23, 24]. In cases where the $L^*$ value is closer to 0, the color of the sample being tested tends to be closer to black; when the $L^*$ value is closer to 100, the color of the sample being tested tends to be closer to white. The $a^*$ indicates the red and green direction. If the $a^*$ value is greater than 0, it indicates the red direction; the $a^*$ value is less than 0, it indicates the green direction. The $b^*$ indicated the direction of yellow and blue. If the $b^*$ value is greater than 0, it indicates the yellow direction; if the $b^*$ value is less than 0, it indicates the blue direction. The total color value $E_0^a$ was calculated according to the formula $E_0^a = (L^* + a^* + b^*)/2$.

Quantitative analysis of the water extract

2.0 g of the different processed PC were soaked in 50 mL of deionized water, then extracted with ethanol, and the extract was then evaporated to dryness to obtain the residue. The residue was dissolved in methanol (10 mL) to prepare a solution with a concentration of 100 mg/mL. The solution was then analyzed using a UV-Visible Spectrophotometer.
water and weighed. After standing for 1 hour, the water bath reflux extraction was carried out for 1 h. After cooling, we reweighed the sample and compensated for the weight loss by adding water. After the removal of insoluble matter, a 25 mL solution was transferred and dried by steaming. The samples were oven-dried at 105 °C for 3 h, cooled for 30 min, and then quickly weighed. The water extract content of three different products was calculated respectively (%).

**Quantitative analysis of the alcohol extract**
The alcohol extract content of PC in different processing methods was determined using the hot dipping method outline in the Chp of 2020, Part I (General rule 2201).

**Quantitative analysis of PCP**
Pieces of different products were taken under '2.3', ground into powder, and then sifted the powder through a No. 5 sieve. About 0.25 g of the powder was accurately weighed into a round-bottom flask with 150 mL of 80% ethanol and boiled for 1 h at 85 °C. After the removal of insoluble matter, 150 mL of water was added and boiled for 1 h at 100 °C. Solutions were collected and diluted with water in a 250 mL volumetric flask. The sample solution was obtained [25, 26].

Accurately pipette 1 mL of the sample solution into the test tube and then add water to make the total volume 2 mL. Then, 8.0 mL of a 0.2% anthrone-sulfuric acid solution was added to the ice water bath. After cooling, the sample was placed in boiling water for 10 min and then transferred to ice water for 10 min. The absorbance was measured at 625 nm according to the UV-visible spectrophotometry guidelines outlines in General Rules 0401 of Chp [8].

**Analysis of the correlation between chromatic value and chemical composition of different products**
The chemical composition of the sample powder was analyzed for Pearson correlation with its chromatic value using SPSS 20.0 software.

**Statistical analysis**
Statistical analysis was implemented using Origin 2021 software. The experimental results were the mean ± standard error (Mean ± SEM) of three experiments. The significance of the differences among groups was analyzed by one-way analysis of variance.

With a post hoc test to determine group differences in the study parameters.

**Results and discussion**

**Description of characteristics of different products**
The results of the characteristics evaluation for three different products are presented in Table 1. The color of the surface gradually deepened, the sweetness increased, and the viscosity strengthened from the crude to the steamed to the wine-processed product. After processing, the numbness disappeared simultaneously. The results of the characteristics showed that the steamed and wine-steamed products could alleviate adverse reactions in the throat and other irritations, while also resulting in changes in other apparent properties such as smell and texture.

**The chromatic value**
The chromatic values and trends of different products were presents in Table 2 and Figure 1. The results of this test showed that the L’ gradually decreased from the crude to the steamed to the wine-processed product. This trend is consistent with the apparent color trend change observed in the three different products in this experiment, shifting from tan to bright black. The a’ became larger after processing, and the a’ of three different products was greater than 0, indicating a shift towards the red direction. At the same time, the steamed product was higher than wine-processed product. The b’ of three different products was greater than 0, indicating a shift towards the yellow direction. The steamed product had the highest quality. The E_a* value gradually decreased from the crude to the steamed to the wine-processed product. This indicates that the apparent color change of the crude, steamed, wine-steamed products was noticeable, transitioning from yellowish-brown to blackish-brown, and finally turning into a bright black.

**Quantitative analysis of the chemical composition**
The contents of PCP, water extract, alcohol extract of three different products are presented in Table 3 and Figure 2. Through the comparative study of the chemical composition of three different products, we found that the PCP content was the highest in the crude product, and the wine-processed product was higher than the steamed product. The content of PCP was significantly reduced after processing, which might be related to the degradation of PCP into monosaccharides or oligosaccharides, as well as the decomposition of oligosaccharides [17, 19]. However, the content of wine-processed PCP had higher levels than that of the steamed product. This might be related to the fact that the Huangjiu used in the processing of wine-processed products contains polysaccharide components, which results in a relatively elevated polysaccharide content in wine-processed products relatively elevated.

The contents of water extract and alcohol extract gradually increased from the crude to the steamed to the wine-processed product. This could be attributed to the increased solubility and higher dissolution rate of the chemical components in PC after being processed by Huangjiu.

<table>
<thead>
<tr>
<th>Table 1 Description of characteristics of different products</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Judging indicators</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Color</strong></td>
</tr>
<tr>
<td>Surface</td>
</tr>
<tr>
<td>Section</td>
</tr>
<tr>
<td><strong>Texture</strong></td>
</tr>
<tr>
<td>Viscosity</td>
</tr>
<tr>
<td>Tongue numbess</td>
</tr>
<tr>
<td><strong>Mouthfeel</strong></td>
</tr>
<tr>
<td>Sweetness</td>
</tr>
<tr>
<td>Bitterness</td>
</tr>
<tr>
<td><strong>Odor</strong></td>
</tr>
<tr>
<td>Wine aroma</td>
</tr>
<tr>
<td>Burnt taste</td>
</tr>
</tbody>
</table>

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Table 2 Measurement results of chromatic value of different products

<table>
<thead>
<tr>
<th>Sample</th>
<th>( L' )</th>
<th>( a' )</th>
<th>( b' )</th>
<th>( E_{ab} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude product</td>
<td>76.34 ± 0.5244</td>
<td>2.116 ± 0.5311</td>
<td>24.03 ± 0.6444</td>
<td>80.07 ± 0.7087</td>
</tr>
<tr>
<td>Steamed product</td>
<td>29.76 ± 0.1819</td>
<td>16.52 ± 0.0346</td>
<td>24.93 ± 0.0208</td>
<td>42.19 ± 0.1392</td>
</tr>
<tr>
<td>Wine-processed</td>
<td>29.66 ± 0.0458</td>
<td>15.27 ± 0.0896</td>
<td>22.24 ± 0.1704</td>
<td>40.17 ± 0.1027</td>
</tr>
</tbody>
</table>

Compared to crude product, \( P < 0.05 \), \( ^* P < 0.01 \); Compared to steamed product, \( ^* P < 0.05 \), \( ^{**} P < 0.01 \).

Figure 1 Trend charts for the chromatic values of different PC products. (A) Trend charts of \( L' \). (B) Trend charts of \( a' \). (C) Trend charts of \( b' \). (D) Trend charts of \( E_{ab} \).

Table 3 The content of the chemical composition for different products

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water extract/%</th>
<th>Alcohol extract/%</th>
<th>PCP/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude product</td>
<td>70.43 ± 1.577</td>
<td>71.08 ± 2.816</td>
<td>9.882 ± 0.113</td>
</tr>
<tr>
<td>Steamed product</td>
<td>71.32 ± 0.484</td>
<td>71.41 ± 0.697</td>
<td>3.132 ± 0.881</td>
</tr>
<tr>
<td>Wine-processed</td>
<td>72.92 ± 0.541*</td>
<td>73.12 ± 2.565*</td>
<td>6.735 ± 0.256</td>
</tr>
</tbody>
</table>

\( ^* \) Compared to crude product, \( P < 0.05 \), \( ^* P < 0.01 \); \( ^* \) Compared to steamed product, \( ^* P < 0.05 \), \( ^{**} P < 0.01 \).

Figure 2 Trend charts for the chemical composition of different PC products. (A) Trend charts of water extract. (B) Trend charts of alcohol extract. (C) Trend charts of PCP.
**Chemical pattern recognition analysis**

**Principal component analysis.** Origin software was used to perform cluster analysis on the chromatic values and the contents of the three chemical compositions, using the intergroup linkage method with the Euclidean squared distance as the measure. The results are shown in Figure 3. When the Euclidean distance was set to 3, the samples of PC could be classified into three classes: the crude products were clustered into one class, the steamed products were clustered into one class, and the wine-processed products were clustered into one class.

**Cluster analysis.** The chromatic values and chemical composition content data of PC different products were imported into SPSS 20.0 software for factor analysis. The results are presented in Table 4. Using the eigenvalue greater than 1 as the extraction criterion, the cumulative variance contribution rate of the first 2 components was 88.808%. This rate could effectively reflect most of the information regarding the different products of PC. PLS-DA divided the samples of different products of PC into three classes. The results are shown in Figure 4. The first class included S1, S2, S3; the second class included samples Q1, Q2, Q3; and the third class included samples J1, J2, J3, which was consistent with the conclusion of HCA.

**Analysis on the correlation between chromatic value and chemical composition of different products**

Pearson's correlation analysis revealed a highly significant positive correlation ($P < 0.01$) between the content of PCP and the $L^*$ and $E_{ab}^*$ values in different chemical composition of PC. It became significantly negatively correlated with $a'$. The results are shown in Table 5. Meanwhile, the results showed that there was no significant correlation between the contents of water extract, alcohol extract, and the chromatic value. This finding is consistent with the ChP of 2020, which uses the PCP content as the sole criterion for assessing quality.

![Figure 3 HCA image of the chromatic values and chemical composition of different PC products](https://www.tmrjournals.com/mhm)

**Table 4 The characteristic value and variance contribution rate of different PC products**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Initial eigenvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>4.774</td>
</tr>
<tr>
<td>2</td>
<td>1.443</td>
</tr>
<tr>
<td>3</td>
<td>0.589</td>
</tr>
<tr>
<td>4</td>
<td>0.177</td>
</tr>
<tr>
<td>5</td>
<td>0.017</td>
</tr>
<tr>
<td>6</td>
<td>0.001</td>
</tr>
<tr>
<td>7</td>
<td>0.000</td>
</tr>
</tbody>
</table>
PC is a traditional Chinese herb used in both food and medicine. Wine-processed PC is commonly used in clinical practice to alleviate adverse effects on the throat and enhance therapeutic outcomes. In this study, we found that the PC processed with Huangjiu could eliminate the irritation of crude products to the throat. Its characteristics and the content of chemical composition were also superior to that of the steamed product. The following speculations could be made by analyzing the combination of chemical composition, appearance characteristics, chromatic value. Firstly, the internal quality of PC had a certain correlation with its chromatic value. Modern studies have reported that L' is a key indicator of chromatic value by comparing the PCA of PCP color data [20]. In our study, L' was significantly correlated with the content of PCP. Therefore, it is speculated that the L' in the chromatic value can be used to objectively characterize the extent of processing on PC. In addition, Huangjiu could increase the polysaccharide content of the wine-processed products and promote the dissolution of chemical components during the processing of PC [27, 28]. Finally, by combining the characteristics of different products, it could be observed that the wine-processed product reached the endpoint of 'color resembling black lacquer, sweetness akin to syrup' earlier than the steamed product through. Therefore, we speculated that Huangjiu had the effect of expediting the process during the processing of PC [29].

Based on the study of the correlation between the apparent color and the chemical composition content of different processing methods, our research integrated different determination indexes, in order to offer a reference for further studying the impact of different processing methods on the quality of PC and to explore the role of Huangjiu in the processing process of PC.

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