Fresh-Cut Processing of Mulberry Branches and Assessment of Their Antioxidant Capacity

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Abstract [**Objectives**] To optimize key fresh-cut technologies for mulberry branches by employing multiple evaluation indicators and to compare their antioxidant capacity with that of traditionally processed slices, thereby providing guidance for the fresh-cut processing of mulberry branch medicinal materials. [**Methods**] A single-factor test method was employed to examine the effects of the drying degree of mulberry branches prior to cutting, cutting thickness, drying method, and drying temperature on the quality of slices. Evaluation indicators included appearance characteristics, alcohol extract content, total polysaccharide content, and total flavonol content, which were used to determine the optimal fresh-cut processing conditions. Additionally, the *in vitro* antioxidant activity of the samples was assessed using the DPPH free radical scavenging assay. [**Results**] The optimal parameters for fresh-cut processing of mulberry branches were identified as follows: A moisture content of (42 ± 5) % prior to cutting, a slicing thickness of 4 mm, and hot air drying at 70 ℃. Slices produced under these conditions exhibited a smooth appearance, with relatively high levels of alcohol extract, total polysaccharides, and total flavonoids. Antioxidant activity assessment revealed that the IC_{50} value of the fresh-cut processed slices against DPPH free radicals was 5.61 mg/mL, demonstrating superior efficacy compared to traditionally processed slices, which had an IC_{50} value of 6.85 mg/mL. [**Conclusions**] The optimized fresh-cut process for mulberry branches is both reasonable and feasible, demonstrating high repeatability. This process significantly enhances the quality of slices and the retention of active components, and improves antioxidant activity, thereby offering a scientific foundation and practical guidance for the initial processing of mulberry branches at their source.

Key words Mulberry branches, Fresh-cut processing, Active component, Quality evaluation

1 Introduction

Mulberry branches refer to the dried young branches of *Morus alba* L., a species belonging to the Moraceae family. In traditional Chinese medicine, mulberry branches are believed to possess properties that dispel rheumatism, enhance joint function, promote diuresis, and stimulate the production of bodily fluids[1]. Contemporary research has identified that mulberry branches contain various bioactive compounds, including polysaccharides, flavonoids, and alkaloids, which contribute to their diverse pharmacological effects such as hypoglycemic, hypolipidemic, antioxidant, and anti-inflammatory activities[2-4]. China is a leading nation in the sericulture industry. Various parts of the mulberry tree, including leaves, fruit, and root bark, are extensively utilized, whereas mulberry branches are frequently discarded as agricultural waste. Consequently, investigating the potential uses of mulberry branches could enhance their value. The conventional processing method for mulberry branches involves removing the leaves post-harvest, sun-drying the branches, cleaning them thoroughly, moistening them adequately with water, and subsequently slicing and drying them. This method is characterized by prolonged processing time, complex techniques, and challenges in ensuring consistent quality. Fresh-cut processing entails cutting and drying freshly harvested medicinal materials, or those that have not been fully dried, based on traditional processing methods to produce decoction pieces. This approach not only minimizes repetitive processing steps but also effectively reduces the loss of active components in the medicinal materials[5-6].

In the 2020 Edition of the *Chinese Pharmacopoeia*, the quality control indicator of mulberry branches is limited to alcohol-soluble extract content, which inadequately reflects the overall quality of mulberry branch decoction pieces. Both total polysaccharides and total flavonoids are recognized as the primary active components of mulberry branches. However, there is a lack of research concerning the fresh-cut processing technology for mulberry branches. Therefore, this study employed appearance characteristics, alcohol extract content, total polysaccharide content, and total flavonol content as evaluation indicators. A single-factor experimental design was utilized to identify the optimal fresh-cut processing method for mulberry branches. The feasibility of this fresh-cut processing approach was also investigated to provide a reference and theoretical basis for the integrated production of mulberry branches.

2 Materials

2.1 Instruments The instruments employed in this study included a BSA124S-CW electronic balance with a precision of 0.000 1 g (Sartorius, Germany), a 200A high-speed multifunctional crusher (Shanghai Sun-Rise Machinery Import/Export Co., Ltd.), a KQ-500E ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.), a DHG-9075A electric thermostatic blast drying oven (Shanghai Yiheng Scientific Instrument Co., Ltd.), an NJL07-3 laboratory-specific microwave oven (Nanjing Jiequan Microwave Equipment Co., Ltd.), and a UV-1800PC ultraviolet-visible spectrophotometer (Shimadzu, Japan).

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2.2 Samples The mulberry branch samples were obtained from the Guilin Academy of Agricultural Sciences, located in the Guangxi Zhuang Autonomous Region. These samples were identified by Professor Tang Hui, a researcher at the Guangxi Institute of Botany, Chinese Academy of Sciences, as fresh and tender branches of *M. alba* L., a species within the Moraceae family.

2.3 Reagents Glucose standard (batch No.: B220912), petroleum ether, anhydrous ethanol, and sulfuric acid were obtained from Xilong Science Co., Ltd. Anthranone (batch No.: M13IS209447) and rutin (batch No.: JB241985) were procured from Shanghai Yuanye Biotechnology Co., Ltd. Sodium nitrite and aluminum nitrate were sourced from Shanghai Macklin Biochemical Co., Ltd. DPPH was acquired from TCI (Shanghai) Co., Ltd., while vitamin C was purchased from Tianjin Fuchen Chemical Reagent Company. Purified water was supplied by Hangzhou Wahaha Group Co., Ltd.

3 Methods and results

3.1 Determination of alcohol extract content The determination was conducted in accordance with the method for assessing alcohol-soluble extracts as outlined in the 2020 Edition (Part IV) of the *Chinese Pharmacopoeia* (General Chapter 2201).

3.2 Determination of total polysaccharide content

3.2.1 Preparation of reference solution. 34.6 mg of anhydrous glucose reference substance was transferred into a 100 mL volumetric flask. Purified water was then added to dissolve the substance and to bring the volume up to the calibration mark. After thorough mixing, a glucose reference solution with a concentration of 0.346 mg/mL was prepared.

3.2.2 Plotting of standard curve. 0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of anhydrous glucose reference solution were accurately pipetted into separate containers. Each was then diluted to a final volume of 1 mL with water and mixed thoroughly. Subsequently, 4 mL of 0.2% anthranone sulfuric acid solution was added to each mixture, followed by thorough mixing. The solutions were allowed to stand at room temperature before being placed in a boiling water bath for 6 min. After cooling to room temperature, the absorbance of each solution was measured at a wavelength of 620 nm. A standard curve was constructed by plotting the mass concentration of glucose against absorbance, yielding the linear regression equation: $A = 7.078\ 8X - 0.014\ 2$, with a coefficient of determination (R^2) of 0.999 9. The results indicate a strong linear relationship between glucose mass concentration and absorbance within the range of 0.034 6 to 0.346 0 mg/mL.

3.2.3 Preparation of test solution. Mulberry branch powder, ground to 40 mesh, was weighed and combined with petroleum ether at a solid-liquid ratio of 1∶10 (g/mL). The mixture was refluxed and defatted at 85 ℃ for 1 h, followed by filtration and drying of the residue. Subsequently, an equal volume of 80% ethanol was added, and the mixture was refluxed at 90 ℃ for 2 h. This extraction process was repeated twice, after which the residue was filtered and dried. Approximately 1 g of pre-treated mulberry branch powder was accurately weighed and combined with 25 mL of distilled water. The mixture was subjected to ultrasonic extraction at 75 ℃ for 80 min, followed by filtration. The filtrate was then concentrated under reduced pressure. Subsequently, anhydrous ethanol was added to the concentrate at a volume four times that of the filtrate to achieve an alcohol concentration of 80%. The solution was allowed to stand overnight at 4 ℃, then centrifuged at 4 ℃ and 8 000 rpm for 15 min to collect the precipitate. The precipitate was dried at 60 ℃ until a constant weight was obtained, yielding crude polysaccharides from mulberry branches. The crude polysaccharides extracted from mulberry branches were dissolved and the solution was adjusted to a final volume of 100 mL in a volumetric flask. Subsequently, 0.5 mL of this solution was accurately pipetted and diluted to 1 mL with water. The absorbance was then measured following the procedure in Section **3.2.2**.

3.3 Determination of total flavonoid content

3.3.1 Preparation of reference solution. 10.5 mg of the rutin reference substance was transferred into a 100 mL volumetric flask. 75% ethanol was added to dissolve the substance, and the volume was adjusted to the mark. After thorough mixing, a rutin reference solution with a concentration of 0.105 mg/mL was obtained.

3.3.2 Plotting of standard curve. 0, 1, 2, 3, 4, and 5 mL of rutin reference solution were transferred sequentially. To each, 0.3 mL of 5% sodium nitrite solution was added, followed by thorough mixing and a standing period of 6 min. Subsequently, 0.3 mL of 10% aluminum nitrate solution was introduced, mixed well, and allowed to stand for an additional 6 min. Then, 4 mL of sodium hydroxide solution was added and mixed immediately. The volume was adjusted to 10 mL with 75% ethanol, mixed thoroughly, and the mixture was left to stand for 15 min. Absorbance measurements were taken at 510 nm, using a solution without the reference substance as the blank. A standard curve was constructed by plotting the mass concentration of rutin on the x-axis and the absorbance on the y-axis. The resulting linear regression equation was $A = 12.387X - 0.000\ 2$, with a coefficient of determination (R^2) of 0.999. This indicates a strong linear relationship between the mass concentration of rutin and absorbance within the concentration range of 0.010 5 to 0.052 5 mg/mL.

3.3.3 Preparation of test solution. The sample extraction method was adapted from reference[7] with slight modifications. Specifically, 2 g of the medicinal powder was accurately weighed and extracted twice by refluxing with 75% ethanol at a solid-to-liquid ratio of 1∶20 (g/mL) for 1.5 h each time. The combined filtrates were concentrated to dryness, then redissolved and diluted to 100 mL in a volumetric flask to prepare the test solution. Subsequently, 1.5 mL of the test solution was precisely transferred into a test tube, and its absorbance was measured following the procedure in Section **3.3.2**.

3.4 Single-factor test

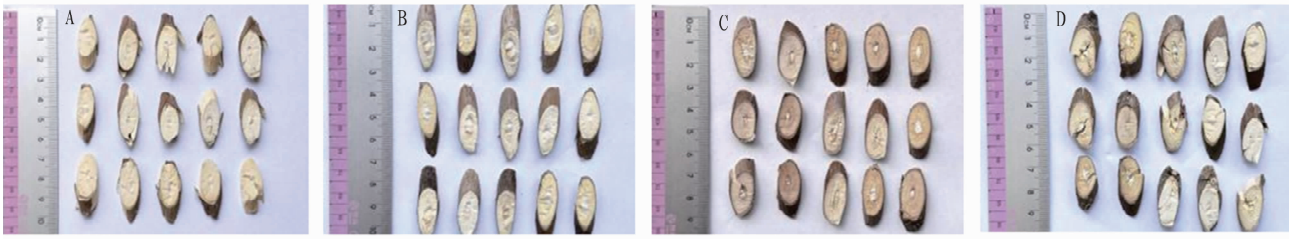
3.4.1 Investigation into the degree of drying of medicinal materials prior to cutting. A batch of fresh mulberry branches, uniform

in thickness, was collected. One group of these branches was used immediately, while the moisture content of the remaining branches was measured at various time intervals. The branches were sliced while still fresh and then dried at 50 ℃ until a constant weight was attained. Subsequently, their appearance characteristics were evaluated.

The research findings regarding mulberry branches with varying moisture contents are presented in Table 1 and Fig. 1. Fresh mulberry branches exhibited a moisture content of 63.6%. Although these branches were crisp at the time of cutting, they tended to adhere to one another during the cutting process. Following drying, the branches displayed noticeable cracking and peeling. After 5 d of air-drying, when the moisture content decreased to approximately (45 ± 5)%, the mulberry branches became softer in texture, easier to cut, with minimal adherence of fragments, and the dried slices remained intact. As the moisture content decreased below 30%, the texture of mulberry branches became increasingly rigid, resulting in greater difficulty during cutting. Once fully dried, the surface of the mulberry branches hardened and desiccated, rendering them uncuttable. Consequently, mulberry branches with a moisture content of (45 ± 5)% were selected for subsequent analysis.

Table 1 Evaluation of the characteristics of mulberry branch slices with different moisture contents

Processing method	Moisture content//%	Cutting situation
Fresh-cut	63.6	The branches can be easily cut; however, they tend to produce numerous adhering fragments and exhibit significant cracking.
Air-drying for 1 d	59.8	The branches can be easily cut; however, they tend to produce numerous adhering fragments and exhibit significant cracking.
Air-drying for 5 d	44.8	The branches are easily cut, exhibiting minimal adhering fragments and producing relatively flat slices.
Air-drying for 7 d	40.4	The branches are easily cut, demonstrating almost no adherence of fragments and resulting in flat slices.
Air-drying for 15 d	26.9	The branches are somewhat easier to cut, demonstrating almost no adherence of fragments and resulting in flat slices.
Air-drying for 20 d	24.2	The branches are relatively difficult to cut, exhibiting no adherence of fragments and yielding flat slices.
Air-drying for 31 d	19.1	The surface of the medicinal materials is dry and hard, which renders them difficult to cut.



NOTE A. Fresh; B. Air-drying for 5 d; C. Air-drying for 15 d; D. Air-drying for 31 d.

Fig. 1 Appearance characteristics of mulberry branch slices with different moisture contents

3.4.2 Investigation of slice thickness. The mulberry branches, which had been air-dried for 5 d, were subsequently cut into slices with thicknesses of 2, 3, 4, and 5 mm, respectively, and then dried at 50 ℃ until a constant weight was attained. Evaluation indicators included appearance characteristics, alcohol extract content, total polysaccharide content, and total flavonoid content.

The study revealed that thinner mulberry branch slices were more difficult to cut. In addition, their larger surface area led to increased juice loss, resulting in relatively lower contents of alcohol-soluble extracts and polysaccharides. Conversely, thicker slices better preserved the components. Notably, the 4 mm slices maintained intact sheet shapes and exhibited higher extract and total flavonoid contents compared to other slice thicknesses (Table 2). Therefore, a thickness of 4 mm was determined to be optimal for slicing.

3.4.3 Investigation on the drying temperature of slices. The mulberry branches, which had been air-dried for 5 d, were subsequently sliced and subjected to drying using microwave drying, air-drying, sun-drying, and hot air drying at temperatures of 50, 60, 70, 80, and 90 ℃. The evaluation indicators included appearance characteristics, alcohol extract content, total polysaccharide content, and total flavonoid content.

Table 2 Determination results of slice components with different cutting thicknesses, drying methods, and temperatures ($n=3$)

Processing method		Alcohol extract %	Total polysaccharides %	Total flavonoids %
Cutting thickness	2 mm	4.238 0	3.308 5	0.551 0
	3 mm	4.207 8	3.361 2	0.575 5
	4 mm	4.953 1	3.818 4	0.611 6
	5 mm	4.833 0	4.577 2	0.533 2
Drying method and temperature	Microwave drying	4.263 0	5.113 4	0.450 7
	Hot air drying at 50 ℃	4.169 0	3.811 5	0.611 2
	Hot air drying at 60 ℃	4.507 2	4.437 8	0.920 7
	Hot air drying at 70 ℃	4.831 7	4.643 1	0.756 3
	Hot air drying at 80 ℃	4.829 2	2.814 3	0.474 1
	Hot air drying at 90 ℃	3.984 0	2.957 4	0.421 1
	Air-drying	4.285 8	3.749 0	0.405 4
	Sun-drying	4.228 3	3.275 6	0.804 8

As presented in Table 2, the alcohol extract content of mul-

berry branch slices subjected to various drying methods and temperatures ranged from 3.98% to 4.83%. The total polysaccharide content varied between 2.81% and 5.11%, while the total flavonol content ranged from 0.40% to 0.92%. Significant differences were observed in the alcohol extract content, total polysaccharide content, and total flavonoid content of mulberry branch slices across the different drying methods and temperatures.

The alcohol extract content of mulberry branch slices dried using hot air at 70 and 80 ℃ was higher compared to other drying temperatures. The total polysaccharide content was highest with microwave drying (5.11%), significantly exceeding that observed with other drying methods, followed by hot air drying at 70 ℃. The total flavonoid content peaked at 0.92% when dried at 60 ℃, whereas it was lowest under air-drying and drying at 90 ℃ conditions. Overall, when the drying temperature was approximately 70 ℃, the alcohol extract content and total polysaccharide content of the resulting mulberry branch slices were comparatively high. Conversely, at a drying temperature of 60 ℃, the total flavonoid content was relatively high. Based on a comprehensive analysis of both appearance characteristics and component content, 70 ℃ was ultimately determined to be the optimal drying temperature.

3.5 Determination of antioxidant capacity

3.5.1 Preparation of test solution. According to the extraction procedure described in reference[8], exactly 0.5 g of mulberry branch powder was weighed and subjected to extraction with 50 mL of 70% ethanol by refluxing for 1 h. This extraction process was repeated once. The combined filtrates were then concentrated to an appropriate volume to obtain the test solution of mulberry branches.

3.5.2 Determination of DPPH free radical scavenging activity. 2.0 mL of the test solution was combined with 2.0 mL of DPPH solution (0.4 mmol/L) and allowed to react in the dark at room temperature for 30 min. Subsequently, the absorbance was measured at a wavelength of 517 nm. Each group was prepared in triplicate, with vitamin C (Vc) solution used as the positive control. The scavenging rate of DPPH free radicals by the sample was calculated using the following formula:

$$\text{Scavenging rate} = [(A_{\text{blank}} - A_{\text{test}})/A_{\text{blank}}] \times 100\%.$$

3.5.3 Determination results. The scavenging capacity of mulberry branch slices against DPPH free radicals is illustrated in Fig. 2. As depicted, the scavenging rates of DPPH free radicals for both fresh-cut and traditional mulberry branch slices exhibited a continuous increase with rising sample concentration. Linear regression analysis was conducted on the scavenging rates, yielding IC_{50} values of 3.12 mg/mL for the fresh-cut slices and 3.31 mg/mL for the traditional slices. These results indicate that the antioxidant activity of the fresh-cut slices is significantly superior to that of the traditional slices.

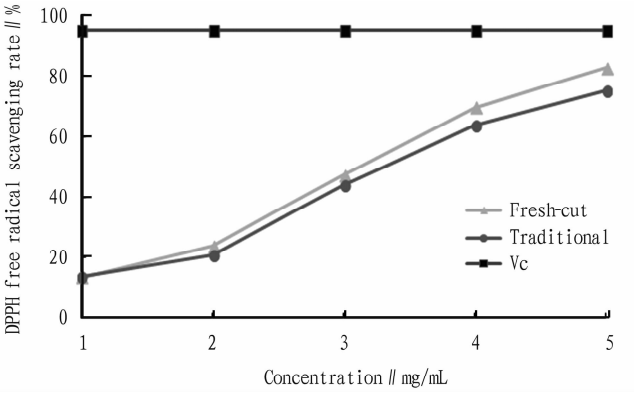


Fig. 2 Scavenging ability of mulberry branch slices against DPPH free radicals

4 Discussion

The integration of on-site processing and preparation of Chinese medicinal materials represents a crucial approach to advancing the modernization and standardization of the traditional Chinese medicine industry. The centre of this approach is the seamless integration of previously disconnected and redundant steps within the traditional processing workflow, thereby achieving multiple objectives, including quality control, enhanced efficiency, and preservation of active components. The critical step of fresh-cut processing not only effectively prevents the loss of medicinal components caused by repeated processing but also ensures that the fresh-cut is performed while the medicinal materials remain in a "fresh state" with higher physiological activity. This approach facilitates the maximal retention of volatile compounds and heat-sensitive active substances, thereby enhancing the uniformity of quality and the stability of efficacy of the decoction pieces from the point of origin. The response letter issued by the National Medical Products Administration in July 2021, addressing issues related to the procurement of locally processed (fresh-cut) Chinese medicinal materials by Chinese herbal decoction piece manufacturing enterprises, exemplifies policy-level guidance and support aimed at optimizing the production processes of traditional Chinese medicine and enhancing quality control throughout these processes. Regions such as Guangxi have also responded proactively, thereby further advancing the standardized development of the fresh-cut processing model at the local level.

The components of traditional Chinese medicine are highly complex, and the overall therapeutic efficacy cannot be adequately represented by analyzing any single active component. Therefore, it is both practically necessary and scientifically significant to develop a multi-indicator comprehensive evaluation system that systematically assesses key parameters, including the moisture content of medicinal materials before cutting, slice thickness, and drying methods.

The moisture content of mulberry branch slices prior to cutting significantly influences their appearance characteristics. Additionally, factors such as slice thickness, drying method, and drying temperature play crucial roles in determining the content of active components in traditional Chinese medicine[9-10]. Experi-

mental results indicate that microwave drying offers distinct advantages in processing mulberry branches. The high-frequency electromagnetic waves facilitate rapid vaporization of internal moisture, enabling uniform heating from the interior outward[11-12]. This method not only enhances drying efficiency but also markedly surpasses traditional sun-drying and air-drying techniques in preserving polysaccharide components. Under controlled temperature conditions, hot air drying is more favorable for the stable retention of flavonoid compounds and alcohol-soluble extracts. Furthermore, slice thickness, as a critical factor affecting drying uniformity and component dissolution in decoction pieces, requires precise regulation throughout the process.

The aforementioned findings not only establish a reliable foundation for optimizing the fresh-cut processing technology of mulberry branches but also provide a valuable reference for the integrated processing of analogous medicinal materials. Future research may incorporate pharmacodynamic evaluations to systematically compare the differences in chemical composition and overall therapeutic effects between fresh-cut and traditional processing methods of decoction pieces. Such investigations would elucidate the advantages of fresh-cut processing more comprehensively and facilitate the precise development and utilization of mulberry branches and other traditional Chinese medicinal materials.

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4 Discussion

This study evaluated the safety of the pilot-scale batch of Dujieqing Pills through acute and chronic toxicity experiments. The acute toxicity results showed that Dujieqing Pills did not cause significant toxic reactions at the maximum administered dose. In the chronic toxicity experiment, although a few parameters related to general state, body weight, and organ coefficients showed statistically significant differences compared to the control group during administration and the recovery period, when considered alongside the histopathological findings and normal fluctuation ranges, these differences were deemed to lack biological significance. The mild lymphocyte infiltration observed in some organs represents a common non-specific change; no drug-related histopathological damage was identified. In summary, no significant toxic reactions were observed with single or long-term use of Dujieqing Pills at the proposed clinical dose. This study provides experimental evidence supporting its safe clinical application.

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