Postprint: Process Study on the Removal of Tannins from Rosa roxburghii Juice Using Ginger Protein

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Abstract

To effectively remove tannins from Rosa roxburghii juice (RRTJ) and reduce its astringency to improve taste, this study employed chemical precipitation method using ginger protein as the tannin removal agent. With tannin removal rate and vitamin C (VC) retention rate as evaluation indicators, single-factor and orthogonal experiments were conducted to optimize the ginger protein tannin removal process and determine the optimal conditions. The results showed: (1) The optimal process conditions for tannin removal from Rosa roxburghii juice by ginger protein were a liquid-solid ratio of 30:1.2 (mL:g), juice pH of 3.0, stirring temperature of 5 °C, and stirring time of 30 min; (2) Orthogonal experimental analysis revealed that the influence degree of each factor on tannin removal from Rosa roxburghii juice followed the order: liquid-solid ratio > stirring temperature > juice pH > stirring time; (3) Under the optimal conditions, the tannin removal rate was (47.451$±0.608)±1.244)±0.662)±$0.297)%, with significantly improved astringency and enriched flavor of Rosa roxburghii juice. In summary, this study provides a novel approach and technical foundation for a new process route to address the common key technical challenges in the deep processing industry of Rosa roxburghii juice, while also establishing a technical basis for expanding the comprehensive utilization of ginger resources.

Full Text

Study on the Process of Removing Tannins from Rosa roxburghii Juice Using Ginger Protein

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Abstract

To effectively remove tannins from Rosa roxburghii juice (RRTJ) and thereby reduce its astringency and improve taste, this study employed chemical precipitation using ginger protein as the tannin removal agent. Tannin removal rate and vitamin C (VC) retention rate were selected as the key evaluation indices, and single-factor and orthogonal experiments were conducted to optimize the tannin removal process. The results demonstrated that: (1) The optimal conditions for tannin removal from RRTJ using ginger protein were a liquid-solid ratio of 30:1.2 (mL:g), juice pH of 3.0, stirring temperature of 5 °C, and stirring time of 30 min; (2) Orthogonal experimental analysis revealed that the influence of various factors on tannin removal from RRTJ followed the order: liquid-solid ratio > stirring temperature > juice pH > stirring time; (3) Under these optimal conditions, the tannin removal rate was (47.451 $\pm$ 0.608)% and the VC retention rate was (75.904 $\pm$ 1.244)%; (4) Under the optimal conditions, juice transmittance increased from (8.44 $\pm$ 0.662)% to (92.47 $\pm$ 0.297)%, with significant improvement in astringency and enhanced flavor. In summary, this study provides a novel approach and technical foundation for addressing common key challenges in the RRTJ deep-processing industry, while also establishing a basis for the comprehensive utilization of ginger resources.

Keywords: Rosa roxburghii juice, ginger protein, tannin, chemical precipitation, vitamin C (VC)

Rosa roxburghii, a perennial shrub of the Rosaceae family, produces flat spherical berries covered with soft spines that turn yellow-brown upon ripening. The plant is primarily distributed across subtropical and warm temperate regions in China, including Guizhou, Sichuan, and Guangxi, with Guizhou being the most abundant region, where it is widely used as an ethnic medicinal material (Liang et al., 2022). The cultivation of Rosa roxburghii and the deep processing of its fruit represent important industries for poverty alleviation and rural revitalization in the region. The fruit contains various bioactive components, including polysaccharides, flavonoids, triterpenoids, VC, and tannins (Fu et al., 2020), and exhibits antioxidant, anticancer, and blood glucose intervention activities (Zhang et al., 2022; Wu et al., 2023). Due to its high VC content, Rosa roxburghii is known as the "King of Vitamin C" (Zhao et al., 2022) and is considered a highly nutritious and medicinally valuable "third-generation fruit" (Hu et al., 2017). However, the fruit also contains high levels of tannins, generally above 0.6% and up to 2.2% in some cases (Luo, 2011), which impart a strong sour and astringent taste to the fruit and its raw juice. This astringency arises because tannins interact with proteins in the oral mucosa, causing saliva to lose its lubricating properties and inducing contraction of epithelial tissues on the tongue tip, resulting in a dry sensation (Soares et al., 2018). Excessive tannin content also leads to browning and precipitation (Zhang et al., 2020), severely affecting the taste, shelf life, and market promotion of Rosa roxburghii products. Therefore, reducing tannin content in raw juice while preserving VC content to improve taste and extend storage period is a common critical challenge facing the industry.

Common methods reported for reducing bitterness and astringency (i.e., tannin removal) in Rosa roxburghii juice include physical adsorption, chemical precipitation, biological hydrolysis, and combined treatments. Physical adsorption primarily employs activated carbon and macroporous adsorption resins (Wang et al., 1994; Yue et al., 2016) to adsorb tannins. Chemical precipitation mainly uses chitosan and gelatin (Liang et al., 2011) to react with tannins and form precipitates. Biological hydrolysis utilizes tannase (Luo et al., 2013; Zhu et al., 2020) to hydrolyze tannins. Combined treatments integrate these three methods (Zhang et al., 2016; Jin et al., 2022). However, current tannin removal methods are imperfect, with most suffering from long processing times, poor clarification effects, or low VC retention rates, limiting their practical application. Therefore, developing a novel tannin removal process is essential. The formation of precipitates through tannin-protein interactions is a unique chemical property of tannins (Szczurek, 2021). Ginger protease is an important plant protein belonging to the papain family, exhibiting antimicrobial, anti-inflammatory, antioxidant activities, and clarification effects (Tang et al., 2021). No studies have yet reported the use of ginger protein for tannin removal from Rosa roxburghii juice to improve its astringency.

This study investigated the use of ginger protein as a tannin removal agent for Rosa roxburghii juice based on the specific precipitation principle of protein-tannin interactions. Single-factor and orthogonal experiments were employed to optimize the process, addressing three key questions: (1) the effectiveness of ginger protein in tannin removal; (2) the impact of the tannin removal process on VC content; and (3) changes in transmittance, color, and taste of the juice before and after tannin removal. This research aims to provide a new technical route for tannin removal and astringency reduction in Rosa roxburghii juice, while offering a novel approach for the comprehensive utilization of ginger resources.

1.1 Materials and Reagents

Raw materials: Raw Rosa roxburghii juice was provided by Guizhou Chuhao Agricultural Science and Technology Development Co., Ltd. (produced in September 2022, stored at 1–4 °C). Ginger residue was obtained in our laboratory following the supercritical CO_2 ginger oil extraction method described by Zhu et al. (2023), using local small yellow ginger from the demonstration planting base of the Chinese Academy of Sciences poverty alleviation project in Shuicheng District, Guizhou Province.

Reagents: Ascorbic acid and gallic acid (analytical grade) were purchased from Yuanye Technology Co., Ltd. Citric acid and sodium bicarbonate (food grade) were obtained from Weifang Yingxuan Industrial Co., Ltd. All other reagents were of analytical grade. Experimental water was purified using a laboratory pure water system.

1.2 Instruments and Equipment

HPLC (1260), Agilent Technologies, USA; U-T6 UV-Vis spectrophotometer, Beijing Purkinje General Instrument Co., Ltd.; WGLL-30BE electric thermostatic drying oven, Tianjin Taiste Instrument Co., Ltd.; DHS-16A moisture analyzer, Ningbo Lichen Technology; TG16-WS high-speed desktop centrifuge, Hunan Michael Experimental Instrument Co., Ltd.; DF-101S constant temperature magnetic stirrer, Shanghai Lichen Bangxi Instrument Technology Co., Ltd.; Model pHS-3C pH meter, Shanghai Shuangxu Electronics Co., Ltd.

1.3 Experimental Methods

1.3.1 Extraction of Ginger Protein Based on the method described by Chen et al. (2016) and preliminary experiments, the ginger protein extraction protocol was determined as follows: Ginger residue was mixed with purified water at a solid-liquid ratio of 1:23 (g:mL) and extracted under stirring for 3 h in an ice bath at 5 °C. The mixture was filtered to remove ginger residue, then centrifuged (6,000 $r \cdot min^{-1}$, 20 min) to remove starch. Citric acid (20 $mmol \cdot L^{-1}$) was added to induce protein aggregation and precipitation, followed by centrifugation (6,000 $r \cdot min^{-1}$, 20 min) to collect the precipitate, which was stored at 4 °C for later use. The ginger protein had a moisture content of (88.52 $\pm$ 1.52)%, extraction yield of (8.53 $\pm$ 0.66)%, and purity of (58.50 $\pm$ 2.23)%. In subsequent tannin removal experiments, ginger protein addition amounts were calculated based on dry weight (i.e., 10.00 g wet protein corresponded to 1.15 g ginger protein).

1.3.2 Tannin Removal from Rosa roxburghii Juice Thirty milliliters of raw Rosa roxburghii juice was measured into a 100 mL beaker, and the pH was adjusted to a specified value using citric acid and saturated sodium bicarbonate solution. Ginger protein extracted according to Section 1.3.1 was added at a specific volume-mass ratio (mL:g), and the mixture was stirred at a constant temperature in a water bath for a set reaction time. For temperatures below room temperature, an ice bath was used. After reaction completion, the mixture was transferred to a high-speed centrifuge and centrifuged at 6,000 $r \cdot min^{-1}$ for 20 min. The supernatant was collected for determination of tannin and VC content.

1.3.3 Index Determination **Tannin content determination:** Conducted according to NY/T 1600-2008 "Determination of tannin content in fruits, vegetables and their products—Spectrophotometric method." The standard curve equation was y = 0.11584x + 0.02842, $R^2 = 0.9982$, with a linear range of 0–10 $mg \cdot L^{-1}$, where x represents the sample absorbance value (Abs 765 nm) and y represents the tannin content in Rosa roxburghii juice.

VC content determination: Conducted according to the HPLC method described by Qian et al. (2021). The standard curve equation was y = 91.1x + 27, $R^2 = 0.9998$, with a linear range of 0–100 $g \cdot mL^{-1}$, where x represents VC content and y represents VC peak area.

Transmittance determination: Conducted according to the method described by Luo (2011), using water as reference and measuring transmittance T_{560} of Rosa roxburghii juice at 560 nm.

1.3.4 Index Analysis Ginger protein extraction rate is calculated using Equation (1):

$$\text{Protein extraction rate} = \frac{\text{Precipitate dry weight}}{\text{Dry ginger powder weight}} \times 100$$

Ginger protein purity is calculated using Equation (2):

$$\text{Protein purity} = \frac{\text{Protein content in precipitate}}{\text{Precipitate mass}} \times 100$$

Tannin removal rate is calculated using Equation (3):

$$E = \frac{S_0 - S_1}{S_0} \times 100$$

where E is the tannin removal rate (%), S_0 is the initial tannin content in Rosa roxburghii juice ($mg \cdot L^{-1}$), and S_1 is the residual tannin content after removal ($mg \cdot L^{-1}$).

VC retention rate is calculated using Equation (4):

$$R = \frac{m_2}{m_1} \times 100$$

where R is the VC retention rate (%), m_1 is the initial VC content in Rosa roxburghii juice ($g \cdot mL^{-1}$), and m_2 is the VC content after tannin removal ($g \cdot mL^{-1}$).

1.3.5 Single-Factor Experimental Design 1.3.5.1 Effect of liquid-solid ratio on tannin removal rate and VC retention rate

Liquid-solid ratios of Rosa roxburghii juice to ginger protein were tested at 30:0.3, 30:0.6, 30:0.9, 30:1.2, 30:1.5, and 30:1.8 (mL:g), with juice pH 3.5, stirring temperature 25 °C, and stirring time 45 min.

1.3.5.2 Effect of pH on tannin removal rate and VC retention rate

At a liquid-solid ratio of 30:0.9 (mL:g), pH values of 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 were tested, with stirring temperature 25 °C and stirring time 45 min.

1.3.5.3 Effect of stirring temperature on tannin removal rate and VC retention rate

At a liquid-solid ratio of 30:0.9 (mL:g) and juice pH 3.5, stirring temperatures of 5, 15, 25, 35, 45, and 55 °C were tested, with stirring time 45 min.

1.3.5.4 Effect of stirring time on tannin removal rate and VC retention rate

At a liquid-solid ratio of 30:0.9 (mL:g), juice pH 3.5, and stirring temperature 5 °C, stirring times of 15, 30, 45, 60, 75, and 90 min were tested.

1.3.6 Orthogonal Experimental Design Based on single-factor experimental results, orthogonal experimental levels were determined using tannin removal rate and VC retention rate as evaluation indices. A four-factor, three-level orthogonal experiment $L_9(3^4)$ was designed with liquid-solid ratio of Rosa roxburghii juice to ginger protein, juice pH, stirring temperature, and stirring time as factors. The orthogonal experimental design is shown in Table 1.

Table 1 Factors and levels of orthogonal test

Factor	Level 1	Level 2	Level 3
Liquid-solid ratio (mL:g)	30:0.6	30:0.9	30:1.2
Juice pH	3.0	3.5	4.0
Stirring temperature (°C)	5	15	25
Stirring time (min)	30	45	60

1.4 Data Processing

All experiments were performed in triplicate, and data are expressed as mean ± standard deviation. Excel 2019 was used for data analysis, and Origin 2018 was used for graphing.

2.1.1 Effect of Liquid-Solid Ratio on Tannin Removal Rate and VC Retention Rate

As shown in Figure 1 [Figure 1: see original paper], the tannin removal rate increased with decreasing liquid-solid ratio, plateauing when the ratio reached a certain level. At a liquid-solid ratio of 30:1.5 (mL:g), the reaction between high-molecular-weight tannins and ginger protein in Rosa roxburghii juice reached equilibrium. Previous studies have shown that proteins and VC spontaneously bind through electrostatic interactions (Liang et al., 2022). Figure 1 demonstrates that VC retention rate decreased with decreasing liquid-solid ratio, as more ginger protein bound with VC. Therefore, a liquid-solid ratio of 30:0.9 (mL:g) was selected as optimal, providing good tannin removal while maintaining relatively high VC retention.

2.1.2 Effect of pH on Tannin Removal Rate and VC Retention Rate

As shown in Figure 2 [Figure 2: see original paper], pH had minimal effect on tannin removal rate with no significant changes, but substantially affected VC retention rate. The original pH of Rosa roxburghii juice is approximately 3.5, and adjusting pH disrupts the native environment, significantly impacting VC retention. During experimentation, large deviations from the original juice pH resulted in increased browning and oxidation. Therefore, pH 3.5 was selected as appropriate.

2.1.3 Effect of Stirring Temperature on Tannin Removal Rate and VC Retention Rate

Temperature is a crucial environmental factor affecting tannin-protein binding, primarily influencing hydrogen bonds and hydrophobic interactions. As shown in Figure 3 [Figure 3: see original paper], temperature significantly affected tannin removal rate, which decreased with increasing temperature. Previous research indicates that low-temperature environments favor tannin-protein binding (Prigent et al., 2003). VC retention rate also decreased with increasing temperature, as VC is relatively stable at low temperatures but degrades at elevated temperatures (Zhang et al., 2017). Therefore, a stirring temperature of 5 °C was selected as optimal.

2.1.4 Effect of Stirring Time on Tannin Removal Rate and VC Retention Rate

As shown in Figure 4 [Figure 4: see original paper], tannin removal rate initially increased then decreased with prolonged stirring time, reaching its maximum at 45 min. Research indicates that tannin-protein binding is a reversible reaction (Li, 2006), suggesting that tannins are released from the polymer after prolonged stirring once equilibrium is reached. VC retention rate decreased gradually with increasing stirring time, as extended protein-juice contact increased protein-VC binding. Therefore, a stirring time of 45 min was selected as appropriate.

2.2 Orthogonal Experimental Results Analysis

Based on single-factor experimental results, an $L_9(3^4)$ orthogonal array was used to further optimize the tannin removal and VC retention process. The results are presented in Table 2 .

Table 2 shows that the fluctuation amplitude of range R represents the influence degree of experimental factors on tannin removal rate and VC retention rate. The primary-to-secondary order of factors affecting tannin removal was A > C > B > D, with the optimal combination being A3B1C1D1, corresponding to a liquid-solid ratio of 30:1.2 (mL:g), juice pH 3.0, stirring temperature 5 °C, and stirring time 30 min. For VC retention, the order was A > B > C > D, with the optimal combination being A1B2C3D1, corresponding to a liquid-solid ratio of 30:0.6 (mL:g), juice pH 3.5, stirring temperature 25 °C, and stirring time 30 min. Since neither optimal combination appeared in the orthogonal array, verification experiments were conducted in triplicate. The optimal tannin removal combination yielded a tannin removal rate of (47.451 ± 0.608)% and VC retention rate of (75.904 ± 1.244)%. The optimal VC retention combination yielded a tannin removal rate of (30.392 ± 0.886)% and VC retention rate of (85.902 ± 1.459)%. The difference in VC retention between the two optimal conditions was approximately 10%, while the difference in tannin removal rate was about 17%. Although both indices were considered, the primary objective was maximum tannin removal to address the critical astringency issue while preserving VC as much as possible. Therefore, the optimal process was determined to be A3B1C1D1.

Table 2 Results of orthogonal experiment

Test No.	Liquid-solid ratio (mL:g)	Juice pH	Stirring temperature (°C)	Stirring time (min)	Tannin removal rate (%)	VC retention rate (%)
1	1 (30:0.6)	1 (3.0)	1 (5)	1 (30)	35.772 ± 0.704	82.761 ± 1.392
2	1 (30:0.6)	2 (3.5)	2 (15)	2 (45)	32.114 ± 1.863	86.897 ± 2.139
3	1 (30:0.6)	3 (4.0)	3 (25)	3 (60)	27.642 ± 1.863	85.827 ± 2.077
4	2 (30:0.9)	1 (3.0)	2 (15)	3 (60)	40.244 ± 1.220	75.536 ± 3.070
5	2 (30:0.9)	2 (3.5)	3 (25)	1 (30)	38.211 ± 1.408	84.218 ± 2.192
6	2 (30:0.9)	3 (4.0)	1 (5)	2 (45)	40.650 ± 0.704	78.621 ± 0.744
7	3 (30:1.2)	1 (3.0)	3 (25)	2 (45)	42.276 ± 1.408	70.976 ± 1.077
8	3 (30:1.2)	2 (3.5)	1 (5)	3 (60)	45.122 ± 1.220	70.593 ± 1.767

Test No.	Liquid-solid ratio (mL:g)	Juice pH	Stirring temperature (°C)	Stirring time (min)	Tannin removal rate (%)	VC retention rate (%)
9	3 (30:1.2)	3 (4.0)	2 (15)	1 (30)	42.683 ± 1.220	70.045 ± 0.352

Optimal combination for tannin removal: A3B1C1D1
Optimal combination for VC retention: A1B2C3D1

2.3 Effects of Ginger Protein Tannin Removal on Rosa roxburghii Juice

Using the optimized ginger protein tannin removal process, detanninized Rosa roxburghii juice was prepared under optimal parameters. Tannin content, VC content, transmittance, color, and taste were analyzed and compared with raw juice, with results shown in Table 3 and color comparison shown in Figure 5 [Figure 5: see original paper].

Table 3 Effect of tannin removal by ginger protein on Rosa roxburghii juice

Test group	Tannin content ($mg \cdot mL^{-1}$)	VC content ($mg \cdot mL^{-1}$)	Transmittance (%)	Color	Taste
Raw juice	16.91 ± 0.101	16.09 ± 0.062	8.44 ± 0.662	Turbid, dark yellow, prone to browning and precipitation	Strong Rosa roxburghii aroma but heavy astringency with obvious dry mouth sensation

Test group	Tannin content $(mg \cdot mL^{-1})$	VC content $(mg \cdot mL^{-1})$	Transmittance (%)	Color	Taste
Detanninized juice	8.93 ± 0.054	12.20 ± 0.092	92.47 ± 0.397	Clear, transparent, golden yellow, minimal color change and essentially no precipitation after long-term storage	Strong Rosa roxburghii aroma with subtle ginger flavor, light astringency, no obvious dry mouth sensation

Figure 5 Sensory effects of ginger protein detannin on Rosa roxburghii juice
[Figure 5: see original paper]
a. Raw juice of Rosa roxburghii; b. Detannin juice of Rosa roxburghii.

3 Discussion and Conclusion

The primary tannins in Rosa roxburghii juice are hydrolyzable tannins of the gallic acid type (Huang et al., 2022). Excessive tannin content causes strong astringency that affects taste, decomposes under certain conditions to produce CO_2 (Nie, 2017), undergoes color changes upon air exposure, and interacts with other macromolecules such as Rosa roxburghii proteins and polysaccharides during storage to form precipitates that further impact sensory quality. These issues—astringency, gas production, browning, and precipitation—represent key technical challenges facing the Rosa roxburghii juice industry, severely affecting market promotion and further processing.

Reported proteins for tannin removal from Rosa roxburghii juice include gelatin and tannase. Liang et al. (2011) studied various additives for tannin removal, achieving a 54.4% tannin removal rate with 0.8% gelatin after 15 h of natural settling, but VC retention was only 42.85%. Although effective for tannin removal, this method suffered from long processing time and low VC retention. Luo et al. (2013) optimized tannase treatment (0.12% tannase, pH 4.5, 45 °C, 100 min), achieving 76.07% tannin removal and 72.13% VC retention. While this method showed high tannin removal and good VC retention, clarification was poor and browning remained unresolved. The flocculation of tannins with proteins to form precipitates is one of the most important chemical properties of tannins (Shi et al., 2022). Ginger protein is a promising plant protein; therefore, this study selected ginger protein to interact with Rosa roxburghii tannins to form ginger protein-Rosa roxburghii tannin precipitates, achieving tannin removal and astringency reduction through precipitation separation.

The optimal process conditions for ginger protein tannin removal were determined to be: liquid-solid ratio 30:1.2 (mL:g), juice pH 3.0, reaction temperature 5 °C, and stirring time 30 min. Under these conditions, tannin removal rate was (47.451 ± 0.608)%, VC retention rate was (75.904 ± 1.244)%, and juice transmittance increased from (8.44 ± 0.662)% to (92.47 ± 0.297)%. The process demonstrated effective tannin removal, high VC retention, significant clarification, and enhanced flavor. Compared with existing studies, the tannin removal rate was relatively lower than that achieved with gelatin or tannase, possibly due to impurities such as starch in the extracted ginger protein, indicating the need for further purification. The relatively high VC retention rate can be attributed to the short processing time and low temperature, as VC is susceptible to oxidative browning at elevated temperatures.

Tannins possess bioactivities including antioxidant, antimicrobial, and blood glucose regulation effects (Huang et al., 2021), making complete removal from Rosa roxburghii juice neither necessary nor desirable, as excessive removal would compromise VC retention. The results demonstrate that the novel ginger protein process effectively reduces tannins and astringency while maximizing VC retention and providing excellent clarification. The technology is feasible, simple to operate, uses small additive amounts from plant extracts with high safety, and provides a new approach and technical foundation for solving the critical industry-wide challenge of astringency in Rosa roxburghii juice, while expanding the scope and technical support for comprehensive ginger resource utilization.

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Note: Figure translations are in progress. See original paper for figures.