

Research Article

Determination of the Content of Phenolic Compounds and the Changes of Polyphenol Oxidase and Each Index during Browning of *Phyllanthus emblica* at Different Storage Temperatures

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In order to further study the early browning of *P. emblica* marker and mechanism, it is necessary for us to measure the changes in the content of some substances to find quality markers during the browning process of *P. emblica*. So, we simulated the storage conditions under different temperatures, including 5°C, 25°C, and 35°C. And, the contents of some of its phenolic compounds were determined by RP-HPLC. Polyphenol oxidase (PPO) activity was determined by PPO reagent kits, and pH was determined with a pH measuring instrument, etc. The experimental results showed that 1-galloyl-glucose was the smoothest at 5°C among the three storage temperature conditions. It fluctuates and decreases in a curve at 35°C and 25°C. The content changes of gallic acid (GA) fluctuated more obviously at 35°C, and it showed a maximum value on the 7th day. The content changes of 1,3,6-tri-O-galloyl-β-D-glucose (TGG) all showed a peak on the 5th day and then showed a gradual decrease. The content changes of ellagic acid (EA) fluctuated more at 35°C. The peak of the content of rutin at both 35°C and 25°C appeared on the 3rd day, and that showed a gradually decreasing trend. The PPO activity varied more significantly at 25°C and 35°C. The browning index increased with time at both 35°C and 25°C. The complete browning of *P. emblica* was already observed on the 13th day. Tips for experimental results 5°C are effective in preventing browning of *P. emblica*. The contents of TGG, 1-galloyl-glucose, and rutin in *P. emblica* are greatly affected by temperature and time, and its contents had a linear relationship with time and temperature and can be used as one of the indicators of early browning, which provides a reference for the storage and transportation of fresh fruit in Chinese herbals.

1. Introduction

Phyllanthus emblica L. (syn: *Emblica officinalis* Gaertn.), commonly known as emblic leaf flower fruit, Indian gooseberry, or Amla, belongs to the genus *Phyllanthus* in the family *euphorbiaceae* [1]. The fruit is usually consumed when it is fresh and is also processed into food [2]. It is often used as food and traditional medicine, which is often used as herbal medicine by Indians because of its strong antioxidant activity and special therapeutic effect [3]. It is widely

distributed in the southwest and south China, including Guangxi and Yunnan. Yunnan is mainly distributed in arid climate areas [4]. *P. emblica* of fruit has been shown to contain many phytoconstituents, such as phenolics, flavonoids, alkaloids, flavone glycosides, and terpenoids [5]. *P. emblica* of fruit has many pharmacological effects, such as antioxidation, antitumor, antidiabetes, protecting the liver, and protecting the stomach [2]. For the effective utilization of traditional Chinese medicine resources, so many people began to study it. According to literature records, the whole

part of *P. emblica*, including roots, bark, leaves, flowers, fruits, and seeds was widely used in the folk medical system, such as the traditional system of Indian medicine (Ayurveda), Tibetan medicine, and traditional Chinese medicine. The minorities living in the southwest China use the root for the treatment of eczema, jaundice, and diarrhea, whereas, in Nepal, it is used as an astringent [2, 6].

Phenolic compounds are the most important active substances in *P. emblica*, and it leads to change of color, taste, and aroma of fresh food. These can reduce the sensory quality of food and change the nutritional properties of food and result in shortened shelf life [7]. At present, the research of fresh fruit postharvest browning mechanism has not been fully elucidated, and some researchers agreed that the fruit browning is mainly by existing in the flesh of PPO and peroxidase (POD) involved in the enzymatic browning occurrences. Besides, the theory of regional distribution of phenol-phenolase has been widely recognized by the academic community. So, according to this theory, there is a finding that PPO and phenolase in fruits are distributed in different cell regions, and if they have no contact under normal circumstances, they will not lead to the browning phenomenon. Therefore, when PPO and the distribution area of phenolic substances are damaged, PPO will catalyze the oxidation of phenolic substances to quinones with the participation of reactive oxygen species, and by polymerization, browning products are generated, causing the browning of flesh [8]. In the area with the large temperature difference between day and night, browning is extremely easy to occur, and the antioxidant components in the fruit are reduced, which make the cell change and easier to induce the enzymatic browning of the fruit. Rayan A and Morsy N reported [9] that processed pomegranates have found that browning is a result of phenolic compounds oxidation, and enzymatic browning can lead to extreme changes in flavor, resulting in nutrient loss and quality decline. Browning of *P. emblica* is common, which will affect the whole production process of its collection, storage, processing, and finished products, resulting in shortening storage time, increasing processing difficulty and quality assurance of finished products, and it can reduce product quality and efficacy. At present, there are few studies on the browning mechanism of *P. emblica* in China, and the means of prevention and control are limited, which restricts the development of related food enterprises and pharmaceutical factories and industries in Yunnan, a province with large resources of *P. emblica*. This experiment is based on previous research and consulting relevant literature [3,10–14], and we found that GA, ascorbic acid, EA, rutin, quercetin, and catechol are the main compounds in these fractions. In order to find more quality control compounds, we choose the 1-galloyl-glucose, GA, TGG, EA, and rutin as detection indicators. And, after many attempts of different experimental schemes, we use the methanol extraction and RP-HPLC detection scheme for sample preparation and detection. According to the climatic characteristics of each region, we set three different temperature conditions to observe

changes, and the indexes of fresh fruit browning of *P. emblica* were analyzed, respectively. The main goal of this study was the effects of browning area on its main chemical component contents, which provide a reference for the storage and transportation of fresh fruit herbs.

2. Materials and Methods (Materials, Devices, and Methods)

2.1. Materials

2.1.1. Plant Material. *P. emblica* was collected from Yunxian County, Lincang City, Yunnan Province, in April 2021. It was picked by Yunxian County Tourism Bureau, Lincang City, Yunnan Province. The base of the fruit stalk and suture line appears slightly sunken, and the fruit tip is mostly yellow-green in colour, belonging to the star-shaped group of wild *P. emblica* in Yunnan [15]. The morphology can be found in Figure 1.

2.1.2. Reagents. Acetonitrile (chromatographic pure), methanol (chromatographic pure), methanol (analytical pure), and phosphoric acid (analytical pure) were all purchased from Yunnan Danchi Trading Co., Ltd. GA was purchased from Sichuan Weiqi Biotechnology Co., Ltd. (batch no.: WKQ16081904), 1-galloyl-glucose was purchased from Shanghai Yuyuan Biotechnology Co., Ltd. (batch no.: K28J12B136898), TGG was purchased from Wuhan Tianzhi Biotechnology Co., Ltd. (batch no.: CFS201802), EA was purchased from Dalian Meilun Biological Technology Co., Ltd. (batch no.: M0104AS), and rutin from Shanghai Yeyuan Biological Technology Co., Ltd. (batch no.: Y24F11Y17051). PPO activity detection kit was purchased from Beijing Solarbio Technology Co., Ltd. Phosphate-buffered saline (PBS, pH 7.2) was supplied by Beijing Solarbio Technology Co., Ltd.

2.1.3. Instruments and Equipment. The RP-HPLC analysis was performed using Agilent 1200 high-performance liquid chromatography, the bonded silica gel column was Agilent Zorbax C18 (150 × 4.6 mm, 5μm) (Agilent, Inc., USA), the ultrasonic cleaning machine was YL-040S road (Shenzhen namely clean ultrasonic technology co., LTD.), the electric thermostatic water bath was D2KW-D (Shanghai Ailang Instrument Co., Ltd.), and rotary evaporator (Shanghai Liangyi Scientific Instrument Co., Ltd.), a T-10900 type electronic balance (Shanghai Puchun Measurement Instrument Co., Ltd.), a PHS-25 pH measuring instrument (Shanghai Instrument Instrument Co., Ltd.), and refrigerator (Hefei Meiling Co., Ltd.).

2.1.4. Treatment Methods of Fresh Fruit. The fresh fruits were divided into three parts, put into brown bottles, sealed away from light, and placed in 5°C, 25°C, and 35°C incubators, respectively. The changes in fruit indexes at three temperatures were detected every day.

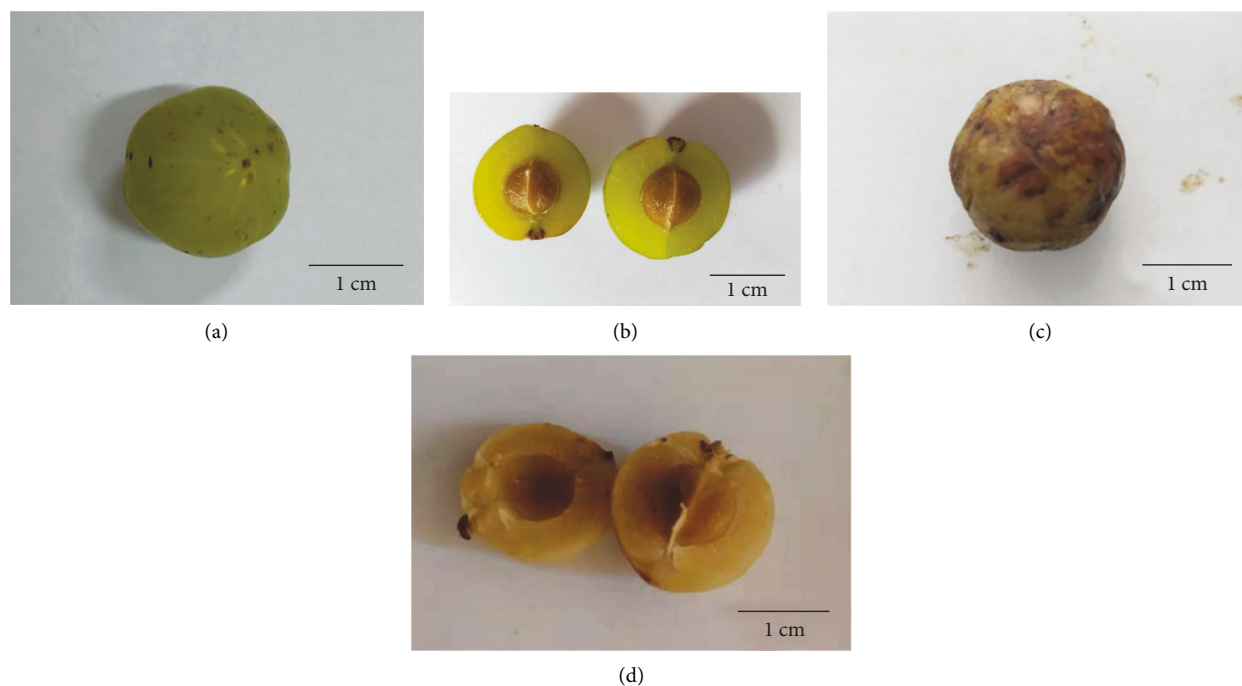


FIGURE 1: The comparison of the browning area ((a, b) grade 0, and (c, d) grade 4). Note: when the browning index is 1, it can be expressed as complete browning.

2.2. Hydrolyze Tannins Assay Methods

2.2.1. RP-HPLC Conditions. Chromatographic conditions are based on a literature review and multiple experimental studies [1, 16]. The chromatographic separation was carried out with the ZORBAX C18 column (250 × 4.6 mm, 5 μm). The flow rate was 1 ml/min. The column temperature was set at 25°C. Ten microliters of each sample were injected into the column. The quantitative wavelength was 270 nm. Elution was conducted using acetonitrile and 0.1% phosphoric acid water (A) and acetonitrile (C). The gradient elution program was 0 ~ 5 min, 5% C; 5 ~ 6 min, 5% C ~ 15% C; 6 ~ 15 min, 15% C ~ 15% C; and 15 ~ 25 min, 15% C ~ 30% C. The chromatogram of standards and sample is shown in Figure 2.

2.2.2. Preparation of Solution

(1) Preparation of Reference Products. 0.17 mg/ml, 0.41 mg/ml, 0.08 mg/ml, and 0.40 mg/ml reference solutions of 1-galloyl-glucose, GA, TGG, and EA were prepared by dissolving accurately weighed 1-galloyl-glucose, GA, TGG, and EA in methanol, respectively.

(2) Sample Preparation. It was extracted according to the description. Put 5 g of accurately weighed samples into a round bottom flask with an appropriate amount of quartz sand and grind them at 5°C, 25°C, and 35°C and then add 50 ml of 50% methanol solution and reflux for 1.5 h. After cooling, it is 45°C ultrasonic for 1 h. To compensate for the loss of weight, we add a 50% solution of methanol, and then the solution is shaken and filtered with a 0.45 μm syringe membrane.

2.3. Flavonoids Assay Methods

2.3.1. RP-HPLC Conditions. The chromatographic separation was carried out with the ZORBAX C18 column (250 × 4.6 mm, 5 μm). The flow rate was 0.8 ml/min. The column temperature was set at 30°C. Ten microliters of each sample were injected into the column. The quantitative wavelength was 327 nm. Elution was conducted using methanol and 0.1% phosphoric acid water (A) and methanol (B). The gradient elution program was 0 ~ 2 min, 22% B; 2 ~ 8 min, 60% B; and 8 ~ 20 min, 22% B ~ 60% B. The chromatogram of standards and sample are shown in Figure 3.

2.3.2. Preparation of Solution

(1) Preparation of Reference Solution. 0.08 mg/ml reference solution of rutin was prepared by dissolving accurately weighed rutin in methanol, respectively.

(2) Sample Preparation. It was extracted according to the description. Respectively, 5 g of accurately weighed sample was placed in the round-bottomed flask at 5°C, 25°C, and 35°C, and then 50 ml of 75% methanol solution was added and refluxed for 1.5 h. After cooling, 75% methanol solution was added to make up for the loss of weight. Then, the solution was shaken and filtered with a 0.45 μm syringe membrane.

2.4. Determination of PPO Activity. According to the pre-selection of PPO as an indicator, we refer to Liu and others' [17] reagent sources, and the experimental method was performed according to the PPO reagent kits instructions.

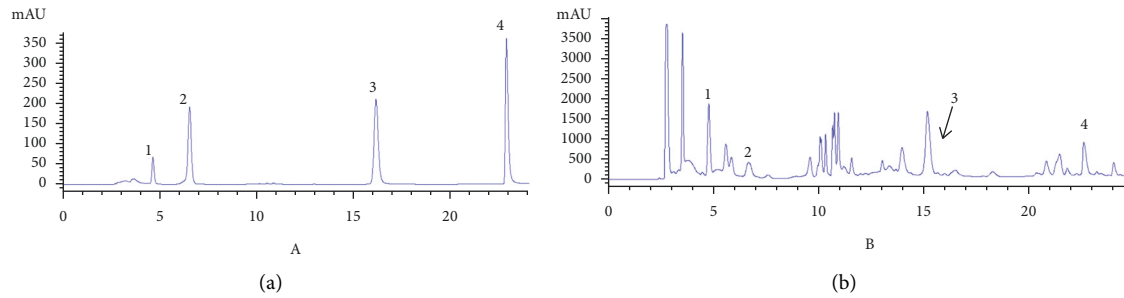


FIGURE 2: RP-HPLC chromatogram of 1-galloyl-glucose, GA, TGG, and EA (Note: A, reference product; B, test product; 1, 1-galloyl-glucose; 2, GA; 3, TGG; 4, EA).

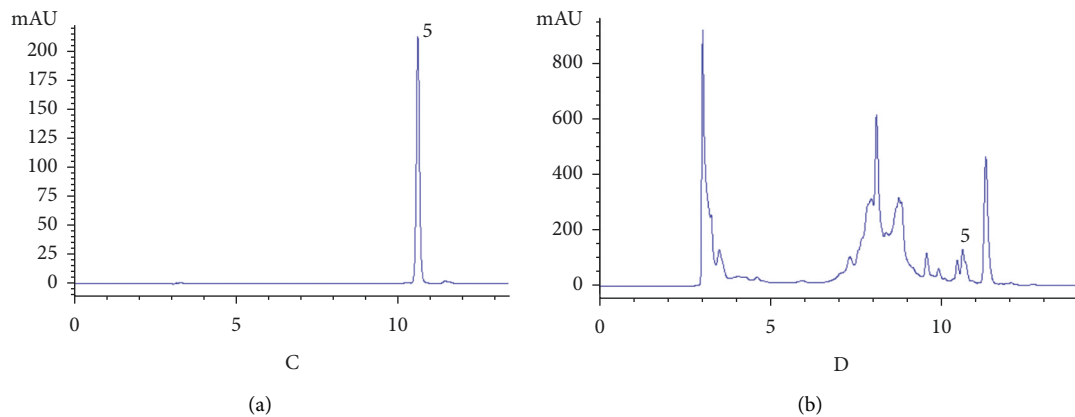


FIGURE 3: RP-HPLC chromatogram of rutin (note: C and D are reference substances; 5, rutin).

Respectively, 0.1 g of cored residual fruit pulp was removed at 5°C, 25°C, and 35°C, placed in a 2 ml EP tube, and then added 1 ml extracting. The supernatant was kept in an ice bath and centrifuged at a high speed (8000 g, 4°C, 10 min). Take 250 μ l supernatant and soak it in the water bath at room temperature for 10 min, followed by a boiling water bath for 10 min. The control group and sample group were set. Control group added reagent A 600 μ l, reagent B 150 μ l, and extract 150 μ l. Sample group added reagent A 600 μ l, reagent B 150 μ l, extract sample solution and mix, and then we set 6 repeated holes, 100 μ l for each hole, PBS edge seal, put on A 96-well plate. Then, the absorbance was measured at 410 nm, and the activity of PPO was calculated. In the experiment, PPO was measured and calculated once every two days, and the calculation formula of PPO is $PPO = \Delta A \cdot 60 / 0.1$ obtained.

2.5. Determination of Browning Index. The purpose of measuring the browning index is to observe the browning degree of the fruit at 5°C, 25°C, and 35°C and compare other indexes to study the changing phenomenon and process according to this index. Crosscutting from the center of the fruit core of *E. molluscera*, grades were classified according to the corresponding browning degree and area of the flesh tissue on the cross section.

The browning area below 20% is classified as grade 2, 20–50% is classified as grade 3, and more than 50% is classified as grade 4 [18], as can be seen in Figure 1. Each time count 10 fruits. Calculate the browning index of *P. emblica*:

$$\text{the browning index} = \frac{\sum \text{browning grade} \times \text{number of grade fruits}}{\text{number of highest grade} \times \text{statistical fruits}} \quad (1)$$

2.6. Determination of pH. Accurately weigh 10 g of enucleated *P. emblica*, take an appropriate amount of quartz sand, grind it evenly at 5°C, 25°C, and 35°C, transfer it to a triangular flask, and add 20 ml of experimental water. The water bath at 80°C for 30 min was cooled and filtered as

reserve liquid. Take the reserve solution to measure the pH with a pH measuring instrument.

2.7. Statistical Analysis. All experiments were repeated at least in triplicate. Results are expressed as the

mean \pm standard deviation. Data were analyzed using SPSS software (IBM, SPSS, Statistics).

3. Results and Discussion

3.1. Hydrolyze Tannins

- (1) Hydrolyzed tannins chromatogram
- (2) Preparation of standard calibration curves and analysis of linearity

Six different concentration levels of mixed reference solutions were analyzed in this research. The six concentrations of 1-galloyl-glucose, GA, TGG, and EA solutions were 0.26, 0.53, 0.79, 1.06, 1.33, and 1.59; 0.81, 1.62, 2.44, 3.25, 4.07, and 4.88; 0.13, 0.27, 0.40, 0.54, 0.68, and 81; 0.79, 1.58, 2.37, 3.16, 3.96, and 4.75 $\mu\text{g}/\text{mg}$, respectively. Calibration curves were generated based on the formula: $Y = aX + b$, among which the Y -axis was the value of peak area (mAU) and the X -axis was the content ($\mu\text{g}/\text{mg}$) injected into the RP-HPLC column of each standard. The regression equations of 1-galloyl-glucose, GA, TGG, and EA calibration curves and the parameters for linearity are presented in Table 1. The correlation coefficients were over 0.998, which indicated good linearity under each range.

- (3) Precision

Precisely absorb the same mixed reference solution under optimized chromatographic conditions, filter with $0.45 \mu\text{m}$ microporous membrane, inject $10 \mu\text{l}$ samples, and continuously inject 6 times according to the chromatographic conditions under Section 2.2.1. Results of RSD of 1-galloyl-glucose, GA, TGG, and EA were 0.64%, 0.22%, 0.20%, and 0.25%, respectively, which indicated good precision of the instrument.

- (4) Stability

The test product solution was prepared according to the method under Section 2.2.2 and placed at room temperature for 0, 2, 4, 6, 8, and 10 h. The chromatographic conditions under Section 2.2.1 were followed for programmed elution using the peak area as the index. The RSD results of these indicators were 3.02%, 3.17%, 3.60%, and 1.81%, respectively, which indicated that the tested products were stable at room temperature for 10 h.

- (5) Repeatability

The same batch of fresh fruits was taken to prepare the test solution according to method (2) under Section 2.2.2, and the chromatographic conditions under Section 2.2.1 were programmed elution. Results of RSD values of 1-galloyl-glucose, GA, TGG, and EA were 0.36%, 0.53%, 2.50%, and 0.67%, respectively. The results showed that the preparation method had good repeatability.

- (6) Sampling recovery

TABLE 1: Linear equation, R^2 , and range of 1-galloyl-glucose, GA, TGG, and EA for quantitative determination.

Compound	Regression equation	R^2	Range (μg)
1-Galloyl-glucose	$Y = 3733.8X - 96.803$	0.9997	0.26–1.59
GA	$Y = 1783X + 226.4$	0.9986	0.81–4.07
1,3,6-Tri-O-galloyl- β -D-glucose	$Y = 1168.2X - 73.014$	0.9991	0.14–0.82
EA	$Y = 1714.3X + 71.053$	0.9992	0.79–4.75

R^2 means coefficient of determination. The above data are all greater than 0 and less than 1, showing that the model fits well.

Six parts of *P. emblica* were carefully weighed respectively, crushed in a mortar, each part was precisely weighed at 5 g, and the sample solution was prepared according to the method in the experimental scheme. The average recovery of 1-galloyl-glucose, GA, TGG, and EA was calculated by adding 1-galloyl-glucose, GA, TGG, and EA into the sample under the same chromatographic condition, and the test solution was prepared according to the method in the experimental protocol. The results are shown in Table 2.

- (7) Changes in the content of hydrolyzed tannins

Fresh *P. emblica* was stored at 35°C , 25°C , and 5°C . The variation of its content is shown in Figure 4. The content of 1-galloyl-glucose reached its maximum on the 1st day at 35°C , and it showed a decreasing trend as the storage time was extended and reached its minimum on the 15th day. The content reached its maximum on the 1st day and showed a decreasing trend as the storage time was extended at 25°C , and it reached its minimum on the 15th day. The content of 1-galloyl-glucose reached the maximum on the 5th day and the minimum on the 3rd day at 5°C , and its content showed a curve fluctuation trend throughout the storage process. The content of 1-galloyl-glucose decreased gradually with storage time, probably due to their unstable nature and susceptibility to decomposition. 1-Galloyl-glucose showed no significant decrease in content at 5°C , indicating that its content has less effect at this temperature. *P. emblica* is one of the main ingredients of the Triphala from Tibetan medicine, which is used to treat diabetes. One researcher found [19] that 1-galloyl-glucose, GA, and others were rapidly identified as potential α -glucosidase inhibitors present in defatted seeds of evening primrose. Therefore, it is necessary to control the quality of the active ingredients in *P. emblica*.

The variation of its content is shown in Figure 5, and the GA content reached the maximum value on the 7th day and the minimum value on the 13th day at 35°C , showing a fluctuating trend. The content reached the maximum value on the 5th day and the minimum value on the 15th day that showed an increasing trend and then with a sudden decreasing

TABLE 2: Recovery of 1-galloyl-glucose, GA, TGG and EA from *P. emblica*.

Composition	Sampling quantity (g)	Sample content (μg)	Amount of addition (μg)	Measured quantity (μg)	Recovery rate (%)	Average recovery (%)	RSD (%)
1-Galloyl-glucose	5.0003	1.5632	1.5452	3.0106	98.84	100.69	3.11
	4.9978	1.6204	1.6050	3.0702	99.04		
	5.1104	1.5811	1.6040	2.9231	101.44		
	5.0639	1.6061	1.5689	2.9703	97.67		
	4.9987	1.5524	1.5521	3.0228	99.98		
	5.0629	1.5229	1.6319	3.0192	107.15		
GA	5.0003	0.8952	0.9547	2.2508	106.64	102.51	3.54
	4.9978	0.9124	0.8964	2.1056	98.24		
	5.1104	0.9703	1.0246	2.1986	105.58		
	5.0639	0.9192	0.9092	2.2570	98.91		
	4.9987	0.9593	1.0256	2.3822	105.58		
	5.0629	0.9680	0.9666	2.2334	99.85		
TGG	5.0003	0.9067	0.9658	2.1184	106.51	101.88	2.52
	4.9978	0.9100	0.9292	2.1058	102.11		
	5.1104	0.9206	0.9173	2.4543	99.63		
	5.0639	0.9094	0.9103	2.4537	100.10		
	4.9987	0.8982	0.9296	2.0560	103.49		
	5.0629	0.9284	0.9234	2.0414	99.46		
EA	5.0003	1.2229	1.2978	2.9731	106.12	97.48	5.36
	4.9978	1.2975	1.2229	3.3873	94.24		
	5.1104	1.2020	1.1287	3.4569	93.89		
	5.0639	1.2121	1.0893	3.4486	89.87		
	4.9987	1.2782	1.2831	3.2424	100.38		
	5.0629	1.2350	1.2396	2.9682	100.37		

RSD means relative standard deviation.

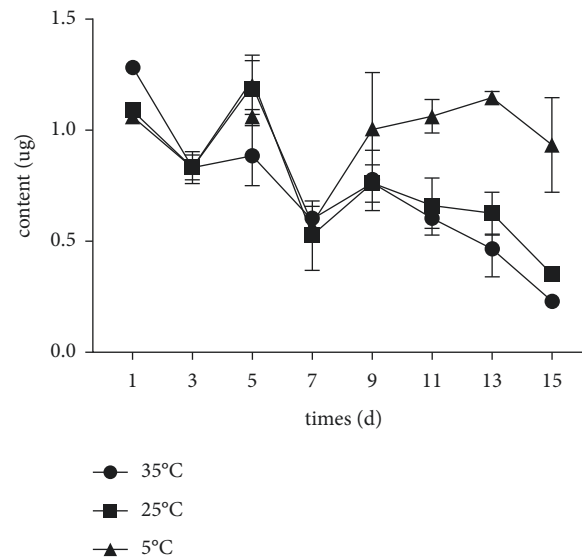


FIGURE 4: The content change curve at different temperatures of 1-galloyl-glucose.

trend on the 5th day at 25°C. GA reached the maximum value on the 11th day and the minimum value on the 1st day, showing a gradually increasing trend, with a decreasing inflection point on the 13th day at 5°C. There was a decreasing inflection point on the 13th day. The results from the previous experiment [20] showed that there is a gradual increase in GA content with increasing storage time, and it may

be due to the decomposition of 1-Galloyl glucose and TGG to produce GA, which differed from the results of this experiment that is possibly due to differences in extraction solvents during the extraction process, or the different sources of residual glucose, which have different composition content and tolerance.

The highest content of TGG on the 5th day, as shown in Figure 6 then gradually declined, the content

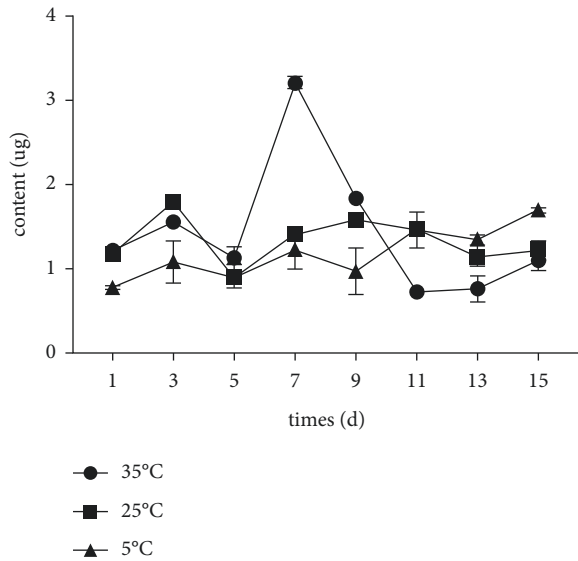


FIGURE 5: The content change curve at different temperatures of GA.

reached the minimum on the 15th day at 35°C, and the content reached the maximum on the 1st day, showing a curve fluctuation; then, the content gradually declined, the content reached the minimum on the 15th day at 25°C, and the content reached the maximum on the 5th day, the lowest content on the 13th day at 5°C, the overall curve fluctuation. The variation of its content is shown in Figure 6. The content of TGG decreased gradually with storage time, and it is probably due to the unstable nature and susceptibility to decomposition. Therefore, the change of the content of TGG was not affected by temperature.

The EA reached the maximum on the 5th day and a minimum on the 15th day, with a trend of curvilinear fluctuations at 35°C. As shown in Figure 7, the maximum was reached on the 5th day and the minimum on the 7th day at 25°C. The variation of its content is shown in Figure 7. There was an overall increasing trend in EA content and no significant difference between the three temperature conditions, indicating that EA is stable in nature, and these changes in content are not affected by temperature.

3.2. Flavonoids

- (1) Chromatogram of flavonoids
- (2) Preparation of standard calibration curves and analysis of linearity

Six different concentration levels of mixed reference solutions were analyzed in this research. The six concentrations of rutin solutions were 0.16, 0.32, 0.48, 0.64, 0.80, and 0.96 ug/mg, respectively. Calibration curves were generated based on the formula: $Y = aX + b$, among which the Y-axis was the value of peak area (mAU) and the X-axis was the content (ug/mg) injected into the RP-HPLC column of each

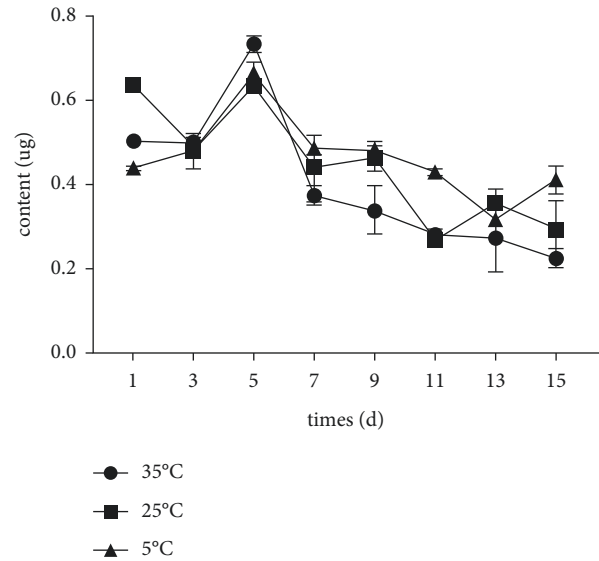


FIGURE 6: The content change curve at different temperatures of TGG.

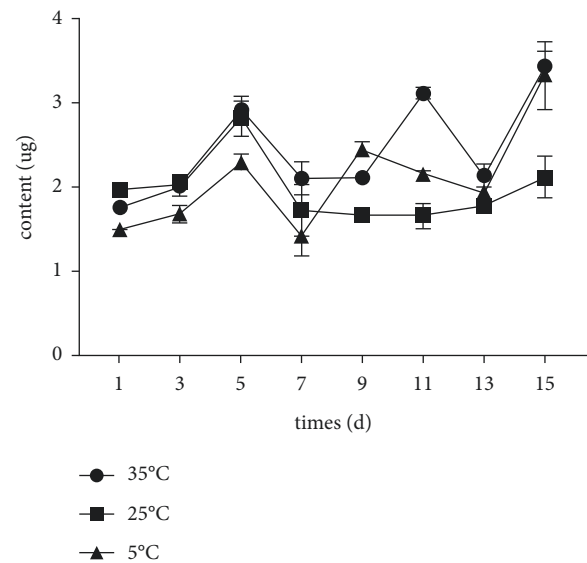


FIGURE 7: The content change curve at different temperatures of EA.

standard. The regression equations of rutin calibration curves and the parameters for linearity are presented in Table 3. The correlation coefficients were over 0.998, which indicated good linearity under each range.

- (3) Precision

The same mixed reference solution under (1) of Section 2.3.2 was accurately absorbed, filtered by 0.45 μm microporous filter membrane, and injected 10 μL . According to the chromatographic conditions under Section 2.3.1, the sample was injected continuously 6 times, and the RSD value of rutin was 0.27%, which indicated that the precision of the instrument was good.

TABLE 3: Linear equation, R^2 , and range of rutin for quantitative determination.

Compound	Regression equation	R^2	Range (μg)
Rutin	$Y = 1513.8X - 9.24$	0.9992	0.16–0.96

R^2 means coefficient of determination. The above data are all greater than 0 and less than 1, showing that the model fits well.

TABLE 4: Rutin's sample recovery experiment.

Composition	Sampling quantity (g)	Sample content (μg)	Amount of addition (μg)	Measured quantity (μg)	Recovery rate (%)	Average recovery (%)	RSD (%)
Rutin	5.0021	0.8027	0.8040	1.6332	100.16	100.73	4.96
	5.0349	0.7671	0.8126	1.7302	105.94		
	4.8830	0.8146	0.8688	1.7240	106.64		
	4.9632	0.7770	0.7962	1.7212	102.47		
	5.1203	0.8173	0.7877	1.7231	96.37		
	3.2361	0.8708	0.8079	1.7244	92.77		

RSD means relative standard deviation.

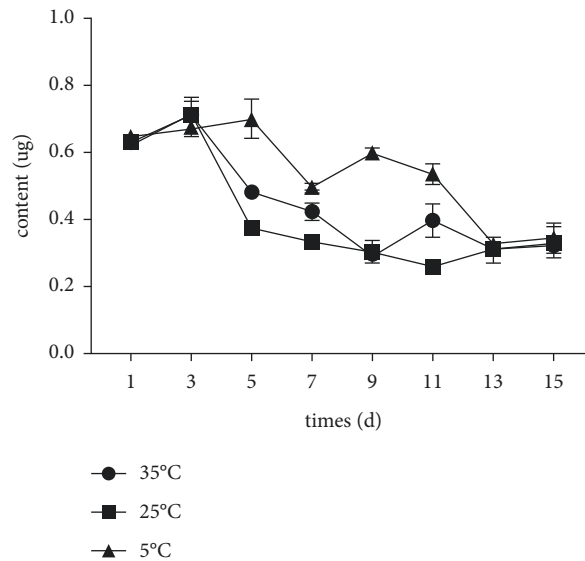


FIGURE 8: Changes in flavonoid content at different temperatures.

(4) Stability

The test solution was prepared according to the method under (2) of Section 2.3.2 and placed at room temperature for 0, 2, 4, 6, 8, and 10 h. The chromatographic conditions under Section 2.3.1 were used for programmed elution using the peak area as the index. The RSD value of rutin was 3.92%, indicating that the test product was stable at room temperature for 10 h.

(5) Repetitive

The same batch of fresh *Radix Eupatorium* L. samples was taken to prepare the test solution according to the method under (2) of Section 2.3.2, and the chromatographic conditions under Section 2.3.1 were programmed to elute. The RSD values of rutin were 0.24%, respectively, indicating that the preparation method had good reproducibility.

(6) Sampling recovery

Six parts of *P. emblica* were carefully weighed, respectively, crushed in a mortar, each part was precisely weighed at 5 g, and the sample solution was prepared according to the method in the experimental scheme. The average recovery of rutin was calculated by adding rutin into the sample under the same chromatographic condition, and the test solution was prepared according to the method in the experimental protocol. The results are shown in Table 4.

(7) Changes in the content of rutin

The content of rutin, a flavonoid, changed at different temperatures, as shown in Figure 8. The rutin content increased first and then decreased, and it reached the maximum on the 3rd day and the minimum on the 9th day at 35°C. The trend was

TABLE 5: Changes of PPO enzyme activity at different temperatures.

Temperature (°C)	Times (d)														
	1	3	5	7	9	11	13	15							
35	35.14 ± 0.0038	17.96 ± 0.0024	23.54 ± 0.0115	18.9 ± 0.0007	93.66 ± 0.0017	90.3 ± 0.0052	77.838 ± 0.0015	65.118 ± 0.0047							
25	24.42 ± 0.0080	18.72 ± 0.0045	18.02 ± 0.0037	32.42 ± 0.0018	54.36 ± 0.0076	77.42 ± 0.0052	69.418 ± 0.0011	85.898 ± 0.0042							
5	18.66 ± 0.0078	12.54 ± 0.0008	9.56 ± 0.0028	34.72 ± 0.0019	35.62 ± 0.0024	23.66 ± 0.0046	16.278 ± 0.0005	15.798 ± 0.0009							

similar to that at 35°C, and the content reached the maximum value on the 3rd day and the lowest value on the 11th day at 25°C. The rutin content showed curve fluctuation, reaching the maximum on the 5th day, and the lowest on the 13th day at 5°C.

The flavonoid compounds showed a gradual decrease during storage. As can be seen from the graph, the two groups at 35°C and 25°C showed a faster change in content than those at 5°C, indicating that the content of rutin is easily affected by the temperature. There was no significant difference in the change of rutin content at 35°C and 25°C, indicating that the two temperatures have little effect on the content change. Flavonoids are also one of the substrates of browning production, but the mechanism of their involvement in browning is not fully understood. In this experiment, rutin was selected as a marker, and the results showed that its content gradually decreased as the storage time increased, probably due to the decomposition of rutin as the temperature increased. So, the results of the browning index means that low temperature is more suitable for the storage of fresh rutin, and the low temperature can effectively prevent the occurrence of browning.

3.3. Change in PPO Activity. As can be seen from Table 5, the PPO activity reached the maximum on the 15th day and the minimum on the 1st day in the whole storage process. The whole thing is curvilinear. The results showed an overall fluctuating upward trend in PPO at 35°C and 25°C. However, there is a large change around the 7th day at 5°C, and the enzyme activity is the highest around the 7th and 9th day and then falls back to a stable trend. Jin and others [19] reported that there is a positive correlation between fresh peach browning and antioxidant enzyme activity, such as superoxide dismutase (SOD), catalase (CAT), and alternative oxidase (AOX), in horticultural crops. Combined with the trend of polyphenol content, it can be explained that, in fact, the 7th day is the key time node of browning.

3.4. Change in Browning Index. During storage for 15 days, the browning index increased gradually at 35°C and 25°C, and the fruits became completely brown on the 13th day. As shown in Figure 9, the browning index is relatively stable at 5°C. The results showed that the browning index showed an increasing trend from the first day of storage, with a sudden drop at 35°C and 25°C on the seventh day, and then followed by a rise, with the more obvious changes in the two groups with higher temperatures, and finally complete browning in both groups. These showed that low-temperature storage was beneficial to prevent browning of fresh traditional Chinese medicine.

3.5. Change in pH. The change in pH is shown in Figure 10. Under the three storage temperatures, the changing trend is relatively closed, and the biggest fluctuation is at 35°C. On the 11th day, three temperature conditions showed a sudden drop followed by a rise. pH trend, in which the results

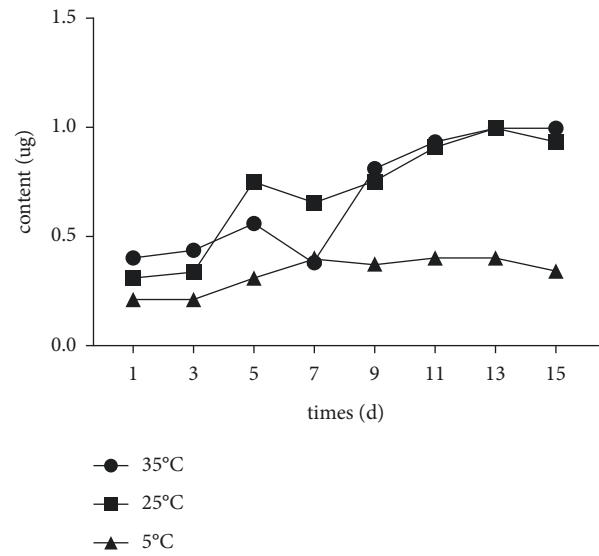


FIGURE 9: The browning index changes.

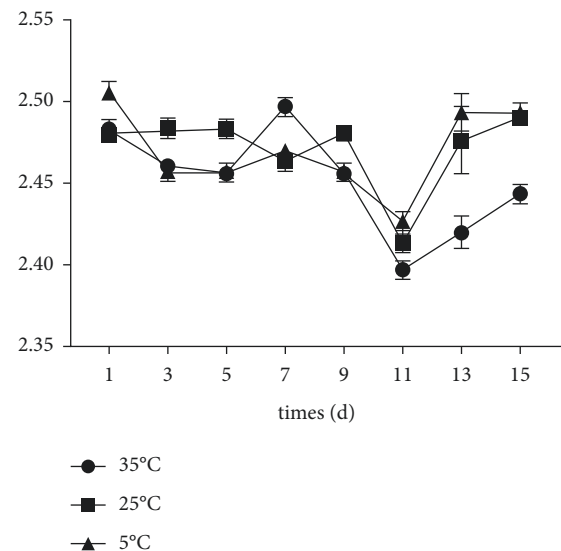


FIGURE 10: Change in pH.

showed a sudden drop on the 11th day, is not yet clear in this experiment and will be further studied.

4. Conclusion

Browning is one of the main reasons for quality loss in *P. emblica* after harvest during processing. The enzymatic browning often occurs, and it is easily leading to discoloration, off-flavor formation, and reduction of nutritional value [21]. Most of the current researches on browning are focused on the field of fresh fruits and vegetables, and researchers mostly use physical or chemical methods to resist browning, such as adding antibrowning agents sulfites, and honey [22,23]. *P. emblica* is widely used as food and medicinal materials in China and southeast Asia. At present, the main methods used to control its browning are physical and

chemical control, but there is little research on its browning mechanism, and fewer studies have been reported on the browning of the fresh herb *P. emblica*. Our group found [24] that GA, tannic acid, 1,2,3,4,6-penta-O-galloyl- β -D-glucose (β -PGG), and the substrates of *P. emblica* could promote enzymatic and nonenzymatic reactions, and it screened GA, tannic acid, β -PGG, and maltose as indicators of early browning of *P. emblica*. Besides, we found that the change of PPO activity in *P. emblica* was related to titratable acid during browning.

This experiment simulated storage conditions under different environments and assayed the content of some of its compounds. We determined the changes of phenolic compounds in the storage of fresh *P. emblica* and analyzed each index. We found that the content of TGG, 1-galloyl-glucose, and rutin in *P. emblica* are greatly affected by temperature and time, and its contents has a linear relationship with time and temperature. In summary, the experimental results show that the browning degree of *P. emblica* was the lowest at 5°C, and the content of browning makers changed stably. So, the compounds of the results can be used as one of the indicators of early browning, and it provides a reference for the storage and transportation of fresh fruit in Chinese herbal medicine.

Data Availability

The data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Rong Li and Shuang Guo contributed equally to this work.

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