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Content Determination of Vitamin C in Durian Endocarp and Optimization of Extraction Process

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Abstract

Purpose: To optimize the extraction process of vitamin C from durian endocarp, and to determine the content of vitamin C in durian endocarp with high performance liquid chromatography (HPLC). **Method**: Optimize ultrasonic extraction conditions by L9 (4^3) orthogonal experiment by using octadecylsilane chemically bonded silica as the stationary phase, using methanol (A): 0.1% oxalic acid (B) = 5:95 (V/V) as the mobile phase, setting the flow rate as 1.0 mL/min, and setting the wavelength as 254 nm for assay. **Result**: The optimal extraction process is as follows: the material-liquid ratio is 1:12, the extraction solvent is 2% oxalic acid aqueous solution, and the extraction time is 30 min; Vitamin C has a good linearity within the concentration range of 5.4 - 108.0 mg·mL^{-1}, and the regression equation is $y = 37698x - 61035$ ($R^2 = 0.9996$); the average recovery rate is 99.03%, the instruments are of high precision with good stability. **Conclusion**: This extraction process performs well in simplicity, costs and extraction efficiency, which may accelerate the development and utilization of vitamin C extraction from durian endocarp, and provide references to relevant studies and practices.

Keywords

HPLC, Orthogonal Experiment, Vitamin C, Durian Endocarp

1. Introduction

Durian [*Durio zibethinus* (L.) Murr.] is the fruit of several tropical deciduous arbors belonging to the Bombacaceae. As the food with great nourishing value, the pulp of durian is well known as the "King of Fruits". It can promote the physical health of people by enhancing spleen and kidney functions, promoting intestinal peristalsis, and harmonizing qi and blood [1]. Durian is hot in property, which can

promote blood circulation and dispel cold, as well as relieve menstrual pain. As a result, it is especially suitable for women suffering from dysmenorrhea. Durian is also an ideal tonic for people with cold corporeity. It can not only relieve abdominal cold symptoms but also accelerate the rise of body temperature.

Vitamin C is also known as ascorbic acid [2]. As one of the essential nutrients for the human body that extensively exists in fresh vegetables and fruits, it has the functions of clearing heat and removing toxicity, resisting cancer, and scavenging free radicals [3] [4]. Vitamin C can prevent colds, accelerate the formation of collagen, reduce the content of low-density lipoprotein and triglyceride, increase the content of high-density lipoprotein and cholesterol, promote the metabolism of cholesterol, reduce the synthesizing rate of cholesterol, prevent lipid oxidation, and other physiological functions [5] [6] [7] [8]. As stated in previous texts, vitamin C extensively exists in many fruits. Specifically, durian contains vitamin A, vitamin B, vitamin C, calcium, potassium, iron, phosphorus and other nutrients. Durian peel accounts for more than 60% of the weight of durian fruit, while few people know that durian endocarp contains a large amount of vitamin C. In this experiment, the orthogonal experiment is conducted to optimize the extraction method of vitamin C in the durian endocarp, and HPLC analytical method is adopted to determine the content of vitamin C in the durian endocarp, which provided a reference and theoretical basis for the development and utilization of durian endocarp.

2. Reagent and Instruments

2.1. Reagent

Durian endocarp (all purchased from a fruit store called "Fresh Fruit Partner" in Baise, Guangxi); L(+)-ascorbic acid (Guangdong Guanghua Sci-Tech Co., Ltd., batch No.: 20200110); methanol (Chengdu Knowles Technology Co., Ltd., chromatographic agent); oxalic acid (Foshan Xilong Chemical Co., Ltd., batch No.: 2008151, analytical reagent); the water adopted is ultra-purified water.

2.2. Instruments

Chromatograph: LC-20A high-performance liquid chromatograph (Shimadzu Corporation, Japan); JJ224BC electronic libra (Changshu G&G Measurement Plant); HC-5002S ultrasonic cleaner (Kunshan Huichao Automation Equipment Co., Ltd.); Chromatographic column: Shimadzu InertSustain C18 (4.60 × 150 mm, 5 μm); Elite Supersil ODS2 (4.60 × 200 mm, 5 μm); 0.22 μm water system micron-level microporous filter membrane (Tianjin Navigator Lad Instrument Co., Ltd.); Pipetting gun (Shanghai Qiujing Biochemical Reagent Instrument Co., Ltd.).

3. Experiment Method

3.1. Solution Preparation

3.1.1. Preparation of Vitamin C Reference Solution Precision

Accurately weigh L(+)-ascorbic acid 0.0050 g in a 50 mL volumetric flask, add a

small amount of purified water, shake the flask to dissolve the solution completely, and use purified water to stabilize the volume. Therefore, the vitamin C reference solution with a concentration of 10 mg·mL^{-1} is obtained. Filter the solution by 0.22 μm aqueous phase filter membrane, and the filtrate is injected into the instrument for assay.

3.1.2. Preparation of Test Solution

Take the durian shell, cut down the durian endocarp, wash it, and absorb excess water with a piece of absorbent paper. Next, accurately weigh 5.0 g of durian endocarp, cut it into pieces, put it in a dry conical flask, add 250 mL oxalic acid solution, and weigh the solution. Next, place it in the ultrasonic instrument for 300 w, then conduct extraction at 25˚C at room temperature for 10 - 30 min, cool it to room temperature, and make up the missing weight with oxalic acid. Finally, filter it, take the supernatant and filter it with 0.22 μm microporous membrane.

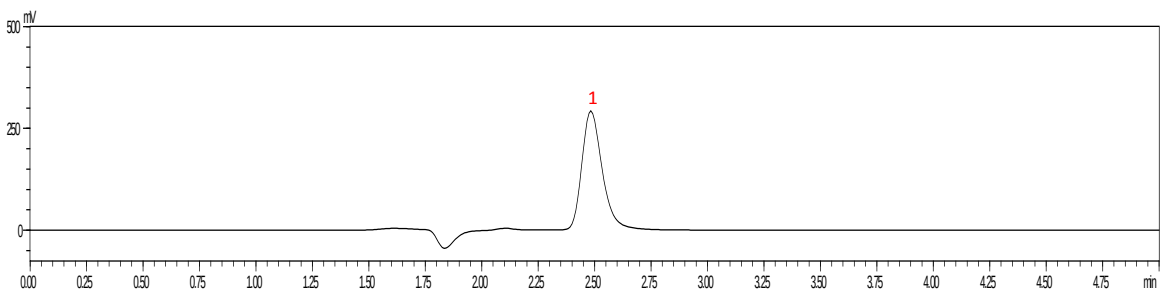
3.2. Chromatographic Conditions and System Applicability Test

The chromatographic column adopted in this experiment is Shimadzu InertSustain C18 (4.60 × 150 mm, 5 μm). Mix methanol (A) and 0.1% oxalic acid (B) in the ratio of 5:95 (V/V) as the mobile phase; control the flow rate as 1 mL·min^{-1}, and conduct constant-gradient elution for 5 min; control the column temperature as 30˚C; control the sample injection amount as 10 μL and the assay wavelength as 254 nm. Under these above chromatographic conditions, all detected components reach baseline separation, and the number of theoretical plates is >2000.

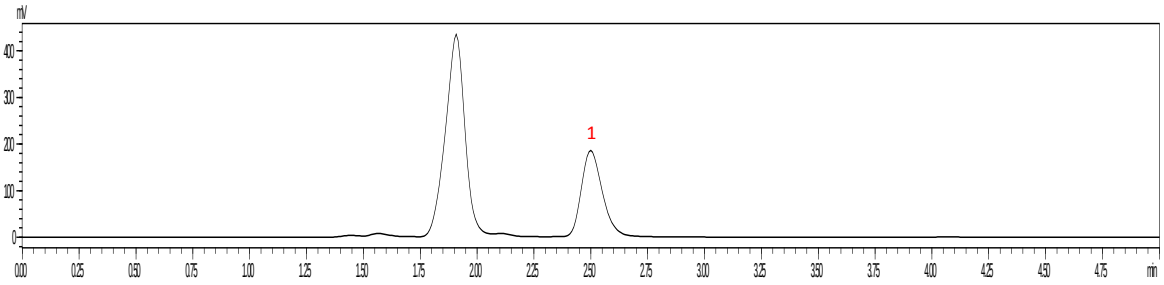
3.3. Methodological Review

3.3.1. Specificity [9]

Conduct precision suction of 10 μL vitamin C reference solution prepared according to the steps in "2.1.1", inject it into HPLC, analysis according to the chromatographic conditions as specified in "2.2", and obtain the corresponding chromatogram, as shown in **Figure 1**. The chromatogram of the test solution presents the chromatographic peak with the same retention time at the same position of vitamin C in the reference substance, which proves that this method has good specificity.



(a)



(b)

Figure 1. HPLC chromatogram of reference substance (a) and test substance (b). Note: 1-Vitamin C.

3.3.2. Investigation of Linear Relationship

Absorb a specific amount of reference solution, and use it to prepare 6 solutions with different concentration coefficients. Determine these solutions under the above chromatographic conditions. Take the injection concentration X ($mg \cdot mL^{-1}$) as the x-coordinate and the peak area Y as the y-coordinate, and then conduct linear fitting and draw the standard curve. The regression equation of vitamin C is $y = 37698x - 61035$, $R^2 = 0.9993$. This result shows that vitamin C has a good linear relationship in the corresponding linear range (5.4 - 108.0 $mg \cdot mL^{-1}$), as shown in **Figure 2**.

3.3.3. Precision

Accurately conduct suction of the vitamin C reference solution, inject it in turn according to the above chromatographic conditions 6 times, and record the peak area and corresponding retention time. According to the chromatographic peaks, the peak retention time is 2.471 min, 2.478 min, 2.473 min, 2.478 min, 2.480 min, and 2.474 min respectively, and RSD is 0.14%. The results indicate that the instruments have good precision.

3.3.4. Stability

Accurately absorb the same test sample solution for HPLC analysis at 0 h, 2 h, 4 h, 6 h and 12 h, detect vitamin C according to the chromatographic conditions as specified in "2.2" project, and record the chromatographic peak area. The calculated RSD is 1.73%, which indicates that the durian endocarp test sample solution has good stability within 12 h, as shown in **Table 1**.

3.3.5. Repeatability Test

Take the same batch of durian endocarp, accurately weigh 6 samples of durian white pulp with 5.0 g for one time, prepare solution according to the method as specified in "2.1.2", record the chromatographic peak area, and calculate the content of vitamin C in durian endocarp samples. The calculation results are 82.44 $mg \cdot g^{-1}$, 84.43 $mg \cdot g^{-1}$, 80.34 $mg \cdot g^{-1}$, 82.34 $mg \cdot g^{-1}$, 83.83 $mg \cdot g^{-1}$ and 84.18 $mg \cdot g^{-1}$ respectively, and the RSD is 1.86%. The above results indicate that this preparation method of the test sample has good repeatability, as shown in **Table 2**.

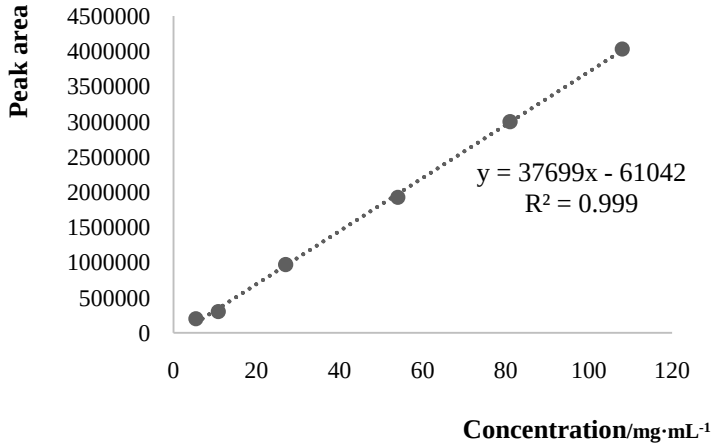


Figure 2. Standard curve of vitamin C.

Table 1. Stability test.

Time (s)	Peak area	RSD
0	106,563	
2	105,899	
4	105,121	1.73%
6	107,180	
12	109,976	

Table 2. Repeatability test.

No.	Peak area	Content (mg·g^{-1})	RSD
1	132,931	82.44	
2	137,987	84.43	
3	133,052	80.34	1.86%
4	135,373	82.34	
5	132,804	83.83	
6	134,244	84.18	

3.3.6. Sample Addition Recovery Test

Take 6 samples of the same batch of durian endocarp with measured content (2.5 g for each sample), accurately weigh them, and prepare the test solution according to the method specified in "2.1.2". Next, take the same test solution of durian endocarp, accurately add 210 mg·g^{-1} reference substance of vitamin C respectively, and detect the chromatographic peak of each sample to calculate the sample addition recovery rate [10] [11]. After calculation, the average recovery rate is 99.03%, and RSD is 1.11%. The above results indicate that the recovery rate of the preparation method is ideal, as shown in **Table 3**.

Table 3. Sample addition recovery test.

No.	Sampling volume/g	Original amount/mg	Added quantity/mg	Measured amount/mg	Sample recovery rate/%	Average recovery rate/%	RSD/%
1	2.5140	217.98	210	86.71	99.40	99.03	1.11
2	2.5089	220.93		88.06	98.70		
3	2.5147	236.75		94.15	101.02		
4	2.5102	222.35		88.58	98.41		
5	2.5127	229.83		91.47	97.75		
6	2.5110	228.32		90.93	98.91		

3.4. Investigation of Extraction Process

3.4.1. Orthogonal Test

L9 $(3)^4$ orthogonal experiment is conducted to investigate the extraction process of vitamin C in durian endocarp. Oxalic acid concentration (A), solid-liquid ratio (B) and ultrasonic extraction duration (C) are taken as investigation factors, and each factor is designed at three levels. Accurately weigh 5.0 g of durian endocarp and conduct ultrasonic extraction according to orthogonal experiment design. Finally, the calculated content of the chromatographic peak area indicates that the highest content of vitamin C is extracted under the conditions of 30 minutes of extraction with 2% oxalic acid concentration and a 1:10 solid-liquid ratio, as shown in Table 4 and Table 5.

Table 5 indicates that the significance of factors affecting the ultrasonic extraction of durian endocarp is ranked as follows: oxalic acid concentration > extraction duration > solid-liquid ratio. Table 6 indicates that there is no significant difference in oxalic acid concentration, solid-liquid ratio and extraction duration in terms of the influence on the extraction efficiency. According to the comprehensive analysis of test results and the variance, it can be concluded that the optimum combination is $A_2B_3C_3$, which means, the concentration of oxalic acid is 2%, the solid-liquid ratio is 1:12, and the extraction duration is 30 min.

3.4.2. Verification

Under the conditions with the oxalic acid concentration of 2%, the solid-liquid ratio of 1:12 and the ultrasonic extraction duration of 30 min, conduct extraction with 5.0 g durian endocarp by HPLC assay method. The same experiment is conducted 3 times in parallel. The average content of vitamin C is 82.24 $mg \cdot g^{-1}$, which indicates that this method is feasible. (Table 7)

4. Investigation of Chromatographic Conditions

4.1. Choice of Chromatographic Column

A proper suitable chromatographic column should be determined through comparison according to the chemical structure, polarity and solubility of the separated substances. Two chromatographic columns from Shimadzu and Elite are inspected in this research. After comparison, Shimadzu's chromatographic column features better resolution and a stable baseline.

Table 4. Factor levels.

Level	Oxalic acid concentration (A)	Solid-liquid ratio (B)	Ultrasonic duration/min (C)	Blank (D)
1	1%	1:8	10	1
2	2%	1:10	20	2
3	3%	1:12	30	3

Table 5. Orthogonal experiment design and results of vitamin C extraction process.

Test No.	A	B	C	D	Content ($mg \cdot g^{-1}$)
1	1	1	1	1	35.36
2	1	2	2	2	29.01
3	1	3	3	3	39.17
4	2	1	2	3	28.99
5	2	2	3	1	38.38
6	2	3	1	2	38.29
7	3	1	3	2	25.00
8	3	2	1	3	21.66
9	3	3	2	1	26.61
K1	34.513	29.783	31.770	33.450	
K2	35.220	29.683	28.203	30.767	
K3	24.423	34.690	34.183	29.940	
R	10.797	5.007	5.980	3.510	

Table 6. Analysis of variance.

Factor	Class III quadratic sum	Degree of freedom	Mean square	F	Significance level
Concentration	218.875	2	109.438	10.833	0.085
Solid-liquid ratio	49.152	2	24.576	2.433	0.291
Duration	54.306	2	27.153	2.688	0.271
Error	20.204	2	10.102		

Table 7. Verification results.

No.	Measured peak area	Content ($mg \cdot g^{-1}$)	Average content ($mg \cdot g^{-1}$)
1	136756	83.12	
2	134685	81.59	82.24
3	132960	82.01	

4.2. Selection of Assay Wavelength

In this experiment, the absorption wavelength of 254 nm, 280 nm and 310 nm are investigated. The results indicate that the absorption wavelength of vitamin C in durian endocarp is relatively large at 254 nm. Therefore, the assay wave-

length of 254 nm is selected in order to ensure the sensitivity and stability of this method.

4.3. Choice of Mobile Phase

Vitamin C features strong instability and is easy to be oxidized and decomposed in aqueous solution and alkaline solutions, while it is more stable when the environment is faintly acidic. Therefore, it is appropriate to choose an acidic mobile phase in order to minimize the oxidative damage in vitamin C determination. However, the stronger the acidity, the more corrosive the mobile phase is for the chromatographic column, liquid chromatograph injection and infusion system [10] [11] [12] [13] [14]. In this experimental research, three mobile phase systems, including methanol-0.1% phosphoric acid, methanol-water, and methanol-0.1% oxalic acid solution, are selected for verification. The verification results indicate that the first mobile phase of 0.1% phosphoric acid and the second mobile phase of water interfere greatly with the detection of vitamin C, and the separation effect is not ideal. However, when the methanol-0.1% oxalic acid solution is adopted in the mobile phase, the target peak is better than others. Therefore, methanol is selected as the organic phase and 0.1% oxalic acid solution as the mobile phase for content determination. The results are shown in Figures 3-5.

4.4. Selection of Flow Rate

The flow rates of 0.5, 0.8 and 1.0 mL·min^{-1} are investigated in this experimental research, and the results indicate that 1.0 mL·min^{-1} has the best effect and the stable peak time.

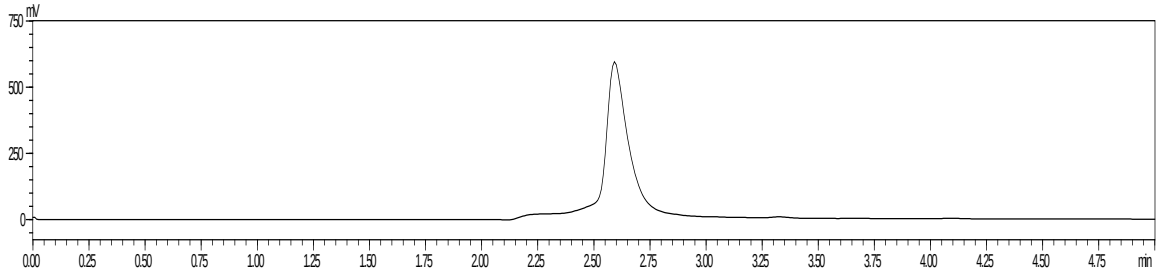


Figure 3. Chromatogram of methanol-0.1% phosphoric acid system.

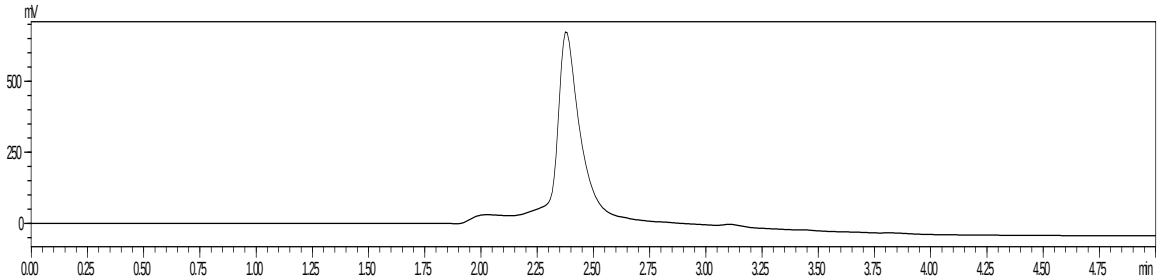


Figure 4. Chromatogram of methanol-water system.

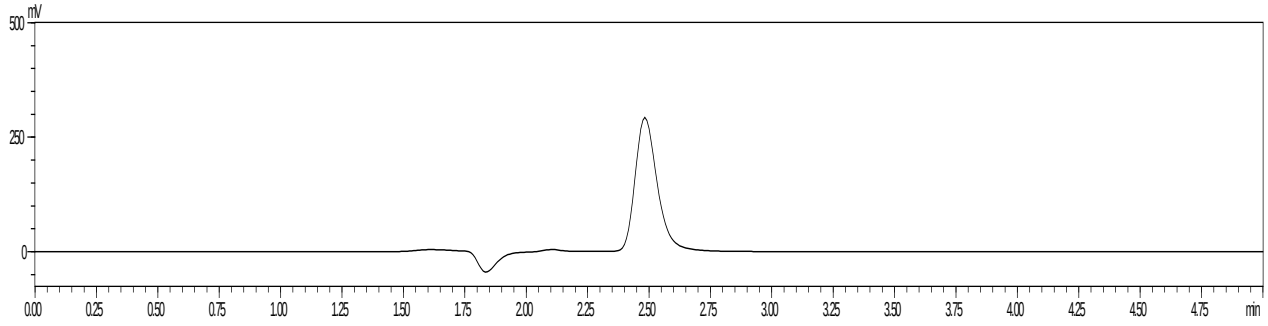


Figure 5. Chromatogram of methanol-0.1% oxalic acid solution system.

4.5. Selection of Column Temperature

The increase in column temperature will cause a decrease in column pressure, thus shortening the retention time of the analyte, but vitamin C is easy to be decomposed when it is exposed to heat. To prevent vitamin C from decomposing in the analysis process, the column temperature is selected at 30˚C.

5. Result and Discussion

The durian endocarp adopted in this experiment is a part of the rind, which is rich in various vitamins. The average content of vitamin C in durian endocarp is 82.93 $mg \cdot g^{-1}$. This is because the experiment time is May and June, which is not the mature season of durian, and the vitamin C in durian endocarp is gradually decreased with the extension of storage time. Through experiments, it is found that vitamin C in durian endocarp will be decomposed in the drying process. Therefore, the fresh durian endoscope is adopted in this experiment.

6. Conclusion

In this experiment, the content of vitamin C in the durian endocarp is extracted by the orthogonal experiment and ultrasonic instrument. As ultrasonic extraction of vitamin C from raw materials has high efficiency and simple operation, it is one of the ideal methods for extracting vitamin C. The content of vitamin C was determined by HPLC, and the column adopted is Shimadzu InertSustain C18 (4.60 × 150 mm, 5 μm); the detection wavelength is 254 nm; the mobile phase is prepared according to methanol: 0.1% oxalic acid = 5: 95 (V/V), and constant-gradient elution is carried out; the flow rate is 1 $mL \cdot min^{-1}$; the column temperature is 30˚C; and the chromatographic peak area is clear and obvious when the sample injection amount is 10 μL. This method is simple, accurate and accurate, and the content of vitamin C has a good linear relationship within the range of 5.8 - 108 $mg \cdot mL^{-1}$. The stability of durian endocarp solution is in good stability within 12 h, which indicates that this experimental method is effective and reliable with the simple and economical extraction process and high extraction efficiency. This method can provide a reference for the extraction, devel-

opment and utilization of vitamin C from durian endocarp.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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