ISSN: 2688-8645

Determination of Ascorbic Acid in Zizyphus Jujube by Ultraviolet Spectrophotometry

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Abstract

To establish a method for the direct determination of ascorbic acid in the *Zizyphus Jujube*, UV spectrophotometry is used to measure ascorbic acid in Jujube. The results show that the calibration curve of ascorbic acid was linear (R^2=0.9998) among 0~20 μg/mL, and the regression equation is y=0.033x+0.0017. The recovery is between 90.3%~112.9%. The method is sensitive, accurate, and the precision is high. It can be applied to determine ascorbic acid in jujube.

Keywords

Zizyphus Jujube; Vitamin C; Ultraviolet spectrophotometry.

1. Introduction

Vitamin C (Ascorbic Acid) also called L-ascorbic acid, is a water-soluble vitamin. Vitamin C has a variety of physiological functions. For example, it plays an important role in the redox reaction process, which is extremely important in life activities. It can promote the absorption of iron in the intestine and turn folic acid into physiologically active tetrahydrofolate. It can also treat scurvy. Therefore, it has an irreplaceable role in maintaining the normal body functions of the human body.

Vitamin C is easily oxidized by light and air under natural conditions. Metal ions such as iron and copper can accelerate its oxidation [1]. It is easy to decompose under alkaline conditions and is more stable under weak acid conditions [2]. The methods for determining vitamin C include iodometry, 2,6-dichloroindophenol titration, 2,4-dinitrophenylhydrazine, fluorescence and high-performance liquid chromatography, etc. [3-6]. Although these methods have their own characteristics, the operation process is complicated, the reagents used are unstable, and the speed is slow. The instruments and equipment required for the latter two are more expensive. At present, there have been research reports on the determination of fruits and vegetables and vitamin C products by ultraviolet spectrophotometry. For example, Zhang Jieli[7] and Li Anrong [8] have shown that Cu^{2+} is a good catalyst, which can accelerate the oxidation of Vc, and use EDTA The background of complexation correction is used as a reference, and the content of vitamin C can be accurately determined according to the absorbance difference (△A) before and after oxidation. Cai Shunxiang[9] used ultraviolet spectrophotometry and 2,6-dichloroindophenol titration to determine the content of vitamin C in oranges, and the results

ISSN: 2688-8645

of the two methods were basically the same. Ultraviolet spectrophotometry is simple, fast and accurate. Vitamin C content is very high in jujube [10], so the study of ultraviolet spectrophotometry to determine the content of reduced Vc in jujube has important practical value.

2. Experimental Part

2.1. Instruments and Reagents

UVmini-1240 ultraviolet-visible spectrophotometer (Shimadzu Corporation); mixed acid solution (30 mL of 3% H3P04 and 70 mL of 8% HAc are mixed and diluted to a 500 mL volumetric flask); oxalic acid; copper chloride; Ethylenediaminetetraacetic acid disodium (EDTA-Na_2) The above reagents are of analytical grade, and the experimental water is double distilled water. Experimental sample: The red date sample was collected from the red date base of Mizhi County, North Shaanxi University of Agriculture and Forestry.

2.2. Experimental Method

2.2.1 Preparation of Vc standard Solution

Accurately weigh 0.0500 g Vc, add mixed acid to dissolve, and dilute to 50 mL with mixed acid, which is a 1.0 mg/mL standard solution.

2.2.2 Preparation of Sample Solution

Take 4.018 g of peeled fresh red dates, add 5.0 mL of mixed acid, grind in a mortar to form a homogenate, add mixed acid to make the volume to 100 mL, centrifuge at 3000 rpm for 5 min at low temperature, pour out the supernatant, which is the sample solution.

2.3. Standard Curve Preparation

Accurately pipet 0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 mL of Vc standard solution into a 50 mL volumetric flask, and dilute to 50 mL with mixed acid to obtain a series of standard solutions. Take 2 parts of this series of solutions, each 1 mL in a 25 mL graduated test tube, add 4 mL of copper chloride solution to the first series, heat in a 70℃ water bath for 3 minutes, and then add Cu^{2+}-EDTA solution 5 ml is used as a reference solution. Accurately add 5 mL of Cu2+-EDTA solution to 10 mL as the solution to be tested in the second series of solutions, measure the absorbance at 243 nm, repeat the measurement 3 times, and take the average. The result shows that the vitamin C standard solution is at 0 Obeying Lambert Beer's law in the range of~20 μg/mL, the regression equation y=0.033x+0.0017, the correlation coefficient R2=0.9998, as shown in Figure 1.

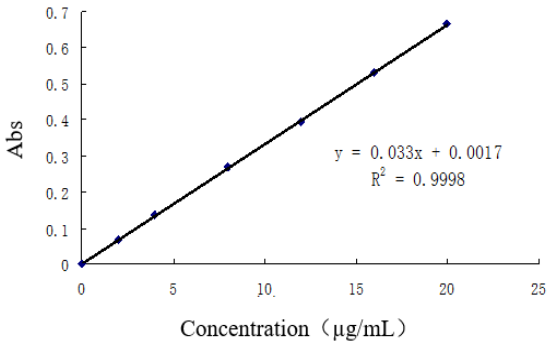

Figure 1. Calibration curve of Vc concentration

2.4. Determination of Vc Content in Samples

Take 4.018 g of peeled fresh red dates, add 5.0 mL of mixed acid, grind in a mortar to form a homogenate, add mixed acid to make the volume to 100 mL, centrifuge at 3000 rpm for 5 min at low temperature, pour out the supernatant, which is the sample solution. Accurately draw

ISSN: 2688-8645

1.0 mL of the sample solution into a 10 ml graduated test tube, add 5 ml of Cu^{2+}-EDTA solution to be the sample solution; take another 1.0 ml of mixed acid solution, add 5 ml of Cu^{2+}-EDTA solution as a blank solution, and dilute to volume to 10 ml. Take the blank solution and the sample solution to be tested to measure the absorbance at the wavelength of 243 nm, obtain the concentration of the sample solution according to the standard curve, calculate the content of vitamin C in the jujube, and compare the result with the 2,6-dichloroindophenol titration method. Compare. The results are shown in Table 1.

Table 1. Determination of vitamin C samples (n=6, mg/g)

Measurement method	Measurement value (mg/g)						Average	RSD%
titration	2.554	2.553	2.549	2.541	2.537	2.531	2.544	1.08
UV method	2.526	2.526	2.518	2.501	2.482	2.482	2.506	

3. Results and Analysis

3.1. Selection of Medium

A certain amount of Vc is dissolved in deionized water, a mixed solution of acetic acid and phosphoric acid, and a 1% oxalic acid solution, and the absorption curve of Vc in different media in the ultraviolet region is scanned every 15 minutes, and the absorption curve of the medium solution in the ultraviolet region is scanned at the same time. The results show that Vc is extremely unstable in water and is easily oxidized, while oxalic acid has an absorption peak in this region, which has great interference, so mixed acid is selected as the medium.

3.2. Wavelength Selections

Although the maximum absorption wavelength of Vc in an aqueous solution is 267 nm, it is easily oxidized in an aqueous solution, and it is not suitable to measure the absorbance at this wavelength. Draw 5.00 mL of Vc standard solution in a 50 mL volumetric flask, dilute to the mark with mixed acid, then respectively take 1.0 ml of this solution in a 10 ml graduated test tube, add 5 ml of Cu^{2+}-EDTA solution to the first part, and dilute to 10 mL is used as the measurement solution. Add 4 ml of Cu^{2+} to the second portion, heat it in a water bath at 70°C for 3 minutes and then cool it down. Add 5 ml of Cu^{2+}-EDTA solution as the reference solution. Record the UV absorption spectrum in the wavelength range of 200~300 nm (Figure 2). It can be seen from the spectrum that Vc has a maximum absorption at a wavelength of 243 nm in a mixed acid medium, so 243 nm is determined to be the measurement wavelength.

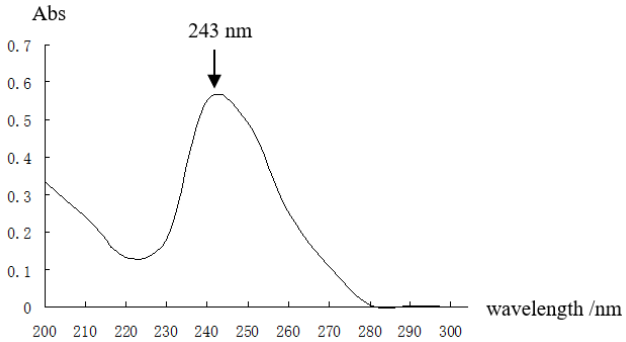

Figure 2. UV absorption curve of Vc

3.3. The Influence of Reaction Temperature on Vc

Take 25 mL graduated test tubes, add 100 μg/mL vitamin C 1.0 ml and 4.0 ml Cu^{2+} ion solution, and dilute to 10 mL, and then add 20, 30, 40, 50, 60, 70, 80, Heat it in a constant temperature water bath at 90 ℃ for 15 min, then take it out to cool, and measure its absorbance. The result is that the absorbance is the highest at 20 ℃ (Table 2), indicating that the Vc destruction speed

ISSN: 2688-8645

is slow at room temperature, and the absorbance drops sharply after 40 ℃. The absorbance of vitamin C measured at 70~90 ℃ is almost the same, indicating that Vc is completely destroyed. So choose the temperature to be 70 ℃.

Table 2. Effect of different temperature application on vitamin C

Temperature (°C)	20	30	40	50	60	70	80	90
Abs	0.327	0.263	0.178	0.089	0.050	0.026	0.024	0.022
RSD (%)	0.64	0.58	0.65	1.12	1.15	2.14	2.31	2.62

3.4. The Impact of Reaction Time on Vc

Take 25 mL graduated test tubes, add 1.0 ml of 100 μg/mL vitamin C solution and 4 ml of Cu2+ ion solution, and dilute to 10 ml. Place 0, 1, 2 in a 70 ℃ water bath. After 3, 4, 5, and 6 minutes, the absorbance value was measured at 243 nm. The results showed that the absorbance value dropped rapidly after heating and stabilized after 3 minutes, indicating that Vc had been destroyed completely, so the selection time was 3 minutes.

Table 3. Effect of different time application on vitamin C

Time (min)	0	1	2	3	4	5	6
Abs	0.326	0.237	0.116	0.027	0.026	0.027	0.026
RSD (%)	0.47	0.48	0.99	2.11	2.19	2.17	2.19

3.5. Reagent Stability

Accurately pipette 4.0 mL of Vc standard solution, place it in a 50 mL volumetric flask, dilute to 50 mL with mixed acid, and then take 1.0 mL into a 25 mL graduated test tube. Add 4 mL of Cu^{2+} solution to the first aliquot. After heating in a water bath at 70 ℃ for 3 min, add 5 mL of Cu^{2+}-EDTA solution as a reference solution. Accurately add 5 mL of Cu2+-EDTA solution to the second solution to make the volume to 10 mL as the solution to be tested. After preparation at room temperature, separate it at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 min. When the absorbance values were measured at the time, they were 0.268, 0.263, 0.256, 0.248, 0.236, 0.225, 0.213, 0.202, 0.191, 0.188. It can be seen that the absorbance value of the Vc solution does not change much within 1 h, and the stability is good.

3.6. Precision Test

Accurately absorb different volumes of sample solution, and measure the absorbance at 243 nm according to the sample determination method. The results are shown in Table 4. The maximum RSD is 1.33%. The RSD value between absorbance values obtained from different volumes is small, indicating good precision.

Table 4. The determination of precision

Sample volume (mL)	Abs measured value					Average value	RSD (%)
0.4	0.136	0.136	0.136	0.134	0.132	0.135	1.33
0.8	0.268	0.267	0.266	0.265	0.263	0.266	0.72
1.2	0.407	0.403	0.401	0.400	0.396	0.401	1.01
1.6	0.537	0.534	0.534	0.532	0.530	0.533	0.49
2.0	0.69	0.698	0.696	0.693	0.690	0.695	0.53

3.7. Determination of Recovery Rate

Use the standard Vc addition method to carry out the recovery test. Accurately draw 1 mL of the sample solution and place them in 6 100 mL volumetric flasks respectively. Add 5 ml of

Cu^{2+}-EDTA solution as the test solution; take another 1 ml of mixed acid plus Cu^{2+}-EDTA solution 5 ml is a blank solution, and the corresponding concentration is obtained according to the standard curve. The calculated recovery rate of standard addition is 90.3%~112.9%, the average recovery rate is 106.8%, and the RSD=1.03%.

4. Conclusion

The determination of vitamin C content mainly adopts the iodometric method [1] and the 2,6-dichloroindophenol titration method [5]. The determination process of the iodometric method is more complicated, and vitamin C is unstable and easily oxidized in the air. The determination result is low [7]; the latter is difficult to determine the titration end point. For colorless or light-colored fruit and vegetable sample liquid, the titration end point is easy to determine, while for jujube, strawberry, hawthorn and other purple, pink or brown color fruits and vegetables The titration end point of the sample solution is difficult to determine [11]. In this paper, the ultraviolet spectrophotometric method is used to determine the content of Vc in red dates. The operation is simple and fast, with high precision, which effectively delays the oxidation time of vitamin C, is not interfered by other reducing substances and colors [12], and the measurement results are accurate. Compared with the 2,6-dichloroindophenol titration method, there is little difference in the measurement results. The method is accurate and has good precision. At the same time, it does not require reference materials and calibration. The reagents used are cheap and easy to obtain. This method has a wide range of Value.

References

[1] Zeng Xiangyun. The physiological function of vitamin C and dietary security [J]. China Food and Nutrition, 2005(4): 52-54.

[2] Zheng Ji, Chen Junhui. General Biochemistry [M]. Beijing: Higher Education Press, 1998.

[3] The Pharmacopoeia Committee of the Ministry of Health of the People's Republic of China. The Pharmacopoeia of the People's Republic of China (Part 2) [M]. Beijing: Chemical Industry Press, 2010.

[4] Edited by Lu Rukun. Soil agricultural chemical analysis method[M]. Beijing: China Agricultural Science and Technology Press, 1999,469-472.

[5] Ruan Li, Zeng Qingxiao, Zhang Wen. Research progress on the detection methods of vitamin C content[J]. Food Safety Guide, 2021, (18): 187-188.

[6] Cao Feng. Optimization of the method for the determination of vitamin C in Zhanhuadongzao by high performance liquid chromatography[J]. Journal of Qilu University of Technology, 2020,34(05):37-42.

[7] Zhang Jieli, Gao Yu, Hou Dongyan. Determination of Vitamin C in Vidokang by Ultraviolet Spectrophotometry[J]. Food Science, 2004, 2(11):234-235.

[8] Li Anrong, Yang Lingli, Xia Daiquan. Determination of Vitamin C in Vitamin C Yinqiao Tablets by Ultraviolet Spectrophotometry [J]. Drug Testing, 2002, 11(8): 44-45.

[9] Cai Shunxiang. Determination of reduced vitamin C in orange by ultraviolet spectrophotometry[J]. Spectroscopy Laboratory, 2009, 26(5): 1091-1094.

[10] Zhao Zidan, Zhang Yan, Yang Chunxia, Yang Jing, Wang Fang, Wang Xiaojing. Research progress on the main nutrients and functional properties of jujube[J]. Ningxia Agriculture and Forestry Science and Technology, 2019,60(12):81-84.

[11] Li Guangru, Wang Chunxia. Quantitative determination of reduced ascorbic acid in fruits and vegetables[J]. Food Development and Research, 1995, 16(1): 43-46.

[12] Siqinge Rile, Ende. Determination of Vitamin C in Huai Medicine[J]. Guangdong Trace Element Science, 2011, 18(5): 45-49.