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Effects of Different Metal Ions on the Stability of Anthocyanins as Indicators

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Effects of Different Metal Ions on the Stability of Anthocyanins as Indicators

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Abstract. In this paper, the stability of anthocyanins in morning glory in the presence of different ions was analyzed by spectrophotometry. The anthocyanin extract was obtained by heating and reflux. Then the maximum absorption wavelength of anthocyanin was obtained by specific characteristic absorbance of anthocyanin, and the concentration of anthocyanin was calculated. Six different metal ions were added to the anthocyanin solution to determine the influence of the stability of anthocyanin by measuring the absorbance value. Through the analysis, we obtained calcium ions which can promote the stability of anthocyanins. Finally, we find the best concentration ratio of calcium ion and anthocyanin. Through the study, we found that when the concentration of anthocyanin and calcium ion is 1:7, the best color retention effect is achieved.

1. Introduction

In previous study, the color of morning glory can change with the change of PH. Through consulting the literature, we know that the anthocyanins in morning glory play a leading role. Anthocyanins are a kind of water-soluble natural pigments widely existing in plants in nature. Among them, morning glory anthocyanins belong to six important anthocyanins in food, which play a unique role in food dyes. In general, anthocyanins are red at pH < 7, purple at pH 7-8 and blue at pH > 11[1]. But at the same time, the literature also mentioned that anthocyanins have better activity when PH is less than 3. When PH is greater than 7, anthocyanins are unstable and easy to decompose. The presence of metal ions can affect the stability of anthocyanins. [2] This experiment was designed to explore the effect of metal ions on anthocyanin under the condition of morning glory as indicator. The stability of anthocyanin was compared by measuring the change of absorbance value. The most suitable ion for increasing stability was selected to explore the best ratio of the two, or the best indicator effect.

2. Methods and Materials

2.1. Materials

All Spectrophotometer, pH meter, round bottom flask (250ml), spherical condensation tube, 1cm colorimetric dish, water bath, thermometer, capacity bottle (25ml), pipette (1ml), measuring cylinder, funnel, electric heating jacket, fresh morning glory, sulfuric acid (0.005mol/L), ammonium sulfate

(0.01mol/L), standard CaSO4 (0.01mol/L), CuSO4 (0.01mol/L), ZnSO4 (0.01mol/L), 2 (SO4) 3 (0.005mol/L). Al2 (SO4) 3 (0.005mol/L) and Na2SO4 (0.005mol/L) were 10 ml each.

2.2. Methods

(1) Preparation of anthocyanin solution

Take 100 g morning glory and put it in 250 ml flask. Take 50 ml sulphuric acid solution and 50 ml distilled water and pour them into flask. Heat them with electric heating jacket and water bath. Control the temperature between 60 and 70 degrees. Condensation reflux 0.5 hours. After reflux, anthocyanin extract was obtained by filtering the solution at room temperature.

(2) Determination of characteristic absorbance and anthocyanin concentration of pigment solution

The anthocyanin solution obtained in step 1 was 5 ml and added with 5 ml sulfuric acid solution. The absorbance of anthocyanin solution was determined by spectrophotometer at the wavelength of 440-560 nm. The characteristic curve of absorbance value-absorbance wavelength was drawn according to the results. The characteristic absorbance wavelength of the pigment was the maximum absorbance wavelength.

Change the amount of anthocyanin solution to 6 ml, 7 ml, 8 ml, 9 ml, 10 ml. The absorbance at the absorbance wavelength was measured. The concentration of anthocyanin solution was obtained by Lambert's law.

(3) Effect of Metal Ions on the Stability of Pigments

Six metal ion solutions of 1 ml each were taken from the pipette and added into six volumetric bottles. 10 ml anthocyanin extract was added and the pH was adjusted to 3 with sulfuric acid and ammonium sulfate solution. The volume of deionized water was set to 25 ml. The absorbance values of six kinds of solutions and background solutions were determined immediately with the absorbance wavelength selected in step (2). The remaining solution was placed in the same environment, and the absorbance values were measured for 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes and 30 minutes. Analyzing the data, the ion which can enhance the stability of anthocyanin solution is obtained.

(4) The optimum concentration ratio of metal ions

0.2 ml, 0.5 ml, 0.8 ml, 1.1 ml, 1.4 ml and 1.7 ml of the target metal ion solution were removed. Prepare according to step 3. The absorbance of the solution was measured after 5 minutes. The optimum metal ion concentration ratio was obtained by fitting the curve.

3. Results

(1) Experimental basis

Spectrophotometry is a method for qualitative and quantitative analysis of the substance measured by measuring the absorbance of light at a specific wavelength or within a certain wavelength range. Any molecule absorbs light selectively [3]. Different molecules have different absorption abilities to light. For the same substance, the maximum absorbance wavelength does not change with the change of concentration and absorbance. Under the condition of dilute solution, the solution conforms to Lambert's law, so the concentration of the solution can be determined by absorbance under the condition of constant absorbance coefficient and material length. In this experiment, we use the standard curve method [4]. Standard curve method is to prepare a series of standard solutions with concentration from small to large, and determine their A values separately. The standard curve is made by using A values as abscissa and concentration as ordinate. When determining the solution to be measured, the operating conditions should be the same as when making the standard curve. The corresponding concentration of the sample can be found from the standard curve by the A value of the solution to be measured.

(2) Determination of Maximum Absorption Wavelength

Step 2 we obtained our absorbance values are as follows:

Table 1. Absorption wavelength

λ/nm	440	450	460	470	480	490	500	510	520	530	540	550	560
A	0.083	0.17	0.182	0.198	0.216	0.225	0.228	0.237	0.245	0.231	0.183	0.119	0.069

From the table, we know that 520 nm has the maximum absorption wavelength. Therefore, our follow-up experiments will be based on 520 nm.

(3) Determination of anthocyanin concentration

In step 2, the absorbance values of anthocyanins are as follows:

Table 2. Absorbance values of anthocyanins

V/ml	5	6	7	8	9	10
A	0.245	0.248	0.251	0.255	0.259	0.262

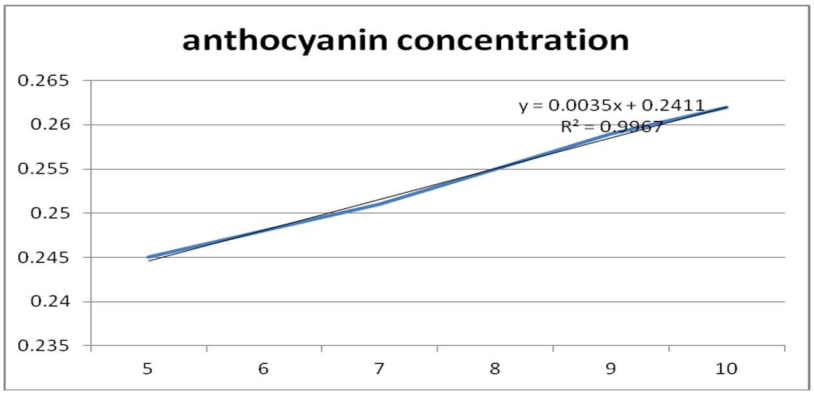

Figure 1. Anthocyanin concentration

From this, we get the fitting curve: y = 0.0315x + 0.2272.

After conversion, the concentration of proanthocyanidin solution c=1.025*10^(-4) mol/L was obtained.

(4) The effect of metal ions on the stability of pigments.

By measuring the data of six different metal ions added immediately, 5, 10, 15, 20, 25 and 30 minutes later, we record the data and draw the table as follows:

Table 3. effect of metal ions on the stability of pigments

	0s	5s	10s	15s	20s	25s	30s
Cu	0.491	0.49	0.475	0.466	0.46	0.457	0.455
Zn	0.21	0.12	0.1	0.07	0.02	0.02	0.01
Fe	0.35	0.28	0.22	0.18	0.16	0.13	0.08
Ca	0.494	0.491	0.488	0.487	0.482	0.48	0.478
Al	0.492	0.48	0.475	0.464	0.462	0.453	0.452
Na	0.49	0.482	0.475	0.466	0.46	0.457	0.455
Contrast	0.488	0.48	0.476	0.465	0.463	0.455	0.454

Among them,we found that when iron ions were added, the black precipitation appeared in the solution, while there was no obvious phenomenon in other solutions. This phenomenon directly shows that iron ions can denaturate anthocyanins and affect the stability of anthocyanins. By comparing the absorbance data, we found that Na, Cu, Al and Ca ions did not affect the stability of anthocyanins. However, Zn and Fe will greatly affect the stability of pigments, not ideal ions. Comparing the four ions

mentioned above, we find that the absorbance of calcium ions decreases more slowly than that of the background, which indicates that calcium ions have a certain color protection effect. So we decided to use calcium as the ideal metal ion.

(5) Optimum metal ion concentration ratio

We add different volumes of calcium ion solution into the volumetric flask and get the following data:

Table 4. Optimum metal ion concentration ratio

V/ml	0.2	0.5	0.8	1.1	1.4	1.7
A	0.489	0.497	0.495	0.493	0.488	0.479

Through polynomial fitting, we get the maximum value when adding 0.7ml calcium ion. By calculating, we find that when the concentration ratio of anthocyanin to calcium ion is about 1:7, the best color retention effect is to maintain the stability of anthocyanin solution.

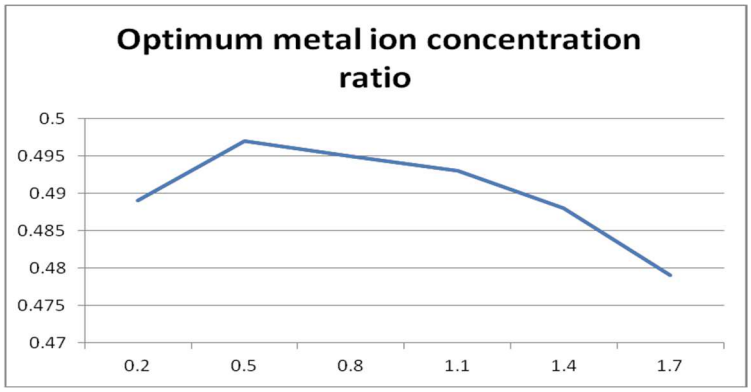

Figure 2. Optimum metal ion concentration ratio

4. Discussions

Spectrophotometry is a commonly used micro-quantitative analysis method, and the experimental method is widely recognized, so the feasibility of the results of several groups of data in this experiment is more assured [5]. The subject of this experiment is novel, and there are few literatures on the study of morning glory anthocyanins. In this paper, fresh morning glory was used to regulate pH strictly, which minimized the possibility of anthocyanin inactivation. In this paper, the effect of six metal ions on anthocyanin was measured, and the specific value data of several groups of stationary samples were measured, which made the data more feasible, and also had reference and reference significance for the stability study of other pigments caused by metal ions. Finally, we selected the strongest calcium ion, and measured the optimum ratio of color retention by measuring the absorbance value. It has a long-term progress and reference value for the future research on anthocyanin as an indicator.

Firstly, the relative error of the quantitative method for trace amounts is relatively large, which is the instrument error, and we can not avoid it. Secondly, when extracting anthocyanins, we only use acid boiling method, the final concentration is low, and the condensation reflux time is long, which may affect the stability and content of anthocyanins. Moreover, the metal ions themselves have a certain absorbance value [6], which we did not design a good control, although the experimental results and comparison have little impact, but from the rigor of the experimental results, we should design the link more carefully to enhance the credibility of the control group. Finally, we use polynomials to fit the curve. More data should be needed to enhance the persuasion of the fitting curve.

5. Conclusion

520 nm is the maximum absorption wavelength of morning glory anthocyanin. The concentration of anthocyanin solution is 1.025*10^(-4) mol/L. Among the six metal ions, Zn and Fe can reduce the stability of anthocyanin, while Cu, Al and Na have little effect on the stability of anthocyanin, while Ca is a suitable stabilizer. When the concentration ratio of anthocyanin to calcium ion is 1:7, the color retention effect is the strongest. Therefore, if anthocyanins are used as indicators, it is better to add 7 times the concentration of Ca ions in the solution.

References

[1] Wang H, Nair M G, Strasburg G M, et al. Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries.[J]. Journal of Natural Products, 1999, 62(2):294-296.

[2] Butelli E, Titta L, Giorgio M, et al. Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors.[J]. Nature Biotechnology, 2008, 26(11):1301-1308.

[3] † T F, Francis F J. Quantitative Methods for Anthocyanins[J]. Journal of Food Science, 2010, 33(3):266-274.

[4] Kähkönen M P, Heinonen M. Antioxidant activity of anthocyanins and their aglycons.[J]. J Agric Food Chem, 2003, 51(3):628-633.

[5] Fuleki T, Francis F J. Quantitative Methods for Anthocyanins. 4. Determination of Individual Anthocyanins in Cranberry and Cranberry Products[J]. Journal of Food Science, 2010, 33(5):471-478.

[6] And J B, Timberlake† C F. Isolation, Identification, and Characterization of New Color-Stable Anthocyanins Occurring in Some Red Wines[J]. Journal of Agricultural & Food Chemistry, 1997, 45(1):35-43.