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POLYSACCHARIDE DETERMINATION IN CORN SILK WITH 3,5-DINITROSALICYLIC ACID COLORIMETRY

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ABSTRACT

Contents of reducing sugars in crude polysaccharides from corn silk were measured by 3,5-dinitrosalicylic acid (DNS) colorimetry with incident wavelength at 492 nm, amount of DNS = 1.5 mL, coloration temperature at 90 °C and coloration time = 7 min. The standard curve equation of glucose was y=3.9163x-0.0292; the glucose solubility ranged from 0.07332 to 0.25662 mg/ml, and the absorbance and glucose content were well linearly related. The DNS colorimetry was outstanding with easiness, fast speed, accuracy, safety, low cost, high stability and reproducibility. The polysaccharide content in the cornstarch (reducing sugar) was 18.16%.

KEYWORDS

Corn silk, 3,5-Dinitrosalicylic acid, Colorimetry, Polysaccharides.

1. INTRODUCTION

Corn silk tastes light and has mild effects of clearing blood heat, diuretics, liver calming, and cholagogue. Corn silk can treat diabetes, jaundice, measles, celiac hematuria, blood collapse and other symptoms [1]. The rich source, low price and easy collection make corn silk a medicinal resource with broad research and development prospects. The major corn silk polysaccharides include glucose, galactose, arabic sugar, galactose, mannose and xylose [2].

Wang Liming used an anthrone sulfate method to measure tea polysaccharides and optimized the conditions as follows: ketone concentration = 0.05%, ratio of sample solution to anthrone-sulfuric acid = 1:4, reaction time of 5 min at 100 °C, immediate cooling in ice water for 30 min, and absorbance at 675 nm [3]. This method was highly reproducible with the average recovery of 94.58%± 1.68% and relative standard deviation (RSD) of 0.548% [3]. Gao Lijun et al. detected polysaccharides in *Radix Cynanchi Auriculati* by using a phenol- sulfuric acid method and found the absorbance at 490 nm was well linearly related with the measured content in the range of 8-64 μg, with the average recovery rate of 100.9% [4]. This method is simple and accurate with high linearity [4].

Wang Hongying et al. used 3,5-dinitrosalicylic acid (DNS) colorimetry to measure polysaccharides in *Ophiopogon japonicas* and confirmed this method was simple, easy-to- implement, and reproducible. DNS colorimetry can be used as a routine method to detect polysaccharide contents in *Ophiopogon japonicus* [5]. Zhang Lihua et al. used the DNS method to measure the reducing sugar and total sugar in AGP and calculated the total polysaccharide content. The method is sensitive, stable and reproducible, which contribute to the study of quality control method of AGP [6]. Hu Xijie et al. optimized the parameters as follows: wavelength at 495 nm, and VDA: V = 1.5: 3.5, reaction temperature at 90 ℃, and time of reaction = 5-7 min and found the method was efficient, accurate and stable in determining reducing sugars [7]. In the present article, we discussed the parameters of DNS colorimetry to detect reducing sugar in corn silk.

2. EXPERIMENTAL CONDITIONS

2.1 Experimental Instruments and Equipment

A ultraviolent (UV) grating spectrophotometer (752N, Shandong Gaomi Rainbow Analysis Instrument Co., Ltd.), a UV visible light photometer (TU-1810, Beijing General Analysis Equipment Co., Ltd.), and an analytical balance (FA2004, Shanghai Fine Technology Instrument Factory) were used.

2.2 Raw materials and reagents

Corn silk (collected in 2016 from the suburbs of Jilin City), phenol (Tianjin Ruijinte Chemicals Co., Ltd., 20060617), glucose (Tianjin Damao Chemical Reagent Factory, 20010724), potassium sodium tartrate (Beijing Chemicals Factory, 20010705), DNS (Sinopec Group Chemical Reagent Co., Ltd., F20080403), NaOH (Tianjin Northern Tianyi Chemical Reagent Factory, 20010308), and anhydrous sodium sulfite (Zhejiang Yongjia County Chemical Reagent Factory, 890311) were used.

3. PROCEDURES AND METHODS

3.1 Reagent configuration

Glucose control stock solution was prepared as follows: 0.1004 g of anhydrous glucose was completely dissolved to a volume of 100 mL (1.004 mg/mL). Glucose standard solution preparation was prepared as follows: 0, 1, 2, 3, 4, 5, 6 and 7 mL of the above stock solution were aspirated and set in 25-mL volumetric flasks for use [8].

DNS rendering liquid was prepared as follows: 6.3 g of DNS and 262 mL of a 2 M NaOH solution were added to 500 mL of a hot aqueous solution containing 185 g of sodium potassium tartrate. Then 5 g of crystalline phenol and 5 g of sodium sulfite were added. After stirring, the solution was cooled and diluted to a constant volume of 1000 mL. The solution was stored in a brown bottle for use [9].

3.2 Determination Conditions of Polysaccharide Content

The optimal conditions for measurement of reducing sugar content in

crude polysaccharides of corn silk were investigated using the DNS colorimetry [10].

3.2.1 Maximum absorption wavelength of the screening

To 1.5 mL of the DNS solution in a test tube, 2 mL 0.28112 mg/mL glucose standard solution was added under shaking. The solution was placed in a boiling water bath and heated for 5 min, removed, and quickly cooled to room temperature with tap water. The solution was diluted to 10 mL with distilled water and allowed to stand for more than 20 min. The DNS was zeroed and the absorbance within 350-650 nm was scanned and measured.

3.2.2 Selection of developer amount

The glucose standard solution (0.28112 mg/mL) was used to select the amount of DNS. Firstly, 2 mL of glucose standard solution was accurately weighed and added into 7 tubes respectively. Then 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, or 2.4 mL of DNS was added to test tubes. The steps were the same as in section 2.2.1. Finally, the volume was adjusted with distilled water. Absorbance was measured in a 25 mL volumetric flask.

3.2.3 Selection of Coloration Temperature

To 5 test tubes, each was added with 2 mL of the 0.16064 mg/mL glucose standard solution and 1.5 mL of DNS to make the reaction systems develop color at 60, 70, 80, 90, and 100 °C respectively. The steps were the same as in section 2.2.1. Finally, the tubes were diluted with distilled water to a 25 mL volumetric flask for measurement of absorbance.

3.2.4 Selection of Color Rendering Time

To 6 test tubes, 2 mL of the 0.16064 mg/mL glucose standard solution and 1.5 mL of DNS were added, followed by color development for 3, 5, 7, 10, 15 and 20 min respectively. The steps were the same as section 2.2.1, and the reaction temperature was 90 °C. The tubes were diluted with distilled water into a 25 mL volumetric flask for absorbance measurement.

3.3 Preparation of glucose standard curve

To a test tube, 2 mL of each concentration of the glucose standard solution was pipetted, and 1.5 mL of DNS was added under shaking, followed by placement in a 90 °C water bath and heating for 7 min. Then the tube was removed, quickly cooled to room temperature with tap water, and diluted to 10 mL with distilled water. After placement for more than 20 min, the absorbance was measured.

3.4 Stability Experiment

The procedure was the same as in section 2.3. The solutions were removed after optimization for 20, 30, 40, or 50 min. Absorbance was measured after 1 and 2 h.

3.5 Precision Experiment

To 5 test tubes, 2 mL of the 0.16064 mg/mL glucose standard was added following the same steps as in section 2.3. After the parameters were optimized, the absorbance was measured after 30 min and the RSDs were calculated.

3.6 Repeatability Experiment

Two clean test tubes were taken at different time periods and added with the 0.16,064 mg/mL polysaccharide solution and then 1.5 mL of DNS. The procedure was the same as in section 2.3, and the absorbance was measured under the optimized parameters. A total of 5 absorbance values were measured and RSDs were calculated.

3.7 Recovery experiment

Five control substances with glucose levels from 0.1 to 0.26 mg/mL were set up and measured under the optimized conditions.

3.8 Sample recovery experiment

To 6 clean test tubes, each was accurately pipetted with 1.0 mL of the sample solution containing 0.06976 mg/mL polysaccharide and added with 1.5 mL of DNS. Every 1 mL of glucose standard solution, with concentration of 0.03665, 0.05499, 0.07328 mg/mL, was measured by the assay and the RSDs were calculated.

3.9 Determination of polysaccharide content

3.9.1 Measurement of Total Polysaccharide Content in Corn Silk

Net content of total polysaccharides in the crude corn silk was measured using the phenol-sulfuric acid method.

3.9.2 Detection of reducing sugar content in crude polysaccharides

To two tubes, each was added with 2 mL of crude polysaccharide solution (2.03 mg/ml), and the blank was added with 2 mL of distilled water. The absorbance (A) was measured under the condition of section 2.3.

4. RESULTS

4.1 Conditions for polysaccharide content measurement

4.1.1 Determination of absorption wavelength

The DNS solution was zeroed and scanned with a UV spectrophotometer (Figure 1.a). The maximum absorption wavelength should be λ_{max} = 492nm. The curve of the glucose content versus the absorbance was plotted. Finally, the appropriate range of measuring reducing sugar was determined.

4.1.2 Effect of optimal amount of color reagent

The amount of DNS was related to the content of reducing sugar in the test solution. When the amount of DNS was less than 1.5 mL, the absorbance of the solution was intensified with the increase of DNS amount (Figure 1.b). When the amount of DNS was larger than 1.5 mL, the absorbance decreased slightly. The optimal amount of DNS was 1.5 mL.

4.1.3 Effect of color temperature

The DNS reacted slowly with reducing sugars at normal temperature and did not even react chemically. In the five reaction temperatures, the general effect was better at higher temperature. The absorbance maximized at 90 °C, but slightly decreased at 100° C (Figure 1.c). This may be because the solution volatilized at too high temperature, which affected the total volume of the reaction and thus its absorbance.

4.1.4 Effect of color rendering time

In the first 6 min, DNS absorbed rapidly due to its rapid reduction (Figure 1.d). After 7 min of color development, the absorbance began to change gently or slightly decreased. In other words, the color development time can be 7 min.

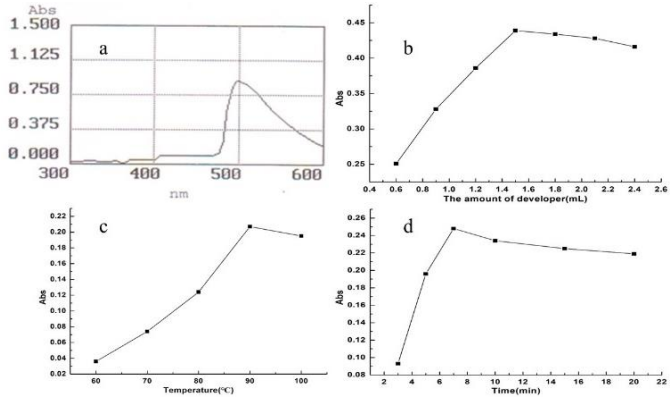

Figure 1: Influences of measurement conditions

4.2 Glucose standard curve

With the glucose concentration as the X-axis and the absorbance as the Y-axis, the standard curve was plotted according to the actual calculated glucose absorbance. The regression equation for the standard curve was y = 3.9163x-0.0292 (R^2 = 0.9981). Experiments showed the glucose concentration ranged from 0.07332 to 0.25662 mg/mL, and the absorbance and glucose content were well linearly related (Figure 2).

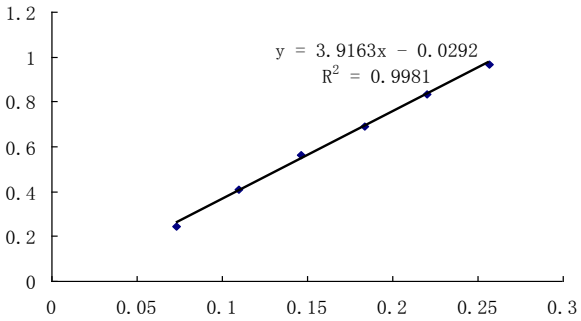

Figure 2: Standard glucose absorbance - content curve

4.3 Stability test results

The absorbance values measured after 20 min, 30 min, 40 min, 50 min, 1 h, and 2 h were 0.408, 0.412, 0.413, 0.414, 0.414 and 0.417, respectively. RSD = 0.66% <1% indicates high stability in 20 – 120 min.

4.4 Precision Experiment Results

The absorbances of five identical samples were 0.409, 0.409, 0.406, 0.412 and 0.404, respectively. RSD = 0.755% < 1% suggest high precision.

4.5 Repeatability Test Results

The absorbances of the five replicates were 0.400, 0.414, 0.400, 0.404 and 0.393, respectively. RSD=0.16% <1% indicates the standard product had high repeatability.

4.6 Recovery Test Results

In the range of 0.1-0.26 mg/mL, the recovery rate increased as the amount of sugar increased (Table 1). The average recovery rate exceeded 98%. Therefore, the experimental method has high accuracy.

Table 1: Recovery experiment

Test group	Absorbance A	Amount of sugar measured (mg/mL)	Actual sugar amount (mg/mL)	Recovery rate(%)	Average recovery rate (%)	RSD(%)
1	0.407	0.10977	0.10998	99.8		
2	0.562	0.15095	0.14664	102.9		
3	0.692	0.18415	0.1833	100.5	100.4	1.46
4	0.832	0.2199	0.21996	100.0		
5	0.966	0.25412	0.25662	99.0		

RSD=0.16% <1% suggests the standard product has high repeatability.

4.7 Sample Recovery Test Results

Table 2: Sample Recovery Test (n=6)

No.	Sample reducing sugar content (mg)	Standard glucose addition (mg)	Absorbance (A)	Measurement (mg)	Recovery rate(%)	Average	RSD (%)
1	0.06976	0.03665	0.384	0.10550	97.5		
2	0.06976	0.03665	0.381	0.10474	95.4		
3	0.06976	0.05499	0.451	0.12262	96.1		
4	0.06976	0.05499	0.451	0.12262	96.1	96.4	0.813
5	0.06976	0.07328	0.520	0.14023	96.2		
6	0.06976	0.07328	0.523	0.14100	97.2		

RSD=0.813% <1% indicates high sample recovery.

4.8 Polysaccharide content results

The total polysaccharide content, reducing sugar content and polysaccharide content in the crude corn silk were 26.3%, 8.14% and 18.16%, respectively.

5. CONCLUSIONS

The DNS colorimetry is convenient, safe, accurate and cheap, and can be used to measure the reducing sugar in corn silk. The optimal measurement conditions were wavelength at 492 nm, amount of DNS = 1.5 mL, and color development time = 7 min. The total polysaccharide content in water-extracted alcohol-coarse corn measured by the sulfuric acid-phenol method was 26.3%. The content of reducing sugar was 8.14% and polysaccharide content was 18.16% detected by DNS colorimetry. The first application of DNS colorimetry to detect the reducing sugar of corn silk has high significance for promoting the development of corn silk polysaccharides and provides scientific basis for improving the quality standards of corn silk polysaccharides.

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