

Determination of tannins content in banana stalk and its silage

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Abstract. Tannins content is one of the main measurement indexes of feedstuff from banana stalks. This study aims to develop a spectrophotometric method to determine tannins content of banana stalk and its silage. The tannins in banana stalk and its silage were extracted by boiling water bath. Using sodium tungstate-phosphomolybdic acid solution as color-developing agent, after 60 min of developing time, the absorbance at 765 nm wavelength was determined, and verified the linear relation between tannins content (C) and absorbance at 765 nm (A) ($C=10.930\times A$, $R^2=0.9992$). The credibility of the method was evaluated by determining detection limit, precision and accuracy. The evaluation demonstrated the spectrophotometric method with sodium tungstate- phosphomolybdic acid is feasible and satisfactory for determination of tannins content in banana stalk and its silage.

Introduction

Banana stalk is a promising feed resource that contains good quantities of crude protein, crude fiber and crude fat. By assuming a straw/grain ratio of 2.4 for banana [1], an annual output of banana stalk reaches 5 million tons in Hainan province. To turn banana stalk into feedstuffs not only can alleviate the shortfall of feed supply, but also provides an effective way for high value-added utilization of the agricultural waste [2,3]. However, the high tannins content in banana stalk makes feeding value discounted greatly, because high tannins content causes a slump in appetite and a decrease in feed digestibility [4]. The inactivation and passivation of tannins is a necessary measure in turning banana stalk into feedstuffs and the tannins content in banana stalk and its feedstuffs is one of the main measurement indexes. Present determination methods of tannins content in grains, fruits and vegetables are mainly based on spectrophotometry with various color-developing agents, including ammonium ferric citrate [5], sodium tungstate- phosphomolybdic acid [6,7], ammonium metavanadate [8], potassium ferricyanide [9]. However, there has not been a standard method for the determination of tannin content in banana stalk and its feed products. This study aims to develop a spectrophotometric method with sodium tungstate- phosphomolybdic acid to determine tannins content of banana stalk and its silage.

Materials and methods

Materials

The banana stalk was collected from a local banana plantation in Hainan. The banana stalk was cut into 15- to 20-cm lengths and squeezed. The solid residue was then dried in the sun. When the moisture content dropped to about 70%, the banana stalk was sealed in plastic bags for 60-day of ensiling.

Extraction of tannins in banana stalk

Ten gram of banana stalk was grinded. Following addition of 100 ml of distilled water or acetone solution (1:1, v/v), the banana stalk was placed in a boiling water for 30~120 min.

Determination of tannins

To 1 ml of supernatant of extract of tannins, 2.5 ml of sodium tungstate- phosphomolybdic acid solution and 5 ml of sodium carbonate solution were added successively. Diluted with distilled water

to 25 ml, the colorimetric tubes were placed stationarily for 60 min. The OD of the supernatant was measured at 765 nm.

Evaluation of determination method of tannins in banana stalk

The theoretical limit of the detection (LOD) was given by the equation, $LOD = K \times Sb / m$, where K is a numerical factor chosen according to the confidence level desired, Sb is the standard deviation of the blank measurements and m is the slope of the calibration curve. When the measurement times of blank is less than 20, $K=3$.

Five samples each of banana stalk and its silage and sap were sampled to determine the tannins content. The precision was assessed by analysis of relative standard deviation (RSD) of repeated measures.

The accuracy was calculated by the recovery yield of tannins from spiked samples. Three concentration levels of tannins (1000, 5000 and 10000 mg/kg) were added to blank reagent, banana stalk, sap of banana stalk and silage of banana stalk. Recovery was determined in triplicate at the three concentration levels.

Data process

Tannins content (mg/kg) = tannins concentration of extract (mg/ml) \times 100 ml / 10 g \times 1000. Statistical analysis was performed using SPSS (Statistical Product and Service Solutions).

Results and discussion

Extraction method of tannins in banana stalk

The extraction solvent significantly affected extraction efficiency ($P < 0.05$) (Table 1). In the same amount of extraction time, the extraction efficiency of tannins by water was higher than that by acetone solution. The extraction time also significantly affected extraction efficiency ($P < 0.05$). However, with the extraction time increasing from 60 min to 120 min, the extraction of tannins didn't increased significantly. According to the experimental results, water was selected as extraction solvent heating in a boiling water bath for 60 min for extracting tannins from banana stalk.

Table 1 The effects of extraction solvent and extraction time on tannins extraction

No.	Extraction solvent	extraction time (min)	OD ₇₆₅ [*]
1	Water	30	0.539 \pm 0.043 ^A
2		60	0.580 \pm 0.052 ^B
3		90	0.587 \pm 0.035 ^B
4		120	0.588 \pm 0.014 ^B
5	Acetone solution	30	0.429 \pm 0.037 ^C
6		60	0.469 \pm 0.044 ^C
7		90	0.494 \pm 0.027 ^C
8		120	0.533 \pm 0.040 ^A

* Values are expressed as mean \pm SEM (n=3). Values in the same row followed by the same capital letter are not significantly different at $P = 0.05$, according to Duncan's multiple range test.

Limit of the detection

A series of concentrations of tannins solution (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 mg/l) were prepared their absorbance at 765 nm were detected following the procedures described above. The linear regression equation was developed between tannins content (C) and absorbance at 765 nm (A) ($C=10.930 \times A$, $R^2=0.9992$).

Ten blank measurements were performed to calculate limit of the detection (LOD) (Table 2). The standard deviation of the blank measurements (Sb) was 148.3, and the slope of the calibration curve (m) was 10.930, and the K was 3. According to the equation, $LOD = K \times Sb / m$, the limit of the detection was 41.9 mg/kg.

Table 2 Test results of limit of the detection

No.	OD ₇₆₅	Tannins content (mg/kg)
1	0.011	318.9
2	0.001	53.2
3	0.005	265.8
4	0.001	53.2
5	0.010	531.5
6	0.003	159.5
7	0.001	53.2
8	0.003	159.5
9	0.003	159.5
10	0.004	212.6
Mean	-	196.7±148.3
Standard deviation	-	148.3
Limit of the detection	-	41.9

Precision analysis

The precision was assessed by analysis of relative standard deviation (RSD) of repeated measures. Five measures each of banana stalk, silage of banana stalk and sap of banana stalk were performed (Table 3). The tannins contents of banana stalk and its silage and sap were 1072.3, 694.2 and 4454.8 mg/kg, respectively. RSD ranged from 1.8 to 13%, indicating good precision of the determination method.

Table 3 Test results of precision analysis

No.	Banana stalk	Silage of banana stalk	Sap of banana stalk
1	996.6	4528.4	691.0
2	936.68	4443.3	677.7
3	1282.2	4496.5	694.9
4	1003.2	4475.2	754.7
5	1142.7	4315.8	652.4
Mean (mg/kg)	1072.3	4451.8	694.2
RSD (%)	13.0	1.8	5.4

Accuracy analysis

Three concentration levels of tannins were added to blank reagent, banana stalk, sap of banana stalk and silage of banana stalk, and the total tannins contents were determined (Table 4). The recovery rates ranged from 95.7 to 108.7 % (RSD 0.3~10.6%), indicating good accuracy of the method.

Table 4 Test results of accuracy analysis

	Addition (mg/kg)	Recovery yield (mg/kg)			Recovery rate (%) [*]	RSD (%)
Blank reagent	1000	1004.5	884.9	1028.5	97.3±7.7	7.9
	5000	4994.8	4994.8	4715.7	98±3.2	3.3
	10000	9822.1	9710.5	9431.5	96.5±2	2.1
Banana stalk	1000	2092.8	2092.8	2292.1	108.7±11.5	10.6
	5000	6188.2	6188.2	6605.8	105.1±4.8	4.6
	10000	11411.6	11685.2	10473.7	101.2±6.4	6.3
Sap of banana stalk	1000	1696.5	1666.8	1674.2	98.5±1.5	1.5
	5000	5469.1	5501.0	5469.1	95.7±0.4	0.4
	10000	10526.4	10571.5	10503.8	98.4±0.3	0.3
Silage of banana stalk	1000	5481.1	5590.5	5428.4	104.8±8.3	7.9
	5000	10807.2	11604.4	11515.8	108.6±1.2	1.1
	10000	16875.1	16210.8	15745.7	103.5±5.2	5.0

* Values are expressed as mean±SEM (n=3).

Conclusions

The tannins in banana stalk and its silage were extracted by boiling water bath. Using sodium tungstate- phosphomolybdic acid solution as color-developing agent, based on the linear regression equation between tannins content and absorbance at 765 nm, the tannins in banana stalk and its silage were quantified. The analysis of detection limit, precision and accuracy indicates that spectrophotometric method is satisfactory for determination of tannins content in banana stalk and its silage.

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