

Determination of Vitamin C Content and Extraction of Pigments from *Fragaria ananassa* (Strawberry)

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Abstract

In the present work, strawberry, *Fragaria ananassa* samples were collected from Say Ta Lone Village, Pyin Oo Lwin Township, Mandalay Region. For chemical analysis of strawberry, *Fragaria ananassa*, vitamin C content was determined by Iodometric method. Extraction of pigment from strawberry sample was carried out by solvent extraction method. And then, pigment, isolated compound was separated and identified by TLC and PC. According to the colour and R_f values, the fruit of strawberry may contain two anthocyanidins such as cyanidin and pelargonidin. Finally, this compound was studied by spectrophotometric method, the maximum absorption spectrum of the colour solution was found at approximately 530 nm.

Keywords: Thin Layer Chromatography, Paper Chromatography, Spectrophotometric method

Introduction

The strawberry, *Fragaria*, is one of the most popular berry fruits in the world. There are native to the temperate regions of the Northern Hemisphere and cultivated varieties are widely grown throughout the world. There are more than 10 species of *Fragaria* that differ in flavour, size and texture. They all have the same characteristics; they are heart-shaped, have red flesh and seeded coat together with small, regal, leafy green caps and stems that adorn their crowns. Strawberries are not actually fruits as their seeds are on the outside. Strawberry plants are runners, and are not produced by seeds. They have an average of 200 seeds per fruit and are actually a member of the rose (rosaceae) family. Strawberries are very perishable and require cool dry storage. Strawberries are an excellent source of vitamins C and K as well as providing a good dose of fibre, folic acid, manganese and potassium. They also contain significant amounts of phytonutrients and flavonoids which makes strawberries bright red. They have been used throughout history in a medicinal context to help with digestive ailments, teeth whitening and skin irritations.

The vibrant red colour of strawberries is due to large amounts of anthocyanidin, which also means they contain powerful antioxidants. The more important antioxidant and anti-inflammatory nutrients are present in fresh, ripe strawberries. They are anthocyanins, flavonols, hydroxy-benzoic acids, hydroxy-cinnamic acids, tannins and stilbenes. Strawberries, a staple fruit of the American diet, are naturally high in anthocyanins. The most common anthocyanin structure in plants is cyanidin-3-glucoside. Anthocyanins are becoming increasingly important as they have antioxidant properties and health benefits, including anti-cancer, anti-inflammatory and vasoprotective effects, preventing coronary heart diseases and improving visual acuity. They are important compounds not only because they have health related properties, but also because they are widely used as colorants in food industry.

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Botanical Description

Botanical name	: <i>Fragaria ananassa</i>
Family name	: Rosaceae
English name	: Strawberry guava
Local name	: Strawberry
Genus name	: <i>Fragaria</i>
Part used	: Fruits



Figure 1. Fruits of *Fragaria ananassa* (Strawberry)

Aim and Objective

Aim

The aim of the present work is determination of vitamin C content, and extraction and separation of pigments from strawberry, *Fragaria ananassa*.

Objectives

- To collect the sample (strawberry) from Pyin Oo Lwin Township, Mandalay Region
- To prepare collected sample for analysis
- To determine vitamin C content
- To extract the pigments from sample
- To separate and identify the pigments from sample

Materials and Methods

Sample Collection

The fruits of strawberry were collected from Say Ta Lone Village, Pyin Oo Lwin Township, Mandalay Region in Myanmar.

Determination of Ascorbic Acid Content in Fruit of *Fragaria ananassa* (Strawberry)

Preparation of Fresh Sample

Fresh fruits of sample *Fragaria ananassa* were washed and dried in air. 100 g of sample was weighed. Then, the sample (100 g) was crushed into a blender for approximately 2 mins. The homogenized sample was transferred into a 250 mL beaker. And then, 50 mL distilled water was added and stirred. Finally, fresh sample solution was obtained.

Preparation of Iodine Solution (0.001 N)

KIO₃ (10 g) was placed in an oven for one hour at 100°C. Then, 0.268 g of KIO₃ and 5 g of KI were dissolved in 500 mL beaker with 200 mL distilled water, 30 mL of 3 M concentrated sulphuric acid was added into a beaker and then dilute with distilled water until 500 mL solution.

Preparation of Ascorbic Acid Solution

Ascorbic acid (0.500 g) was dissolved with 100 mL of distilled water in the beaker. The solution was transferred into 500 mL beaker and diluted with distilled water until 500 mL solution.

Preparation of Starch Indicator Solution

Starch (1 g) was dissolved in 100 mL boiling distilled water.

Standardization of Ascorbic Acid Content

The ascorbic acid content, was determined by iodometric titration. 25 mL of ascorbic acid solution was placed into a 250 mL conical flask and starch indicator (10 drops) was added into a conical flask. And then, the solution was standardized with (0.001 N) iodine solution. When the solution became pale-blue colour, ascorbic acid content was completed.

Analyses of Vitamin C Content of the Fresh Sample

25 mL of fresh sample solution was taken in 25 mL of pipette and it was transferred into a 250 mL conical flask. 10 drops of starch solution was added. And then, this solution was titrated with iodine solution (0.001 N). When the solution became reddish brown, analyses of vitamin C content of the sample was completed. This analysis was repeated three times for a sample. Finally, the vitamin C content of sample could be calculated.

Qualitative Tests for Anthocyanins

Extraction of Anthocyanins from Fruit of *Fragaria ananassa* (Strawberry)

Anthocyanins containing in the fresh fruit of *Fragaria ananassa* (Strawberry) were extracted with amyl alcohol for about one week. This extract was filtered and stored in a refrigerator for several weeks.

Test for Detection of Anthocyanins

The presence of anthocyanins in the fruit of *Fragaria ananassa* (Strawberry) was generally tested by three common reagents: (1) Sulphuric Acid (2) Dilute Hydrochloric Acid and (3) Ferric Chloride.

Test for Detection of Anthocyanins Containing in Fruit of Strawberry

Table (1) Test for Detection of Anthocyanins

No.	Experiment	Observation	Inference
1.	H ₂ SO ₄ Test : Dilute sulphuric acid was added to 2 mL of extracted solution. This solution was warmed gently, cooled and shaken with amyl alcohol.	The anthocyanin was separated in the alcohol layer.	A general test for anthocyanin
2.	Dilute HCl Acid Test : 2 % HCl was added to 2 mL of extracted solution. 5 % NaOH was added to the above solution. The solution was acidified again.	A red colour was produced. The colour was changed to green. Red colour was reappeared.	Pelargonidin may be present Cyanidin may be present Pelargonidin may be present.
3.	Ferric Chloride Test : The fruits were extracted with amyl alcohol. A little sodium acetate and a small quantity of 5 % ferric chloride solution were added to 2 mL of extracted solution.	Violet colour was developed	Cyanidin and pelargonidin may be present



H₂SO₄ Acid Test

Dilute HCl Acid Test

Ferric Chloride Test

Figure 2. Test for Detection of Anthocyanins Containing in the Fruit of *Fragaria ananassa* (Strawberry)

Hydrolysis of Anthocyanins

Procedure

The extracted samples (1 mL), 1 % HCl in absolute methanol extract and 1 mL of 4 M HCl were placed in a test tube, and the test tube was heated on a water bath of approximately 80°C for varying time intervals. The test tube was covered with a glass marble during hydrolysis, to prevent the escape of vapours. Acid hydrolysis was carried out in a fume hood. The addition of acid without concurrent heating of the anthocyanin extracts results in very little hydrolysis in a 24 hr period. When the hydrolysis was complete, the tube was stored in the cold for up to a week with little evidence of additional hydrolysis. After hydrolysis for each time interval, a small sample was removed from the heated mixture and spotted on the TLC plate and the spots were checked.

Separation of Anthocyanidins by Thin Layer Chromatography (TLC)

Procedure

The hydrolysate was spotted on the plate of Thin Layer Chromatography. The spotted plate was placed in a chromatographic jar containing the solvent mixture butanol : acetic acid : water (4 : 1 : 5). After the development was completed, the plate was quickly dried with a dryer (cool air) and determined the R_f values of the coloured spots.



Separation of Anthocyanidins by Paper Chromatography (PC)

Procedure

1 × 2.5 inches Whatmann No. 1 paper was cut off for paper chromatographic separation of the anthocyanidin component. The sample solution was spotted on the chromatographic paper by using a capillary tube. After drying the spot, paper was placed in the chamber and was allowed to run by ascending method at room temperature. The solvent system for this ascending paper chromatography was n-butanol : acetic acid : water (3 : 1 : 1 v/v). The solvent was allowed to travel up the sheet until the solvent under it has traveled 85 % of the ways to the top. The paper chromatogram was removed from the tank and immediately drawn a thin pencil line at the solvent front. And then, it was dried with a dryer. The colour and R_f value of common anthocyanidin in paper chromatograms (PC) were detected.



Table (2) The Colour and R_f Values of Anthocyanidins Containing in Strawberry

Name	Colour in visible light	R _f value (TLC) BAW	R _f value (PC) BAW
Cyanidin	Magenta	0.69	0.64
Pelargonidin	Orange	0.81	0.77

Spectrophotometric Study
Separation of Anthocyanidins by Paper Chromatogram for Spectrophotometric Study

The anthocyanidins extract was separated by Paper Chromatogram method for the spectrophotometric study.



Elution of Coloured Bands for Spectrophotometric Study

The developed paper chromatogram was dried quickly in an oven and coloured spots were made with a pencil. The coloured part was removed into a test tube and dissolved in 10 ml of 1 % HCl in methanol. The mixture was shaken and centrifuged. The absorption spectra of coloured solution were determined at the ultraviolet and visible regions of the spectrophotometer.

Results and Discussion

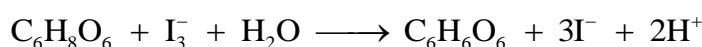
Determination of Vitamin C Content by using Iodometric Titration

Vitamin C content in fruit of strawberry was determined by iodometric method. In this case, the determinations was done three times and the mean value was taken. The result is shown in the following Table (3) and (4).

Table (3) Titration of 25 mL Fresh Juice Sample of Strawberry with 0.001 N Iodine Solution by using Starch Indicator

No.	Initial volume (mL)	Final volume (mL)	Volume used (mL)
Rough	0	14	14
1	14	27.2	13.2
2	27.2	40.5	13.3
3	0	13.2	13.2
Mean value			13.2

Calculation



Titration of fresh juice sample with I₂ solution,

$$\begin{aligned}
 25 \text{ mL of sample solution} &= I_2 \ 13.2 \text{ mL} \times 0.0081 \text{ M} \\
 &= 0.1069 \text{ mmol of } I_2 \\
 &= 0.1069 \text{ mmol of Ascorbic acid} \\
 &= 0.1069 \times 10^{-3} \text{ mol} \times 176 \text{ gmol}^{-1} \\
 &= 18.81 \times 10^{-3} \text{ g ascorbic acid}
 \end{aligned}$$

$$\begin{aligned}
 25 \text{ mL of sample solution} &= 18.81 \times 10^{-3} \text{ g ascorbic acid} \\
 100 \text{ mL} &= ? \\
 &= \frac{100 \text{ mL} \times 18.81 \times 10^{-3} \text{ g}}{25 \text{ mL}} \\
 &= 75.24 \times 10^{-3} \text{ g} \\
 &= 0.07524 \text{ g} \\
 &= 75.24 \text{ mg} \\
 100 \text{ g of sample} &\text{ contains } 75.24 \text{ mg of ascorbic acid.}
 \end{aligned}$$

Table (4) The Amount of Ascorbic Acid in Strawberry

No.	Sample	Content of Ascorbic Acid (mg/100 g)
1.	Strawberry	75.24

Extraction and Separation of Sample

Strawberry fruits were collected from Say Ta Lone Village, Pyin Oo Lwin Township, Mandalay Region. The pigment from the collected sample was extracted by amyl alcohol. And then the extracted solution was filtered and centrifuged. The solution was decanted and checked for qualitative test for anthocyanins. According to qualitative tests these selected fruits of strawberries may contain cyanidin and pelargonidin respectively.



H₂SO₄ Acid Test



Dilute HCl Acid Test



Ferric Chloride Test

And then the extracted sample was digested with 4 M HCl. After digestion, the anthocyanidin constituent of the strawberry fruits were determined by Thin layer and paper chromatography methods. After separation, anthocyanidins containing in this sample were identified by comparing their colour and R_f values of coloured spots (0.69, 0.81 (TLC) and 0.64, 0.77 (PC)) with literature values (0.68, 0.80). From the comparison of these data, it was found that the fruits of strawberry contain two anthocyanidins which are cyanidin and pelargonidin respectively.



Separation of Anthocyanidins by TLC
B:A:W (4:1:5)



Separation of Anthocyanidins by PC
B:A:W (3:1:1)

Furthermore, these coloured spots were removed from paper for spectrophotometric study. The absorption spectra of the colour solution obtained, were determined at the (ultraviolet and) visible regions of spectrophotometer. The determination of absorption spectra, the absorption maximum of these prepared anthocyanidin solution show characteristic maximum of absorption in the visible region approximately 530 nm.

Conclusion

In this research work, strawberry fruits were collected for the determination of vitamin C content and extraction of pigment from these sample. For chemical analysis of strawberry, *Fragaria ananassa*, vitamin C content was determined by iodometric method. From the study, it was found that (75.24 mg/100 g) vitamin C is present in the collected sample. The extracted sample was qualitatively detected by Dilute H₂SO₄ Acid test, Dilute HCl Acid test and Ferric Chloride test. The result obtained from these tests, showed that this selected sample has cyanidin and pelargonidin. Extraction of pigment from strawberry fruits was carried out by solvent extraction method. The extracted sample (pigment) was separated and identified by TLC. Butanol : acetic acid : water (4:1:5 v/v) was used as a solvent. The chromatogram showed that the pigment was found to be present in sample with R_f value (0.69, 0.81). By comparing the colour and R_f values of coloured spots on TLC with literature values (0.68, 0.80), it was identified that this selected sample consists of two anthocyanidins (cyanidin and pelargonidin) respectively. According to the spectrophotometric study, the presence of anthocyanins in the fruits of strawberry was confirmed by the observation of absorption maximum of anthocyanidin at approximately 530 nm in visible region.

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