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Rafiya Tazeen
Department of Biochemistry,
Shri Shivaji College of Arts,
Commerce and Science Akola,
Maharashtra, India

SM Mular
Department of Biochemistry,
Shri Shivaji College of Arts,
Commerce and Science Akola,
Maharashtra, India

ZH Khan
Department of Biochemistry,
Shri Shivaji College of Arts,
Commerce and Science Akola,
Maharashtra, India

Nazia D Khan
Department of Biochemistry,
Shri Shivaji College of Arts,
Commerce and Science Akola,
Maharashtra, India

Correspondence
Rafiya Tazeen
Department of Biochemistry,
Shri Shivaji College of Arts,
Commerce and Science Akola,
Maharashtra, India

Comparative analysis of sugar and protein in three different fresh and decaying fruits Pomegranate, Orange and Sweet lime (musambi)

Rafiya Tazeen, SM Mular, ZH Khan and Nazia D Khan

Abstract

The research was done on determining the concentration of sugar protein in three different fresh fruits extract Pomegranate, Orange and Sweet lime (citrus sine sis) musambi. The concentration of sugar and protein present in fresh and decaying fruit extracts. The sugar content is analyzed by DNSA method while Protein content will be determined by Folin Lowry method in fresh and decaying fruits. The result showed by plotting graph that sugar content present in fresh fruit extracts in which is Pomegranate (20mg/ml), Orange (15mg/ml) and in Sweet lime (10mg/ml) while protein contents Pomegranate (4mg/ml), Orange (10mg/ml) musambi (20mg/ml).

Keywords: Sugar, protein, fresh fruits, decaying fruits

1. Introduction

The purpose of the study to determined the concentration of sugar by DNSA (Dinitro salicylic acid). Fruit production has been consistently notching up impressive growth year after year despite because fruit is a key component of a healthy balanced diet [1]. On a commercial note very little of fruit is exported in the absence of adequate processing and refrigeration/storage/cold chain facilities the situation is made worse by callous and antiquated post-harvesting practices, staggering quantity of the produce rots in the fields or dangerously decays while in the process of transportation, storage and in the last leg of reaching the consumer [2].

Protein concentration will determined by Lowry method after decaying fruits it should important to know the concentration of protein because it is good for health or not, so the study will show the protein content in 10 days decayed fruits that are Pomegranate Orange and musambi.

2. Material and Methods

2.1 Collection of the fruit

The fruits were collected from city market, brought in a sterilized plastic bag to the laboratory and stored in a refrigerator until further usage.

2.2 Preparation of Fruit Juice Extract

The collected fresh fruits were surface sterilized by washing in 5% Potassium Permanganate and rinsed well in distilled water. About 10g of decaying fruit sample was taken in a pestle and mortar, grinded well with the addition of about 20.0 mL of Distilled water. The liquid extract was obtained by filtering the grinded content using a cheese cloth. The liquid extract was suitable diluted to 1:100 with distilled water (v/v) [3].

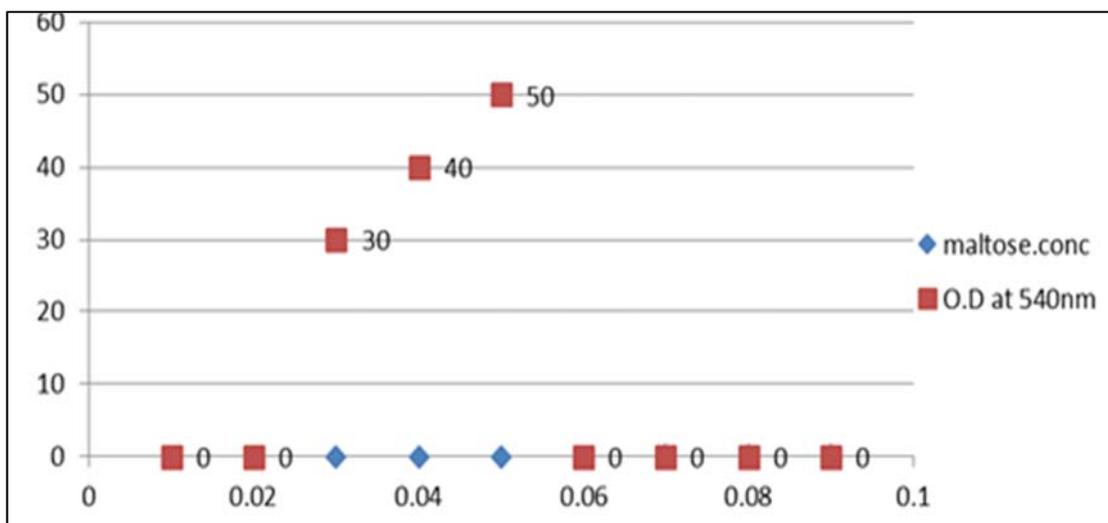
2.3 Estimation of Sugar content

Estimation was done both before and after decaying to determine the reducing concentration of sugar in different extract, DNS Method was followed. Standard graphs have been plotted by using Glucose solution (200µg/mL of working standard). To the above samples 2 ml of DNS reagent was added into a lightly capped test tube.(To avoid the loss of liquid due to evaporation, cover the test tube with a piece of paraffin film if a plain test tube is used).

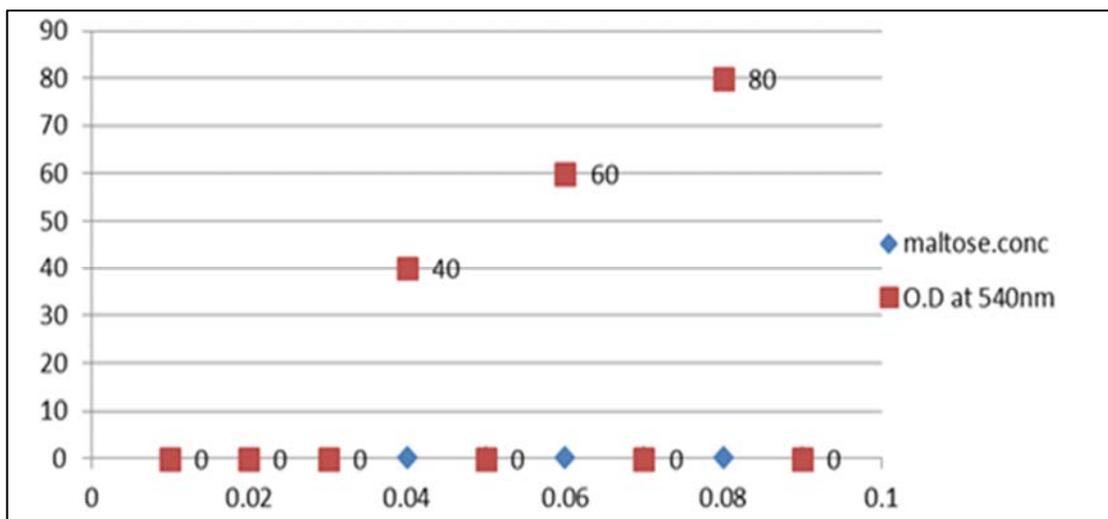
The mixture was heated at 90°C for 15 minutes to develop the red brown color. Thereafter 16 ml of distilled water was added to stabilize the color. After cooling it to room temperature in cold water bath, absorbance was measured with a spectrophotometer at 540nm. Hence, the above

procedure was repeated for 1.0mL of extract and 1.0mL of water has been taken for unknown estimation [4, 5]. After 10 days decayed fruits the procedure for estimation of reducing sugar in decaying fruits followed as above and reading will be noted by calorimetrically at 540nm.

Sr. No	Reagents	Blank	Std1	Std2	Std3	Std4	Sample
1	Std. maltose		0.1	0.2	0.3	0.4	
2	Dist. water	0.5	0.4	0.3	0.2	0.1	
3	Sample						0.5
4	DNSA	0.5	0.5	0.5	0.5	0.5	0.5
5	Keep in boiling	Water bath for 10 min					
6	Dist water	5	5	5	5	5	5
7	O.D at 540 nm	0.00	0.01	0.02	0.03	0.04	0.04(Sweet lime)
8	O.D at 540nm	0.00	0.02	0.04	0.06	0.08	0.06(Orange)
9	O.D at 540nm	0.00	0.04	0.08	0.12	0.16	0.08(Pomegranate)



Estimation of reducing sugar in decaying fruits by DNSA method



Estimation of reducing sugar in fresh fruits by DNSA method

2.4 Estimation of protein by Lowry method

2.4.1 Reagents

1. BSA stock solution (1mg/ml),
2. Analytical reagents: (a) 50 ml of 2% sodium carbonate mixed with 50 ml of 0.1 N NaOH solution (0.4 gm in 100 ml distilled water.) (b) 10 ml of 1.56% copper sulphate solution mixed with 10 ml of 2.37% sodium

- potassium tartar ate solution. Prepare analytical reagents by mixing 2 ml of (b) with 100 ml of (a)
3. Folin - Ciocalteu reagent solution (1N) Dilute commercial reagent (2N) with an equal volume of water on the day of use (2 ml of commercial reagent+2 ml distilled water)

2.4.2 Procedure

1. Different dilutions of BSA solutions are prepared by mixing stock BSA solution (1mg/ml) and water in the test tube as given in the table. The final volume in each of the test tubes is 5 ml. The BSA ranges 0.05 to 1 mg/ml.
2. From these different dilutions, pipette out 0.2 ml protein solution to different test tubes and add 2 ml of alkaline copper sulphate reagent (analytical reagent). Mix the solutions well.
3. This solution is incubated at room temperature for 10 mins.

Sr. No	Reagent	Blank	Std1	Std2	Std3	Std4	Std5	Sample
1	Dist water		0.8	0.6	0.4	0.2	0.0	0.5
2	BSA		0.2	0.4	0.6	0.8	1	
3	Alkaline Cuso4	5	5	5	5	5	5	5
Mix well and wait for 10 min at room temp								
4	Folin ciocalteu	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mix well and wait for 30 min at room temp blue colour develop								
5	O.D at 660nm	0.00	0.04	0.08	0.12	0.16	0.09	0.13 Musambi
6	O.D at 660nm	0.00	0.02	0.04	0.06	0.08	0.11	0.6 Orange
7	O.D at 660nm	0.00	0.02	0.04	0.06	0.09	0.12	0.7 Pomegranate

Estimation of protein different decaying fruits will followed as above protocol

2.5 Calculations

Formula: Amount of protein in fresh fruits in mg /ml = O.D of test /O.D of std x 20 x 25

1. $=0.13/0.16*20*25$
 $=406\mu\text{g/ml} = 0.406\text{mg/ml}$ (Sweet lime)
2. $=0.7/0.9*20*25$
 $=388\mu\text{g/ml} = 0.388\text{mg/ml}$ (Pomegranate)
3. $=0.6/0.8*20*25$
 $=375\mu\text{g/ml} = 0.375\text{mg/ml}$ (Orange)

Amount of protein in decaying fruits in mg /ml = O.D of test /O.D of std x 20 x 25

1. $=0.11/0.15*20*25$
 $=366.6\mu\text{g/ml} = 0.366\text{mg/ml}$ (Sweet lime)
2. $=0.6/0.9*20*25$
 $=333.3\mu\text{g/ml} = 0.333\text{mg/ml}$ (Pomegranate)
3. $=0.5/0.8*20*25$
 $=312.5\mu\text{g/ml} = 0.312\text{mg/ml}$ (Orange)

3. Results and Discussion

The standard graph for the estimation of reducing sugar have been plotted by using glucose as working standard solution (200 $\mu\text{g/ml}$) and the values have shown in table 1 and figure 1 and 2. The sugar content of fresh fruit extracts have been calculated by comparing their optical density values at A 540 with the standard graph. The individual values were taken in triplicates and the mean values have been entered. Among the three extracts used for the analysis of reducing sugar content (glucose level), the result shows that the glucose level was high in Pomegranate (210 $\mu\text{g/ml}$) when compared to Orange (200 $\mu\text{g/ml}$) and *Citrus sinensis* (170 $\mu\text{g/ml}$). Since the extracted juices were high in sugar content, they had been diluted to 1:100 with distilled water.

Hence, the actual values of sugar concentrations in decaying fruits are Pomegranate , Orange and musambi were 21mg/mL, 20mg/mL and 17mg/mL respectively (Table 1 and figure 1)

4. Then add 0.2 ml of reagent Folin Ciocalteu solution (reagent solutions) to each tube and incubate for 30 min. Zero the colorimeter with blank and take the optical density (measure the absorbance) at 660nm.
5. Plot the absorbance against protein concentration to get a standard calibration curve.
6. Check the absorbance of unknown sample and determine the concentration of the unknown sample using the standard curve plotted [6, 7, 8].

4. Conclusion

From the result of this work, it can be concluded that fresh fruits are high both in sugar and protein conc. while decaying fruits are less in sugar and protein conc.

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