

Biochemical estimations of different fruits and vegetable samples

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Abstract: Carbohydrate, Protein, Flavonoid and Phenol are important component of fruits and vegetable. for knowing its actual amount present in different fruits and vegetable sample we take 10 different fruits and vegetables sample. The samples are collected from vegetables and fruit market of Patna. All the above samples are consumed by the people, and biomolecules present in it have major importance to life, and many biological aspects dealing vital in science. The total protein content was determined using the Lowry method. The total protein content was also measured by spectrophotometrically. The estimation showed that the *Malus domestica* (Apple) contain highest protein content among all other selected samples, it records an optical density of 0.214 at 750 nm which indicate that it contains total 516 $\mu\text{g/ml}$ of protein content. The total carbohydrates content was determined using by DNSA method. The total carbohydrates content was also measured by spectrophotometrically. The estimation showed that *Musa acuminata* (banana) contain highest carbohydrates among all other selected sample, it recorded an optical density of 1.649 at 540 nm which means banana contain 555.793 μg of carbohydrate content in per ml of sample extract. The total Flavonoid content was determined using by aluminium chloride colorimetric method. The estimation showed that the *Malus domestica* (Apple) contains highest flavonoid content, it record an absorbance of 0.214 at 510 nm and contain 516.75 $\mu\text{g/ml}$ of flavonoid content. The Phenolic content estimation is done by Folin-Ciocalteu method. The estimation showed that the *Malus domestica* (Apple) record an absorbance of 0.370 at 650 nm, and contains 44.30 $\mu\text{g/ml}$ of phenolic content. It is the highest phenol containing sample among all other sample.

Keywords: protein, carbohydrates, flavonoids, phenol

INTRODUCTION

Most people are aware that proper nutrition and exercise can help them maintain a healthy weight. However, the advantages of good nutrition extend beyond weight loss. Nutritional support can be beneficial. Reduce your risk of certain diseases, such as heart disease, diabetes, stroke, certain cancers, and osteoporosis; lower your blood pressure; improve your well-being; improve your ability to fight illness; improve your ability to recover from illness or injury. People eat a lot of fruits and vegetables from their local market for good nutrition. Is it healthy or safe to eat fruits and vegetables without knowing the exact amount of biomolecules present? It's possible that the amount is less or more than we need, and it's also possible that it's toxic. Because toxic and health-hazardous chemicals have been reported. Chemicals such as calcium carbide / ethephon and oxytocin are allegedly used in fruit and vegetable mandis / farms to artificially ripen fruits and increase the size of fruits and vegetables (1). Biomolecules (carbohydrates, proteins, flavonoids, and phenols) are essential for living organism function and can be found in fruits and vegetables.

The goal of this research was to determine the total carbohydrate, protein, flavonoid, and phenolic content of ten different fruits and vegetables

Carbohydrates: Carbohydrates are an important source of energy, accounting for roughly 40% to 80% of our total energy consumption. Several health organisations advise that carbohydrates be the primary source of energy rather than other foods. Carbohydrates are a convenient source of energy for oxidative metabolism and energy production.

Fibre is beneficial in maintaining normal and healthy gastrointestinal function. Unlike fat and protein, a high carbohydrate intake is not linked to adverse health effects when the carbohydrates are derived from a variety of sources. Compared to fat- and protein-rich diets, carbohydrate-rich diets lower the risk of obesity and its complications. (2)

Protein:- Protein is one of three macronutrients, or nutrients that the body requires in greater quantities Fat and carbohydrates are the other macronutrients. Protein is found in every cell of the body, and getting enough of it is essential for keeping muscles, bones, and tissues healthy. Protein is involved in a variety of bodily functions, such as blood clotting, fluid balance, immune system responses, vision, hormones, and enzymes. Protein is important for growth and development, especially during childhood, adolescence, and pregnancy. (3)

Flavonoids Flavonoids are plant compounds that have a number of health benefits. Flavonoids come in six different types, each with different health benefits These are the following • Anthocyanidins • Isoflavones • Flavonoids • Flavones • Flavan-3-

Consuming a variety of fruits and vegetables is the best way to get all six types of flavonoids. Flavonoids can be found in a variety of plant-based foods and beverages, such as tea and wine. Numerous studies have demonstrated the numerous

advantages of these phytonutrients, Researchers discovered that eating a flavonoid-rich diet lowers the risk of cardiovascular disease, diabetes, and certain cancers. (4)

Phenol:- Natural phenolic compounds have gotten a lot of attention in recent years because a lot of them can be found in plants, and eating vegetables and beverages with a lot of them may reduce the risk of developing a variety of diseases due to their antioxidant power, among other things (5). The present work is to find out amount of Carbohydrates, proteins, Phenol and Flavonoids present in :- Spinacia oleracea (Spinach), Solanum tuberosum (Potato), Daucus carota subsp. sativus (Carrot), Lagenaria siceraria (Bottle gourd), Brassica oleracea var. botrytis (cauliflower), Citrus X sinensis (Orange), Musa acuminata (Banana), Vitis vinifera (Grapes), Malus domestica (Apple), Ipomea batatas (Sweet potato)

MATERIALS AND METHODS

Samples are collected from patna markets . 10 different fruits or vegetables taken for this experiment . for this work required some equipment are Hot air oven ,

Centrifuge, Incubator, Water bath, UV-vis. Spectrophotometer, Mortar and pestle, Weighing machine, sample preparation- The samples were properly collected and washed. Then, using a clean Mortar and Pestle, a small portion of each sample was taken and crushed properly. 1 gm puree of sample was placed in a centrifuge tube with 10 ml distilled water, followed by the same procedure for each sample and left for 24 hours. 1 gm puree of sample was placed in a centrifuge tube with 10 ml distilled water, followed by the same procedure for each sample and left for 24 hours. After 24 hours, centrifuge it for 10 minutes at 1000rpm, then carefully collect the supernatant in a falcon tube. The sample extract from the supernatant will be used in the experiment. Estimation of Carbohydrate is done by DNSA method, Estimation of Protein is done by Lowry method, Flavonoid estimation is done by Aluminium chloride colorimetric method, And Phenolic content estimation is done by Folin–Ciocalteu method.

Determination of Carbohydrate Content - Prepare 20 mL of 2N NaOH. Weigh 1 g DNSA and dissolve in 20 mL NaOH with the help of a magnetic stirrer. Weigh 30 g of sodium potassium tartrate and dissolve in 50 mL dH₂O. Slowly pour sodium potassium tartrate solution in the DNSA and NaOH solution and made the volume up to 100 mL (Note: Wait for the two to mix properly), Decant the contents in a brown bottle. 10 test tubes taken and 1 ml of each sample extract were added in respective test tubes and opening of tubes has been covered by aluminium foil . All test tubes was placed in the boiling water bath for 15 minutes at 65 C. When tests has cooled down, 3 ml of DNSA reagent were added in all tubes. Again it has been covered by aluminium foil and placed in the boiling water bath for 15 minutes at 65C. After cooling to room temperature the absorbance were recorded with a spectrophotometer at 540nm against blank. (6)

Standard curve of absorbance vs concentration of glucose was prepared

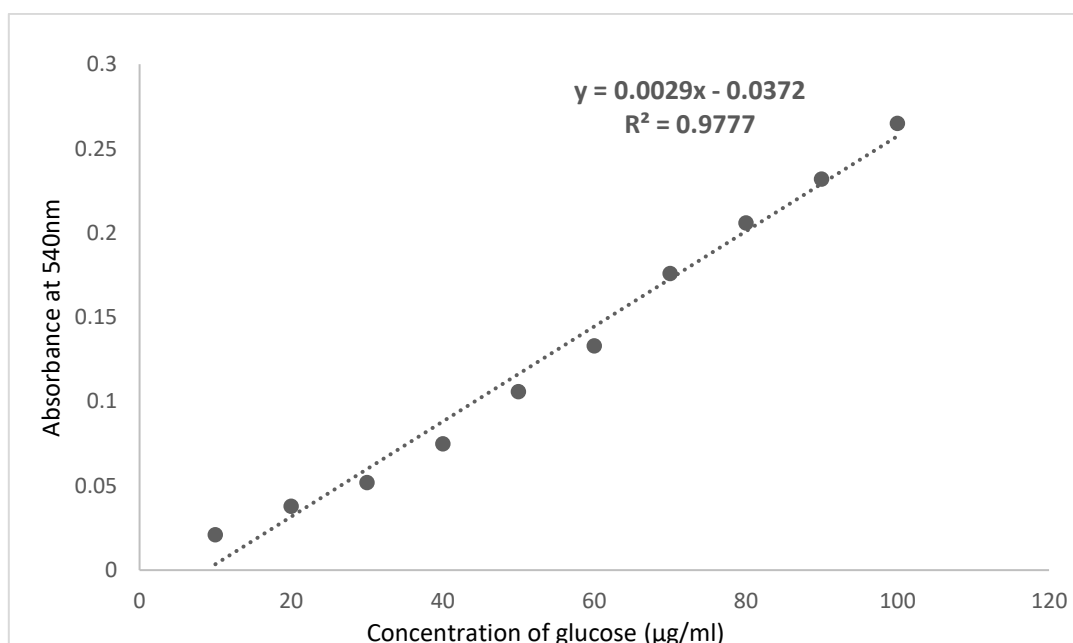


Fig.1-Standard curve of glucose

Determination of protein Content - For protein estimation we required 4 types of solution . 1. Solution A- 2gm Na₂CO₃ dissolved in 100ml distilled water and 0.4gm NaOH added in to it, mix well. Solution A is prepared. 2. Solution B- 0.5% CuSO₄ solution mixed with 1% Na-K-tartrate in same ratio. 3. Solution C- 50ml of Solution A is mixed with 1ml of solution B total 51ml solution will be prepared that is Solution C. 4. Solution D- is FC reagent. 10 test tubes taken and 1 ml of each sample extract were added in respective test tube. Then 5ml solution C was added in each tubes. 0.5ml FC reagent was added . All test tubes was kept in dark area for 30 minutes and then OD was taken using Spectrophotometer at 750nm against blank. (7)

Standard graph .of absorbance vs BSA concentration prepared.

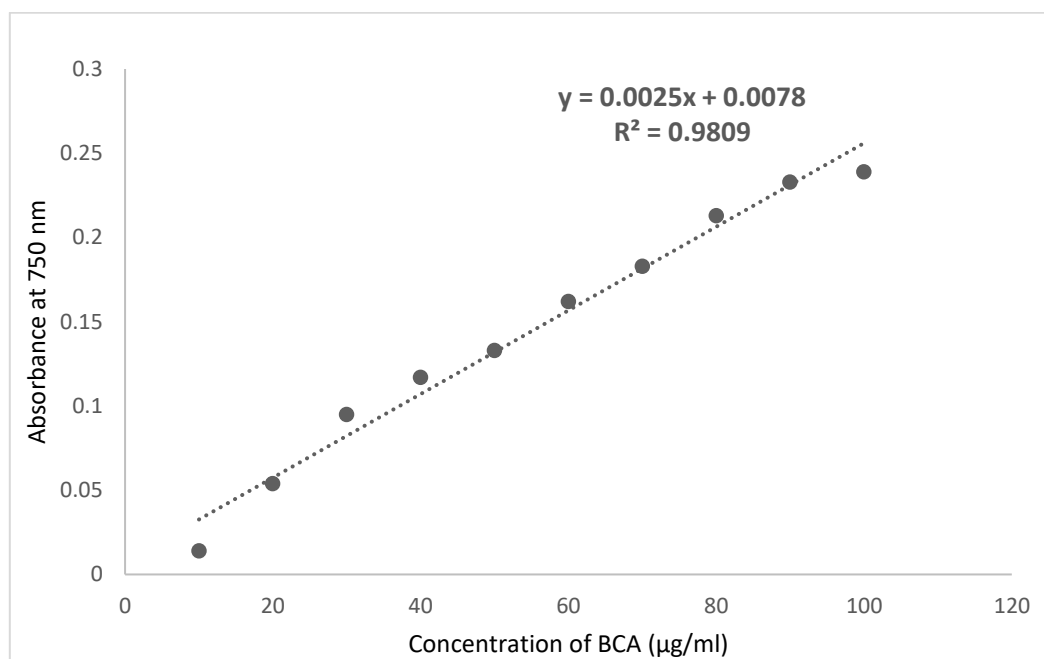


Fig. 2- Standard curve of BSA

Determination of flavonoid Content - 200µl sample extract was taken and 800µl Methanol was added so that volume become 1ml. Then 4ml distilled water was added so that volume become 5ml. Now 0.3ml of 5% NaNO₂ solution was added. Incubated at room temperature for 5 minutes. 0.3ml of 10% AlCl₃ solution was added. Again incubate at room temperature for 6 min. 2ml (1M NaOH) solution was added. Now by added distilled water total solution were made 8ml. Incubate for 15 minutes at room temperature. Then OD was taken using Spectrophotometer at 510nm against blank. Same procedure was performed for all samples.

Standard curve of absorption vs rutin concentration prepared

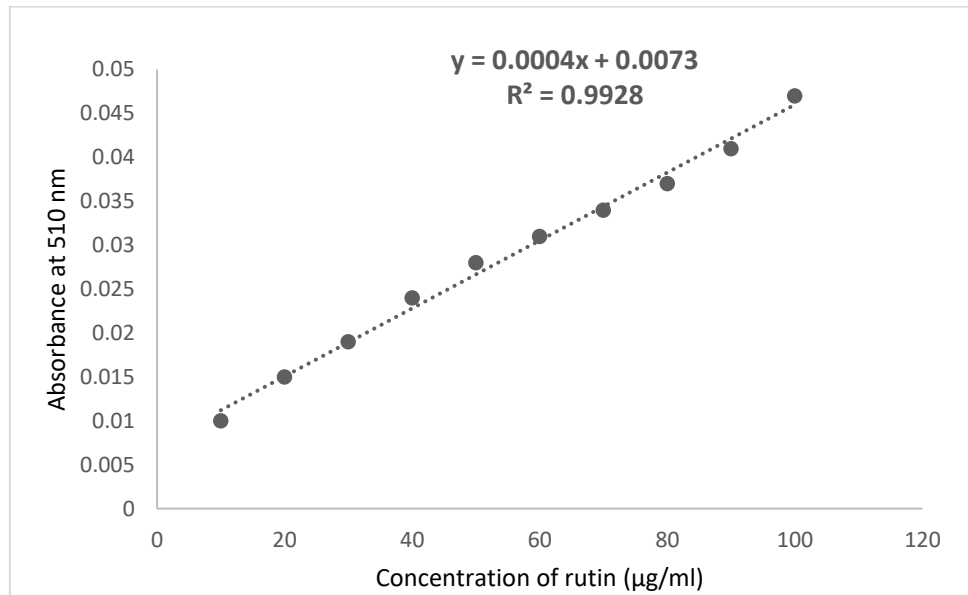


Fig. 3- Rutin standard curve

Determination of phenolic Content- 200µl of sample extract was taken in attest tube. Make volume up to 3ml by adding distilled water. 0.5ml Folin & Ciocalteu's phenol reagent were added. Leave for 3 minutes. 2ml Sodium carbonate (20% W/V) were added. Incubated for 60 minutes in dark area at room temperature. And then OD was taken at 650nm against blank.

Standard curve of absorption vs concentration of gallic acid prepared

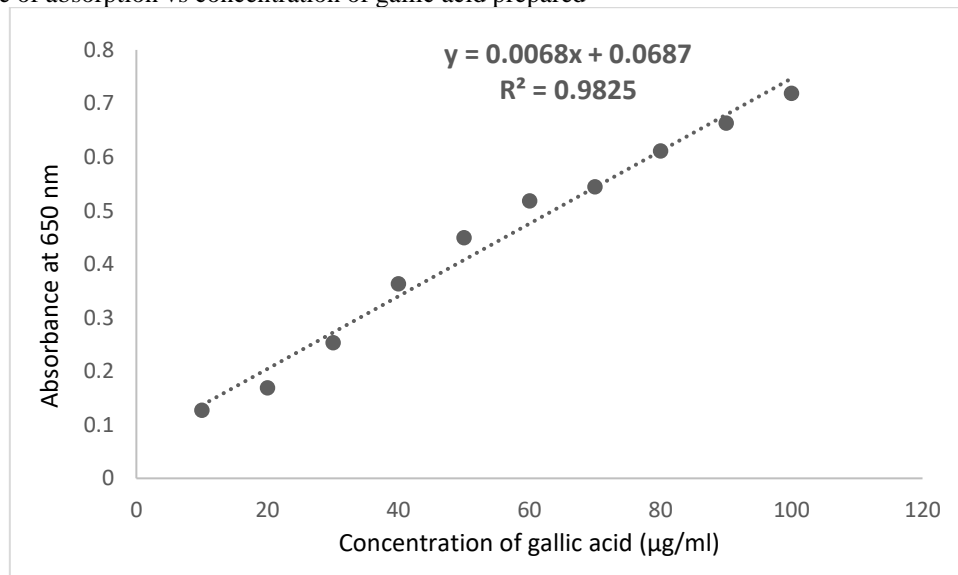


Fig. 4- Standard graph of gallic

RESULT AND DISCUSSION

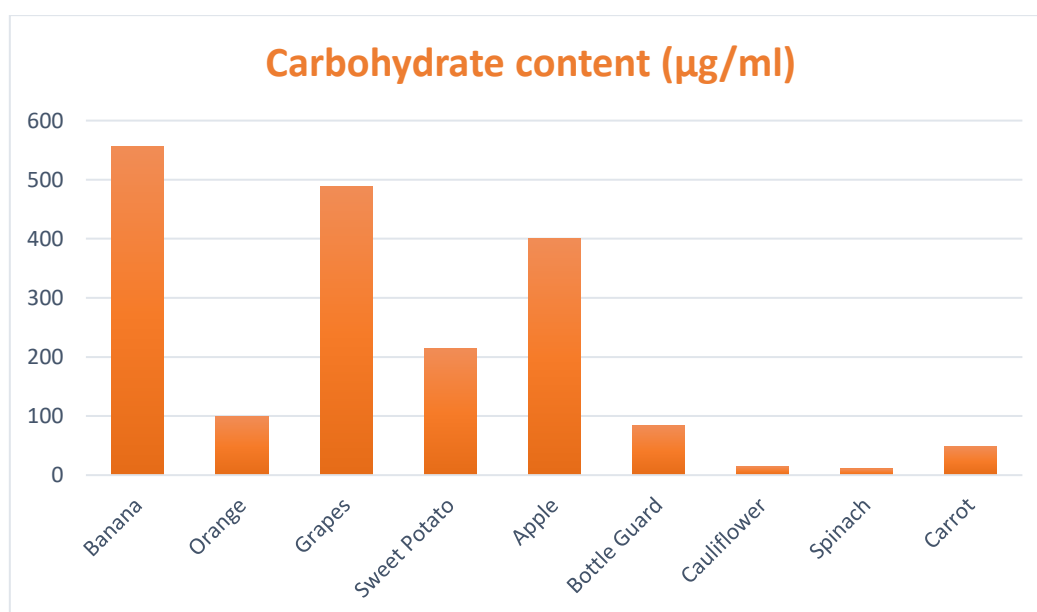
The biomolecular content which were observed in the selected fruits andvegetables sample are tabulated below in Table no. 1, 2, 3, and 4. Following bar graph shows comparative content of biomolecules are representedbelow in Bar graph 1, 2, 3, and 4.

Carbohydrate content- As shown in Table: 1 Banana recorded an optical density of 1.649 at 540 nm which meansbanana contain 555.793 µg of carbohydrate content in per ml of sample extract. It shows highest carbohydrate content among rest the sample. In group of vegetable sweet potato contain highest carbohydrate content i.e; 214.068 µg/ml. spinach

contain very low amount of carbohydrate approximately 11 μ g/ml. Fruit contains high carbohydrate content as compare to vegetables. The amount of carbohydrate content in different samples are listed below.

Sample	Absorbance at 540nm	Carbohydrate content (μ g/ml)
Banana	1.649	555.793
Orange	0.326	99.586
Grapes	1.453	488.206
Sweet Potato	0.658	214.068
Apple	1.199	400.620
Bottle Guard	0.28	83.724
Cauliflower	0.081	15.103
Spinach	0.069	10.965
Carrot	0.18	49.241
Potato	0.117	27.517

Table no. 1:- Amount of Carbohydrate content in μ g/ml and absorbance recorded in spectrophotometer at 540 nm



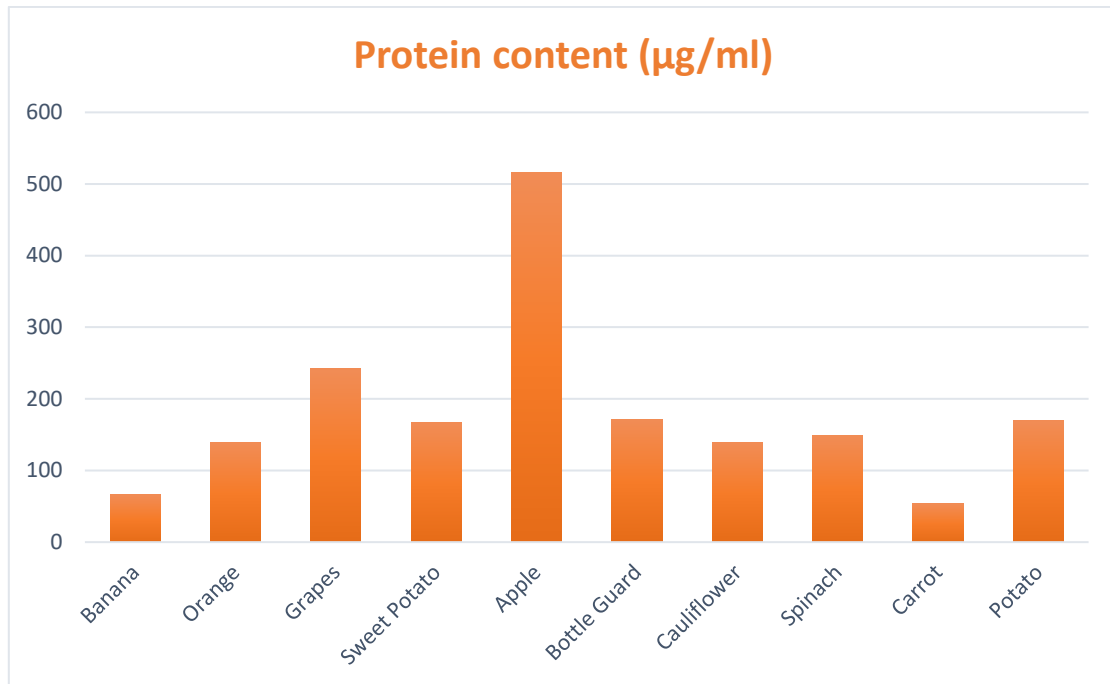
Bar graph no. 1:- Shows amount of carbohydrate in different samples.

Protein content - As shown in Table: 2 Apple contain highest protein content among all other selected samples, it record an optical density of 0.214 at 750 nm which indicate that it contains total 516 μ g/ml of protein content. Selected samples contain as much amount of protein content are listed below.

Sample	Absorbance at 750nm.	Protein content (μ g/ml)
Banana	0.034	66.75
Orange	0.063	139.25
Grapes	0.104	241.75
Sweet Potato	0.074	166.75
Apple	0.214	516.75
Bottle Guard	0.076	171.75
Cauliflower	0.063	139.25
Spinach	0.067	149.25
Carrot	0.029	54.25

Potato	0.075	169.25
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Table no. 2:- Amount of Protein content in µg/ml and absorbance recorded in spectrophotometer at 750 nm.

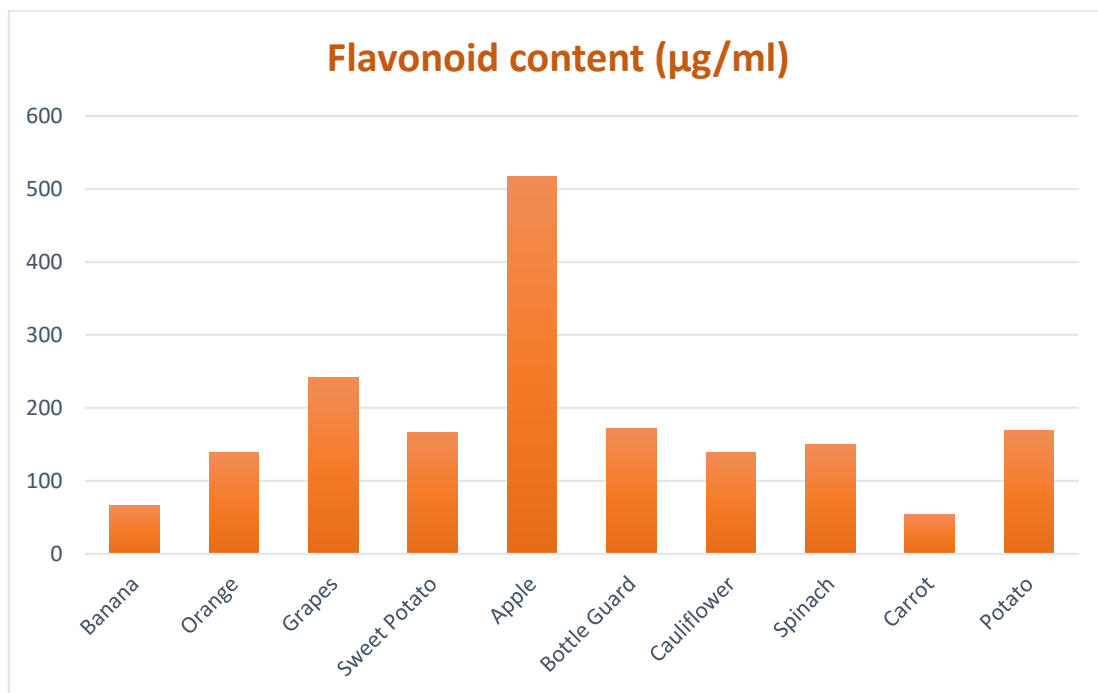


Bar graph no. 2:- Shows amount of protein in different samples.

Flavonoid content - As shown in Table: 3 Apple contains highest flavonoid content, it record an absorbance of 0.214 at 510 nm and contain 516.75 µg/ml of flavonoid content. Whereas carrot contains lowest amount of flavonoid content with absorption of 0.029 recorded by spectrophotometer which means it contain 54.25 µg/ml of flavonoid content. Rest of samples and their flavonoid content are as follow in the table.

Sample	Absorbance at 510 nm.	Flavonoid content (µg/ml)
Banana	0.034	66.75
Orange	0.063	139.25
Grapes	0.104	241.75
Sweet Potato	0.074	166.75
Apple	0.214	516.75
Bottle Guard	0.076	171.75
Cauliflower	0.063	139.25
Spinach	0.067	149.25
Carrot	0.029	54.25
Potato	0.075	169.25

Table no. 3:- Amount of Flavonoid content in µg/ml and absorbance

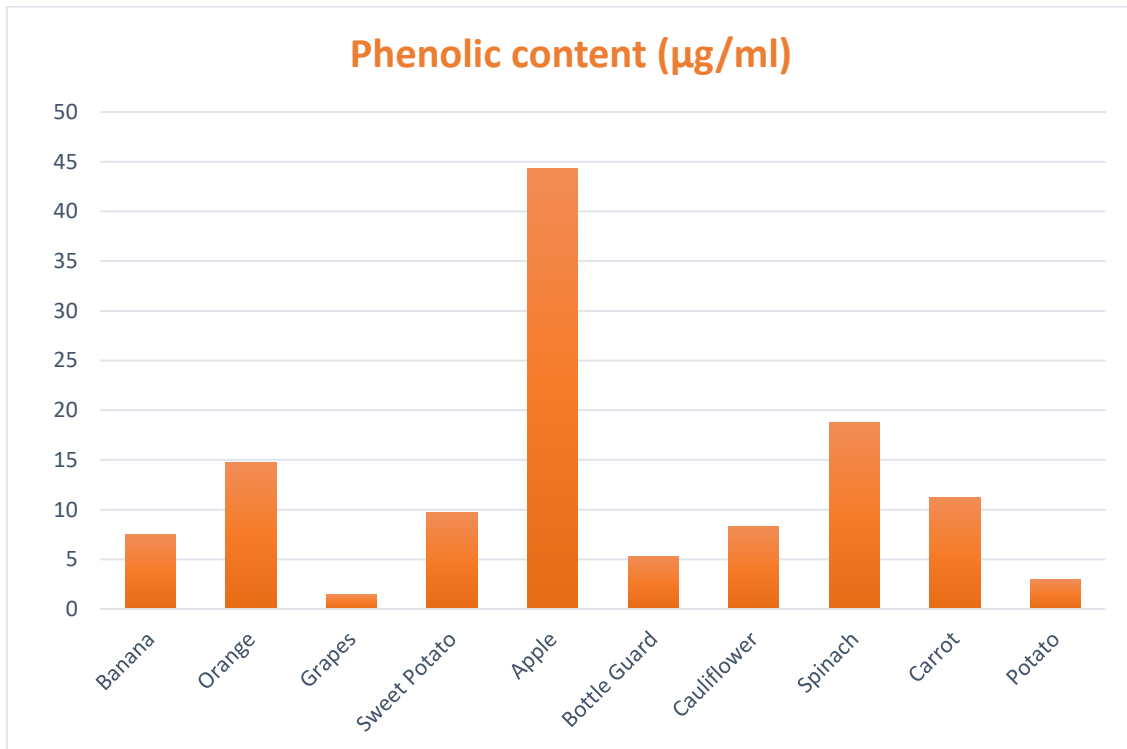


Bar graph no. 3:- Shows amount of flavonoid in different samples.

Phenolic content - As shown in table no. 4 Apple record an absorbance of 0.370 at 650 nm, and contains 44.30 µg/ml of phenolic content. It is the highest phenol containing sample. The lowest phenol containing sample is grapes, it contains 1.51 µg/ml of phenolic content. And othersample and it's phenolic content are tabulated below.

Sample	Absorbance at 650 nm.	Phenolic content (µg/ml)
Banana	0.12	7.54
Orange	0.169	14.75
Grapes	0.079	1.51
Sweet Potato	0.135	9.75
Apple	0.37	44.30
Bottle Guard	0.105	5.33
Cauliflower	0.125	8.27
Spinach	0.196	18.72
Carrot	0.145	11.22
Potato	0.089	2.98

Table no. 4:- Amount of Phenolic content in µg/ml and absorbance recorded inspectrophotometer at 650 nm.



Bar graph no. 4:- Shows amount of phenol in different samples.

CONCLUSION

The results of this study show a link between the carbohydrate, protein, flavonoid, and phenolic content of the vegetables and fruits studied. Banana had the highest carbohydrate level in the fruit group, and sweet potato had the highest carbohydrate content in the vegetable group. As a result, fruit was shown to have a larger carbohydrate content than vegetables. In terms of protein, flavonoid, and phenolic content, apples outperformed all other dietary products tested thus far.

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