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DETERMINATION AND QUANTIFICATION OF IRON AND CALCIUM IN DIFFERENT TYPES OF BELL PEPPER

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ABSTRACT: *Simple and accurate ultraviolet-visible spectrophotometer and flame photometer were developed for the determination of iron and calcium in different types of bell pepper. The spectrophotometric method was depending on the intensity of the color product by using potassium thiocyanate for iron determination. But for calcium no need to make any reagent to make color, direct method utilizing flame photometer. Initially the moisture content of all types of pepper was determined. Overall the selectivity and specificity of the proposed methods were very excellent. This method can be applied for the determination iron and calcium in different fruits as well as in vegetables.*

KEYWORDS: Bell pepper; Iron; Calcium; Spectrophotometer; Flame Photometer

INTRODUCTION

Healthy foods are interested for producers as well as for consumers due to its essential elements and nutrients for our health that continues to promote energy. The capsicum is a precious vegetable due to the importance in respect to biological value and economic significance cultivated in many countries around the world [1–3]. The bell pepper is a family member of Solanaceae [4–5]. This is also well known in our daily life as capsicum. Different colors mainly green, yellow, red, orange, purple and white bell pepper are found worldwide [6–7]. This bell pepper represents multiple plant group and consists of twenty-seven components [8] and five domesticated and remaining are un domesticated. These five common bell peppers are capsicum baccatum, C. frutescens, capsicum annuum, capsicum pubescens and capsicum genus.

Iron is very important nutritional element for the body. Due to its deficiency may cause anemia and still this is a big challenge for any country to solve the problem of particular disease [9]. So, for this reason, researcher and governmental authorities has always been an interest to determine iron in local foods. Previously the general methods for determination of iron are gravimetric and spectrophotometer, used ammonium nitrosophenyl hydroxylamine, thioglycolic acid and ortho-phenanthroline [10–12] as colorimetric reagent. Calcium is an important trace element in living organisms. It is the amplest metal by mass in many vegetables, fruits [13]. It is very important constituent of teeth, bone and shells.

Due to its (calcium and iron) nutritional capability and unavailability of literature for the determination of particular elements in bell pepper, it is necessary to develop method for the quantification.

The proposed method was developed to determine calcium and iron in bell pepper using flame photometer and uv visible spectrophotometer.

EXPERIMENTAL

Instrument and Materials

- UV–Visible spectrophotometer (Jenway 6300, UK).
- Flame photometer (Jenway PFP7, UK).
- Potassium thiocyanate (KSCN) was purchased from Sigma Aldrich, USA.
- Ferric nitrate nonahydrate [$Fe(NO_3)_3 \cdot 9H_2O$] was purchased from Sigma Aldrich, USA.
- Calcium carbonate ($CaCO_3$) was purchased from Sigma Aldrich, USA.
- Hydrochloric acid (HCl, 37%) was purchased from Sigma Aldrich, USA.
- Nitric Acid (HNO_3, 70%) was purchased from Sigma Aldrich, USA.
- Sulfuric acid (H_2SO_4, 95–98%) was purchased from Sigma Aldrich, USA.
- Double distilled water from Jubail Industrial College.
- All chemicals were of analytical grade and used without any further purification.

Solutions

Nitric Acid Solution (2M)

The volumetric flask (1000 ml) was filled water and added 128 ml concentrated HNO_3 with it. (128 ml) was put in 1000 ml volumetric flask. Then, distilled water was added up to the mark.

Ca^{+2} Stock Solution (100 ppm)

$CaCO_3$ (0.125 g) was diluted with 5 ml of HNO_3 (2M) into 500 ml volumetric flask and made up to the mark with HNO_3 (2M).

Nitric and Hydrochloric Acids Solution (0.05M)

3.2 ml of concentrated HNO_3 and 4.16 ml of concentrated HCl were added into one-liter volumetric flask and completed up to mark with distilled water.

Mixture of Concentrated Sulfuric and Nitric Acid

125 ml of concentrated H_2SO_4 and 125 ml of concentrated HNO_3 was added into 250 ml volumetric flask.

Fe^{+3} of Stock Solution (100 ppm)

0.3617g of $Fe(NO_3)_3.9H_2O$ was dissolved and completed with 0.05M mixture of nitric acid and hydrochloric acid solution up to the mark.

Potassium Thiocyanate Solution (5M)

Potassium thiocyanate (48.59 g) was transferred into 100 ml volumetric flask and dissolved with distilled water.

Standard Solutions

Calcium

Five standard solutions (2.5 ppm, 7.5 ppm, 12.5 ppm, 17.5 ppm and 22.5 ppm) were prepared separately by adding (2.5 ml, 7.5 ml, 12.5 ml, 17.5 ml and 22.5 ml respectively) from stock solution (100 ppm) into 100 ml volumetric flasks and completed the final volume with 2M HNO_3 solution.

Iron

A blank and five standard solutions (0.0, 0.5, 1.5, 2.5, 3.5 and 4.5 ppm Fe^{+3}) were prepared by adding required volume from Fe^{+3} stock solution (100 ppm). 10 ml of potassium thiocyanate solution (5M) was added to each of 100 ml volumetric flask and made up to the mark with 0.05M HNO_3 and HCl solution.

Percentage of Water in Pepper

10 g of each pepper (red, green and yellow) was heated in an oven for 10 hours at 105°C. The masses after evaporation were measured and the percentage of water in each type was calculated.

Sample Preparation

Calcium

- The green bell pepper (5g) was transferred into 100 ml beaker covered with watch glass and added 25 ml conc. HNO_3 in it. The mixture was heated to reflux on an electric hot plate at 80–100°C.
- The mixture was heated for hour and again treated with additional 25 ml conc. HNO_3. The contents were heated without watch glass and volume reduced. 25 ml HNO_3 (2M) was added with it and heated for 2 minutes. Then the mixture was cooled and filtered through filter paper into 100 ml volumetric flask and completed up to the mark with HNO_3 (2M).
- The same procedure was followed to prepare solutions with red and yellow pepper.
- The blank, standard and sample solutions were aspirated into a flame photometer for all samples.

Iron

- 5g green pepper was dissolved with 25 ml of concentrated sulfuric and nitric acid mixture and boiled on hot plate for 4 hours.

- The solution was filtered by gravity and transferred into a volumetric flask. 10 ml KSCN (5M) solution were added and diluted with nitric acid and hydrochloric acid (0.05 M) solution up to the mark.
- The same procedure was followed to prepare solutions with red and yellow pepper.
- The absorbance of standards and samples were measured at 481 nm using uv-visible spectrometer.

RESULTS AND DISCUSSION

Moisture content

The moisture content of all type of pepper were determined (Table 1) and in the range of 91.38–94.16 %.

Table 1: Moisture Content of all Type of Pepper

Type	Mass of Sample (g)	Mass of Dried Sample (g)	Mass of Water (g)	Percentage (%)
Green	11.635	0.679	10.956	94.16
Yellow	10.604	0.914	9.690	91.38
Red	10.186	0.825	9.361	91.90

Calibration Curve

The flame photometry was used for determination of calcium content in bell peppers. Atomic emission was fast, simple, and sensitive method for the determination of trace concentration for elements in solution. A calibration curve method was used in analytical chemistry to determine the concentration of an unknown sample. The graph generated by experimental results and its corresponding concentration. The concentration of solution plotted on the x-axis and the observable variable, for example emission intensity plotted on the y-axis. The curve was constructed by measuring the concentration and emission of several prepared solutions, called calibration standards. Once the curve has been plotted, the concentration of the unknown solution can be determined by placing it on the curve based on its emission or other observable variable.

The calibration curve (Figure 1) was constructed for calcium using the emission intensity of standard solutions (Table 2). The linear equation was Y=0.0585X with correlation coefficient (r^2) = 0.9935 for Ca^{2+}.

Table 2: Data of Calcium for Standard Solutions

Standard concentration (ppm)	Emission intensity
0.0	0.00
2.5	0.20
7.5	0.50
12.5	0.75
17.5	1.00
22.5	1.30

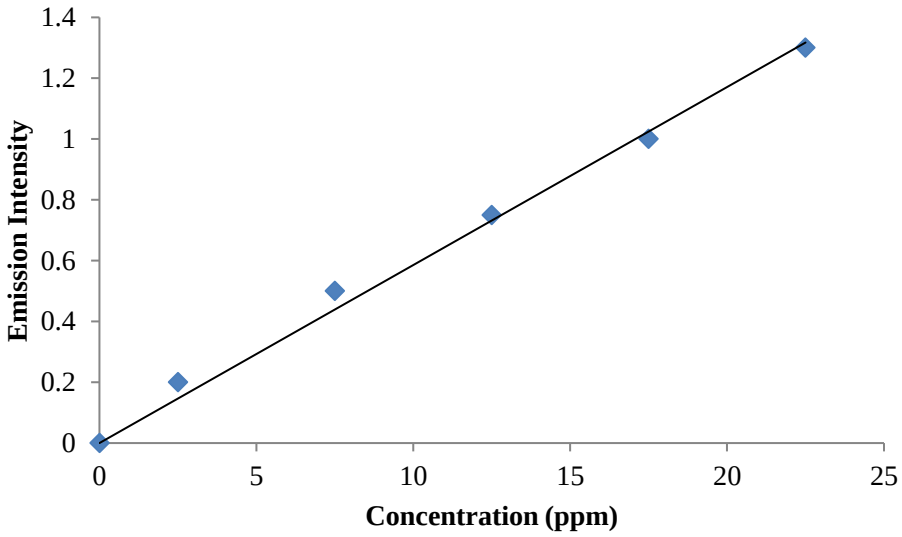

Figure 1: Calibration Curve for Standard Ca^{2+} Solutions

The colorimetric reagent used for the analysis was potassium thiocyanate and λ_{max} value obtained 481 nm by UV-Visible spectrophotometer. The basic reaction when thiocyanate reacts with iron (III) was as follows:

Fe^{3+} (aq) + $6SCN^-$ (aq) ↔ $[Fe(SCN)_6]^{3-}$ (aq)

The thiocyanate complex, $[Fe(SCN)_6]^{3-}$ had a deep red color and its intensity was directly related to the concentration of solution. The absorbance (Table 3) of standard solutions of Fe^{3+} were used to construct the calibration curve (Figure 2). The linear equation was Y=0.0701X and correlation coefficient (r^2) 0.9912.

Table 3: Data of Iron for Standard Solutions

Standard concentration (ppm)	Absorbance
0	0.000
0.5	0.050
1.5	0.110
2.5	0.175
3.5	0.230
4.5	0.294

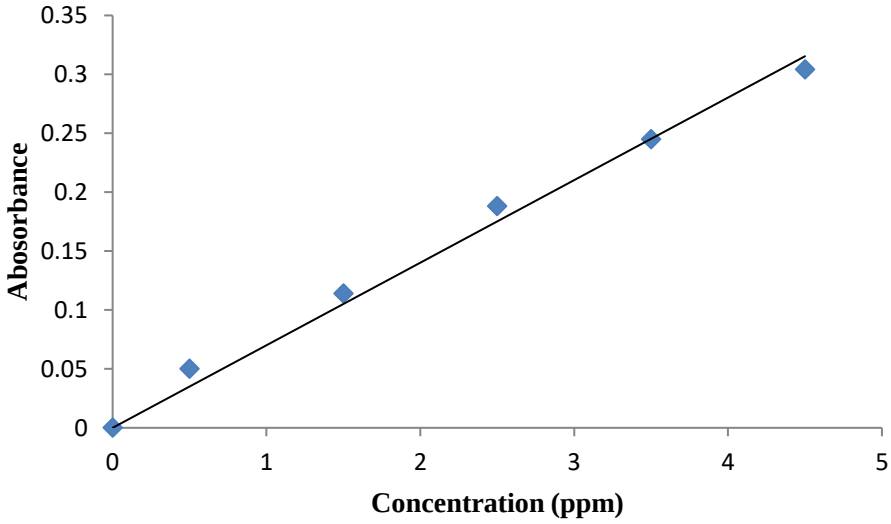

Figure 2: Calibration Curve for Standard Fe^{3+} Solutions

Determination for Pepper Samples

The unknown concentration of different types of peppers were studied using flame photometer and uv visible spectrophotometer and tabulated in Table 4. The amount of calcium and iron were found respect to 100 g of sample. The concentration of calcium and iron were in the range of 6.8–10.3 and 0.83–1.48 mg respectively.

Table 4: Results of calcium and iron content in pepper samples

Type	Ca (mg)	Fe (mg)
Green	6.8	0.83
Yellow	8.5	1.48
Red	10.3	1.08

CONCLUSION

Two simple and low-cost methods were proposed for the determination of calcium and iron in different types of peppers (green, yellow and red). These methods can be applied to determine the quality of food for routine analysis due to its excellent analytical performance. The proposed method may be applied to quantify calcium and iron in different vegetables.

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