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UV/Vis Spectrophotometric Quantification of Vitamin C in Leafy Vegetables Under Different Storage Conditions

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ABSTRACT

Leafy vegetables (LVs) have health promoting phytochemicals and are rich source of many nutrients such as Vitamin C (Vit. C). The aim of this study was to design an easy, accurate, sensitive and fast method to determine concentration of vitamin C (ascorbic acid) from leafy vegetables. So, concentration of Vit. C was measured spectrophotometrically by making a red color complex between 1, 10 Phenanthroline and Vit. C in the presence of ferric chloride ($FeCl_3$). This colored product has maximum absorption at lambda max (λmax= 430 nm). Ascorbic acid was estimated from leafy vegetables placed at three different conditions such as firstly, from fresh vegetables as available in market, secondly stored for 2 days at room temperature (25±2 ºC) and thirdly, stored in refrigerator (4 ºC) for 2 days. It was observed that vegetables stored at room temperature was significantly affected by loss of Vit. C as compared to refrigerated LVs. The average % age loss of ascorbic acid observed in refrigerated and room temperature stored vegetables was 34.18 % and 40.9 % respectively, as compared to fresh one. The highest concentration of vitamin C in fresh LVs was shown by spinach (268.7 mg/L) and least by lettuce (98.03 mg/L). This study shows that fresh vegetables are good source of vitamin C as compared to stored vegetables.

Keywords: Leafy vegetables, Vitamin C, Spectrophotometer

INTRODUCTION

Vitamins are significant nutrients that cannot be prepared by our body so they must be supplied through our diet for biochemical and physiological processes. Casimir Funk originally coined the term “vitamine” in 1912 (Semba *et al*., 2012). Vitamins are further classified into two categories i.e fat soluble and water soluble vitamins. Vitamins A, D, E and K are fat soluble whereas vitamin C and vitamin B complex are water soluble vitamins. The chemical name of vitamin C is ascorbic acid. It is L- enantiomer of ascorbic acid. It is a strong reducing agent and antioxidant. Many factors effects Vit. C stability such as temperature, oxygen and light (Dave & Patil, 2017). The structural formula of Vit.C is shown in Fig 1 (Elgailani *et al.,* 2017).

Due to its biological importance Vit. C is very important and has great significance in many body functions, including formation of collagen, absorption of iron, the proper functioning of the immune system, wound healing, and the maintenance of cartilage, bones, and teeth. Many healthy benefits are of the Vit. C such as necessary for the growth, development and repairing of all body tissues.

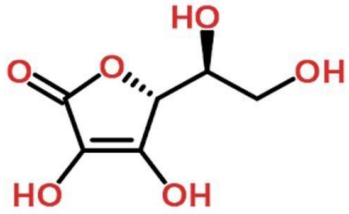

Fig 1: Structure of vitamin C (Ascorbic acid)

It also helps in preventing pathological disease scurvy, provides relief from common cold and is involved in wound healing (Iqbal *et al.,* 2004). It can be used in treatment of many chronic type of

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cancer such as colon, oral and prostate cancer (Levine, 1986; Block, 1991). It aids in the formation of collagen and fabulous healthy skin, cartilage, ligaments, corneas etc. Because of antioxidant property, it act as free radicals scavenger (Mazloum Ardakani *et al.,* 2018). HIV- patients are often prescribed Vit. C to protect their defending system and plays its important role in defending chronic diseases like tuberculosis disease and AIDS (Makinde *et al.,* 2017). Detoxifing ability of toxic substances of liver can be enhanced by Vit. C (Matei & Birghila, 2001; Mushtaq *et al*., 2022).

Fruits are primary and significant sources of Vit. C such as citrus fruits, peaches, red grapes, pineapple, pomelo, grape fruit contain vitamin C in great range (Matei & Birghila, 2001). A significant amount of Vit. C is also found in green leafy vegetables such as in Parsley, coriander, spinach, fenugreek etc. (Matei *et al.,* 2004).

Shelf life of vegetables can be increased by refrigeration which helps in retention of Vit. C content. Vit. C components depend upon different conditions such as weather, ripeness, processing, storage and preparation of each type of food. Cutting, cooking, washing, thermic processes and exposure to atmospheric oxygen cause a significant loss of Vit. C content. Vit.C concentration is gradually varied by season, transportation to market, time of storage, cooking practices and shelf life (Vina & Chaves, 2006).

Various classical and instrumental methods such as Titration, potentiometry, amperiometry, fluorimetry, voltammetry, HPLC, TLC etc. can be applied to quantify the concentration of ascorbic acid. Spectrophotometric methods for determination of ascorbic acid depend upon oxidation-reduction reaction because of its reducing property. UV radiations can be easily absorb by Vit. C that's why spectrophotometry is one of the most frequently used simple methods and is considered one of the best method with less chances of error (Silva, 2005; Timothy 2021).

The purpose of present study was to measure spectrophotometrically quantity of Vit. C in selected LVs available in Shalimar Link road, Lahore (Pakistan). The LVs included in this study were *Spinacia oleraceae , Coriandrum sativum, Mentha spicata, Trigonella foenum- graecum , Brassica oleraceae, Lactuca sativa*. The amount of Vitamin C from these LVs, was estimated in three modes such as fresh available in market, stored at room temperature (25±2 ºC) for 2 days and stored in refrigerator (4 ºC).

MATERIALS AND METHODS

Reagents and Chemicals

Analytical grade level reagents and chemicals are used in this procedure. Ferric Chloride ($FeCl_3$, BDH 99%), ascorbic acid, 1-10 Phenanthroline (BDH 99%), Hydrochloric acid (HCl). To determine the amount of ascorbic acid, UV/visible spectrophotometer (Germany, UV-3000) was used with range of 300nm-800nm.

Experimental Procedure
Preparation of Samples

Selected LVs such as spinach, coriander, mint, lettuce, fenugreek (Methi) and cabbage were purchased from market as fresh vegetables. These vegetables were washed three times with tap water to remove dirt. 50 g of each vegetable were ground in mortar and pestle separately to get the extract. The obtained extract was sieved to get pure filtrate of each vegetable in the form of juice. Finally, 10 ml of each vegetable filtrate was added in three test tubes separately, and labelled them as extract 1, 2 and 3 for fresh, room temperature (25 ºC) stored and refrigerator stored (4 ºC) vegetables respectively. The quantity of Vit. C in fresh vegetables was measured immediately but for stored vegetables, the amount was measured after two days of storage.

To measure ascorbic acid concentration, 5 mL of each vegetable extract was taken separately in a test tube. Then 1 mL of ferric chloride (FeCl3) and 4 to 5 drops of 1,10 Phenanthroline solution were added in each test tubes. After continuous stirring for two minutes, a dark red colour solution was obtained whose absorbance was noted on spectrophotometer at 430 nm.

RESULTS

Calibration Curve

To measure the concentration of ascorbic acid in samples, a standard calibration curve was drawn for different dilutions (20-300 mg/L) of ascorbic acid. The absorbance of dilutions was measured at λmax of 430 nm. The standard curve was plotted between concentration and the corresponding absorbance as shown in Figure 3.1. The straight line indicated that absorbance is directly related to ascorbic acid concentration.

With the help of calibration curve, the measured concentration of ascorbic acid in fresh, room temperature stored (25 °C, 2 days) and refrigerated stored (4 °C, 2 days) vegetables are

shown in Fig 3.2 – 3.4.

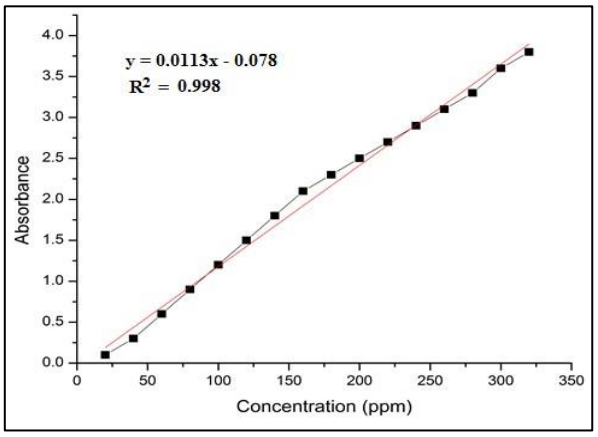

Fig 3.1: Calibration curve for ascorbic acid

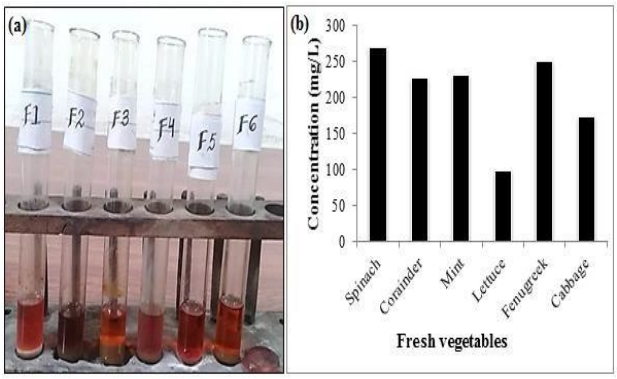

Fig 3.2 (a-b): Fresh vegetables extract showing ascorbic acid concentration F1(spinach), F2(fenugreek), F3(mint), F4(coriander), F5(cabbage), F6(lettuce)

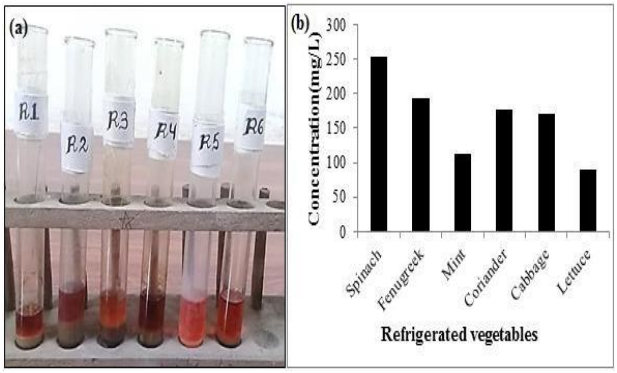

Fig 3.3 (a-b): Refrigerated vegetables extract showing ascorbic acid concentration;
R1 (spinach), R2 (fenugreek), R3 (mint), R4 (coriander), R5 (cabbage), R6 (lettuce)

The graph plotted between % age loss between market fresh vegetables and refrigerated vegetables at 4ºC storage for 2 days is shown in Fig 3.5.

The graph plotted between % loss of Vit. C between fresh and vegetables stored for 2 days under room temperature is shown in Fig 3.6.

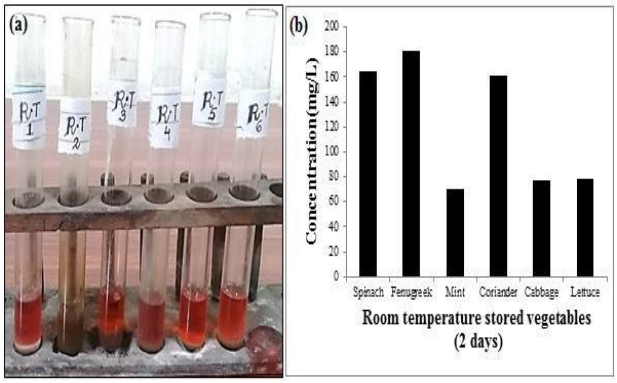

Fig 3.4 (a-b): Room temperature stored vegetables extract showing ascorbic acid concentration test, RT1 (cabbage), RT2(fenugreek), RT3 (mint), RT4 (spinach), RT5 (coriander), RT6(lettuce)

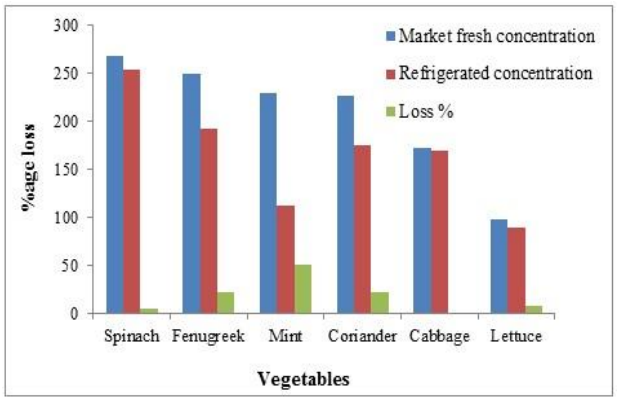

Fig 3.5: Quantity of % age loss (Vit. C) between market fresh and refrigerated vegetables

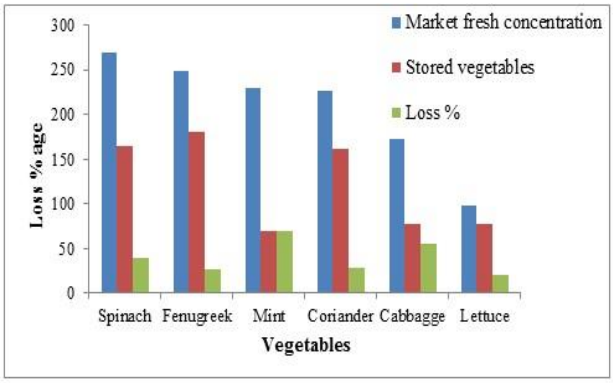

Fig 3.6: Quantity % age loss of Vit. C between market fresh and stored vegetables for 2 days at room temperature

DISCUSSION

Vit. C is usually quantified by various methods such as HPLC, TLC, LC, potentiometric, voltametric, amperometric and titrimetric method. But there are some limitations with such methods such as in titrimetric method, food colour interferes with the concentration of Vit. C. Chromatographic method is very costly. So the best method for the quantification is spectrophotometric method. It is more accurate and reliable. As titration and chromatographic methods take more time for to the detection of Vit. C, we choose spectrophotometric method. UV/Vis Spectrophotometry is one of the most unique and frequently used simple method (Grosso *et al.,* 2013; Loria *et al.,* 2000; Moeslinger *et al.,* 1995). Spectrophotometric method described here is based on the principle of reduction of Ferric to Ferrous ions and to form a complex called Ferroin by using ascorbic acid in presence of 1,10-phenanthroline. Absorbance of the resulting Fe(II)-phenanthroline red complex (Ferroin) was measured at 430 nm.

The concentration of Vit. C detected here in fresh vegetables is higher as compared to refrigerated vegetables and those which are stored at room temperature. A loss of Vit. C is observed in refrigerated vegetables and stored vegetables for 2 days.

Vit. C is a water soluble compound which can easily leach into water and then degraded by heat. Vit. C is unstable molecule which can easily be denatured by heat. It is very sensitive to oxidation and can be depleted easily. Refrigeration reduces the rate of respiration of vegetables and that's why refrigeration is often recommended for increased shelf life of vegetables (Khan *et al.,* 2006; Wonsawat, 2014; Rickman *et al.,* 2007; Murcia *et al*., 2000; Bessey & King, 1933; Kifer & Munsell, 1932; Wasson,1931; Langley *et al.,* 1933).

Leafy vegetables wrapped in polythene was very effective in reducing weight and moisture and helpful in retention of Vit. C content (Podsedek, 2007). Quality of fertilizer also effects the Vit. C content of the vegetable (Vina & Chaves, 2006). Factors which effect Vit. C content are oxygen, temperature and light because Vit. C is easily oxidized with the exposure with oxygen. Temperature also affects as it is water soluble and dehydrated by heat easily. Some vegetables lose half of their vitamin C content when allowed to stand at room temperatures for 3 days, and their rate of degradation is determined lower when allowed to stand under refrigeration (Mushtaq *et al*., 2022). Depletion of Vit.C occurs gradually after harvest and with the passage of time it steadily degrades. (Souzan & El-Aal, 2000).

Results indicated that the concentration of Vit. C in market fresh vegetables was 98.035 mg/L in lettuce, 268.7 mg/L in spinach, 230.24 mg/L in mint, 226.619 mg/L in coriander, 249.45 mg/L in fenugreek and 172.19 mg/L in cabbage. The order of concentration of Vit. C in market fresh vegetables was;

Spinach > Fenugreek > Mint > Coriander > Cabbage > Lettuce

The spectrophotometric quantification indicated that the high concentration of Vit. C was found in spinach (268.7 mg/L) and the least in lettuce (98.035 mg/L). The concentration of Vit. C in refrigerated vegetables that are stored under temperature 4ºC for 2 days are 254.5 mg/L in spinach, 192.548 mg/L in fenugreek, 111.92 mg/L in mint, 176mg/L in coriander, 169.71 mg/L in cabbage and 89.90 mg/L in lettuce. The concentration of Vit. C in vegetables that are stored at room temperature for 2 days was measured as 164.7 mg/L in spinach , 181.04 mg/L in fenugreek, 69.982 mg/L in mint, 161.66 mg/L in coriander, 77.23 mg/L in cabbage and 78.03 mg/L in lettuce.

Higher concentration is seen in fresh vegetables as compared to refrigerated and vegetables stored at room temperature for 2 days. A significant loss of Vit. C was observed in refrigerated and stored vegetables. According to Von Hahn & Gorbing study, the % age loss in beans, cauliflower, lettuce, parsley, peas and spinach was 33%, 8%, 36%, 13%, 10% and 32% respectively when stored at 4 ºC for the duration of two days. Whereas the %age loss of Vit. C was further increased by increasing temperature at 20 ºC (Von Hahn & Gorbing, 1933). In the present study, the percentage losses determined for refrigerated vegetables were 5.28% (spinach), 22.81% (fenugreek), 51.39% (coriander), 22.33% (coriander), 1.44% (cabbage), 8.3% (lettuce) and for stored vegetables (for 2 days, 25 ºC) were 38.70% (spinach), 27.42% (fenugreek), 69.60% (mint), 28.66% (coriander), 55.148% (cabbage) and 20.41% (lettuce).

Overall, the decrease in concentration of Vit. C was noted for refrigerated vegetables. The highest percentage loss was shown by refrigerated samples of *Mentha spicata* (mint). The average % age loss observed in between fresh and refrigerated LVs is 37.18 % age whether the % age loss observed between fresh and vegetables stored for 2 days at room temperature is 40.9 % age. This average % age loss indicates that highest loss is seen under room temperature storage as it is very

labile to the action of heat heat and oxygen and therefore Vit. C activity slowly decreases during its storage. As described earlier that we should consume fresh vegetables rather than the stored vegetables at 4 ºC in refrigerator or at normal room temperature stored for 2 days. Market fresh vegetables found to be the greatest source of Vit. C. Refrigerated vegetables were found to lose less % age loss as compared to vegetables stored at room temperature for 2 days (Souzan & El-Aal, 2000). This study shows that fresh vegetables are good source of Vit. C as compared to stored vegetables.

CONCLUSION

It can be easily concluded from the present work that performed UV/Vis Spectrophotometric method for determination of Vit. C is an easy, simple, accurate, precise, selective, safe and more reliable. All the reagents used were cheap and readily available. So, by using this procedure quantity of ascorbic acid can be measured in different samples quite rapidly. A significant loss in ascorbic acid concentration was found for refrigerated vegetables. It can be concluded that we should give preference for the consumption of fresh LVs as Vit. C source over refrigerated vegetables at 4ºC as well as stored vegetables at room temperature.

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