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SPECTROPHOTOMETRIC DETERMINATION OF VITAMIN C IN UNDERGROUND VEGETABLES AND KINETIC MODELLING TO PROBE THE EFFECT OF TEMPERATURE AND pH ON DEGRADATION OF VITAMIN C

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Abstract

Ascorbic and ascorbate, also known as Vitamin C, which is an essential dietary component. In present study, the concentration of vitamin C was determined in different vegetables such as beetroot (*Beta vulgaris*), carrot (*Daucus carota*), onion (*Allium cepa*), potato (*Solanum tuberosum*) and radish (*Raphanus raphanistrum*) using a benign spectrophotometric method. The quantity of vitamin C in fresh vegetable such as beetroot, carrot, onion, potato and radish (25°C) as purchased from market was 212.74 mg/L, 148.27 mg/L, 139.19 mg/L, 155.13 mg/L and 248.22 mg/L respectively. To monitor the effect of temperature on chemical stability of ascorbic acid, all selected vegetables were stored under three different temperature (0°C, 25°C and 50°C) for up to 8 days. To evaluate the quantitative effect of temperature on degradation of Vitamin C, kinetic modeling was applied. The regression factor (R^2> 0.99) and rate constant (k) values indicated that chemical instability of Vit. C follows first order kinetics. The effect of pH (2-12) on stability of Vit. C was also examined. It was analyzed that fresh vegetables at normal temperature (18°C±7) in optimum acidic medium (2.8±1.2) are good source of vitamin C compared to stored one.

Key words: Underground vegetables, Vitamin-C, Spectrophotometer, Kinetic modeling.

Introduction

Vitamin C (Vit. C) is an essential dietary organic compound. It occurs in white solid form but can show slightly yellow appearance due to impurity. It is a water soluble compound having antioxidant properties and used to treat tissue repairing and scurvy like diseases. It has a significant role to strengthen the immune system (Rahman *et al*., 2007). Role of Vit. C in collagen production assist faster healing of wounds and injuries. In diabetics blood sugar level and improving metabolisms can be controlled by Vit. C supplementation. Vit. C enhance the rate of white blood cell and important to immune system balance. Sodium ascorbate and calcium ascorbate derived form of vitamin C are used as dietary supplements. As per protocols of WHO and RDA for Vit. C intake, a normal men and women should take 90mg and 75mg per day respectively to avoid from common and chronic diseases. Vit. C contributes in growth of various biochemical processes such as bone formation and tissue repairing (Milivojevic *et al*., 2010; Rafique *et al*., 2011). Fruits and vegetables are good source of Vit. A, C and E. Ascorbic acid has ester (–COOR) and alcoholic (–OH) functional group.

Various analytical methods such as Spectrophotometry, Chromatography, Titration, Voltammetry, Fluorometry, Potentiometry, Spectrometry and Amperometry have been designed for the quantification of Vit. C (Pathy 2018; Khan *et al*., 2006; Arya *et al*., 2000; Ogunlesi *et al*., 2010). The selection of analytical technique is based upon product application, nature of study, cost, number of samples, product application, apparatus accessibility and time (Heudi *et al*., 2005). Khan R. and contributors determined ascorbic acid quantity in different vegetables and fruits of Sylhet area (Bangladesh) using simple UV-Spectrophotometric method (Khan *et al*., 2006). Dilgin and Gurel (2005) designed a sensitive experiment to measure ascorbic acid concentration by using fluorimetric method, principally in refined materials. In spectrofluorometric technique, spectra are recorded on the basis of fluorescence (Dilgin *et al*., 2005).

Ascorbic acid is an unstable compound (Okatan, 2020) and its stability is vastly affected by certain environmental dynamics such as light, moisture content, pH and temperature (Khan *et al*., 2006; Hiatt*et al*., 2010; Gundesli *et al*., 2019). Rivelli and co-workers reported that radish tissues showed more than 48% loss of Vit. C when frozen and stored at -20°C for 2 days (Rivelli *et al*., 2017). Within one month of freezing at -22°C, the beans and peppers showed more than 97% loss and degradation of Vit. C (Carr *et al*., 2020). Langlois and his co-workers recorded a little change in concentration of Vit. C (25 %) in broccoli and cauliflower when stored at low temperature for a month (Langlois *et al*., 2016).

In our present study, three significant parameters including pH, temperature and storage time duration were applied to examine their effect on chemical stability of ascorbic acid. All selected vegetables such as beetroot (*Beta vulgaris*), carrot (*Daucus carota*), onion (*Allium cepa*), potato (*Solanum tuberosum*) and radish (*Raphanus sativus*) were stored under three different temperature (0°C, 25°C and 50°C) for up to 8 days. In order to probe the effect of pH on stability of Vit. C, various pH 2, 4, 6, 8, 10 and 12 was adjusted for two days storage time at

25°C. In each case concentration of vitamin C was determined by using our newly developed easy spectrophotometric method. At the end kinetic modeling was also performed.

Material and Methods

Chemicals and apparatus: For extraction of vitamin C from vegetables, pestle - mortar and electrical juicer was used. To make colored precipitates of Vit. C, analytical grade chemicals Ferric Chloride (FeCl3, BDH 99%), ascorbic acid (Sigma-Aldrich 99%), 1-10 Phenanthroline (BDH 99%), Hydrochloric acid (Sigma-Aldrich) and sodium hydroxide (BDH 99%) were used. The quantity of Vit. C was measured by UV/visible spectrophotometer (Germany, UV-3000) having range of 300-800 nm.

Experimental procedure: Selected fresh vegetables including carrot, beetroot, onion, potato and radish were purchased and washed multiple times with tap water to remove dirt. To obtain extract of these vegetables, each vegetable was grinded in pestle-mortar separately. The collected extract was sieved to get pure filtrate juice of each vegetable. To quantify the concentration of Vit. C, about 10 ml of each extracted vegetable juice was taken in different test tubes separately at 25°C. 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 of two minutes, a dark red color solution was obtained whose absorbance was noted on spectrophotometer at 430 nm.

Three set of each vegetable (beetroot carrot, potato, onion, red radish and white radish) was stored at 0°C, 25°C and 50°C separately, for 8 days. After duration of 2 days, the concentration of Vit. C was measured by the same spectrophotometric method as mentioned above.

In order to probe the effect of pH on stability of Vit. C, 10 ml of each fresh vegetable juice was taken in different test tubes separately at 25°C. The pH 2, 4, 6, 8, 10 and 12 in test tubes was adjusted using 0.2 M HCl and 0.2 M NaOH. After two days storage time, concentration of Vit. C was measured in each test tube by the same 1,10-Phenanthroline solution and spectrophotometric method as mentioned above.

Kinetic modelling: The data collected by using UV/Visible spectrophotometry was used to monitor the chemical instability and degradation of Vit. C. The following first order equation 1 (Hassan *et al*., 2018), was applied to study kinetic mathematical models for ascorbic acid.

$$ln\frac{x}{x_0} = -kt \quad \text{.................................... } 1$$

In equation 1, $\boldsymbol{x}$ shows concentration at corresponding time t and $\boldsymbol{x_0}$ is the original concentration of Vit. C. Where k represent the rate constant of reaction per day. To find k, the graph was plotted between ln x/x_0 and time. The quantitative effect of temperature T on rate constant k was studied using Arrhenius equation 2 (Valter *et al*., 2019).

$$k = Ae^{-\frac{E_a}{RT}} \quad \text{....................................... } 2$$

In equation 2, A is called Arrhenius or frequency constant (often found to be temperature independent quantity), E_a is energy of activation, R called general gas constant having value (S.I) 8.3145 $J.mol^{-1}\cdot K^{-1}$.For degradation modeling, color changes of Vit. C was noted after fixed interval of time. In literature, first order, zero and fractional first order kinetic model has been used to describe the stability and color changes of ascorbic acid (Lipasek *et al*., 2011, Vikram *et al*., 2005).

Results and Discussion

Standard curve: The unknown concentration of Vit. C in vegetable extracted juices was determined by drawing a standard calibration curve of various known concentrations (20-300 mg/L) of ascorbic acid. The change in color intensity of these dilutions changes the absorbance and the absorbance was measured at maximum wavelength (λmax, 430 nm) of ascorbic acid as determined in (Fig. 1). The standard straight line was obtained by drawing a graph between absorbance and concentration (Fig. 2).

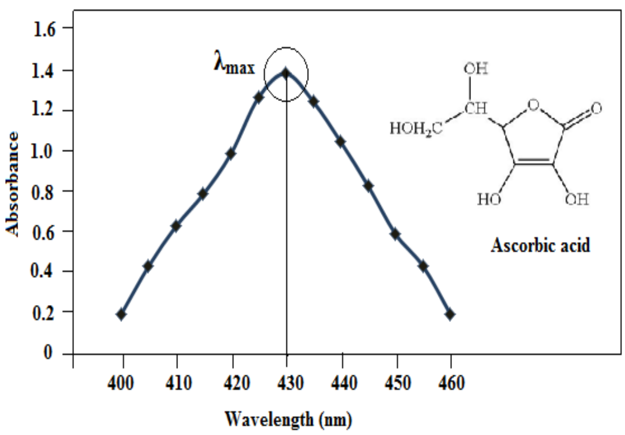

Fig. 1. Determination of maximum wavelength (λ_{max}) of ascorbic acid.

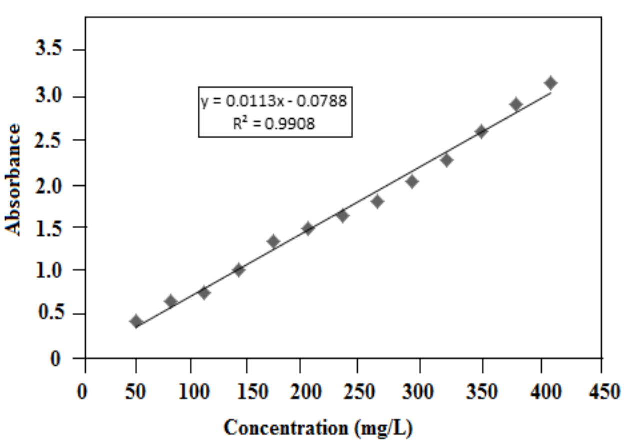

Fig. 2. Calibration curve for standardized solutions of Vit. C.

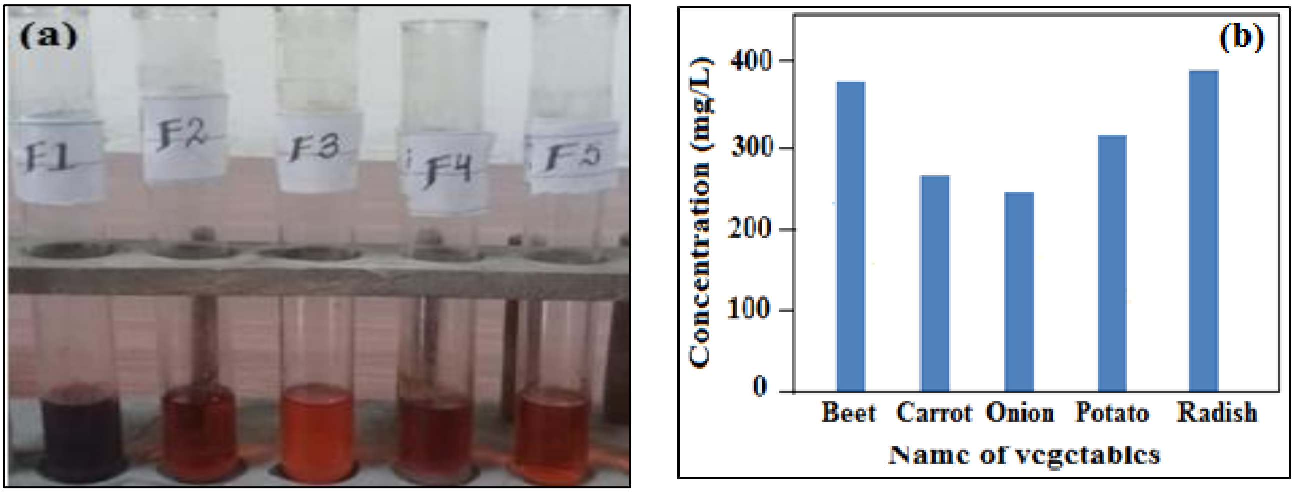

Fig. 3 (a-b) Fresh vegetables extract showing ascorbic acid concentration F1(Beet), F2(Carrot), F3(Onion), F4(Potato), F5(Radish).

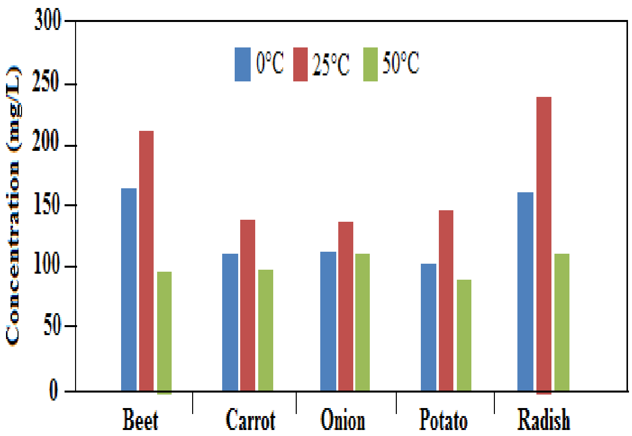

Fig. 4. The concentration of Vit. C in vegetables after 8 days storage at various temperature (0°C, 25°C, 50°C).

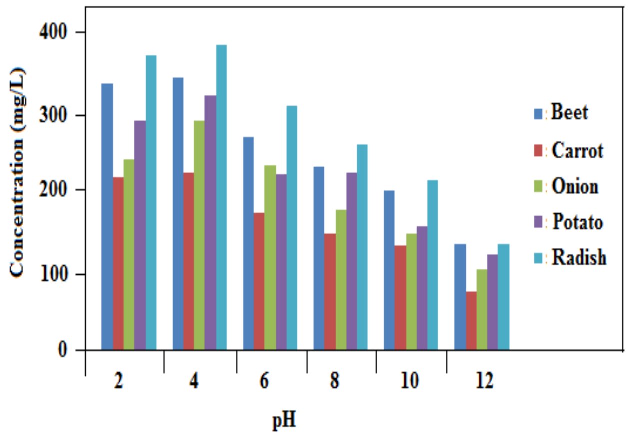

Fig. 5. Concentration of Vit. C in selected vegetables with respect to pH.

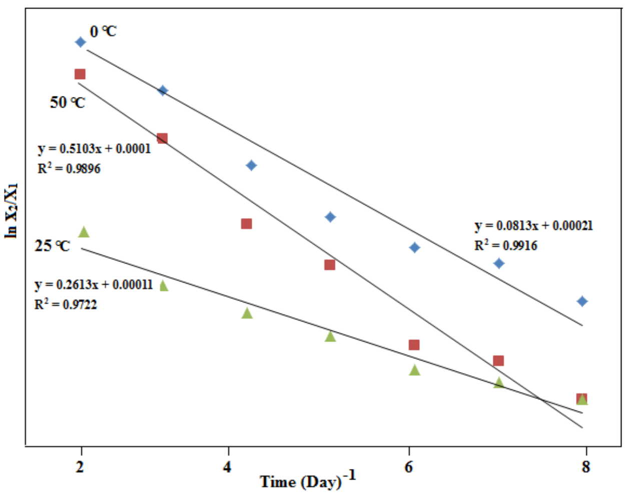

Fig. 6. Showing kinetic modelling of Vit. C degradation at various temperatures.

By using standard curve, the quantity of Vit. C was determined at 25°C in fresh vegetables purchased from the market. The chemical test to quantify ascorbic acid concentration in fresh vegetables and its graphical form are shown in (Fig. 3) (a) and (b) respectively. The concentration of Vit. C in fresh vegetables and its chemical degradation at different temperatures (0°C, 25°C and 50°C) after storage of 8 days is discussed in Table 1 and shown in (Fig. 4). While, the decrease in Vit. C concentration with change of pH is shown in (Fig. 5).

The kinetic modelling results of Vit. C are shown in Table 2 and (Fig. 6). The continuous degradation in concentration of Vit. C is following the 1st order reaction kinetics as reported in literature also (Schleicher *et al*., 2009). By putting the value of rate constant (k), temperature and gas constant (R) in Arrhenius equation, activation energy was determined as shown in Table 3 In all findings, regression (R^2) values are greater than 0.97, representing significant correlation to the applied models.

Discussion

In the whole study, it has been observed that fresh vegetables are good source of ascorbic acid compared to stored ones. In all selected fresh vegetables, the concentration of Vit. C was greater than 280 mg/L which decreased upto 140 mg/L after storage of 8 days at room temperature (25°C). As Vit. C is highly sensitive to oxidation, moisture content, pH, temperature etc. so, it may leach into water soluble media during storage

(Rickman *et al*., 2007). The loss of Vit. C from all these vegetables is most likely dominated by enzyme-induced oxidation. However, the variation in the rate loss of Vit. C in different vegetables was due to the involvement of different physical condition of these vegetables such as surface area, mechanical damage, sulphydryl content, as well as their differing enzymatic potential (Fadhel, 2012). Many climatic factors specially temperature and total available heat affects Vit. C level in plants. The low temperature zone produce citrus fruits with higher Vit. C level as compared to hot tropical areas (Padayatty *et al*., 2003; Krawiec *et al*., 2019; Jedrszczyk *et al*., 2019).

According to health expert organizations (FAO/WHO), the stability of Vit. C is highly sensitive pH change. In our performed experiment, it was observed that Vit. C showed chemical stability in acidic environment (pH: 2 to 4) but became unstable in neutral and alkaline pH. As long as exposure of Vit. C increased in higher pH (> 4), greater loss of Vit. C was noted. At acidic pH 2 to 4, an average quantity of Vit. C was greater than 270 mg/L which was decreased upto 100 mg/L in neutral and alkaline pH. The reason behind this significant loss was due to faster oxidation of Vit. C in higher pH. During oxidative degradation of Vit. C, hydrolysis of the dehydroascorbic acid (DHAA) lactone produce 2,3-diketogulonic acid (DKGA). This hydrolysis is more supported by alkaline pH. As DHAA lactone is most likely to stable at acidic pH (4.5) and becomes unstable as pH of the medium increases (Levine *et al*., 1996).

With respect to temperature effect, it has been noted that high temperature is more vulnerable for Vit. C stability. The slope and kinetic modelling data as shown in Figure 6, representing that the degradation of Vit. C was slow and constant during storage at temperature 25°C, which became medium at 0°C but very fast at 50°C. On the average, the percentage loss of Vit. C after 8 days of storage at 50°C was greater than 45% as shown in Table 2.In fact, ascorbic acid degrades by following aerobic and anaerobic decomposition as reported in literature. In the presence of oxygen, it is easily oxidized and finally hydrolyzed to 3-deoxy-pentosone (3DP) and 2,3-diketo-gulonic acid (DKGA). This aerobic path is called the primary route of chemical degradation of ascorbates in fruits and vegetables. As ascorbic acid is an organic compound, so the end product produces in the form of gasses CO_2 and H_2 either aerobic or anaerobic degradation (Nath *et al*., 2005).

Table 1. Vit. C concentration in stored vegetables at different temperature.

Fresh vegetables (25 ˚C)	Vit. C (mg/L)	Storage Temperature ˚C	Concentration (mg/L) of Ascorbic acid remaining over time (day)					Total % age loss
			Stored vegetables	Day 2	Day 4	Day 6	Day 8	
			Beetroot	342.9	287.2	228.5	163.8	57.47
Beet	385.2		Carrot	241.6	293.2	221.9	118.5	56.19
		0 ˚C	Onion	279.4	227.6	178.8	106.6	65.70
			Potato	233.6	201.2	159.2	112.4	55.46
Carrot	270.5		Radish	369.3	341.5	276.4	168.2	57.61
			Beetroot	358.3	329.1	296.6	215.6	44.03
			Carrot	252.2	221.6	195.5	151.3	44.06
Potato	252.4	**25 ˚C**	Onion	298.2	271.6	219.8	141.7	54.40
			Potato	243.6	219.8	191.5	153.8	39.06
			Radish	379.8	344.4	296.6	248.4	37.39
Onion	310.8		Beetroot	323.8	272.3	210.9	100.7	73.85
			Carrot	228.7	192.4	145.6	92.80	65.69
		50 ˚C	Onion	278.3	236.8	195.8	114.6	63.12
Radish	396.8		Potato	218.8	271.5	172.5	67.20	73.37
			Radish	364.4	316.6	238.8	122.4	69.51

Table 2. The data collected for kinetic modeling.

Sr. No.	Temperature	Rate Constant k	Regression factor R^2	Half life $t_{1/2}$	E_a
1.	0 °C	0.0183	0.9916	147 ± 4.0	56 ± 4.4
2.	25 °C	0.2613	0.9896	217 ± 3.3	107 ± 3.0
3.	50 °C	0.5103	0.9722	92 ± 2.8	41 ± 5.6

Conclusion

It can be easily concluded from the present work that the spectrophotometric method adopted for the determination of ascorbic acid using 1,10-Phenanthroline, is an easy, simple, safe and more reliable method. So, by using this procedure quantity of ascorbic acid can be measured in different samples quite rapidly and precisely. The sustainability of Vit. C is significantly affected by certain surrounding conditions including storage time, photochemical light, moisture content, pH and temperature. The fresh vegetables are good supplementary source of Vit. C compared to stored one. Room temperature (25°C) and acidic pH (2 to 4) are the ambient conditions for the stability of Vit. C, however high temperature and higher pH are the prime reasons to degrade the Vit. C. Finally, it can be concluded that the fresh vegetables and the vegetables stored at room temperature in acidic pH are good source of Vit. C.

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