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Determination of Vitamin C (Ascorbic Acid) Contents in Vegetable Samples by UV-Spectrophotometry and Redox Titration Methods and Estimation the Effect of Time, Cooking and Frozen on Ascorbic Acid Contents

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Abstract - The concentrations of Vitamin C, also known as L-ascorbic acid, in twenty two different types of fresh vegetables were determined by spectrophotometric and Back Redox Titration methods. The effects of some conditions such as time, cooking and frozen on the Vitamin C contents were studied. The samples were collected from different markets in Benghazi city from different places. Each sample or a part of it was weighed, squeezed in a juicer machine and centrifuged; the volume and the weight of the supernatant liquid were taken. The supernatant liquid was homogenized with 5% metaphosphoric acid-10% acetic acid solution. The ascorbic acid was oxidized to dehydroascorbic acid by bromine water in presence of acetic acid and the excess of bromine was removed by a few drops of 10% thiourea . A coupling was done with 2,4-dinitrophenyl hydrazine by putting the solution in a water bath at 37°C temperature for three hours, it was cooled in an ice bath and was treated with chilled 85% H_2SO_4 . A red colour complex was produced and the absorbance was measured spectrophotometrically at 521 nm. Beer's law was obeyed. Another method for the determination of ascorbic acid was done by an iodimetric back-titration in which an excess measured amount of iodine was generated in the sample and then the unreacted iodine was titrated with a standard solution of sodium thiosulfate. The estimation of ascorbic acid contents was done for fresh, after 4 and 7 days, cooking and frozen samples.

Key words - Vitamin C, vegetables, spectrophotometry, an iodimetric back-titration

I. INTRODUCTION

Vitamin C or L-ascorbic acid has the chemical formula $C_6H_8O_6$. It consists of two inter-convertible compounds: L-ascorbic acid, which is a strong reducing agent, and its oxidized derivative, L-dehydroascorbic acid $C_6H_6O_6$

Vitamin C is a water-soluble vitamin that is naturally present in some foods, added to others, and available as a dietary supplement. Humans, unlike most animals, are unable to synthesize vitamin C endogenously, so it is an essential dietary component [1].

Vitamin C is required for the biosynthesis of collagen, L-carnitine, and certain neurotransmitters; vitamin C is also involved in protein metabolism [1,2]. Collagen is an essential component of connective tissue, which plays a vital role in wound healing. Vitamin C is also an important physiological antioxidant [3] and has been shown to regenerate other antioxidants within the body, including alpha-tocopherol (vitamin E) [4]. Ongoing research is examining whether vitamin C, by limiting the damaging effects of free radicals through its antioxidant activity, might help prevent or delay the development of certain cancers, cardiovascular disease, and other diseases in which oxidative stress plays a causal role. In addition to its biosynthetic and antioxidant functions, vitamin C plays an important role in immune function [4] and improves the absorption of nonheme iron [5], the form of iron present in plant-based foods. Insufficient vitamin C intake causes scurvy, which is characterized by fatigue or lassitude, widespread connective tissue weakness, and capillary fragility [1,2,4,6].

Most fruits and vegetables especially leafy ones rich in ascorbic acid [7]. Animal sources of this vitamin such as meat, fish, poultry, eggs and dairy products contain smaller amounts and are not significant sources. Most food–based dietary guidelines are similar in that all recommend consumption of 5 servings of fruits and vegetables daily. If this recommendation is followed, daily intake of ascorbic acid will be 210 to 280 mg, depending on food content factors [8]. Ascorbic acid is the least stable of all vitamins and is easily destroyed during processing and storage. Exposure to oxygen, prolonged heating in the presence of oxygen, contact with minerals (iron and copper) and exposure to light are destructive to the ascorbic acid content of foods.

Many analytical techniques are mentioning in the literature for the determination of vitamin C in different matrices, such as: titrimetric [9], fluorimetric [10], spectrophotometric [11], high-performance liquid chromatography [12], and enzymatic [13] techniques.

In this work, vitamin C was determined by two methods: Redox Back Titration and UV- Visible Spectrophotometry.

1. Redox Back Titration
   This method determines the vitamin C concentration in a solution by a redox titration with potassium iodate in the presence of potassium iodide.
   When iodate ions (IO_3^-) are added to an acidic solution containing iodide ions (I^-), an oxidation-reduction reaction occurs; - the iodate ions are reduced to form iodine as illustrated in the following two equations:

$$IO_3^- + 5\,I^- + 6\,H^+ \rightarrow 3\,I_2 + 3\,H_2O \qquad (1)$$

   The iodine that is formed by this reaction oxidises the ascorbic acid to dehydroascorbic acid as the iodine is reduced to iodide ions.

$$C_6H_8O_{6(aq)} + I_2 \rightarrow 2\,I^- + C_6H_6O_{6(aq)} \quad .\ (2)$$

   Due to this reaction the iodine formed is immediately reduced to iodide as long as there is any ascorbic acid present. Once all the ascorbic acid has been oxidised, the excess iodine is free to react with the starch indicator, forming the blue-black starch-iodine complex. This is the endpoint of the titration. The method is suitable for use with Vitamin C tablets, fresh or packaged fruit juices and solid fruits and vegetables
2. UV- Visible Spectrophotometery.
   We used spectrophotometric determination of total ascorbic acid (ascorbic acid and dehydroascorbic acid) based on oxidation of ascorbic acid to dehydroascorbic acid by Bromine water in presence of acetic acid. After coupling with 2,4-dinitrophenyl hydrazine at 37°C temperature for three hours, the solution was treated with 85% H_2SO_4 to produce a red color complex and the absorbance was spectrophotometrically measured at 521 nm. DNPH procedure is one of the most simple, accurate and applicable method for determination of total ascorbic acid in fresh food, such as fruits and vegetables.

II. Materials and Method

Reagents:

All reagents were of analytical grade:

1. For Redox Back Titration:
   0.04 N KIO_3, 10% KI, 0.2M H_2SO_4, 0.03N $Na_2S_2O_3$, Na_2CO_3 solid, 5% Starch
2. For UV- visible spectrophotometer:
   - 500 ppm Ascorbic acid standard solution (dissolve 0.0500 g of ascorbic acid in 100 ml distilled water),
   - 3% Meta phosphoric acid (dissolve 15 g meta phosphoric acid in warm distilled water, add 40 ml of glacial acetic acid (BDH) then transfer the solution to 500 ml volumetric flask and dilute the solution to the mark with distilled water).
   - Bromine water (dissolve 55 ml of bromine water in 500 ml distilled water).
   - 2,4-dinitrophenylhydrazine (dissolve of 1.0 g 2,4-dinitrophenylhydrazine in 2 ml conc. sulfuric acid after cooling, add 20 ml methanol).
   - Thiourea (dissolve 50 g of Thiourea in 500 ml distilled water).

   Instrument

A Double beam UV-Visible spectrophotometer (Model GESEYS 10uv) with 1 cm cell was used.

Twenty two kind of vegetables were studied individually. Vegetables were Pumpkin, Carrots, Tomatoes, Paprika, Hot Red Pepper, Hot Green Pepper, Cucumber, Radishes, Onion, Broccoli, Cabbage, Squash, Potato, Parsley, Cauliflower and Coriander. Also some leafy vegetables were Coriander, Dill, Parsley, Spinach, Celery, Mint, Arugula and Green onions. They were collected from different markets in Benghazi city. The analysis was done in the same day of the purchase except that for studying the effect of time, cooking and frozen of the vegetables.

Procedures:

1. Preparation of Samples:

Each vegetable sample or a part of it was weighed, squeezed in a juicer machine and centrifuged. Then the volume and weight of the supernatant liquid were taken.

2. Analysis of the Samples by Titration Method:

i. Standardization of Sodium Thiosulfate Solution:

This standardization was done by pipetting 5.00 ml of KIO_3 solution + 10 ml of 10 % KI solution + 10 ml of dilute H_2SO_4 into a conical flask. The liberated I_2 was titrated against $Ns_2S_2O_3$ solution from a burette until a faint yellow colour was reached. Then 10 drops of starch solution was added and the titration was continue until the blue-black colour of the starch - triiodide complex just disappeared. The titration was repeated until concordant results were obtained. The normality of the sodium thiosulfate solution was calculated.

ii. Titration of Standard Solution of Ascorbic Acid:

5.00 ml of the sample solution was pipetted and the steps for the standardization of sodium thiosulfate solution were repeated. The concentration of the ascorbic acid was calculated from the relation:

Milliequivalent of sodium iodate - milliequivalent of sodium thiosulfate = milliequivalent of ascorbic acid

iii. Titration of vegetable samples:

5.00 ml of the treated sample was pipetted in to a conical flask and the steps were repeated as with standard ascorbic acid.

3. Analysis of the Samples by Spectrophotometric Method:

i. Preparation of standard calibration curve:

1. From 500 ppm stock solution of ascorbic acid, 0.00 ml, 0.25 ml, 0.50 ml, 0.75 ml, 1.00 ml, 1.25 ml, 1.50 ml, 1.75 ml and 2.00 ml were taken in 9 of 25 ml volumetric flasks to prepare 0 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm 25 ppm, 30 ppm, 35 ppm and 40 ppm respectively.
2. To each flask few drops of bromine water was added to oxidize the ascorbic acid to dehydroascorbic acid. Then a few drops of thiourea was added to remove the excess bromine and thus the clear solution was obtained.
3. 1 ml of glacial acetic acid and then 1 ml of 2,4-DNPH solution were added to all standards and blank. For the completion of the reaction, all the flasks were kept at 37°C temperature for 3 hours in a water bath (thermostatic).
4. After the incubation, the flasks were cooled in an ice bath for half an hour and then treated with 5 ml of chilled 85% H_2SO_4 with constant stirring. Then the volume was completed to the mark with distilled water.
5. Scanning of the spectra was done for determining the λ_{max} for the complex formed. And the calibration curve was drawn at the proper wavelength.

ii. Analysis of vegetable samples:

5 ml from the vegetable sample was taken in a 25 ml volumetric flask and the volume was completed to the mark with 5% metaphosphoric acid 10% acetic acid solution. In another 25 ml volumetric flask, 5 ml from the above solution was taken and the steps 3 and 4 were repeated as with the standard solutions. Finally, the concentration of the ascorbic acid in the vegetable samples are determined from the calibration curve and then calculated in the original samples.

III. Results And Discussion

Our purpose is to determine the Vitamin C content in some vegetables, and studying the correlation between the content of Vitamin C and the state of vegetables (stored in a refrigerator for 4 days and 7 days , cooking and frozen).The samples were collected from different markets in Benghazi city.

Vegetables were Pumpkin, Carrots, Tomatoes, Paprika, Hot Red Pepper, Hot Green Pepper, Cucumber, Radishes, Onion, Broccoli, Cabbage, Squash, Potato, Parsley, Cauliflower and Coriander. Also some leafy vegetables were Coriander, Dill, Parsley, Spinach, Celery, Mint, Arugula and Green onions.

The titration method determines vitamin C in the form of ascorbic acid while the spectrophotometric method determines the total vitamin C (ascorbic acid + dehydroascorbic acid). The amount of vitamin C is expressed in mg/100g for each vegetable.

1.1. Determination of the amount of Ascorbic Acid obtained from the titration method:

Ascorbic acid was determined in different types of vegetables by the titration method as was described in the experimental part and the effect of keeping them at 10°C for 4 days and 7 days was studied and also the amount of the vitamin was measured after cooking the vegetables and frozen them.

3.1.1 Effect of Time

Table 3.1. : Amount Of vitamin C of some vegetables and effect of time on them

Kind of vegetable	Fresh mg/100g	4 days mg/100g	7 days mg/100g	12 days mg/100g
Broccoli	120.85±0.25	80.85±0.11		28.47±0.22
Paprika	85.53±0.08	44.99±0.11	23.89±0.04	
Coriander	80.69±0.46	15.96±0.08		
Cauliflower	73.78±0.40	10.29±0.52		
Cabbage	64.25±0.25			
Radishes	61.54±0.06	50.45±0.08		
Squash	60.82±0.42	20.31±0.08		
Potato	51.77±0.34	30.27±0.00	13.20±0.10	
Hot Red Pepper	47.84±0.32			
Parsley	38.42±0.52	12.58±0.32		
Pumpkin	38.23±0.27	19.80±0.00	18.23±0.23	
Carrots	28.93±0.05			
Cucumber	27.90±0.41			
Spinach	25.64±0.05			
Onion	24.89±0.30			
Tomatoes	15.05±0.07	10.32±0.00		

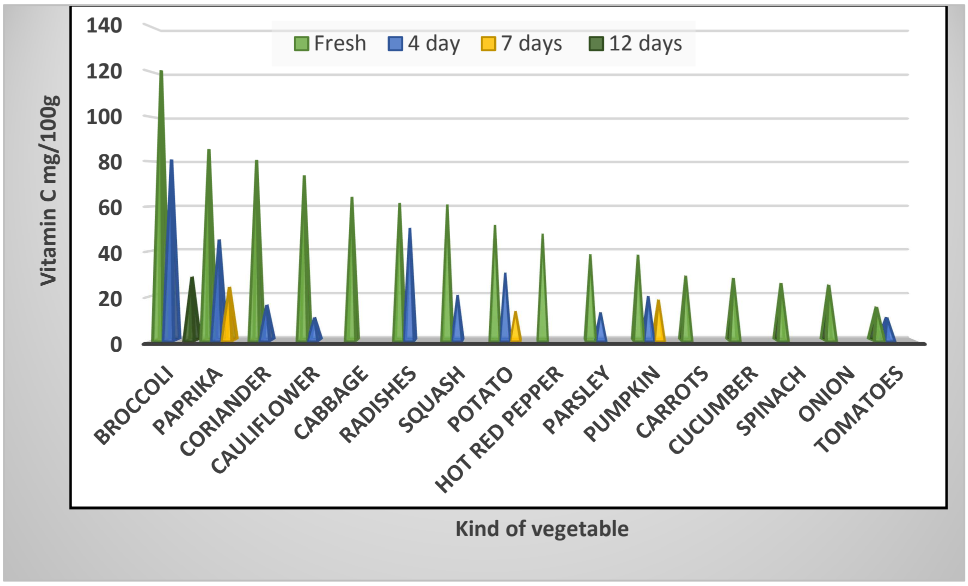

Figure 3.1. : Amount Of vitamin C of some vegetables and effect of time on them

From the table and figure above, the broccoli has the highest value and the tomato has the least one; the effect of time is clear in the destroying of vit. C especially for the broccoli.

Effect of time on leafy vegetables

Table 3.2. : Amount Of vitamin C of some leafy vegetables and effect of time on them

Kind of vegetable	**Fresh** mg/100g	**4 day** mg/100g	**7 days** mg/100g
Spinach	51.57	44.64	22.53
Celery	49.07	27.75	24.12
Mint	39.47	15.46	10.48
Arugula	28.4	20.33	12.11
Green onions	25.38	18.8	10.16

Table 3.2. and figure 3.2. show the amount of vitamin C in some leafy vegetables and the effect of leaving them in s refrigerator for 4 and 7 days . It is clear that vitamin C is destroyed with the time and about 50% of it was destroyed after 7 days.

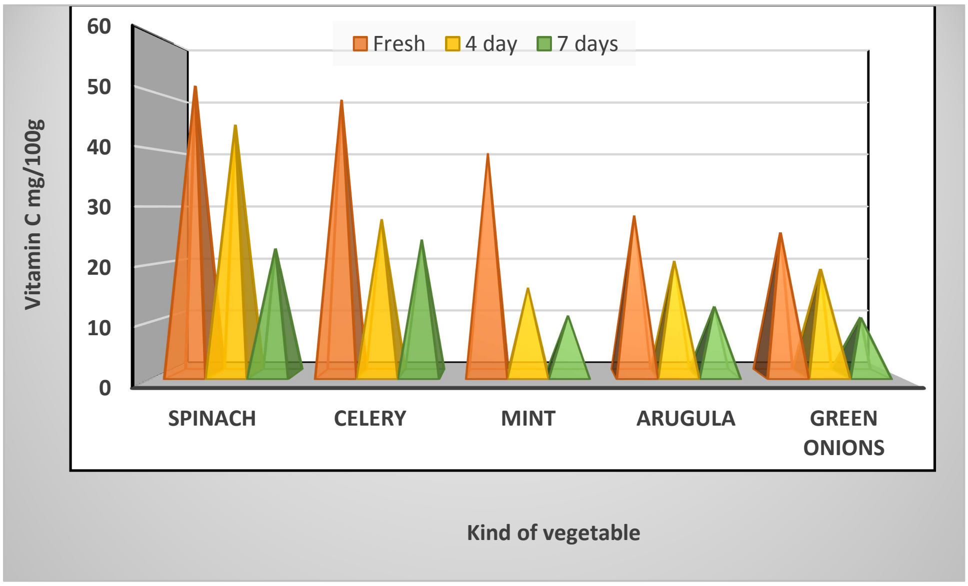

Figure 3.2. : Amount Of vitamin C of some vegetables and effect of time on them

3.1.2 Effect of Cooking

The effect of cooking the fresh and the stored vegetables for 15 min at 100°C on the amount of vitamin C present was studied as shown in table 3.2.

Table 3.3: Effect of cooking the vegetables for 15 min at 100°C on the amount of vitamin C

Kind of vegetable	Fresh mg/100g	4days mg/100g	7 days mg/100g
Broccoli	63.55 ±0.25		
Paprika	50.52 ±0.18		
Cabbage	47.14 ±0.23		
Parsley	38.42 ±0.52	15.96 ±0.72	
Cauliflower	32.00 ±0.33		
Coriander	25.33 ±0.67	12.58 ±0.08	
Pumpkin	15.64 ±0.25		
Potato	14.62 ±0.10	5.30 ±0.11	
Carrots	13.61 ±0.16		
Tomato	12.50 ±0.07	9.13 ±0.35	2.64 ±0.30
Onion	8.36 ±0.20		

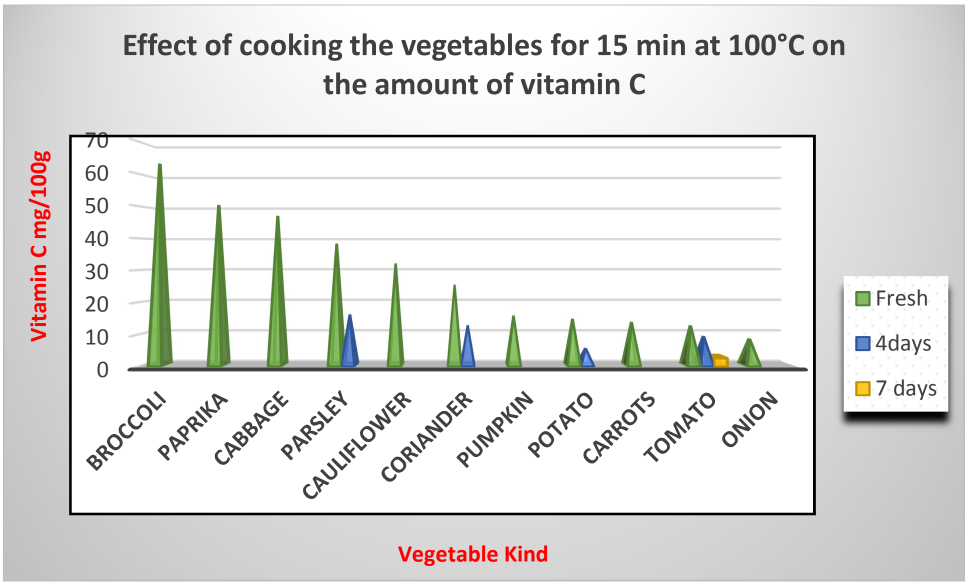

Figure 3.3: Effect of cooking the vegetables for 15 min at 100°C on the amount of vitamin C

From tables 3.3 and figure 3.3, it is clear that the cooking destroys vitamin c even cooking in a short time. For example broccoli lost about 50% of its vit. C content and this lost increases with the time.

3.2. Determination of amount of Ascorbic Acid obtained from Spectrophotometric Method

3..2.1 Estimation of λ_{max} and construction of Standard Curve

Scanning of the complex in a wavelength range from 350 nm to 650 nm showed a maximum absorbance (λ_{max}) at 521 nm and then the absorbance of all standards (converted to colored complex) was taken to construct a calibration curve by plotting the concentration versus the corresponding absorbance at 521 nm as shown in figure 3.3.

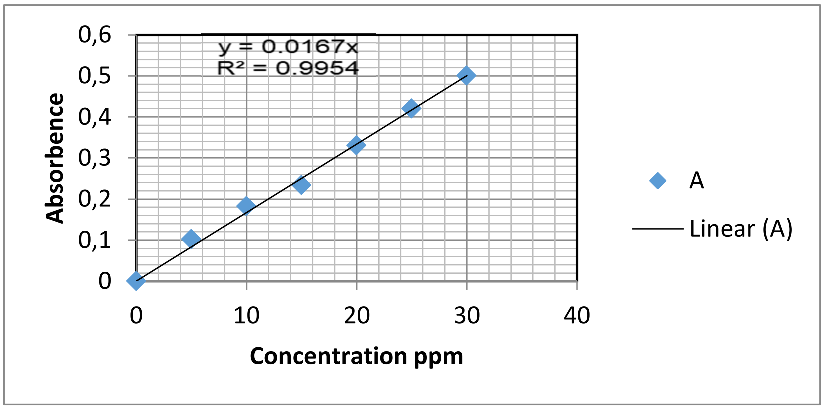

Figure 3.4: Calibration Curve of standard Vitamin C at 521 nm

It is clear from the above figure that Beer`s law is obeyed within the range of the concentrations in the studying

Total vitamin C in vegetables and the different effects on it were also studied spectrophotometrically

3.2.2. Effect of Time

Table 3.5 shows the amount of vitamin C of some vegetables , their sources and effect of storing them in the refrigerator for 4, 7 and 12 days.

Table 3.4: Amount of vitamin C of some vegetables and effect of time on them

Kind of Veg.	Fresh mg/100g	4 days mg/100g	7 days mg/100g	12 days mg/100g
Broccoli	154.11± 2.44	148.76 ±0.42	142.93±4.91	**54.02±5.37**
Hot Red Pepper	125. 59± 2.82	72.81± 1.53	53.21 ±2.34	
Paprika	107.73± 2.85	82.00±1.34	36.81±0.65	
Cauliflower	91.22± 1.03	53.20± 0.5	48.95±0.98	
Potato	84.137 ± 6.510	46.81±2.62	36.73±1.89	
Cabbage	89.34±1.23	69.11±0.89	58.19±1.09	
Squash	75.56±4.80	25.35±2.28	20.87±2.08	
Radishes	65.07±0.26	53.05±2.28	35.87±1.43	
Green chilli peppers	50.55±2.23	37.78± 1.27		
Pumpkin	48.19±0.67	34.33±0.54	30.82±0.20	

Cucumber	45.98±0.98	23.08±2.40	19.34±0.58	
Carrots	43.72±0.52	34.93±0.99	19.84±1.06	
Onions	34.83±1.11	30.76± 0.45	10.47±0.55	
Tomatoes	16.46±0.48	12.45±0.47	10.0.69±0.16	

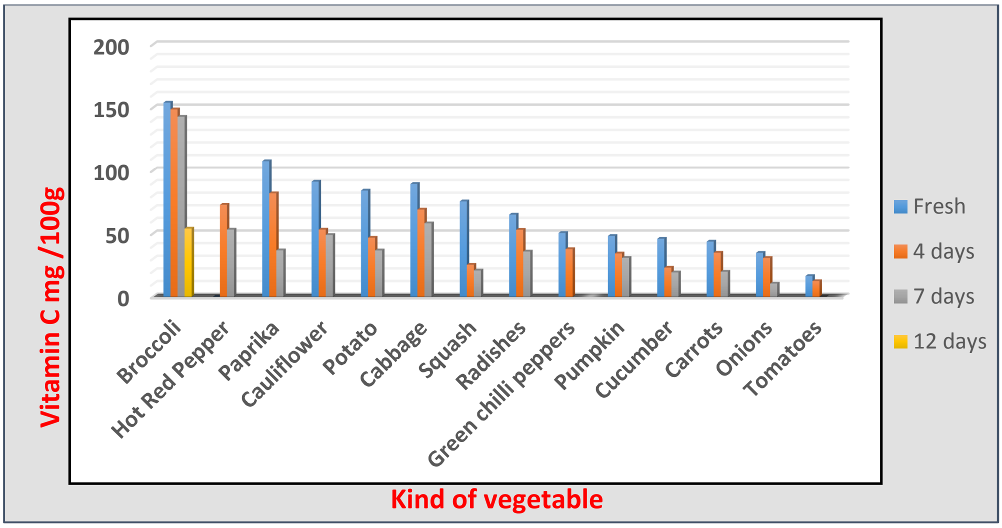

Figure 3.5: Amount of vitamin C of some vegetables and effect of time on them

It is clear from Table 3.4 and Figure 3.5 that vitamin C is destroyed with time.

Table 3.5 shows the amount of vitamin C in leafy vegetables for the fresh and after 4 and 7 days in mg/100g

Table 3.5: Amount of vitamin C in leafy vegetable sand effect of time on it

Kind of Veg.	Fresh mg/100g	4 days mg/100g	7 days mg/100g
Coriander	159.39±6.31	87.53±2.26	**28.77±2.41**
Dill	79.37±0.73	25.33±0.78	**20.32±1.37**
Parsley	67.43±4.72	23.46±1.53	**19.25±0.85**
Spinach	51.57±4.29	44.64±4.18	**22.53±1.22**
Celery	49.07±1.83	27.75±3.20	**24.12±1.32**
Mint	39.47±1.24	15.46± 0.91	**10.48±1.16**
Arugula	28.40±1.16	20.33±2.11	**12.11± 0.86**
Green onions	25.38± 2.21	18.80±0.56	**10.16±0.71**

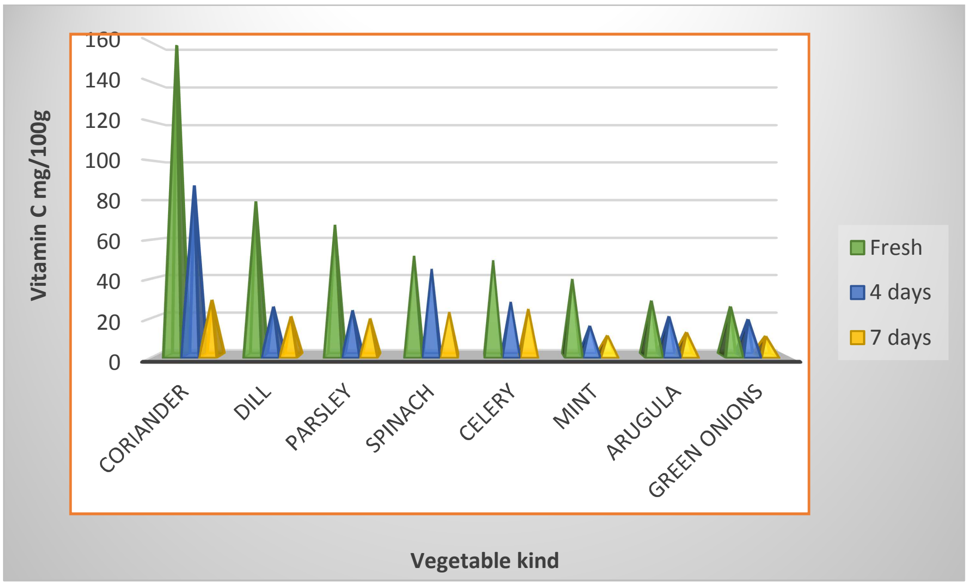

Figure 3.6: Effect of time for some leafy vegetables

It is clear from table 3.5 and figure 3.6 that the amount of vitamin C decreased strongly with time especially in coriander

3.2.3: Effect of Cooking

The effect of cooking of the vegetables at 100°C for 15 min on the amount of vitamin C was studied for the fresh and the stored samples for 4,7 and 12 days as shown in tables 3.6 and 3.7 and figures 3.7 and 3.8 .

Table 3.6: Effect of cooking vegetables on the amount of vitamin C

Kind of Veg.	Fresh mg/100g	4 days mg/100g	7 days mg/100g	12 days mg/100g
Paprika	87.65±1.33	50.44±0.87	14.24±0.44	
Broccoli	86.40±2.54	43.69±0.49	31.99±4.24	**28.25±3.29**
Squash	38.48±1.21	21.23±0.91	15.87±0.80	
Cauliflower	35.20±0.39	28.56±0.54	25.76±0.77	
Pumpkin	30.41±0.84	28.22±0.61	9.50±0.43	
Coriander	25.33 ± 0.67			
Potato	21.94 ± 2.11	8.98 ±1.96	6.23±1.96	
Carrots	20.79±0.31	10.62±1.85	8.92±0.31	
Onions	9.683 ± 0.81	7.55± 0.72	5.62±0.82	
Tomatoes	6.26±0.56	5.40±0.21	4.46±0.41	

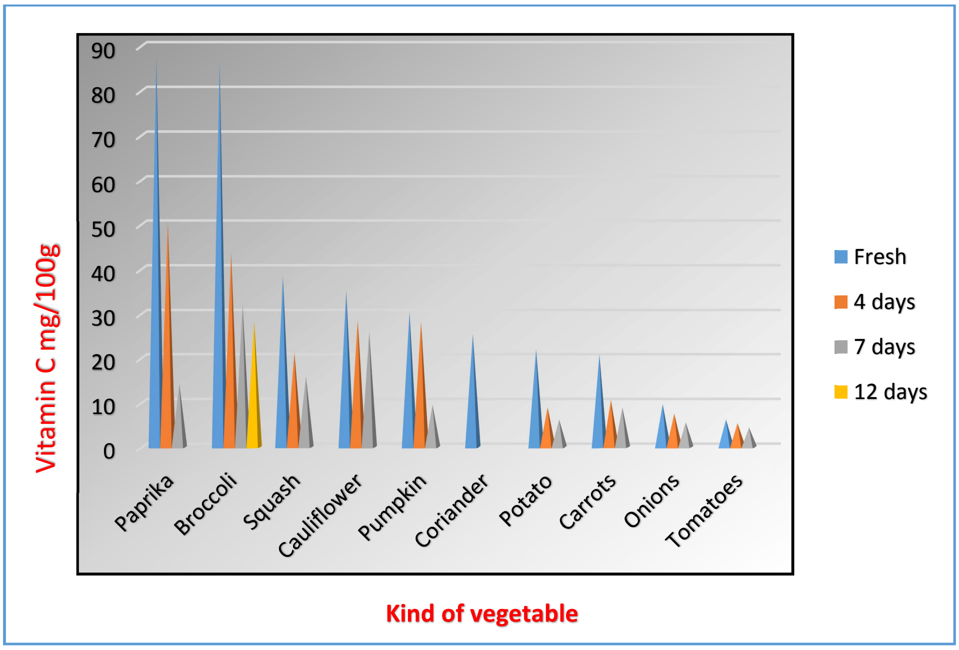

Figure 3.7: Effect of cooking vegetables on the amount of vitamin C

Table 3.7: Effect of cooking leafy vegetables on the amount of vitamin C

Kind of Veg.	Fresh mg/100g	4 days mg/100g	7 days mg/100g
Green onions	21±0.90	8.4± 0.54	**5.47± 0.98**
Coriander	29.77±0.55	17.08±0.45	**8.15±1.43**
Parsley	26.05±0.80	17.50±1.76	**15.23 ±0.72**
Celery	26.41±1.29	19.38±1.16	**14.86±2.71**
Spinach	28.80±0.23	20.94±2.72	**14.76±0.95**
Dill	15.44±0.53	11.79±0.66	**10.73±2.20**

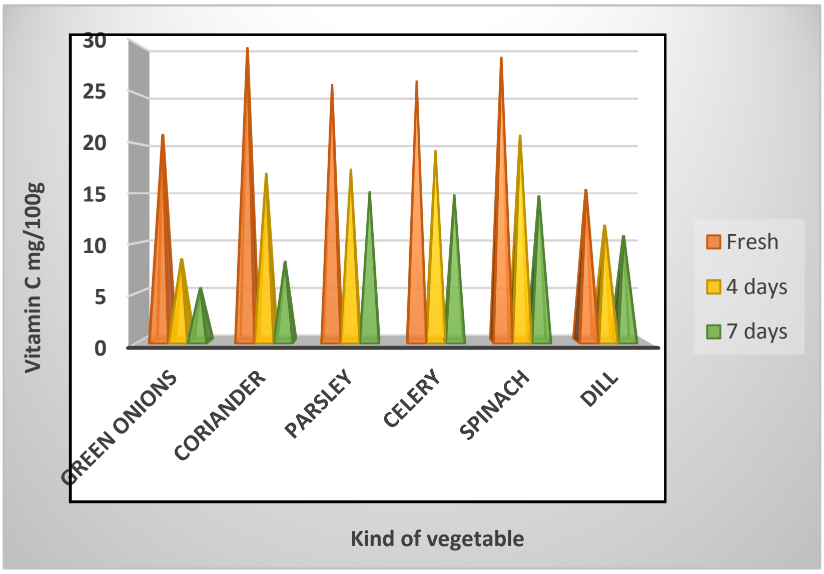

Figure 3.8: Effect of cooking leafy vegetables on the amount of vitamin C

It is clear that vitamin C is destroyed by cooking especially paprika, broccoli and green onions

3.2.4. Effect of Frozen

Effect of frozen vegetables at -5°C for one, two, and three weeks and cooking them at 100°C for 15 min. were studied. Tomato as an example was used to measure the amount of vitamin C in many cases as illustrated in table 3.8 and figure 3.9

Table 3.8: Effect of cooked and frozen the tomatoes on the amount of vitamin C

State of Tomatoes	Fresh mg/100g	4 days mg/100g	7 days mg/100g	1 week mg/100g	2 weeks mg/100g	3 weeks mg/100g
Uncooked & Unfrozen	16.46	12.45	10.69	-	-	-
Cooked	6.26	5.4	4.46	-	-	-
Frozen	-	-	-	8.68	7.54	5.19
Frozen & Cooked	-	-	-	2.3	1.65	0.95

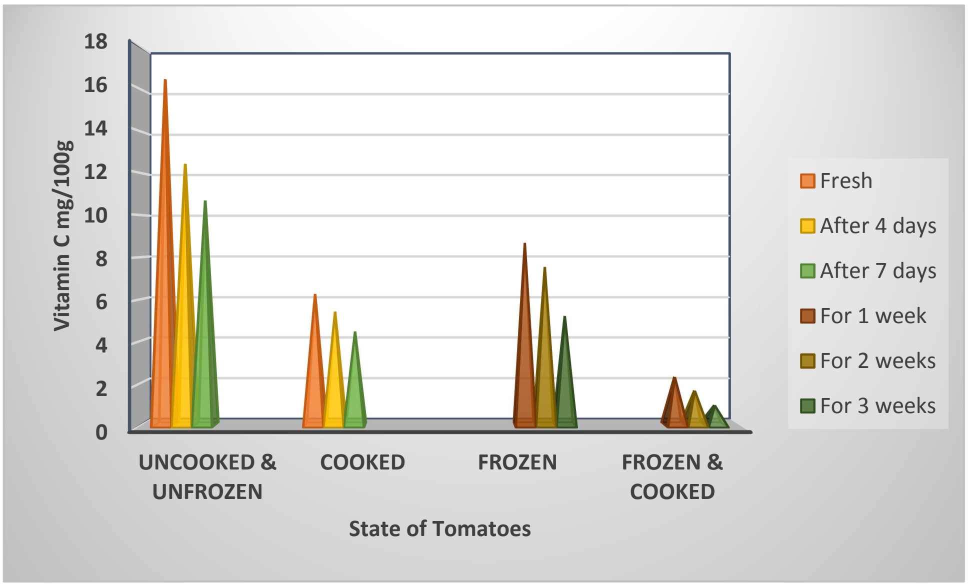

Figure 3.9: Effect of cooked and frozen the tomatoes on the amount of vitamin C

From table 3.7 and figure 3.8, it is clear that the highest amount of vitamin C is present in the fresh state (16.46 mg/100g) and the least amount (or almost no) is present in the frozen and cooked after 3 weeks state (0.95 mg/100g). Also it is clear that vitamin C is destroyed by cooking more than frozen but as we know that the frozen tomatoes should be cooking before eating, so there is almost no vitamin C in such cases.

IV. Conclusion

The results showed that amount of vitamin C which determined by Redox Titration was always less than by spectrophotometric method (e.g. in broccoli by titration the result was121mg/100g while by spectrophotometry was 154.11 mg/100g). This is because by titration only L-ascorbic acid, is determined but by spectrophotometer the total of L-ascorbic and, L-dehydroascorbic are determined.

In vegetables, the results showed that the highest concentration of vitamin C is in broccoli (154.11 mg/100g) and the lowest concentration of vitamin C is in tomatoes (16.46 mg/g).

In herbs, the highest concentration of vitamin C is in coriander (159.39 mg/100g) and the lowest concentration is in green onion (25.38 mg/100g) and the concentration of vitamin C decreases with time, it is lost by boiling and frozen also lost most of it by frozen. then cooking

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