Comparison of Two Methods for The Determination of Vitamin C (Ascorbic Acid) in Some Fruits

American Journal of Chemistry
Vol. 2, No. 1, 1-7, 2017

(Corresponding Author)

Isam Eldin H. Elgailani[1]
Mohamed A. M. Gad-Elkareem[2]
Elnoor A. A. Noh[3]
Omer E. A. Adam[4]
Ahmed M. A. Alghamdi[5]

[1,2,3,4,5]*Department of Chemistry, Faculty of Sciences and Arts at Baljurashi, Albaha University, Albaha, Saudi Arabia*

ABSTRACT

The present research deals with the comparison of the two methods for the determination of vitamin C (ascorbic acid) content in some fruits namely apples, oranges, lemons, tangerines and grapes. The fruits were collected from local market in Baljurashi city. Vitamin C content of fresh fruit were determined by titrimetric and spectrophotometric methods using potassium permenganate as a chromogenic reagent. The absorbance is measured spectrophotometrically at 530 nm. The titrimetric method was carried out by an iodimetric back-titration. The results obtained from this study revealed that there is no significant difference between the two methods, but the spectroscophotometric method has been preferred to determine the amount of vitamin C than the titrimetric method.

Keywords: *Spectrophotometric, Titrimetric , Vitamin C, Ascorbic acid, Determination.*

DOI: 10.20448/812.2.1.1.7
Citation | Isam Eldin H. Elgailani; Mohamed A. M. Gad-Elkareem; Elnoor A. A. Noh; Omer E. A. Adam; Ahmed M. A. Alghamdi (2017). Comparison of Two Methods for The Determination of Vitamin C (Ascorbic Acid) in Some Fruits. American Journal of Chemistry, 2(1): 1-7.

Funding: This study received no specific financial support.
Competing Interests: The authors declare that they have no competing interests.
History: Received: 11 February 2017/ Revised: 27 February 2017/ Accepted: 7 March 2017/ Published: 11 March 2017
Publisher: Online Science Publishing

1. INTRODUCTION

Vitamins as general help the human to maintain a healthy diet (Rahman *et al.*, 2006). Vitamin C, more properly called ascorbic acid. The chemical formula of ascorbic acid is (R)-5-((S)-1,2-dihydroxyethyl)3,4- dihydroxyfuran-2(5H)-one, molar mass 176.12 g mol−1, Density 1.65 g/cm3, melting point 190-192 °C, with solubility in water about 33 g/100 mL (Rahman *et al.*, 2006; Fadhel, 2012) (Figure 1). Vitamin C is an essential antioxidant needed by the human body. Ascorbic acid is of great importance in biochemical reactions as a reducing agent (Moeslinger *et al.*, 1995; Rahman *et al.*, 2006; Wonsawat, 2006; Fadhel, 2012). Vitamin C is the L-enantiomer of ascorbic acid, it is a water-soluble vitamin used by the body for several purposes. It is the most important vitamin in fruits and vegetables. Most animals can synthesize their own vitamin C, except human and other primates (Kumar *et al.*, 2013).

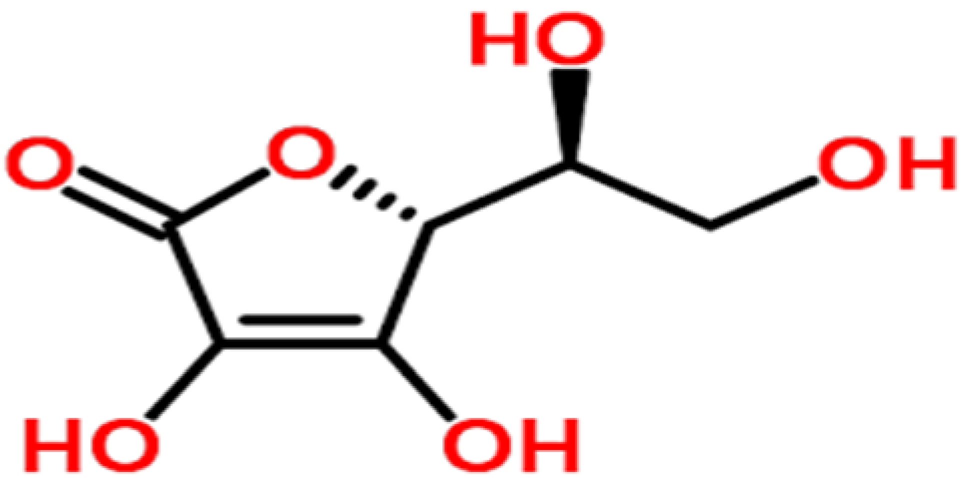

Figure-1. The structure formula of Ascorbic acid.

Source: Fadhel (2012)

We get vitamin C from the food we eat, particularly fruit and vegetables. Our bodies need vitamin C to make a substance called collagen which is required for the health and repair of our skin, bones, teeth and cartilage (Rahman *et al.*, 2006). Vitamin C was first isolated in 1928, and in 1932 it was proved to be the agent, which prevents scurvy. The common sources of vitamin C are citrus fruits, (lime, lemon, orange, grape fruit) and many other foods such as tomatoes papaya, broccoli, brussels sprouts, blackberries, strawberries, cauliflower, spinach, cantaloupe, and blueberries. Several analytical methods have been reported for the determination of vitamin C using spectrometry and amperometry (Arya *et al.*, 1998; Arya *et al.*, 2000). The development of rapid, simple, and inexpensive analytical methods is one of areas of growing interest, especially since quick decisions are needed in environmental, medical, and industrial fields (Shephard *et al.*, 1999). Many analytical methods were used for ascorbic acid determination, including Titrimetric spectrophotometry and Chromatography (Rahman *et al.*, 2006; Selimović *et al.*, 2011; Fadhel, 2012) titrimetry, voltammetry, fluorometry, potentiometry as an analytical techniques (Shephard *et al.*, 1999; Shephard *et al.*, 1999; Shephard *et al.*, 1999; Kabasakalis *et al.*, 2000; Ruedas *et al.*, 2004). Similarly, liquid chromatography (Zerdin *et al.*, 2003; Franke *et al.*, 2004) capillary electrophoresis (Versari *et al.*, 2004) and gas chromatography (Silva, 2005) were also used for the the determination of ascorbic acid from different species of citrus fruits.. Spectrophotometry is one of the most frequently used simple methods, because Vitamin C is able to absorb UV ray (Moeslinger *et al.*, 1995; Rahman *et al.*, 2006; Wonsawat, 2006; Fadhel, 2012). The method is suitable for use with vitamin C tablets, fresh or packaged fruit juices and solid fruits and vegetables. In this work, vitamin C in some fruits was determine by titrimetric and spectrophotometric method . The two methods were also compared in order to determine a simple methods for the determination of vitamin C in some local fruits.

2. MATERIALS AND METHODS

2.1. Samples Area and Preparations

Fruit samples werebcollected from different markets of Baljurashi city, southwest of Saudi Arabia. The samples used in this study were apples, oranges, lemons, tangerines and grapes which are considered to be the most popular fruits. Samples were thoroughly cleaned with water to remove dust and unwanted particles. The samples were prepared by cutting them into small pieces for each sample separately, and have been dealt with a manner to prevent them from contamination by chemicals or any other pollutants. Each of the samples has been mixed thoroughly homogenous mixture and stored in containers at room temperature for instant analysis.

2.2. Chemicals, Reagents and Apparatus

All chemicals and reagents used in this study were of analytical grade. UV-Visible Spectrophotometer (PD-303UV, APEL CO., LT) was also used for specrophotometric measurements.

2.3. General Procedure for Preparation of Fruit Samples

The queous extracts for each of the five samples were prepared by weighing accurately 100 g of the freshly prepared fruit sample in a 500 ml beaker and blended vigoursly to obtain the fruit juice by adding 30 ml of oxalic acid (0.5% w/v) in order to prevent the oxidation of ascorbic acid (vitamin C). Each of the mixtures were filtered through a precleaned cloth and receiving the filtrate in a 250 ml Erlenmeyer flask. The aliquot of each sample was transferred to a 100 ml volumetric flask and then completed to the mark with oxalic acid solution (0.5%). Blank was prepared by the same manner as except for the addition of fruit samples. Each of the five fruit samples was treated separately as described under the general procedure.

2.4. Preparation of Stock and Standard Solutions of Ascorbic Acid

Standard solution of ascorbic acid was prepared by dissolving an accurate weight of 0.01g of standard ascorbic acid in small amount of oxalic acid solution (0.5%.) and then completed to 100 ml with the same solution to obtain a concentration of 100 μg/mL. A series of dilutions 1.0, 4.0, 8.0, 12 and 16 μg/mL were prepared from the stock ascorbic acid solution.

2.5. Prepare Potassium Permanganate Solution

A solution of $KMnO_4$ of concentration of 100 μg/mL was prepared by dissolving an accurately 0.01 g of $KMnO_4$ in H_2SO_4 solution (5.0M) , then transferred into a 100 mL volumetric flask and completed to the mark with distilled water and thoroughly mixed. This solution was used a chromogenic reagent for the determination of ascorbic acid (vitamin C) by spectrophotometer.

2.6. Preparation of Standard Calibration Curve of Ascorbic Acid

Standard calibration curve of ascorbic was established by graphing concentrations versus absorbance of ascorbic standard solutions by taking 10 mL of each of standard solutions and put in a test tube, then 1 mL of $KMnO_4$ solution (100 μg/mL) was added. This solution was let to stand for 5 minutes. The absorbance of this standard solutions were read at 530 nm against blank.

2.7. Preparation of Fruit Samples for Analysis by UV-Visible Spectrophotometer

Each of the five of fruit samples were accurately taken as 10.0 mL for each sample, and then transferred into a test tube, and 1.0 mL of $KMnO_4$ (100 µg/mL) was added for each. The contents of each test tube were mixed well and stand for 5 minutes. The prepared solutions were were read at 530 nm against blank by spectrophotometer using a suitable concentration for the analysis.

2.8. Determination of Ascorbic Acid in the Fruit Samples by Titration Method

Accurate 1 ml of each of the freshly prepared fruit sample solution was transferred and then diluted to 200 ml with distilled water.Then 10 ml of each of these solutions were put into conical flask. To this flask 5.0 ml of KI solution (0.2 M) , 2.5 ml of hydrochloric acid HCl (1.0 M) and few drops of starch solution were added. Each of the five solutios was then titrared against KIO_3 (0.015 M) from the burette until the appearance of blue -black colour which indicate the end point of the reaction. The titration was repeated three time for each of the fruit samples. The results were recorded, tabulated and calculated for ascorbic acid determination for each samples.

2.9. Statistical Analysis

Averages and standard deviations from sample studies and linear regressions for calibration curves were determined using Microsoft Excel 2007. Statistical analysis was performed with one-way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1. Determination of the Absorption Spectra

The maximum absorption wavelength (λ_{max}) for the products which obtained by the reaction of ascorbic acid of prepared fruit samples and $KMnO_4$ solution (100 µg/mL) occurred at 530 nm against the blank as in (Figure 2).

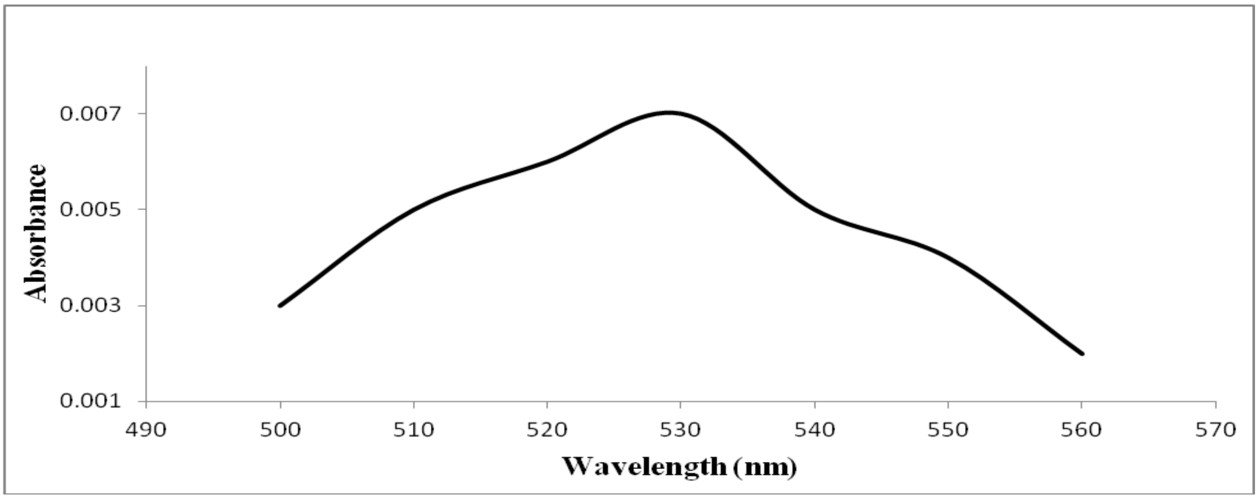

Figure-2. The λmax of reaction of prepared ascorbic acid (10 µg/mL) with $KMnO_4$ solution (100 µg/mL).
Source: (UV-Visible Spectrophotometric analysis).

3.2. Preparation of the Calibration Curve of Ascorbic Acid

Calibration curves for the determination of ascorbic acid in the prepared fruit samples and ($KMnO_4$ solution) were constructed by plotting the absorbances as a function of the corresponding concentrations as shown in (Figure 3). There was observed a proportional increase in absorbance at the respective λ_{max} with increasing concentration of each ascorbic acid standard solutions.

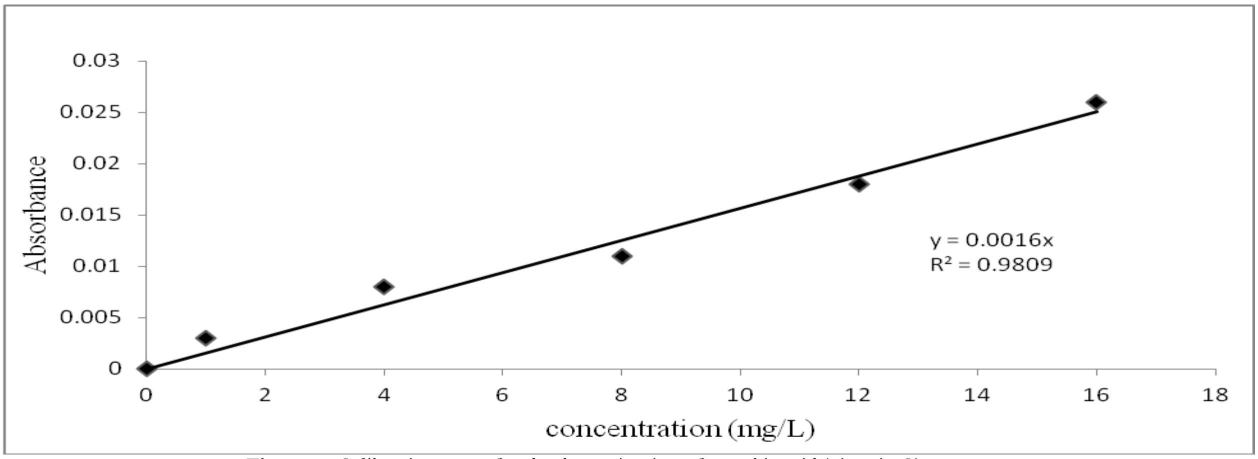

Figure-3. Calibration curve for the determination of ascorbic acid (vitamin C).
Source: (UV-Visible Spectrophotometric analysis).

3.3. Determination of Ascorbic Acid (Vitamin C) in the Fruit Samples by Spectrophotometer

Each of unknown fruit samples solutions were treated in the same way for ascorbic acid determination. The absorbance of unknown sample solutions were measured. These absorbance values were referred to their related concentrations in the calibration curves (Table 1). The results obtained by UV-Visible Spectrophotometer reveal varying amounts of vitamin C in fruit samples.

Table-1. Amount of vitamin C in fruit samples by spectrophotometric method:

Fruit samples	Absorbance	Vitamin C (mg/L)	Vitamin C (g/L)*
apples	0.015	15.0	7.50
oranges	0.013	13.0	11.70
lemons	0.012	12.0	10.80
tangerines	0.015	15.0	3.00
grapes	0.019	19.0	15.00

* The corresponding values after multiplication by dilution factor.

3.4. Determination of Ascorbic Acid (Vitamin C) in the Fruit Samples by Titrimetric Method

In the determination of ascorbic acid content of fruit samples by titrimetric method using potassium iodate. Iodine, I_2, is a weak oxidizing agent. It would only oxidize vitamin C ($C_6H_8O_6$) as far as desired. But iodine is almost insoluble in water (0.0013 M). The iodine produced immediately react with Vitamin C. Its concentration will never be large enough to precipitate. Iodides like potassium iodide, KI, are quite soluble. Iodides are easily oxidized to $\mathbf{I_2}$. A convenient, and common, oxidizing agent is iodate ion, IO_3^-

IO_3^- (iodate) oxidizes I^- (iodide) to I_2 (iodine)

$IO_3^- + 5\ I^- + 6\ H^+ \rightarrow 3\ I_2 + 3H_2O$

Liberated I_2 (iodine), oxidizes $C_6H_8O_6$ (Reduced Ascorbic Acid)

$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2\ H^+ + 2\ I^-$

Overall reaction:

$IO_3^- + 3\ C_6H_8O_6 \rightarrow 3\ C_6H_6O_6 + 3\ H_2O + I^-$

1 mol IO_3^- = 3 mol $C_6H_8O_6$

The amount of vitamin C in the fruit samples were determined by using the below relationship can be found as shown in (Table 2):

$$\frac{M_1V_1}{n_1} = \frac{M_2V_2}{n_2}$$

Where, M = molar, V = volume (Palml), and n = number of moles of the equation.

Table-2. Amount of vitamin C in fruit samples as determined by titremetric method

fruit samples	volume of KIO_3 required for the titration with vitamin C (ml)				amount of vitamin C (mole /L)	amount of vitamin C (g/L)*
	I	II	III	Average volume		
apples	12.5	12.7	12.7	12.6	0.0567	9.986
oranges	13.6	13.3	13.3	13.4	0.0603	10.620
lemons	12.5	12.3	12.1	12.3	0.0554	9.757
tangerines	12.7	12.6	12.7	12.7	0.0571	10.056
grapes	12.5	12.4	12.3	12.4	0.0558	9.827

* Equal molar concentration multiplied by 176.12 (molecular weight)

Comparison of the two methods was carried out, it was found that there is no large differences between the two method except in the case of tangerines and grapes in which they were showed differences in the amount of vitamin C as seen in (Figure 4).

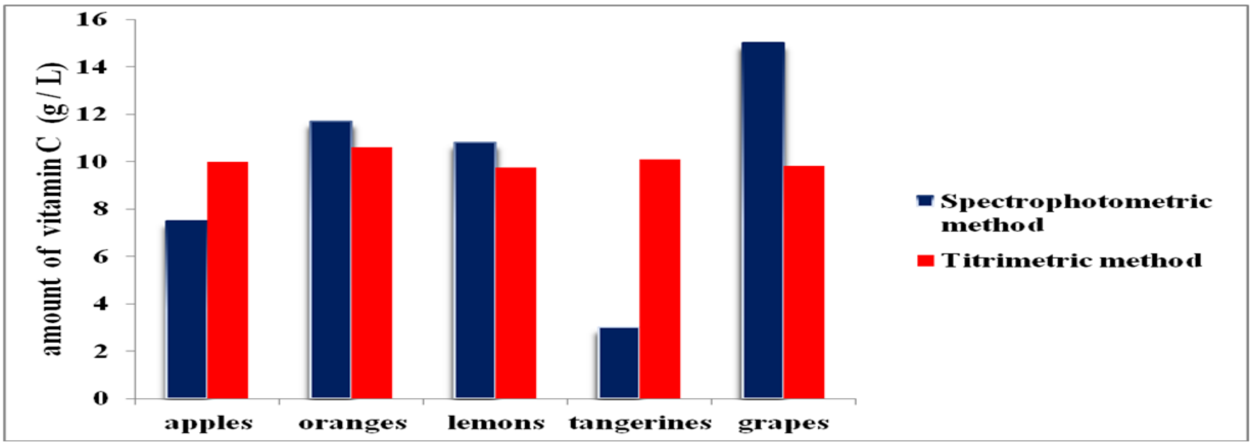

Figure-4. Comparison of specrophotomeric and titrimetric methods in some fruits.
Source: (results obtained and compared from the investigation).

4. CONCLUSIONS

We can conclude that the current study revealed that there is a good agreement with results of the amount of vitamin C in some fruits obtained by the spectrophotometric and titrimetric method. Spectrophotometric method has been preferred because it is simple and fast method.

5. ACKNOWLEDGMENTS

Special thanks to the Department of Chemistry, Faculty of Science and Arts at Baljurashi, Albaha University where this evaluation and investigation have been carried out, for laboratory facilities and valuable assistance in the use of various equipments.

REFERENCES

Arya, S.P., M. Mahajan and P. Jain, 1998. Photometric methods for the determination of vitamin C. Analytical Sciences, 14(5): 889-895. *View at Google Scholar* | *View at Publisher*

Arya, S.P., M. Mahajan and P. Jain, 2000. Non-spectrophotometric methods for the determination of vitamin C. Analytica Chimica Acta, 417(1): 1-14. *View at Google Scholar* | *View at Publisher*

Fadhel, D.H., 2012. Spectrophotometric determination of ascorbic acid in aqueous solutions. Journal of Al-Nahrain University, 15(3): 88-94. *View at Google Scholar*

Franke, A.A., L.J. Custer, C. Arakakiand and S.P. Murphy, 2004. Vitamin C and flavonoid levels of fruits and vegetable foods consumed in Hawaii. Journal of Food Composition and Analysis, 17(1): 1-35. *View at Google Scholar* | *View at Publisher*

Kabasakalis, V., D. Siopidou and E. Moshatou, 2000. Ascorbic acid content of commercial fruit juices and its rate of loss upon storage. Food Chemical, 70(3): 325- 328. *View at Google Scholar* | *View at Publisher*

Kumar, G.V., A.K. Kumar, P.G.R. Raghu and S. Manjappa, 2013. Determination of vitamin C in some fruits and vegetables in Davanagere city, (Karanataka) - India. International Journal of Pharmaceutical and Life Sciences, 4(3): 2489-2491. *View at Google Scholar*

Moeslinger, T., M. Brunner and I.V.P.G. Spieckermann, 1995. Spectrophotometric determination of ascorbic-acid and dehydroascorbic acid. Clinical Chemistry, 41(8): 1177-1181.

Rahman, K.M.M., M.M. Rahman, M.S. Islam and S.A. Begum, 2006. A simple UV-spectrophotometric method for the determination of vitamin C content in various fruits and vegetables at Sylhet area in Bangladesh. Journal of Biological Sciences, 6(2): 388 – 392. *View at Google Scholar* | *View at Publisher*

Ruedas, R.M.J., M.A. Ruiz and D.A. Molina, 2004. A Prussian blue-based flow-through renewable surface optosensor for analysis of ascorbic acid. Microchemical Journal, 78(2): 157-162. *View at Google Scholar* | *View at Publisher*

Selimović, A., M. Salkić and A. Selimović, 2011. Direct spectrophotometric determination of lascorbic acid in pharmaceutical preparations using sodium oxalate as a stabilizer. International Journal of Basic & Applied Sciences, 11(2): 106 - 109. *View at Google Scholar*

Shephard, A.B., S.C. Nichols and A. Braitawaite, 1999. Moisture induced solid phase degradation of l-ascorbic acid Part 1: A kinetic study using tristimulus colorimetry and a quantitative HPLC assay. Talanta, 48(3): 585-593. *View at Google Scholar* | *View at Publisher*

Shephard, A.B., S.C. Nichols and A. Braitawaite, 1999. Moisture induced solid phase degradation of l-ascorbic acid part 2, separation and characterization of the major degradation products. Talanta, 48(3): 595-606. *View at Google Scholar* | *View at Publisher*

Shephard, A.B., S.C. Nichols and A. Braitawaite, 1999. Moisture induced solid phase degradation of l-ascorbic acid part 3, structural characterisation of the degradation products. Talanta, 48(3): 607-622. *View at Google Scholar* | *View at Publisher*

Silva, F.O., 2005. Total ascorbic acid determination in fresh squeezed orange juice by gas chromatography. Food Control, 16(1): 55-58. *View at Google Scholar* | *View at Publisher*

Versari, A., A. Mattioli, G.P. Parpinello and S. Galassi, 2004. Rapid analysis of ascorbic and isoascorbic acids in fruit juice by capillary electrophoresis. Food Control, 15(5): 355-358. *View at Google Scholar* | *View at Publisher*

Wonsawat, W., 2006. Determination of vitamin C (Ascorbic Acid) in orange juices product. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 8(6): 623-625. *View at Google Scholar*

Zerdin, K., M.L. Rooney and J. Vermue, 2003. The vitamin C content of orange juice packed in an oxygen scavenger material. Food Chemistry, 82(3): 387-395. *View at Google Scholar* | *View at Publisher*