Titrimetric Determination of Ascorbic Acid Contents in Plant Samples by 2, 6-Dichlorophenolindophenol Method

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Summary: The aim of this study was to investigate the levels of ascorbic acid (vitamin C) in various plant samples, which were available in Iran markets, by redox titration method, using2,6-dichlorophenolindophenol. The obtained data were used for determination of vitamin C amounts at the 95% confidence level (student's t-test). The order of ascorbic acid contents in samples was leaves of parsley, kiwifruit, broccoli, orange, red pepper, lemon, leaves of geranium, lime, leaves of spinach, green pepper, potato, tomato, white part of scallion, leaves of leek, apricot, peach, green part of scallion, white onion, lettuce and leaves of coriander.

Keywords: Vitamin C, Redox titration, 2,6- dichlorophenolindophenol, Plant samples.

Introduction

Ascorbic acid (AA) or vitamin C is a white crystalline compound, which is highly soluble in water. It has significant functions in human body such as its role in syntheses of collagen, carnitine and neurotransmitters, acting as antioxidant and improvement of the body resistance to infectious diseases. It is applied for the treatment of some diseases and disorders, including scurvy (caused by lack of vitamin C), common cold, anemia, hemorrhagic disorders, infertility and wound healing. Human body is not able to synthesize vitamin C, therefore the daily necessary amount of it (60 mg) should be obtained from dietary sources such as fruits and vegetables or pharmaceutical preparations. Ascorbic acid can be utilized for industrial purposes as an antioxidant as well as a reducing agent [1-3]. Among different methods for measurement of vitamin C, such as electrophoresis [4], chromatography [5], voltammetry [2], biamperometry [6], spectrophotometry [7], high-performance liquid chromatography [8] and titration by iodine [9], the simple redox titration of AA by 2,6-dichlorophenolindophenol (DCPIP) is accepted as a standard method for determination of vitamin C [9, 10]. The UV-spectrophotometric, voltammetric and titrimetric (using N-bromosuccinimide) determinations of AA in plant samples have been reported [2, 7]. However, there is no report on the ability of DCPIP method for comparative study of vitamin C contents in a group of plant samples.

The intention of this study was to determine the amount of vitamin C in 20 plant samples using DCPIP method and comparison of vitamin C amounts in the natural plant samples.

Results and Discussion

Calibration of DCPI solution by standard AA solution

A Solution of DCPIP was calibrated by a standard solution of AA (2 mL), which had 1.08 mg of AA. Based on the obtained data each mL of DCPIP solution was equivalent to 0.093 mg of AA.

Measurement of vitamin C in plant samples

The liquid extracts of samples were titrated by calibrated titrant. After titrations, results were expressed as mean ± confidence limits at 95% confidence level (student's t-test) [11]. Then, the amount of AA (mg) could be calculated in 100 g of the natural plant sample utilizing equation (1).

Amount of AA (mg) in 100 g of the plant sample=

$$\frac{v \times m \times 10000}{V \times M} \qquad (1)$$

where v is the consumed volume of DCPIP (mL), m is equivalent amount of DCPIP (1 mL) based on AA (mg), V is the volume of titrated extract (mL) and M is the weight of sample (g). The obtained data for various samples were shown in Table-1, which is in accordance with the decrease of their vitamin C content.

The vitamin C contents which were found in this study were compared (Table-1) with the other existing data reported in the USA and Pakistan by the United States Department of Agriculture (USDA) [12] and Iqbal *et al*. [13], respectively. As can be seen from Table-1, there is a good agreement in

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vitamin C levels in orange, lime, spinach, potato, tomato, leek, apricot, peach and onion from the US and Iran. The same tendency was observed in vitamin C contents in spinach, tomato, onion and lettuce from Pakistan and Iran. The differences in vitamin C amounts in plant samples from mentioned countries can be attributed to various regional varieties, cultivation systems and harvest time [10, 13].

Experimental

Materials

2,6- dichlorophenolindophenol (purity> 97%) and L-ascorbic acid (Puriss) were purchased from Fluka. Sodium dihydrogen phosphate (Puriss), dipotassium hydrogen phosphate (Puriss) and glacial acetic acid (Puriss) were obtained from Merck. Double distilled water was used for preparation of all solutions.

Plant Samples and Extraction Procedure of Vitamin C

Natural fresh plant samples including apricot, broccoli, green pepper, green (top) and white (bulb) parts of scallion, kiwifruit, lemon, lime, lettuce, orange, peach, potato, red pepper, tomato, white onion and coriander, geranium, leek, parsley and spinach leaves were gathered from local market of Tabriz and analyses were done instantly upon arrival. The plant samples were identified by Dr. M.B. Hassanpouraghdam in Horticultural Sciences Department, University of Maragheh, Iran.

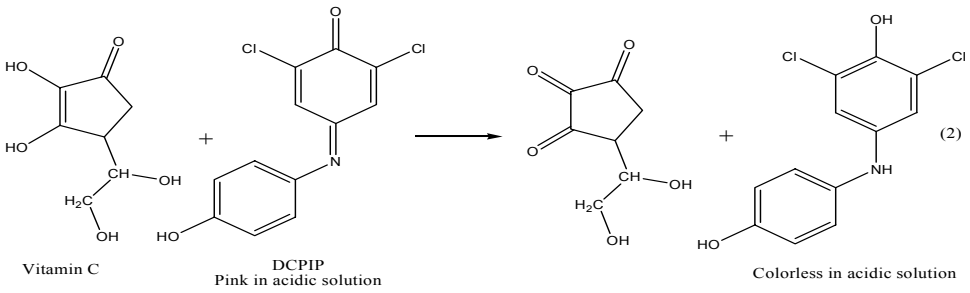
A weighed amount of a sample was chopped and squeezed (if it was juicy). Then mixed with acid acetic 3% (v/v) up to total volume of 90 mL with stirring for 10 min. Afterwards, the mixture of mentioned solution and pulp was centrifuged to separate from each other. The liquid extract was transferred to a volumetric flask and its volume made up to 100 mL with acetic acid 3% (v/v) solution.

DCPIP and standard AA solutions

200 mg of DCPIP, 106 mg of K_2HPO_4 and 90 mg of NaH_2PO_4 were dissolved in 1000 mL of double distilled water. This solution was stable for 30 days, and calibrated by a standard AA solution before using for titrations

Table-1: Vitamin C amounts in plant samples at confidence level of 95%.

Plant sample (exact weight, titrated volume)	Botanical name	ascorbic acid (mg/100 g)	ascorbic acid (mg/100 g) (the USA, Pakistan)
Leaves of parsley (11.36 g, 5 mL)	*Petroselinum crispum* (Miller) A.W.Hill	145.72±0.98	(133.0, -)
Kiwifruit (18.90 g, 5 mL)	*Actinidia chinensis* Planch.	80.99±0.50	(92.7, -)
Broccoli (19.21 g, 5 mL)	*Brassica oleracea* L.	76.49±0.58	(89.2, -)
Orange (20.51 g, 5 mL)	*Citrus sinensis* (L.) Osbeck	53.51±0.45	(53.2, 32.1)
Red sweet pepper (23.87g, 5 mL)	*Capsicum annum* L.	50.96±0.62	(127.7, -)
Lemon (18.36 g, 5 mL)	*Citrus limon* (L.) Burm	41.54±0.60	(53.0, 28.7)
Leaves of geranium (16.72 g, 10 mL)	Geranium maculatum	36.68±0.25	(Not reported, geranium not eaten by human)
Lime (19.15 g, 5 mL)	*Citrus aurantifolia* (Christm.) Swingle	34.97±0.48	(30.0, -)
Leaves of spinach (26.41 g, 10 mL)	*Spinacia oleracea* L.	28.52±0.11	(28.1, 33.8)
Green sweet pepper (40.00 g, 5 mL)	*Capsicum annum* L.	26.74±0.28	(80.4, 73.5)
Potato (25.61 g, 10 mL)	*Solanum tuberosum* L.	20.34±0.18	(19.7, 7.9)
Tomato (46.41 g, 5 mL)	*Lycopersicum esculentum* Mill.	13.40±0.35	(13.7, 9.6)
bulb of scallion (25.62 g, 10 mL)	*Allium ascalonicum* L.	12.64±0.19	(18.8, -) (includes top and bulb)
Leaves of leek (35.56 g, 10 mL)	*Allium ampeloprasum*	10.62±0.26	(12.0, -)
Apricot (35.14 g, 10 mL)	*Prunus armeniaca* L.	10.06±0.13	(10.0, 3.7)
Peach (36.70 g, 10 mL)	*Prunus persica* (L.) Batsch	7.85±0.16	(6.6, 3.5)
top of scallion (29.17 g, 10 mL)	*Allium ascalonicum* L.	5.85±0.14	(18.8, -) (includes top and bulb)
White onion (40.93 g, 10 mL)	*Allium cepa* L.	5.54±0.12	(7.4, 5.4)
Lettuce (32.01 g, 10 mL)	*Lactuca sativa* L.	4.94±0.17	(9.2, 5.0)
Leaves of coriander (20.33 g, 10 mL)	*Coriandrum sativum* L.	4.76±0.23	(27.0, -)



Scheme: Reaction between vitamin C and DCPIP reagent.

54 mg of AA was dissolved in 100 mL of acetic acid 3% (v/v) aqueous solution as a standard solution.

Titration Procedure

DCPIP acts not only as an oxidant but also functions as a visual acid-base indicator for the detection of the end point of titration, where the pink color is observed in the solution, which is generated by the excess of DCPIP reagent (equation-2).

The definite volume of vitamin C containing solution was transferred into the conical flask. If mentioned volume is less than 10 mL, acid acetic 3% (v/v) was added up to total volume of 10 mL, and then titrated against the calibrated DCPIP solution up to equivalent point. The above procedure was repeated four times for each sample.

Conclusion

In this investigation the amounts of vitamin C, which were in a wide range depending on the plant samples, were measured by redox titration method using 2,6-dichlorophenolindophenol at the 95% confidence level (student's t-test). The amount of vitamin C in 20 plant samples was in the following order: parsley leaves, kiwifruit, broccoli, orange, red pepper, lemon, geranium leaves, lime, spinach leaves, green pepper, potato, tomato, white part of scallion, leek leaves, apricot, peach, green part of scallion, white onion, lettuce and coriander leaves. Even in the same samples, the accumulation of vitamin C in its different parts could be different from each other; this claim can be proven by scallion where the amount of vitamin C differs in its white (bulb) and green (top) parts.

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