

## Effect of Temperature Variations during Cooking and Storage on Ascorbic Acid Contents of Vegetables: A Comparative Study

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**Summary:** Vegetables are generally boiled for cooking or stored in refrigerators. This results in loss of their nutritional values. Ascorbic acid is one of the important nutrients for human health. In this study, Ascorbic acid (vitamin-C) content of various vegetables of Pakistan was determined, and effect of boiling and freezing were compared with natural Ascorbic acid contents by HPLC. The maximum concentration of Ascorbic acid was found in green chilli: i.e. 105 mg /100 g in fresh state; while in boiled and frozen state its concentration is comparatively less: i.e. 85 and 92 mg/100 g respectively. The other vegetables like: cabbage, tomato, turnip, potato, spinach, onion, garlic, green pea, green beans and cauliflower contained greater amount of Ascorbic acid in their fresh state i.e. 30, 20, 25.3, 20, 30, 24.3, 31, 28.5, 30, 42 mg/100 g as compared to frozen (23.4, 13, 23.6, 15, 23.4, 14.1, 25, 26.5, 27.0, and 39 mg/100g respectively) and boiled state (11.6, 9.3, 22.5, 10.0, 20.3, 13.1, 23, 25.2 and 35 mg /100g respectively). The minimum amount of Ascorbic acid was found in boiled state of carrot and lettuce: i.e. 4.0 mg/100 g. These results showed that freezing or boiling of vegetables causes significant loss of available Ascorbic acid contents, especially boiling.

Key words: Ascorbic acid, HPLC, vegetables, boiling, freezing.

### Introduction

Ascorbic acid (Vitamin-C) belongs to nutrient class "vitamins". Vitamins are the important constituents of nutrition for mankind and other living creatures; for proper growth and healthy life. They are classified into fat soluble and water soluble categories. Examples of fat soluble vitamins are A, D, E and K, whereas Ascorbic acid belongs to water soluble group [1]. Ascorbic acid is a less stable species among other vitamins. It is very sensitive to light, heat and air, which can stimulate its oxidation. It is vital for living creatures, because it is required for several developmental, enzymatic and metabolic processes like: supports the activity of collagen, and works as an anti-histamine and anti-inflammatory compound [2]. It is also found that Ascorbic acid act synergistically with other vitamins / nutrients e.g. vit. E [3]. Its structural formula is given in Fig. 1. L-ascorbic acid is the biologically-active form of Ascorbic acid [4]. Ascorbic acid is quickly oxidized to dehydro-ascorbic acid (DHA) because of two hydroxyl groups in its structure. Furthermore, oxidation results in the production of diketo-gluconic acid (DKG), which has no biological function. Oxidation reaction is triggered by: increased temperatures, severe fluctuation in pH, and presence of oxygen / metals light and enzymatic action [5].

In last few decades, consumption of vegetables has amplified considerably due to their benefits for good human health and socio-economic nature. Some examples of the most significant

antioxidant compounds usually found in vegetables are: polyphenols, carotenoids and vitamins [6].

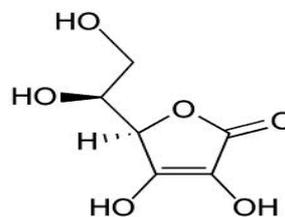


Fig. 1: Structural formula of Ascorbic acid.

Many biochemical and epidemiological researchers had found that vegetables lead to the diminution of several diseases like: cardiovascular, neurological and carcinogenic destruction of different body parts [7]. There are various systems for classifying fruits / vegetables on the basis of: food composition, botanic speciation, colors and edible parts of plants [8]. In this study, we have classified vegetables into three groups, on the basis of edible parts of plant: i.e. leafy, green and root vegetables.

Numerous methodologies for analysis of Ascorbic acid in vegetable samples based on the reversible redox reaction of Ascorbic acid oxidation / dehydro-ascorbic acid reduction were developed in the past. But they lacked true specificity and were prone to interferences by other reducing agents. HPLC methods preferred mostly because they

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provide superior selectivity than other ways without any need of derivatization like: spectrophotometric, titration or enzymatic methods [9-11].

The main objective of this research work was to seek the comparative effect of severe temperature changes during boiling and freezing on Ascorbic acid contents of vegetables. Reversed phase HPLC method with C18 column and UV/Vis detector was employed for this analysis. The C18 (ODS) column was used because it is compatible with aqueous / alcoholic matrices of samples and gives fast equilibration in short intervals of time, when alterations of composition in the mobile phase take place due to fluctuation.

### Results and Discussion

A chromatographic method i.e. reversed phase HPLC was adopted to determine the concentration of ascorbic acid in different vegetables which are generally found in Pakistan to study the effect of nutrient losses especially Ascorbic acid attributed to freezing and boiling. This selected analytical method for Ascorbic acid analysis used during present study is an economical, rapid, sensitive, accurate and efficient way of analyzing Ascorbic acid in vegetable samples [12-14].

Fig. 2 is showing the calibration line for standard solutions of Ascorbic acid used. Concentration of Ascorbic acid in all samples was calculated by means of regression analysis of calibration line and resulting values were mentioned in Table-1. Average values were used for comparative purpose. Standard deviation values were also calculated and are in the range of 0.011-0.045. For comparative purpose, the results were presented in graphical form in Fig. 3.

It is observed that maximum concentration of Ascorbic acid was found in fresh state of green chilli i.e. 105 mg/100 g and the minimum amount was found in boiled state of carrot and lettuce i.e. 4.0 mg/100 g. Cabbage, tomato, turnip, potato, spinach, onion, garlic, green pea, green beans and cauliflower also showed same variations in their fresh, boiled and frozen states. It is also observed that loss of Ascorbic acid was more in case of leafy vegetables (cabbage, spinach and lettuce) as compared to root (turnip, potato, onion, garlic and carrot) and green vegetables (green chilli, green pea and green beans) and more loss is caused by boiling as compared to freezing. The concentration values of Ascorbic acid found in fresh samples of vegetables are also comparable with the reported values of

Giannakourou and Rodrigues [12, 13]. More losses in case of leafy vegetables are due to the structural aspects of storage site of Ascorbic acid in plant tissues. These vegetables are juicier and contain more Ascorbic acid in them as compared to root or stem vegetables. Little variation found in the values is attributed to several factors including: genetic and environmental influences, climatic conditions, storage and transportation methodologies of traders, and soil type etc. Harvesting stage variations of the vegetable samples also influence it. The results also demonstrate the potential value of vegetables as a dietary source of antioxidants (Ascorbic acid) [4-9].

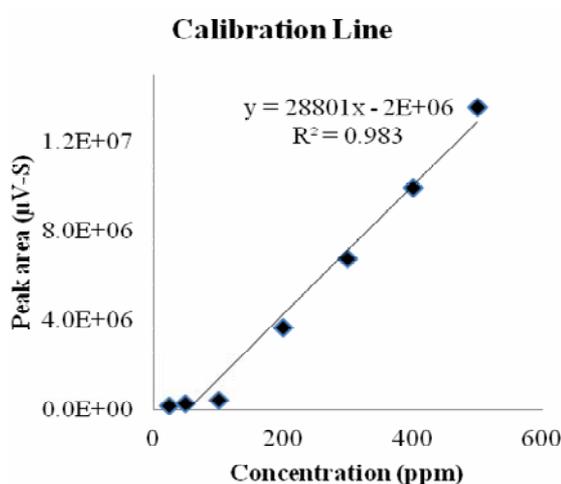


Fig. 2: Calibration curve for standard solutions of ascorbic acid.

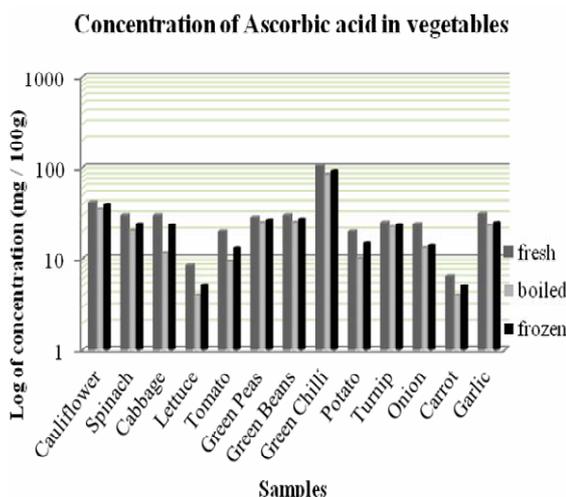


Fig. 3: Comparative concentration of vegetables in their fresh, boiled and frozen states.

Table-1: Concentration of Ascorbic acid in various vegetables.

Class	Samples	Concentration (mg / 100g) ± S.D*		
		Fresh	Boiled	Frozen
Leafy/stem	Cauliflower	42.0 ± 0.021	35.0 ± 0.031	39.0 ± 0.031
	Spinach	30.0 ± 0.032	20.3 ± 0.027	24.1 ± 0.011
	Cabbage	30.0 ± 0.027	11.6 ± 0.011	23.4 ± 0.027
	Lettuce	8.5 ± 0.045	4.0 ± 0.012	5.1 ± 0.023
	Tomato	20.0 ± 0.031	9.3 ± 0.022	13.0 ± 0.031
Green	Green Peas	28.5 ± 0.027	25.1 ± 0.026	26.5 ± 0.027
	Green Beans	30.0 ± 0.022	25.2 ± 0.023	27.0 ± 0.025
	Green Chilli	105.0 ± 0.032	85.0 ± 0.032	92.0 ± 0.037
Root	Potato	20.0 ± 0.011	10.0 ± 0.016	15.0 ± 0.019
	Turnip	25.3 ± 0.017	22.5 ± 0.018	23.6 ± 0.016
	Onion	24.3 ± 0.012	13.1 ± 0.034	14.1 ± 0.012
	Carrot	6.5 ± 0.023	4.0 ± 0.033	5.0 ± 0.029
	Garlic	31 ± 0.041	23.0 ± 0.019	25.0 ± 0.031

The present study also pointed out the fact that some preventive measures should be taken to avoid nutrient losses like: short storage interval; peeling and slicing vegetables near to serving time and in bigger slices to reduce exposure to atmospheric oxygen. Vegetable hygiene should be ordered enough to remove dust and contamination. However, washing time should be monitored to avoid unnecessary contact with water and leaching losses of nutrients [13-16].

## Experimental

### Chemicals and Reagents

Meta Phosphoric Acid (MPA), L-Ascorbic Acid and Methanol (HPLC grade) were purchased from Fisher scientific.

### Sample Collection:

These vegetables were selected on the basis of availability and their popular consumption in Pakistan like: cabbage (*Brassica oleracea*), tomato (*Solanum lycopersicum*), green chilli (*Capsicum annum*), carrot (*Daucus carota L.*), turnip (*Brassica rapa*), potato (*Solanum tuberosum*) spinach (*Spinacea oleracea*), onion (*Allium cepa*), garlic (*Allium sativum*), lettuce (*Lactuca sativa L.*), green peas (*Pisum sativum*), green beans (*Phaseolus vulgaris, L*) and cauliflower (*Brassica oleracea, var. botrytis L.*). They were purchased from local market.

### HPLC Specifications

The HPLC system used was a Perkin Elmer 200 Series with same specifications as described earlier [17].

### Preparation of Standard Solutions

For preparing 1000 ppm stock solution of Ascorbic acid, 0.1g was dissolved in 100 mL of

methanol. Then standard solutions of variable concentration (25, 50, 100, 200, 300, 400 and 500 ppm) were prepared by proper dilution of this stock solution with methanol.

### Sample Preparation

The fresh healthy vegetables were washed under tap water. After drying, 4.0 g of edible portion of each vegetable sample was sliced separately into small pieces and crushed with the help of pestle and mortar. Then, 20 mL of 5% of m-phosphoric acid was added into them and grinding was continued until smooth slurry was formed. All of them were centrifuged at 4000 rpm for 20 minutes, at room temperature. All the clear supernatant liquids were filtered through 0.45- $\mu$ m filters membrane and 5 mL of these filtrates was diluted up to 25 mL by methanol. These samples were labeled and stored in dark cool place to inhibit oxidation. Before injection, the samples were again filtered through 0.45- $\mu$ m filter membrane. For the preparation of sample for boiled state of vegetable, 4g of sample was weighed and boiled for 30 minutes and all other steps were performed in the similar fashion as for fresh sample. Same procedure was followed for preparing sample for frozen state of vegetable after keeping them in freezer for two days [16-18].

### Quantification and Statistical Analysis of Ascorbic Acid Contents

Ascorbic acid content of vegetables was measured by reversed phase HPLC with ultraviolet (UV) detector working at 238 nm ( $\lambda_{max}$  of Ascorbic acid). For calibration of HPLC system, 20  $\mu$ L of standards ranging from 25-500 ppm were injected into a reversed-phase C18 column (25cm  $\times$  4.6 mm, 5 mm i.d.). Methanol was used as mobile phase during analysis with a flow rate of 1.0 mL min<sup>-1</sup>. Same procedure was followed with sample solutions. All the solutions were run in triplicate and average values were used in further calculations. Standard

calibration line was obtained by plotting average peak area versus concentration of standard solutions in Microsoft Excel-2007. Ascorbic acid recognition in the vegetable samples was done by comparing standard and sample retention times under the analogous conditions i.e. 3.04 min. Column purging was done with methanol for removing impurities and residues left behind in HPLC system. Ascorbic acid concentration was determined from regression analysis of calibration curve and calculating the dilutions performed [6, 17].

### Conclusion

The severe variations in temperature during boiling and cooking considerably reduce the level of vitamins present in vegetables. This is proved in the following study by comparative analysis of Ascorbic acid contents of various vegetables in fresh, boiled and frozen form by adopting reversed phase HPLC methodology. It is observed that the concentration of Ascorbic acid in fresh vegetables vary between 6.5 - 105 mg / 100 g. This is higher than the frozen range: 5-92 mg / 100 g; while the boiled state of vegetables contains 4 - 85 mg / 100g. Comparative analysis has shown that boiling severely affects the Ascorbic acid contents of vegetables, especially in case of leafy vegetables. This study suggests that we should consume vegetables in fresh form in food to get maximum nutrition; with regard of Ascorbic acid (Vitamin C) especially.

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