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**Effect of Cold Storage on the Vitamin C Contents of Fruits and
Vegetables**

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ABSTRACT: The vitamin C contents of 5 fruits and 7 vegetables was determined as a whole and diced during cold storage. Vitamin C contents were measured by HPLC. Results showed a decrease in vitamin C contents during 15 days refrigeration (7°C) as well as freezing at -20 °C. It was found that fruits are more stable than vegetables as it was also observed that rate of degradation of vitamin C was higher in vegetables as compared to fruits either during freezing or refrigeration. During 15 days freezing fruits showed a decrease of 41.05 - 51.44% where as this loss augmented to 54.12 – 89.10% in vegetables. In addition to this it was also observed that fruits and vegetables which have peels are less perishable to vitamin C degradation as ratio of degradation of vitamin C in all fruits studied and potatoes was not more than 51.44%. In fruits apples were more susceptible and in vegetable potatoes are more stable to vitamin C degradation. The information is of interest to the general public as well as to the food industries because basically we eat fruits and vegetables as a major source of vitamins/antioxidants, mineral and dietary fiber and if we use stored fruits and vegetables we will not get the health benefits.

Keywords: fruits/vegetables, vitamin C, refrigeration, freezing

Introduction

Vitamin C is a water soluble vitamin, essential for the synthesis of collagen and inter-cellular material. Several substances have vitamin C activity, notably ascorbic acid and its sodium and calcium salt. A daily dietary intake of about 30-100mg of vitamin C has been recommended for adults (Sweetman, 2007). Vitamin C functions in a number of biochemical reactions, mostly involving oxidation. Thus it is required for or facilitate the conversion of certain proline residue in collagen to hydroxylproline in the course of collagen synthesis (Myllyda and others 1978), the oxidation of lysine side chains in proteins to provide hydroxytrimethyllysine for carnitine synthesis (Hulse and others 1978), the synthesis of steroids by the adrenal cortex (Deana and others 1975), the conversion of folic acid to folinic acid etc. (Stokes and others 1975).

Humans are unable to form their own vitamin C, so a dietary source is necessary. Most dietary vitamin C obtained from fruit and vegetable sources, only small amount present in milk and animal tissues. Relatively rich source includes rose hips, black current, citrus fruits, leafy vegetables, tomatoes, potatoes, green and red peppers. Vitamin C is readily destroyed during cooking processes. Considerable losses may also occur during storage (Sweetman, 2007). Studies indicated that vitamin C is by far the least stable nutrient during processing due to its sensitivity to oxidation and leaching into water soluble media during processing, storage and cooking of fresh, frozen and canned fruits and vegetables (Franke and others 2004, Lathrop and Leung 1980). In past various studies have determined the effect of processing and of storage on the vitamin C contents in a variety of fruit products

(de-Dios and Alvarado 1991, Lima and others 1999, Gil others, 2002), losses in vitamin C after six days at 5°C were 5- 25 % in various fruits and vegetables (Gil and others 2006). Vitamin C contents of the lemon juice were reduced to about 36% of the initial value over a period of 12 weeks storage at various storage conditions (Abbassi and Niakousari). No considerable losses in vitamin C contents of green beans were occurred during the blanching and freezing processes (Martins and Silva 2003).

There has been an increasing demand for fresh cut fruits and vegetable because of their convenience as ready to eat products, along with their nutritional values requisite for healthy living (Block and others 1992, Liu and others 2000, Martin and others 2002). A major benefit from a higher intake of fruits and vegetables may be the increased consumption of vitamins and their antioxidant activity may reduce the risk of cancer, heart diseases as well as prevent degenerative diseases (Doll 1990; Rimm and others 1996; Tee 1992; Grassmann and others 2002; Gaziano and Hennekens 1993). Among all other valuable nutrients like phenols, carotenoids and vitamin etc. vitamin C is most perishable (Gil and others 2006). Degradation of vitamin C proceed both aerobic and anaerobic pathways and depends upon many factors such as oxygen, heat, light, storage temperature and time (Sedas and others 1994; Uddin and others 2002; Nunes and others 1998). In addition fruits and vegetables are typically over 90% water and once they harvested begin to undergo higher rates of respiration, resulting in moisture loss and quality deterioration. Harvesting separates the fruits and vegetables from its source of calories and energy required for above mentioned activities utilized from fruits and vegetables resulting in decreasing in nutritional values of fruits and vegetables. Storage

and processing technologies have been utilized for centuries to transform these fruits and vegetables into safe, delicious and stable products (Fennema 1982). Refrigeration slow down degradation rate and allow for long shelf life of fruits and vegetables (Rickman and others 2007). The primary objective of the present study was to determine the vitamin C contents of various fruits and vegetables (whole and diced) at various storage temperatures. The study comprises of two parts, in the first part whole and diced fruits and vegetables were stored at various temperatures for a definite period of time and in 2nd part the diced fruits and vegetables were stored at fixed temperature and vitamin C contents were determined after one week interval and study was prolonged to three weeks.

Materials and Methods

Materials

Fruits and vegetables including mangoes, grapefruit, papaya, lemon, apple, spinach, potato, bitter ground, capsicum and broccoli were purchased from the local market of Lahore city.

Chemicals and reagents

Ascorbic acid (BDH, England), 1-octanesulphonic acid sodium salt (Fisher Scientific, UK), Citric acid (Scharlau, Spain), di- Sodium EDTA, NaF, HPLC grade Methanol (Fisher Scientific, UK), Tri-ethanol amine (Merck, Germany), Glacial Acetic Acid (Merck, Germany)

Preparation of samples

All fruits and vegetables under study were grouped into four lots and stored at various temperatures and vitamin C was analyzed for whole fruits and vegetables and fresh diced at room temperature, at refrigeration (7°C) and at freezing temperature (-20 °C). One lot was immediately analyzed for vitamin C contents as a whole and diced and the other lots were stored as a whole and diced on the above mentioned temperatures. In the 2nd part of the study pre-weighed diced fruits and vegetables were kept in a refrigerator at 7°C and vitamin C contents were analyzed after a fixed interval of one week and study was prolonged to three weeks.

Extraction and analysis

Procedure used was based on the method of (Zapata and Dufour 1992) with some changes. 10 g of sample was taken and 10 ml of extraction solution (0.1M Citric acid, 0.05% EDTA di-sodium salt, 5% methanol and 4 mM NaF) was added. The contents were homogenized on an ice bath and filtered through Whatmann I filter paper. The filtrate was collected in a centrifuge tube and centrifuged on 10,000 rpm for 10 min at 3 °C. The pH of the filtrate was adjusted to 2.35 - 2.40 and passed through an activated Sep-Pak C₁₈ cartridge and then filtered through a 0.22µm filter paper. After 35 min analysis were performed with HPLC (Perkin Elmer) with Total Chrom Workstation (TCW) software and Perkin Elmer Series 200 UV/Vis detector, a reverse phase C₁₈ Nucleosil column (25cm x 4.6mm ID, 5µm particles), flow rate was 1ml/min with a mobile phase prepared by dissolving 1.1g of 1-octanesulphonic acid sodium salt in 800ml of water and by adding 24 ml of glacial acetic acid, 5 ml of tri-ethanolamine and 150 ml of methanol and total volume was made up to 1000 ml with distilled water. The pH of the mobile phase was adjusted to 3.61 ± 0.1 with acetic acid or tri-ethanol amine. The detection was made at 348 nm for dehydroascorbic acid (DHAA) and 261 for ascorbic acid (AA). The vitamin C contents (DHAA +AA) were expressed in mg/100g of fresh weight.

Results and Discussion

Vitamin C contents of fruits and vegetables remained stable during storage either in the form of ascorbic acid or de-hydroascorbic acid for a particular period of time on specific temperature. Beyond that time and temperature vitamin C contents drastically decreased though the ratio of degradation varied in various fruits and vegetables.

Effect of various temperatures on the vitamin C contents

When various fruits and vegetables were frozen at -20°C for fifteen days, diced and whole it was observed that vitamin C contents (AA+DHAA) decreased up to 90% during 15 days storage in vegetables while this loss was limited to 51 % in the fruits. As Table. 1 indicated broccoli showed maximum loss after fifteen days storage at -20°C for diced and whole with slight variation. Minimum vitamin C loss was observed in grape fruit which showed 41.05% loss in vitamin C contents during above mentioned storage conditions. A very contradictory situation was observed about the refrigerated samples. It was observed that vitamin C increased in refrigerated fruits samples and a tremendous increase was observed in diced samples due to the moisture losses. As Table. 2 indicated, maximum increase was observed in vitamin C contents of diced lemons which showed an increase of 64.37%, after two days refrigeration and minimum increase was observed in papaya which showed an increase of 4.64 % in vitamin C contents. Similarly fruits stored without dicing either showed an increase or maintained vitamin C contents. On the other hand vegetables stored at 7°C for two days showed a decrease in vitamin C contents. Capsicum showed

maximum loss in diced samples, which was 36.8 % and minimum losses was observed in spinach which showed a decrease of 2% in its vitamin C contents. Based on these observations 2nd part of the studies was conducted.

Effect of storage time on the vitamin C contents

When pre-weighed diced samples were stored at 7 °C for two weeks it was observed that vitamin C contents of both fruits and vegetables reduced, though the ratio of degradation was higher in vegetables as compared to fruits as fig. 1 indicated that in case of fruits maximum loss of vitamin C reached up to 50.62 % for apple after one week storage and in vegetables this loss amplified to 77.04 %, 75.20 % and 74.76 % in broccoli, capsicum and bitter ground respectively. During 1st week of study diced samples lost about 30-77 % of vitamin C and this loss increased to 98.04 % during 2nd week of study. Only traces of vitamin C remained during 3rd week of study Fig. 1.

Conclusion

Due to much losses of moisture contents of stored fruits and vegetables vitamin C contents increased when analyzed on the basis of current weight, but in case of pre-weighed samples a decrease in vitamin C contents happened. It was also noticed that the fruits and vegetables with removable peels are less susceptible to vitamin C degradation. Further studies are needed to confirm the role of peels in the stability of fruits and vegetables

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Table 1- Effect of freezing at -7°C for 15 days on the vitamin C contents of the whole and diced fruits and vegetables

Fruits/Vegetables	Vitamin C contents of fresh Fruits/Vegetables				Vitamin C contents of samples stored at -7°C			
	Whole (mg/100g)	RSD* (%)	Diced (mg/100g)	RSD* (%)	whole (mg/100g)	Loss (%)	Diced (mg/100g)	Loss (%)
Mango	12.30	0.98	13.19	0.62	5.97	51.44	8.32	32.30
Grape fruit	23.88	2.54	22.95	0.81	14.08	41.05	13.8	42.2
Papaya	37.08	0.67	36.73	1.22	19.55	47.29	19.83	46.52
Apple	8.72	0.42	8.03	0.56	4.25	51.26	4.20	51.83
Tomato	41.06	1.02	42.35	3.02	10.62	74.12	11.34	72.38
Lemon	49.34	1.82	49.86	1.80	11.96	75.76	10.37	78.98
Bitter ground	87.16	0.38	85.94	0.29	26.88	69.16	20.34	76.66
Broccoli	47.29	2.37	46.73	0.86	5.15	89.10	5.12	89.17
Capsicum	31.25	1.04	30.72	1.09	5.16	83.47	5.10	83.68
Potato	5.82	1.15	5.79	2.11	2.67	54.12	2.85	51.03
Spinach	9.55	0.67	9.60	1.69	3.11	67.43	2.95	69.10
Cabbage	8.55	0.49	8.48	2.54	1.93	77.42	1.95	77.19

*Every reading is an average of three independent measurements

Table 2- Vitamin C contents of fruits and vegetables refrigerated at 7°C for two days

Fruits/Vegetables	Vitamin C contents					
	Whole (mg/100g)	RSD* (%)	% vitamin C increase/decrease as compared to fresh**	Diced (mg/100g)	RSD* (%)	% vitamin C increase/decrease as compared to fresh**
Mango	14.40	2.13	15.85 I	14.25	1.54	15.85 I
Grape fruit	24.59	1.46	3.0 I	27.79	0.84	16.39 I
Papaya	35.26	0.57	4.9 I	38.807	1.21	4.64 I
Apple	8.85	1.58	1.49 I	9.35	0.43	7.22 I
Tomato	37.49	1.05	8.69 d	31.16	0.94	24.11 d
Lemon	79.65	0.76	61.0 I	81.10	1.02	64.37 I
Bitter ground	70.76	1.69	51.05 d	19.75	0.86	16.46 d
Broccoli	31.33	0.76	33.74 d	46.73	1.25	1.2 d
Capsicum	15.29	2.02	51.05 d	19.75	0.73	36.8 d
Potato	5.85	0.56	0	5.88	0.92	1.81 I
Spinach	9.05	0.41	5.2 d	9.35	1.61	2.0 d
Cabbage	8.79	2.87	3.4 d	8.05	0.48	8.41 d

*Every reading is an average of three independent measurements

** I --- Increase & d --- decrease

grape										
fruit	papaya	apple	tomato	lemon	bitterground	broccoli	capsicum	potato	spinach	cabbage
100	100	100	100	100	100	100	100	100	100	100
72	59.25	49.38	26.8	65.82	25.24	22.96	24.8	95.28	39.54	40.01
67.39	29.56	23.69	11.23	59.2	2.5	18.3	1.96	80.25	6.01	8.31
8.79	4.8	2.3	0.89	16.2	0.5	0.97	0.38	10.1	3.1	2.5