IJFANS
International Journal of Food And Nutritional Sciences

Volume 7, Issue 2, April 2018, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

IJFANS

International Journal of Food and Nutritional Science

e-ISSN 2320 -7876 www.ijfans.com
Vol. 7, No. 2, April 2018

Research Paper ***Open Access***

ANALYSIS OF VITAMIN C CONTENTS OF SOME TROPICAL FRUITS AND VEGETABLES: THEIR DIETARY RELEVANCE IN CANCER

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Received on: 25th December, 2017 Accepted on: 8th February, 2018

To determine the concentrations of vitamin C (ascorbic acid) in some tropical fruits and vegetables, fresh fruits such as grapefruit, mango, kiwi, lemon, orange, pineapple and the vegetables included broccoli, bell pepper (red), bell pepper (green), tomatoes and green chilies were purchased from Danoube in Jazan and brought to the biology laboratory of Faculty of Science and Arts in Farasan. The fruits were thoroughly washed with normal tap water; juice from each sample was squeezed out manually without applying heat or any solvent, and filtered with a muslin cloth to get rid of pulp and seeds. Collected samples of vegetables were also treated the same way to get required amount of juice for analysis. Ascorbic acid content was analyzed by iodometric titration method which is a simple and precise method. Titrimetric analysis revealed that to derive maximum benefits for cancer patients' kiwi, lemon, orange, red and green bell peppers, and broccoli should be served as high dose vitamin C dietary supplement to stress cancer cells as it has also been reported in various previous studies that high doses of vitamin C are deadly for cancer cells. Among the vegetables analyzed, bell peppers should be preferred over other vegetables as this is generally consumed raw and thus minimizes the chance of destruction and leaching of vitamin C by cooking and washing.

Keywords: Vitamin C, Diet, Cancer, Titrimetric analysis, Fruits, Vegetables

INTRODUCTION

Vitamin C (ascorbic acid, ascorbate) is a basic compound that is of great importance with its role in various enzymatic reactions including the synthesis of collagen, as well as with its redox functions (Ahmet Unlu *et al.*, 2016). Vitamin C is also known as ascorbic acid or L-ascorbic acid or antiscorbutic vitamin because deficiency of vitamin C causes scurvy which results in dermal hemorrhages of skin, inefficient and prolonged wound healing, edema and weakness. In ancient times, scurvy was common among the sailors who go on long vouyage in the sea and thus do not get the dietary source of vitamin C as long as they travel in the sea (Wedad and Fatma, 2017). Despite being protective against scurvy, vitamin C has multifunctional roles which include participation in collagen and carnitine syntheses, promotion of iron absorption and the more recently discovered participation in noradrenaline synthesis, inactivation of free radical chain reactions and many more. Researchers have also suggested that vitamin C-rich foods play a protective role against development of cancer and plasma concentrations of ascorbate have been shown to be inversely associated with cancer risk (Frömberg *et al.*, 2011; and Ahmet unlu *et al.*, 2016). Given the many extra-antiscorbutic functions of the vitamin, the Recommended Dietary Allowances (RDA) should not just prevent deficiency disease but should aim at providing sufficient amounts for all vitamin C-dependent functions to operate at full capacity. Vitamin C can be produced by most animals

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and plants including gold fish to some extent from D-glucose and D-galactose, whereas humans are unable to synthesize this vitamin due to the lack of L-gulonolactone oxidase enzyme; and hence, its dietary supplementation becomes necessary (Naidu, 2003).

The World Health Organization (WHO) recommends a daily intake of 45 mg vitamin C per day for healthy adults (WHO, 2004). Vitamin C is believed to have many beneficial effects, from strengthening the immune system and making the common cold go away faster to being one of the Nature's best anticancer compounds. Uetaki *et al.* (2015) have published their findings on Intravenous administration of high-dose vitamin C that has recently attracted attention as a cancer therapy. In their study they proved that high-dose vitamin C induces pro-oxidant effects and hence selectively kills cancer cells. However, the anticancer mechanisms of vitamin C are not fully understood. Yun*et al.* (2015) present the evidence that high levels of vitamin C are indeed deadly to human cancer cells (Chemistry and Biology, 2015). Now researchers say there may be a way to make those treatments work even better. In a study published in*Cancer Cell*, scientists say that giving people high doses of vitamin C during treatment may weaken cancer cells and make them more vulnerable to the effects of chemotherapy and radiation (Park, 2015). In view of above, present study was conducted to ascertain the vitamin C contents in some tropical fruits and vegetable so that they could be used as high dose vitamin C food supplements by the dieticians and clinicians who are dealing with the cancer patients.

Vitamin C content of the collected fruit and vegetable and samples was analyzed using redox-titration method as this is simple and authentic method that determines the vitamin C concentration in a sample solution by a redox titration involving iodine solution. This approach has also been utilized by various other workers, some with slight modification and some with almost the same (Okiei *et al.*, 2009; Katz, 2013; Tareen *et al.*, 2013; and Wedad and Fatma, 2017).

Since there are reports available in literatures that that high levels of vitamin C is indeed deadly to cancer cells (Park, 2013), present study was conducted to estimate the vitamin C contents of a variety of vegetables and fruits so that those with high levels of vitamin C may be recommended in the diets for cancer patients for immune boost up. Data generated will be of high use for clinicians in general and dieticians in particular.

Information generated in this study will be of high use in clinical nutrition.

MATERIALS AND METHODS

Procurement of the Sample

Fruits and vegetable samples as have been listed in Table 1, were purchased from the supermarket Danaoube in Jazan and brought to the Biology Laboratory of Faculty of Science and Arts in Farasan. The choice of the fruits and vegetables were based on their availability, cost-effectiveness and demand or acceptability in the area.

Sample Preparation

The collected fruits and vegetables were washed thoroughly with normal tap water and the juice was extracted by manual squeezing. The juice samples thus squeezed were then filtered using a muslin cloth to remove pulp and seeds, and transferred in already labeled test-tubes. The study was carried out in Biology laboratory of Faculty of Science and Art in Farasan. All samples were fresh and the titration reaction was done within 15 minutes of juice extraction.

Preparation of Titrimetric Reagents

Iodine Solution

An iodine solution was freshly prepared to titrate the ascorbic acid. A 1:10 solution was made by diluting 30 mL of

Table 1: List of the Collected Fresh Fruits and Vegetables Samples

Fresh Fruits	Scientific Names
Mango	*Mangifera indica*
Lemon	*Citrus limon*
Orange	*Citrus nobilis*
Kiwi	*Actinidia species*
Pineapple	*Ananas comosus*
Grapefruit	*Citrus paradisi*
Vegetables	**Scientific Names**
Bell Pepper (red)	*Capsicum species*
Bell Pepper (green)	*Capsicum species*
Broccoli	*Brassica oleracea*
Green chilies	*Capsicum annuum*

Lugol's Iodine with 270 mL of distilled water. This solution was stored in amber glass bottles.

Starch Indicator Solution

To indicate the titration of ascorbic acid, a 0.5% starch solution was made by dissolving 1 g of soluble starch into 200 mL of boiling distilled water on a water bath.

Vitamin C Standard Solution

To measure the mL of iodine needed to titrate a standard amount of ascorbic acid, a vitamin C standard solution was prepared. A pack of vitamin C tablet was purchased from a local Pharmacy. From this, 500 mg was carefully weighed on an electronic balance (Radwag, 0.1 g) and was dissolved in a known volume of distilled water. Once the tablet is completely dissolved, the volume is top up to a total volume of 500 mL using distilled water. This resulted in a 1 mg/mL vitamin C solution. Reagent grade distilled water obtained by GFL 2004 Water Distillation Unit (Made in Germany) was used for all preparations.

Analysis of Vitamin C Content of the Juice Samples

Vitamin C content of the packed and fresh fruit juice samples was determined by iodometric titration method as has been done by Wedad and Fatma Sayed (2017). Briefly, in a 5 mL juice sample, 2 mL of starch indicator solution was added. The resulting solution was then titrated with the dropwise addition of standard iodine solution near the end point. Following reaction occurs during this:

$$\text{Ascorbic acid } (C_6H_8O_6) + I_2 \rightarrow 2I + \text{Dehydroascorbic acid } (C_6H_6O_6)$$

$$\text{(Reduced form)} \rightarrow \text{(Oxidized form)}$$

After the entire the ascorbic acid was titrated, the iodine reacted with starch to create a dark blue color (Figure 2) which was read as the end point of the titration and at this moment the reading of iodine solution in burette was noted down.

Calculations

The mL of iodine solution used in the titration of standard vitamin C solution and that of the juice samples are then noted down. The vitamin C contents of the samples were calculated in terms of the mL of titrant (standard iodine solution) used for standard vitamin C solution and for the juice samples. The amount of vitamin C is easily calculated for every unknown juice samples as per following equation:

$$\text{Vitamin } C_1 = \text{Iodine}_2/\text{Iodine}_1 \text{ x Vitamin } C_1$$

where, Iodine_1 is the average amount of iodine (in mL) used to titrate the vitamin C standard solution; Vitamin C_1 is the amount of vitamin C in the standard solution (in mg).

Iodine_2 is the average amount of iodine (in mL) needed to titrate the orange juice sample. Vitamin C_2 is the amount of vitamin C in the orange juice sample (in mg).

Statistical Analyses

All the analyses were done in triplicates (n = 3). Statistical evaluation and graphical representation of the data were done using MS Excel (Office, 2008).

RESULTS

Freshly squeezed fruit and vegetable samples exhibited significant differences in their vitamin C content. The results summarized in Table 3 clearly indicate that each juice sample has a different titre value to attain its end point. This is due to the differences of the amount of vitamin C in different fruits. The volume of iodine used to titrate 5 mg of vitamin C standard solution was 8.5±0.1 mL. The volume of iodine required to titrate the ascorbic acid in various fruits and vegetable samples ranged between 0.30±0.01 mL to 6.90±0.03 mL, respectively (Table 2). The juice with lower amount of vitamin C required less of the titrant to attain its end point whereas the juices with higher amounts of vitamin C required more of the titrant to attain the permanent end point-colour. This is due to the fact that the colour changes when all the vitamin C of the sample has been completely reduced and only starch is in the solution which combining with the iodine from the titrant produces purplish-blue colour. All the analyses were done in triplicates (n = 3). The titrimetric analysis revealed that between fruits and vegetables examined, highest vitamin C content was found in the kiwi fruit (81.8 mg/100 mL), followed by the lemon (58.6 mg/100 mL), orange (56.3 mg/100 mL), pineapple (51.3 mg/100 mL), grapefruit (42.4 mg/100 mL) and mango (41.5 mg/100 mL). Among the vegetable samples analysed highest vitamin C content was recorded for bell pepper (red) (59.3 mg/100 mL), broccoli (58.4 mg/100 mL), bell pepper green (57.9 mg/100 mL), tomatoes (13 mg/100 mL) and green chilies (3 mg/100 mL). The lowest vitamin C content was recorded for mango and chilies among the fruits and vegetables analyzed. Data have been graphically presented as Figure 1. Figure 2 exhibits example of the orange juice before and after attaining the end point with iodine solution.

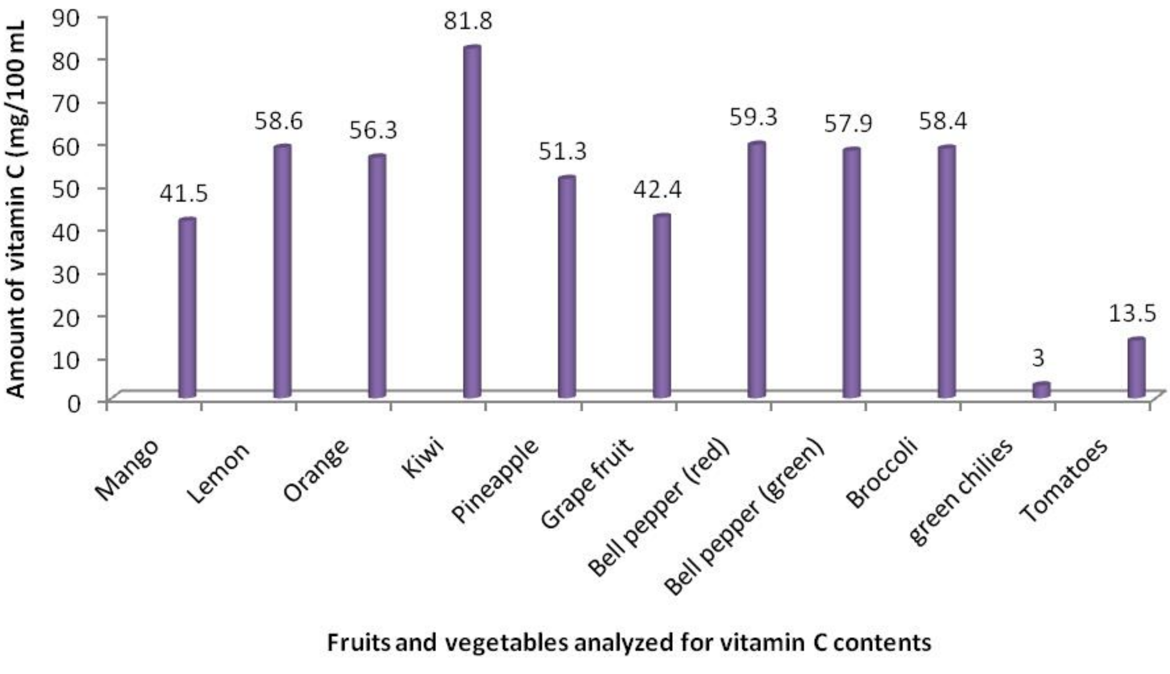
DISCUSSIONS

Risk of chronic diseases can be reduced by frequent

Table 2: Amount of Vitamin C in the Collected Fruits and Vegetable Samples

Samples	In. Reading of Burette	Fn Reading of Burette[1]	Volumes of Iodine Solution Used[1]	mg of Vitamin C/100 mL
Mango	25.00	21.50±0.12	3.50±0.03	41.5±0.11
Lemon	25.00	20.10±0.05	4.90±0.14	58.60±0.23
Orange	25.00	20.20±0.13	4.80±0.11	56.30±0.05
Kiwi	25.00	18.10±0.07	6.90±0.03	81.80±0.17
Pineapple	25.00	20.70±0.02	4.36±0.01	51.3±0.09
Grapefruit	25.00	21.40±0.05	3.60±0.05	42.4±0.03
Vegtables				
Bell pepper (green)	25.00	20.1±0.02	4.92±0.04	57.90±0.07
Bell Pepper (red)	25.00	19.9±0.11	5.10±0.11	59.30±0.01
Broccoli	25.00	20.10±0.07	4.90±0.21	58.40±0.03
Green chillies	25.00	24.71±0.03	0.30±0.12	03.00±0.14
Tomattoes	25.00	23.80±0.14	1.20±0.01	13.50±0.05

Figure 1: Analyzed Values of Vitamin C Contents of Collected Fruits and Vegetable Samples



consumption of fruits and vegetables (Rafiq *et al.*, 2016). The biological importance of vitamin C is that it plays a cofactor role, as a reducing agent, in various enzymatic reactions. Due to its low redox potential, it is likely to react with almost all other oxidized free radicals. Therefore, is used as an antioxidant. Vitamin C is also a compound that

Figure 2: Collected Samples and Colour of Juice Sample Before and After Titration with I2 Solution

plays an important role in collagen synthesis (Naidu, 2003), responsible in the synthesis of carnitine, various neurotransmitters as well as in tyrosine and microsomal metabolism (Gropper *et al.,* 2005). Vitamin C cannot be synthesized by the body cells and it is not stored in the body. It is therefore important to include plenty of vitamin C-containing foods in daily diet. Vitamin C toxicity is very rare, because the body cannot store the vitamin however, amounts greater than 2000 mg/day are not recommended because such high doses can lead to stomach upset and diarrhea. Also, the WHO (2004) also recommends 45 mg vitamin C/day for a healthy adult.

Because it is available in high concentrations in the immune cells, and is rapidly consumed in the body in case of any infection, it is thought to be associated with the immune system, as well. However, its mechanism has yet to be clearly elucidated (Preedy *et al.,* 2010). Some researchers assert that high doses of vitamin C can be used in the treatment of cancer.

There is increasing evidence that vitamin C (ascorbate) is selectively toxic to some types of tumor cells, functioning as a pro-oxidant (Aboul-Enein *et al.,* 1999; Naidu, 2003; and WHO, 2004). Studies have established that the growth of leukemic progenitor cells from patients with Acute Myeloid Leukemia (AML) and myelodysplastic syndromes (MDS) can be significantly modulated by vitamin C (Padayatty *et al.,* 2010). Intravenous administration of Sodium 5,6-Benzylidene-l-Ascorbate (SBA) to inoperable cancer patients induced a significant reduction in tumor volume without any adverse side effects (Sakagami *et al.,* 1991). Furthermore, recent clinical studies have reported that manipulation of vitamin C levels *in vivo* can result in clinical benefit for patients with AML and solid tumors (Park *et al.,* 1999).

Vitamin C is absorbed by cells where it breaks down into hydrogen peroxide, which is capable of damaging body tissue and DNA. Cancer cells are much less efficient in removing hydrogen peroxide than normal cells, as has been stated by Professor Garry Buettner, a member of the Holden Comprehensive Cancer Centre at the University of Iowa.

Thus, cancer cells are much more prone to damage and death from a high amount of hydrogen peroxide. Very high levels of vitamin C used in our clinical trials do not affect normal tissue, but can be damaging to tumour tissue. Normal cells have several ways to remove hydrogen peroxide while cancer cells are defenseless. One way normal cells destroy hydrogen peroxide is by producing a chemical capable of breaking down other molecules called catalase.

The researchers discovered that cancer cells, which produce low amounts of catalase, were more susceptible to damage and death when they were exposed to high amounts of vitamin C than normal cells. This suggests that the vast majority of cancer cells may lack the biochemical machinery needed to detoxify higher fluxes of H_2O_2 efficiently. While in general, the levels of catalase are low in cancer cells, catalase activity appears to vary greatly across different cancer cell lines (Chen *et al.,* 2005; and Verrax and Calderon, 2009).

Doskey *et al.* (2016) have also suggested that ascorbate delivered at pharmacological concentrations has shown selective toxicity to several different tumor cell types. It has also been suggested by Allen *et al.* (2014) that feeding ketogenic diets that are high in fats and low in glucose and other carbohydrates, would selectively cause metabolic oxidative stress in cancer versus normal cells. Increased metabolic oxidative stress in cancer cells would in turn be predicted to selectively sensitize cancer cells to conventional radiation and chemotherapies. The main purpose of kinetogenic diet is to stress the cancer cells and thus to make them weak. In the same line, the vitamin C rich diets can also be used to sensitize cancer cells and thus make them weaker. Gerster (1987) using the kinetic models have shown that the body pool is saturated with a daily intake of 100 mg vitamin C in non-smokers and 140 mg in smokers, amounts that may be regarded as optimal RDA values. Certain disease states may be accompanied by still higher vitamin C requirements but the exact amounts however, are not yet known. The analyzed vegetables and fruits have high vitamin C contents and therefore, can be used as a high vitamin C supplement for immune boost up and to stress the cancer cells. Therefore, the results of this study are of high significance for those admitted in hospitals for cancer therapy. The outcomes of this study are useful in reducing the duration of hospital stay for cancer patients and making the chemotherapy more effective in shorter durations.

CONCLUSION

Results of vitamin C analyses clearly indicates that kiwi, lemon, oranges, pineapples and red bell pepper, broccolis and green bell pepper are the high vitamin C fruits and vegetables and should be included in the diets as their ascorbic acid content is above that recommended daily intake for this vitamin (45 mg/day). The above mentioned fruits and the vegetables such as bell peppers should be considered over other vegetables as most of these analysed vegetables are cooked before served and cooking the ascorbic acid destroys. Therefore, the study further recommends that fruits should be preferred over vegetables as they are rich sources for this vitamin and one can get an intact vitamin from these.

ACKNOWLEDGMENT

The author is highly thankful to the Dean, Dr. Afaf Babeir and the vice-Dean of Faculty of Science and Arts in Farasan, Jazan University, KSA for providing necessary laboratory facilities for this study.

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