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Effects of Cooking on Content of Vitamin C in Green Leafy Vegetables

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Abstract: This study was designed to determine the effect of processing on the Vitamin C content of five Indian green leafy vegetables namely, Spinach (*Spinacia oleracea*), Methi (*Trigonella foenum-graecum*), Lal maath (*Amaranthus cruentus*), Chauli (*Thotakur avepudu*) and Bhathua (*Chenopodium album*). The Vitamin C content of the lemon sample was also estimated using DNPH colorimetric assay, DCPIP, Iodometry and N-Bromosuccinimide titration methods. Among the methods used for Vitamin C estimation of lemon, N-Bromosuccinimide method was found to be most accurate and sensitive. Hence, it was further used to estimate the Vitamin C content in the above mentioned vegetables. Processing methods employed were boiling, blanching and microwave heating the vegetables for particular time interval. The vegetable extracts were subjected to 0.01% N-Bromosuccinimide titration to estimate their Vitamin C content before and after the treatment. Results revealed that maximum reduction in Vitamin C activity of vegetable samples were observed with microwave heating followed by boiling and blanching. Vitamin C content of boiled vegetable samples showed 40-50% reduction in their vitamin C activity, microwave heated samples resulted into 50-75% reduction and blanching lead to minimum reduction i.e. 20-35% as compared to unprocessed vegetable samples. Vitamin C is easily destroyed by excessive heat and water as well as exposure to air. For retention of Vitamin C in cooked foods, it is recommended that foods containing Vitamin C be cooked as fast as possible with less heat and small amount of water.
Keywords: Vitamin C, Lemon, Green leafy vegetables, Boiling, Blanching, Microwave heating

INTRODUCTION

Vegetables and fruits are valuable components of the daily diet contributing carbohydrate in form of dietary fibre, vitamins and minerals to the body [1]. Most tropical countries diet contains a diversity of leafy vegetables such as spinach, amaranth, lettuce. The main protective action of vegetables has been attributed to the antioxidants present in them. The potential cancer inducing oxidative damage might be prevented or limited by dietary antioxidants found in vegetables [2]. Researchers had found that vegetables lead to the diminution of several diseases like: cardiovascular, neurological and carcinogenic destruction of different body parts [3].

Vitamins are essential to maintain normal metabolic processes and homeostasis. The amount of specific vitamin required by an individual varies considerably [4].Vitamin C (Ascorbic Acid) is a water-soluble antioxidant. It was first isolated in 1928 by the Hungarian biochemist and Nobel Prize winner Szent-Gyorgyi. Vitamin C is available in reduced form (L-ascorbic acid) and oxidized form (L-dehydroascorbic acid). It is an unstable, easily oxidized acid and can be destroyed by oxygen, alkali and high temperature [5]. Great interest has been seen in the clinical roles of Vitamin C because of evidence that oxidative damage is a root cause and mostly associated with many diseases. Population studies have shown that individuals with high intakes of Vitamin C have lower risk of chronic diseases including, heart disease, cancer, eye diseases and neurodegenerative conditions [6]. Diet supplies more than 90% of the Vitamin C in human by fruits and vegetables [7]. Except human and other primates, most of the higher animals can synthesize Vitamin C (L-ascorbate). Humans lack the enzyme (L-gluconolactone oxidase, GLO) which is needed to convert glucose to Vitamin C [8]. Ascorbic acid is widely distributed in fresh fruits and vegetables like orange, lemons, grapefruit, watermelon, papaya, tomatoes, broccoli, green and red peppers [9]. Vitamin C cannot be synthesized through body cells nor does stores it. It is therefore important to include plenty of Vitamin C-containing foods in daily diet [10].

Vitamin C plays significant functions in the body. The biochemical functions of Vitamin C includes: Stimulation of certain enzymes, collagen biosynthesis, hormonal activation, antioxidant, detoxification of histamine, phagocytic functions of leukocytes, formation of nitrosamine and proline hydroxylation amongst others. Vitamin C has been associated with reduction of incidence of cancer, blood pressure, immunity and drug metabolism and urinary hydroxyproline excretion, tissue regeneration [11]. Study published in Anticancer Research in 1992 reported that use of high-dose antioxidants in conjunction with chemotherapy and radiation prolonged survival in patients with small-cell lung cancer [12]. The recommended dietary allowance (RDA) for Vitamin C for non smoking adult male and female was 60 mg/day [6]. The new higher Recommended Dietary Allowance (RDA) for Vitamin C is 75mg for women and 90mg for men because it acts as an antioxidant as well as for protection from deficiency [13]. Since smokers suffer increased oxidative stress there recommended dietary allowance is increased by 35mg/day.

Most vegetables are commonly cooked before consumption. In general, vegetables are cooked at home on the basis of convenience and taste preference of consumer rather than retention of nutrient and health-promoting compounds. Study shows that cooking methods affect the contents of nutrient and health-promoting compounds such as Vitamin C, carotenoids, polyphenols and glucosinolates in broccoli [14]. It is known that cooking of vegetables induces significant changes in chemical composition which influences the concentration and bioavailability of bioactive compounds. However, both positive and negative effects have been reported depending upon differences in process conditions and morphological and nutritional characteristics of vegetable species. Physical properties are greatly affected by heat treatments to vegetables. Although consumption of fresh unprocessed plant food is widely advocated to obtain maximum nutrition but evidence is emerging that *in vivo* bioavailability of many protective compounds present in vegetables is enhanced when they are cooked. Along with positive attributes of boiling vegetables before consumption, consumers are also deprived of important nutrients as the nutritional properties of the vegetables are greatly affected after cooking [15]. The degree of vitamin loss is influenced by various factors including: type of food, variety of vegetables, method of cutting, duration and processing of cooking [16]. Ascorbic acid is sensitive to heat and oxygen and is a water soluble vitamin, therefore leaching into the cooking water may occur during processing resulting in the potential loss during industrial processing or domestic cooking. In addition, leaves of the vegetables may absorb a large amount of cooking water and this can lead to dilution and further reduction in the level of vitamins in the cooked product [17]. Research has demonstrated that thawing of vegetables before cooking is useless and leads to more Vitamin C loss. Therefore, frozen vegetables must not be thawed before cooking. To prevent Vitamin C from destruction, use of double based stainless steel pan, minimum amount of water and cooking of frozen vegetables are recommended [18]. Microwave cooking has gained considerable importance as an energy-saving, convenient, and time-saving cooking method. A large amount of data is available on the effects of microwaves on vitamins. It is concluded that microwave and conventional cooking have only slight difference on vitamin retention in foods [19].

The recent scientific literature indicates that beyond protecting against scurvy, Vitamin C contributes to many aspects of human health regulating many metabolic processes. The data indicates that the Vitamin C requirement of smokers is higher by at least 60mg per day than that of non-smokers due to increased metabolism. Important functions such as, immune response, pulmonary function and iron absorption are related to Vitamin C intakes in diet. Daily Vitamin C intake of at least 150-200mg per day is required for these functions. Vitamin C also play critical roles in the prevention of CHD, cancer and cataract [20].

Studies from the UK and North America have reported Vitamin C deficiency in around 1 in 5 men and 1 in 9 women in low income groups [21]. The prevalence of Vitamin C deficiency is highest among Indians and people of South Asian origin compared to other races except the Mexican population. Lower intake of fresh fruits and vegetables and over-cooking of food by South Asians might be the reason for higher prevalence of Vitamin C deficiency in these populations. Also this deficiency of Vitamin C may be the one of the reason for higher rates of cardiovascular disease and cancer among South Asians individuals in Pakistani, Indian, Malay and Chinese populations compared to most Western populations [22]. The research done on prevalence of Vitamin C deficiency in India clearly highlights that serum ascorbic acid deficiency is associated with elevated markers of chronic disease in this population of young adults which may have long-term adverse health consequences [23]. The age, sex and season standardized prevalence of Vitamin C deficiency was 73.9% in 2668 people in north India and 45.7% in 2970 from south India. Only 10.8% in the north and 25.9% in the south met the criteria for adequate levels of Vitamin C. Deficiency was more prevalent in men with increasing age, users of tobacco and biomass fuels with poor nutrition and with lower intakes of dietary Vitamin C [21].

The purpose of this study is to investigate the effects of different cooking processes like boiling, blanching and microwave cooking on Vitamin C content of green leafy vegetables namely spinach, methi, lal maath, bhathua and chauli. Also to find the most suited and accurate method to determine the Vitamin C content in vegetables among the various chemical methods available.

MATERIAL AND METHODS

Estimation of Vitamin C by various methods using lemon as sample

Various methods were examined to accurately determine the Vitamin C content in lemon and using Vitamin C tablet as a standard. Methods used are as follows:

1) Iodometry [24]

Vitamin C can be determined in food by use of an oxidation-reduction reaction. The redox reaction is preferable to an acid-base titration because a number of other species in juice can act as acids but relatively few interfere with the oxidation of ascorbic acid by iodine. The solubility of iodine is increased by complexation with iodide to form tri iodide. Tri iodide then oxidizes Vitamin C to dehydroascorbic acid:

$$C_6H_8O_6 + I_3 - + H_2O\ C_6H_6O_6 + 3I- + 2H+ \quad (2)$$

The endpoint is indicated by the reaction of iodine with starch suspension which produces a blue-black product. Take 25ml of standard Vitamin C solution (1mg/ml) into 150ml conical flask. Add 10 drops of 1% starch solution and titrate it against iodine solution till end point is reached, take 3 readings. Repeat the titration taking 25ml of lemon juice and calculate Vitamin C gm% of lemon.

***2)* DNPH method [25]**

Extract 5gm of sample in 25-50ml 4% oxalic acid as described. Transfer an aliquot (10ml) to a conical flask and add bromine water drop wise with constant mixing. The enolic hydrogen atoms in ascorbic acid are removed by bromine. When the extract turns orange-yellow due to excess bromine, expel it by blowing in air. Make up to a known volume (25 or 50ml) with 4% oxalic acid solution. Prepare stock solution of Vitamin C using tablet of 100mg/ml. Take aliquots in the range of 0.2-1ml, make up the volume with distilled water to 3ml. Add 1ml of 2% DNPH solution and 2ml of 10% thiourea. Incubate in waterbath for 1hr at 60^o C. The dehydroascorbic acid is then reacted with 2, 4-dinitrophenyl hydrazine to form osazone and dissolved in sulphuric acid to give an orange-red color solution which is measured at 540nm. Take 1ml of the processed lemon juice sample as unknown. Calculate the ascorbic acid content in the sample using the standard graph.

C) DCPIP (Dichlorophenoliodophenol) titration [26]

Standard: 5ml of standard solution was pipetted out into conical flask, 10ml of 4% oxalic acid was added and titrated against the dye. The end point appeared as pink color which persists for few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid.

Test: 5ml of test sample (lemon juice) was pipetted out into a 100ml of conical flask. 10ml of 4% oxalic acid was added and titrated against the dye. The endpoint appeared as pink color which persists for sometimes and reading was noted.

Calculations: The amount of ascorbic acid present in the given sample is

[volume of dye required to titrate test/Volume of dye required to titrate standard] * conc. of std * Dilution factor

D) N-Bromosuccinimide [27]

Stock solution of ascorbic acid was prepared freshly by dissolving 0.05g in $100cm^3$ of 0.5% oxalic acid solution. Serial dilutions were made from the stock solution (50mg ascorbic acid/$100cm^3$) to give the working solutions of 40mg, 30mg, 20mg and 10mg of ascorbic acid in $100cm^3$ of 0.5% oxalic acid solution. $10cm^3$ each of the standard ascorbic acid solutions were transferred to a standard flask and the volume made up to $100cm^3$ with de-ionized water. $10cm^3$ of this solution was then titrated against 0.01% N-bromosuccinimide solution according to the method earlier reported. All titrations were performed in triplicate and the results used to construct a calibration curve for ascorbic acid.

Titration of lemon sample to determine ascorbic acid content

Take approximately 20gm of lemon and squeeze to obtain sufficient amount of juice. Take 10 ml of lemon juice and titrate against 0.01% N-bromosuccinimide. Note the reading and estimate the amount of Vitamin C present in 10gm of sample using standard ascorbic acid curve obtained above.

B) Estimation of vitamin C in vegetables using N-Bromosuccinimide titration method

Sample collection and preparation

Vegetables were selected on the basis of availability and their popular consumption. Fresh vegetables were purchased from the local market and were used on the same day. These were washed thoroughly with water, leafs of the vegetables were grinded using a pestle and mortor and a buffer as specified by the individual assay methods. Filtrate was collected after crushing and used for Vitamin C assay. The samples used for analysis of Vitamin C were Spinach, Methi, Lal maath, Chauli and Bhathua.

Processing treatments

5g of vegetable samples were taken and subjected to boiling (10mins), blanching (2mins) and microwave heating (10mins). Then the vegetables were crushed in a mortor and pestle along with 0.5% oxalic acid, it was filtered with muslin cloth and the volume was made upto 100ml using measuring cylinder. The filtrate obtained was refiltered using filter paper for obtaining more clear solution. The treated samples were extracted in 0.5% oxalic acid and volume of the filtrate was made up to 100ml, 10ml of the sample solution was titrated against 0.01% N-bromosuccinimide. The content of Vitamin C was determined before and after the extracts were exposed to blanching, heating and microwave radiation was determined using standard calibration curve of ascorbic acid.

RESULTS

Table1: Estimation of Vitamin C by various methods using lemon as sample

Method	Weight of Lemon sample taken (g)	Volume of Juice extracted (ml)	Amount of Vitamin C/10g of Lemon sample (mg)	Mean Value (mg/10gm) of lemon sample
Iodometric Titration	75.63	40	7.616	7.62
	09.44	45	5.35	
	99.22	45	9.88	
DNPH Method	6.510	2.9	46.39	42.99
	6.11	2.5	41.90	
	5.07	2	40.68	
DCPIP Dye Titration Method	24.13	12	42.0	48.86
	29.66	17	52.3	
	28.92	14	52.3	
By N-Bromosuccinimide Method	20.3	11	60.3	61.47
	21.6	13	59.4	
	19.8	11	64.7	

From the above methods used to determine the Vitamin C content in lemon juice sample, Iodometry method is least sensitive followed by DNPH colorimetric assay followed by DCPIP dye titration method. N- bromosuccinimide titration method gave best results to estimate Vitamin C content.

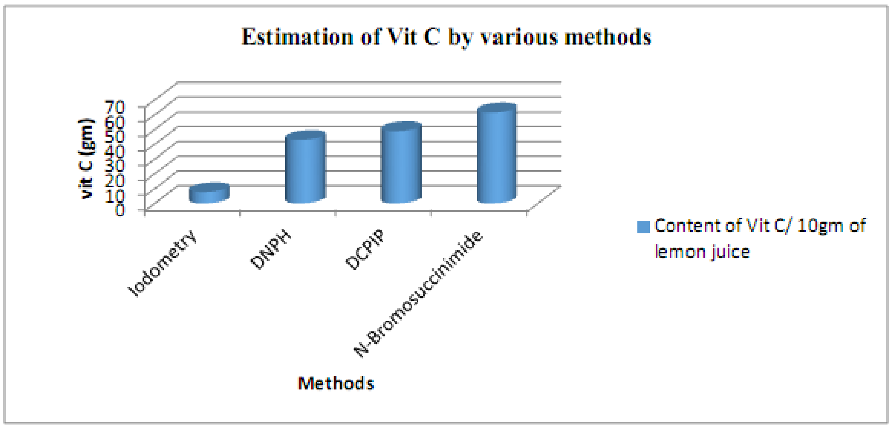

Fig-1: Comparison of the results obtained by various method for estimation of Vitamin C in lemon juice sample

Fig-2: DNPH method: standard and sample tubes

Table 2: Vitamin C content in vegetables before and after processing

Sample	Vitamin C content (mg%) after processing															
	Before (raw)			Mean	Boiling			Mean	Blanching			Mean	Microwave heating			Mean
Spinach	55.37	67.31	61.21	61.30	17.56	35.56	35.6	29.56	34.87	61.21	49.02	48.36	14.14	12.43	16.09	14.22
Methi	66.0	59.4	65.29	63.56	32.5	30.0	31.47	31.32	38.8	39.4	36.62	38.27	27.8	29.2	23.38	26.79
Lal maath	223.4	215.96	219.56	219.64	136.8	102.8	140.6	126.73	148.2	147.4	181.0	158.86	57.8	60.0	61.58	59.79
Bhathua	217.28	146.32	151.43	171.67	118.25	94.95	56.11	89.77	133.78	110.5	89.12	115.94	100.7	81.35	79.4	87.15
Chauli	46.8	54.93	48.6	50.11	22.8	27.09	29.62	26.50	33.2	44.8	34.68	37.56	13.2	20.75	22.02	18.67

The above table shows that Vitamin C content of various green leafy vegetables considered for study when they are unprocessed and also the amount of Vitamin C available after subjecting to various cooking processes including boiling, blanching and microwave heating. The amount of vitamin C was calculated in mg of Vitamin C present in 100gm of the vegetable sample.

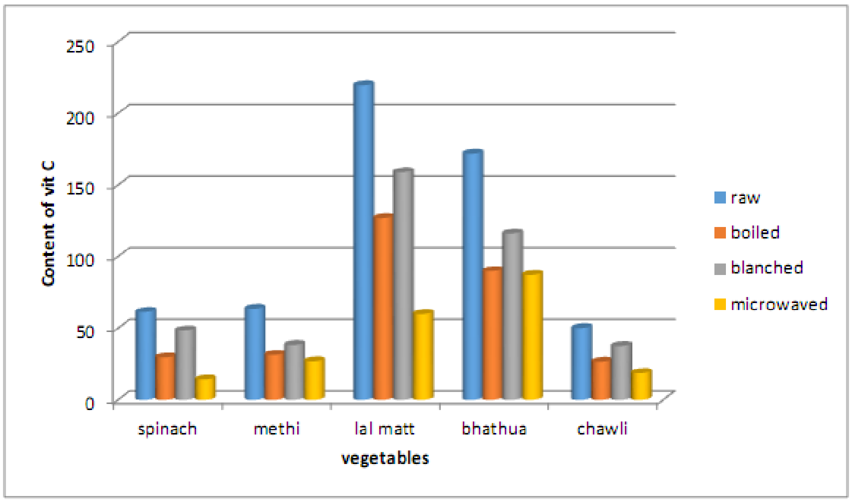

Fig-3: Vitamin C content in vegetables before and after processing

Table 3: Comparative values of Vitamin C after various treatment

Sample		% reduction in the content of vitamin C		
		Boiling	Blanching	Microwave heating
Spinach	61.29	52.42%	22.03%	76.57%
Methi	63.56	50.72%	39.61%	57.65%
Chauli	50.11	46.59%	25.39%	62.10%
Lal maath	219.64	42.33%	33.75%	72.77%
Bhathua	172.62	48.30%	35.18%	48.89%

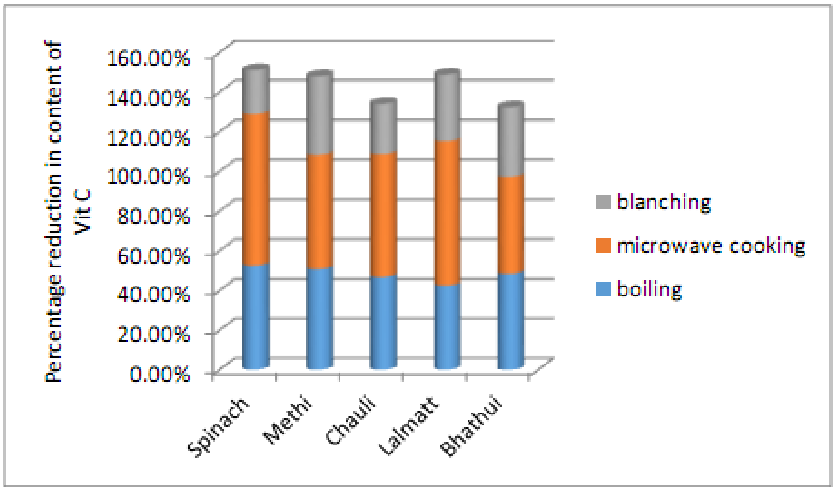

Fig-4: Percentage reduction in content of Vitamin C after subjecting to various cooking methods

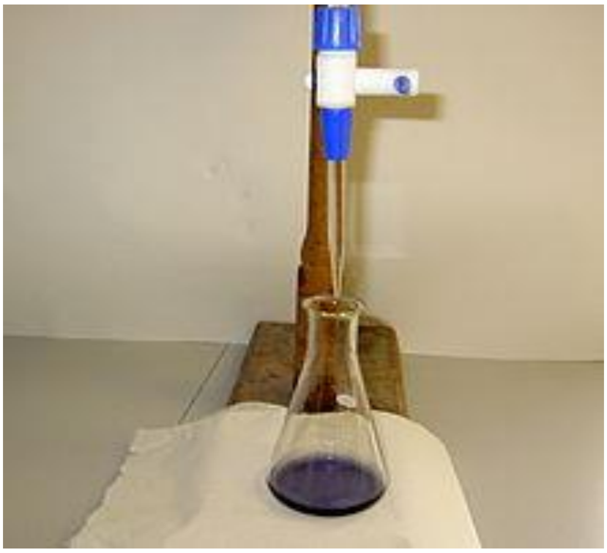

Fig-5: DCPIP Titration

DISCUSSION

Vitamin C content was estimated by using various methods: Iodometry, DNPH method, DCPIP dye and N- Bromosuccinimide titration method by using lemon juice as sample and ascorbic acid tablet as standard (Table 1, Figure 1, 2 & 5). The results obtained showed N- Bromosuccinimide method estimation was most efficient and sensitive in estimating the amount of Vitamin C as it quantified both ascorbic acid and dehydroascorbic acid present in the sample. The other techniques showed lower quantification of Vitamin C as the methods were specific only for estimation of ascorbic acid. Hence, N-Bromosuccinimide method was chosen for estimation of Vitamin C in vegetables as it was most sensitive method for estimating the total ascorbic acid present in the sample and also due to easy and simplicity of method.

We report here the Vitamin C content of raw, boiled, blanched, microwave radiation exposed samples of vegetables namely Spinach, Methi, Lal maath, Chauli and Bhathua (Figure 4). The retention of Vitamin C is often used as an estimation for overall nutrient retention of food products because it is highly sensitive to oxidation and leaching into water during cooking. It begins to degrade immediately after harvest and degrades steadily during storage and other processes. The study done clearly highlights that Vitamin C activity drops on treatment with heat. The loss of Vitamin C in green leafy vegetables is due to the processing method employed in its preparation. The losses observed in this study are high most especially when the vegetables were subjected to boiling and microwave heating as compared to blanching (Table 2). Loss as a result of boiling, microwave heating and blanching can be justified since Vitamin C is water-soluble and heat labile. Thus, Vitamin C is easily leached into the boiling medium, the rate of destruction of vitamin was not uniform. The percentage of Vitamin C activity loss was 40-50% on boiling, 20-40% on blanching and 50-75% on microwave heating (Table 3 & Figure 3). The observations put forth that microwave cooking of the said vegetables does not seems to be a good cooking practice.

CONCLUSION

It has been observed from the analytical results that Vitamin C content is conserved maximum on blanching as compared to boiling and microwave heating. This attribute needs further investigation in terms of loss of other nutrients as well as on microwave cooking. This study focused on these five leafy vegetables as they are used commonly in our everyday cooking. The study endorses that traditional method of cooking that involve blanching rather than boiling is the correct way to cook these vegetables. The study needs to be validated by HPLC method for estimation of Vitamin C to ascertain whether there is loss of minerals and other nutrients due to exposure to heat on these 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. Also appropriate cooking methods which are having minimum destruction of Vitamin C and other nutrients in food should be followed to overcome the Vitamin C deficiency prevailing in our society.

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