International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.5, pp 112-121, 2017

Titrimetric quantification of stability of ascorbic acid in fruits and vegetables

N.Madhavi*[1], B.Jyothi[2],S.Kalpana[3]

[1]P.G department of chemistry, J.K.C College, A.P, Guntur
[2]Department of Chemistry, SwarnaBharathi Institute of Science and Technology, Khammam, Telangana, India
[3]Department of Chemistry, S.D.M.S.M Kalasala, Vijayawada, India

Abstract : Ascorbic acid is an essential water soluble vitamin, required for biosynthesis of neurotransmitters. It is necessary to take fruits and vegetables because humans cannot synthesize ascorbic acid due to lack of gulonolactone oxidase enzyme. Vitamin C is a six carbon lactone.It has antioxidant activity in biological fluids and therapeutic properties. A redox titre method using copper sulphate is employed to determine the concentration of vitamin C in some fruits and leafy vegetables. From the present study it is concluded that refrigeration stabilizes and enhances ascorbic acid quantity. Exposure to heating causes decrease in its concentration.

Introduction:

Fruits and vegetables are important dietary food items for humans [1].Vitamins are crucial for all life processes and are also called as co-enzymes .L-ascorbic acid is commonly called asvitamin-C, a necessary nutrient and widely distributed in nature. It has chemical formula $C_6H_8O_6$ and molecular weight of 176.12g per mole..It is a natural antibiotic, enhances W.B.C and interferon levels, works as a vital electron donor, antioxidant, therapeutic and used as a reducing agent. It helps in synthesis of collagen and carnitine [2].It is awhite crystalline [3], water-soluble vitamin, structural component of blood vessels, tendons, ligaments and bones [4, 5, and 6]. Non-haeme food sources iron absorption is enhanced with vitamin C [6].It is the least stable or labile of all the vitamins [8, 9]. Its concentration helps in determining the nutritional quality of the fruit [10]. It protects us from many acute or chronic diseases such as commoncold and infertility [11]. Biologically it acts as a reducing agent by donating electrons in various enzymatic and few non-enzymatic reactions. The presence of glutathione in cells and extracellular fluids helps in maintaining of ascorbatein reduced state [12]. It is absorbed in the body by active transport and simple diffusion. Deficiency of vitamin C causes follicular hyperkeratosis, dryness of mouth and eyes, loss of hair, scurvy and itchy skin. Large doses of ascorbic acid may cause indigestion, particularly when taken on an empty stomach [13]. Prolonged heating, exposure to sunlightand oxygen destructs it [14, 15]. Refrigeration stabilizes it upto some extent. Green leafy vegetables may be eaten raw, boiled or dried. Usage of boiled vegetables and leaves is the most common in all parts of the world. This process eliminates potential pathogens, sometimes poisonous or irritating substances are neutralized and spoilage is brought to a halt.

Papaya lowers cholesterol, helps in weight loss, easing menstrual pain, reduces stress, boostsimmunity, rich source of vitamin A, protects against arthritis, improves digestion ,prevents signs of ageing and reduces the risk of colon and prostate cancer.

The health benefits of phyllanthus emblica includes protection of heart by reducing cholesterol, controls diabetes and its complications, reduces gastric problems and protects liver by preventing oxidative damage. The studies based on the epidemiology confirm significant association between vitamin C intake and protection against cardiovascular mortality. The other role of it includes promoting healthy teeth and gums, helps in absorption of iron, aids in maintenance of normal connective tissues, promotes wound healing, and lessens risk of developing high blood pressure.

Grapes are actually berries. They grow in bunches in temperate climates throughout the world. Grapes are also known as the "Queen of Fruits". Green grapes are the sweetest and most commonly found grape variety. Popular green grapes varieties are Thompson seedless, Sugarone, and Calmeria.Delicious taste of black grapes is due to their deep and rich black color.

Black grapes help to cure diabetes and to improve concentration and memory. The polyphenols present in them also aid in curing migraine, dementia and preventing Alzheimer's. The phytochemicals present in them help in reducing damage of the heart muscles and also aid in reducing and regulating cholesterol levels in the body thereby preventing heart attacks and other cardio vascular diseases. Antioxidant properties of black grapes help in releasing the unwanted toxins accumulated in the body which results in loss of weight. Resveratrol, in black grapes, is an excellent bactericide and fungicide and therefore helps to prevent pathogenic infections and inflammations. It has antiviral properties which are effective against diseases like polio and herpes. It also helps to cure asthma by increasing the level of moisture present in the lungs. Black grapes are well known for their effect on the eyesight. These grapes contain Lutein and Zeaxanthin which help to maintain a good eyesight and proper vision.

Green grapes are low in calories, contain zero grams of fat and pack a nutritional punch. It is simple to add these tiny fruits to a variety of healthy dishes to enhance the flavor and to add nutrition.

Tomatoes contain a chemical called lycopene, which is a natural antioxidant that works effectively to slow down the growth of cancerous cells. They are helpfulfor maintaining strong bones. Tomatoes also contain coumaric acid and chlorogenic acid that work to protect the body from carcinogens that are produced from cigarette smoke. Because of the Vitamin B and potassium in tomatoes, they are effective in reducing cholesterol levels and lowering blood pressure, reduce the risk of kidney stones also. They are packed full of the valuable mineral known as chromium. It works effectively to help diabetics keep their blood sugar levels under better control.

Rumex vesicarius (Bladder Dock)is an edible plant and used as a sorrel.As it is rich in fiber, can help to reduce total cholesterol in the body and thereby protecting heart health. The presence of polyphenolic compounds, flavonoids and anthocyanins help in preventing cancer. The significant level of iron in sorrel boosts red blood cell production and prevents anemia.

Malabar spinach also called as basella.It holds good amounts of vitamins, minerals and antioxidants. Regular consumption of basella in the diet helps in preventing osteoporosis.

Yam is a tuber and is full of starch. There are about 200 varieties of this vegetable. It is long and cylindrical in shape, and is rough to touch. Yam is a rich source of energy and also Vitamin B Complex, Vitamin C, antioxidants, minerals, fiber and complex carbohydrates.

Roselle (Hibiscus sabdariffa) is a species of Hibiscus native to West Africa, used for the production of bastfibre and as an infusion, in which it may be known as carcade. It is an annual or perennial herb or woody-based sub shrub, the roselle leaves and calyces have been used as food and the flowers steeped for tea. Hibiscus has been used in folk medicine as diuretic and mild laxative, as well as in treating cancer, cardiac and nerves diseases.

Ascorbic acid is easily destroyed by oxidation, particularly in the presence of heat and alkalinity, because it is highly soluble in water. Therefore the best sources are fruits and vegetables, preferably acidic, fresh and when necessary, rapidly cooked in very little water and served immediately.

The objective of this work is to investigate the vitamin C content of various fruits and leafy vegetables available in the Guntur local market and to note at what point of maturity ascorbic acid is at its peak and when it is the best to harvest.

Materials and Method:

Sample Collection:

Seedless grapes, amla, hybrid amla, papaya,tomatoes, Malabar spinach, yam,rumex and roselle leaves are the samples taken for present study.

Sample Extraction:

The juice was squeezed out of the fruits manually in a clean glass beaker and remaining portion of the juice squeezed through layers of muslin cloth. To eliminate certain impurities the extract was filtered through another clean layer of muslin cloth. A portion of juice was kept in a refrigerator for twenty hours prior to the analysis, another portion of juice was exposed to intense sunlight for the same length of time and third portion was left at ambient temperature. Then the ascorbic acid present in these portions were determined.

The juice was squeezed out of the vegetables manually in a clean glass beaker and remaining portion of the juice squeezed through layers of muslin cloth. To eliminate certain impurities the extract was filtered through another clean layer of muslin cloth. A portion of juice was kept on a heating top and its content is determined for every 5 minutes upto 30 minutes. Another portion of juice was kept in a refrigerator for twenty hours prior to the analysis, another portion of juice was exposed to intense sunlight for the same length of time and fourth portion was left at ambient temperature. Then the ascorbic acid present in these portions were determined.

Preparation of Reagents:

2.5g of $CuSO_4 5H_2O$was dissolved in a beaker using distilled water. The solution was then transferred into a 100ml volumetric flask in order to make 0.01M $CuSO_4$solution.

30% KI solution was prepared by dissolving 30g of KI in 100ml distilled water.

Determination of Ascorbic Acid:

5g of juice is taken in a 250ml conical flask and 10 ml of 30% KI was added. It is mixed well and then four drops of 1% starch solution is added. The mixture was titrated against 0.01M $CuSO_4$solution until a black color appeared. Similarly blank analysis is carried out using distilled water.

1ml of $CuSO_4$ = 0.88 mg of vitamin-C

Vitamin c mg/100g = $\frac{100}{W} \times 0.88(B - T) \times \frac{VT}{VA}$

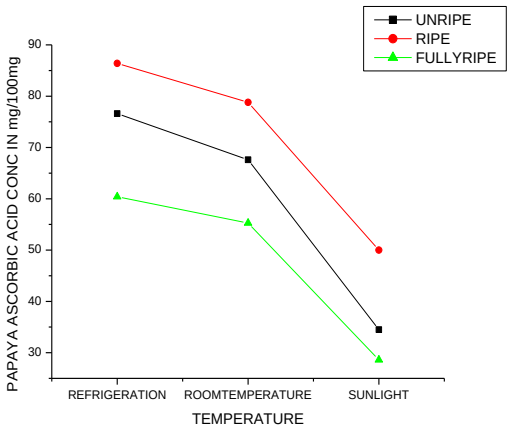
Where,
W=weight of sample
T=titre value of sample
B=titre value of blank
VT=total extract volume
VA=volume of extract titrated

Results:

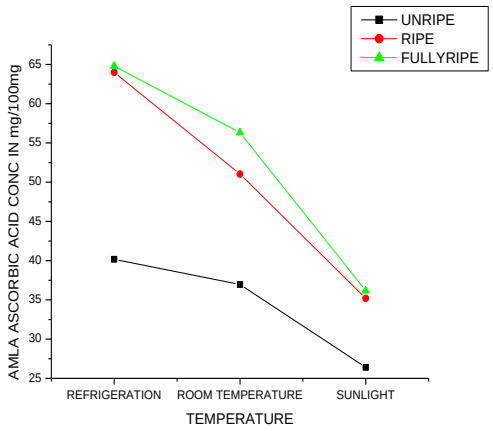
This method determines the vitamin C concentration in a solution by a redox titration. On titration of copper sulphate solution with sample extract, potassium iodide and starch indicator, the potassium iodide reacted with copper sulphate forming cupric iodide (CuI_2). Excess iodide ions oxidize the ascorbic acid to dehydroascorbic acid. Once all the ascorbic acid has been oxidized, the excess iodide is free to react with the starch indicator, forming the blue-black starch-iodine complex. This is the endpoint of the titration.

Table 1: Fruits

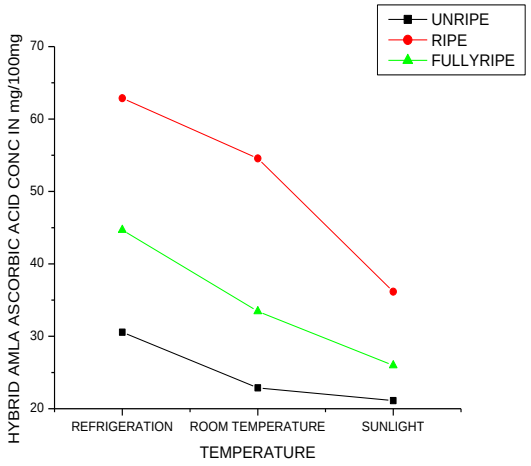
FRUIT	**Type of Fruit**	**Room Temperature (20 Hrs)**	**Refrigeration (20 Hrs)**	**Sunlight (20 Hrs)**
		ascorbic acid content in mg/100mg	**ascorbic acid content in mg/100mg**	**ascorbic acid content in mg/100mg**
Papaya *(*Carica papaya)	unripe	67.6	76.6	34.5
	Ripe	78.8	86.4	50.0
	Fully ripe	55.28	60.4	28.6
Amla(Phyllanthus emblica)	unripe	36.96	40.48	26.4
	Ripe	51.04	64	35.2
	Fully ripe	56.32	64.8	36.16
Hybrid amla(Phyllanthus emblica)	unripe	22.88	30.56	21.12
	Ripe	54.56	62.88	36.16
	Fully ripe	33.45	44.68	25.98
Seedless grapes(Citrus grandis)	Black	6.232	10.4	4.64
	Green	5.88	8.936	3.584
Amla (Emblicaofficinalis)	Fresh	53.44	70.40	40.44
	Fry	12.32	17.60	10.00



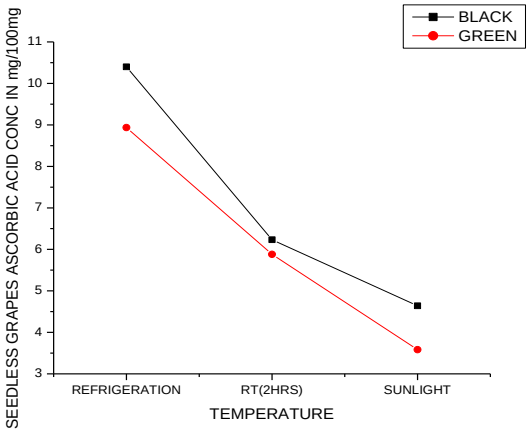
Graph 1



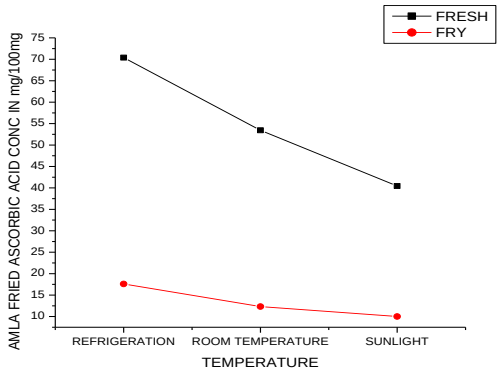
Graph 2



Graph 3



Graph 4

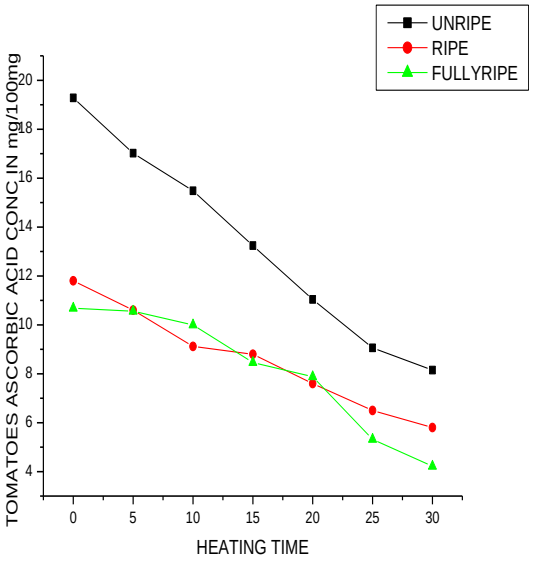


Graph 5

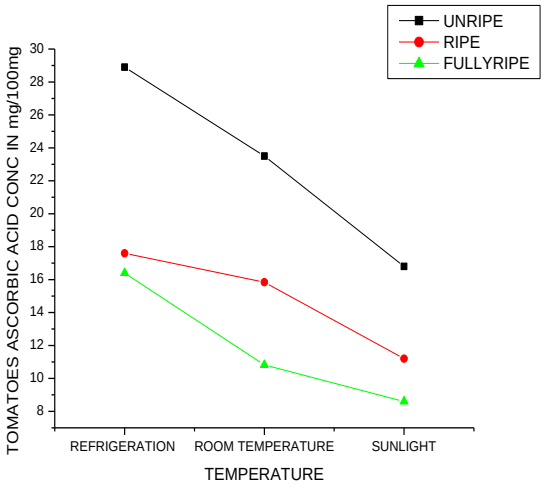
Table 2: Vegetables

Fruit	Type of Fruit	Room Temperature (20hrs)	Refrigeration (20 Hrs)	Heating		Sunlight(20 Hrs)
		ascorbic acid content in mg/100mg	ascorbic acid content in mg/100mg	heating time	ascorbic acid content in mg/100 mg	ascorbic acid content in g/100mg
TOMATOES(Solanumlycopersicum)	unripe	23.5	28.9	0	19.28	16.8
				5	17.02	
				10	15.48	
				15	13.24	
				20	11.04	
				25	9.06	
				30	8.15	
	ripe	15.84	17.6	0	11.8	11.2
				5	10.6	
				10	9.12	

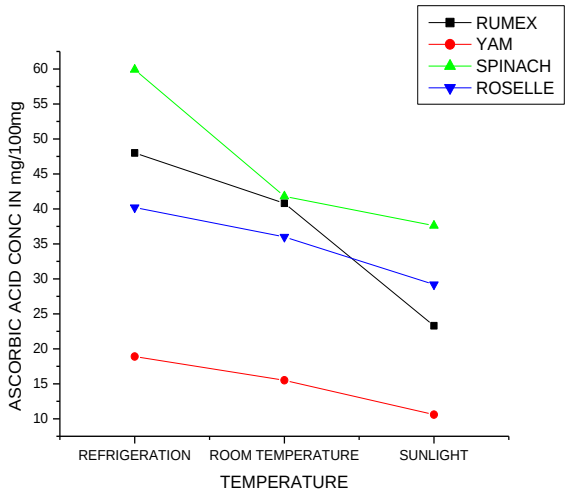
				15	8.8	
				20	7.6	
				25	6.5	
				30	5.8	
	fully ripe	10.82	16.4	0	10.68	8.6
				5	10.56	
				10	10	
				15	8.46	
				20	7.88	
				25	5.32	
				30	4.22	
Rumex(Rumexcrispus)		40.8	48	0	26.1	23.3
				5	21.7	
				10	16.9	
				15	14.2	
				20	11.4	
				25	8.6	
				30	4.2	
Yam(Dioscoreaalata)		15.5	18.9	0	14.6	10.6
				5	14.1	
				10	10.4	
				15	7.8	
				20	6.78	
				25	5.46	
				30	4.09	
Malabar spinach(Basella alba)		41.8	59.9	0	28.9	37.6
				5	24.1	
				10	20.4	
				15	17.8	
				20	14.78	
				25	12.46	
				30	10.09	
Roselle (Hibiscus sabdariffa)		36	40.2	0	31.7	29.2
				5	25.7	
				10	19.3	
				15	14.5	
				20	13.7	
				25	13.02	
				30	10.2	



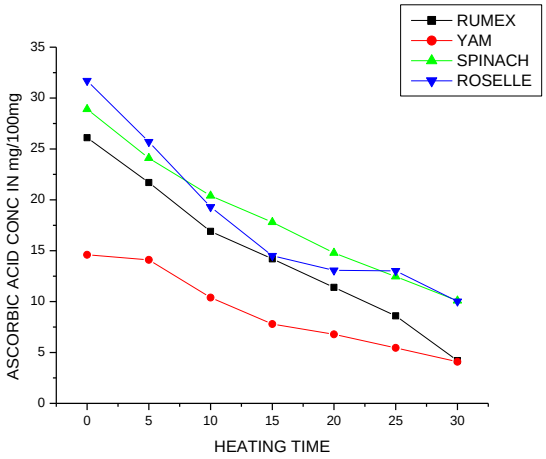
Graph 6



Graph 7

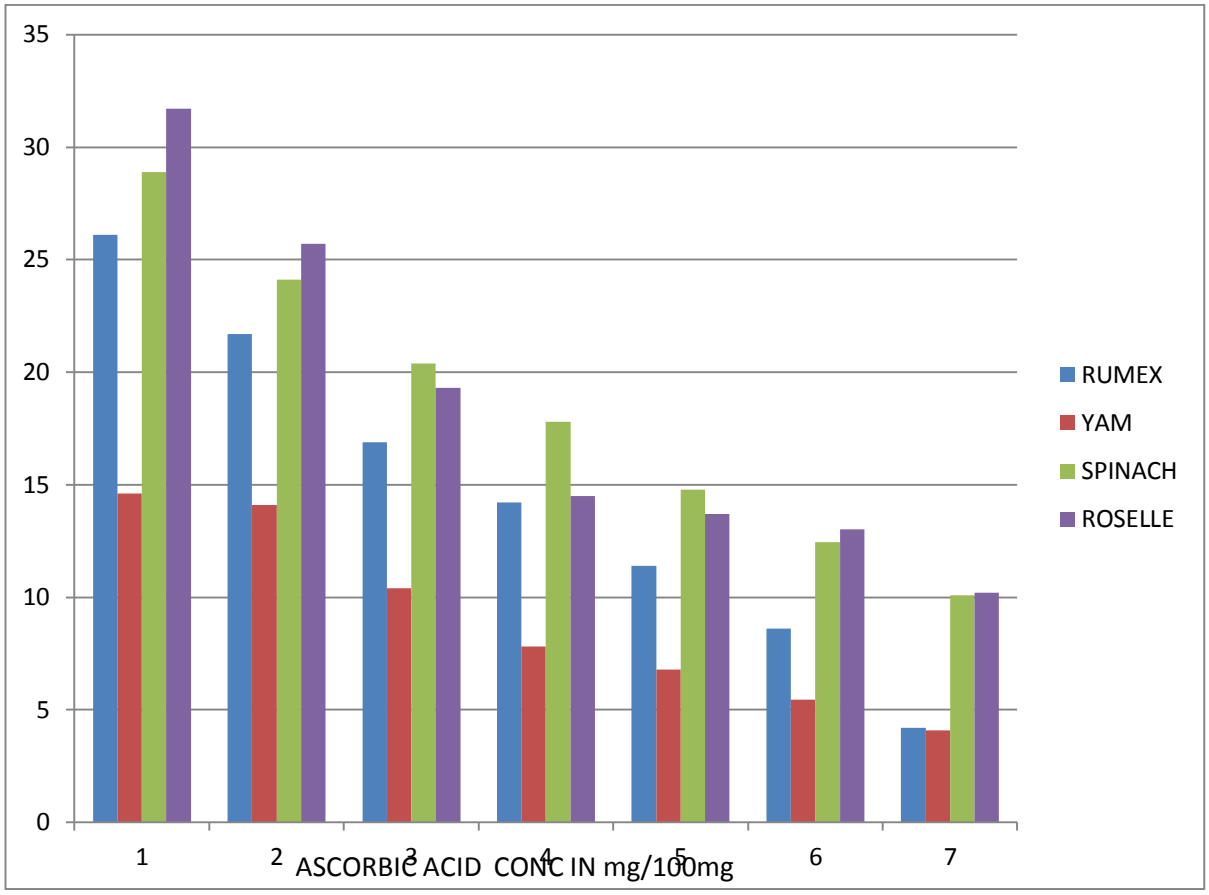


Graph 8



Graph 9

Bar graph to show variation of ascorbic acid conc for vegetables on heating



Discussion:

Vitamin C chemically decomposes under certain conditions, many of which may occur during the cooking of food. Its concentration in various food substances decreases due to increased enzymatic destruction as it may be more significant at sub-boiling temperatures. The rate of destruction was faster initially but dropped drastically for longer duration readings.

It is highly prone to oxidation during exposure to sunlight. It is reversibly oxidized to dehydroascorbic acid and further oxidation leads to the irreversible formation of physiologically inactive diketogluconic acid. Degradation is enhanced with increase in temperature. Sun drying reduces some nutrients and this affects the nutritional value of leafy vegetables. Sunlight increases vitamin C during growth of fruits and vegetables but diminishes its overtime in juice form.

Ascorbic acid is more in fruits and vegetables during refrigeration than at room temperature. Refrigeration stabilizes and enhances vitamin C.

By observing how temperature affects the vitamin C in fruits and vegetables, it will be easy to conclude the range of temperature suitable for its consumption.

Ripe papaya has high concentration of ascorbic acid when compared to unripe and fully ripe papayas. Its concentration of papaya is enhanced after refrigeration and decreased during exposure to sunlight.

The ascorbic content of grapes differs under different temperature conditions in case of all species. Black grapes contain more of it than green grapes. Fresh juice exhibited more quantity in these two species. At room temperature and when exposed to sunlight more concentration of ascorbic acid is present in black grapes. After refrigeration its concentration is enhanced in both species of grapes on exposure to sunlight.

Ripe amla has more ascorbic acid content than unripe and fully ripe amla at room temperature. Incase of Hybridamla, its concentration is more in ripe amla and fully ripe amla than unripe amla. It is more during refrigeration than during heating in hybrid amla and amla.

In emblica officinalis more concentration of vitamin C is exhibited in fresh species than the species dried during room temperature, refrigeration and on exposure to sunlight.

Unripened tomatoes have higher vitamin C concentration when compared to fully ripe and ripen tomatoes. Higher concentrations of ascorbic acid were identified at room temperature than at heating and on exposure to sunlight. Heating reduces the concentration of it. Its concentration is stabilized during refrigeration and further decreased on exposure to sunlight than during heating.

Rumex has more vitamin C concentration at room temperature and when exposed to sunlight. Its concentration decreases with increase in temperature. Its concentration is also increased after refrigeration.

Yam has higher vitamin C concentration at room temperature when compared to heating in yam .Its concentration is decreased during heating. Its concentration is further decreased on exposure to sunlight. Its concentration is more at refrigeration.

Malabar spinach has higher vitamin C concentration at room temperature when compared to heating in yam .Its concentration is decreased during heating. Its concentration diminished on exposure to sunlight. At refrigeration its concentration is high.

Roselle leaves has more vitamin C concentration at room temperature and when exposed to sunlight. Its concentration is increased after refrigeration and decreased during heating at regular intervals.

Conclusion:

From the present investigation it is concluded that this is an accurate and easier method to determine the concentration of ascorbic acid. In vegetables like yam, malabar spinach, rumex, roselle and tomatoes have high concentration of vitamin C during refrigeration. Vitamin C content in grapes, hybrid amla, papaya and amla is stabilized and enhanced by refrigeration. Heating combined with leaching (boiling), reduces significant concentration of it. For high retention of vitamin C while cooking it is recommended that the vegetables are cooked in low heat and small amounts of water for short periods to minimize the loss of it. Therefore it recommended to intake fruits and vegetables which are of natural source of Vitamin C rather than synthetic tablets.

References:

1. Madhavi N, Pravallika D. D, Determination of physico chemical parameters of various fresh fruit juices, International Journal of Organic and Bioorganic Chemistry, Universal Research Publications, 2015 March 20 5(1),13-14.
2. Robert K.M., Daryl K.G, Peter A.M. and Victor W.R, Structure and functions of water -soluble vitamins, Harper's Biochemistry 25th ed., McGraw-hill New York, 2000, 640-641.
3. Okenwa UchennaIgwe ,Quantitative estimation of ascorbic acid levels in citrus fruits at variable temperatures and physico chemical properties, International journal of chemical and biochemical sciences 2014 January 31,5,67-71.
4. Afroz M, Bhothinard B, Etzkorn JR, Horenstein S, McGarry JD, Vitamins C and B12 [letter], JAMA, 1975, 232-246.
5. Aronow WS, Serum ferritin is not a risk factor for coronary artery disease in men and women aged 62 years, Am J Cardiol, 1993 ,72,347–348.
6. Baer DM, Tekawa IS, Hurley LB, Iron stores are not associated with acute myocardial infarction Circulation, 1994, 89, 2915–2920.
7. FAO/WHO, Vitamin C, In: Human Vitamin and Mineral Requirements. Report of a Joint FAO/WHO Expert Consultation, FAO, Rome, 2002, 73-86.
8. Sies H & Stahl W, Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. Am J Clin Nutr62: 1995, 1315S-1321S.

9. Weber P, Bendich A &Schalch , Ascorbic acid and human health – a review of recent data relevant to human requirements, Int J VitNutr Res, 1996,66,19-30.
10. Julia R. Esch, Jeffrey R. Friend, and James K. Kariuki ,Determination of the Vitamin C Content of Conventionally and Organically Grown Fruits by Cyclic Voltammetry ,International Journal of electrochemical science ,2010 October 1, 5 ,1464 – 1474.
11. Madhavi N, Swathi G, Padmaja E, Determination of chemical parameters of various fresh fruit juices, International Journal of Institutional Pharmacy and Life Sciences 2015 March 01, 5(2), 35-37.
12. Gropper SS, Smith JL, Grodd JL (2005). Advanced nutrition and human metabolism. Belmont, CA: Thomson Wadsworth, 260–275.
13. Pauling, Linus, Vitamin C and the Common Cold (1 Ed.), San Francisco: W. H. Freeman and Company, 1970.
14. Atinmo T, Ogunta T, Okorie A.U and T.M.Kamalu T.M, Nutrition and good health,Nigerian Journal of Nutritional Sciences, 1981,2(2),80-85.
15. Madhavi N, jyothi B, Colorimetric determination of vitamin c in fresh and dilute fruit juices and effect of thermal exposure on concentration at various stages, International Journal of Pharma and Bio Sciences, 2016 Oct , 7(4), 197 – 211.

*****