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Determination of Ascorbic acid content in conventionally grown fruits and vegetables by colorimetry

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Abstract : Plants and their products have a wide range of nutrients and phytochemicals. They play an important role in protection of humans against risk of oxidative stress related diseases such as cardiac diseases and cancers. Ascorbic acid content has been determined by colorimetric method in Citrullus lanatus, Phyllanthus emblica, Musa paradisiaca, Cucumis sativus, Coccinia grandis,(Teegabachali), (Kadabachali), Hibiscus sabdariffa and Rumex crispus. From the present investigation it is revealed that the ascorbic acid concentration is more in unripe banana, amla and fully ripen cucumber. The concentration of vitamin C is diminishing with increase in temperature in watermelon, kadabachali, teegabachali, roselle, rosellekonda and rumex. The present study suggests that it is advised to consume them with less processing time by thermal exposure to absorb more concentration of vitamin C and to avoid more destruction. Because fresh vegetables and fruits have higher level of vitamin C than cooked vegetables.
Keywords: Ascorbic acid, Colorimetry, ripening, cancers, diminishing, thermal exposure.

Introduction:

Vegetables and fruits are essential components of the daily diet contributing carbohydrates in the form of dietary fibre, vitamins and minerals to the body [1].These are good dietary sources of natural antioxidants for humans, containing many different antioxidant components that provide protection against harmful free radicals [2].Vegetables lead to the diminution of several diseases like: cardiovascular, neurological and carcinogenic destruction of different body parts [3], helps in treating asthma, allergies, high blood pressure, cataracts, Parkinson's disease, skin ulcers, wound healing and pregnancy related conditions[4]. Vitamins are important to maintain normal metabolic processes and homeostasis [5]. L-ascorbic acid ($C_6H_8O_6$) is the trivial name of vitamin C. The chemical name is 2-Oxo-L-threo-hexono-1, 4-lactone-2, 3-enediol [6].Most plants and animals synthesize it for their own requirement but the human body is unable to synthesize vitamin C due to lack of an enzyme gulonolactoneoxidase [7]. Hence, ascorbic acid has to be supplemented mainly through fruits and vegetables [8]. Ascorbic acid is one of the most important water soluble vitamins [9].It is essential for collagen, carnitine and neurotransmitters biosynthesis [10], functions as a vital electron donor and is an important antioxidant, helps protection against cancers, heart disease, stress, and provides energy. It is also essential for sperm production, and for making the collagen protein involved in the building of cartilage, joints, skin,blood vessels, prevents oxidative damage to proteins and DNA [11], and most potent enhancer of non-heme iron absorption. Vitamin C helps in maintaining a healthy immune system, aids in neutralizing pollutants, and needed for antibody production. Ascorbic acid is generally non-toxic but at high doses (2-6g/day) it causes

gastrointestinal disturbances or diarrhoea [12, 13].It is a labile molecule and losts from foods during cooking even though it has the ability to preserve foods by virtue of its reducing property. Green leafy vegetables may be eaten raw, boiled or dried, mostly it is in practice of taking boiled green leafy vegetables. Heating process eliminates potential pathogens, sometimes poisonous or irritating substances are neutralized and spoilage is brought to a halt [14].Fresh vegetables have higher level of vitamin C than cooked vegetables.Vitamin C content can be decreased by exposure to oxygen, oxidized readily in light, high temperature and leached out of the food into the water. It is oxidized, first to dehydroascorbic acid and then to diketogulonic acid. Cooking diminishes the vitamin C content of vegetables by around 60% possibly partly due to increased enzymatic destruction as it may be more at sub-boiling temperatures [15, 16].Longer cooking times also add to this effect, as will copper food vessels, which catalyze the decomposition.

Amla(Phyllanthus emblica) is a good source for vitamin C which is essential in human nutrition. It enhances food absorption, balances stomach acid, fortifies the liver, nourishes the brain and mental functioning, supports the heart, strengthens the lungs, anti-inflammatory ,regulates elimination of free radicals, enhances fertility, helps the urinary system, increases skin health, promotes healthier hair, acts as a body coolant, flushes out toxins, increases vitality, strengthens eyes, improves muscle tone and, acts as an antioxidant.

Bananas (Musa paradisiaca) are among the most and widely consumed fruits on the planet, source of vitamins and minerals, especially vitamin C which is essential for human nutrition. They have high potassium and low sodium content and also help to protect cardiovascular system from high pressure. It helps the body to develop resistance against infectious agents. According to" World's healthiest foods ", Musa paradisiaca is the best source of vitamin C and are exist throughout the year. It is helpful in overcoming depression due to high levels of tryptophan, which the body converts to serotonin, the mood-elevating brain neurotransmitter. Nutritionally, the green banana is a good source of fibre, vitamins and minerals, and contains a starch that manages tocontrol blood sugar, weight and decreases blood cholesterol levels.

Cucumber (Cucumis sativus) is a good source for vitamin C which is essential in human nutrition.They contain an anti-inflammatory flavonol called fisetin that plays an important role in brain health.It contains polyphenols called lignans, reduces the risk of breast, uterine, ovarian, and prostate cancers. They have phytonutrients called cucurbitacins, which have anti-cancer properties. It containspotassium, which helps in maintaining lower blood pressure levels. These are used as anti-oxidants and relieve stress, maintain health.

Courgelet (Coccini agrandis) the ivy gourd, also known as scarlet gourd.It has antipyretic, anti-inflammatory, antimicrobial, antiulcer, antidiabetic, antioxidant, hypoglycemic, hepatoprotective, and antimalarial, and antidyslipidemic, anticancer, antitussive and mutagenic properties. Its low concentrations of crude extract show an inhibitory effect on the cervical cancer celland fruit mucilage was shown to reduce water turbidity by 77.67%.

Water melon (Citrullus lanatus)has high water content. This makes humans hydrating and helps in feel. Decreases the risk of obesity and overall mortality, diabetes, heart disease, reduces blood pressure and promotes a healthy complexion and hair, increases energy, overall lowers weight. It protects eyes from macular degeneration, reduces risk of kidney disorders and effective in repairing damaged tissues.

Basella Alba (Teegabachali) Indian Spinach is an annual and perennial climbing herb with red or green vines and leaves. The leaves are thick, fleshy, pointed at the tip, and arranged alternately along the vine. Antioxidant nature of spinach protects us from aging, cancer and nourishes eye. High fibre present in it improves digestion and boosts detoxification, keeps healthy pregnancy. Its fibre and antioxidant content boosts cardiovascular health. Spinach has high vitamin C, calcium and magnesium content which helps to develop healthy bone and get better immunity.

Basella Alba **(**Kadabachali)Malabar spinach fresh leaves, particularly of basella rubra, are rich sources of several vital carotenoid pigment anti-oxidants such as ß-carotene, lutein, Zeaxanthin**.** These compounds act as protective scavengers against oxygen-derived freeradicals and reactive oxygen species that reduces in aging and various disease processes. Its thick, fleshy leaves are an excellent source of non-starch polysaccharide, mucilage and its mucilaginous leaves help inproper digestion. Fibre diets bring a reduction in cholesterol absorption, and help to prevent bowel problems.

Roselle plant (Hibiscus sabdariffa) is primarily cultivated for the production of bastfibre from the stem of the plant. Itis used in folk medicine as a diuretic and mild laxative. It reduces hyper tension, helps in weight loss and aids in treating thrist, cold and cough.

Rumex (Rumex crispus),yellow dockis used as a wild leaf vegetable. Dock leaves are an excellent source of both vitamin A and C, as well as a source of iron and potassium. It is often used for treating anemia, due to its high level of iron. It can be powdered and given in the form of capsules.

Materials:

The reagents oxalic acid, bromine water, 2, 4-DNP, thiourea and sulphuric acid are used are of analytical grade.

Method:

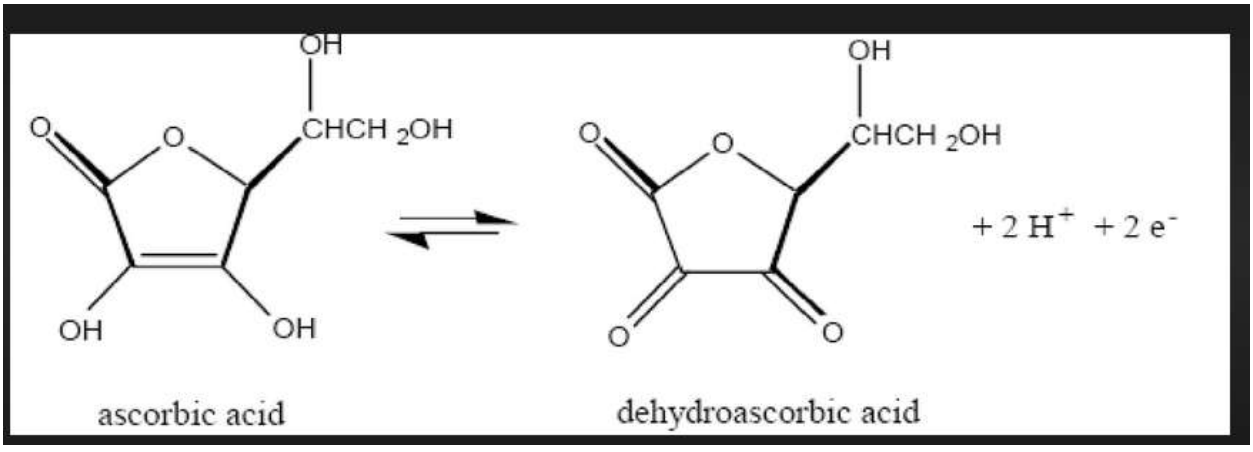
Extraction preparation:

Two different types of fresh phyllanthus emblica, roselleleaves (two types), basella alba (two types-teegabachali and kadabachali), watermelon, rumex, three types of banana (musa paradisiaca,chakkarakeli banana, raw banana), two types of cucumbers were collected from Guntur local markets.These were washed thoroughly with water, cut into pieces and weremade into paste by using mixer.

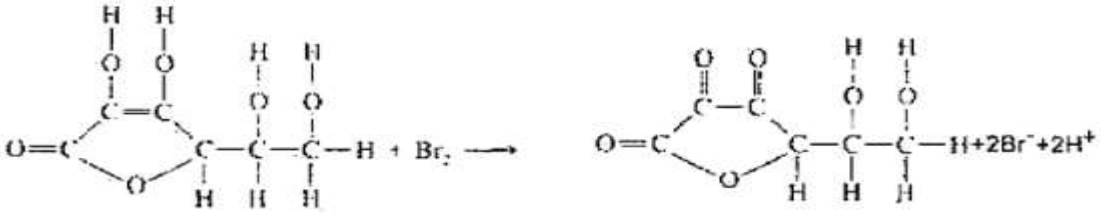
Analysis of Vitamin C:

Vitamin C analysis was carried out using colorimetric method since it gives accurate analysis of ascorbic acid content.5 g of the sample and 50ml of 4% oxalic acid were taken into a beaker and swirled. Thesolution containing sample was filtered using filter paper and funnel over another beaker to collect the liquid.10ml of the collected liquid was taken using a 10ml pipette into 50ml volumetric flask and bromine water was added drop wise with constant shaking till the solution turned into orange-yellow due to excess bromine water. The enolic hydrogen atoms in ascorbic acid were removed by bromine. The volume was made upto the 50ml with 4% oxalic acid. A volume of 1ml of the liquid in the 50ml volumetric flask was taken using graduated pipette into 100ml conical flask. To this 1ml of Thiourea, 1ml of 2, 4-dinitrophenylhydrazine solution and 1ml of water were added. The contents of the conical flask were shaken thoroughly and placed on hot plate at 37^0C for 3 hours. The solution in the conical flask was allowed to cool and 7ml of 80% sulphuric acid was added. Same analysiswas carried out using standard Ascorbic acid. Similarly the ascorbic acid stock solution was converted into dehydro form by bromination. Ascorbic acid is first dehydrogenated by bromination. 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 450 nm.

STEP 1:



STEP 2:



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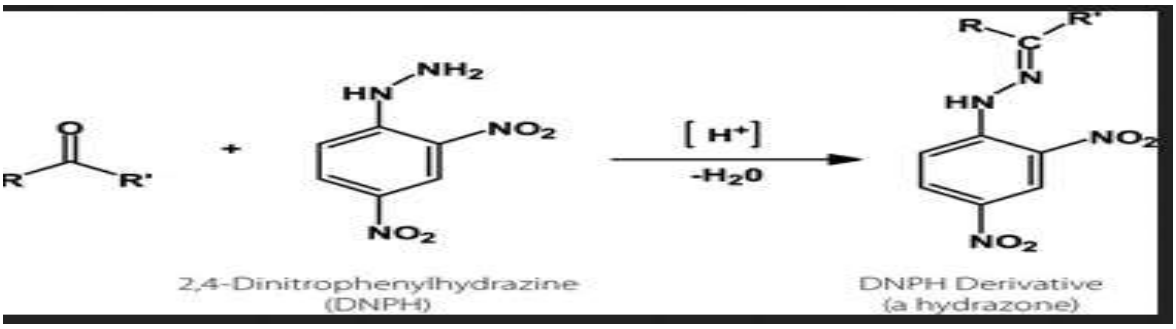

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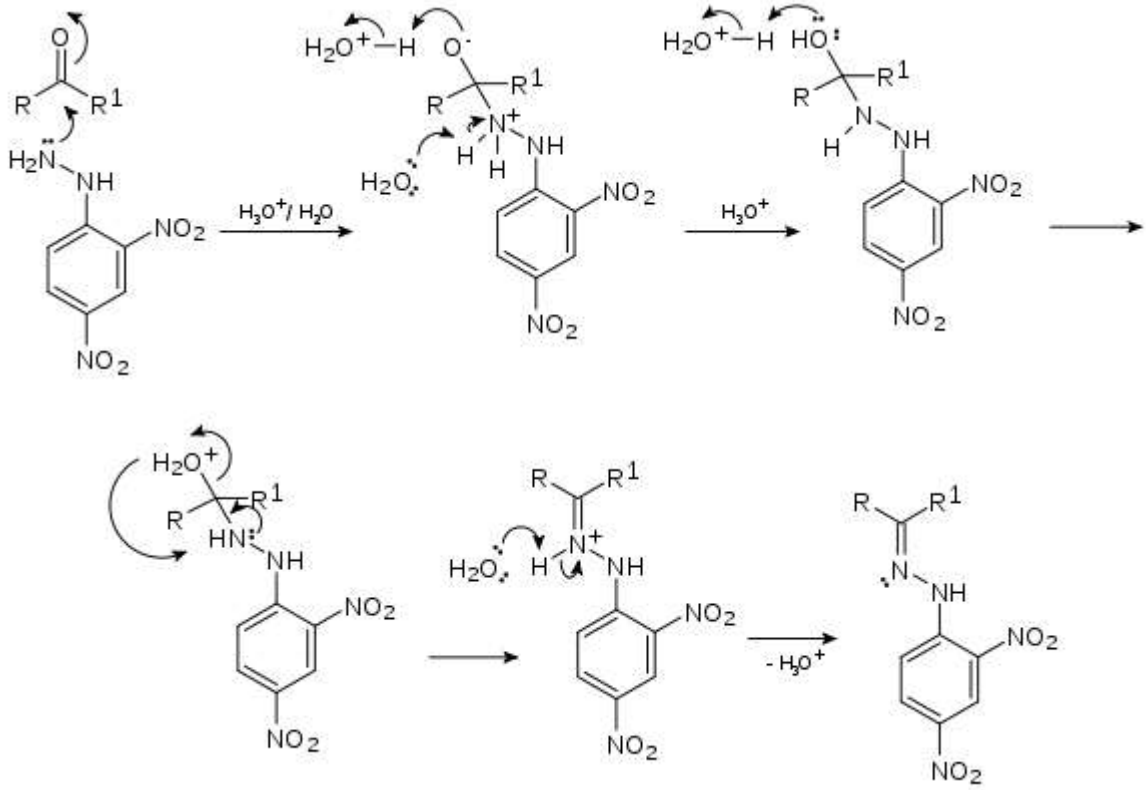
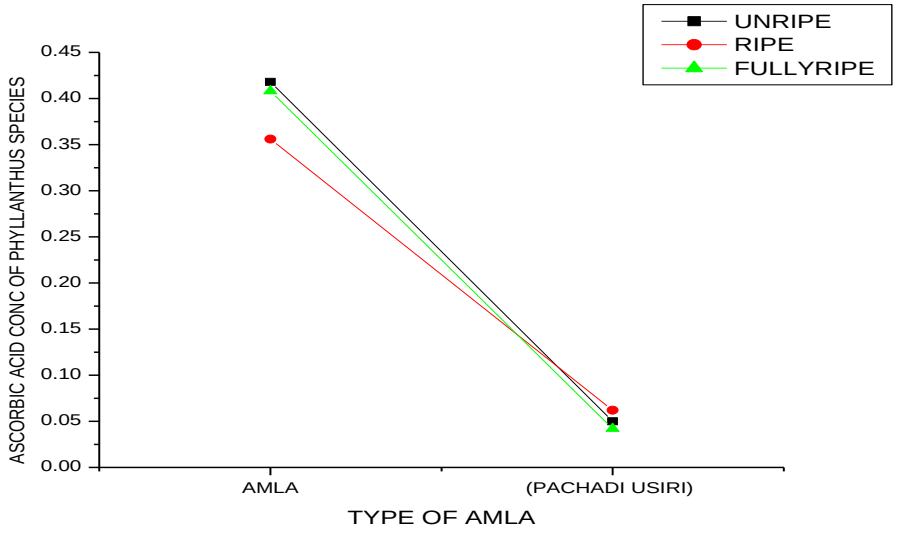


Determination of Vitamin C:

By using colorimeter the absorbance was measured for all samples and from that their concentrations were determined at wavelength 450nm.

Table 1:

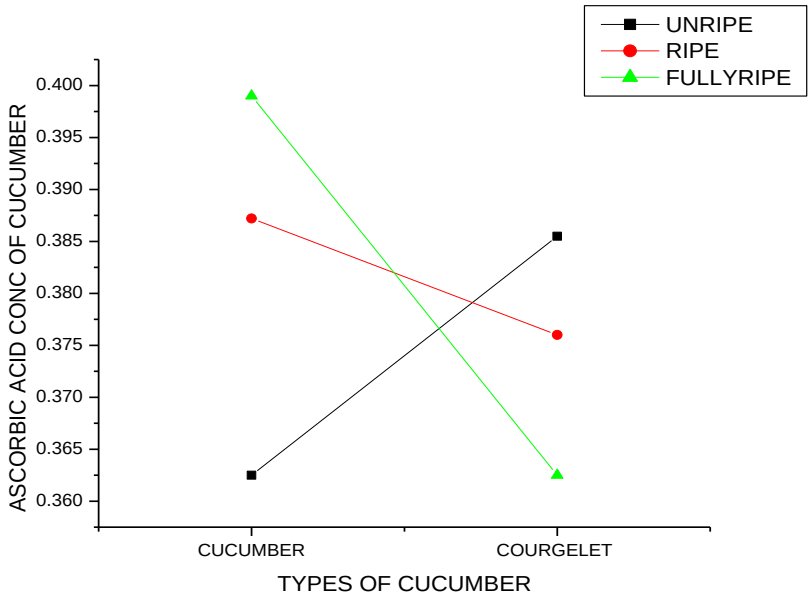
Types of Phyllanthus Species	Concentration of Vitamin C In Unripe Amla (g/mol)	Concentration of Vitamin C In Ripe Amla (g/mol)	Concentration of Vitamin C In Fully Ripe Amla (g/mol)
Amla (Phyllanthus emblica)	0.418	0.356	0.408
Indian Goose Berry (Pachadi Usiri) (Emblica officinalis)	0.05	0.062	0.042



Graph 1

Table 2:

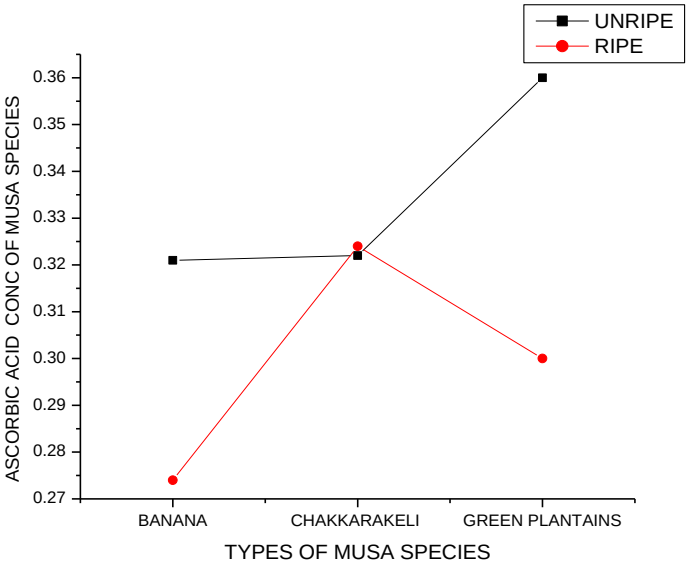
Types Of Cucumber Species	Concentration of Vitamin C In Unripe Cucumber(g/mol)	Concentration of Vitamin C In Ripe Cucumber(g/mol)	Concentration of Vitamin C In Fully Ripe Cucumber(g/mol)
Cucumber (Cucumis sativus)	0.3625	0.3872	0.399
Courgelet (Coccinia grandis)	0.3855	0.376	0.3625



Graph 2

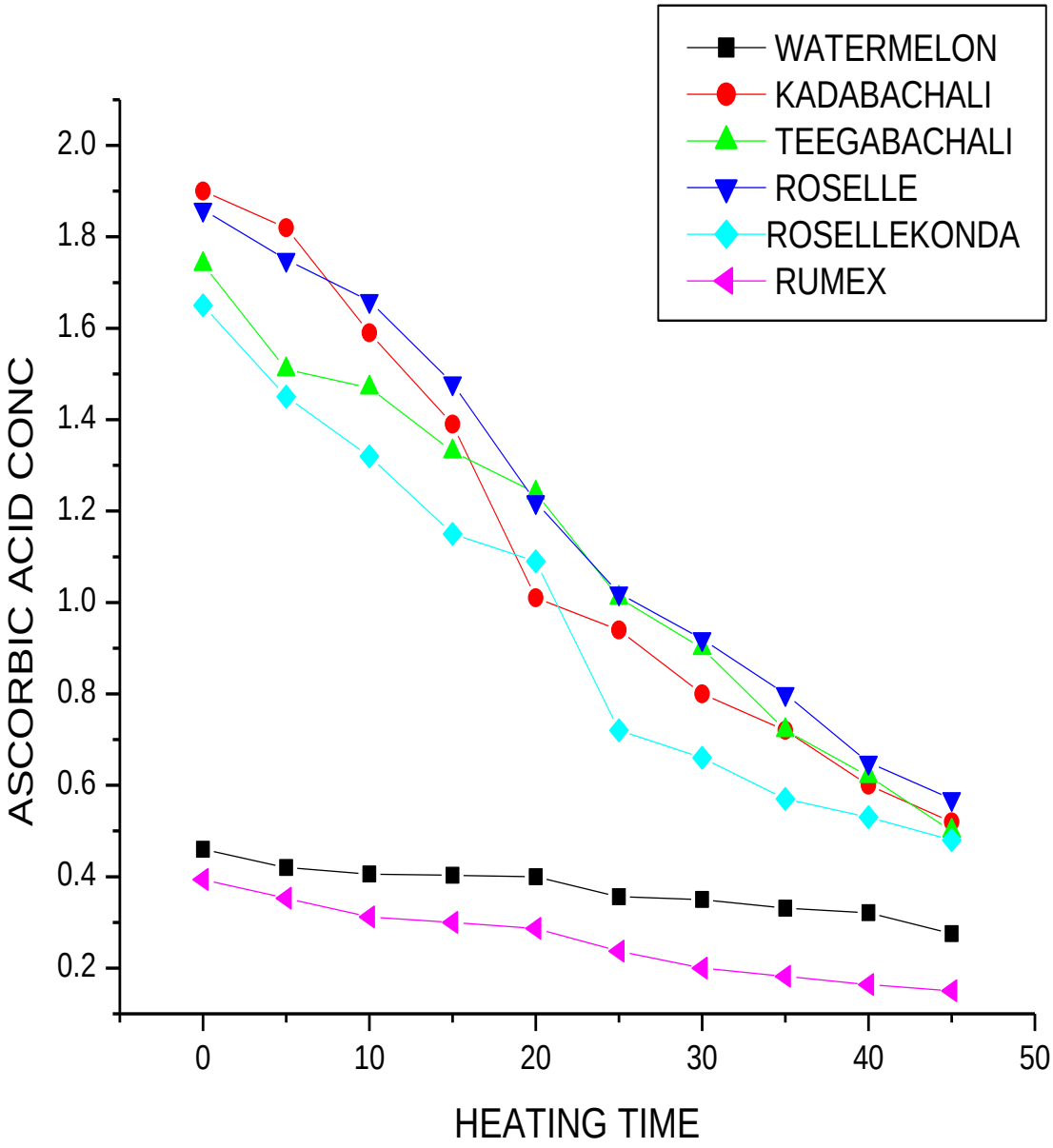
Table 3:

Types of Musa Species	Concentration of Vitamin C in Unripe Fruit (g/mol)	Concentration of Vitamin C in Ripe Fruit (g/mol)
Banana(Musa paradisiaca)	0.321	0.274
Chakkarakeli Banana	0.322	0.324
Green Plantains (Raw Banana)	0.36	0.3

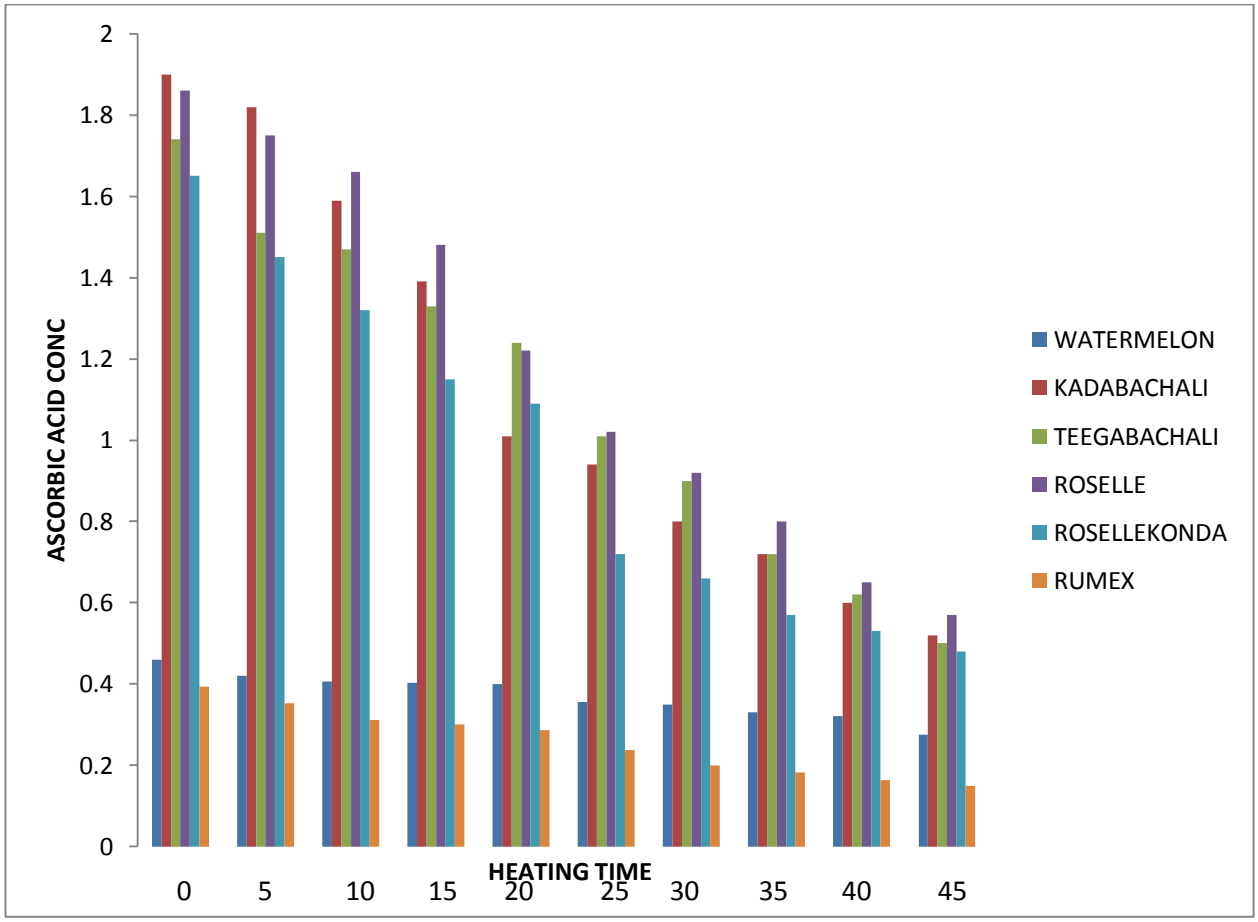


Graph 3

Graph showing ascorbic acid concentration of fruits and vegetables variation with heating time



Graph 4



Graph 5

Table 5 : Water Melon:

S.no	Temperature (in degree centigrade)	Concentration of Vitamin C (g/mol)
1	0min	0.46
2	5 min	0.42
3	10min	0.406
4	15min	0.403
5	20min	0.4
6	25min	0.356
7	30min	0.35
8	35min	0.331
9	40min	0.321
10	45min	0.275

Table 6 : Basella Alba (kadabachali):

S.no	Temperature (in degree centigrade)	Concentration of Vitamin C (g/mol)
1	0 min	1.9
2	5 min	1.82
3	10min	1.59
4	15min	1.39
5	20min	1.01
6	25min	0.94
7	30min	0.80
8	35min	0.72

9	40min	0.60
10	45min	0.52

Table 7: Basella Alba (teegabachali):

S.no	Temperature (in degree centigrade)	Concentration of Vitamin C (g/mol)
1	0 min	1.74
2	5 min	1.51
3	10min	1.47
4	15min	1.33
5	20min	1.24
6	25min	1.01
7	30min	0.90
8	35min	0.72
9	40min	0.62
10	45min	0.50

Table 8 : Roselle (Hibiscus sabdariffa):

S.no	Temperature (in degree centigrade)	Concentration of Vitamin C (g/mol)
1	0 min	1.86
2	5 min	1.75
3	10min	1.66
4	15min	1.48
5	20min	1.22
6	25min	1.02
7	30min	0.92
8	35min	0.80
9	40min	0.65
10	45min	0.57

Table 9: Roselle konda (Hibiscus cannabinus):

S.no	Temperature (in degree centigrade)	Concentration of Vitamin C (g/mol)
1	0 min	1.65
2	5 min	1.45
3	10min	1.32
4	15min	1.15
5	20min	1.09
6	25min	0.72
7	30min	0.66
8	35min	0.57
9	40min	0.53
10	45min	0.48

Table 10: Rumex:

S.No	Temperature (in degree centigrade)	Concentration of Vitamin C (g/mol)
1	0 min	0.394
2	5 min	0.353
3	10min	0.312
4	15min	0.3
5	20min	0.287
6	25min	0.237
7	30min	0.20
8	35min	0.182
9	40min	0.164
10	45min	0.15

Results:

The concentration of ascorbic acid in amla, banana and cucumber can be determined by using colorimetric method. By taking standardascorbic acid values, plot was drawn and then the concentrations of samples were determined by using calibration curve.

By observing the graph given above concentration of vitamin Cis more in unripe amlathan fully ripe and ripe, whereas in Indian goose berry the ripe amla has more ascorbic acid than fully ripe and unripe.

The concentration of ascorbic acid in bananas is more in unripe fruit than ripe fruit, whereas in chakkarakeli banana the ripefruit has more ascorbic acid than unripe fruit and in green plantains (raw banana) the unripe fruit has more ascorbic acid than ripe one.

Thequantity ofvitamin C in the fully ripen cucumber is more than unripe and ripe, whereas the in unripe courgeletits ascorbic acid concentration is more than fully ripe and ripe.

The quantity of ascorbic acid in water melon, roselle, roselle (konda), teegabachali, kadabachali and rumex decreases with increase in temperature during blanching.

Discussion:

The present work includes determination of ascorbic acid concentration by colorimetric method. In this method, the absorbance of sample and ascorbic acid were measured at wavelength 450nm.Then drawn a graph for concentration vs absorbance and determined the concentration of ascorbic acid in taken samples.

Bananas:

From the above table, the concentrations of ascorbic acid in unripe and ripe bananas were 0.321 and 0.274g/mol. Similarly in ckakkarakeli bananas and green plantains (raw bananas) were 0.322, 0.324 g/mol and 0.360, 0.300 g/mol respectively. Hence the vitamin C in Musa helps the body to resistant against infectious agents and scavenges harmful oxygen-free radicals.

Amla:

Its ascorbic acid concentration in unripe and ripe and fully ripe amla was 0.418, 0.356 and 0.408 g/mol. Similarly in Indian gooseberry were 0.05, 0.062 and 0.042 g/mol respectively. So taking of amlaleads to maintain vitamin C percentage in the body.

Cucumber:

Ascorbic acid concentration inunripe, ripe and fully ripen cucumber were 0.3625, 0.3875 and 0.399 g/mol. Similarly in courgelet were 0.3855, 0.376 and 0.3625 respectively. So wide use ofthe cucumbers helps to maintain water and Vitamin C in the body.

Watermelon:

The concentration of vitamin C in water melon is 0.42 g/mol. It diminishes with increase in temperature at regular intervals of time 0, 5, 10, 15,20,25,30,35,40,45 minutes slowly upto 0.27g/mol. So it should be consumed without any processing. It reduces body temperature.

Kadabachali:

Ascorbic acid in kadabachali is 1.9g/mol. It decomposes with increase in temperature at regular intervals of time 0, 5, 10, 15,20,25,30,35,40,45 minutes slowly upto 0.52g/mol. It should be taken with less blanching time to provide mucilage to body.

Teegabachali:

In teegabachalithe vitamin C concentration is 1.74g/mol. It decreases with increase in temperature at regular intervals of time 0, 5, 10, 15,20,25,30,35,40,45 minutes slowly upto 0.50g/mol. So it is consumed with less heating timeto attain more fibre.

Roselle:

The concentration of ascorbic acid is 1.86g/mol. It declines with increase in temperature at regular intervals of time 0, 5, 10, 15,20,25,30,35,40,45 minutes minimallyupto 0.57g/mol. To attain more in its concentration it is consumed with less processing time to reduce hypertension.

Roselle (konda):

Its ascorbic acid concentration is 1.65g/mol. It decreases with increase in temperature at regular intervals of time 0, 5, 10, 15,20,25,30,35,40,45 minutes slowly upto 0.48g/mol. It is good to consume with low processing time to protect against cold, thrist etc.

Rumex:

The concentration of ascorbic acid in rumex is 0.394g/mol. It diminishes with increase in temperature at regular intervals of time 0, 5, 10, 15,20,25,30,35,40,45 minutes minimally upto 0.15g/mol. So it is consumed with less heating time to attain more absorption of iron.

Conclusion:

The colorimetric method is the most reliable, accurate and easy method to determine the ascorbic acid concentration. Itis concluded that in bananas the unripe fruit has more ascorbic acid than ripen fruit, whereas in chakkarakeli banana the ripe fruit has more ascorbic acid than unripe fruit and in green plantains (raw banana) the unripe fruit has more ascorbic acid than ripe one by applying the colorimetric method. So it is advisable to take Musa species which are needed for the growth and repair of tissues in all parts of human body and also as an antioxidant. Therefore taking of bananas decreases the risk of heart diseases and reduces the constipation problems.

It isconfirmed that in case of phyllanthus emblica the unripe amla has more ascorbic acid than fully ripe and unripe by applying the colorimetric method. So it is needy thing to take phyllanthus species because it is a potent source of vitamin C and also contains iron and calcium. Consumption of amla helps in reducing arthritis related pain, cures sore throat, cold and manages constipation troubles.

In case of cucumbers, the fully ripen cucumber has more ascorbic acid than unripe and ripen, whereas the unripe courgelet has more ascorbic acid than fully ripe and ripe which is identified by the colorimetric

method. Cucumbers contain an anti-inflammatory flavonol called fiestin that appears to play an important role in brain health. In addition to improving memory and protecting nerve cells from age-related decline, fiestin has been found to prevent progressive memory and learning impairments in mice with Alzheimer'sdisease. By taking the cucumbers during summer it protects from dehydration because cucumbers are having 95% of water content and are good sources of phytonutrients which have anti-inflammatory and antioxidant activities according to "World's healthiest foods."

Taking of watermelon gives vitamin C and causes soothing effect to the body.

In leafy vegetables like rumex, roselle, roselle (konda), teegabachali, and kadabachali it is preferred to consume them with less processingtime by thermal exposure to absorb more concentration of vitamin C and to avoid more destruction.The present work concludes the quantity of ascorbic acid in sample vegetables and fruits and also gives an idea at what stage they are preferred to take and get higher concentration of vitamin C.

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