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# DETERMINATION OF AMOUNT OF ASCORBIC ACID PRESENTIN CITRUS FRUITS AVAILABLE IN BUTWAL SUB-METROPOLITAN CITY NEPAL

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## ABSTRACT

*In this study, five samples of citrus fruits; Lemon, Orange, Sweet orange, Tangerine, and lime were collected from the local market of Butwal City and analyzed for pH and ascorbic acid contents in them. All these studies were carried out in the laboratory of Butwal Multiple Campus from 10 to 15 December 2021. The study compared the concentration of ascorbic acid in different citrus fruits. Determination of ascorbic acid was carried out by iodometric titration and DCPIP titration method while pH was measured by a digital pH meter. The highest amount of Ascorbic acid was found in Tangerine and the least in lime. The amount of ascorbic acid (mg) found in 100 ml of fresh juice of the lime, lemon, Tangerine, sweet orange,and orange citrus fruits were 39.09±1.08, 49.81±0.86, 70.4±1.6, 63.6±2.13, 52.8±2.44respectively.Similarly lowest pHis found in lime and the highest in sweet orange.*

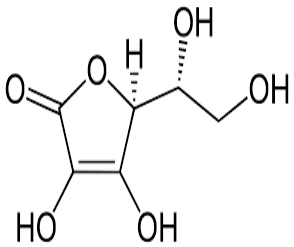
**KEYWORDS:***Ascorbic acid, DCPIP, iodine titration, citrus fruits, Ph.*

## INTRODUCTION

Vitamin C or Ascorbic Acid is a citrus fruit, a white crystalline substance; it is easily soluble in water and easily oxidized, especially in an alkaline medium and exposure to heat and light. It is fairly soluble in cold acidic solutions. The water-soluble carbohydrate-like substance involved in the certain metabolic process of animals. Although most animals can't synthesize Vitamin C, it is necessary forthe diet of some including men and other primates. To prevent scurvy disease characterized by hemorrhage, especially in skin and mucus membranes Vitamin C was identified as a curative agent for scurvy in 1928. Ascorbic Acid is also known as Vitamin C or L-ascorbic acid or antiscorbutic vitamin.

Ascorbic Acid (AA) is the most reducing agent known to occur naturally in living tissue and is easily reversibly oxidized to Dehydroascorbic Acid (DHA). Vitamin C plays important role in controlling infectious and the body's response to stress. It is also found to be a powerful antioxidant that can neutralize harmful free radicals and helps more collagen, a tissue needed for healthy bones and teeth, gums, and blood vessels. Exposure to oxygen, light, metals, and heat destroys Ascorbic Acid. So, it must be stored in a dark, cold, and non-metal container. The L-

Ascorbic Acid is known as Vitamin C. The name Ascorbic Acid came from its property of preventing and curing scurvy.Ascorbic Acid and its sodium, potassium, calcium, and salt are commonly used as antioxidant food additives. For this purpose, the fat-soluble ester of ascorbic Acid with long chain fatty acid can be used as food preservatives.The structure of Ascorbic acid ($C_6H_8O6$) is given below:




Citrus trees and their fruits are very popular in Nepal. Citrus trees grow on clay as well as on sandy soil with properties like fertile, acidic, and good water drainage to infertile,alkaline, and poor water drainage (Reuther et al., 1973). The citrus species are evergreen and medium-sized trees that give fruit of different forms and sizes. They belong to the Rutaceae family containing full of fragrance.Flavor and juice. Citrus fruit has rough robust and bright color skin which protects the fruit from damage (Okwu et.al., 2007). There is three temperature parameter that strongly influences the quality and consumption of citrus fruits. They are total available heat, the extent of low and high temperature duringthe maturation and growth period of fruits. Among this temperature parameter total available heat is the most important factor in determining the growth rate and time of ripening of citrus fruits (Jones1961). Considerable research has been conducted to clarify the biochemical functions of Vitamin C in humans and animals.Albert Szent-Gyorgyi in1928 was successful to accumulate less amount of an off-white crystalline substance from the cortex of cattle. After the discovery of this off-white crystallinecompound, he had done a series of test on it.The result showed that the substances can decolorizeiodine. Next, it also lowers the vapor pressure of the water, which gave the relative molecular mass of the crystal to about 180g/mol.He also did the combustion analysis on the compound,which gave 40.7% carbon,4.7% hydrogen, and 54.6% oxygen. This data enables him to deduce the $C_6H_8O_6$ as the molecular formula for this compound. Later he named the compound ‘Hexuronic Acid’ but finally changed to Ascorbic acid which means prevention of scurvy. Davies in 1991and Townsend Chet in1999 studied the Vitamin C in citrus juices. They found that orange juice made from frozen concentration orange juice (FCOJ) has the highest Vitamin C levels as compared to freshly squeezed. This is probably because Vitamin C degrades over time in fresh but doesn’t degrade as much as in FCOJ due to it being frozen until reconstitution. Ascorbic Acid is the most abundant Vitamin in orange, lemon, and grapefruit (Ralph and Bender 2007. More than 90% of the Vitamin C in the diet is supplied by fruits and vegetables. It is synthesized by most organisms from glucose but man and other primates and various other species must obtain it from their diet (Alibone, 2000). Vitamin C is an essential nutrient that plays an essential role in protecting the body from infection and diseases.It is necessary forthe synthesis of collagen in connective tissue, neurotransmitters, a steroid hormone, and conversion of cholesterol to bile acid and enhances iron bio-availability (Robert, 2000). The health benefits of citrus fruits have mainly been attributed to the presence of bioactive compounds, such as ferulic acid; hydrocinnamic acid;

cyanidin-glucoside; hesperidin; vitamin C; carotenoid, and naringin contents (Abeysinghe et al., 2007; Xu et al., 2008)

Nowadays, health has become the most important property of human life. Commonly, diets with high content of antioxidants and phytochemicals in fruits are protective against several human diseases such as cardiovascular disease and even cancer. Therefore, people are putting more and more attention on anti-oxidant substances such as Vitamin C. Vitamin C is probably one of the most highly well-known.Furthermore, people have become more aware of the importance of Vitamin C.Hence,this causesthe global market flooded with Vitamin C fortified foods.(Arya, Mahajan and jain,2000).The great importance of Vitamin C has gained increased significance in several areas of analytical chemistry such as pharmaceutical and food applications (Yusuf and Gruel,2005).Studies on Vitamin C content in food are important for control of nutritional levels, the update of food databases, and the establishment of dietary reference intake, orange juice is probably the most globally accepted fruit juice and is recognized worldwide as a good source of ascorbic Acid (Sharma, Singh and Saxena 2006).

Huma Tareen and her co-workers (2007) studied the determination of vitamin C content in citrus fruits and Non-citrus fruits by titrimetric method, with special reference to their nutritional importance in the human diet. A.Izugie and F.O.Izugie (2007) studied the Iodometric determination of ascorbic acid in citrus fruits to know which fruit would best supply the Ascorbic Acid need for the body. Igwe et al., 2013 showed that Vitamin C concentration of unripe fruits decreased when the temperature and length of exposure of fruit were increased. Storage temperature and handling also are important in maintaining their Vitamin C content in fruit samples in addition, citrus fruits are grown in a fully irrigated system during flowering, and the fruit growing stage and ripening showed increased concentration of Vitamin C. Fereshteh Khosravi and HamidehAsadollahzadeh (2014) studied the determination of ascorbic acid in different citrus fruit under reversed phase condition with UPLC. Shrestha et.al. (2015) studied the determination of Ascorbic Acid in the citrus fruit of Kathmandu valley. Iodine Titration method, indophenol titration method, and spectrophotometric method were used by them. In their study Ascorbic Acid was found highest in Pomelo juice followed by grapefruit, Lemon, Sweet orange, and Citron. This difference may be the reason that the Ascorbic Acid content of citrus fruit is never stable but varies with some factors which include position on the tree, climatic/environmental condition, ripening stage, species, and variety of citrus fruits as well as temperature. (Holcombe, 1992)

The differences in the content of Ascorbic acid might be a result of variation in maturity stage and regional varieties of fruits. Different techniques of measuring and squeezing processes may also affect the ascorbic acid content of the juice.Factors including climate, temperature, amount of fertilizer used in growing plants, and various physical conditions such as light can also affect the concentration of Ascorbic Acid in fruits. The amount of Ascorbic Acid content in juice can also be affected by the type and duration of storage. KebenaGebeyehuMotura (2017) studied the Ascorbic Acid content of fruit juice by Iodometric titration. She found thatamount of Vitamin C varied between different samples of the same species. Different techniques of measuring and squeezing processes may also affect the Vitamin C content of fruit juices. Factors including temperature, climate and amount of Nitrogen fertilizers used in growing the plant, and climatic conditions such as light type of storage, can affect the concentration of Ascorbic Acid. Fruit juice must be stored at a cool temperature storing fruit at a low temperature doesn’t lose Vitamin C

while a high-temperature results in loss of Vitamin C content. FatinNajwa.R. and Azrina, (2017) from their study on the topic "Comparison of Vitamin C content in citrus fruit by titration and HPLC method" stated that the Vitamin C content in fruit sample was higher in the titration method compared with HPLC method. The significant difference between the two methods could be affected due to many factors such as lack of specificity, presence of reducing substances, time consumption, and exposure to air.

Stress: It is also found to be a powerful antioxidant that can neutralize harmful free radicals and helps more collagen, a tissue needed for healthy bones and teeth, gums, and blood vessels.Exposure to oxygen, light, metals, and heat destroys Ascorbic Acid. So it must be stored in a dark, cold, and non-metal container. The L-enantiomer of Ascorbic Acid is known as Vitamin C.The name Ascorbic Acid came from its property of preventing and curing scurvy.Ascorbic Acid and its sodium, potassium, calcium, and salt are commonly used as antioxidant food additives.These compounds are water-soluble and thus cannot protect fact from oxidation. For this purpose, the fat-soluble ester of ascorbic Acid with long chain fatty acid can be used as food preservatives.

A recent survey of USAID shows that the average intake of Vitamin C by American adults was over the AI for Vitamin C. Women tended to consume less than men of the same age. Taking too much Vitamin C is reported to cause side effects such as nausea and diarrhea. Vitamin C is lost from food during preparation, cooking, or storage. To prevent loss of Vitamin C, serve fruits and vegetables raw whenever possible.Refrigerate prepared juice and store them for no more than two to three days.Store, and cut raw fruits and vegetables in an airtight container and refrigerate. Do not soak or store in water.Citrus fruits have been very long valued as part of (https://www.medicalnewstoday.com/articles/301506)a nutritious and tasty diet. Theflavors provided by citrus fruits are most preferred in the world and is increasingly evident that citrus not only tastes good but is also good for people. It is well established that citrus and citrus products are a rich source of vitamins, Minerals, and dietary fiber that are essential for normal growth and development and overall nutritional well-being. Citrus is most commonly thought of as a good source of vitamin C. Citrus fruits are used to treat cardiovascular diseases, and a diet low in saturated fat and cholesterol and rich in fruits and vegetables reduces the risk of heart disease. Heart disease appears to be a high level of oxidized low-density lipoprotein (LDL) i.e.bad cholesterol. Significantly a recent study has shown that a high intake of Vitamin C (500mg/day)obtained from the juice of freshly squeezed oranges, prevented a rise in the level of oxidized LDL, even in the presence of a high saturated fat diet (Harats et. al.1998).Vitamin C also protectsfrom cancer. The epidemiological studies showed that protective effects are more closely associated with the consumption of fruits and vegetables rather than the enormous level of Vitamin C. Anemia is the most serious nutrient-related public health problem, resulting in poor growth, impaired, psychomotor development, reduced physical performance, and decreased cognitive function. Consuming citrus fruits rich in Vitamin C can help prevent anemia and its devasting consequences.Scurvy is a disease resulting from a lack of vitamin C. Early symptoms include weakness, feeling tired and sore arms and legs. Scurvy can be prevented by a diet that includes vitamin c rich foods such as amala, broccoli, bell peppers, guava, kiwifruit, and parsley. Other sources rich in vitamin C are fruits such as lime lemons orange etc. Scurvy currently is rare. It occurs more often in the developing world in association with malnutrition. Vitamin C (Ascorbic Acid) is the main nutrient. Our body needs to form blood vessels, cartilages, muscles,

and collagen in bones. Vitamin C is also vital to our body's healing process. It acts as an antioxidant and helps to protect our cells against the effect of free radicals so it protects us from different diseases.

The main objective of the study was to determine the amount of Vitamin C in different fruits, to give baseline information about deficiency of Vitamin C and its effects, and tocomparethe reliability of the iodine titration method and DCPIP titration method for Ascorbic Acid determination. Some limitations of the study are:constraint budget and time, shortage of chemicals, and well-equipped instrument, the study is carried out taking limited samples.Experimental error observational error and calculation error may cause deviation in the actual result.

## MATERIALS AND METHOD

### Samples collection

After fixing the study sites,differentcitrus fruits had been collected from the different regions of Butwal city. Five different types of citrus fruitsviz: Lime, lemon, Sweet orange,Tangerine, and Orange were collected from the local market of Butwal city from December 5 to 10. 2021. The samples were analyzed after a few hours of collection.

### Data collection, Analysis, and Interpretation

The primary data were collected from the lab afterthe experiment. The analysis was done as per Steel and Torrie (1980), using Completely Randomized Design (CRD). The raw data was edited properly and organized in the form of tables, and later on,the calculation was done and the results were again tabulated. The data were analyzed using appropriate statistical tools such as bar diagrams; line graphs pie charts, etc.

### Chemicals required and Reagents

All reagents used are analytical grades such as Iodine, Potassium Iodide, Distilled water, Starch, DCPIP(2,6-dichlorophenolindophenol)(Merck India), Ascorbic acid,$H_2SO_4$,6% metaphosphoric acid,etc.

**Apparatus required:** Conical flask, Volumetric flask, Muslin cloth, Burette, Pipette, Squeezer, Measuring cylinder**,** Electronic balance, breaker**,** Watch glass, etc.

### Preparation of Iodine solution

0.254g of solid iodine was weighed and poured into a dry volumetric flask. Then 4gm ofpotassium iodide was added and dissolved by adding distilled water and making a 100ml iodine solution. That solution has a molarity of 0.01M.

### Preparation of DCPIP solution

Accurately Weighed out 0.2-gram DCPIP (mol.wt=268.1g) dissolved in 1000 ml distilled water.This was very hard to dissolve,left overnight, and filtered.The molarity was 0.000895.It was standardized with std. ascorbic acid solution(0.00116M)

**Starch Solution:**To prepare a starch solution, a spatula of starch was added to 100ml of distilled water and subsequently boiledand filtered.

**Extraction of Juice fromFruits**

The fruits of five different samples were collected,cut in half using a knife, and juice was extracted using a squeezer and then the juice from the fruits was filtered by using a muslin cloth.Fresh juice was stored in the refrigerator until they were analyzed.

**METHODS USED FOR DATA ANALYSIS**

| **Parameters** | **Methods Employed** |
|---|---|
| **pH** | **Auto digital pH meter (HI 98107, HANA Romania)** |
| **Determination of Ascorbic acid** | **Iodometric titration Method** |
| **Determination of Ascorbic acid** | **DCPIP Titration method** |

**PH Measurement of Sample**

At first, the juice extracted from the citrus fruits was kept in a beaker. After that PH meter was calibrated with a buffer solution of PH 9 and PH 4.Then PH meter was dipped in juice and PH was noted after some minutes of dipping. The process was repeated for each sample solution.

**Iodine Titration of a Sample**

The iodinetitration method by Nwezeet al., (2015) was used to determine the concentration ofVitamin C followed in freshly prepared fruitjuices. Iodine solution of 0.01M is kept in burette.5ml of prepared juice sample was taken in each of six 100ml conical flasks, 2ml of 1M H2SO4 was added and titrated using standard iodine solution using 1ml of starch solution as indicator till the appearance of dark violetcolor and then amount of Ascorbic Acid was calculated(Table3).

**DCPIP dye Titration Method for Determination of Ascorbic Acid**

Ascorbic acid was determined according to the method Mau et al.,(2005). Citrus fruit juice was mixed with an equal volume of 3% metaphosphoric acid solution and then filtered through fresh cotton. 5 ml of this filtrate was taken in each of six 50 ml conical flasks and 2 ml of Metaphosporic acid acetic acid ($HPO_3$-HOAC) was added and titrated with Indophenol dye and the amount of ascorbic acid was calculated (Table 4).

**CALCULATION**

**Preparation of 100ml 0.01M of Iodine solution**

Accurately0.254gm (mol.wt=254) solid Iodine and 4 gram of KIwas taken and dissolved in 100ml distilled water in a volumetric flask.

Molarity= No.of moles of Iodine in 100ml of solution of iodine =$\frac{0.254\times1000}{254\times100}$=0.01M

**Calculation of the Amount of Ascorbic Acid by the Iodine TitrationMethod**

The volume of Iodine solution used ($V_1$) =Xml

Molarity of Iodine solution ($M_1$) = 0.01M

The volume of juice used ($V_2$) = 5ml

Let, Molarity of juice be ($M_2$) = $\frac{0.01 \text{ x } x\text{ml}}{5 \text{ ml}}$

$=0.002x$ml

Wt. of ascorbic acid (mg) present in 5ml of juice= 5×mol.wt of AA×molarity of juice

$=5\times176\times0.002$ xs

Mass of Ascorbic Acid (mg) per 100 ml of juice= $\frac{\text{Wt of ascorbic acid in 5 ml juice}}{\text{volume of juice}}$ x 100

**Calculation of Mass of Ascorbic Acid by DCPIP Titration Method**

Mass of ascorbic acid (mg) per 5 ml of juice= mol.wt of AAx M(DCPIP)x Vol.of DCPIP

**DETERMINATION OF ASCORBIC ACID IN FRUIT JUICES BY IODINE TITRATION METHOD**

| Fruit samples | Vol.of Fruit Juice | vol. of iodine soln consumed(ml) | Amount of AA per 100 ml juice mean±SD |
|---|---|---|---|
| Lime | 5 ml | 1.1 | 38.72±4.23 |
| Lemon | 5ml | 1.3 | 45.76±2.11 |
| Sweet Orange | 5ml | 1.8 | 63.36±2.13 |
| Tangerine | 5ml | 2 | 70.4±1.6 |
| Orange | 5ml | 1.5 | 52.8±2.44 |

**TABLE 1: CALCULATION OF THE AMOUNT OF VITAMIN C IN DIFFERENT FRUIT JUICES, AA=ASCORBIC ACID**

**DETERMINATION OF ASCORBIC ACID IN CITRUS FRUITS JUICES BY DCPIP DYE TITRATION METHOD**

| Fruit Samples | Vol.of samples(ml) | Vol. of DCPIP soln consumed(ml) | Amount of AAper 100ml juices mean±SD |
|---|---|---|---|
| Lime | 5ml | 12.4 | 39.09±1.08 |
| Lemon | 5ml | 15.8 | 49.81±0.86 |
| Sweet Orange | 5ml | 19.5 | 61.47±1.72 |
| Tangerine | 5ml | 22.2 | 69.98±0.6 |
| Orange | 5ml | 16.3 | 51.38±1.2 |

**Table 2: Calculation of the amount of Ascorbic acid in Different Fruit Juices,AA=Ascorbic acid**

**RESULT AND DISCUSSION**

In this research work, five different samples of citrus fruit juice were tested to determinethe concentration of Vitamin C and their PH levels. The samples of fresh juice were analyzed forascorbic acidbythe Iodine titration method and DCPIP titration method.Lime and lemon

showed more ascorbic acid in the iodine titration method, while tangerine, sweet orange, and orange showed more ascorbic acid in the DCPIP titration method.Tangerine showed max.ascorbic acid(70.4±**1.6**),followed bysweet orange(63.6±**2.1),** andlime showed the least(39.09±1.08).The lime juice has the lowest pH(more acidic), followed by lemon(2.2), while sweet orange has the highest pH 4.1(less acidic). PH ofdifferent fruit juices are shown below(table 3).

**TABLE 3: PH MEASUREMENT OF DIFFERENT CITRUS FRUIT JUICE**

| S.N | Different Samples of Fruit Juice | pH |
|---|---|---|
| 1 | Lime | 1.9 |
| 2 | Lemon | 2.2 |
| 3 | Tangerine | 4 |
| 4 | Sweet Orange | 4.1 |
| 5 | Orange | 3.8 |

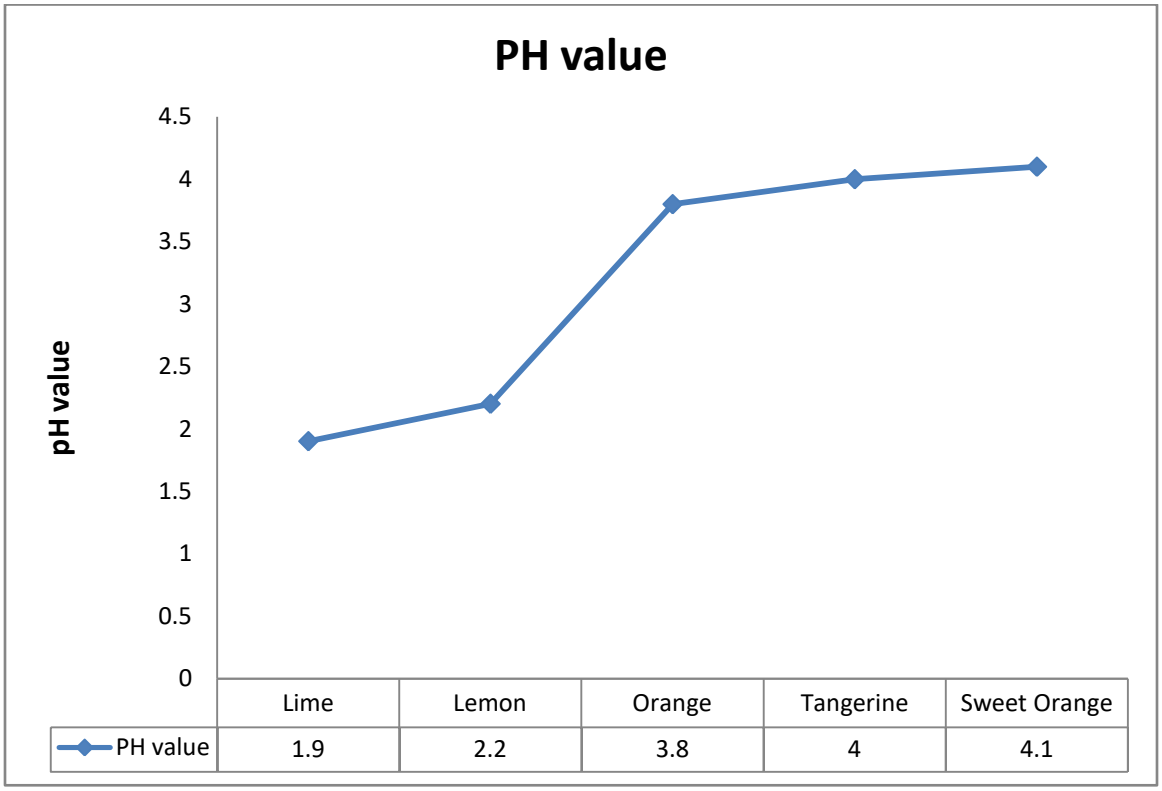



**Fig1: graphical representation ofthe variation of pH in different citrus fruit juices.**

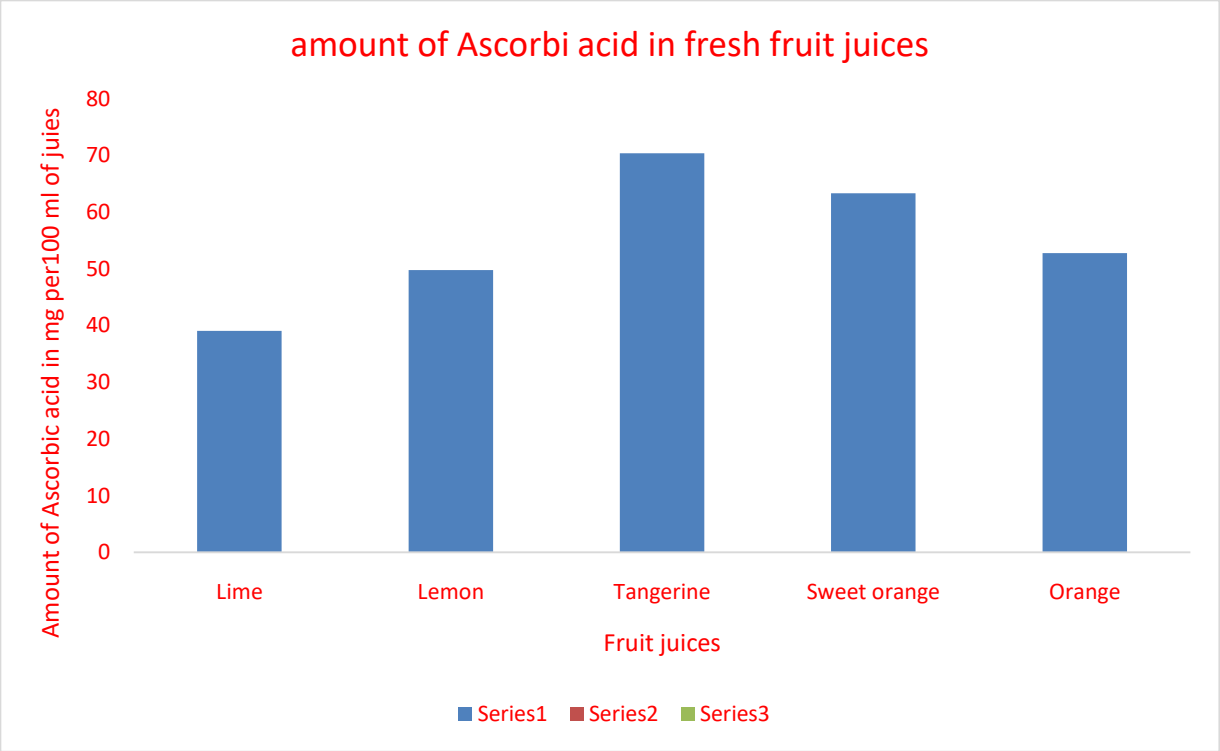



**Fig2: graphical representation of variation in concentration of Vitamin C in different fruit juices.**

From the pH measurement table, Lime is the most acidic with PH 1.9 and sweet orange is the least acidic with pH 4.1 among five samples of citrus fruits. It can be also deduced that all five samples of juice have different titer valuesfor attaining their respective endpoints. Tangerine would require a significantly greater amount than that lime.

From table 1, the concentration of Vitamin C in fresh fruit juice Tangerine is higher than in Sweet Orange followed by Orange, Lemon, and Lime. It was well known that fresh fruit juice normally contains more Vitamin C compared to commercial fruit juice. The amount of Ascorbic Acid content in fruit juice is affected by different factors. Therefore, fruit juice can be stored at a cool temperature for its Ascorbic Acid content does not decrease.

## CONCLUSION

The concentration of ascorbic acid varies from one citrus fruit to another citrus fruit and also varies from one method to another method of determination. The iodine titration method and DCPIP titration method are simple and easy methods. The average concentration of ascorbic acid is about 70.4±1.6 mg/100 ml for tangerine and about 39.09±1.08mg/100ml for lime.Lime is the most acidic (pH1.9), while sweet orange is the least acidic (pH 4.1) among five citrus fruits collected fromButwal city of Nepal.

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