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Determination of Ascorbic Acid in Different Citrus Fruits of Kathmandu Valley

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ABSTRACT

This study compared the concentration of ascorbic acid between citrus fruits collected from the area of Kathmandu valley of Nepal. Ascorbic acid was determined for six species of citrus: *Lemon* (*Citrus limon*), Bitter Orange (*Citrus aurantium*), Sweet Orange (*Citrus aurantium var. sinensis*), Pomelo (*Citrus maxima)*, Grapefruit (*Citrus paradisi*) and Citron (*Citrus medica*). Determination of ascorbic acid was carried out by iodometric titration, dye titration and spectrophotometric methods. In all three methods, the average concentration of ascorbic acid was found to be maximum in pomelo about 61.29 mg/100 ml and the least in citron about 17.4 mg/100 ml. The amount of ascorbic acid found in lemon, bitter orange, grapefruit and sweet orange were about 34.8 mg/100 ml, 29.89 mg/100 ml, 39.80 mg/100 ml, and 25.11 mg/100 ml respectively. The aim of this study is to compare the concentration of ascorbic acid among the citrus fruits collected from the area of Kathmandu valley of Nepal and also comparison is done with available literatures.

Key words: Ascorbic acid, Iodometric titration, Dye titration and Spectrophotometric methods

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INTRODUCTION

Citrus trees and their fruits are very popular in Nepal. Citrus trees grow on clay as well as on very sandy soils with properties fertile, acidic and good water drainage to infertile, alkaline and poor water drainage (Reuther, 1973). The citrus species are evergreen and medium sized trees that give fruits of different forms and sizes. They belong to the Rutaceae family containing full of fragrance, flavour and juice. Citrus fruits have a rough, robust and bright color skin, which protects the fruits from damage (Okwuand, 2007).

There are three temperature parameters which strongly influence on the quality and composition of citrus fruit. They are the total available heat, the extent of low and high temperatures during the maturation and the growth periods of the fruit. Among three temperature parameters, total available heat is the most important factor in determining the growth rate and the time of ripening of citrus fruit (Jones, 1961). The citrus fruits contain anti-oxidant, anti-inflammatory, anti-tumor, anti-fungal and blood clot inhibition activities (Abeysinghe et al., 2007). The consumption of citrus juice is found to be beneficial in preventing coronary diseases and chronic

Table 1. Determination of ascorbic acid content in each fruits according to their weight.

Name of Fruits	Weight (g)	Juice (ml)	Amount of ascorbic acid per (mg/piece)
Lemon	27.70	13	4.16
Bitter orange	86.50	34	9.86
Pomelo	99.93	43	25.80
Sweet orange	95.52	45.8	10.53
Grapefruit	599	170.5	75.65
Citron	180	47	7.20

Table 2. Standardization of reagents and standard deviation.

Titrants	Ascorbic acid Taken (mg)	Average volume of titrant consumed (ml)	Standard deviation (σ)
Iodine solution	100	15.46	0.047
DCPIP solution	0.5	3.8	0.057

asthma (Abd-Ghafar et al., 2010). It is also found that the regular consumption of citrus fruit juice protects the organisms from oxidative stress (Ebrahimzadeh et al., 2004; Fernandez-Lopez et al., 2005). So, over the last decades, the medicinal benefits of citrus fruits have been discovered besides the anti-scurvy property (Rapisarda et al., 1999).

These health benefits of citrus fruits have mainly been attributed to the presence of bioactive compounds, such as ferulic acid; hydrocinnamic acid; cyanidin-glucoside; hisperidin; vitamin C; carotenoid and naringin contents (Abeysinghe et al., 2007; Xu et al., 2008). We can classify citrus fruits as acid fruits because they are soluble solids are composed mainly of organic acids and sugars. For determination of ascorbic acid contents, (Arya et al., 1998, 2000) used spectrometry and amperometry techniques respectively, whereas Kabasakalis et al. (2000) used titrimetry as an analytical technique. Similarly, the different group (Ke et al., 1994; Zerdin et al., 2003; Franke et al., 2000) used liquid chromatography and then (Versari et al., 2004) used capillary electrophoresis and Silva, 2005 used gas chromatography. In this study, the determination of ascorbic acid from different species of citrus fruits from the Kathmandu Valley in Nepal such as: lemon; bitter orange; sweet orange; pomelo; grapefruit and citron, was determined using an iodometric titration; dye titration and spectrophotometric methods.

MATERIALS AND METHODS

Six species of citrus fruits were collected from different area of Kathmandu Valley, Nepal and were squeezed to extract juice and then filtered through fresh cotton. 0.05 M Iodine solution (Merck, India), 6% Metaphosphoric acid solution (Ranbaxy, India), Indophenol standard solution [DCPIP(2, 6-dichlorophenolindophenol), Merck, India], Standard thiosulphafate solution (Merck, India), 4% Oxalic acid (Ranbaxy, India), 1mg/ml of Ascorbic acid solution in Oxalic acid (Merck, India), concentrated H_2SO_4 (Ranbaxy, India), Starch (Merck, India) was prepared by using pure doubled distilled water.

Availability of Ascorbic Acid in Each Fruits Samples

The amount of ascorbic acid present in each single fruit has been shown in Table 1. From this table, we can deduce the number and size of fruits that should be taken to balance the daily need of ascorbic acid in the human body. 99.93 g of pomelo contained 25.80 mg which is the lowest ratio of weight to the amount of ascorbic ratio (3.87) whereas 180 g of citron contained 7.2 mg of ascorbic acid, which is the highest ratio of weight to the amount of ascorbic ratio (25.00). For the determination of ascorbic acid in different citrus fruits, first the standardization of the reagents has been carried out by titration. For the titration methods, Iodine solution and DCPIP dye solution have been standardized with authentic ascorbic acid as shown in Table 2. During the standardization of Iodine solution, 15.46 ml of iodine solution was required to oxidize 100 mg of ascorbic acid. Similarly, 3.8 ml of DCPIP dye was reduced to colorless by 0.5 mg of ascorbic acid. The ascorbic acid of lemon; bitter orange; sweet orange; pomelo; grapefruit and citron, was determined using an iodometric titration; dye titration and spectrophotometric methods.

Iodine Titration Method

Ascorbic acid was determined according to the method of Nweze et al. (2015). 25 ml of prepared juice was taken in

Table 3. Determination of ascorbic acid in citrus fruit juices by Iodine Titration method.

Fruits	Sample juice (ml)	* Volume of I_2 solution consumed (ml)	*Amount of ascorbic acid per100 ml juice mean ± SD
Lemon	25	1.2	31.05 ± 4.23 [a]
Bitter orange	25	1.1	28.46 ± 2.11
Pomelo	25	2.2	56.93 ± 2.13
Sweet orange	25	1.0	25.88 ± 1.46
Grapefruit	25	1.53	39.68 ± 3.22[b]
Citron	25	0.6	16.38 ± 2.44

[a](Swisher and Swisher, 1977), [b] (Krezdorn and Cain,1952) and *Average of three measurements.

Table 4. Determination of ascorbic acid in citrus fruits juices by DCPIP dye titration method.

Fruits	Volume of sample (ml)	*Volume of DCPIP solution consumed (ml)	*Amount of ascorbic acid per100 ml juice mean ± SD
Lemon	5	6.23	33± 1.08 [a]
Bitter orange	5	5.80	30.63 ± 0.86
Pomelo	5	12.63	66.70± 1.72
Sweet orange	5	4.16	22 ± 0.65
Grapefruit	5	7.03	37.5 ± 0.67 [b]
Citron	5	2.90	15.5 ± 0.42

[a](Swisher and Swisher, 1977), [b] (Krezdorn and Cain, 1952) and *Average of three measurements.

Table 5. Spectrophotometric determination of ascorbic acid.

Fruits sample	*Amount of ascorbic acid/100 ml (Mean ± SD)
Lemon	40.35 ± 0.22 [c]
Bitter orange	30.60 ± 0.78
Pomelo	60.25 ± 0.25
Sweet orange	27.45 ± 0.14 [d]
Grapefruit	42.23 ± 0.52[e]
Citron	20.32 ± 0.10

c(Dawes, 1969), d(Mudambiand, 1977), e(Edrissi and Kooshkabadi, 1975) and *Average of three measurements.

each of six 100 ml conical flasks. 10 ml of 1 M H_2SO_4 was added and titrated with a standard iodine solution using 2 ml starch as indicator till the appearance of blue color and then the amount of ascorbic acid was calculated (Table 3).

Indophenol (DCPIP) Titration Method

Ascorbic acid was determined according to the method of Mau et al. (2005). Citrus fruit juice was mixed with 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 Metaphosporic acid acetic acid (HPO_3-HOAC) was added and titrated with Indophenol dye and the amount of ascorbic acid was calculated (Table 4).

Spectrophotometric Method

Ascorbic acid was determined according to the method of Simona et al. (2011). 20 ml of citrus juices of each fruit was mixed with equal volume of 0.4% oxalic acid solution and then filtered through fresh cotton. Ascorbic acid was determined in Jenway, 6150 model UV-Vis spectrophotometer (India) by monitoring the pink color of indophenol dye produced in acidic medium at λ_{max} = 518 nm which was reduced to colorless by ascorbic acid, so intensity of color decreases with the increased amount of ascorbic acid (Table 5).

RESULTS AND DISCUSSION

Figure 1 illustrates the calibration curve for the

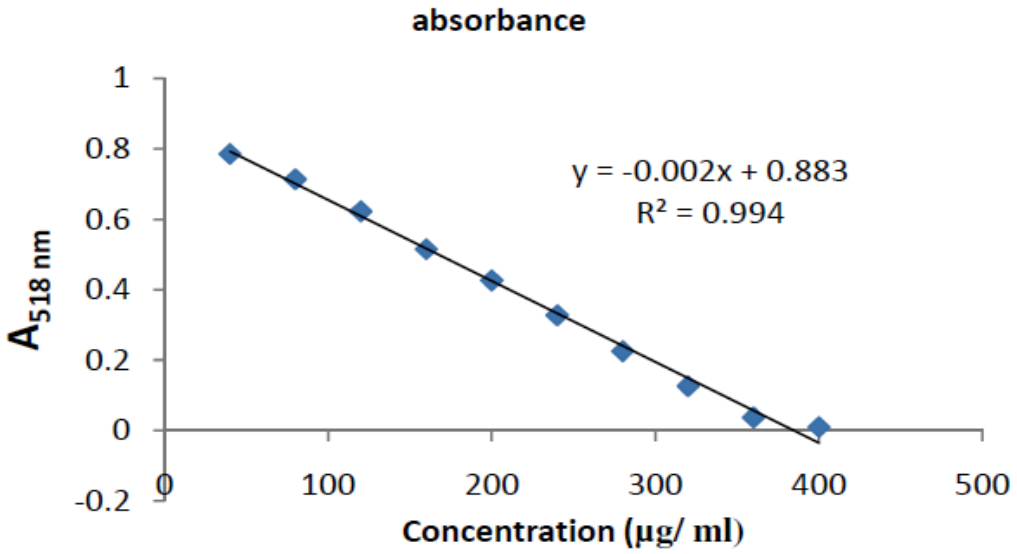

Figure 1. Calibration curve of ascorbic acid with absorbance, Y-axis versus concentration, X-axis.

determination of ascorbic acid, by monitoring the absorbance of residual dye at 518 nm in acidic medium. The absorbance due to the dye decreased with the increase in conc. of ascorbic acid, owing to the reduction of the pink color of the dye to colorless by ascorbic acid. The molar absorption coefficient of dye at λ_{max} was found to be $6.52 \times 10^{3} mol^{-1}$ cm^{-1}. It was found that pomelo contained the highest amount of ascorbic acid that is, 57 mg/100 ml of juice among the six citrus fruits included in Iodine Titration method (Table 3). Ascorbic acid in grapefruit and lemon was found to be 39.68 mg/100 ml of juice and 31.05 mg/100 ml of juice, respectively. Similarly, bitter orange and sweet orange contained 28.46 mg/100 ml of juice and 25.88 mg/100 ml of juices, respectively. On the other hand, citron contained the lowest amount of ascorbic acid, 16.38 mg/100 ml of juice. Results from dye titration showed that, pomelo contained the highest amount of ascorbic acid that is, 66.70 mg/100 ml of juice than other citrus fruits (Table 4). The results of iodine titration and dye titration were found to be close to each other. On the other hand, citron contained the lowest amount of ascorbic acid, 15.5 mg/100 ml of juice.

In spectrophotometric method, pomelo contained the highest amount of ascorbic acid that is, 60.25 mg/100 ml of juice whereas the least amount of ascorbic acid that is, 20.32 mg/100 ml compared with the other citrus fruits (Table 5). Also, results of the spectrophotometric methods were found to be close to the results of dye and iodine titration methods. The average ascorbic acid was the highest in lemon juice followed by sweet orange juice, sweet and white grapefruit in the study of Nour et al. (2010). But in the current investigated results, the average ascorbic acid was found to be highest in pomelo juice followed by grapefruit, lemon, sweet orange and citron. Nweze et al. (2015) found the ascorbic acid of sweet orange as 10.13± 0.10 mg/ 100 ml by Iodometric titration which was lesser value than our findings 25.88± 1.46 mg/100 ml from the same method.

This difference may be the reason that ascorbic acid content of citrus fruits is never stable but varies with some factors which include position on the tree; climatic/environmental conditions; ripening stage; species and variety of the citrus fruits as well as temperature (Holcombe, 1992).

The values of ascorbic acid found in nutrient composition tables of Malaysians Foods stated that ascorbic acid content of edible portion of pomelo was 44.8 mg/100 g FW stands for fresh weight (Tee et al., 1997), where as in the current study, the average ascorbic acid for pomelo was found to be 61.29 mg/100 ml. Ascorbic acid of citron fruit was found for to be 884 to 11,130 ppm and for fruit juice 300 to 445 ppm (Simona et al., 2011) whereas in our case, the average ascorbic acid for citron was about 17.4 mg/100 ml. Aurelia et al. (2011) reported ascorbic acid content of lemon juice 54.74 mg/100 ml and orange juice 39.25 mg/ 100 ml by using voltammetry performed at Carbon Paste Electrodes, whereas in our methods, the average ascorbic acid of lemon juice was found about 34.8 mg/100 ml and the bitter orange juice contained about 29.89 mg/100 ml. Okiei et al. (2009) reported that the ascorbic acid content of freshly prepared lemon juice is 48.61 mg/100 ml. Conversely, there are considerable

differences in the values of ascorbic acid obtained in the present study with those reported by several other studies for some fruit juice samples. All such differences in the contents of ascorbic acid in the present study and previous studies might be as a result of variations in maturity stage and regional varieties of fruits. Different techniques of measuring and squeezing process may also affect the ascorbic acid content of fruit juices.

The amount of ascorbic acid could even vary between the different fruit samples of the same species (Mahdavi et al., 2010; Tareen et al., 2015; Bekele and Geleta, 2015). Factors including climate; temperature; amount of nitrogen fertilizers used in growing the plant and various physical conditions such as light can also affect the concentration of ascorbic acid in fruits. The amount of ascorbic acid content in fruit juices can also be affected by the type and duration of storage. Therefore, it is necessary that fruit juices be stored at cool temperature in order for its ascorbic acid contents not to decrease.

CONCLUSION

The following conclusions have been drawn from the above results and discussion. 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 average concentration of ascorbic acid is about 61.29 mg/100 ml for pomelo and is about 17.4 mg/100 ml for citron. It is found that the average concentration of ascorbic acid in lemon; bitter orange; grapefruit and sweet orange are about 34.8 mg/100 ml, 29.89 mg/100 ml, 39.80 mg/100 ml, and 25.11 mg/100 ml, respectively. Hence the average concentration of ascorbic acid calculated from iodometric titration, dye titration and spectrophotometric methods are found to be maximum in pomelo whereas minimum in citron among six citrus fruits collected from the Kathmandu Valley of Nepal.

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