Comparative Analysis of Ascorbic Acid Concentration in Two Varieties of Citrus (*Citrus sinensis*, *Citrus limetta*) Collected from Different Tehsils of District Sargodha, Pakistan

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Abstract

Citrus is one of the most commonly consumed fruits in Pakistan. The present study was conducted to evaluate ascorbic acid concentration in *Citrus sinensis* and *Citrus limetta* collected from different tehsils of District Sargodha. All the varieties have good source of vitamin c, but the highest concentration was reported in *Citrus sinensis*, which was collected from tehsil Sahiwal and that was 89.69 mg/100 mL and lowest reported in *Citrus limetta* 33.155 mg/100 mL, which was collected from Tehsil Silanwali.

Keywords: Vitamin C; *Citrus sinensis*, *Citrus limetta*; District Sargodha

Introduction

Vitamin C, commonly famous as ascorbic acid, which is an important food constituent due to its antioxidant as well as therapeutic activities. It helps in the formation of collagen (a type of connective tissue) and imparts a key role as an antioxidant that acts as body defense system against reactive oxygen species and free radicals, thus preventing tissue damage and destroy cancer cells and overall improve the health of the body [1,2]. It is generally used in the treatment of many diseases such as scurry, common cold, anemia, hemorrhagic disorders, wound healing as well as infertility. Animals and humans obtain vitamin C from fruits and vegetables [1].

Citrus fruit and their products are one of the largest source of vitamin C. There are many factors affecting vitamin C concentration in citrus fruits, including production factors, climatic conditions, maturity stage, position of fruit on the tree where it is present on the tree, type of citrus fruit i.e., sweet lemon (*Citrus limetta*), sweet orange (*Citrus sinensis*) etc. Vitamin C concentration higher when the fruit is immature and declines as the fruit mature. Excessive heat and cold also affect vitamin C concentration in fruits [3,4]. Daily recommendation of vitamin C includes 75 mg/day and for adults it includes 90 mg/day, for children 45 mg/day [5]. A daily dose of 90-100 mg can prevent us from serious diseases like cancer, heart diseases, and cataracts [4]. More than 90% of the vitamin C in our diet comes from food including vegetables, fruits [6]. In animals vitamin C is important in wound healing and preventing bleeding from capillaries [7].

Global production of citrus is reported to be around 120 million tons and generate about 105 billion dollars per year all over the world. The size, color, juice quality and shape vary due to any factor. Seeds of citrus vary in color from greenish to pale whitish and size of flowers vary from 2-4 cm, calyx contain 4-5 lobes, generally five petals and contain some oil glands. Size of leaves vary from 2-8 cm, dark green in color. Medical uses of orange include anti-oxidant property, Protection against Cardiovascular Diseases, anti-carcinogenic property, reduce the risk of kidney stones, anti-ulcer property, anti-anxiety, anti-typhoid, anti-bacterial, larvicidal, anti-diabetic, anti-fungal, anti-inflammatory, healing, anti-arthritis activity. Other medical use of citrus include arthritis, asthma, alzheimer’s disease, parkinson’s disease, muscular degeneration, diabetes, gallstones, multiple sclerosis, cholera, gingivitis, optimal lung function, cataracts, ulcerative colitis, crohn’s disease, help to citrus plant including fruits, leaves, flowers, peels and juice are used as in homeopathic (Desi) medicines and people prefer these medicines because these are long term effective and not too much expensive. Overall citrus contain Vitamin B1, B2, B3, B5, B6, A, C, Flavonoids, terpenes, potassium and calcium [8]. Traditional uses of citrus include its juice help to eliminate toxins from the body, maintain hydration, general tonic, anxiety disorder, stress, treatment of tuberculosis, cough, cold, respiratory problems, in the treatment of obesity, innocence, fertility, angina, hypertension, constipation, diarrhea, menstrual disorder, palpitation. Citrus fruits can be stored at room temperature for 2-3 days but in refrigerator stored up to 14 days [8,9].

Materials and Methods

Sample collection

The different Tehsils of District Sargodha were selected for the purpose of sample collection. Fruits of two citrus varieties (*Citrus sinensis*, *Citrus limetta*) were collected from different Tehsils (Bhehra, Silanwali, Sargodha city and Sahiwal) for analysis (Tables 1-4). Each sample comprised of three replicates. Each sample was randomly handpicked, wrapped in a specific brown envelop and labeled (Figures 1-4).

Chemicals: The chemicals used for the experimental purpose in the experiment includes distilled water, potassium iodide 5 grams, potassium iodate 0.268 grams, conc. Sulphuric acid 30 mL, 1% starch solution, standard ascorbic acid 0.25 grams.

Sample preparation: The fruits were blended with a blender each and was filtered by using muslin cloth and made up to 100 mL with distilled water.

Preparation of reagents and estimation of ascorbic acid

First of all Starch indicator (1%) was prepared, adding 0.50 g starch to 50 mL of distilled water. Iodine solution was prepared by mixing...
5.00 g potassium iodide and 0.268 g potassium iodate was dissolved into 500 mL beaker with 200 mL of distilled water. 30 mL of 3 molar sulfuric acid was added into beaker and then diluted with distilled water until 500 mL solution.

Vitamin C standard Solution was prepared by dissolving 0.250 g ascorbic acid in the beaker with 100 mL of distilled water. The solution was transferred into 250 mL volumetric flask and diluted to 250 mL with distilled water.

Standardization of iodine solution with vitamin C standard solution by pipetting 25 mL of vitamin C solution a 125 mL Erlenmeyer flask. 10 drops of 1% starch solution were added and then titrated against iodine solution until blue black color was observed. Titration was repeated three times. The volume of each fruits sample used was measured and concentration of ascorbic acid per 100 mL fruits was calculated using:

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\text{Concentration of ascorbic acid} = \frac{\text{Concentration (mg/ml) standard}}{\text{weight of sample in grams}} \times 1000
\]

Results and Discussion

Ascorbic acid content in two varieties of citrus fruit Varied significantly by all selected tehsils of District Sargodha. Ascorbic acid concentration in sweet orange variety in various tehsils was 89.6975-46.885 mg/100 mL. The highest ascorbic acid concentration of sweet orange was observed in tehsil Sahiwal 89.6975 mg/100 mL and lowest concentration was observed in Bhehra which was 46.885 mg/100 mL. While ascorbic acid concentration in Sweet lemon collected from different tehsils of Sargodha was 83.985-33.155 mg/100 mL. The Highest Ascorbic acid concentration in sweet lemon was observed also in Sargodha city and that was 83.985 mg/100 mL and on the other side the lowest concentration of ascorbic acid was observed in Silanwali and that was 33.155 mg/100 mL (Figure 5; Table 5).

Conclusion

Fluctuation in ascorbic acid concentration in both varieties, collected from different Localities may be due to environmental factors (soil composition, water, temperature, light, etc.) or due to special variation. like lemon and sweet orange from Bhehra, Silanwali, Sargodha city, Sahiwal but overall the highest concentration (89.6975 mg/100 mL) of ascorbic acid was observed in Tehsil Sahiwal orange while the lowest concentration (46.885 mg/100 mL) of ascorbic acid in Tehsil Bhehra orange. And overall the highest concentration of ascorbic acid in lemon of Sargodha city was observed (83.985 mg/100 mL) and the lowest concentration of ascorbic acid was found in lemon Tehsil Silanwali i.e., (33.155 mg/100 mL).

Difference in random samples vary a lot due to some environmental factors, soil, height of fruit from ground or at the top and samples were taken from different citrus orchids even in a single tehsil.
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Table 1: Variation in Bhehra samples.

| Orange | Lemon |
|--------|-------|
| Sample 1 | 32.27 mg/mL | Sample 1 | 49.13 mg/mL |
| Sample 2 | 76.02 mg/mL | Sample 2 | 46.33 mg/mL |
| Sample 3 | 44.78 mg/mL | Sample 3 | 19.36 mg/mL |
| Sample 4 | 34.47 mg/mL | Sample 4 | 44.65 mg/mL |

Table 2: Variation in Silanwali samples.

| Orange | Lemon |
|--------|-------|
| Sample 1 | 68.74 mg/mL | Sample 1 | 66.39 mg/mL |
| Sample 2 | 39.36 mg/mL | Sample 2 | 24.64 mg/mL |
| Sample 3 | 51.88 mg/mL | Sample 3 | 22.28 mg/mL |
| Sample 4 | 109.02 mg/mL | Sample 4 | 19.31 mg/mL |

Table 3: Variation in Sargodha city Samples.

| Orange | Lemon |
|--------|-------|
| Sample 1 | 22.09 mg/mL | Sample 1 | 77.41 mg/mL |
| Sample 2 | 89.10 mg/mL | Sample 2 | 77.39 mg/mL |
| Sample 3 | 76.14 mg/mL | Sample 3 | 102.04 mg/mL |
| Sample 4 | 102.52 mg/mL | Sample 4 | 79.10 mg/mL |

Table 4: Variation in Sahiwal Samples.

| Orange | Lemon |
|--------|-------|
| Sample 1 | 94.89 mg/mL | Sample 1 | 43.09 mg/mL |
| Sample 2 | 78.92 mg/mL | Sample 2 | 58.79 mg/mL |
| Sample 3 | 110.21 mg/mL | Sample 3 | 39.61 mg/mL |
| Sample 4 | 74.77 mg/mL | Sample 4 | 41.51 mg/mL |

Table 5: Comparison of average concentration of Ascorbic acid in orange and Lemon with respect to different Tehsils.

| Varieties | Tehsils |
|-----------|---------|
|           | Bhehra  | Silanwali | Sargodha city | Sahiwal |
| Sweet orange | 46.885  | 67.25   | 72.4625   | 89.6975 |
| Lemon | 39.8675 | 33.155  | 83.985    | 45.75   |

Table 6: Comparison of average concentration of Ascorbic acid in orange and Lemon with respect to different Tehsils.