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*Research Article*

# Effect of cooking temperature and storage period on preservation of water soluble vitamin C content in *Citrus macroptera* and *Moringa oleifera lunk*

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## Abstract

The aim of the present work was to study the effect of temperature and storage period on the preservation of vitamin C in leaves and whole plants (leaves with petioles and stems) of *Moringa oleifera lunk* (Sajna) and *Citrus macroptera* (Shatkara). Changes in the levels of these compounds in the process of freezing and refrigerated storage were also determined. Fresh *Moringa oleifera lunk* (Sajna) leaves contained 120 mg/100g vitamin C, whole plant contained 107 mg/100g and *Citrus macroptera* (Shatkara) contained 42 mg/100g of vitamin C respectively. Treatment by blanching of *Moringa oleifera lunk* (Sajna) resulted in a decrease in the level of vitamin C by 21-50%. After blanching smaller losses were recorded in whole plants, more so than in leaves. Freezing induced a decrease in the levels of the vitamin. Approximately 21–50% of the ascorbic acid contents were lost in leaves by water in Sajna and 24-83% ascorbic acid lost in Shatkara following cooking. A loss of about 53-97% for Sajna and 5-61% for Shatkara were observed at 2-5°C after 2 month storage period. The lower storage temperature favourably affected only the preservation of vitamin C.

**Keywords:** leafy vegetables, blanching, freezing, refrigeration, Bangladesh

## Introduction

Human health is very important for survival. Vitamins help humans to maintain a healthy diet. Among the vitamins, vitamin C (ascorbic acid) is an essential micronutrient required for normal metabolic function of the body [1]. The importance of vitamin C to the human body is widely acknowledged throughout the globe. It lowers blood pressure and cholesterol levels [2] and is also a major water- soluble antioxidant within the body. It is widely used in the treatment of certain diseases such as scurvy, common cold, anemia and even infertility.

Vitamin C occurs in different concentrations in a variety of natural samples. A variety of citrus fruit are available in Bangladesh and these are a good source of vitamin C. In Sylhet, *Citrus macroptera* (Shatkara) is one of the renowned locally available citrus fruit. *Citrus macroptera* (Shatkara) belongs to the genus Citrus and has leaves 10-12 inches long and

edible fruit as large as sweet oranges. In Bangladesh it is available only in Sylhet region due to the different properties of soil and climate from the rest of the country. Shatkara is a well-known fruit in Sylhet for its extensive use. It is used in curries with both fish and meat and also used to make pickles.

*Moringa oleifera lunk* (Sajna) belongs to the group of leafy vegetables and is high in vitamins. It is very popular vegetable used for food in most parts of Bangladesh and is found in almost all districts. It is commonly prepared by boiling for a long period of time by frying with oil.

Processing conditions such as temperature, pH, moisture content and food preparation steps like cutting, macerating, washing etc. are known to affect nutrient retention during processing of food. Cooking temperature is one such factor which dramatically affects the nutrient retention. The degree of vitamin C loss also depends on the quantity of cooking water used. Oke [3], reported the changes in vitamin C content during various stages of growth of some Nigerian leafy vegetables. Vitamin C is also reduced by cooking [4, 5].

During the harvest season, there are significant post-harvest losses of leafy vegetables due mainly to spoilage resulting from a lack of adequate storage facilities. Little research has been conducted on the fate of ascorbic acid during processing and a prolonged period of storage of leafy green vegetables in most tropical countries.

The purpose of the present study was to determine the level of vitamin C in locally available *Citrus macroptera* (Shatkara) and green vegetable *Moringa oleifera lunk* (Sajna) grown in Bangladesh and also to determine the appropriate cooking time and to asses the ascorbic acid levels under different storage conditions for a specific period. Two storage temperatures were also taken into consideration.

## Materials and Methods

### *Raw materials and source*

The leafy green vegetables used in this study were *Moringa oleifera lunk* (Sajna) obtained from Amborkhana, Sylhet and *Citrus macroptera* (Shatkara) obtained from Jaflong, Sylhet. Samples were processed within two hours after harvesting.

The investigation concerned two types of raw material.
1. (a) leaves alone and whole Sajna plant and (b) Shatkara.
2. Different temperatures of refrigerated storage, e.g., at 2-5°C and –10 to -15°C.
Time of refrigerated storage for two months and the frozen samples being analysed after 7 days interval.

### *Determination of ascorbic acid*

There are a number of different methods employed for analysis, such as biological method, chromatographic method [6, 7, 8, 9], with some limitations for different purposes such as biological samples, food products and pharmaceuticals for the quantitative determination of vitamin C. Here a well established chemical method (2,4-dinitrohydrazine methods-DNPH) [10], was used as a simplified approach for the simultaneous determination of the total vitamin C content.

***Sample preparation***

About 10g of blended sample was homogenized with about 50 ml of 5% metaphosphoric acid-10% acetic acid solution. Then it was quantitatively transferred into a 100 ml volumetric flask and was shaken gently until a homogeneous dispersion was obtained. It was then diluted up to the mark by the 5% metaphosphoric acid-10% acetic acid solution. Then the solution was filtered and the clear filtrate was collected for the determination of vitamin C in each sample.

***Estimation of vitamin C***

*Procedure*

A few drops of bromine water were added to the 4 ml of the filtered sample solution until the solution became coloured (to confirm the completion of the oxidation of ascorbic acid to dehydroascorbic acid). Then 2 to 3 drops of thiourea were added to it to remove the excess bromine and thus the clear solution was obtained.

Standard solutions of ascorbic acid (5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm) were prepared from 500-ppm stock solution of ascorbic acid by proper dilution. Then 1 ml of 2,4-DNPH solution was added thoroughly with all standards and also with the oxidized ascorbic acid. For the completion of the reaction, all the standards, samples and blank solutions were kept at 37°C for 3 hours in a water bath (thermostatic). After this incubation all of the samples were cooled in an ice bath and treated with 5 ml of 85% $H_2SO_4$ with constant stirring. As a result, a coloured solution was obtained whose absorbance was taken at 521 nm. To obtain a calibration curve the absorbance of the standards were plotted against the concentration and consequently the content of vitamin C in the sample was calculated by UV-spectrophotometry.

*Cooking procedure (hot water blanch)*

The fresh leaves were washed by distilled water, spread on a gauze wire tray, and allowed to drain dry under a fan for 15 min at ambient temperature (27-30ºC). The sample was immersed into boiling water which covered the leaves. The leaves were blanched for 15 or 30 min. A sample of 10 g of blanched leaves was ground in a blending machine and the sample was analysed for estimation of vitamin C by 2,4-DNP method.

**Results and Discussion**

Separate parts of plants can considerably differ in their chemical composition, this is particularly so with leaves, petioles, or stems [11]. Ishida *et al* [12], found that the leaves of sweet potato contained 78-130% more vitamin C, four to eight times more thiamine and five to seven times more riboflavin than the shoots. According to Ottosson [13], kale and parsley leaves contain much more vitamin C than the shoots.

In the presented work the content of vitamin C in 100 g fresh matter of Sajna leaves was 120 mg, in whole plants significantly less, i.e. 107 mg, the difference reaching 13% in favour of the leaves (Table 1). In the case of Shatkara, vitamin C in the fresh sample was 43 mg/100g.

Heat, water, air, light, and storage can destroy vitamin C. Therefore the sample was cooked at different temperatures with the amount of water also varied. The volume of water did not change the vitamin C content significantly. However, cooking temperature affected the percentage of vitamin C significantly. It was found that 30ºC and 100 ml of water vitamin C retention was at its highest for Sajna after boiling. In case of Shatkara, vitamin C retention

was maximum when it was boiled with water at 40ºC and 50 ml. Blanching reduced the ascorbic acid. This was due to the leaching of ascorbic acid into the blanch water.

**Table 1**. **Ascorbic acid content of fresh vegetables/fruit.**

| Entry | Fruit/ vegetable species (local name) | Fruit/ vegetable species (scientific name) | Ascorbic acid, mg/100g |
|---|---|---|---|
| **1** | Sajna leaves | *Moringa oleifera lunk* | 120 ± 2.65 |
| **2** | Sajna whole plant | *Moringa oleifera* | 107 ± 0.04 |
| **3** | Shatkara | *Citrous macroptera* | 42.50 ± 0.44 |

Table 2 and Figure 1 present the ascorbic acid content of the samples at different temperature.

**Table 2. Blanching time (in minutes) of vegetables/fruit.**

| Entry | Sample | Scientific name | Temp. °C | Time/ min. | Amount of water | Ascorbic acid mg/100g | |
|---|---|---|---|---|---|---|---|
| **1** | Sajna leaves | *Moringa oleifera lunk* | 30 | 15 | 100 | 44.88 ± 0.345 | |
| | | | | 15 | 150 | 42.56 ± 0.326 | |
| | | | | 30 | 100 | 38.75 ± 0.224 | |
| | | | | 30 | 150 | 36.07 ± 0.350 | |
| | | | 60 | 15 | 100 | 24.96 ± 0.294 | |
| | | | | 15 | 150 | 23.79 ± 0.031 | |
| | | | | 30 | 100 | 18.21 ± 0.120 | |
| | | | | 30 | 150 | 17.35 ± 1.341 | |
| | | | 90 | 15 | 100 | 16.17 ± 0.198 | |
| | | | | 15 | 150 | 14.38 ± 0.191 | |
| | | | | 30 | 100 | 12.51 ± 0.35 | |
| | | | | 30 | 150 | 11.97 ± 0.286 | |
| **2** | Shatkara | *Citrous macroptera* | 40 | 15 | 50 | 32.30 ± 1.88 | |
| | | | | 30 | 50 | 30.54 ± 0.235 | |
| | | | | 15 | 100 | 31.17 ± 0.159 | |
| | | | | 30 | 100 | 29.58 ± 0.248 | |
| | | | 60 | 15 | 50 | 28.76 ± 0.625 | |
| | | | | 30 | 50 | 25.35 ± 0.360 | |
| | | | | 15 | 100 | 27.05 ± 0.561 | |
| | | | | 30 | 100 | 24.41 ± 0.165 | |
| | | | 80 | 15 | 50 | 22.92 ± 0.231 | |
| | | | | 30 | 50 | 20.68 ± 0.488 | |
| | | | | 15 | 100 | 21.78 ± 0.198 | |
| | | | | 30 | 100 | 19.72 ± 0.246 | |
| | | | 90 | 15 | 150 | 13.14 ± 0.330 | |
| | | | | 30 | 50 | 9.63 ± 0.246 | |
| | | | | 15 | 100 | 10.96 ± 0.221 | |
| | | | | 30 | 100 | 7.10 ± 0.321 | |

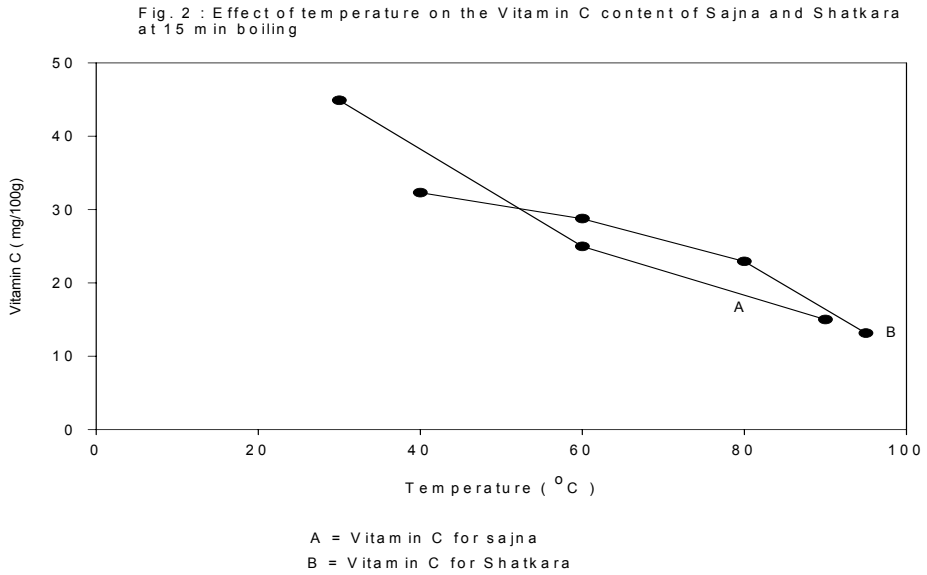


**Figure 1. Effect of Temperature on Vitamin C Content of Sajna and Shatkara at 15 min Boiling.**

The chief reasons for losses in the content of the vitamin C are the solubility in water, thermic destruction and enzymatic oxidation during the technological process [14]. The results on the ascorbic acid content of fresh and water-blanched samples of both Sajna leaves and Shatkara after two months at different storage conditions are presented in Table 3 and Figure 2. The quantity of ascorbic acid present varied with species. It is observed that at 2-5°C storage of both Sajna and Shatkara the ascorbic acid decreased significantly, about 97% for Sajna and 61% for Shatkara after two months storage but storage at –10 to –15°C ascorbic acid retention was 40% for Sajna and 64% for Shatkara. Vitamin C content of fruit and vegetables slowly decreases with time as the storage period increases, even at low temperature.

**Table 3**. **Ascorbic acid content of vegetables/fruit after 2 months storage & various temperature.**

| Entry | Fruit/Veg. (local name) | Fruit/Veg. species (scientific name) | Ascorbic acid, mg/100g | | |
|---|---|---|---|---|---|
| | | | Days | Storage at 2-5°C | Storage at –10 to -15°C |
| 1 | Sajna leaves | *Moringa oleifera lunk* | 7 | 65.66 ± 0.105 | 102.43 ± 0.777 |
| | | | 14 | 38.91 ± 0.175 | 89.32 ± 0.958 |
| | | | 21 | 23.92 ± 0.197 | 80.43 ± 1.50 |
| | | | 28 | 15.30 ± 0.162 | 72.92 ± 0.83 |
| | | | 35 | 9.90 ± 0.095 | 64.90 ± 1.20 |
| | | | 49 | 4.18 ± 0.171 | 55.43 ± 0.923 |
| | | | 60 | 1.76 ± 0.160 | 48.32 ± 0.820 |
| 2 | Shatkara | *Citrous macroptera* | 7 | 40.42 ± 0.110 | 41.40 ± 0.120 |
| | | | 14 | 38.54 ± 0.141 | 40.81 ± 0.148 |
| | | | 21 | 36.13 ± 0.141 | 39.42 ± 0.103 |
| | | | 28 | 34.10 ± 0.034 | 38.83 ± 0.302 |
| | | | 35 | 32.26 ± 0.140 | 37.40 ± 0.127 |
| | | | 42 | 29.78 ± 0.047 | 35.21 ± 0.115 |
| | | | 49 | 24.91± 0.14 1 | 31.30 ± 0.046 |
| | | | 60 | 16.47 ± 0.053 | 26.87 ± 0.066 |

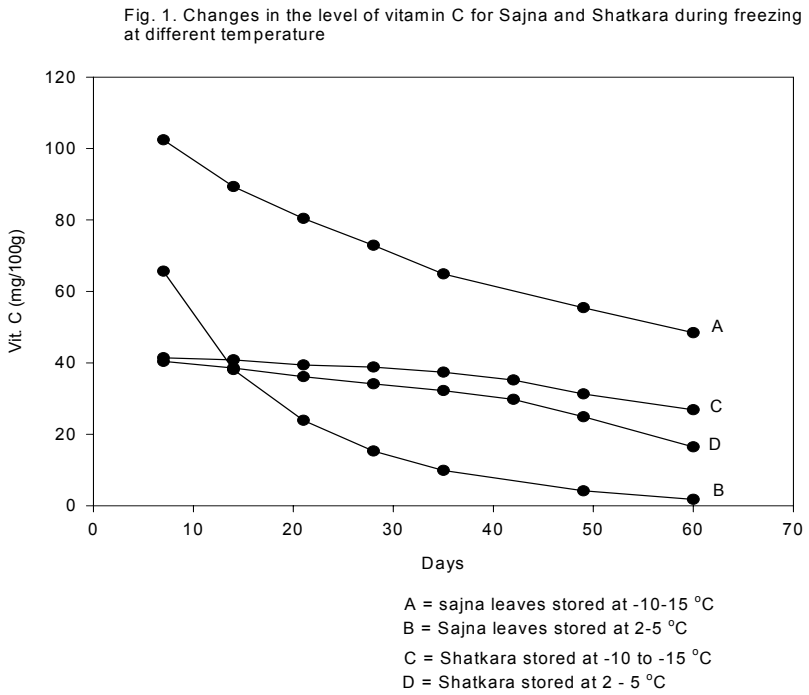


**Figure 2. Changes in Level of Vitamin C for Sajna and Shatkara during Freezing at Different Temperatures.**

## Conclusions

Vitamin C is important to human health and a necessary dietary source. However, it is very delicate and easily destroyed by many things, particularly heat. Fresh Sajna and Shatkara contained 120 mg/100g and 43 mg/100g of vitamin C, respectively. The treatment by blanching resulted in a decrease in the levels of vitamin C by as much as 85%. Freezing induced a decrease in vitamin C in fresh samples. It can, therefore, be concluded that a lower cooking temperature of fruit/vegetables resulted in higher retention of vitamin C. Since most leafy vegetables are cooked prior to consumption, it is recommended that the vegetables are cooked in small amounts of water for short periods to minimize the loss of vitamin C. A lower storage temperature favourably affects only the preservation of vitamin C.

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