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Assessment of Ascorbic Acid Values of Fresh, Shade and Sun-Dried Vegetables

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Abstract: Green leafy vegetables are rich and suitable source of micronutrients and vitamins. The utilization of most of the vegetables is limited in the backward areas and villages due to lack of information and knowledge of its nutritive importance. In addition to this, the local inhabitants of Gilgit-Baltistan (G-B) are used to store most of the vegetables in summer by drying them for winter season because of unavailability of fresh vegetables during off-season due to harsh and severe weather conditions. This study amid to quantify the ascorbic acid (AA) contents of fresh, sun-dried and shade-dried samples of rape mustard: *Brassica rapa-sylvestris*, common mallow: *Malva sylvestris* and fenugreek: *Trigonella Foenum-graecums* (26.24, 6.40, and 18.40 mg/100g, respectively) by iodometry. The percent retention and loss of AA contents of sun-dried and shade-dried sample were analyzed with respect to the fresh vegetables. It was found that the sun drying caused the loss of AA more than the shade drying in all analyzed vegetables from 77.79, 21.79% in *B. rapa-sylvestris;* 70.51, 50.83% in *M. sylvestris*, and 76.08, 54.34% in *T. Foenum-graecums* for sun-dried and shade dried, respectively. This shows that the dried vegetables are inadequate in order to obtain recommended AA allowance daily. Therefore, it is recommended that the local community should use either fresh or shade dried vegetables as food at non-production season and encouraged the increased consumption of these vegetables to avoid many physiological disorders related to the deficiency of ascorbic acid.

Key words: Ascorbic acid, common mallow, vegetables, *Brassica rapa-sylvestris*, *Malva sylvestris*, *Trigonella Foenum-graecums*.

INTRODUCTION

Vitamins are complex organic compounds which are essential for normal growth, metabolism and reproduction. Vitamins are neither produced nor stored by the human body, rather they are required to supply on regular bases in a small quantity.[1,2] Vitamins are biologically active micronutrients which are necessary for metabolism and physiological processes in the human body. They are found in natural foods.[3-5]

Ascorbic acid (AA) is white crystalline water soluble substance isolated in 1928. It has sour taste and is a hexose derivative.[6] Vitamin C is found in green leafy vegetables e.g. *Brassica rapa-sylvestris*, *Malva sylvestris*, citrus fruits and other barriers.[7-9] The fruits and vegetables rich in AA contents provide 60 mg of ascorbic acid while low vitamin C fruits provide 30 mg of ascorbic acid per serving. The deficiency of AA in human body may be caused due to less consumption of vegetables and fruits.[10,11] In order to avoid the AA acid deficiency, the health professional recommends at least one whole fruit or vegetable as a part of daily food.[12] Ascorbic acid is also important for the lactating mothers and infants for normal growth and protection against AA related physiological disorders.[13,14] The recommended daily allowance was established from 10 to 60 mg per day which is now increased to 90 to mg per day to prevent cardiovascular disease and cancer.[15,16]

Vitamin C is an antioxidant and protects the body from harmful effects of toxins, pollutants and free radicals produced as a result of normal redox reaction in the human body, while no side effects and toxicity of AA has been observed yet.[17,18] The most appropriate source of antioxidants and AA to overcome oxidative stress in human body are vegetables and fruits with high concentration in polyphenols, vitamins, carotenoids, flavonoids, etc.[19-21] Different research studies have shown that high intake of fruits and vegetables lower the risk of different types of cancer. In the presence of higher concentration of AA the cancer cells either do not grow or it shrinks the tumor.[22] Vitamin C rich foods play an important role in protection against matel induced hepatotoxicity.[23] People who use tobacco products are at risk for hypo-vitamins and much need for dietary intervention. The average serum concentration of AA in non-smokers is 25% higher than smokers. Therefore the RDA for smokers is 200 mg to maintain serum concentration of vitamin C as compare to non-smokers.[24] Ascorbic acid also helps in conversion of cholesterol into bile acid and lowers the blood cholesterol level.[25]

During present investigation, three different green leafy fresh and dried vegetables i.e. rape mustard: *Brassica rapa-sylvestris*, common mallow: *Malva sylvestris*, and Fenugreek: *Trigonella Foenum-graecums* were analyzed for AA estimation. These vegetables are commonly cultivated and consumed by local population in Gilgit Baltistan region. The process was carried out through iodometric titration.

MATERIALS AND METHODS

Sample collection: The selected vegetables were *Brassica rappa-sylvestris*, *Malva sylvestris* and *Trigonella Foenum-graecum*. These vegetables were collected from Nomal Valley of district Gilgit, Pakistan during June 2014. The collected vegetables were identified by plant taxonomist Dr. Sher Wali Khan, Department of Biological Science, Karakoram International University, G-B. The shade dried sample of the vegetables was prepared by cutting the fresh vegetables into small pieces and then kept the sample under shadow to dry for eight days. The sun-dried sample was prepared by cutting sample vegetables into small pieces and kept in open air to dry for two days.

Preparation of 0.005 mol L^{-1} iodine solution: Accurately weight 2 g of KI and 1.3 g of I_2, dissolved in few mL of water and shake until dissolving. Transfer iodine solution to a 1 L volumetric flask, making sure to rinse all traces of solution in to the volumetric flask using distilled water, completed the volume up to the mark.

Preparation of 0.5% starch indicator solution: Soluble starch (0.25 g) to a 100 mL conical flask and 50 mL of distilled water was added. Solution heated with stirring at 80 ^{0}C for 5 minutes, ensuring not to exceed the stated temperature. Allow solution to cool to room temperature.

Preparation of vegetables sample: Two parallel methods were adopted to extract AA contents from *B. rapa-sylvestris*, common mallow: *M. sylvestris* and Fenugreek: *T. Foenum-graecums*. In first instance, 100 g of sample were chocked into small pieces and grinded by using a mortar and pestle. Mixed 10 mL portions of distilled water seven times at different intervals while grinding the sample. Each time decanted off the liquid extract into a 100 mL volumetric flask. Finally, squished the ground vegetable pulps through cheesecloth, rinsed the pulp with a few 10 mL portions of water, and collected all filtrate and washings in the volumetric flask. The final volume of the extracted solution was made up to 100 mL with distilled water. Alternatively the 100 g sample of vegetable was blended in a food processor together with about 50 mL of distilled water. After blending, the pulp was squished through cheesecloth, washed it with a few 10mL portions of distilled water, and made the extracted solution up to 100 mL in a volumetric flask.

Standardization of the iodine solution with the ascorbic acid standard solution: Iodine solution changes rapidly because I_2 is a halogen gas that evaporates quickly. Therefore, iodine solutions need to be standardized all the time during the experimental procedure. Ascorbic acid is also susceptible to oxidation by atmospheric oxygen over time. For this reason, the sample was prepared immediately before the titrations. In addition to this, a small amount of oxalic acid (2 mL) was added to standard ascorbic acid solution in order to minimize the probability of oxidation of ascorbic acid.

Titration

1. Aliquot of the vegetable sample (100 mL) solution prepared above transferred into a 250 mL conical flask, 2 ml of oxalic acid, about 100 mL of distilled water and 1 mL of starch indicator solution.
2. Samples were titrated with 0.005 mol.L^{-1} iodine solution. The endpoint of the titration was identified as the first distinct trace of a dark blue-black color due to the formation of starch-iodine complex.
3. Titration were repeated with further aliquots of sample solution until concordant results (titres agreeing within 0.1 mL) were obtained.

Calculations

$$\text{mg of vitamin C in 1 mL of solution} = \frac{\text{mg of vitamin C (standard value)}}{100\ \text{mL}} \times 1\ \text{mL}$$

$$\text{mg of vitamin C oxidized by 1 mL of iodine reagent} = \frac{\text{mg of vitamin C in flask}}{\text{average volume (mL) of iodine reagent}}$$

$$\text{mg of vitamin C in vegetable sample} = 100\ \text{mL} \times \frac{\text{mg vitamin C}}{1\ \text{mL of sample solution}}$$

RESULTS AND DISCUSSION

Ascorbic acid is an important water soluble nutrient which is found in green vegetables and citrus fruits. This vitamin is a powerful antioxidant and controls enzymatic and metabolic functions in the human body. This helps to maintain its necessary value regularly. It is an established truth that there are many environmental and agronomic factors which directly or indirectly effects the concentration of AA in green vegetables. Among them, temperature, sunlight, salinity, and air pressure play an important role. Therefore it is of utmost importance to know about the quantity of this ingredient in natural food to recommend its use to local population in particular.

The local communities of Gilgit-Baltistan (G-B) are used to store most of the vegetables in summer by drying them for winter season because of unavailability of fresh vegetables during off-season due to harsh and severe weather conditions. This study amid to quantify the ascorbic acid (AA) contents of fresh, sun-dried and shade-dried samples of *Brassica rapa-sylvestris,* common mallow: *Malva sylvestris* and Fenugreek: *Trigonella Foenum-graecums* by iodometry. The percent retention and percent loss of AA contents of sun-dried and shade-dried samples were analyzed with respect to the fresh vegetables.

The amount of AA was estimated from three different vegetables i.e. *B. rapa-sylvestris, M. sylvestris* and *T. Foenum-graecums*. The amount of AA in of fresh *B. rapa-sylvestris* was found to be 26.24 mg /100 g of sample while for shadow-dried and sun-dried samples the calculated AA contents were 20.40 and 5.72 mg /100 g, respectively **(Figure 1)**. While the percentage retention values for shade-dried and sun-dried samples of *B. rapa-sylvestris* were 77.70 and 21.79%, respectively **(Table 1**, **Figure 2a)**. The AA values for the fresh, shade and sun-dried samples of *T. Foenum-graecums* were calculated 18.4, 14.0 and 10.0 mg/100 g, respectively. The percentage retention of AA values for shade-dried and sun-dried samples of *T. Foenum-graecums* was 76.08 % and 54.64 %, respectively **(Figure 2b).**

Table 1: Ascorbic acid contents of fresh, shade and sun-dried *B. rapa-sylvestris, M. sylvestris,* and *T. Foenum-graecums*

Name of vegetable	Ascorbic acid value (mg/100 g)				
	fresh	shade-dried	sun-dried	% retention	% loss
Brassica rapa-sylvestris	26.24	20.40	05.72	77.70 21.79	22.30 88.21
Malva sylvestris	06.24	04.40	03.17	70.51 50.83	29.49 49.17
Trigonella Foenum-graecums	18.40	14.00	10.00	76.08 54.34	23.92 45.66

The results showed 6.26, 4.4 and 3.2 mg of AA for fresh, shade-dried and sun-dried samples of *M. sylvestris*. The calculated values for percentage retention of AA contents of shade-dried and sun-dried samples of *M. sylvestris* were 70.51 and 50.83 %, respectively (**Figure 2c**). In comparison, the results showed that the fresh sample of *B. rapa-sylvestris* have highest level of ascorbic acid contents followed by *T. Foenum-graecums* whereas *M. sylvestris* contains very small amount of AA contents **(Table 1**, **Figure 1)**.

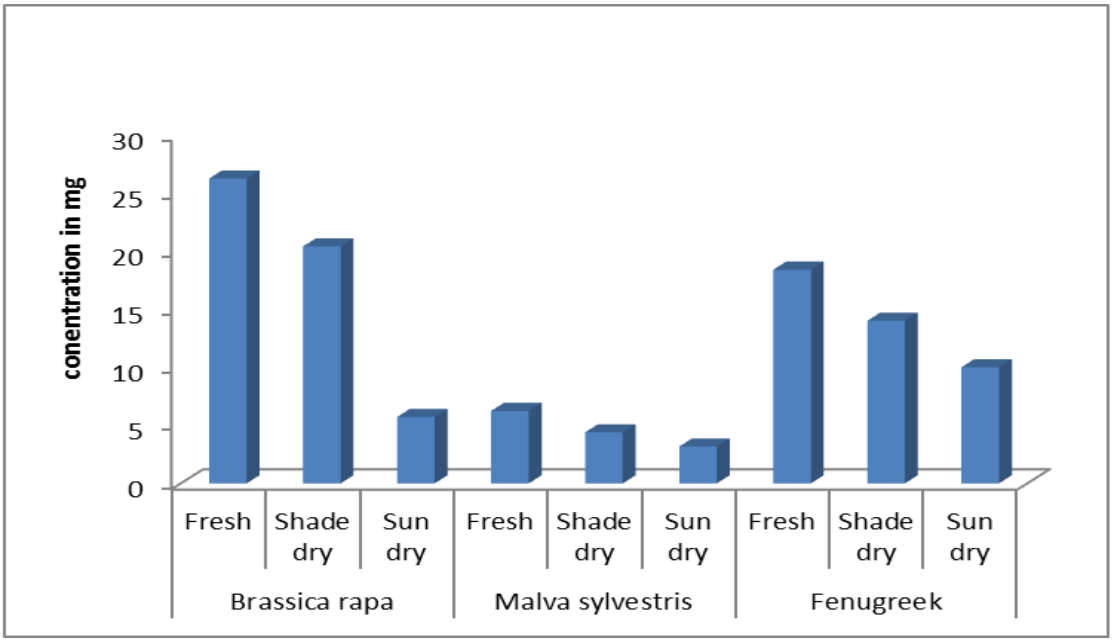

Figure 1: AA contents of ripe mustard, common mallow, and fenugreek.

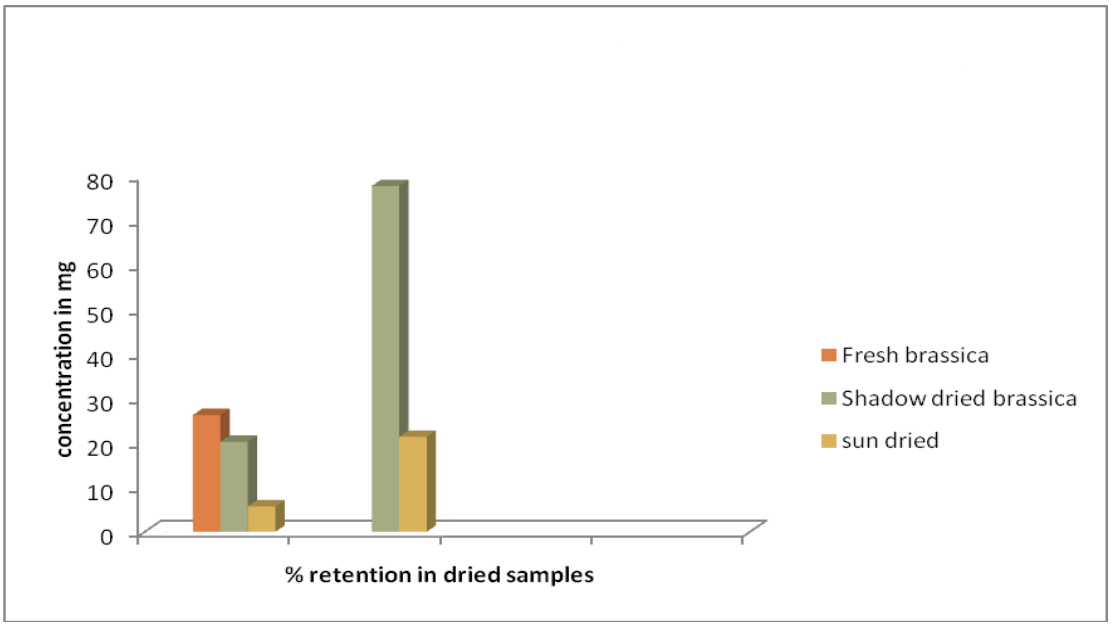

Figure 2a: Percent retention of AA in shade and sun-dried samples of ripe mustard.

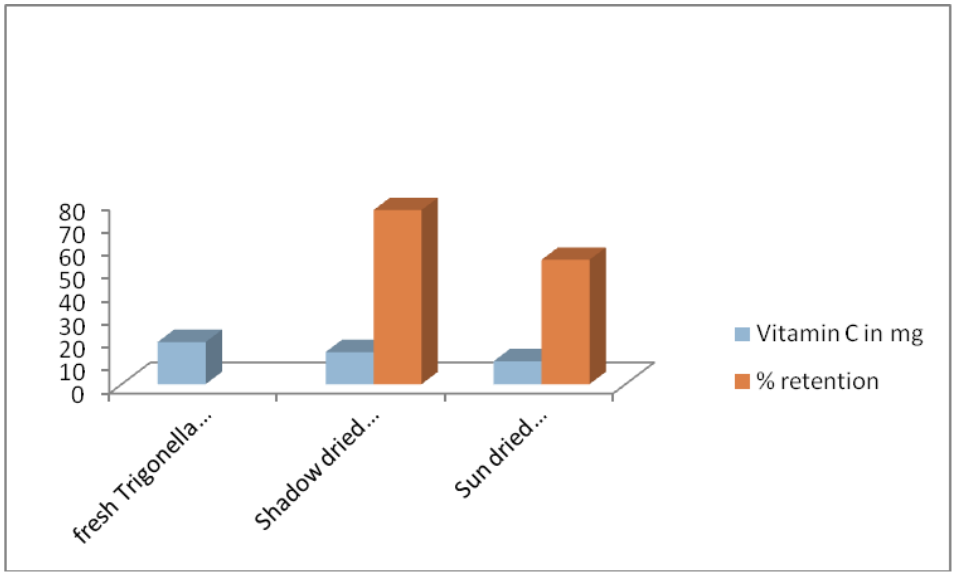

Figure 2b: Percentage retention of AA in shade and sun-dried samples of fenugreek.

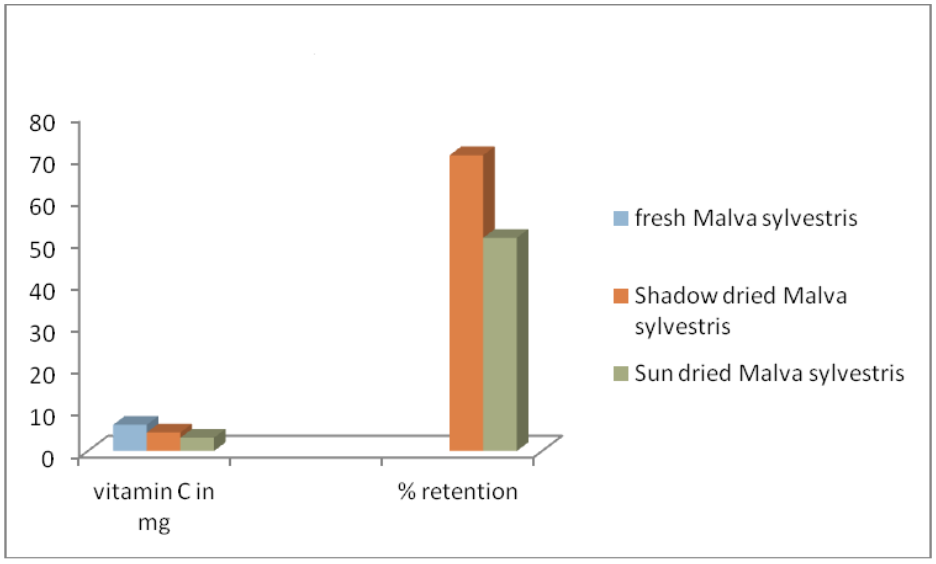

Figure 2c: Percentage retention of AA in shade and sun-dried samples of common mallow.

It is revealed by this investigation that the direct sunlight has drastic effect on AA level in vegetables, whereas *B. rapa-sylvestris* (88.21%, **Figure 3a)** was badly affected by direct sunlight than that of *M. sylvestris* (49.17%, **Figure 3b**), and *T. Foenum-graecums* (45.66%, **Figure 3c).** Among the selected vegetables, retention of AA contents in shade-dried method was highest in *B. rapa-sylvestris* (77.70%), while in sun-dried method *T. Foenum-graecums* (54.34%). showed highest retention level. Overall, shadow-dried vegetables showed more retention of AA than conventional sun-dried method. Direct sun-drying method affects AA contents in vegetables more than that of shade-drying method.

On the basis of these interesting findings, it is suggested that, the local communities should sort a way to grow fresh vegetables to ensure its availability during off season by establishing green-houses culture rather than relying on dried ones.

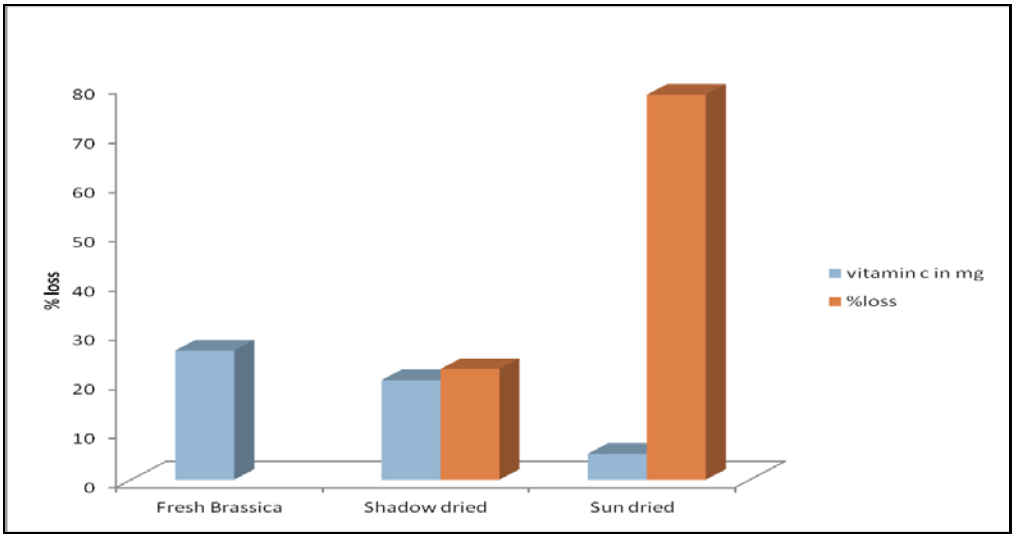

Figure 3a: Percentage loss of AA **i**n shade and sun-dried sampled of ripe mustard.

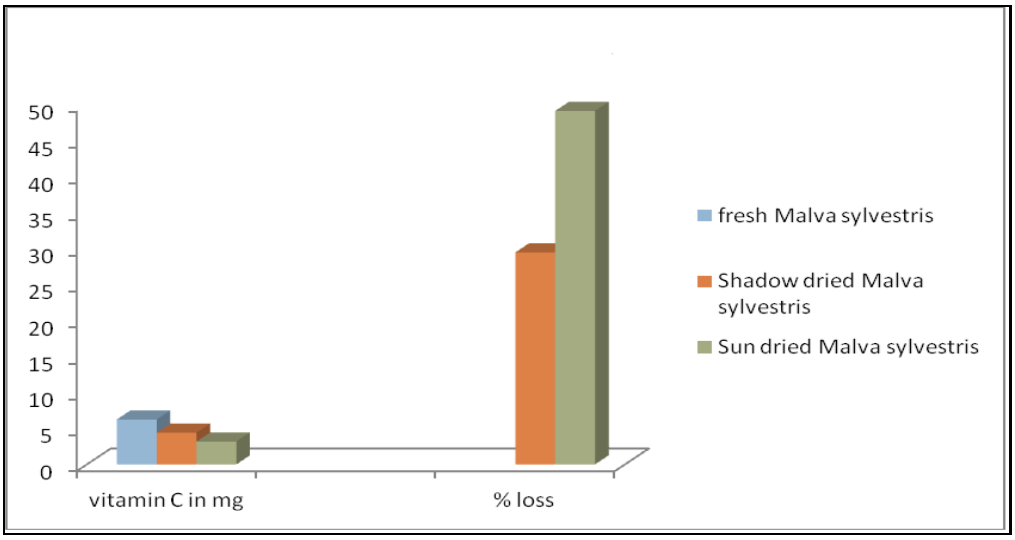

Figure 3b: Percentage loss of AA in shade and sun-dried samples of common mallow.

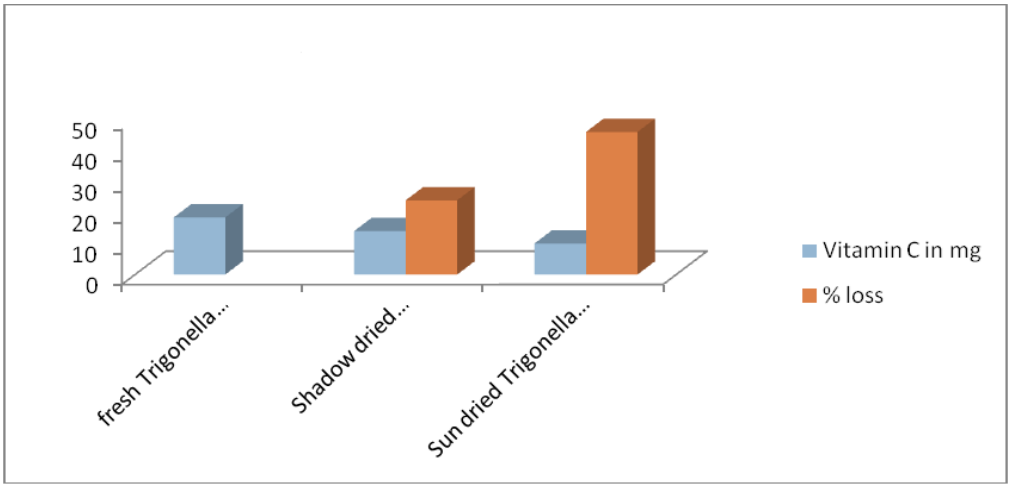

Figure 3c: Percentage loss of AA in shade and sun-dried samples of fenugreek.

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