COMPARATIVE EVALUATION OF DRYING METHODS ON VITAMIN C AND PROTEIN CONTENT OF *MORINGA OLEIFERA* LEAVES

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ABSTRACT

Moringa oleifera is a source of abundant proteins, carbohydrates, vitamins, and minerals. Proteins and vitamin C are important for human growth, survival, development and it participates in various biological processes.The body can store proteins, whereas the body doesn't store vitamin C. Since vitamin C is eliminated in the urine and proteins are mainly used by the muscles, our diet requires an uninterrupted daily supply of both proteins and Vitamin C. Malnutrition is more prominent in poor masses caused by a dietary protein deficiency that is characterized by muscle loss, tiredness, depression, fluid retention or edema. In the present investigation, comparative study was carried out for the air, oven and microwave dried leaves for estimation of Vitamin C and protein. The amount of vitamin C and protein in dry air method was observed to be higher compared to dried samples in the oven and microwave.Therefore, due to the increased preservation of vitamin C and the protein content of the dried samples, it is recommended that *Moringa oleifera* leaves be dried at ambient conditions.

***Keywords*:** *Moringa oleifera* leaves, Drying methods, Vitamin C, Protein quantification

INTRODUCTION

Moringa oleifera is the most widely grown species in the genus Moringa, the only genus in the Moringaceae plant family, a fast-growing, multipurpose, drought-resistant tree, high-economic crop (Paliwal and Sharma, 2011), native to India, now widely grown throughout the world's tropical and subtropical regions. *Moringa oleifera*, common names includes *Moringa*, in English language Drumstick (from the long, slender, triangular seed-pods), horseradish tree (from the taste of the roots, which resembles horseradish) and ben oil tree or benzoil tree (from the oil which is derived from the seeds). *Moringa* leaf extract, being rich in K, Ca, Fe, amino acids, carotenoids, phenols, ascorbate (ascorbic acid mineral salt), growth regulating hormone and ideal enhancer of plant growth (Makkar and Becker, 1996). *M. oleifera* displayed essential medicinal properties such as anti-inflammatory, antioxidant,

antimicrobial, cardiovascular, antihyperlipidemic, CNS depressant, antifertility, anticancer, anti-hepatotoxic, and antiulcer (Goyal *et al.*, 2007) that enabled humans to avoid and treat diseases. Therefore, the *Moringa oleifera is popularly* known as 'The Miracle Tree' (Fuglie, 1999). Ayurvedic traditional medicine states that *Moringa oleifera* will resist 300 diseases, and its leaves have been used for both preventive and cultivation purposes (Ganguly, 2013). Ascorbic acid and glutathione act as oxidants which are found in chloroplasts of *Moringa* and other cellular compartments at high concentrations.Those oxidants are vital to the defence of plants against oxidative stress. *Moringa* plant species can be used either as a food or food supplement providing nutrients and biochemicals like carbohydrates, fats and proteins which play vital roles in satisfying human needs for energy and living processes.

Two different types of vitamins are the fat soluble and water soluble. Fat-soluble vitamins are vitamin A, vitamin D, vitamin E, vitamin K and water-soluble vitamins that include the vitamin B complex group and vitamin C (Li and Schell, 2007; Carr and Frei, 1999).Vitamin C is used in cure of certain diseases such as scurvy, common cold (Hemila, 1992) anaemia and even infertility. Due to mutation in the enzyme-coding pseudogene (L-gulonolactone oxidase) required for the biosynthesis of vitamin C via the pathway of glucuronic acid, humans and other primates have lost their ability to produce vitamin C (Abdalla, 2003). Thus, vitamin C should be obtained through diet. Besides, Moringa is also a good alternative protein source. Protein as an essential nutrient should be an inescapable part of a diet to combat malnutrition. Keeping in view the importance of *Moringa* plant, vitamin C using titration method and protein were quantified using Lowry method in leaves.

MATERIALS AND METHODS

In the preparation of reagents, chemicals of analytical grade purity and distilled deionised water were used. *Moringa* leaves sample was collected from the campus of VPM's B.N. Bandodkar College of Science, Thane (Maharashtra).

Material collection and sample preparation of *Moringa* leaves: Fresh plant leaves were collected from the campus of V.P.M's B. N. Bandodkar College of Science, Thane (Maharashtra). *Moringa* leaves were washed with water and rinsed with distilled water. Leaves were dried using drying methods such as air, oven, and microwave. Microwave drying was performed in a microwave oven at 180°C for 8 minutes, oven drying at 40 ± 2° C for 24 hours and air drying was performed at room temperature in a sterilized laboratory for 7 days. All dried samples were then homogenized into a powder.

General procedure for determination of following:

1. **Vitamin C (Ascorbic acid) in *Moringa oleifera* leaves by using redox titration method:**

Moringa oleifera leaves (1 g) were ground to fine powder and transferred to 100 cm^3 beaker. Then 20 cm^3 distilled water with 2 cm^3 of 2 M HCl solution was added. The mixture was warmed for 5 minutes. The mixture was then cooled and filtered with whatman filter paper No. 40. The whole solution was then transferred to the 100 cm^3 standard measuring flask and diluted upto the mark with distilled water. 10 cm^3 of the sample aliquot was pipetted out in a conical flask and 15cm^3 of 2 M sulphuric acid was added. The sample was then titrated using 2 percent Ferroin indicator against standardized 0.1 N ceric ammonium sulphate till color changed from red to pale blue to green colour (Rao and Sastry, 1971). In acidic medium, ascorbic acid is oxidized by Ce (IV) ion solution to dehydroascorbic acid (Figure 1).

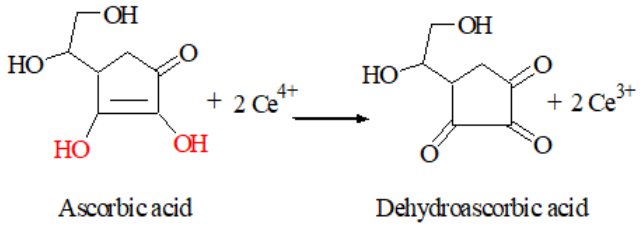

FIGURE 1: Ascorbic acid is oxidised to dehydroascorbic acid by Cerium (IV) ion solution.

2. Estimation of Protein in *Moringa oleifera* by using Folin-Ciocalteu reagent (Lowry method):

a. **Preparation of standard protein BSA solution:** 0.5 mg/ml

b. **Preparation of alkaline complex reagent:** Added 48 ml 2% Na_2CO_3 in 0.1 N NaOH, 1 ml of 1% Sodium potassium Tartrate in H_2O, 1 ml of 0.5% $CuSO_4.5H_2O$ in H_2O to prepare alkaline complex reagent. By using a standard solution and alkaline complex reagent, prepared the calibration curve (Table 1), kept for incubation for 15 minutes before adding Folin-Ciocalteau reagent. Measured the absorbance at 660 nm on the spectrophotometer.

c. **Preparation of sample for estimation:** The sample powder (0.5 g) was dissolved in 10 cm^3 of distilled water and filtered by using Muslin cloth. Filtrate was collected in 100 cm^3 standard measuring flask and diluted upto the mark with distilled water. Pipetted

out 50 cm^3 of the sample solution and transferred it to 100 cm^3 standard measuring flask. Prepared sample as per Table 1, measured absorbance values of the standard BSA protein and sample solution (Table 1).

Table 1: Preparation of calibration curve and sample of *Moringa oleifera* leaves solution for protein analysis

Standard protein Solution in cm^3	Distilled water in cm^3	Alkaline complex reagent in cm^3	Folin-Ciocalteu reagent in cm^3	Concen-tration of BSA Protein (mg/ml)	O.D.
0.2	0.8	5.0	0.5	0.1	0.17
0.4	0.6	5.0	0.5	0.2	0.29
0.6	0.4	5.0	0.5	0.3	0.40
0.8	0.2	5.0	0.5	0.4	0.50
1.0	0.0	5.0	0.5	0.5	0.62
Sample A(0.4) [a]	0.6	5.0	0.5	-	0.34
Sample B(0.4) [b]	0.6	5.0	0.5	-	0.22
Sample C(0.4) [c]	0.6	5.0	0.5	-	0.17

[a]Sample A: Air dried, [b]Sample B: Oven dried, [c]Sample C: Microwave dried

RESULTS AND DISCUSSION

The powder samples obtained from air, oven and microwave drying of *Moringa oleifera* leaves were used for the further determination of vitamin C and protein. It was observed that varying the drying methods altered the amount of vitamin C. Blanching in hot water in fruits can cause an appreciable loss in thermally labile vitamin C. Ascorbic acid oxidase must be inactivated during processing; this avoids the enzyme-catalyzed reaction (Abubakar and Obirinakem, 2015, Igwemmar *et al.*, 2013). The concentration of sample proteins is determined by comparing their assay responses with that of a series of standards solutions whose concentrations are known. The standard responses are used to plot a calibration curve.

The effect of various drying methods in the *Moringa oleifera* leaves on the vitamin C and protein content showed varying amounts of vitamin C and protein (Table 2). Air dried sample shows the largest amount of vitamin C and protein in the three. Excessive heat and water easily destroyed the vitamin C and protein, as well as exposure to air. It occurred due to the heat-labile nature of vitamin C and also depends on the irradiation time of microwave ovens (Khraisheh *et al.*, 2004, Santos and Silva, 2008). For vitamin C preservation in cooked foods, it is recommended that vegetables be stored at a temperature of 70°C in order to avoid the loss caused by heat (Clement *et al.*, 2017).

Table 2: Vitamin C and protein amount in *Moringa oleifera* leaves by using ceric ammonium sulphate titration and Lowry method

Sr. No.	Sample Drying methods	mg of Vitamin C/ 100 g of powder	mg of protein /100 g of powder
1	Air	359	10.2
2	Oven	278	6.6
3	Microwave	269	5.2

CONCLUSIONS

Moringa oleifera is abundantly available, inexpensive leaves that can serve as a pool house of nutrients and can be used to combat nutrient deficiencies in developing countries. The drying process of highly seasonal and perishable green leafy vegetables is one of the most possible strategies.The effect of different drying methods on the content vitamin C and protein in *Moringa oleifera* leaves has been studied. In the present investigation the method of redox titration provides amounts of vitamin C in the leaves of *Moringa oleifera*. The Folin-Ciocalteu reagent (Lowry method) was the simplest and most effective method of determining the protein content in leaves of *Moringa*. Air dried samples show higher amounts of vitamin C and protein than dried samples from microwaves and ovens. That is because at elevated temperatures vitamins and proteins are denatured. It was also observed that air dried *Moringa* took longer (7 days) time to dry than oven-dried and microwave methods. Although the microwaves and ovens are faster and better methods for drying it is advisable to preserve material using air drying methods to prevent vitamin C and protein loss.

ACKNOWLEDGMENT

The authors are thankful to V.P.M's B. N. Bandodkar College of Science, Thane for providing laboratory faculties for research work.

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