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Research Article

Comparative analysis of preservation techniques on *Moringa oleifera*

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Abstract

The medicinal and therapeutic properties of *Moringa oleifera* have led to its application as a cure for diverse ailments, diseases and physiological disorders. In eastern allopathic medicine it is also reported to possess various pharmacological actions. So the need to preserve this medicinal entity is of great interest. The aim of the present study is to analyse the self-life of the fruit, leaves and pods of *Moringa oleifera* preserved using various techniques. The microbial analyses of the preserved samples are undertaken to identify the efficiency of the preservation techniques. Various biochemical assays are also done to assess the biochemical properties of the sample employed to different preservation techniques. The results obtained are satisfactory and the preservation techniques engaged are useful in increasing the shelf-life of *Moringa Oleifera* to a great extent.

Keywords: Therapeutic properties, traditional medicine, shelf-life, India

Introduction

Moringa (*Moringa spp*.) belongs to a monogenetic family, the Moringaceae. *Moringa oleifera* is also known as "Miracle Tree" [1]. *Moringa oleifera* has a host of other country specific vernacular names, an indication of the significance of the tree around the world [2]. It is a small fast-growing ornamental tree originally belongs to India. The trees originated from Agra and Oudh in North Western region of India to South of the Himalayan Mountains [3] now widely cultivated throughout the tropics and is found in many countries of Africa, Asia and South America regions and the Middle East, Central and South America, Sri Lanka, India, Mexico, Malaysia and the Philippines [4]. As the species are drought resistant it can also tolerate a wide range of soil and rainfall conditions. Various varieties of *Moringa oleifera* have been developed to meet the tastes of local populations [5, 6, 7].

Cited as one of the world's most useful plants, Moringa leaves are edible and are of high nutritive value. It is consumed throughout Asian countries. It is a cosmopolitan tropical drought tolerant tree, available throughout the year and is documented in eastern allopathic medicine [7, 8] to possess various pharmacological actions [9], such as analgesic, antihypertensive activity, and anti-inflammatory effects [1, 10, 11, 12]. Leaves of this plant are reported to possess various biological activities, including hypocholesterolemic, antidiabetic, hypotensive agent and [13, 14, 15, 16], regulate thyroid hormone [17], central nervous system, digestive system, nutrition and metabolism eye, ear nose throat Genito-urinary system [18], to treat gastric ulcers [19] and scurvy [20]. Reports

have also described the plant to be highly potent anti-inflammatory agent [21] and antitumour activity [22]. The plant has also been reported to be hepatoprotective against antitubercular drug such as isoniazid and rifampicin [23, 24]. Furthermore, it is an important source of the glucosinolate precursors of the isothiocyanate group of chemopreventives [25] that can inhibit carcinogenesis. *Moringa oleifera* is also being studied for its anti-inflammatory, antimicrobial, diuretic [10, 26, 27], antibiotic [28], hypotensive [15], and antimicrobial properties [29, 30]. An immune enhancing polysaccharide [31] and niaziminin, having structural requirement to inhibit tumour promoter induced Epstein Barr virus activation have been reported from the leaves [22]. The alcoholic extract of leaves of *Moringa oleifera* were reported to have analgesic activity [32]. *Moringa oleifera* pod extract is bioenhancing and are niaziridin rich fraction. Pods enhance the bioactivity of commonly used antibiotics such as rifampicin, tetracycline and ampicillin against Gram positive and negative bacteria and also facilitate the absorption of drugs, vitamins and nutrients through the gastro-intestinal membrane thus increasing their bio-availability [33]. Therefore, niaziridin can be used in combination therapy with drugs and nutrients resulting in reduced drug associated toxicity, reduced cost and duration of chemotherapy.

It has been estimated that about one-fourth of all *Moringa oleifera* produce harvested is spoiled before consumption. Spoilage of fresh vegetables usually occurs during storage and transport. The need for preservation of *Moringa oleifera* is very essential due to its medicinal and therapeutic properties. Preservation is a mere word which means "to save something from being harmed or destroyed". Fresh vegetables may be dried, frozen, fermented, pasteurized or canned [34]. Generally, the process of preservation is classified as drying, heating, refrigerating, irradiation, and the use of chemicals or natural agents. The preserved form can be useful to make it available throughout the world. Hence the objective of the study is to find the best optimised preservative method which is simple and cost effective, besides keeping a check on microbial growth.

Materials and Methods

Sample collection

Fresh *Moringa oleifera* were collected in the month of January 2010 from a nearby village farm and identified by experts in VIT University. The collected samples were stored at room temperature in a closed environment.

Sample pre-treatment

The collected *Moringa oleifera* samples of fruits and leaves were washed with distilled water to remove dirt and other impurities, fruits were then chopped in length of 5 cm and separated in approximately 50 sticks in each packet and the leaves were also separated in different packet by weighing 250 grams in each packet. The distilled water treated sample is considered as control.

Preservative treatment

The fruit and leaves samples were treated with different preservatives and in combination [35].

- Germicide treatment: The fruit and leaf sample were dipped in 25% glutaraldehyde for 5 min and then taken out and subjected for dehydration.
- Germicide and Salt treatment: Samples of fruit and leaf were dipped in 25% glutaraldehyde for 5 min and then taken out then dipped in brine (10% salt solution) and subjected for dehydration.
- Salting treatment: Fruit and leaf samples were dipped in brine (10% salt solution) for 5 min and subjected for dehydration.
- Turmeric and Salt treatment: Samples of fruit and leaf were dipped in turmeric (2%) with brine (10% salt solution) for 5 min and subjected for dehydration.

Drying
The following type of drying process is employed for the samples respectively [36].

- Sun drying: Sun drying was done by exposing the sample in sun between day times between 10.00 am to 5.00 pm daily till the sample attained constant weight.
- Oven drying: The sample was dried at 600°C using hot air oven for 24 hours to obtain a completely dried uniform sample.
- Wind drying: The sample was subjected for wind drying by exposing it in open ground between 10.00 pm to 5.00 am daily till the sample attained constant weight.

Microbiological analysis
Nutrient agar and potato dextrose agar were used for bacteria and fungi respectively [37]. Nutrient agar and potato dextrose agar was prepared and autoclaved at 121°C for 15 min. It is then poured in sterile petri plates under laminar air flow and allowed to solidify. The fruit and leaf samples were incubated for the duration of seven days. The colony morphology of isolates in plates was observed and stained.

Gram staining
A loop full of the culture was smeared on the slide, thin smear was made and air dried. The slide was show in the flame to heat fix the smear. The primary stain (crystal violet) was added to the smear surface and left for 1 minute. The slide was washed gently in running tap water and blot dried using blotting paper. Then grams iodine was added to the smear surface and left for 1 minute, the slide was washed under gently running tap water and blot dried. The decolourizer was added to the smear surface and immediately washed with tap water and blot dried. Finally the counter stain (Saffranin) was added to the smear surface and left for 1 minute and blot dried. The slide was examined at 100X under microscope to visualize the structure of Gram + ve and Gram -ve bacteria [35].

Catalase test
The purpose of the catalase test is to determine the ability of microorganism to degrade H_2O_2 by producing the enzyme catalase. The culture from the plate was transferred to a clean glass slide with the use of inoculation loop under laminar air flow. Few drops of 3% hydrogen peroxide (H_2O_2) to glass slide containing smear was added. The formation of bubbles of free oxygen gas represents a positive reaction.

Oxidase test
The purpose of oxidase test is to distinguish among groups of bacteria on the basis of cytochrome oxidase activity. The oxidase disc containing p-amino dimethylaniline oxalate (HI-MEDIA) was placed in a glass slide.A full loop of culture was taken by sterile inoculation loop and placed on the disc. The formation of purple colour indicates positive.

Indole test
Peptone broth (HI-MEDIA) was prepared according to the manufacturer instruction. 2-3 ml of broth was dispensed in test tubes and autoclaved at 121°C for 15 min. The bacteria were inoculated in the tubes containing peptone broth and incubated at 37°C for 24 hrs. The change in colour of tube was observed by adding Kovac's reagent.

Methyl red (MR) and Voges Proskauer (VP) test
MR/VP broth was prepared and dispensed in test tubes. The test tubes were autoclaved at 121°C for 15 min. The bacteria were inoculated in two test tubes of MR/VP broth, and incubated at 37°C for 24 hrs. After incubation, 0.5 ml of MR reagent was added in one test tube. 0.2 ml of VP reagent A

and 0.2 ml VP reagent B was added to another test tube. The tubes were gently mixed and allowed to stand for 15 min.

Citrate utilization test

Simmons citrate agar (HI-MEDIA) was prepared and dispensed into the test tubes of 2-3 ml. Test tubes were autoclaved at 121°C for 15 min and test tubes were solidified in slanting position. The bacteria were streaked at the slant and incubated at 37°C for 24 hrs.

Urease test

Urea agar (HI-MEDIA) was prepared and autoclaved at 121°C for 15 min. The medium was cooled to 50°C and then 5 ml of filter sterilized 40% urea solution were added. The medium were mixed and dispensed into the test tubes of 2-3 ml. The test tubes were solidified in slanting position. The bacteria were streaked at the surface of the slant and incubated at 37°C for 24 hours.

Dehydration and rehydration ratio

Dehydrated sample (dried sample) was weighed. Rehydration was carried out by steeping the dried samples (5g) in water (120 ml) at 27°C for 2 hours and the sample is weighed [38]. Dehydration and rehydration ratio was calculated by formula mentioned below.

$$\text{Dehydration and rehydration ratio} = \frac{\text{Weight of the rehydrated sample}}{\text{Weight of the dried sample}}$$

Moisture analysis

About 5g of sample was stored at 110°C for 2 hours, and difference in weight between the dry condition and wet condition was reported as moisture content [39].

Chlorophyll estimation

Chlorophyll was estimated by the method followed by Sadavasivam [40]. 1g of the finely cut sample was weighed and grinded into mortar and pestle using 20 ml of 80% acetone. The grinded pulp was transferred into centrifuge tube and centrifuged at 5000 rpm for 5 min and the supernatant was transferred to volumetric flask. The residue was again grinded with 20 ml of 80% acetone and again centrifuged and supernatant was added to same volumetric flask. The process was repeated until and unless the residue was colourless. The mortar and pestle was washed using 80% acetone and the clear washings was collected in the volumetric flask. Then the volume was made up to 100 ml using 80% acetone. Absorbance was read at 645,663 and 652 nm against solvent 80% acetone as blank.

Ascorbic acid estimation

Sample preparation: 5 gram sample extracted in 4% oxalic acid and volume was made up to 100 ml and centrifuged. Supernatant was used as sample. 5 ml of the working standard solution was taken in 100 ml conical flask and 10 ml of 4% oxalic acid was added and it was titrated against the dye (2,6-dichloro phenolindophenol), end point was appearance of pink colour which persists for few minutes.5 ml of sample is taken and 10 ml of 4% oxalic acid was added and titrated against the dye and ascorbic acid content was calculated. Then by comparing the sample with the working standard, the ascorbic acid content is estimated [41].

Carbohydrate estimation

Carbohydrate estimation was done by the method as followed by Sadasivam [40]. About 100 mg of the sample was extracted using 5 ml 80% hot ethanol in mortar and pestle, it is then centrifuged at 8000 rpm for 10 minutes. And the process was repeated with the pellet. After centrifugation the

supernatant obtained was collected and evaporated by keeping it in water bath at 80°C, after evaporation10 ml of distilled water was added in it. 1 ml of the extracts was pipetted in the test tube. And a series of working standard was also prepared by pipetting 0.2, 0.4, 0.6, 0.8 and 1 ml in test tubes. Then the volume was made to 2 ml using distil water in all the test tubes. After that 3 ml of DNS (Dinitrosalicylic acid) reagent was added and kept in water bath for 5 min, 1 ml of 40% potassium sodium tartarate was in all the tubes and allowed to be cooled. Absorbance of dark red colourformed was read at 510 nm. And using standard graph, carbohydrate content in sample was determined.

Protein assay

Protein assay was performed using Lowry method [42]. 0.5mg of sample was grinded in mortar and pestle with 10 ml of phosphate buffer and centrifuged at 8000 rpm for 10 minutes then 0.1 and 0.2 ml of its supernatant was pipetted in the test tubes. Beside this a series of working solution such as 0.2, 0.4, 0.6, 0.8 and 1 ml was pipetted in test tubes. After that all the tubes volume was made up to 1 ml using distilled water and a tube with 1 ml distilled water serves as blank. 5 ml of alkaline copper solution reagent was added in all the tubes, then mixed and allowed to stand for 10 minutes. Then 0.5 ml of folin ciocalteau reagent was added and incubated at room temperature in dark for 30 minutes. The blue colour developed was measured at 660 nm. The protein content in sample was determined by using standard graph.

Results and Discussion

Microbiological analysis

The effect of dehydration, preservative processing and micro-organism present on their surface was studied. Moisture content provide a favourable environment for the growth of microorganism, thus it is reduced during the process of dehydration. Sun-drying in direct sunshine and under shade is the common practices used in most parts of the world to preserve vegetables for dry season consumption [43]. However, ways of food preparation and preservation may affect significantly the concentration and availability of minerals, vitamins and other essential compounds in food. Some reports have documented the losses of nutrients from vegetables during drying [44] and cooking [45, 46].

Different number and types of microorganisms was found to be present on the surface of *Moringa oleifera* leaves and pods. Bacterial and fungal colonies were found when it was plated on Nutrient agar media and Potato Dextrose mediarespectively. The results obtained are shown in Tables 1, 2, and 3 respectively for bacterial colonies and 5, 6 for fungi. Figure 1 represents the morphology of the microbes obtained in pods and leaves of *Moringa oleifera*.

Table 1. Bacterial colonies (cfu) found in pods exposed to different preservative treatment and dehydration during storage time period on nutrient agar media.

Treatment	Fresh	Oven Dried (cfu)				Wind Dried (cfu)				Sun Dried (cfu)			
	(cfu)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled Water (Control)	55	10	12	12	14	50	45	50	53	15	16	16	20
Germicide	4	5	6	7	7	6	6	7	7	2	4	4	4
Germicide and Salt	4	3	3	4	4	4	6	6	7	1	3	3	5
Salt	12	5	4	5	6	13	13	14	16	3	4	4	5
Turmeric and Salt	10	2	4	4	5	12	14	14	15	3	4	4	5

Table 2. Bacterial colonies (cfu) found in leaves exposed to different preservative treatment and dehydration during storage time period on nutrient agar media.

Treatment	Fresh	Oven Dried (cfu)				Wind Dried (cfu)				Sun Dried (cfu)			
	(cfu)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled Water (Control)	10	0	1	1	2	2	2	3	3	6	6	6	6
Germicide	3	0	1	1	2	2	2	3	3	5	6	6	6
Germicide and Salt	3	0	1	1	2	2	2	3	3	5	6	6	6
Salt	5	0	1	1	1	2	2	3	4	6	7	7	7
Turmeric and Salt	4	0	1	1	1	2	2	3	4	6	7	7	7

Table 3. Morphological characterization of bacteria colonies.

Tests	Distilled Water	Germicide	Germicide and Salt	Salt	Turmeric and Salt
Colony morphology	Round, flat/ irregular	Round, flat/ irregular	Round, flat/ irregular	Round, flat/ irregular	Round, flat/ irregular
Colony colour	White and yellow	White	White	White	White
Gram Staining	Gram (+)ve and Gram (-)ve rods and cocci	Gram (+)ve and Gram (-)ve rods and cocci	Gram (+)ve and Gram (-)ve rods and cocci	Gram (+)ve and Gram (-)ve rods and cocci	Gram (+)ve and Gram (-)ve rods and cocci

Table 4. Biochemical characterization of bacteria.

Tests	Bacillus	Staphylococcus	Streptococcus	Pseudomonas	Neisseria
Catalase	+ve	-ve	-ve	+ve	+ve
Oxidase	-ve	+ve	-ve	+ve	+ve
Indole	-ve	-ve	-ve	-ve	-ve
Methyl red	+ve	+ve	+ve	-ve	-ve
Vogesproskauer	-ve	+ve	-ve	-ve	-ve
Citrate utilization	+ve	-ve	-ve	+ve	-ve

Note: +ve = positive; -ve = negative

Biochemical characterisation

The biochemical characterization are analysed by performing catalase, oxidase, indole, methyl red, vogesproskauer, citrate utilization test and the results are tabulated in Table 4.

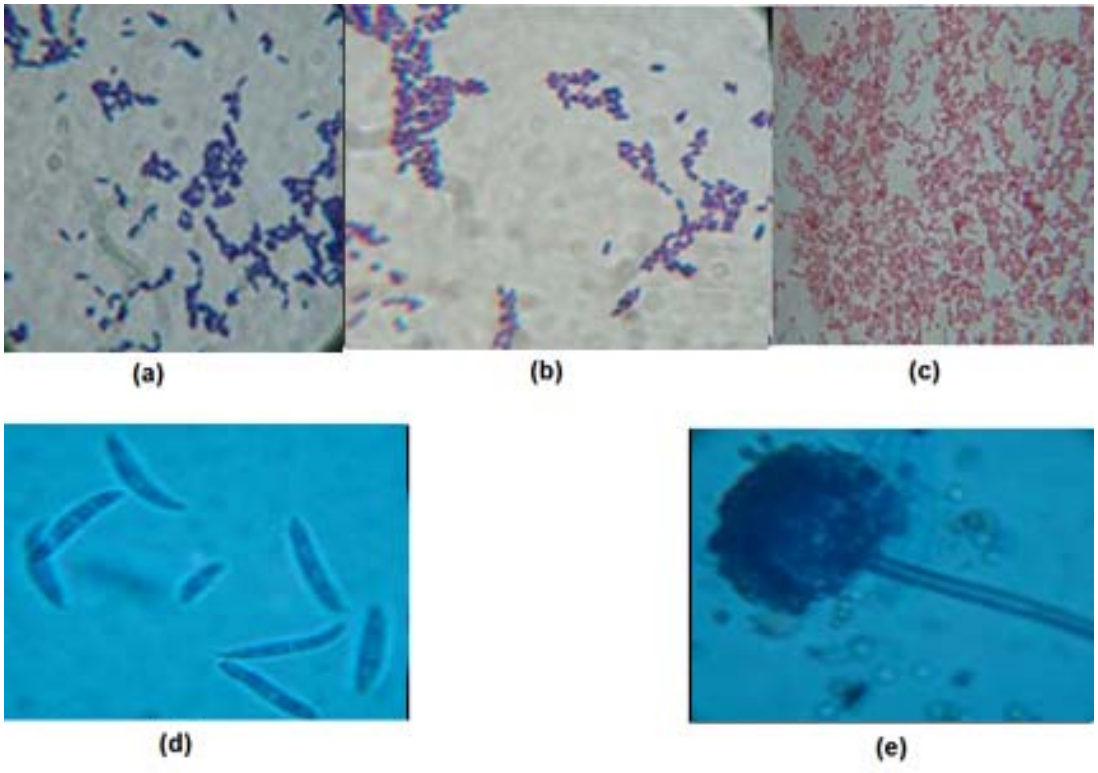

Figure 1. Morphological analysis of microbes present in pods and leaves of *Moringa oleifera*
(a) Gram positive rods (b) Gram positive cocci (c) Gram negative rods and cocci (d) Microscopic picture of Fusarium (e) Microscopic picture of Aspergillus.

The morphological and biochemical characterization of bacteria resulted to identify the bacteria. The bacterial colonies found in pods and leaves were of *Streptococcus, Staphylococcus, Bacillus, Pseudomonas* and *Neisseria*.

Table 5. Fungal colonies (cfu) found in pods exposed to different preservative treatment and dehydration during storage time period on potato dextrose agar.

Treatment	Fresh	Oven Dried (cfu)				Wind Dried (cfu)				Sun Dried (cfu)			
	(cfu)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled Water	4	1	2	2	2	5	5	5	6	2	3	3	3
Germicide	0	0	2	2	3	2	3	4	4	0	1	2	3
Germicide and Salt	0	0	2	2	2	2	3	4	4	0	0	2	3
Salt	0	0	0	0	2	3	3	3	3	0	2	2	4
Turmeric and Salt	0	0	0	2	2	2	2	4	4	1	2	2	4

Table 6. Fungal colonies (cfu) found in leaves exposed to different preservative treatment and dehydration during storage time period on potato dextrose agar.

Treatment	Fresh	Oven Dried (cfu)				Wind Dried (cfu)				Sun Dried (cfu)			
	(cfu)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled Water	5	2	2	2	2	4	5	5	5	2	2	2	3
Germicide	0	0	0	1	1	2	2	3	3	0	0	0	2
Germicide and Salt	0	0	0	1	1	2	2	2	3	0	0	1	1
Salt	1	0	0	1	1	1	2	3	3	1	1	1	2
Turmeric and Salt	1	0	0	1	1	1	2	2	3	1	1	1	2

Dehydration and rehydration ratio

Dehydration and rehydration ratio was found to be highest in case of sun drying, rather than oven and wind drying. The dehydration and rehydration ratio was analysed for samples treated with distilled water, germicide, germicide + salt, salt, turmeric + salt (Tables 7& 8). The dehydration and rehydration ratio was found to be maximum in sun drying in both pods and leaves.

Table 7. Dehydration and Rehydration Ratio in Pods.

Treatment	Oven Dried (Dry Wt /Rehydrated Wt.)		Wind Dried (Dry Wt /Rehydrated Wt.)		Sun Dried (Dry Wt /Rehydrated Wt.)	
	Cold Water	Hot Water	Cold Water	Hot Water	Cold Water	Hot Water
Distilled Water	3.1 : 1	2.8 : 1	2.2 : 1	2 : 1	3.2 : 1	3: 1
Germicide	3.1 : 1	2.8 : 1	2.2 : 1	2 : 1	3.2 : 1	3: 1
Germicide and Salt	3.1 : 1	2.8 : 1	2.2 : 1	2 : 1	3.2 : 1	3: 1
Salt	3.1 : 1	2.8 : 1	2.2 : 1	2 : 1	3.2 : 1	3: 1
Turmeric and Salt	3.1 : 1	2.8 : 1	2.2 : 1	2 : 1	3.2 : 1	3: 1

Table 8. Dehydration and Rehydration Ratio in Leaves.

Treatment	**Oven Dried** (Dry Wt/Rehydrated Wt.)		**Wind Dried** (Dry Wt/Rehydrated Wt.)		**Sun Dried** (Dry Wt/Rehydrated Wt.)	
	Cold Water	Hot Water	Cold Water	Hot Water	Cold Water	Hot Water
Distilled Water	3 : 1	2.4 : 1	2 : 1	1.9 : 1	3.2 : 1	3 : 1
Germicide	3 : 1	2.4 : 1	2 : 1	1.9 : 1	3.2 : 1	3 : 1
Germicide and Salt	3 : 1	2.4 : 1	2 : 1	1.9 : 1	3.2 : 1	3 : 1
Salt	3 : 1	2.4 : 1	2 : 1	1.9 : 1	3.2 : 1	3 : 1
Turmeric and Salt	3 : 1	2.4 : 1	2 : 1	1.9 : 1	3.2 : 1	3 : 1

Moisture content

Maximum moisture content was measured in pods and leaves. Moisture content in pods was given in table 9, and moisture content of leaf was shown in table 10. Fuglie [47] reported that *Moringa oleifera* leaves contain 75.00% moisture. This is in contrast with the moisture and protein levels obtained in the study. This may be due to the difference in the environment where *Moringa oleifera* tree was grown.

Table 9. Moisture content in pods.

Treatment	Oven Dried	Wind Dried	Sun Dried
Distilled water	86%	79.6%	83%
Germicide	86.4%	77.2%	82.8%
Germicide and Salt	88%	78.4%	84%
Salt	86%	82.5%	85%

Table 10. Moisture content in leaves.

Treatment	Oven Dried	Wind Dried	Sun Dried
Distilled water	78.46%	72.30%	76.92%
Germicide	78.46%	72.30%	76%
Germicide and Salt	78.46%	70%	75%
Salt	80%	73%	78%
Turmeric and Salt	75%	74%	76.92%

Ascorbic acid estimation

The ascorbic acid content in all preservation treatments was found to be 100 mg in pods (Table 11) and 200 mg (Table 12) in leaves. There is no significant change in the ascorbic acid level in treated sample.

Table 11. Ascorbic acid in pods before and after drying at regular intervals.

Treatment	Control	Oven dried				Room dried				Sun dried			
	(mg/g)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled water	100	100	100	100	100	100	100	100	100	100	100	100	100
Germicide	100	100	100	100	100	100	100	100	100	100	100	100	100
Germicide and salt	100	100	100	100	100	100	100	100	100	100	100	100	100
Salt	100	100	100	100	100	100	100	100	100	100	100	100	100
Turmeric and salt	100	100	100	100	100	100	100	100	100	100	100	100	100

Table 12. Ascorbic acid in leaves before and after drying at regular intervals.

Treatment	Fresh	Oven Dried (mg/100g)				Wind Dried (mg/100g)				Sun Dried (mg/100g)			
	(mg/g)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled water	200	100	100	100	100	100	100	100	100	100	100	100	100
Germicide	200	100	100	100	100	100	100	100	100	100	100	100	100
Germicide and salt	200	100	100	100	100	100	100	100	100	100	100	100	100
Salt	200	100	100	100	100	100	100	100	100	100	100	100	100
Turmeric and salt	200	100	100	100	100	100	100	100	100	100	100	100	100

Chlorophyll estimation

Chlorophyll is heat sensitive and easily degrades in processed foods. Chlorophyll content of samples continuously declined during the dehydration treatment and storage. Tables 13 and 14 represents the chlorophyll content in pods and leaves respectively. The chlorophyll retention was maximum in room drying as compared to oven drying and was least in sun drying.

Table 13. Chlorophyll in pods before and after drying at regular intervals.

Treatment	Fresh	Oven Dried (mg/g)				Wind Dried (mg/g)				Sun Dried (mg/g)			
	(mg/g)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled water	0.8	0.45	0.45	0.41	0.38	0.49	0.46	0.44	0.40	0.43	0.40	0.40	0.37
Germicide	0.70	0.44	0.44	0.42	0.38	0.47	0.43	0.40	0.38	0.41	0.41	0.39	0.37
Germicide & salt	0.74	0.44	0.44	0.40	0.38	0.47	0.42	0.38	0.38	0.41	0.40	0.39	0.38
Salt	0.8	0.45	0.44	0.40	0.35	0.48	0.45	0.40	0.39	0.43	0.42	0.40	0.39
Turmeric & Salt	0.75	0.43	0.43	0.40	0.37	0.47	0.43	0.40	0.37	0.42	0.40	0.39	0.38

Table 14. Chlorophyll in leaves before and after drying at regular intervals.

Treatment	Fresh	Oven Dried (mg/g)				Wind Dried (mg/g)				Sun Dried (mg/g)			
	(mg/g)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled water	0.75	0.45	0.43	0.40	0.38	0.48	0.46	0.43	0.41	0.42	0.39	0.37	0.36
Germicide	0.80	0.43	0.41	0.39	0.37	0.45	0.44	0.42	0.40	0.40	0.38	0.36	0.35
Germicide & salt	0.79	0.43	0.40	0.37	0.37	0.44	0.43	0.41	0.40	0.41	0.40	0.38	0.36
Salt	0.75	0.45	0.42	0.41	0.40	0.48	0.45	0.42	0.41	0.42	0.40	0.37	0.36
Turmeric & Salt	0.76	0.43	0.43	0.41	0.38	0.44	0.42	0.40	0.40	0.40	0.37	0.36	0.36

Carbohydrate estimation

Green vegetables and leaves are important sources of carbohydrates. Carbohydrate content of samples continuously got degraded during the dehydration treatment and storage in pods while in case of leaves it got increased. It has been reported that the carbohydrate level in the leaf of *Moringa oleifera* is comparatively higher when compared to other vegetables. This perfectly coincides with the carbohydrate levels obtained in the study. Table 15 represents the carbohydrate level in pods and table 16 represents the carbohydrate level in leaves. Carbohydrate was found to be higher in leaves as comparative to pods.

Table 15. Carbohydrate level in pods before and after drying at regular intervals.

Treatment	Fresh	Oven Dried (g/100g)				Wind Dried (g/100g)				Sun Dried (g/100g)			
	(g/100g)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled Water	3.5	1.7	1.5	1.5	1.0	2.5	2.5	2.3	2	2.5	2.2	2.0	1.5
Germicide	3.4	1.7	1.5	1.5	1.0	2.5	2.5	2.3	2	2	2	1.5	1
Germicide and Salt	3.7	1.5	1.5	1.5	1.0	2.5	2.5	2.25	2	2	2	1.5	1
Salt	3.5	1.5	1.3	1.0	0.80	2.25	2.25	2.2	2	2.5	2.5	1.3	1.3
Turmeric and Salt	3.5	1.5	1.3	1.0	0.80	2.5	2.5	2.3	2	2.25	2.25	1.2	1.2

Table 16. Carbohydrate level in leaves before and after drying at regular intervals.

Treatment	Fresh	Oven Dried (g/100g)				Wind Dried (g/100g)				Sun Dried (g/100g)			
	(g/100g)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled Water	10	34	32	30	29	30	28	28	28	32	30	30	29
Germicide	10	32	32	32	30	29	28	28	28	30	30	29	28
Germicide and Salt	10	32	32	32	30	29	28	28	27	30	30	29	29
Salt	10	32	32	32	30	28	28	27	27	29	30	30	29
Turmeric and Salt	10	34	34	34	31	28	28	28	26	29	28	28	27

Protein assay

Protein content of samples continuously declined during the dehydration treatment and storage in pods while in the case of leaves it got increased. Table 17 and table 18 represents the protein levels in pods and leaves respectively. Protein level was found to be higher in leaves as comparative to pods. Fuglie [47] reported that *Moringa oleifera* leaves contain protein level of 10% which is also in contrast with the results obtained this may be due to protein degradation during the storage period.

Table 17. Protein in pods before and after drying at regular intervals.

Treatment	Fresh	Oven Dried (g/100g)				Wind Dried (g/100g)				Sun Dried (g/100g)			
	(g/100g)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled Water	2.4	1.8	1.8	1.8	1.6	2.0	2.0	1.8	1.7	1.8	1.8	1.7	1.7
Germicide	2.6	1.6	1.6	1.6	1.5	2.0	2.0	1.7	1.7	1.7	1.7	1.5	1.5
Germicide and Salt	2.6	1.6	1.5	1.5	1.5	1.8	1.7	1.7	1.6	1.8	1.7	1.5	1.6
Salt	2.4	1.6	1.6	1,5	1.5	1.8	1.8	1.6	1.5	1.8	1.8	1.6	1.4
Turmeric and Salt	2.4	1.6	1.5	1.5	1.4	2.1	1.8	1.7	1.7	1.8	1.7	1.6	1.6

Table 18. Protein in leaves before and after drying at regular intervals.

Treatment	Fresh	Oven Dried (g/100g)				Wind Dried (g/100g)				Sun Dried (g/100g)			
	(g/100g)	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr	Jan	Feb	Mar	Apr
Distilled Water	6.4	25	24	24	22	18	18	17	16	24	24	22	22
Germicide	6.0	25	25	22	22	18	18	17	16	23	23	22	21
Germicide and Salt	6.0	26	24	24	23	18	18	17	15	24	24	22	22
Salt	6.4	26	25	22	22	17	16	16	15	25	24	24	22
Turmeric and Salt	6.4	25	25	22	22	17	17	16	15	25	25	22	22

Conclusion

It was observed that dehydrating *Moringa oleifera* samples by means of oven drying, wind drying and sun drying yielded more or less similar results when employed with combination treatments with germicide, salt and turmeric treatment. The process of moisture removal prevents favourable environment for the growth of microbes, thus it can be stored for longer period of time. Thus it is concluded that dehydration can be effectively used to extend the shelf life of *Moringa oleifera* without alteration in the nutritional value.

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