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Effect of drying methods and storage on bioactive compounds of *Moringa oliefera* leaf powder

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Abstract
Moringa oleifera (Drumstick tree) is well known for its nutritional quality but usage of leaf powder as a functional ingredient is very less. Hence, the leaves were subjected to hot-air, solar-tunnel and shade drying methods and stored under ambient condition. Solar tunnel drying had 89.58 and 16.66% lesser drying time compared to shade and hot-air drying, respectively. The study showed that solar-tunnel dried leaf powder had high ascorbic acid (471 mg /100g), total carotenoids (2.49 mg/g), total polyphenols (39.29 mg gallic acid equivalent /g) and total chlorophyll (7 mg/g) content compared to other drying methods. Higher retention of bioactive compounds was found in solar-tunnel dried leaf powder during storage. Aerobic plate count was higher in shade dried sample throughout the storage period. It can be concluded from the study that solar-tunnel drying is the best green technology to obtain moringa leaf powder with high retention of bioactive compounds which can be utilized as a functional ingredient in food preparations.

Keywords: *Moringa oleifera*, bioactive compounds, drying, storage studies

Introduction
Moringa oleifera (Moringaceae) grows wildly in tropical and subtropical regions of the world. It is native to the sub-Himalayan tracts of India, Pakistan, Afghanistan and Bangladesh. *Moringa oleifera* is commonly called as drumstick tree in India. It has an impressive range of medicinal uses with high nutritional value. Drumstick pods, leaves and seeds are rich source of protein, vitamins, minerals, dietary fibre, carotenoids and polyphenols (Gopalan *et al*. 2009; Verma *et al*. 2009; EI Sohaimy *et al*. 2015) [10, 41, 4]. Moringa leaves have been used to combat malnutrition among infants, children, pregnant and lactating mothers. Carotene from drumstick leaves is effective in overcoming vitamin A deficiency when male albino rats were fed with fresh or dehydrated moringa leaves (Nambiar and Seshadri, 2001) [26]. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers, exudates and immature pods act as cardiac and circulatory stimulants, antihypertensive, antitumor, antiulcer, antipyretic, antiepileptic, anti-inflammatory, antispasmodic, diuretic, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being used for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia (Anwar *et al*. 2007; Mishra *et al*. 2011; Tejas *et al*. 2012) [1, 20. 39].
As drumstick pods and its leaves have been used for food preparation, its dried form has not got so much importance in food preparations or for medicinal purpose. A very few studies are available on moringa leaf powder. As reported by Teixeira *et al*. (2014) [38] moringa leaf powder posses a high amount of protein (28%), fat (7%) and minerals (11%). The major phytochemicals were found to be carotenoids and polyphenols. It contains high amount of mono- and polyunsaturated fatty acids and low amount of saturated fatty acids which is a good combination for benefit of health (Saini *et al*. 2014) [34]. The hypoglycaemic effect of phytochemicals present in moringa leaf powder was evaluated on rats and it was found to reduce blood glucose significantly when administered orally (Ndong *et al*. 2007) [27]. Similarly, the anticancer properties of moringa leaf extracts was tested against human breast cancer cell lines (MCF 7 and MDA MB 231), leukaemia (AML and ALL) and hepatocarcinoma cell lines (HpG2). It was found that leaf extract can reduce the cancer cells on dose dependent manner (Ghosh N. 2014; Khalafalla *et al*., 2010) [1, 15].

The multiple benefits of moringa leaves have been reported by many of the researchers (Palada, 1994; Tejas *et al*. 2012) [30, 39]. Replacement of wheat flour with 5, 10 and 15% drumstick leaf powder in the preparation of cookies increased protein, iron, calcium, β-carotene and dietary fiber content (Dachana *et al*. 2010) [5]. Although it is widely cultivated for its pods in many parts of the world, its leaves have been sparsely used in food application. Leaves of *M. oleifera* have been consumed by tribal groups and in some parts of Africa and southern India. Hence, this study was undertaken to evaluate the effect of different drying methods on bioactive compounds of moringa leaves after drying and during storage period.

Materials and Methods

Fresh, matured leaves of cv. Bhagya were harvested in early hours of morning from the experimental field at ICAR-Indian Institute of Horticultural Research during April month. The leaves were separated manually, washed with potable water and allowed to drain. Each batch containing 500 g of leaves were separated and subjected to different methods of drying such as hot-air, solar-tunnel and shade drying in duplicates. The solar tunnel drier consisted of a floor area of (6m×3m) and 2.7m height. The dryer was provided with galvanised iron frame covered with Ultra-Violet stabilised polyethylene sheet of 200µ size. Moringa leaves in trays of size (0.8m×0.4m×0.03m) were placed in platform of size 2.7m x1m x 0.96m for drying. Moisture laden air from the dryer was removed by two exhaust fans (each 50 watt capacity) fitted at the front side of the dryer. The solar-tunnel was oriented towards north-south direction and the temperature ranged between 30 - 43.2 °C. The hot-air drying was performed in cabinet tray drier which consists of 24 trays of size (0.8m x 0.4m x 0.03m). The temperature was maintained at 50 °C. Shade drying was performed at room condition (30-34 ºC). Weight of samples at an hourly interval was recorded to calculate the drying time. The samples were removed and powdered using laboratory type steel blade blender and was packed in aluminium pouches of size 13.3 x 8.5 cm^2 and stored under room temperature. The samples were analyzed for bioactive compounds and microbial parameters after drying and during storage period up-to 120 d in triplicates. Total carotenoids content were analyzed spectrophotometrically (Ranganna, 2000) [20] and ascorbic acid was done by titrimetric method using 2, 6-dichlorophenol indophenol dye (Ranganna, 2000) [20]. Total polyphenols content was estimated by Folin-Ciocalteau method at 700 nm, and expressed as gallic acid equivalents (GAE) (Singleton et al., 1999) [36]. Total chlorophylls were estimated spectrophotometrically as per the method described by Arnon (1945) [2] and Witham *et al*. (1971) [43].

The samples were analyzed for their microbiological quality during their storage period by serial dilution and plating in standard selective and differential media. For this, 25 g of sample was transferred to stomacher bags containing 225 ml sterile water and blended in a stomacher (Seward 400 Circulator®, Seward UK) for two minutes. Serially diluted suspensions from the blended sample were pour plated on different media *viz*., plate count agar (aerobic plate count), Mann Rogosa Sharpe agar (lactic acid bacteria), yeast extract peptone dextrose agar (yeasts), violet red bile agar (coliforms) and tryptone bile glucuronic acid agar (Escherichia coli). Violet red bile agar plates were incubated at 37 ºC for 24 h, while all the other media plates were incubated at 28 ºC for 48 h. Colony enumeration was done using a digital colony counter (LA663 Digital colony counter®, Hi media Laboratories, India). All analyses were conducted in triplicate and the microbial counts are reported as log 10 colony-forming units (CFU) per gram.

MS Excel was used for calculation of mean and SE values. A completely randomized blocking experimental design was used for this study and data were analyzed using WASP statistical software (Web Agri Stat Package, Version 2.0, ICAR Research Complex for Goa, Ela, Goa, India.) (Jayade *et al.* 2015) [13].

Results and Discussion

Weight loss of moringa leaves was found higher in hot-air drying during initial hour of drying compared to solar-tunnel drying (Fig 1). After 2 hrs, the weight loss was found higher and faster in solar-tunnel drying. Samples dried under solar-tunnel found to have shorter drying time (5 h) compared to hot-air (6 h) and shade drying (48 h). The results showed that solar tunnel drying had 89.58 and 16.66% lesser drying time compared to shade and hot-air drying, respectively. The final moisture content of the sample dried under solar-tunnel, hot-air and shade drying was 4.04, 3.85 and 5.65%, respectively. Similar results were observed in bitter gourd and capsicum drying under solar drying by Mehta *et al*. (2017) [19]; in moringa leaf powder dried under oven, sun and shaded condition (Emilike and Ebere, 2016) [6]. Bioactive compounds such as ascorbic acid, total carotenoids, total chlrorophyll; and polyphenols was found higher in dried moringa leaves compared to fresh leaves.

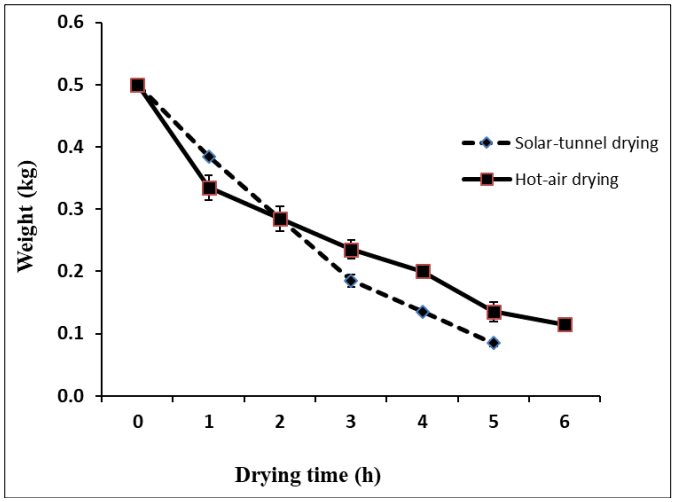

Fig 1: Drying time of moringa leaves under solar-tunnel and hot-air drying methods. Bars represent standard error (n=2)

Ascorbic acid

Ascorbic acid plays many roles in human body. It is essential for maintenance of collagen tissue, cartilage tissue, carnitin, skin, bones; teeth etc., Absorption of heam iron can be enhanced in the presence of ascorbic acid in foods (Naidu, 2003) [24]. Ascorbic acid was found significantly higher in solar-tunnel dried moringa leaf powder followed by hot-air dried and least was found in shade dried powder on dry weight basis (Table 1). Similar observations of high ascorbic acid content in solar dried amaranth and fenugreek leaves compared to shade drying was made by Negi and Roy (2000) [28]. During storage period of 120 d, about 36.95% retention was observed in shade dried samples whereas in solar dried samples 46.26% was retained. An important observation was made by Munyaka *et al*. (2010) [21] on the effect of ascorbic acid oxidase (AAO) enzyme on retention of vitamin C due to

heating in broccoli vegetable. It was observed that heat shock treatment effectively reduced the activity of an enzyme resulting in retention of more vitamin C as L-ascorbic acid (L-AA). Whereas in samples which were not heat treated showed more dehydroascorbic acid (DHAA) which means the enzyme was actively involved in conversion of L-AA in to DHAA. So, higher retention of AA in heat treated samples was may be due to inactivation of AAO (Leong and Oye., 2012, Munyaka *et al*., 2010, Howard *et al*., 1999) [17, 21, 11]. Similar observations can be made in the present study also, high ascorbic acid content can be observed in heat treated samples *viz*. hot-air dried and solar-tunnel dried, whereas less ascorbic content was observed in shade dried samples in which heat was not applied. This means, the AAO might have oxidized most part of L-AA into DHAA in shade dried samples. High ascorbic acid content was believed to be due to inactivation of ascorbic acid oxidase during heating which might have protected ascorbic acid from oxidation.

Total carotenoids

Carotenoids play an important role in human health. The biological role of carotenoids such as antioxidant activity, pro-vitamin A activity, immunity response, gap junction communication and in prevention of many chronic diseases have been reported well (Rao & Rao 2007; Johnson 2002) [33, 14]. Several epidemiological studies showed that disorders caused by reactive oxygen species can be significantly reduced by intake of carotenoids (Fiedor and Burda, 2014) [8]. In the present study, total carotenoids content was found higher in solar-tunnel dried (2.49 mg/g) moringa leaf powder compared to hot-air (2.05 mg/g) and shade dried (1.76 mg/g) leaf powder after drying (Table 1). After storage period of 120 d, only 42% of carotenoids were retained in both solar-tunnel and hot-air dried moringa leaf powder. Even though the retention has reduced to half but the value is much higher when compared to fresh fruits such as mango, orange and papaya (Gopalan *et al*. 2009) [10]. Similarly, high β-carotene content was observed in leaves of savoy beet, amaranth and fenugreek after drying under solar and cabinet drying compared to sun and shade drying conditions (Negi and Roy 2000) [28]. High content of all-trans-β-carotene (662-353 µg/g dm), all-trans-α-carotene (21-8 µg/g dm) and 9-cis- β-carotene (101-63 µg/g dm) were observed in solar dried compared to open sun-drying of eight types of green leafy vegetables compared to open sun drying (Mulokozi and Svanberg, 2003) [22]. Low content of total carotenoids in shade dried leaf powder may be attributed to oxidation and isomerisation of carotenoids due to long exposure of leaves to atmospheric oxygen and light (Thane and Reddy, 1997) [40].

Total polyphenols

Polyphenols are secondary metabolites of plants and it gives a taste of bitterness and astringency to the fruits and vegetables. Many studies suggests that long term consumption of fruits and vegetables which are rich in polyphenols benefits human health by reducing the risks for development of certain types of cancers, cardiovascular diseases, hypertension and neurodegenerative diseases (Nardini *et al*. 2007; Pandey and Rizvi 2009; Li *et al*. 2015) [25, 31, 18]. High polyphenol content was observed in solar-tunnel dried moringa leaf powder after drying. However, polyphenol content in leaf powder obtained from hot-air drying was at par with shade dried leaf powder (Table 1). It was observed that storage period did not have any impact on phenol content in shade dried powder and more than 60 per cent polyphenol retention was observed in both solar-tunnel and hot air dried leaf powder.

Total chlorophyll

Chlorophylls are the plant pigments which are available in large quantity and consumed in higher amount through intake of leafy vegetables which are rich source of chlorophylls. A number of studies have been conducted to examine the health benefits of chlorophylls as antimutagenic, anti-inflammatory and anticancerous agent (Okai *et al.* 1996; Ferruzi and Blakeslee 2007; Subramoniam *et al*. 2012) [29, 17, 37]. In the present study, high chlorophyll content was observed in solar-tunnel dried (7 mg/g) leaf powder and the least was observed in shade dried (4.77 mg/g) leaf powder (Table 1).

Table 1: Changes in bioactive compounds in moringa leaf powder after drying and during storage period.

			Drying methods		
Bioactive compound	**Storage period (d)**	**Fresh leaves***	**Hot-air drying**	**Solar-tunnel drying**	**Shade drying**
Ascorbic acid (mg/100g)	0	166.83^{c}	223.20^{b}	471.20^{a}	124.00^{d}
	60	-	140.00^{b}	271.60^{a}	58.66^{c}
	120	-	100.00^{b}	218.00^{a}	45.83^{c}
Total carotenoids (mg/g)	0	1.49^{d}	2.05^{b}	2.49^{a}	1.76^{c}
	60	-	1.84^{b}	2.40^{a}	1.70^{b}
	120	-	1.00^{a}	1.05^{a}	0.61^{b}
Total Polyphenols (mgGAE/g)	0	11.97^{c}	32.67^{b}	39.29^{a}	34.28^{b}
	60	-	44.85^{ns}	44.45^{ns}	45.99^{ns}
	120	-	25.88^{b}	26.21^{b}	34.08^{a}
Total Chlorophylls (mg/g)	0	7.81^{a}	5.81^{c}	7.00^{b}	4.77^{d}
	60	-	2.75^{b}	3.13^{a}	2.23^{c}
	120	-	1.70^{b}	2.51^{a}	1.46^{b}

Values (dry weight basis) are the means of three replications and values in each row with different superscript are significant from each other.

* Fresh weight basis

After 120 d of storage period, about 36% retention was observed in solar-tunnel dried powder which is significantly high compared to hot-air dried and shade dried powder where in about 30% retention was observed. It was observed in apricots, cherries, nectarines, peaches, plums and carrots that heating increased anthocyanins in some fruits while carotenoids content was maintained in cherries, peaches, plums and red bell peppers (Leong and Oye, 2012) [17]. It was hypothesized by the author that an increased amount of phytochemicals might be due to the fact that disruption of cell membranes due to heating in which phytochemicals were embedded leads to higher extraction into the solvent and also diffusion of phytochemicals from cells into water medium.

Microbial quality

Molds, lactic acid bacteria, yeasts and E coli were not detected in moringa leaf powder throughout the storage period irrespective of the drying methods (Table 2). However, aerobic plate count was higher in shade dried samples throughout the storage period. The microbial load in shade dried samples was more than the safety limit ($40x10^{3}$cfu/g)

during initial period of storage as per FSSAI (Food Safety and Standard Authority of India) specification for dehydrated vegetables. Most of the other countries have specifications based on food borne pathogens *viz. Staphylococcus aureus*, *Salmonella*, *Bacillus cereus* etc., but not for microbes associated with spoilage. The samples were found to be free of such public health significance organisms also (data not shown). However, storage reduced the total plate count in shade dried samples. This is probably due to the hostile environment for the organisms such as antimicrobial properties of moringa and low water activity. Highly contaminated (fungi ranged between 6.5×10^5 to 9.4×10^5 CFU/g) leaves of spinach and pumpkin were observed in home dried samples which are mostly dried under open sun drying condition. These were also contaminated with *Salmonella*, *Shigella* and *Bacillus* sps. This may be due to the fact that these leafy vegetables are close to ground level and the pathogen may easily get transferred to the plants (Victor *et al*. 2017) [42]. It is also important to mention here that since moringa leaves are not grown on the ground level, contamination by pathogenic microbes is very limited.

Table 2: Microbial load of moringa leaf powder obtained from different drying methods during storage period

Storage period	Sample	Total aerobic plate count (Log CFU/g)	Mold	Yeasts	Lactic acid bacteria	*E. coli*
0 d	Hot-air dried	1.30	ND	ND	ND	ND
	Solar-tunnel dried	ND	ND	ND	ND	ND
	Shade-dried	5.28	ND	ND	ND	ND
60 d	Hot-air dried	3.70	ND	ND	ND	ND
	Solar-tunnel dried	ND	ND	ND	ND	ND
	Shade-dried	3.99	ND	ND	ND	ND
120 d	Hot-air dried	3.62	ND	ND	ND	ND
	Solar-tunnel dried	2.95	ND	ND	ND	ND
	Shade-dried	3.83	ND	ND	ND	ND

ND-not detected

Conclusion

It can be concluded from the study that *Moringa oleifera* leaf powder with high bioactive compounds can be obtained by solar-tunnel drying with safety level of microbial load as green technology compared to hot-air and shade drying methods. Moringa leaf powder can be used as functional ingredient in preparation of food products as it provides high amount of ascorbic acid, carotenoids, chlorophylls and poplyphenols.

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