Drying Kinetics and Physico-Chemical Analysis of Drumstick Leaves (*Moringa oleifera)*

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By

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Under the guidance of

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CERTIFICATE

This is to certify that the project entitled **"Drying kinetics and physico-chemical analysis of drumstick leaves (*Moringa oleifera*)",** is a bonafide record of work carried out by **Mr. Abhishek Kumar,** under my supervision and guidance for the partial fulfillment of the requirements for the award of the degree of Master of Technology in Food Process Engineering during the academic session 2021-22 in the Agricultural and Food Engineering Department at the Indian Institute of Technology Kharagpur. The experimental works and results embodied in this thesis have not been submitted to any other University or Institute for the award of any degree or diploma.

Date: 25-04-2022 **Prof. P. Srinivasa Rao**

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DECLARATION

I, **Abhishek Kumar,** hereby declare that the thesis entitled **"Drying kinetics and physico-chemical analysis of drumstick leaves (*Moringa oleifera*)"** submitted to Indian Institute of Technology Kharagpur for the degree of **Master of Technology in Food Process Engineering,** is a result of the original research work doneby me. This written submission represents my own ideas, presented in my own words. Others' ideas have been considered in completing the above work. I have adequately cited and thoroughly referenced the original sources. I also declare that I have completely adhered myself to all principles of the academics and completed the work with complete honesty and integrity. Also, to the best of my knowledge, the thesis or part thereof has not been published earlier elsewhere in any manner.

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Place: IIT Kharagpur

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Date: 25-04-2022 **Abhishek Kumar**

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LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviations	Descriptions
%	Percentage
°C	Degree Celsius
AC	Antioxidant Capacity
cm	Centimeter
db	Dry Basis
wb	Wet Basis
et al.	and others
DPPH	2,2-diphenyl-1-picrylhydrazyl
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
&	And
mL	Milliliter
min	minute
Fig.	Figure
QE/g	Quercetin equivalent per gram
GAE/g	Gallic acid equivalent per gram
mm	Millimeter
RT	Room temperature
ΔE^*	Total color change
mg	Milligram
m/s	Meter per second
viz	namely
dm	Dry matter

ABSTRACT

Drumstick (*Moringa Oleifera*) is known for its amazing nutritional content and medicinal properties. Moringa leaf powder can be utilised in preparation of food products and diet supplements. It can play an important role in tackling the problems or diseases caused by lack of nutrition. Preservation and storage of leaves for future utilization is the first and most important step in the leaf processing units. Drying, the process of removal of free moisture is very economical and vast employed method to enhance shelf life of biological produce. Therefore, drying becomes important for the conservation and storage of the moringa leaves until its further processing. The present study aimed to evaluate the drying kinetics of moringa leaves, as well as to understand the diffusion phenomenon during the drying process under various drying methods. Quantitative analysis of phytochemicals and antioxidant capacity under various drying methods. The study was extended to study the storage stability of the dried leaf powder at different storage conditions. Moringa leaves were dried using three methods, *viz.*, hot air oven dryer (55 65, and 75 °C), microwave dryer (200, 400, and 600 W) and freeze dryer. Moisture content of moringa leaves was found in the ranges of 75-80% (wb). It was observed that the drying time for the leaves varied between 4.5-6.5 h, 3-13 min, and 24 h in hot air oven dryer, microwave dryer, and freeze dryer, respectively. The effective moisture diffusion coefficient ranged from 2.88×10^{-9} to 3.04×10^{-9} m²/s in case of hot air oven dryer and 1.01×10^{-7} to 3.45×10^{-7} m²/s in case of microwave dryer. Drying kinetics revealed that drying occurs in falling rate period which indicates the moisture removal from leaves was governed by diffusion process. There was a significant change in the physico-chemical properties of the leaves upon drying. Higher drying temperature was the main cause of reduction of quality attributes. TPC of samples dried under microwave at 200 W was 57.42 ± 2.49 mg GAE/g-dm and was the highest. TPC of samples dried under hot air oven at 65 °C was 24.62 ± 1.10 mg GAE/g-dm and was the least. Antioxidant capacity of the samples dried in freeze dryer was 5.94 ± 0.25 mg GAEAC/g-dm and was the highest. From the study, it can be stated that microwave drying at 200 W power level resulted better retention of physico-chemical properties of the moringa leaves. The water activity was found below the critical water activity (0.6) and other parameters were also in acceptable condition over the observation period (one months) in both packaging materials. Water activity of samples stored in LDPE in deep freezer (-30 °C) increased by 2.07 % and was minimum.

Key words: Moringa leaves, drying kinetics, diffusion, phytochemicals, storage stability.

Chapter 1

Introduction

Drumstick *(Moringa oleifera),* commonly known as moringa. It belongs to the monogeneric family. Moringa is the most widely known, cultivated, and utilized of all 14 known species in this genus. It is native to the Sub-Himalayan tracts of India, Bangladesh, and Afghanistan. Moringa leaves are known to cure several diseases with tremendous health benefits (Ali et al. 2014). Due to the incredible medicinal advantages of moringa leaves, it is now in high demand as a functional food supplement. The leaves are rich in minerals, vitamins and other essential phytochemicals. Extracts from the leaves are used to treat malnutrition, augment breast milk in lactating mothers. It is used as potential antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial agent (Gopalakrishnan et al. 2016). For ease of dissemination of the functional properties of moringa leaves, it needs to be converted in a convenient form (such as powder) using suitable technologies for consumption and year-round availability.

Moringa leaves has long been used in herbal medicine by Indians and Africans. It is often referred as a panacea and can be used to cure more than 300 diseases. It has also been incorporated into chocolates. It is also used to treat dementia, as it has been shown to be a promoter of spatial memory. It can decrease acidity in gastric ulcers by a percentage of 86.15% and 85.13% at doses of 500 mg and 350 mg, respectively and therefore can be used as an antiulcer agent. Moringa leaves are suggested to be included in the diet, with the view of boosting the immune system of HIV positive individuals. Moringa dried leaves powder can be used as a fortificant with the hope of adding additional nutrients to snacks.

1.1 Nutritional and medicinal values of moringa leaves

The moringa leaves are rich in minerals, vitamins and other essential phytochemicals. Extracts from the leaves are used to treat malnutrition. Moringa leaves is said to provide seven times more vitamin C than oranges, ten times more vitamin A than carrots, 17 times more calcium than milk, nine times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach (Gopalakrishnan et al. 2016; Yadav & Srinivasamurthy, 2020). As per Gopalakrishnan et al. (2016) eight ounces of milk can provide 300-400 mg, while moringa leaves can provide 1000 mg and moringa leaves powder can provide more than 4000 mg of Calcium. Moringa leaves powder can be used as a substitute for iron tablets, hence

as a treatment for anaemia. Beef has only 2 mg of iron while moringa leaves leaf powder has 28 mg of iron (Table 1.1). It has been reported that moringa leaves contains more iron than spinach. Boiling increased the availability of iron and antioxidant content. Phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugar present along with anti-cancerous agents like glucosinolates, isothiocyanates, glycoside compounds and glycerol-1-9 octadecenoate. It is used as potential antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial agent (Gopalakrishnan et al. 2016).

1.2 Potential food application of moringa leaves

With increased knowledge of consumers about the relationship between food, health and nutrition, it has also increased the demands of nutritional food, as well as the need to develop foods with functional ingredients from plant source such as *moringa oleifera*, which is well known as 'natural nutrition of the tropics'. Deriving simple, acceptable and nutritionally rich foods made of moringa leaves would help in fighting nutritional deficiencies across nations. Specially, in developing countries where the number of people living in poverty is high, identification of affordable, readily available food items which are rich in nutrients will be a boon to them. The leaves, pods and seeds of moringa oleifera tree possess enormous nutritional and antioxidant properties. Drying makes leaves easy to handle and store as drying increase their self-life. Also, after drying, nutrients get concentrated, making leaves more nutrient richer and hence more valuable.

Soups can be made out of moringa leaves alone or in combination with spinach, melon etc. Moringa paneer, Paneer with extract of moringa leaf of different concentration were investigated and it was found to have better nutrient content than normal paneer. Moringa incorporated chocolates were prepared and estimated for its nutritional content, it was found that the protein, crude fibre and ash content increased appreciably with increasing concentration of moringa leaf powder. Herbal biscuits, biscuits incorporated with moringa oleifera leaf powder at the rate of 5% was reported to increase protein content by 14% (Alam et al. 2014). Bread fortified with 5% of moringa oleifera was found to have 17% and 88% increase in protein and dietary fibre content (Chinma et al. 2014). Moringa leaves are considered to be more palatable. The use of moringa leaves as an ingredient in foods to add functionality has increased recently. Many studies have shown the potential use of different

parts of moringa oleifera in food applications such as discussed in the following paragraph. It was found that moisture, crude protein, crude fibre and total ash showed an increase while total fat and carbohydrate content decreased with increasing concentration of moringa (Chinma et al. 2014).

Moringa Muffin: Moringa oleifera dried powder has also been used in the production of muffin, where up to 12% concentration of dried leaves powder were incorporated. At this concentration, the muffin can be produced successfully with enhanced nutritional qualities and acceptable sensory qualities. The values for ash content increased significantly from the controlled muffin. Moringa muffin was found to contain significantly high amount of protein, fat, beta carotene, and vitamin C. The mineral content was also high for moringa muffin. Calcium, iron and potassium content of the moringa muffin was found to increase significantly than the controlled one. Phosphorus content also increased in the moringa muffin though not significantly (Srinivasamurthy et al. 2017).

1.3 Drying

Drying is one of the oldest preservation techniques utilized for moisture content reduction of biological materials to a level where microbial growth is inhibited, and deteriorative chemical reaction rates are minimized. Besides extending shelf life, drying also facilitates production of variety of value-added products from agricultural produce.

Drying is defined as a process of moisture removal due to simultaneous heat and mass transfer. In food engineering terms, drying can be considered as a coupling phenomenon of simultaneous heat and mass transfer. Heat is transferred directly or indirectly to the plant material, resulting in the evaporation of water with two forms: either as surface moisture (both the initial surface moisture and the moisture transferred by diffusion to the surface) or as vapor that is internally evaporated and subsequently transported to the surface. Water moving by diffusion from the interior of the plant material to the surface may drift essential oil and volatile substances (Orphanides et al. 2016).

The main goal of the drying process is to lower water levels to less than 15% in order to inhibit microbial growth and to minimize biochemical changes. Dehydration is the most common preservation method for prolonging the shelf-life of herbs and spices. Drying also facilitates easier handling and transportation. Drying allow longer periods of storage, minimize

packaging requirements, and reduce shipping weights. The dried product can easily be milled or ground and can be used in the nutraceuticals, baking and culinary industries.

Drying not only affects the water content of the product, but also alters other physical, biological, and chemical properties such as enzymatic activity, microbial spoilage, viscosity, hardness, aroma, flavour, and palatability of foods. Principally high temperature causes the decomposition of bioactive ingredients and changes in colourful components. A potent hepatotoxin, in *Mentha longifolia* subsp. capensis was significantly decreased when oven drying was applied. It is also observed to decrease bitterness in bay leaves.

1.4 Research gaps

Moringa is best known for its medicinal and nutritional values and has enormous potential uses but is very less explored. It can be utilized to make foods which could be a step towards curbing malnutrition. But there is a knowledge gap in potential uses of moringa and its use in food fortification. In making of most of the products moringa leaves has to be dried first to incorporate with the base materials, so there is need to find out the optimum drying conditions which should be economic as well as able to preserved its nutritional and medicinal value. Drying of leaf not only ensure the storage stability but also helps availability throughout the year. Moreover, fewer studies available on storage stability of dried leaf powder of moringa.

1.5 Objectives

1) To analyse the drying kinetics of *Moringa oleifera* leaves under various drying methods.
2) To evaluate the effect of drying on physico-chemical properties of *Moringa oliefera* leaves dried using different drying methods.
3) To study the storage stability of dried *Moringa oleifera* leaves powder under different storage conditions.

Table 1.1: Nutritional composition per 100 g of fresh leaves, dried leaves and powder of dried moringa Leaves.

Nutrients	**Fresh Leaf**	**Dried Leaf**	**Leaf Powder**
Calories	92	329	205
Protein(g)	6.7	29.4	27.1
Fat (g)	1.7	5.2	2.3
Carbohydrate (g)	12.5	41.2	38.2
Fiber (g)	0.9	12.5	19.2
Vitamin B1 (mg)	0.06	2.02	2.64
Vitamin B2 (mg)	0.05	21.3	20.5
Vitamin B3 (mg)	0.8	7.6	8.2
Vitamin C (mg)	220	15.8	17.3
Vitamin E (mg)	448	10.8	113
Calcium (mg)	440	2185	2003
Magnesium (mg)	42	448	368
Phosphorus (mg)	70	252	204
Potassium (mg)	259	1236	1324
Copper (mg)	0.07	0.49	0.57
Iron (mg)	0.85	25.6	28.2

Source: Gopalakrishnan et al. (2016)

Chapter 2

Review of Literature

Several studies have been conducted and published related to the drying of medicinal herbs but limited studies are available on the intermittent or hybrid drying of medicinal herb. This chapter reviews on the utilization of different herbs and spice with special focus on moringa oleifera, bioactive compounds inplants and their importance, drying methods and their effects.

2.1 Medicinal herbs: Antioxidative properties and their utilization

Herbs are generally considered as rich source of antioxidants. Moringa powder extensively used to incorporate flavours, colours and aroma in food. The phytochemicals present in these herbs have preservative effect on food. Since the antioxidants found in these herbs are of natural origin, their importance and demands are always observed among the consumers (Embuscado, 2015). Different parts of various plants show antioxidative properties and are utilized in food applications. For example, antioxidative compounds are found in the leaves of Moringa, Basil, Bay leaf, Tarragon, Thyme; bark of Cinnamon, Cassia; seeds of Fennel, Dill mustard, Fenugreek; fruits of Black pepper, Clove, Chilly; bulbs of Leek, Garlic, Onion; roots of Ginger, Turmeric, etc. Numerous plants have been identified with potential antioxidative properties, and so further research need be carried out to find new plants with important antioxidative properties along with development of protocols for their proper extraction and isolation.

2.2 Microwave drying

Microwaves are defined as electromagnetic waves with a frequency band of about 300 MHz to 300 GHz. The microwave drying is possible because water molecules present in the wet material are electric dipoles. This means that they have a positive charge at one end and a negative charge at the other and therefore rotate as they try to align themselves with the alternating electric field induced by the magnetron. This molecular movement creates heat by friction as the rotating molecules hit other molecules and put them into motion (Sutar & Prasad, 2008). According to Figiel (2010) during microwave drying, the temperature of the dried material depends on the balance between the energy generated by water dipoles in the microwave field and the energy absorbed by water molecules evaporated from the surface of

the material. Dev et al. (2011) studied *moringa oliefera* and found that the samples dried at 50 °C using microwave assisted hot air drying were the best in terms of all of the quality attributes tested in the study. He also reported that drying was five times faster (more than 80% reduction) in comparison to the simple hot air drying. Microwave-assisted drying at 60 °C with a power density of 1 W/g is the optimum drying condition in terms of colour change.

Alibas (2014) studied basil leaves and reported dramatic losses in protein and nutrients can be attributed to the increase in the product's oxidation due to the longer drying time rather than the high temperature resulting from the high microwave power. The most successful drying method was microwave-drying at 700 W. By increasing the microwave output power from 180 to 900 W the drying time decreased from 34 to 8 min that reduced the moisture content of celery leaves from 6.65 to 0.1 kg/kg dry basis (-76%) (Demirhan & Özbek, 2011). Microwave drying of savoury leaves at 700 W and 2450 Hz shortened the drying time over 99% when compared to the sun drying (Arslan & Özcan, 2012).

Motevali et al. (2016) experimented on chamomile under eight microwave power levels varied from 200 W to 900 W and found the optimum drying at 400 W, 50 °C and 0.5 m/s. Also found that moisture diffusivity initially increases but after sometimes it decreases and drying time decrease with increase in power. Drying rate decrease because moisture content decrease with drying time since water is responsible for energy absorption. Energy consumption increase continuously with decreasing moisture content.

2.3 Oven and vacuum oven drying

When savoury leaves were dries at 50 °C for 12 hours it was found that the drying time reduced over 70% when compared to the sun. Also mineral content of oven-dried savoury were higher than the sun and microwave drying (Arslan & Özcan, 2012). Lemon verbena leaves were oven drying at 40 °C is often used for drying of medicinal plants, vacuum drying at 60 °C seems to be an effective approach for the conservation of total essential oil content as well as of specific oil components in a significantly shorter drying time. And twofold reduction of drying time was reported in vacuum compared with oven drying irrespective of drying temperature (Ebadi et al. 2015).

Divya et al. (2012) worked on coriander, she evaluated the changes in growth phases and highest foliage biomass was recorded before flowering stage in all varieties of coriander. Also,

the oven drying of coriander foliage, even at a very low temperature of 45 °C resulted in substantial loss of both chlorophylls (65%) and carotenoids (35%).

2.4 Hot air oven drying

Dev et al. (2011) dried moringa leaves sample at 50 °C using microwave assisted hot air oven drying and found the results best in terms of all the quality attributes tested in this study. And drying time reduced more than 80% in comparison to simple hot air oven drying. Microwave-assisted drying at 60 °C with a power density of 1 W/g is the optimum drying condition in terms of colour change. Premi et al. (2010) dried moringa leaves at 60 °C was found better quality attributes as compared to the samples obtained at 50, 70 and 80 °C because at 50 °C retention time was more and at 70 and 80 °C retention time was less but colour degradation is more. Shaw et al. (2007) reported that 300 minutes is required to dry coriander leaves to a final moisture content of 4.17% (dry basis) at 50 °C and 1.1 m/s air velocity, using a convective dryer.

2.5 Sun, shade and freeze drying

Freeze drying (FD) has also been used to dry many herbs. In this technology, firstly the plant material is frozen and subsequently the ice is removed by sublimation. Because of the absence of liquid water and the low temperature required for the process, most of the deterioration and microbiological reactions are prevented, which gives the final product with an excellent quality. Fresh and dried savoury leaves had high amounts of minerals but the drying time required was approximately 70.4% more than the time needed to reach the same level of moisture content in hot air oven drying (Arslan & Özcan, 2012). When lemon verbena dried using sun, shade, hot air oven and freeze drying it was observed that the structure of trichome was conserved only in shade and freeze drying (Ebadi et al. 2015).

2.6 Intermittent Drying

In an energy intensive industry like heating or drying, improving energy efficiency by 1% could result as much as 10% increase in profit. Drying causes changes in the food properties including discolouring, aroma loss, textural changes, nutritive value, and changes in physical appearance and shape. Intermittent drying is one of the technical solutions to this because it reduces effective drying time and improve quality of the product. Intermittent drying can be accomplished by controlling the supply of thermal energy, which can be achieved by varying

the air-flow rate, air temperature, humidity, or operating pressure (Kumar et al. 2014). Intermittent drying is a drying method where drying conditions are changed with time. It can be achieved by varying drying air temperature, humidity, pressure or even mode of heat input. Intermittency should be selected based on heat and mass transfer involved in the drying process and material properties of the product to be dried. Intermittent drying gives good quality product, decreases the effective drying time and drying air utilization thus it reduces energy consumption.

Intermittency ratio (α) is defined as the ratio of tempering time (off time) to total drying time. where T_{on} and T_{off} are the on and off period of each cycle, respectively. So, alpha = 0 refers to continuous drying and higher intermittency (α) refers higher tempering period.

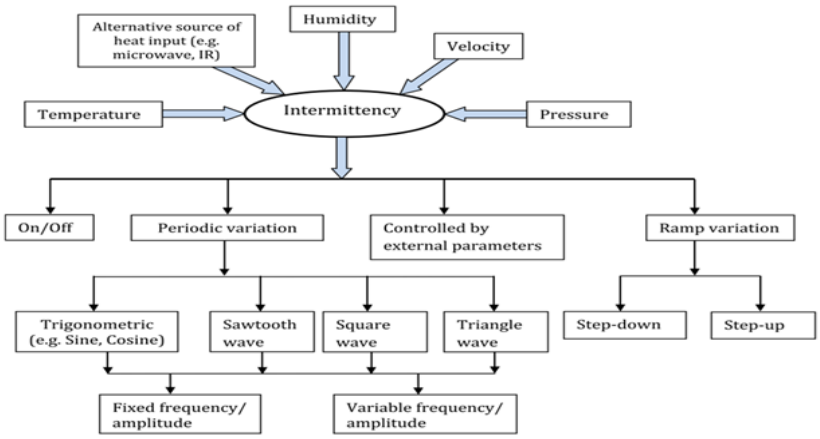


Fig 2.1 A general classification scheme for intermittent drying

Source: Kumar et al. (2014)

It was observed that, for the same intermittency (e.g. a = 0. 33) energy saving was increased from 14% to 21% as drying air temperature was increased from 28.4 °C to 40.6 °C. Pan et al. (1998) studied continuous and intermittent drying of squash slice at 100 °C in a vibrated fluidized bed dryer. They found that after 95 min of continuous drying moisture content was reduced to 14.75% (wb). On the other hand, if the squash slices were tempered in ambient air after 40 min of drying, it took only 60 min of effective drying time to reach the same moisture content. May be because of intermittency allows moisture content to redistribute. According to their study total energy consumption was 1.15 kWh for variable temperature compared to 1.5 kWh for the constant temperature drying condition. However, in case of variable humidity, energy consumption was 3.30 kWh which was significantly higher as huge amount of energy was consumed by the dehumidifier.

Table 2.1 Drying effects on leaf attributes under various drying methods

Drying Method	Color	Chlorophyll content	Essential oil content	Aroma compounds profile	Structural properties	Bioactive compounds
Sun drying (Hassanpouraghdam et al., 2010; Mohamed Hanaa et al., 2012; Pirbalouti et al., 2013; Rahimmalek & Goli, 2013)	caused substantial color degradation in basil, parsley, coriander, and thyme	data not available	decreased essential oil content in roman chamomile, basil and lemon grass	caused major degradation of aroma compounds in roman chamomile	increased shrinkage compared to shade drying in Vernonia amygdalina	decreased content of antioxidant compounds in menthe x piperita L
Shade drying (Alara et al., 2018; Ghasemi Pirbalouti et al., 2013; Hassanpouraghdam et al., 2010; Rahimmalek & Goli, 2013)	better at preserving color of rosemary, thyme, mint, and sage dried with sun drying, hot air drying, microwave drying and freeze drying	good retention of chlorophyll content in Mentha x piperita L. and Origanum vulgare	better preservation compared to sun drying in many herbs such as rosemary, mint and sage	preserved most aroma compound components of thyme similar to low temperature hot air drying (50 °C) and sun drying	better at preserving trichome structure of lippia citriodora compared to hot air and vacuum drying	showed good preservation of bioactive compounds in orthosiphon aristatus, lemon, balm, peppermint and rosemary
Hot air drying (Antal et al., 2011; Calín-Sánchez et al., 2012; Oboh, 2005; Szumny et al., 2010)	caused significant color degradation in basil, parsley, coriander and thyme at higher temperature	caused major chlorophyll degradation in coriander, basil, and parsley	decreased essential oil amount in most herbs, especially with drying temperature higher than 60 °C	caused major degradation of aroma compounds at temperature higher than 60 °C	caused major degradation of structure at temperature higher than 60 °C	caused major loss of bioactive compounds especially with drying temperature higher than 60 °C
Freeze drying (Antal et al., 2011; Consuelo et al., 2002; Mary et al., 1954; Orphanides et al., 2016; Pirbalouti et al., 2013; Rahimmalek & Goli, 2013;	Excellent preserving color of many types of herbs such as basil, coriander, bay leaf, rosemary, and thyme	Caused minor loss in chlorophyll content in basil	better preservation of essential oil content in many types of herbs compared to most other drying methods	Caused the loss of major aroma compounds in parsley	Preserved in structure Andrographis paniculate, Lippia citriodara, orhtosiphon aristatus	preserved bioactive compounds in thyme and spearmint. Caused major loss of bioactives in rosemary, aregano, sage, basil
Microwave drying (Alibas et al., 2021; Divya et al., 2012; Soysal, 2004; Sukadeetad et al., 2018)	Better preservation of color in parsley, basil, and rosemary basil, and compared to hot-air drying	Caused lesser loss in the chlorophyll types in parsley, coriander	Better preservation oil of essential content in basil and coriander compared to hot-air drying	Good preservation of aroma compounds in coriander and basil	Data not available	Better preservation of bioactive compounds of peppermint and spearmint compared to hot air drying

Chapter 3

Materials and Methods

This chapter deals with the detailed description of experimental methodologies conducted in the study and includes details of the selection of raw material and sample preparation, instruments, chemicals, analytical methods and experimental design followed for specific results.

3.1 Sample preparation

Fresh leaves were harvested freshly before each series of experiments. Leaves were selected based on visual assessment for their uniform colour and size. Leaves were always picked in the morning after morning dew has dried and kept in cooled bags before reaching the laboratory followed by storage in the deep freezer as suggested by (Miraei Ashtiani et al. 2017).

Fig 3.1 Preparation of sample for drying

3.2 Chemicals, reagents and instruments

All chemicals and laboratory reagents used in the experimental work were of analytical grade and were procured from Himedia and Merck, India.

The chemicals and reagents were 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteau (FC) reagent, sodium hydroxide, sodium carbonate, sodium nitrite, sodium acetate, acetic acid, ferric chloride, hydrochloric acid, 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ), aluminium chloride, and ethanol. The instruments used were weighting balance (from Denver Instrument, model: SI-234), probe sonicator (Model: ATP 500, 500 W power, 40 kHz frequency, 5-500 mL capacity; Athena Technology, India), centrifuge (Model: C-24BL, make: Remi

Instruments Ltd., India), vertexer, freeze dryer (IIC-INSTIND, Kolkata, India), spectrophotometer (UV Plus; Motras Scientific, India), colorimeter (BYK Gardner, Geretsried, Germany), hot air oven dryer (Reico Equipments, Kolkata India).

3.3 Mathematical Modelling

The goodness of fit of the models can be determine by statistical parameters between experimental and model data.

a) Reduced chi-square (χ^2) – Lower the better (<0.000651)
b) Coefficient of determination (R^2) – Higher the better (>0.96)
c) Root mean square error (RMSE) – Lower the better (<0.02521)

$$\chi^2 = \frac{\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2}{N-z} \quad (3.1)$$

$$R^2 = \frac{\sum_{i=1}^{N}(MR_i - MR_{pre,i}) * \sum_{i=1}^{N}(MR_i - MR_{exp,i})}{\sqrt[2]{\left[\sum_{i=1}^{N}(MR_i - MR_{pre,i})^2\right]\left[\sum_{i=1}^{N}(MR_i - MR_{exp,i})^2\right]}} \quad (3.2)$$

$$RSME = \sqrt[2]{\frac{1}{N}\left[\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2\right]} \quad (3.3)$$

MR_{exp} and MR_{pre} are dimensionless moisture ratios obtained from experiment and modelling

N = number of observations

z = number of constants in a model

i = number of terms

3.4 Drying characteristics of moringa leaves

3.4.1 Moisture content

The moisture content of the fresh moringa leaves was measured by hot air oven method (AOAC, 2005). Moringa leaves sample of 15 ± 0.002 g was taken and the initial weight was measured (W_1). Then it was kept in a hot air oven at 105 °C for 24 hours to get the dry matter weight (W_2) (Rout et al., 2021). The moisture content of the sample was measured using the formula:

$$Moisture\ content\ _{(wet\ basis)}\% = \frac{W_1 - W_2}{W_1 - W_0} \quad (3.4)$$

$$Moisture\ content_{(dry\ basis)}\% = \frac{W_1 - W_2}{W_1 - W_0} \quad (3.5)$$

Where, W_0 = weight of empty vessel (g)

W_1 = weight of vessel with sample before drying (g)

W_2 = weight of vessel with sample after drying (g)

3.4.2 Drying rate

Drying is defined as the removal of moisture from a material through thermal treatment. Drying is one of the main steps in the particle board production process. Consumption of a high amount of energy, in addition to environmental impacts, makes it one of the most energy intensive operations (Aremu & Akintola, 2016). Drying rate of the moringa leaves is given by the below equation:

$$DR = \frac{M_{t+dt} - M_t}{dt} \quad (3.6)$$

M_t and M_{t+dt} are moisture content at t and t + dt respectively (kg moisture/kg dry matter)

dt is the time difference in minutes

The dimensionless moisture ratio (MR) of leaves given by the following equation:

$$MR = \frac{M_t - M_e}{M_o - M_e} \quad (3.7)$$

M_t = MC at any given time

M_o = initial MC

M_e = equilibrium MC

All measurements are in % dry basis.

For longer drying time, M_e becomes negligible compared to M_t and M_o so the equation becomes:

$$MR = \frac{M_t}{M_o} \quad (3.8)$$

3.4.3 Effective moisture diffusivity

For the solution of Fick's diffusion equation, the leaves were assumed as a slab because of the thickness of the sample. The following assumptions were made for the slab-shaped body of celery leaves (Demirhan and Özbek, 2011):

- Moisture is initially uniformly distributed throughout the mass of a sample.
- Mass transfer is symmetric with respect to the centre.
- Surface moisture content of the sample instantaneously reaches equilibrium with the condition of the surrounding air.
- Resistance to mass transfer at the surface is negligible compared to internal resistance of the sample.
- Mass transfer is represented by a diffusional mechanism.
- Diffusion coefficient is constant, and shrinkage is negligible.
- The effective moisture diffusivity was therefore calculated by the following equation

Effective moisture diffusivity can be determine using following equation:

$$MR = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2} * Exp\left[\frac{-(2n+1)^2\pi^2 D_{eff}t}{4L^2}\right] \quad (3.9)$$

D_{eff} = effective diffusivity coefficient (m^2/s)

L = half of the thickness of the sample (m)

n = a positive integer

t = duration of the drying process (s)

For longer Drying time only first term if the series is considered and equation becomes –

$$\ln(MR) = \ln\left(\frac{8}{\pi^2}\right) - \left(\frac{\pi^2 D_{eff}}{4L^2}\right) *t \quad (3.10)$$

This equation also known as simplified Fick's Diffusion equation. The effective moisture diffusivity can be extracted from a diagram showing the drying data from the experiments as ln(MR) against time. The ln(MR)-time diagram would present an inclined line with its slope (K) given by the following equation:

$$K = \frac{\pi^2 D_{eff}}{4L^2} \quad (3.11)$$

The effective moisture diffusivity is also determined using this equation.

3.4.4 Activation energy

The bonding potential of moisture is quantified by the value of activation energy, which is the energy required for eliminating 1 mol moisture from the substance with constant compositions and given MC. The activation energy of the hot-air technique is obtained from the simple Arrhenius equation:

$$D_{eff} = D_o Exp\left(-\frac{E_a}{R(T+273.15)}\right) \quad (3.12)$$

or

$$lnD_{eff} = lnD_o - \frac{E_a}{R(T+273.15)} \quad (3.13)$$

T = drying air temperature (°C)

R = universal gas constant (8.314 × 10-3 kJ mol^{-1} K^{-1})

D_o = pre-exponential factor of the Arrhenius equation (m^2/s)

E_a = activation energy (kJ mol^{-1})

E_a is derived from the slope of a straight line when ln(D_{eff}) is plotted against the multiplicative inverse of absolute temperature (1/(T + 273.15))

A modified form of the Arrhenius equation is used if it is not possible to accurately measure the drying temperature:

$$D_{eff} = D_o Exp\left(-\frac{mE_a}{P}\right) \quad (3.14)$$

m = sample weight (g)

E_a = activation energy (W/g)

P = output power(infrared) (W)

3.4.5 Energy consumption

While energy consumption calculation vacuum pump, heater and exhaust fan (Tarafdar et al., 2021) can be taken into considerations. Total energy consumption (ET, kWh) = Energy

consumed by the pump (EP) + Energy consumed by the heater (EH) + Energy consumed by the exhaust fan (EF):

$$E_p = V * I_p * t_d \quad (3.15)$$

$$E_H = V I_H \cos \emptyset * t_d \quad (3.16)$$

$$E_F = P_F * t_d \quad (3.17)$$

V = voltage (240 V, 50 Hz)

I_P = electric current in the pump (A)

I_H = electric current in the heater (A)

t_d = drying time (h)

P_F = power consumed by fan (kW).

3.5 Physio-chemical characterization of dried leaves powder

3.5.1 Colour

Colour parameters of the dried samples were measured using a BYK colorimeter (BYK Spectro guide 45/0 gloss; Make: BYK Gardner, Geretsried, Germany). The standard colours like white, green and gloss were first standardized using standard colour plates. Then, colour of the samples was measured in CIE L* a* b* scale (CIE - *Commission Internationale de I'Echairages;* L* for luminosity, denoting lightness; a* and b* for the colour-opponent dimensions, a* representing red/green value and b* representing yellow/blue value) by keeping the samples on the surface of the standard white plate. Colour parameters like chroma (C*), hue angle (h*), whiteness index (WI), yellowness index (YI) and browning index (BI) were measured for all the samples in triplicate. The overall colour difference was given by total colour change (ΔE^*) and was calculated by equation given as follows:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3.18)$$

$$\Delta L^* = (L_1^* - L_0^*) \quad (3.19)$$

$$\Delta a^* = (a_1^* - a_0^*) \quad (3.20)$$

$$\Delta b^* = (b_1^* - b_0^*) \quad (3.21)$$

Where, subscript '0' denotes the colour value of reference sample and subscript '1'represents the colour value for the sample being analysed.

The colour parameters are described as follows:

$$Chroma = \sqrt{(a^*)^2 + (b^*)^2} \tag{3.22}$$

$$Whiteness\ index\ (WI) = L^* - 3b^* + 3a^* \tag{3.23}$$

$$Hue\ angle = tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{3.24}$$

$$Yellowness\ index\ (YI) = \frac{142.86 * b^*}{L^*} \tag{3.25}$$

$$Browning\ index\ (BI) = \left(\frac{x - 0.31}{0.17}\right) * 100 \tag{3.26}$$

$$where, x = \frac{a^*}{5.645L^* + a^* - 3.012b^*} \tag{3.27}$$

The chroma (C*) is the quantitative attribute of colour, which is used to determine the degree of difference of a hue in comparison with a grey colour of same lightness. Higher chroma values indicate higher colour intensity of the samples perceived by humans. Hue angle (h*) is the qualitative attribute of colour, which is used to define the difference of a certain colour with reference to the differences in absorbance corresponding to different wavelengths. The Whiteness Index (WI) mathematically combines the lightness and yellow-blue colour into a single term. It represents overall whiteness of the sample which indicates the extent of discoloration due to processing conditions. The Yellowness Index (YI) is generally used to quantify different types of degradation of food products which may be caused due to light, chemical exposure or processing. The Browning Index (BI) is used to characterize the overall changes in brown colour of the sample.

3.5.2 Water activity (a_w)

Water activity is expressed as the ratio of partial vapor pressure of water in a substance to that of pure water at same temperature and pressure. Water activity was measured using a water activity meter (Rotronic Hygrolab 3, USA). The samples were kept in a clean, dry and transparent sample holder, leaving a little headspace and it was kept under the sensor of the water activity meter. When the relative humidity of the sample came in equilibrium with that

of the surrounding environment (here, the headspace), the water activity was displayed on the digital display unit. The measurements were done at room temperature of 30 ± 2 ℃.

3.5.3 Solubility

The solubility of the powder was determined following the protocol given by Tran & Nguyen, (2018) with slight modifications. Briefly, sample and deionized water in the ratio of 1:10 were taken in a beaker and were stirred for one hour at room temperature.

(a) (b)

Fig 3.2 (a) Samples stirring (b) Supernatant after hot air oven drying

Then it was centrifuged at 1500 rpm for 10 minutes. The supernatant was filtered and collected in a moisture can, then dried by using hot air oven dryer at 105 ℃ until reaching a constant moisture content. The weight of the solids remained was used to calculate the solubility of the samples. The solubility was expressed as:

$$Solubilty\ (\%) = \frac{W_f}{s} * 100 \qquad (3.28)$$

Where, W_f = final weight of supernatant after drying (g)

S = total weight of the sample taken (g)

3.5.4 Bulk density, tapped density, flowability and cohesiveness

The bulk density was measured by using a graduated measuring cylinder. A known mass of sample was put inside the cylinder and its corresponding volume was measured, and the ratio of mass of sample to the volume occupied gives the bulk density of the sample ρ_b (w/v). The tapped density was measured in a similar way, but the volume was measured after tapping the cylinder gently for 50 times. The tapped density was denoted as ρ_t (w/v). The cohesiveness and flowability of the encapsulated powder were determined in terms of Hausner Ratio (HR)

and Carr Index (CI), respectively. The CI and HR of leaf powder were calculated based on the method given by Hao (2015), Rout et al. (2021) using the following formulae:

$$Carr\ Index\ (CI) = \frac{\rho_t - \rho_b}{\rho_t} * 100$$

$$Hausner\ Ratio\ (HR) = \frac{\rho_t}{\rho_b}$$

3.6 Extraction of bioactive compounds from moringa leaves

For extraction powder has been prepared from dried leaves using mortar and pestle and then sieved through BS 95 sieves to obtain a particle size less than 0.2 mm. A mixer has been prepared of 1 g of dried powder of moringa leaves with 20 mL pure (99.99%) ethanol. After this mixer was vortexed for 5 minutes, probe type sonicator was used for extraction of bio-actives from the moringa leaf powder at 8 second treatment interval with 2 second off time for 10 minutes.

Fig 3.3 Extract preparation

After extraction, samples were centrifuged at 10000 rpm for 10 minutes, and filtered through Whatman No. 4 filter paper. The extract obtained was collected in 10 mL plastic vials and stored in deep freezer until further use.

(a) (b) (c) (d)

Fig 3.4 (a) Sieved Powder (b) Vertexing (c) Sonication process (d) Extract

3.6.1 **Total phenolic content (TPC)**

The total phenolic content of the extract was determined by Folin Ciocalteau reagent (FCR) method proposed by Singleton et al. (1999) with minor modifications. Briefly, 0.2 mL of the extract sample were taken in a test tube and 2.8 mL of deionized water was added to it, followed by the addition of 0.5 mL of FCR (10 times diluted) and 0.75 mL sodium carbonate (Na_2CO_3, 20 % w/v). The samples were properly mixed and then incubated in dark for 90 minutes. Absorbance was measured at wavelength of 760 nm using UV–Vis spectrophotometer (UV Plus; Motras Scientific, India). Zeroing was done by blank sample, which contained all chemicals except the extract. Gallic acid was taken as the standard and TPCwas expressed in terms of mg gallic acid equivalent (GAE) / g sample.

3.6.2 Total flavonoids content (TFC)

TFC was determined using $AlCl_3$ colorimetric method proposed by Vuong et al. (2013) with slight modifications. Briefly, 0.5 mL of diluted extract was taken in a test tube. 2 mL of deionized water was added to it, followed by addition of 0.15 mL $NaNO_2$. After 4-5 minutes, 0.15 mL of 10% $AlCl_3$ solution was added to it. 5 minutes later, 1 mL of 1M NaOH was added, followed by addition of 1.2 mL deionized water. The sample was incubated for about 10 minutes. The absorbance was measured at a wavelength of 510 nm using a UV–Vis spectrophotometer. Quercetin was taken as the standard with TFC recorded as mg quercetin equivalents per gram of sample (mg QE / g sample). Zeroing was done by control sample.

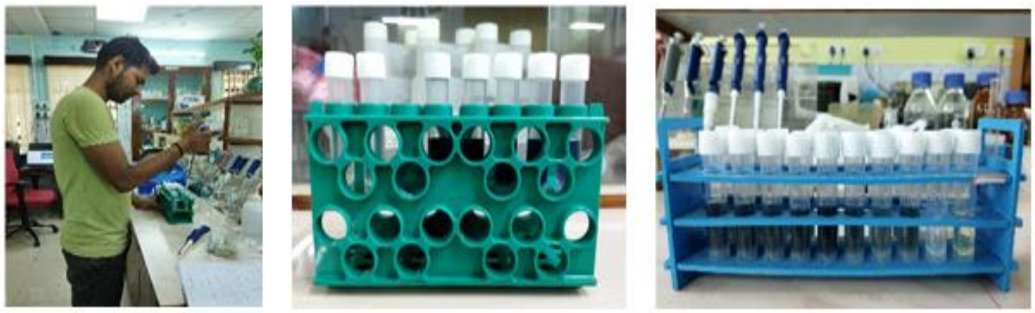


Fig 3.5 Sample preparation for bioactive compound analysis

3.6.3 Gallic acid equivalent antioxidant capacity (GAEAC)

DPPH method as proposed by Vuong et al. (2013) was followed with slight modifications. Briefly, 0.2 mL of the diluted extract was taken in a test tube and to it, 0.95 mL of DPPH (2, 2–Diphenyl–1–Picrylhydrazyl) solution and 2.85 mL of pure methanol were added. The sample was incubated for half an hour in dark. Absorbance was measured at a wavelength of 517 nm using UV-Vis spectrophotometer. Zeroing was done by pure methanol. Gallic acid was taken as the standard and the results were expressed in terms of mg gallic acid equivalents antioxidant capacity per gram of sample (mg GAEAC / g sample). It was measured using the formula:

$$GAEAC\ \left(mg\frac{GAEAC}{g}sample\right) = \frac{\Delta Abs_{sample}}{\Delta Abs_{standard}} * C * \frac{v}{w} \qquad (3.29)$$

Where, C = concentration of gallic acid (g mL^{-1})

V = volume of sample taken (mL)

W = weight of powder taken in the sample (g)

IC50 value is the concentration of sample require to scavenge 50% of DPPH free radical and is calculated from the graph of radical scavenging activity against the concentration of extracts. Statistically, the correlation between antioxidant activity and total phenolic content is determined by plotting IC50 (µg/mL) against TPC (mg/g).

3.7 Storage study of freeze-dried moringa leaves powder

In the present storage study of dried moringa leaf powder, changes in four parameters total phenolic content, solubility, water activity and moisture content were measured. Fresh leaves were dried using freeze dryer. Dried leaves were then ground to powder using mixer grinder to

particle size less 0.2 mm. Four samples were prepared from two different packaging material (Tarsons HDPE and LDPE), for desiccator and deep freezer (Fig 3.6 a). Two samples were stored in deep freezer at around -30 °C and two were kept in desiccator at room temperature (around 30 °C). Observations were taken at an interval of 10 days.

(a) (b)

Fig 3.6 (a) Samples prepared for storage study (b) Freeze dried leaves

Chapter 4

Results and Discussion

This chapter deals with the findings obtained by various experimentations on the basis of predetermined objectives. The results are well supported by published literature in concerned fields. Discussion will be on drying and drying kinetics of freeze drying, microwave drying, hot air oven drying, diffusivity under different drying methods, drying effects on the physio-chemical properties of moringa leaves.

4.1 Drying kinetics

Initially, the moringa leaves were dried using freeze dryer, it took 24 hours to dry leaves to bone dry level at – 44 °C and 0.024 mbar. Freeze-dried moringa leaves were ground to fine powder of particle size less than 0.2 mm using pestle and mortar. Extraction of bioactive compounds from dried leaves powder was done using ultrasound assisted extraction (UAE).

4.1.1 Moisture content

The moisture content of the moringa leaves was determined by hot air oven method. Samples were dried at 105 °C for 24 hours. The values were in the range of 75 ± 0.02 % to 80 ± 0.02 % (wet basis)

(a) (b)

Fig 4.1 (a) Samples placed in hot air oven dryer (b) Dried sample

4.1.2 Drying kinetics of moringa leaves under hot air oven drying

Drying rate and moisture ratio of the samples were calculated using the equations (3.6) and (3.7). The drying rate curves of moringa leaves at 55 °C, 65 °C and 75 °C temperature has shown in Fig 4.2(b). The drying rate decreased continuously throughout the drying period. It can be observed from the figure that the constant rate period was absent, and the drying process occurred in the falling rate period. These results were in good agreement with the

earlier studies on moringa leaves by (Premi et al. 2010). The drying has taken 270 to 390 minutes to reach the bone dry level in hot air oven dryer. The variations of drying rate and moisture ratio with drying time at 55 °C, 65 °C, and 75 °C temperature is given in Figures 4.2(b) and 4.2(c), respectively.

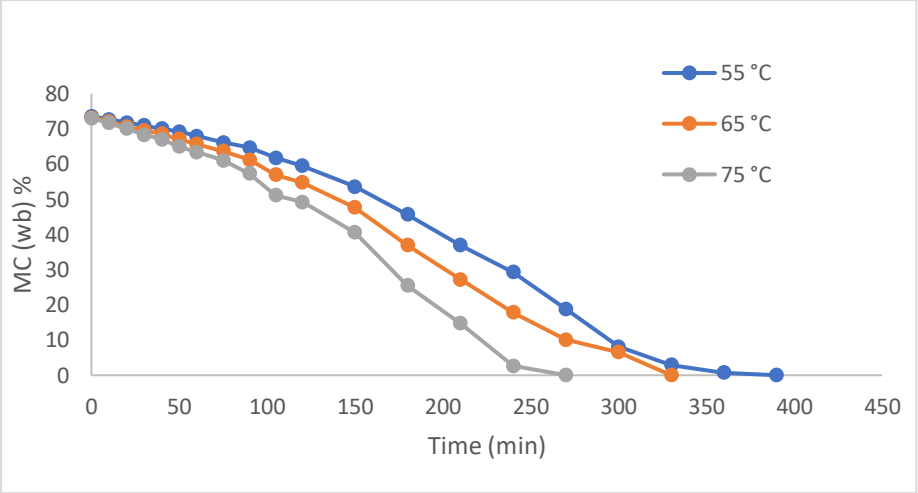


Fig 4.2 (a) Moisture content vs Time under different drying temperatures

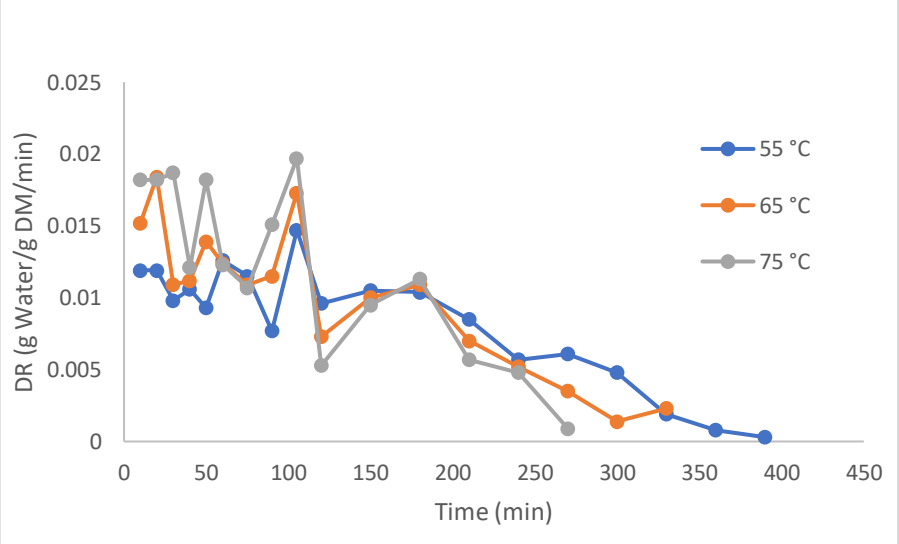


Fig 4.2 (b) Drying rate vs Time under different drying temperatures

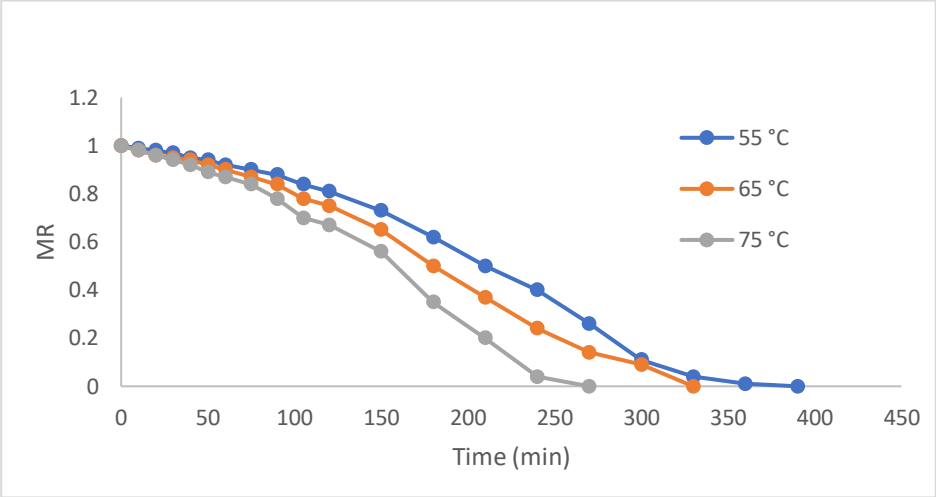


Fig 4.2 (c) Moisture ratio vs Time under different drying temperatures

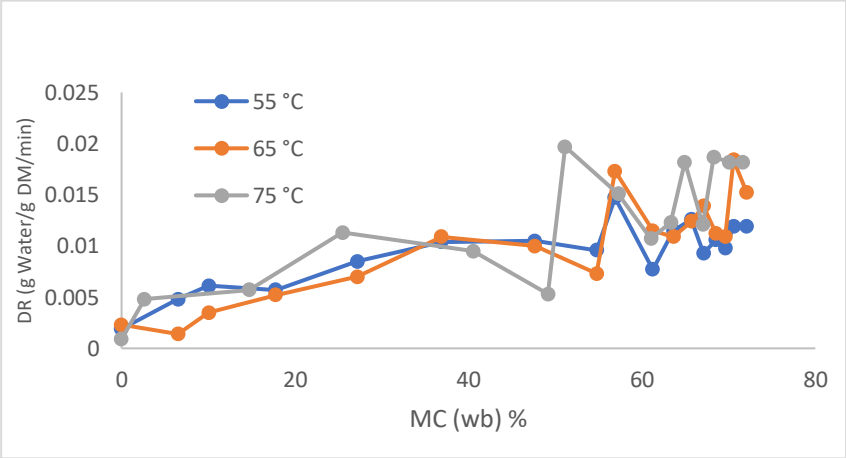


Fig 4.2 (d) Drying rate vs Moisture content under different drying temperatures

The moisture ratio reduced exponentially as the drying time increased. Continuous decrease in moisture ratio indicates that diffusion has governed the internal mass transfer. A higher drying air temperature decreased the moisture ratio faster due to the increase in heat supply rate to the leaves and the acceleration of moisture migration (Demir et al. 2004).

Experimental results showed that drying temperature is one of the most effective parameters for the drying. At higher moisture content, the increase in temperature has more considerable effect on the drying rates as compared to lower temperatures, which is almost negligible towards the end. It was further observed that the drying rate or moisture loss was faster at the beginning than that at the end. The reduction in the drying rate at the end of drying may be due to the reduction in moisture content as drying advances (Sharma and Prasad, 2001). Thus,

a higher drying air temperature produced a higher drying rate and consequently the moisture ratio decreased Fig. 4.2 (b) and Fig 4.2 (c). (Lahsasni et al. 2004; Abdullah et al. 2012; Ali et al. 2014, 2017; Arslan & Özcan, 2012; Consuelo et al. 2002; Ebadi et al. 2015; Pirbalouti et al. 2013; Rababah et al. 2015; Sousa et al. 2018).

4.1.3 Drying kinetics of moringa leaves under microwave drying

It can be seen in Fig. 4.3 (b) that all curves have two stages except drying 600 W. The drying rate rapidly increased and then slowly decreased as drying progresses except in case of 600 W power level in which first stage was missing because of instant heating. In general, it's observed that drying rate reduces with time or with the reduction of moisture content. The leaves moisture content reduces over time which was one of the important reasons why drying process took place in the falling rate period. Lahsasni et al. (2004) reported that the drying occurs during the falling rate period is so governed by water diffusion in the solid. The moisture content of the leaves was very high during the initial phase of the drying which resulted in a higher absorption of microwave power and higher drying rates due to the higher moisture diffusion. As the drying progressed, the loss of moisture in the leaves caused a decrease in the absorption of microwave power and resulted in a fall in the drying rate. Higher drying rates were obtained at higher microwave output powers; a sharp fall can be observed in Fig 4.3 (b) for 600 W power. Thus, the microwave output power had a crucial effect on the drying rate. Similar findings were reported in previous studies (Alibas, 2014; Altay et al. 2019; Rahimmalek & Goli, 2013; Soysal et al. 2006; Zambra et al. 2021).

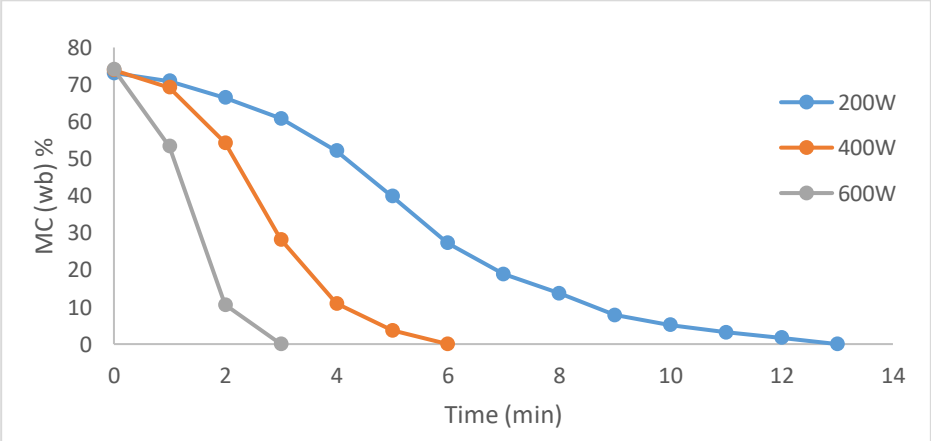


Fig 4.3 (a) Moisture content vs Time under different microwave power levels

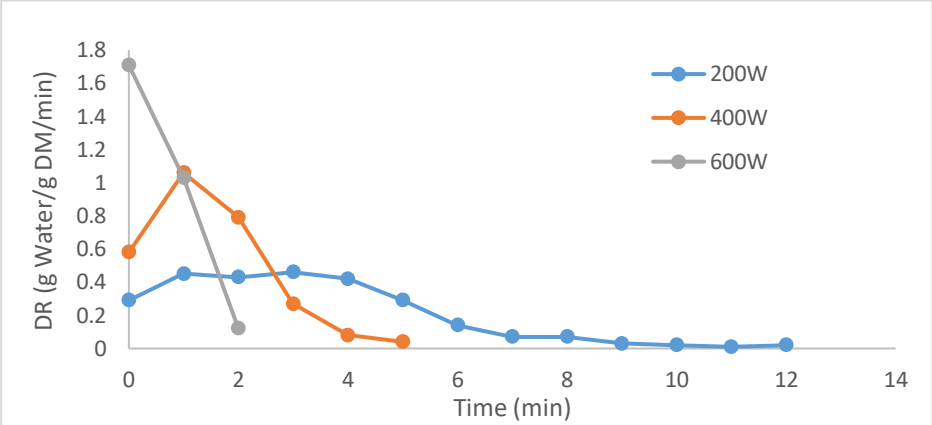


Fig 4.3 (b) Drying rate vs Time under different microwave power levels

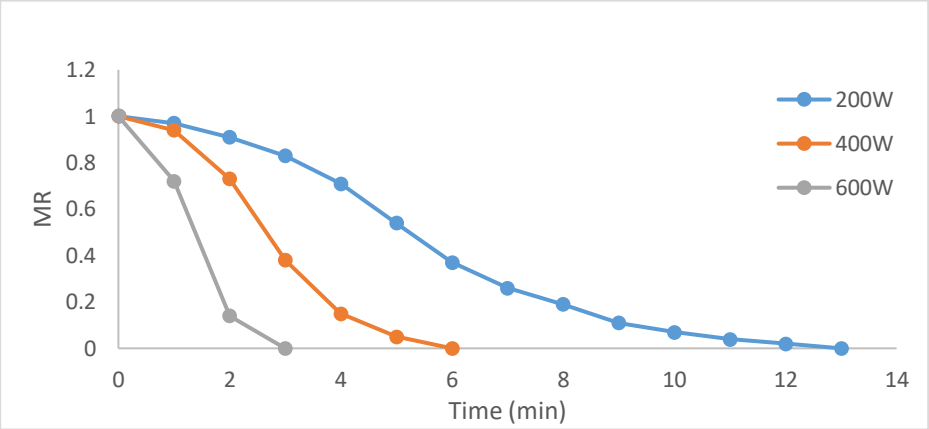


Fig 4.3 (c) Moisture ratio vs Time under different microwave power levels

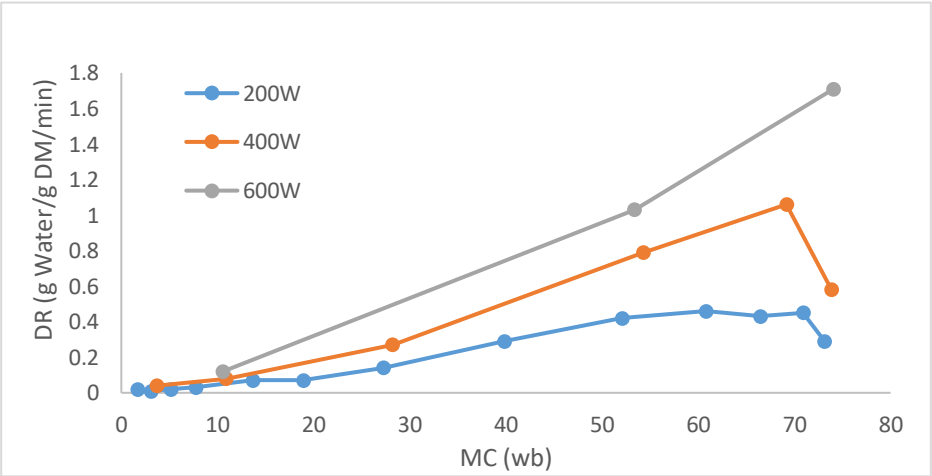


Fig 4.3 (d) Drying rate vs Moisture content under different microwave power levels

4.1.4 Effective moisture diffusivity

The results obtained have shown that internal mass transfer resistance due to presence of falling rate drying period controls drying time. Therefore, experimental results can be interpreted by using Fick's diffusion equation assuming leaf as infinite slab. The effective moisture diffusivity D_{eff} is determined by using the analytical solutions of Fick's second law Crank (1975), represented as equation (3.9). Simplifying the equation (3.9) by taking the first term of the series solution, equation (3.10) has been obtained. Effective moisture diffusivity is also typically calculated by using slope of Equation (3.10), when natural logarithm of MR versus time was plotted, Fig. 4.4(a) and (b) straight line with a slope (K) as in equation (3.11) is obtained.

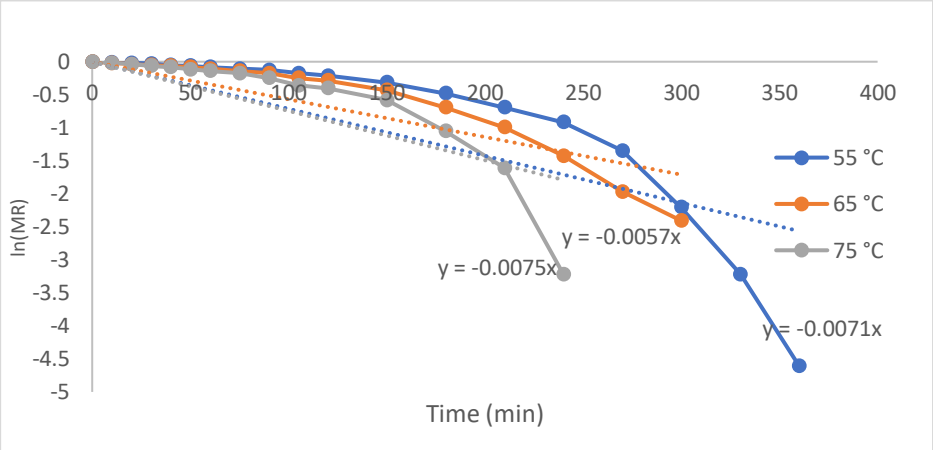


Fig 4.4 (a) ln(MR) vs Time curve under hot air oven dryer

The calculated values of effective moisture diffusivity vary from 2.88×10^{-9} to 3.04×10^{-9} m^2/s in case of hot air oven drying (Table 4.1). The values obtained lie in the general range of 10^{-11} to 10^{-9} m^2/s for food materials (Zogzas et al., 1996). Effective moisture diffusivity varies from 1.01×10^{-7} to 3.45×10^{-7} m^2/s in case of microwave drying (Table 4.1). Similar results were found out by (Darvishi et al. 2014). Modified Arrhenius equation is generally used to model the effect of temperature on the effective moisture diffusivity as equation (3.12). The energy level of the molecule to initiate a chemical reaction is represented as activation energy (E_a).

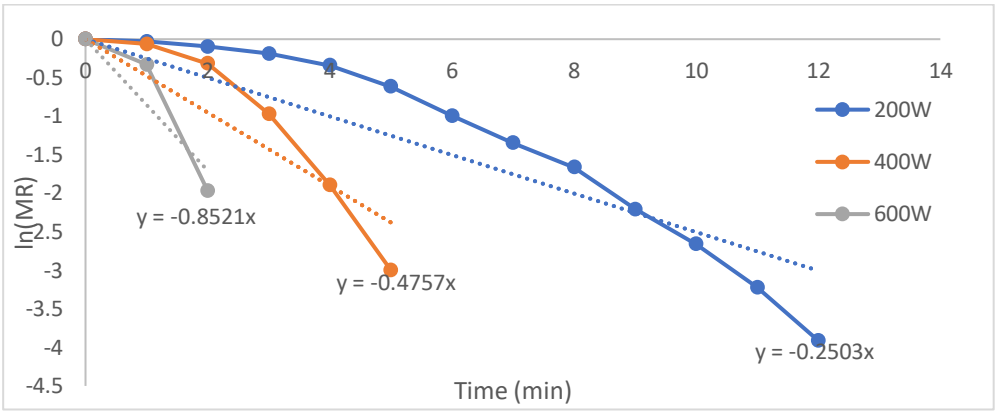


Fig 4.4 (b) ln(MR) vs Time curve under microwave dryer

Variation in effective moisture diffusivity of samples with moisture content at different microwave power levels is shown in Fig. 4.4 (b) The effective moisture diffusivity increased with decrease in moisture content. However, the moisture diffusivity further was higher at any level of moisture content at a higher microwave power level, resulting in a shorter drying time. This may indicate that as the moisture content decreased, the permeability to vapour increased, provided the pore structure remained open. The temperature of the product rises rapidly in the initial stages of drying, due to more absorption of microwave heat, as the product has a high loss factor at higher moisture content. This increases the water vapour pressure inside the pores and results in pressure induced opening of pores. In the first stage of drying, liquid diffusion of moisture could be the main mechanism of moisture transport. As drying progressed further, vapour diffusion could have been the dominant mode of moisture diffusion in the latter part of drying. Effective moisture diffusivity was studied by various researchers and reported the similar trends (Alibas, 2014; Altay et al. 2019; Darvishi et al. 2014; Demirhan & Özbek, 2011; Kadam et al. 2011; Miraei Ashtiani et al. 2017; Mouhoubi et al. 2019; Premi et al. 2010; Sousa et al. 2018; Torki-Harchegani et al. 2016; Youssef & Embaby, 2012; Zambra et al. 2021).

4.2 Colour Measurement

Colour parameters were measured of all the samples dried in freeze dryer, hot air oven dryer and microwave dyer to analysed the effect of drying method on colour, which indicates the presence of chlorophyll in leaves and the severity of the drying process. The measured parameters are recorded in Table 4.2. Each colour parameter is discussed in details in the following paragraphs.

Table 4.1 Effective moisture diffusivity values under hot air drying and microwave drying method

Sample		Slope (K)	Thickness (m)	D_{eff} (m^2/s)
Hot air oven drying	T = 55 °C	-0.0068 ± 0.0003 [a]	0.001 ± 0.0001 [a]	$2.07 \times 10^{-9} \pm 1.53 \times 10^{-10}$ [b]
	T = 65 °C	-0.0059 ± 0.0002 [b]	0.001 ± 0.0001[a]	$1.80 \times 10^{-9} \pm 1.02 \times 10^{-10}$ [a]
	T = 75 °C	-0.0074 ± 0 [a]	0.001 ± 0.0001[a]	$2.26 \times 10^{-9} \pm 2.34 \times 10^{-10}$ [b]
Microwave drying	P = 200 W	-0.4508 ± 0.347 [b]	0.001 ± 0.0001[a]	$1.82 \times 10^{-7} \pm 1.40 \times 10^{-7}$ [a]
	P = 400 W	-0.4807 ± 0.013 [a]	0.001 ± 0.0001[a]	$1.95 \times 10^{-7} \pm 5.48 \times 10^{-9}$ [b]
	P = 600 W	-0.6423 ± 0.339 [b]	0.001 ± 0.0001[a]	$2.60 \times 10^{-7} \pm 1.37 \times 10^{-7}$ [a]

* Tukey test applied at 95% confidence interval to test the significance of different parameters for samples (different letters indicate significant differences among the data)

L* values represent lightness of the samples, which vary from 0 (pure black) to 100 (pure white). L* value of fresh leaves was 27.92 ± 0.77 which was increased to 47.05 ± 0.57 in case of freeze drying and 45.92 ± 0.28 in case of microwave drying. This indicates the colour changes from darker green to light green. Again, the L* value of hot air oven dried leaves falls down to 26.25 ± 0.46 which was because of the colour changes from green to light brown. a* represents the red-green hue of the material. Positive values indicate red hue while negative values indicate green hue. a* values of all the samples was negative which is quite obvious because of green colour of leaves except hot air oven dried leaves because their colour change to light brown where positive a* values indicate yellow colour. In hot air oven dried leaves the green completely disappear which depicts the loss of chlorophyl. While a little change in a* values of freeze-dried leaves was observed in comparison to fresh leaves (Fig. 4.5).

Fig 4.5. Effect of drying methods on color of the dried leaves (a) Fresh leaves, (b) Freeze dried, (c) Microwave dried at 600 W, and (d) Hot air oven dried at 75 °C

b* represents the yellow-blue hue of the material, positive values indicate yellow hue while negative values indicate blue. b* also showed positive values for all the samples (Table 4.2) highest value observed in case of microwave drying while lowest for hot air oven drying. The positive values show yellowish hue in dried leaves. The total colour difference index (ΔE) indicates the overall colour change which was quite high means it can be observed by human eye easily (Fig. 4.5). All the values colour difference index lied in the range of 9 - 22 (Table 4.2), suggesting that the colours are more similar than opposite (Mokrzycki & Tatol, 2011).

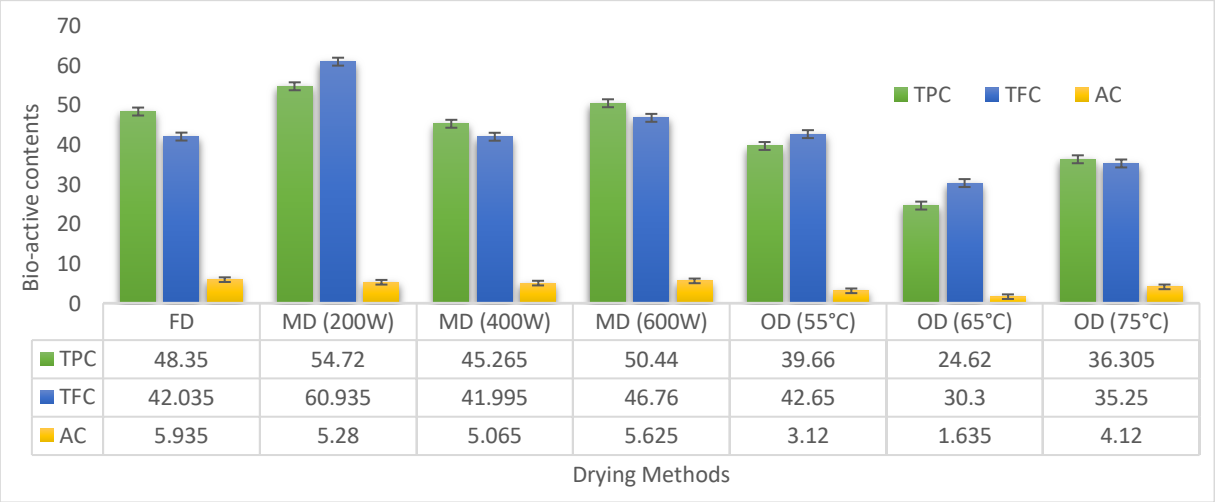
Table 4.2 Colour parameter values of fresh leaves and leaves dried under different drying methods

Sample	L*	a*	b*	ΔE*	Chroma (C*)	Hue angle (h*)	WI	YI	BI
Fresh leaves	27.48 ± 0.27	-4.51 ± 0.21	10.34 ± 0.19	-	11.28 ± 0.23	-1.16 ± 0.016	-17.08 ± 0.85	53.75 ± 0.75	32.2 ± 0.91
Freeze dried	47.05 ± 0.57	-4.57 ± 0.24	17.35 ± 0.08	20.78± 0.26	117.94 ± 0.14	-1.31 ± 0.009	-18.73 ± 1.06	52.7 ± 0.66	36.78 ± 0.0.64
Microwave dried (200 W)	42.98 ± 0.28	-2.23 ± 0.78	17.11 ± 0.67	17.09 ± 0.40	17.28 ± 0.75	-1.44 ± 0.042	-1507 ± 4.57	56.90 ± 2.60	45.01 ± 1.52
Microwave dried (400 W)	46.25 ± 0.30	-5.69 ± 0.18	21.51 ± 0.30	21.88 ± 0.48	22.26 ± 0.24	-1.31 ± 0.014	-35.37 ± 0.65	66.46 ± 1.31	49.90 ± 1.83
Microwave dried (600 W)	46.80 ± 0.08	-6.95 ± 0.00	20.31 ± 0.03	21.88 ± 0.39	21.47 ± 0.03	-1.24 ± 0.00	-35.00 ± 0.20	62.00 ± 0.23	42.50 ± 0.24
Oven dried (55 °C)	26.25 ± 0.46	2.44 ± 0.08	14.49 ± 0.25	8.21 ± 0.17	14.70 ± 0.23	1.4 ± 0.008	-9.90 ± 0.90	78.89 ± 1.37	83.73 ± 1.79
Oven dried (65 °C)	25.43 ± 0.21	3.31 ± 0.13	13.5 ± 0.25	8.71 ± 0.14	13.09 ± 0.27	1.33 ± 0.008	-5.12 ± 0.36	75.83 ± 0.88	82.49 ± 1.47
Oven dried (75 °C)	24.75 ± 0.077	4.46 ± 0.31	15.62 ± 0.24	10.77 ± 0.56	16.2 ± 0.30	1.29 ± 0.016	-8.72 ± 0.60	90.18 ± 1.34	107.05 ± 2.93

Note: All values are expressed as mean ± standard deviation

4.3 Functional properties of moringa leaves

The phenolic contents, flavonoids contents and antioxidant activity of drumstick (*Moringa oleifera*) leaves were investigated and found that the quantities varied significantly with different drying methods and drying parameters (Fig. 4.6). There is high correlation between the phenolic content, flavonoid content, and antioxidant activity in the leaf extracts, since phenolic compounds contribute directly to antioxidant activity (Maisuthisakul et al., 2007). The same can be observed from the Fig. 4.7 to Fig. 4.9.



	FD	MD (200W)	MD (400W)	MD (600W)	OD (55°C)	OD (65°C)	OD (75°C)
TPC	48.35	54.72	45.265	50.44	39.66	24.62	36.305
TFC	42.035	60.935	41.995	46.76	42.65	30.3	35.25
AC	5.935	5.28	5.065	5.625	3.12	1.635	4.12

Fig 4.6 Comparison of TPC, TFC, and AC under various drying methods

The TPC Values ranged from 24.62 ± 1.10 mg GAE/g-dm (hot air oven drying at 65 °C) to 54.72 ± 5.44 mg GAE/g-dm (microwave drying at 200 W). TFC ranges from 30.3 ± 1.75 mg QE/g-dm (hot air oven drying at 65 °C) to 60.94 ± 1.02 mg QE/g-dm (microwave drying at 200 W). Antioxidant capacity (AC) varied significantly from 1.63 ± 0.13 mg GAEAC/g-dm (hot air oven drying at 65 °C) to 5.94 ± 0.25 mg GAEAC/g-dm (freeze drying) (Table 4.3). The overall comparison between TPC, TFC, and AC observed in dried samples using different drying methods can be seen in the Fig. 4.6.

4.3.1 Total phenolic content

Phenolic compounds are very important plant constituents as they have antioxidant property, they can inactivate the lipid free radicals or prevent the decomposition of hydroperoxides into free radicals (Pokorny, 2001). The highest TPC was recorded in case of microwave dried samples at 200 W power level (54.72 mg GAE/g db) whereas the lowest was found in hot air oven dried samples at 65 °C (24.62 mg GAE/g db) Fig. 4.7. The higher phenolic content in

freeze dried and microwave dried samples could be because of no heating and lower temperature respectively. Moreover, freeze drying promotes the preservation of heat sensitive compounds of the samples. The TPC of hot air oven dried samples found at temperature 55°C was greater than TPC at temperature 65 °C and 75 °C might be because of the lower temperature. The phenolic content increased in samples dried at 75 °C temperature from samples dried at 65 °C temperature could be because of the lesser drying time.

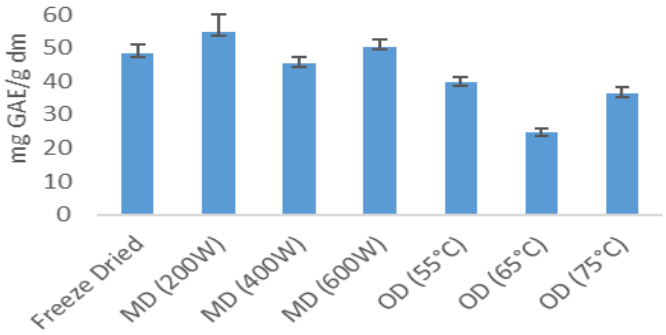


Fig 4.7 TPC under various drying methods

TPC loss after drying might be caused by enzymatic reactions. The drying process did not immediately deactivate the responsible enzymes such as polyphenol oxidases therefore, they were able to degrade the phenolic compounds present in the leaves before the plant materials are completely dry (Abdullah et al. 2012; Maisuthisakul et al. 2007; Saikat et al. 2014; Sukadeetad et al. 2018; Tummanichanont et al. 2017).

4.3.2 Total flavonoid content

The results showed the significant differences in flavonoid content of dried samples using various drying methods. The highest amount of flavonoids content was found in microwave dried sample at 200 W (60.94 ± 1.02 mg GAE/g dm). Lesser quantity of flavonoids content was found in hot air oven dried samples in comparison to freeze dried and microwave dried samples. This can be attributed to the heating effect and longer drying time which leads to the destruction of flavonoid content. The increase of flavonoid content in case of hot air oven dried samples at 75 °C in comparison to 65 °C can be explained as heating may breakdown some phytochemicals which affect cell wall integrity and cause a migration of some components flavonoids. The similar effect can be seen in microwave dried samples at 600 W and 400 W (Fig. 4.8). In addition, the loss in flavonoids may due to breakdown or leakage by

chemical reactions includes oxygen, enzymes and light (Hamrouni-Sellami et al. 2013; Maisuthisakul et al. 2007; Riyad & Elkholany, 2020; Sukadeetad et al. 2018).

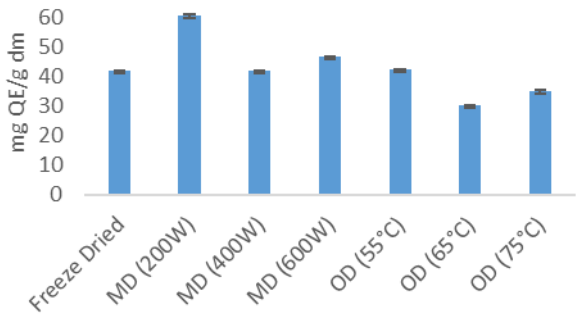


Fig 4.8 TFC under various drying methods

4.3.3 Antioxidant activity

Antioxidant activity in terms of DPPH is based on reduction reaction and electron transfer. DPPH is a stable free radical, when antioxidant reacts with DPPH free radical then electron is paired off and the DPPH solution is decolorized. The scavenging activity of the antioxidant or the bleaching of the colour stoichiometrically depends on the number of electrons taken up. The strong scavenging capacity of the extracts of *moringa oliefera* leaves on DPPH radical might be due to the hydrogen donating ability of the polyphenolic compounds present in the extracts (Shirwaikar et al. 2011).

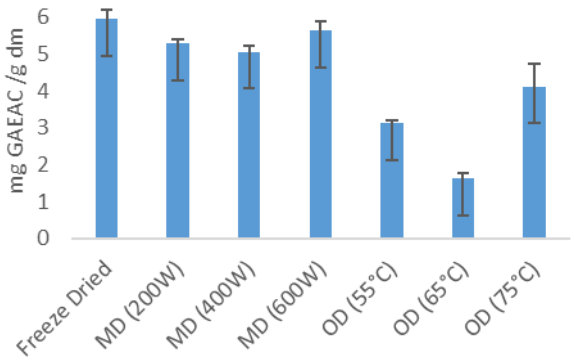


Fig 4.9 AC under various drying methods

The overall highest AC (5.94 ± 0.25 mg GAEAC /g dm) was found in freeze dried leaves this might be because of no heating and preserved quality attributes. Overall lowest AC (1.63 ± 0.13 mg GAEAC /g dm) was observed in hot air oven dried leaves at 65 °C and this can be explained in terms of the combined effect of high temperature and longer drying time. Also, lesser AC observed in case of hot air oven dry at 55 °C this could be because of hot air oven drying process at low temperature which leads to longer drying time that might have caused

a decrease of AC. In hot air oven dried leaves AC was found better in drying at 75 °C temperature than at 55 and 65 °C (Fig. 4.9). This might be because of lowest drying time. In microwave dried leaves, no significant difference in AC was noticed but the antioxidant activity was comparable to freeze dried leaves (Abdullah et al. 2012; Choo et al. 2020; Klungboonkrong et al. 2018; Maisuthisakul et al. 2007; Riyad & Elkholany, 2020; Tao et al. 2016; Tummanichanont et al. 2017).

4.4 Storage study of dried moringa leaves powder

Moringa leaves are used as herbal medicines, food supplement and fortificant because of their high nutritional content and natural antioxidant compounds such as phenolics, flavonoids and vitamin C (Verma et al. 2009, Sahakitpichan et al. 2011). According to Subadra et al. (1997) factors including temperature, humidity and storage time influence the antioxidant activity of products. The physical and chemical characteristics changed unfavourably over passing of days (Table 4.3). Initial (at day 0) value of moisture content of the powder was 5.2% (wb), total phenolic content was 71.86 mg GAE/g-dm, water activity was 0.31, solubility 11.83 %, bulk density 0.32 g/mL and tapped density was 0.39 g/mL. The water activity remains below 0.6 which is considered to be safe because above 0.6 water activity there may be appearance of fungal growth. The water activity might be increased due the exposure to open environment while sample preparation for taking observations. No significant changes in solubility as well as colour was observed. Total phenolic content decreased between 4 - 5% in all the samples. Better preservation of desired attributes was notices in case of deep freeze storage at -30 °C. However, both the packaging materials selected were found to be safe for the storage of moringa powder over the observation period as no undesirable traits were found to be developed. Further, absorption isotherm, equilibrium moisture content (EMC) and glass transition temperature (T_g) can be study to be more specific on the storage stability of the powder.

Table 4.3 Change in quality attributes of moringa powder during storage

	Sample	MC % (wb)			Water activity (a_w)			Solubility (%)			TPC (mg GAE/g dm)		
		I	II	% change	I	II	% change	I	II	% change	I	II	% change
Desiccator	HDPE	6.3	7.18	13.9	0.402	0.489	21.64	11.45	11.27	-1.57	61.83	59.34	-4.02
	LDPE	6.18	7.23	16.99	0.342	0.428	25.14	11.5	11.7	1.73	62.48	59.38	-4.96
Deep Freezer	HDPE	5.4	6.12	13.33	0.345	0.392	13.62	11.86	11.7	-0.75	65.57	62.46	-4.74
	LDPE	5.7	6.24	9.47	0.386	0.394	**2.07**	11.34	11.38	0.35	69.14	66.24	-4.19

I - Readings taken after 10 days, II – Readings taken after 20 days, HDPE – High density polyethylene, LDPE – Low density polyethylene

Chapter 5

Summary and Conclusions

The current chapter presents the summary of major findings from the present study and valid conclusions drawn from the same. Future scopes of the research work is also given which provide insights of the fields on the further exploration in order to understand the mechanism of operations associated in the work in a better and simpler way.

Drumstick (*Moringa oleifera*), grows in the tropical and subtropical regions of the world. Since moringa can tolerate both drought and frost conditions, it is widely cultivated throughout the world. Being highly nutritious tree, every part it is suitable for either nutritional or commercial purposes. The leaves are rich in minerals, vitamins and other essential phytochemicals. Extracts from the moringa leaves can be used to treat malnutrition, augment breast milk in lactating mothers. It is used as potential antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial agent (Gopalakrishnan et al. 2016).

5.1 Summary of results

5.1.1 To analyse the drying kinetics of *moringa oleifera* leaves under various drying methods

Moisture content of moringa leaves was found in the range of 75-80% (wb). Freeze dryer took 24 hours to dry the leaves to bone dry level at – 44 °C and 0.024 mbar. The drying time for the leaves varied between 4.5-6.5 h, and 3-13 min, in hot air oven dryer and microwave dryer, freeze dryer, respectively. Experimental results showed that drying temperature is one of the most influencing parameters for the drying. At higher moisture content, the increase in temperature has more considerable effect on the drying rates as compared to lower temperatures, which is almost negligible towards the end. In all the cases, the drying occurred in falling rate period. The calculated values of effective moisture diffusivity varied from 2.88×10^{-9} to $3.04 \times 10^{-9}\ m^2/s$ in case of hot air oven drying. Similarly, the effective moisture diffusivity varied from 1.01×10^{-7} to $3.45 \times 10^{-7}\ m^2/s$ in case of microwave drying.

5.1.2 To evaluate the effect of drying methods on physio-chemical properties of the leaves.

The TPC values ranged from 24.62 ± 1.10 mg GAE/g-dm (hot air oven drying at 65 °C) to 54.72 ± 5.44 mg GAE/g-dm (microwave drying at 200 W). TFC values ranged from 30.3 ± 1.75 mg QE/g-dm (hot air oven drying at 65 °C) to 60.94 ± 1.02 mg QE/g-dm (microwave drying at 200 W). Antioxidant capacity (AC) varied significantly from 1.63 ± 0.13 mg GAEAC/g-dm (hot air oven drying at 65 °C) to 5.94 ± 0.25 mg GAEAC/g-dm (freeze drying). TPC of samples dried under microwave at 200 W was 57.42 ± 2.49 mg GAE/g-dm and was the highest. TPC of samples dried under hot air oven at 65 °C was 24.62 ± 1.10 mg GAE/g-dm and was the least. Antioxidant capacity of the samples dried in freeze dryer was 5.94 ± 0.25 mg GAEAC/g-dm and was the highest. From the study, it can be stated that microwave drying at 200 W power level resulted better overall retention of physicochemical properties of the moringa leaves.

5.1.3 To study the storage stability of dried *moringa oleifera* leaves powder under different environment conditions

Initial (at day 0) moisture content of the powder was 5.2% (wb), total phenolic content was 71.86 mg GAE/g-dm, water activity was 0.31, solubility 11.83%, bulk density 0.32 g/mL and tapped density was 0.39 g/mL. The water activity was found below critical water activity (0.6) throughout the storage study and other parameters were also in acceptable condition over the observation period (one months) in both packaging materials. Water activity of samples stored in LDPE in deep freezer (-30 °C) increased by 2.07% which was minimum.

5.2 Conclusions

High drying temperature can be considered the main cause of significant loss in bioactive compounds and other important food constitutes that found naturally in plants. Furthermore, antioxidant depletion may be due to grinding of dried leaves which could induce rapid oxidation of enzyme and also because of the heat generated due to the friction. The drying rate decreased continuously with increasing drying period. Constant rate period was absent since no surface moisture was present and the drying process of moringa leaves took place in falling rate period. Drying time decreased with increased temperature in hot air oven drying and also with increase in microwave output power the drying time decreased

considerably. Microwave dryer took the shortest time for drying to similar level of end moisture content. The colour parameters of freeze-dried leaves were close to that of the fresh moringa leaves. While chlorophyll seems to worst hit in hot air dryer because of the high temperature and longer drying time. Also colour parameters were better in microwave dried leaves than in the hot air oven dryer. Microwave drying method (200 W) found to be best for drying of moringa leaves in terms of overall quality attributes. The water activity was found below 0.6 and other parameters were also in acceptable range over the observation period in both packaging materials. Since storage study done over a short period so there is need to further study the storage stability of dried leaves powder and need to include more important parameters (like varying temperature, and humidity). More responses can be worked out like absorption isotherm, hygroscopicity, equilibrium moisture content (EMC), and glass transition temperature. On the basis of results from the present study, deep freeze storage (-30 °C) in LDPE can be suggested to store dried leaf powder.

5.3 Future scope of the research

i. HPLC analysis of moringa leaf extract for isolation of particular compound of interest.

ii. Preparation of food products (like RTE snack food, RTC foods like noodles, pasta, macaroni, baked products like cake, bread, cookies, etc.) incorporated with base material in a desired proportions and analyze their structural and physico-chemical properties.

iii. In the present study, storage stability of dried leaves powder has not been observed over sufficient period. So, it can be studied further under accelerated storage using Arrhenius Equation.

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APPENDIX I

List of chemicals

Chemical	Manufacturer
Acetone	Merck Limited, India
Aluminium chloride	Merck Limited, India
DPPH (pure)	Sigma-Aldrich
Ethanol	CS reagent, India
Ferric chloride hexahydrate	ACS Chemicals, India
Folin Ciocalteau reagent (FCR)	Merck Limited, India
Glacial acetic acid	Merck Limited, India
Gum Arabic	Merck Limited, India
Hydrochloric acid	Merck Limited, India
Maltodextrin	Merck Limited, India
Methanol (Leishman stain)	Finar chemicals
Sodium acetate	Merck Limited, India
Sodium carbonate	ACS Chemicals, India
Sodium hydroxide	Merck Limited, India
Sodium nitrite	Merck Limited, India
TPTZ (pure)	Sigma-Aldrich
Trolox	Sigma-Aldrich

APPENDIX II

List of equipments

Equipment	Model manufacturer
BOD incubator	Reico Equipment and Instrument Pvt. Ltd., India
Hot air oven	Reico Equipments, Kolkata India
Laboratory freeze dryer	IIC-INSTIND Laboratory Freeze Dryer, India
Magnetic stirrer with thermostat	2MLH, Remi Laboratory Instruments, India
Mixer-grinder	Phillips India Limited, India
Portable colorimeter	Spectro-guide 45/0 gloss, BYK Gardner, Germany
Precision weighing balance	SI234, Denver Instruments, Germany
Refrigerated centrifuge	C-24 BL, Remi Laboratory Instruments, India
Ultrapure water purification system	Synergy UV, Millipore Co., India
Ultrasound assisted extraction system	ATP 500, Athena technology, India
UV-Visible spectrophotometer	UV Plus; Motras Scientific, India
Vortex shaker	Spinix MC-0, Tarsons Product Pvt. Ltd., India
Water double-distillation unit	3362041, Borosil Glass Works Ltd., India