EFFECT OF DRYING TEMPERATURE ON BIOACTIVE COMPONENTS OF MORINGA LEAVES AND EVALUATION OF STORAGE STABILITY

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A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirement for the degree of B. Tech. in Food Technology

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Approval Letter

This ***dissertation*** entitled ***Effect of Drying Temperature on Bioactive Components of Moringa Leaves and Evaluation of Storage Stability*** presented by **Prashamsa Pandey** has been accepted as the partial fulfillment of the requirement for the **B. Tech. Degree in Food Technology**.

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Abstract

The study was aimed to determine the effect of drying temperatures on the phytochemicals of moringa leaf and the effect of packaging materials on the storage stability of moringa leaf powder. The fresh moringa leaves were collected from Dharan-14, Vijayapur, Nepal. The leaves were passed through the destalking and cleaning procedures. It was dried at 45℃, 55℃ and 65℃ in cabinet drier and then powdered to a fine consistency. Both fresh and dry moringa leaves were analyzed for the bioactive components such as total phenolic content, total flavonoid content, tannin content, chlorophyll and antioxidant activity. The leaf powder dried at optimum temperature were then stored in three packaging materials i.e., HDPE pouches, LDPE pouches, PET bottle at room temperature. During storage, moisture content and Total Plate Count of leaf was analyzed at the interval of 1 week and 5 weeks respectively.

The proximate composition of fresh moringa leaf was found to be 86.896 ± 0.25997%, 7.043 ± 0.47%, 19.923 ± 0.32%, 9.173 ± 0.345%, 8.51 ± 1.304% and 55.574 ± 1.159% for moisture, crude fat, crude protein, crude fiber, Ash and carbohydrate respectively on the dry basis except for moisture content. Result showed that drying decreased the levels of phytochemicals content and antioxidant activity significantly ($p<0.05$) as compared to the fresh leaves. However, drying at 55℃ showed a lesser loss of bioactive components with the value of TPC, TFC, chlorophyll, tannin and antioxidant activity to be 23.42 mg GAE/g, 25.53 mg QE/g, 3.72 mg/g, 8.12 mg/g and 60.33 % RSA respectively. The sensory analysis showed that the sample dried at 55℃ has higher acceptability as compare to other sample. The sample packaged in HDPE pouches was found superior in term of moisture content, total plate count and sensory evaluation followed by PET bottle with PP cap. The highest moisture content and total plate count was found in sample packaged in LDPE pouches. Leaves dried at 55℃ was found superior in term of bioactive component and sensory analysis.

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Abbreviations	Full forms
AA	Anti-oxidant activity
ANOVA	Analysis of variance
AOAC	Association Of Analytical Communities
db	Dry basis
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FAO	Food and agriculture organization
GAE	Gallic acid equivalent
HDPE	High density polyethene
LDPE	Low density polyethene
LSD	Least significant difference
PAL	Phenylalanine ammonia-lyase
PET	Polyethylene terephthalate
QE	Quercetin equivalent
RDA	Recommended dietary allowance
TFC	Total flavonoid content
TPC	Total polyphenol content
WHO	World health organization

Part I

Introduction

1.1 General introduction

Moringa oleifera is a perennial tree, which belongs to the Moringaceae family and is still regarded as one of the underappreciated plants (Singh and Prasad, 2013). Moringa is the only genus in the Moringaceae family, with roughly 13 recognized species. *Moringa oleifera*, on the other hand, is the most frequently cultivated and popular moringa species. Moringa can be either a deciduous or an evergreen perennial tree, depending on its growing environment and climate (Ciju, 2019).

Moringa oleifera is a versatile and extremely nutritious vegetable tree with numerous possible applications. Its leaves are an excellent source of nutrients for people of all ages. Moringa leaves have a high concentration of vital, disease-fighting minerals, according to nutritional research. They even have all of the necessary amino acids, which is remarkable for a plant source. The young leaves are edible and are typically cooked and eaten like spinach, as well as used in soups and salads. They are high in provitamin A, vitamins B and C, minerals (especially iron and calcium), and the sulfur-containing amino acids methionine and cystine. Because the dried leaves are condensed, the amino acid content of the leaf protein is well balanced. Dried leaves contain even more micronutrient content; (ten times the vitamin A of carrots), (17 times the calcium of milk), (15 times the potassium of bananas), (25 times the iron of spinach) and (nine times the protein of yogurt) (Mishra *et al.*, 2012).

Because of the presence of numerous antioxidant chemicals, this plant's leaves are a valuable source of natural antioxidants as well as a good source of nutraceuticals and functional components (Singh and Prasad, 2013). Fresh leaves of moringa are good source of vitamins such as vitamin A, vitamin C and vitamin E. The dried leaves of moringa are great source of polyphenol compounds, such as flavonoids and phenolic acids. The leaves are rich in alkaloids, glucosinolates, isothiocyanates, tannins and saponins. MO leaves are generally utilized for medical purposes as well as for human nutrition because they are high in antioxidants and other nutrients that are typically deficient in people living in undeveloped nations. MO leaves have been used to treat a variety of ailments ranging from malaria and typhoid fever to hypertension and diabetes (Vergara-Jimenez *et al.*, 2017).

Drying is a common method for increasing the shelf life of vegetables. It is a processing device that removes moisture from food material through heat and mass transfer. Drying involves the use of several temperature regimes, which may impact the nutritional content of vegetables (Olabode *et al.*, 2015). The nutrients and anti-nutrients in Moringa oleifera leaves are affected by different drying processes. All drying methods increase the protein, fiber, carbohydrate, vitamin B1, vitamin A, calcium, and zinc content of the leaf. The different drying methods reduce the anti-nutrients (tannin, oxalate, and saponin) in *Moringa oleifera* while increasing tannin (Mbah *et al.*, 2012).(Rajput *et al.*, 2017) Found that fresh leaves contained lower amounts of total mineral, crude protein, ether extract, total carbohydrate and crude fiber, 4.59, 5.29, 6.72, 10.57 and 5.75 respectively than dried leaves.

1.2 Statement of the problem

Every portion of the moringa tree is valuable, very edible, and has significant economic and medical significance (Ciju, 2019). The leaves are an excellent source of vitamin A. They are high in B vitamins and are one of the greatest plant sources of minerals. For a plant, the calcium level is extremely high. Phosphorus levels are low, as they should be. The iron content is excellent. They have a high protein content and a low fat and carbohydrate content. The leaves are an exceptional source of the sulfur-containing amino acids methionine and cystine, both of which are often in short supply (martin, 1985). Moringa trees have traditionally been used to fight malnutrition, particularly in infants and nursing mothers. One rounded tablespoon (8 g) of leaf powder provides 14% of a child's protein, 40% of calcium, 23% of iron, and nearly all of their vitamin A needs. During pregnancy and breast-feeding, six rounded spoonful of leaf powder will meet virtually all of a woman's daily iron and calcium needs. Small amounts of leaf powder have no discernible effect on the flavor of a product. Moringa leaves will be readily available in this manner to boost nutritious intake on a daily basis (Mishra *et al.*, 2012).

Fresh leaves contained almost 75% moisture content which makes them highly perishable. Drying of Moringa leaves has been carried out commercially for microbial decontamination, minimize packaging, reduce shipping cost, and increasing shelf life (Ali *et al.*, 2017). It is generally believed and advised that M. oleifera leaves dried under shade is the way to preserve nutrient content. However, it may become increasingly difficult to produce sufficient leaf powder by drying naturally under the shade to meet the growing

demand. Therefore, it is needed to conduct a laboratory study on the effect of drying temperature on the nutrient content of moringa leaves (Alakali *et al.*, 2015).

If special technologies are applied, Moringa leaves can be a source of valuable bioactive compounds. Despite the fact that much study has been conducted on the prospect of incorporating moringa leaves into food products, fresh leaves have a short self-life. Drying and proper packaging can improve stability, and dried powder is easier to incorporate into various food products than fresh leaves. As a result, it is required to select acceptable technology for preparing it with the least amount of bioactive component loss and to determine the influence of storage conditions on self-life.

1.3 Objectives

1.3.1 General objectives

The general objective of the dissertation work is to study effect of drying temperature on bio-active components of moringa leaf and evaluation of storage stability.

1.3.2 Specific objectives

The specific objectives are as follows:

1. To determine the bioactive components (chlorophyll, tannin, polyphenol, flavonoid and antioxidant activity) of mature and fresh moringa leaves.
2. Drying of moringa leaves at different drying (45℃, 55℃ and 65℃) temperature.
3. To determine the bioactive components (chlorophyll, tannin, polyphenol, flavonoid and antioxidant activity) of dried moringa leaf powder.
4. Study of storage stability using different packaging materials at room temperature.

1.4 Significance of the study

1. This study will open the chances of moringa leaves to incorporate in any food all seasons.
2. The underutilized moringa leaves will be used as a nutritious food supplement.
3. People can easily prepare, use and sold moringa leaf powder for income.
4. This study might help in evaluation of effective packaging material for storage of moringa leaf powder with best quality.

1.5 Limitation of the study

1. Vitamins are not studied due to lack of facilities.

Part II

Literature review

2.1 Introduction

M. oleifera, sometimes known as drumstick in English, is one of 13 species in the Moringa genus. It is a small, thin, deciduous, drought-resistant, perennial Moringaceae tree that grows to a height of around 10 m. Humans and animals both consume the leaves (Atawodi *et al.*, 2010). It is a small native tree of North West India's Sub-Himalayan areas, but it is now native to numerous regions in Africa, Arabia, South East Asia, the Pacific and Caribbean Islands, and South America (Mouminah, 2015).

Moringa thrives in hot, semi-arid climates. It is drought resistant and flourishes with annual rainfalls ranging from 250-1500 mm (10-60 in). Moringa grows best at elevations less than 600 m (2000 ft). Moringa trees thrive in well-drained sandy or loam soil. It can withstand clay soil but not water logging (Kannan and Thahaaseen, 2016). Depending on the environment and climate where it grows, the moringa tree can be either deciduous or evergreen. Its crown is open and curved like an umbrella. When fully grown, plants can reach heights of 7 to 12 meters and trunk diameters of up to 40 to 45 centimeters (Ciju, 2019). Moringa leaves are compound, pinnately double, and have a small rounded oval at the tips. Yellowish white flowers bloom all year. The drumstick fruit is long and angular, with sides that form a triangle and a length of 15-45 cm, with about 20 fragile and brittle stems (Mutiara *et al.*, 2013).

Taxonomic classification of moringa:

Kingdom:	Plantae
Super kingdom:	Tracheobionta
Super division:	Spermatophyta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Dilleniidae
Order:	Capparales
Family:	Moringaceae
Genus:	*Moringa*
Species:	*oleifera* lam

(Mallenakuppe *et al.*, 2019)

2.1.1 Origin and distribution

Moringa is a genus with thirteen identified species in the Moringaceae family. *Moringa oleifera, M. arborea, M. borziana, M. concanensis, M. drouhardii, M. hildebrandtii, M. ovalifolia, M. peregrine, M. pygmaea, M. rivae, M. ruspoliana, and M. stenopetala* are the species. *Moringa arborea, M. borziana, M. longituba, M. pygmaea, M. rivae,* and *M. ruspoliana* are all unique to Africa and are found nowhere else on the planet. *M. drouhardii* and *M. hildebrandtii* are also native to Madagascar. These two, as well as *M. ovalifolia* and *M. stenopetala,* each native to Africa, are referred to as "bottle trees." The three-valved fruits, the three-winged seeds, the stalked glands at the base of the leaves and the rachis articulations, as well as the strong horseradish scent coming from the leaves, are all characteristics of *M. oleifera L*. *Moringa oleifera* can be found all across the tropics and subtropics of the planet. Asia, Africa, North America, Central America, the Caribbean, South America, and Oceania are all home to the species (Thakur and Bajagain, 2020).

M. oleifera L. is a native of the northern foothills, which include Nepal, Pakistan, and Northern India (Leone *et al.*, 2015). Particularly in the Tarai region of Nepal and India, there is a lot of genetic variation available. As a result, *M. oleifera* has gained widespread acceptance, recognition, and utility across Nepal's many ethnic groups. In Nepal, M. oleifera L. is grown on 67 ha of land in 13 different districts, and 554 metric tons are produced annually (Thakur and Bajagain, 2020).

2.2 Consumption pattern of moringa

Even in large concentrations, the moringa plant does not cause harm. It is simple to use as a supplement or on most foods, is simple to conserve, and is simple to digest. Because the moringa plant and its processed derivatives don't contain caffeine like other beverages, they don't have any negative health consequences (Egwui *et al.*, 2013). The tree's young green "drumsticks" are the component that is most prized and frequently used. They are frequently eaten in India, are typically served similarly to green beans, and have a mild asparagus flavor. From fully developed pods, the seeds are occasionally extracted and either eaten as peas or roasted like nuts. When cooked, the blooms are edible and are said to taste like mushrooms (Kannan and Thahaaseen, 2016).

The leaves are very nutrient-dense and a good source of protein, iron, beta-carotene, vitamin C, and potassium. The leaves are prepared like spinach and then utilized. In addition to being substituted for spinach when used fresh, its leaves are frequently dried, ground into a powder, and added to soups and sauces (Kannan and Thahaaseen, 2016). For pregnant women, nursing mothers, infants, young children, as well as adults of all ages, leaves have been used successfully in their dried state or powdered form to enhance and make delectable meals and porridge diets. Malnourished children have made large weight gains when nursing moms and caregivers add *M. oleifera* leaves to their diets in Africa, where studies have shown that doing so significantly increases the amount of milk nursing mothers make (Alakali *et al.*, 2015). It has also been used into biscuits, porridges, and other food products. Moringa tea is often made by steeping dried leaf powder in hot water for a period of time before drinking (Tetteh, 2009).

2.3 Uses of moringa

The Moringa tree is grown and used as a vegetable (leaves, green pods, blossoms, roasted seeds), spice (mostly roots), cooking and cosmetic oil (seeds), and medicinal plant (all plant organs). Currently, the young seeds and pods are used as vegetables, the kernel oil is extracted for industrial use, the water extract is used to purify water, the seed cake is used as fertilizer and animal feed, and various plant parts (such as the roots, bark, sap, leaves, oil, and flowers) are used in traditional medicine in several different countries (Egwui *et al.*, 2013). *Moringa oleifera* seed oil, commonly known as Ben oil, is a sweet, non-sticking, non-drying oil that resists rancidity (yield 30–40% by weight). In addition to being utilized in the

production of perfume and hair care products, it has also been employed in salads and as a fine machine lubricant (Tsaknis *et al.*, 1999). According to reports, moringa oleifera leaves are a good source of natural antioxidants and a rich source of beta-carotene, protein, vitamin C, calcium, and potassium. Because they contain a variety of antioxidant compounds, including ascorbic acid, flavonoids, phenolics, and carotenoids, they also extend the shelf life of foods that contain fat (Mutiara *et al.*, 2012).

2.4 Nutritional composition of moringa

M. oleifera has essential nutrients and antinutrients in every region of the plant. Minerals such as calcium, potassium, zinc, magnesium, iron, and copper are abundant in the leaves of *M. oleifera*. *M. oleifera* contains vitamins such as beta-carotene, folic acid, pyridoxine, and nicotinic acid, as well as vitamins C, D, and E. Anti-cancerous substances such glucosinolates, isothiocyanates, glycoside compounds, and glycerol-1-9-octadecanoate are found along with phytochemicals like tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids, and reducing sugar (Gopalakrishnan *et al.*, 2016). Regarding human micronutrient and macronutrient needs, *M. oleifera* quantitatively provides more nutrients per gram of plant material than many other plant species. For example, gram-for-gram comparisons of *M. oleifera* leaves (fresh and dried) and other common nutritional plant sources reveals that *M. oleifera* provides more than seven times the vitamin C found in oranges, 10 times the vitamin A found in carrots, 17 times the calcium found in milk, nine times the protein found in yogurt, 15 times the potassium found in bananas and 25 times the iron found in spinach (Rockwood *et al.*, 2013).

The most abundant nutrient in all *M. oleifera* sections is protein, which accounts for 25% of dry matter. In contrast, the lipid content is higher in seeds (30% of dry matter) and lower in fruit, leaves, and pods. *M. oleifera* fruit, leaves, and pods (20% of dry mass) also contain a significant amount of nutritional fiber. Including globulin, albumin, glutein, and prolamin, *M. oleifera* leaves contain all necessary amino acids. The predominant unsaturated fatty acid in *M. oleifera* seeds is oleic acid, while the saturated fatty acids in the seeds are palmitic, stearic, arachidic, and behenic (Godinez-Oviedo *et al.*, 2016). Essential elements like calcium and iron are abundant in the leaf and fruit pods of the moringa plant, which also contains vitamins A, B, and C. Additionally, they are a great source of sulphur containing amino acids like methionine and cystine (Hamza and Azmach, 2017).

2.5 Phytochemistry and nutritive quality of moringa leaves

The leaves are a great source of protein and have little fat or carbohydrate content. As a result, leaves are among the greatest plant foods available. Additionally, leaves are the richest source of the sulfur-containing amino acids methionine and cystine, which are usually in short supply in the plant kingdom (Subadra *et al.*, 1997). Leaf powder contains tannins, saponin, alkaloids, phenols, flavonoids, and glycosides as the main phytochemicals (Mensah *et al.*, 2012). According to numerous researchers, moringa leaf is an excellent source of protein since *M. oleifera* leaves contain 20-30% of their dry weight of protein. Furthermore, leaves contain unsaturated fatty acids such as linoleic acids, which are uncommon in plant sources. *M. oleifera* leaves are high in almost all vitamins, including vitamin A, vitamin B (containing folic acid, pyridoxine, and nicotinic acid), vitamin C, vitamin D, and vitamin E (Qi *et al.*, 2016). Because it contains all the essential amino acids in adequate amounts, including arginine (1325 mg/100 g dry leaf) and histidine (613 mg/100 g dry leaf), which are crucial for newborns who cannot produce enough protein for growth, moringa is a great non-animal source of protein (Dhakar *et al.*, 2011).

Arise *et al.* (2014) reported a greater protein content of 25%, a fat content of roughly 1.5%, a fiber content of around 7.5%, and an ash level of about 6%, respectively. According to Hasaballa *et al.* (2017), there are variations in protein and fat composition, with protein as low as 19% and fat as high as 8.80%; fiber and ash content were respectively at 6.75% and 8%. Calcium was found to be 1255.31mg/100 g, and iron was found to be 47.13 mg/100 g. It has also been claimed that moringa leaf has the greatest calcium and iron content of any portion of the plant. TPC and total flavonoids in leaves were 28.61 mg GAE/g and 19.41mg/g, respectively. In addition, the IC50 value for leaves was 77.71 g/ml. Additionally, the saponins in moringa leaves, such as triterpenoid glucoside and steroids, produce a disagreeable aroma and a bitter flavor (Indriasari and Kumalaningsih, 2016).

The anticancer potentials of *M. oleifera* leaves are directly influenced by the significantly high antioxidant levels. Aside from acting as a free-radical scavenger and contributing to the production of vitamin E, which can prevent and treat scurvy symptoms like rashes, gum infections, and ozostomia, vitamin C is abundant. M. oleifera leaves can aid in the treatment of night blindness since vitamin A serves as a form of important nutrient for regular eyesight, particularly in low-light conditions. The acetylcholine esterase activity can be decreased by M. oleifera leaf extracts, which enhances cholinergic function and memory (KC *et al.*, 2022).

2.5.1 Composition of *moringa oleifera* leaves

Table 2.1: Nutritional value of Moringa Leaves

Parameter	Dried leaf
Moisture %	7.96
Crude protein (%, db)	22.14
Crude fat (%, db)	7.19
Crude fiber (%, db)	10.44
Ash content (%, db)	9.12
Iron (mg/100g, db)	47.13
Calcium (mg/100g, db)	1255.31
Tannin (mg/g)	9.18
Chlorophyll (mg/g)	3.06
Carbohydrate (%, db)	51.11
Energy (Kcal/100g)	357.71
Antioxidant activity(%RSA)	58.62
TFC (mg QE/100g 70% ethanol extract)	19.41
TPC (mg GAE/g 70% ethanol extract)	28.61

(Hasaballa *et al.*, 2017)

2.5.2 Polyphenols

Polyphenols, which have a variety of complex structures, are naturally found in foods made from plants. Phenolic rings, which are the fundamental monomer in polyphenols, are typically categorized as phenolic acids and phenolic alcohols. Polyphenols can be divided into numerous classes based on the strength of their phenolic rings, but their main subclasses include phenolic acids, flavonoids, stibins, phenolic alcohols, and lignans. The collection of

biologically active substances found in foods made from plants is known as polyphenols (Abbas *et al.*, 2017).

The most prevalent antioxidants in our diet are polyphenols, which are found in large quantities in a variety of foods and beverages, including tea, coffee, wine, dried beans, grains, fruits, vegetables, and olives. Polyphenols are the antioxidants that we consume most frequently. These prevent the oxidative modification of low density lipoprotein, which is the fundamental mechanism underlying the endothelial lesions that occur in atherosclerosis (D Archivio *et al.*, 2007). Antioxidants like polyphenols may shield cellular components from oxidative damage and lower the risk of oxidative stress-related degenerative illnesses. A function for polyphenols in the protection of cardiovascular disease, cancer, osteoporosis, diabetes mellitus, and neurodegenerative illness is in fact highly supported by experimental studies (Scalbert *et al.*, 2005).

2.5.3 Flavonoids

Plants produce flavonoids, a subgroup of polyphenolic chemicals with a benzo-pyrone structure, in reaction to microbial diseases, which is why they are so common in nature. The diets of both humans and animals contain flavonoids. Flavonoids cannot be produced by either humans or animals because they are phytochemicals. As a result, animal flavonoids are not biosynthesized in situ, but rather originate from plants (Kumar and Pandey, 2013). Epidemiological studies have repeatedly demonstrated the preventive effects of a high flavonoid intake against a wide range of infectious (bacterial and viral infections) and degenerative diseases, including cancer, cardiovascular disease, and other age-related disorders (Pandey and Rizvi, 2009). The leaves of the Moringa oleifera are an interesting source of flavonoids chemicals. The concentration of total flavonoids in dried leaves varies from 5.059 to 12.16 mg/g of DW (Yang *et al.*, 2008). The primary flavonoids present in Moringa oleifera leaves are myricetin, quercetin, and kaempferol (Leone *et al.*, 2015).

2.5.4 Tannins

The floral composition of plants contains a lot of tannins. They include high molecular weight phenolic compounds. In the root, bark, stem, and outer layers of plant tissue, tannins are present that are soluble in both water and alcohol. Tannins' distinctive ability to tan, or turn materials into leather, is one of their most notable traits. As a result of the presence of phenolics or carboxylic groups, they undergo an acidic reaction (Doughari, 2012). There are

two different categories of tannin. Although the two forms of tannins share the majority of their features, hydrolysable tannins are less stable and have a higher potential for toxicity. With the size of the tannin molecule, the water solubility is constrained and generally declines. In cases of diarrhea, skin bleeding, and transudates, larger tannins are employed as astringents since they indiscriminately bind to proteins (Bernhoft, 2010).

2.5.5 Chlorophyll

Chlorophyll is the most common natural pigment, found in the leaves and other portions of practically all plants (Humphrey, 1980). In some species, it can reach levels as high as 1000 to 2000 ppm wet weight. These blue-green pigments have frequently been disregarded with regard to their potential physiological impact and role in the prevention of chronic disease because of the primary function of chlorophyll in photosynthesis and its close association with yellow/orange carotenoid pigments well known for their bioactivity. Chlorophyll and its many derivatives are thought to belong to a family of phytochemical molecules that may be responsible for preventing chronic diseases like cancer, which has sparked interest in plant food phytochemicals as physiologically active dietary components. The majority of dietary chlorophyll is made up of lipophilic derivatives, such as chlorophylls a and b, which are found in fresh fruits and vegetables, metal-free pheophytins and pyro-pheophytins, which are found in thermally processed fruits and vegetables, and Zn-pheophytins and Zn-pyro-pheophytins, which are found in thermally processed green vegetables. A commercial-grade derivative called sodium copper chlorophyllin (SCC), along with other water-soluble derivatives like chlorophyllides and pheophorbides, add to the variety of dietary chlorophyll derivatives (Ferruzzi and Blakeslee, 2007). Natural chlorophyll and commercial-grade derivatives such as sodium copper chlorophyllin (SCC) have been extensively researched for a variety of positive biological functions such as wound healing (Bowers, 1947).

2.5.6 Anti-oxidants

Antioxidant compounds are substances that stop free radicals from damaging cells in a way that could potentially damage DNA and result in the development of cancer. Whereas a biological antioxidant is "any substance that, when present in low concentrations relative to those of an oxidizable substrate, significantly retards or prevents oxidation of that substrate"(Halliwell and Gutteridge, 1995). Free radicals can come from a variety of sources, including pollution, cigarette smoke, UV light, ionizing radiation, some organic solvents,

and industrial waste (Boonchum *et al.*, 2011). Numerous chronic human diseases, including as cancer, neurodegenerative diseases, diabetes mellitus, arthritis, atherosclerosis, and aging, are caused by oxidative damage (Jahan *et al.*, 2015). The most effective antioxidants are those that can stop the chain reaction of free radicals and prevent the harm that can result from their effects (Brewer, 2011).

Some vegetables, fruits, and a variety of other foods contain natural antioxidants (Moon and Shibamoto, 2009). Flavonoids, alkaloids, phenols, and tannins are some of the essential phenolic compounds that are frequently found in medicinal plants and perform antioxidant activities. The natural antioxidants in the body purify reactive oxygen species (ROS), such as oxygen (O2) and hydrogen peroxide (H2O2), which are created as free radicals. This may result in a balance between the ROS formed and the antioxidants already present. The discovery of some natural antioxidants, including vitamin C, flavonoids, tocopherols, and other phenolic compounds, made it clear how efficient Moringa oleifera is as an anti-oxidant (Jahan *et al.*, 2015). Moringa oleifera is a fantastic source of natural antioxidants that can be used to prevent the advancement of various diseases, according to numerous research (Siddhuraju and Becker, 2003). The antioxidants present in moringa leaves are vitamin A, vitamin C, flavonoids, phenolic acids, alkaloids, tannin and saponin (Vergara-Jimenez *et al.*, 2017).

2.6 Moringa leaf powder

Moringa Leaf Powder can be mixed into any dish or beverage to boost the vitamin, mineral, and protein content. A few spoonsful of Moringa Leaf Powder can be added to any meal to make it more nutritious for healthy people. Because the nutrient content of Moringa Leaf Powder reduces when heated, add it after the food or drink has been made, shortly before serving (Doerr and Cameron, 2005). Even though a significant number of water-soluble vitamins are lost during drying and storage, the leaf powder remains a very rich nutritional supplement that is concentrated in the dried leaves (Emelike and Ebere, 2016). Because Moringa leaf powder can be added to food after cooking, it helps to preserve nutrients. Moringa powder can be used as a vitamin supplement in practically any cuisine (Makkar and Becker, 1996).

Dried Moringa powder can boost the nutritional value of pancakes, cereals, and drinks, and it can even be used to fortify pastries (Emelike *et al.*, 2015). Moringa Leaf Powder has

the biggest influence on people who are most vulnerable: malnourished children, pregnant or breastfeeding women, youngsters of weaning age, HIV/AIDS patients, and the elderly. Malnourished toddlers aged 1-3 years should take three rounded tablespoons (25g) of Moringa Leaf Powder every day. Pregnant or lactating women should take six rounded tablespoons (50g) of Moringa Leaf Powder each day (Doerr and Cameron, 2005).

Thus, the usage of Moringa oleifera leaf powder has the potential to address protein-energy malnutrition and micronutrient deficiencies in developing nations (Karim *et al.*, 2015). The use of moringa leaves in food can be regarded as cost-effective because they are a readily available plant resource, can be preserved for a long time after drying, require little storage space and processing, are accessible all year round, and have a low cost of production because they can be grown in hot climates and tolerate poor soil (Kar *et al.*, 2013).

2.7 Drying

Food drying is a significant and commonly used method of food processing (Koyuncu *et al.*, 2007). Every year, roughly one-third of worldwide food output is lost due to a lack of efficient and timely processing (Gustavsson *et al.*, 2011). One of the oldest techniques for preserving food is drying. Microbiological deterioration and detrimental chemical reactions can be reduced by lowering the moisture content of fruits and vegetables. Drying reduces the weight and volume of the finished product, in addition to preserving it, which saves the cost of packaging, storage, and transportation. But when foods are dried at high temperatures, the product's color, flavor, and nutritional qualities could be affected. As a result, numerous research have examined how drying affects the active components and phytochemical content of fruits, vegetables, herbs, and medicinal plants (Potisate *et al.*, 2015).

A higher drying temperature shortens the drying time but may lead to an inferior finished product, surface damage from heat, and increased energy use. On the other side, gentle drying conditions with a lower temperature may enhance the product's quality while reducing the rate of drying, lengthening the drying period (Kumar *et al.*, 2014). The final product's quality is affected by the drying process, temperature, and final water activity. Therefore, the choice of drying technique depends on a number of variables, including the type of product, the accessibility of a certain dryer, and the desired characteristics of the desiccated product (Potisate *et al.*, 2014).

2.7.1 Effect of Drying on Phytochemicals

Vegetables are frequently preserved via drying, a processing unit that removes moisture from food material through heat and mass transfer. Different temperature regimes are used for drying, which may have an impact on the nutritional value and bioactive components of vegetables. According to reports, depending on the drying technique, operational conditions, and kind of plant, such losses in medicinal plants range from 0 to 100% (Olabode *et al.*, 2015; Potisate *et al.*, 2015).

According to the drying techniques, there were substantial differences in the degradation of total phenolics and flavonoids. It was discovered that fresh leaf extracts always had higher concentrations of total phenolics and flavonoids than dried leaf extracts did. The drying process parameters, particularly the temperatures and time used, may be responsible for the loss of phenolics and flavonoids. It has been observed that thermal processing can damage phytochemicals by affecting the integrity of the cell structure, resulting in the migration of components, leading to losses through leakage or disintegration by various chemical reactions involving enzymes, light, and oxygen (Youssef and Mokhtar, 2014). Other mechanisms associated with the reduction in total phenolic content include the binding of phenolic compounds to proteins, changes in chemical structures, or poor extraction efficiency. On the other hand, different drying techniques increased the overall phenolic content of apples by up to a factor of ten. The release of phenolic chemicals attached to the plant cell wall can account for these increases in the overall phenolic content. (Gümüşay *et al.*, 2015).

Due to chemical, enzymatic, or thermal decomposition, biologically active substances degrade at high temperatures, which results in a decrease in antioxidant activity as a result of drying (Kamiloglu *et al.*, 2016). The overall antioxidant capacity of dried fruits and vegetables was raised by some drying techniques, particularly freeze-drying (Vidinamo *et al.*, 2021). The fact that partially oxidized polyphenols have higher antioxidant activity than non-oxidized polyphenols may be the cause of the enhanced antioxidant activity in fruits and vegetables after drying (Dalla Nora *et al.*, 2014). Additionally, the Maillard reaction products, which can result from heat treatment or extended storage and which typically exhibit strong antioxidant qualities, may be connected to increase in antioxidant capacity after drying (Kamiloglu and Capanoglu, 2015).

2.8 Storage stability

The basic goal of storage is to keep an item that is more or less perishable in a salable and edible state for as long as it is economically feasible to do so. In addition to keeping perishable products in marketable shape throughout the season, good storage also ensures a more consistent market supply (Stuart, 1930). The best way to keep moringa leaf powder is in airtight containers away from heat, humidity, and light. Insufficient drying or storage of the powder could promote the formation of molds or mildews, which could result in issues ranging from unpleasant to dangerous. If powder that has been stored is exposed to heat or light, it will deteriorate and lose some of its nutritional value. Under the following circumstances, moringa leaf powder can be maintained for up to a year: clean, dry powder preserved in airtight containers, shielded from light and humidity, and kept below 24 °C (75 °F) (Doerr and Cameron, 2005).

2.8.1 Changes during storage

Foods are different from other types of products; they are complex living systems where physicochemical, microbiological, and enzymatic activities are occurring at the same time. Understanding this fact is essential to understanding how long foods may be stored. These interactions have a significant impact on the shelf life, flavor, and texture of foods (Singh and Cadwallader, 2004). The term "shelf life" refers to the time between a product's production date and the point at which it is no longer able to meet the necessary standards for safety and quality. All foods contain moisture, whether in small amounts in dehydrated meals or in large amounts in cold or hot beverages. Water content is crucial for food stability and shelf life because it directly correlates with how quickly food spoils (Arendse and Jideani, 2022).

During the storage period, physical, chemical, and microbiological changes are employed as indicators of the quality of the commodities. Examples of physical changes in food include sugar crystallization in dried fruit and the absorption of moisture by dry items resulting in mushiness. According to studies, oxidation, enzymatic browning, and non-enzymatic browning are the primary chemical changes responsible for food degradation and a shorter shelf life. In foods, microbial growth and activity can result in gas production, pH changes, discoloration, bad flavors, and odors (Robertson, 2016; Singh and Cadwallader, 2004).

Regardless of the packaging materials, it was generally observed that the crude fiber, carbohydrate, vitamin (A, C, and E), sodium, and calcium contents of the powdered moringa leaves decreased as storage duration increased. However, the highest reduction in vitamin A and C, sodium, and calcium contents were observed. However, factors such as the length of storage, temperature, light exposure, and oxygen content could be responsible for the overall loss in nutritional component of the powdered moringa leaves as storage progresses (Fuglie, 1999), whereas the levels of crude protein, crude fat, and ash rose with storage (Adejumo and Dan, 2018). The results demonstrated that moisture content and water activity increased from the day of storage to 90 days later (Gnana *et al.*, 2021).

2.9 Packaging

Packaging preserves the advantages of food processing long after the process is finished, allowing food to travel over large distances securely and retain its nutritional value when consumed. Food packaging aims to effectively contain food while meeting industry standards, consumer demands, preserving food safety, and minimizing environmental impact. Glass, metals (aluminum, foils and laminates, tinplate, and tin-free steel), paper and paperboards, and polymers have all historically been used in food packaging. Additionally, a greater range of polymers in both rigid and flexible forms have been introduced (Marsh and Bugusu, 2007b).

2.9.1 PET (Polyethylene Terephthalate)

PETE, which is created when terephthalic acid and ethylene glycol combine, acts as an effective barrier to moisture and gases (oxygen and carbon dioxide). It also resists heat well, mineral oils, cleaners, and acids well, but not bases. As a result, PETE is increasingly being used as packaging material for a variety of food items, especially drinks and mineral water. PETE is increasingly used to create plastic bottles for carbonated beverages (Willige *et al.*, 2002). Most bottled items are packaged with polyethylene terephthalate, which has many benefits like being lightweight, unbreakable, handy, affordable, resealable, and recyclable (Kodama *et al.*, 2006).

2.9.2 LDPE

Low-density polyethylene is adaptable, robust, easy to seal, and moisture-resistant. Low-density polyethylene is typically utilized in film applications and in situations where heat

sealing is required because it is relatively clear. Low-density polyethylene products include squeezable food bottles, flexible lids, and bread and frozen food bags. In the supermarket industry as well as other types of retail, polyethylene bags are occasionally reused (Marsh and Bugusu, 2007b). However, due to its low oxygen barrier qualities, this form of plastic is not suitable for food goods that are susceptible to oxidation (Kim *et al.*, 2014).

2.9.3 HDPE

High-density polyethylene is stiff, strong, tough, impermeable to gases, resistant to chemicals and moisture, and simple to process and form. It is used to create grocery, trash, and retail bags as well as bottles for milk, juice, and water, cereal box liners, and margarine tubs (Marsh and Bugusu, 2007). This PEs has excellent water vapor barrier properties, which are needed for many water-sensitive food goods, such as dried and liquid food products, in addition to superior processability (e.g., can be made into bags, films, bottles) (Alter, 1962).

2.9.4 Effect of packaging materials during storage

The powder of moringa leaves is frequently packaged in glass, polythene, and different plastic containers with various qualities. Prior to use, the powder is packaged so that its nutrients and health advantages can be utilized as an addition to pap, water, soup, and other food products (Omobolanle *et al.*, 2015).

After storage, moringa leaf powder packaged in PET bottles had the lowest moisture content, while due to poor oxygen barrier moringa leaf powder packed in LDPE 200gauge poly bags had the highest moisture level. This was caused by the varying water vapor permeability of the packaging materials. similarly, moringa leaf powder packed in PET bottles had the highest calcium, protein, and anti-oxidant levels, while moringa leaf powder packed in LDPE 200gauge poly bags had the lowest levels (Gnana *et al.*, 2021).

Part III

Materials and methods

3.1 Materials

Unless otherwise noted, AR grade chemicals were employed, and calibrated glassware and equipment were used throughout the project.

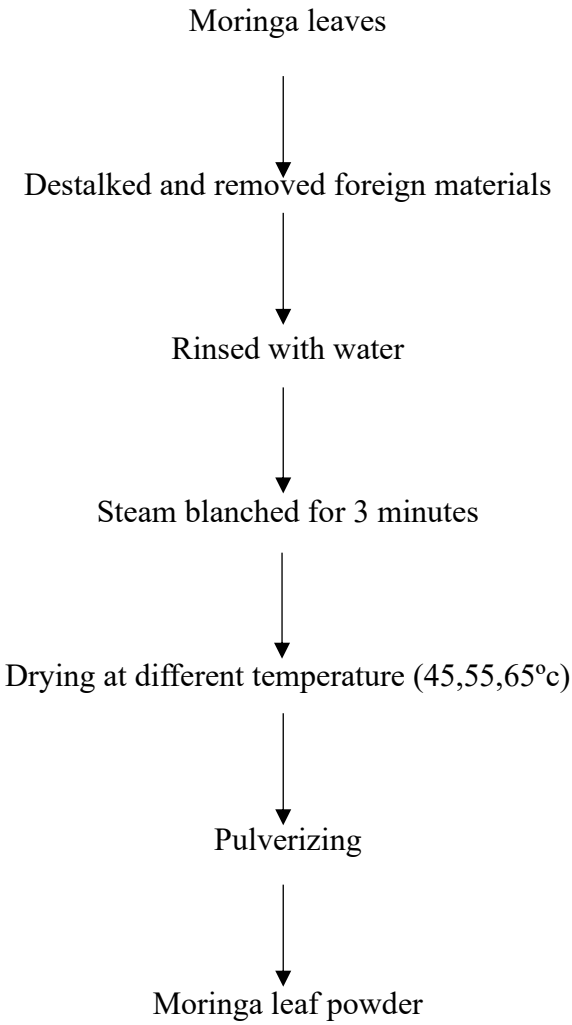
3.1.1 Collection of raw material

The leaves of moringa (*Moringa oleifera*) of about 1.79cm length and 1.22 cm breadth were collected from vijayapur, Dharan-14, Nepal. The packaging materials i.e. HDPE bag(8”×12”), LDPE bag(4”×5”) and PET bottle were purchased from local market of Dharan.

3.2 Methods

3.2.1 Drying of moringa leaves

The leaves were separated from the stems after collection. Any damaged or discolored leaves were set aside during this process. The leaves were then rinsed with clean water and steam blanched for 3 minutes and cooled in cool water (Wickramasinghe *et al.*, 2020). The blanched leaves were dried at different temperature. The dried leaves were then powdered to a fine consistency and sieved via a 40 mesh size. After the determination of optimum temperature, the powder prepared by drying the leaves at optimum temperature were stored in different packaging materials.



Wickramasinghe *et al.*, (2020)

Fig:3.1 Flow-chart for preparation of moringa leaf powder

3.3 Analytical methods

3.3.1 Determination of moisture content

The sample's moisture content was evaluated by weight loss during heating in a thermostatically controlled oven at 105 °C using the hot air oven method described in Rangana (1986).

3.3.2 Determination of crude fiber

The crude fiber content of raw materials was evaluated in accordance with AOAC (2005).

3.3.3 Determination of crude protein

The crude protein content of raw material samples was tested in the same manner as reported in AOAC (2005).

3.3.4 Determination of crude fat

The crude fat content of the sample was measured using the solvent extraction method described by Rangana (1986).

3.3.5 Determination of Ash content

The ash content of raw materials is determined as described by AOAC (2005).

3.3.6 Determination of carbohydrate

The carbohydrate content was calculated using the difference method.

3.3.7 Total polyphenol content

Total phenol was determined using the Folin-Ciocalteau reagent as described by Sadasivam and Manickam (1996). In the Folin-Ciocalteau reagent in alkaline medium, phenols react with phosphomolybdic acid to form a blue complex (molybdenum blue). 0.5 ml of the extract was combined with 1 ml of the Folin-Ciocalteau reagent and incubated at room temperature for 15 minutes. Then 2.5 ml of saturated sodium carbonate was added and incubated for 30 minutes at room temperature before measuring absorbance at 760 nm.

3.3.8 Total flavonoid

The total flavonoids content was assessed using a small modification of the aluminum chloride assay method published by Samatha *et al.* (2012). 10g powdered material was extracted overnight in 100 ml 80% methanol. The extract was filtered and heated to 45 degrees Celsius on a hot plate. To 2 ml of extracted material, 0.2 ml of 5% $NaNO_2$ was added and allowed to stand for 6 minutes. After 6 minutes, 0.2 ml of 10% $AlCl_3$ was added. Finally, 2 ml of 1N NaOH was added, and the volume was increased to 5 ml with 80% methanol and stand for 15 minutes before measuring absorbance at 510 nm. In place of the sample, 80% methanol was utilized as a blank. 2ml of varied concentrations (20%, 40%, 60%, 80%, and 100%) of quercetin in 80% methanol was prepared for standard curve.

3.3.9 Tannin

The tannin was measured using the Folin-Ciocalteu technique. A volumetric flask (10 ml) was filled with 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, 1 ml of 35% Na2CO3 solution, and diluted to 10 ml with distilled water. The mixture was thoroughly shaken and left at room temperature for 30 minutes. Gallic acid reference standard solutions (20, 40, 60, 80, and 100 g/ml) were prepared. An UV/Visible spectrophotometer was used to measure the absorbance of test and standard solutions against a blank at 725 nm. The tannin concentration was calculated as (mg of GAE /g) of extract (Haile and Kang, 2019).

3.3.10 Anti-oxidant activity

The antioxidant activity of sample extracts was evaluated using the method described by Hawa *et al.* (2018), with minor variations. Various concentrations of samples were made using 80% methanol. 1 ml of obtained extract was combined with 2 ml of 0.1 mM DPPH solution and left in the dark for 30 minutes before measuring absorbance at 517 nm. Methanol and DPPH were used to prepare blank samples.

The percentage inhibition was calculated as follows:

$$\%\text{Inhibition} = \frac{(\mathrm{A_b} - \mathrm{A_s})}{\mathrm{A_b}} \times 100$$

Where Ab is the absorbance of the control sample and As is the absorbance of the test sample. The 50% inhibitory concentration (IC50) was calculated as the quantity of extracts required to react with half of the DPPH.

3.3.11 Chlorophyll content

Chlorophyll was extracted in 80% acetone and the absorbance was measured at 663 and 645nm in spectrophotometer (KC and Rai, 2007). Using the absorbance coefficients, the chlorophyll content was calculated by the empirical formula:

$$\text{chl a, mg/gtissue} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{\mathrm{V}}{1000 \times \mathrm{W}}$$

$$\text{chl b, mg/gtissue} = 22.9(\mathrm{A_{645}}) - 4.68(\mathrm{A_{663}}) \times \frac{\mathrm{V}}{1000 \times \mathrm{W}}$$

$$\text{Total chlorophyll, mg/gtissue} = \text{chl a} + \text{chl b (calculated above)}$$

Where A is the absorbance at specific wavelength, V is the final volume of chlorophyll extract and W is the fresh weight of tissue extracted.

3.4 Sensory evaluation

Sensory analysis was evaluated by ten semi trained panelists and sensory parameters analyzed were color, smell, taste, mouthfeel, and overall acceptability using Hedonic rating scale (Rangana, 1986). Samples were prepared using 2 gram of each sample in 200ml of hot water and left for 5 minutes (Madukwe *et al.*, 2013). Sensory analysis score card is given in Appendix B.

3.5 Microbiological examination

Total Plate count (TPC) was determined by pour plate technique on Plate Count Agar (PCA) medium (incubated at 30℃/48 h) (AOAC, 2005).

3.6 Storage studies

The moringa leaf powder dried 55℃ was filled in HDPE (8" ×12"), LDPE (4" ×5") and PET with PP cap and were stored at room temperature. The samples were drawn at the interval of 7 days and moisture content and microbial analysis was evaluated.

3.7 Statistical analysis

The triplicate data of each experimental analysis were analyzed by one- way analysis of variance (ANOVA) and this was carried out by using software GenStat Release 12.1 (Copyright 2009, VSN International Ltd.). Means were compared using fisher's unprotected LSD ($P<0.05$).

Part IV

Results and discussion

The moringa (*Moringa oleifera*) leaves collected from Dharan were used to make the moringa leaf powder. The blanched leaves were subjected to drying at different temperature (45℃, 55℃ and 65℃) and their phytochemicals and antioxidant activity were analyzed. The leaves then dried at best temperature were used to make powder and stored in different packaging materials (HDPE, LDPE, PET).

4.1 Proximate and bioactive composition of moringa leaf

The proximate and chemical composition of fresh moringa leaves is given in table 4.1 and table 4.2 respectively.

Table 4.1 Proximate composition of *Moringa oleifera* leaves

Parameter	Values
Moisture (%, wb)	86.89 ± 0.26
Protein (%, db)	19.92 ± 0.32
Fat (%, db)	7.04 ± 0.47
Fiber (%, db)	9.17 ± 0.35
Ash (%, db)	8.51 ± 1.30
Carbohydrate (%, db)	55.57 ± 1.16

Values represent the mean of triplicate determination ± standard deviation. All the values are expressed in dry basis except moisture content.

The moisture, protein, crude fiber, fat and ash of fresh Moringa leaf was found from the analysis as shown in table 4.1. The proximate values of the fresh moringa leaves were found to be 86.896 ± 0.25997% moisture with the value of crude protein, crude fat, crude fiber, total ash and carbohydrate to be 19.923%, 7.043%, 9.713%, 8.51% and 55.574% on dry weight basis respectively which is similar with the study performed by Nweze and Nwafor

(2014) , though the value of fat was higher. The higher value of fat may be due to geographical variation and development stage of *Moringa oleifera.* The values for the proximate of moringa leaves were in range with several other studies (Rajput *et al.*, 2017; Sultana, 2020).

Table 4.2 Bioactive composition of *Moringa oleifera* leaves.

Parameters	**Values**
TPC (mg GAE/g, dry matter)	35.31 ± 2.53
TFC (mg QE/g, dry matter)	44.8 ± 4.057
Tannin (mg/g, dry matter)	23.28 ± 3.08
Chlorophyll (mg/g, db)	28.09 ± 3.51
Anti-oxidant activity (%RSA)	79.58 ± 3.42

Values were the mean of determinations ± standard deviations.

The values for all the parameter where in range with several studies (Du Toit *et al.*, 2020; González-Romero *et al.*, 2020; Jahan *et al.*, 2015).Jahan *et al.* (2015) reported that the TPC of fresh moringa leaves lies between 30.83 to 35.51 mg GAE/g which is similar to the value of TPC obtained from this study.(Jahan *et al.*, 2015) also reported that the TFC of fresh moringa leaves lies between 32.74 to 98.67 mg QAE/g . The value of TFC obtained from this study lies with in this range. The variation of TPC and TFC may be due to the maturity and chlorophyll content of leaf. Du Toit *et al.* (2020) found that the tannin content of moringa leaves lies between 16 to 36 mg/g and the variation on tannin content is due to maturity and severity of harvesting. The value of tannin obtained from this study lies with-in the range. According to González-Romero *et al.* (2020) the chlorophyll of moringa leaves is 244.2 mg/100 g fresh weight. The slight variations may be due to location, season and maturity. The anti-oxidant activity of fresh moringa leaves was found to be 79.58 ± 3.42, while (Jahan *et al.*, 2015) found 75.73 ± 1.10 %RSA. The slight variation may be variation in maturity of leaves and location.

4.2 Effect of temperature on bioactive components of moringa leaf

4.2.1 Effect on Total Polyphenol Content (TPC)

Fresh moringa leaf was found to have a greater total phenolic content i.e.,35.3067 ± 2.53 mg GAE/g than the other samples as shown in the figure 4.1. From the figure it is found that there are significance differences by P<0.005 among the samples i.e., fresh, 45℃, 55℃ and 65℃. The TPC of dried moringa leaf varied from 19.026 to 23.422 mg GAE/g dry matter. The study conducted by Wickramasinghe *et al.* (2020) found similar data of TPC at 65℃ i.e.20.42 mg GAE/g while the finding of this study was 19.026 mg GAE/g. This difference may be due to difference in maturity, location and cultivar (Jahan *et al.*, 2015).

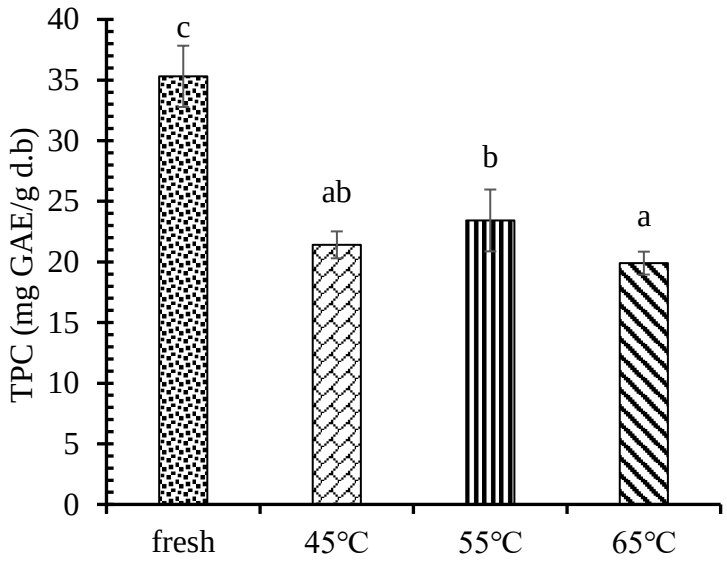


Fig. 4.1 Total Polyphenol Content of Moringa Leaf

Values represent the mean of three determination. Bar sharing the different letter are significantly different by P<0.005.

The figure shows that slight increase in temperature caused less decline in TPC up-to maximum until further increase led to drastic decline in TPC of moringa leaves. Similar result was observed by Pandidurai *et al.* (2022). The initial lower TPC value could be attributed to the activation of the enzyme polyphenol oxidase (PPO) at temperatures ranging from 35 to 45°C, which induces oxidative destruction of polyphenols. The increase in TPC value with moderate temperature increase was attributed to the deactivation of enzyme PPO,

which is thermally unstable and loses activity after 60°C (Prathapan *et al.*, 2009). The dramatic decrease in TPC values at higher temperatures could be attributed to nonenzymatic oxidation of polyphenols. Thermal processing in general is likely to reduce TPC (Wang *et al.*, 2013).

4.2.2 Effect of temperature on TFC (Total Flavonoid Content)

The TFC of fresh moringa leaf was found to be 44.8 ± 4.05708 mg QE/g which is greater in comparison with other dried samples as shown in figure 4.2. The TFC of dried moringa leaf was found between 19.552 to 25.533 mg QE/g. The similar result was found by Wickramasinghe *et al.* (2020) i.e.21.21 ± 0.24 mg QE/g while the finding of this study was 19.552 mg QE/g. The slight difference may be due to maturity, location and different extraction method (Jahan *et al.*, 2015). The figure shows that slight increase in temperature leads to less decrease in TFC up-to maximum at 55°C until further increase in temperature reduces the TFC of moringa leaves. Similar result was observed by (Pandidurai *et al.*, 2022); Siskawardani *et al.* (2021).The figure 4.2 displays that there is significance difference(P<0.005) between the samples.

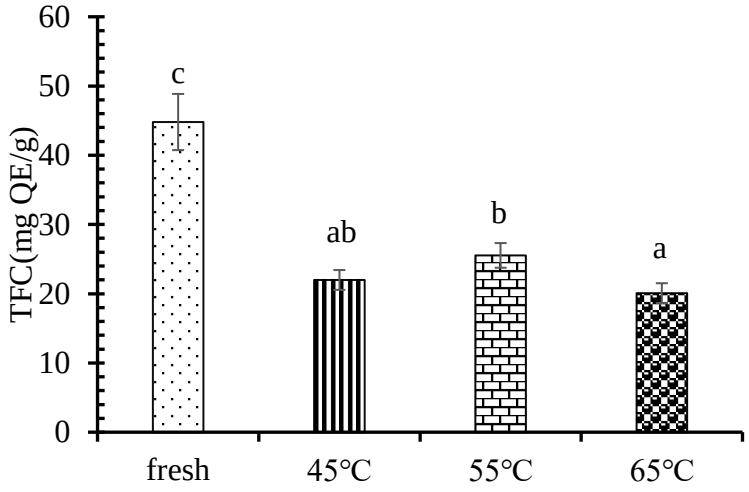


Fig.4.2 Total Flavonoid Content of Moringa Leaf

Values represent the mean of three determination. Bar sharing the different letter are significantly different(P<0.005).

Although drying at low temperatures might protect active components from degradation, prolonged drying time can readily lead to oxidation and thermal damage of flavonoids during the drying process. The retention of TFC was facilitated by appropriately raising the drying temperature, which may be related to the fact that phenylalanine ammonia-lyase (PAL) is a crucial enzyme in the synthesis of flavonoids (Ai *et al.*, 2023). It is found that heat treatment can increase PAL activity and that PAL activity and TFC during drying stress had a positive connection. While the thermal degradation of flavonoids will be accelerated by a greater internal temperature of the material as a result of increased drying temperature(above 55°C) which causes the decrease in TFC at higher temperature (Zhao *et al.*, 2019).

4.2.3 Effect of temperature on chlorophyll

Figure 4.3 shows the total chlorophyll content of fresh and dried moringa leaf. In comparison to dried sample fresh moringa leaf has higher chlorophyll content. The chlorophyll content of fresh moringa leaf was found to be 28.0847 mg/g which is similar to the research conducted by González-Romero *et al.* (2020). The chlorophyll content of dried leaf was found between 2.34 to 7.51 mg/g. Whereas Wickramasinghe *et al.* (2020) found the chlorophyll content of 3.06 mg/g at 55°C and 1.36 mg/g at 65°C. The variation may be due to difference in maturity, cultivars and harvesting season of leaves (Dubey and Kapoor, 2017).

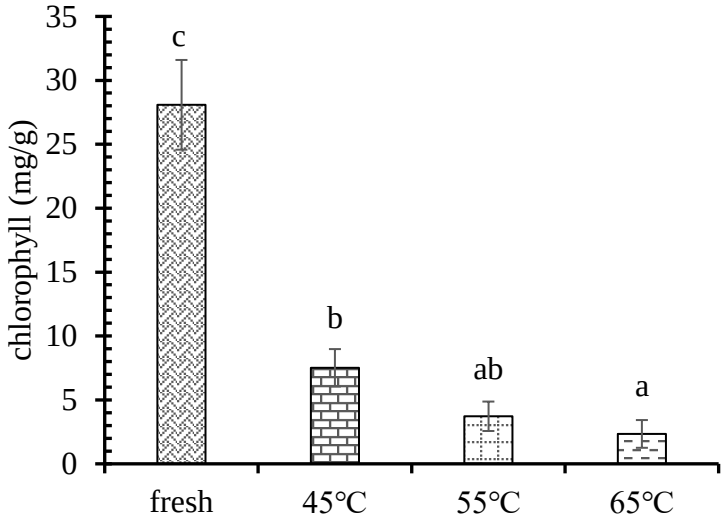


Fig.4.3 Total Chlorophyll Content of Moringa Leaf

Values represent the mean of three determination. Bar sharing the different letter are significantly different(P<0.005).

Figure 4.3 shows that there is significance difference among the samples i.e., fresh, 45℃, 55℃ and 65℃. The chlorophyll content of moringa decreases drastically with increase in temperature. Heat treatment has been determined to have a deleterious impact on chlorophyll. The conversion of chlorophyll to pheophytin is thought to be the cause of the decrease in chlorophyll content (Guan *et al.*, 2005).

4.2.4 Effect of temperature on Tannin

As shown in figure 4.4 the tannin content of the fresh moringa leaves is higher than other dried sample. The tannin content of fresh moringa leaf was found to be 23.2752 ± 3.0825 mg/g dry matter. The tannin content of dried moringa leaf was found between 8.12 to 13.44 mg/g while drying at 45-55℃. While the similar research conducted by Yadav (2018) found the tannin content of 9.810 mg/g at 50℃. The difference in result may be due to different extraction method, maturity of leaf, cultivar and different drying temperature (Du Toit *et al.*, 2020).

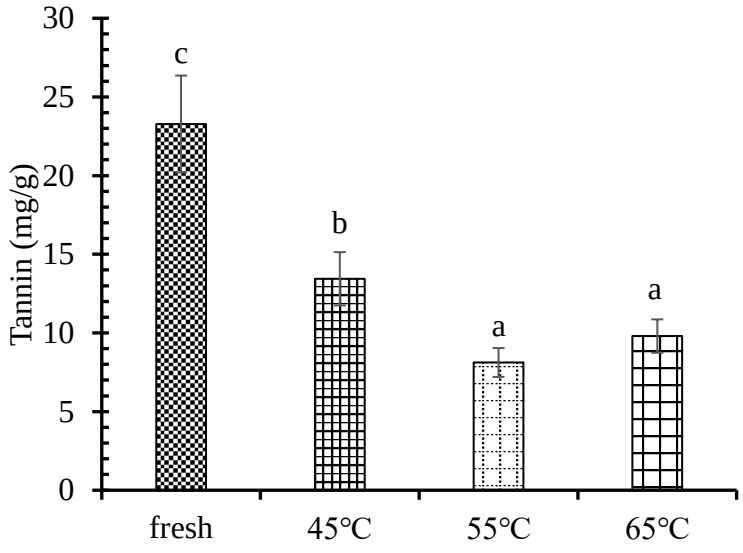


Fig 4.4 Tannin content of Moringa leaf

Values represent the mean of three determination. Bar sharing the different letter are significantly different(P<0.005).

Figure 4.4 shows that with the increase in drying temperature the tannin content decreases. However, after passing 55℃ temperature the tannin content tends to rise again. The decrease in tannin content may be due to the thermal degradation and condensation of tannin at higher temperature. The tannin content may also decrease due to the reduction in tannin extractability with increasing drying temperature (Hove *et al.*, 2003), while the increase in tannin after 55℃ temperature may be due to inactivation of catechol oxidase enzyme at higher temperature (Wahyuni *et al.*, 2020). Similar result was observed by Nguyen *et al.* (2021).

4.2.5 Effect of temperature on Anti-oxidant Activity

The anti-oxidant activity of fresh moringa leaves was found to be 79.579 ± 3.4177 %RSA as shown in table 4.5. While the study conducted by Jahan *et al.* (2015) found 75.73 ± 1.10 %RSA. The slight variation may be due to different maturity and location. From the figure it is clear that the anti-oxidant activity of fresh moringa leaf is greater than other sample.

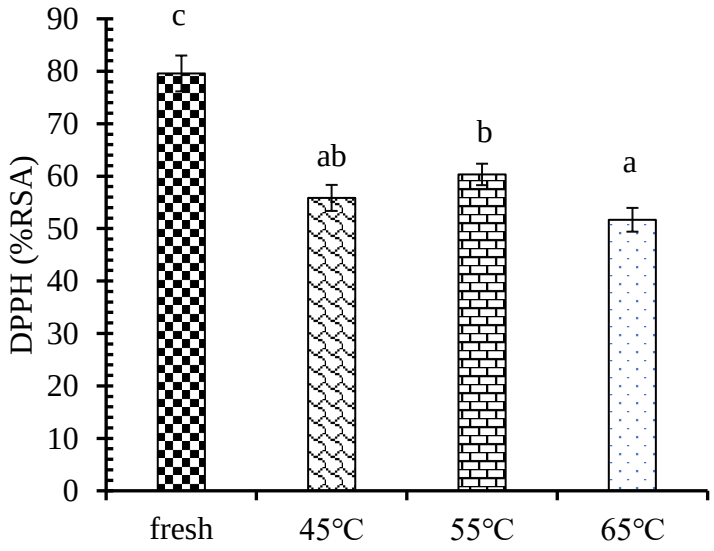


Fig.4.5 Anti-oxidant Activity of Moringa Leaf

Values represent the mean of three determination. Bar sharing the different letter are significantly different($P<0.005$).

The anti-oxidant activity of dried moringa leaves was found between 51.677 to 60.325 %RSA. While Abdulkadir *et al.* (2015) found 58.62 ± 1.13 %RSA at 45℃. The variation may be due to maturity of moringa leaves and location (Abdulkadir *et al.*, 2015).

As the figure 4.5 shows, the slight increase in temperature increases the anti-oxidant activity after that the rise in temperature further decrease the anti-oxidant activity of dried moringa leaves. The occurrence of antioxidant substances or phenolic compounds by thermal reactions, such as non-enzymatic browning reactions, or due to phenolic compounds from other thermal reactions, such as the thermal degradation of insoluble and bound phenolic compounds, may be responsible for an increase in antioxidant activity (Wangcharoen and Gomolmanee, 2013). While the antioxidant value was found to decrease with the increasing temperature above 55℃, which maybe because of the destruction of the chemical composition of the polyphenolic compounds, ascorbic acid and chlorophyll content of the Moringa leaf (Hossain *et al.*, 2020).

4.3 Sensory analysis of dried leaf

Three sample of moringa leaves dried at temperature 45℃, 55℃ and 65℃ were prepared as sample A, B and C respectively. The sensory score collected from ten semi-trained panelists was statistically analyzed using a 9-point hedonic rating test (9=like extremely, 1=dislike extremely). The sample was prepared as tea infusion. Color, Taste, Smell, Mouthfeel, and Overall Acceptability were used to evaluate the samples as shown in fig 4.6.

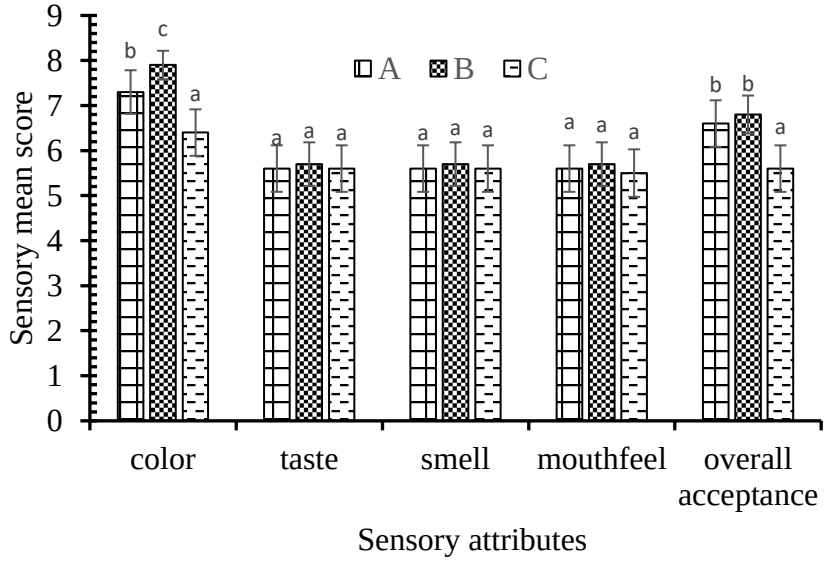


Fig 4.6 Mean sensory evaluation of moringa leaf dried at different temperature

From the sensory evaluation for color of three different sample A, B and C of moringa dried at temperature 45℃, 55℃ and 65℃ mean sensory score was found to be 7.3, 7.9 and 6.4 respectively. Statistical analysis showed significant difference ($p<0.05$) of variation of

drying temperature of moringa leaf in color at 5% level of significance. Sample B was found superior in color.

The mean sensory score for taste and smell of three sample A, B and C was found to be 5.6, 5.7 and 5.6 respectively. While the mean sensory score for mouthfeel of sample A, B and C was found to be 5.6, 5.7 and 5.5 respectively. Statistical analysis showed that sample were not significantly different in taste, smell and mouthfeel on variation of drying temperature.

From the sensory evaluation of overall acceptability of sample, A, B and C mean sensory score was found 6.6, 6.8 and 5.6 respectively. The overall acceptability of sample was significantly different ($p<0.05$). LSD showed that sample A and C, B and C are significantly different while sample A and B are not significantly different from each other at 5%level of significance. sample B was found superior on the basis of overall acceptability from statistical analysis. Overall acceptability is the reflection of other sensory attributes. On the basis of color, flavor, taste, mouthfeel sample B was most liked by panelists. Statistical analysis showed higher acceptability for sample B.

4.4 Storage Stability

4.4.1 Effect on moisture content during storage

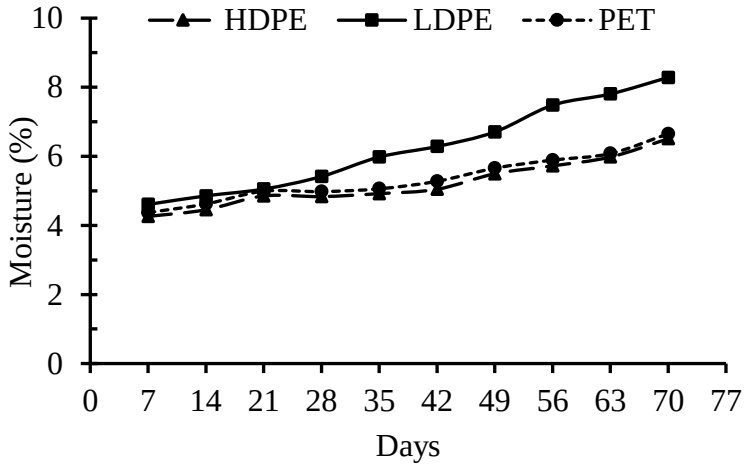


Fig.4.7 Effect of packaging materials on moisture during storage of Moringa Leaf Powder

The figure.4.6 shows the data on the moisture content of moringa leaf powder after storage as affected by different packaging materials. The various packing materials used where PET bottle with PP cap, HDPE and LDPE pouches at room temperature and product where regularly evaluated at interval of 7 days. From figure it can be concluded that there were significant changes in the moisture content of moringa leaf powder packed in LDPE, followed by PET bottle with PP cap and HDPE.

The minimum moisture content was recorded in moringa leaf powder packed in HDPE from initial day after storage (4.1%) to final day of storage (6.50%) and the maximum moisture content was recorded in moringa leaf powder packed in LDPE poly bags from initial day after storage (4.1%) to final day of storage (8.28%). The maximum increase in moisture content was found in LDPE 200gauge polybags due to poor oxygen barrier properties followed by PET bottle with PP cap. Gnana *et al.* (2021); (Seevaratnam *et al.*, 2012) found least increase in moisture content in PET bottle. This was due to the differential permeability of packaging materials to water vapor. However the figure 4.6 shows least increase of moisture content in HDPE pouches which may be due to use of PET bottle with PP cap.

4.4.2 Effect of packaging material on Total Plate Count

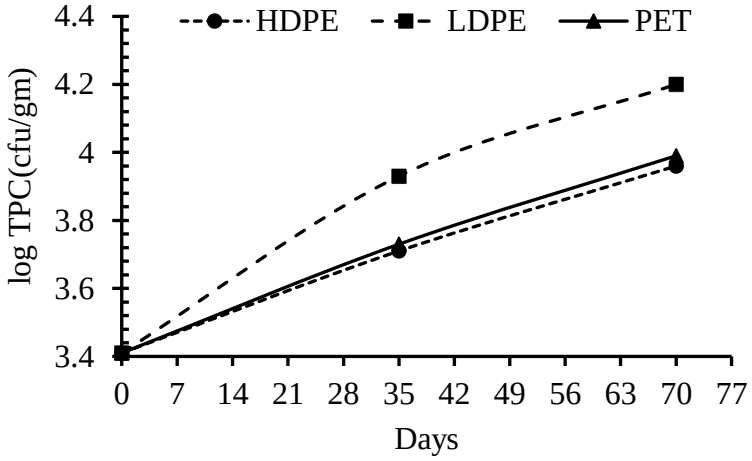


Fig.4.8 Effect of packaging material on Total Plate Count

In microbial analysis Total Plate Count was performed and the changes in microbial count during storage is shown in fig 4.8. Lowest microbial growth was found in moringa leaf

powder stored in HDPE followed by PET bottle with PP cap and highest microbial count was found in LDPE pouch as shown in figure. The total plate count of moringa powder packed in different packaging materials was found between 2.57×10^3 and 15.89×10^3 which lies within the limit($<10^4$) (FDA, 2013).

4.4.3 Sensory Analysis

The moringa leaf powder stored in packaging material PET with PP cap, HDPE and LDPE were prepared as sample D, E and F respectively. The sensory score collected from ten semi-trained panelists was statistically analyzed using a 9-point hedonic rating test (9=like extremely, 1=dislike extremely). The sample was prepared as a tea infusion. Color, Taste, Smell, Mouthfeel, and Overall Acceptability were used to evaluate the samples as shown in fig 4.9.

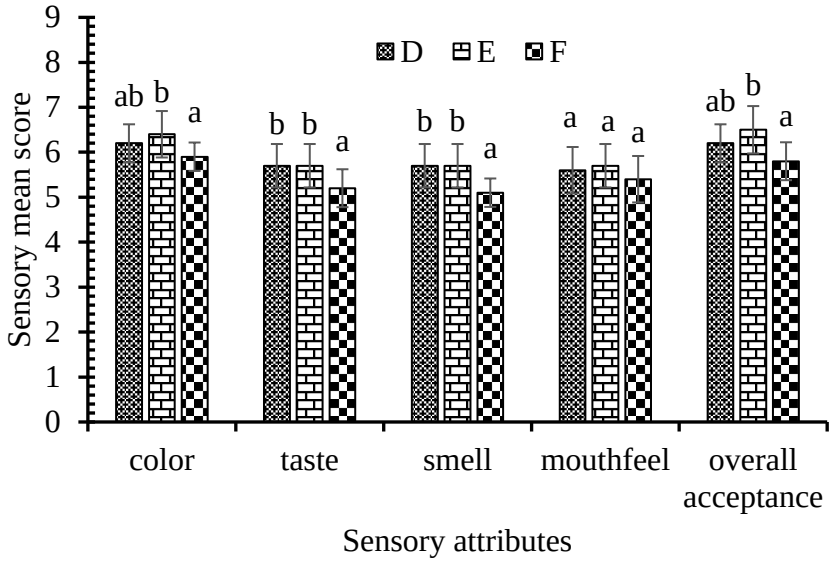


Fig 4.9 Mean sensory evaluation of moringa leaf powder stored in different packaging materials

From the sensory evaluation for color of three different sample D, E and F of moringa leaf powder stored in PET, HDPE and LDPE mean sensory score was found to be 6.2, 6.4 and 5.9 respectively. Statistical analysis showed significant difference ($p<0.05$) of variation of packaging materials on moringa leaf powder in color at 5% level of significance. Sample E was found superior in color.

The mean sensory score for taste of three sample D, E and F was found to be 5.7, 5.7 and 5.2 respectively and the mean sensory score for smell of sample D, E and F was found to be 5.7, 5.7 and 5.4 respectively. Statistical analysis showed that samples D and E were not significantly different in taste and smell on variation of packaging materials while the sample D and F, E and F were significantly different.

The mean sensory score for mouthfeel for sample D, E and F was found to be 5.6, 5.7 and 5.4 respectively. Statistical analysis showed that the sample were not significantly different in mouthfeel on variation of packaging materials.

From the sensory evaluation of overall acceptability of samples D, E and F mean sensory score was found 6.2, 6.5 and 5.8 respectively. LSD showed that sample E and F are significantly different while other sample are not significantly different from each other at 5%level of significance. sample E was found superior on the basis of overall acceptability from statistical analysis. On the basis of color, flavor, taste, mouthfeel sample E was most liked by panelists. Statically analysis showed higher acceptability for sample E.

4.5 Optimum drying temperature for bioactive components

From one way ANOVA analysis within the level of confidence ($P<0.05$) of above data of different physiochemical properties and sensory analysis at different drying temperatures, the optimum temperature for moringa leaf was found to be 55℃. Figures 4.1, 4.2, 4.3, 4.4, 4.5, and 4.6 indicate that the values of parameters at 55°C are higher than at other temperatures.

Part V

Conclusion and recommendation

5.1 Conclusion

Based on the result of present study following conclusion can be drawn:

1. Proximate analysis showed that moringa leaves are nutritious in term of protein, fiber, fat, ash, carbohydrate and rich in bioactive components.
2. Drying at temperature 55℃ is superior in term bioactive components and sensory analysis. Total phenolic content, flavonoids, anti-oxidant activity were maximum while chlorophyll and tannin content decreased at 55℃.
3. The best packaging material considering moisture, total plate count and sensory analysis was found to be HDPE followed by PET bottle with PP cap.

5.2 Recommendation

1. Drying of moringa leaves can be performed at 55℃ with minimum loss of bioactive components and can be commercially produced.
2. More research can be conducted on effect of storage conditions on bioactive components and evaluation of the possible application of moringa leaf powder dried at various temperatures in various food products.

Part VI

Summary

M. oleifera Lam., known as drumstick in English, is one of 13 species belonging to the genus Moringa. It is widely farmed throughout tropical and subtropical regions of the world and comes originally from sub-Himalayan plains of Northern India. Moringa oleifera is a multipurpose and exceptionally nutritious vegetable tree with a variety of potential uses. The presence of numerous antioxidant chemicals makes moringa leaves a rich source of natural antioxidants as well as a good source of nutraceuticals and functional components.

The proximate composition of fresh moringa leaves was found to be 86.896% moisture with the value of crude protein, crude fat, crude fiber, total ash and carbohydrate to be 19.923%, 7.043%, 9.713%, 8.51% and 55.574% on dry weight basis respectively. The bioactive components of fresh and dried moringa leaves at temperature 45°C, 55°C and 65°C were analyzed during the analysis. The bioactive components including flavonoid content, polyphenol content, tannin content, chlorophyll and antioxidant activity of fresh and dried moringa leaves were analyzed in dry basis. According to the analysis, drying at 55°C results in less substantial loss of bioactive components than drying at other temperatures. It was found that slight rise in temperature leads to increase in bioactive components except for tannin and chlorophyll up-to maximum until further increase in temperature results in decline in bioactive components. However, rise in temperature was accompanied with a fall in concentration of chlorophyll content. The moringa leaves powder dried at optimum temperature i.e.,55°C was stored at room temperature in HDPE, LPDE bag and PET bottle with PP cap. The sample packaged in HDPE has the lowest moisture and total plate count followed by PET bottle with PP cap.

According to the sensory analysis the leaves dried at 55°C has the best appearance while other parameter doesn't differ significantly with the study temperature. The study of all the sample revealed that the sample dried at 55°C is best for retention of bioactive components and HDPE bag is superior packaging material in term of moisture, total plate count and sensory analysis.

References

Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, T., Bashir, M. S., Ishtiaq, A., Hussain, S. and Suleria, H. A. R. (2017). Natural polyphenols: An overview. *Int. J. Food Prop.* **20** (8), 1689-1699. doi: https://doi.org/10.1080/10942912.2016.1220393.

Abdulkadir, A. R., Zawawi, D. D. and Jahan, M. S. (2015). DPPH antioxidant activity, total phenolic and total flavonoid content of different part of Drumstic tree (Moringa oleifera Lam.). *J. Chem. Pharm. Res.* **7** (4), 1423-1428.

Adejumo, B. A. and Dan, E. J. (2018). Nutritional composition of packaged Moringa oleifera leaves powder in storage. *Ann. Food Sci. Technol.* **19** (2), 225-231.

Ai, Z., Xiao, H., Zhang, Y., Lei, D., Peng, Z., Li, M. and Liu, Y. (2023). Effect of hot air impingement drying on drying behavior, volatile components profile, shell burst ratio, flavonoid contents, microstructure of Amomum villosum fruits. *Dry. Technol.* **41** (1), 107-121.

Alakali, J. S., Kucha, C. T. and Rabiu, I. A. (2015). Effect of drying temperature on the nutritional quality of Moringa oleifera leaves. *Afr. J. Food Sci.* **9** (7), 395-399.

Ali, M. A., Yusof, Y. A., Chin, N. L. and Ibrahim, M. N. (2017). Processing of Moringa leaves as natural source of nutrients by optimization of drying and grinding mechanism. *J. Food Process Eng.* **40** (6), e12583.

Alter, H. (1962). A critical investigation of polyethylene gas permeability. *J. Polym. Sci.* **57** (165), 925-935.

AOAC. (2005). "Official Methods of Analysis of the Association of Official Analytical Chemists" (18th ed. ed.). Association of Official Analytical Chemists. Arlington, Virginia, USA.

Arendse, W. and Jideani, V. (2022). Storage stability and consumer acceptability of dried apple: Impact of citric acid, potassium sorbate and moringa oleifera leaf extract powder. *Foods*. **11** (7), 984. doi: https://doi.org/10.3390/foods11070984.

Arise, A. K., Arise, R. O., Sanusi, M. O., Esan, O. T. and Oyeyinka, S. A. (2014). Effect of Moringa oleifera flower fortification on the nutritional quality and sensory properties of weaning food. *Croat. J. Food Sci. Technol.* **6** (2), 65-71.

Atawodi, S. E., Atawodi, J. C., Idakwo, G. A., Pfundstein, B., Haubner, R., Wurtele, G., Bartsch, H. and Owen, R. W. (2010). Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of Moringa oleifera lam. *J. Med. Food*. doi:10.1089/jmf.2009.0057.

Bernhoft, A. (2010). A brief review on bioactive compounds in plants. *In:* "Bioactive Compounds in Plants-Benefits and Risks for Man and Animals" (Vol. 50).). pp. 11-17. Oslo, Norway. The Norwegian Academy of Science and Letters. [ISBN 978-82-7099-583-7].

Boonchum, W., Peerapornpisal, Y., Kanjanapothi, D., Pekkoh, J., Pumas, C., Jamjai, U., Amornlerdpison, D., Noiraksar, T. and Vacharapiyasophon, P. (2011). Antioxidant activity of some seaweed from the Gulf of Thailand. *Int. J. Agric. Biol.* **13** (1).

Bowers, W. F. (1947). Chlorophyll in wound healing and suppurative disease. *Am. J. Surg.* **73** (1), 37-50.

Brewer, M. S. (2011). Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Compr. Rev. Food Sci. Food Saf.* **10** (4), 221-247. doi: https://doi.org/10.1111/j.1541-4337.2011.00156.x.

Ciju, R. J. (2019). "Moringa: The Drumstick Tree". Agrihortico

D Archivio, M., Filesi, C., Di Benedetto, R., Gargiulo, R., Giovannini, C. and Masella, R. (2007). Polyphenols, dietary sources and bioavailability. *Ann. Ist. Super. Sanita*. **43** (4), 348.

Dalla Nora, C., Müller, C. D., de Bona, G. S., de Oliveira Rios, A., Hertz, P. F., Jablonski, A., de Jong, E. V. and Flôres, S. H. (2014). Effect of processing on the stability of bioactive compounds from red guava (Psidium cattleyanum Sabine) and guabiju (Myrcianthes pungens). *J. Food Compos. Anal.* **34** (1), 18-25. doi: https://doi.org/10.1016/j.jfca.2014.01.006.

Dhakar, R. C., Maurya, S. D., Pooniya, B. K., Bairwa, N. K., Gupta, M. and Sanwarmal. (2011). Moringa: The herbal gold to combat malnutrition. *Chron. Young Sci.* **2** (3), 119-126.

Doerr, B. and Cameron, L. (2005). "Moringa Leaf Powder". ECHO. USA.

Doughari, J. H. (2012). "Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents". INTECH Open Access Publisher Rijeka, Croatia. [ISBN 9535102966].

Du Toit, E. S., Sithole, J. and Vorster, J. (2020). Leaf harvesting severity affects total phenolic and tannin content of fresh and dry leaves of Moringa oleifera Lam. trees growing in Gauteng, South Africa. *S. Afr. J. Bot.* **129**, 336-340. doi: https://doi.org/10.1016/j.sajb.2019.08.035.

Dubey, S. U. and Kapoor, M. k. (2017). Monthly variation and impact of pollution on the nutritional value of Moringa oleifera (Lam.) leaves. *Int. J. Bioassays*. **6** (10), 5535-5542. doi: http://dx.doi.org/10.21746/ijbio.2017.10.5.

Egwui, P. C., Mgbenka, B. O. and Ezeonyejiaku, C. D. (2013). Moringa plant and it use as feed in aquaculture development: a review. *Anim. Res. Int.* **10**, 1672-1680.

Emelike, N. J. T. and Ebere, C. O. (2016). Effect of drying techniques of moringa leaf on the quality of Chin-Chin enriched with Moringa leaf powder. *J. Environ. Sci., Toxicol. Food Technol.* **10** (4), 65-70. doi: 10.9790/2402-1004016570.

Emelike, N. J. T., Uwa, F. O., Ebere, C. O. and Kiin-Kabari, D. B. (2015). Effect of Drying Methods on the Physico-Chemical and Sensory Properties of Cookies Fortified with Moringa (Moringaolelfera) Leaves. *Asain J. Agric. Food Sci.* **3** (4).

FDA. (2013). Revised Guidelines for the Assessment of Microbiological Quality of Processed Foods [Circular No. 2013-010]. Retrieved from https://members.wto.org/crnattachments/2021/TBT/PHL/21_3930_00_e.pdf. [Accessed 31 July, 2023].

Ferruzzi, M. G. and Blakeslee, J. (2007). Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutr. Res.* **27** (1), 1-12.

Fuglie, L. J. (1999). "The Miracle Tree: Moringa oleifera, Natural Nutrition for the Tropics". Church wood service.

Gnana, D. G., Sudha Vani, V., Vinayakumar Reddy, P., Viji, C. P. and Sujatha, R. V. (2021). Influence of different drying methods and packaging material on the quality of moringa leaf powder. *Phram. Innov. J.*

Godinez-Oviedo, A., Guemes-Vera, N. and Acevedo-Sandoval, O. A. (2016). Nutritional and phytochemical composition of Moringa oleifera lam and its potential use as nutraceutical plant: A review. *Pak. J. Nutr.* **15** (4), 397-405.

González-Romero, J., Arranz-Arranz, S., Verardo, V., García-Villanova, B. and Guerra-Hernández, E. (2020). Bioactive compounds and antioxidant capacity of moringa leaves grown in Spain versus 28 leaves commonly consumed in pre-packaged salads. *Processes*. **8** (10), 1297. doi: https://doi.org/10.3390/pr8101297.

Gopalakrishnan, L., Doriya, K. and Kumar, D. S. (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. *Food Sci. Hum. Wellness*. **5** (2), 49-56. doi: https://doi.org/10.1016/j.fshw.2016.04.001.

Guan, T. Y., Cenkowski, S. and Hydamaka, A. (2005). Effect of drying on the nutraceutical quality of sea buckthorn (Hippophae rhamnoides L. ssp. sinensis) leaves. *J. Food Sci.* **70** (9), E514-E518. doi: https://doi.org/10.1111/j.1365-2621.2005.tb08312.x.

Gümüşay, Ö. A., Borazan, A. A., Ercal, N. and Demirkol, O. (2015). Drying effects on the antioxidant properties of tomatoes and ginger. *Food Chem.* **173**, 156-162.

Gustavsson, J., Cederberg, C., Sonesson, U. L. F., Van Otterdijk, R. and Meybeck, A. (2011). Global food losses and food waste: FAO Rome.

Haile, M. and Kang, W. H. (2019). Antioxidant activity, total polyphenol, flavonoid and tannin contents of fermented green coffee beans with selected yeasts. *Fermentation*. **5** (1), 29. doi: https://doi.org/10.3390/fermentation5010029.

Halliwell, B. and Gutteridge, J. M. (1995). The definition and measurement of antioxidants in biological systems. *Free Radic. Biol. Med.* **18** (1), 125-126.

Hamza, T. A. and Azmach, N. N. (2017). The miraculous moringa trees: From nutritional and medicinal point of views in tropical regions. *J. Med. Plants Stud.* **5** (4), 151-162.

Hasaballa, M. A., Elsohaimy, S. A., Shaltout, O. E. and Zeitoun, M. A. M. (2017). Chemical composition and bioactive compounds of leaves, flowers and seeds of moringa plant. *J. Adv. Agric. Res.* **22** (2), 662-674.

Hawa, A., Satheesh, N. and Kumela, D. (2018). Nutritional and anti-nutritional evaluation of cookies prepared from okara, red teff and wheat flours. *Int. Food Res. J.* **25** (5), 2042-2050.

Hossain, M. A., Disha, N. K., Shourove, J. H. and Dey, P. (2020). Determination of antioxidant activity and total tannin from drumstick (Moringa oleifera lam.) leaves using different solvent extraction methods. *Turk. J. Agric.-Food Sci. Technol.* **8** (12), 2749-2755.

Hove, L., Ndlovu, L. R. and Sibanda, S. (2003). The effects of drying temperature on chemical composition and nutritive value of some tropical fodder shrubs. *Agrofor. Syst.* **59**, 231-241. doi: https://doi.org/10.1023/B:AGFO.0000005224.64208.42.

Humphrey, A. M. (1980). Chlorophyll. *Food Chem.* **5** (1), 57-67.

Indriasari, Y. and Kumalaningsih, S. (2016). Effect of blanching on saponins and nutritional content of moringa leaves extract. *J. Food Res.* **5** (3), 55-60. doi: 10.5539/jfr.v5n3p55.

Jahan, M. S., Zawawi, D. D. and Abdulkadir, A. R. (2015). Effect of chlorophyll content and maturity on total phenolic, total flavonoid contents and antioxidant activity of Moringa oleifera leaf (Miracle tree). *J. Chem. Pharm. Res.* **7** (5), 1147-1152.

Kamiloglu, S. and Capanoglu, E. (2015). Polyphenol content in figs (Ficus carica L.): Effect of sun-drying. *Int. J. Food Prop.* **18** (3), 521-535. doi: http://doi.org/10.1080/10942912.2013.833522.

Kamiloglu, S., Toydemir, G., Boyacioglu, D., Beekwilder, J., Hall, R. D. and Capanoglu, E. (2016). A review on the effect of drying on antioxidant potential of fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* **56** (sup1), S110-S129. doi: 10.1080/10408398.2015.1045969.

Kannan, K. and Thahaaseen, A. (2016). Process optimization for drying of drumstick leaves. *Indian J. Sci.*, 275-288.

Kar, S., Mukherjee, A., Ghosh, M. and Bhattacharyya, D. K. (2013). Utilization of Moringa leaves as valuable food ingredient in biscuit preparation. *Int. J. Appl. Sci. Eng.* **1** (1), 29-37.

Karim, O., Kayode, R., Oyeyinka, S. and Oyeyinka, A. (2015). Physicochemical properties of stiff dough 'amala'prepared from plantain (Musa Paradisca) flour and Moringa (Moringa oleifera) leaf powder. *Foods Health Dis.* **4** (1), 48-58.

KC, J. B. and Rai, B. K. (2007). "Basic Food Analysis Handbook" (1st ed. ed.). Prompt printers pvt.Ltd. Kathmandu, Nepal.

KC, Y., Bhattarai, S., Shiwakoti, L. D., Paudel, S., Subedi, M., Pant, B. R., Paudel, M., Dhugana, S., Bhattarai, S. and Tiwari, T. (2022). Sensorial and chemical analysis of biscuits prepared by incorporating Moringa flower powder and leaf powder. *Int. J. Food Prop.* **25** (1), 894-906.

Kim, Y. T., Min, B. and Kim, K. W. (2014). General characteristics of packaging materials for food system. *In:* "Innovations in Food Packaging".). pp. 13-35. Elsevier.

Kodama, H., Nakaya, M., Shirakura, A., Hotta, A., Hasebe, T. and Suzuki, T. (2006). Synthesis of practical high-gas-barrier carbon films at low and atmospheric pressure for PET bottles. *New. Diam. Front. Carbon Technol.* **16** (2), 107-119.

Koyuncu, T., Tosun, I. and Pınar, Y. (2007). Drying characteristics and heat energy requirement of cornelian cherry fruits (Cornus mas L.). *J. Food Eng.* **78** (2), 735-739.

Kumar, C., Karim, M. A. and Joardder, M. U. H. (2014). Intermittent drying of food products: A critical review. *J. Food Eng.* **121**, 48-57. doi: https://doi.org/10.1016/j.jfoodeng.2013.08.014.

Kumar, S. and Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *Sci. World J.* **2013**.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J. and Bertoli, S. (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera leaves: An overview. *Int. J. Mol. Sci.* **16** (6), 12791-12835. doi: https://doi.org/10.3390/ijms160612791.

Madukwe, E. U., Ezeugwu, J. O. and Eme, P. E. (2013). Nutrient composition and sensory evaluation of dry Moringa oleifera aqueous extract. *Int. J. Basic Appl. Sci.* **13**, 100-102.

Makkar, H. P. S. and Becker, K. (1996). Nutrional value and antinutritional components of whole and ethanol extracted Moringa oleifera leaves. *Anim. Feed Sci. Technol.* **63** (1-4), 211-228. doi: https://doi.org/10.1016/S0377-8401(96)01023-1.

Mallenakuppe, R., Homabalegowda, H., Gouri, M. D., Basavaraju, P. S. and Chandrashekharaiah, U. B. (2019). History, Taxonomy and Propagation of Moringa oleifera-A Review. *SSR Inst. Int. J. Life Sci*. **5** (3), 2322-2324. doi: 10.21276/SSR-IIJLS.2019.5.3.7.

Marsh, K. and Bugusu, B. (2007a). Food packaging-roles, materials, and environmental issues. *J. Food Sci.* **72** (3), R39-R55.

Marsh, K. and Bugusu, B. (2007b). Food packaging—roles, materials, and environmental issues. *J. Food Sci.* **72** (3), R39-R55.

martin, L. P. (1985). "The Moringa Tree". ECHO.

Mbah, B. O., Eme, P. E. and Paul, A. E. (2012). Effect of drying techniques on the proximate and other nutrient composition of Moringa oleifera leaves from two areas in Eastern Nigeria. *Pak. J. Nutr.* **11** (11), 1044.

Mensah, J. K., Ikhajiagbe, B., Edema, N. E. and Emokhor, J. (2012). Phytochemical, nutritional and antibacterial properties of dried leaf powder of Moringa oleifera (Lam) from Edo Central Province, Nigeria. *J. Nat. Prod. Plant Resour.* . **2** (1), 107-112.

Mishra, S. p., Singh, P. and Singh, p. (2012). Processing of Moringa oleifera leaves for human consumption. *Bull. Environ. Pharmacol. Life Sci.* **2** (1), 28-31.

Moon, J. and Shibamoto, T. (2009). Antioxidant assays for plant and food components. *J. Agric. Food Chem.* **57** (5), 1655-1666.

Mouminah, H. H. S. (2015). Effect of dried Moringa oleifera leaves on the nutritional and organoleptic characteristics of cookies. *Alex. Sci. Exch. J.* **36**, 297-302.

Mutiara, K. T., Estiasih, T. and Sriwahyuni, E. (2012). Nutrient content of kelor (Moringa oleifera Lamk) leaves powder under different blanching methods. *Food Public Health*. **2** (6), 296-300.

Mutiara, T. K., Estiasih, T. and Sriwahyuni, E. (2013). Effect of blanching treatments against protein content and amino acid drumstick leaves (Moringa oleifera). *J. Food Res.* **2** (1), 101.

Nguyen, P. T. N., Nguyen, V. H., Bach, L. G., Pham, T. N., Dao, T. P., Nguyen, D. V., Nguyen, V. M., Do, V. Q., Tran, T. Y. N. and Tran, T. T. (2021). Effects of tannin, ascorbic acid, and total phenolic contents of cashew (Anacardium occidentale L.) apples blanched with saline solution. *Food Res.* **5**, 409-416. doi: https://doi.org/10.26656/fr.2017.5(1).454.

Nweze, N. O. and Nwafor, F. I. (2014). Phytochemical, proximate and mineral composition of leaf extracts of Moringa oleifera Lam. from Nsukka, South-Eastern Nigeria. *J. Pharm. Biol. Sci.* **9** (1), 99-103.

Olabode, Z., Akanbi, C. T., Olunlade, B. and Adeola, A. A. (2015). Effects of drying temperature on the nutrients of Moringa (Moringa oleifera) leaves and sensory attributes of dried leaves infusion. *Direct Res. J. Agric. Food Sci.* **3** (5), 177-122.

Omobolanle, o., Adefunke, B. and Oluwatoyin, R. (2015). Effect of blanching and packaging material on the colour and microbial load of ben oil (Moringa oleifera) leaf powder (pp. 311-320).

Pandey, K. B. and Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev.* **2**, 270-278. doi: https://doi.org/10.4161/oxim.2.5.9498.

Pandidurai, G., Amutha, S., Kanchana, S., Vellaikumar, S. and Prabhakaran, K. (2022). Optimization of Heat pump-assisted dehumidified air drying (HPD) temperature on

phytochemicals, drying characteristics and nutrient compound retention of drumstick by GC-MS and principle component analysis. *J. Appl. Nat. Sci.* **14** (SI), 119-128.

Potisate, Y., Kerr, W. L. and Phoungchandang, S. (2015). Changes during storage of dried Moringa oleifera leaves prepared by heat pump-assisted dehumidified air drying. *Int. J. Food Sci. Technol.* **50** (5), 1224-1233.

Potisate, Y., Phoungchandang, S. and Kerr, W. L. (2014). The effects of predrying treatments and different drying methods on phytochemical compound retention and drying characteristics of Moringa leaves (Moringa oleifera Lam.). *Dry. Technol.* **32** (16), 1970-1985.

Prathapan, A., Lukhman, M., Arumughan, C., Sundaresan, A. and Raghu, K. G. (2009). Effect of heat treatment on curcuminoid, colour value and total polyphenols of fresh turmeric rhizome. *Int. J. Food Sci. Technol.* **44** (7), 1438-1444.

Qi, N., Gong, X., Feng, C., Wang, X., Xu, Y. and Lin, L. (2016). Simultaneous analysis of eight vitamin E isomers in Moringa oleifera lam. Leaves by ultra performance convergence chromatography. *Food Chem.* **207**, 157-161. doi: https://doi.org/10.1016/j.foodchem.2016.03.089.

Rajput, H., Prasad, S. G. M., Srivastav, P., Singh, N., Suraj, L. and Chandra, R. (2017). Chemical and phytochemical properties of fresh and dried Moringa oliferiea (PKM-1) leaf powder. *Chem. Sci. Rev. Lett.* **6**, 1004-1009.

Rangana, S. (1986). "Handbook of Analysis and Quality Control for Fruits and Vegetable Products" (second ed. ed.). Tata McGraw-Hill Education, 1986. [ISBN 978-0-074-5185-9].

Robertson, G. L. (2016). Packaging and food and beverage shelf life. *In:* "The stability and shelf life of food". (n. ed., Ed.). pp. 77-106. Woodhead Publishing

Rockwood, J. L., Anderson, B. G. and asamatta, D. A. (2013). Potential uses of *moringa oleifera* and an examination of antibiotic efficacy conferred by m. Oleifera seed and leaf extracts using crude extraction techniques available to underserved indigenous populations *IntL. J. Phytother. Res.* **3** (2), 61-71.

Sadasivam, S. and Manickam, A. (1996). Peroxidase. *Methods Biochem.*, 108-110.

Samatha, T., Shyamsundaracharya, R., Srinivas, P. and Swamy, N. R. (2012). Quantification of total phenolic and total flavonoid contents in extracts of Oroxylum indicum L. Kurz. *Asain J. Pharm. Clin. Res.* **5** (4), 177-179.

Scalbert, A., Manach, C., Morand, C., Rémésy, C. and Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sci. Nutr.* **45** (4), 287-306.

Seevaratnam, V., Banumathi, P., Premalatha, M. R., Sundaram, S. P. and Arumugam, T. (2012). Effect of packaging materials on retention of quality characteristics of selected dehydrated green leafy vegetables during storage. *World J. Dairy Food Sci.* **7** (2), 190-194.

Siddhuraju, P. and Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. *J. Agric. Food Chem.* **51** (8), 2144-2155.

Singh, T. K. and Cadwallader, K. R. (2004). Ways of measuring shelf-life and spoilage. *In:* "Understanding and Measuring the Self-Life of Food".). pp. 165-183. Woodhead Publishing Limited.

Singh, Y. and Prasad, K. (2013). Moringa oleifera leaf as functional food powder. *Int. J. Agric. Food Sci. Technol.* **4**, 317-324.

Siskawardani, D. D., Winarsih, S. and Khawwee, K. (2021). The antioxidant activity of kelor (Moringa oleifera lam.) leaves based on drying method. *Jordan J. Biol. Sci.* **14** (2), 291-295.

Stuart, W. (1930). "Potato storage and storage houses". US Government Printing Office.

Subadra, S., Monica, J. and Dhabhai, D. (1997). Retention and storage stability of beta-carotene in dehydrated drumstick leaves (Moringa oleifera). *Int. J. Food Sci. Nutr.* **48** (6), 373-379.

Sultana, S. (2020). Nutritional and functional properties of Moringa oleifera. *Metab. Open*. **8**, 100061. doi: https://doi.org/10.1016%2Fj.metop.2020.100061.

Tetteh, O. N. A. (2009). Effects of blanching and dehydration methods on the quality of Moringa leaf powder used as herbal green tea. M. Tech. Thesis. Kwame Nkrumah Univ. of Science and Technology,

Thakur, S. B. and Bajagain, A. (2020). Moringa: alternative for the food security, climate resilience and livelihood improvement in Nepal. *Intl. J. Res. Granthaalayah*. **8** (3), 190-200. doi: https://doi.org/10.5281/zenodo.3734215.

Tsaknis, J., Lalas, S., Gergis, V., Dourtoglou, V. and Spiliotis, V. (1999). Characterization of Moringa oleifera variety Mbololo seed oil of Kenya. *J. Agric. Food Chem.* **47** (11), 4495-4499.

Vergara-Jimenez, M., Almatrafi, M. M. and Fernandez, M. L. (2017). Bioactive components in Moringa oleifera leaves protect against chronic disease. *Antioxidants*. **6** (4), 91. doi: https://doi.org/10.3390/antiox6040091.

Vidinamo, F., Fawzia, S. and Karim, M. A. (2021). Effect of drying methods and storage with agro-ecological conditions on phytochemicals and antioxidant activity of fruits: a review. *Crit. Rev. Food Sci. Nutr.* **62** (2), 353-361.

Wahyuni, R., Wignyanto, W., Wijana, S. and Sucipto, S. (2020). Optimization of protein and tannin extraction in Moringa oleifera leaf as antioxidant source. *Food Res.* **4** (6), 2224-2232.

Wang, Y., Liu, Y., Huo, J., Zhao, T., Ren, J. and Wei, X. (2013). Effect of different drying methods on chemical composition and bioactivity of tea polysaccharides. *Int. J. Biol. Macromol.* **62**, 714-719.

Wangcharoen, W. and Gomolmanee, S. (2013). Antioxidant activity changes during hot-air drying of Moringa oleifera leaves. *Maejo. Int. J. Sci. Technol.* **7** (3).

Wickramasinghe, Y. W. H., Wickramasinghe, I. and Wijesekara, I. (2020). Effect of steam blanching, dehydration temperature & time, on the sensory and nutritional properties of a herbal tea developed from Moringa oleifera Leaves. *Int. J. Food Sci.* **2020**. doi: https://doi.org/10.1155/2020/5376280.

Willige, R. V., Linssen, J. P. H., Meinders, M. B. J., Stege, H. v. d. and Voragen, A. G. J. (2002). Influence of flavour absorption on oxygen permeation through LDPE, PP, PC and PET plastics food packaging. *Food Addit. Contam.* **19** (3), 303-313.

Yadav, A. (2018). Effect of drying and blanching (steam and lye) on the phytochemicals composition of sitalchini (Moringa oleifera) leaves and its sensory attributes. B. Tech. Thesis. Tribhuvan Univ., Nepal.

Yang, R., Lin, S. and Kuo, G. (2008). Content and distribution of flavonoids among 91 edible plant species. *Asia. Pac. J. Clin. Nutr.* **17**.

Youssef, K. M. and Mokhtar, S. M. (2014). Effect of drying methods on the antioxidant capacity, color and phytochemicals of Portulaca oleracea L. leaves. *J. Nutr. Food Sci.* **4** (6), 1.

Zhao, D., Wei, J., Hao, J., Han, X., Ding, S., Yang, L. and Zhang, Z. (2019). Effect of sodium carbonate solution pretreatment on drying kinetics, antioxidant capacity changes, and final quality of wolfberry (Lycium barbarum) during drying. *LWT-Food Sci. Technol.* **99**, 254-261. doi: https://doi.org/10.1016/j.lwt.2018.09.066.

Appendices

Appendix A

Equipment

i. Weighing balance

ii. Hot air oven

iii. Muffle furnace

iv. Spectrophotometer

v. Cabinet dryer

vi. Grinder

vii. Heating mantle

viii. Refrigerator

ix. centrifuge

Chemical used

i. NaoH

ii. Oxalic acid

iii. Boric acid

iv. Indicators (Methyl red, Bromocresol green, Phenolphthalein)

v. Folin-ciocalteau reagent

vi. Methanol

vii. Sodium carbonate

viii. Sodium nitrite

ix. Aluminium chloride

x. Ethanol

xi DPPH

Appendix B

Sensory Analysis Score Card

Name of the Panelist:

Date:

Name of product: Moringa leaf

Dear panelist, you are given 3 coded samples of moringa leaf. please give points for your degree of preference on the following parameter using the table given;

Sample code	Color	Taste	Smell	Mouth feel	Overall acceptance
A					
B					
C					
D					
E					
F					

Judge the characteristics on the 1-9 scale as below:

Like extremely – 9	Like slightly – 6	Dislike moderately – 3
Like very much – 8	Neither like nor dislike – 5	Dislike very much – 2
Like moderately – 7	Dislike slightly – 4	Dislike extremely – 1

Any comments: Signature:

Appendix C

ANOVA result for analysis of different parameter of moringa leaf

Table C.1 one way ANOVA (no blocking) for Total Phenolic Content

Source of variation	Degree of freedom	Sum of square	Mean squares	Variance ratio	F pr.
Temperature	3	471.475	157.158	40.22	<.001
Residual	8	31.256	3.907		
Total	11	502.731			

Table C.2 one way ANOVA (no blocking) for Total Flavonoid Content

Source of variation	Degree of freedom	Sum of square	Mean squares	Variance ratio	F pr.
Temperature	3	1171.058	390.353	65.79	<.001
Residual	8	47.465	5.933		
Total	11	1218.523			

Table C.3 one way ANOVA (no blocking) for Chlorophyll

Source of variation	Degree of freedom	Sum of square	Mean squares	Variance ratio	F pr.
Temperature	3	1292.024	430.675	101.52	<.001
Residual	8	33.937	4.242		
Total	11	1325.960			

Table C.4 one way ANOVA (no blocking) for Tannin content

Source of variation	Degree of freedom	Sum of square	Mean squares	Variance ratio	F pr.
Temperature	3	414.113	138.038	38.50	<.001
Residual	8	28.682	3.585		
Total	11	442.795			

Table C.5 one way ANOVA (no blocking) for Anti-oxidant Activity

Source of variation	Degree of freedom	Sum of square	Mean squares	Variance ratio	F pr.
Temperature	3	1368.503	456.168	67.13	<.001
Residual	8	54.361	6.795		
Total	11	1422.864			

Appendix D

ANOVA result for sensory analysis of moringa leaf dried at different temperature

Table D.1 Two way ANOVA (no blocking) for color of dried moringa leaf at different temperature

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
sample	2	11.4000	5.7000	31.41	<.001
panelist	9	2.1333	0.2370	1.31	0.300
Residual	18	3.2667	0.1815		
Total	29	16.8000			

Table D.2 Two way ANOVA (no blocking) for smell of dried moringa leaf at different temperature

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
sample	2	0.0667	0.0333	0.18	0.834
panelist	9	3.6333	0.4037	2.22	0.071
Residual	18	3.2667	0.1815		
Total	29	6.9667			

Table D.3 Two way ANOVA (no blocking) for taste of dried moringa leaf at different temperature

Source of variation	Degree of freedom	Sum of square	Mean squares	Variance ratio	F pr.
sample	2	0.0667	0.0333	0.18	0.834
panelist	9	3.6333	0.4037	2.22	0.071
Residual	18	3.2667	0.1815		
Total	29	6.9667			

Table D.4 Two way ANOVA (no blocking) for mouthfeel of dried moringa leaf at different temperature

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
sample	2	0.2000	0.1000	0.47	0.630
panelist	9	3.2000	0.3556	1.68	0.166
Residual	18	3.8000	0.2111		
Total	29	7.2000			

Table D.5 Two way ANOVA (no blocking) for overall acceptance of dried moringa leaf at different temperature

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
sample	2	8.2667	4.1333	19.93	<.001
panelist	9	2.6667	0.2963	1.43	0.248
Residual	18	3.7333	0.2074		
Total	29	14.6667			

Appendix E

ANOVA result for sensory analysis of moringa leaf powder

Table E.1 Two way ANOVA (no blocking) for color of moringa leaf powder stored in different packaging materials

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
sample	2	1.2667	0.6333	4.17	0.032
panelist	9	2.1667	0.2407	1.59	0.194
Residual	18	2.7333	0.1519		
Total	29	6.1667			

Table E.2 Two way ANOVA (no blocking) for smell of moringa leaf powder stored in different packaging materials

Source of variation	degree of freedom	Sum of squares	Mean squares	Variance ratio	F pr.
sample	2	2.40000	1.20000	13.50	<.001
panelist	9	3.50000	0.38889	4.38	0.004
Residual	18	1.60000	0.08889		
Total	29	7.50000			

Table E.3 Two way ANOVA (no blocking) for taste of moringa leaf powder stored in different packaging materials

Source of variation	Degree of freedom	Sum of squares	Mean square	Variance ratio	F pr.
sample	2	1.66667	0.83333	9.00	0.002
panelist	9	4.13333	0.45926	4.96	0.002
Residual	18	1.66667	0.09259		
Total	29	7.46667			

Table E.4 Two way ANOVA (no blocking) for mouthfeel of moringa leaf powder stored in different packaging materials

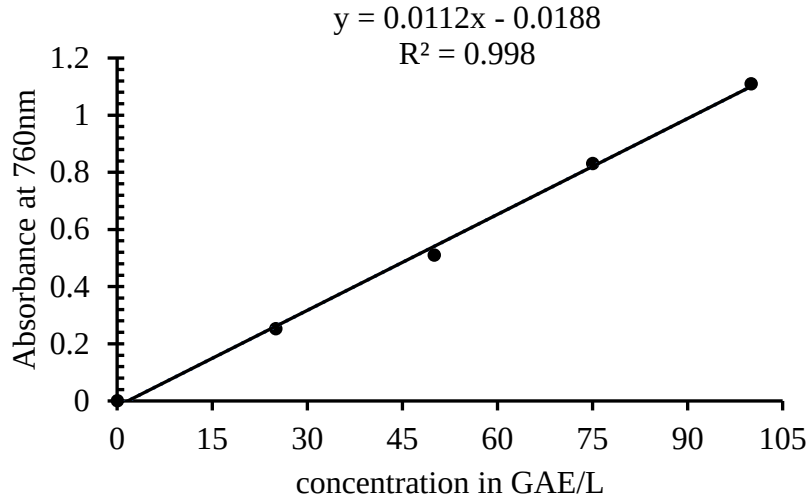
Source of variation	Degree of freedom	Sum of squares	Mean square	Variance ratio	F pr.
sample	2	0.4667	0.2333	1.19	0.327
panelist	9	3.3667	0.3741	1.91	0.117
Residual	18	3.5333	0.1963		
Total	29	7.3667			

Table E.5 Two way ANOVA (no blocking) for overall acceptance of moringa leaf powder stored in different packaging materials

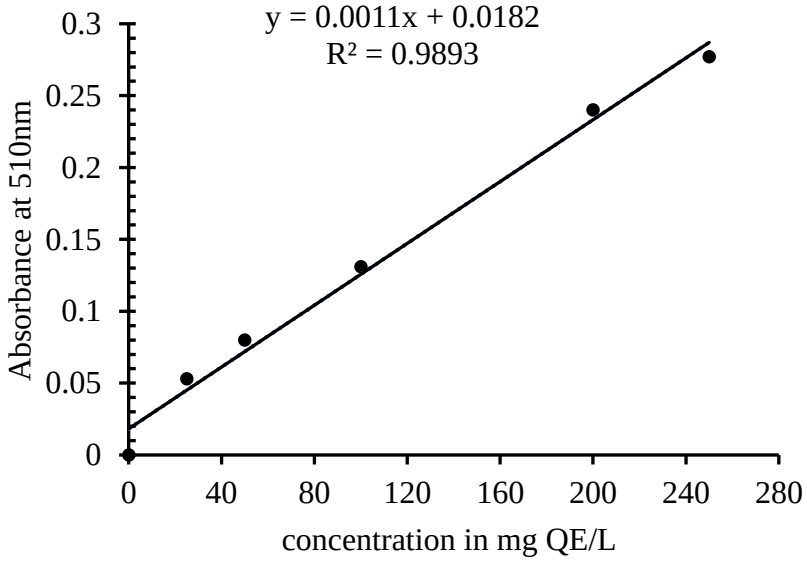
Source of variation	Degree of freedom	Sum of squares	Mean square	Variance ratio	F pr.
sample	2	2.4667	1.2333	6.28	0.009
panelist	9	2.1667	0.2407	1.23	0.339
Residual	18	3.5333	0.1963		
Total	29	8.1667			

Appendix F

1. standard curve for total phenolic content determination



2. standard curve for total flavonoid content determination



3. standard curve for tannin determination

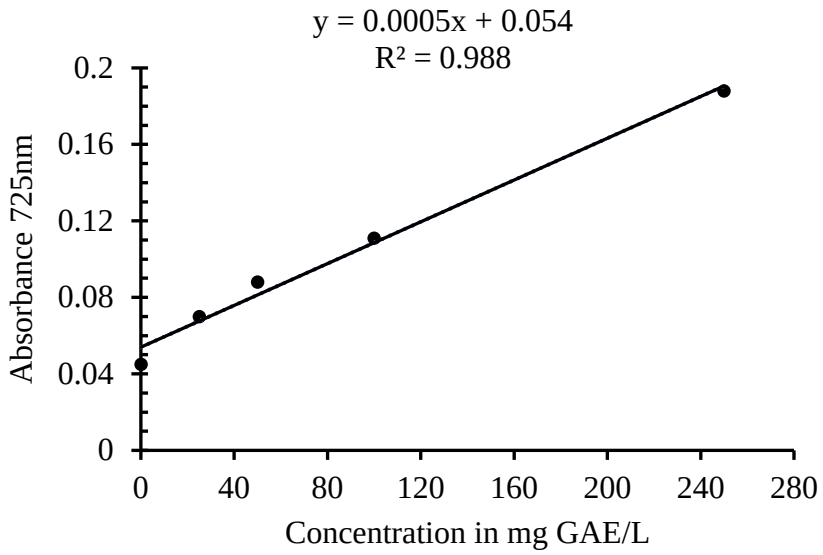


Photo gallery

plate 1: Sample preparation for analysis

plate2: moringa leaves after destalking

Plate 3: Drying of moringa leaves

plate 4: protein determination

Plate 5: Extract preparation for chlorophyll

plate 6: spectrophotometer analysis

Plate 7: HDPE, LDPE pouches and PET bottle

plate 8: sensory analysis

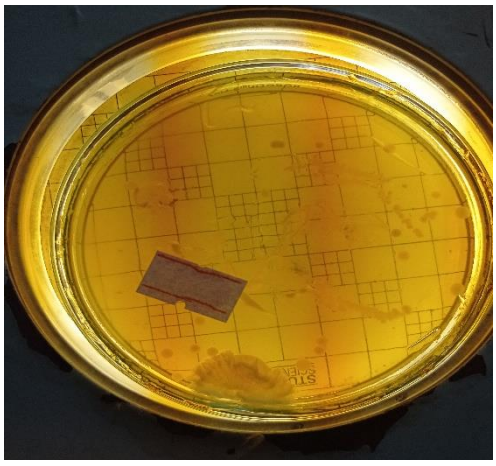


Plate 9: colony counting