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Fluidized bed dryer processing of *Moringa Oleifera* leaves and its impact on the phytochemical compounds

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ABSTRACT

An experiment was performed to evaluate and standardize the various drying methods such as shade drying, cabinet drying, and fluidized bed drying and their impact on the physicochemical properties and bioactive compounds of dried moringa powder by GCMS. Drying by Fluidized bed drier exhibited antioxidant activity of per cent of 62.81 and bioactive compounds such as lauric acid, palmitic acid, and linolenic acid ester that possess various therapeutic properties were ascertained. About 11 compounds from the ethanolic extract of the M. oleifera leaves sample have been characterized. They were 9,12,15-dienooctadecadienoic acid ethyl ester (z,z,z) with the highest peak area of percent of 23.12. The next major compound characterized was undecanoic acid, 10-bromo with a peak area of 16.53 percent. Phytol with the peak area of 16.01 percent and a retention time of 14.13 minutes. Palmitic acid, ethyl esters, and hexadecanoic acid were the other compounds exhibited. A fluidized bed dryer was proven to retain the antioxidant and physicochemical properties of moringa leaf powder.

Keywords: *Moringa oliefera, fluidizedbed dryer, ethanolic extract, phytochemicals, GCMS*

INTRODUCTION

Moringa oleifera contains essential nutrients, enzymes, omega oils, minerals, antioxidants, and phytochemical compounds. It possesses the most important nutrient-rich properties on the planet with an outstanding source of nutritional components [1]. The quality and storage shelf life behavior of moringa leaf powder prepared from moringa leaves, flowers, and pods was highly improved by the use of pre-processing techniques such as blanching and dehydration by drying. Blanching is the pre-processing heat treatment method followed by dehydration through different drying methods like sun drying, shade drying, cabinet drying, freeze-drying, and fluidized bed drying *etc*., for good consumer acceptability and preservation. Post-harvest processing of moringa leaves for increasing storage stability includes drying by different methods. Amongst all methods, the fluidized bed drying process effectively reduced the moisture gradually without affecting much of the nutrient properties. A simple processing technique with a steady rate of moisture reduction and drying method for long time preservation of the moringa leaves was proved to be fluidized bed drying [2]. With this above fact, the present investigation was concentrated to exploit the phytochemical and bioactive properties of fluidized bed-dried moringa leaf powder.

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MATERIALS AND METHODS

Processing of moringa leaves

To assess the suitable drying method, for obtaining better nutrient-rich leaf powder of moringa and for further processing of value-added products, different drying methods were evaluated. Drying of moringa leaves was carried out by three drying methods such as shade drying, cabinet drying, and fluidized bed drying.

Preparation of moringa leaves for drying

Selection of leaves

Fresh, green leaves of moringa were separated from the thin branches. Discolored yellow and decayed leaves that lead to loss of nutrients were discarded. Only good-quality leaves were selected for the drying process.

Washing and quality control of moringa leaves

The selected leaves were washed with water to remove dust and dirt particles. Excess water was thoroughly drained off. The residual moisture was evaporated for 2-3 hours at room temperature (32°C) by even spreading the cleaned leaves on the absorbent paper with constant turning over to avert fungal growth.

Blanching of moringa leaves

The cleaned leaves were blanched for 3 minutes before subjecting to the process of different drying methods. Besides killing the deteriorating microorganisms, blanching softens the cell walls and speeds up the process of drying, and further deactivates the lip oxidases enzyme, which affects the nutritional quality and off-flavor production of the leaves and helps to improve the shelf life of the dried leaf powder.

Dehydration methods of moringa leaves
Blanched moringa leaves were dehydrated and dried by three different methods namely, shade drying, cabinet drying, and fluidized bed drying and the phytochemical compounds obtained were identified using GCMS.

Shade drying
The blanched leaves were spread on a clean dry tray and covered with a clean muslin cloth, the trays were kept for shade drying in a well-ventilated room for 48-50 hours at a temperature of 32°C.

Cabinet drying
The blanched leaves were loaded on the trays forming one single layer and were dried in the dehydrator by forced air technique. The loaded trays with leaves were incubated for 3-4 hours in a cabinet dryer oven at 60°C [3].

Fluidized bed drying
The blanched leaves were dried in a fluidized bed dryer at 55°C for 35-45 minutes which clean air passes over the electric heaters. The clean hot air penetrates the moringa leaves and dries the product by fluidization.

Estimation of the phytochemical and antioxidant content of dried moringa leaves
The powdered moringa leaves (300g) that were dried by different methods were subjected to ethanol extraction using a Soxhlet apparatus for 10 hours. The last traces of the solvent were removed and concentrated in a rotary evaporator [4]. The concentrated extract was subjected to the evaluation of tannin, phenolic compounds, alkaloids, flavonoids and antioxidant activity. The phytochemical content of moringa leaf powder was done by adopting the procedure suggested by [5], [6]. The following methods were adopted for the estimation of tannins, polyphenols, alkaloids, flavonoids, and antioxidant activity.

Estimation of tannin content (Indigo Sulphonic Acid - Titrimetric Method (U.S.S.R.P)
About 1gm of the powdered moringa leaf powder sample (W) was weighed and transferred into a 250 ml glass stoppered flask and added water (100 ml), It was kept in a shaker for 1 hr and kept overnight. Allowed the solid material to settle and filter the liquid through a filter paper of 12 cm diameter, discarding the first 20 ml of the filtrate.
Transferred 10 ml of the filtrate to one liter conical flask, added 750 ml of distilled water and Indigo Sulphonic acid solution (25 ml). Then titrated against 0.1N potassium permanganate solution and vigorously shaken till a golden-yellow endpoint (T_2) is reached. A blank determination (T_1) was performed to make the necessary correction.
Quantity of Total Tannins (%) = T_2-T_1 X Actual normality X Strength X 1000/W X 0.1

Estimation of total polyphenol (Folin- Ciocalteau method)
An aliquot of methanolic extract 5 ml was mixed with 0.2 ml of Folin Ciocalteau reagent and 2 ml of distilled water into a 10 ml test tube. The mixture was warmed and vortexed for 20 sec and kept aside for 10 minutes and 1ml of 20 % solution sodium carbonate was added. The mixture was incubated in a water bath at 40°C for 30 minutes. After cooling to room temperature, the absorbance was taken at 670nm using UV-spectrophotometer. The gallic acid solution was used as standard. The total polyphenol was calculated by using the formula,

$$\text{The total amount of polyphenol} = \frac{\text{O.D of Test solution}}{\text{O.D of standard solution}} \times 100$$

Estimation of total alkaloids (titre method)
A sample of 5 gm of methanolic extract was treated with 50 ml of methanol chloroform (2:1) and filtered. The extract was mixed with 100 ml of 0.8% Sodium sulfate. The upper chloroform layer was separated and dried and the residue was dissolved in 15 ml of 2N Sulphuric acid. Then the solution was heated for 2 hrs and made basic with 10 ml of 4N sodium hydroxide. The alkaline layer was extracted with 30 ml of benzene in 3 aliquots and the benzene layer was collected and evaporated to dryness until it forms a residue. The residue was dissolved with 5ml of methanol. The samples were titrated with a solution of 0.067 % bromophenol blue and 10 % phenol in absolute methanol. A blank was performed without extract.

$$\text{Percentage of alkaloids} = \frac{\text{(Assay- blank)} \times \text{Eq. Wt. factor} \times \text{strength of phenol}}{\text{Weight taken}} \times 100$$

Estimation of total flavonoid (colorimetric method)
An aliquot of 5 ml methanolic extract was taken in a test tube containing 4 ml of distilled water. Added 0.5 ml of 5% sodium nitrate solution to the test tube. After 5 minutes added 0.5 ml of 10% aluminium chloride and stay for 6 minutes before the addition of 2 ml of 1.0 M NaOH and adjust the total volume to 10 ml with distilled water and then vortexed for 10 seconds. The absorbance was measured at 510 nm. Rutin solution was used as standard. The total flavonoid content was calculated from the values of Optical Density (O.D) of the test and standard solution by using the formula.

$$\text{Percentage of flavonoid} = \frac{\text{O.D of test solution}}{\text{O.D of standard solution}} \times 100$$

Antioxidant activity (Free radical scavenging activity by DPPH method)
The DPPH (1,1- diphenyl- 2- picryl hydroxyl) radical scavenging activity was measured by the spectrophotometric method. The methanolic and ethanolic solution of extracts (100 mcg/ml) was mixed with 400 µl of DPPH ethanol solution at a ratio of 1:3. The mixture was kept in the dark at room temp for 90 min. the absorbance of the resulting solution was measured at 517 nm. The capability of scavenging DPPH radical was calculated by the following equation. Analyses of all samples were run in triplicate. Ascorbic acid was used as standard (100 mcg/ml).
Abs – Absorbance. Control abs = 0.640
Percentage of scavenging activity = (1- abs of sample/abs of control) × 100

Preparation of powder for extraction
Ethanolic extracts of moringa leaf powder as subjected to the characterization of bioactive compounds using GCMS analysis. About 2 µl of the ethanolic extract of moringa leaf powder was employed for GCMS analysis.

Gas Chromatography-Mass Spectrometry (GCMS) analysis
The analysis was carried out using GC Clarus 500 Perkin Elmer equipment. Compounds were separated on Elite 1 Capillary column (5 % Diphenyl and 95 % Dimethylpolysiloxane) operating in electron energy 70 ev: the carrier gas at constant flow 1 ml/1 min with the split ratio of 10:1. The oven temperature was assembled from 110°C (2 minutes hold) with the increasing of 10°C / min increased up to 200°C, then 5°C /

minute increased up to 280°C of 9 minutes hold. The total GC running time is 36 minutes. The detector (Mass detector Turbo Mass Gold- Perkin Elmer) was used with the 5.2 Turbo Mass Software. For the MS program, the NIST version – 2.1 (2005) library was used for data preparation.

RESULTS AND DISCUSSION

Quantitative analysis of phytochemicals in moringa leaves

The weight of moringa leaf powder obtained from 100 g of cleaned fresh moringa leaves after drying obtained by three different drying methods such as shade drying, cabinet drying and fluidized drying of the leaves were 27.0, 24.0, and 27.0 g, respectively.
From this study, it was found that one kg of fresh leaves yields 230-270 g of dry powder. The initial nutrient content of moringa leaf powder obtained from the selected drying method was assessed. The antioxidant test conducted by [7]illustrated that ascorbic acid content decreased by heat drying method and hence fluidized bed drying was proved to be an effective method of drying exhibiting superior qualities for phytochemical compounds (Table 1).

Table 1. Phytochemical and antioxidant activity of moringaleaves

Phytochemical contents	Shade drying	Cabinet drying	Fluidized bed drying
Tannins (%)	3.09	3.05	2.97
Total polyphenols (%)	1.19	1.19	1.02
Total alkaloids (%)	1.34	1.34	1.31
Total flavonoids (%)	1.20	1.00	1.00
Antioxidant activity (%)	57.96	60.93	62.81

From Table 1, it was observed that the presence of tannins, polyphenols, alkaloids and flavonoids was increased to various extents, depending on the type of drying methods. The presence of tannins, polyphenols, alkaloids and flavonoids was high in fluidized -bed-dried moringa leaf powder samples when compared to other drying methods [8]. The tannin composition varied from 2.97 to 3.30 per cent. Shade-dried samples exhibited higher tannin content of 3.30 percent followed by fluidized bed-dried samples. [9] studied the total flavonoid content in moringa leaves and expressed as catechin equivalents, they reported that the sun-dried moringa leaves had 0.44% of flavonoids. The plant flavonoids in general are highly effective free radical scavengers and antioxidant properties with the highest content of tannins among the other medicinal plants studied. The presence of polyphenols was higher in moringa leaves dried in shade drying followed by cabinet drying. The fluidized bed-dried leaves exhibited low total polyphenolic content of 1.02 percent. This might be due to the processing techniques of leaves. Dietary phenolic compounds have generally been considered as non-nutrients but their strong antioxidant property has medicinal and nutritional interest. The presence of phytochemicals in the moringa extracts such as tannins, steroids and triterpenoids, flavonoids, saponins, anthraquinones, alkaloids, dietary antioxidants, and reducing sugars were reported by [10, 11].
The data obtained in the present study suggests that the extracts of moringa leaves have potent antioxidant activity. [12] studied the preliminary phytochemical screening and antioxidant activity of five different extracts methanol, ethanol, petroleum ether, n-hexane, chloroform, ascorbic acid, and butylated hydroxyl toluene of moringa leaf, that exhibited significant DPPH free radical scavenging activity with the ethanol and methanol extract of 62.09 and 68.32 respectively of dried moringa leaves powder. The results obtained in the present study conformed with the above findings.

Quantitative analysis of bioactive compounds in moringa leaves by GCMS

The active principles with their retention time (RT), molecular formula (MF), Molecular weight (MW) and concentration (%) in the ethanol extract of moringa leaves powder are presented in Table 2

Table 2. Compounds identified in ethanol extract of Moringa leaf powder

S.No	RT	Name of the compound	Nature of the compound	Molecular formula	MW	Peak area (%)
1.	7.88	Dodecanoic acid, 3- hydroxyl	Lauric acid	$C_{12}H_{24}O_3$	216	1.66
2.	8.91	Undecanoic acid, 10- bromo	Undecyclic acid	$C_{11}H_{21}BrO_2$	264	16.53
3.	10.90	10-Undecenoic acid, octyl ester	Carboxylic acid	$C_{19}H_{36}O_2$	292	7.95
4.	11.36	1,2, Cyclopentane dicarboxylic acid 4-1,1 –(dimethyl ethyl) dimethyl ester(1a.2a.4a)	Carboxylic acid	$C_{13}H_{22}O_4$	242	7.32

5.	12.67	Hexadecanoic acid, ethyl ester	Palmitic acid	$C_{18}H_{36}O_2$	284	15.54
6.	14.13	Phytol	Diterpene alcohol	$C_{20}H_{40}O$	296	16.01
7.	14.86	9,12,15-Octadecatrieonic acid ethyl ester (z,z,z)	Linolenic acid ester	$C_{20}H_{34}O_2$	306	23.12
8.	15.25	3'8'8- Trimethoxy- 3-piperidyl-2-2–binpthalene-1,1',4,4' tetrone	Alcohol	$C_{28}H_{25}NO_7$	487	5.57
9.	0.50	Cholestan-3-ol-2 methylene –(3a, 5a)	Coprostanol	$C_{28}H_{48}O$	400	1.17
10.	24.96	9- octadecenoic acid (2)-hexyl ester	Oleic acid	$C_{24}H_{46}O_2$	366	0.13
11.	30.73	E-8- Methyl-(tetradecen-i-ol- acetate	Terpene alcohol	$C_{17}H_{32}O_2$	268	5.00

Eleven compounds were identified in the *moringa* leaf sample. The prevailing bioactive compounds were identified as 9,12,15-dienooctadecadienoic acid ethyl ester (z,z,z) which is a linoleic acid ester compound found in the highest peak area of 23.12 % and 14.86 minutes of retention time. The second major compound, undecanoic acid, 10-BBromo is a carboxylic acid and has 16.53 % of peak area at a retention time of 10.90 min. [13]identified 71 types of volatile compounds in methanolic extracts of moringa leaves and stalks and found that benzyl aldehyde was more predominant in leaves rather than stalks. The results of the present GCMS studies of moringa leaf powder obtained by fluidized bed drying exhibited only 11 unique compounds.

Phytol, acyclic diterpene alcohol which is a constituent of chlorophyll was detected at a peak area of 6.01 % and retention time of 14.13 minutes. [14] had reported that phytol acts as an antimicrobial, anticancer, antidiuretic and anti-inflammatory compound and proved to be effective at different stages of arthritis. Palmitic acid was detected at the peak area of 15.54% and 12.67 minutes of retention time. Earlier studies had proved that palmitic acids, hexadecanoic acid, and ethyl esters have the property of -anti-oxidant, hypocholesterolemic effect, nematicide, pesticide, hemolytic, exhibits alpha-reductase inhibitor activity [15]. The presence of saturated and unsaturated fatty acids (oleic acid, palmitic acid and lauric acid) and its derivatives of moringa may be the response to the flavoring activity. An experiment carried out to study the effect of different blanching and drying methods of moringa leaf extract by [16] indicated that steam blanching and cabinet drying of moringa leaves retained maximum quantities of crude protein, fiber, fat and carbohydrates. GCMS analysis of leaf powder of the present study also showed the presence of various bioactive compounds.

A study conducted by [17] indicated that an optimum steam blanching conditions of 2.5 min air drying of moringa leaves and microwave heating and drying at 270 Watt for 3 min can be used for retaining the ascorbic acid content and antioxidant activity to produce better quality dried moringa leaf extracts at industrial point of view.

[18] elucidated that *Moringa* herbal tea produced by steam blanching and drying at 65°C for 4 hours serves to beneficial as a functional food with numerous therapeutic effects and health benefits. The study highlighted that steam blanching significantly increased the DPPH scavenging activity, carbohydrates, fat, Mn, Fe, vitamin A, and vitamin E, and also unaffected the vitamin C, Zn, Cu, and Mg contents.

CONCLUSION

The present research aimed to characterize the utility of fluidized bed drying in moringa leaf powder preparation and characterization of phytochemicals and bioactive compounds substantially highlighted that fluidized bed drying exhibited maximum antioxidant activity of 62.81% and the presence of bioactive compounds such as lauric acid, palmitic acid, a-linolenic acid ester that possess various therapeutic properties.

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