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Effect of Drying Method on Volatile Nutraceuticals and Microbial Growth in *Moringa oleifera*

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ABSTRACT

Fresh *Moringa oleifera* leaves are very rich in phytonutrients, however the leaves are also highly perishable and require processing for increased shelf-life. The method of processing, specifically drying affects the nutritional value of the product. The present study therefore, analyzed the nutraceutical value and growth of toxic microbes when the leaves were dried under different conditions i.e. room temperature, greenhouse, 50% shade net, and in the oven at 60 ºC for 4 h. The experiments were carried out at the Jomo Kenyatta University of Agriculture and Technology (JKUAT). The treatments were applied in triplicate and arranged on a completely randomized design (CRD). Data on nutritional value of dried Moringa leaves was subjected to analysis of variance (ANOVA) for parameterization and means separated using protected $LSD_{0.05}$. The study showed that drying Moringa leaves under shade, room and greenhouse conditions significantly ($P<0.05$) affects the nutritional value of the product. The results showed highest levels of vitamin C, vitamin A, polyphenols and terpenoids when the leaves were dried under 50% shade net and room temperature conditions. In contrast, the glucosinolate content was significantly ($P<0.05$) higher when the leaves were dried instantly in the oven (9.1%/wt), followed by drying under greenhouse conditions (8.7%/wt) before oven drying. However, drying of Moringa leaves under shade before oven drying resulted in growth of toxic microbial organisms such as staphylococcus, yeast, *E. coli* and molds that can potentially affect the safety of the product. Finally, the drying conditions also significantly ($P<0.05$) affected the moisture content of the powder obtained. The results of this study form an important reference for small-holder Moringa growers and processors in the development of an optimal processing regime for high value Moringa powder.

Introduction

Moringa plant (*Moringa oleifera)* Lamarck, 1785 (Fig. 1) as a member of the family Moringaceae is an ever-green and drought resistant tree propagated from seed or vegetative materials. The plant is native to the Indian sub-continent from where it has gained worldwide distribution (CABI, 2019). It is also known as drumstick tree or ben oil tree. It displays polymorphism in color and structure.

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Two main morphotypes including green stemmed branches and red or brown stemmed branches with many color-shades exist. The crop is a C4 and does well under tropical conditions. Moringa plant is prolific in growth for production of leaves, seedpods and seeds all of which are of great nutritional importance (Olson, 2010). Production of Moringa plant in Kenya has been widely promoted by a great number of non-governmental organizations (NGOs) and government agencies due to its environmental conservation properties such as drought tolerance and resistance to most of the pests and diseases. The plant has great potential value due to its nutritional, phytochemical, biomass, water-purification, agro-forestry, biofumigation and medicinal qualities (Atawodi et al., 2010). Moringa is known to contain high quantities of vitamins (C, A, B-complex, E, K, D), minerals (Ca, Fe, Mg, K, P, Mn, S, Zn and Se) and phytochemicals (glucosinolates, polyphenols, chlorophyll and terpenoids) (Yameogo et al., 2011). Consequently, Moringa plant products and extracts have been referred to as superfoods.

However, during processing a lot of the volatile nutrients such as vitamins, proteins and secondary metabolites are lost resulting in a product of lower nutritional value (Begum et al., 2009). This study therefore, sought to determine the most effective drying practices in terms of obtaining a dry product with high nutritional value and freedom from microbes. Different methods of drying were investigated. Harvested Moringa leaves were dried under different conditions i.e. 50% shade net, at room temperature, inside the greenhouse and in the oven (Abdullah, 1997). These conditions were selected because most farmers in Kenya and globally apply at least one of the methods for drying prior to processing (Kasolo et al., 2010).

This study is unique because, despite the availability of nutritional and medicinal information about *M. oleifera* leaves, there is dearth of information comparing nutritional and microbial properties of Moringa powder produced under different processing regimes. Results from this study can be adopted by processing companies particularly those employing out-grower production models where the contracted growers are scattered and have low production and processing capacities. If adopted, the study would therefore provide simple techniques in enhancing shelf-life of Moringa produce with concomitant phytonutrients conservation.

Fig. 1. *Moringa oleifera* plants before and after harvesting (Authors archive, 2020)

Materials and Methods

Materials

Fresh Moringa leaves were obtained from small scale growers in Central Kenya. To reduce variation, leaves were obtained from the red stemmed morphotype of Moringa trees. The leaves were dried under room temperature, 50% shade net, greenhouse and oven before being subjected to physical and chemical analysis. Analytical grade solvents and reagents were used in the analysis. The experiments were carried out at the Jomo Kenyatta University of Agriculture and Technology (JKUAT) for a period of 3 months. The treatments were applied in triplicate and arranged on a completely randomized design (CRD) due to the homogeneity of the environmental conditions in the greenhouse and laboratory.

Under room conditions

Bunches of 1 kg were spread on a table in JKUAT chemistry laboratory at 22 ºC for two days. The wilted leaves were later dried at 60 ºC for 4 h when a constant moisture content was obtained and cooled in a desiccator. The dried leaves were then ground using a milling machine. A sample (20 g) was drawn for analysis.

Under shade net (50% shade) conditions

Bunches of 1 kg were spread on a table in a 50% shade net structure in the department of Horticulture farm, JKUAT at 22 ºC for two days. The wilted leaves were later dried at 60 ºC for 4 h, cooled in a desiccator and ground into powder. A sample (20 g) was drawn for analysis.

Under greenhouse conditions

Bunches of 1 kg were spread on a table inside a greenhouse at the department of Horticulture farm, JKUAT. The daytime temperature was ranging between at 15 and 35 ºC for the two days with relative humidity (RH) of 70–74%. The wilted leaves were later dried at 60 ºC for 4 h, cooled in a desiccator and ground into powder. A sample (20 g) was drawn for analysis.

In oven

Bunches of 1 kg were directly placed in an oven after harvesting in the chemistry laboratory, JKUAT. The oven was set at a temperature of 60 ºC for 4 h, cooled in a desiccator and ground into powder. A sample (20 g) was drawn for analysis.

Moringa leaf powder preparation

Moringa leaflets were separated from stalk and then ground into fine powder using an electric mill. The powder was then sieved through a 60 mesh sieve before chemical analysis was done.

Vitamin A

Standard solutions of beta carotene including 1 ppm, 2 ppm, 4 ppm, 8 ppm and 10 ppm were prepared and their absorbance on SHIMADZU Ultraviolet–visible (Uv-vis) spectrometer were used in calibration. A 10 g sample of Moringa powder was placed in 100 mL flask, heated with acetic acid and acidified sand for extraction. The extract was concentrated with the rotary evaporator and re-extracted with 10 mL petroleum ether. The ethereal extract was measured by ultraviolet–visible spectrometer at 440 nm to obtain absorbance values. The procedure was repeated for all the samples and replicates. A calibration curve was obtained as a linear equation from which the beta-carotene values in the samples were obtained with a coefficient of determination ($R^2 = 0.996$) (AOAC, 1990).

Vitamin C

Standardization of the indophenol solution was done and the ascorbic acid equivalent calculated. A 10 g sample of Moringa powder was placed in a flask and acidified with trichloroacetic acid (TCA) solution. Filtrated and 10 mL of each extracted sample titrated with indophenol solution. The amount of the filtrate used for the color of the indicator to turn pink was taken and used to obtain the concentration of the ascorbic acid in the samples. The titration was repeated and the results agreed within 0.1 mL. The procedure

was repeated for all the samples and replicates and concentration of ascorbic acid determined (AOAC, 1990).

Kjedahl method

A 10 g sample of Moringa powder was placed in the digestion flasks, mixed with sulphuric acid and copper sulphate as the catalyst. A blank test was prepared separately and sulphuric acid added. The two, that is, the sample and blank tests were heated under a digestion heater until the color changed to green like the blank. The samples were then transferred to distillation using the Marknan method and Titration done using hydrochloric acid until the orange color occurred. The color change was used to determine the amount of nitrogen. The nitrogen content was converted to proteins using a factor of 6.25 (AOAC, 1990).

Total Polyphenol (TPP) estimation

A 0.2 g of the ground sample was weighed into a centrifuge tube and 10 mL of 80% ethanol added. The mixture was vortexed for 5 min and centrifuged at 4500 rpm for 5 min. The supernatant was collected and diluted 10 times with distilled water. Initially, 0.2, 0.4, 0.6, 0.8 as well as 1 mL aliquots of working standard solution was pipetted into a series of test tubes marked M1, M2, M3, M4 and M5, respectively. 50 µL of phenolics extract of sample was then pipetted into another series of test tubes. The contents of all the standard and sample test tubes were made up to 1 mL with distilled water. Another test tube marked 'B' with 1 mL of distilled water served as the blank. 0.5 mL of Folin-Ciocalteu reagent (1 N) was then added to each test tube including the blank. The test tubes were vortexed well and allowed to stand for 5 min at room temperature. 2.5 mL of 5% sodium carbonate was then added to all the test tubes including the blank. The test tubes were vortexed again and incubated in the dark at room temperature for at least 40 min. The absorbance of the blue colour developed against the reagent blank was measured at 725 nm using a SHIMADZU Ultraviolet (Uv-vis) spectrophotometer (AOAC, 1990).

Terpenoids analysis

A 2 g sample of the powdered foliage material was extracted with 10 mL of methanol in water bath at 80 ℃ for 10 min. The concentration of constituent terpenes in the extract was determined by HPLC (SHIMADZU model LC-20A) following the procedure of Sanjay et al. (2013). The terpenes were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. After 24 h, the extract was removed with a Pasteur pipette and filtered through glass wool. The sample was washed in 400 µL hexane to remove any remaining terpenes (AOAC, 1990).

Glucosinolate analysis

Total glucosinolates were determined by the sulfate method. A 2 g of the powdered foliage material was extracted with 70% ethanol on a rotary shaker at 180 rpm for 10 h. 70% lead acetate was added to the filtrate and centrifuged after adding 6.3% sodium carbonate at 100 rpm for 10 min. The glucosinolates were separated using ethyl acetate-methanol-water (80:10:10) solvent mixture. A calibration curve was obtained as a linear equation from which the glucosinolates values in the samples were obtained with a coefficient of determination ($R^2 = 0.989$) and refractive values of the spots were recorded under SHIMADZU Ultra-violet visible (Uv-vis) spectrophotometer at 254 nm (AOAC, 1990).

Determination of moisture content

A (2) gm of finely ground sample was put in a Mettler's HB43 Infra-Red Moisture balance to determine the moisture content. The readings were done in triplicates and the average taken to indicate the actual powder moisture content.

Microbial colonies count

Escherichia coli microbial colonies were determined following the FDA, 2002 protocol

and Khodaeeyan (2008). EMB selective agar was obtained from Sigma Aldrich.

Total mould and yeast counts were done following ISO 21527, (2008). Sabourand 2% glucose agar was used. Enumeration of *Staphylococcus aureus* was done following FDA, 2001 protocol. Aureus ChromoSelect agar was used. Estimation of *Salmonella spp.* was done following ISO, 6579; 2002 protocol. Salmonella ChromoSelect agar was used.

Determination of Aflatoxin B1

The sample was prepared based on Yang and An (2011) protocol. Aflatoxin B1 standard was obtained from Sigma Aldrich. A calibration curve was obtained as a linear equation from which the aflatoxin values in the samples were obtained with a coefficient of determination ($R^2 = 0.9986$). The sample was analyzed using HPLC (Shimadzu model LC-20A), with Quaternary pump (LC-20AD), PDA detector (SPD-M20A). Quantification was by multi-wavelength monitoring done with a band width of 4nm and absorbance spectra recorded from 200-400 nm (Yang and An, 2011).

Data Analysis, validation and presentation

The data on nutritional and phytochemical in Moringa powder dried under different conditions were subjected to ANOVA and means separated using protected $LSD_{0.05.}$ All data (nutritional, phytochemical, microbial colonies and moisture content) were presented in tables for ease of comparisons. The results were compared with Kenya Bureau of Standards for black tea (KS 65) recommended values because there are no standards for Moringa powder in Kenya.

Results

Phyto-nutraceutical levels of Moringa powder dried under different conditions

The study illustrated that the method of processing had significant ($P < 0.05$) effect on the nutritional quality of the obtained Moringa powder (Table 1). Direct drying of Moringa leaves in the oven had a negative effect on the labile nutrients such as vitamins (A and C), total polyphenols and terpenoids.

Table 1. Nutritional and volatile phytochemicals in Moringa powder dried under different conditions

	Nutrients					
Drying conditions	**Vit. C (%)**	**Vit.A (%)**	**Protein (%/wt)**	**Total Polyphenols(%/wt)**	**Terpenoids (%/wt)**	**Total Glucosinolates (%/wt)**
Room tempt	5.2 c	0.21 b	26.4 a	22.2 b	10.8 ab	6.8 a
50% shade	4.7 ab	0.19 b	27.5 a	28.4 c	12.7 b	6.4 a
Greenhouse	4.5 ab	0.18 ab	27.1 a	19.7 ab	9.4 a	8.7 b
Oven	3.1 a	0.11a	25.8 a	14.4 a	7.5 a	8.1 b

Values followed by similar letters in a column are not significantly different ($LSD_{0.05}$).

Microbial growth and moisture content of Moringa powder dried under different conditions

The method of drying employed had a bearing on the microbiological quality of the Moringa powder obtained (Table 2). The Moringa powder dried under room conditions had higher yeasts, while more moulds were produced under the 50% shade net suggesting optimal conditions for the growth of yeast. It was also observed that *E. Coli* only proliferated under the 50% shade net conditions and was absent in all the others. *Staphylococcus aureus* was also high in the 50% shade net conditions (Fig. 2).

Table 2. Microbial and moisture content analysis of Moringa powder dried under different conditions

Test	Room conditions cfu/g	50% shade cfu/g	Greenhouse cfu/g	Oven dried cfu/g	Maximum allowable KS 65)
Staphylococcus aureus	0	9.5	0	0	Absent
E. coli	0	4.2	0	0	Absent
Yeast	200	150	40	35	10^2
Moulds	180	275	60	45	10^3
Salmonella	0	0	0	0	Absent
Aflatoxins B1	<1.0	<1.5	<1.0	<1.0	5 ppb
Moisture content %/wt.	8.3	7.8	6.5	6.1	7-8

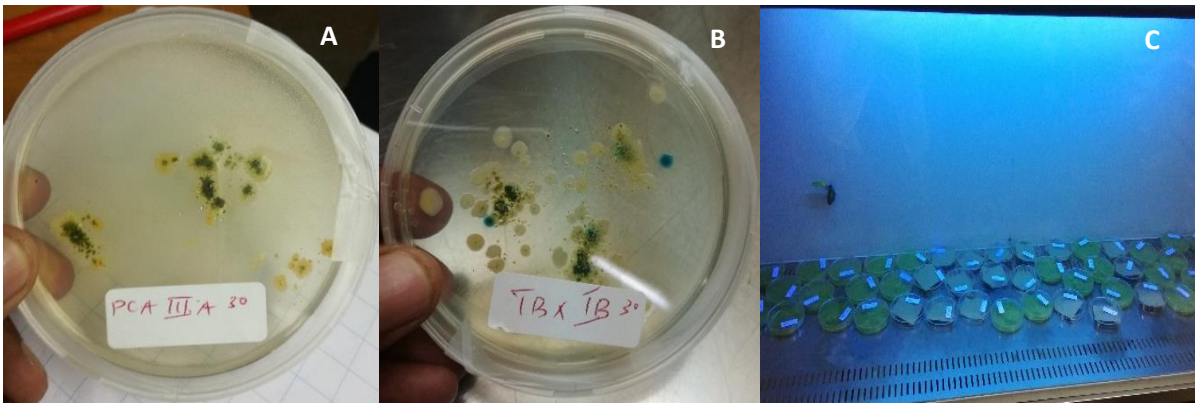

Fig. 2. Fungal growth on Moringa powder (A), bacterial colonies on Moringa powder (B), microbial culturing in biosafety model clean bench

Discussions

Phyto-nutraceutical levels of Moringa powder dried under different conditions

Drying conditions had significant effect on the nutraceutical quality of processed Moringa powder. The significantly ($P < 0.05$) higher levels of total polyphenols and terpenoids in Moringa leaves dried under 50% shade net can be attributed to the gradual loss of surface moisture and preservation of the labile phytochemicals at relatively lower temperature (Baruah et al., 2012; Begum et al., 2009), which indicate that drying Moringa leaves at cooler conditions before eventual drying in the oven can enhance the nutraceutical value of the product. It is also clear that the polyphenols are more enhanced at cooler conditions prevalent under shade net and room temperature. This can be compared to the increase in polyphenols in black tea processing where the level of polyphenols increases during the process of chemical withering that takes place at room temperature for some time. This is because polyphenol oxidase enzymes function best under such conditions provided oxygen is not limiting (Baruah et al., 2012). This is in sharp contrast to the effect of drying under shade net or room temperature as opposed to greenhouse or oven on glucosinolates level. This demonstrated that glucosinolates require to be fixed by prompt drying at optimal temperature. This can possibly be attributed to the loss of surface moisture which took place over the extended drying period at low temperature (Jensen et al., 1995; Song and Thornalley, 2007).

Drying under optimum temperature before processing resulted in physical withering of Moringa leaves, which reduced the moisture content before processing. Pre-drying of the leaves before oven drying reduced the moisture level in Moringa leaves, the estimated moisture before oven drying was about 50-65% for greenhouse, room and shade dried leaves. While, fresh leaves had a moisture content of about 70%. Withering of leaves reduces moisture thus increasing the concentration of nutrients (Omiadze et al., 2014), this was manifested by the difference in

vitamins and phytochemicals from Moringa powder dried at different conditions. Thus, withering can be optimised to enhance the nutraceutical value of Moringa powder.

Microbial growth and moisture content of Moringa powder dried under different conditions

There was a nexus between the level of moisture content and the extent of microbial growth, which was consistent with the findings of Negi and Roy, (2001) that showed drying conditions of *Moringa oleifera* leaves affects the quality of the product. In all the treatments it was noted that there was no growth of the Salmonella bacteria possibly due to the antibacterial activity of the polyphenols in Moringa powder (Kasolo et al., 2010).

Despite of all the treatments showing growth of moulds, the extent of toxins produced was assessed through determination of the levels of aflatoxins i.e. secondary metabolites produced by the moulds (Bargah, 2015). In this case, the level of aflatoxins was within the allowable limits of less than 5 ppb (KS 65). However, when the leaves were dried under 50% shade, the level of aflatoxin was slightly higher (1.5 ppb) than under the other conditions (1.0 ppb), this shows the ease of growth of moulds under such conditions.

The results also showed that glucosinolates impacted microbial growth. Indeed, the high levels of glucosinolates obtained by direct drying of leaves in the oven resulted in the least microbial growth for the treatment. This can be attributed to the antimicrobial activity against microbes (Aires et al., 2009; Sreelatha and Padma, 2009), which is because of antimicrobial activity of glucosinolates on most bacterial and fungal agents. Antimicrobial inhibition is associated with glucosinolates breakdown, whose products impairs necrotrophic fungi and bacteria (Singh and Sharma, 2012). This property of glucosinolates may explain the reducing number of bacterial and fungal growth with increased levels of Glucosinolates as can be seen from the results (Singh and Sharma, 2012). Glucosinolate hydrolysis products and specifically, the isothiocyanates SFN and BITC have significant antimicrobial activity against Gram-positive and Gram-negative bacteria thus could be useful in controlling human pathogens through the diet (Fahey, 2005).

Conclusion

From the study, it is established that the method of drying has a direct effect on the quality of the product. It is important that a balance between conservation of volatile nutrients, microbial growth conditions and cost of dying be considered and optimized to ensure the integrity of the product, which is maintained thorough the processing cycle. Pre-wilting the leaves in the greenhouse achieved the least moisture content in the product, while oven drying immediately after harvesting had the least microbial colonies on the product. Shade dried and room dried leaves had the highest microbial colonies and the highest volatile nutrients too. It is recommended that ideal conditions for pre-drying of Moringa leaves before oven drying enhances the nutraceutical and microbial quality of the product.

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Conflict of Interest

The authors declare no competing interests.

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