

Effect of drying type on the physicochemical and phytochemical properties of young and mature *Moringa oleifera* leaves

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Abstract

Biochemical differences in response to dehydration methods in *Moringa oleifera* leaves were studied in moringa young and mature leaves. Sun drying, oven drying and Shade-drying were applied to leaves samples. After drying, leaves were powdered and subjected to physicochemical and phytochemical analyses. The results showed a lower concentration of physicochemical components in shade-dried moringa leaves excepted moisture content. However, shade-dried *M. oleifera* leaves showed higher phytochemical components than sun and Oven-drying leaves without significant difference ($p < 0.05$) between young and mature leaves. Furthermore, shade-dried drying leaves had better antioxidant activity ($IC_{50} = 0.072$ mg / ml for mature leaves and $IC_{50} = 0.049$ mg / ml for young leaves) than sun and oven-dried leaves. The shade-dried technique would be therefore the most recommended for preserving physicochemical and phytochemical quality. However, the higher moisture content of shade dried leaves could be a source of micro-organism proliferation when the powders are stored.

Keywords: *Moringa oleifera*; Drying; Phytochemicals; Physicochemical; Young Leaves; Mature Leaves.

1. Introduction

Moringa oleifera is a tree belonging to the *Moringaceae* family. It is a popular plant that is widely cultivated in tropical areas [1]. All parts of this tree have important nutritional and medicinal properties. This places it among the world's most important trees [2]. *M. oleifera* leaves are used for their richness in protein, calcium, iron, β carotene (converted to vitamin A in the human body), vitamin C, and vitamin E [3]. They are also a good source of natural antioxidants.

M. oleifera leaves also contain a good number of bioactive compounds, such as coumarins, phenolic acids, tannins, terpenes, and flavonoids [4]. In the medical field, *M.oleifera* leaves are used for the treatment of several diseases due to their therapeutic virtues particularly in the treatment of inflammatory and infectious diseases [5], [6].

In animal feed, *M. oleifera* leaves are used as good-quality feed for cattle and fish [7]. They have positive effects on the growth of captive animals [8] and increased milk production in cows [9]. Incorporating moringa leaf powder in chicken rations has shown a significant improvement in yolk coloration [10].

When it comes to human consumption, *M. oleifera* leaves or their derivatives remain the easiest way to benefit from their nutritional properties. They are used in the preparation of culinary dishes in many cultures because of their slightly spicy, herbaceous flavor. They are added to soups, sauces, and other food preparations to enhance the nutritional value

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and flavor of the food [11]. In some culinary practices, *M. oleifera* leaves are also dried and ground into a fine powder to be added to almost all foods and other cereal grains as a nutritional supplement [3].

Consumption of *M. oleifera* leaves leads to an increase in beneficial intestinal bacteria, reduced intestinal inflammation and improved immune function [12]. In pregnant women, *M. oleifera* leaf consumption leads to improved fetal growth, good lactation, and maternal nutritional status with reduced anemia [11].

In these various fields of application, leaves are often used in powder form. *M. oleifera* leaf, if properly dried, packaged and stored, can help increase its availability. Various drying methods have been studied and evaluated based on leaf quality, including air drying, Oven-drying, microwave drying, vacuum drying, freeze drying. However, according to several works, the type of drying to produce *M. oleifera* powder has a considerable impact on the biochemical composition of the powders [1], [4]. Mansour et al. (2016) concluded that Shade-drying is the most recommended as it preserves physicochemical properties. Similarly, young leaves showed higher antioxidant potential and total phenolic content according to the work of [13]. The aim of this study is to evaluate the effect of drying type on the physicochemical and phytochemical properties of young and mature *M. oleifera* leaves. It will identify the type of drying likely to preserve the nutrients in *M. oleifera* leaves.

2. Materials and methods

2.1. Material

The plant material used in this study consisted of *Moringa oleifera* leaves at different physiological stages (young and mature). The leaves were harvested from a private field in the Azaguié area of Côte d'Ivoire. Young leaves are characterized by a pale green color and mature leaves by a dark green colour.

2.2. Methods

2.2.1. Leaf drying procedures

Three types of drying tests were carried out on mature and young *M. oleifera* leaves during this study: sun-drying, shade-drying and oven-drying. Three hundred grammes (300g) of leaf were weighed for each sample. Sun and Shade-drying was carried out on black plastic tarpaulins measuring 200 x 100 cm. Sun-drying was carried out at a temperature varying between 30° C and 35° C for 4 days. Drying in the shade was carried out at a temperature varying between 28°C and 29°C for 14 days. Oven-drying, were carried out at a controlled temperature of 45° C for 24 hours. A total of six (6) samples were collected and dried. These were young, dried leaves (sheltered from the sun, in the sun and in oven) and mature dried leaves (sheltered from the sun, in the sun and in oven). The various samples were ground and used for analysis in triplicate.

2.2.2. Biochemical analysis

Proximal composition

Proximal composition was determined according to the method [14]. The moisture content of the powders was determined by drying in an oven at 105° C to a constant weight. The ash content was determined by weighing the residue obtained by incineration at 550° C for 8 to 12 hours. The total crude protein content was determined by the Kjeldahl method. Lipid content was determined gravimetrically after Soxhlet extraction. Fiber content was determined after acid boiling under reflux cooling.

Mineral composition

Calcium (Ca) and iron (Fe) contents were determined by atomic absorption spectroscopy after wet mineralization [14].

Determination of Phenolic Compounds

2.3. Extraction method

Sample (10 g) were weighed and placed in 500 ml beakers. One hundred (100) mL of 80% (v/v) ethanol was added. The mixture was homogenized and left to macerate for 2h. The supernatant was filtered through white filter paper and transferred to porcelain plates. The plates containing the extracts were steamed for 48 h. The dry extracts from each porcelain plate were collected and packed in hermetically sealed boxes.

2.3.1. Determination of total polyphenols

Determination of total polyphenols (in mg Eq. A.G / g) was carried out using the method described by [15]. To 1 ml of each, 1/10th diluted ethanolic extract, 1 mL of Folin-Ciocalteu reagent (0.5N) was added. After 3 min, 1 ml of an aqueous sodium carbonate solution (20%, w/v) was added, and the volume adjusted to 10 mL with distilled water. The tube was then placed in the dark for 30 min. Absorbance was read on a UV spectrophotometer (AQUALYTIC AL800, Germany) at 725 nm against blank. Finally, a calibration curve was performed using a range of gallic acid concentrations from 0 to 1 mg / ml. The results were expressed as mg of gallic acid equivalent (GAE) / g of extract.

2.3.2. Total flavonoids assay

Total flavonoids were determined according to the method described by [16]. To a volume of 0.5 mL of each 1:10 diluted extract was successively added 0.5 mL distilled water, 0.5 mL aluminum chloride (10% w/v), 0.5 mL sodium acetate (1 M) and 2 mL distilled water. The contents were then left to stand for 20 min in the dark, and the absorbance was read on a UV spectrophotometer (AQUALYTIC AL800, Germany) at 415 nm against a blank. Finally, a calibration curve was made using a range of quercetin concentrations from 0 to 0.1 mg / ml. Results were expressed as milligrams of quercetin equivalent (QE) / g of extract.

2.3.3. Determination of Condensed Tannins

The determination was carried out according to the method described by [17]. To a volume of 400 µL of each 1/10th diluted extract was added 3 ml of vanillin reagent (3 mL of a 4% methanolic vanillin solution and 1.5 mL of concentrated hydrochloric acid). The tube was then left to stand for 15 min in the dark and the absorbance was read on a spectrophotometer (PG Instruments, UK) at 500 nm against the blank. Finally, a calibration curve was performed using a range of catechin concentrations of 0 to 300 µg/mL. The results were expressed as mg of catechin equivalent (CE) / g of extract.

Total antioxidant capacity

Antioxidant capacity was determined by slightly modifying the method of [18], which uses 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Various quantities of the dissolved samples in methanol were added to 5 ml of a 0.004% DPPH (2,2-diphenyl-1-picrylhybride) methanol solution. After 30 minutes incubation at room temperature, absorbance was read against a blank at 517 nm. Vitamin C was used as a positive control. The percentage of inhibition of DPPH free radicals (I%) was calculated according to the equation below. Linear regression between inhibition percentages and concentrations was used to determine the median inhibitory concentration (IC50).

$$I \% = (\text{Absorbance control} - \text{Absorbance sample} / \text{Absorbance control}) \times 100$$

2.4. Statistical analysis

The results obtained were subjected to an analysis of variance (ANOVA) at a significance level of 0.05 using XLSTAT software (version 19.6). The Tukey Test was used to determine significant differences between samples.

3. Results

3.1. Physicochemical Characteristics

Table 1 shows the physicochemical characteristics of young and mature *M. oleifera* leaves after applying different drying techniques. The biochemical parameters measured are moisture, ash, minerals (iron and calcium), fiber, and protein content. The results obtained revealed significant differences ($p < 0.05$) only between the different drying techniques on all the different biochemical parameters studied. There were no significant differences ($p > 0.05$) between drying techniques in terms of leaf physiology. The highest moisture content was observed in young and mature leaves ($p < 0.05$) was observed after drying in the shade ($14.45 \pm 0.51\%$ for mature leaves and $14.69 \pm 0.07\%$ for young leaves) and the lowest in sun- and oven dried samples ($10.01 \pm 0.11\%$ for mature leaves and $10.05 \pm 0.07\%$ for young leaves). Conversely, in the shade, other components such as ash, fiber, protein, calcium and iron were higher ($p < 0.05$) in oven-dried leaves than in sun-dried ones.

3.2. Photochemical Characteristics

Table 2 shows the phytochemical compounds of young and mature *M. oleifera* leaves. The polyphenol, flavonoid and tannin content of young and mature leaves is significantly different ($p < 0.05$) according to the drying method applied and the physiological nature of the leaf.

Table 1 Physicochemical characteristics of young and mature *M. oleifera* leaves

Parameters	Drying type					
	Shade-drying		Sun-drying		Oven-drying	
	FM	FJ	FM	FJ	FM	FJ
Moisture content (%)	14.45±0.51 ^a	14.69±0.07 ^a	10.35±0.02 ^b	10.43±0.03 ^b	10.01±0.11 ^c	10.05±0.07 ^c
Ash (%)	4.87±0.02 ^c	4.82±0.05 ^c	8.75±0.07 ^b	8.62±0.04 ^b	9.84±0.08 ^a	9.63±0.10 ^a
Fiber (%)	6.06±0.02 ^c	5.82±0.65 ^c	9.20±0.83 ^b	9.14±0.33 ^b	10.51±0.02 ^a	10.23±0.74 ^a
Protein (%)	24.32±0.04 ^c	24.96±0.14 ^c	26.70±0.19 ^b	26.51±0.14 ^b	28.59±0.19 ^a	28.36±0.03 ^a
Calcium(mg/100g)	1181.68±2.60 ^c	1139.267±2.31 ^c	1605.51±2.36 ^b	1599.05±2.64 ^b	1683.87±2.80 ^a	1671.32±2.70 ^a
Iron (mg/100g)	34.37±3.21 ^c	33.85±3.02 ^c	37.56±2.27 ^b	37.29±2.60 ^b	42.06±2.42 ^a	41.83±1.43 ^a

Values in the same line are assigned the same letter and are not significantly different according to the Tukey test at the 5% threshold. Values are expressed as Mean ± Standard deviation (n=3 trials), FM= mature leaves; FJ= young leaves.

Table 2 Phytochemical components of young and mature *M. oleifera* leaves

Parameters	Drying type					
	Shade-drying		Sun-drying		Oven-drying	
	FM	FJ	FM	FJ	FM	FJ
Polyphenol (mg EAG/g)	159.93±0.10 ^a	148.84±1.95 ^b	137.26±0.09 ^c	133.77±0.55 ^d	127.95±0.70 ^e	122.33±0.11 ^f
Flavonoids (mg EQ /g)	64.61±0.11 ^a	63.58±0.08 ^b	42.64±0.04 ^c	40.17±0.05 ^d	39.54±0.07 ^e	38.78±0.03 ^e
Tannins (mg EC /g)	11.98±0.6 ^a	11.58±0.02 ^b	11.40±0.09 ^c	11.37±0.05 ^d	5.76±0.07 ^e	5.61±0.03 ^e

Values in the same line are assigned the same letter and are not significantly different according to the Tukey test at the 5% threshold. Values are expressed as Mean ± Standard deviation (n=3 trials), FM= mature leaves; FJ= young leaves.

Polyphenol (159.93 ± 0.10 mg EAG/g and 148.84 ± 1.95 mg EAG/g), flavonoid (64.61 ± 0.11 mg EQ/g and 63.58 ± 0.08 mg EQ/g) and tannin (11.98 ± 0.6 mg EC/g and 11.58 ± 0.02 mg EC/g) respectively of young and mature shade-dried *M. oleifera* leaves were significantly higher ($p < 0.05$) than in leaves dried by other techniques. The lowest levels of these compounds were observed in oven-dried leaves. With this drying technique, the contents of flavonoid polyphenol and tannin were respectively 127.95 ± 0.70 mg of EAG/g; 39.54 ± 0.07 mg EQ /g and 5.76 ± 0.07 mg of CE / g for mature leaves and 122.33 ± 0.11 mg of EAG/g ; 38.78 ± 0.03 mg EQ /g ; 5.61 ± 0.03 mg of CE / g for young leaves.

3.3. Antioxidant Activity

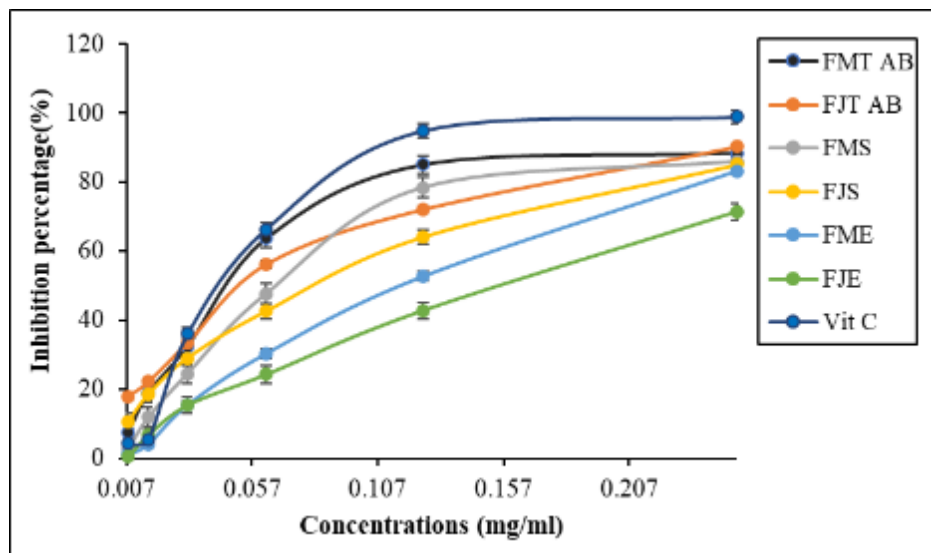


Figure 1 In vitro DPPH free radical scavenging activity of *M. oleifera* leaves

Vit C: vitamin C; FMT AB: mature leaves dried in the shade, FJT AB: young leaves dried in the shade, FMS: mature leaves sun dried, FJS: young leaves sun-dried, FME: mature leaves oven dried, FJE: young leaves oven-dried.

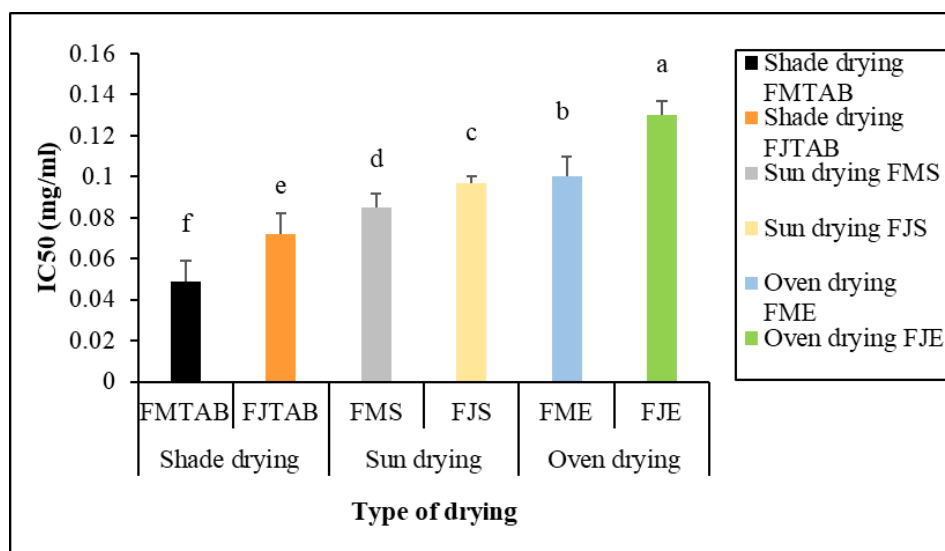


Figure 2 IC₅₀ of *M. oleifera* leaves

FMT AB: mature leaves dried in the shade, FJT AB: young leaves dried in the shade, FMS: mature leaves sun dried, FJS: young leaves sun-dried, FME: mature leaves oven dried, FJE: young leaves oven-dried.

The antioxidant activity of sun-dried, shade-dried and oven-dried young and mature leaves is shown in Figures 1 and 2 respectively. Antifree radical activity profiles show that sun-dried young and mature dried in the shade have higher antioxidant activities than sun- and oven-dried leaves. However, these activities are lower than the standard (vitamin

C). Furthermore, the 50% DDPH free radical inhibitory concentration (IC₅₀) in this shade-dried young and mature leaves (0.072 ± 0.01 mg / ml and 0.049 ± 0.01 mg/ml) are lower than those in sun and oven-dried young and mature leaves.

4. Discussion

Variations in moisture in the different drying techniques are related to the difference in temperature to which the leaves were subjected. In fact, the higher the temperature, the more water the leaves lose. These results agree with those of [19], who after drying the *M. oleifera* leaves at different temperatures observed a drop in the moisture content of the *M. oleifera* leaves with increasing temperature. Low moisture content in foods reduce their microbial load and extends their shelf life during storage [20]. For example, oven-dried leaves will tend to keep longer than sun- and shade-dried leaves.

Drying further reduces water content and increases dry matter content. The protein, lipid, fiber, calcium, and iron contents of dried leaves were higher with increasing drying temperature. This increase was also observed by [21]. In their study, the protein, ash, and carbohydrate content of sun-dried *M. oleifera* leaves was higher than those of shade-dried leaves, indicating that Oven-drying with a high heat input (45°C) optimized the contents of physicochemical compounds compared with sun-drying (30-35°C) and Shade-drying (28-29 C).

In contrast to the high content of physicochemical compounds, concentrations of phytochemical compounds were lower in oven-dried leaves than in sun-dried ones. This is because phenolic compounds are thermosensitive. The lower the temperature used, the better these compounds are preserved [1]. These results corroborate those obtained by [22] on basil leaves. Like polyphenols, flavonoid and tannin concentrations were lower in high-temperature dried leaves. In general, the presence of these phytochemical compounds could explain the advertised medicinal properties of these leaves in various diseases such as atherosclerosis, arthritis, diabetes, nausea, asthma, skin antiseptic, diarrhea, dysentery, colitis and cancer [23].

The low concentration of phytochemicals has a negative impact on leaves with a significant reduction in antioxidant activity [1]. As found in this study, the antioxidant activity of *M. oleifera* leaves dried at relatively higher temperature (>30°) was the lowest. Phenolic compounds are strongly involved in antioxidant activity. Thus, altering their concentration strongly impacts antioxidant activity [21], [22].

5. Conclusion

At the end of this study, we note that the type of drying type influences the biochemical quality of *M. oleifera* leaves. Shade-drying remain the best type to preserves the phytochemical properties of *M. oleifera* leaves. This study is of particular interest to consumers and manufacturers alike. It will also serve as a guide to good moringa leaf-drying practice and gives some pointers on how to preserve the leaves. However, Shade-drying showed a higher moisture content, which could be a source of micro-organism proliferation when the powders are stored.

Compliance with ethical standards

Disclosure of conflict of interest

All authors have no conflict of interests to declare.

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