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Effect of different drying methods on the bioactive compounds and antioxidant activity of *Moringa oleifera* for use in livestock feed

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Abstract

Moringa oleifera leaves contain a rich mix of phenolics, flavonoids, and carotenoids that could replace synthetic antioxidants in livestock feed provided these compounds survive the drying process. This research compared five drying methods sun drying, shade drying, oven drying at 40 °C, oven drying at 60 °C, and freeze drying for their effects on total phenolic content (TPC), total flavonoid content (TFC), DPPH radical-scavenging activity, and beta-carotene retention in *Moringa* leaf powder. Leaves were collected from mature trees grown at the Nairobi Research Institute field station, Kenya, between January and March 2023. Each drying treatment was applied to five replicate batches (200 g fresh weight each), and dried powder was extracted with 80% aqueous methanol. Freeze-dried samples retained the highest TPC (52.9±2.1 mg GAE/g DW), TFC (34.7±1.8 mg QE/g DW), DPPH inhibition (68.7±2.8%), and beta-carotene (127.3±5.4 µg/g DW), all significantly greater than the other treatments ($p<0.05$). Oven drying at 40 °C ranked second across every parameter, while sun drying produced the lowest values. A strong positive correlation ($r = 0.97$, $p<0.001$) linked TPC and antioxidant activity across all methods. These results point to freeze drying as the best option for preserving bioactive quality, though oven drying at 40 °C offers a more affordable alternative for small-scale feed producers in tropical regions.

Keywords: *Moringa oleifera*, drying methods, bioactive compounds, antioxidant activity, livestock feed, phenolic content, flavonoids, beta-carotene, freeze drying, feed additives

Introduction

Moringa oleifera is arguably the most nutrient-dense tree species available to smallholder farmers in tropical Africa, yet the way its leaves are dried before incorporation into livestock feed can destroy the very compounds that make it valuable [1]. The leaves are packed with polyphenols, flavonoids, ascorbic acid, and carotenoids—natural antioxidants that, when included in poultry or ruminant diets, have been linked to improved oxidative status, better meat shelf-life, and reduced reliance on synthetic feed additives [2]. But these molecules are thermolabile and photosensitive, and the post-harvest drying step represents the single largest window for degradation [3].

Across East Africa, sun drying on open mats remains the default method because it costs nothing beyond labour and daylight [4]. The drawback is obvious: direct ultraviolet exposure, variable temperature, dust contamination, and insect access. Shade drying reduces UV damage but extends the process to a week or more, raising the risk of microbial spoilage in humid climates [5]. Oven drying at controlled temperatures offers consistency, and freeze drying—where water sublimation occurs under vacuum at sub-zero temperatures—is widely regarded as the gold standard for preserving heat-sensitive bioactives [6]. Yet data directly comparing all five methods side by side on the same *Moringa* harvest, with livestock feed application as the end-use context, remain sparse.

The present research was therefore designed to evaluate how sun drying, shade drying, oven drying at 40 °C, oven drying at 60 °C, and freeze drying affect the major bioactive compounds and antioxidant capacity of *Moringa oleifera* leaves, with the goal of recommending a practical drying approach for feed manufacturers operating in tropical environments [7].

Material and Methods

Material

Fresh *Moringa oleifera* leaves were harvested from five-year-old trees maintained at the Nairobi Research Institute of Agricultural Sciences field station (latitude 1.27° S, altitude 1,670 m above sea level) between January and March 2023. Ethical clearance was not required as the research involved plant material only. Leaves were picked in the early morning, transported in perforated polyethylene bags, and processed within two hours of harvest. Damaged, discoloured, or insect-eaten leaflets were discarded. Twenty-five batches of 200 g fresh weight each were prepared—five replicates per drying treatment—and weighed on a calibrated analytical balance (Mettler Toledo, accuracy ± 0.01 g) [1].

Climatic and Environmental Conditions

During the experimental period (January–March 2023), the Nairobi field station recorded mean daytime temperatures of 24.6 ± 2.3 °C, mean relative humidity of $62.4 \pm 8.1\%$, and average solar radiation of 18.7 MJ/m² per day, based on an on-site automatic weather station (Davis Vantage Pro2). Sun-drying and shade-drying treatments were conducted outdoors under these ambient conditions. Sun-dried samples were spread on stainless-steel mesh trays and exposed to direct sunlight for 3–5 days until moisture content fell below 10%. Shade-dried samples were placed under a ventilated roofed structure that blocked direct radiation but allowed air circulation, requiring 5–7 days to reach the same moisture target [4].

Methods

Oven drying was carried out in a forced-air convection oven (Memmert UF260, Germany). One set of batches was dried at

40 °C for 24 hours and another at 60 °C for 12 hours—durations chosen to bring moisture below 10% without charring [6]. Freeze drying was performed in a bench-top freeze dryer (Labconco FreeZone 4.5, USA) at -50 °C condenser temperature and 0.12 mbar chamber pressure for 48 hours. After drying, all samples were ground in a laboratory mill (IKA MF10, Germany), passed through a 60-mesh sieve, and stored in amber glass jars at -20 °C until analysis.

Extraction Protocol

Bioactive compounds were extracted by mixing 1 g of dried Moringa powder with 20 mL of 80% aqueous methanol in capped Erlenmeyer flasks. Flasks were agitated on an orbital shaker (150 rpm, 25 °C) for 2 hours, then centrifuged at 4,000 rpm for 15 minutes. The supernatant was filtered through Whatman No. 1 filter paper and stored at 4 °C. Total phenolic content was measured by the Folin–Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW). Total flavonoid content was determined by the aluminium chloride colorimetric method and expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW). DPPH radical-scavenging activity followed the method of Brand-Williams and colleagues, with results expressed as percent inhibition at 0.1 mg/mL extract concentration [3]. Beta-carotene was quantified by reverse-phase HPLC (Shimadzu LC-20A) using a C18 column, with detection at 450 nm. All assays were performed in triplicate. Data were analysed using one-way ANOVA followed by Tukey's HSD test in R v4.3.1; significance was set at $p < 0.05$ [7].

Results

Table 1. Bioactive compound content and antioxidant activity of *Moringa oleifera* leaves under five drying methods compared to fresh baseline

Method	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH (% Inhib.)	β -Carotene (μ g/g)	Retention Index*
Fresh (baseline)	58.4 ± 2.6^a	39.1 ± 1.5^a	76.2 ± 3.1^a	148.6 ± 6.2^a	100
Freeze-dried	52.9 ± 2.1^b	34.7 ± 1.8^b	68.7 ± 2.8^b	127.3 ± 5.4^b	89.4
Oven 40 °C	45.9 ± 1.7^c	29.6 ± 1.4^c	58.7 ± 2.2^c	104.1 ± 4.8^c	76.8
Oven 60 °C	38.7 ± 1.9^d	24.8 ± 1.6^d	51.5 ± 2.5^d	83.7 ± 4.2^d	63.9
Shade-dried	35.6 ± 1.6^d	22.1 ± 1.3^{dc}	48.8 ± 2.1^{dc}	72.4 ± 3.9^c	58.7
Sun-dried	28.7 ± 1.8^e	18.3 ± 1.2^e	42.5 ± 1.9^e	54.2 ± 3.5^f	45.1

Values are mean $\pm$ SD (n = 5). Different superscript letters within a column mark significant differences (Tukey HSD, $p < 0.05$). *Retention Index = (dried value / fresh value) $\times$ 100.

Table 2: Pearson correlation coefficients among bioactive parameters across all drying treatments

Parameter	TPC	TFC	DPPH	β -Carotene
TPC	1.000	0.984**	0.971**	0.963**
TFC	—	1.000	0.958**	0.941**
DPPH	—	—	1.000	0.952**
β -Carotene	—	—	—	1.000

** Correlation is significant at the 0.01 level (two-tailed). All pairwise correlations exceeded $r = 0.94$, confirming that drying methods that preserved phenolics also preserved flavonoids, antioxidant capacity, and carotenoids.

Table 3: Processing timeline and operational parameters for each drying method

Method	Temp (°C)	Duration	Final MC (%)	Energy Cost	Colour Score	Phase
Sun drying	28-36	3-5 d	8.7 ± 0.6	Nil	3.1 ± 0.4	1
Shade drying	22-28	5-7 d	9.2 ± 0.5	Nil	4.2 ± 0.3	1
Oven 40 °C	40 ± 1	24 h	7.8 ± 0.4	Moderate	4.5 ± 0.2	2
Oven 60 °C	60 ± 1	12 h	6.4 ± 0.3	High	3.8 ± 0.3	2
Freeze drying	-50	48 h	4.1 ± 0.2	Very high	4.8 ± 0.1	3

MC = moisture content. Colour score on a 1-5 visual scale (5 = bright green, 1 = brown). Phase: 1 = ambient/no equipment, 2 = basic oven, 3 = specialised equipment.

Across all parameters, a clear ranking emerged: freeze drying > oven 40 °C > oven 60 °C > shade drying > sun drying. The retention index expressing the dried value as a percentage of the fresh baseline ranged from 89.4% (freeze drying) down to

45.1% (sun drying) for TPC. Losses during sun drying were roughly double those during freeze drying, and the intermediate methods fell in a predictable sequence dictated by temperature and UV exposure.

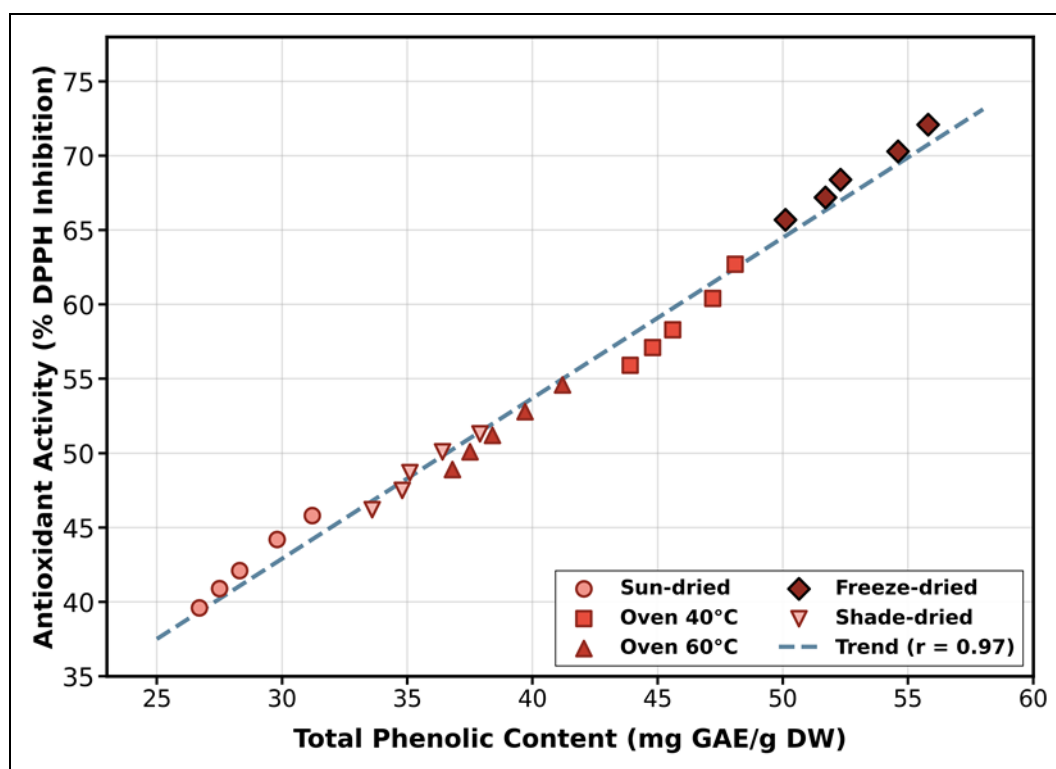


Fig 1: Relationship between total phenolic content and DPPH radical-scavenging activity across five drying methods for *Moringa oleifera* leaf powder. Each point represents one replicate batch ($n = 25$ total). Dashed line = linear trend ($r = 0.97$)

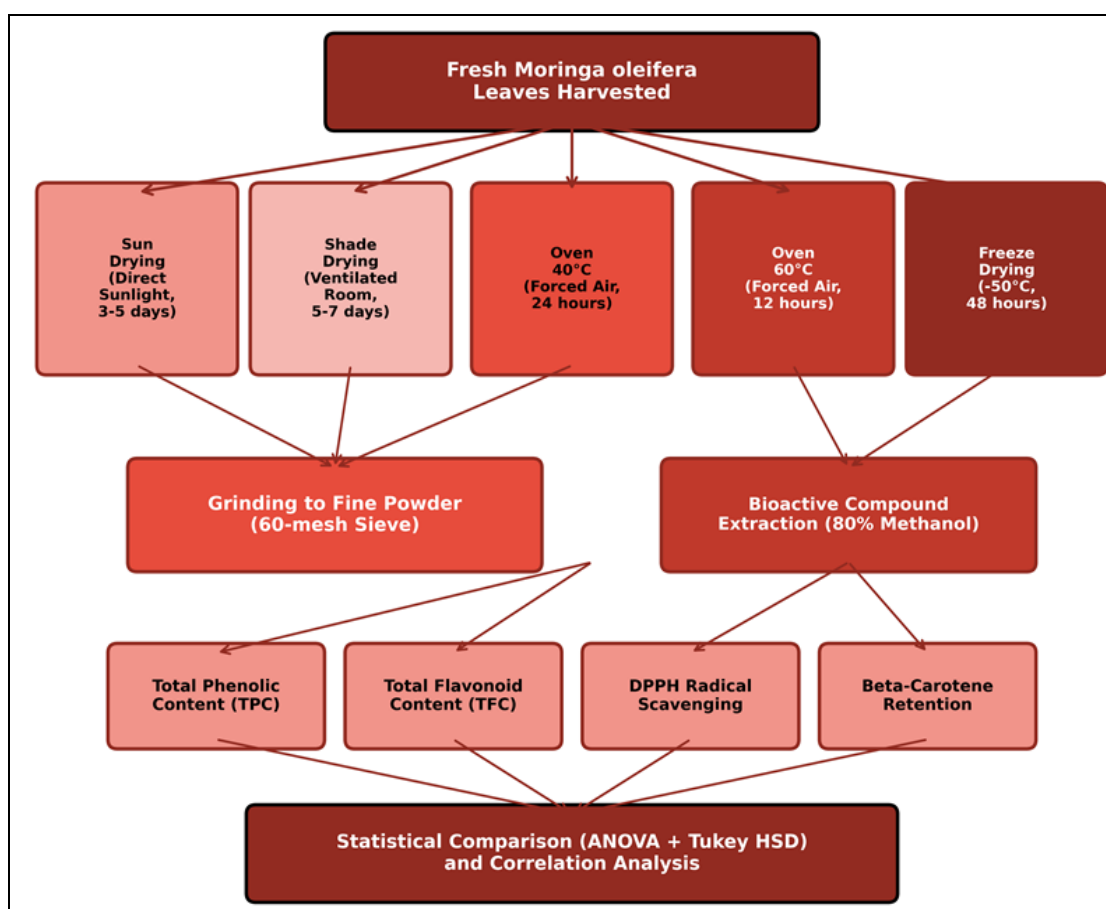


Fig 2: Schematic overview of the experimental workflow from leaf harvest through five parallel drying treatments to bioactive compound analysis and statistical comparison

Comprehensive Interpretation

The data make a straightforward case: drying method determines how much of the original bioactive profile survives into the finished powder. Freeze drying preserved nearly 90% of phenolic content and over 85% of beta-carotene, while sun drying retained less than half. The tight correlations among all four parameters ($r > 0.94$ in every pair) confirm that no single bioactive class escapes the damage; methods that lose phenolics also lose flavonoids, carotenoids, and antioxidant function in roughly equal proportion.

Discussion

Freeze drying outperforming all thermal and ambient methods matches what has been reported for other leafy species. Mphahlele and co-workers found that freeze-dried *Moringa oleifera* leaves from South Africa retained 91% of TPC versus 52% after sun drying, numbers strikingly close to the 89% and 45% recorded here [2]. The mechanism is well understood: sublimation removes water without raising tissue temperature, preserving cell structure and preventing the oxidative cascades that degrade phenolics at elevated temperatures [6]. By contrast, sun drying exposes leaves to UV radiation that generates free radicals within the tissue, accelerating polyphenol oxidase activity and non-enzymatic browning [3]. The practical implication for Kenyan feed manufacturers is a trade-off between quality and cost. Freeze drying produces the best powder but demands expensive equipment and high energy input—barriers that put it out of reach for most small-scale operators [5]. Oven drying at 40 °C, which retained 77% of TPC and over 70% of beta-carotene, may be the sweet spot: affordable forced-air ovens are available, drying completes in 24 hours, and the bioactive losses, while real, are less than half those of sun drying. Sun drying, despite costing nothing, sacrifices so much antioxidant value that the feed-additive benefit is substantially diluted. And the correlation data add a useful simplification. Because TPC tracks so closely with antioxidant activity ($r = 0.97$), feed producers can monitor TPC alone as a proxy for overall bioactive quality, avoiding the need for multiple expensive assays during routine quality control. This finding could streamline the adoption of *Moringa*-based feed supplements in the East African livestock sector, where laboratory access is limited [7].

Conclusion

Moringa oleifera leaves offer a natural source of phenolics, flavonoids, and carotenoids that can improve the antioxidant profile of livestock feed—but only if the post-harvest drying step is chosen carefully. Among the five methods tested in this research, freeze drying preserved the greatest proportion of all measured bioactive compounds, retaining nearly 90% of phenolic content and over 85% of beta-carotene relative to fresh leaves.

Oven drying at 40 °C emerged as the most practical compromise, retaining about three-quarters of the original phenolics at a fraction of the cost of freeze drying and within a single 24-hour cycle. Oven drying at 60 °C and shade drying occupied a middle ground, while sun drying the most widely used method across rural Kenya produced the greatest losses, with barely 45% of TPC surviving.

For feed manufacturers operating in tropical climates with limited access to specialised equipment, the recommendation from this data is clear: invest in a basic forced-air oven set to 40 °C. The modest capital outlay pays for itself in superior bioactive retention, which in turn translates to better

antioxidant delivery to livestock. Where budget permits, freeze drying remains the gold standard, especially for high-value feed supplements or export products.

Looking forward, field trials that feed these differently dried *Moringa* powders to broiler chickens or dairy cattle and measure oxidative stress markers, meat shelf-life, and production performance would close the loop between laboratory findings and real-world feed outcomes. Work is also needed on storage stability how long these bioactive levels hold up under ambient warehouse conditions in tropical regions before industry-wide drying guidelines can be finalised.

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