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EFFECT OF PROCESSING METHODS ON THE PROXIMATE AND PHYTOCHEMICAL CONSTITUENTS OF *MORINGA OLEIFERA* (LAMARCK, 1785) LEAVES

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ABSTRACT
Due to the presence of active phytochemical metabolites, *Moringa oleifera* a fast-growing deciduous plant provides protein, vitamins, and amino acids that enhance growth and treat illnesses. The aim of the study was to compare the proximate and phytochemical composition of *Moringa oleifera* leaves after air and sun drying. The leaves were sourced from Millennium City, Kaduna, Nigeria and sent to Kaduna State University's Biological Sciences Department for identification and authentication. A specimen number; KASU/BSH/754, was assigned and recorded. The leaves were divided into two groups and treated to air drying and sun drying after being cleaned in water to remove dust. The leaves were kept in a well-ventilated room at a temperature of 25±2^{o}C for six days. Leaves were also exposed to direct sunshine for 3days. With a mortar and pestle, the treated leaves were pulverized before being analyzed for proximate and phytochemical components using standard techniques. The proximate analysis was evaluated using a method developed by the Association of Analytical Chemist. The proximate analysis of air dried leaves recorded 6.58±0.32 moisture content, 12.09±0.44 ash content, 24.5±0.62 crude protein, 8.70±0.35 crude lipid, 12.5±0.60 crude fibre and 35.7±2.45 carbohydrate. While sundried leaves produced 7.59±0.33 moisture content, 13.76±0.86 ash content, 24.4±0.13 crude protein, 8.98±0.18 crude lipid, 10.7±0.49 crude fibre and 34.6±0.65 carbohydrate. The qualitative analysis of the phytochemical constituents shows that there is high concentration of phytosterol, alkaloid and saponin, moderate concentration of flavonoid and low concentration of tannin for air dried leaves. While sundried leaves produced high concentration of only alkaloid and flavonoid. And low concentration of phytosterol, saponin and tannin. Statistical analysis shows that air drying is the most suitable method of processing *Moringa oleifera* leaves.

Keywords: *Moringa, Proximate, Phytochemical, Air Dry, Sun Dry*.

INTRODUCTION

The most frequently grown species of the thirteen-species-strong tropical flowering plant family Moringaceae is *Moringa oleifera* (Lam.) Native to South Asia. *Moringa oleifera* grows in the foothills of the Himalayas from North-Eastern Pakistan to North-Western Bengal, India. Other regions of the world, including East and West Africa, the species was also introduced (Paliwal et al., 2011). However, the *Moringa oleifera* tree is a diminutive, quickly-growing evergreen or deciduous tree that typically reaches heights of 10 or 12 m. It features a feathery foliage with tripinnate leaves, an open, spreading crown, and thick, corky, and whitish bark (Zaku *et al.,* 2015). Traditional uses for every part of the species vary, although leaves are typically the most utilized(Sivasankari *et al.*, 2014).

Although all of the plant's parts have purportedly been used alone or in combination with other plants to treat a variety of illnesses and disorders, *Moringa* is said to be particularly rich in medicinal qualities (Pamok *et al.*, 2011).The leaves are utilized to cure a variety of conditions in traditional medicine, including diabetes, hypertension, genito-urinary problems, arthritis, swellings, wounds, typhoid fever, parasite illnesses, and malaria (Leone *et al.*, 2015). In addition to treating HIV/AIDS-related illnesses, they are also utilized to induce lactation and strengthen the immune system (Popoola and Obembe, 2013; Sivasankari et al., 2014).
The imperative to establish an appropriate processing procedure is necessitated by the presence of some active phytochemical metabolites in *Moringa* leaves, some of which may be lost during drying.
The aim of the study was to evaluate the effect of different processing methods on the proximate contents and phytochemical constituents of *Moringa* leaves so as to determine the most appropriate drying method which will minimize loss of nutrients.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in the Chemistry Laboratory of Food Technology Department, Kaduna Polytechnic, Kaduna.

Sample Collection

Moringa oleifera leaves were collected from New Millennium city Kaduna, Nigeria. In order avoid the effect of soil variation on the micro nutrient contents, leaves were obtained from the same tree and then taken to Biological Sciences Department for identification and authentication. Specimen number; KASU/BSH/754 was assigned and documented.

Preparation of *Moringa oleifera*

The leaves were washed with distilled water to remove dust, divided into two groups and each was subjected to air drying and sun drying.

Drying Methods

The drying methods used in the present study were
(i) Sun drying
(ii) Air drying.

Sun Drying

The leaves were spread out on a cotton sheet and positioned on a roof in direct sunshine. They were transferred indoor at night when the temperature decreases and occasionally turned to guarantee even drying since rapid temperature changes could put moisture back into the leaves and prolong the drying process. The leaves

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dried in three days (Pallavi and Dipika, 2010).

Air Drying

The leaves were spread out on a piece of cloth and kept at a temperature of 25 ± 2^0C for six days in a well-ventilated space indoor. The leaves were dried in the air using natural air currents and took six days to dry entirely before becoming crisp and brittle to the touch.

Proximate analysis of the processed *Moringa oleifera* leaves

The Association of Analytical Chemist's (AOAC, 2010) method was used to undertake quantitative studies of the various macronutrients of the processed *Moringa oleifera* leaves. Moisture content, ash, crude protein (also known as Kjeldahl protein), crude lipid, crude fiber, and nitrogen-free extracts were among the components of *M. oleifera* leaves that have been identified (Digestible Carbohydrates).

Moisture content

All other nutrients are converted to a dry matter basis using moisture determination. At 100°C and a pressure of 100 mmHg, a sample containing 2g of dry matter was dried to a constant weight. The desiccators held the dry samples as they cooled and were reweighed. Moisture was cited as the cause of weight loss which was achieved by taking the weight of the sample before and after drying. The following formula, created by AOAC (2010), was used to determine moisture content:

Moisture (%) = initial mass – final mass ×100.

Ash content

Samples were weighed into crucibles and cooked for three hours at 100^{o}C in a moisture extraction oven. It spent around 6 hours in a muffle furnace being heated until it turned powdered white. Ash = initial mass - final mass 100/initial mass was used to calculate the final mass after it had been cooled and placed in a desiccator for additional cooling at ambient temperature (AOAC, 2010).

Crude protein

The Micro Kjeldahl Procedure as suggested by AOAC (2010) was adopted. In a heating tube, two grams of each sample were combined with 10 milliliters of concentrated sulfuric acid. The tube was filled with one table of selenium catalyst, and the mixture was heated in a fume cupboard. The digest is released and interacts with distilled water and 40% sodium hydroxide. The mixture was distilled and the distillate was collected into 2% boric acid and quantified by titration against 0.2m hydrochloric acid. Crude protein was calculated by multiplying the nitrogen content by 6.26.

This is given as

Percentage nitrogen = (100×N×14×VF)T. 100×Va.

Where N= normality of the titrate (0.1N)

VF= total volume of digest= 100ml, T= Titre value, Va= Aliquot volume distilled.

Lipid content

The Soxhlet technique was used to extract the lipid from the samples in order to calculate the crude lipid content. One gram (1g) of the sample was placed loosely in the cellulose thimble, and 150ml of acetone solvent was used to extract it. In a water bath, the device was heated for 5hrs after which the solvent was evaporated using rotary evaporator. The flask was placed in a drying oven for 1hr to remove water. After cooling, the flask was weighed and the lipid content was calculated using:

Lipid content (gm) = final flask mass – initial flask mass × 100 (AOAC, 2010).

Crude fibre content

Two grams of the sample and one gram of asbestos were added to 200ml of 1.25% sulphuric acid and the mixture was then heated for 30 minutes. After that, the solution was added to a Buchner funnel lined with muslin material. After filtering, the residue was added to 200ml of boiling NaOH and left to boil for 30 minutes. This was again filtered and washed with alcohol. The Crude fibre content was then calculated using the formula below as adopted by AOAC (2010).

Fibre (mg) = initial mass – final mass ×100 Initial mass

Nitrogen free extract (NFE)

The nitrogen free extract method as described by (AOAC 2010) was used. The carbohydrate is calculated as weight by difference between 100 and the summation of other proximate parameters as:

NFE = 100 – (Protein + Moisture + Fat + Ash + Fibre)

Phyto-Chemical screening:

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide).Formation of brown/reddish precipitate indicates the presence of alkaloids (Tulika and Mala, 2017).

Detection of Saponins

Foam Test was used to make the determination. It involved shaking 0.5gm of extract with 2ml of water. Saponins are present when foam forms and lasts for 10 minutes (Brijesh *et al*., 2023).

Detection of phytosterols

Salkowski's Test was used to identify phytosterol, the sample was treated with chloroform before filtering of the extracts. A few drops of concentrated sulfuric acid were added to the filtrates, which were then agitated and left to stand. The presence of phytosterols is indicated by a golden yellow appearance (Kokilananthan *et al*., 2022).

Detection of tannins

Gelatin Test was used to make this determination. It involved adding 1% sodium chloride-containing gelatin solution to the extract. The presence of tannins is shown by the formation of white precipitate (Subhendu *et al*., 2022).

Detection of flavonoids

Lead acetate Test: Extracts were treated with a few drops of lead acetate solution in order to identify it. The presence of flavonoids is indicated by the precipitate's yellow color (Abdullahi and Mainul, 2020)

RESULTS

Proximate Constituents of *Moringa oleifera* Leaves Subjected to Air and Sun Drying

Proximate analysis of *Moringa oleifera* leaves subjected to two different drying methods shows that there is high crude fibre, carbohydrate and protein content in air dried leaves than sun dried. It also indicated that there is high ash content and crude fat in sun

dried than the air dried leaves (Table 1).

Table 1: Proximate Contents of *Moringa oleifera* Leaves Subjected to Air and Sun Drying

Sample	Moisture content	Ash content	Protein content	Crude fat	Crude fibre	Carbohydrate
A	6.58±0.32^{a}	12.09±0.44^{b}	24.5±0.62^{c}	8.70±0.35^{d}	12.5±0.60^{e}	35.7±2.45^{f}
B	7.59±0.33^{a}	13.76±0.86^{b}	24.4±0.13^{c}	8.98±0.18^{d}	10.7±0.49^{e}	34.6±0.65^{f}
p value	0.090	0.134	0.852	0.429	0.080	0.598

Values are given as mean ± standard deviation. In each column, mean values with different superscripts have statistically significant difference ($p < 0.05$)

Key: Sample **A** – Air dried leaves; Sample **B** – Sun dried leaves

Qualitative Phyto-chemicals of *Moringa oleifera* leaves subjected to Air and Sun drying

Table 2 presents the qualitative anti-nutrient composition of *Moringa oleifera* leaves subjected to two drying techniques used in this research. Saponin and Phytosterol composition was observed to be high in sample A (Air dried treatment) than sample B (sun dried treatment). Flavonoid composition in sample B is higher than that of sample A. While Alkaloids composition in both treatments were observed to be the same (+++).

Table 2: Qualitative Phyto-chemicals of *Moringa oleifera* leaves at Different Drying Methods

Photochemical	Sample A (air drying)	Sample B (sun drying)
Tannin	+	+
Flavonoids	++	+++
Saponin	+++	+
Alkaloids	+++	+++
Phytosterol	+++	+

KEY:– :- Absent, + :- Low Concentration, ++ :- Moderate Concentration, +++ :- High Concentration

DISCUSSION

Sun-dried leaves' low levels of crude fiber, carbohydrate, and protein indicated that the value of macronutrients declines with rising temperatures. This outcome is consistent with that of Mongi *et al*. (2017), who investigated the impact of solar drying on the nutritional content of tomatoes and came to the conclusion that heat lowers the nutritional value of tomatoes as temperature rises. The stability of the bonds involved in macro nutrients may also be responsible for their considerable reduction during drying. According to Morris et al. (2004), the application of heat caused losses of these macronutrients, particularly protein. The results of Pallavi and Dipika (2010), who studied the effects of dehydration on the nutritional value of drumstick leaves, are in agreement with the finding that the greater lipid contents in sun dried treatments could not be classified as a good source of lipid.

According to Yi-Jui *et al*. (2020), who investigated the impact of thermal processing on the saponin profile of *Momordica charantia* L. and found that saponin content decreases with increasing temperature, the high content of saponin and alkaloids observed in air dried leaves as compared to that of sun dried treatment is consistent with this finding. According to Tan *et al*. (2014), thermal treatment reaching Bitter gourd's total saponin concentration would drop at 40^{o}C.

The high concentration of flavonoids found in sun-dried leaves is consistent with Hind *et al*. (2017) findings, which examined the impact of heat processing on six flavonoids' thermal stability and antioxidant activity and came to the conclusion that some flavonoids are heat-resistant. While low levels of phytosterol were found in sun-dried leaves, Soupas *et al*. (2004), who studied the effects of sterol structure, temperature, and lipid medium on phytosterol oxidation, came to the same conclusion. They found that temperature and heating time, as well as sterol structure and lipid matrix composition, all had an impact on phytosterol.

Conclusion

Proximate analysis of *Moringa oleifera* leaves subjected to two different drying methods shows that there is high crude fibre (12.5±0.60), carbohydrate (35.7±2.45) and protein content (24.5±0.62) in air dried leaves than sun dried. High content (+++) of Saponin and Phytosterol was observed only in air dried leaves when compared with sun dried treatment. Flavonoid composition in sun dried is higher than that of air dried. While Alkaloids composition in both treatments were observed to be the same (+++).

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