

Nutritional properties and hygienic quality of a processed dried organic product from *Moringa oleifera* leaves

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Abstract. Nowadays, people are getting aware of the detrimental effects of synthetic products. That is why they are overselling natural food products rather than synthetics/artificial food products. The use of natural products from plants has few or no side effects on human health when used correctly and has gained the attention of the public over the years. There is immense scope for natural products that can imitate health benefits beyond traditional artificial nutrients. *Moringa oleifera* is one of such plants having nutritional and medicinal benefits. This study is focused on the quality assessment of the processed dried product obtained from *M. oleifera* leaves. Fresh leaves were collected from Dhaka city which is the most densely industrialized region in Bangladesh. Then the collected leaves were washed, dried (mechanically at 40°C for 6 h with 30% relative humidity), and grinded. The light green dried powder had the following proximate compositions 5.2% moisture, 29% protein, 38.2% carbohydrate, 2.3% fat, 19.2% fiber. Nine essential amino acids were detected. In addition, a prompt amount of calcium was found (2 g/100 g), with a considerable amount of magnesium (0.368 g/100 g) and beta carotene (50269.29 µg/100 g). No aflatoxin (B₁, B₂, G₁, and G₂) and heavy metals (Cd, Cr, and Pb) were detected in the processed sample. It is concluded that the mechanical dried *M. oleifera* leaves powder means a good quality natural food product with potential industrial prospects for Bangladesh.

Keywords: *Moringa oleifera*, mechanically dried leaf powder, proximate composition, amino acid, hygienic quality.

INTRODUCTION

Moringa oleifera tree is locally known as the “Sajna tree” in Bangladesh and is a widely known plant species because of its diversified health benefits (Fahey, 2005). When it comes to the health benefit of Moringa leaf, there are almost too many to count. Among 13 different species, *M. oleifera* is one of the most nutrient-rich and useful trees in the world (Khalafalla *et al.*, 2010). Yang *et al.* (2006) reported that Moringa leaves are used as an alternative nutrient source to fight malnutrition.

Moringa is grown and naturalized in some countries of south-east Asia, such as Bangladesh, Afghanistan, Pakistan, Sri Lanka and India (Gupta, 2010). All parts of the plant have different uses. But the leaves have multiple uses (as food, medicine, nutraceuticals, etc.)

because of their unique phytochemical and nutritional properties (Fahey, 2005). Moreover, Ibeh *et al.* (2013) reported the use of *M. oleifera* leaves in the treatment of various diseases.

Recently, the use of the powdered form of Moringa leaves has increased in non-native countries because of its high nutritional properties (Oduro *et al.*, 2008). It is now used to fight malnutrition of children and nursing mothers as well (Moyo *et al.*, 2011). Moringa leaves have contributed to boosting up animal immunity through fulfilling its nutritional demand (Anwar *et al.*, 2007). As its uses are increasing day by day, it is high time to develop some value-added products with ensuring food safety. Various toxic substances accumulate in plants depending

on the type of species, soil, and environmental conditions. So it is important to ensure the nutritional properties with hygienic before producing a product.

Bangladesh is a developing country. The rates of malnourished children are decreasing day by day. Still, more than 28% of preschool-aged children are stunted and malnourished (UNICEF, 2018). In India, Moringa leaves have been used and sold as tablets that reportedly improve health. The United Nations World Food Programme (WFP) had used Moringa leaves as a nutritional source for the malnourished children and women of Africa. But, here in Bangladesh considering the availability of these nutritionally diversified plants, no diversified uses have been reported. However, few health-conscious people have added the nutritious Moringa leaves to their diet as a vegetable.

This study is designed to produce *M. oleifera* dried leaves powder through a mechanical drying process and its quality assessment. If its quality is found suitable, the further aim of the study is to the proper adaptation of the process which will support to fulfill the nutritional demand of the country. In this study, native plants leaves were used for powder preparation. The leaves were collected from Dhaka which is one of the most densely industrialized regions in Bangladesh. Rahman *et al.* (2012) reported excess heavy metals and metalloids in the soil of Dhaka city. Significant concentrations of As, Fe, Mn, Cd, Sb, Pb, Zn, Cu, Ni, and Cr, etc. were also detected in the atmosphere of Dhaka city (Islam *et al.*, 2015). In addition, vehicular emissions also cause heavy metal pollution which impacts the environment and alongside vegetation. Plants in such an atmosphere naturally accumulate heavy metals (Altaf *et al.*, 2021). Besides regular use, it will also be necessary to preserve powder for future use. In this case, mycotoxins are also a great factor in creating huge contamination which leads to food insecurity (Udomkun *et al.*, 2017).

Every technology comes through a trial-error method. Presently, plants and their different parts are formulated in so many ways to develop different organic products. Because, plants generally consist of various highly nutritional properties and various biologically active secondary metabolites (Soetan and Oyewole, 2009; Gafar and Itodo, 2011). Developing various kinds of plant-based products is important to fulfilling the nutritional demands of the present world.

The development of different organic products by using plants and their proper formulation is important to store and for future use. It is also important to develop different value-added products. Production of Moringa leaves powder by using mechanical dryer, preservation and its uses could be great technology. But there is no precise data available of Moringa leaves powder which was prepared by using a mechanical dryer. Hence the study was designed to develop Moringa leaves powder by using the mechanical dryer and determine its nutritional properties in order to provide scientifically some experimental proof for its further beneficial use and

research.

MATERIALS AND METHODS

Source of *Moringa oleifera* leaves

Raw leaves were collected from the *M. oleifera* cultivation land of Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh. Mature leaves were used for powder preparation. Note that the area is one of the densest industrial areas and vehicles cities in the world. The area is evident by heavy metal contamination in the air and soil.

Powder production process

Initially, the fresh mature leaves were collected. Sorting, washing, drying and grinding; simply these steps were followed to produce *M. oleifera* dried leaves powder. Figure 1 illustrates the methods of *M. oleifera* dried leaves powder production process.

Nutritional properties determination

Proximate composition

Moisture content: Moisture content was determined by using Shimadzu Moisture Analyzer (Moc63u). The auto mode was configured for analysis at 105°C, where 0.05% moisture loss per 30 s was set as the ending point. A definite amount of sample was placed in the sample tray, and the auto mode was selected. Finally, the moisture result was recorded from the screen.

Crude protein content: The protein content of dried leaves powder was determined according to the Dumas (1831) method. An innovative TCD auto-calibrating detector was used as a detector where Helium gas was used as carrier gas.

Sample preparation

Initially, the samples were ground properly (<0.5 mm). Then the samples were dried at 105°C for 2-3 h using a forced hot air oven and cooled at desiccator. Then the dried sample was weighed into tin foil and folding the tin foil like a capsule. Finally, the capsules were placed into the autosampler.

Analysis

Sample name, weight, method, sample type, calibration number, etc. were filled into the database. The method



Figure 1. Production process flowchart of moringa leaves powder.

“Plant sources” proceed with the following parameters, protein factor: 5.70, O₂ flow rate: 400 ml/min and O₂ factor 1.8 ml/mg. Finally, the protein content was recorded by pressing the start button.

Total carbohydrate

Total carbohydrate was determined according to Dubois *et al.* (1956). Initially, a 5 mg dried sample was taken in a test tube. Then the samples were mixed thoroughly with distilled water by using a tissue homogenizer to prepare a 25 ml well-mixed solution. After that, 1 ml of sample was pipetted in a sample tube, and 5% phenol solution + 5 ml concentrated Sulfuric acid was added into the solution. Then the samples were cooled by using a cold water bath. After cooling, absorbance was determined of each sample by using a spectrophotometer at 488 nm wavelength. Finally, total carbohydrate content was calculated using a calibration graph. For the calibration graph, the stock solution was prepared by using glucose (1000 µg/L). At the same time, a series of the standard of different concentrations were prepared from the stock solution (20, 40, 60, 100 and 140 µg/L). For the standard graph, the identical procedure was repeated for carbohydrate analysis and applied to the standard series. Observing the standard results absorbance, a standard graph was produced, and the carbohydrate composition was determined for each sample.

Crude fat

Fat was extracted according to the official method AOCS Ba 3-38 by American Oil Chemists’ Society (Revised 2017). As by Twisselmann (1923), extraction of the samples was set by using Fat Extractor E-500 ECE. Initially, all the beakers were dried for 30 min at 102°C.

Then, cooled at ambient temperature in a desiccator for at least one hour and weighed.

Crude fiber

Crude fiber content was estimated by the insoluble formic method (Deymie *et al.*, 1981). A total of 100 ml of formic acid 80% (V/V) were taken in a vial with 5 g of sample. The vial was placed in boiled water for 75 min. After cooling, the insoluble portion was separated through filtration and transfer into a crucible. The crucible was dried and weighed (W1). Then the crucibles were incinerated into an oven, and the weight of ash was determined (W2). Finally, the fiber content was calculated from the differences.

Minerals

Calcium and Magnesium were determined using the method AOAC (2005).

Beta-carotene

Beta-carotene was measured according to AOAC methods 974.29, 992.04 and 992.06 and the method of Thompson and Duval (1989).

Amino acid determination

A solution of 6M Hydrochloric acid in addition to one gram of 0.1% phenol was prepared. Besides, another solution of 1M Sodium hydroxide was prepared by using distilled water. Each of the samples of Pyrex flasks was filled with 200 mg of Moringa leaves dried powder (≤ 0.5 mm) in addition to 25 ml acidic hydrolysis solution. The

Table 1. Nutritional composition (%) of dried *Moringa* leaves powder.

Properties	Amount (%)
Moisture content	5.2 ± 0.11
Crude protein	29 ± 1.12
Total carbohydrate	38.2 ± 0.76
Total fat	2.3 ± 0.23
Crude fiber	19.2 ± 2.57
Calcium	2 ± 1.01
Magnesium	0.3 ± 0.01

Values are mean ± SD (n=3)

powder was hydrolyzed in an oven at 110 ± 2°C for 24 h. Then the hydrolyzed was transferred into the beakers with as little as possible SDB/Na (Sample Dilution Buffer). The temperature was maintained at less than 40°C during pH was an adjustment (2.1-2.3) using basic neutralization solution with a gentle magnetic stirring. Finally, the defined amount of aliquot of the hydrolysate was transferred into the injection vial and dilute with SDB/Na. Give the author of that method

Amino acid analysis was done by (SYKAM S 433 Amino Acid Analyzer). The analysis was done by maintaining the gas flow rate of 0.5 ml/min at 60°C where the reproducibility was 3%. The amino acid content was analyzed by comparing against the standard and expressed as a percentage of the total protein.

Hygienic quality determination

Microbiological analysis

Total plate count, *Staphylococcus aureus* count, yeast and mould counts, *Escherichia coli* count and total coliform count, were done for microbiological investigations. The standard bacteriological analytical manual was followed to enumerate all these counts. Total plate count was calculated using the pour plate method. Baird-parker agar medium and potato dextrose agar medium were used to calculate *Staphylococcus aureus*, yeast, and mould through the spread plate method. The computing of *Escherichia coli* count, Total Coliform count were done by using the three-tube most probable number (MPN) method (BAM, 2002).

Aflatoxin determination

Sample preparation and extraction

Aflatoxin was determined according to the method of Leszczynska *et al.* (2001).

Initially, the dried powder was screened properly using a low micron (<20) sieve. Then 20 g samples were weighed and put in a clean tube with 100 ml of 70/30

(v/v) methanol/water for extraction. After that, the mixture was vortex for three minutes and allowed to settle. Then the supernatant was collected and filtered using Whatman filter paper. Finally, the filtrate was collected for aflatoxin analysis.

Analysis

For aflatoxin analysis, the AgraQuant Total Aflatoxin kit was used. The analysis was done according to the manual described in the kit manual. The absorptions were determined by using an ELISA microwell reader at a 450 nm absorbance filter.

Chemical substances

Heavy metal detection

The sample was digested according to the procedure of Allen *et al.* (1974) and Blowes (2002) by using a mixed acid solution (nitricperchloric-sulphuric). Here, 0.2 g each sample was digested in 7.0 ml of the digestion mixture. Finally, the sample was used to determine the heavy metal (Cd, Cr, and Pb) content by using an ICP-MS 12 (High-Resolution ICP-MS Model: Agilent 7500).

Statistical analysis

Mean, and standard deviation of mean were calculated using MS excel. Each parameter analysis was done in triplicate.

RESULTS

Nutritional properties

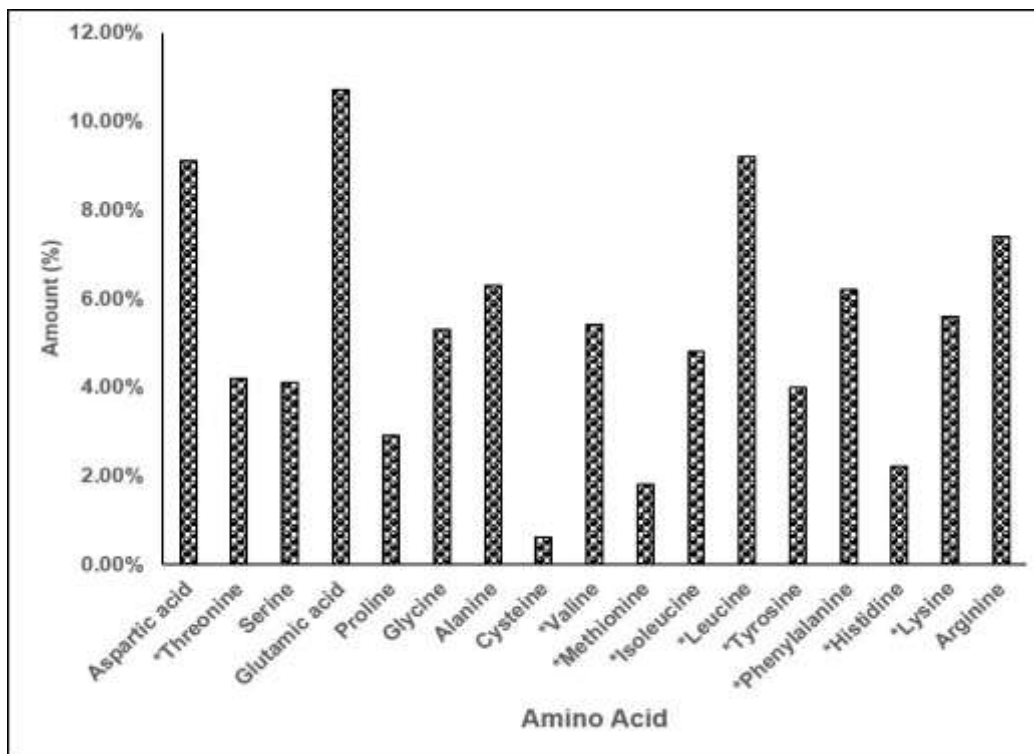
The nutritional composition of dried *Moringa* leaves powder is shown in Table 1. The result showed that dried *Moringa* leaves powder contains an appreciable amount of protein content (29 ± 1.12%). In addition, *Moringa* leaves dried powder also contain a representative amount of other nutrients.

Beta-carotene

A commendable amount of 50.27 mg/100 g beta carotene was observed in the dried powder.

Amino acid profile

A significant number (17) of the amino acid were detected in the leaves powder, where nine were



*Essential amino acid

Figure 2. Amino acids composition (%) of dried Moringa leaves.

Table 2. Mean microorganisms count detected in *Moringa oleifera* leaves powder.

Total plate count (cfu/g)	<i>Staphylococcus aureus</i> (cfu/g)	Yeast and Mould (cfu/g)	<i>Escherichia coli</i> (MPN/g)	Total coliform Count (MPN/g)
1.19×10^1	Nil	Nil	Nil	Nil

belonging the group of essential amino acids (Figure 2). Among these 17 amino acids, glutamic acid was found as the maximum amount (10.7%), where cysteine (0.6%) was found as the minimum. Considering the essential amino acid profile, Leucine (9.2%) was found as the maximum, where Methionine (1.8%) was found as the minimum contributor.

Hygienic quality of the powders

Microbiological status

Microbiological counts of different organisms in *M. oleifera* leaves powder are shown in Table 2.

Aflatoxin quantity

Aflatoxin was not found in the studied powders (Table 2).

Heavy metals concentration

The Cadmium, Chromium and Lead concentration was found in the acceptable limit (Table 3)

DISCUSSION

Moringa oleifera is well known to be is a nutrient-rich plant. It is considered a miracle tree because of its nutritional properties. Every part of this plant has different nutritional benefits. In the present global market, there are various types of products that have already been produced from different parts of the plant. In this case, plenty of research works had been carried out to develop a processed product of *M. oleifera* leaves. In this study, a mechanical drying method (Mechanical Heat Pump Dryer) was carried out to dry the raw leaves. Finally, the finely ground powder was prepared, and its nutritional properties and hygienic quality were determined.

Table 3. Aflatoxin and heavy metal status (mg/kg) of dried *Moringa* leaves powder.

S. no	Test parameter	Amount
1	Aflatoxin	Not Detected
2	Cadmium (Cd)	Not Detected (<0.25 mg/kg)
3	Chromium (Cr)	Not Detected (<0.50 mg/kg)
4	Lead (Pb)	Not Detected (<0.25 mg/kg)

The nutritional properties of *M. oleifera* leaf powder is shown in Table 1. The moisture content of the powder is an important attribute. Low moisture content ensures higher shelf life. Here the moisture content of the powder was detected at $5.2 \pm 0.11\%$ which falls within the limit (below 6.5%) of Owuor (2003). Moreover, the moisture content is one of the most important measurements for food processing, preservation and storage (Onwuka, 2005). The crude protein content of the powder was recorded $29 \pm 1.12\%$ based on dry matter. The protein content of *Moringa* leaves varies depending on their location and processing methods. The crude protein percentage was within the range (16 to 40%) described by Nouala *et al.* (2006). Carbohydrate is essential for healthy diet also essential to fulfill the caloric requirements of the body. The total carbohydrate content of the produced powder was $38.2 \pm 0.76\%$. On the other hand, the fresh *Moringa* leaves contain $1.12 \pm 9.26\%$ (Adeyemi *et al.*, 2014). The higher amount of carbohydrate content observed could be due to moisture reduction and drying. The leaf powder contained a very low-fat content of $2.3 \pm 0.23\%$. Mbah *et al.* (2012) reported higher fat content in fresh *Moringa* leaves than dried *Moringa* leaves. The low-fat content in dried leaves powder is advantageous, which reduces rancidity and ensures longer storage life. The *Moringa* leaves powder contained a higher amount of crude fiber that is $19.2 \pm 2.57\%$. The appropriate fiber content in food increases digestibility and helps to prevent constipation (Okonlawon, 2000). The Calcium content and Magnesium content of the powdered leaves were found at $2 \pm 1.01\%$ and $0.3 \pm 0.01\%$. Price (1997) reported a range of 3-5.5% calcium and 0.8-1.2% Magnesium in fresh *Moringa* leaves. Emelike and Ebere (2016) reported no significant effect of the drying method on the mineral content of *Moringa* leaves.

Moringa leaves are a great source of beta-carotene, where 50.27 mg/100g beta-carotene amount was found in the powdered sample. Subadra *et al.* (2009) reported a similar range of beta carotene content in *Moringa* leaves powder.

Amino acids have a direct effect on the quality and quantity of protein. The powdered *Moringa* leaves comprised 17 amino acids which are between 16 and 18, were found by Sanchez-Machado *et al.* (2009) and Foidl *et al.* (2001). Amino acids are two types (essential and-non essential) that differs depending on species

types and production system (Swanepoel *et al.*, 2010). Among these 17 amino acids, 9 (Threonine, Valine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Histidine, and Lysine) were classified as essential amino acids. Each amino acid has its special function in the body. Here Glutamic acid presence was reported as the highest amount 10.70%, which is different from the findings of Moyo *et al.* (2011). Cystine reported as lowest amount 0.60%, which is commonly deficient in green leaves. Methionine, Proline, and Cystine are powerful antioxidants that protect against radiation (Brisibe *et al.*, 2009). Moreover, amino acids function on various nutrients in the animal's body, which helps in the body's various physiological functions. Diets rich in proteins and amino acids are important to maintain a balanced life (Kyriazakis and Houdijk, 2006).

The exoteric uses of *Moringa* leaves create the necessity of testing the safety issues regarding health hazards. Five microbial parameters of the *Moringa* leaves were carried out. Because the excess load of microbial content in the powder can create food-borne illness. Microbial loads are probably enhanced by oxidation and the presence of various active compounds in the substances (Donia, 2008). Whatever it is, all the microbial counts of the *Moringa* leaves powder were within the limit according to the International Microbiological Standard recommendations (Awe *et al.*, 2009). Aflatoxins were tested on the produced powder sample. No toxins were detected. This contamination can occur in plants during collection, processing, transport and storage (Kader and Hussein, 2009). Post-harvest treatment must have to implement to control the occurrence. In addition, the heavy metals concentration were also tested, especially the Cd, Cr, and Pb in the powdered sample. As Bangladesh is a densely industrial country, there is a high risk of heavy metal accumulation in the plant part. Moreover, a significant amount of heavy metal in soil and atmosphere had been reported by Mottalib *et al.* (2016). Whatever it is, the results showed that the concentrations were within the permissible limits and in normal ranges.

The source of leaves, plant cultivation process, the genetic background of the plant, environmental factors, soil, and climate, etc. have direct effects on nutritional properties (Sanchez-Machado *et al.*, 2009). Moreover, the way of processing in between harvesting and drying might impact the nutritional profile of the powder (Broin, 2006). As *Moringa* leaves is a promising nutrient source,

it is necessary to develop various such new technique to utilize this kind of rich nutrient source. Adaptation and availability of such valuable nutrient sources could be a great addition to our country.

CONCLUSION

The nutritional profile of Moringa leaves powder implies its potentiality as a feed additive or nutrient supplement. The potential features e.g protein, amino acid and minerals content of the product indicate the great quality of the powder. Besides, other proximate properties of the powder were also found in considerable amounts. In addition, the hygienic quality of the leaves powder was found within the acceptable limits. All the characters indicate the novelty of the Moringa leaves powder. Moreover, the dried form of the leaves will help to increase the powder shelf life. So that, it could be easier to store the dried powder (low moisture) for future use. On the other hand, one can easily distribute the products through commercialization anywhere in the world. Culture of the Moringa tree, harvesting, drying, and processing, etc. by using mechanical drier will also create job opportunities in rural and urban areas of people. So, proper optimization and adaptation of the technology could be a good addition to the modern industrial sector.

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