

Effect of drying method and irradiation on the microbiological quality of moringa leaves

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To cite this article:

Abraham Adu-Gyamfi, Tahiru Mahami. Effect of Drying Method and Irradiation on the Microbiological Quality of Moringa Leaves. *International Journal of Nutrition and Food Sciences*. Vol. 3, No. 2, 2014, pp. 91-96. doi: 10.11648/j.ijnfs.20140302.21

Abstract: Effect of drying method and irradiation on the microbiological quality of dried moringa leaves (DML) was investigated. Moringa leaves dried by 3 methods (mechanical, solar and room drying) was assessed for microbiological quality in addition to 6 different retail samples of DML. Mechanical, solar and room-dried moringa leaves were irradiated at doses of 0, 2.5, 5.0, 7.5, 10.0 kGy with gamma radiation from a Co-60 source and the microbiological quality was assessed using the methods of serial dilution and pour plating. Range of mean counts of total viable cells, coliforms, moulds and yeasts, *E. coli* and *Pseudomonas* spp. of the retail samples of DML were respectively 5.92 – 8.44, 4.85 – 7.25, 1.65 – 3.69, 3.71 – 4.78 and 1.84 – 4.22 log₁₀cfu/g. *Salmonella* spp. was detected in 2 out of the 6 retail samples analysed. Room-dried moringa leaves relatively had high counts of total viable cells (6.45); coliforms (6.18); moulds and yeasts (3.46); *E. coli* (1.58) and *Pseudomonas* spp. (3.32) compared to samples dried by mechanical or solar methods. Irradiation dose of 2.5 kGy reduced the counts of all microorganisms to ≤ 3.57 and no survivors were detected in the samples of DML at 5, 7.5 and 10 kGy. High counts of total viable cells, coliforms, *E. coli*, *Pseudomonas* spp. and presence of *Salmonella* spp. in retail samples of DML raise food safety concerns. Solar-dried moringa leaves had improved microbiological quality compared to mechanical or room-dried leaves. Gamma irradiation significantly improved the microbiological quality of DML. A minimum irradiation dose of 5 kGy is recommended to enhance the quality of dried moringa leaves to meet standards of domestic and export markets.

Keywords: Drying Methods, Microbiological Quality, Moringa Leaves, Irradiation

1. Introduction

Moringa products are obtained from the plant *Moringa oleifera*. This is a rapidly-growing tree widely cultivated in most parts of the tropics. *Moringa oleifera* has many uses, including utilisation as animal forage, domestic cleaning agent, fertilizer, foliar nutrient, green manure, honey and sugar cane juice-clarifier, medicine, bio-pesticide and water purifier [1]. In recent times moringa products have been advocated as outstanding indigenous sources of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many developing countries; and therefore have been used to combat malnutrition, especially among infants and nursing mothers [2, 3, 4]. Studies have also shown moringa products as having antibiotic, anti-trypanosomal, hypotensive, anti-spasmodic, anti-ulcer, anti-inflammatory, hypo-cholesterolemic, and hypoglycemic activities [5, 6, 7, 8, 9].

Currently, the production of moringa products in Ghana is

largely a cottage industry characterized by small scale productions. Although national standards have been recently developed [10], the overall production, processing and packaging of moringa products lacks adequate regulation. The code of practice for the production of moringa products in the country [11] strongly recommends effective drying to reduce moisture content to less than 10%. However, since the products easily attracts and reabsorbs moisture during milling and storage, contamination with moulds and other potential pathogens is a serious concern. In Ghana, other parts of West Africa and elsewhere, studies on herbal products in general have identified contaminants that present serious health hazards such as *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp and other Gram positive and Gram negative strains of bacteria [12, 13, 14, 15, 16, 17].

Decontamination of dried herbal and food products have been achieved over the years using methods such as fumigation with ethylene/propylene oxide, ozone, irradiation and saturated steam [18, 19, 20, 21]. With the

exception of irradiation, these procedures are increasingly becoming unacceptable because they result in losses of colour, flavour and aroma properties as well as deposit chemical residues in various products. Several studies have proved gamma irradiation as an effective decontaminating process that also preserves the sensory and organoleptic qualities of the products [22, 23, 24].

The objectives of this study were to:

- assess the microbiological quality of retailed dried moringa leaves,
- assess the effect of drying methods (mechanical, solar and room drying) on the microbiological quality of moringa leaves,
- assess the effect of gamma irradiation on the microbiological quality of moringa leaves dried by the methods of mechanical, solar and room drying.

2. Materials and Methods

2.1. Samples

Dried moringa leaves (DML) used for the study were obtained from the Food Research Institute, Accra. Retail samples of DML were obtained from 6 different retail outlets in Accra.

2.2. Drying

Moringa leaves were dried by 3 methods at the Food Research Institute. Mechanical drying was undertaken using electric hot-air dryers at a temperature of 50°C for 30 minutes. Solar drying was undertaken by drying the leaves in a solar dryer at a temperature range of 35°C–55°C for 4 hours. Room drying was undertaken by drying the leaves at ambient temperatures (28 - 32°C) for 4 days.

2.3. Irradiation

Samples of DML were sealed in polyethylene pouches and exposed to gamma-radiation doses of 0, 2.5, 5.0, 7.5 and 10.0 kGy using a Cobalt-60 source at the Radiation Technology Centre of Ghana Atomic Energy Commission. The dose rate of the irradiation process was 2.50 kGy/hr and the absorbed dose was confirmed by Fricke's dosimetry.

2.4. Microbiological Analysis

Samples were analysed for total viable cells, coliforms, moulds and yeasts, *Escherichia coli*, *Salmonella* spp. and *Pseudomonas* spp. by using the methods of serial dilution and pour plating [25]. Ten grams of each samples was added to 90-ml peptone water (1% peptone + 0.5% NaCl) and placed on a mechanical shaker (Junior Orbital Shaker, Lab-line Instruments, USA) for 15 minutes. The mixture was then allowed to settle for about 5 minutes to allow coarse material to settle down. Microbial load determination was carried out on the supernatant by using serial dilution and pour plate methods. Total viable cells were estimated on Plate count agar (Oxoid, UK) at 37°C for 48 hrs, coliforms on Violet red bile agar (Oxoid, UK) at 37°C for 48 hrs. *Escherichia coli* was estimated on Eosin Methylene Blue Agar (Oxoid, UK) at 37°C for 48 hrs. *Pseudomonas* spp. was enumerated on Pseudomonas Agar Base and C-N Supplement (Oxoid, UK) at 37°C for 48 hrs. Moulds and yeast were estimated on oxytetracycline glucose yeast extract agar (Oxoid, UK) with incubation at 28°C for 48 hours. The presence of *Salmonella* spp. was shown by plating on Xylose Lysine Deoxycholate Agar (Difco, USA) with incubation at 37°C for 48 hours. For each sample, duplicate plating was carried out. Three independent experiments were conducted.

2.5. Data Analysis

The microbial counts (colony forming units, cfu/g) were transformed into logarithms (\log_{10}) and means were determined.

3. Results and discussion

3.1. Microbiological Quality of Retail Dried Moringa Leaves

The range of mean counts of total viable cells, coliforms, moulds and yeasts, *E. coli* and *Pseudomonas* spp. of the retail samples of DML were respectively 5.92 – 8.44, 4.85 – 7.25, 1.65 – 3.69, 3.71 – 4.78 and 1.84 - 4.22 \log_{10} cfu/g (Table 1). *Salmonella* spp. was detected 2 out of the 6 samples analysed.

Whiles International standards require that total aerobic bacteria and fungi should not exceed 10^4 and 10^2 cfu/g

Table 1. Microbiological profile of retail samples of dried moringa leaves.

Samples	Total viable Count	Coliforms	Moulds and Yeasts	E. coli	Pseudomonas sp	Salmonella sp
1	6.28 ± 1.46	6.20 ± 0.64	3.69 ± 0.22	4.56 ± 0.08	2.67 ± 0.32	^b +
2	5.92 ± 0.61	4.85 ± 0.18	1.95 ± 0.04	3.72 ± 0.32	1.84 ± 0.04	^c ND
3	8.44 ± 1.48	5.59 ± 1.21	1.65 ± 0.21	4.78 ± 0.61	4.22 ± 0.44	ND
4	6.83 ± 1.41	7.25 ± 1.12	2.08 ± 0.04	4.72 ± 0.22	2.46 ± 0.04	+
5	7.54 ± 1.55	5.36 ± 0.82	2.61 ± 0.04	3.92 ± 0.22	3.58 ± 0.41	ND
6	6.69 ± 1.24	6.95 ± 0.82	3.56 ± 0.08	3.88 ± 0.16	3.15 ± 0.14	ND

^a mean (\log_{10} cfu/g) \pm SD (n=3), ^b + = detected, ^c ND = not detected

respectively [26], recently developed domestic standards for moringa products in Ghana require the acceptable counts of

total viable cells to be $\leq 1.0 \times 10^2$ cfu/g and that of moulds and yeasts to be $\leq 5.0 \times 10$ cfu/g [10]. In this study, all the retail

samples of dried moringa leaves (DML) had mean counts of total viable cells and moulds and yeasts in excess of both domestic and international standards. Counts of coliforms, *Pseudomonas* spp and *E. coli* spp were also relatively high and the presence of *Salmonella* spp. in some of the samples of DML indicates unacceptable microbiological quality. Like most herbal products, dried moringa products are prone to contamination by microorganisms from the soil, the droppings of insects and birds which adhere to the leaves and stems of the plant before harvest and from the environment, water and handlers during drying. In addition to this, contamination could have been facilitated by prolonged drying of the leaves in shade to prevent loss of vitamins and other essential nutrients as recommended by the national code of practice [11].

3.2. Effect of Drying Method on the Microbiological Quality of Moringa Leaves

Samples of room-dried moringa leaves relatively had high counts of total viable cells (6.45); coliforms (6.18); moulds and yeasts (3.46); *E. coli* (1.58) and *Pseudomonas* spp. (3.32) compared to samples dried by mechanical or solar methods (Table 2). The corresponding counts for mechanically-dried moringa leaves were; total viable cells (3.12); coliforms (0.56); moulds and yeasts (2.66) while *E. coli* and *Pseudomonas* spp. were not detected. In the case of solar-dried moringa leaves, the counts were; total viable cells (2.61), while coliforms, moulds and yeasts, *E. coli* as well as *Pseudomonas* spp. were not detected. *Salmonella* spp. was also not detected in all the samples of DML (Table 2).

The processing of DML involves important steps such as washing, draining and drying which together ensure acceptable product quality. The process of drying (to <10% moisture content) is therefore a critical step which prevents the proliferation of moulds and bacteria as well as other contaminating organisms. According to local and international codes of practice, 3 drying methods are recommended for processing moringa products depending on the scale of production and availability of resources. While mechanical drying is recommended for large scale production, room drying is suitable for small scale production. Solar drying is recommended for both small and large scale production or in instances where electricity is unavailable.

The results show that room-dried moringa leaves had relatively higher counts of total viable cells, coliforms; moulds and yeasts, as well as *E. coli* and *Pseudomonas* spp. compared to mechanically or solar-dried moringa leaves. This observation could be due to contamination by

microorganisms during the relatively longer period (4-6 days) for room drying. It is also important to note that the temperature of room drying (28-32°C) is lower than those of mechanical drying (50-55°C) and solar drying (35-55°C), thus increasing the available moisture that could have supported microbial growth. Despite these limitations, most producers of moringa products continue to utilize room drying because of the leaching of nutrients associated with mechanical and solar drying methods. It is however important to note that solar-dried moringa leaves had a relatively better microbiological quality compared to mechanically-dried leaves. This observation might probably be due to the longer duration of exposure (4 hrs) of the solar-dried leaves compared to that of mechanically-dried leaves (30 min) as recommended by the code of practice [11].

3.3. Effect of Irradiation on the Microbiological Quality of Dried Moringa Leaves

As shown in Tables 3-7, gamma radiation dose of 2.5 kGy reduced the count of total viable cells to ≤ 3.57 ; coliform to ≤ 2.61 ; moulds and yeasts to ≤ 2.61 and eliminated all *Pseudomonas* spp and *E. coli*. Doses of 5, 7.5 and 10 kGy eliminated all total viable cells, coliforms, moulds and yeasts, as well as *Pseudomonas* spp and *E. coli* from all the samples of mechanical, solar and room-dried moringa leaves.

Irradiation at a dose of 2.5 kGy reduced the counts of total viable cells to $\leq 3.57 \log_{10}$ (Table 3). In a previous study, an irradiation dose of 2.5 kGy reduced the count of total viable cells in moringa seed powder to 1.3×10^4 cfu/g [27]. No microorganisms were detected in all samples of mechanical, solar and room-dried moringa leaves after gamma irradiation at doses of 5, 7.5 and 10 kGy. These findings are to be expected since gamma irradiation has been shown to be generally effective in reducing the microbial load of dehydrated ingredients, spices and dried herbal products [22, 28]. Therefore, in order to meet national and international standards, irradiation should be integrated into processing protocols of moringa products in the country. It is accordingly recommended that DML should be irradiated at a minimum dose of 5 kGy to ensure acceptable microbiological quality for both domestic and export markets. It is important to further note that gamma irradiation overcomes shortcomings such as chemical residues, loss of heat-sensitive active ingredients and discoloration associated with conventional technologies such as the use of chemical preservatives, heat, fumigants [29].

Table 2. Microbiological counts of moringa leaves produced by mechanical, solar and room drying methods.

Drying method	Total viable Count	Coliforms	Moulds and Yeasts	<i>E. coli</i>	<i>Pseudomonas</i> sp
Mechanically-dried	3.12 ± 0.83	0.56 ± 0.34	2.66 ± 0.16	^b ND	ND
Solar-dried	2.61 ± 0.42	ND	ND	ND	ND
Room-dried	6.45 ± 0.35	6.18 ± 0.08	3.46 ± 0.45	1.58 ± 0.15	3.32 ± 0.31

^a mean (\log_{10} cfu/g) \pm SD (n=3), ^bND = not detected

Table 3. Microbiological counts of total viable cells of dried moringa leaves before and after gamma irradiation.

Drying method	Dose (kGy)				
	0 kGy	2.5 kGy	5.0 kGy	7.5 kGy	10 kGy
Mechanically-dried	^a 3.20 ± 1.41	^b ND	ND	ND	ND
Solar-dried	3.76± 1.19	2.36±0.18	ND	ND	ND
Room-dried	6.20± 0.42	3.57± 0.04	ND	ND	ND

^amean (log₁₀ cfu/g) ±SD (n=3), ^bND = not detected**Table 4.** Microbiological counts of coliforms of dried moringa leaves before and after gamma irradiation.

Drying method	Dose (kGy)				
	0 kGy	2.5 kGy	5.0 kGy	7.5 kGy	10 Gy
Mechanically-dried	^a 0.49±0.86	^b ND	ND	ND	ND
Solar-dried	ND	ND	ND	ND	ND
Room-dried	6.83±0.06	2.61±0.08	ND	ND	ND

^amean (log₁₀ cfu/g) ±SD (n=3), ^bND = not detected**Table 5.** Microbiological counts of moulds and yeasts of dried moringa leaves before and after gamma irradiation.

Drying method	Dose (kGy)				
	0 kGy	2.5 kGy	5.0 kGy	7.5 kGy	10 kGy
Mechanically-dried	^a 2.63±0.19	^b ND	ND	ND	ND
Solar-dried	ND	ND	ND	ND	ND
Room-dried	2.99±0.65	2.61±0.07	ND	ND	ND

^a mean (log₁₀ cfu/g) ± SD (n=3), ^bND = not detected**Table 6.** Microbiological counts of *Pseudomonas* spp. of dried moringa leaves before and after gamma irradiation.

Drying method	Dose (kGy)				
	0 kGy	2.5 kGy	5.0 kGy	7.5 kGy	10 kGy
Mechanically-dried	^b ND	ND	ND	ND	ND
Solar-dried	ND	ND	ND	ND	ND
Room-dried	^a 3.52±0.21	ND	ND	ND	ND

^a mean (log₁₀ cfu/g) ± SD (n=3), ^bND = not detected**Table 7.** Microbiological counts of *E. coli* of dried moringa leaves before and after gamma irradiation.

Dryingmethod	Dose(kGy)				
	0kGy	2.5kGy	5.0kGy	7.5kGy	10kGy
Mechanically-dried	^b ND	ND	ND	ND	ND
Solar-dried	^a 0.72±0.15	ND	ND	ND	ND
Room-dried	1.34±0.21	ND	ND	ND	ND

^a mean (log₁₀ cfu/g) ± SD (n=3), ^bND = not detected

4. Conclusion

Microbiological quality of most dried moringa leaves retailed in Accra does not meet domestic and international standards. Mechanical or solar methods of drying moringa leaves improved the microbiological quality to a greater

extent compared to room drying. However, the microbiological quality of solar-dried moringa leaves was superior to that of mechanically-dried leaves. Gamma irradiation significantly improved the microbiological quality of dried moringa leaves. A minimum irradiation dose of 5 kGy is recommended to enhance the quality of dried

moringa leaves to meet standards of domestic and export markets.

Acknowledgements

We wish to express our profound gratitude to all technicians, technologists, trainees and national service personnel of Radiation Technology Centre of the Ghana Atomic Energy Commission.

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