IMPACT OF DEBITTERING AND SOLID-STATE FERMENTATION ON THE ANTINUTRITIONAL FACTORS ANDANTIOXIDANT ACTIVITIES OF*MORINGA OLEIFERA* SEED FLOUR

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Abstract

Moringa seeds are rich in proteins, micronutrients and bioactive constituents but its consumption is limited due to its high concentration of bitter alkaloids and other antinutritional factors (ANFs) that impede nutrient digestibility. In recent times, there is increasing interest in finding low-cost and effective processing technology for reducing ANFs in *moringa* seeds for improved nutrition and promotion of health. The aim of this study was to determine the impact of debittering (aqueous heat treatment) alone or in combination with fermentation on the antinutritional compounds and antioxidant activities of *moringa* seed flour. Results obtained revealed that debittering alone, and combined debittering and fermentation treatments significantly ($p \leq 0.05$) decreased phytic acid (61.65% and 92.85%, respectively), tannin (51.32% and 71.24%, respectively), trypsin inhibitors (57.95% and 81.54%, respectively), saponin (57.22% and 86.57%, respectively), nitrates (92.45% and 93.73%, respectively), urease activity (88.33% and 95.00%, respectively) and total alkaloids (67.49% and 92.93%, respectively), increased phenolics, antioxidant activities (ABTS, DPPH, FRAP and ascorbic acid)compared to the raw flour. The use of combined debittering and fermentation with *Saccharomyces cerevisiae* is thus recommended for debittering of *moringa* seeds.

Keywords: *Moringa Oleifera* seed, Debittering, Solid-state fermentation, Antinutrients, Antioxidant potential

1.0 Introduction

Moringa oleifera is an important traditional vegetable tree that grows in the tropics producing leaves and seeds of immense value in human nutrition. *Moringa oleifera* seeds are underutilized crops grown widely in the tropics. *Moringa seed* flour contain about 36.18% protein, rich in essential amino acids and micronutrients than most pulses (pigeon pea, cowpea, bambara nut, lentils and chickpea) but comparable to soybean flour (35 to 40%) (Ogunsina *et al.*, 2015). *Moringa* seed flour is usually blended with wheat flour to obtain a well-balanced protein diet. However, like other plant protein sources, *moringa* seeds contain antinutritional factors such as phytic acid, tannin, oxalate, saponin, trypsin inhibitor, alkaloids, among others (Sardabi *et al.*, 2022). Antinutritional factors limit the utilization of plant foods for human nutrition by impeding nutrient digestibility. The bitter taste of *moringa* seed flour

limits its food and industrial application. However, a debittering process has been applied to remove antinutritional factors and bitter compounds in legume seeds such as lupin making its flour safe for consumption (Villacrés *et al.*, 2020). Similarly, a few traditional debittering methods such as germination, natural fermentation and boiling have been used for debittering *moringa oleifera* seeds (Sardabi *et al.*, 2022). It has been established that heat treatment decreases antinutritional factors and increases nutrient digestibility in cereals and legumes. Particularly, aqueous heat treatment (AHT) is one of the debittering used in reducing bitter alkaloid content and antinutritional factors to safe level in *L.upinus campestris* seeds(Jiménez-Martínez *et al.*, 2001); the effect of debittering treatment using AHT method on the antinutritional factors in *moringa* seeds has not been studied. On the other hand, solid-state fermentation (SSF) with yeast (*Saccharomyces cerevisiae*) has been applied to reduce antinutritional factors and improve the nutritional quality of cereals and legumes (Chinma *et al.*, 2020). According to Villacrés *et al.* (2020), SSF with *Rhizopus oligosporus strain* increases the polyphenol content and improves antioxidant activity, because microbial action enables the breakdown of cell walls and allows the release or synthesis of antioxidant compounds that act as metal chelators or hydrogen donors to free radicals. Nevertheless, the impact of SSF with *Saccharomyces cerevisiae* on the antinutritional factors and antioxidant activities of *moringa* seed flour has not been determined. It can be hypothesized that debittering alone or combined debittering and SSF may modify antinutritional factors, total phenolics and antioxidant activities of *moringa* seed for improved nutrition. The objective of this study was to evaluate the impact of debittering or in combination with solid-state fermentation on the antinutritional factors and antioxidant activities of *moringa* seed flour.

2.0 Materials and methods

2.1 Materials

*Moringa*seeds and *Saccharomyces cerevisiae* (baker's yeast, Angel Yeast Company, Yichang Hubei, China) were procured from an Agro seed company and bakery shop, respectively, at Kubwa Main market Abuja, Nigeria. All chemicals used were of analytical grade.

2.2 Sample preparation

2.2.1 Preparation of raw moringa seed flour

Cleaned *moringa* seeds were washed with tap water, drained, and dried in air draft-oven (Gallenkamp, Cheshire, UK) at 50 °C for 24 h. The dried seeds were milled and sieved (screen diameter 100 μm) to produce raw *moringa* seed flour (RMOSF). The flour was defatted with n-hexane to obtain raw *moringa* seed flour which served as control.

2.2.2 Preparation of debittered flour

Debittered moringa seed flour was prepared following the aqueous heat treatment method described by Villacres *et al.* (2020) with slight modification in terms of cooking time and washing period used. A 500 g of cleaned *moringa* seeds were soaked (1:3 w/v) at an initial temperature of 80 °C for 16 h. Thereafter, the samples were cooked in water (at grain to water ratio of 1:3, at 100 °C for 1 h) and washed with distilled water. The aqueous washing

of the grain was carried out in a stirring system (maintaining a grain to water ratio of 1:15). The first washing step was carried out with distilled water (at 35 °C for 5 h) while the second washing step involved washing at 18 °C for 5 h. Afterwards, the debittered *moringa* seeds were dried at 50 °C for 24 h, milled and defatted using n-hexane. The aqueous heat treatment of seeds was carried out in three batches. The defatted meal was blended and sieved (through 100 µm sieve) to obtain debittered *moringa* seed flour (DMOSF). The flour sample was stored in plastic containers with lids prior to subsequent analysis. The debittered *moringa* seed flour was divided into two portions. One portion was packed in polypropylene bags, kept in airtight container and stored at 4 °C prior to analyses, while the remaining portion was used for the subsequent preparation of another sample batch.

2.2.3 Debittered- fermented moringa seed flour

Debittered-fermented *moringa* seed flour was prepared according to the method described by Villacres *et al.* (2020) and Chinma *et al.* (2020). Briefly, 1 g of dry yeast (*S. cerevisiae*) was mixed with 65 mL distilled water and the suspension was poured into 100 g DMOSF and gently mixed for 5 min. Afterwards, the mixture was covered with aluminum foil and fermented at 28 °C for 16 h in a fermentation cabinet (National MEG Company, Lincoln, USA). The fermented batter was oven dried (Gallenkamp, Cheshire, UK) for 24 h at 40 °C. The dried meal was blended and sieved (100 µm mesh sieve) to obtain debittered-fermented *moringa* seed flour (DFMOSF). The DFMOSF was packed in polypropylene bags, kept in airtight container and stored at 4 °C prior to analyses.

2.3 Analysis of antinutritional factors (ANFs)

Phytic acid and tannin content were assayed as described by AOAC (2005). Trypsin inhibitor activity and oxalate content were assayed as reported by Chinma *et al.* (2022). Total nitrates, residual urease activity and total alkaloid were determined as previously described by Villacrés *et al.* (2020).

2.4 Analysis of total phenolic content and antioxidant activities

A methanolic extract (ME) was prepared from the respective samples as described by Chinma*et al.* (2014). Briefly, 0.2 g of sample was mixed with 4 mL 80% methanol (4 mL) and the mixture was centrifuged at 4000 × g for 20 min. Thereafter, the supernatant was transferred into test tubes, evaporated (under nitrogen stream) and stored at 4 °C. The extraction of samples was done in triplicate. The MEs of the samples were used for the analysis of total phenolic content (TPC) and antioxidant activities (except vitamin C). The TPC was determined by the Folin–Ciocalteau reagent method as previously explained by Chinma *et al.* (2014) and expressed as mg GAE)/100 g. The ABTS [free radical scavenging 2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid)] was determined as explained by Chinma *et al.* (2022) and presented as mg TE/g (dry basis). The DPPH (1,1-diphenyl-2-picryl-hydrazil) radical scavenging activity and ferric reducing antioxidant power (FRAP) were determined as described by Chinma *et al.* (2014) and expressed as mg TE/ g.Ascorbic acid (vitamin C) content was assayed using 2,6 dichlorophenol-Indophenol method (AOAC, 2005).

2.5 Statistical analysis

Data analysis was carried out using analysis of variance (ANOVA) with a statistical software (SPSS 20 , BM, Armonk, NY, USA) and Tukey's test was used to determine significant differences (5% probability) among means.

3.0 Results and Discussion

3.1 Effect of debittering, and combined debittering and fermentation on ANFs of moringa seed flours

Table 1 shows the antinutritional composition of raw, debittered and debittered-fermented *moringa* seed flour. The phytic acid (PA) content of raw *moringa* seed recorded in this study is lower compared to the value (78.33 mg/100 g) recorded by Ijarotimi *et al.* (2013). The initial concentration of PA (74.83 mg/100 g) in raw *moringa* seed decreased by 61.65% and 92.85% after debittering, and combined debittering and fermentation treatments, respectively. The decrease in PA content may be ascribed to hydrothermal treatment during debittering (prior to fermentation), and the production of phytase by microbial strains in addition to activation of endogenous phytase during fermentation due to favourable conditions (Yakubu *et al.*, 2022).

Tannin content of the raw *moringa* seed was 268.25 mg/100g which is higher than the value (241.67 mg/100 g) reported by Ijarotimi *et al.* (2013). The tannin content was decreased by 51.32% and 71.24% following debittering, and combined debittering and fermentation treatments, respectively. The significant ($p \leq 0.05$) reduction in tannin content following debittering may partly be attributed to high leaching loss of tannin in cooking water caused by breakdown of protein-tannin complex (Villacres *et al.* 2020) as well as microbial synthesis of tannase during fermentation (Yakubu *et al.*, 2022).

Debittering, and combined debittering and fermentation treatments caused significant ($p \leq 0.05$) reduction in trypsin inhibitors (57.95% and 81.54%, respectively), saponin (57.22% and 86.57%, respectively), total nitrates (92.45% and 93.73%, respectively), urease activity (88.33% and 95.00%, respectively) and total alkaloids (67.49% and 92.93%, respectively) content compared to raw *moringa* seed flour (Table 1), probably due to the effect of heat treatment prior to fermentation, and increased activities of microorganisms during fermentation. It was observed that combined debittering and fermentation treatment had a substantial effect in the reduction of antinutritional factors and improvement of antioxidant activities compared to debittering treatment alone. These results are in agreement with a previous report where debittering, and combined debittering and solid-state fermentation *with Rhizopus oligosporus* caused significant reduction in antinutritional factors in lupin seed flour than the raw flour (Villacrés *et al.*, 2020).

3.2 Effect of debittering, and combined debittering and fermentation on the TPC and antioxidant potential of moringa seed flours

Raw *moringa* seed flour contained 7.22 mg GAE/g, which is close to the value (7.8 mg GAE/g) reported by a previous worker that assayed TPC in raw *moringa* seed using ME (Singh *et al.*, 2013). It was observed that TPC content of the samples was in the order: DFMOSF > DMOSF> RMOSF. The higher TPC recorded in debittered seeds may be attributed to the effect of hydrothermal treatment during the debittering process in facilitating the extraction of phenolics from the *moringa* seed matrix. On the other hand, the increased TPC following combined debittering and fermentation process, could be ascribed

to activation of the enzymes that enabled the formation of phenolic compounds; thus, the observed significant increase in ABTS, DPPH, FRAP and ascorbic acid content in the processed *moringa* seed flour compared to the raw flour. It has been reported that increased antioxidant activities in foods are usually associated with increased phenolics (Chinma *et al.*, 2021). These results are in agreement with increased TPC and antioxidant properties of debittered, and debittered-fermented lupin seed flours (Villacrés *et al.*, 2020).

4.0 Conclusions

This study demonstrated that debittering, and combined debittering and solid-state fermentation with *S. cerevisiae* significantly ($p \leq 0.05$) decreased the antinutritional factors, increased TPC and antioxidant potential of *moringa* seed flour. The study also established that combined debittering and solid-fermentation caused a substantial reduction in antinutritional factors and improvement in antioxidant potential of *moringa* seed flour than debittering treatment alone. Consequently, the low level of residual antinutrients in *moringa* seed following debittering, and combined debittering and fermentation is expected to improve the nutritional composition of the flour. In view of this, studies are on-going in our laboratory on the impact of debittering alone or in combination with solid-state fermentation on the nutritional composition and techno-functional properties of *moringa* seed flour.

Table 1. Antinutritional composition of raw, debittered, and debittered-fermented *moringa* seed flours

Parameter	Raw flour	Debittered flour	Debittered-fermented flour
Phytic acid (mg/100 g)	74.83 ± 1.05^{a}	28.70 ± 0.34^{b}	9.35 ± 0.10^{c}
Tannin (mg/100 g)	268.25 ± 0.89^{a}	130.59 ± 0.75^{b}	77.16 ± 0.11^{c}
Trypsin inhibitors (TIU/mg sample)	1.95 ± 0.04^{a}	0.82 ± 0.01^{b}	0.36 ± 0.01^{c}
Saponin (mg/100 g)	$10.73\ \pm0.10^{a}$	4.59 ± 0.05^{b}	1.44 ± 0.02^{c}
Total nitrates (mg/100 g)	$36.\ 54\pm0.17^{h}$	2.76 ± 0.03^{a}	2.29 ± 0.01^{a}
Urease activity (pH difference)	$0.62\ \pm0.01^{a}$	0.09 ± 0.00^{b}	0.05 ± 0.00^{b}
Total alkaloids (mg/100g)	$16.55\ \pm0.13^{a}$	5.38 ± 0.09^{b}	1.17 ± 0.12^{c}

The data from triplicate experiments are expressed as a mean ± standard deviation.
Values with different superscripts in a row are significantly different ($p \leq 0.05$).

Table2. Total phenolic content and antioxidant activities of raw, debittered, and debittered-fermented *moringa* seed flours

Parameter	Raw flour	Debittered flour	Debittered-fermented flour
TPC (mg GAE/g)	7.22 ± 0.01^{c}	8.79 ± 0.01^{b}	9.06 ± 0.01^{a}
Vitamin C (mg/100 g)	4.35 ± 0.02^{c}	5.60 ± 0.05^{b}	6.23 ± 0.03^{a}
ABTS (mg/100g)	91.70 ± 0.13^{c}	127.95 ± 0.16^{b}	148.61 ± 0.20^{a}
DPPH (mg TE/g)	$2.44\ \pm0.17^{c}$	3.16 ± 0.10^{b}	6.75 ± 0.13^{a}
FRAP (mg TE/g)	$3.\ 15\pm0.04^{c}$	5.38 ± 0.02^{b}	8.10 ± 0.01^{a}

The data from triplicate experiments are expressed as a mean ± standard deviation.
Values with different superscripts in a row are significantly different ($p \leq 0.05$).
TPC= Total phenolic content, ABTS+ [free radical scavenging 2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid)], DPPH (1,1-diphenyl-2-picryl-hydrazil) radical scavenging activity, and FRAP = Ferric reducing antioxidant power.

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