Full Paper

COMPARATIVE STUDY OF THE FUNCTIONAL AND PHYSICO-CHEMICAL PROPERTIES OF DEBITTERED MORINGA SEEDS AND SOYBEANS FLOURS

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Abstract

The physico-chemical and functional properties of debittered moringa seeds flour were compared with soybean flour following AOAC method. The results show that ordinary defatted moringa seeds (DFMS) and debittered moringa seeds (DBMS) flours were higher in crude protein and crude fibre than defatted soybean (DFSB) flour. *In vitro* digestibility increased from 60.1 to 77.5% for DFMS and DBMS respectively; but these are less than that of soybean (83%). Increasing and decreasing trends of nitrogen solubility was observed for DFMS flour as pH increased giving minimum solubility at pH 4.0 and 9.0. Emulsion capacity reduced significantly for DBMS flour at all ionic concentrations; whereas for DFSB flour, the trend was similar to nitrogen solubility. Minimum gelation concentrations of the flours were 14% for DFMS flour and 10% for both DBMS and DFSB flours. Foaming capacity of DFMS and DFSB flours increased with pH, whereas for DBMS flour foaming reduced significantly and no visible change was observed as pH increased. Scanning electron micrographs showed no noticeable difference in the microstructure of DFMS and DFSB flours; however the particles of DBMS flour formed a continuous protein matrix that were devoid of air cells. Trypsin inhibitor activity was not detected in moringa seed flours but phytic acid was 0.38, 0.24 and 1.53 g/100 g for DFMS, DBMS and DFSB flours respectively. Successful removal of the bitter principle in moringa seeds holds good prospect for its utilization as an alternative source of vegetable protein; however, detailed toxicological properties still needs to be evaluated regarding the chemistry of moringa seeds protein and its interaction with other food constituents such as starch and lipids.

Keywords: Debittering, *Moringa oleifera* Seeds and Soybean Flours, Functional and Physico-Chemical Properties

1. Introduction

The need to further augment global protein supply to the low income group has triggered research interests on several lesser-known and under-utilized seeds as viable alternatives to soybeans [1] known to be the richest and most explored source of vegetable protein. In this regard, a number of oil seeds and legumes such as canola, jatropha, pumpkin seeds, madhuca and linseed have been identified, studied and found to possess good functionality as alternative sources of vegetable protein [2-6]. Many plants contain chemical compounds with anti-nutritional properties some of which act as natural defense against animal predators [7]; this is partly why primitive hunters-gatherers were only able to exploit a small part of plant materials that are available in their habitat and by evolution, adapted their digestive abilities to limited number of plant foods. In most cases, detoxification of food proteins from these sources is required in order to reduce the interference of anti-nutrients with functionality and nutrition.

Apart from the known use of crushed *Moringa oleifera* seeds as flocculent for water purification in developing countries, it is seldom eaten especially because of its bitter and astringent taste. Although defatted moringa flour contains 54.41 g of protein per 100 g of sample [8,9], the presence of alkaloids, phytates, saponins, cyanogenic glucosides and glucosinolates have been reported [10]. Bitterness and the presence of anti-nutritients hinder the utilization of some oilseeds in functional foods, but the nutraceutical perspectives of bitter food materials make the need to remove bitter principles from certain food materials debatable. However, consumers' negative attitude and sensitivity towards bitter taste in food formulations make the debittering process important. Some of the methods that have been widely used to debitter, and/or remove anti-nutritional constituents from food materials include soaking, fermentation, germination, boiling, microwave cooking and autoclaving [11-13]. It is generally important that protein flours from unconventional sources be investigated for their interaction with water, oil and other food constituents in relation to their nutritional properties and functionality in food formulations [14]. In this study, the physico-chemical and functional properties of the debittered moringa seeds flour were compared with that of soybeans to provide useful insight regarding the suitability of moringa seeds as an alternative source of vegetable protein.

2. Materials And Methods

2.1. Materials

About 10 kg of mature and dry moringa (*Moringa oleifera*) seeds procured from Veg-Indian Exports, Erode, Tamil Nadu, India and 2 kg of soybeans (*Glycine max*) from a local market in Mysore, Karnataka, India were used for this investigation. The moringa seeds were dehulled and cleaned to remove extraneous and foreign matters. Reagents of analytical grade and triple-distilled water were used for the experiment.

2.2. Debittering Of Moringa Seeds

A simple, non-chemical, heat-assisted process for debittering moringa seeds (Fig. 1) had earlier been established [15]. The procedure involves 35 min of ordinary boiling or 25 min of microwave cooking using seeds/water ratio of 1:30 w/v. For the purpose of this study, 3 kg of moringa seeds were debittered using the ordinary boiling method. The gruel was decanted and the debittered seeds were oven-dried at 80 °C for 8 h.

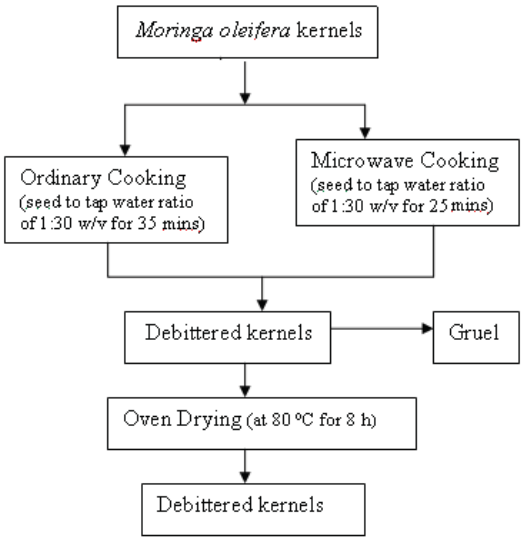

Fig. 1. Process for debittering moringa seeds

2.3. Preparation Of Flour Samples

A portion of 2 kg each of raw and debittered moringa seeds were flaked using flaking machine (Model J #6725, Kvarnmaskiner, Malmo, Sweden). One kg of soybean seeds was also cleaned, dehulled and flaked. The flakes were dried at 55 °C for 4 h and defatted afterwards by repeated washing (4–5 times) with hexane until the fat content of each sample reduced below 1%. The defatted flakes were dried and milled separately in a Quadrumat mill (Buisberg, Germany) each to 100 microns. The three samples that were consequently obtained are defatted moringa seeds (DFMS); debittered and defatted moringa seeds (DBMS) and defatted soybean (DFSB) flours. The samples were stored in airtight containers until the time of use.

2.4. Proximate Analysis And *In Vitro* Protein Digestibility

Protein, fat, ash and crude fibre content were determined by standard methods of analysis [16]. Percent nitrogen was estimated by micro-Kjeldhal method using automated nitrogen distiller and protein content was calculated by multiplying the nitrogen value with 6.25 [17]. Carbohydrate content was obtained by difference.

In vitro protein digestibility was carried out according to Akeson and Stahman [18]. A known amount of sample was incubated with 1.5 mg pepsin in 15 mL of 0.1 N HCl at 37 °C for 3 h. After neutralization with 2 M NaOH and addition of 4 mg pancreatin in 7.5 mL of phosphate buffer (pH 8.0) which contains 0.005 mol/L of sodium azide to prevent microbial growth, the solution was incubated for an additional 24 h at 37 °C. After 24 h, the enzyme was inactivated by addition of 10 mL of tri-chloro acetic acid to precipitate the undigested protein. The volume was made up to 100 mL and centrifuged at 5000 rpm for 20 min. The protein content of the clear supernatant was determined by Kjeldahl method. *In vitro* protein digestibility was expressed as the percentage of the total protein solubilized after enzyme hydrolysis and calculated as shown below.

$$\textit{In vitro}\ \text{protein digestibility} = \frac{\text{Nitrogen content in the supernatant}}{\text{Total nitrogen content in the sample}} \times 100 \quad (1)$$

Values were averages of three separate determinations

2.5. Determination Of Functional Properties

Water and oil absorption capacity (WAC and OAC) were determined following the procedure of Beuchat [19]. This involves mixing 1 g of with 10 mL of distilled water (or refined groundnut oil for OAC) in a 15 mL centrifuge tube at 27.5 °C for 30 min. The mixture was centrifuged at 2000 rpm for 30 min and the supernatant was quantified using a 10 mL graduated measuring cylinder. WAC or OAC was expressed as grams of water or oil bound per 100 g of flour; taking the density of water and that of groundnut oil as 1 and 0.9 g/mL respectively.

Wettability is the time it takes the flour particles to sink down the water surface after absorbing moisture. This was estimated by placing about 1 g of each flour sample in a test tube of about 10 mm diameter. With a finger placed over the open end, the test tube was inverted at a height of about 10 cm from the surface of 250 mL of distilled water contained in a 500 mL beaker. The finger was removed to allow the test sample fall onto the water surface [20]. Values were averages of five determinations.

Nitrogen solubility of the sample was determined according to the method of Chobert *et al.* [21] considering pH ranges 2.0 to 10.0 Samples were dispersed in distilled water (1% w/v) and mixed thoroughly at the room temperature for 5 min using a magnetic stirrer. The pH of the solution was adjusted with 0.1 M HCl or 0.1 M NaOH. After a 45 min of stirring at room temperature, the pH was measured (or readjusted if necessary) and the samples were centrifuged at 10,000 rpm for 30 min. The total nitrogen content in the supernatant was determined and NS was calculated as:

$$\text{NS}\ (\%) = \frac{\text{Nitrogen content in the supernatant}}{\text{Total nitrogen content in the sample}} \times 100 \quad (2)$$

NS profile was obtained by plotting average values of nitrogen solubility (%) against pH.

Foaming capacity and stability of the flour samples were determined using the method of Kinsella [22] as simplified by Booma and Prakash [23]. A 2% aqueous dispersion of the protein sample was mixed thoroughly in a kitchen blender for 3 min. The contents were immediately transferred into a 250 mL graduated cylinder, and the foam volume was noted. Foaming capacity was calculated as the percentage increase in volume of the protein dispersion upon mixing. Foaming stability was estimated as the relative volume of foam left after 30 min standing.

$$\text{Foaming capacity (\%)} = \frac{\text{Vol. after whipping} - \text{Vol before whipping}}{\text{Vol. before whipping}} \times 100 \quad (3)$$

Emulsification capacity was determined according to the method of Beuchat [19]. About 2 g flour sample was dispersed in 50 mL distilled water (and different concentrations of NaCl ranging from 0.02–1.0 M) and homogenized in a blender at high speed to disperse the proteins thoroughly. As sample was being homogenized, refined groundnut oil was added at about 0.5 mL/sec portions from a burette. This process was interrupted intermittently after each addition of 5–10 mL, there was a pause to prevent sudden rise in temperature. Oil addition continued until the emulsion became thick and attained maximum viscosity; oil addition rate was reduced afterwards and gradually as homogenization progressed until the emulsion breakpoint was reached at which there was separation of oil and water into two phases. Emulsion capacity was measured as mL of oil emulsified and held per gram of flour. Determinations were carried out at room temperature (27.5 °C) and reported values were means of triplicates.

The ability of proteins to form gels is the minimal protein concentration required for inverting a tube without the gel siding at the walls [24] known as the minimum gelation concentration (MGC) was determined according to Coffman and Garcia [25] as modified by Alobo *et al.* [26]. Sample suspensions of 2, 4, 6, 8, 10, 12, 14, 16 and 30% (w/v) were prepared in distilled water. The suspensions were heated inside a boiling water bath for 1 h; and afterwards subjected to rapid cooling in a cold water-bath and further cooling at 4±1 ºC for 2 h.

2.6. Scanning Electron Microscopy

The micro-structural differences in the flour samples were studied by scanning electron microscopy. Scanning electron microphotographs were taken using scanning electron microscope LEO 435 VP, Cambridge model. Samples were coated with gold using poloron coating system E-5000 within 2–3 min. The coating thickness was calculated as 200–300 nm, using the formula: $T = 7.5I\,t$, where I = current (mA), t = time (min), T = thickness (A°). The coated samples were loaded on to the system and the image was viewed under 20 kV potential using a secondary electron image and captured using a 35mm Ricoh camera (LEO 435 VP Operator Manual Version V2.04, Issue I, March 1996).

2.7. Colour Measurement

Colour measurement of flour samples was carried out using a Labscan XE colour meter (Hunter Associates Lab. Inc., Reston, VA). Colour readings were expressed as L, a, b and ΔE colour units; where L is the degree of lightness (100 represents perfect white and 0 perfect black), a is the intensity of colour in the direction of green (−) to red (+) with grey as zero and b is the intensity of the colour in the direction of blue (−) to yellow (+) with grey as zero. Total colour (ΔE) is the difference between the sample and the reference standards. The standard reference white tile had L, a and b values of 90.69, −1.08 and 0.61 respectively.

2.8. Determination Of Trypsin Inhibitor Activity And Phytic Acid Content

Trypsin inhibitor activity was determined according to Radha *et al.* [14]. The procedure involved extracting 1 g of sample with 50 mL of 0.01 mol/L NaOH (with pH adjusted, when required, to 8.4 – 10.0) for 3 h. The suspension was centrifuged and diluted so that 2 mL of the extract inhibited 40–60% of the trypsin used as a standard in the analysis. Benzoyl-DL-arginine *p*-nitroanilide (BAPA) hydrochloride was used as the substrate. One trypsin unit (TU) is an increase of 0.01 absorbance units at 410 nm per 10mL of the reaction mixture at the conditions used. Trypsin inhibitor activity is expressed in terms of trypsin units inhibited (TUI) per gram of sample. Phytic acid content of the samples was determined according to the method of Thompson & Erdman [27] as documented by Gupta *et al.* [28]. The conversion factor of 3.55 for converting phytin phosphorus to phytic acid was used.

2.9. Statistical Analysis

All values were computed as a mean of three determinations ± standard deviation. Based on the analysis of variance of the data, where significant difference was indicated, means were separated by Duncan multiple range tests [29].

3. Results And Discussion

3.1. Proximate Composition And *In Vitro* Protein Digestibility

In Table 1, the proximate composition of the three flour samples differed significantly ($p<0.05$). The results show that both DFMS and DBMS flours were richer in crude protein and crude fibre than DFSB flour. Although crude protein was more in DFMS than DBMS flour, the reverse was the case for crude fibre but total ash was highest in DFSB flour followed by DFMS and DBMS flours. These results agree with the report of Foidl *et al.* [10] for DFMS flour and Radha *et al.* [30] for defatted soybean meal. After debittering, *in vitro* protein digestibility of moringa seed flours increased from 60.1 to 77.5% but was less than that of soybean (83%). Improved protein digestibility of oil seeds and legumes had earlier been attributed to the inactivation of trypsin inhibitors that occurs during heat treatment [30], but since trypsin inhibitor was not detected in moringa seeds [10]; impaired protein digestibility may be attributed to other anti-nutritional constituents.

Table 1. Proximate composition of DFMS, DBMS and DFSB flours

Component	Proximate composition (g/100g)		
	DFMS	DBMS	DFSB
Protein	59.66±0.16^{b}	57.51±0.42^{b}	53.30±0.24^{a}
Crude fibre	4.9±0.13^{b}	5.98±0.11^{c}	3.58±0.12^{a}
Ash	6.52±0.32^{b}	3.97±0.44	7.17±0.30
Fat	2.67±0.07^{a}	1.79±0.03^{c}	2.27±0.07^{b}
Carbohydrate	26.25±2.5^{b}	30.75±3.33^{b}	33.68±3.56^{c}

All values are means of triplicates ± standard deviations

^{a,b,c}Means with same letters on same row are significantly different ($p<0.05$)

3.2. Functional Properties

The functional properties of DFMS, DBMS and DFSB flours are shown in Table 2. Significant difference ($p<0.05$) was observed in the WAC of the three flour samples. Debittering improved the WAC of moringa flour although DFSB flour showed the highest WAC. The higher WAC of DBMS flour may be due to the denaturation of protein that exposes additional water binding sites [31]. It may also be due to gelation of carbohydrates and swelling of crude fiber due to heat treatment as reported for winged bean by Narayana & Narasinga Rao [32]. The ability of the flour to absorb and retain water and oil may help to improve binding of the structure, enhance flavor retention, improve mouth feel and reduce moisture and fat losses of food products [33]. For most oil seed proteins, heat treatment increases WAC significantly compared to the ordinary

flour. In bread baking, high WAC enables the addition of more water to the dough in order to improve handling characteristics and maintain freshness [34]. The highest OAC (208 g) was observed for DFMS flour. After debittering, the value decreased to 122.2 g which is lower than 130 g recorded for DFSB flour. The trend shown by emulsification capacity of DBMS flour corresponds to that of OAC and this may be due to increased concentration of hydrophobic amino acids causing a reduction in the ability of the proteins to interact with oil. The ability of a food component to entrap oil is an important characteristic in food formulations because oil retains flavor and enhances mouth feel [22]. Dench, *et al.* [35] had earlier reported a negative correlation between WAC and OAC of alfalfa leaf and sesame proteins. This was noticed in this study as DFMS which had the lowest WAC showed the highest OAC. Kinsella [36] associated increase in OAC with heat dissociation of the proteins and denaturation, which is expected to unmask the non-polar residue from the interior of protein molecules.

Table 2. Functional properties DFMS, DBMS and DFSB flours

Property	DFMS	DBMS	DFSB
In vitro digestibility (%)	60.1±0.18[a]	77.5 ±3.14[b]	83±1.0[c]
Water absorption capacity (g of water/100 g of sample)	83.5±2.4[a]	138±3.0[b]	211±4.2[c]
Oil absorption capacity (g of oil/100 g of sample)	208.2± 2.6[c]	122.2±0.4[a]	130.4±3.1[b]
Emulsification capacity (mL of oil/g of sample)	97±2.0[a]	92±3.0[b]	92±1.0[b]
Wettability (seconds)*	189±4.0[b]	75±2.3[a]	252.1±3.62[c]

All values are means of triplicates ± standard deviations

[a,b]Means with same letters on same row are significantly different (p<0.05)

From Table 2, DFMS and DBMS flour got wet in 189 and 75 s respectively whereas under the same ambient conditions, it took DSFSB our 252 s. Generally, proteins have the ability to form hydrogen bonds with water molecules and polar groups on the polypeptide chains thereby binding large quantities of water. When the flour particles are largely made up of protein and carbohydrates, these may have become more closely packed such that it takes more time for water to get absorbed. This may be the reason why it took a longer time for DFSB flour to get wet; however, Igene *et al.* [37] reported that the microstructure of protein particles and factors such as texture, size, topography, surface area also affect wettability.

The NS profiles of DFMS, DBMS and DFSB flour proteins in water are shown in Fig. 2. For DFMS flour, NS showed increasing and decreasing trends as pH varied from acidic to alkaline. Minimum NS occurred at an acidic pH 4.0 and an alkaline pH 9.0 showing two isoelectric points; suggesting that defatted moringa seeds proteins are recoverable at both acidic and alkaline pH. This behaviour is quite different from that of soybean which showed a U-shaped pattern with minimum NS (24%) occurring between pH 4.0 and 5.0 (Fig. 2). This is similar to earlier reports by Shanmugasundaram & Venkataraman [5]. The NS of DBMS flour had a similar U-shape as that of soybean but the values were lower. This may be due to the removal of non-protein nitrogen fraction or denaturation of proteins which might have occurred during debittering. A reduction in the NS by heat treatments has been reported earlier for linseed meal [38], ethanol-treated guar meal [39] winged bean [31] and detoxified madhuca seed flour [5]. Khattab and Arntfield [2] reported reductions in NS of Canola, soybean and flax seed meals after water boiling and roasting. The dissociation of the high molecular weight proteins of water-boiled flaxseed meal and low solubility of denatured proteins had been documented by Madhusudhan & Singh [6]. Higher solubility is related to the presence of a low number of hydrophobic residues, the elevated charge, electrostatic repulsion and ionic hydration occurring at pH values above and below the isoelectric pH. Due to alterations in the hydrophobicity / hydrophilicity balance of proteins as a result of denaturation, Nitrogen soubility reduces [24]. Although highly soluble proteins may be needed in applications in which emulsification, whipping and film formation are important, low NS may be desirable in applications with high protein levels and when limited emulsification or protein–protein interactions are required [40].

The effect of ionic strength and salt concentration on the EC of DFMS, DBMS and DFSB flours are presented in Fig. 3 and 4. In Fig. 3, it is observed that emulsification versus pH profiles of the three flour samples showed trends similar to NS. Radha *et al.* [14] reported that for most legumes and oil seeds a strong positive linear relationship exists between the NS and emulsifying properties. The effect of pH on the EC of moringa flours was not much; though debittering caused significant reduction in the EC at all ionic concentrations; whereas for soybean, the trend of EC was similar to NS. In Fig. 4, the emulsification profile of DFSB was higher than DFMS and DBMS flours at different NaCl concentration. Many chemical and physical factors are involved in the formation, stability, and textural properties of protein–fat–water emulsions. Emulsifying capacity varies with the type of protein, its concentration, pH, ionic strength, and viscosity of the system. Kinsella [30] had earlier established that high NS and high fat-adsorption values are closely related to the formation and stabilization of emulsions. In the same context, the lower EC values of DBMS may be due to its low NS. Shanmugasundaram & Venkataraman [5] reported that saponins can act as emulsifying agents hence, the presence of saponins in the defatted moringa flour may have accounted for its higher emulsification values than the debittered flour. Eke & Akobundu [41] had reported that application of heat reduces the EC of legume proteins.

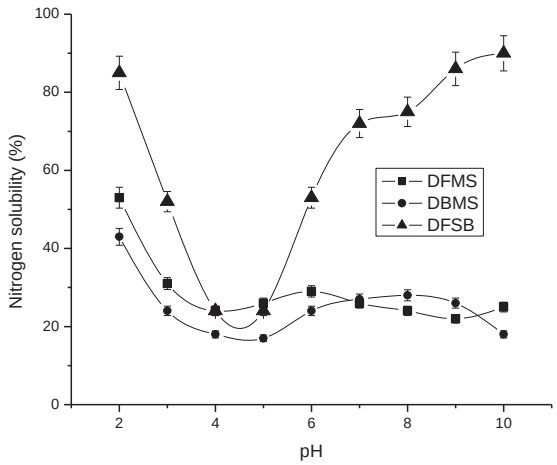

Fig. 2: Nitrogen solubility profile of DFMS, DBMS and DFSB flours

The MGC of the flours was 14% for DFMS and 10% for both DBMS and DFSB (Table 3). It was observed that the DFMS gelled slightly and showed evidence of slipping when the concentration was 8% whereas for DBMS and DFSB, similar behaviour was observed at 4 and 6% respectively. Narayana & Narasinga Rao [31] linked gelation to structured aggregation of denatured molecules. The gel strength of oil seed flours have been reported to increase with protein and starch concentration; as the proteins undergo dissociation by heat, carbohydrates become gelatinized and crude fibre, swollen [35,26,42]. This ability provides the required structural matrix for holding water, flavors, sugars and food ingredients and enhances the functionality of flours in food products development.

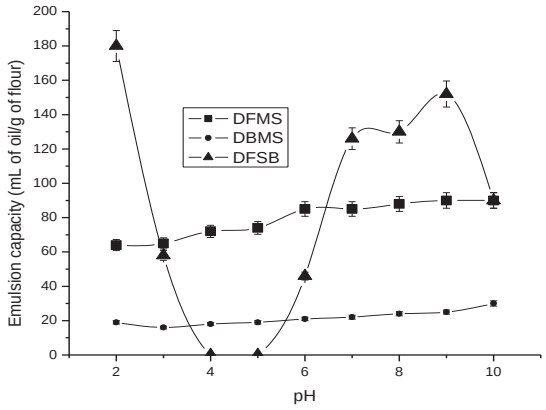

Fig. 3: Effect of pH on the emulsification capacity of DFMS, DBMS and DFSB flours

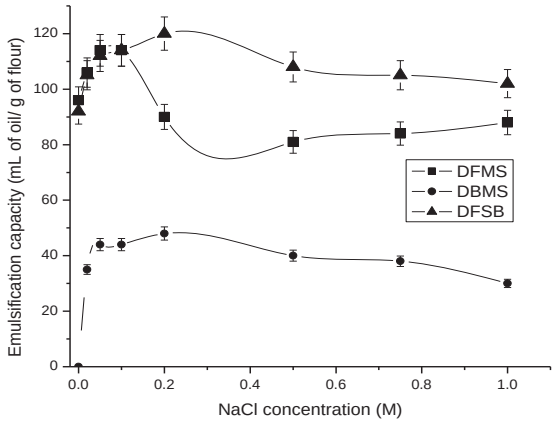

Fig. 4: Effect of NaCl concentration on the emulsification capacity of DFMS, DBMSand DFSB flours

Table 3. Gelation properties of DFMS, DBMS and DFSB flours

Flour sample	Flour concentration (% w/v)								
	2	4	6	8	10	12	14	16	30
DFMS		–	–	±	±	±	+	+	+
DBMS	–	±	±	±	+	+	+	+	+
DFSB	–	–	±	±	+	+	+	+	+

-not gelled; ±, slightly gelled but slipped; +, gelled

Fig. 5 shows the FC and FS of the flour samples in water. Although DFMS had higher foaming properties that DFSB flour, sequel to debittering (which may have included removal of saponins), foaming reduced markedly. Fig. 6 and 7 show the FC of the flour samples at different pH and NaCl concentration respectively. It was observed that the trend of FC for DFMS was similar to NS profile which shows 2 iso-electric pH. For both DFMS and DFSB flours, FC increased with pH, whereas debittering reduced the FC of moringa flour considerably and no noticeable change was observed as pH increased. This may be due to possible removal of saponins and denaturation of proteins by heat during debittering. At different NaCl concentration, the FC of the flours increased (Fig. 7); attaining the highest at 0.2 M. At higher salt concentration, FC decreased slightly. Increase in FC with increasing salt concentration has been attributed to high protein solubility. Akintayo *et al.* [43] linked high FC with the flexible protein molecules which reduce surface tension, while low FC is due to highly ordered globular protein, which are relatively difficult to denature. Denaturation and aggregation of proteins during whipping owing to a large increase in the surface area in the liquid/air interphase is an important property regarding the suitability of flours in baked foods [32]. Due to its reduced NS, it was observed that DBMS flour showed lower foaming and emulsification properties than DFSB flour.

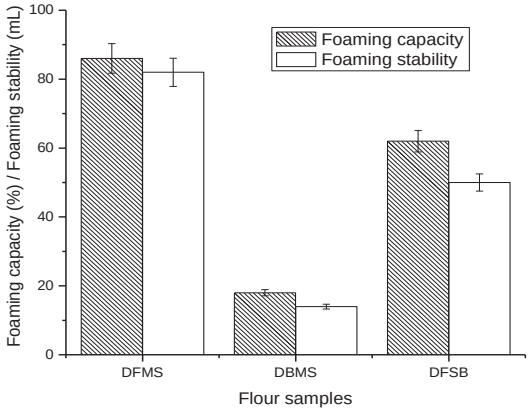

Fig. 5: Foaming capacity and foaming stability of DFMS, DBMS and DFSB flours

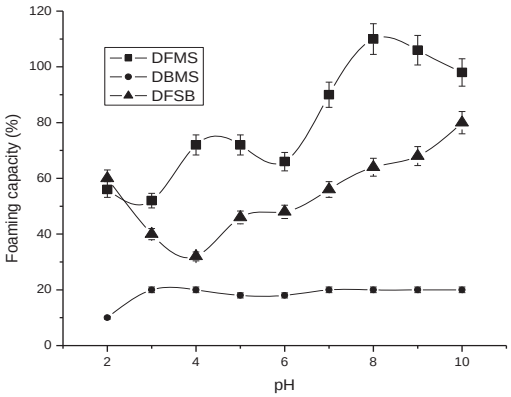

Fig. 6. Effect of pH on the foaming capacity of DFMS, DBMS and DFSB flours

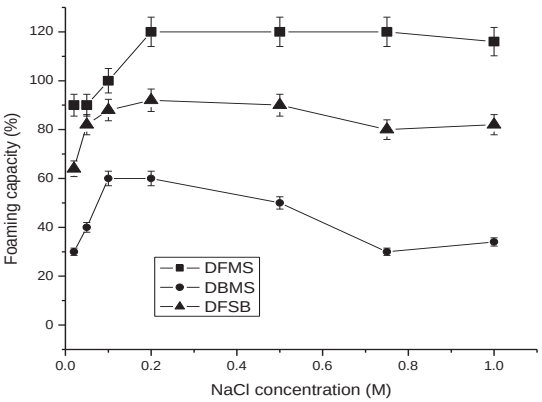

Fig. 7. Effect of NaCl concentration on the foaming capacity of DFMS, DBMS and DFSB flours

3.3. Scanning Electron Microscopy (SEM)

The scanning electron micrographs of the flour samples are shown in Fig. 9. In Fig. 9A and 9C, no noticeable difference was

observed in the structure of defatted soy and moringa flours. It can be seen that the defatted moringa seeds flour particles are rounded granules, separated by air vacuoles. However, Fig 9B shows that the heat-assisted debittering process caused the particles to form a continuous protein matrix devoid of air cells. An increase in inter protein bonding between the particles may be responsible for these micro-structural changes. This buttresses the report of Anderson [44] and Radha *et al.* [29] that processing of proteins by moist heat can cause aggregation of particles.

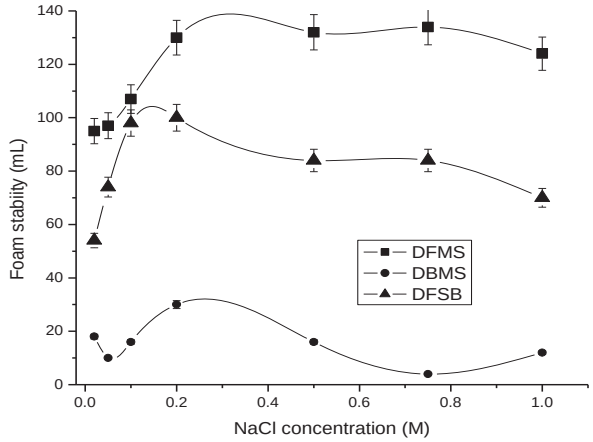

Fig. 8: Effect of NaCl concentration on the foaming stability of DFMS, DBMS and DFSB flours.

3.4. Colour

The colour characteristics of the investigated flour samples are presented in Table 4. The L –value for colour lightness of the DBMS and DFMS flours were not significantly different, but that of DFSB flour was significantly higher ($p<0.05$). This implies that DFSB is whiter than the other two.

3.5. Trypsin Inhibitor Activity And Phytic Acid Content

Trypsin inhibitor activity has been reported extensively in soybean [14, 43, 20] but in this study, it was not detected in moringa seeds. This agrees with previous findings by other researchers [10, 9].

The phytic acid content of DFMS, DBMS and DFSB flour samples were found to be 0.38, 0.24 and 1.53 g/100 g respectively. This compares favourably with the results of Foidl *et al.* [10] for defatted moringa seeds and Hidvegi and Lasztity [45] for soybean. It is observed that debittering reduced the phytate content of moringa seeds. Phytate as phytic acid is a major phosphorus storage compound in plants and can account for up to 80% of the total phosphorus in seeds. At acidic pH, phytic acid reacts with proteins and upon neutralization, insoluble complexes are precipitated. The strong ability of phytic acid to chelate multivalent metal ions, especially zinc, calcium and iron also results in poor bioavailability of such ions [46].

Table 4. Colour characteristics of DFMS, DBMS and DFSB flours

Flour sample	Flour concentration (% w/v)			
	L	a	b	DE
DFMS	83.89	–0.85	–0.47	11.91
DBMS	83.24	–0.43	11.72	13.40
DFSB	86.29	–0.62	14.50	14.57

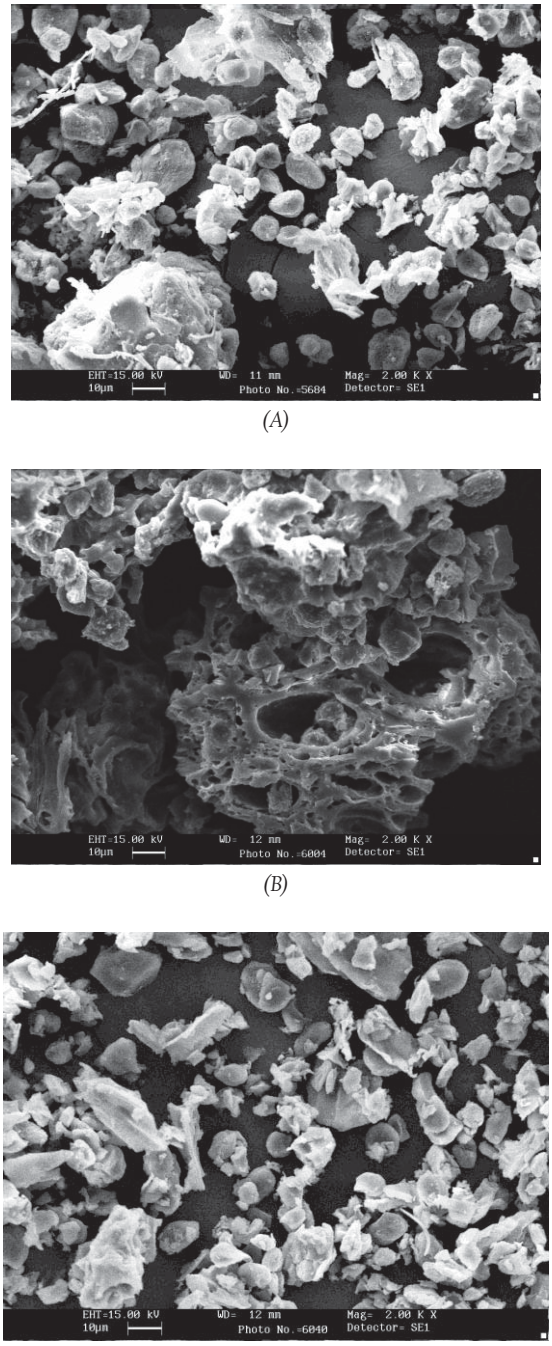

Fig. 9: Scanning electron micrographs of (a) DFMS flour; (b) DBMS flour; (c) DFSB flour. LEO 435 VP, Cambridge model scanning electron microscope was used. For all flour samples the scale bar represents 10 µm; magnification 2000×

4. Conclusion

A process for debittering *Moringa oleifera* seeds has been developed. This paper compares the functional properties of defatted raw moringa seeds and debittered moringa seeds flours with that of defatted soybean flour. With the removal of the bitter principle in moringa seeds which presents great limitation for its utilization, debittered moringa seeds flour showed relatively higher emulsification, foaming and gelation functional properties than soybean. This holds good prospect for the utilization of moringa

seeds flour as an alternative source of vegetable protein in value-added products development; however, detailed toxicological properties needs to be evaluated regarding the chemistry of moringa seeds protein and its interaction with other food constituents such as starch and lipids.

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