Volume 13 Number 1 JUNE 2011

IFE JOURNAL OF SCIENCE

A Journal of the Faculty of Science
Obafemi Awolowo University, Ile-Ife, Nigeria

ISSN 0794-4896

Ife Journal of Science

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PROTEIN EXTRACTABILITY FROM DEFATTED *MORINGA OLEIFERA* LAM. SEEDS FLOUR

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(Received: December, 2010; Accepted: May, 2011)

ABSTRACT

Protein extractability from defatted *Moringa oleifera* seed flour was studied under various conditions of pH (2-10), time (5-60 minutes), salts (NaCl and $CaCl_2$), salt concentrations (0-2 M) and solvent to flour ratio (10:1-30:1). Results showed that protein extractability was dependent on pH, type of salt, salt concentrations and extraction time. Salts extracted more proteins from the moringa seed flour than water. Maximum extraction of protein was 85.06% and 84.72% with 0.5 M $CaCl_2$ and 0.75 M NaCl respectively. On varying the pH, maximum extraction of protein from defatted moringa flour was obtained at pH 4 with 0.5 M $CaCl_2$. Solvent to flour ratio had no significant effect on the protein extractability.

Keywords: Moringa Seed, Protein Extractability, Solubility, Salt Concentration

INTRODUCTION

Plant proteins play significant roles in human nutrition, particularly in developing countries where average protein intake is less than required (Khalid *et al.,* 2003). Due to inadequate supplies of food proteins, there has been a constant search for unconventional legumes as new protein sources for use as both functional food ingredients and nutritional supplements (Onweluzo *et al*., 1994). In recent years, there has been serious worry over lack of adequate protein to meet the nutritional requirements of a large segment of world's population. Modern research has thus focused more on oilseed crops as largely unexploited sources of food proteins.

Extractability of proteins of various legumes in aqueous salt solutions has been widely studied and different results were obtained due to the large number of factors involved. Munasinghe and Sakai (2004) demonstrated that NaCl had a significant increase in protein extractability as compared to KCl and LiCl salts. Prakash (1986) studied the effect of NaCl on the extractability of proteins from sesame seeds. The effects of various factors on protein extraction from conophor seeds (Ekpenyong, 1986). Kinsella (1979) listed pH and ionic strength of the medium as having significant influence on the functional properties of proteins. Ragab *et al.* (2004), Lawal (2004), Inyang and Iduh (1996) and Badifu and Akubor (2000), studied the effect of NaCl and pH on the functional properties of cowpea proteins, locust bean protein isolate, sesame seed concentrate and African breadfruit kernel flour respectively. El Tinay *et al.* (1980) studied the extraction of protein from cottonseed flour at different pH and salt concentration. Molina *et al.* (1974) worked on the extraction of nitrogen constituents of jack bean. Aluko and Yada (1995) determined the physicochemical and functional properties of cowpea globulin isolate as a function of pH and ionic strength. Solubility characteristics, under various conditions, are very useful in selecting optimum conditions for extracting proteins from natural sources (Mahgoub and Elbashir, 2009).

Drumstick (*Moringa oleifera* Lam.) is an important commercial vegetable crop throughout India and some other parts of the world; the immature green pods, apart from being widely eaten as vegetable curry are sliced and canned in brine for export to Europe and America. Hitherto, studies on *Moringa oleifera* seeds was limited to high oleic acid oil and water purification property (Katayon *et al.,* 2006; Foid *et al.,* 2001 and Folkard *et al.,* 1993), whereas it contains up to 332.5 g of crude protein per kg of sample (Jose *et al.,* 1999). Studies to characterize the interaction effects of pH and salts on the extraction of

protein from moringa seed flour will provide useful information for food processors wishing to use it for specific food applications. No report on the effect of several variables like pH, time, solvent to flour ratio and salt concentration on the extractability of defatted moringa seed flour have been documented, hence, this study was carried out to investigate the effects of various factors such as pH, time, solvent to flour ratios, salt type and salt concentrations on protein extractability.

MATERIALS AND METHODS

Collection and preparation of defatted Moringa flour

Matured and dry seeds (10 kg) that were botanically identified as *Moringa oleifera* and purchased randomly from a local market in Mysore, India were dehulled and cleaned to obtain the kernels. The kernels were conditioned to an optimized moisture content of 8.5 g /100 g of sample (w.b.) and flaked at a drum clearance of 0.85 mm using a flaking machine (Model J #6725, Kvarnmaskiner Malmo, Sweden). The flakes were dried at 50 - 55 °C for 4 h to a moisture content of 5.5 g /100 g of sample (w.b.) and subsequently defatted by repeated washing with hexane until the fat content was below 1%. The defatted sample was air dried at room temperature (30 °C) for about 24 h and milled in a Quadrumat mill (Brabender, Duisburg, Germany) to pass through a 100 μm mesh stainless steel sieve and designated as defatted moringa seed flour. The flour sample was stored in an airtight container under refrigeration until when needed.

Proximate analysis

Analysis sample of moringa seed and its defatted flour for fat, and ash contents was carried out. Moisture content of moringa seeds was determined using the American Society of Agricultural and Biological Engineers standard S410.1 (ASABE, 1998). Approximately 10 g of sample was oven dried at 130 °C for six hours after which it is cooled in desiccators and weighed. The percent difference in weight before and after oven-drying was calculated as the moisture content per 100 g of sample. Crude fat was estimated by extracting 20 g of the kernels in petroleum ether (b.p. 4060 °C) under reflux for 8 h using a soxhlet unit. The extract was calculated per 100 g of sample as crude fat (Ogunsina *et al.,* 2010). Ash content was determined by charring 5 g of sample and incinerating the residue in a muffle furnace (Lenton Model AF 11/6B, Made in England) at 550 °C for 6 hrs. The weight of the greyish white residue formed was calculated and expressed as total ash per 100 g of sample (AOAC, 2000). Total nitrogen content was estimated using the Kjeldahl procedure and crude protein was obtained by multiplying the nitrogen content by 6.25. Total carbohydrate was calculated as = 100 - (protein + fat + ash). All values were expressed as mean of triplicates.

Effect of time on protein extraction

The effect of time on the extractability of protein from defatted moringa seed flour was determined by dispersing 1 g of sample in 10 ml of water. The extraction periods that were investigated are 10, 20, 30, 40, 50 and 60 and 120 min. Samples were stirred on a mechanical stirrer for specified periods. The solubilized liquor was separated from insoluble material by centrifugation at 6000 rpm for 30 min at room temperature (25° C). The aliquots were taken for protein estimation according to AOAC micro-Kjeldahl method (AOAC, 2000).

Effect of different salt concentrations on protein extraction

Protein extractability was determined by using either NaCl or $CaCl_2$ with 0.05 to 2.0 M concentrations; solvent to flour ratio, 20:1 and extraction time, 45 min. The homogenate was separated from insoluble residue by centrifugation and the aliquots were taken for protein estimation.

Effect of different solvent to flour ratios on protein extraction

The effect of solvent to flour ratio on the extraction of protein was studied. The selected salt concentrations were 0.5 M $CaCl_2$ and 0.75 M NaCl, as they gave highest protein extraction. Samples of different solvent to flour ratios (10:1, 15:1, 20:1, 30:1) with $CaCl_2$ and NaCl were prepared and stirred on a mechanical stirrer for 45 min. The solubilized liquor was separated from insoluble material by centrifugation and aliquots were taken for protein estimation.

Effect of pH on protein extraction

The effect of pH on the protein extractability of defatted moringa seed flour was determined by dispersing 1 g flour in 50 ml of

distilled water and 0.5 M $CaCl_2$ with pH adjustments over the range of 2-10 by adding either 0.2 N NaOH or 0.2 N HCl. The suspensions were stirred for 45 min and centrifuged at 6000 rpm for 30 min at room temperature (25° C). The protein content of each of the supernatants was determined by Kjeldahl method. The percentage of soluble nitrogen was calculated and plotted against corresponding pH values.

Statistical Analyses

All the experiments were conducted in triplicate and means ± SD are reported. For proximate analysis, based on the analysis of variance, means were separated by Duncan grouping where significant difference were indicated (SAS, 2002).

Table 1. Proximate Composition of *Moringa oleifera* Seed and its Defatted Flour

Composition (g/100g)	Seed	Defatted flour
Protein	36.18±0.04[b]	62.76±0.16[a]
Ash	3.73±0.04[b]	6.75±0.03[a]
Fat	43.58±0.08[a]	0.08±0.0015[b]
Carbohydrate (by difference)	16.51±0.07[b]	30.42±0.05[a]

Values are means of triplicates ± standard deviations
Means with different letters on same row are significantly different.

RESULTS AND DISCUSSION

The chemical composition of moringa seed and its defatted flour are reported in Table 1. Fat constituted substantial portion of the kernel weight (39.36 g of fat/100g of sample). Protein content of the flour increased significantly ($p<0.05$) from to 32.7 and 54.4 g/100 g as a result of defatting. A similar significant increase was also observed for carbohydrate and ash contents. Earlier reports by Egbekun and Ehieze (1997) and Alobo *et al.* (2009) showed similar changes in soybeans, beniseed and cashew nut. The high-protein content of the moringa seed and its good balance of essential amino acids (Foidl, 2001) suggest that it can supplement cereal and tuber flours which are not only low in protein but deficient in amino acids.

The influence of extraction time on protein extractability from defatted moringa seed flour in three different solvents viz: water, 0.5 M $CaCl_2$ and 0.75 M NaCl is shown in Fig. 1. Virtually all of the protein was extracted within 10 to 20 min, which indicated that the bulk of moringa seed protein solubilized within a relatively short period of time. With water (26.11%) and NaCl (58.66%), most of the protein extracted in the initial 10 min compared to $CaCl_2$ where maximum protein extraction was obtained at 20 min

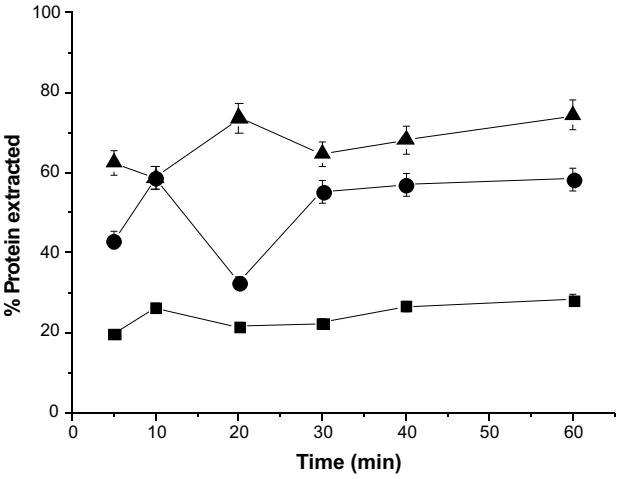

Figure 1 Effect of Time on the Extractability of Proteins from the Defatted Moringa Flour. (-■ - Water, -● - NaCl, -▲ - $CaCl_2$)

(73.67%). In the next 10 min., extraction of protein decreased in all solvents which later on increased gradually. This result agreed with the findings of Thompson (1977) for mung bean proteins, reporting that the time of extraction did not have much influence on nitrogen extractability. Jyothirmayi *et al.* (2006) had also reported that extraction of proteins increased till 35 min after which it remained constant.

Protein extractability profile of defatted moringa seed flour in two different salts of various concentrations is shown in Fig. 2. The increase in ionic strength of the salt solutions

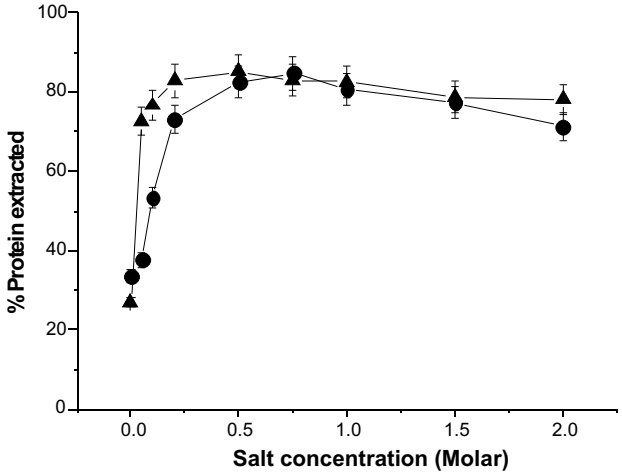

Figure 2 Effects of Different Concentration of Nacl and $Cacl_2$ on the Extractability of Protein from Defatted Moringa Flour (-●- NaCl, -▲- $CaCl_2$)

increased the protein solubility. With NaCl, protein extraction at 0.05, 0.1, 0.2, 0.5, 0.75, 1.0, 1.5 and 2.0 M concentration were 37.74, 53.28, 73.01, 82.52, 84.72, 80.63, 77.38 and 71.21% respectively. Increase in protein extraction was observed from 33.63% at 0.0 M concentration to 84.72 at 0.75 M concentration. With **C**a**C**l_2 the protein extraction at 0.05, 0.1, 0.2, 0.5, 0.75, 1.0, 1.5 and 2.0 M concentration was 72.71, 76.46, 82.71, 85.06, 82.9, 82.55, 78.72 and 78.04% respectively. Increase in protein extraction was also indicated from 33.63% at 0.0 M concentration to 85.06% at 0.5 M concentration. When the salt concentration exceeded optimum levels (eg. 0.75 M and 0.5 M in the case of NaCl and $CaCl_2$ respectively), protein extractability decreased gradually showing that the extractability of moringa seed proteins depend on the concentration of salt used. El Tinay *et al.* (1980) had earlier shown that protein extractability from cotton seed flour increased as the salt concentration increased with maximum extraction of 75% occurring at 1.0 M $CaCl_2$. Similar increases in protein solubility with increase in ionic strength have been reported by McWatters and Holmes (1979) for soy flour. It can be observed in Figure 2 that protein extraction with $CaCl_2$ is more efficient than NaCl. The improvement of protein extraction caused by salts may be due to the fact that moringa seed protein is composed of more salts soluble globulins. Similar results have been reported for defatted karkade seed flour [Khalid *et al.,* 2003; Gheyasuddin *et al.,* 1970; Hamza, 1995).

Protein extractabilities of defatted moringa seed flour with different solvent to flour

Table 2. Effect of Solvent to Flour Ratio on the Extractability of Proteins from Defatted *Moringa oleifera* Flour

Solvent to flour ratio	% Protein extractability		
	Water	NaCl	$CaCl_2$
10:1	39.51±0.021	72.34±0.056	83.52±0.021
15:1	32.70±0.056	74.16±0.056	83.25±0.028
20:1	30.28±0.084	78.19±0.056	82.33±0.035
30:1	26.60±0.056	78.66±0.070	84.40±0.056

ratios are presented in Table 2. It is evident from the data that solvent to flour ratio had very little influence on protein extractabilities with two different solvents (0.5 M $CaCl_2$ and 0.75 M NaCl). With water, increasing the solvent to flour ratio from 10:1 to 30:1, decreased the percent extractability of protein from 39.51 to 26.60%. However with NaCl, there was a slight increase in nitrogen solubility as the ratio increased from 10:1 to 20:1. Increasing the solvent to flour ratio from 20:1 to 30:1 decreased the extractability of protein from 39.51% to 26.60%. Adel and Mohammed (1981) showed that solvent to flour ratio of 10:1 or less than that had little influence on nitrogen extractability and the percent extractable nitrogen was found to remain more or less constant. Maximum extractability obtained was 83.52% and 78.19% with 0.5 M $CaCl_2$ and 0.75 M NaCl respectively. These results agree with the findings of Gheyasuddin *et al.* (1970) and Thompson

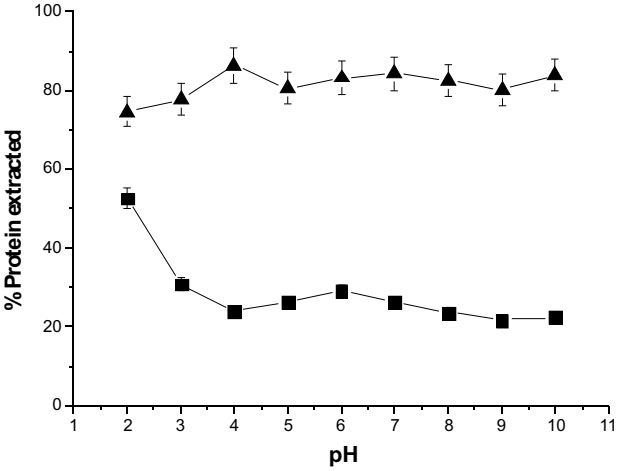

Figure 3 Effect of pH on the Extractability of Proteins from the Defatted Moringa Flour (-▲- Water, -■- $CaCl_2$)

(1977) for sunflower seed and mung bean proteins respectively.

Nitrogen solubility profiles of defatted moringa seed flour dispersed in water and 0.5 M $CaCl_2$ are shown in Fig. 3. The response of protein extraction to changing levels of pH in 0.5 M $CaCl_2$ differed significantly from that of the water suspension. At pH 4.0, protein extraction in water was significantly lower than that of $CaCl_2$ suspension. McWatters and Holmes (1979) reported similar findings for soy flour protein except that the salt concentration of 0.5 M $CaCl_2$ was used in the present study. Jyothirmayi (2006) reported pH 3.0-4.0 as the common point of minimum dispersion for proteins of defatted erythrina variegate flour. Kanu *et al.* (2007) and Arogundade *et al.* (2006) also reported pH 4.0 as the isoelectric point for sesame protein and broad bean (*Vicia faba* L.) protein extraction. This pattern was unique for defatted moringa seed protein in comparison with many other proteins showing a drop in the protein extractability in the acidic pH range 4.0 - 5.0.

In $CaCl_2$ sample, the percentage of soluble proteins increased from pH 2.0 - 4.0 followed by a decrease at pH 5.0 and then a gradual increase from pH 5.0 - 7.0. As pH increased further, the soluble protein decreased slightly till pH 9.0 followed by an increase at pH 10.0. Solubility behaviour provides a good index of the potential applications of proteins. Protein solubility at various pH values is a useful indicator of the behaviour of protein materials when incorporated in food systems (Okezie and Bello, 1988).

CONCLUSION

This work indicates that pH, salt type and concentration and extraction time affect protein extractability from moringa seeds flour. The highest yield of moringa seed flour proteins was extracted at $CaCl_2$ concentration of 0.5 M and NaCl concentration of 0.75 M. It is established that changing the electrovalent properties of the protein, either by adjusting the pH or modifications in salt concentration influences the functional characteristics of moringa seed flour. This study offers valuable information on the potential of moringa seed flour as a functional agent in a variety of formulated foods.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. V. Prakash, Director, Central Food Technological Research Institute (CFTRI), Mysore, India and Dr. A.G. Appu Rao, Head, Protein Chemistry and Technology Department for their keen interest and support during this investigation. The third author expresses profound gratitude to the United Nations University, Tokyo Japan for granting a fellowship at CFTRI where this work was carried out.

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Tables of Contents: June Edition 2011; Vol. 13, No. 1