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Evaluation of some Physicochemical and Functional Characteristics of Proteins from *Mucuna pruriens* Bean

Yemisi A. Adebowale[1,2*] • Thomas Henle[2] • Uwe Schwarzenbolz[2]

[1] Department of Food Technology, University of Ibadan, Ibadan, Nigeria
[2] Fakultät für Mathematik und Naturwissenschaften, Institut für Lebensmittelchemie, Technische Universität Dresden, Germany

Corresponding author: * **yemiadebo@yahoo.co.uk**

ABSTRACT

Proteins isolated from *Mucuna pruriens* bean were characterized and the foaming properties of both the flour and protein isolates determined. The pH-dependent protein solubility profile of the flour and isolates indicated that the isoelectric point ranged between 4.0 and 5.0. At all concentrations, the foaming capacity of both the flour and protein isolates increased with concentration increase; increase in the ionic strength from 0.1 to 0.4 M and declined afterward as the ionic strength increase from 0.6 to 1.0 M. It reduced as the pH increased from 2 to 4 (or 5) after which it increased as pH increased to a maximum value at pH 10. The highest foam stability was observed at pH 4 or 5, following which it reduced as the pH increased. Incorporation of carbohydrates at a level of 0.25g/g of protein increased the foaming capacity and stability compared with the control sample. In the flours, the starch resulted in the highest improvement of the capacity and stability against protein isolates where lactose resulted in the highest increase. Two peaks were distinguished in Differential Scanning Calorimetry (DSC) thermograms: 7S-vicilin and 11S-legumins. In 7S-vicilin the stability of the proteins was highest in *cochichinensis* and least in *rajada*. The trend of stability of the 11S-legumins was highest in *Veracruz mottle* and least in *rajada* and *deerigeana*. Gel filtration chromatographic studies of the protein isolates show two major fractions with molecular masses of 40 and 15.3 kDa and minor components' molecular weight 913.5, 35.8, 3.9 and 1.2 kDa. Polyacrylamide gel electrophoresis revealed a similar pattern of molecular weights. Finally, a major band consisting of a broad zone with molecular weights 36 ± 7 and 17 ± 3 kDa appeared in all the samples under both reducing and non-reducing conditions.

Keywords: flour, foaming properties, gel filtration chromatography, legume, seed proteins

INTRODUCTION

Increasing demand for plant protein, particularly in developing countries, as a substitute for scarce and expensive animal protein has been emphasized in our previous publications (Adebowale and Lawal 2003, 2004; Lawal and Adebowale 2004, 2005; Lawal *et al*. 2005; Lawal and Adebowale 2006). There has also been renewed awareness on the utilization of legumes in developed countries, as they are now regarded as versatile functional ingredients which could serve as replacement for animal proteins already implicated in a number of diseases (Guillion and Champ 1966; Sirtori and Lovati 2001).

Legumes are target crops in this regard because they are one of the most important sources of proteins, carbohydrates and dietary fibre for human nutrition. Generally legumes have protein contents between 20 and 40% and a few range between 40 and 60% (Emenalom and Udedibie 1998; Maneepun 2000). One such crops is mucuna beans (*Mucuna* spp.). Mucuna beans, an underutilised legume, which belongs to the family *Fabaceae*, is primarily used as green manure cover crops (GMCCs) (Buckles *et al*. 1998; Carsky *et al*. 1998). The seed is lesser known and neglected because of the lack of information on the composition as well as its utilisation particularly for food and other uses (Prakash and Misra 1987; Ravindran and Ravindran 1988). Limited studies have revealed that *Mucuna* is not only rich in proteins but also carbohydrates, fats, minerals and other nutrients. However, it is limited by the presence of anti-metabolic/ anti-physiological substances such as protease inhibitors, phenolic substances, non-protein amino acids, lecithins, saponins, flatulence and non-starch polysaccharides (Siddhuraju *et al*. 2000; Vidivel and Janardhanen 2001; Pugalenthi and Vadivel 2007). The level of some of these factors has been evaluated in earlier studies (Adebowale *et al*. 2005a, 2005b).

For plant proteins to be useful and successful in food application, they should ideally possess several desirable characteristics, referred to as functional properties, as well as providing essential amino acids (Kinsella 1976). These properties are intrinsic physicochemical characteristics, which affect the behaviour of proteins in food systems during processing, manufacturing, storage and preparation (Kinsella 1976). They include solubility, water and oil absorption capacity, gelation, foaming and emulsifying properties.

Foam can be defined as a two-phase system consisting of air cells separated by a thin continuous liquid layer called the lamella phase (Zayas 1997). Foam is produced when air is injected into a liquid and entrapments in the form of bubbles occur. Such a system is very unstable and there is a need of some surfactant molecules to orient at the air-water interface. The most critical requirement for the formation of foams during whipping is rapid reduction of the free energy (interfacial tension) of the newly created interface, with the aid of a surfactant. The surfactants tend naturally to migrate towards interfaces where the global free energy of the molecules is lower than at either of the phases.

In the case of proteins, which are amphiphilic, adsorption proceeds through sequential attachments of several polypeptide segments. In most cases a greater portion of the molecule remains suspended in the aqueous phase in the form of loops and tails. Proteins are ideally suited than small molecular weight surfactants to act as macromolecular surfactants in foam-type products. This is because in addition to lowering the interfacial tension, proteins can

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form a continuous and highly viscous film at interfaces via complex intermolecular interactions (Damodaran 1996). The basic function of protein in foams is to decrease interfacial (surface) tension, to increase viscous and elastic properties of the liquid phase and to form strong gels. Although all proteins are amphiphilic, they differ remarkably in their surface-active properties.

Foaming or whipping (i.e., the capacity to form stable foams with air) is an important functionality of proteins in several products. Foaming properties include whippability and foamability, with the two terms being used interchangeably. These properties are measured as foam expansion or foam capacity. Foaming power measures the increase in volume upon introduction of a gas into protein solution or dispersions. Foam stability refers to the ability of foam to retain its maximum volume over time and it is usually determined by measuring the rate of leakage of fluid from the foam (Lin *et al*. 1974; Kinsella 1976). In whippability measurements the volume of foam is usually expressed as the percentage volume increase. Elgridge *et al*. (1963) expressed the volume of foam as foam expansion, which indicated the increase in volume.

Food foams usually consist of gas droplets dispersed in and enveloped by a liquid containing soluble surfactants. The surfactant lowers the surface tension of the liquid thereby facilitating deformation of the fluid and the marked expansion in its total surface area against its own surface tension. The surfactant also lowers interfacial tension. Food foams must also be stable, and thus protein-forming foams exhibit a critical balance between their ability to engage in limited intermolecular cohesion required to form a stable membrane and the tendency to self-associate excessively, which would result in the aggregation and breakdown of foam (Bora 2002).

The basic requirements for a protein to be a good foaming agent are the ability to (i) absorb rapidly at the air-water interface during bubbling; (ii) undergo rapid conformational change and rearrangement at the interface during bubbling and (iii) form a cohesive visco-elastic film via intermolecular interaction. The first two criteria are important for foamability whereas the third is important for the stability of the foam (Damodaran 1996).

Apart from these there are a variety of other factors which may influence the absolute and apparent foaming properties of proteins. These include protein source, method of preparation, composition, solubility, concentration, pH, temperature, ionic strength and duration of heating, the presence of salts, sugars, lipids and of course method of measurements (Buckingham 1970).

In the search for novel plant proteins, this paper considers the foaming characteristics of *Mucuna* proteins both in the flour and the isolate. This work involves studies on the effect of pH, ionic strength and concentration of proteins on the foaming capacity and stability of the flour and isolate. The pH dependent nitrogen solubility profiles were presented. The proteins present have also been characterized using gel filtration chromatography and polyacrylamide gel electrophoresis, granular structure was studied using scanning electron microscopy and the identity of the proteins studied using differential scanning calorimetry. This work is part of our comprehensive research aimed at the eventual incorporation of protein isolates into food products to produce natural, cost-effective and adaptable functional foods.

MATERIALS AND METHODS

Six species of *Mucuna* bean seeds – *deeringeana*, *rajada*, *pruriens*, *veracruz mottle*, *veracruz white* and *cochinchinensis* – were obtained from the International Institute of Tropical Agriculture/ International Livestock Research Institute IITA/ILRI, Ibadan-Nigeria. All the chemicals and reagents used are of analytical grade (analar grade). They were purchased from Sigma-Aldrich.

Preparation of defatted flour

Mucuna bean seeds were dehulled manually using a pestle and mortar. The seeds were ground in a Christy Laboratory Mill (Cheff Food Processor, Japan) and thereafter sieved through a screen of 20 mesh sizes before extraction for 9 hrs with hexane in a Soxhlet apparatus at a flour: hexane ratio of 1: 10. The defatted flour was air-dried at room temperature (approximately 28°C) and subsequently kept in air-tight plastic containers at 4°C prior to use.

Preparation of protein isolates

The procedure for isolate preparation was as described by Lqari *et al*. (2002) with some modifications which involved the use of different extractants as mentioned below. The basic steps are as follows.

The slurry (1: 20, flour: water ratio) at pH 6.37 was first extracted for 10 min, thereafter the slurry was stirred for 2 hrs using a Gallenhamp magnetic stirrer; the pH was adjusted to the desired pH using 1 M NaOH or 1 M HCl. Different extractants [ascorbic acid (0.5% w/v); EDTA + 0.25% ascorbic acid; cystein (0.5%); sodium sulphite (0.25%) and water] were added singly. Each extractant was centrifuged in a Sorvall RC5C automatic super speed refrigerated centrifuge at 10,000 × *g* for 30 min at 5°C. After centrifugation and recovery of supernatant, three additional extractions were carried out with half of the volume of the initial water. The supernatants were pooled and precipitated at pH 5.0, the isoelectric point (IEP). The precipitate formed was subsequently recovered by centrifugation at 10,000 × *g* for 15 min at 5°C. The precipitate was washed twice with distilled water, adjusted to pH 5.0 with HCl, and then freeze dried. The precipitate was neutralised by the addition of 1 M NaOH. The final protein isolate was obtained by lyophilisation. A schematic diagram of the isolation procedure is shown in **Fig. 1**.

Protein solubility

Protein solubility was determined by the method of Sathe *et al*. (1982) with some modifications. The suspensions (0.2%) of the flour in distilled water were adjusted to pH 2-11 using 1 M HCl and 1 M NaOH. The percent nitrogen in each supernatant was determined by the micro Kjedahl method according to the method already described in AOAC (1990). The percentage of soluble protein was calculated as the percentage of nitrogen determined, multiplied by 6.25, and expressed on wet weight basis according to the AOAC (1990).

Determination of foaming properties

The foam capacity and stability were determined by the method of Coffman and Garcia (1977). A known weight of the *Mucuna* sample was dispersed in 100 ml distilled water. The resulting solution was homogenized for 5 min at high speed. The volume of foam separated was noted. The total volume remaining at intervals of 0, 0.30, 1, 2, 3, 4 up to 24 hr was noted for the study of foaming stability. Studies were carried out to consider the effect of concentration on the foaming properties by whipping 2, 4, 6, 8, and 10% (w/v) of the dispersion as described above. The influence of ionic strength was studied by dispersing 2 g of the flour and protein isolates in 100 ml KCl solutions of various ionic strengths which ranged from 0.1-1.0 M before whipping vigorously. The effect of pH on the foaming properties was studied by preparing flour and isolate dispersions at various pH values ranging from 2-10 using 0.5 M NaOH and 0.5 M HCl, followed by vigorous whipping as described earlier.

$$\% \text{ Foaming Capacity} = \frac{\text{Volume after homogenisation} - \text{Volume before homogenisation}}{\text{Volume before homogenisation}} \times 100$$

$$\% \text{ Foaming Stability} = \frac{\text{Foaming volume after time } t}{\text{Initial foam volume}} \times 100$$

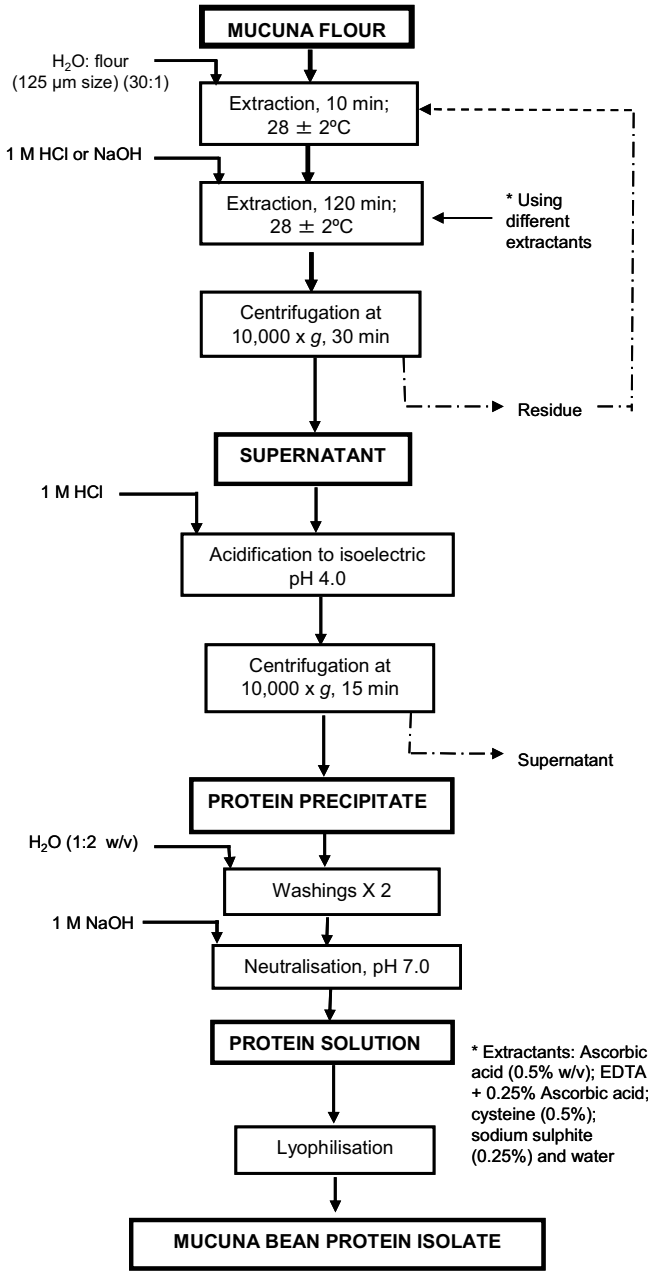

Fig. 1 Preparation of *Mucuna* bean protein isolate.

Gel eletrophoretic studies

The molecular weight profile for the protein fractions was established using sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis as described by Laemmli (1970). 100 mg of the sample was added to 2.5 ml of the buffer containing 0.5 M Tris-HCl (pH 6.8), 0.5% bromophenol blue, 10% glycerol and 2% SDS. 5% 2-mercaptoethanol (2-ME) was added to another preparation to effect reduction of disulphide bonds. The preparation was centrifuged at 20,000 × *g* for 15 min at 4°C and the supernatant was employed for electrophoresis. Protein samples (10 µl) were added onto the gel using a model 491 cell (Bio-rad Laboratories, California, USA). The electrode buffer contained a mixture of 0.025 M Tris buffer; 0.192 M glycine and 10% SDS to a final pH of 8.7. The separating gel contained 12.5% acrylamide (final concentration), while the stacking gel contained 4.5%. Electrophoresis was carried out at a constant current of 60 mA at a maximum voltage of 600 V until the dye reached the bottom of the gel. The gel was stained with Coomassie brilliant blue G-250 and de-stained with 25% methanol solution. The gel was then transferred to 25% ammonium sulphate solution for storage. The molecular weight of protein sub-units for each sample was determined using the broad spectrum molecular weight standard from Sigma-Aldrich containing the following: aprotinin, bovine lung, 6.5 kDa; α-lactalbumin, bovine milk, 10.2 kDa; trypsin inhibitor, soybean, 20.0 kDa; trypsinogen, bovine pancreas, 24.0 kDa; carbonic anhydrase, bovine erythrocytes, 29.0 kDa; glycerol-3-phosphate dehydrogenase, rabbit muscle, 36.0 kDa; ovalbumin, chicken egg, 45.0 kDa; glutamic dehydrogenase, bovine liver, 55.0 kDa; albumin, bovine serum, 66.0 kDa; fructose-6-phosphate kinase, rabbit muscle, 84 kDa; phosphorylase b, rabbit muscle, 97 kDa; rabbit muscle phosphorylase b, 97.4 kDa; β-galactosidase, *Escherichia coli*, 116.0 kDa; myosin, rabbit muscle, 205.0 kDa.

Gel filtration chromatography

Gel filtration chromatography of the samples were carried out according to the modified method of Lqari *et al*. (2002). Lyophilized samples (0.15 g) were dissolved in 10 ml of 0.05 M K_2HPO_4 and 0.15 M Na_2SO_4 buffer (pH 7.0). Gel filtration was then carried out in the HPLC system equiped with Macrophere GPC 300A 7 µm (Alltech Associate Ltd.). Volume injection and concentration of samples were 200 µL and 1.6 mg protein per ml, respectively. The eluent used was the buffer mentioned earlier at a flow rate of 0.3 $ml.min^{-1}$. Protein elution was monitored at 280 nm and the approximate molecular masses were determined using blue dextran 2000E (2000 kDa); bovine serum albumin (67 kDa); ribonuclease (13.7 kDa); ovalbumin (43 kDa) and chymotrypsinugen A (25 kDa) as molecular weight standards.

Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) was performed according to the method described by Escamilla-Silva *et al*. (2003) using a Perkin-Elmer DSC 7 analyser calibrated with indium and zinc over a range of 25 and 450°C. Thermograms were obtained using slurries of the protein isolate prepared on a 20% (w/v) basis. Samples of 20 to 25 mg were weighed accurately to the nearest 0.01 mg on DSC stainless steel capsules and scanned at a heating rate of 10°C. min^{-1} from 30 and 130°C. Peak temperature and enthalpy were recorded using the DuPont thermal analyst 2000 system software.

Scanning electron microscopy

The granular structure of each of the protein isolate and commercial soybean isolate was determined using high vacuum scanning electron microscope (Cambridge Stereoscan S360 SE equipped with Oxford IWCA energy dispersed X-ray system). Freeze-dried protein isolate was mounted on aluminium stubs with double-sided sticky conductivity tape and coated first with 40-60 nm of gold. They were then photographed on the microscope at an accelerating voltage of 10 kV.

Statistical analysis

All experiments in this study are reported as mean of three replicate analyses. One-way analysis of variance (ANOVA) was carried out to compare between the mean values of different species of the seeds. Differences in the mean values were determined at $P < 0.05$ with Duncan's Multiple Range Test using (SAS 1990).

RESULTS AND DISCUSSION

Protein solubility

The pH-dependent protein solubility profile is presented for the flour and isolates in **Fig. 2**. It was found that the isoelectric point of the proteins was between 4.0 and 5.0. Generally, the solubility reduced as the pH increased until it reached the isoelectric point; this was followed by progressive increase in solubility with further increase in pH. Similar observation was reported for winged bean and chickpea (Sathe *et al*. 1982; Sanchez *et al*. 1999).

The solubility profile of a protein provides some insight into the extent of denaturation or irreversible aggregation and precipitation which might have occurred during the isolation process.

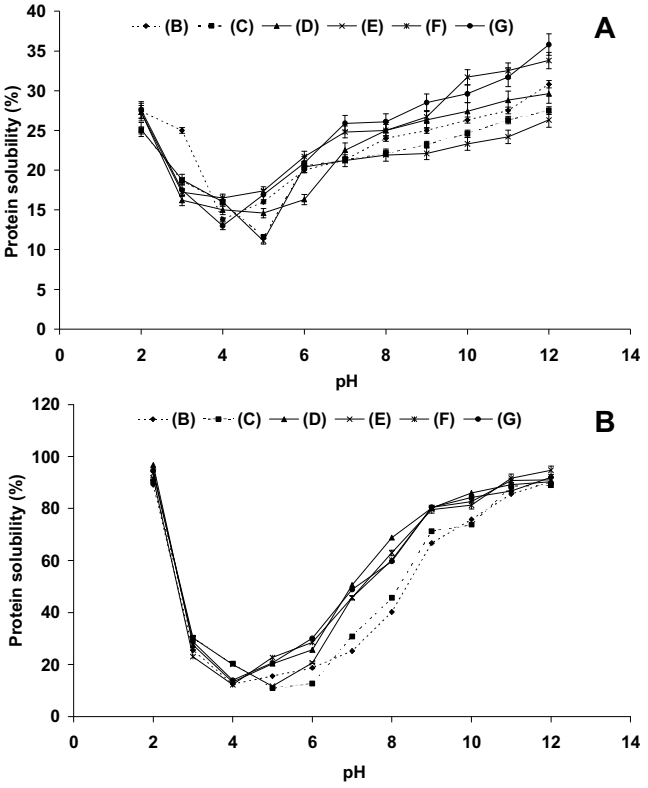

Fig. 2 (**A**) Protein solubility of *Mucuna* flours. (**B**) Protein solubility of *Mucuna* protein isolates. (B) = *Mucuna veracruz mottle*; (C) = *M. rajada*; (D) = *M. cochinchinensis*; (E) = *M. deerigeana*; (F) = *M. pruriens*; (G) = *M veracruz white*.

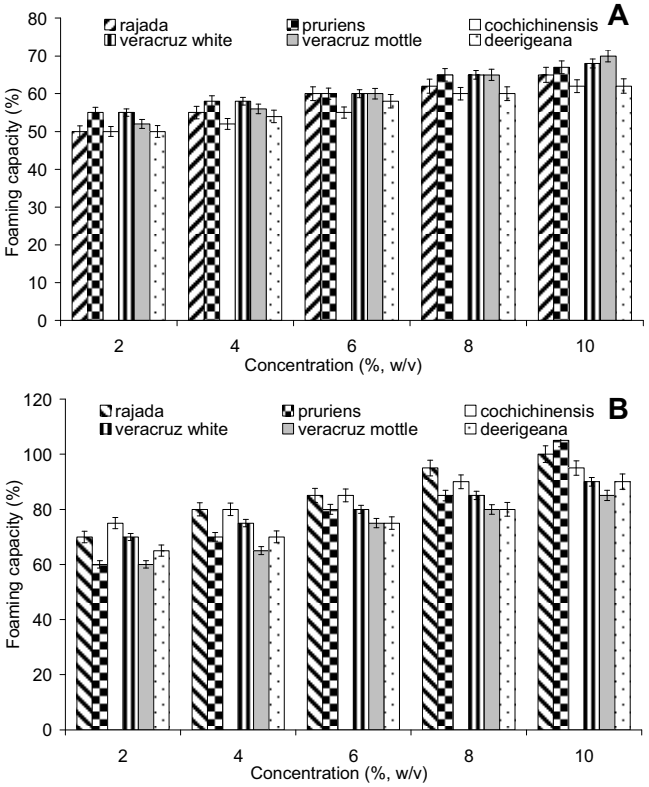

Fig. 3 The effects of concentration on the foaming capacity of *Mucuna* flours (A) and (B) protein isolates. Error bars are the standard deviation of three replicate studies. Samples in the same group with different letters are significantly different at $p<0.05$ according to DMRT.

It also gives an indication of the types of foods or beverages into which the protein could be incorporated. Factors such as concentration, pH, ionic strength and the presence of other substances influence the solubility of protein. The characteristics described above can be understood on the basis of the overall ionic charge of the protein with the pH. At low pH values, most of the carboxyl and amino groups from the lateral amino acid chains are protonated in the –COOH and $–NH_3^+$ forms respectively, and the overall charge of most protein molecules is positive. As the pH increases some of the carboxyl groups are dissociated into –COO and $–H^+$, according to their dissociation constants, and the positive charges associated with the proteins diminish up to the isoelectric point, where these are neutralized.

At this point, the protein cannot be hydrated by water molecules, due to the modification of its tertiary and quaternary structures and its solubility reaches a minimum value (Sathe *et al*. 1982). As the pH increases even more, the amino groups dissociate into $–NH_2$ and $–H^+$, and the overall protein charge becomes negative due to the presence of –COO groups and can consequently be hydrated and dissolved in water.

Foaming capacity and stability of flour and protein isolate

1. Effect of sample concentration on foaming characteristics

Data on the effect of concentration on the foaming capacity and stability of *Mucuna* flour and protein isolate is presented in **Figs. 3** and **4**, respectively.

At all the concentrations studied, the foaming capacity increased with increasing concentration for both the flour and protein isolates. For example, the foaming capacity for *M. rajada* flour increased from 50% at a concentration of 2% (w/v) to 65% at a concentration of 10% (w/v). The foaming capacity ranged between 62-70% for the flours at a concentration of 10% (w/v) and between 85 and 105% for the protein isolates at the same concentration. The values obtained for the flour are higher than the 32% reported for lupin seed flour (Sathe and Salunkhe 1981).

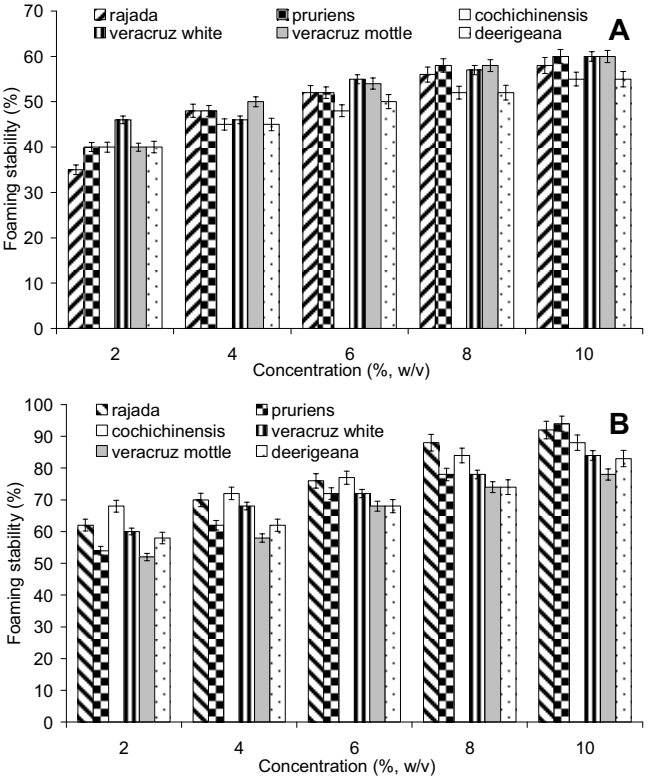

Fig. 4 The effects of concentration on the foaming stability for *Mucuna* flours (A) and (B) protein isolates. Error bars are the standard deviation of three replicate studies. Samples in the same group with different letters are significantly different at $p<0.05$ with DMRT.

The results of the foaming stability are shown in **Fig. 4**;

the foaming stability of all the protein isolates and flours increased with an increase in concentration. Foaming stability was higher for the protein isolates than for the flours.

Foam is a two-phase system consisting of air cells separated by a thin continuous liquid layer called the lamella phase (Zayas 1997). The ability of protein to form and stabilise foams depends on several parameters such as types of proteins, degree of denaturation, presence or absence of calcium ions, pH, temperature and whipping methods (Eleousa *et al*. 2006). Foam collapse takes place by any of the three mechanisms:

- Dispropotionation of bubbles;
- coalescence of bubbles due to instability of the film between them; and
- Drainage of water from the surface of the bubbles down the liquid layer thereby leading to the removal of proteins from film around the bubble. The protein on the film then becomes too thin to support the bubble (Adebowale and Lawal 2003).

Increase in concentration enhances greater protein-protein interaction, which increases viscosity and facilitates formation of multilayer cohesive protein film at the interface. The formation of a cohesive multilayer film offers resistance to dispropotionation and coalescence of bubbles. The availability of more proteins, as the level of protein isolates are increased, enhances foam formation (Ahmanda *et al*. 1999; Bongkosh *et al*. 2001; Lawal *et al*. 2005).

2. Effects of pH value on foaming characteristics

The effects of pH on the foaming capacity and stability of the defatted flours and protein isolates is presented in **Figs. 5** and **6**, respectively. The foam capacity of both the flour and protein isolates was reduced as the pH increased from 2 to 4 (or 5). The lowest foam capacity was therefore observed at pH 4 or 5. Thereafter the foaming capacity increased as the pH increased with a maximum value recorded at pH 10. On the other hand, the highest foam stability was observed at pH 4 or 5 (isoelectric region), following which the stability reduced as the pH increased. A similar observation was reported by earlier studies on lupin bean - *Lupinus mutabilis* (Sathe *et al*. 1982a), winged bean – *Psophocarpus tetragonolobu* L. (Sathe *et al*. 1982b), sunflower seeds - *Helianthus annuus* (Lin *et al*. 1974), cowpea seeds - *Vigna unguiculata* (Aluko and Yada 1995) and *Phaseolus lunatus* seeds (Chel-Guerrero *et al*. 2002). In the present study, a high foam capacity was obtained at both pH 2 and 10, although a higher value was observed at pH 10. A decrease in the attractive hydrophobic forces among the protein molecules occurs at high acidic and alkaline regions. At these extreme pH regions, the protein molecules become positively and negatively charged, respectively. This development leads to repulsion, which facilitates the flexibility of the protein molecules (Aluko and Yada 1995). Increase in the foaming capacity at the two extremes could therefore be attributed to an increase in the flexibility of the protein which induces its diffusion rapidly to the air-water interface to encapsulate air particles leading to enhanced foaming capacity (Chau and Cheng 1998). The highest foam stability was observed near the isoelectric region. In the past, studies have revealed that protein-stabilised foams are more stable in the neighbourhood of the isoelectric pH of the protein other than any other pH (Buckingham 1970; Aluko and Yada 1995).

The increase in foam stability is due to the formation of stable molecular layers in the air-water interface of the foams. Protein adsorption and viscoelasticity at an air-water interface is highest near the isoelectric pH because protein is not strongly repelled. In addition, the protein possesses low net charge near isoelectric pH, which may contribute to the formation of stable molecular layers in the air-water interface, a development that improves foam stability (Akintayo *et al*. 1999; Adebowale and Lawal 2003).

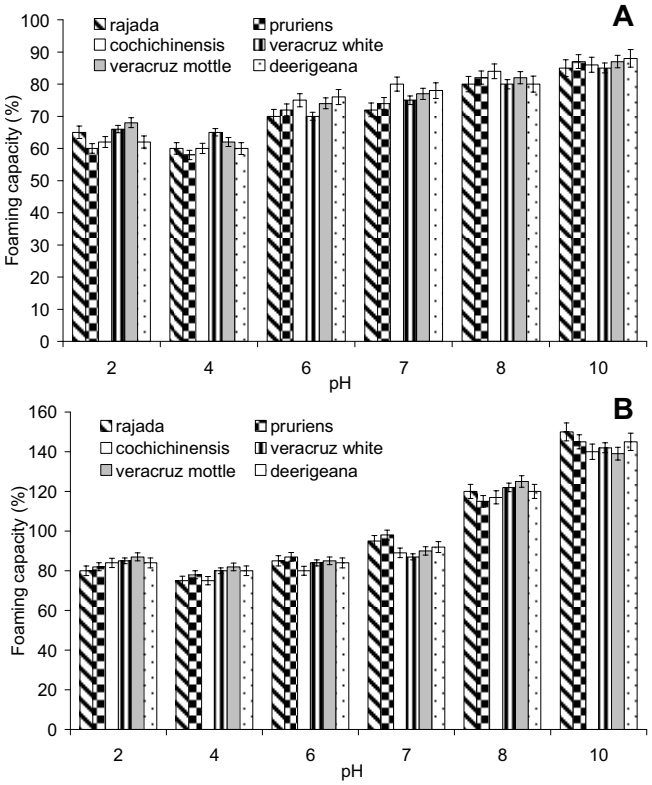

Fig. 5 The effects of pH on the foaming capacity of *Mucuna* flours (A) and (B) protein isolates. Error bars are the standard deviation of three replicate studies. Samples in the same group with different letters are significantly different at p<0.05 with DMRT.

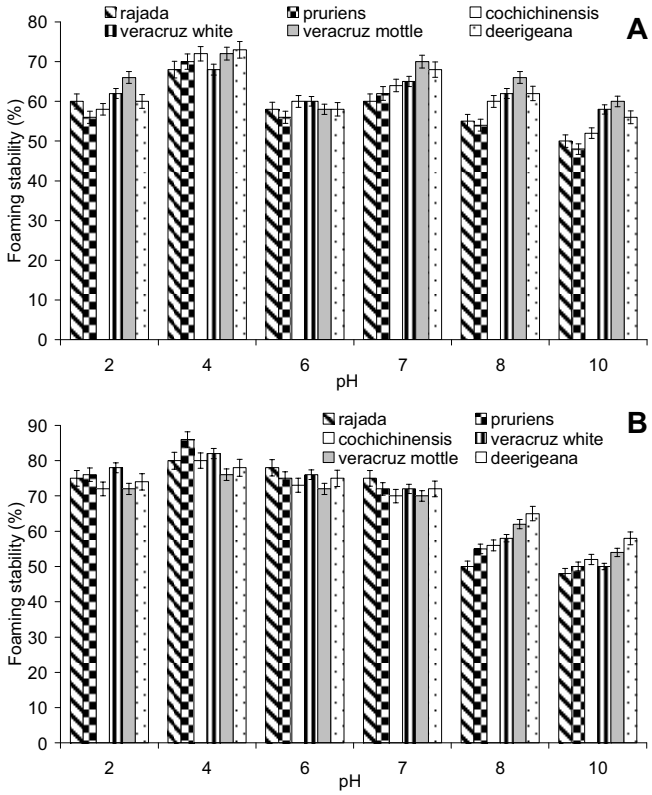

Fig. 6 The effects of pH on the foam stability of *Mucuna* flours (A) and (B) protein isolates. Error bars are the standard deviation of three replicate studies. Samples in the same group with different letters are significantly different at p<0.05 with DMRT.

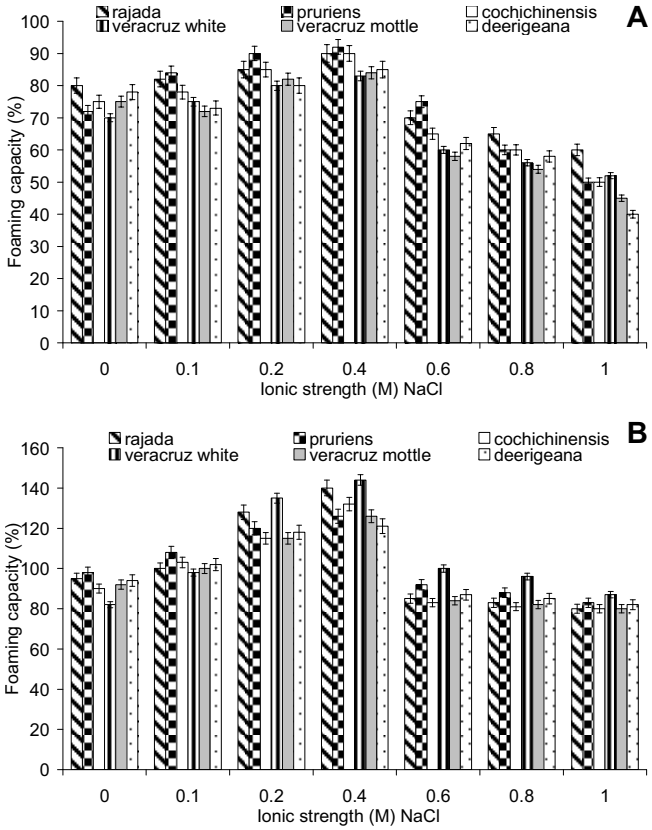

Fig. 7 The effects of ionic strength on the foaming capacity of *Mucuna* flours (A) and (B) protein isolates. Error bars are the standard deviation of three replicate studies. Samples in the same group with different letters are significantly different at $p<0.05$ with DMRT.

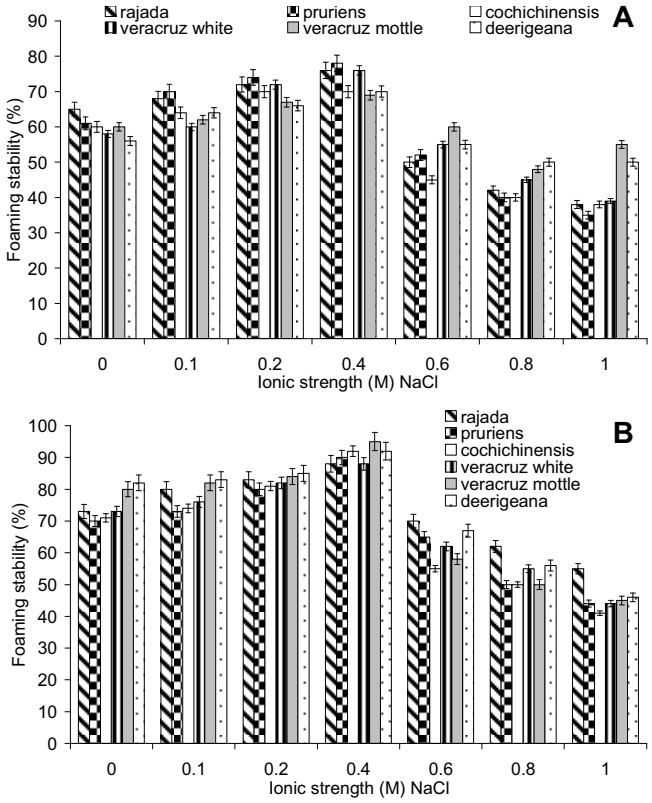

Fig. 8 The effects of ionic strength on the foaming stability of *Mucuna* flours (A) and (B) protein isolates. Error bars are the standard deviation of three replicate studies. Samples in the same group with different letters are significantly different at $p<0.05$ with DMRT.

Effects of ionic strength on foaming characteristics

The effects of ionic strength on the foaming capacity of flours and protein isolates are shown in **Fig. 7**; similar results for the foaming stability are shown in **Fig. 8**. There was an increase in the foaming capacity of the samples as the ionic strength increased progressively from 0.1 to 0.4 M, after which a decline in the foaming capacity was observed as the ionic strength was increased from 0.6 to 1.0 M. Similarly, enhanced foaming was observed in solutions of ionic strength ranging from 0.1 to 0.4 M, which thereafter reduced at higher ionic strength (0.6-1 M). Foaming in protein dispersions is enhanced by an increase in the solubility of the proteins. Low ionic strength (0.1-0.4 M) improved the solubility of proteins in the flour and the isolates in the dispersions. This facilitates the formation of stable cohesive films around the air vacuoles. A further increase in the ionic strength, from 0.6 to 1.0 M resulted in charge screening, which enhanced the hydrophobic interaction. This increase in hydrophobic interaction can lead to a "salting out" effect and a consequent reduction in the foaming capacity and stability (Lawal and Adebowale 2005).

Effects of carbohydrates on foaming characteristics

The effects of carbohydrates on the foaming capacity and stability are summarised in **Tables 1** and **2** for the *Mucuna* flours and protein isolates, respectively. Incorporation of carbohydrates at a level of 0.25 g/g of protein increased the foaming capacity and stability compared with the control sample. In the flours, it appeared that starch resulted in the highest improvement of the capacity and stability, while in the protein isolates, lactose resulted in the highest increase. Sathe *et al*. (1982b) reported an increase in the foaming capacity and stability of winged bean flour and protein isolates after the addition of sucrose, amylose, amylopectin, potato starch, gum Arabic and pectin at a level of 0.25 $g.g^{-1}$. The addition of sucrose has been shown to increase the stability of alfafa leaf protein (Wang and Kinsella 1976) and to increase the foaming capacity of whey protein (Sorgentini and Wagner 2002). Foam capacity and stability are related to the viscosity, density and surface tension of the liquid phase foam. Positive effect of the added carbohydrates might be due to increased bulk phase viscosity which reduced the rate of drainage of the lamella fluid (Subagio 2005).

In general, foam contributes to smoothness, lightness, flavour dispersions and palatability of foods. Foaming of proteins in solutions can also be desirable in many food applications. Results obtained in this study indicate that the protein isolates could serve as potential replacements for known proteins in food applications requiring high formability and stability, such as in cakes, breads, marshmallow, whippings, toppings, ice creams and desserts.

Characterization of the *Mucuna* protein isolates

1. Differential scanning calorimetric studies

The Differential Scanning Calorimetry (DSC) thermograms of the six isolates are presented in **Fig. 9**, while the values of the onset temperature T_m, peak or denaturation temperature T_d and the transition enthalpy ΔH are given in **Table 3**. Two peaks were observed in the thermograms; the first peak has a smaller intensity than the second peak. According to previous authors (Derbyshire *et al*. 1976; Murray *et al*. 1985; Paredes-Lopez 1988), the first peak corresponds to the 7S-vilicins in the isolate while the second peak corresponds to the 11S-legumins in the isolate. The T_m and the T_d are measures of the degree of protein denaturation and are influenced by the heating rate and the protein concentration. The DSC transition heat change, which results from the difference in the uptake of energy, is usually calculated by integrating the area below the endothermic peak and it is

Table 1 Effects of carbohydrates on the foaming capacity and stability (%) of *Mucuna* flours.

Sample	Carbohydrates	Capacity (%)	Stability (%)				
			10 min.	20 min.	40 min.	60 min.	120 min.
Control	-	55	52	50	45	40	38
M. rajada	MF + sucrose	65	60	58	55	52	50
	MF + lactose	68	65	62	60	58	55
	MF + maltose	70	65	60	58	55	52
	MF + starch	80	78	76	73	70	68
M. pruriens	MF + sucrose	60	58	56	54	52	50
	MF + lactose	65	62	60	58	55	53
	MF + maltose	68	64	62	59	57	54
	MF + starch	74	70	68	66	62	60
M. cochinchinensis	MF + sucrose	62	60	58	56	54	50
	MF + lactose	60	58	57	55	53	51
	MF + maltose	65	62	60	58	56	52
	MF + starch	76	72	68	65	62	58
M. veracruz white	MF + sucrose	63	60	58	55	52	50
	MF + lactose	58	55	52	50	48	46
	MF + maltose	65	60	58	56	54	52
	MF + starch	77	74	72	70	68	66
M. veracruz mottle	MF + sucrose	60	58	56	55	52	50
	MF + lactose	65	62	60	58	55	52
	MF + maltose	63	60	58	55	54	53
	MF + starch	72	70	69	67	65	62
M. deerigeana	MF + sucrose	66	64	62	60	58	54
	MF + lactose	61	59	57	54	52	50
	MF + maltose	65	63	60	58	56	54
	MF + starch	78	75	72	70	67	65

Table 2 Effect of carbohydrates on the foaming capacity and stability of *Mucuna* protein isolates.

Sample	Carbohydrates	Capacity (%)	Stability (%)				
			10 min.	20 min.	40 min.	60 min.	120 min.
Control	-	92	90	87	85	82	80
M. rajada	MF + sucrose	98	96	94	90	88	86
	MF + lactose	120	118	116	115	110	107
	MF + maltose	105	100	98	96	94	92
	MF + starch	110	107	105	102	100	98
M. pruriens	MF + sucrose	95	92	90	88	87	84
	MF + lactose	125	122	120	118	117	115
	MF + maltose	107	102	101	98	96	94
	MF + starch	112	108	106	104	102	100
M. cochinchinensis	MF + sucrose	97	95	92	90	88	86
	MF + lactose	122	120	118	116	113	109
	MF + maltose	105	102	100	97	94	92
	MF + starch	102	100	98	96	94	90
M. veracruz white	MF + sucrose	95	91	89	87	86	84
	MF + lactose	120	117	115	112	109	107
	MF + maltose	102	98	96	94	92	90
	MF + starch	103	101	100	98	96	92
M. veracruz mottle	MF + sucrose	91	89	88	86	84	82
	MF + lactose	115	113	111	110	108	106
	MF + maltose	100	98	96	94	92	90
	MF + starch	107	105	102	100	98	96
M. deerigeana	MF + sucrose	98	96	94	92	90	88
	MF + lactose	117	115	112	109	107	105
	MF + maltose	102	100	95	92	90	88
	MF + starch	109	106	104	102	98	96

used to monitor the proportion of the protein that does not denature. A decrease in the enthalpy value of DSC transitions reflects the degree of denaturation due to treatments of the isolates and/or greater tendency of aggregation during the DSC scan. The aggregation can be ascribed to an increase in the surface hydrophobicity of the proteins as a result of structural modifications. Since all the samples are subjected to the same conditions and treatments, the contributions from the latter would apply to all the samples to the same extent. More importantly, the contribution of the energy from the DSC scan is small compared with the denaturation effect (Provalov and Khechinashvili 1974).

If a protein is partially denatured, the magnitude of the transition enthalpy ΔH is decreased and it will be zero if the protein is completely denatured. A high value of the T_d or T_m indicates that lower amount of the protein has been denatured. This will be coupled by a relatively high ΔH value. The 11S values of ΔH for the *M. rajada* and *M. deerigeana* isolates obtained in the current studies compare well with the value of 27 J/g reported for soybean isolate (Parades-Lopez 1988).

In comparison with results from an earlier author (Parades-Lopez 1988), the identity of the *Mucuna* protein isolates were relatively preserved during the extraction process. Mwasaru *et al*. (1999) reported DSC studies of *Cajanus cajan*, and *Vigna unguiculata* protein isolates. Our results compare favourably with the isolates obtained from isoelectric precipitation. According to the authors, a Td value of 92

Table 3 Differential Scanning Calorimetry (DSC) data of protein isolates from *Mucuna* beans.

Sample	Peak I (7S)			Peak II (11S)		
	T_{dI} (°C)	T_{mI}(°C)	ΔH_I(J.g^{-1})	T_{dII}	T_{mII}	ΔH_{II}
M. rajada	95.03	88.67	8.3	107.51	101.91	24.16
M. pruriens	94.81	94.06	17.1	107.37	102.05	13.6
M. cochichinensis	94.33	90.46	6.2	107.71	102.7	14.28
M. veracruz white	91.83	89.12	4.6	108.46	102.97	17.8
M. veracruz mottle	96.39	86.54	22.6	108.35	102.28	7.4
M. deerigeana	95.45	83.36	4.8	107.8	101.82	24.49

* ΔH_{II} = 27 J/g for soy flour (Paradez-Lopez 1988)

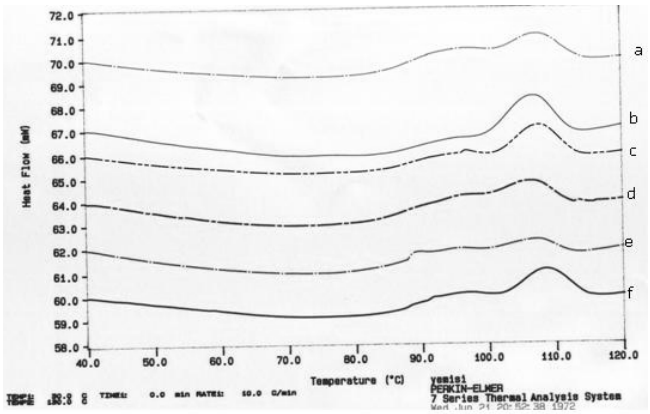

Fig. 9 Differential Scanning Calorimetry (DSC) thermograms of *Mucuna* protein isolates. a – *M. cochichinensis*; b – *M. rajada*; c – *M deerigeana*; d – *M. pruriens*; e – *M. veracruz mottle*; f – *M. veracruz white*; I- Peak for 7S; II- Peak for 11S.

and 91.5°C were obtained for *Cajanus cajan* and *Vigna unguiculata* protein isolates, respectively.

2. Evaluation of the sub-unit patterns in the isolates

In order to characterize the proteins in the isolates, the subunit patterns were studied by means of gel filtration chromatography and gel electrophoresis SDS-PAGE. The latter was performed in the presence and absence of a reducing agent (mercaptoethanol). This allowed us to distinguish between free polypeptide chains and the chains linked by disulphide bridges.

The results of gel filtration chromatographic studies of the protein isolates are depicted in **Fig. 10a-e**. *M. pruriens* isolate consisted of a two major fractions (peak 2) with a molecular mass of 40 kDa and (peak 4) with a molecular weight of 15.3 kDa. The molecular weight of other minor components were 913.5 kDa (peak 1), 35.8 kDa (peak 3), 3.9 kDa (peak 5) and 1.2 kDa (peak 6). *M. rajada*, *M. veracruz* white and *M. veracruz* mottle had similar molecular weight patterns in the fractions. Only five peaks were identified in *M. cochinchinensis* and *M. deerigeana* isolates. Their molecular weights were roughly similar.

More detailed information can be obtained with gel electrophoresis SDS-PAGE, as shown in **Fig. 11a** and **11b**. A major band consisting of a broad zone with molecular weight 36 ± 7 kDa and 17 ± 3 kDa appeared in all the samples under both reducing and non reducing conditions. This indicated that this major polypeptide were free from interchain disulphide bonds and possibly represented typical subunits of vilicin-like storage proteins, as reported by earlier authors (Derbyshire *et al*. 1976; Rahma *et al*. 2000). In addition some minor polypeptide chains gave similar patterns under both reducing and non-reducing conditions. These included the polypeptide chain with 55, 84, 97 and 116 kDa. In contrast, two peaks which occurred as minor fractions (molecular weights, 205 and 66 kDa) disappeared after reduction which resulted in the formation of a smaller polypeptide chain with 24 kDa. A similar observation was reported by Rahman *et al*. (2000), in their gel electrophoresis studies of mung bean. The authors reported similar disappearance of a polypeptide chain with molecular weight of 61.5 ± 1.3 kDa and the appearance of another polypeptide chain of molecular weight 23.3 ± 1.0 kDa. Therefore in these studies, the parent subunit might belong to the legume-like 11S legumin type storage proteins which are characterized by disulphide α-β subunits. A smaller polypeptide fraction also disappeared after reduction. Legume seeds have been shown to contain high molecular weight oligomeric storage proteins which are the major components in protein isolates prepared from seeds. While some legumes such as soy beans (Thanch and Shibasaki 1976; Carsey and Domoney 1984; Iwabuchi and Yamauchi 1987); and faba beans (Muntz *et al*. 1986) contain two major storage proteins 11S legumins and 7S vicilin, there are a number of other legumes which contain a 7S fraction as the major protein component and other storage proteins in trace amounts: legumes like *Phaseolus vulgaris* (Sakakibara *et al*. 1979), cowpea (Sefa-Dedeh and Stanley 1979), pigeon pea (*Cajanus cajan*) (Krishna and Bhatia 1985), jack bean (*Carnavalia gladiata*) (McPherson 1980) and winged bean (*Psophacarpus angularis*) which contains 7S and 2.5S vicilin proteins (Yanagi 1985).

The present results provide evidence that the *Mucuna* bean species belong to the latter group of legumes, having a 7S fraction as the predominant storage proteins. Other storage proteins which might be assigned to the 11S legumins type, composed of larger and smaller units of disulphide linked polypeptide chain, are present only in marginal amounts in the *Mucuna* bean isolates and extracts. This was supported by our preliminary studies of the isolation and characterisation of 7S vicilin and 11S legumin proteins in the isolates. Work is already in progress on the 2-dimensional gel electrophoresis and ultracentrifugal analysis of the fractions, the results of which will be published in the near future.

Microstructural studies

The microstructure of the *Mucuna* bean isolates is given in **Fig. 12A-E** to **Fig. 13A-E**. In *M. pruriens*, the protein particles formed irregular flat compact flakes with sizes ranging from 35 μm × 60 μm to 100 μm × 200 μm. The enlarged view of one of the flakes shows a spongy-like surface with non-uniform size distribution. A similar structure was observed for *M. cochinchinensis*. In *M. rajada*, the protein particles occurs as nearly spherical shapes (average size, 18 μm) with some relatively much smaller flakes attached to the surface of the spheres. In the *M veracruz* white, the protein particles occur in the form of rectangular parallelogram shapes averaging (140 μm × 40 μm × 200 μm). The longitudinal face consists of a spongy surface with non-uniform size distribution. This result is similar to microstructural study of dry beans (*Phaseolus vulgaris*) (Agbo *et al*. 1987). In *M. veracruz mottle*, the protein particles are spongy with heterogeneous lump sizes. A detailed view of one the lumps of *M. veracruz mottle* showed an undulating surface (**Fig. 13c, 13d**). The microstructure of *M. deerigeana* is similar to that of *M. pruriens* and *M. cochinchinensis*; however, the flakes are much larger in size and the surface pores are smaller. For comparison, a microstructural study was also conducted on a commercial soybean isolate. The micrographs of the commercial soybean isolate are shown in **Fig. 14**. Various intact and deformed globules

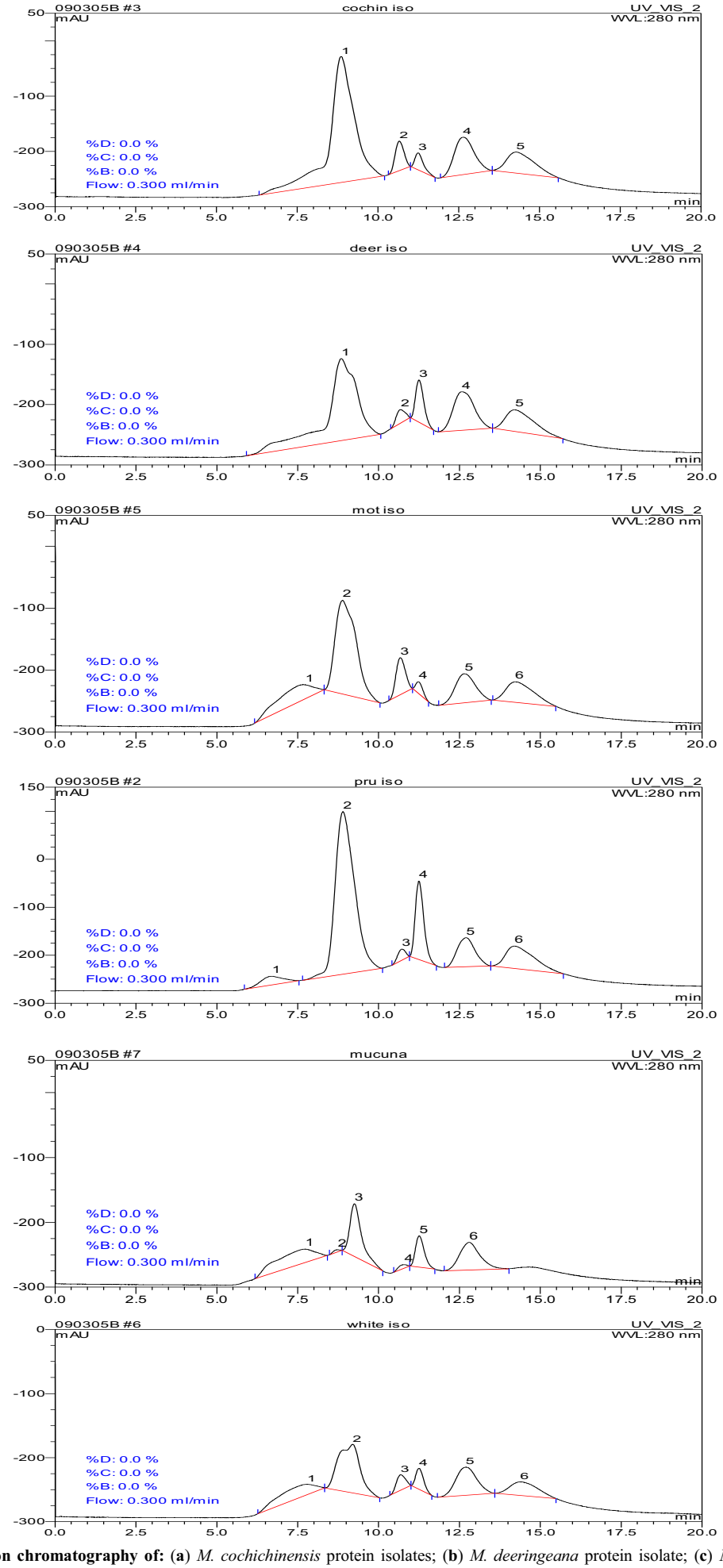

Fig. 10 Gel filtration chromatography of: (**a**) *M. cochichinensis* protein isolates; (**b**) *M. deeringeana* protein isolate; (**c**) *M. veracruz mottle* protein isolate; (**d**) *M. pruriens* protein isolate; (**e**) *M. rajada* protein isolate; (**f**) *M. veracruz* white protein isolate.

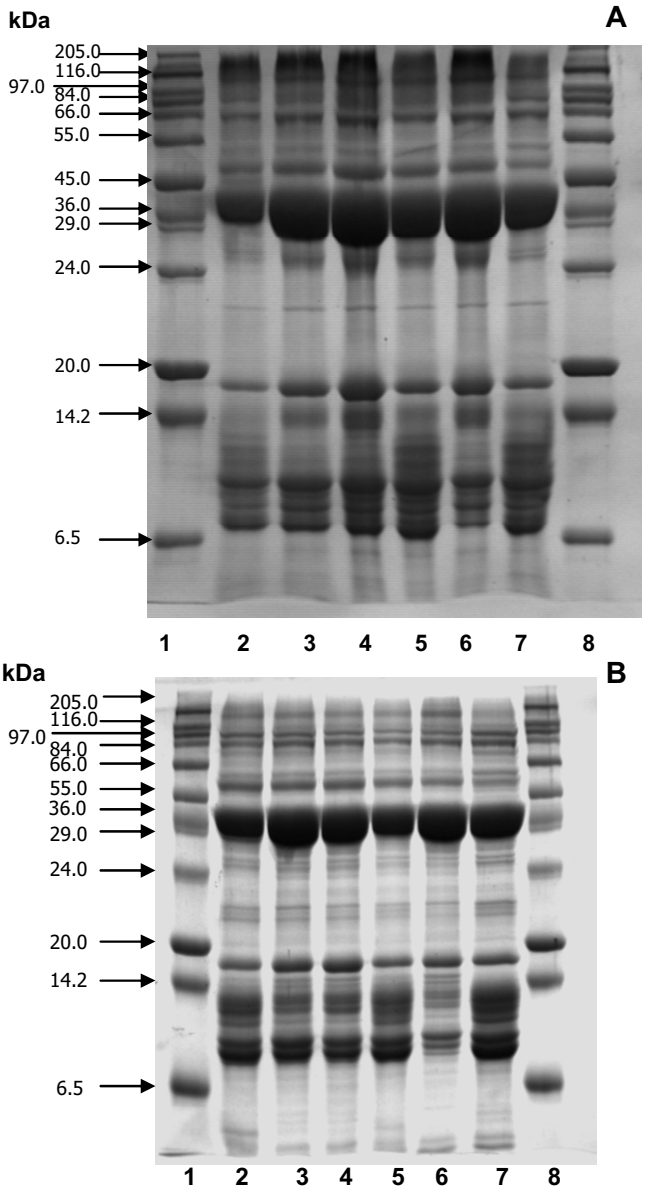

Fig. 11 Gel electrophoresis SDS-PAGE without (**A**) or with (**B**) β-mercaptoethanol, ME of six *Mucuna* protein isolates. Lanes 1 and 8 are standard markers. Lane 2: *M. veracruz mottle*; Lane 3: *M. vera cruz white*; Lane 4: *M. deerigeana*; Lane 5: *M. cochichinensis*; Lane 6: *M. rajada*; Lane 7: *M. pruriens*.

unevenly distributed were observed in the isolate. It also contains irregular particles. These structures differ from those of *Mucuna* isolates. This might be due to differences in the origin and procedure used for the isolation such as the addition of ascorbic acid into the slurry (Aluko and Yada 1995). The microstructure for soybean observed in this study is similar to that for safflower and a commercial soybean protein isolate as reported by Paredes-Lopez *et al*. (1988).

The microstructures of the *Mucuna* bean flours are shown in **Figs. 15** and **16**. The micrograph for each of the flour samples showed a mixture of starch granules and protein flakes. The starch granules are embedded in the protein flakes; the flakes are attached to the surface of the granules. It appeared that the average size of the starch granules is independent of the flour species. A detailed examination at the surface of one of the granules revealed surface indentations, which are locations of the point of attachment between the starch granules and the protein matrix.

CONCLUSIONS

Results obtained from the Differential Scanning Calorimetric studies indicates that the identity of the *Mucuna* protein isolates were relatively preserved during the isoelectric precipitation technique employed for the isolation of proteins.

Foam contributes to smoothness, lightness, flavour dispersions and palatability of foods. Foaming of proteins in solutions can also be desirable in many food applications. Results obtained in this study indicate that the protein isolates could serve as potential replacements for known proteins in food applications requiring high formability and stability, such as in cakes, breads, marshmallow, whippings, toppings, ice creams and desserts.

The present results provide evidence that the *Mucuna* bean species belong to the group of legumes, having a 7S fraction as the predominant storage proteins. Other storage proteins which might be assigned to the 11S legumins type, composed of larger and smaller units of disulphide linked polypeptide chain, are present only in marginal amounts in the *Mucuna* protein isolates and extracts.

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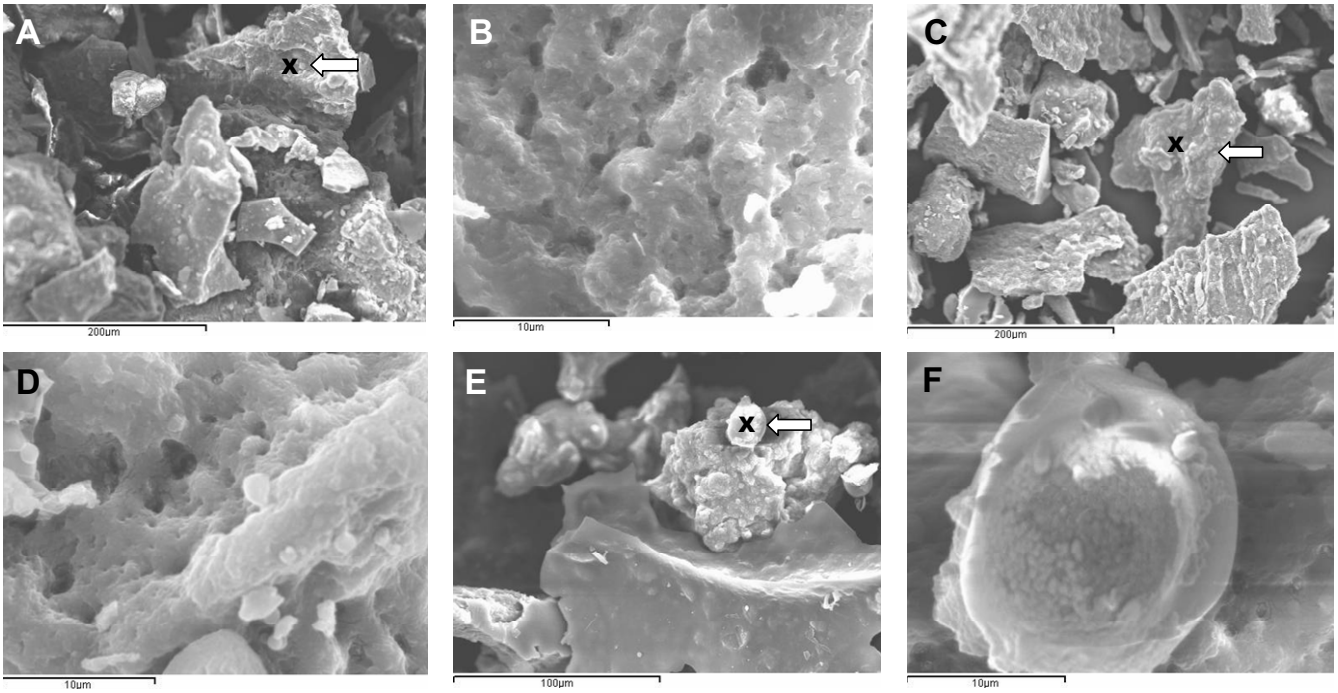

Fig. 12 Scanning electron micrographs for *Mucuna* bean isolates. (**A**) *M. pruriens*; (**B**) enlarged view of the region labelled X in (**A**) for *M. pruriens*; (**C**) *M. cochinchinesis*; (**D**) enlarged view of the region labelled X in (**C**) for *M. cochinchinesis*; (**E**) *M. rajada*; and (**F**) enlarged view of the region labelled X in (**E**) for *M. rajada*.

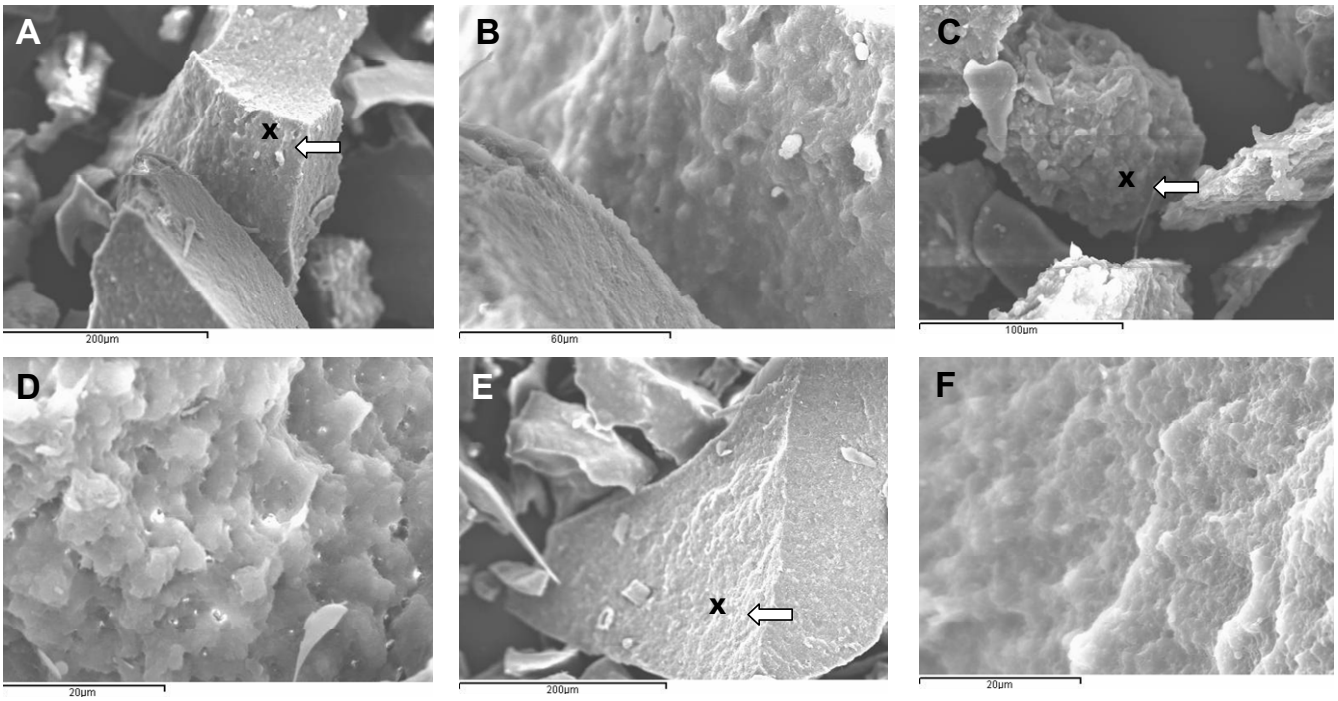

Fig. 13 Scanning electron micrographs for *Mucuna* bean isolates. (**A**) *M. veracruz white*; (**B**) enlarged view of the region labelled X in (**A**) *for M. veracruz white*; (**C**) *M. veracruz mottle*; (**D**) enlarged view of the region labelled X in (**C**) for *M. veracruz mottle*; (**E**) *M. deeringeeana*; and (**F**) enlarged view of the region labelled X in (**E**) for *M. deerigeeana*.

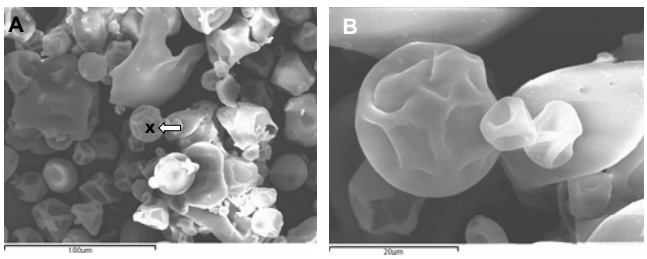

Fig. 14 Scanning electron micrographs for soybean isolates (A), and enlarged view of the region labelled X (B).

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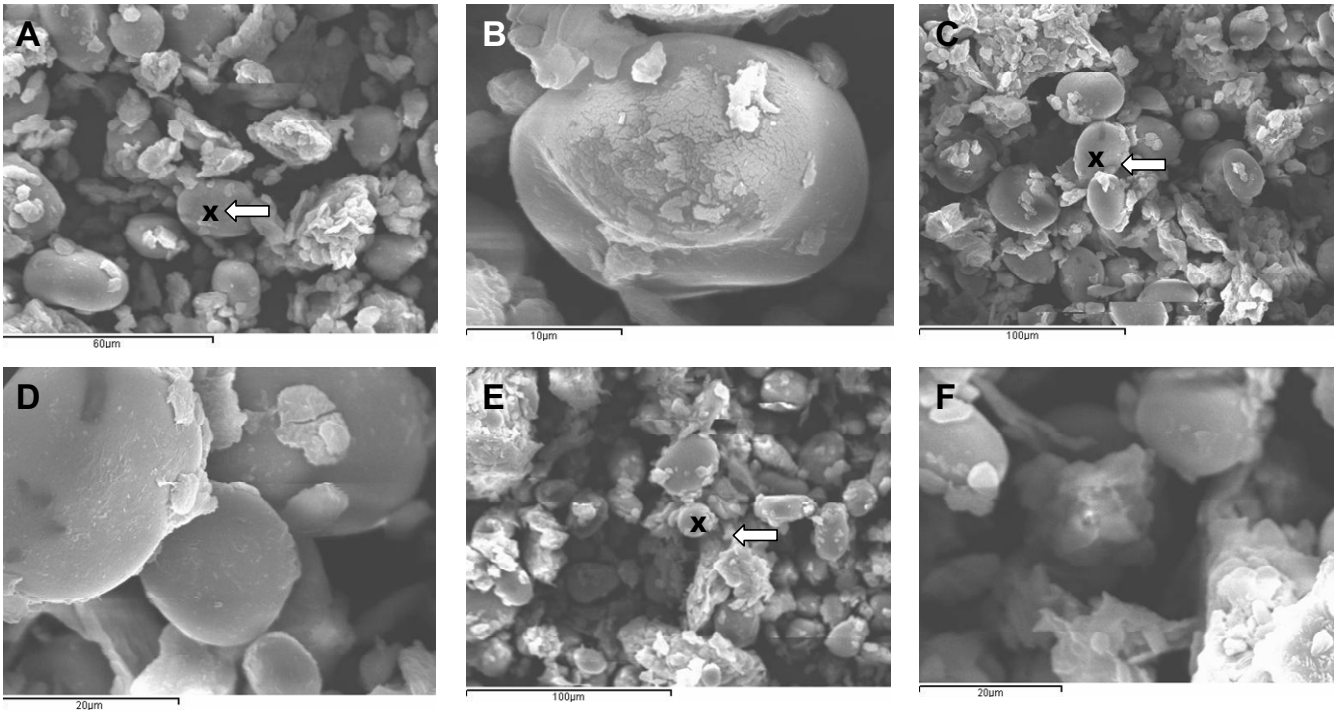

Fig. 15 Scanning electron micrographs for *Mucuna* bean flour. (**A**) *M. pruriens*; (**B**) enlarged view of the region labelled X in (**A**) for *M. pruriens*; (**C**) *M. cochinchinesis*; (**D**) enlarged view of the region labelled X in (**C**) for *M. cochinchinesis*; (**E**) *M. rajada*; and (**F**) enlarged view of the region labelled X in (**E**) for *M. rajada*.

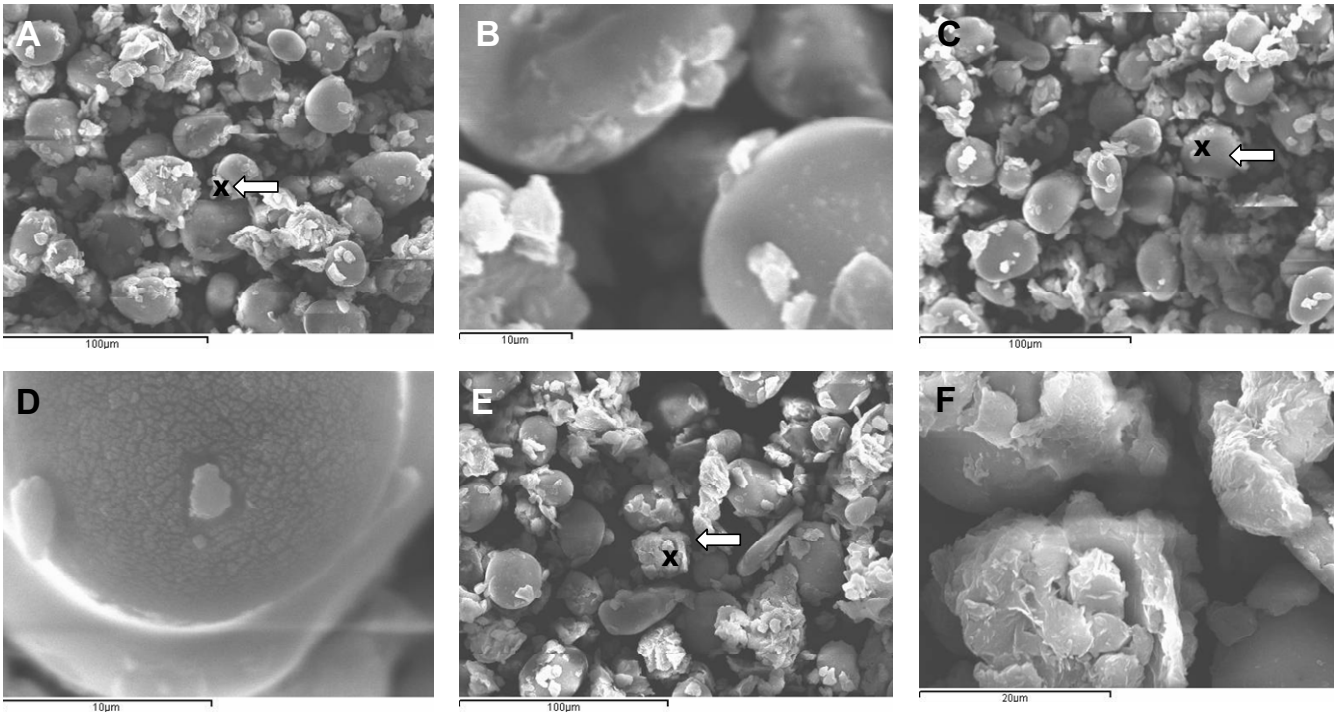

Fig. 16 Scanning electron micrographs for *Mucuna* bean flour. (**A**) *M. veracruz white*; (**B**) enlarged view of the region labelled X in (**A**) *for M. veracruz white*; (**C**) *M. veracruz mottle*; (**D**) enlarged view of the region labelled X in (**C**) for *M. veracruz mottle*; (**E**) *M. deeringeeana*; and (**F**) enlarged view of the region labelled X in (**E**) for *M. deerigeeana*.

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