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Emulsifying Properties of Mucuna Flour and Protein Isolates

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Abstract: The emulsifying properties of the flour and protein isolates from Mucuna Bean (*Mucuna* sp.) have been investigated. The pH dependent protein solubility profiles indicated that the isoelectric point of the proteins was between 4 and 5 depending on the species. Generally the solubility reduces as the pH increased until it reaches the isoelectric poiunt. This was followed by a progressive increase in the solubility with further increase in pH. The emulsifying capacity of the flours increases as the concentration of the flour samples increased up to 3% concentration of sample. Subsequent increase in the concentration of the samples reduced the emulsifying activity. The emulsion stability of the flour follow a similar trend except that the increase in the stability was up to 6% sample concentration, after which further increase in the level of the flour reduced the emulsifying stability. Similar trend was observed in the protein isolates, except that a much higher values were obtained. The trends of the emulsifying capacity and stability were similar in *M. rajada* and *M. cochinchinensis* for both the flours and protein isolates. There was an increase in the emulsifying capacity and stability as the pH was increased from 2-4. However at pH 5, there was a rapid reduction in the emulsifying capacity and stability while in all other samples (whether flour or protein isolate), there was a corresponding rapid reduction at pH value of 4. The result parallels the trend recorded for the protein solubility because the region of minimum solubility of proteins (isoelectric region) was the region of minimum emulsifying capacity and solubility of the samples. It was found that the emulsifying capacity and stability of the flours and protein isolates increased as the ionic strength was increased from 0.0-0.4M of KCl solution. Thereafter the values of the parameters decreased as the ionic strength was increased to 1.0M. Minimal values of the emulsifying capacity and stability were obtained at ionic strength of 1.0M. The Emulsion Activity Index (EAI) as well as the Emulsion Stability Index (ESI) for protein isolate were determined to allow for comparison of emulsifying properties which were determined using the same technique for similar seeds. The highest EAI value (123 $m^2 g^{-1}$) and ESI value (72 h) was obtained for *M. pruriens* while corresponding values for *M. veracruz white* was 98 m^2/g and 48 h, respectively. Data for Mucuna isolate as reported in this study are higher than those reported for Soy protein isolate with values of 118 $m^2 g^{-1}$ and 52h for EAI and ESI, respectively. As the temperature was increased from 25-60°C, the viscosity of the flours and the isolates increased. On cooling again to 25°C the viscosity increased further at all pH values. However, minimum viscosity was observed at either pH 4 or 5 (region of isoelectric point) for both the flours and protein isolates. The result of gel electrophoresis SDS-PAGE indicated the presence of a major band consisting of a broad zone with molecular weight 36±7 and 17±3 kDa appeared in all the samples under both reducing and non-reducing conditions. In addition, some minor polypeptide chains (55, 84, 97 and 116 kDa.) gave similar patterns under both reducing and non-reducing conditions. In contrast, a 66 kDa minor fraction disappeared after reduction which resulted in the formation of a smaller polypeptide chain with 24 kDa.

Key words: Mucuna flour and protein isolate, emulsifying properties, gel electrophoresis

INTRODUCTION

Legume seeds are one of the most important sources of protein, carbohydrates and dietary fibre for human nutrition. The renewed interest on legumes in developing countries arises from the fact that animal proteins are relatively scarce and expensive. Similar awareness applies to developed countries where plant proteins are now regarded as versatile functional ingredients and biologically active components rather than essential nutrients (Marcello and Gius, 1997). The partial replacement of animal foods with legumes has been shown to improve nutritional status due to lower cholesterol level in plant foods (Guillion and Champ, 1966). In addition, plant food diets increase the level of fibre intake which reduces the risk of bowel diseases,

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including cancer of the colon and also reduction in incidence of osteoporosis (Sirtori and Lovati, 2001).

Mucuna beans, an underutilised legume, which belongs to the family Fabacea, 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). The level of some of these factors has been evaluated in earlier studies (Adebowale *et al*., 2005a and b).

Interest in the production of new food products from protein rich seeds are constantly increasing. In order to decide upon the most effective ways and conditions for incorporating the protein extracted from the seeds into food products, functional properties must be investigated. Functional properties such as solubility, gelation, foaming and emulsifying properties are crucial to the production of these new ingredients.

Emulsions are highly unstable systems and protein isolates play some important roles during emulsification. They aid the formation of oil-in-water emulsions and stabilize the emulsions once they are formed. Since they are surface active, they collect at oil-water interfaces and lower surface tension, thereby making it easier to form emulsions. The emulsified oil droplets are then stabilized by collection of proteins at the surface of the droplets to form a protective barrier that prevents their coalescence and emulsion breakdown. Emulsion stability is important since success of an emulsifier depends on its ability to maintain the emulsion in subsequent processing steps such as cooking and canning (Williams, 1999; Akintayo *et al*., 1998; Tsaliki *et al*., 2004). However at low protein concentrations or low protein/oil ratios, there is insufficient protein present to saturate the interface created during emulsification and the resulting emulsion is highly unstable and flocculation occurs. The degree to which an emulsion is flocculated depends on the structure of the adsorbed layer and the thermodynamic quality of the intervening solvent. If the stabilizing film at the oil-water interface ruptures, coalescence occurs and oil droplets merge into larger spherical globules. Factors that influence this phenomenon are viscosity, of the dispersed and continuous phases, the droplet deformability, the droplet size and the inter-droplet forces, the interfacial tension and the mobility of the adsorbed film (Eleousa and Doxastakis, 2006).

Therefore, the objective of this study is to evaluate the emulsifying properties of Mucuna bean flours and protein isolates prepared by isoelectric precipitation. The proteins will be characterized and the effect of protein concentration, pH and ionic strength on the emulsifying properties will also be studied. This study is part of our comprehensive research aimed at incorporating the protein isolate into food product to produce natural, cheap and adaptable functional foods.

MATERIALS AND METHODS

Seeds of *Mucuna rajada, M. pruriens, M. veracruz mottle, M. veracruz white, M. cochinchinensis* and *M. deeringeana* were collected from the International Institute of Tropical Agriculture/International Livestock Research Institute IITA/ILRI, Ibadan, Nigeria. The seeds were air-dried (at an average temperature of 30±2°C) for 48 h. The immature seeds and extraneous materials were first removed and the remaining seeds were stored in plastic containers at room temperature (30±2 °C).

Preparation of flours: Cleaned Mucuna seeds were cracked with a hammer mill followed by winnowing of the seed coats before milling into flour in a hammer mill. The meal was sieved to pass 0.5 mm mesh sieve and kept in air-tight plastic container in a refrigerator at 4°C prior to use. The fraction collected (< 0.5 mm) is referred to as flour in this thesis. A portion of the flour was defatted by extracting with n-hexane in a soxhlet extractor for 9 h, followed by air-drying in the fume cupboard for 24 h. The full fat and defatted samples were then used for the analysis.

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. The basic steps are as follows.

The slurry (1:20, flour-to-water ratio) at pH 6.37 was first extracted for 10 min, thereafter the slurry was stirred for 2 h using a Gallenhamp magnetic stirrer; the pH was adjusted to the desired pH using 1M NaOH or 1M HCl. Different extractants (ascorbic acid (0.5% w v^{-1}); 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 xg for 30 min

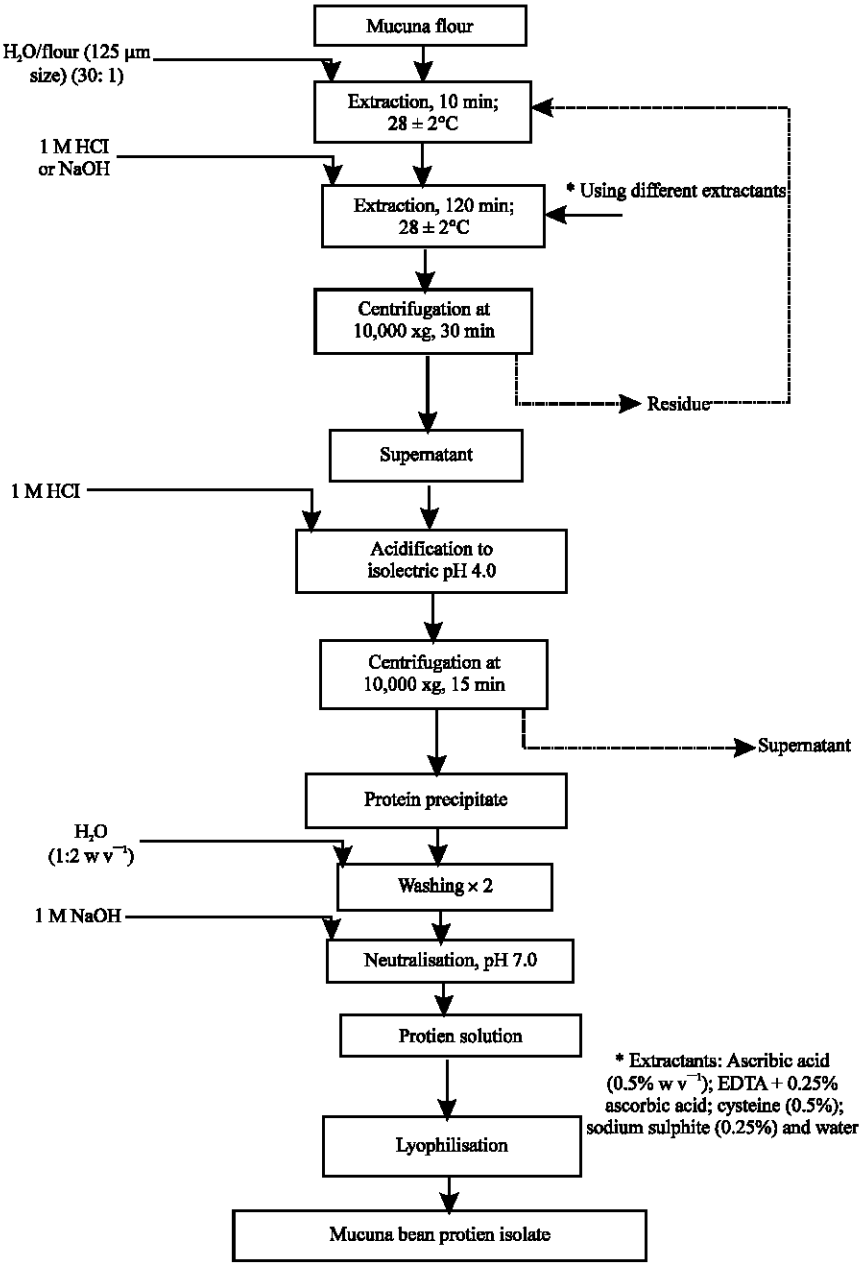

Fig. 1: Preparation of *mucuna* bean protein isolate

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 xg 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 1M NaOH. The final protein isolate was obtained by lyophilisation. A schematic diagram of the isolation procedure is shown in Fig. 1.

Functional properties of flours and protein isolates

Protein solubility: Protein solubility was determined by the method of Sathe *et al.* (1982) with some modifications stated below. The suspension (0.2%) of the flour in distilled water was adjusted to different pH values of between 2 and 11 using either 1M HCl or 1M NaOH. Percent nitrogen in each supernatant was determined by

micro Kjedahl method according to the method already described in the AOAC. Percent soluble protein was calculated as percent nitrogen multiplied by 6.25 on wet basis.

Emulsifying properties: The emulsifying properties of the samples were determined using two different techniques. The first technique involves the determination of the emulsion capacity and stability as described by Akintayo *et al.* (1998) while the second involves the determination of the Emulsion Activity Index (EAI) and the Emulsion Stability Index (ESI) by the turbidimetric technique as described by Pearce and Kinsella (1978) with some modifications.

Emulsion capacity and stability: Emulsions were formed inside a 600 mL beaker using a continuous stirring apparatus. The apparatus consisted of a regulated/stabilised 6 V power supply, a burette, a stirrer, a beaker with emulsion and a digital milliameter. The stirrer was made up of stainless steel rod holding a Perspex bridge and fixed to a 6 V D.C motor spindle by means of a plastic adaptor. The motor itself was driven by a regulated and stabilized 6 V D.C power supply. The milliameter monitored the current drop by the stirrer motor to maintain a constant speed. The greater the viscosity of the emulsion, the greater will be the current drawn. The protein sample (0.25, 0.5, 0.75, 1.00 and 1.25 g) was dissolved in 25 mg mL^{-1} of distilled water making 1, 2, 3, 4 and 5% slurries w v^{-1}, respectively. Necessary pH adjustment was made to ensure maximum solubilisation of the protein. The mixture was stirred for 30 min. In order to disperse the sample. Oil was then added at a rate of 1.00 mL s^{-1} from a burette until emulsion collapsed indicated by a sharp fall in motor current. The volume of oil added up to inversion point was noted and the emulsion capacity expressed as ml oil per gram of sample. The emulsion stability was determined by allowing the emulsion prepared to remain in a graduated cylinder and the volume of oil separated at time of 0, 0.5, 1, 2, 3 up to 24 h. was noted in each case. The emulsion stability was determined by following the procedure used for emulsion capacity, except that 100 mL of oil was added rather than adding oil until the emulsion breakdown. The emulsion stability was therefore determined from

$$\% \text{ Emulsion stability} = \frac{\text{Height of the emulsified layer}}{\text{Height of the total content}} \times 100$$

The effect of concentration on the on the emulsifying activity and stability of flour and isolates was studied by preparing 2-10% w v^{-1} solutions before conducting experiments as described above. The influence of ionic strength was studied by preparing flour and isolate dispersions in water 10 mg mL^{-1} of water in solution of various ionic strength of 0.1-1.0M KCl solutions. Influence of pH was studied by preparing flour dispersions in water at 10 mg mL^{-1} of water in solutions at various pH between 2-10.

Emulsion activity index: Emulsion of each protein dispersion was prepared according to the method described by Lqari *et al.* (2001). Protein sample (3.5g) was homogenised for 30 sec in 50 mL water using a Model A Polytron homogeniser (Brinkmann, Wesbury, N.Y.) at 10,000 rpm). Canola oil, 25 mL (Nobisco Foods, Winston-Salem, N.C., USA) was added to the mixture and homogenised again for 30 sec. Then another 25 mL of canola oil was added and the mixture homogenised for 90 sec. One gram of the emulsion was weighed into a 100 mL standard flask and made up to mark with 0.1% solution of dodecyl sodium sulphate. The solution (1 mL) was withdrawn and 4 mL of the 0.1% dodecyl sodium sulphate (0.002%) was added. The absorbance of the diluted emulsions was measured by a spectrophotometer (UV/VIS Spectrophotometer Lambda 3B; Perkin-Elmer Norwalk, Conn., USA) at 500 nm in 1 cm path length cuvettes. The absorbance was read initially and turbidity, T, was calculated using the formula:

$$T = \frac{2.303A}{I}$$

Where A is the absorbance at 500 nm and I is the path length of cuvette (cm).

The oil volume fraction of emulsion was estimated by drying 5 g of the emulsion in an IsoTemp oven (Fischer Scientific) to a constant weight at 105°C. The oil volume fraction was calculated from:

$$\varphi = \frac{W_d - (E \times W_1)}{W_d + W_1\left[\left(((1+E)\times D_o)/D_m\right) - E\right]}$$

D_o = Density of oil.
D_m = Density of protein solution.
E = Concentration of solutes (mass per unit mass solvent).
W_1 = Loss of weight of emulsion on heating/weight of emulsion.
W_d = Dry weight/weight of emulsion.

The Emulsion Activity Index (EAI) was then calculated as follows:

$$EAI = \frac{2T}{\varphi C}$$

Where T is the turbidity (calculated from the equation above); ϕ is the oil volume fraction (mL); C is the weight (in grams) of the protein per unit volume of aqueous phase before emulsion is formed. EAI has units of area of interphase stabilised per unit weight of protein (i.e $m^2 g^{-1}$).

Emulsion stability index: The emulsions were held at 4 °C for 24 h and reanalyzed for emulsion activity as described above. An emulsion stability index was calculated by the formula:

$$ESI = \frac{T\,\Delta t}{\Delta T}$$

Where T is the turbidity value at 0 h; ΔT is the change in turbidity during the 24 h period and Δt is the time interval (24 h).

Viscosity measurements of the flour and isolate: The viscosity of the flour and protein isolates was determined using the method of Idouraine *et al.* (1997). Suspensions (10% w v^{-1}) with pH values ranging from 2 to 10 were stirred for 30 min at 25°C. Viscosity was measured using a Brookfield LVT synchro-electric viscometer (Brookfield Engineering Lab., Stoughton) at 100 rpm, adapting spindle number 21 and the data expressed in centipoises (cP). The protein sample was first heated to 60°C at a rate of 1.5°C min^{-1} and the viscosity was measured. The samples was thereafter cooled to 25°C and the viscosity was again measured.

Gel eletrophoretic studies: The molecular weight profile for the protein fraction was evaluated using sodium dodecyl sulphate polyacrylamide gel electrophoresis SDS-PAGE as described by Aminlari and Majzoobi (2002). A known weight of the sample (100 mg) was added to 2.5 mL of the buffer containing 0.5M Tris-HCl (pH 6.8), 0.5% bromophenol blue, 10% glycerol and 2% SDS. To another preparation, 5% of 2-Mercaptoethanol (2-ME) was added to effect reduction of disulphide bonds. The preparation was boiled and then centrifuged at 20,000 xg for 15 min. at 4°C and the supernatant was employed for electrophoresis. Protein sample (10 μL) was added onto the gel using a model 491 cell, Bio-rad Laboratories , CA, USA. The electrode buffer contained a mixture of 0.025M Tris buffer; 0.192M glycine and 10% SDS adjusted to a final pH of 8.7. The separating gel contains 12.5% final acrylamide concentration, while the stacking gel contains 4.5% concentration. Electrophoresis was carried out at a constant current of 60 mA at a maximum voltage of 500V until the dye reaches 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. Broad spectrum molecular weight standard from SIGMA, USA containing 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, 29.0kDa; 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; Phosphrylase b, rabbit muscle, 97 kDa; rabbit muscle phosphorylase b, 97.4 kDa; β-galactosidase, Escherichia coli, 116.0 Da; Myosin, rabbit muscle, 205.0 kDa was used for the determination of sample molecular weights of protein sub-units.

Statistical analysis: All experiments in this study are reported as mean of 3 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 $p<0.05$ (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); Jackbeans and Bambarra groundnut (Adebowale and Lawal, 2004; Lawal and Adebowale, 2006) and African locust bean (Lawal *et al*., 2005).

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. 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

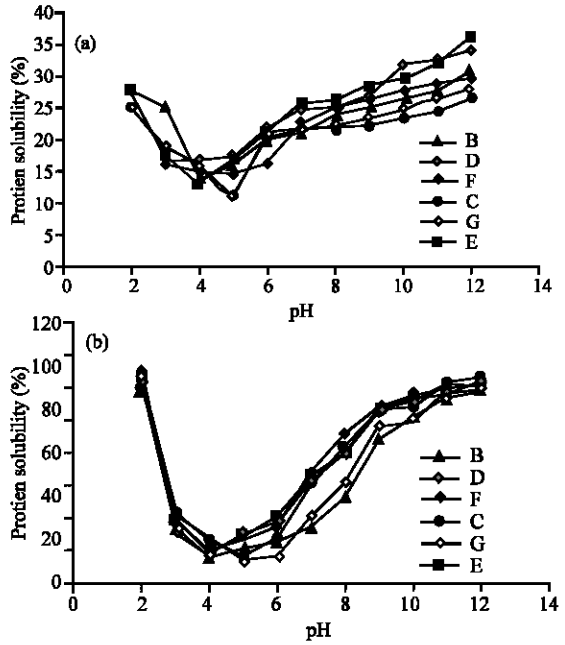

Fig. 2: Protein solubility of (a) mucuna flours and (b) protein isolates.In the legend, (B) = *Mucuna veracruz mottle*; (C) = *M. rajada*; (D) = *M. cochinchinensis*; (E) = *M. deerigeana*; (F) = *M. pruriens*; (G) = *M veracruz white*

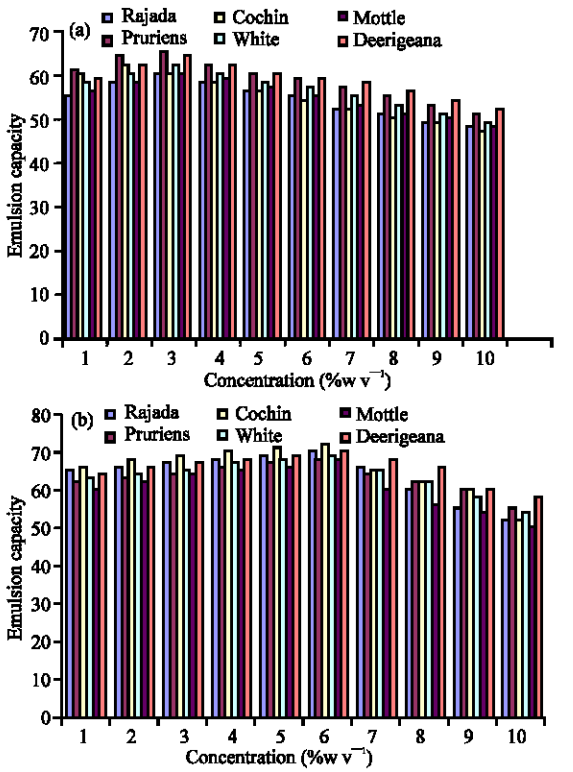

Fig. 3: Effects of concentration on (a) the emulsifying capacity and (b) the emlusifying stability of Mucuna bean flours

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 quatenary 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.

The high solubility of these isolates in the acidic pH range indicates that these isolates may be useful in the formulation of acidic food like protein rich carbonated beverages (Kinsella, 1979). Since protein solubility affects other functionalities like emulsification, foaming and gelation (Kinsella, 1976), the high solubility of the proteins indicates that they could have promising food applications.

Emulsifying properties

Effect of sample concentration on emulsification: The effect of concentration on the emulsion capacity and stability of the flour is presented in Fig. 3. The emulsifying capacity of the flours increases as the concentration of the flour samples increased up to 3% concentration of sample. Subsequent increase in the concentration of the samples reduced the emulsifying activity. The emulsion stability of the flour follow a similar trend except that the increase in the stability was up to 6% sample concentration, after which further increase in the level of the flour reduced the emulsifying stability.

The influence of concentration on the emulsion capacity and stability of the protein isolates is presented in Fig. 4. The emulsifying capacity and stability of the protein isolates were much higher than the corresponding value for the flours. For example, the emulsifying capacity of *M. pruriens* protein was 30% higher than that for the corresponding flour. As for the flours, there was an increase in the emulsifying capacity of the isolates as the concentration of samples was increased up to 5%, after which further increase resulted in a decrease in the emulsifying capacity. A similar trend was observed for the emulsifying stability of the isolates; the stability increased up to 4% concentration of the samples, after which there was a reduction in the stability of the emulsions. At 5%

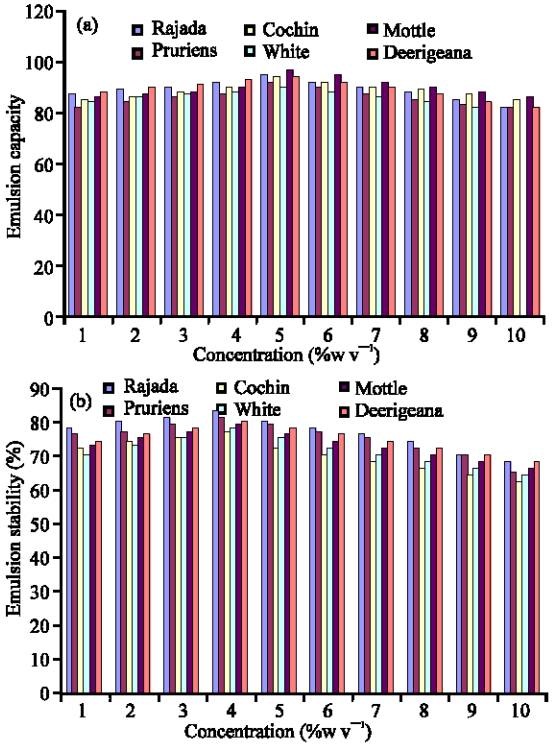

Fig. 4: Effects of concentration on (a) the emulsifying capacity and (b) the emulsifying stability of Mucuna bean protein isolate

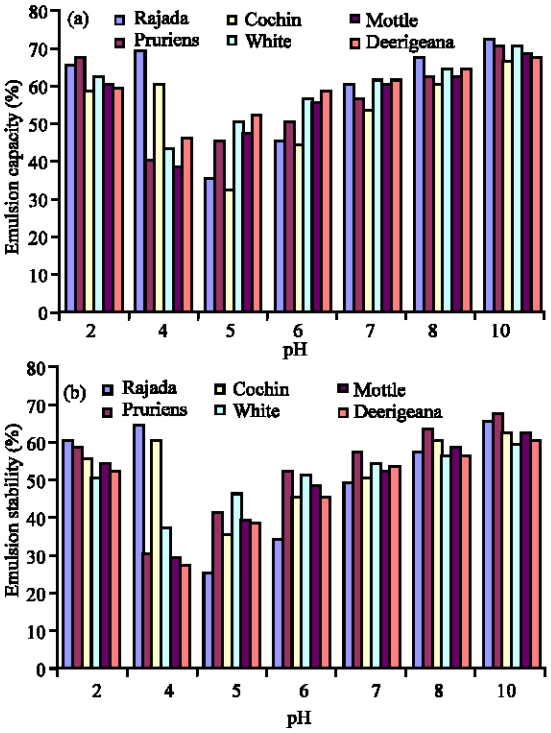

Fig. 5: (a) The emulsifying capacity versus pH and (b) emulsifying stability versus pH for Mucuna bean flour

level of concentration of protein isolate, the range obtained for the emulsifying capacity and stability were 90-95 and 72-80%, respectively.

The effects of concentration on the emulsifying capacity and stability of flours and isolates presented above compared favourably with those reported by other researchers. For example, Ahmendna *et al.* (1999) reported an emulsifying capacity of 57.5% for dried egg white protein isolate; 59.8% for nonfat dry milk protein isolate and 87.9% for soy bean protein isolates; while Dzudie reported an emulsion capacity of 85% for chickpea protein isolate. The ability of flour and the isolate to emulsify oil and protein suspension into a mixture of fine globule dispersion can be attributed to the soluble proteins (Ahmendna *et al.*, 1999). Soluble proteins are inherently surface active due to their amphiphilic nature and tendency to adsorb at oil-water interfaces. At low protein concentration, protein adsorption at the oil-water interface is diffusion controlled, whereas at high protein concentration, activation energy barrier does not allow the system to be diffusion controlled, instead it is controlled by mass transfer mechanism (Kinsella, 1976). Initial increase in protein concentration facilitates enhanced interaction between the oil phase and the aqueous phase. However, as the concentration increases, a point is reached where further increase in protein concentration leads to the accumulation of proteins in the aqueous phase, leading to a decrease in the emulsifying capacity. This is consistent with the results obtained in the present investigation.

Effect of pH value on emulsification: The effect of pH on the emulsifying capacity and stability of the flours and protein isolates are presented in Fig. 5 and 6, respectively. The trends of the emulsifying capacity and stability were similar in *M. rajada* and *M. cochinchinensis* for both the flours and protein isolates. There was an increase in the emulsifying capacity and stability as the pH was increased from 2-4. However at pH 5, there was a rapid reduction in the emulsifying capacity and stability while in all other samples (whether flour or protein isolate), there was a corresponding rapid reduction at pH value of 4. The result parallels the trend recorded for the protein solubility because the region of minimum solubility of proteins (isoelectric region) was the region of minimum emulsifying capacity and solubility of the samples.

This is in agreement with the general correlation between emulsifying capacity and nitrogen solubility found in previous studies, which gives V-shaped pattern

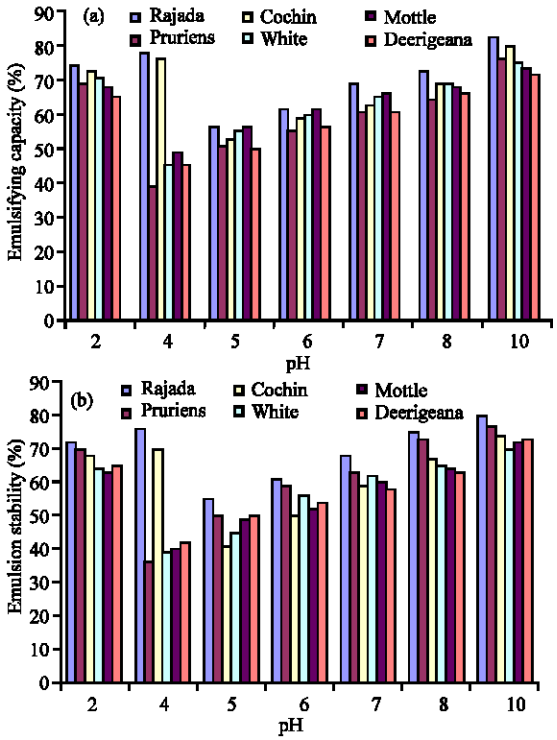

Fig. 6: (a) The emulsifying capacity versus pH and (b) emulsifying stability versus pH for Mucuna bean protein isolates

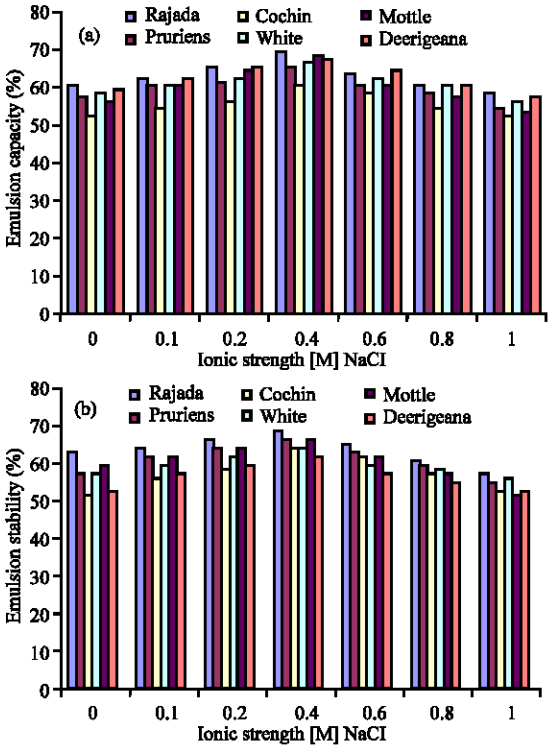

Fig. 7: Effects of ionic strength on (a) the emulsifying capacity and (b) the emulsifying stability of Mucuna flour

(Crenwelge *et al*., 1974; Hung and Zayas, 1991). The emulsifying capacity of proteins depends on the hydrophilic-lipophilic balance, which is affected by the pH (Chi-Fai and Yum-Shing, 1997). At the oil-water interphase, the protein orients lipophilic residues to the oil phase and hydrophilic residues to the aqueous phase. The net charge of the lipophilic-hydrophilic interphase may impede or facilitates emulsifying capacity and stability. In previous studies, pH dependent emulsifying capacity and stability have been reported by several authors for proteins of *Cajanus cajan*, *Vigna unguculata Phaseolus lunatus*, *Carnavalia einseformis* and flours of lablab and soybeans (Mwarsaru *et al*., 1999; Chau and Cheng, 1998; Lawal and Adebowale, 2006).

Effect of ionic strength on emulsification: The effect of ionic strength on the emulsifying capacity and stability of the flours and protein isolates are shown in Fig. 7 and 8. It was found that the emulsifying capacity and stability of the flours and protein isolates increased as the ionic strength was increased from 0.0-0.4M of KCl solution. Thereafter the values of the parameters decreased as the ionic strength was increased to 1.0M. Minimal values of the emulsifying capacity and stability were obtained at ionic strength of 1.0M. The maximal emulsifying capacity of the flours at ionic strength of 0.4M ranged between 60-69%, while the emulsion stability also at the same concentration ranged between 54-69%. However in the isolates, both the emulsifying capacity and the stability were much higher than the value recorded for the flours. The emulsifying capacity for the protein isolates at an ionic strength of 0.4M ranged from 81-94% while the corresponding emulsifying stability was between 68-74%. The lower values recorded for the flours might be due to interaction of other components in the flours with emulsification (Wanasundra and Shahadi, 1997). Enhanced emulsifying capacity and stability with the initial increase in the ionic strength is due to the rapid migration to and aided adsorption of the protein molecules at the water-oil interphase. This adsorption of proteins in turn lowers the interfacial tension between the water and oil, thereby stabilising the protein-oil emulsion. Further increase in the ionic strength results in charge screening which could facilitate protein-protein interaction. This effect limits the protein solubility and it accounts for the reduction in the emulsion capacity and stability at high ionic strength. Aluko and Yada (1995) reported the influence of ionic strength on emulsifying properties of cowpea protein, while Chavan *et al*. (2001) reported the ionic strength dependent of emulsifying

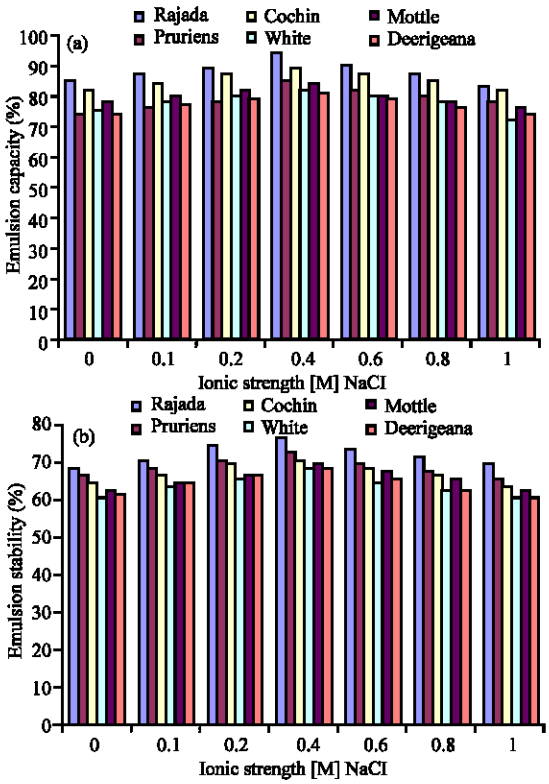

Fig. 8: Effects of ionic strength on (a) the emulsifying capacity and (b) the emulsifying stability of Mucuna protein isolates

properties of beach pea. Wagner and Guegeon (1999) attributed the higher emulsifying stability of soy protein at low ionic strength to the dissociation of oligomeric structure of 11S glycinin and subsequent improvement of the surface properties. Emulsifying properties as stated by the authors depend on two factors: A substancial decrease in interfacial energy due to adsorption of the protein at the oil-water interphase and the electrostatic, structural and mechanical energy barrier caused by the interfacial layer that opposes destabilisation processes. Initial increase in ionic strength of the solutions up to 0.4M enhanced the formation of charged layers around the fat globules and this resulted in mutual attraction among them. In addition, at low ionic strength, formation of hydrated layer around the interface resulted in lower interfacial energy and retarded droplet coalescence. However at higher ionic strength (0.6-1.0M), protein unfolding decreases, thereby limiting the adsorption of protein on the oil-water interface.

Emulsion activity and stability indices: In the present study, The Emulsion Activity Index (EAI) as well as the Emulsion Stability Index (ESI) for protein isolate were

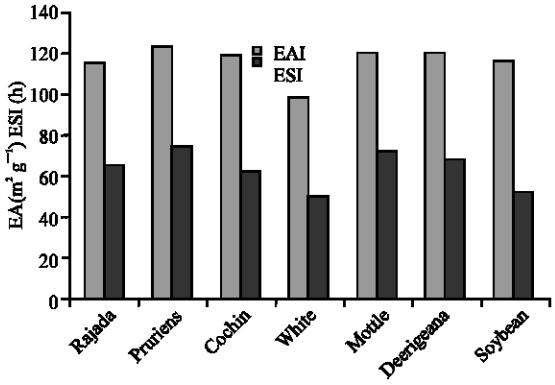

Fig. 9: Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI) of Mucuna bean protein isolates in comparison with soy bean isolate. The results for Soybean (Subagio, 2005) are included for comparison purposes

determined to allow for comparison of emulsifying properties which were determined using the same technique for similar seeds. The results are presented in Fig. 9. The highest EAI and ESI values of 123 $m^2 g^{-1}$ and 72 h, respectively was obtained for *M. pruriens* while corresponding values for *M. veracruz white* was 98 $m^2 g^{-1}$ and 48 h, respectively. Data for mucuna as reported in this study are higher than those reported for Soy protein isolate with values of 118 $m^2 g^{-1}$ and 52h for EAI and ESI. Our results compare favourably with the data on soybean displayed alongside our results. Most of the isolates have higher EAI and ESI than the commercial soy isolates. The EAI reflects the ability of the sample to rapidly adsorb at the water-oil interphase during the formation of emulsion, thereby preventing flocculation and coalescence; while ESI reflects the ability to maintain a stable emulsion over a period by preventing the flocullation and coalescence of the oil globules (Subago, 2005).

From the EAI and ESI values of the isolates obtained in the present study, Mucuna protein isolates could serve as potential ingredient in many food formulations such as salad dressing, sausages, comminuted meats, ice creams, cake batters and mayonnaise.

Viscosity measurements: The viscosity of the flours and protein isolates was determined as a function of pH at a temperature of 25 °C, using a viscometer. The results are presented in Table 1 and 2 (a-c), respectively. As the temperature was increased to 60°C, the viscosity of the flours and the isolates increased. Heating of protein in aqueous solution produces gel due to changes in the conformational structure of protein molecules as a result of protein denaturation at higher temperature (Idouraine *et al*., 1991). On cooling again to 25°C the

Table 1: Effects of pH on the viscosity[a] of Mucuna bean flour

	M. rajada			*M. pruriens*		
	Temperature			Temperature		
pH	25 °C[b]	60 °C[c]	25 °C[d]	25°C[b]	60 °C[c]	25°C[d]
2	3.56	4.85	5.00	3.89	4.00	4.34
3	3.35	4.76	5.88	3.55	3.78	4.78
4	3.05	4.88	5.60	2.70	2.03	3.65
5	2.89	3.50	4.25	3.89	4.00	4.85
6	4.04	5.76	6.44	4.54	4.75	5.78
7	3.25	3.70	4.60	3.60	4.28	4.90
8	5.35	4.50	5.75	4.80	4.97	5.25
9	4.85	5.15	5.95	4.64	4.85	5.00
10	3.65	4.88	5.00	4.01	4.12	4.95
	M. cochichinensis			*M. veracruz white*		
	Temperature			Temperature		
pH	25 °C[b]	60 °C[c]	25 °C[d]	25 °C[b]	60 °C	25 °C[c]
2	3.55	3.60	4.00	3.90	4.00	4.40
3	3.25	3.90	4.95	3.40	3.87	4.30
4	3.00	3.87	3.95	3.00	3.81	4.70
5	2.60	3.41	4.30	3.65	4.30	5.25
6	4.00	4.85	5.55	4.40	5.25	5.95
7	3.25	3.60	4.50	3.65	4.00	4.75
8	5.05	5.55	6.05	5.05	5.95	6.15
9	4.70	3.75	4.95	5.10	6.00	6.90
10	3.60	3.75	4.70	4.00	4.90	5.05
	M. veracruz mottle			*M. deerigeana*		
	Temperature			Temperature		
pH	25°C[b]	60°C[c]	25°C[d]	25°C[b]	60°C[c]	25°C[d]
2	3.70	3.95	4.20	3.60	3.75	4.30
3	3.20	3.70	4.45	3.70	4.60	5.55
4	2.95	3.75	4.50	2.10	3.65	4.60
5	3.95	4.95	5.25	3.85	4.85	5.00
6	4.25	5.00	6.00	4.15	5.10	6.10
7	3.45	4.70	5.70	3.30	3.60	4.80
8	5.25	5.75	6.20	5.15	5.65	6.60
9	4.95	5.95	6.34	4.75	5.80	6.50
10	3.70	4.70	5.25	3.60	3.90	4.45

[a]Viscosity-adapting spindle no 21 at 100rpm; [b]heating stage; [c]Cooling stage

Table 2: Effects of pH on the viscosity[a] of Mucuna bean protein isolates

	M. rajada			*M pruriens*		
	Temperature			Temperature		
pH	25 °C[b]	60 °C[c]	25 °C[d]	25°C[b]	60 °C[c]	25°C[d]
2	26.65	30.7	32.7	25.65	28.75	29.05
3	6.95	7.60	10.65	7.10	8.23	10.20
4	5.95	6.98	6.60	4.00	6.98	8.10
5	3.75	5.75	6.60	5.14	7.65	9.40
6	10.54	10.10	12.30	10.60	12.80	14.70
7	3.46	22.5	24.45	3.95	21.70	22.35
8	24.98	26.9	29.98	25.60	27.15	30.98
9	30.7	32.7	38.45	27.65	29.35	31.35
10	11.23	13.50	18.5	10.95	12.80	14.25
	M. cochichinensis			*M. veracruz white*		
	Temperature			Temperature		
pH	25 °C[b]	60 °C[c]	25 °C[d]	25°C[b]	60 °C[c]	25°C[d]
2	23.45	24.55	26.55	20.70	22.35	23.45
3	6.50	5.11	10.45	5.98	7.85	9.80
4	3.25	5.50	7.60	3.00	5.99	6.95
5	5.60	6.90	8.25	5.15	7.60	8.40
6	10.90	11.50	14.15	10.30	12.90	14.45
7	3.45	20.70	24.65	2.90	19.85	20.45
8	20.60	22.50	26.65	22.70	24.45	28.75
9	22.75	24.65	28.25	23.70	25.35	30.45
10	11.05	12.70	14.25	10.20	12.20	14.35
	M. veracruz mottle			*M. deerigeana*		
	Temperature			Temperature		
pH	25 °C[b]	60 °C[c]	25 °C[d]	25°C[b]	60 °C[c]	25°C[d]
2	20.50	24.70	25.75	21.70	23.75	26.75
3	5.90	7.55	9.05	6.05	8.00	10.90
4	3.00	5.97	7.30	2.90	5.00	8.20
5	5.15	6.60	8.70	5.00	7.20	10.60
6	10.65	12.60	14.80	10.05	12.90	14.00
7	2.95	18.50	22.75	2.75	18.80	20.78
8	20.60	22.70	26.75	21.75	20.60	17.85
9	21.35	23.50	28.70	22.70	24.78	28.30
10	8.75	10.60	14.50	9.70	10.20	14.24

[a]Viscosity-adapting spindle no 21 at 100rpm; [b]heating stage; [c]Cooling stage

viscosity increased further at all pH values. This additional increase in viscosity observed upon cooling might be due to progel formation (Betancur *et al*., 2001). Minimum viscosity was observed at either pH 4 or 5 (region of isoelectric point) for both the flours and protein isolates.

For the flours, the values observed at pH 7 were between 3.25-3.65 cP at 25°C before heating to 60°C and 4.60-5.70 cP at 25°C after thermal treatments. However in the isolates, viscosity values at pH 7 before heating ranged from 2.95-3.95cP at 25 °C to 20.78-24.65 cP at 25°C after heat treatments.

Similar results have been reported in the studies using the same protein concentration (10%) at pH 7 with values of 12.3, 12.5, 10.2 and 23 cP for *P. angularis, D. lablab, P. calcaratus* and soybean isolate, respectively (Chau *et al*., 1998).

It was noted that the viscosities of the protein isolates were higher than for their respective flours. The difference in viscosity between the flour and protein is due to the fact that flours is composed of starch and other components unlike the protein isolates. As a result, the viscosity values of the flour is influenced by the physical competition of water for starch, preventing it from reaching gelatinisation range where gels are produced which provides the viscosity (Betancur *et al*., 1997). It is noteworthy from the results of changes in viscosity with concentration presented in Fig. 10, there is an indication that the viscosity of mucuna isolate compares favourably with soybean isolate. This further demonstrates that mucuna isolates and, to a lesser extents, their flours, can be used in food systems as thickening agents such as dry foods and soup mixes to obtain certain consistency when reconstituted with water.

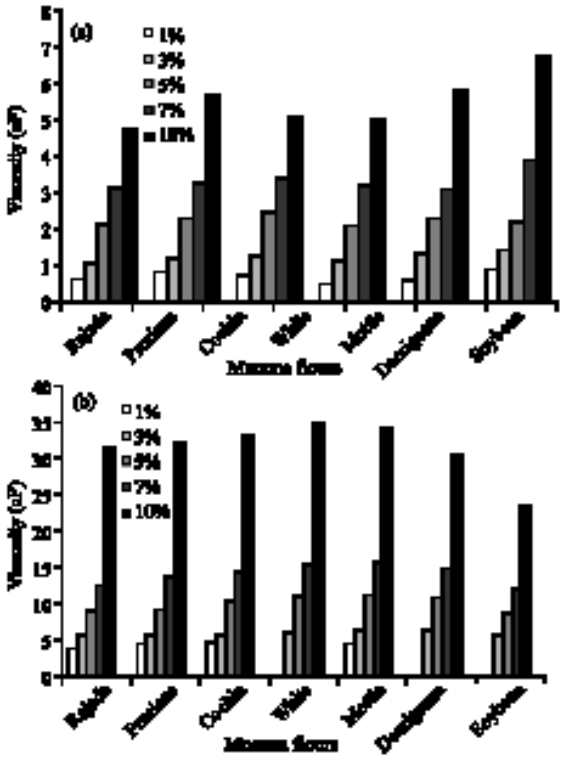

Fig. 10: Effect on concentration on the viscosity (centipoise) off(a) Mucana bean flours and (b) Mucuna protein isolates. The viscosity of commercial soy isolate obtained by Chi-Fai Chau *et al*. (1997) is included for comparison

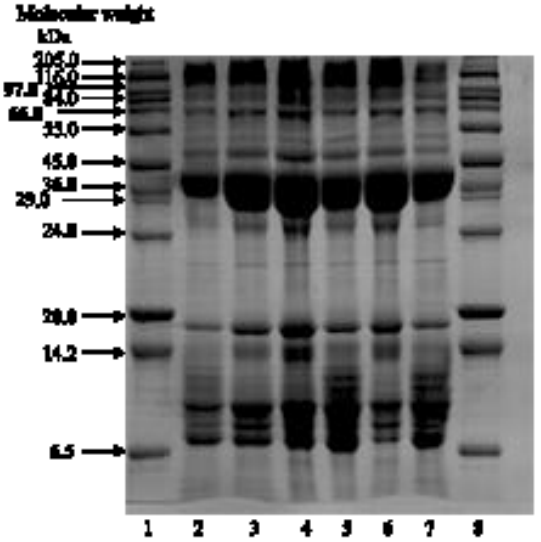

Fig. 11: Gel electrophoresis SDS-PAGE (Without Mercaptoethanol ME) of six Mucuna protein isolates. Lanes 1 and 8 are standard markers. Lane 2: *M. veracrus mottle*; Lane 3: *M. vera cruz white*; Lane 4: *M. deerigeana*; Lane 5: *M cochichinensis*; Lane 6: *M. rqiada*; Lane 7: *M pruriens*

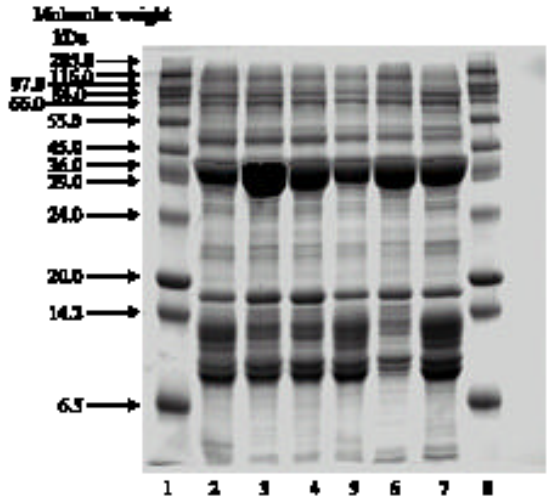

Fig. 12: Gel electrophoresis SDS-PAGE (With Mercaptoethanol ME) of six Mucuna protein isolates. Lanes 1 and 8 are standard markers. Lane 2: *M. veracrus mottle*; Lane 3: *M. vera cruz white*; Lane 4: *M. deerigeana*; Lane 5: *M cochichinensis*; Lane 6: *M. rqiada*; Lane 7: *M pruriens*

Evaluation of the protein sub-unit patterns in the flour and protein isolates: In order to characterize the proteins in the isolates, the sub-unit patterns were studied by gel electrophoresis (SDS-PAGE). This was performed in the presence and absence of a reducing agent (Mercaptoethanol) in order to distinguish between those polypeptide chains which are linked by disulphide bridges and those free of polypeptide chains.

The result of the gel electrophoresis SDS-PAGE as shown in Fig. 11 and 12 indicated the presence of a major band consisting of a broad zone with molecular weight 36±7 and 17±3 kDa appeared in all the samples under both reducing and non-reducing conditions. This shows that this major polypeptide are free from interchain disulphide bonds and might represent typical subunits of vilicin-like storage proteins as reported by other 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 include the polypeptide chain with 55, 84, 97 and 116 kDa. In contrast, a 66 kDa minor fraction disappeared after reduction which resulted in the formation of a smaller polypeptide chain with 24 kDa. A

similar observation was reported by Rahma *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 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 soybeans (Thanh and Shibasaki, 1976; Iwabuchi and Yamauchi, 1987); field bean (Carsey and Domoney, 1984) and faba beans (Muntz *et al*., 1986) contain 2 major storage proteins: 11S legumin and 7S vicilin, there are a number of other legumes which contain a 7S fraction as the major protein component and other storage protein in marginal amount. To these belong legumes like *Phaseolus vulgaris,* cowpea, pigeon pea, jack bean and winged bean which contains 7 and 2.5S proteins (Sakakibara *et al*., 1979; Sefa-Dedek and Stanley, 1979; Krishna and Bhatia, 1985; McPherson, 1980; Yanagi, 1985).

Our 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 legumin type composed of larger and smaller units of disulphide linked polypeptide chains which are present only in marginal amounts in the Mucuna bean isolates and extracts. Our preliminary studies of the isolation and characterization of 7 and 11S proteins in the isolates supported this proposition. Work on the isolation and characterization of both the 7 and 11S proteins is already in progress, the results of which will be published in the near future.

CONCLUSION

The nature of the proteins as well as the emulsifying properties of Mucuna flours and isolates has been investigated.

The high protein solubility of the flour and isolates in the acidic pH range indicates that Mucuna bean may be useful in the formulation of acidic food like protein rich carbonated beverages. In addition the high emulsion capacity and stabilities recorded in both the flour and isolate indicates that Mucuna bean could serve as potential ingredient in many food formulations such as salad dressing, sausages, comminuted meats, ice creams, cake batters and mayonnaise.

It is noteworthy from the results of changes in viscosity with concentration there is an indication that the viscosity of Mucuna isolate compares favourably with soybean isolate. This further demonstrates that Mucuna isolates and, to a lesser extents, their flours, can be used in food systems as thickening agents such as dry foods and soup mixes to obtain certain consistency when reconstituted with water.

Our 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 legumin type composed of larger and smaller units of disulphide linked polypeptide chains which are present only in marginal amounts in the Mucuna bean isolates and extracts.

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