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Foaming, gelation and electrophoretic characteristics of mucuna bean (*Mucuna pruriens*) protein concentrates

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Abstract

The influences of pH, ionic strength and carbohydrates on the foaming and gelation properties of mucuna bean protein concentrate (MPC) were investigated. The protein concentrate was extracted by alkaline solubilization at pH8, followed by isoelectric precipitation at pH 4. The protein solubility–pH profile showed minimum solubility (19.4%) at pH 4.0 (iso electric point) and maximum solubility (96%) was obtained at pH 12. Foaming capacity increased as the sample concentration increased. Increase in sample concentration also enhanced foaming stability at the various times studied. pH had a pronounced effect on the foaming properties of MPC. At pH 4, MPC exhibited minimum foaming capacity and maximum foaming stability. At lower pH values, there was enhanced foaming capacity and a reduction in the foaming stability. Alkaline media (pH 8 and 10) enhanced foaming but the foams were less stable. Sucrose, maltose, lactose and potato starch improved the foaming capacity and stability of the protein concentrate. Increase in ionic strength, from 0.1 to 0.4 M, improved foaming capacity and stability, while further increase beyond the ionic strength resulted in a reduction of the foaming properties. In all cases studied, gelation improved with increases in concentration of the protein concentrate in the media. Gelation properties were reduced in alkaline and acidic media, except at pH 4, where least gelation concentration endpoint (LGE) was 8. Gelation properties of MPC improved in the presence of carbohydrates in the mixture. Gel-forming properties also increased with increases in ionic strength of the media from 0.1 to 0.4 M, while further increase, from 0.6 to 1.0 M, reduced the gelation properties of MPC. Five polypeptide protein sub-units, at apparent molecular weights of 200, 116, 82, 63, and 59 kDa, were obtained from polyacrylamide gel electrophoresis under non-reducing conditions (without 2-mercaptoethanol). In addition, two other sub-units, at apparent molecular weights of 97 and 40 kDa, were obtained under reducing conditions (with 2-mercaptoethanol).

Keywords: Functional properties; Protein concentrates; Mucuna; Electrophoretic characteristics

1. Introduction

The problem of protein malnutrition in the developing countries cannot be over-emphasized, as a large number of people in this part of the world still do not have access to cheap protein sources. This has therefore called for a continuous search for cheap plant proteins. Mucuna bean (*Mucuna pruriens*), a tropical underutilised legume, could be a potential candidate in this regard. Apart from being underutilised, it is rich in proteins (about 35 g/100 g of seed flour) (Josephine & Janardhanan, 1991).

Before a legume protein can be properly integrated as a food component, information about the functional properties of the protein, as well as the effect of pH, temperature, ionic strength and presence of other food components, such as lipids, sugars and starches, are important considerations (Damodaran, 1989; Myers, 1988). Incorporation of legume seed flour into most foods is limited because of restricting antinutritional factors and impairment of flavour (Akintayo, Oshodi, & Esuoso, 1999). Attention is now focussed on the use of protein concentrates rather than the seed flour of legume seeds, since they have superior functional properties, low flavour profiles and relative freedom from toxic factors and indigestible carbohydrates (Neto, Narain, Silva, & Bara, 2001).

In recent years, studies on functional properties of protein concentrates of some legumes, in relation to environmental factors, and the presence of other food hydrocolloids have been reported for cowpea (Aluko & Yada, 1995; Mwasaru, Muhammed, Bakar, & Cheman,

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2000), lupin seed (Pozami, Doxastakis, & Kiosseoglu, 2002) *Cajanus cajan* (Akintayo et al., 1999; Mwasaru et al., 2000) and soybean (Boulet, Britten, & Lamarche, 2000; Puppo & Anon, 1999).

As far as we know, there are no reports on either the study of mucuna bean protein or protein concentrate. The goal of this study, therefore, was to investigate the effects of pH, ionic strength, concentration and carbohydrates on the foaming properties and gelation behaviour of mucuna protein concentrate. The data obtained from this work could provide a guide to determination of parameter values for use in further studies involving the functionality of mucuna protein or protein concentrates in different food systems.

2. Materials and methods

2.1. Materials

Mucuna bean seeds (*Mucuna pruriens*) were obtained from the International Institute of Tropical Agriculture, IITA, Ibadan. Manual screening was used to remove foreign materials from the seeds. After that, the seeds were ground to pass through a BS-60 mesh screen using a household flourmill (Braun Multimix Deluxe, Germany). The flour was kept in a refrigerator at about 4 °C until used.

2.2. Preparation of mucuna bean protein concentrate

The method of preparation is outlined schematically in Fig. 1. One kilogramme of mucuna bean flour was dispersed in 10 l of distilled water and the pH was adjusted to 8.0 with 1 M NaOH, to facilitate protein solubilisation. The pH of the supernatant obtained after centrifugation was adjusted to 4.0 to precipitate the protein concentrate, which was recovered by centrifugation at 5000×*g* for 30 min. The average yield of protein concentrate from the mucuna bean flour was 25.4% and the percentage protein content of the concentrate was 78.3% (Kjeldahl method) (AOAC, 1985).

2.3. pH Solubility profile

This was determined according to the method of Were, Hettiarachchy, and Kalapathy (1997). One-hundred and twenty-five milligrammes (125 mg) of the sample were dispersed in 25 ml distilled water and the solution pH was adjusted to 2, 3, 4, 5, 6, 7, 8, 9 and 10 using 0.1 M NaOH. The slurries were mixed for 1 h at 24 °C using a magnetic stirrer before centrifuging at 12,000×*g* for 20 min at 4 °C. The supernatant was filtered through glass wool to obtain a clear solution. Nitrogen content in the supernatant was determined by the Kjeldahl method. Triplicate determinations were carried out and solubility profile was obtained by plotting

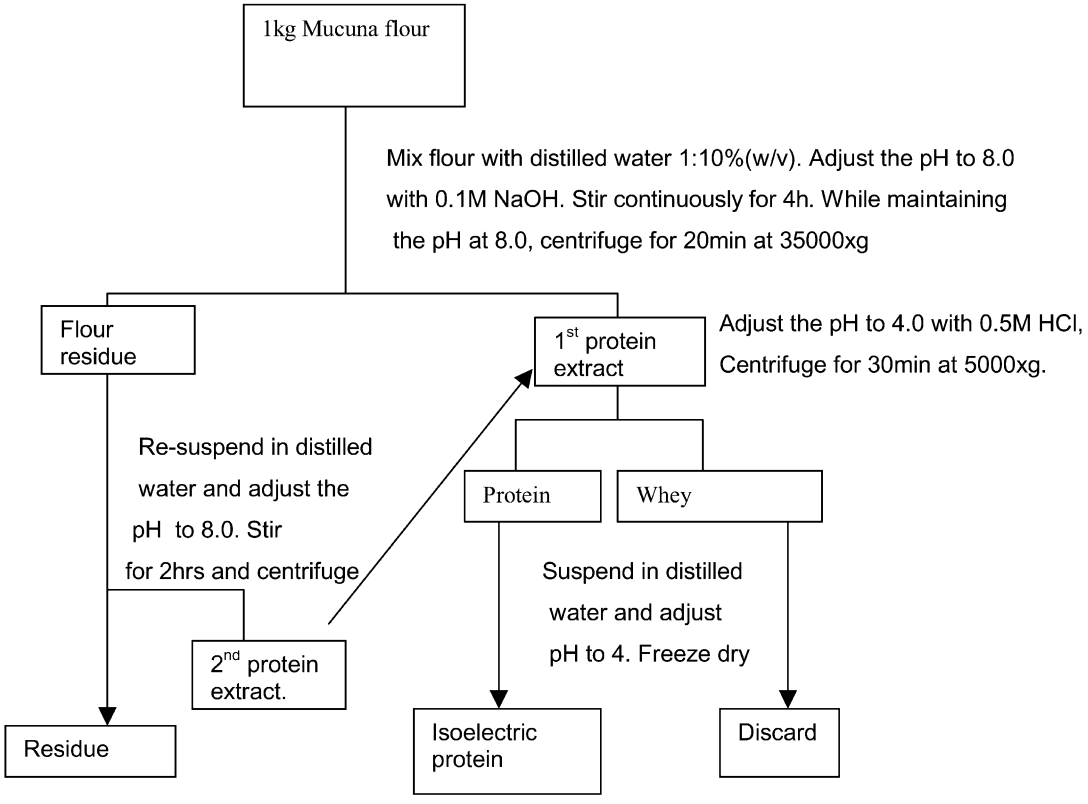

Fig. 1. Schematic diagram for preparation of protein concentrate from mucuna bean.

averages of protein solubility (%) against pH. The percentage soluble protein was calculated as follows:

$$\text{Solubility (\%)} = \frac{\text{amount of nitrogen in the supernatant}}{\text{amount of nitrogen in the sample}} \times 100.$$

2.4. Foaming properties

The foaming capacity and stability were studied according to the method of Coffman and Garcia (1977). A weighed amount of protein concentrate was dispersed in 100 ml distilled water. The resulting solution was whipped vigorously for 2 min in a Phillips Kitchen blender set at speed 2. Volumes were recorded before and after whipping. The percentage volume increase was calculated according to the following equation

$$\%\ \text{Volume} = (V_2 - V_1)/(V_1) \times 100$$

where V_2 = volume of protein solution after whipping; V_1 = volume of solution before whipping.

Effects of concentration on the foaming properties were investigated by whipping 2, 4, 6, 8, 10% w/v of the dispersions, as described above. The effect of pH on foaming properties was found by adjusting 2% w/v dispersions to the desired pH range from 2.0 to 10.0, using either 1 M HCl or 1 M NaOH, followed by vigorous whipping, as described above.

Influence of ionic strength was evaluated by dispersing 2 g of protein concentrate in 100 ml KCl solution of known ionic strength. Studies were conducted in solutions of ionic strength (μ) of 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 M, after which they were whipped vigorously.

Effects of some carbohydrates on foaming properties were investigated using 2 g of protein concentrate. Lactose, maltose, sucrose and potato starch were added at a concentration of 0.25 g/g of protein concentrate. All experiments were performed in triplicate.

2.5. Gelation properties

Gelation properties were investigated, using the method described by Coffman and Garcia (1977). Sample suspensions of 2–20% were prepared in distilled water. Ten millilitres of each of the prepared dispersions was transferred into a test tube. It was heated in a boiling water bath for 1 h, followed by rapid cooling in a bath of cold water. The test tubes were further cooled at 4 °C for 2 h. The least gelation concentration was determined as the concentration when the sample from the inverted test tube did not slip or fall.

Studies on the effect of pH were conducted on the sample at various concentrations by adjusting the pH to the desired value, from 2.0 to 10.0, prior to heating, using either 0.5 M HCl or 0.5 M NaOH. Least gelation concentration was determined as described above.

Effect of ionic strength was investigated by preparing sample suspensions (2–20% w/v) at various concentrations in KCl solution of known ionic strength (μ). The pH was adjusted to 7.0 in each case. Studies were conducted at ionic strengths (μ) of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 M.

Studies were conducted to investigate the effects of carbohydrates on gelation properties of mucuna protein concentrate, by adding maltose, sucrose, lactose and potato starch at 0.25 g/g of the protein concentrate. Sample suspensions (2–20% w/v) were prepared and the pH was adjusted to 7.0 in each case.

2.6. Electrophoresis

The molecular weight profiles for the protein fractions were established using sodium dodecyl sulphate polyacrylamide gel electrophoresis, according to the method of Laemmli (1970), as described by Aminlari and Majzoobi (2002). 100 mg of sample were added to 2.5 ml of buffer containing 0.5 M Tris–HCl (pH 6.8), 0.5% bromophenol blue, 10% glycerol and 2% SDS. To another preparation, 5% 2-mercaptoethanol (2-ME) was added to effect reduction of disulphide bonds. The preparations were centrifuged at 20,000 *g* for 15 min at 4 °C and the supernatant was employed for electrophoresis. Protein samples (10 μl), were loaded onto the gel using a model 491 cell, Bio-rad Laboratories, CA, USA. Electrode buffer contained 50 mM Tris and 25 mM borate, pH 8.7. The separating gel had 12% final acrylamide concentration, while the stacking gel had 3.75% concentration. Electrophoresis was carried out at a constant 200 V for 8 h at 4 °C and gel was stained with Coomassie Brilliant Blue R-250 and de-stained with 10% trichloroacetic acid/7.0% methanol solution. Molecular weight standard from Promega, Madison, USA, containing myosin 212.0 kDa, *Escherichia coli* β-galactosidase, 116.0 Da; rabbit muscle phosphorylase *b*, 97.4 kDa; bovine serum albumin, 66.0 kDa; catalase, 57.5 kD and aldolase, 40.0 kDa, was used for the determination of sample molecular weights of protein subunits.

3. Results and discussion

3.1. pH solubility profile

The protein solubility profile of the mucuna protein concentrate (MPC) was pH-dependent (Fig. 2). The profile showed a decreasing solubility with increasing pH until it reached minimum solubility (19.4%) at the iso-electric point (pH 4.0), followed by increase in solubility with increasing pH values after the iso electric

point. The result is similar to those reported for other common legumes such as cowpea (Prinyawiwatkul, Beuchat, Mcwatters, & Phillips, 1997) and chick pea (Sanchez-Vioque, Clement, Vioque, Bautista, & Millan, 1999). MPC recorded higher solubility at the alkaline pH (8–12) than at the acidic pH (2–6). A maximum value of 96% was recorded at pH 12, compared with 78% at pH 2. A similar observation has been reported for Gila bean (Siddhuraju, Becker, & Makkar, 2001) and winged bean (Sathe, Despande, & Salunhe, 1982b). Protein solubility is important in food systems because it affects other functional properties, such as foaming, emulsification and gelation (Kinsella, 1976). By this result, it may be suggested that MPC is similar to other legume protein concentrates in terms of solubility and electrical charge distribution. This makes it potentially useful in food applications where high solubility profiles are needed to impart certain characteristics. Such applications include baby food, baked products and deserts (Chel-Guero, Perez-Flores, Bentacur-Ancona, & Davila-Ontiz, 2002).

3.2. Foaming properties

Foaming capacity was dependent on sample concentration (Fig. 3), showing 94% increase in volume at 10% (w/v) concentration compared to 58% at 2% (w/v). A progressive increase in foaming capacity was

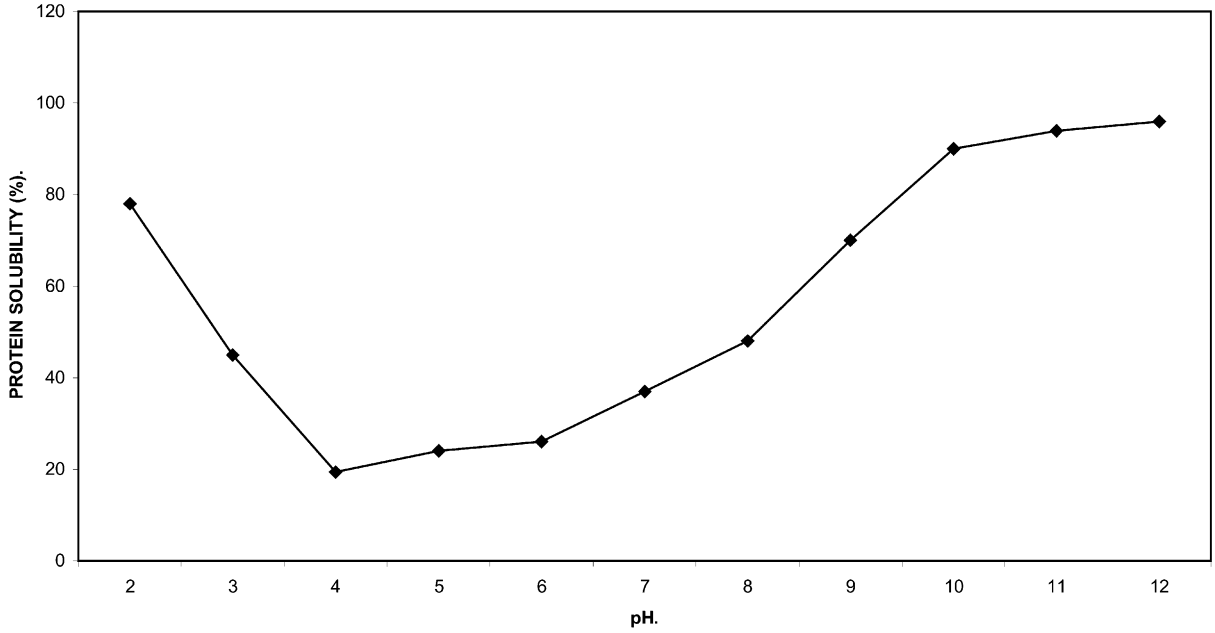

Fig. 2. Effect of pH on protein solubility profile.

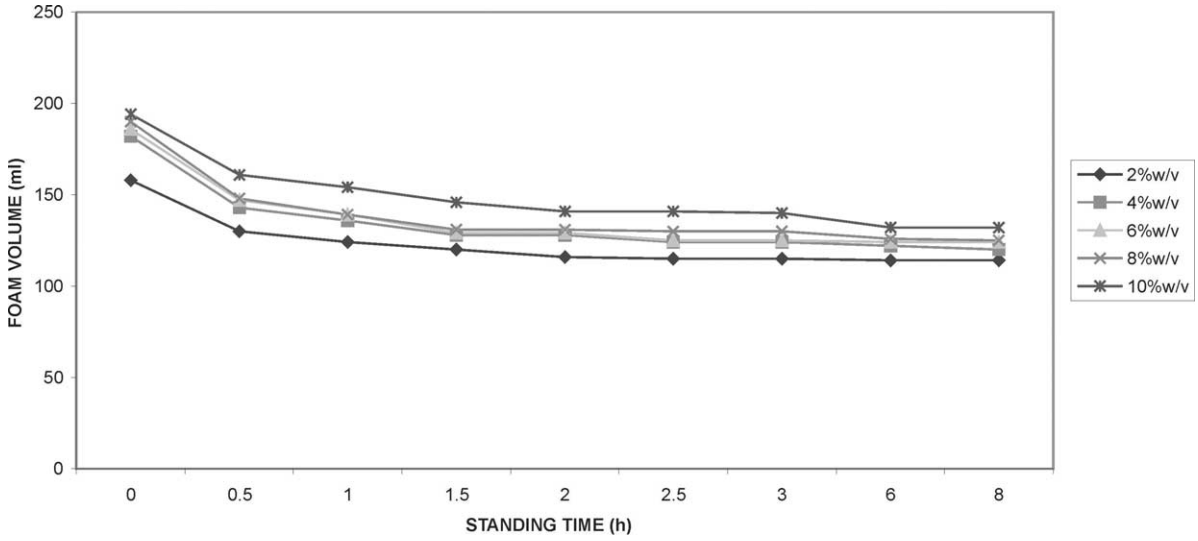

Fig. 3. Effect of concentration of sample on the foaming capacity and stability of mucuna protein concentrate.

observed as the concentration of the protein concentrate increased. The results are in agreement with the report of earlier workers on the foaming properties of lupin seed proteins and protein concentrates (Sathe et al., 1982b) and pigeon pea protein concentrate reported by Akintayo et al. (1999). The foaming capacity of 58% at 2% (w/v) reported in this work is high compared to the 32% reported for lupin seed (Sathe et al., 1982b) but lower than the 80% increase reported for pigeon pea (Akintayo et al., 1999). However, MPC showed a higher stability after 8 h (114 ml) than the 100 ml (liquid layer) reported for pigeon pea (Akintayo et al., 1999). The variations are possibly due to physiological differences in the protein used. Foam collapse takes place by any of these three mechanisms:

1. disproportionation of bubbles;
2. coalescence of bubbles due to instability of the film between them; and
3. drainage of water from the surface of the bubbles down to the liquid layer, thereby leading to the removal of protein from film around the bubble. The protein on the film then becomes too thin to support the bubble.

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 disproportionation and coalescence of bubbles. In addition, increase in concentration could lead to formation of thicker films, which limits the effect of drainage of protein from films. The availability of more protein, as the level of protein concentrate increases in the aqueous dispersion, enhances foam formation.

The results of effect of pH on foaming capacity and stability of the protein concentrate are presented in Fig. 4. The results indicated 35% increase in the foaming capacity at pH 4 (corresponding to minimum foaming capacity) while the maximum foaming capacity of 134% was recorded at pH 10. Also, an increase in foaming capacity was observed at pH 2. The foaming capacity at alkaline extreme (pH 10) was, however, higher than the value obtained at pH 2. After 8 h, the highest foam stability (122 ml) was observed at pH 4 (isoelectric region), while the least value of 110 ml was recorded at pH 10. Earlier workers (Aluko, & Yada, 1995; Lin, Humbert, & Sosulski, 1974; Sathe, Despande, & Salunkhe 1982a; Sathe et al., 1982b) reported a pH-dependency of foaming capacity and stability for lupin seeds, winged bean, sunflower and cowpea proteins, respectively. In the present study, maximum foaming capacity was observed at pH 10. A decrease in attractive hydrophobic forces among the protein molecules occurs at the high acidic and alkaline regions, in which cases the protein molecules become net positively charged and net negatively charged, respectively. This development leads to repulsion, which facilitates the flexibility of the protein molecules. Increase in foaming capacity of MPC at the two extremes of pH could be attributed to increase in the flexibility of the protein, which diffuses more rapidly to the air–water interface to encapsulate air particles, leading to enhanced foaming (Chau & Cheung, 1998). In the past, studies have revealed that protein stabilized foams are more stable in the neighbourhood of the isoelectric pH of the protein than at any other pH (Aluko, & Yada, 1995; Buckingham,

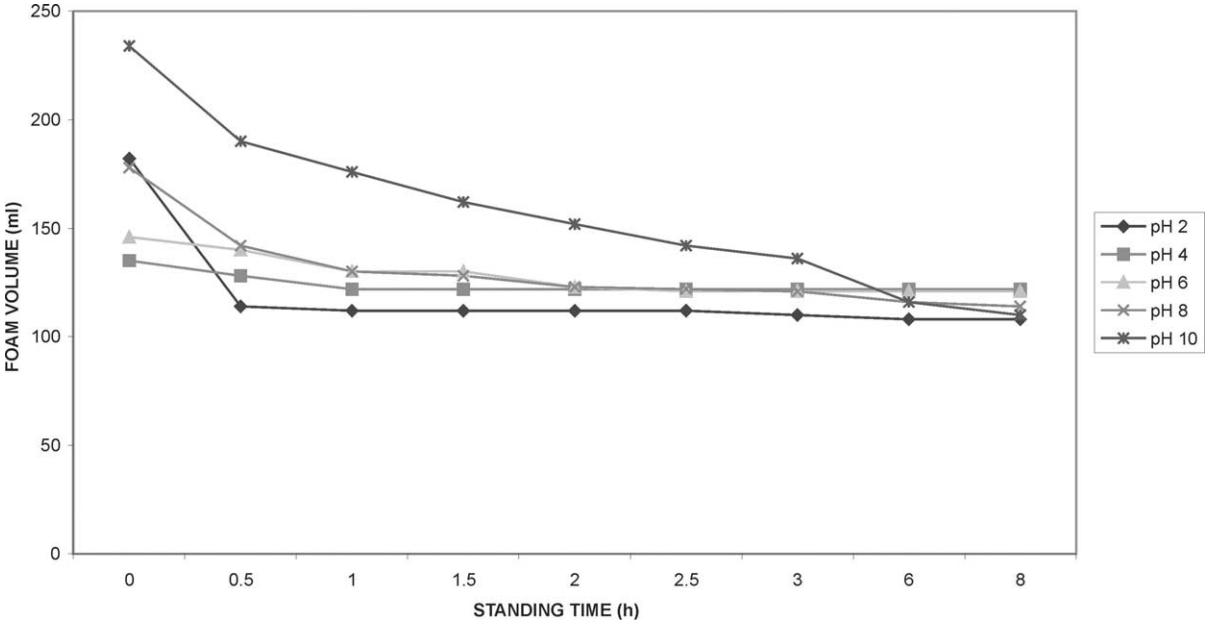

Fig. 4. Effect of pH on foaming capacity and stability of mucuna protein concentrate.

Table 1
Effects of carbohydrates on the foaming capacity and stability of Mucuna protein concentrate*

Carbohydrates	Volume after whipping (ml)	% increase in vol.	Volume (ml) at room temperature (30±2 °C) at time intervals (h)							
			0.5	1.0	1.5	2.0	2.5	3.0	6.0	8.0
Control	158.0±1.0	58	130.2±2.0	124.0±1.0	120.0±1.3	116.0±2.1	115.0±2.0	114.8±2.4	113.6±1.9	111.0±1.0
Mp+Su	174.0±1.3	74	144.0±1.6	130.0±1.8	127.9±2.0	124.0±2.0	124.0±2.0	124.0±1.0	115.2±2.1	114.0±2.0
Mp+L	191.0±1.1	91	152.0±2.0	147.8±2.4	147.0±1.5	132.0±4.4	130.0±1.2	130.0±1.0	130.0±2.2	123.8±2.0
Mp+M	177.2±2.4	77	143.8±3.2	138.0±2.3	136.0±2.0	135.0±2.0	135.0±2.0	135.0±1.6	124.0±2.0	111.2±1.6
Mp+St	176.0±1.5	76	172.0±1.5	160.2±3.0	152.0±1.0	152.0±1.0	151.0±2.3	142.0±2.0	133.0±2.1	118.0±1.0

Results reported as mean±SD of triplicate determinations. All experiments were conducted at pH 7.0. Mp+Su, Mucuna protein concentrate plus sucrose; Mp+L, Mucuna protein concentrate plus lactose; Mp+M, Mucuna protein concentrate plus maltose; Mp+St, Mucuna protein concentrate plus starch.

1970). Lack of repulsive interactions enhances favourable protein–protein Interactions and formation of a viscous film at the interface. Also, the amount of protein adsorbed at the interface increases around the isoelectric pH because of lack of repulsion between the interface and adsorbing molecules. This may also have contributed to the formation of stable molecular layers in the air– water interface, which imparted stability to the foam. Lowering of foaming capacity around the isoelectric pH has been attributed to decrease in protein solubility in this region (Aluko & Yada, 1995).

Effects of carbohydrates on foaming properties of mucuna bean protein concentrate are presented in Table 1. Incorporation of carbohydrates, at 0.25 g/g of protein, increased the foaming capacity and stability compared with the control. Sathe et al. (1982b) reported increase in foaming capacity of winged bean protein concentrate after the addition of sucrose, amylose, amylopectin, potato starch, gum arabic and pectin at 0.25 g/g of protein. Wang and Kinsella (1976) also reported increase in stability of Alfafa leaf protein on addition of sucrose. Similarly, DeVilbiss, Holsinger, Poasti, and Pallanski (1974) reported a marked improvement in foaming stability of whey protein concentrate and ovalbumin when sucrose was added to the mixture. Foam stability and leakage are related to the viscosity, density and surface tension of the liquid phase foam. Hence, the positive effect of sucrose, lactose and starch on foam stability might be due to increased bulk-phase viscosity, which reduced the rate of drainage of the lamella fluid.

Foaming properties are dependent on the ionic strength of the dispersion. These results are presented in Fig. 5. Increase in foaming capacity of mucuna bean protein concentrate MPC was observed as the ionic strength increased progressively from 0.1 to 0.4 M, after which a decline in the foaming capacity occurred in solutions prepared in ionic strengths of 0.6–1.0 M. Similarly, enhanced foam stability was observed in the range 0.1–0.4 M, whereas, 0.6–1.0 M ionic media

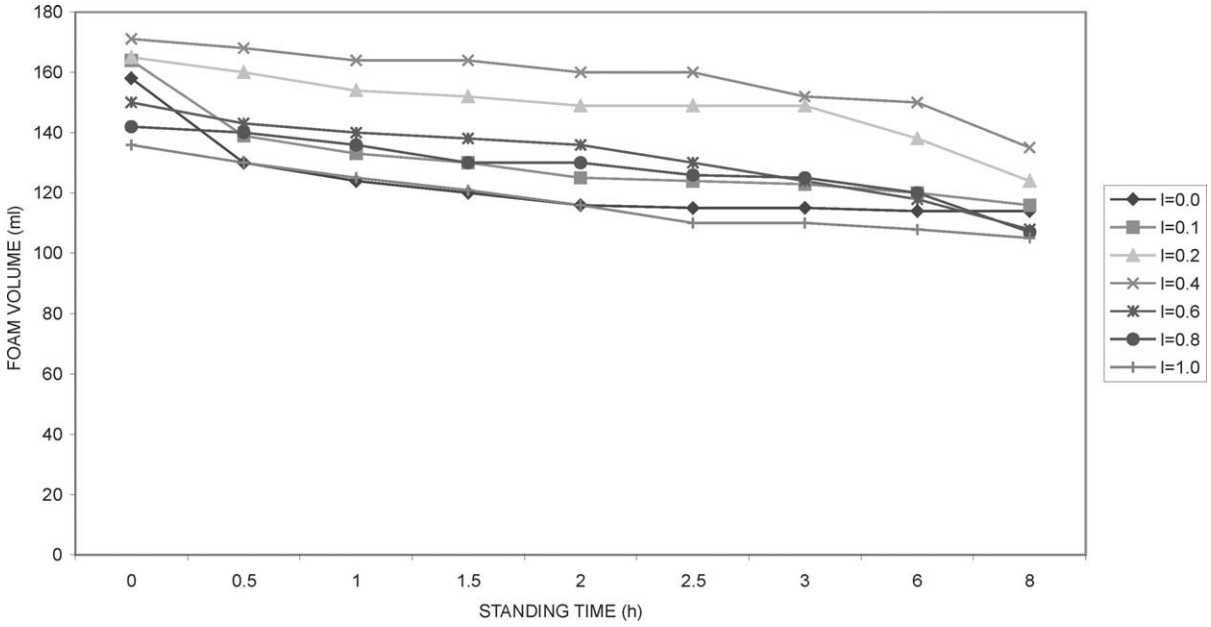

Fig. 5. Effect of ionic strength (m) on the foaming capacity and stability of mucuna protein concentrate.

reduced the stability of MPC foams. Foaming in protein dispersions is enhanced by increase in solubility of the proteins. Low ionic strength (0.1–0.4 M) improved the solubility of MPC in the dispersion and this facilitates the formation of stable cohesive films around the air vacuoles. Further increase in ionic strength, from 0.6–1.0 M, resulted in charge-screening, which enhanced hydrophobic interaction. This increase in hydrophobic interaction can lead to a "salting out" effect, leading to a reduction in the foaming capacity and stability.

3.3. Gelation

In the gelation experiments, the least gelation concentration endpoint (LGE) was taken as an index of gelation capacity. The lower the LGE, the better the gelating ability of the composition. The results presented in Table 2 show that pH has a pronounced effect on the LGE of Mucuna bean protein concentrate MPC. The minimum LGE, 8% (w/v) was recorded at pH 4, while the maximum value, 16% (w/v), which indicates least tendency to form gel, was recorded at pH 2. When the pH was adjusted to 7 and 8 the LGE value of 12% (w/v) was constant, but the samples showed different characteristics in terms of strength at 6% (w/v) concentration. At pH10, the LGE increased further to 14% (w/v), suggesting a lower tendency toward gel formation. The LGE of 12% (w/v) at pH 7, which serves as the control, is lower than the 14% (w/v) reported for lupin seed proteins (Sathe et al., 1982a, 1982b). This indicates that mucuna beans will be a better gelating food component than lupin seed proteins. Protein gels are formed by intermolecular interactions, which produce a continuous, three-dimensional network exhibiting structural rigidity. Gelation of proteins can be induced by chemical, physical (which includes heating) and enzymatic treatments (Sato, Nakamura, Kawanari, & Nakajima, 1995). In the case under study, the pH had a pronounced effect on the heat induced gelation reactions by influencing the balance of polar and non-polar residues. At pH 4, enhanced protein–protein interaction increased the bond formations necessary for the building of a gel macro-structural molecular network. At pH 2 and 10, the lowering of gel strength could be as a result of increased electrostatic repulsion among the protein molecules, over the electrostatic attraction forces that form part of the bonding forces in the molecular network.

The effect of carbohydrates on the gelation of Mucuna protein concentrate MPC is presented in Table 3. All the carbohydrates, at the concentrations studied, improved the gelation properties of MPC over the control. Sucrose and lactose produced the least improvement in the gelation properties of MPC. Maltose and starch improved the gelation properties more than sucrose and lactose. However, starch showed better improvement in gelation as the concentration of the additives increased. Bryant and McClements (2000) reported an increase in gelation of whey protein solutions with the addition of sucrose. The reduction in LGE after the addition of carbohydrate might be due to the decrease in thermodynamic affinity of proteins for the aqueous solution, caused by the presence of carbohydrates. On the other hand, improvements can be attributed to the magnitude of the protein-protein interactions, which enhances gelation.

Among the effects of ionic strength on the gelation properties, as presented in Table 4, the LGE at 0.1 M, 12% (w/v) was higher than the 6% (w/v) observed for 0.2 and 0.4 M ionic strengths. A progressive increase in gelation concentration was observed from ionic strength of 0.6–1.0 M. This result indicate that increase in ionic strength from 0.1–0.4 M improved gelation, while further increase in the ionic strength led to formation of gels of lower strength. Earlier, Akintayo et al. (1999) reported increase in least gelation concentration for pigeon pea, when the ionic strength of the medium was increased from 0.5 to 1.0 M. Otte, Schumacher, Ipsen,

Table 2
Effect of sample concentration and pH on the gelation capacity of Mucuna protein concentrate[a]

Sample conc. (% w/v)	PH 2.0		pH 4.0		pH 7.0		pH 8.0		pH 10.0	
	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance
2	–	Liquid	–	Liquid	–	Liquid	–	Liquid	–	Liquid
4	–	Liquid	–	Liquid	–	Liquid	–	Liquid	–	Liquid
6	–	Liquid	–	Viscous	–	Viscous	–	Liquid	–	Liquid
8	–	Viscous	+	Gel	–	Viscous	–	Viscous	–	Viscous
10	–	Viscous	+	Gel	–	Viscous	–	Viscous	–	Viscous
12	–	Viscous	+	Firm gel	+	Gel	+	Gel	–	Viscous
14	–	Viscous	+	Firm gel	+	Firm gel	+	Ffirm gel	+	Gel
16	+	Gel	+	Firm gel	+	Firm gel	+	Firm gel	+	Firm gel
18	+	Firm gel	+	Very firm gel	+	Very firm gel	+	Very firm gel	+	Very firm gel
20	+	Firm gel	+	Very firm gel	+	Very firm gel	+	Very firm gel	+	Very firm gel

[a] Results of triplicate determinations. (–) indicates no gelation. (+) indicates gelation.

Table 3
Effects of carbohydrates on the gelation of Mucuna protein concentrate[a]

Sample conc. (% w/v)	Control		Mp + maltose		Mp + lactose		Mp + sucrose		Mp + starch	
	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance
2	–	Liquid	–	Liquid	–	Liquid	–	Liquid	–	Viscous
4	–	Liquid	+	Liquid	–	Liquid	–	Liquid	+	Gel
6	–	Viscous	+	Gel	–	Liquid	–	Liquid	+	Gel
8	–	Viscous	+	Gel	+	Viscous	+	Viscous	+	Firm gel
10	–	Viscous	+	Firm gel	+	Gel	+	Gel	+	Firm gel
12	+	Gel	+	Firm gel	+	Gel	+	Gel	+	Firm gel
14	–	Gel	+	Very firm gel	+	Firm gel	+	Firm gel	+	Very firm gel
16	+	Firm gel	+	Very firm gel	+	Firm gel	+	Firm gel	+	Very firm gel
18	+	Firm gel	+	Very firm gel	+	Very firm gel	+	Very firm gel	+	Very firm gel
20	+	Very firm gel	+	Very firm gel	+	Very firm gel	+	Very firm gel	+	Very firm gel

[a] All experiments were carried out at pH 7.0. (–) indicates No gelation. (+) indicates gelation.

Table 4
Effects of concentration and ionic strength (m) on the gelation of mucuna bean protein concentrate[a]

Sample conc. (%w/v)	$\mu = 0.1$		$\mu = 0.2$		$\mu = 0.4$		$\mu = 0.6$		$\mu = 0.8$		$\mu = 1.0$	
	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance	Gelation	Appearance
2	–	Liquid	–	Liquid	–	Liquid	–	Liquid	–	Liquid	–	Liquid
4	–	Liquid	–	Viscous	–	Viscous	–	Liquid	–	Liquid	–	Liquid
6	–	Viscous	+	Gel	+	Gel	–	Viscous	–	Liquid	–	Liquid
8	–	Viscous	+	Gel	+	Gel	–	Gel	–	Liquid	–	Liquid
10	–	Viscous	+	Gel	+	Gel	+	Gel	+	Viscous	–	Viscous
12	+	Gel	+	Firm gel	+	Firm gel	+	Gel	+	Gel	–	Viscous
14	+	Gel	+	Firm gel	+	Firm gel	+	Gel	+	Gel	+	Gel
16	+	Firm gel	+	Very firm gel	+	Very firm gel	+	Firm gel	+	Gel	+	Gel
18	+	very firm gel	+	Very firm gel	+	Very firm gel	+	Firm gel	+	Gel	+	Gel
20	+	very firm gel	+	Very firm gel	+	Very firm gel	+	Firm gel	+	Firm gel	+	Gel

[a] All experiments were performed at pH 7.0. (–) indicates no gelation. (+) indicates gelation.

Ju, and Qvist (1999) also gave an account of reduction of gel firmness of whey proteins, when the NaCl content of the mixture was increased. Castimopoolas and Meyer (1970) also reported a reduction in gelation properties of soybean globulins in solutions of high ionic strength. Increase in ionic strength from 0.1 to 0.4 M improved solubility of protein in the dispersion, thereby making more proteins available for formation of bonding in the gel molecular network. Further increase in ionic strength from 0.6 to 1.0 M influences the gel forming process negatively by decreasing protein unfolding, which is the vital denaturation stage necessary for gelation.

3.4. Polyacrylamide gel electrophoresis

The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) pattern of Mucuna bean protein concentrate is depicted in Fig. 6. This indicates the presence of five polypeptide protein sub-units of apparent molecular weights 200, 116, 82, 63, and 59 kDa. Similar ranges of polypeptide molecular weight distribution have also been reported for several other legume proteins (Bhatty, 1982; Chan & Phillips, 1994). Variations in the number of sub-units and molecular weight distribution obtained for various legumes have been attributed to physiological differences among the legumes (Gillespie & Blagrov, 1975). The band patterns obtained under reducing conditions (addition of 2-mercaptoethanol) and none-reducing conditions (without

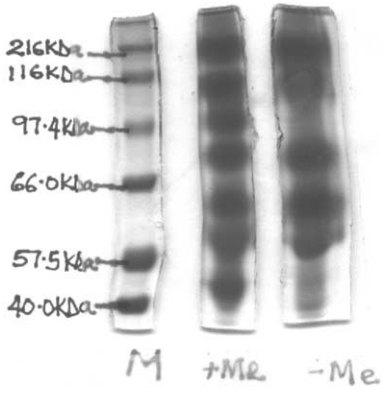

Fig. 6. SDS-PAGE of Mucuna protein concentrate. Molecular weight marker (M), band obtained in the presence of mercaptoethanol (+Me)and band obtained in the absence of mercaptoethanol (–Me).

2-mercaptoethanol) were compared. After the addition of 2-mercaptoethanol, two additional sub-unit bands were obtained at approximately 97 and 40 kDa. Appearance of new bands at approximately 97 and 40 kDa on addition of 2-mercaptoethanol is a result of reduction of disulphide bonds and this suggests that these bands are sub-units of larger holoproteins. Similar observations have been reported previously (Machuka, 2000).

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