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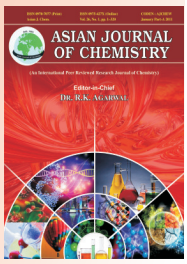

Functional Properties of *Alstonia scholaris* Seed Protein Isolates

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Functional properties of total protein isolate (TPI) prepared from *Alstonia scholaris* seeds were investigated. The moisture content and ash content of total protein isolate were found to be 9.13 and 2.12 % respectively. The protein contents of the seed protein concentrate was found to be 68.93 %. The minimum protein solubility was observed at pH 4 and maximum at pH 12. Oil- and water-holding capacities of the seed protein concentrate were 6.21 and 3.75 g/g respectively. The emulsifying activity and emulsion stability as well as foaming capacity and foam stability were greatly affected by pH. Lower values were observed at slightly acidic pH (pH 4). Total protein concentrate was moderately viscous and dependent on concentration. Viscosity was also affected by increased pHs. The lowest gelation concentration of the seed protein concentrate was found to be 14 % when protein was dissolved in distilled water.

Keywords: *Alstonia scholaris*, Seed protein isolate, Nitrogen solubility, Functional properties.

INTRODUCTION

The new challenge of providing adequate protein for an expanding world population has made the study of proteins obtained from non-conventional plant seeds for use as functional food ingredient and nutritional supplement[1] an important and interesting one. These studies are of particular importance in developing countries like India where protein intake is less than that is required[1]. Exploitation of such non-conventional resources can boost economic development and during the last four decades our food industry has placed special attention on the utilization of seed protein. In addition to providing essential amino acids, the ultimate success of utilizing any seed protein depends on its desirable functional properties[2,3]. The functional properties of proteins denote any physico-chemical property which affects the processing behaviour of protein in food systems as judged by the quality attributes of the final product.

Present study revolves around the isolation and study of functional properties of seed protein of *Alstonia scholaris* R.Br. (Chatim) belonging to the family Apocynaceae. This plant is found almost throughout India upto an altitude of 600 m. Unfortunately seeds of this plant are thrown away each year inspite of its appreciable amount of nitrogen and protein content. This study will determine the solubility, viscosity, water absorption, foaming, emulsification and gelation capacity of the seed protein and its potential as an alternative food ingredient.

EXPERIMENTAL

Fresh mature seeds of *Alstonia scholaris* were collected from the Burdwan Divisional Forest Department, Burdwan, West Bengal, India and authenticated by Prof A. Mukherjee, Department of Botany, The University of Burdwan, Burdwan, West Bengal, India. Voucher specimen Burdwan, Mita 199 has been deposited at the herbarium of the Department of Botany, The University of Burdwan bearing the Acronym BURD. The seeds were air dried and then used for protein extraction. All reagents used in this investigation were of analytical grade. Corn oil used was obtained from National Chemicals, Vadodara, India.

Preparation of protein isolates: The air dried seeds were crushed to powder by a hand crusher and the seed flour (500 g) was then defatted by extracting with 3L hexane at room temperature (20 °C) for 21 days. It was then washed thrice with $CHCl_3$: MeOH (3:1) and then air dried. The defatted flour was extracted by stirring with distilled water using a flour to solvent ratio 1:20 for 45 min and the pH was adjusted to 7 by 0.5M NaOH/0.5M HCl. The suspension obtained was first filtered through cotton and finally centrifuged at 10,000 g for 20 min. The pH of the supernatant was lowered to minimum solubility (4.0) of the seed protein by 10 % aq. trichloroacetic acid. This pH is probably near the isoelectric point of the protein. The precipitate was redissolved with deionized distilled water of pH 7, dialyzed against distilled water for

72 h at 4 °C, freeze dried and finally stored in refrigerator at 4 °C for further use.

Proximate analysis: Nitrogen content of the protein isolate was determined by the micro-Kjeldahl method following the method of the AOAC[4]. Percentage of the nitrogen was converted to crude protein by multiplying with 6.25. The moisture content and the ash of the protein isolates were determined according to AOAC procedures[4]. All results are given in Table-1.

Nitrogen solubility: Nitrogen solubility was determined using the method of Were *et al.*[5] with slight modification. 125 mg of the sample was dispersed in 25 mL of distilled water and the pH of the solution was adjusted to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 using either 0.5 M HCl or 0.5 M NaOH. The dispersions were agitated for 0.5 h at room temperature (20 °C) and then centrifuged at 10000 g for 15 min. After freeze-drying, nitrogen content of the supernatant was determined by Kjeldahl method[4]. Determinations were carried out in triplicate and the solubility profile was obtained by plotting averages of protein solubility (%) against pH. The per cent soluble protein was calculated as follows:

$$\text{Solubility } \% = \frac{\text{Amount of nitrogen in the supernatant}}{\text{Amount of nitrogen in the sample}} \times 100$$

Water and oil-holding capacity: The method of Carcea Bencini[6] was used with slight modification. 1 g of protein sample in each case was stirred in 10 mL of distilled water of pH 7 or corn oil for 1 min. The samples were then allowed to stand separately at room temperature (20 °C) for 0.5 h and centrifuged at 5000 g for 15 min. The volume of the supernatant was noted in a graduated cylinder. The water holding capacity is expressed as the number of grams of water held by 1 g protein sample. The oil holding capacity is expressed as the number of grams of oil held by 1g protein sample. Density of corn oil was found to be 0.92 g/mL. Values of water and oil-holding capacities are placed in Table-2.

Emulsifying activity (EA) and emulsion stability (ES): Emulsions were prepared according to the method of Sathe *et al.*[7] with few modifications. 5 mL of 2 % seed protein concentrate suspension at different pHs (2 to 10) was homogenized at high speed for 30s. 5 mL of corn oil was then added and homogenized for another 1 min. The emulsions were centrifuged at 3000 g for 5 min and the volume of the emulsion left was measured.

$$\text{EA } (\%) = \frac{\text{Volume of emulsified layer}}{\text{Volume of whole layer of centifuged tube}} \times 100$$

For determination of the emulsion stability (ES), emulsions prepared by the above method were heated at 80 °C for 0.5 h, then cooled to room temperature and centrifuged at 3000 g for 5 min.

$$\text{ES } \% = \frac{\text{Volume of remaining emulsifed layer}}{\text{Original emulsion volume}} \times 100$$

Variation of emulsion activity (EA) and emulsion stability (ES) with pH is represented in Figs. 2 and 3 respectively.

Foaming properties: Foam capacity and stability at different pH levels were determined according to the method of Venktesh & Prakash[8] with slight modification. 1.5 g of protein concentrate was dispersed in 100 mL of distilled water with the help of an ultrasonic vibrator (model no. Transsonic T660/H, Elma, Germany) then it was whipped in an electric stirrer for 5 min at medium speed and was poured in a 250 mL graduated cylinder and foam volume was recorded. Foaming capacity was expressed as percent increase in foam volume measured at 30 s. Foam stability was determined according to residual foam volume at 5, 30 and 120 min after whipping. Percent increase in foam volume was calculated as follows:

$$\text{Foam capacity } (\%) = \frac{\text{Volume after whipping (mL)} - \text{Volume before whipping (mL)}}{\text{Volume before whipping (mL)}} \times 100$$

Viscosity of seed protein: Relative viscosity at different protein concentration and different pH concentrations were determined according to the method of Hazra and Laskar[9]. The sample was dispersed in distilled water with the aid of an ultrasonic vibrator at room temperature (20 °C) prior to viscosity measurement. Each sample was prepared at concentrations of 1, 2, 4, 8 and 10 % (w/v) and 2 % (w/v) solution of different pH levels (2, 4, 6, 8 and 10). Relative viscosity (relative to water) was measured at room temperature (20 °C) employing an Ostwald type viscometer. All the calculations were done in triplicate and data are presented as mean ± standard error of the mean in Table-3.

Gelation capacity: Gelation capacity of the seed protein was determined according to the method of Coffmann and Graciaj[10] with slight modification. Appropriate sample suspensions of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 % (w/v) were prepared in 5 mL distilled water with the use of ultrasonic vibrator. The test tubes containing these suspensions were then heated for 1 h in a boiling water bath, followed by rapid cooling under running cold tap water. The test tubes were then cooled for 2 h at 4 °C. The lowest gelation concentration was determined as the concentration when the sample from the inverted test tube did not fall down or slip.

RESULTS AND DISCUSSION

The moisture, ash and protein content of the seed protein isolated from the seeds of *A. scholaris* are presented as mean ± standard error of the mean (n = 3) in Table-1.

TABLE-1
CHEMICAL COMPOSITIONS OF THE TOTAL PROTEIN ISOLATE OF *A. scholaris* SEEDS

Parameter	Value (%)
Moisture content	9.13 ± 0.011
Ash content	2.12 ± 0.020
Protein (Nx6.25)	68.93 ± 0.075

Nitrogen solubility: The nitrogen solubility profile (Fig. 1) of the protein concentrate indicates that the minimum nitrogen solubility was observed at a pH of 4. On either side of pH 4 there was a sharp increase in the nitrogen solubility of the protein isolate. At pH 2, 52.76 % of the nitrogen was soluble and at pH 11, 87.54 % of the nitrogen was soluble. Thus nitrogen solubility studies showed good solubility in both acidic and alkaline pH regions which is important characteristic

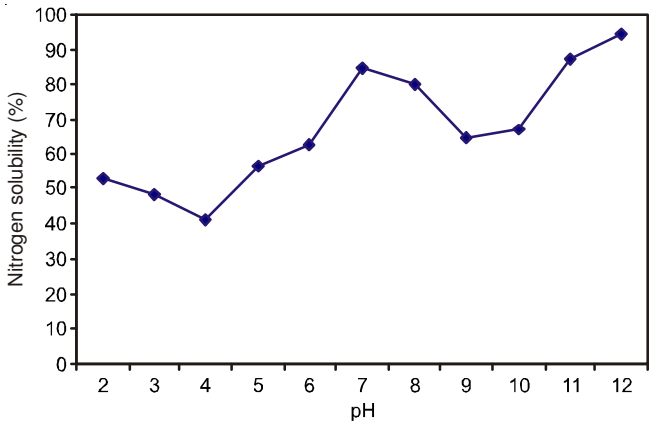

Fig. 1. Effect of pH on the nitrogen solubility % of *A. scholaris* seed protein isolate

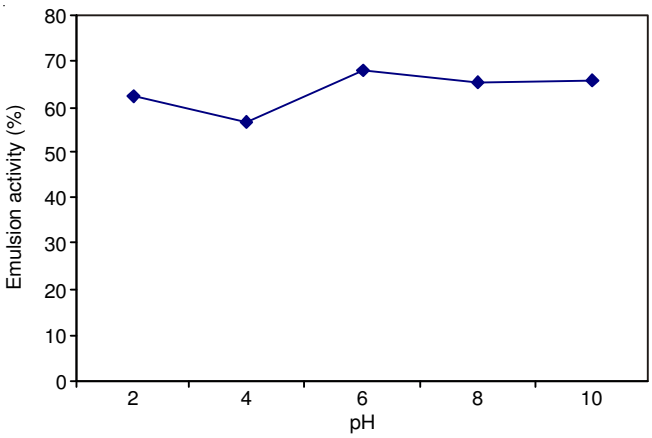

Fig. 2. Variation of emulsion activity of *A. Scholaris* seed protein isolate with pH

for food formulation[11]. Seena and Sridhar[12] reported that at highly acidic and alkaline pH, the protein acquires net positive and negative charges respectively, which favours the repulsion of molecules and thereby increase the solubility of the protein.

Water and oil holding capacity: Water holding capacity (WHC) and oil holding capacity (OHC) of the protein isolates were 3.75 g/g and 6.21 g/g respectively. All the calculations were done in triplicate and data are presented as mean ± standard error of the mean in Table-2.

TABLE- 2
WATER AND OIL HOLDING CAPACITY OF
A. scholaris SEED PROTEIN ISOLATE

Parameter	Value
Water holding capacity	3.75 ± 0.015 (g water/g protein)
Oil holding capacity	6.21 ± 0.010 (g oil/g protein)

The water holding capacity is comparable to soy bean protein concentrate (3.46 g/g)[13] but lower than three legume seed protein concentrate *P. angularis*, *P. calcartus* and *D. lablab* (5.05, 5.25, 5.08 g/g respectively)[13]. Different proteins exhibit different water holding capacities due to the difference in protein conformations and variations in the number and nature of water binding sites on protein molecules[14].

The oil holding capacity of the seed protein isolate of *A. scholaris* was found to be higher than soybean protein concentrate (3.06 g/g) and three legume seed protein concentrate (4.38, 4.71 and 4.77 g/g, respectively)[13]. The high oil holding capacity may be attributed to the higher level of non-polar side chains in their molecules. Such high oil holding capacity make the protein potentially useful in structural interactions in food, especially in flavour retention, improvement of palatability and extension of shelf life in meat products through reduction of moisture and fat loss.

Emulsifying activity (EA) and emulsion stability (ES): Emulsifying activity and emulsifying stability of total protein isolate are shown in Figs. 2 and 3, respectively. The emulsifying properties of proteins were influenced by charge adsorption and film formation at the interface. Nakai[15] reported that emulsifying properties not only depend on the protein solubility but also on the hydrophilic-lipophilic balance of the particular protein. Emulsion activity and emulsion stability of the protein under our investigation are both seen to be minimum at pH 4.

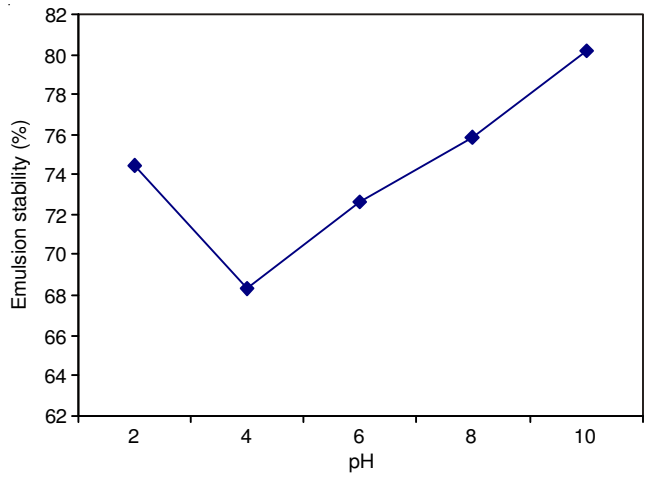

Fig. 3. Variation of emulsion Stability of seed protein isoloate of *A. Scholaris* with pH

The protein isolate exhibited good emulsifying activity values (56.7-65.6 %) at different pH levels.

Like emulsion activity, emulsion stability of this protein isolate was higher at both acidic and alkaline pH (Fig. 3). Hung and Zayas[16] suggested that various factors including pH, droplet size, net charge, interfacial tension, viscosity and protein conformation could influence the value of emulsion stability. In this case, the high emulsion stability (in different pHs), which evolved during heating of protein at 80 °C for 0.5 h, might be attributed to the dissociation of some protein molecules giving rise to more hydrophilic groups, which showed stronger interaction with the lipid phase.

Foaming properties: The results obtained from the study of foaming capacity (FC) of *A. scholaris* seed protein concentrate as shown in Table-3 and Fig. 4 indicated that its lowest foaming capacity was obtained at pH 4 which is probably the isoelectric point of the protein. Protein in dispersion lowers and reduces the surface tension at the water-air interface, thus creating foaming capacity[17]. On either side of pH 4.0, foaming capacity increased significantly and showed a V shaped pattern. The higher foaming capacity at pH 2 and pH (6-10) was due to increased net charges on the protein which allowed the protein to diffuse more rapidly to the air-water interface to encapsulate air particles and then enhance foam formation[3].

TABLE-3
FOAMING CAPACITY AND STABILITY OF *A. scholaris* SEED PROTEIN

1.5 % protein concentrate solution at different pH	Volume after whipping (mL)	(%) volume increase	Volume after whipping (mL)		
			5 min	30 min	120 min
2	168	68	150	140	120
4	150	50	135	128	102
6	165	65	155	130	122
8	185	85	170	155	138
10	200	100	190	185	140

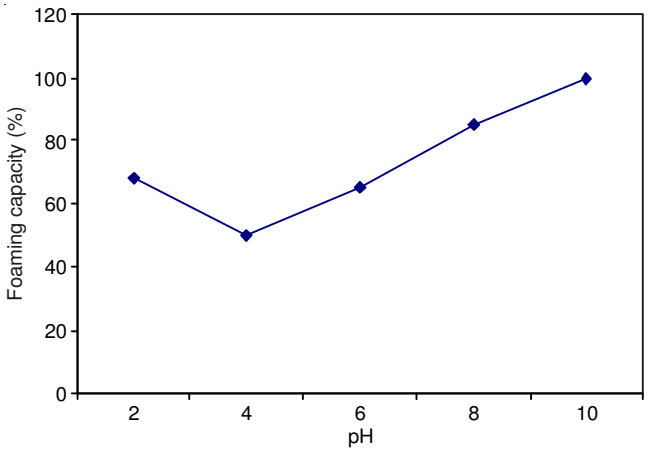

Fig. 4. Variation of Foaming capacity of seed protein isolate of *A. Scholaris* with pH

The effect of pH on foam stability as a function of time is shown in Fig. 5. It showed that foam stability decreased with time. Result of study of foam stability from pH 2 to pH 10 indicated that foam stability decreased with time. Such pH and time dependence of foam stability was also reported for soybean and sunflower protein[18].

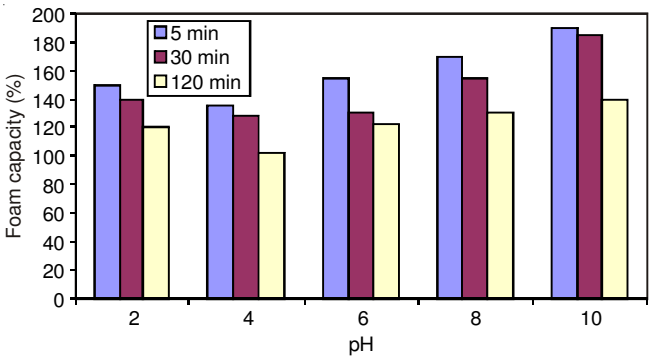

Fig. 5. Variation of foaming capacity of seed protein isolate of *A. Scholaris* with time at different pH

Viscosity of seed protein: The results of viscosity of *A. scholaris* seed protein isolate at different concentrations at pH 7 and also at different pH levels at room temperature (20 °C) are presented in Table-4. All the calculations were done in triplicate and data are presented as mean ± standard error of the mean. A marked increase in the viscosity with increasing protein concentration was observed. Concentration dependence of viscosity was also reported for sunflower protein concentrate and soybean protein isolates[19]. At room temperature, the viscosity of protein solution (2 %) showed marked increase in viscosity with increase of pH. Results of present study indicate that the viscosity of protein depends on the pH, which is likely to affect the conformational characteristics of protein, since viscosity is conformation dependent[11].

TABLE-4
EFFECT OF PROTEIN CONCENTRATION AND pH ON VISCOSITIES OF *A. scholaris* SEED PROTEIN ISOLATES AT ROOM TEMPERATURE (20 °C)

Protein concentration (g/100 mL)	Viscosity (Centipoise)	pH	Viscosity (centipoise)
1	0.95 ± 0.015	2	0.81 ± 0.011
2	1.07 ± 0.015	4	1.04 ± 0.010
4	1.75 ± 0.020	6	1.24 ± 0.011
6	2.35 ± 0.020	8	1.42 ± 0.010
8	3.42 ± 0.058	10	1.90 ± 0.011
10	4.76 ± 0.017	–	–

Gelation capacity: Gelation capacity of this seed protein is shown in Table-5. The least gelation concentration of *A. scholaris* seed protein was found to be 14 % (w/v), when it was dissolved in distilled water. According to Schmidt[20], considerably higher protein concentrate is usually required for the gelation of globular proteins. So it can be concluded that gelation is not only a function of protein quantity but seems also to be related to the type of protein as well as to the non protein components and protein solubility. A similar conclusion was reached by Sathe and Salunkhe in their study[21].

TABLE-5
GELATION CAPACITY (GC) OF *A. scholaris* SEED PROTEIN ISOLATE IN WATER AT DIFFERENT CONCENTRATIONS

Protein concentration (g/mL)	Gelation capacity in water
2	–
4	–
6	–
8	–
10	–
12	–
14	+
16	+
18	+
20	+

Conclusion

The functional properties of *A. scholaris* seed protein were evaluated to assess their effective use in food systems. It was found that *A. scholaris* seed protein showed good solubility as the solution approached neutrality and alkaline pH. The oil holding capacity and water holding capacity are also higher than any other standard seed protein, which is very important for preparation of sausages, doughnuts (requiring high oil holding capacity) in food industries. The high foaming capacity and foam stability of the protein makes it a potent functional ingredient in food systems such as whipped toppings, chiffon deserts, angel and sponge cakes *etc*. and its good emulsifying activity abd emulsion stability makes it a potent ingredient in meat analogs.

These properties make the seed protein isolate of *A. scholaris* attractive as functional ingredients in food systems after toxicological screening.

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