Physicochemical Properties of Rapeseed (*Brassica campestris* Var. Toria) Seed Proteins: Viscosity and Ionizable Groups

Amita Mahajan and Saroj Dua*

Department of Botany, Panjab University, Chandigarh 160014, India

Albumins and globulins are the major seed proteins of rapeseed. The relative viscosities of both of these protein fractions increased with an increase in the protein concentration up to 0.6%. It was minimum at pH 6 and was high in acid and alkaline conditions. The presence of sodium lauryl sulfate and urea increased the viscosity. Sodium lauryl sulfate was effective at low concentrations (0.1–0.9 M) and urea at higher concentrations (1–9 M). The viscosity of the albumin fraction suffered more than globulin by these denaturing reagents. The carboxyl group was the principal functional group ($173/10^5$ and $174/10^5$ g of protein, respectively) followed by guanidinium, amino, and imidazole groups.

INTRODUCTION

Proteins not only provide a nutritional component in a food system but also perform a number of other functions. The physical and chemical natures of a protein have major effects on the functional and physicochemical properties which determine its food and industrial uses. A major factor to be considered in using proteins for glues, adhesives, paints, etc., is that the viscosity of a solution must remain fairly constant for a reasonable length of time. A major determinant of the physicochemical properties of the proteins is their amino acid composition, in terms of both electrostatic and hydrophilicity properties. The electrically charged groups of protein molecules play an important role in determining the nature of interactions of proteins with other substances (Klotz, 1953) in foods or for industrial purposes. Therefore, a detailed knowledge of the structure and properties of the proteins is advantageous.

The functional groups of proteins and their characterization have been determined by electrometric titration curves of animal proteins (Creamer, 1972) and milk proteins (Puri *et al.*, 1968). Although good information is available on isolation, characterization, and physicochemical properties of linseed proteins (Madhusudan and Singh, 1985), mustard and rapeseed proteins (Rao and Rao, 1983), and soybean proteins (Ishino and Okamoto, 1975), no systematic procedure for electrometric titration of plant proteins is reported in the literature except some findings reported by Mourge *et al.* (1956), Polit and Sgarbieri (1976), and Puri and Bala (1975). In this paper, we report the changes in viscosity measurements of rapeseed proteins along with various factors affecting it. Also, the number of ionizable groups is determined by electrometric titration curves to have a better picture of the physical and chemical state of the proteins.

MATERIALS AND METHODS

Pure line seeds of rapeseed (*Brassica campestris* var. Toria) were obtained from Punjab Agricultural University, Ludhiana, India.

Defatted meal was prepared by extracting rapeseeds with petroleum ether six times, each time changing the solvent and finally evaporating the solvent at room temperature. The meal was finely powdered and passed through an 80-mesh sieve.

Protein was extracted from defatted meal according to the following method: Ten grams of meal was extracted with 100 mL of distilled water for 1 h with intermittent stirring and centrifuged at 7000 rpm for 15 min at 4 °C. Residue was re-extracted twice with water as given above, and supernatants were pooled. This water extract was precipitated with solid ammonium sulfate at 100% saturation [ratio of extract and solid $(NH_4)_2SO_4$ is 10:7] to separate albumins. Precipitates were dialyzed exhaustively against water, dissolved in distilled water, and taken for experimentation as "albumins". Residue was extracted three times with 1 M NaCl, and from the supernatant globulins were precipitated at 50% saturation of ammonium sulfate [ratio of extract and solid $(NH_4)_2SO_4$ is 10:3]. After centrifugation, the precipitates were dissolved in 1 M NaCl, dialyzed, and used for studying the different properties under the name "globulins".

Viscosity Measurements. Viscosity of the proteins was determined using an Ostwald viscometer at 30 ± 1 °C. Protein solution with concentration ranging from 0.1% to 0.6% was put into the viscometer, and flow time of each solution was calculated, using the equation

$$\eta_{rel} = \eta/\eta_0 = (t/t_0)(d/d_0)$$

where t is the time of flow of proteins, t_0 is that of distilled water, d is the density of the protein solution, and d_0 is the density of distilled water.

Specific viscosity was calculated as follows:

$$\text{specific viscosity} = \eta_{rel} - 1$$

For both of the protein fractions, graphs were plotted of the specific viscosity as a function of the volume fraction of protein (designated ϕ). The volume fraction is calculated by multiplying the concentration (C), in grams per 100 g, by the partial specific volume ($\bar{v}$) of the solute, i.e.

$$\text{volume fraction } (\phi) = c\bar{v}$$

The partial specific volume is given by

$$\bar{v} = w - (l - h)/eh$$

where w is the weight of the solvent, l is the weight of the solution, h is the weight of the protein, and e is the density of the solvent.

To calculate axial ratios, specific viscosity was plotted vs volume fraction and was further determined by applying Kuhn's equation (Kuhn, 1933)

$$K = 2.5 + {}^1/_{16}(a/b)^2$$

where a and b are the major and minor axes, respectively, of molecules and K is the slope of the graph plotted between specific viscosity and volume fraction.

The effect of pH, sodium lauryl sulfate, and urea was also studied on the viscosity of both protein fractions. Concentration of protein taken was 0.4%.

0021-8561/94/1442-1411$04.50/0

Table 1. Relative Viscosity and Volume Fraction of Albumin and Globulin Fractions of Rapeseed Proteins

	albumins				globulins			
concn of protein (%)	time of flow of protein (s)	density of protein (g/cm³)	vol fraction (ϕ)	rel viscosity	time of flow of proteins (s)	density of protein (g/cm³)	vol fraction (ϕ)	rel viscosity
0.1	20.1	1.002	0.079	1.017	20.6	1.034	0.058	1.013
0.2	20.4	1.004	0.157	1.034	21.2	1.036	0.135	1.033
0.3	21.4	1.006	0.237	1.087	22.0	1.038	0.211	1.074
0.4	22.9	1.008	0.317	1.165	22.8	1.040	0.288	1.116
0.5	23.1	1.015	0.352	1.184	23.4	1.042	0.374	1.148
0.6	23.5	1.022	0.381	1.212	24.6	1.044	0.405	1.182

Electrometric Titrations. Electrometric titrations of proteins were carried out using 0.1 N NaOH as titrant in the alkaline range and 0.1 N HCl in acidic range. Twenty-five milliliters of protein solution of known concentration (4 mg/mL) was taken in a small beaker and kept in a thermostat at 30 ± 1 °C for 1 h. The pH of this solution was noted on a Beckman pH meter having an accuracy of ±0.1 pH unit. This pH was taken close to the isoionic point of the protein. Increasing amounts of 0.1 N NaOH or 0.1 N HCl were added to different flasks containing protein solutions, and the pH was noted after each addition.

Experiments were also conducted in the presence of formaldehyde (1%) when NaOH was used as titrant. All of the above measurements were done in duplicate, and experiments were repeated twice.

RESULTS AND DISCUSSION

Albumins and globulins were found to be the predominant proteins in rapeseed, comprising 46% and 42% of total proteins, respectively. Therefore, albumins also serve as important storage proteins in rapeseeds along with globulins. The presence of albumins as storage proteins had been shown in castor seeds (Youle and Huang, 1981; Dua *et al.*, 1984) and fenugreek seeds (Sauvaire *et al.*, 1984).

The relative viscosity of albumins and globulins increased progressively with increase in protein concentration from 0.1% to 0.6% (Table 1). It was found to be 1.212 for albumins and 1.182 for globulins at maximum concentration of proteins (0.6%). It has been observed that viscosity depends upon the molecular shape and orientation of the protein molecules in solution. At higher protein concentration, the protein molecules are closely packed, some of which orient perpendicular to the direction of fluid flow. The frictional force against the fluid is increased, and therefore, viscosity increases. In salt-soluble globulins, viscosity was lower than that in water-soluble albumins. When salt is added, neutralization of some of the charges of the proteins takes place and molecules align parallel to the direction of flow, which decreases their viscosity. Also, a neutral chain is more likely to coil up than the charged one, because the charged segments of the latter will tend to repel each other. Salts, therefore, affect the viscosity of charged macromolecules not only by lowering the electrical potential but also by causing a change in the shape of the molecule in solution.

A good correlation between viscosity and pH was observed in both protein fractions (Table 2). Relative viscosity of albumins and globulins was maximum (1.105) at pH 12 and minimum (1.048 for albumins and 1.068 for globulins) at pH 6. Below and above this pH, there was an increase in specific viscosity. This minimum pH corresponds to the isoelectric point of both protein fractions. At very low and high pH values, there is dissociation of oligomeric proteins to smaller subunits. Since the particle size affects the hydrodynamic volume, it therefore decreases the viscosity of the protein.

The relative viscosity of albumins and globulins increased linearly with increase in concentration of sodium lauryl sulfate (0.1–0.9 M concentration) (Table 3). Albumins showed a maximum value (1.216) at 0.9 M concentration of this detergent. In the presence of urea, the same pattern was observed and the relative viscosity of both protein fractions increased progressively with increase in concentration from 1 to 9 M (Table 4). These observations indicate that, under denaturant conditions, increase in viscosity is due to unfolding of the protein, which increases the axial ratios, as well as interactions between the protein molecules via hydrogen bonding and hydrophobic and electrostatic interactions (Roberts and Briggs, 1963). A steady increase in reduced viscosity suggests a gradual conformational change in protein due to unfolding or swelling of protein molecules. Similar findings are given for soybean proteins (Roberts and Briggs, 1963; Sateeja, 1988) and for rapeseed and mustard proteins (Rao and Rao, 1983).

The axial ratio of albumins was calculated to be 5.09, and that of globulins was 5.35 (Figure 1), showing that protein molecules are either rod-shaped or ellipsoidal.

Ionizable Groups in Proteins. The isoionic points of albumins and globulins were found to be 5.47 and 5.37,

Table 2. Effect of pH on the Relative Viscosity of Albumin and Globulin Fractions of Rapeseed Proteins

	albumins			globulins		
pH	time of flow (s)	density (g/cm³)	rel viscosity	time of flow (s)	density (g/cm³)	rel viscosity
2	21.4	1.008	1.078	21.3	1.023	1.100
4	20.9	1.008	1.053	21.1	1.001	1.077
6	20.8	1.008	1.048	21.0	1.007	1.068
8	21.0	1.026	1.078	21.2	1.009	1.080
10	21.2	1.024	1.084	21.3	1.022	1.099
12	21.5	1.029	1.105	21.4	1.023	1.105

Table 3. Effect of Different Concentrations of Sodium Lauryl Sulfate on the Viscosity of Albumin and Globulin Fractions of Rapeseed Proteins

	albumins			globulins		
concn (M)	time of flow (s)	density (g/cm³)	rel viscosity	time of flow (s)	density (g/cm³)	rel viscosity
0.10	22.4	1.015	1.098	22.0	1.007	1.065
0.20	22.9	1.029	1.103	22.3	1.007	1.069
0.30	24.3	1.043	1.123	22.1	1.008	1.074
0.40	26.5	1.087	1.145	23.1	1.009	1.084
0.60	28.0	1.091	1.162	23.4	1.012	1.091
0.70	29.0	1.093	1.181	23.7	1.013	1.101
0.85	31.0	1.098	1.215	24.0	1.014	1.106
0.90	32.0	1.098	1.216	24.2	1.015	1.106

Table 4. Effect of Different Concentrations of Urea on Viscosity of Albumin and Globulin Fractions of Rapeseed Proteins

	albumins			globulins		
concn of urea (M)	time of flow (s)	density (g/cm³)	rel viscosity	time of flow (s)	density (g/cm³)	rel viscosity
1	22.9	1.009	1.103	22.0	1.002	1.059
3	24.3	1.046	1.120	22.3	1.004	1.071
5	25.6	1.065	1.135	22.7	1.007	1.088
7	27.3	1.087	1.153	23.0	1.013	1.099
9	28.8	1.096	1.155	23.2	1.014	1.099

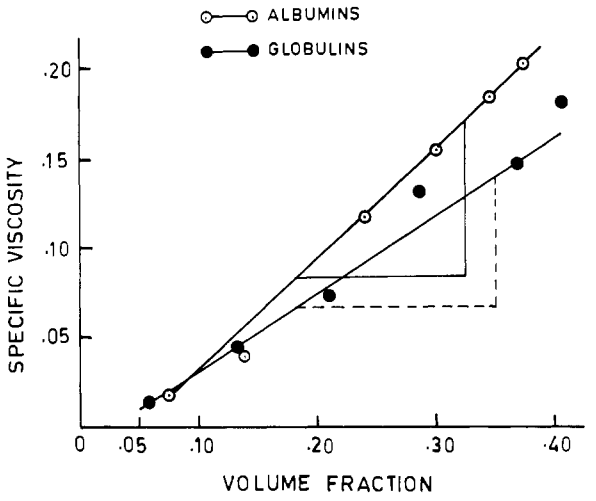

Figure 1. Specific viscosity vs volume fraction graph of albumin and globulin of rapeseed proteins.

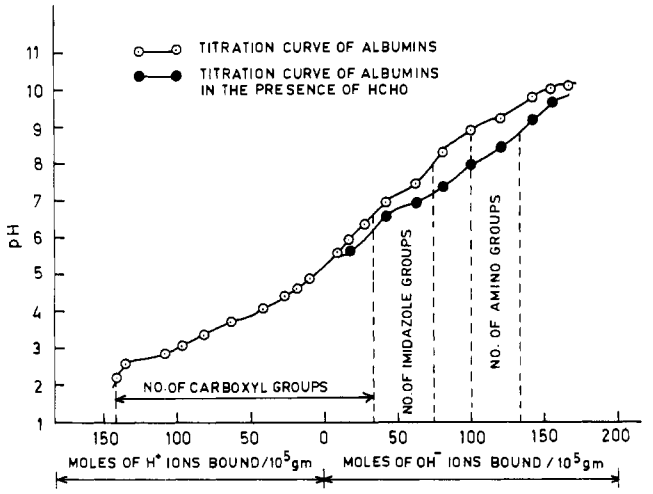

Figure 2. Electrometric titrations of albumin fraction of rapeseed proteins.

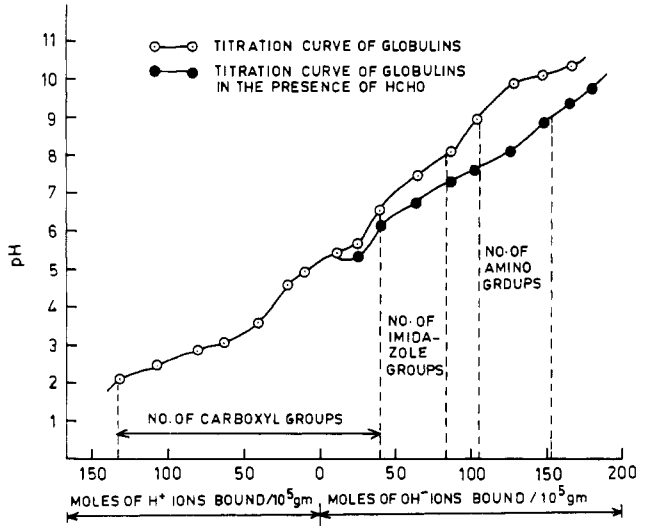

Figure 3. Electrometric titrations of globulin fraction of rapeseed proteins.

respectively. The pH values obtained during titrations were plotted against the amounts of H^+ and OH^- ions actually bound by the proteins per 10^5 g of protein (Figures 2 and 3). When HCl was added as titrant, the pH measured at any stage was used to calculate concentration of free ions (H^+) or m_{H^+}. Similarly, when NaOH was used as

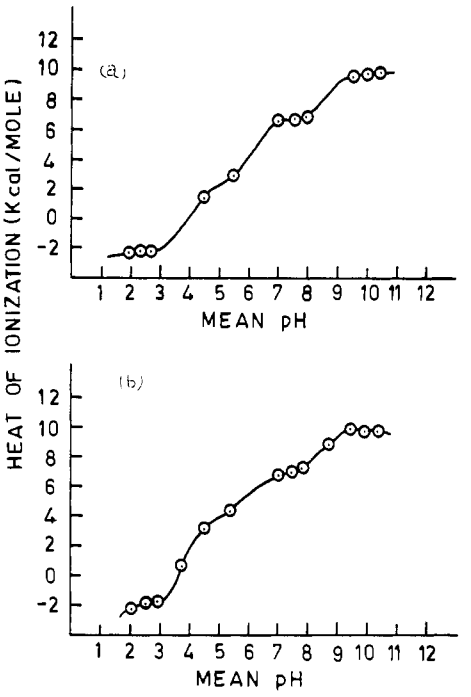

Figure 4. pH vs heat of ionization of rapeseed proteins: (a) albumins; (b) globulins.

titrant, the free OH^- or m_{OH^-} ions were calculated by the following equations:

$$pH = -\log m_{H^+}\gamma_{H^+} \quad (1)$$

$$pOH = -\log m_{OH^-}\gamma_{OH^-} \quad (2)$$

Subtracting m_{H^+} or m_{OH^-} from the molality of H^+ or OH^- ions added, the amount of hydrogen or hydroxyl ions was calculated. The values of activity coefficient were taken from the tables of Robinson and Harned (1941).

Titration curves of proteins revealed that the amount of H^+ ions required for albumins and globulins showed a continuous increase, and this increase was 13-fold for albumins and 11-fold for globulins. In albumins and globulins, no more acid bound after pH 2.2 and 2.1, respectively. The titration curve in the basic range remained incomplete because proteins become denatured after pH 10, and the results of the calculations above this pH become anomalous.

From the above titration curves, maximum acid and base binding capacities of albumins and globulins were calculated. Acid binding capacities were $140.8/10^5$ and $131.6/10^5$ g of protein, respectively (Figures 2 and 3).

The number of electrically charged groups can be calculated from the titration curve provided the pH range in which these groups ionize and the heat of ionization are known. To calculate heat of ionization (φ), the experiment was carried out at three different temperatures, i.e., 15, 30, and 45 °C, and the following equation was employed:

$$\varphi = -4.579T_1T_2(pH_2 - pH_1)/T_2 - T_1$$

pH_2 and pH_1 are the values of pH corresponding to the same amount of acid or base added at temperatures T_2 and T_1, respectively. The apparent heats of ionization (φ) for albumins and globulins were plotted against mean pH values [$(pH_1 + pH_2)/2$] (Figure 4). Results indicate that there were three horizontal zones between pH 2 and 11 in each case. The apparent heats of ionization of albumins and globulins between pH 2 and 6.5 were about −1.5 to −2.0 kcal, which is the range characteristic of carboxylic

groups. The number of carboxylic groups was almost the same (173 and 174) for albumins and globulins. The values between pH 6.5 and 8 were between 6.5 and 7.0 kcal, which correspond to imidazole groups. These were found to be 35 and 40 in albumins and globulins, respectively. The groups still left untitrated were amino, phenolic hydroxyl, and sulfhydryl groups which were ionized in the third and last horizontal zone of curve (pH 9.5–10.3).

Results in the presence of formaldehyde indicated that most of the acid part of the curve remained particularly unaffected, while there were different values in the basic part of the curve.

The numbers of equivalents of amino groups were calculated from the difference between the equivalents of alkali bound in the presence and in the absence of formaldehyde at pH 9 and were found to be 33.0 and 46.0 for albumins and globulins, respectively (Figures 2 and 3).

The guanidinium groups are not fully neutralized even up to maximum pH value since their pK value lies beyond pH 12. These groups were, however, determined in another way. The equivalents of acid required from isoionic points to pH 2.0 gave the total number of cationic groups, i.e., the sum total of amino, imidazole, and guanidinium groups. The number of guanidinium groups was calculated as follows:

guanidinium groups = total number of cationic groups − (number of amino + number of imidazole groups)

This number was 70 in albumins and 53 in globulins.

The present findings show that the number of carboxylic groups in albumins and globulins (173 and 174, respectively) was more than the sum of imidazole, amino, and guanidinium groups (138 and 139.6, respectively), which confirms that aspartic and glutamic acids are present in high amount. When comparison is made between the rapeseed protein and casein, which is considered to be a protein of high nutritive value, it is found that the number of imidazole groups and guanidinium groups was higher in rapeseed than in casein. The number of amino groups was less compared to casein, showing that lysine is less in rapeseed.

ACKNOWLEDGMENT

A.M. is thankful to CSIR, New Delhi, for financial assistance.

LITERATURE CITED

Creamer, L. K. Hydrogen ion equilibria of Bovine Casein. *Biochim. Biophys. Acta* **1972**, *271*, 252–261.

Dua, S.; Sareen, K. N.; Amma, M. K. P. Effect of intact embryo and gibberellic acid on the water soluble proteins of 96 hours germinated castor seeds. *Indian J. Plant Physiol.* **1984**, *27*, 96–98.

Ishino, K.; Okamoto, S. Molecular interaction in alkali denatured soybean protein. *Cereal Chem.* **1975**, *52*, 9–21.

Klotz, I. M. In *The Proteins*; Neurath, H., Bailey, K., Eds.; Academic Press: New York, 1953; Vol. I, p 739.

Kuhn, W. The shape of fibrous molecules in solutions. *Kolloid Z.* **1933**, *62*, 269.

Madhusudan, K. T.; Singh, N. Effect of heat treatment on the functional properties of linseed meal. *J. Agric. Food Chem.* **1985**, *33*, 1222–1226.

Mourge, M.; Dokhen, R.; Reynand, J. Proteins of seeds of *Ricinus communis*. *Bull. Soc. Chem. Biol.* **1956**, *38*, 123–128.

Polit, P. F.; Sgarbieri, V. C. Some physicochemical and nutritional properties of castor bean (*Ricinus communis*) proteins. *J. Agric. Food Chem.* **1976**, *24*, 795–798.

Puri, B. R.; Bala, Neelam. Physicochemical properties of vegetable proteins—Electrometric titration curves. *Indian J. Chem.* **1975**, *13*, 144–148.

Puri, B. R.; Toteja, K. K.; Malik, R. C. Physicochemical properties of casein. I. pH titration curves. *J. Indian Chem. Soc.* **1968**, *45*, 889–896.

Rao, G. A.; Rao, N. M. S. Effect of SDS on the high molecular weight protein fractions of mustard (*Brassica juncea*) and rapeseed (*B. campestris*) by guanidinium chloride. *J. Biol. Sci.* **1983**, *5*, 301–309.

Roberts, R. C.; Briggs, D. R. Isolation and characterization of the 7S component of soybean globulins. *Cereal Chem.* **1963**, *42*, 71–85.

Robinson, R. A.; Harned, H. S. Some aspects of the thermodynamics of strong electrolytes from electromotive force and vapour pressure measurements. *Chem. Rev.* **1941**, *28*, 419–476.

Sateeja, V. Physicochemical properties of 7S fractions of soy proteins. M.Sc. thesis, Punjab Agricultural University, Ludhiana, India, 1988.

Sauvarie, Y. D.; Baccou, J. C. F.; Kobrehel, K. Solubilization and characterization of fenugreek seed protein. *J. Agric. Food Chem.* **1984**, *32*, 41–47.

Youle, R. L.; Huang, H. C. A. Occurrence of low M.W. and high cysteine containing albumin storage proteins in oilseeds of diverse species. *Am. J. Bot.* **1981**, *68*, 44–48.

Received for review October 12, 1993. Revised manuscript received March 10, 1994. Accepted March 28, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, May 1, 1994.