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Effect of Sodium Chloride on the Extractability of Proteins from Sesame Seed (*Sesamum indicum* L.)

V. Prakash

The effect of various concentrations of sodium chloride on the extractability of sesame seed proteins has been investigated. The extractability of total protein increases to nearly 80% until 5% sodium chloride concentration after which it remains constant. The extractability is also investigated as a function of pH in all the sodium chloride concentrations. The results indicate that the pH of minimum extractability drifts toward acidic pH as the sodium chloride concentration increases from 0.05 to 2.0 M. The observed results are explained due to preferential extractability of the various protein fractions with increasing concentration of sodium chloride and also due to the binding of sodium ions to these protein fractions.

Sesame seed (*Sesamum indicum* L.) contains nearly 25% protein, and the defatted meal contains nearly 50% protein. The earliest work on sesame seed proteins was by Ritthausen (1880) who extracted the protein from the sesame seed cake under variable conditions of alkali, NaCl, and temperature. Later Adolph and Lin (1936) determined the solubility of sesame seed proteins from the fat-free meal in NaCl, NaOH, and Na_2CO_3 solution. Basu and Gupta (1947) carried out similar solubility studies in water at various pH values and in the presence of NaCl and $NaHSO_3$. Nath and Giri (1957 a,b) carried out peptization studies of sesame seed proteins in NaCl and in acidic and alkaline pH media. Later, Guerra and Park (1975) carried out similar extractability studies of the protein in $CaCl_2$, Na_2SO_3, and Na_2HPO_4. They observed that high salt concentration increased the solubility of the proteins. Prakash and Nandi (1978) extracted the total protein in 1 M NaCl in order to isolate the major fraction α-globulin. Even though work on the protein α-globulin has advanced considerably (Prakash, 1985; Prakash and Narasinga Rao, in press), it is felt that no systematic work is available on the effect of different concentrations of NaCl on the extractability of total proteins. In most of the seed proteins it is an established fact that during extraction if more of NaCl is present, the point of minimum extractability is generally shifted toward acidic pH (Prakash and Narasinga Rao, in press). The explanation given is only empirical, and no systematic data are available, both experimentally as well as the analysis of data to explain the above phenomenon.

In this study data are presented on the extractability of sesame seed total proteins in various concentrations of NaCl. The shift in the pH of minimum extractability of

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the proteins in the presence of various concentrations of NaCl is explained with the available data on ion binding parameters and protein–salt interactions along with preferential extraction of various protein fractions.

MATERIALS AND METHODS

Sesame seeds (*S. indicum* L.) were obtained from National Seeds Corp., Bangalore, India. The seeds were flaked, dried, and extracted with *n*-hexane, with a solvent to flaked seed ratio of 1:1, six times. A meal containing less than 1% fat was obtained. This defatted meal was air-dried in a cabinet drier at 45 °C for 6 h, after which it was powdered and passed through a quadramat mill. The flour obtained thus was passed through 60-mesh sieve and was used for extraction of total protein (Prakash and Nandi, 1978).

Extraction of Total Protein. The total protein in the flour was extracted by rotary shaking with the solvent at 1:10 solute to solvent ratio for 1 h at ≃27 °C. The slurry was centrifuged at 6000*g* for 30 min in a Hitachi 55P preparative centrifuge. The nitrogen value of the supernatant obtained was determined and expressed as percent protein extracted (AOAC, 1984).

Protein Concentration. The concentration of the total protein was determined by the Kjeldahl procedure (AOAC, 1984). A calibration curve relating the milligrams of nitrogen in the protein sample to ultraviolet absorbance at 280 nm was obtained for routine determination of protein concentration. A value of 13.0 was used as the absorption coefficient E of 1% protein solution at 280 nm, i.e., $E^{1\%}_{1cm,280nm}$. The pH of minimum extractability was determined on the basis of the midpoint of a regression line if the region was flat and the experimental pH minimum if the region was sharp.

The pH of the slurry was measured immediately after the addition of the solvent (10 min) and at the end of the extraction time (1 h) on an Orion 901 microprocessor ionalyzer. The average of the two values was calculated, and data were analyzed. The pH meter was standardized with buffers of appropriate pH before each experimental determination. All solutions were prepared with double distilled deionized water. The extracted solutions were diluted with corresponding NaCl concentrations to 25 times, and the absorbance was monitored at 280 nm. The pH was adjusted with either dilute HCl or NaOH.

Analytical Ultracentrifugation. Sedimentation velocity experiments were carried out at 27 °C in a Beckman Model E analytical ultracentrifuge equipped with a phase plate Schlieren optics and a rotor temperature indicator and control (RTIC) unit. For a typical run a standard 12-mm Kel F centerpiece cell was used. Protein sample of 1.6% was routinely used and centrifuged at 59 780 rpm unless otherwise stated. Photographs were taken at frequent intervals, plates were read on a Gaertner microcomparator, and $S_{20,w}$ values were calculated by the standard procedure (Schachman, 1959). The percent compositions of the various peaks were calculated from the sedimentation velocity patterns after enlarging them on a Gaertner microcomparator and accurately weighing the various fractions on a tracing paper. In all the runs, the pH was kept constant at 7.0 and the concentration of NaCl varied.

RESULTS AND DISCUSSION

Figure 1 shows the extractability of sesame seed total protein as a function of sodium chloride concentration. Figure 2 shows the extractability profile of the total protein as a function of pH in water and in various concentrations of NaCl. From Figures 1 and 2 it can be observed that (i)

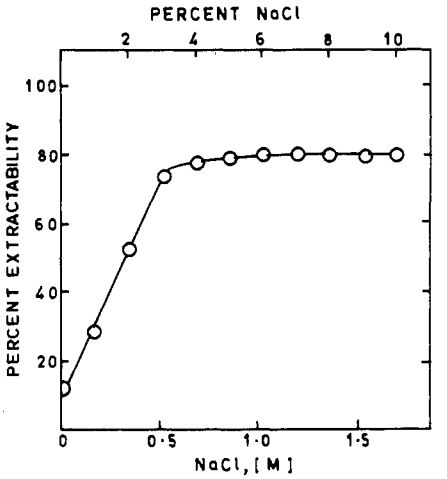

Figure 1. Percent extractability of sesame seed total protein as a function of sodium chloride concentration. Experimental details are given under Materials and Methods.

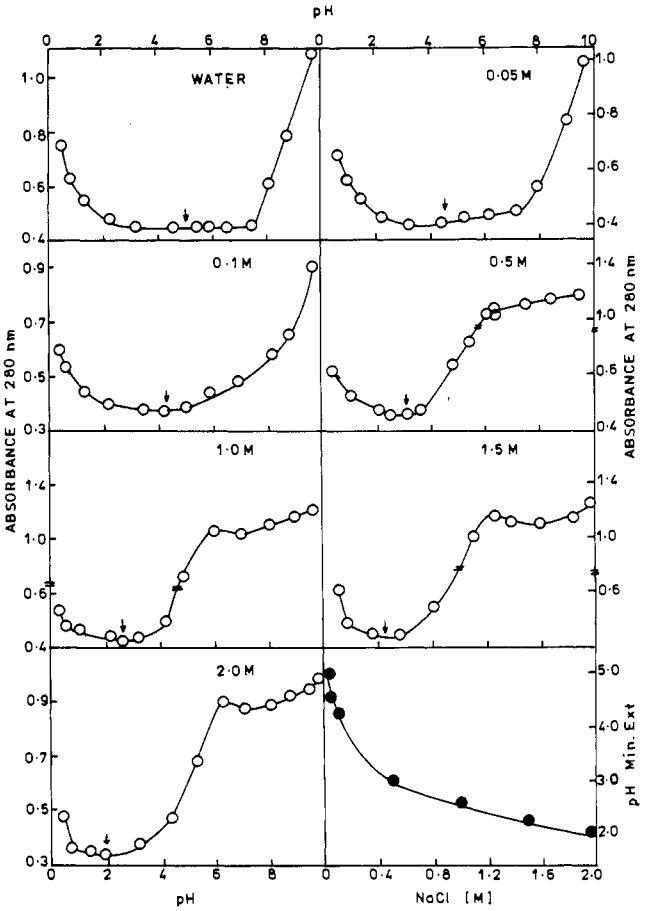

Figure 2. Extractability of sesame seed total protein as a function of pH in various concentration of sodium chloride. At the right-hand bottom corner a plot of molarity of sodium chloride vs. pH of minimum extractability is shown.

as the concentration of NaCl increases from 0.05 to 2 M, the extent of extractability of the proteins increases (see Figures 1 and 2), and (ii) the range of pH of minimum extractability is narrowed as the NaCl concentration increases (Figure 2), and (iii) the pH of minimum extractability shifts toward acidic pH as the concentration of NaCl increases (Figure 2). In Figure 2 (lower right) is shown the shift in the pH of minimum extractability with increase in the molarity of NaCl. The value tapers to a fairly constant value after 0.8 M NaCl concentration.

In order to find a possible explanation for the results

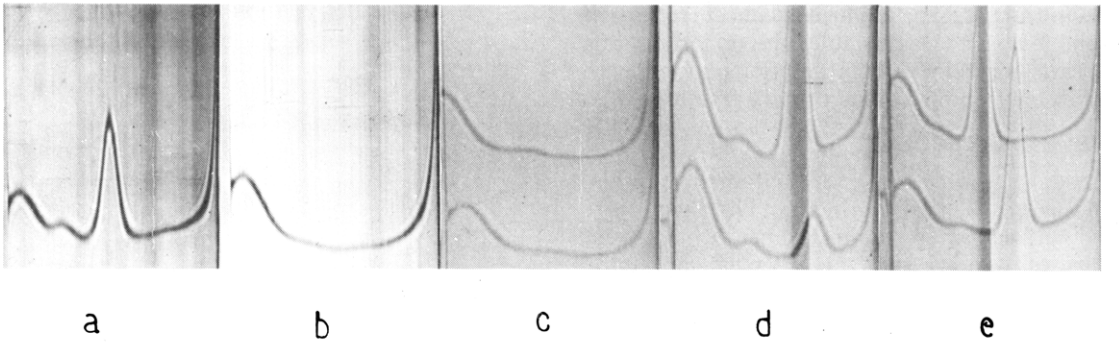

Figure 3. Sedimentation velocity patterns of sesame seed total protein extracted: (a) phosphate buffer at pH 7.0, 0.02 M containing 1 M NaCl; (b) in water at pH 7.0; (c) (lower), 0.05 M NaCl, (upper) 0.1 M NaCl; (d) (lower) 0.25 M NaCl, (upper) 0.5 M NaCl; (e) (lower), 1 M NaCl, (upper) 2 M NaCl. All the solutions were adjusted to pH 7.0 during extraction. The runs are performed at ≃27 °C and at 59780 rpm in Kel F cells.

observed above, the sedimentation velocity pattern of total protein was obtained. In Figure 3 is shown the sedimentation velocity pattern of sesame seed total protein at various concentrations of sodium chloride. In Figure 3a is shown the sedimentation velocity pattern of sesame seed total protein in phosphate buffer of pH 7.0, 0.02 M containing 1 M NaCl. The pattern is represented by two major peaks of 2S and 11S components constituting 30 and 60%, respectively, and two minor peaks of sedimentation values 7S and 16S constituting 5% each. The results agree with earlier reports by other workers (Sinha and Sen, 1962; Ventura and Lima, 1963; Prakash and Nandi, 1978). Under these conditions nearly 80% of the protein is extracted from the flour. In water only (Figure 3b) the 2S component appears to be preferentially extracted. As the concentration of NaCl increases, the 11S component, i.e., α-globulin, gets more and more solubilized (Figure 3c,d). After 1 M NaCl (Figure 3c) the pattern almost remains constant without any variation in the percent fraction of the individual components. In Figure 4 is shown the variation of percent fraction extracted (2S and 11S components) as a function of NaCl concentration. It can be seen that initially around 0.05 and 0.1 M NaCl nearly the whole pattern is represented by 2S component. As the NaCl concentration increases, α-globulin (11S) gets solubilized and both the 2S and 11S components reach a plateau with 2S:11S = 40:60. However, since the other components, i.e., 7S and 16S components, constitute ~5% of the total protein pattern, for the purposes of bringing out generalities we have assumed that the whole pattern is constituted by low molecular weight component, 2S, and high molecular weight components constituting 7S, 11S, and 16S and plotted accordingly.

From the above results it is apparent that as the concentration of NaCl is increased at neutral pH the extractability of proteins increases up to nearly 0.8 M NaCl after which it plateaus. During this process, initially the low molecular weight protein component (2S) is preferentially extracted up to a NaCl concentration of 0.2 M, above which the high molecular weight protein component (11S) solubilizes and reaches a plateau around 0.8 M NaCl and no change is observed after 1 M NaCl concentration. Concomittantly, one can see in the extractability profile as a function of NaCl that the pH of minimum extractability shifts toward acidic pH (Figure 1). We have analyzed the above data in a more quantitative way in terms of binding of the sodium ions to the various groups on the proteins.

Nozaki et al. (1959) have shown that with the addition of salt to β-lactoglobulin solution there is observed a displacement in pH, as a result of cation binding at carboxyl-rich locus. Similar results are observed here also. The

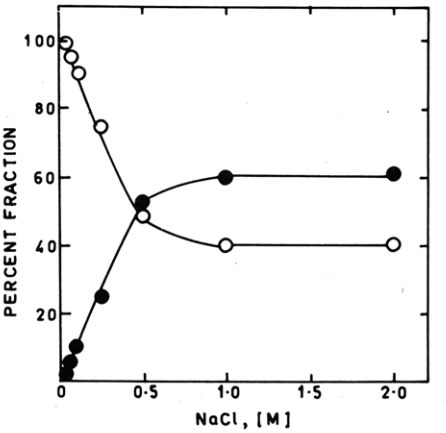

Figure 4. Percent fraction of low molecular weight protein fraction (2S) (○) and high molecular weight protein fractions (7S, 11S, 16S) (●) as a function of molarity of sodium chloride as observed in sedimentation velocity experiments.

number of ions bound per protein molecule, ν_{Na^+}, can be calculated by the equation of Scatchard and Black (1949).

$$\nu_{Na^+} = \Delta pH/0.868W \quad (1)$$

Since we are dealing with total protein it may not be very reasonable to calculate the values of W. However, we have made an attempt to calculate the value of ν_{Na^+} by using a value of $W = 0.07$ the electrostatic interaction parameter for α-globulin obtained at a given salt concentration by the equation (Tanford, 1962)

$$W = (e^2/2DTbK)\left(1 - \frac{kG}{1 + ka}\right) \quad (2)$$

where e is the electronic charge, D is the dielectric constant of the medium, K is Boltzmann's constant, T is the thermodynamic temperature, k is the Debye–Hückel parameter, a is the radius of closest approach of small ions to the macroion, and b is the radius of a sphere with a volume equal to the hydrated volume of the protein molecule. Using the experimental radius of α-globulin (Prakash, 1985), the major protein fraction of sesame seed, b, is found to be 45 Å. From these values, a value of $W = 0.07$ is estimated. By substituting this value in eq 1 a value of 12 is obtained for the value of ν_{Na^+} at an ionic strength of 0.1. However, if one uses an average experimental value of $W = 0.0055$ from the hydrogen ion equilibria of α-globulin (Prakash and Narasinga Rao, 1985) one gets a value of $\nu_{Na^+} = 147$ that is an enormously large value compared to many other proteins in literature (Tanford, 1962). For example β-lactoglobulin A binds nearly six K^+ ions as being reported by Basch and Timasheff (1967). It is to be borne in mind that the above value of $\nu_{Na^+} = 147$

is only a minimum value for the major fraction α-globulin and the value of ν_{Na^+} for the total protein would be much higher if one considers the amount of Na^+ bound to the 2S component also. However, at this point we have no definite information on the characterization of 2S component from sesame seed from the point of view of its hydrogen ion equilibria and amino acid composition. Nevertheless, the point is clear that this enormous amount of Na^+ bound to the total protein obviously need to shift the pH of minimum extractability to a more acidic region as the sodium chloride concentration increases. However, as the concentration of NaCl increases above 0.8 M the binding sites are all saturated and all the fractions are extracted. Hence, one does not see much change either in pH of minimum extractability or in the percent fraction of 2S and 11S components extracted above 0.8–1.0 M NaCl. Since initial studies from our laboratory have also indicated that the 2S component has much higher isoelectric pH (~6.0), it is conceivable that at low concentration of NaCl only the 2S component is extracted and the pH of minimum extractability is near pH 5.0. At higher NaCl concentration since α-globulin is preferentially extracted and has a lesser isoelectric pH (~4.5), it shifts the pH of minimum extractability toward acidic pH. It is shown by Nozaki et al. (1959) that the cations are bound at a carboxyl-rich locus, and since sesame total protein contains nearly 35% of acidic amino acids (Prakash and Nandi, 1978), it is conceivable that the value of ν_{Na^+} is large. These two effects in conjunction could be responsible for the drift in pH of minimum extractability as the NaCl concentration is increased during extraction of the protein.

The above result is only an indication of how sensitive some of these seed proteins are toward minor changes like ionic strength and effecting the macroscopic constants like "isoelectric pH" of the protein. However, other effects like conformational changes and association–dissociation reactions (Prakash and Nandi, 1977) might also be playing a dominant role in such interactions. These can only be investigated in pure systems and the results extrapolated to obtain meaningful interpretations.

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Effect of Wilting on the Ascorbate Content of Selected Fresh Green Leafy Vegetables Consumed in Sri Lanka

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The loss of ascorbate in eight green leafy vegetables due to wilting, from harvest up to a period of 24 h at environmental temperatures (24.7–25.8 °C) and under refrigeration (4.4 °C), was studied. The ascorbate content in the fresh leaves ranged from 4 to 86 mg/100 g. The loss of ascorbate was appreciably reduced when the leafy vegetables were wilted under refrigerated conditions compared to that under environmental temperatures. The greatest loss in ascorbate content was observed in Gotukola (*Centella asiatica*) whereas Nivithi (*Basella alba*) showed the lowest loss during the 24-h wilting period. Wilting of these fresh leaves for 4, 8, 12, 16, 20, and 24 h lowered their ascorbate contents under environmental temperatures on the average by 30, 50, 58, 66, 72, and 80%, respectively. However, when refrigerated for the same periods, they lost 21, 41, 49, 55, 64, and 74% of ascorbate, respectively.

INTRODUCTION

Vitamin C (ascorbic acid) is required in the diet in greater amounts than all the other vitamins combined (FAO/WHO, 1974). However, it is notably labile and is more readily lost than most other food constituents.

Temperature and humidity are major factors in the shelf life of fresh vegetables (Ezell and Wilcox, 1959). Green leafy vegetables wilt rapidly in unfavorable environments due to their extensive and rather permeable surfaces. The amount of vitamin C available to the consumer depends on the environmental conditions the green leaves are subjected to, between harvest and the time of purchase. The effect of varying temperature and of slow, moderate and rapid wilting on the loss of vitamin C in vegetables have been reported by Ezell and Wilcox (1959). The loss of vitamin C in various vegetables on standing in different environmental temperatures, including refrigeration, has been reported in the literature (Cadwell and Gim Sai, 1973;

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