

Full Length Research Paper

Effect of processing on functional properties of *Spirulina* protein preparations

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The present investigation has been undertaken selecting a protein rich blue green alga *Spirulina platensis* in relation to change in functional properties of its proteins on chemical treatment with succinic anhydride, acetic anhydride and formaldehyde for succinylation, acetylation and methylation respectively. Modified proteins have been studied for their functional properties such as protein solubility, foaming properties; emulsification properties and viscosity. Protein solubility in unmodified water soluble *Spirulina* protein fraction was found to be 23%. It decreased considerably on treatment with all the three modifying reagents. Emulsification activity (EA) increased slightly on methylation, whereas succinylation and acetylation resulted in a decreased EA and emulsion stability (ES). Foam capacity (FC) increased on treatment with succinic anhydride at all the concentrations used, whereas acetylation and methylation could show an increase in FC only at lower concentrations. FC was found to be maximum on succinylation and minimum on acetylation. Foam Stability (FS) was found to be much higher with methylation and acetylation. The protein fraction modified with succinic anhydride has shown the maximum viscosity followed by acetylation. Methylation however, caused a rapid decrease in viscosity and it was more pronounced at lower concentrations.

Key words: Acetylation, functional properties, methylation, proteins, *Spirulina platensis*, succinylation.

INTRODUCTION

To meet the protein need of our growing population, it is important to include non-conventional protein sources in our diet. Important non – conventional sources are oil seed proteins, leaf protein concentrate, (LPC) fish protein concentrate (FPC) and single cell proteins (SCP) or biomass protein (BMP). Single cell protein recently attracted attention and holds a major potential for increasing protein supply. Proteins not only provide a nutritional component in a food system but also perform a number of other functions (Mahajan and Dua, 1995).

The protein obtained from microbial source is designed as "Single Cell Protein" (SCP) (Vincent, 1969; Becker and Venktaraman, 1982). Green and Blue-green algae are widely used as source of single cell protein. However, blue-green algae, where cell wall lacks cellulose, are easily digestible and are the most frequently used orga-

nism (Lipinski and Litchfield, 1974). Among blue-green algae, various species of *Spirulina* have attained the maximum attention. This organism has been hailed as one of the "Greatest superfood on Earth" by (WHO). *spirulina* is a prokaryotic photoautotroph organism forming spirally coiled uniseriate filaments. It is an exceptionally important edible blue - green alga in view of its high nutritional value and in having a long history of safe human consumption and over 30 years of safety checking. According to Clement (1971) even in 16th century, *Spirulina* was consumed as a major source of protein. This alga contains up to 70% proteins, many vitamins, mineral salts and fatty acids. It contains most of the essential amino acids, making it a unique vegetarian source of complete protein. *Spirulina* protein is 90% digestible and contains enzymes, which assist the digestion process. Besides nutritional value, a protein should have desirable functional properties also for its incorporation in food. Functional properties of proteins vary with the source, composition, method of preparation/extraction, prevailing environment etc.

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A great deal of work has been centered on the functional properties of algal proteins but there is hardly any study available on the modified functional properties of algal proteins. Due to high protein content and its nutritional value, *spirulina* has been taken as the experimental material for the present investigation. The present work deals with the effects of chemical processing on functional properties of *spirulina* protein preparations.

MATERIALS AND METHODS

Powered form of *spirulina* (Norst.) Gomont used in the present study was obtained as a gift from "Parrys Nutraceutical Limited". In addition to this, *Spirulina platensis*, a filamentous non heterocystous blue-green alga belonging to family Oscillatoriaceae, was also cultured and grown in the laboratory to be used for the present study.

The cultures of *S. platensis* were grown in CFTRI medium (3). Culture medium contained in suitable Erlenmeyer flasks was sterilized in an autoclave at a pressure of 15 - 17 lb inch² for 15 - 20 min.

The filaments of *Spirulina* were harvested from exponentially growing cultures by centrifugation. Exponentially growing *spirulina* cultures (7 days old) were centrifuged at a speed of 3000 rpm for 5 min, washed with sterilized distilled water and then pellet was sun dried. Following this, it was ground in a pestle and mortar and the ground powder along with the gifted powder was used for experiments.

Chemical modification

To investigate chemical modification, acetic anhydride, succinic anhydride and formaldehyde were used for acetylation, succinylation and methylation respectively. 5% suspension of *Spirulina* powder was prepared in distilled water at room temperature and its pH was adjusted to 8.0 with 1N NaOH. Succinic anhydride was slowly added to the suspension at the levels of 0.2, 0.4, 0.6, 0.8 and 1.0 g/g protein (separately for each) with constant stirring for 1 h. During succinylation, pH of the mixture was maintained at 8.0 by periodic addition of 1N NaOH and slurry was kept for 2 h at room temperature. The extract was collected after centrifugation at 5000 rpm for 30 min at 4°C. Residual meal was re-extracted. Both the extracts were pooled together and dialysed against distilled water at 4°C for 48 h. Distilled water was changed twice a day.

Same procedure was adopted for acetylation and methylation for which acetic anhydride and formaldehyde were used, respectively, at the levels of 0.2, 0.4, 0.6, 0.8, 1.0 g/g protein. Weight of acetic anhydride and formaldehyde were determined by specific gravity (specific gravity of acetic anhydride and formaldehyde is taken as 1.081 and 1.080 g/ml, respectively).

Analytical procedures

Chemically modified preparations of *Spirulina* protein were studied for various functional properties such as: 1) Protein solubility; 2) Emulsification; 3) Foaming properties; 4) Viscosity.

Protein solubility

Protein solubility was determined by estimating the amount of soluble proteins in chemically modified preparation by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

Emulsification properties

Emulsification properties of the protein were studied by the procedure of Yasumatsu et al. (1972).

Emulsifying activity: To 5 ml of each chemically modified preparation, 5 ml of the groundnut oil was added and the mixture was homogenized for 3 min. The emulsion so obtained was centrifuged at 500 rpm for 5 min. The height of the emulsion layer was noted in the graduated centrifuge tube and emulsifying activity was expressed as

$$EA = \frac{\text{Height of the emulsified layer}}{\text{Height of the total contents in the tube}} \times 100$$

Emulsion stability (ES): Emulsion stability was determined in a similar manner as above but it involved heating the emulsion before centrifugation at 80°C for 30 min in a water bath. It was then kept for cooling under running water for 15 min. The emulsion stability was expressed as the percentage of the emulsifying activity remaining after heating.

$$ES = \frac{\text{Height of the emulsified layer after heating}}{\text{Height of the total contents in the tube}} \times 100$$

Foaming properties

Foaming capacity (FC) and Foaming stability (FS) was estimated as given by Lawhon et al. (1972) and Ahmed and Schmidt (1979), respectively.

20 ml of each chemically modified preparation was whipped at 1600 rpm for 5 min. The mixture was poured immediately into 100 ml graduated cylinder and the foam volume was recorded after different time intervals ranging from 30 s to 60 min.

Foam capacity (FC) was calculated using formula:

$$FC = \frac{\text{Volume after whipping} - \text{volume before whipping}}{\text{Volume before whipping}} \times 100$$

Foam stability (FS) was calculated by the following formula:

$$FS = \frac{\text{Foam volume after time } t}{\text{Initial foam volume}} \times 100$$

Viscosity

Procedure of Mahajan and Dua (1994) was used for the determination of viscosity.

Time of flow of chemically modified protein solution was determined using Ostwald's viscometer of 15 ml capacity. Density of the chemically modified protein solution was determined using bicapillary pycnometer of 10 ml capacity. Density of the protein solution was then calculated by the following formula.

$$\text{Density of the protein} = \frac{\text{Weight of the protein solution}}{\text{Weight of the distilled water}} \times 100$$

Here 1 (one) is the density of distilled water.

Determination of relative (REL) and specific (sp) viscosity

Viscosity was determined by the equation:

$$\eta_{rel} = \frac{\eta}{\eta_0} = \frac{t}{t_0} \times \frac{d}{d_0}$$

Where; t , η , d represent coefficient of viscosity, time of flow and density of the protein solution respectively and t_0 , η_0 , d_0 are corresponding values of the pure solvent that is pure water.

Specific viscosity was given by:

$$\eta_{sp} = \eta_{rel} - 1$$

Chemicals used were of analytical grade. Data was analysed statistically.

RESULTS AND DISCUSSION

The present investigation is confined to evaluate the effect of chemical processing on some functional properties of Spirulina protein preparations. The basic idea of this study was to assess the use of this algal protein in industrial preparations after modification with various concentrations of different chemicals e.g. succinic anhydride, acetic anhydride and formaldehyde. Functional properties were studied after modification of the protein.

Data regarding the protein solubility is given in Figure 1 and indicate maximum solubility (23%) in the unmodified water soluble fraction. Chemical treatment with the reagents decreased the protein solubility. This decrease was more pronounced in succinylated and acetylated water – soluble fraction as compared to methylated one. The solubility went on decreasing with increasing level of modification. This decrease indicates the denaturation of proteins thereby disturbing the protein – solvent interactions and hence the solubility. However, it suggests that degree of denaturation varies with the type of treatment involved. Similar observations have also been reported for wild oats due to heating (Chang and Sosulski, 1985) and soybean flours (Mcwaters and Holmes, 1979).

Low solubility is considered desirable for applications with high protein levels and when limited emulsifying properties and protein- protein interactions are required. A highly soluble protein is considered desirable for applications such as emulsification, whipping and film formation.

Since an increase in solubility of a protein is due to dissociation of a larger subunit into smaller subunits, it seems that Spirulina protein has behaved differentially on its modifications with the three reagents. However, protein- protein interaction might have been responsible for this decreased solubility.

Emulsification properties

The emulsification properties of proteins are of great importance for its utilization in salad dressing, comminuted meat products, cakes and coffee whiteners (Borton et al., 1968). Efficiency of emulsification varies with the

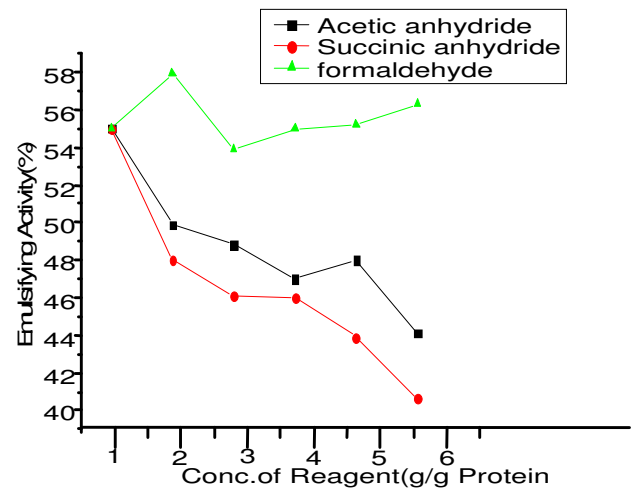


Figure 1 Effect of Modification on Protein Solubility Of Spirulina Proteins.

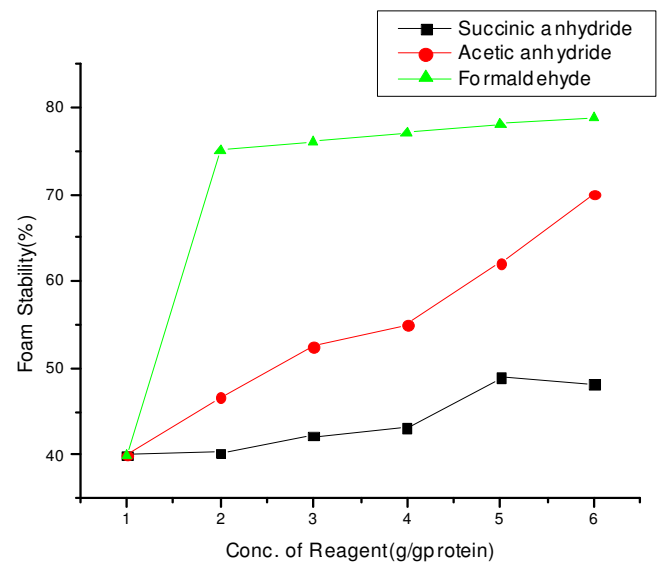


Figure 2 Effect of Modification on emulsifying Activity Of Spirulina Proteins.

type of protein, its concentration, solubility, pH, ionic strength, temperature and method of preparation of emulsion (Saffle, 1968).

Results as depicted in Figures 2 and 3 indicate that methylation caused a slight increase in Emulsifying Activity (EA) whereas succinylation and acetylation resulted in decrease of Emulsifying Activity (Figure 2). Pearce and Kinsella (1978) demonstrated a significant correlation between emulsification properties and surface hydrophobicity of proteins. Protein with a high relative hydrophobicity tends to be surface active. Nakai et al. (1980) also found that net surface hydrophobicity of a protein influences emulsion formation and stability. Dua et al. (1996)

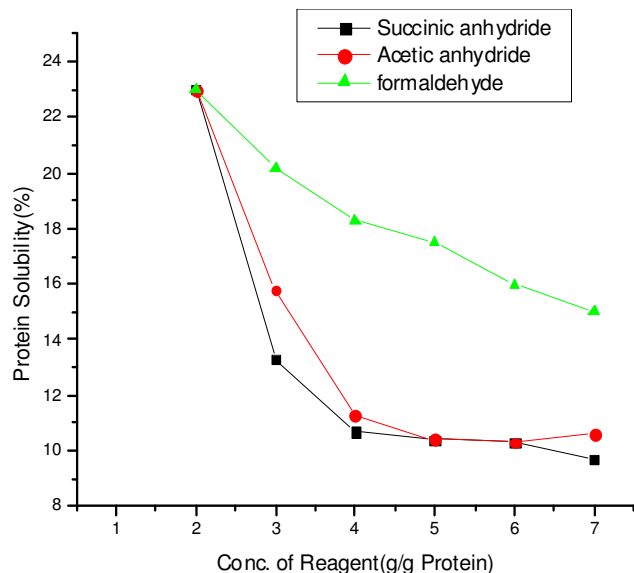


Figure 3 Effect of Modification on the Emulsion Stability Of Spirulina Proteins.

also found a decrease in Emulsifying activity and Emulsion stability in rapeseed on succinylation and acetylation. The findings in case of algal proteins in the present study are in tune with the earlier studies conducted with different non- algal protein systems (Schwenke et al., 1991; Dua et al., 1996).

Foaming properties

Foaming refers to the capacity of a protein to form stable foam with air. Foam capacity is due to solubilised protein. The ability of proteins to rapidly form a film during whipping is an important property for use in cakes, confectionaries, ice-creams etc. Foam Stability is of significance since the usefulness of whipping agents depends on their ability to maintain the whip as long as possible. The formation of protein based foams involves the diffusion of soluble proteins towards the air – water interface and rapid conformational change and rearrangement at interface (Damodaran, 1994).

The ability of a protein to form and stabilize foams depends on several parameters such as type of protein, degree of denaturation, presence or absence of calcium ions, pH and whipping methods (Townsend and Nakai, 1983). Data presented in the present study indicate that foaming capacity (FC) increased on treatment with succinic anhydride at all the concentrations, whereas acetylation and methylation could cause an increase in FC only at lower concentrations (Figure 4). A decrease was recorded at higher levels of such modifications. FC was found to be maximum with succinylation and minimum with methylation. Improvement in foaming capacity with succinylation seems to be due to negative charges imparted during modification causing folding of protein mole-

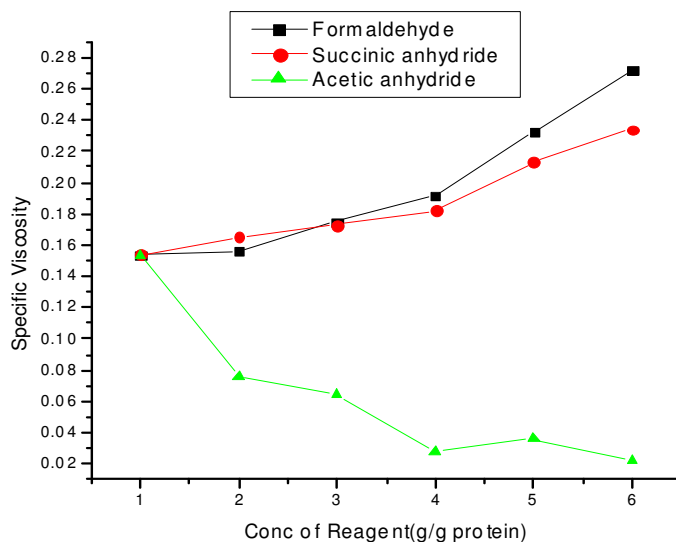


Figure 4 Effect of Modification on Foam Capacity Of Spirulina Proteins.

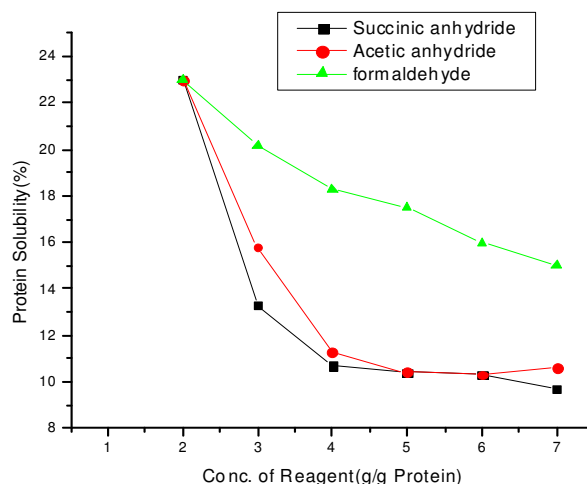


Figure 5 Effect of Modification on Foam Stability Of Spirulina Proteins.

cules (Townsend and Nakai, 1983).

Childs and Park (1976) have also shown an increased FC with increasing acylation in glandless and glanded cottonseed flour (Rahma and Narasinga, 1983). An increased FC with succinylation and acetylation was also observed in rapeseed preparations by Dua et al. (1996). Present study on algal *S. platensis* has also exhibited a similar trend.

Stability of foam formation was recorded to be much higher with methylation and it was closely followed by acetylation, however, succinylation proved to be largely ineffective (Figure 5). The steric obstruction caused by polar residues of the proteins at the interface and by solutes causing hydrogen bonding has been reported to

decrease the rate of drainage and improvement of foam stability (Mac Ritchie, 1970).

It appears that protein molecules of *Spirulina* are unable to dissociate on treatment with the modifying chemicals and hence show higher foam stability. Poor stability of foam is often indicative of the dissociation of proteins (Narayana and Narasinga, 1984). Strong association of protein molecules results in continuous intermolecular polymers enveloping the air bubbles thus providing rigidity to the interfacial film for foam stabilization (Mac Ritchie, 1970; Mita et al., 1978). This function is important for film formation by the proteins.

Viscosity

Viscosity reflects the flow behavior of a substance in the liquid state. The hydrodynamic volume influences the flow behavior of a protein solution and molecular shape of the protein and it is dependent on the physicochemical state of the protein. As the protein is dispersed in water, it imbibes water and swells and increases its hydrodynamic volume and induces long range effect on the flow behavior of the solvent molecules (Kuntz, 1971).

Succinylated water soluble fraction of *Spirulina* protein has shown the greatest viscosity followed by acetylated one (Figure 6). Methylation, however, caused a rapid decrease in viscosity and was more pronounced at low concentrations. An increase in viscosity of *Spirulina* protein preparations on modifications with succinic and acetic anhydrides was observed and this increased with higher levels of modifications. During modification, addition of these chemicals caused a conformational change due to unfolding or swelling of protein molecules. This resulted in changes in the viscosity as well as interaction between the protein molecules via hydrogen bonding and hydrophobic and electrostatic interactions (Ishino and Okamoto, 1975). The viscosity of succinylated rapeseed preparations may be attributed to the unfolding of the protein (Dua et al., 1996). Schwenke et al. (1991) have also shown an increase in the intrinsic viscosity of albumin at higher levels of succinylation. Jayarama and Rao (1978) reported a small but continuous increase of viscosity with succinylation of peanut protein. To date, algae have been a poorly studied group for these preparations.

Hydrodynamic volume and shape of protein are the most important factors governing the flow behavior of proteins (Frisch and Sinha, 1956). This volume is dependent on molecular size and degree of hydration of the molecule. Lee and Rha (1979) reported that particle size distribution of soy protein dispersion affected hydrodynamic volume and viscosity of the dispersion.

The present data for viscosity can be interpreted as indicating an increase in volume of significant number of polymer molecules with succinylation, while conformational changes in protein along with hydrophobicity are responsible for the decreased viscosity with methylation. This decrease on methylation reflects thinning of the

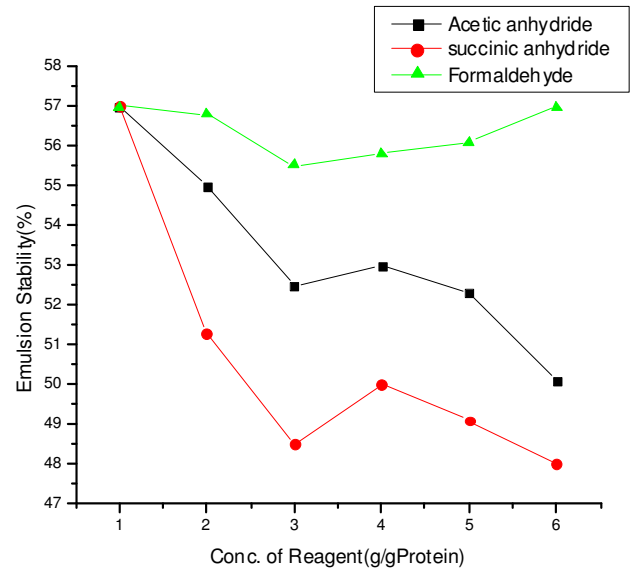


Figure 6 Effect of Modification on Viscosity Of *Spirulina* Proteins.

proteins in solution.

To conclude, the algal protein has many potential applications in new product formulations and fortifications and hence offers an exciting alternative protein source for use in various food products. Fundamental properties like foaming, viscosity and emulsification of this algal protein have the potential to find use in meat, ice cream, bakery, food, pharmaceuticals and baby food formulations.

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