Studies on the Proteins of Mass-Cultivated, Blue-Green Alga (*Spirulina platensis*)

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The characteristics of the protein of fresh-water, mass-cultured *Spirulina platensis* have been studied. The solubility of this algal protein in water and various aqueous solvents has been estimated. The total protein content of the blue-green algae was ~50–55% of which nearly 9.9% was nonprotein nitrogen. About 80% of the total protein nitrogen can be extracted by three successive extractions with water. The isoelectric point of this algal protein is found to be 3.0. The total proteins were characterized physicochemically by standard techniques. In the ultracentrifuge total proteins resolve into two major components with $s_{20,w}$ values of 2.6 and 4.7 S. The polyacrylamide gel electrophoretic pattern of the total protein showed seven bands including three prominent ones. The in vitro digestibility of the total protein of fresh algae was found to be 85% when assayed with a pepsin–pancreatin system.

Much interest has been evidenced from the theoretical and applied angles, in the mass production of algae, as biomass protein (BMP) has been identified as a promising protein source in the past two decades (Burlew, 1964; Setlik et al., 1970). Only a few species of algae have been sufficiently studied from the view point of their growth and production characteristics. Among them are the green algae *Scenedesmus acutus* and *Chlorella pyrenidosa* and the blue-green alga *Spirulina platensis*. These algae are considered as a protein source of good quality. Most researchers have concentrated primarily on the technology of production and nutritional and toxicological evaluation. No detailed information is available on the physicochemical properties of algal proteins. The extraction of protein, after cell wall rupture, from *Scenedesmus acutus* has been reported (Venkataraman and Shivashankar, 1979). The present paper details some of the studies on the extractability and characteristics of mass-cultured *Spirulina platensis* which is a potential poultry and cattle feed in India.

MATERIALS AND METHODS

The Alga. A pure culture of *Spirulina platensis* was mass cultured in fresh water in outdoor PVC tanks. The volume of cultures ranged from 500 to 1000 L. Nutrients that supplied major and minor elements were added to the culture (Venkataraman et al., 1979). Bicarbonate was supplied as a carbon source at the rate of 8 g/L. The cultures were agitated by mechanical brushing for 30 min/day. pH of the medium was maintained between 9.0 and 10.0 during the growth period of 8–10 days. Daily monitoring of growth was done by measuring the optical density at 560 nm in a Medico photometer. At the log phase of growth, the culture was concentrated by cloth filtration (80-mesh size) on a Deck filter and dried in a cross-flow drier. Freeze-dried and sun-dried samples were also used, as indicated in the text. The other details of culture conditions, cultivation, and processing not mentioned here have been reported elsewhere (Venkataraman et al., 1979).

Analytical Procedure. Total nitrogen and nonprotein nitrogen (Cl_3AcOH extractable) were estimated by Kjeldahl's method (Pearson, 1970) and protein nitrogen was obtained by difference.

Extractability of Nitrogen. Freeze-dried algal powder was ground to 60-mesh size and defatted with *n*-hexane. The defatted algal powder was successively extracted with distilled water 3 times, for a total period of 2 h. The solute:solvent ratio was 1:20, 1:15, and 1:10, respectively. The pH was adjusted suitably with 1 N HCl or 1 N NaOH (Figure 1). Each extract was centrifuged at 4000 rpm (~2300*g*), for 20 min, and pooled together for protein studies. The percentage of the total protein nitrogen in the pooled extract was estimated by micro-Kjeldahl method. Different molarity of NaCl (0.1–2.0 M) was also used in protein extractability studies.

Preparation of Protein Sample. The aqueous extract of N thus obtained was dialyzed, for 48 h, against 0.01 M phosphate buffer of pH 7.5, to remove nonprotein N and smaller peptides. The dialyzed sample was used for protein characterization studies as detailed below.

Gel Filtration. Sepharose 6B-100 (Sigma Chemicals) was packed into a 2.0 × 85 cm column. Four milliliters of the dialyzed sample, containing ~100 mg of protein, was loaded on the column, and the protein was eluted with a buffer of pH 7.8 containing 0.5 M NaCl solution. Fractions (3.0 mL) were collected in an automatic fraction collector, and absorbance was measured at 280 nm.

Polyacrylamide Gel Electrophoresis. Electrophoresis was carried out in a Shandon electrophoresis apparatus. Polyacrylamide gels (7.5%) in 0.02 M phosphate buffer of pH 7.5 were prepared by the standard procedure. The same buffer was used during electrophoresis which was continued for 1.5 h at 3 mA/tube (tube size, 7.5 × 0.5 cm). Separated protein components on the polyacrylamide gel were identified by staining the gel column for 1 h with 0.5% Amido Black, followed by diffusion in 7.5% acetic acid medium.

Ultracentrifugation. Experiments were carried out in a Spinco Model E analytical centrifuge equipped with a rotor temperature indicator unit and phase plate Schlieren optics. A standard, duraluminium cell centerpiece was used. The experiments were carried out at 28 °C with 1% protein solution using speeds of 59 780 rpm and a bar angle of 60°. The enlarged tracings of the photographs were used for the calculation of the relative percentage of each fraction. The $s_{20,w}$ value of each peak was calculated by the standard procedure (Schachman, 1959).

In Vitro Digestibility. This was determined by the method of Akeson and Stahman (1964), incubating with pepsin (Sigma Chemicals, St. Louis, MO) for 3 h, followed by pancreatin (Centron Research Laboratory, Bombay, India) for a total period of 24 h at 37 °C. Measurements

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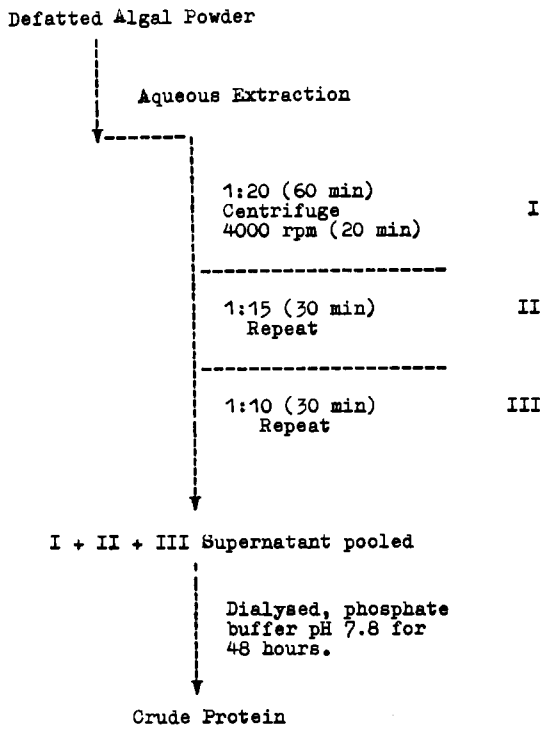

Figure 1. Extraction of *Spirulina* protein.

Table I. Composition of the Nitrogen Constituents of Processed Algae and Legumes[a]

material	g/100 g of dry weight: total N	protein N	non-protein N
algae			
Spirulina platensis, freeze-dried	8.56	6.98	1.58
Scenedesmus acutus,[b] freeze-dried	7.3	6.30	1.00
legumes[c]			
cowpea	3.5	3.2	0.3
chickpea	3.8	3.4	0.4
greengram	4.4	4.1	0.4

[a] Values represent mean of four independent observations. [b] Venkataraman and Shivashankar (1979). [c] Ganesh Kumar and Venkataraman (1975).

were taken at 4-h intervals. Undigested protein was precipitated with 10% Cl_3AcOH, and N content during the proteolytic period was determined by the micro-Kjeldahl procedure.

RESULTS AND DISCUSSION

The total crude protein content of this alga was found to be 50–55% ($N \times 6.25$) of which nonprotein nitrogen accounts for about one-sixth (Table I). *Spirulina* has a higher crude protein content as compared to *Scenedesmus* and other algae. The nonprotein nitrogen (NPN) is reported to be ~13% in *Scenedesmus* of which nucleic acid is a major component (Venkataraman and Shivashankar, 1979). The other three constituents of NPN are the smaller peptides, free amino acids, and pigments. In *Scenedesmus* the RNA content was found to be higher than DNA. The presence of a nitrogen-containing pigment, phycocyanobilin, which forms the prosthetic group of biliprotein may be responsible for the higher NPN in *Spirulina*. This is a characteristic of the blue-green algae and is absent in green algae. In algae, the NPN is higher than in other protein sources like legumes (Ganesh Kumar and Venkataraman, 1975) as is evident from Table I.

Table II. Extractability of Nitrogen Using Different Cell Disintegration Procedures and Different Solvents

material	solvent	cell disintegration method	extractable N, %[a]
Spirulina platensis, fresh/freeze-dried	water, pH 8.0 (first extraction)	handgrinding	65.0
	water, pH 8.0 (successive extraction)	handgrinding	85.0
	1 M NaCl	handgrinding	47.0
	0.5 M NaOH	handgrinding	40.0
	phosphate buffer, pH 8.0	handgrinding	45.5
	0.25% Na_2CO_3	handgrinding	58.0
Scenedesmus acutus,[b] fresh alga	water, pH 8.0	French press	7.8
	0.25% Na_2CO_3	French press	5.5
	1 M NaCl	French press	7.8
	1 M NaOH	French press	21.5
	phosphate buffer, pH 8.0	French press	15.0
Scenedesmus acutus,[b] drum-dried	1 M NaOH	French press	36.0
	2 M NaOH	French press	40.0

[a] Extractable nitrogen expressed as percent of total N which is 7.2 g/100 g of dry algae *Scenedesmus* and 7.8 g/100 g of dry algae *Spirulina*. French press cell (Inch Aminco), 15 000 lb/in.^{2} in the hydraulic press. Values represent mean of four independent observations. [b] Venkataraman and Shivashankar (1979).

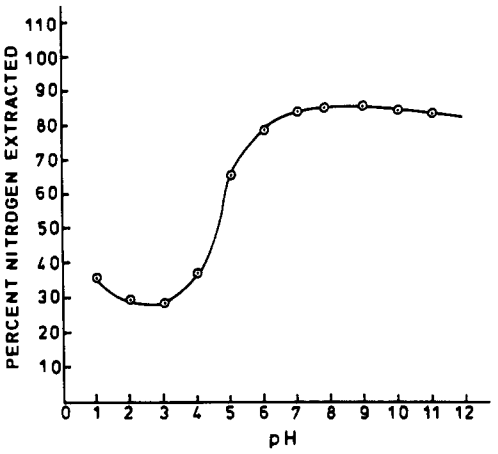

Figure 2. Isoelectric point of algal protein.

The absence of a cellulosic cell wall and the presence of only a thin cell wall membrane in *Spirulina* may account for the easier extractability of protein. The extractability of protein of *Scenedesmus* has been reported to be difficult for this reason. Apparatus like the French press, sonicator, and cell mill have been used to break the cell wall.

The isolated cell wall of *Scenedesmus* constitutes ~5.8% of the total algae dry weight (Northcote et al., 1958; Venkataraman and Shivashankar, 1979). In spite of severe cell wall rupturing methods, only ~40% of the total N could be extracted in *Scenedesmus* (Table II).

Water is the most efficient solvent in extracting the nitrogenous constituents present in *Spirulina*. About 61% of the total nitrogen can be extracted with water of pH 8.0, by the first extraction. By three successive extractions nearly 85% of the total N can be extracted in water alone (Figure 1).

pH 8.0 has been found to be most favorable for extracting while solubility decreases at other pH values. The isoelectric point of the total protein is found to be 3.0 (Figure 2). The pattern of the solubility curve obtained

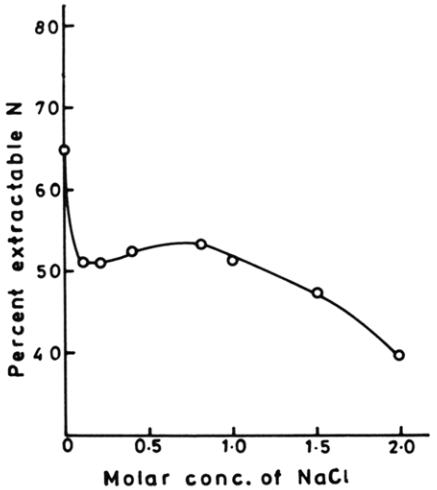

Figure 3. Extractable nitrogen with increasing concentration of sodium chloride.

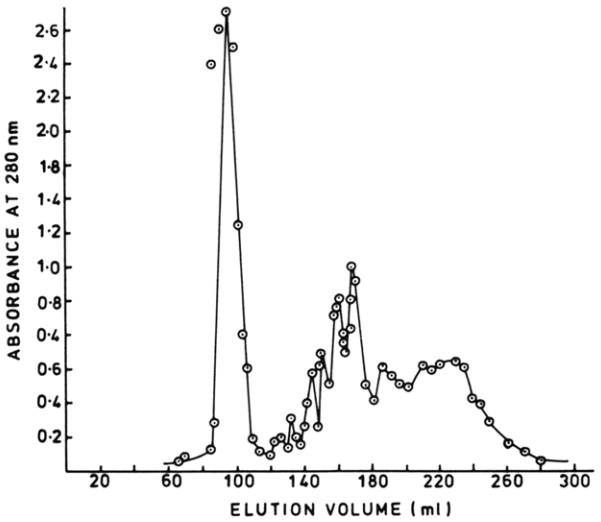

Figure 4. Gel filtration pattern of total *Spirulina* proteins.

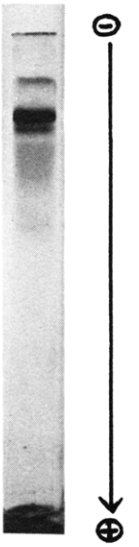

Figure 5. Polyacrylamide gel electrophoretic pattern of *Spirulina* proteins in 0.02 M phosphate buffer of pH 7.5. (The arrow shows the direction of migration.)

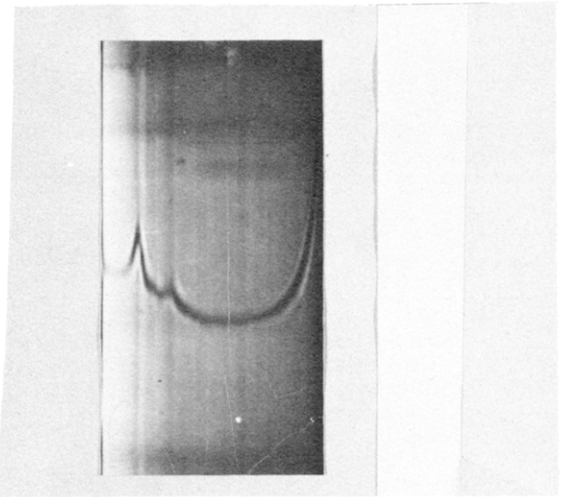

Figure 6. Sedimentation velocity pattern of *Spirulina* proteins in water. Photographs were taken at 30 min of centrifugation at 59780 rpm. Sedimentation proceeds from left to right.

for *Spirulina* is similar to that of *Scenedesmus* which has the isoelectric point of 3.5 (Venkataraman and Shivashankar, 1979). In the case of *Spirulina*, at the isoelectrical point of pH 3.0, ~28% of the total nitrogen could be extracted, whereas at pH 8.0, ~85% of the nitrogen was solubilized.

The extractability of nitrogen with NaCl did not show a sharp rise at any particular concentration, but there was a definite drop at the concentration of 1.5 N NaCl (Figure 3). It is interesting to note that, in the presence of NaCl, the solubility of the algal protein in water decreased, unlike that of the other proteins. This may be due to the presence of the pigment–protein complexes existing in the algae.

The major part of the algal protein usually exists in the form of complexes with protein, and a chlorophyll–protein complex has been isolated from algae (Thronber, 1971). The biliprotein, which is an important photosynthetic plant protein in blue-green algae, exists in the form of a protein–pigment complex. These are chromoproteins in which the prosthetic group is a bile pigment tightly bound by covalent linkage (Crespi et al., 1968). As the proteins are already in the form of complexes, the force of attraction between the protein ion and the salt ion is probably less, and this accounts for its low solubility.

Both in the method of isolating the protein and in its characterization, there seems to be major differences between oilseed proteins, which have been studied extensively, and the algal proteins which are less well understood.

The gel filtration pattern of algal protein showed seven peaks (Figure 4). The proportion of the seven fractions were 34, 3, 5, 9, 13, 17, and 19%, respectively. Since the protein solutions are colored, readings were taken at 660 nm for chlorophyll pigment and 620 nm for phycocyanobilin pigment. These readins were found to be negligible when compared to readings at 280 nm.

The polyacrylamide gel electrophoresis pattern of the total protein is shown in Figure 5. There are seven bands in the gel of which three are prominent. There are fast moving, low molecular weight fractions. Algae are generally reported to contain several low molecular weight proteins such as cytochrome *c*, ferridoxin, and flavoprotein (Crespi and Katz, 1972). Work is in progress to isolate these low molecular weight proteins in the pure form for further characterization.

Sedimentation velocity pattern of the water extract showed a presence of two peaks having $s_{20,w}$ values of 2.6 and 4.7 S (Figure 6). Certain standard techniques for protein separation (ion-exchange chromatography; solvent precipitation) proved to be inapplicable for the characterization of total protein of algae. This may again be due

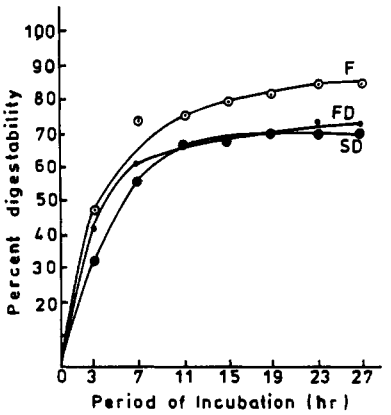

Figure 7. In vitro digestibility of *Spirulina* proteins.

to the presence of a chlorophyll–protein complex which is soluble in the detergent sodium dodecyl sulfate (Thronber, 1969). unlike oilseed proteins which are primarily storage proteins in nature, algal proteins are metabolically active and are difficult to characterize by the usual physicochemical methods which are applicable to other proteins.

In Vitro Digestibility. Digestibility of algal proteins is an important criterion in its practical use as a supplementary protein source in feed and food. In the pepsin–pancreatin enzyme system, the protein hydrolysis reached a maximum level in ~11-h time (Figure 7). The fresh algal samples were most digestible (85%), while the sun-dried and freeze-dried algal samples showed only 70% digestibility by the same proteolytic system. Tamiya (1963) had shown that in vitro digestibility of decolorized *Chlorella* was 66%, while it was only 55% in the case of freeze-dried ones. The values obtained in the present study are comparable to that of Lipinsky and Litchfield (1974), who have reported 84% in vitro digestibility for *Spirulina*.

As compared to *Spirulina*, *Scenedesmus* had comparatively lower digestibility in view of its cellulosic cell wall (Subbulakshmi et al., 1976). In drum-dried *Scenedesmus*, in which the cell wall was ruptured, the digestibility was found to be higher (Subbulakshmi et al., 1976).

The preparation of the purified *Spirulina* protein complexes and the nature of binding between the two are under study and will be reported later.

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