

## Amino acid composition and protein efficiency ratio (PER) of *Spirulina platensis*

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**Abstract.** The amino acid composition of the blue-green alga *Spirulina platensis* was determined and compared with other algal, vegetable and animal protein sources. The alga with 58.5% protein is rich in lysine and tryptophan, but deficient in sulphur containing amino acids. Of the three species of *Spirulina*, *S. platensis* has a higher content of sulphur containing amino acids. The PER of this alga is much higher than that of other algae like *Chlorella* and *Scenedesmus*.

**Keywords.** *Spirulina platensis* ; protein efficiency ratio ; amino acid composition ; single cell protein.

### 1. Introduction

Several microalgae like *Chlorella*, *Scenedesmus*, *Coelastrum* and *Spirulina* are being promoted as single cell protein (SCP) sources (Kihlberg 1972), of which the species of *Spirulina* are receiving greater attention. In Mexico, the tribes use a species of *Spirulina* locally called "Tecuitlatl", harvested from the lake Texcoco as a food additive (Farrar 1966). In Chad (Africa), even today *S. platensis* is harvested, dried in the form of flat cakes and sold under the name *Dihe* or *Douhe* (Leonard 1966). Production of *Spirulina* has now assumed industrial dimension in many countries (Seshadri *et al* 1980). In India, attempts are being made to grow this alga on a variety of substrates such as digested slurry from biogas plants (Seshadri *et al* 1980 ; Rao and Venkataraman 1980), domestic sewage and inorganic medium (Venkataraman 1979a, 1980).

The present paper evaluates the amino acid composition and protein efficiency ratio (PER) of *S. platensis* grown in an outdoor semi-pilot plant (Venkataraman 1979b) using a defined inorganic medium.

## 2. Material and methods

### 2.1 Production of the alga

The alga was a gift from Mr. Ripley D. Fox of the Laboratoire de la Roquette, France, and was grown in an outdoor production unit of 30m<sup>2</sup> capacity at the Indian Agricultural Research Institute, New Delhi. The nutrient medium contained (g/l) : NaHCO<sub>3</sub> 15.0 ; K<sub>2</sub>HPO<sub>4</sub> 0.5 ; NaNO<sub>3</sub> 2.5 ; K<sub>2</sub>SO<sub>4</sub> 0.6 ; NaCl 0.2 ; MgSO<sub>4</sub>·7H<sub>2</sub>O 0.04 ; CaCl<sub>2</sub>·2H<sub>2</sub>O 0.008 ; Fe-EDTA 0.1 ml (Jacobson 1951) and A<sub>5</sub> solution 1ml (Arnon 1938). The pH of the medium was around 9.0. For harvesting, the algal suspension was pumped out by a hand pump onto a series of cloth filters (meshes in the weave of ca 150 μ<sup>m</sup>) fitted to wire mesh baskets suspended in a frame. The filtered biomass was repeatedly washed with freshwater to remove the salts and spread thinly on polyethylene sheets and sun-dried. The dried algal flakes were collected and stored for further use.

### 2.2 Chemical analysis

The algal sample and skim milk powder (reference protein) were dried in an oven at 105°C for 6 hr to determine the moisture. Protein content was computed from Kjeldahl nitrogen values. Carbohydrates were estimated by anthrone method and fat and crude fibre by AOAC methods (AOAC 1965). Amino acid analysis was done by using an automatic amino acid analyser according to Eggum (1968). DNA and RNA were estimated by the diphenylamine (Burton 1956) and orcinol (Mejbaum 1939) methods respectively.

### 2.3 Chemical score

Chemical score (CS) of the alga was calculated from the essential amino acid data using FAO/WHO (1973) reference scoring pattern. The percentage deficiency of the most limiting amino acid compared to FAO/WHO (1973) pattern gave the value of chemical score.

### 2.4 Protein efficiency ratio (PER)

The algal (test) and skim milk (standard) diets were prepared at 10% protein level. Salt mixture and vitamin mixture (Eggum 1970) constituted 4% and 1% of the diets respectively. The dry matter was made up with nitrogen free diet (g/kg) : sucrose 90 ; cellulose powder 52 ; potato starch 806 and groundnut oil 52. The potato starch was mixed with one-third water and autoclaved at 15 lb for 30 min and then dried for 3 hr before grinding. Autoclaved starch was used since crude starch has a negative effect on digestibility.

PER was determined by the rat growth method of Osborne *et al* (1919). Weaning male albino rats about 22 day old and weighing 28 to 33 g were divided into three groups with 5 rats in each. All groups had the same average initial weight. The rats were kept in individual cages. Weighed amounts of diets were given along with measured amounts of water and feeding was done *ad libitum*. Food intake and increases in body weight of each rat were carefully recorded for a period of 4 weeks. PER was calculated as weight gained per g protein consumed.

Table 1. Essential amino acid composition (g/16 g N) of *S. platensis* compared with other protein sources and FAO/WHO 1973 pattern.

Source	Val	Leu	Ile	Phe	Lys	Met + Cys	Trp	Thr	Ms (meth + Cyst)	Reference
<i>Spirulina platensis</i>	5.70	7.95	4.93	3.63	4.34	2.78	0.88	4.02	76	Present work
<i>S. maxima</i>	6.50	8.02	6.03	4.97	4.60	1.77	1.40	4.56	50	Clement <i>et al</i> 1967
<i>S. fusiformis</i>	5.33	8.33	5.00	4.00	5.33	1.66	..	3.66	49	Seshadri <i>et al</i> 1980
<i>Chlorella phreoidosa</i>	2.70	1.20	1.70	2.10	2.40	0.60	0.40	1.90	17	Combs 1952
<i>Scenedesmus acutus</i>	7.00	6.60	4.20	3.60	5.00	2.10	1.20	5.80	52	Soeder 1970
<i>Ulothrix</i> sp.	2.60	1.40	0.60	3.40	1.50	..	..	1.80	..	Priestley 1975
<i>Uronema gigas</i>	6.80	10.50	4.00	4.70	6.30	..	..	4.00	..	Priestley 1975
Rice	5.43	7.95	4.46	5.22	3.49	3.37	0.60	3.25	..	Eggum 1968
Whole wheat	4.58	6.79	3.38	4.41	2.55	3.63	1.12	3.02	..	Eggum 1968
Corn	5.01	10.60	3.77	4.52	2.73	4.62	0.68	4.00	..	Eggum 1968
Barley	5.33	7.11	3.68	4.91	3.69	4.12	1.28	3.16	..	Eggum 1968
Peas	4.02	5.28	3.32	3.16	5.36	1.91	0.80	4.09	..	Eggum 1968
Soybean meal	5.02	7.48	4.53	5.21	5.98	3.17	1.29	3.73	..	Eggum 1968
Casein	6.96	9.70	5.23	5.20	8.41	3.67	1.68	4.35	..	Eggum 1968
Skimmed milk	6.40	10.66	5.85	5.30	7.76	3.15	1.47	5.17	..	Eggum 1968
Whole egg	7.54	8.90	5.76	6.69	6.65	5.34	1.49	5.14	..	Eggum 1968
FAO/WHO	4.96	7.04	4.00	6.08	5.44	3.52	0.96	4.00	..	FAO/WHO 1973

(FAO/WHO-1973 scoring pattern contains the values of phenylalanine + tyrosine. The values for *S. platensis* and other samples are for phenylalanine only. Phenylalanine + tyrosine is not a limiting amino acid in alga).

Table 2. Protein efficiency ratio (PER) of *Spirulina platensis* as compared with other algae and skimmed milk (for *S. platensis* protein level 10% ; duration of experiments 4 weeks ; values average of 5 rats).

Diet	Initial wt. (g)	Gain in wt. (g)	Protein consumed	PER	References
Skim milk	30.40	33.50	11.65	2.87	Present study
<i>Spirulina platensis</i>	30.50	33.20	15.97	2.07	Present study
<i>S. maxima</i>	...	...	...	2.30	Anonymous 1975
<i>Scenedesmus acutus</i>	...	...	...	1.27	Becker <i>et al</i> 1976
<i>Chlorella ellipsoidia</i>	...	...	...	0.94	Priestley 1975
<i>C. pyrenoidosa</i>	...	...	...	1.38	Priestley 1975

### 3. Results and discussion

The chemical composition of *S. platensis* showed a protein content of 58.5%, carbohydrate 8.5%, lipid 6.5%, crude fibre 5%, ash 90% and nucleic acids 4%. The high content of protein in the alga compares favourably with animal proteins. The alga has a well balanced amino acid spectrum with sulphur containing amino acids as the most obvious deficit (table 1), but the deficit of sulphur amino acids in this alga was of a lower order as compared to other algae. The chemical score was found to be 76, which was higher than all other algae (table 1).

The PER of 2.07 for this alga is better than that of pulses and cereals, except opaque 2 maize and high lysine sorghum (IARI 1971). In general, the PER of *Spirulina* species is much higher than that of other algae like *Chlorella* and *Scenedesmus* (table 2) which are promoted as sources of single cell protein.

PER can also reveal toxicity, since growth is extremely sensitive to any toxic factor present in protein foods (Mauron 1973). In the present study, no growth retardation was observed in the rats. In fact, the rats relished the algal diets as could be seen from the higher intake of food as compared to skim milk (table 2).

The safe history of this alga for human usage for centuries in Chad and Mexico, its high protein with a well balanced amino acid pattern and easy digestibility together with its low cost of production in waste waters indicate its suitability as a high quality protein supplement for poultry in our country. An average increase of 49% in the body weight, 15% in egg laying capacity, 20% in vitamin A and 18% in protein contents and two to three fold increases in blood carotene have been observed in poultry birds fed with *Spirulina* (Tulaganov and Zarinov 1974).

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## References

- Anonymous 1975 *Spirulina*. In: Under-exploited tropical plants with promising economic values ; *Natl. Acad. Sci. Wash.* pp. 162-168
- AOAC 1965 *Official Methods of Analysis of the Assoc. Official Agricul. Chemists.* (ed. W Horowitz) Washington Ed. 10.
- Arnon D I 1938 Microelements in culture solution experiments with higher plants ; *Am. J. Bot.* **25** 322-25
- Becker W E, Venkataraman L V and Khanum P M 1976 Digestibility coefficient and biological value of the proteins of the alga *Scenedesmus acutus* processed by different methods ; *Nutr. Rep. Int.* **14** 457
- Burton K 1956 A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid ; *Biochem. J.* **62** 315-323
- Clement G, Giddey C and Menzi R 1967 Amino acid composition and nutritional value of the alga *Spirulina maxima* ; *J. Sci. Fd. Agr.* **18** 497-501
- Combs G F 1952 Algae (*Chlorella*) as a source of nutrients for the chick ; *Science* **116** 453
- Eggum B O 1968 Aminosyrekoncentration proteinkvalitet ; *Stougaard Forlag, Kobenhavn* **9**
- Eggum B O 1970 Current methods of nutritional protein evaluation In : Improving plant protein by nuclear techniques ; *Proc. Symp. IAEA/FAO, Vienna* pp. 289-302
- Farrar W V 1966 Tecuilitlatl, a glimpse of Aztec food technology ; *Nature (London)* **211** 341-342
- FAO/WHO 1973 Energy and protein requirements ; *Rept. Joint FAO/WHO Nutrition Rept. FAO Rome Ser.* **52**
- IARI 1971 Recent research on the improvement of protein and nutritive properties of food and feed plants ; *Indian Agricultural Research Institute, New Delhi, Res. Bull.* No. 6 p. 116
- Jacobson L 1951 Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium ethylenediamine tetracetate ; *Plant Physiol.* **26** 411-413
- Kihlberg R 1972 The microbe as a source of food ; *Annu. Rev. Microbiol.* **26** 427-466
- Leonard J 1966 The 1964-65 Belgian trans-Saharan expedition ; *Nature* **209** 126-128
- Mauron J 1973 The analysis of food proteins, amino acid composition and nutritive value. In *Proteins in human nutrition* (eds.) J W G Porter and B A Rolls (London : Academic Press) pp. 139-154
- Mejbaum W 1939 Estimation of small amounts of pentose especially in derivatives of adenylic acid *Z. Physiol. Chem.* **258** 117-120
- Osborne T B, Mendel L B and Ferry B L 1919 A method of expressing numerically the growth promoting value of proteins ; *J. Biol. Chem.* **37** 223
- Priestley G 1975 Algal proteins. In : *Food from wastes* (eds.) G G Birch, K J Parker and J T Worgan, *Appl. Sci. Pub.*, England pp. 114-138
- Rao D L N and Venkataraman G S 1980 Growth potential of *Spirulina platensis* on animal wastes ; *Curr. Sci.* **49** 478
- Seshadri C V, Thomas S, Manoharan R, Jeeji Bai N and Raja G 1980 Mass culture of *Spirulina platensis* ; *MCRC Monographs Ser.* No. 5 pp. 1-61
- Soeder C J 1970 Aspects for the use of microalgae in feeding humans and animals ; *Ber. Dtsch. Bot. Ges.* **83** 607-625
- Tulaganov A T and Zarinov I Z 1974 Application of biomass of *Spirulina* in poultry. In *Proc. Conf. Microbiologists, Algologists and Mycologists, 50th Ann. Uzbek Rep. USSR ; Fan Uzbek SSR, Tashkant* pp. 125-126
- Venkataraman G S 1979a Indian experience with algal ponds ; *Food and Nutrition, UNU Suppl.* **2** 68-71
- Venkataraman G S 1979b All India Coordinated Project on Algae ; *AICPA Annual Report 1978-79*, New Delhi pp. 1-119
- Venkataraman G S 1980 All India Coordinated Project on Algae. *AICPA Annual Report 1979-80*, New Delhi pp. 1-128