NON-CONVENTIONAL APPROACHES TO FOOD PROCESSING IN CELSS. I-ALGAL
PROTEINS; CHARACTERIZATION AND PROCESS OPTIMIZATION.

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ABSTRACT

Protein isolate obtained from green algae (Scenedesmus obliquus) cultivated under controlled
conditions was characterized. Molecular weight determination of fractionated algal proteins
using SDS-polyacrylamide gel electrophoresis revealed a wide spectrum of molecular weights
ranging from 15,000 to 220,000. Isoelectric points of dissociated proteins were in the range
of 3.95 to 6.20. Amino acid composition of protein isolate compared favorably with FAO
standards. High content of essential amino acids leucine, valine, phenylalanine and lysine
makes algal protein isolate a high quality component of CELSS diets. To optimize the removal
of algal lipids and pigments supercritical carbon dioxide extraction (with and without
ethanol as a co-solvent) was used. Addition of ethanol to supercritical CO_2 resulted in
more efficient removal of algal lipids and produced protein isolate with a good yield and
protein recovery. The protein isolate extracted by the above mixture had an improved water
solubility.

INTRODUCTION

There is a need for suitable feeding systems for extended manned space missions. The poten-
tial utilization of algal biomass in a non-conventional food supply in space, is the subject
of our study. Unique multifunctional characteristics of algae such as high photosynthetic
activity, lack of need for organic carbon and nitrogen in the growth media and the ability
to control algal chemical composition by varying cultivation conditions make algae an
attractive system for biological regeneration of the CELSS environment /1,2,3,4,5,6,7,8,9/.
Consumption of untreated and unpurified algal biomass, due to associated physiological pro-
blems (e.g. nondigestible components of cell wall and disturbing carbohydrates, excess
nucleic acids, unknown toxins and allergens) and poor flavor or color limits the amount of
algae in the diet. Conversion of algal biomass into physiologically and organoleptically
acceptable components requires suitable methodology. Previously developed methods eliminated
or largely reduced three known undesirable components of algae (cell walls, nucleic acids,
and pigments) and gave an "algal protein isolate" with a reasonably high yield /10/.

Green algae (Scenedesmus obliquus) grown in Constant Cell Density Apparatus, under con-
trolled conditions were used in this study. They were obtained through the courtesy of Drs.
Richard Radmer and Paul Behrens of MARTEK Corp. Columbia, MD. Figure 1 shows the stepwise
procedure for preparation of various fractions from algae. Major nutritional components of
the algae flour (freeze-dried ruptured cells) were determined. Upon cell rupture and removal
of the cell wall, the nucleic acid content of the algae was reduced by 92%, using an enzy-
matic procedure. This increased the safe consumption level of algal protein concentrate
(fraction #6) from 20g to approximately 250g per day and for algal protein isolate (fraction
#8A) from 15g to 192 g per day. Generally accepted safe level of nucleic acids intake in
human is 2g per day /11/. Ethanol extraction of algal pigments (mostly chlorophyll A) from
the protein concentrate (fraction #6) resulted in removal of most of the green color and
consequently the algal protein isolate (fraction #8A) had a "light olive" color upon freeze-
drying. Removal of the pigments enhanced the color and flavor, but caused protein denatura-
tion and insolubility of the final product.

In order to further improve the quality of algal proteins we have experimented with the re-
moval of algal pigments and lipids using supercritical fluids. The results are reported
here.

Characterization of isolated algal proteins was a major part of our recent study. This
knowledge can be useful in designing further processing and fabrication of algal proteins

as a food component in CELSS. The present paper also reports the results of electrophoretic studies and amino acid analyses of algal proteins.

METHODOLOGY

Preparation of algal protein fractions

Green algae (Scenedesmus obliquus) were grown in Constant Cell Density Apparatus (CCDA). Conditions were: Algal density in medium: 0.55 mg dry weight/ml.; 32°C; pH= 7.0; nitrogen source: KNO_3 2.0g/l; carbon source: 2% CO_2 in air. The algae were grown, harvested, and supplied to us by Martek Corp. (Columbia, MD).

SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was used to determine the molecular weights (M.W.) of algal proteins. Freeze-dried algal protein concentrate (fraction #6) was used. The experimental details of SDS-PAGE were reported previously /12/.

Isoelectric focusing (IEF)

IEF in polyacrylamide gel was used to determine the isoelectric points (pI) of algal proteins. Ampholine pH 3-10 was used to produce pH gradient across the gel. The IEF conditions were as previously reported /12/. Freeze-dried algal protein isolate (fraction #6) was used in this study.

Amino acid analysis

Amino acid composition of algal protein isolate was determined using a recently developed pre-column derivatization method which has been originally demonstrated by Koop, Morgan, Tarr and Coon /13/ for analysis of free amino acids and later modified by Bidlingmeyer, Cohen and Tarvin /14/ for application to acid-hydrolysates of proteins.

The method which is called "Pico-Tag" is based on derivatization of amino acids with phenyl-isothiocyanate (PITC). PITC reacts with free amino acids to yield phenylthiocarbamyl (PTC) amino acid, which can then be separated on a reversed-phase HPLC column. Strong UV absorbance of PTC-amino acids (254 nm) makes it possible to detect quantities as small as 1 picomole /14/. Because PITC forms the same chromophore with primary and secondary amino acids no extra treatment is necessary for detection of proline. Excellent reproducibility, derivatives stability (6-10 hrs at R.T.) and rapid analysis (12 min.) of amino acids made the Pico-Tag the method of choice for our purpose.

Upon HCl hydrolysis destruction of Trp, conversion of Asn and Gln to ASP and Glu , also oxidation of cysteine to cystine and consequently partial destruction of cystine occur /15/. Hence, in order to obtain complete amino acid profile, algal protein isolate (fraction #8A) was treated in 3 different ways: 1) HCl hydrolysis and PITC derivatization to detect all PTC-amino acid derivatives except for Cys and Trp, 2) Performic acid oxidation followed by HCl hydrolysis and derivatization to quantify the amount of cysteine in the form of cysteic acid, and 3) methane-sulfonic acid hydrolysis followed by derivatization to obtain the amount of tryptophan. Amino acid standard used was Pierce standard H. Least square treatment of data and calculated regression lines were used for quantification of amino acid content of algal proteins.

Supercritical Fluid Extraction

Supercritical fluid extraction of algal pigments and lipids was carried out in two steps. The 1st extraction was with supercritical carbon dioxide (SC -CO_2) and the 2nd extraction was with carbon dioxide and anhydrous ethanol (22%) as a co-solvent. Freeze-dried algal protein concentrate (fraction #6), 4.86g was charged alternatively between layers of glass wool to an extraction vessel, a 1.8 cm diameter X 30 cm long stainless steel tube (Autoclave Engineers, Inc.), and connected to the system shown in Figure 2; the glass wool served to keep the algae powder from compacting during passage of gas through the extractor. Carbon dioxide (Airco, Inc., Grade 2.8) was supplied at about 87 bar pressure and 313°K to the suction side of a double-end diaphragm compressor (Superpressure, Inc.) and was compressed to the measurement pressure. The pressure was controlled by a back-pressure regulator (Circle Seal, BPR) which diverted the bulk of the compressed gas from the surge tank back to the suction side of the compressor resulting in an almost pulse-free flow of gas to the extractor. The high-pressure gas passing downstream of the compressor was heated in a tube preheater to about 338°K and was passed through the extraction vessel which was maintained at 338°±2 K by a temperature indicator/controller which measured the temperature via an iron constantan thermocouple (Superpressure, Inc.) positioned in the bed of algae powder and which regulated power to a heater (Glascol tapes) on the extractor.

The solution (consisting of carbon dioxide and dissolved materials) leaving the extraction vessel was passed through a heated, flow-regulating, pressure let-down valve and was expanded to ambient pressure. The materials which were dissolved by the gas passing through the extraction vessel precipitated during the pressure-reduction step and were separated from the gas in a U-tube collector (Kimble, 200 mm) whose exit junction was fitted with a glass wool filter to prevent fine-particle solids from passing through the tube; a second U-tube with a more tightly packed glass wool filter was positioned downstream of the first collector and served to trap any fine particles which might have passed through the first filter.

The ambient gas leaving the collection system passed through a rotameter, (Fischer-Porter, Inc., Series 10A 35) for flow rate measurement and through a dry test meter (Singer, Inc., DTM-200) for flow volume integration. The flow rate of the carbon dioxide through the extractor was maintained at 3 SLPM (standard liters per minute). A total of 0.66 g of green semi solid materials, SC-CO_2 extract (fraction #7B) was collected (in the U-tube) during the passage of 720 g of carbon dioxide at 380 bar pressure. After the extraction with carbon dioxide a fraction of the charge of algae (SC-CO_2 residue, fraction #8B) was removed from the vessel to be used for solubility tests, the vessel was resealed and connected to the system. The 2nd extraction was carried out with SC-CO_2 at 380 bar pressure at 338° K, with anhydrous ethanol added as a co-solvent. It was pumped into the system at the entrance to the extractor. The ratio of ethanol to carbon dioxide was 100 to 360 g (22%). The SC-CO_2 + EtOH extract (dark green ethanol solution) was collected in a container (fraction #7C). The SC-CO_2 + EtOH residue (fraction #8C) was used for solubility test (Figure 3).

Solubility Test

Algal protein isolate (fraction #8A), SC-CO_2 residue (fraction #8B) and SC-CO_2 + EtOH residue (fraction #8C) were used. To 100 mg of each fraction, 10 ml, 0.05 M phosphate buffer, pH 8.0 (based on pH-solubility profile of plant proteins, Wolf /17/) was added. The samples were mixed and stored at room temperature for about 1 hr. They were then centrifuged at 15,000 RPM for 10 min. at 2° C. The supernatant was collected and the pellet was washed with another 2 ml of phosphate buffer and recentrifuged. The wash was added to supernatant (kept at 5° C) and the pellet was freeze-dried (Virtis Research Equipment, Gardiner, NY). Protein concentration of the supernatants and the pellets were determined using the micro-kjeldahl method A.O.A.C. /18/. Solubility was defined as:

$$\% \text{ Solubility} = \frac{\text{Protein in supernatant (mg) x 100}}{\text{(sum of protein in supernatant and pellet) mg}} \tag{1}$$

Absorption Spectra

Absorption spectra of SC-CO_2 extract (fraction #7B) and SC-CO_2 + EtOH extract (fraction #7C) in the range of 700 to 240 nm were obtained and compared with that of fraction 7A, which is the boiling-ethanol extract. Since SC-CO_2 extract (fraction #7B) is in the form of semi solid materials, an aliquot was dissolved in hexane, filtered (Millex-SLSR025NS) and used for this study.

Analysis of Algal Lipids

Isolation of algal lipid components (neutral, glyco and phospholipids) using sequential solvent extraction, gel filtration and thin layer chromatography, as well as determination of fatty acid composition using GC is underway. SC-CO_2 and SC-CO_2 + EtOH extracts (fractions #7B and 7C) and the corresponding residues (fractions #8B and 8C) will be analyzed.

RESULTS AND DISCUSSION

Molecular Weight Determination

Literature on molecular weights of algal proteins is limited. Lee and Picard /19/ studied the electrophoretic pattern of Oocystis algal proteins using three systems: Tris-glycine pH 8.7, SDS-gel and SDS-gel with 2-mercaptoethanol. Using the SDS-gel along with dissociating agents they observed a wide spectrum of molecular weights (M.W.) ranging from 12,500 to 250,000. In our study using SDS-PAGE we also obtained wide range of M.W. for Scenedesmus obliquus proteins. Figure 4 shows the electrophoretic pattern of protein concentrate (fraction #6). The M.W. of proteins ranged from 15,000 (band #20) to 220,000 (band #1). Major protein bands correspond to bands # 7, 14, 15, 18, 19 and 20 with M.W. of 55,000, 30,000, 28,000, 18,000, 16,500 and 15,000 respectively. Band #21 corresponded to chlorophyll and other algal pigments (M.W. below 1,000) which migrated to the front of the gel. A comparison of SDS-PAGE pattern of protein isolate obtained from ethanol extraction (fraction #8A) with that of protein isolate resulting from supercritical fluid extraction (fractions #8B and 8C) will enable us to detect the extent of changes such as oligomerization and/or partial fragmentation of treated algal proteins.

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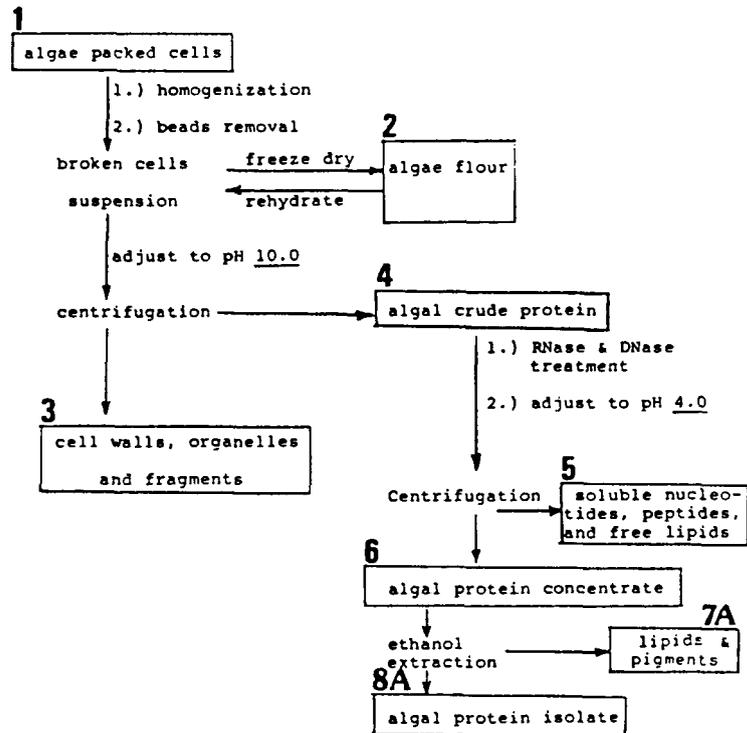
Fig. 1) Stepwise procedure for preparation of various
algae fractions.
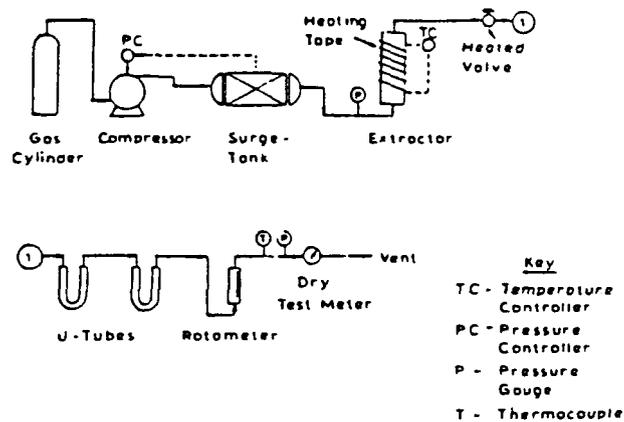
Fig. 2) Schematic diagram of experimental for supercritical
fluid extraction (from /16/).
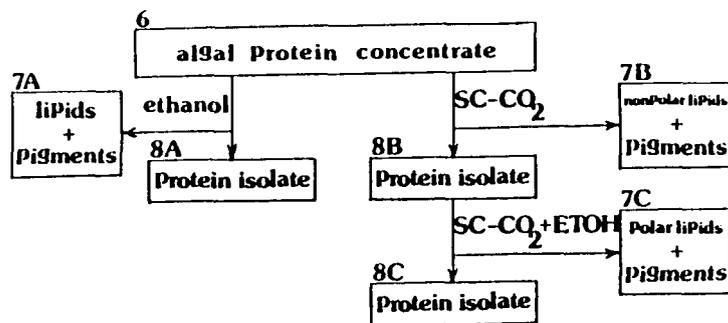

Fig. 3) Procedure for extraction of algal lipids and
pigments using supercritical fluids (at about
380 bar pressure and 338°K).

Isoelectric Point Determination

There is no report available on isoelectric points of algal proteins. The IEF pattern of algal proteins and pH profile of IEF gel for determination of the PI's of focused proteins are shown in Figure 5. The isoelectric points of the algal proteins ranged from 3.95 (band # 15) to 6.20 (band #2). Major protein bands corresponded to bands #7, 9, 11 and 13 with PI's of 5.45, 5.20, 4.85 and 4.35 respectively. Band #1 corresponded to chlorophyll and other pigments which did not migrate in the IEF gel.

Amino Acid Composition

Nutritional value of proteins is primarily determined by their amino acid composition. Hence amino acid profile of algal protein (Scenedesmus obliquus) was studied and aminograms were obtained (Figure 6).

The amino acid composition of algal proteins compared favorably with FAO standards, Table 1. Analysis of amino acid composition revealed high content of leucine, valine, phenylalanine and lysine and low content of methionine and tryptophan. (The high values for cysteine contrary to reported low values in the literature is because we analyzed cysteine in the form of cysteic acid which yielded in about 70% more compared to values we obtained for cystine).

There is no data reported on amino acid composition of Scenedesmus obliquus cultivated under controlled conditions (CCDA). However, there are reports on amino acid composition of Scenedesmus obliquus cultivated under open air conditions according to German technique of Dortmund /20, 21, 22, 23/ cultivation ponds /24/ and unspecified conditions / 5 /. The comparison of the aminograms from Scenedesmus obliquus cultivated under different conditions revealed a similar pattern of high content of valine, leucine,and lysine and low content of methionine and tryptophan. Our results, showed relatively higher values for isoleucine, leucine and phenylalanine.

The nutritional value of green algae depends on digestibility which depends on pretreatment of algae and disruption of the cell wall. Low nutritional value is to be expected if algae is not sufficiently processed /25/. Nutritional value (biological value, BV, digestibility, D, net protein utilization, NPU, and protein efficiency ratio, PER) of pretreated Scenedesmus obliquus cultivated under open air conditions (Dortmund technique) has been studied in a series of different and independent investigations in animals and also in human volunteers. The results repeatedly and unequivocally confirmed high nutritive value of algal proteins /25, 26, 27, 28/. Drum-dried Scenedesmus obliquus was studied as a diet component in rats. High nutritional value due to improved protein digestibility (as a result of cell wall disruption) was reported, Table 2 /30, 31/. Fortification of wheat and rice with proteins from Scenedesmus acutus in rats' diets resulted in significantly higher PER value than cereal diet alone /32/. They also concluded that Scenedesmus proteins supplemented wheat proteins to a greater extent than rice.

In summary, algal proteins, because of their high content of essential amino acids (valine, leucine, lysine and phenylalanine) upon cell wall rupture and removal of undesirable components, can significantly improve the nutritional value of other plant proteins e.g. cereals (wheat, rice and corn with lysine as their limiting amino acid), legumes, etc. and serve as a high quality component of CELSS diets. Wheat and soybeans are considered as two sources of macronutrients in CELSS diet scenario /33/.

Supercritical Fluid Extraction

In our previous study boiling ethanol was used to remove pigments and lipids. Ethanol treatment of algal proteins (regardless of extraction temperature) improves the color and flavor of the isolate, but causes denaturation and consequently aggregation of algal proteins with loss of solubility. Supercritical fluids are receiving increasing attention as extraction solvents. Because of their pressure-dependent dissolving power properties often display the fractionation ability of multicomponent solutes /34/. Supercritical carbon dioxide (SC-CO_2) has been considered by many investigators as the ideal fluid for extraction and separation processes. It is reported to behave very much like hydrocarbon solvent with very low polarizability /35/. Therefore, the use of SC-CO_2 for extraction of lipids or lipid soluble materials while giving comparable yields offers several advantages.

Organic solvents are flammable and explosive. They might also contain traces of higher boiling fractions that may be left in the extract and pose a potential health hazard /36/. On the other hand carbon dioxide is nontoxic, nonflammable and rather easily separatable from the extracted materials.

There are an increasing number of reports on replacement of lipophilic extraction by SC-CO_2. Extractions of oil from corn /36, 37/, soybeans /38, 39, 40/, cottonseed /41/, lupine seeds

SDS/PAGE

STANDARDS ALGAE   M.W.'s detected

M.W.'s detected
220,000
170,000
100,000
86,000
78,000
62,000
55,000
50,000
44,000
41,000
38,000
36,000
32,000
30,000
28,000
26,000
22,000
18,000
16,500
15,000
Chlorophyll

Standards: 94,000 · 67,000 · 43,000 · 30,000 · 20,100 · 14,400
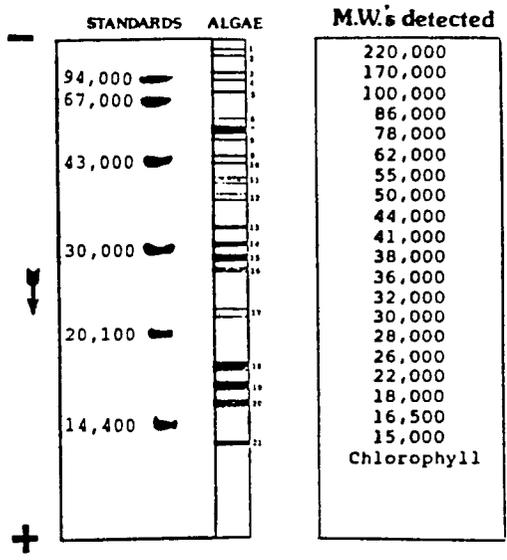
Fig. 4) SDS-PAGE pattern of algal protein concentrate (fraction #6).

IEF

P.I.'s detected
Chlorophyll
6.20
6.10
6.05
5.95
5.70
5.45
5.30
5.20
4.95
4.85
4.52
4.35
4.00
3.95

Gel Length (Cm) vs pH
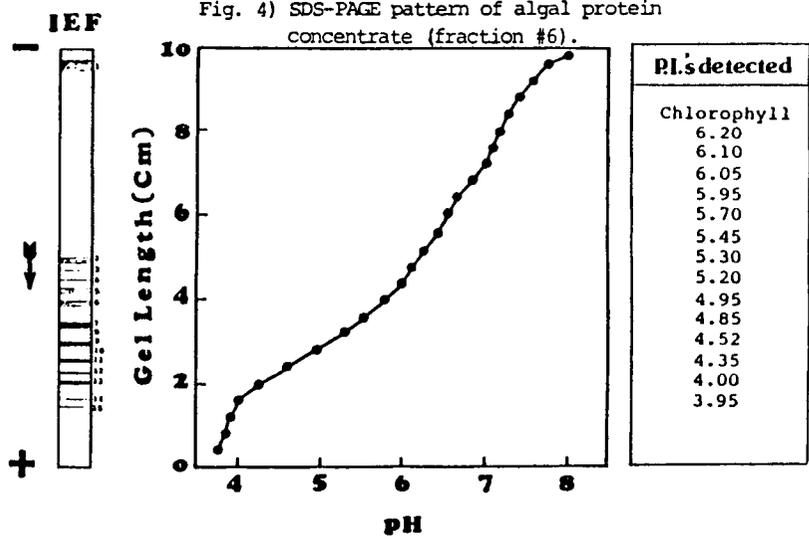
Fig. 5) IEF pattern of algal protein concentrate (fraction #6) and pH profile of IEF gel for determination of pI of focused proteins.

Standards

Algae

1. ASP		10. NH$_3$	
2. GLU		11. TYR	
3. SER		12. VAL	
4. GLY		13. MET	
5. HIS		14. CYS	
6. ARG		15. ILE	
7. THR		16. LEU	
8. ALA		17. PHE	
9. PRO		18. LYS	

0 min   TIME   13 min
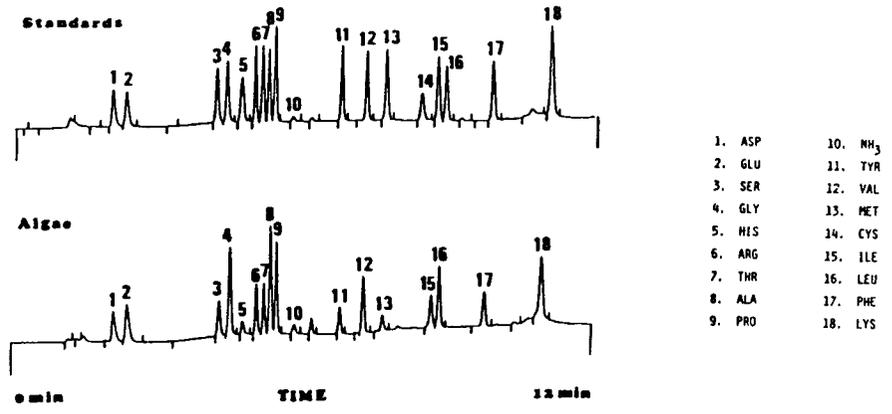
Fig. 6) HPLC chromatograms of amino acid standards (Pierce standard H) and of algal protein isolate (fraction #8A)

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/42/ and animal oils and fats /43/ using supercritical CO_2 has been reported.

Comparative study on the storage ability of defatted corn germ flour using hexane and SC-CO_2 showed significant reduction of peroxidase activity (apparently as a result of protein denaturation under the extraction conditions, 344 to 551 bar pressure and 323° K) and high flavor quality upon accelerated storage tests for SC-CO_2 extracted corn germ flour /37/. Similar study on oil extraction of soybean flakes using SC-CO_2 (730 to 854 bar pressure, 353-373° K and 5-13% moisture) and hexane also resulted in better flavor quality and shelf life for SC-CO_2 defatted soybean flour /40/. In a comparative study on oxidation stability of "oil" extracted from soybean flakes using either SC-CO_2 at 344 bar and 323° K or hexane, SC-CO_2 extraction yielded a product comparable to a hexane-extracted degummed soybean oil. Phosphatides were essentially absent in SC-CO_2 and this may be the reason for lower oxidative stability of SC-CO_2 extracted soybean oil /39/.

There is no report on supercritical extraction of algal lipids and/or pigments. The extraction of algal protein concentrate (fraction #6) using (SC-CO_2) resulted in removal of green semi solid materials believed to contain nonpolar lipids and lipid-soluble pigments (such as chlorophyll and carotenoids). Chlorophylls are classified as lipid- and water-soluble and carotenoids as lipid-soluble pigments /44/. The yield of SC-CO_2 extract (fraction #7B) was 7%. The absorption spectra of fraction #7B (dissolved in hexane) indicated the predominance of chlorophyll A (with absorption maximum, λ_{max} at 410 nm) and some other minor peaks probably due to extracted carotenoids. The yield of SC-CO_2 residue (fraction #8B) which had an "olive green" color was 44.6%. Further extraction of protein isolate (fraction #8B) using SC-CO_2 and 22% ethanol resulted in a dark green ethanol solution believed to contain polar lipids and chlorophylls (fraction #7C) with the yield of 12%. The absorption spectra of SC-CO_2 + EtOH extract indicated mainly the presence of chlorophyll A and traces of chlorophyll B (λ_{max} at 470 nm). The absorption spectra of SC-CO_2 + EtOH extract (fraction #7C) were almost identical to those of ethanol extract (fraction #7A). The SC-CO_2 + EtOH residue (fraction #8C) had a "light olive" color similar to ethanol extract residue (fraction #8A). Yield of algal protein isolate obtained from SC-CO_2 + EtOH extraction was 33%. The remaining balance as compared to previously reported value for fraction 8A which was 36.6%, is due to the removal of 19% total lipids (nonpolar and polar) and pigments (fraction #7B and 7C) compared to 15% (fraction #7A). We believe that SC-CO_2 extraction resulted in more efficient removal of nonpolar lipids. Isolation and determination of lipid components (neutral, glyco and phospholipids) and fatty acid composition of various extracts (ethanol, SC-CO_2 and SC-CO_2+ EtOH) and the residues are underway and will be reported in our next paper. Protein concentration of SC-CO_2 + EtOH residue (protein isolate 8C) was 69.1%, similar to the ethanol residue (protein isolate 8A) which was 70.5%. Table 3 shows the yield, protein concentration and recovery of various algal fractions.

Protein Recovery

Recovery of Oocystis algal proteins was studied by Lee and Picard /19/. Using successive ammonium sulfate fractionations they recovered 44% of algal proteins. The protein recovery increased to 66% when the fractionation was done in the presence of ascorbic acid and insoluble PVP (polyvinylpyrrolidone). The presence of endogenous proteolytic and oxidative enzymes may partially impair the recovery of plant proteins.

Phenoloxidase and peroxidase catalyze the reduction of O-diphenols to quinones; subsequently the quinones polymerize and form complexes with proteins thus impairing the solubility /19, 45, 46, 47/. The formation of these complexes is prevented by extracting the plant proteins in the presence of reducing agents (such as ascorbic acid) and by removing the phenols with substances such as PVP which forms hydrogen bonds with phenols. Nonspecific aggregation of plant proteins is also reported in the literature and believed to be the result of sulphydryl interactions /48/.

Solubility of Algal Proteins

Solubility of algal proteins is shown in Table 3. Under our experimental conditions, the solubility of algal protein (fraction #6) prior to removal of pigments and lipids was 63%. Upon extraction with ethanol the solubility decreased to 20% (protein isolate 8A). The solubility of SC-CO_2 residue (protein isolate 8B) was 45% and of SC-CO_2 + EtOH residue was 41%.

Partial protein denaturation as a result of "anhydrous" SC-CO_2 extraction (344 to 551 bar pressure and 323° K) of dry-milled corn germ flour /37/ and "humid" SC-CO_2 (730 to 854 bar, at 373° K and 5-13 % moisture) extraction of soybean flakes /40/ has been reported. Protein denaturation has been considered to be the result of high pressure extraction /37/. However, this partial denaturation resulted in reduction of peroxidase activity (heat-resistant oxidative enzyme) and consequently better storage stability of the extracted corn germ flour and soybean flakes. Weder /49, 50/ studied the effect of humid supercritical CO_2 and N_2 at 300 bar, 353° K and room temperature on the structure and amino acid composition of ribonuclease and lysozyme. The amino acid composition and TNBS-reactive lysine (trinitrobenzene

sulfonic acid) remained unaltered. Partial oligomerization (due to disulfides) and some fragmentation of protein molecules in protein extracted at 353° K was detected. No oligomers were detected using SDS-PAGE when extraction was at room temperature. The occurrence of the changes in the protein molecules was concluded to be the result of heating proteins in the presence of water regardless of the nature or pressure of the gas used. Tryptic digestion of the treated proteins indicated a better digestibility than untreated proteins.

In our system, decrease in the solubility of protein isolate (41%) as compared to protein concentrate (63%) could possibly be due to the extraction temperature (338° K). In our previous study on enzymatic reactions of polyphenol oxidase in supercritical fluids the enzyme was found to be catalytically active at 344 bar, 307° K and 309° K /51/ indicating that no protein denaturation has occurred due to the exposure to supercritical fluids.

In summary: SC-CO$_2$ + EtOH extraction of green algae resulted in : 1) more efficient removal of algal lipids and pigments (19% vs 15% ethanol extract). 2) Fractionation of nonpolar and polar lipids. 3) Protein isolate with a good yield and protein recovery. 4) Increased solubility of the protein.

The above results suggest that supercritical fluid extraction is a useful extraction method to be used in the space habitats.

REFERENCES

1. R.P. Casey and J.A. Lubitz. Food Technology. 17(11); 48 (1963).

2. G. Clement, C. Giddey and R. Menzi. J. Sci. Food Agric. 18: 497 (1967).

3. P.A. Lachance. Single Cell Protein, R. Mateles and S. Tannenbaum, MIT Press, Cambridge, MA, 1968.

4. E.S. Lipinsky and J.H. Litchfield. CRC Crit. Rev. Food Tech. 12: 581 (1970).

5. C.I. Waslien. CRC Crit. Rev. Food Sci. Nutr. 6:77 (1975).

6. G. Clement. Single Cell Protein, Vol. II, S.R. Tannenbaum and D.I.C. Wang, MIT Press, Cambridge, MA, 1975.

7. C.I. Waslien, J. Myers, B. Koh and W. Oswald. Protein Resources and Technology: Status and Research Needs, M. Milner, N. Scrimshaw and D. Wang, AVI Publ. Co., Inc. Westport, CT, 1978.

8. W.J. Oswald. Algae Biomass, G. Shelef and C.J. Soeder, Elsevier/North Holland Biomedical Press, N.Y. 1980.

9. M. Piorreck, K. Baasch and P. Pohl. Phytochemistry. 23(2): 207 (1984).

10. A.R. Kamarei, Z. Nakhost and M. Karel. SAE Technical Paper Series, 851388. Society of Automotive Engineers, Inc., Warrendale, PA (1985).

11. N.S. Scrimshaw. Single Cell Protein, Vol. II, S.R. Tannenbaum and D.I.C. Wang, MIT Press, Cambridge, MA, 1975.

12. Z. Nakhost and M. Karel. J. Food Sci. 48(4): 1335 (1983).

13. D.R. Koop, E.T. Morgan, G.E. Tarr and M.J. Coon. J. Biol. Chem. 257: 8472 (1982).

14. B.A. Bidlingmeyer, S.A. Cohen and T.L. Tarvin. J. Chromatogr. 336: 93 (1984).

15. R.H. Haschemeyer and A.E.N. Haschenmeyer. Proteins, Wiley-Interscience Publication, N.Y., 1973.

16. V.J. Krukonis and R.T. Kurnik. J. Chem. Eng. Data 30: 247 (1985).

17. W.J. Wolf. Soybeans: Chemistry and Technology, Vol. 1: Proteins. A.K. Smith and J. Circle, AVI Publ. Co., Inc., Westport, CT, 1978.

18. A.O.A.C. Official methods of analysis. Association of Official Analytical Chemists. W. Horwitz (ed.),(1980).

19. B.H. Lee and G.A. Picard. Can. Inst. Food Sci. Technol. J. Vol. 15(2): 120 (1982).

20. H.D. Bock and J. Wunsche. Dtsch. Akad. Landw. Wiss. 16(9): 113 (1967).

21. C.J. Soeder and W. Pabst. Ber. Dtsch. Bot. Ges. 83: 607 (1970).

22. O.P. Walz, F. Koch and H. Brune. Tierphysiol, Tierern, Futtermittelkd. 35: 55 (1975).

23. F. Kugler and B. Pithakpol. Algae Project Fourth Report. Kaestsart University, Bangkok (1975).

24. E.W. Becker. Process Biochem. 16(5): 10 (1981).

25. H.D. Payer, W. Pabst and K.H. Runkel. Algae Biomass, G. Shelef and C.J. Soeder, Elsevier/North-Holland Biomedical Press, N.Y. 1980.

26. W. Pabst. Arch. Hydrobiol. Suppl. 11: 65 (1978).

27. E. Kofranyi and F. Jekat. Hoppe-Seyler's Z. Physiol. Chemie. 348: 84 (1967).

28. H. Muller-Wecker and E. Kofranyi. Hoppe-Seyler's Z. Physiol. Chemie. 354: 1034 (1973).

29. I.E. Liener. Soybean: Chemistry and Technology, Vol. I: Proteins. A.K. Smith and J. Circle, AVI Publ. Co., Inc., Westport, CT, 1978.

30. W. Pabst. Z. Ernaehrungswiss. 13: 73 (1974).

31. A. Richmond. Biotechnology. Vol. 3. H.J. Rehm and G. Reed, Verlag Chemie, Weinheim, 1983.

32. L.V. Ventkatamavan, W.E. Becker, P.M. Khanum and I.A.S. Murthy. Nutr. Rep. Int. 15: 145 (1977).

33. M. Karel and A.R. Kamarei. NASA Cooperative Agreement No. NCC 2-231, NASA CR-177329. Ames Research Center, Moffett Field, CA (1984).

34. V.J. Krukonis. Presented at 1984 Annual Meeting, ACS, Philadelphia, PA (1984).

35. J.A. Hyatt. J. Org. Chem. 49: 5097 (1984).

36. D.D. Christianson, J.P. Friedrich, E.B. Bagley and G.E. Inglett. MAIZE: Recent progress in chemistry and technology. Academic Press, Inc. 1982.

37. D.D. Christianson, J.P. Friedrich, G.R. List, K. Warner, E.B. Bagley, A.C. Stringfellow and G.E. Inglett. J. Food Sci. 49: 229 (1984).

38. K.W. Quirin. Fette , Seifen, Anstrichmittel. 85(12); 460 (1982).

39. G.R. List and J.P. Friedrich. JAOCS. 62(1): 82 (1985).

40. A.C. Eldridge, J.P. Friedrich, K. Warner and W.F. Kwolek. J. Food Sci. 51(3): 584 (1986).

41. G.R. List, J.P. Friedrich and J. Pominski. JAOCS. 61(4): 1847 (1984).

42. E. Stahl, K.W. Quirin and H.K. Mangold. Fette, Seifen, Anstrichmittel. 83: 472 (1981).

43. E. Stahl. Fette, Seifen, Anstrichmittel. 84: 444 (1982).

44. F.M. Clydesdale and F.J. Francis. Principles of Food Science. Part 1 Food Chemistry. O.R. Fennema, Marcel Dekker, Inc., N.Y. 1976.

45. W.D. Loomis and J. Battaile. Phytochem. 5: 423 (1966).

46. A. Betschart and J.E. Kinsella. J. Agric. Food Chem. 21: 60 (1973).

47. F. Cremer and C. Van De Walle. Analytical Biochemistry. 147: 22 (1985).

48. M.A. Stahman. Ann. Rev. Plant Physiol. 14: 137 (1963).

49. K.P. Weder. Z. Lebensm Unters Forsch. 171: 95 (1980).

50. K.P. Weder. Food Chemistry. 15: 175 (1984)

51. D.A. Hammond, M. Karel, A.M. Klibanov and V.J. Krukonis. Applied Biochemistry and Bio-Technology. (1985).

Table 1   Amino Acid Composition of Algal Protein Isolate (Fraction #8A)

Amino Acid	(g/100g protein) FAO Standard	(g/100g protein) Scenedesmus obliquus
ASP		8.0
Glu		8.3
Ser		3.6
Gly		6.1
His		1.8
Arg		5.8
Thr*	4.0	4.6
Ala		8.3
Pro		5.8
Tyr		3.5
Val*	5.0	7.3
Met*	2.7	2.2
Cys		7.0
Ile*	4.0	5.0
Leu*	7.0	11.1
Phe*	3.4	6.0
Trp*	1.0	a
Lys*	5.5	5.7
%Essential	32.6	42.0

*Essential amino acid. a. not reported here.

Table 2   Nutritional Value of Algae in Comparison with Some Representative Food Proteins (from /29/).

	PER	BV	NPU	C.S.	limiting a.a.
Egg	3.8	87-97	91-94	100	none
Soybeans	0.7-1.8	58-69	48-61	69	S
Rice	1.9	75	70	57	Lys.
Corn	1.2	60	49-55	55	Lys.
Wheat	1.0	52	52	57	Lys.
Algae [a]	3.21	81	68	57 [b]	Met. Trp.

(a), Drum-dried Scenedesmus obliquus (from /31/).
(b), from /51/.
PER, Protein Efficiency Ratio.
BV, Biological Value.
NPU, Net Protein Utilization.
C.S., Chemical Score.
S, Sulfer-containing amino acid.

Table 3   Yield (%), Protein Concentration (%) Protein Recovery (%) Solubility (%) of Various Algae Fractions Without Enzymatic Treatment.

Fraction #	Yield (%)	Protein Concentration (%)	Protein Recovery (%)	Solubility (%)
2 (algae flour)	100	52.6	100	---
4 (algal crude protein)	65	57.6	71.2	---
6 (algal protein concentrate)	51.6	53.4	52.4	63
8A (algal Protein Isolate)	36.6	70.5	49.1	20
8B (SC-CO$_2$ Protein Isolate)	44.6	66.5	56.4	45
8C (SC-CO$_2$ + EtOH Protein Isolate)	33.0	69.1	43.3	41