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Utilization of Non-Conventional Systems for Conversion of Biomass to Food Components

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CELSS

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A. Introduction:

The subject of our study has been the investigation of potential use of micro-algae in yielding useful macronutrients (primarily proteins and lipids) for the CELSS. We have previously reported the isolation and characterization (amino acid analysis and electrophoretic studies SDS-PAGE and IEF) of algal proteins from green algae (Scenedesmus obliquus) grown under controlled conditions (Nakhost et al. 1987). Characterization of algal proteins revealed a high content of essential amino acids leucine, valine, phenylalanine and lysine. The algal lipids showed high content of total unsaturated fatty acids. To optimize the removal of algal lipids and pigments and to minimize protein denaturation, we used supercritical fluid (SCF) extraction using carbon dioxide with and without ethanol as a co-solvent, which resulted in more efficient removal of algal lipids and improved water solubility of protein isolate.

B. Work Accomplished:

The present work was focused on two areas; 1) Determination of chemical composition of blue-green alga, Synechoccus 6311 which provided by Dr. Packer (U.C. Berkeley); 2) Large scale preparation of

protein isolate from green algae, S. obliquus and its incorporation into food products.

1. Study on Blue-Green Algae Synchococcus 6311:

Methodology:

Synechococcus 6311 was grown in KMC medium, in a two litre Bethesda Research Laboratories Airlift Fermentor at 30°C, 150 u $ES^{-1}m^{-2}$ light (using Bethesda Research Laboratories 2201 LB day light white 300-700nm) with an airflow rate of two litres/min, supplemented with 0.5% CO_2. 200ml aliquots were withdrawn daily, the fermentor volume made up by an addition of 200ml of sterile medium. Cells were centrifuged at 10,000xg/10min and resuspended to 2ml in KMC medium supplemented with 10mM Tes buffer pH 7.0.

Cells grown under controlled conditions were ruptured by the method previously used for S. obliquus (Fig. 1). Comparison of the scanning electron micrographs (SEM) of intact and homogenized cells (Fig. 2) indicated the rupturing of the cell walls as a result of homogenization. Upon freeze-drying, the algal flour was used for determination of algal chemical composition (Fig. 3). Microkjeldahl method (A.O.A.C., 1980) was used to determine the protein concentration of algal flour.

Extraction of lipids and pigments from blue-green algal flour was achieved using boiling ethanol in a Soxhlet apparatus. Fatty acid composition of the lipid fraction was determined by gas-liquid

chromatography (GLC), the details of the procedure was reported previously (Choi et al., 1987). Determination of nucleic acids (DNA and RNA) was achieved using the modified procedure of Schmidt-Thannhauser (1945) and Burton (1968). Amino acid composition of protein fraction was done using "Pico-Tag" method (Bidlingmeter et al., 1984). The method is based on derivatizations of amino acids with Phenylisothiocyanate (PITC) and measurement of the absorbance at 254 nm. Ethanol (EtOH) and water extractions of the algal flour were performed (Fig. 4). Ethanol extractions (boiling ethanol for 3 hours in a Soxhlet apparatus) yielded a green extract and a blue-gray residue. The residue was air dried (room temperature) and dissolved in water and filtered to obtain a clear solution. Absorption spectra of the ethanol extract (Spectra D) and dissolved blue-gray residue (Spectra B) were obtained. Water extraction of algal flour resulted a dark blue extract and a residue. The residue was extracted with boiling ethanol (for 3 hrs in a Soxhlet apparatus). This resulted in a green ethanol extract. The absorption spectra of water extract (Spectra A) and ethanol extract (Spectra C) were obtained.

Results and Discussions:

Major chemical components of freeze-dried algal flour from Synechococcus 6311 are shown in Table 1. The comparison of the composition of Synechococcus 6311 with that of Scenedesmus obliquus showed similar values for proteins, lower values for nucleic acids

and "lipids" plus "lipid-soluble pigments" for Synechococcus 6311. Our values for Synechococcus 6311 is in good agreement with values cited in the literature for prokaryotic micro-algae (Robinson and Toerien, 1982). The fatty acid composition of total lipid fraction is shown in Table 2. The main fatty acids in Synechococcus 6311 were $C_{16:1}$, $C_{16:0}$, and $C_{18:1}$. We found lower amount of total unsaturated fatty acids (TUSFA) for Synechococcus 6311 as compared to S. obliquus. The only unsaturated fatty acids found were the monounsaturated ones. Our results are in a good agreement with those reported (Holton and Blecker, 1970; Kenyon, 1972; Piorreck et al., 1984).

To determine the nutritional value of the algal proteins the amino acid composition was obtained. The aminograms of Synechococcus 6311 algal flour and of amino acid standards (Pierce Standard H) are shown in Figure 5. There is no data on amino acid composition of Synechococcus 6311 cultivated under the controlled conditions. However, there are reports on amino acid content of prokaryotic algae. The total value for the essential amino acids content of Synechococcus 6311 (32.3%) compared with that of FAO standard (32.6%) Table 3. We found high leucine and low methionine and tryptophan contents in Synechococcus 6311. Similar results for prokaryotic algae were reported (Soeder, 1976; Robinson and Toerien, 1982). The pigments of cyanobacterium Synechococcus 6311 were studied (Nakhost and Karel, 1987). Figure 6 shows the comparison of the colors of freeze-dried blue-green and green algae. Water

extraction (for water-soluble pigments) and ethanol extraction (for lipid-soluble pigments) of algal flour were performed. The major pigments in blue-green algae are chlorophyll A; c-phycocyanin; allophycocyanin; c-phycoerythrin; beta-carotene and several xanthophylls (Bold and Wynne, 1985). The amount of these pigments for Spirulina Platensis (another cyanobacterium) is reported to be as following; phycocyanin 1-3%, chlorophyll A 1%, beta-carotene 0.2% and xanthophylls 0.2% of the dry weight (Tel-or et al., 1980). Water extraction of algal flour (freeze-dried ruptured cells) resulted in extraction of biliprotein phycocyanin (a photo synthetic water-soluble blue pigment). Phycocyanin also functions as a reserve source of nitrogen and amino acids (Tel-or et al., 1980).

The absorption spectra of water-extracted pigmets from algal flour (Spectrum A) and of water-extracted pigments from blue-gray residue (Spectrum B) are compared in Fig. 7. Spectrum B clearly shows the predominance of c-phycocyanin with absorption maximum at about 620 nm (Tel-or et al., 1980). Because chlorphylls are water- and lipid-soluble, the coelution of chlorophyll A (absorption maxima at about 410 and 670 nm) with phycocyanin is indicated by Spectrum A. Absorption spectra of ethanol-extracted pigments from water-extracted residue (Spectrum C) and from algal flour (Spectrum D) are shown in Fig. 8. Overlapping of the absorption maxima of these ethanol-extracted pigments resulted in a spectrum which is not specifically representative of any individual pigment.

In summary, the study of blue-green algae and determination of its chemical composition showed that Synechococcus 6311 contains 52.3% protein (dry weight basis) which is high in essential amino acids leucine, threonine and phenylalanine as compared to FAO standards. Relatively lower amount of nucleic acids in blue-green algae (3.6% of dry weight vs. 6% in green algae) is an advantage in processing the blue-green algae.

2. Fabrication of Food Products Containing Scenedesmus obliquus (Green Algae) Protein Isolate:

Initial steps towards preparation of model foods for potential use in CELSS were taken. Our goal was to fabricate food products which contain isolated algal macronutrients such as proteins and lipids and also some components derived from higher plants including wheat flour, soy flour, potato powder (flakes), soy oil and corn syrup.

Large scale preparation of protein isolate from Scenedesmus obliquus was achieved. Ethanol extraction of lipids and pigments from protein concentrate was completed overnight. The isolate was air dried (at room temperature) and ground to a fine powder. The isolate which had a light olive color (Fig. 9) was used for incorporation into food products.

The food items that we prepared were; bran muffins (ingredients: wheat flour, bran, algal protein isolate, milk, eggs,

butter, molasses, salt, sugar, baking soda and grated orange rind); chocolate chip cookies (ingredients: wheat flour, algal protein isolate, sugar, brown sugar, butter, eggs, chocolate chips, baking soda and vanilla); Fettuccine (spinach noodles imitation) (ingredients: wheat flour, algal protein isolate, eggs, vegetable oil, water and salt). The baking or cooking recipes were obtained from the book, "Joy of Cooking" (I.S. Rombauer and M.R. Becker, 1981). As a comparison, we also incorporated commercially available spray-dried Spirulina (Earthrise™, The Earthrise Co., Berkeley, CA) into food samples. The amounts of incorporated algal protein isolate for muffins and cookies were 5% of the total flour weight (i.e. 95% wheat flour) and for fettucini we had 5 and 10% isolate. Same percentages were used for commercial spirulina samples. The control samples were made without algae. Considering the protein concentration of algal isolate to be 70% (as we reported previously, Nakhost et al., 1987) and that of wheat flour about 12%, the percentage of plant proteins supplied from algae, for each product was calculated and is shown in Table 4.

Sensory Evaluation:

We conducted some preliminary sensory evaluations. The members of our sensory panel were graduates and undergraduates in our department. For testing the acceptability of the food samples the panelists were asked to taste the food materials and write their descriptive terms which represented their rating of the food

samples. Also scores 1 to 4 were assigned to the following terms; 1 = poor, 2 = fair, 3 = good and 4 = very good. The results are shown in Table 5. The description of the taste of the control cookies and muffins was "regular" (standard). The panel described the taste of the cookies containing protein isolate as slightly grassy (algal flavor) and the taste of the cookies containing commercial spirulina as a slightly bitter "over processed" flavor. The spray-drying of the commercial spirulina (Switzer, 1980) could have probably contributed to such a burnt flavor. Similar taste descriptions were given to muffins, but the tastes were stated to be stronger in muffins. This is because in cookies the above tastes were masked by the chocolate and vanilla flavors. The greenish color of the cookies and muffins was not found to be objectional. However, the color of the S. obliquus cookies (Fig. 10) and muffins (Fig. 11) were lighter than those of commercial spirulina. The texture of all the cookies and muffins were moist and pleasant.

Cooked fettuccine was tasted with and without (plain) cheese sauce (Alfredo sauce). The taste of the plain control (Lambert's Spinach Fettuccine) was described as spinachy or slightly grassy. The plain 5% S. obliquus fettuccine had an almost bland taste and the 10% one had a slightly grassy flavor. For commercial spirulina (5%) plain fettuccine an after taste was detected which was stronger as the level of algae increased to 10%. When the panelists had a cheese sauce with the fettuccine the scores were improved. Fettuccine containing 5 and 10% S. obliquus protein isolate were

rated very good and no grassy taste could be detected. When commercial spirulina fettuccine (5 and 10%) tasted with the sauce the after taste was still detectable. The color of the fettuccine made from isolate was lighter than that made from commercial spirulina (Fig. 12 and 13) and compared well with the green color of spinach fettuccine (control). The texture of all different fettuccine products were satisfactory.

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Table 1

Chemical Composition Calculated on a Dry Weight Basis

Algal Flour Freeze-Dried	Proteins %	Nucleic Acids %	Lipids and Lipid-Soluble Pigments (%)
Scenedesmus obliquus (green alga)	52.6	6.0	15.0
Synechococcus 6311 (blue-green alga)	52.3	3.6	12.6

Table 2 Fatty acid composition (% total of fatty acids) of total lipid fraction of Synechococcus 6311.

Fatty Acids	Synechococcus 6311
12:0	0.12
14:0	1.27
14:1	1.58
16:0	38.21
16:1	46.74
16:2	-
16:3	-
16:4	-
17:0	0.24
17:1	0.45
18:0	2.35
18:1	9.04
18:2	-
18:3(γ)	-
18:3(α)	-
18:4	-
T.S.F.A.	42.19
T.U.S.F.A.	57.81
EFA	0

TSFA = Total saturated fatty acids
TUSFA = Total unsaturated fatty acids
EFA = Essential fatty acids

Table 3

Amino Acid Composition of Algal Flour (Fraction #2) from Blue-Green Algae (*Synechococcus* 6311)

Amino Acid	FAO Standard (g/100g protein)	*Synechococcus* 6311 (g/100g protein)
ASP		6.7
GLu		15.0
Ser		5.7
Gly		4.9
His		0.1
Arg		5.5
Thr*	4.0	4.7
Ala		11.1
Pro		2.9
Tyr		3.0
Val*	5.0	4.6
Met*	2.7	0.9
Ile*	4.0	3.3
Leu*	7.0	11.2
Phe*	3.4	4.4
Trp*	1.0	a.
Lys*	5.5	3.2
%Essential	32.6	32.3

*Essential amino acid.

a. Not reported here.

Table 4

The Percentage of Plant Proteins Supplied from Algae (*Scenedesmus obliquus*)

Food Product	% Algal Proteins
Cookies (5% isolate)	23.6
Bran muffins (5% isolate)	19.6
Fettuccini: (5% isolate)	23.5
(10% isolate)	39.3

Table 5

The Results of Sensory Evaluation of Algal Protein Isolate (*Scenedesmus obliquus*) Incorporated Food Products

Food Product	Descriptive Rating
COOKIES:	
Protein isolate	slightly grassy (algae) flavor
Comm. spirulina	slightly bitter "over processed" flavor
Control	regular (standard) flavor
MUFFINS:	
Protein isolate	mild grassy flavor
Comm. spirulina	burnt after taste
Control	regular (standard) flavor
FETTUCCINE:	
Plain:	
5% Protein isolate	almost bland
10% Protein isolate	slightly grassy flavor
5% Comm. spirulina	after taste
10% Comm. spirulina	strong after taste
Control	spinachy, slightly grassy (standard) flavor
With sauce:	
5% Protein isolate	very good flavor
10% Protein isolate	very good flavor
5% Comm. spirulina	mild after taste
10% Comm. spirulina	after taste
Control	very good (standard) flavor

Table 6

Some Food Products that can Potentially Contain Various Levels of Incorporated Algal Proteins and/or Lipids

Food Product	Descriptions
Baked goods:	
Breads	Dark specialty bread (caramel added)
Doughnuts	Chocolate flavored
Muffins	Cinnamon flavored
Crackers	Cinnamon flavored
Brownies	
Cookies	Chocolate flavored
Cereals	Cracked wheat
Pizza	Topped with tomato sauce
Pasta	Spinach pasta imitation
Soups	Pea or Spinach flavored
Tofu	Prepared by precipitation of soy milk (dispersion of soy protein isolate in water) using calcium sulfate or a comparable coagulating agent.
Deserts	Gelatin desert, lime flavored, pudding, chocolated flavored
Dips	Guacomole type
Drinks	Vegetable juices
Sauces	Tomato sauce (prepared from freeze-dried tomato paste supplied from earth)
Gravies, Spreads Dressings	

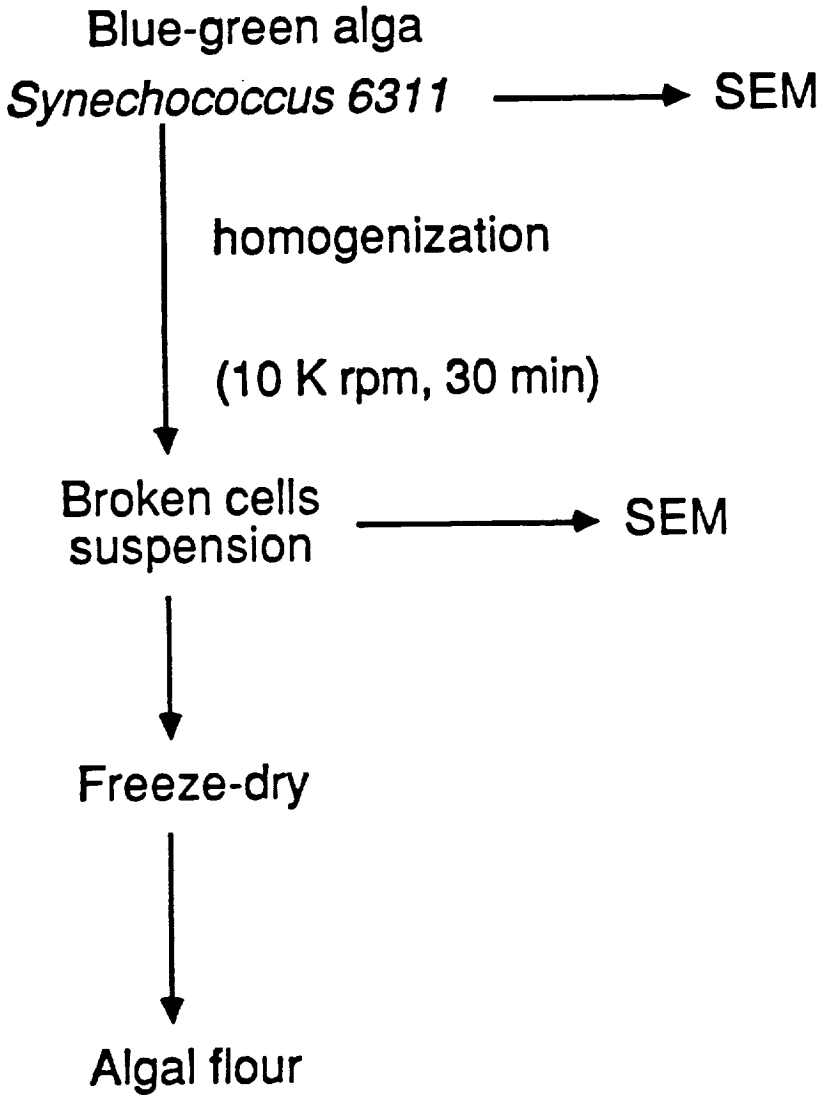


Figure 1. Procedure for prepartion of algal flour from blue-green algae Synechococcus 6311.

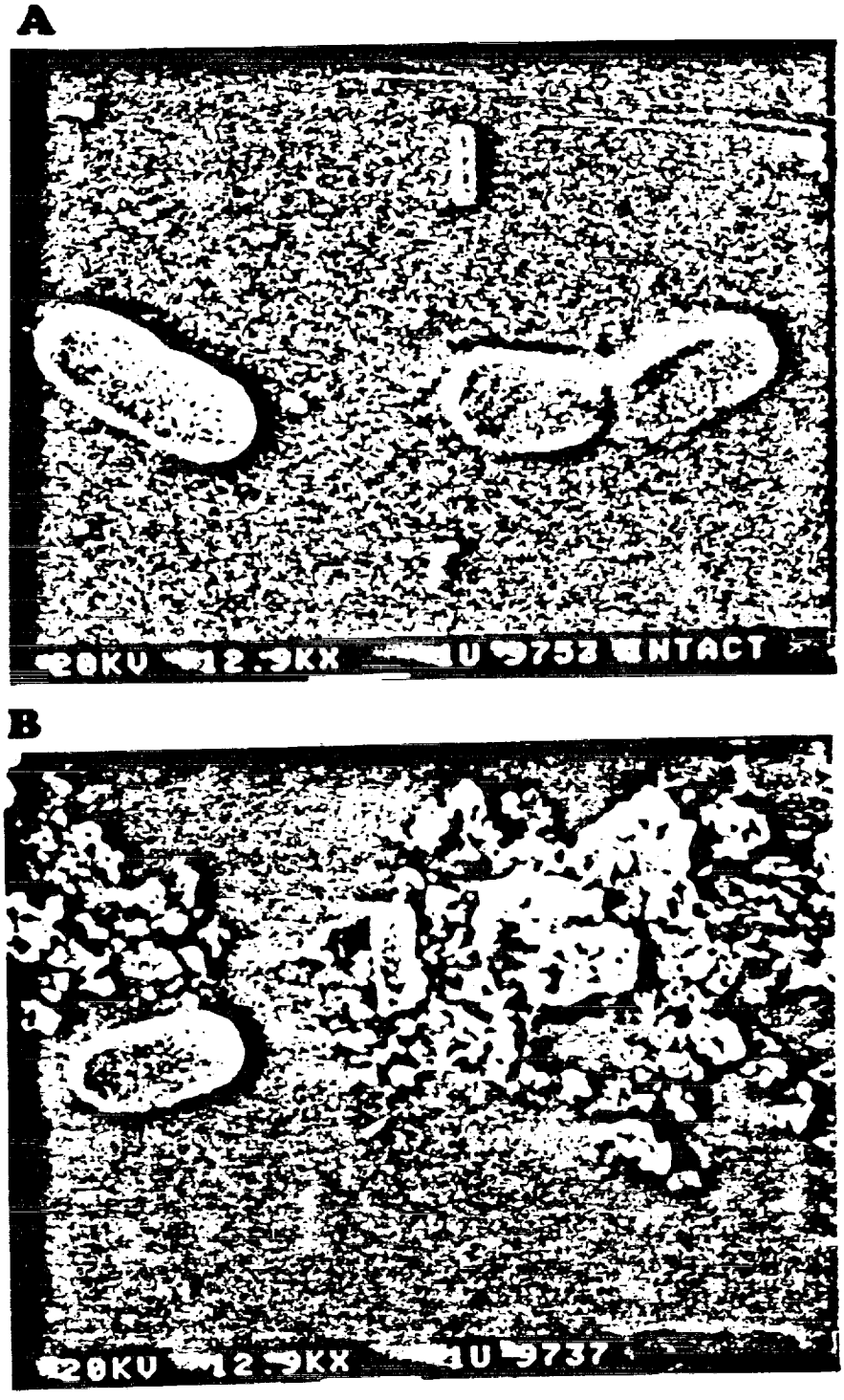


Figure 2.

Scanning electron micrograph of blue-green algae *Synechococcus* 6311 (A) and homogenized cells for 30 min (B).

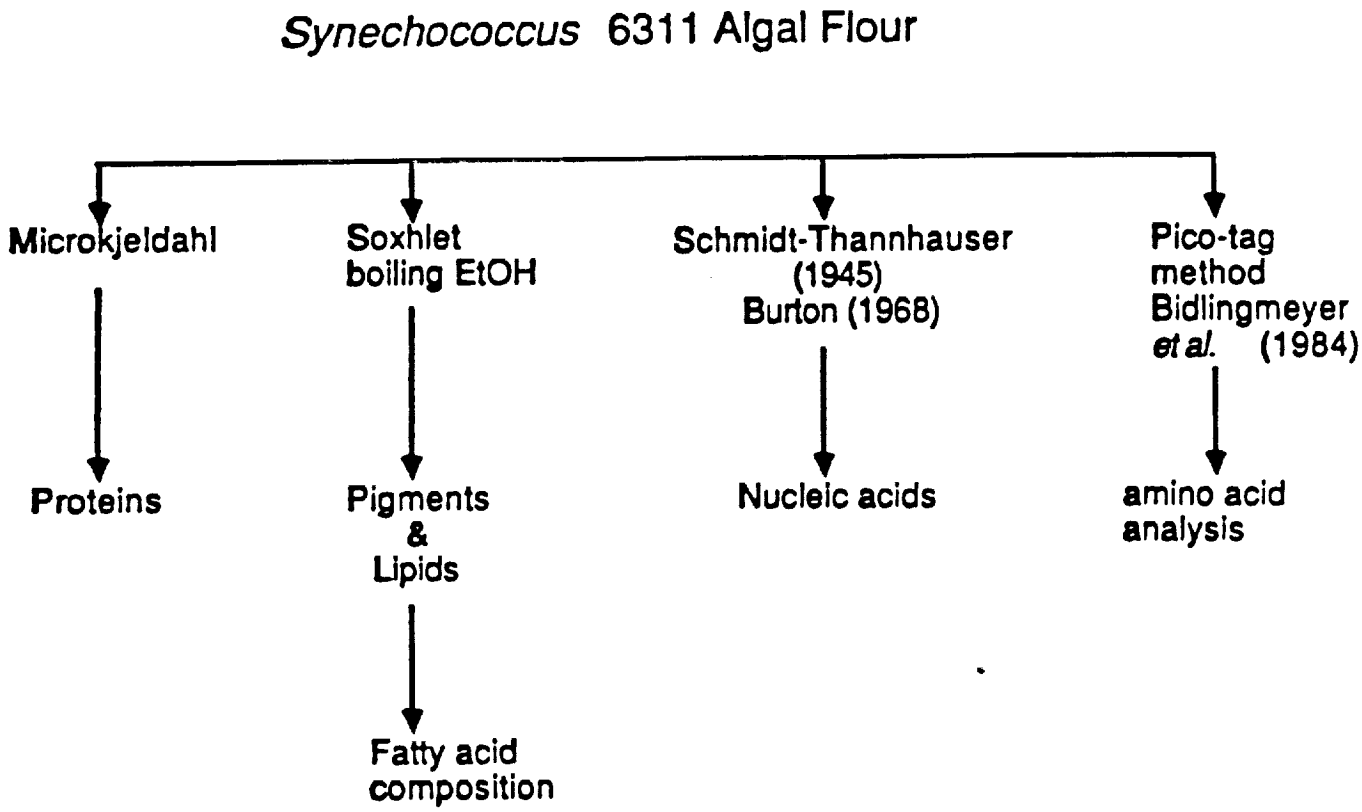


Figure 3. Flow chart for determination of chemical composition.

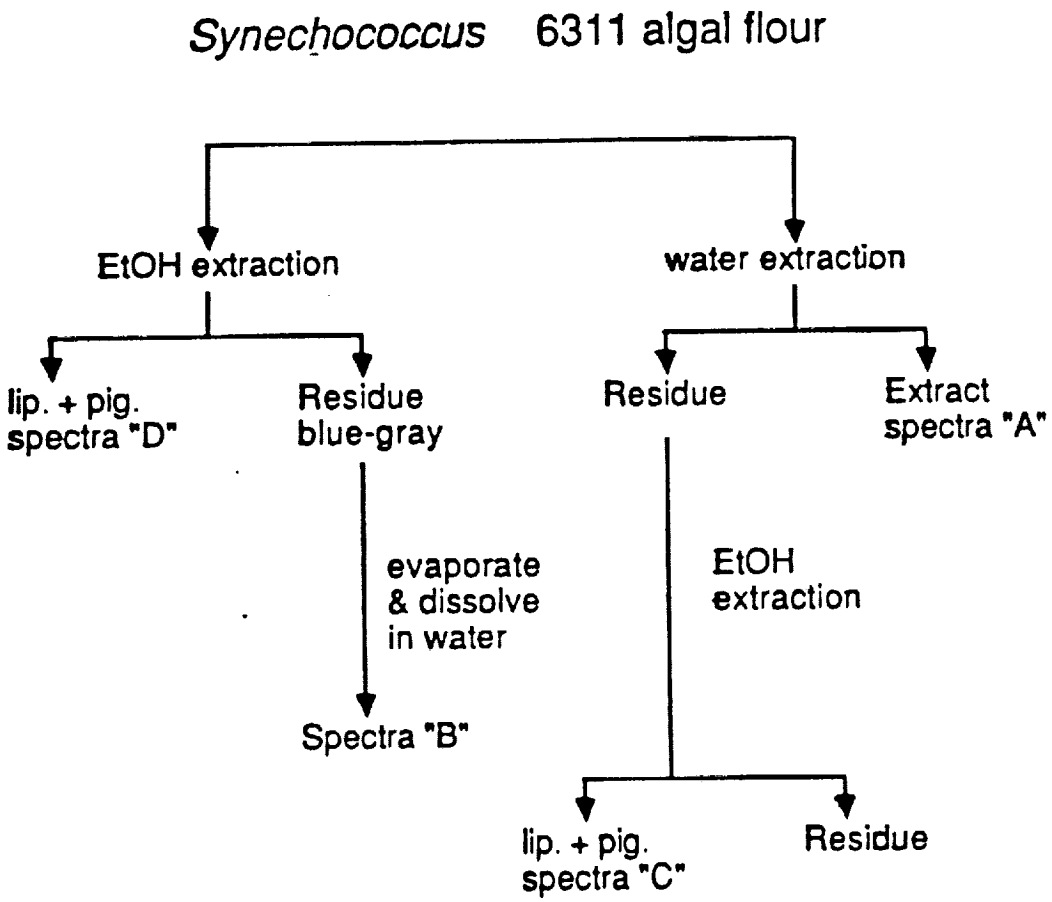


Figure 4. Procedure for extraction of lipids and pigments from Synechococcus 6311 algal flour.

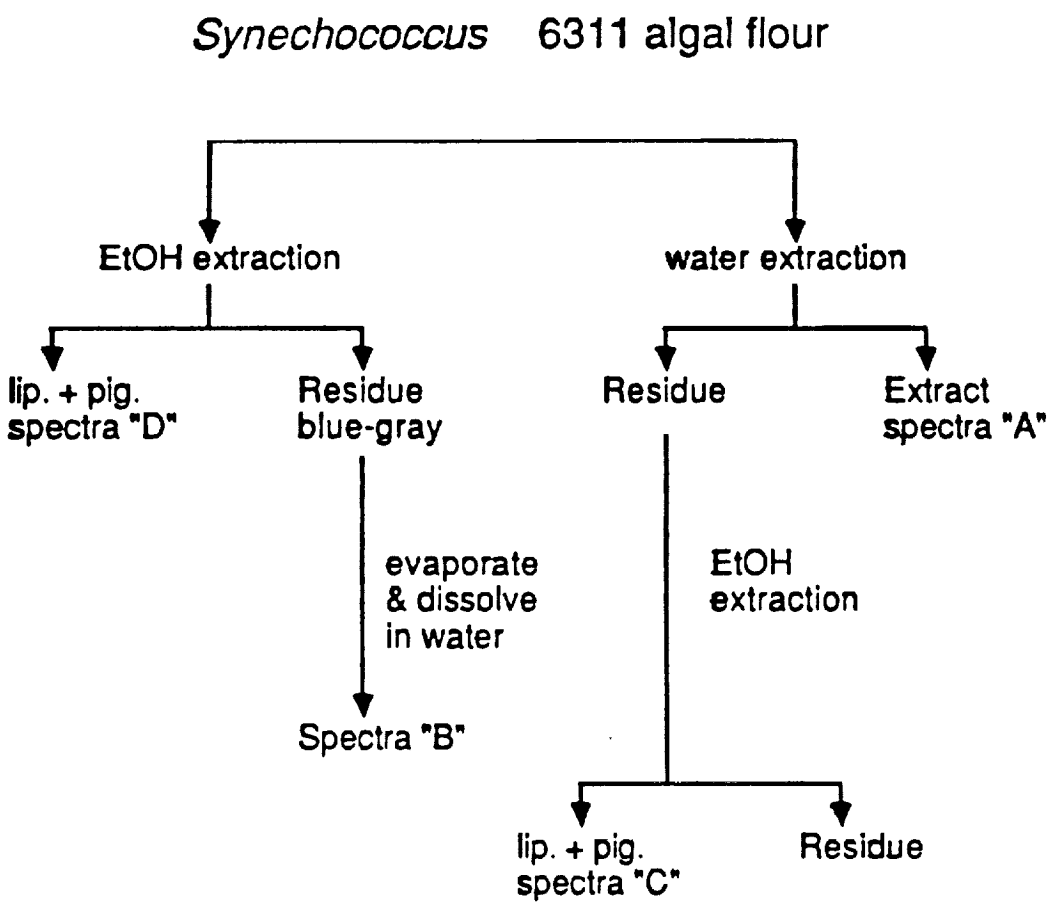


Figure 4. Procedure for extraction of lipids and pigments from Synechococcus 6311 algal flour.

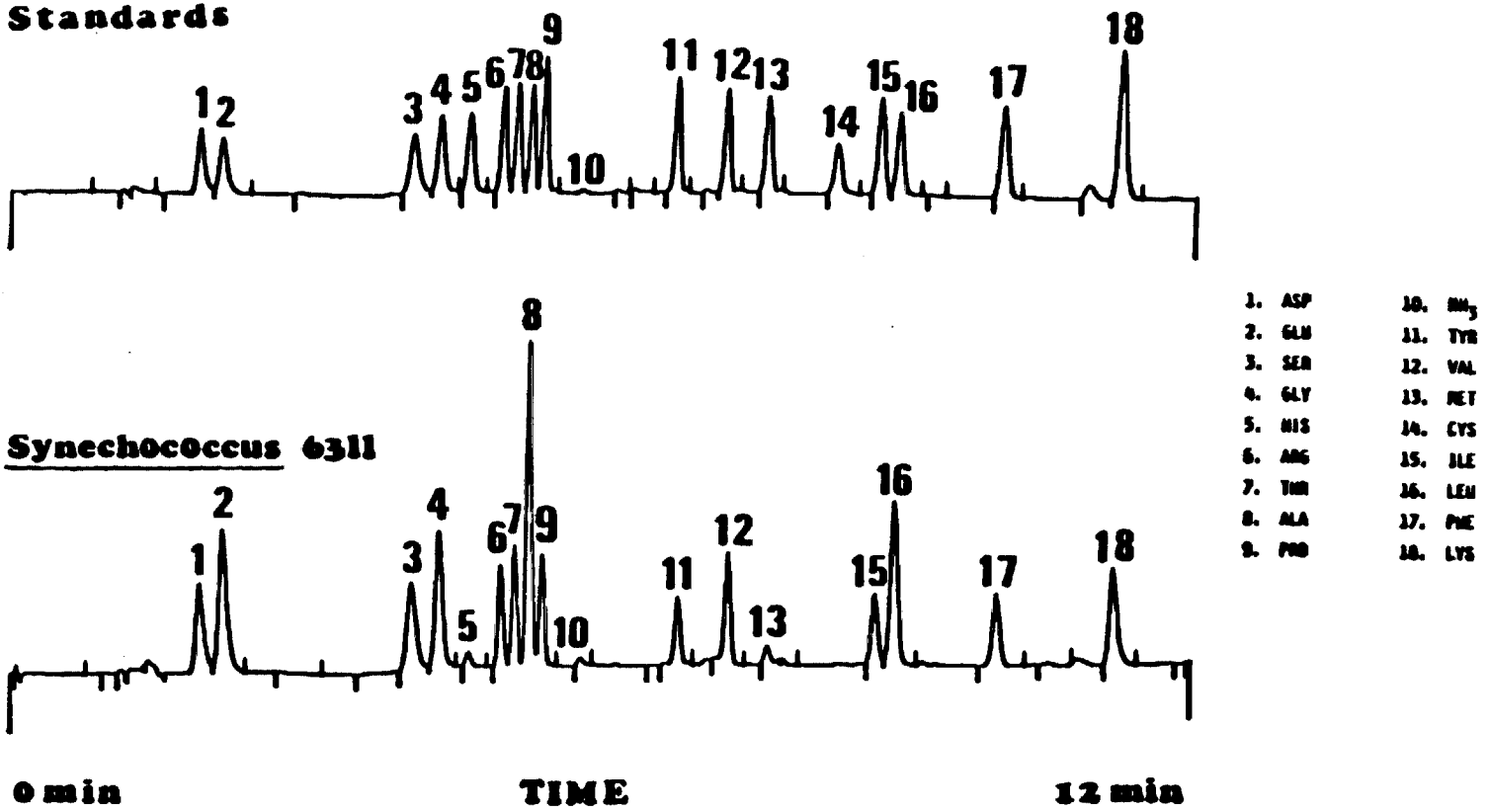


Figure 5. HPLC chromotagrams of Synechococcus 6311 algal flour and of amino acid standars (Pierce Standard H).

Figure 6. Freeze-dried blue-green, Synechococcus 6311 and green, Scenedesmus obliquus algal flours.

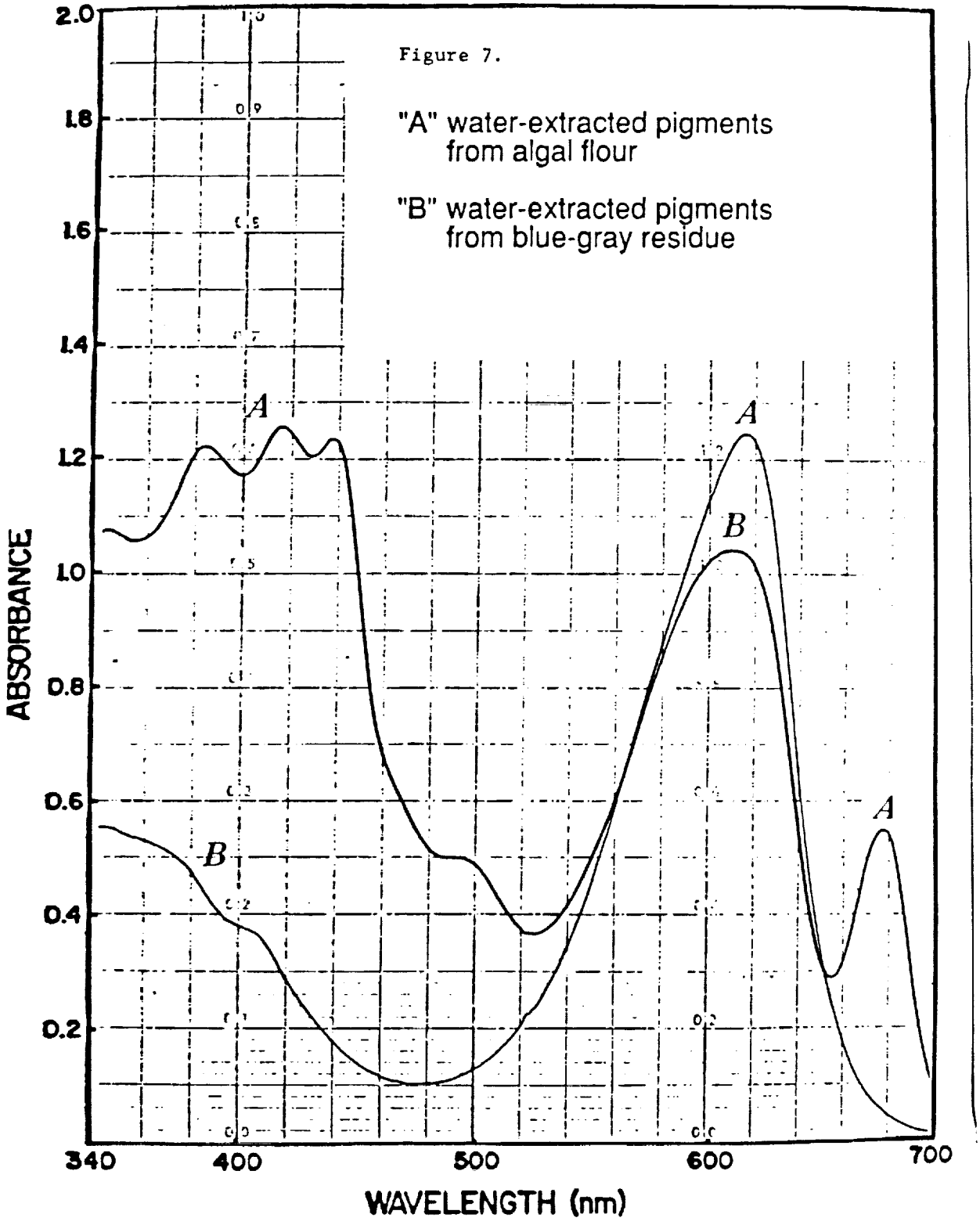

"A" water-extracted pigments from algal flour
"B" water-extracted pigments from blue-gray residue
A
B
A
B
A
ABSORBANCE
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
340
400
500
600
700
WAVELENGTH (nm)

Figure 7.

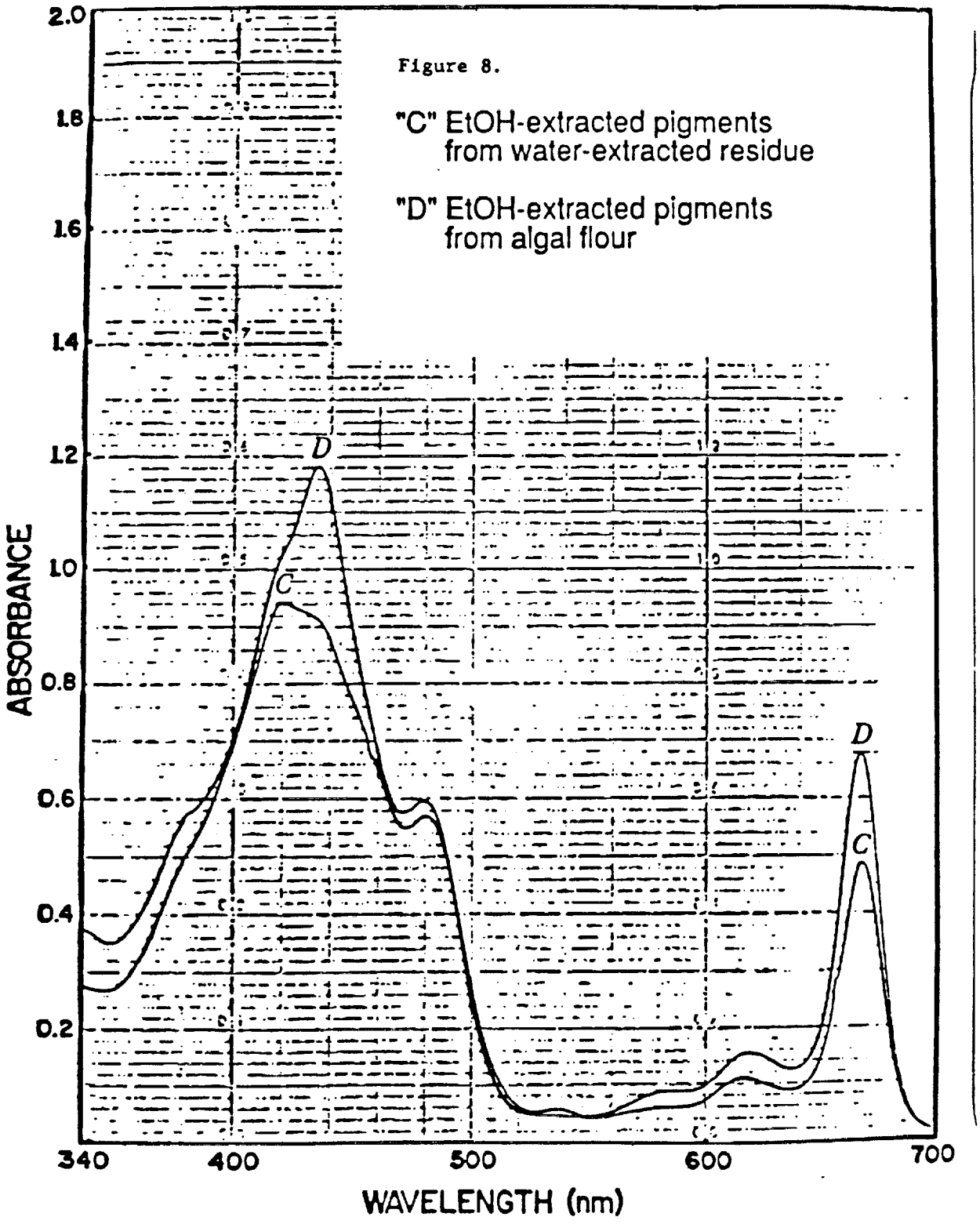


Figure 8.
"C" EtOH-extracted pigments from water-extracted residue
"D" EtOH-extracted pigments from algal flour
ABSORBANCE
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
D
C
D
C
340
400
500
600
700
WAVELENGTH (nm)

Figure 9. S. obliquus protein concentrate (at left) and protein isolate (after removal of the pigments and lipids).

Figure 10. Comparison of "S. obliquus protein isolate" and "Commercial Spirulina" incorporated chocolate chip cookies (5% algae and the balance wheat flour) with control (without algae).

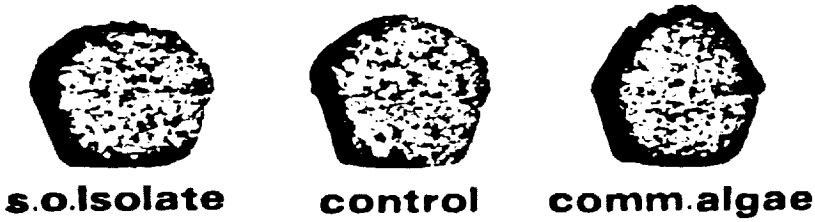


Figure 11. Comparison of S. obliquus protein isolate and "Commercial Spirulina" incorporated bran muffins (5% algae and the balance wheat flour) with control (without algae).

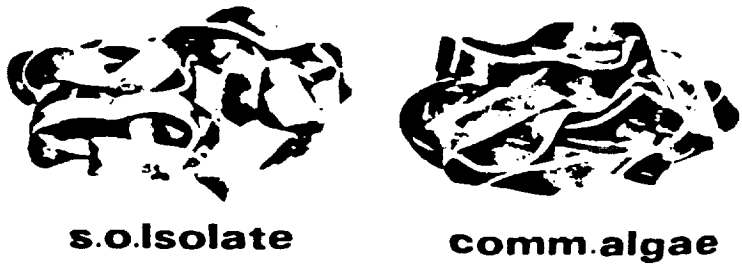
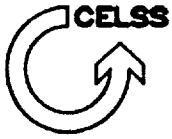


Figure 12. Comparison of "S. obliguus protein isolate" and "Commercial Spirulina" incorporated noodles (5% and 10% algae and the balance wheat flour).

Figure 13. Comparison of "S. obliquus protein isolate" and "Commercial Spirulina" incorporated noodles (5% algae and the balance wheat flour) with control (Lambert's Spinach fettuccini).



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16. Abstract

This report describes work accomplished in investigating the potential use of micro-algae in yielding useful macronutrients for closed ecological life support systems in space habitats. Analysis of the chemical composition of the blue-green alga Synechoccus 6311 was done in the present work, and was compared to values found in previous work on the green alga Scenedesmus obliquus. Similar values were obtained for proteins, and lower values for nucleic acids and lipids. A second part of the work involved fabrication of food products containing various levels of incorporated algal (S. obliquus) proteins and/or lipids. Protein isolate was incorporated into a variety of food products such as bran muffins, fettuccine (spinach noodle imitation), and chocolate chip cookies. In the sensory analysis the greenish color of the bran muffins and cookies was not found to be objectionable. The mild spinachy flavor was less detectable in chocolate chip cookies than in bran muffins. The color and taste of the algae noodles were found to be pleasant and compared well with commercially available spinach noodles.

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