

Full Text for the paper Presented at the 1st International Congress of Food Science and Technology, September, 1962, London

UTILIZATION OF CHLORELLA FOR FOOD

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INTRODUCTION

When we envisage the future of Japan with but a small land and limited natural resources on one hand and with a population about to reach one hundred million on the other, where to find protein resource poses one of the most serious problems to us. Our natural conditions are not favourable for stockraising, consequently its rapid development to meet the demand cannot be expected. We are not optimistic about the future of our fishing industry either in the face of deterioration of coastal fishing grounds and the ever increasing severity of international limitation on our deep-sea fishing. One may say that increased products of these animal proteins are important indeed, but utilization of vegetable proteins of high quality such as soy bean protein which is comparable with animal proteins, should be of equal importance. However, our supply of soybean is again heavily dependent on import. Pressed by these situations we have attempted to culture chlorella and torula yeast on a large scale, and accumulated basic data for rational utilizations of these resources for food. A part of these results is presented in the present paper.

Studies on the utilization of chlorella for various purposes trace back to war time researches during both of the world wars in Germany and the United States. After the last war, the systematic research by Milner (1948) and Spoehr and Milner (1949) revealed the possibility of utilizing the algae for food if produced on a large scale, and stimulated studies on its mass culture in various countries. In Japan Tamiya's group (Tamiya, Hase, Shibata, Mituya, Iwamura, Nihei and Sasa, 1953 and Tamiya, 1957) have succeeded in obtaining an yield of 15 to 19 grams of chlorella or scenedesmus per day per square meter in an open outdoor field with circulation.

On the other hand studies on the nutritional aspect of chlorella, conducted in Japan by the Committee on The Essential Amino Acid Studies were rather disappointing since the dried algae was found to be hardly digestible and to cause diarrhea and other gastro-intestinal troubles due to its tough cell wall. Moreover, its dark greenish colour, due to an extremely high content of chlorophyll, and a strong unpleasant flavour seem to disqualify it as food. The results of experiments by Powell, Nevels and McDowell (1961) fully concur with the above findings. They have reported that chlorella, supplemented to any food gave a bitter, strong spinach-like flavour, and caused prominent gastro-intestinal symptoms. Methanol extraction, which has been employed to decolour the algae, has not been found effective in improving the digestibility either. Consequently for a satisfactory utilization of chlorella for food, the following must be achieved.. (1) Removal of the cell wall. (2) Removal of the strong colour and flavour to make it more palatable. (3) Thorough utilization of various components by separately extracting precious components such as folic acid, vitamin B12 chlorophyll, carotene etc. beside protein.

DISRUPTION OF THE ALGAL WALL

In these lines various treatments were tried to break the cell wall as indicated in Table 1 (Mitsuda, Kawai, Shikanai and Nakazawa, 1959)

Table 1

The extractabilities of algal protein by various treatments

Treatment	Fresh chlorella			Lyophilized chlorella		
	Extract		Residual -N(%)	Extract		Residual N(%)
	Protein - N(%)	Non Protein- N(%)		Protein	Non- Protein -N	

					(%)	
Water extraction	0	0	100,0	3,2	11,0	83,0
Hot water extraction				6,5	16,1	77,4
Ball mill treatment, water extraction				5,6	15,9	68,3
Grinding, water extraction	4,7	7,5	87,8	3,4	16,1	70,5
Grinding, 0,1% NaOH extraction	6,6	14,7	78,7			
Grinding, 1,0% NaOH extraction	20,5	18,1	61,4			
Phosphate buffer extraction, pH 7,0		1,4	97,3			
Phosphate buffer extraction, pH 10.0	1,4	2,1	96,5	7,2	9,3	83,5
Freezing melting with liquid air, water extraction	21,0	43,0	36,0			
Freezing melting with liquid air, hot water extraction	7,0	24,2	68,6			
Alkaline extraction, 0.1% NaOH				20,5	10,7	63,5
Alkaline extraction, 0,5% NaOH	22,1	8,1	69,8	23,5	14,0	62,5
Alkaline extraction, 0,1% NaOH (24 hrs)				25,3	13,4	61,6
Alkaline extraction, 0,5% NaOH (24 hrs)				29,5	19,5	51,6
Butanol Treatment	25,1	17,9	57,0			
Autolysis (toluol 1:1, 37°, 24hrs)	17,5	25,0	57,1	7,5	22,2	70,3
Autolysis butanol treatment	23,6	36,4	40,0			

In the butanol treatment, which is shown in Scheme 1 a 0.4 volume of *n*-butanol was added drop wise to an algal suspension then stirred. On centrifuging the suspension, four layers separated, namely from the top, a butanol layer (A), a broken cell layer (B), a water layer (C) and an unbroken cell layer (D). The butanol layer contained

lipids and pigments. In the water layer 16% of the total algal nitrogen was extracted, while a larger part of the nitrogenous materials remained with the cells and went into the layers B and D. The combined layers B, C and D were mixed and an equal volume of an ethanol-ether mixture (4:1) was added drop-wise and stirred. Cell particles were dispersed into the medium by this treatment and more nitrogenous materials could be extracted. The autolysis was carried out by incubating a well stirred mixture of equal volumes of toluol and fresh algal paste or suspension at 37 to 38° for a period indicated. Then 10 volumes of water or other solvents were added and stirred at room temperature. Extract was separated from residues by centrifugation.

In the combination autolysis-butanol treatment, the above described autolysis was carried out first. After suspending the autolysate in 10 volumes of water, the butanol treatment was applied to it.

When one looks at the results summarised in Table 1, one will see the followings (Mitsuda and Shikanai, 1960). Water or buffer alone cannot extract nitrogenous material, unless chlorella has been pretreated by the above method.

A mechanical treatment such as grinding or repetition of freezing and melting is effective.

Extraction with an alkali solution was very effective. Nearly all the nitrogen could be extracted at a higher temperature or with a higher alkali concentration. However, proteins thus obtained is thickly coloured and could not be decoloured by extraction with organic solvents. Therefore, these proteins are of little use for food.

The butanol treatment alone is of comparable effectiveness with the freezing-melting or with the dilute alkali extraction. Proteins extracted by this method accounted for 25% of the total algal nitrogen and 29% of the total protein-nitrogen. This method seems to suit the present purpose because proteins as well as non-protein nitrogenous materials and pigments such as chlorophyll and carotene can be fractionally isolated, thus making an exhaustive utilization of the algae possible. When examined by electron microscopy the protein isolated by this method was completely free from cells and cell walls.

The autolysis works quite differently from the mechanical or chemical treatments. The result of this treatment strongly depended on the length and the temperature of incubation. Although a longer incubation increased the extractable nitrogenous materials, the yield of the protein decreased due to an accompanying degradation.

The combination of autolysis and butanol treatment gave more nitrogenous materials than either of these treatments alone.

Scheme 1 Preparation of cell-free chlorella protein

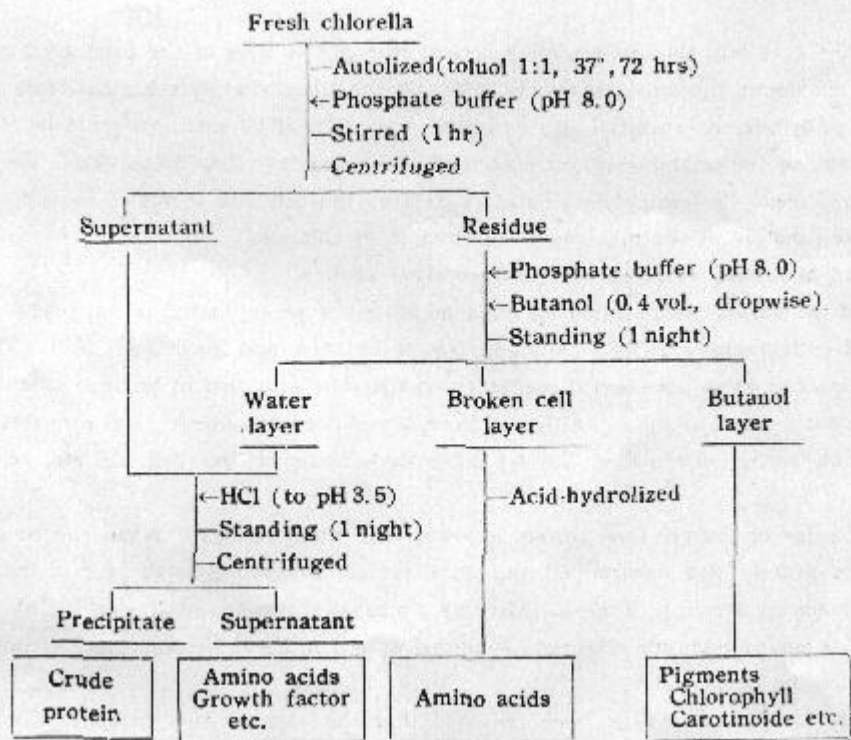


Table 2 Comparison of digestibilities and biological values of chlorella protein reported by several workers (a: present author, b: Tamura, c: Kandachi, d: Nakamura)

<u>Digestibility with trypsin <i>in vitro</i></u>			
Casein	96.9 ^a	99.5 ^b	91.8 ^{c***}
Cell-free chlorella protein*	92.7		
Cell-free scenedesmus protein**	93.1		
Dried chlorella	27.4	62.5-65.5	43.9
Decolored chlorella	75.1	26.3	
Lyophilized chlorella	59.7	27.6	
Fresh chlorella		46.2	
<u>Digestibility with rat <i>in vivo</i></u>			
Casein	94.8 – 97.5 ^a	95.9 – 98.0 ^b	
Cell-free scenedesmus protein**	85.6 – 87.4		
Dried chlorella	47.8 – 65.5	49.1 – 58.4	67.0-62.0 ^c
Decoloured chlorella		77.1 – 84.4	
Decoloured scenedesmus		66.6 – 73.0	
<u>Biological value</u>			
Dried chlorella	51.0 ^a	7.7 ^d	73.7-80.1 ^c
Decolored chlorella		26.0	
		25.4	70.7
		48.8	
		51.5	
Cell-free chlorella protein	60.9		

* Isolated by the autolysis-butanol treatment
 ** Isolated by the altered autolysis-butanol treatment
 *** The value with pepsin digestion

These results show that only 25% of the total nitrogen could be isolated as protein and the remaining 60 to 70% goes into the non protein fraction. Since 70 to 90% of the total algal nitrogen is of protein, the maximum protein yield is only 30%. On the other hand by shearing stress method of Fowden (1952) or by our concentrated alkali method practically all of nitrogen could be isolated, while the yield of any of the milder methods described was less than 60 to 70%. Therefore, a more drastic treatment preferably enzymatic to destroy the cell wall seems to be necessary to achieve a complete isolation of the nitrogenous substances of chlorella.

DIGESTIBILITY AND BIOLOGICAL VALUE OF THE CELL-FREE ALGAL PROTEIN

Digestibility of the various preparations of the algal protein were estimated *in vitro* with trypsin and are compared with casein in Table 2 (Mitsuda, Kawai, Shikanai and Murakami, 1959). The cell-free protein preparations show excellent digestibility comparable with that of purified casein, while the dried chlorella has a very low value. Although the hot methanol treatment gives an intermediate value, it is not high enough for a food, besides indigestible materials like cell wall still remain in the preparation.

The biological value of the cell-free protein is lower than that of casein. When the amino acid composition of the protein was determined, the algal protein was found to be poor in the sulfur containing amino acids as shown in Table 2. (Mitsuda, Shikanai, Yoshida and Kawai, 1961a). Since the sulfur containing amino acids are essential for animal nutrition, these become the limiting factor and result in the low biological value.

An interesting result has, however, been obtained that the algal protein, in spite of its lower biological value, is more effective in restoring the body weight of fasted rats. In interpreting this puzzling result, it should be understood that an increase in the body weight depends not only on the increase in the tissue protein but on

various other factors. And for a protein to be of a higher biological value does not necessarily mean to be more effective in increasing the body weight. As a matter of fact Fink and Ross (1952) also have reported that the chlorella protein is just as effective as casein in supporting growth of young rats. These results suggest a possible presence of some unknown growth stimulating substance in the cell-free protein preparation. On the other hand, we have confirmed the finding that the growth promoting substance for a lactic acid bacteria is present in the acid soluble fraction of chlorella (Figure 1). A similar substance was found to be present in torula yeast by us, and its composition and structure are now being investigated as a contribution to our microbiological industry (Figure 1). (Mitsuda, Shikanai and Yoshida, in press).

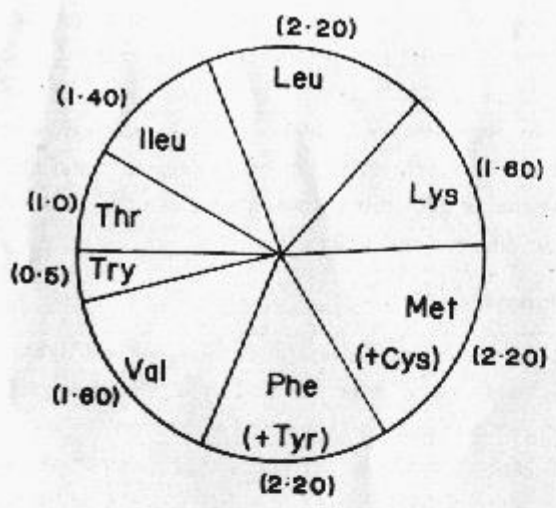
UTILITY OF THE ALGAL PROTEIN AS FOOD

The nutritive value of the algal protein itself is not very high. However, since the algal protein is rich in lysine, threonine and tryptophan (Table 3), while cereal proteins are usually poor in these essential amino acids, the algal protein is very useful to cover the weak point of the cereal protein (Mitsuda, Shikanai, Yoshida and Kawai, 1961b)

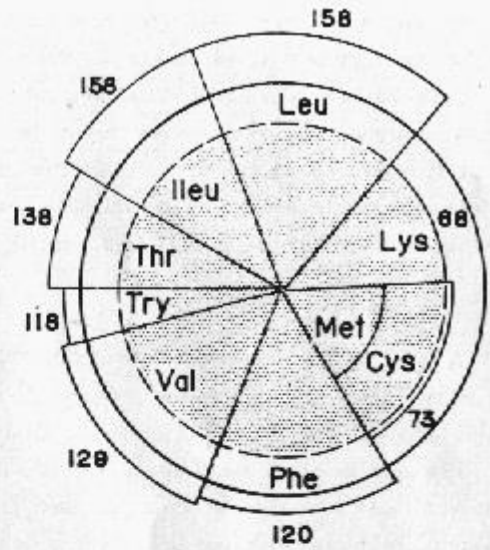
Although the standard intakes of the essential amino acids are still subjects of controversy, the standard proportion of FAO (1955) and the safe intakes, which are the double amounts of the minimum requirements, of Rose, Wixom, Lockhart and Lambert (1955) may be taken as reasonable standard for the proportion and the amounts of the essential amino acids respectively.

As a method to illustrate the nutritive value of a protein, while taking these two factors, proportion and amounts, into consideration in a simple, clear-cut way, we have devised a circular diagram representation and name it "circular explanatogram of the essential amino acids" (Mitsuda *et al.* 1961b). The central angle of the circular diagram is divided in proportion to the amounts of the safe intakes of the eight essential amino acids.

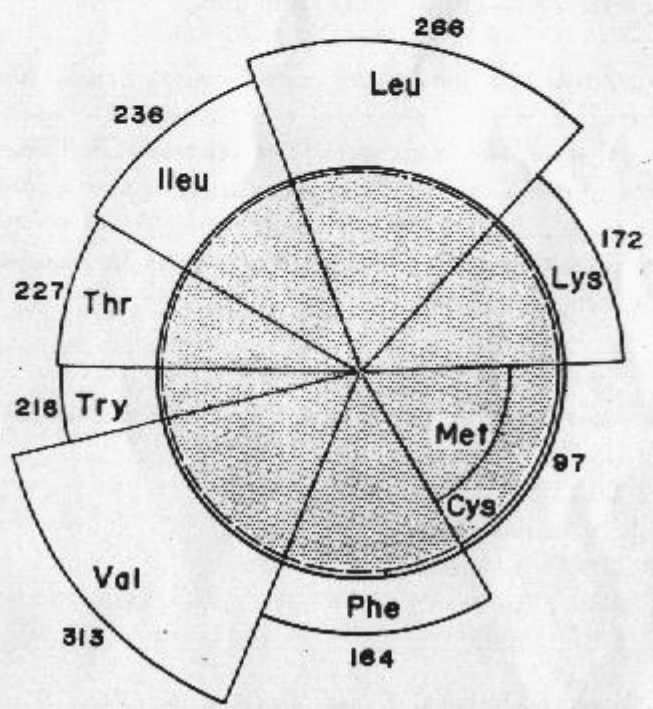
Consequently the areas of the resulting eight semicircles are also in proportion to the safe intakes. A standard, therefore, can be represented by fixing the radius of this complete circle to a certain definite value, an example of which is shown in Figure 2A.



(A) Standard (Rose's Safe Intakes)



(B) 600g of Bread (Protein 48g)



(C) 600g of Bread Supplemented by 4% of Chlorella Protein (Protein 72g)

In order to represent nutritive value of a certain amount of a given protein, a semicircle for each essential amino acid is drawn with the same central angle as that of the standard, but with area, or radius, corresponding to its amount in the protein. Since the radii of these eight semi-circles are generally not uniform, when put together, the resulting circular diagram may consist of indented and projectile parts. From an example for 600 grams of bread prepared from 480 grams of wheat flour, shown by Figure 2B, it can be readily seen that the semi circle of the smallest radius is lysine and 600 grams of bread can supply only 68% of the safe intake of this amino acid. Thus lysine limits the nutritive value of the wheat protein to a very low value, while other essential amino acids

such as phenylalanine, threonine, valine and isoleucine are utilized at very low efficiencies.

From this example, it can be understood that the smoother the periphery of the circle, the better is the quality of a protein. In wheat protein, lysine and the sulfur containing amino acids are short. On supplementing 100 grams of wheat flour with 5 grams of chlorella protein (Figure 2C is obtained for 600 grams of bread. It clearly indicates a marked improvement in the amino acid composition of the wheat protein, which makes the 600 grams of bread sufficient to cover the safe intakes.

Chlorella is a very efficient and fine chemical plant which utilizes solar energy to synthesize versatile organic compounds from carbon dioxide and water exclusively. Therefore, considerations must be given to an exhaustive utilization of not only protein but of other organic materials like chlorophyll, carotene and growth stimulating substances. For this purpose we believe the combination of the autolysis and the butanol treatment is an efficient and practical method.

SUMMARY

A vigorous and limitless growth of Chlorella can be sustained simply by water, air and light. 50% of an air dried chlorella cell is protein, 6% is made up of fatty substances, 19% is of sugars and approximately 10% consists of minerals. Besides it contains an abundance of vitamins.

However, being an unicellular algae, the cell is protected by an extremely tough membrane, which has withstood numerous attempts to make the algae a food source and left it to be utilized as a fodder. In Japan, with but a limited protein resource, utilization of the algae as food has been urgently demanded, and it has been established that a combination autolysis-butanol treatment method is most suitable for an exhaustive utilization of protein as well as of pigments such as chlorophyll and carotene.

When the digestibility of the cell free protein was examined with trypsin *in vitro*, it was found to be far superior to dried or decoloured chlorella, only to be equaled by casein. Although the biological value of the cell-free algal protein is lower than that of casein due to the limit posed by its low content of the sulfur containing amino acids, its high content of lysine makes it an ideal supplement to cereal proteins for improving their nutritive value. The circular diagram method, devised by us to illustrate the nutritive value of a protein is employed to indicate possibilities for utilization of the chlorella protein.

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