PRELIMINARY EXPERIMENTS IN THE USE OF CHLORELLA AS HUMAN FOOD

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The use of unicellular algae such as chlorella as a food supplement is discussed, since the production of these algae by industrial methods may be feasible. Algae powder increases as the protein and vitamin content of foods to which it is added and palatable bread, noodles, ice cream, soups, etc., thus fortified are described. Acceptable "soy sauce" can be prepared by processing chlorella.

Among human sensations, perhaps the most complicated and intangible are those of taste. Closely associated with other physiological functions as well as with multifarious psychological factors, the gustatory sense is affected not simply by the palatability of substances, but also by traditional prepossessions of individuals as well as by capricious fads and propaganda. It is, indeed, a most difficult undertaking to introduce a new item of human food, unless its taste and appearance are quite similar to those of other popular foods or unless it has some special nutritive or medicinal effect without any disagreeable taste or appearance. Theoretically, we may, by proper processing, extract palatable substances from almost all natural organic materials around us, even those that have an abominable taste in the raw state. Obviously, however, it is the cost of processing that determines the usability of such raw organic materials as sources of human food.

For unicellular algae to be readily acceptable as human food, they must either in the raw state or after being processed in the simplest possible way, either have a taste, odor, and appearance that are similar to, or reminiscent of, those of some pre-existing popular food; or have no peculiar taste, odor, or appearance, so that they can be mixed with other foods without being noticed. We have attempted to test the usability of chlorella as food by adding it in the raw dried state to some common foods, and also by processing it by certain simple methods.

We will discuss the origin and nature of chlorella powder and describe some food enriched with it. The nutritional value of its addition to bread, noodles and ice cream as well as the occurrence of vitamins, amino acids and carbohydrates in chlorella will be listed. In addition to the direct addition of chlorella powder to foods, a processing method for the preparation of "soy sauce" from chlorella will be described.

Material used

In most of the experiments we used dried and powered cells of *Chlorella ellipsoidca* which has been grown The "urea-EH" medium described by Tamiya *et al*, (*2)*. The algal cells, separated from the culture medium, were washed once or twice with water by centrifuging, and the thick paste of cells obtained was dried at room temperature with infrared lamps and an electric fan. The dried mass of cells as then crushed and ground to find powder in a mortar.

The powder is dark green and a little hygroscopic. Kept in an ordinary atmosphere, it absorbs water to about 8.5 to 10% of the total weight. Its appearance is similar to, although its color is a little darker than that of the powdered green tea which is commonly used in the traditional tea ceremony in Japan. Its taste, though not exactly the same, is very similar to that of powdered dry "aonori" a seaweed *(enteromorpha compressa)* which is commonly used as seasoning in Japanese cookery. Cells dried at temperatures lower than 15° C. have a milder taste and odor than those dried at higher temperatures.

Addition of chlorella powder to foods

Tests were made to determine to what extent chlorella powder can be added to various common foods without causing queer or disagreeable taste sensations. In each case, tasting was

a. *Powered green tea (*"hiki'cha") To powdered green tea of ordinary quality, chlorella powder can be added without causing significant change in taste, up to as much as 20% of the total weight. To mix well with tea powder, algal cells must be ground as finely as possible.
b. *Soups*

Western style. One or 2 teaspoons of chlorella powder added to bean, mushroom, or beef soup or to consomme was excellent, but in chicken soup the taste was not so pleasant. The green color of algae is less apparent in cream soupe than in clear ones.

Japanese style. A Japanese-style soup was prepared using not only chlorella powder and a dried seaweed, "kombu" (*Lami-naria japonica)*, but also a substitute "soy sauce" made of chlorella cells. (For this sauce, see a later section of

this paper.) A piece of dried "kombu" 3 ½ inches square is boiled with 4 cups of water for 3 minutes, after which ½ cup of "katsuo-bushi" flakes (flakes of dried meat of bonito), 2 tablespoons of ""sake""(Japanese rice wine) and 1½ teaspoon of sodium glutamate are added successively at 3-minute intervals. To 1 cup of the supernatant of the above solution are added 1 or 2 teaspoons of chlorella powder, ¼ teaspoon of chlorella "soy sauce", and ¼ teaspoon of salt.

c. *Noodles.*

Western style. Tasty green noodles were prepared by kneading 4 teaspoons (9.4g) of chlorella powder with 1 cup (142g) of flour, ¼ teaspoon (3.3g) of salt and ¼ cup (60ml) of water.

*"Soba", a buckwheat noodle of Japanese Tele. "*Soba*"* is one of the most popular foods in Japan and is made with 30 to 50% buckwheat flour and 70 to 50% wheat flour. By adding 2 to 5% Chlorella powder to this mixture of flours we obtained a green noodle almost identical, both in appearance and in taste, with the so-called "cha-soba" a choice type of "soba" containing powdered green tea as an ingredient.

d. *Bread and rolls.* Green-colored French bread having an interesting and agreeable taste was prepared by adding 8 tablespoons (56g) of chlorella powder to 6 cups (852g) of white flour, the mixture being baked in the usual way after adding 1 tablespoon (14g) of sugar, 2 teaspoons (27g) of salt, and one yeast cake (5g dry weight)

Richer in taste were rolls prepared with the following materials: 8 tablespoons (56g) of chlorella powder, 4 cups (568g) of flour, ½ cup (110g) of butter, 1 cup (237ml) of milk, 7 tablespoons (99g) of sugar, 2 eggs, 1 teaspoon (13g) of salt, and one yeast cake. Addition of nuts increased the attractiveness of the rolls in taste, texture, and appearance. Fancy looking rolls with variegated colors were prepared by layering ordinary dough alternately with that containing chlorella.

e. *Cookies*. Different kinds of green-colored cookies were prepared by mixing chlorella powder with flour. Appropriate proportions of ingredients for a simple recipe are 2 teaspoons (47g) of chlorella powder, 2 cups (226g) of cake flour, ½ cup (114g) of sugar, ¼ pound of butter, and 1 small egg.

f. *Ice cream.* Surprisingly large amounts of chlorella powder can be added to ice creams. Not only is the dark green color of the algae diluted to a pleasant light green, but also the characteristic taste of the algae effectively accentuates the basic taste of the ice cream. To one cup of vanilla ice cream, more than 5 teaspoons of chlorella powder may be added. The proportion of ingredients in such an ice cream is, for example, as follows: 10 tablespoons (71g) of chlorella powder, 4 cups (1000g) of evaporated milk, 2 cups (473ml) of milk, 1 tablespoon (89g) of flour, 1 cup (27g) of sugar, 2 eggs (98g) and 2 tablespoons (30ml) of vanilla extract.

Table 1
Estimated quantities of principal nutrient substances in some chlorella-containing foods
(The composition of chlorella cells was tekn, following Ketchum *et al (1)* as being 42% protein 22% fat, 24% carbohydrate, and 12% ash, in dry weight)

Composition (Per 100g dry Weight	Protein (g)	Fat (g)	Carbo-Hydrate (g)	Ash (g)	Vit.A (I.U.)	Vit.B1 (mg)	Vit B2 (mg)	Vit.C (mg)
French bread:								
Chlorella (5.0g)	2,5	1,3	1,4	0,7	30,000	0,02	0,15	21
Wheat flour (89,3g)	12,3	1,7	74.6	0,7	0	0,3	0,05	0
Yeast (0,5g)	0,23	0,03	0,2	0,04	0	0,05	0,02	0
Sugar (1,5g)	0	0	1,5	0	0	0	0	0
Salt (2,8g)	0	0	0	2,8	0	0	0	0

Noodles								
Chlorella (6.1g)	2,6	1,3	1,5	0,7	30,000	0,02	0,15	21
Wheat flour (91,8g)	12,6	1,7	76,7	0,7	0	0,3	0,5	0
Salt (2,1g)	0	0	0	2,1	0	0	0	0
Ice Cream:								
Chlorella (9.9g)	4,2	2,2	2,4	1,2	50,000	0,04	0,25	35
Evaporated milk (37.0g)	9,6	11,3	13,8	2,3	270	0,1	0,5	1,4
Milk (7,5g)	2,0	2,0	3,0	0,5	53	0,03	0,1	1,3
Flour (10,8g)	1,5	0,2	9,0	0,1	0	0,03	0,01	0
Eggs (3,4g)	1,7	1,5	0	0,2	110	0,01	0,04	0
Sugar (31,4g)	0	0	31,4	0	0	0	0	0

Table 2
Amino Acids in chlorella cells

Amino Acids	Fractions		
	Water Soluble		Water Insoluble Combined
	Free	Combined	
Essential			
Leucine Iso-leucine	+++	++++	++++
Valine	++	+++	++++
Lysine	++	+++	+++
Threonine	++	+++	+++
Histidine	++	+++	++
Phenylalanine	++	++	+++
Areginine	+++	+	++
Tryptophane	--	++	++
Methionine	+	+(?)	++

Tyrosine	++	++	++
Cystine	+	+	+
Nonessential:			
Alanine	++++	++++	++++
Glycine	+++	+++	++++
Glutamic Acid	++	+++	++++
Proline	+++	+++	+++
Aspartic acid	++	++	++
Serine	++	+++	++
y-Amino-butyric acid	+++	+	++(?)
b -Alanine	+	+	++
A-Amino butyric acid	--	--	++
Citrulline	--	?	+
Unknown	+	+	+

Estimated quantities of nutrient substances in chlorella containing foods

To what extent does the addition of chlorella powder increase the nutritional values of the foods described above? Table 1 shows the estimated content of various substances in French bread (containing 8 tablespoons of algal powder per 6 cups of flour), noodles (Western style, containing 4 teaspoons of algal powder per 1 cup of flour) and ice cream (containing 4 ½ teaspoons of algal powder per 1 cup of ice cream)

As may be seen from these figures, additions of chlorella causes an increase of 20% in protein and 75% in fat in the case of bread and noodles and about 30% in protein and 15% in fat in the case of ice cream. Noteworthy is the fact that by the addition of algae the foods are made considerably richer in vitamins A and C, which are lacking in ordinary breads and noodles. The amino acid, sugar, and vitamin contents of the chlorella powder are given in Tables 2, 3 and 4 respectively

Some attempts at processing chlorella powder

Simple methods of processing chlorella cells are suggested by those used in processing soybeans in Japan.

One of the most popular foods in both China and Japan is "tofu" or soybean curd, which is a clot of proteins (called "glycinin") prepared by coagulating the water extract of soybean by the action of magnesium or calcium salts (chlorides or sulfates). "Tofu" is white and by itself almost tasteless, although it contains, besides easily digested proteins, some fat and carbohydrate. Its appeal as food is largely due to it soft and delicate texture, as well as its neutrality, which permits it to harmonize with and set off the taste of other foods mixed with it. For these reasons it is used in almost all kinds of dishes in far Eastern countries. In the hope.of getting a "tofu"-like product, the method used with

soybeans was tested with water decoction of chlorella powder, but unfortunately, it proved totally unsuccessful. Obviously, this failure is due to the difference in nature of the proteins contained in different plants.

Another suggested Japanese method is that of preparing a substitute "soy sauce" by hydrolysis of soya beans. Soy sauce is indispensable in preparing almost all kinds of Japanese foods; the authentic way of preparing it is by fermenting steamed soybeans (together with some wheat or rich) with salt and a mold. *As pergillus oryzae*.

Besides this mold, a number of bacteria and yeast's participate in the process of fermentative decomposition of various substances, which requires about one year for producing the typical color and taste of soy sauce Since this method is rather complicated and time consuming, a short-cut chemical method is now being widely used in parallel with the authentic method. They soybeans are hydrolyzed with hydro-chloric acid which is later neutralized with sodium hydroxide or sodium carbonate to give the appropriate amount of sodium chloride. The resulting sauce is almost identical in appearance and taste with genuine ones, although it lacks some essential savor. To conceal this defect, the sauce manufactured in this way is usually sold mixed with genuine soy sauce.

Using powdered chlorella cells, we attempted the chemical method of preparing a substitute for soy sauce. One hundred g. of chlorella powder was suspended in 200ml of 25 to 20% solution of hydrochloric acid in an Erlenmeyer flask provided with a condenser, and heated on a sand bath. Analysis of the solution for nitrogen indicated that about 5 hours' heating was sufficient for they hydrolysis to proceed to a desirable stage. After 5 to15 hours' hydrolysis, the mixture was filtered and neutralized with sodium bicarbonate to pH 4.5-5.4. The solution thus obtained was almost exactly similar in appearance to real soy sauce, and showed the composition presented in Table 5. In this table the composition of typical genuine soy sauce as well as the prescribed standard for commercial "soy sauce" is also given for comparison.

Table 3
Sugars in chlorella cells

Sugar	Fractions		
	Water Soluble	Water Soluble 80% alcohol insoluble	Water Insoluble
Glucose	++	+++	++
Fructose (ketcheptose)	+++	±	+++
Xylose	+++	--	+++
Galactos	++	?	++
Ribose and Ribalose	+	--	++
Triose	+	+	+
Sucrose	+	.....	--
Phosphate esters	++	.....	--

Gluconic acid	+	.....	++
Ketogluconic Acid	-	±	++
Galacturonic acid	+	.....	++
Glucuronic acid	+	.....	+

Table 4
Some vitamins fond in chlorella cells

	Content per gram dry weight
Vitamin A	5000 International Units (as provitamin)
Vitamin B1	4g
Vitamin B2	21~28g
VitaminB6	9g
Niacin	176g
Folic Acid	485g
Vitamin C	2000-5000g

Table 5
Composition of chlorella hydrolysate as compared with that of genuine soy sauce and the standard for commercial "soy sauce"

	Genuine Soy Sauce	Chlorella Hydrolysate	Standard for commercial "soy sauce"
Nonaqueous substances	36.9%	41.2%	-
Total Nitrogen	1.22%	2.29%	>0.7%
Amino nitrogen	0.42%	1.42%	-
Sodium Chloride	18.9%	22.1%	.17.0%
Specific Gravity	23.2Be	25,5 Be	.17.5 Be

When properly diluted, the hydrolysate of chlorella powder tastes almost the same as sauce prepared from soybeans by the chemical method. The taste was considerably improved when the following sub substances were added: lactic acid (0.2%), acetic acid (0.05%), succinic acid (0,0005%), ethanol (0.01% and sucrose (0,05%)/

These experiments show that the preparation of a substitute for soy sauce is a very promising way of

using chlorella cells.

Taste of other unicellular algae

A few experiments conducted with dried powder of *Chlorella pyrenoidosa,* Emerson's strain, showed that it tastes almost exactly the same as the strain of C *ellipsoidea* we have used. We also found that dried materials of scenedesmus, chlorococcum, and even a sea-water alga, *Dunaliella salina,* tastes almost the same as chlorella. It may well be expected that the methods used with chlorella may also be applied successfully to these different kinds of unicellular algae.

Summary

Dried powder of chlorella cells shows a taste and flavor similar to those of powered green tea and powdered dry "aonori" a seaweed (*Enteromorpha compressa)* commonly used as a seasoning in Japanese cookery. It was found that the algal powder can be added to various kinds of food, both Western and Japanese, giving them an agreeable taste and appearance and increasing their protein, fat, and vitamin content. An attempt at extracting a "tofu" –like material from chlorella cells failed, but by hydrolysis of algal powder a solution was obtained which may be used as a substitute for soy sauce.

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a Some of the experiments reported were carried out at the Carnegie Institution of Washingtons's Department of Plant Biology, Standford, California, Members of the Department staff assisted in testing the products

[b]Besides this seaweed, there are a number of algae, from both fresh and sea water, which are popular in Japanese cookery. Some examples are:suizenji-nori(*Phyllodorma sacumI),kawanori I(Prasiola japonica)* hitoegusa *(Monostroma nitidum);* makombu (*Laminaria japonica)* wakame *(Undaria pinnatifida)*, hijiki *(Hijikia fusiformis)*arame *(Eisenia bicyclis),*mozuku*(Cladosiphone decipiens)*

[c]This kind of tea is served not as a water extract, as are other kinds of tea, but as a suspension of tea powder in hot water, prepared by beating the mixture with a small bamboo whisk immediately before serving. The somewhat bittersweet taste of raw young tea leaves is much enjoyed by Japanese people, but does not appeal to most Western people.