Microstructure of Spray-Dried and Freeze-Dried Microalgal Powders

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Abstract

Chlorella and Spirulina algae were spray-dried and freeze-dried under several different conditions, and the morphological changes induced were studied by scanning electron microscopy. The internal structure of the particles (granules) was revealed by cryofracturing. The cellular composition of the two algae, method of drying, cell concentration, feed conditions, temperature, and drying times all affected the external morphology of the resulting powders. In the case of spray-drying, at a temperature of 160°C for 6 sec, the particles shrunk or collapsed; if the drying time was over 10 sec at 160°C, similar results were observed. The cell concentration affected the thickness of the dried particle wall; a high concentration produced thicker and heavier particles, lower concentrations produced thinner and lighter particles. The rate and pressure at which the liquid was fed into the drying chamber affected the proper atomization of the liquid. When freeze-dried, the algal powder structure was different from particles formed by spray-drying. Spray-dried particles were individual spheres with a void space in the center. Each particle was composed of a few thousand cells in the case of Chlorella and several trichomes in the case of Spirulina. In contrast freeze-dried algae formed sheets of cells that were no longer spherical and which adhered together in a linear fashion.

The cryogenic process used in this study to fracture and study the structure of spray-dried microalgal powders should prove to be very helpful in selecting optimal conditions for the production of high quality health food products.

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Introduction

Mass algal cultivation began in Germany during World War II when experiments were performed to grow certain algal strains as a source of lipids and proteins. During the late 1940's and 1950's, particularly in Japan, microalgae were produced as a potential source of food or animal feed (4). Subsequently, Chlorella species were produced on a commercial scale in Japan as a food or feed additive. In the U.S., algal products have also been used to treat waste and sewage in oxidation ponds in California (10), and they have also been used in conjunction with bioregenerative designs for long-duration space missions. Spirulina has been studied extensively under many different growth conditions (2,6,7).

The production of Spirulina began in the early 1960's with the work of the Institute Français du Petrole (IFP). Clement and her coworkers, as mentioned by Dubinsky's review (4), studied Spirulina after observing that tribes along the shores of Lake Chad used S. maxima as a substitute for meat source. Today, there are several commercial plants that produce Spirulina for the health food industry.

Spirulina is a cyanobacterium with relatively wide filaments normally coiled in large, lax helices. It can tolerate high intensities of sunlight, can grow rapidly in a warm, shallow, brackish lakes, and will reach high filament densities, making Spirulina relatively easy to harvest. It is one of the most abundant algae found in the many alkaline lakes of Africa and the Americas (4). The mass cultivation of S. maxima is now being practiced in alkaline waters at Lake Texcoco in Mexico, where production is 1 metric ton per day (10). Spirulina platensis is cultivated in Japan and Taiwan by using bicarbonate as a source of carbon and sunlight as a source of energy. Extensive research has also been performed in Israel (17). There have been several ultrastructural studies of Spirulina spp. (1,5,20).

Recently, Chlorella and Spirulina algae have been produced on a large commercial scale in Japan and Taiwan. Dried algal powders and tablets are used as health foods, and a concentrated water-soluble extract called "Chlorella Growth Factor" has also been produced by boiling the dry algal powder. The specific chemical composition of this extract has not yet been fully described, but it is already being used as a health food additive in a wide variety of foods.

A thorough understanding of the microstructure of dried Chlorella and Spirulina would be of great value for the improvement of processing techniques. In order to make a uniform,
dense, and pure dry form of these algae, the heating temperatures, duration times, feed conditions, cell concentrations, and cell densities during the drying process must be carefully regulated. When properly regulated, the quality and nutritional value of the algal powders will be greatly enhanced. In addition, when the health food industry uses these dried algae to make tablets, they cannot use cementing materials to make the particles adhere together as would a vitamin manufacturer or other non-health food industry. Instead, they must produce dry algae of sufficient density and appropriate structure or the tablet will disintegrate. Thus, an analysis of dried algal microstructure is essential for the health food industry.

The purpose of this paper is to examine the microstructure of spray-dried and freeze-dried microalgal powders in order to improve the manufacturing techniques available for use with these microalgae.

**Materials and Methods**

**Algal cultures**

*Chlorella pyrenoidosa* was used throughout this study. The inoculum was prepared in a modified Allen's medium containing mg/L: urea-600; MgSO4·7H2O-300; KH2PO4-300; FeSO4·7H2O-5; EDTA-7. Each liter contained 70 mL of glacial acetic acid (6); this medium was transferred into open culture pools of 2 m in diameter, and then gradually scaled up to pools of 30 m in diameter. The cells were grown in autotrophic conditions; mineral nutrients and glacial acetic acid were periodically replenished to create favorable growth conditions (6,7).

*Spirulina platensis* was cultivated in a mineral medium (16) using two liter flat-flasks. The incident light intensity from cool white fluorescent lamps (40W) ranged from 0.3 to 21 Klux, and the culture temperature ranged from 25 to 30°C. Mass culturing was performed on the same scale as the *Chlorella* culturing; *Spirulina* was fed with bicarbonate as the source of carbon.

**Drying**

The cells were harvested and washed by continuous centrifugation at 5,000 g. Spray-dried powders were prepared in a Koshiwa spray dryer by means of centrifugal atomization (4,500 g) at an inlet temperature of 140-150°C and an outlet temperature of 80-85°C. The central portion of the chamber was maintained at 130-135°C. The powder was formed within 6-8 sec after being injected into the chamber. Two procedures were used for freeze-drying: slow-freezing and rapid-freezing. One mL samples of suspended *Chlorella* cells at a concentration of 10% (w/v) were placed in 15 mL screwcap bottles of methanol maintained at -60°C and were slowly frozen in 1 min Liquid nitrogen (-196°C) was used for rapid-freezing. In both cases, a VirTis freeze dryer (Model 10030) with a vacuum of 6 × 10⁻² Torr was used for the 12 h drying period. The final moisture content was usually less than 3%.

**Preparation of original algal cells for electron microscopy**

Samples were periodically withdrawn from the bottles or pools. Cells were then harvested by centrifugation at 600 g for 15 min and washed with distilled water three times. The cells were fixed in 2.5% osmium tetroxide solution for 2 h, then washed again in buffer. After fixation, all specimens were dehydrated by transferring them through a series of 10 min alcohol baths with concentrations ranging from 60, 70, 80, 90, 95, and 100% ethyl alcohol. They were then embedded in Epon 812 mixtures. Ultrathin sections were cut with a DuPont diamond knife on a Sorvall MT2-B "Porter Blum" ultramicrotome, and sections were picked up on uncoated grids. The sections were doubly-stained with uranyl acetate and lead citrate. Photomicrographs were taken with a Hitachi HU-12 electron microscope at an acceleration voltage of 75 kV; initial magnifications were from 4,000 to 20,000.

**Scanning electron microscopy (SEM)**

Fresh cells were fixed in 0.1 M phosphate buffered (pH 7.4) 1% glutaraldehyde for 2 h at 5°C. Specimens were dehydrated by transferring them through 10 min alcohol baths as described above; the alcohol was removed by a 10 to 15 min bath in iso-amylacetate, and the specimens were dried with an HCP-1 critical point dryer (Hitachi Koki, Tokyo, Japan). The specimens were coated with a thin conductive layer of gold, and were then examined in a Hitachi S-550 scanning electron microscope using an acceleration voltage of 20 kV. Micrographs were taken on Fuji film (125 ASA), and the initial magnifications ranged from 500 to 5,000.

**Examination of dried algal powders**

In order to examine the external structure, dried *Chlorella* and *Spirulina* powders were attached to double-sided adhesive tape on SEM stubs, and were then sputter-coated with gold (20 to 40 nm) in an Eiko Ion Coater, Model IB-2. The specimens were kept in a dessicator (filled with silica gel) until placement in the SEM chamber. The specimens were examined in a Hitachi S-550.

The process used to examine the internal structure is outlined in Fig. 1. Spray dried powders were first encapsulated in an agar gel; this assured that the original particle structure would remain intact during the process of preparing the cells for electron microscopy. A slice was then cut from the coagulated gel, rinsed in distilled water, fixed in 1% osmium tetroxide for 2 h, and then dehydrated by immersion in the series of alcohol baths as mentioned above. The next stage was to freeze the dehydrated sample in liquid nitrogen; it was first wrapped in a cellophane film so that the small samples were more easily retrieved after fracturing. An alternate method would be to place the samples on small pieces of aluminum foil. The liquid nitrogen hardened the gel so that it could be gently cracked open with a razor blade while still in the liquid nitrogen after the boiling stopped. If done carefully, the original structure of the particle was not distorted. This fractured material was then stored in a bath of 100% ethyl alcohol until critical point drying. Critical point drying and ion coating procedures for SEM were the same as mentioned above.

**Chemical and bacteriological analysis**

Algal powders used in the health food industry must also be examined for purity and safety. We therefore performed preliminary chemical and bacteriological analysis on the dried powders in order to evaluate and compare the quality of various samples.

The water content was determined by drying the sample at 105°C for 12 h. The total chlorophyll content was determined by the methanol extraction method described by MacKinney (13). The relative activity of chlorophyllase was determined by the method described by Tamai, et al. (19); when spray-dried at 160°C for 6 sec, chlorophyllase was deactivated. The content
of toxic pheophorbide was estimated according to the method described by Wickliff and Arnoff (21).

The total bacterial count was estimated by using the conventional plate count method; coliform bacteria were counted by using deoxycholate media according to the Difco manual (3).

**Results and Discussion**

*Chlorella pyrenoidosa* grows autotrophically on CO₂, but as the rate of cellular growth is much too slow for industrial purposes, acetic acid may be added to the solution and the *Chlorella* will grow heterotrophically, thus rapidly increasing the rate of cell growth. As this alga grows both by means of autotrophy and heterotrophy, it may be called mixotrophic. Grown under these conditions, the cells (Fig. 2) were spherical, 2–8 μm in diameter with a relatively thin cell wall. The chromatophore was cup-shaped and a pyrenoid was occasionally present in the cell. The chloroplast was clearly differentiated and contained abundant photosynthetic storage material. Starch granules surrounded the pyrenoid and were also located in the interlamellar spaces of the chloroplasts. The SEM micrograph (Fig. 3) shows that the cells were spherical and measured 3 to 6 μm in diameter; there was no significant contamination of the cultures. In contaminated cultures, photosynthetic purple bacteria of the *Rhodopseudomonas* variety were usually stuck to the surface of the algal cells (1).

*Spirulina* was in the shape of a helix (Fig. 4), the pitch of which was proportional to the size of the cells. A transverse section through the trichome is shown in Fig. 5. The cell wall was divided into four layers and the septum into three layers. The photosynthetic lamellae were closed, irregularly shaped discs, and the membranes consisted of two parallel lines. Mesosomes were often located near the cross wall and were connected to the photosynthetic lamellae. These have been observed by Allen (1) and by van Eykelenburg (5). Polyhedral bodies (polysaccharide-like granules) were observed in some preparations. These bodies were located near or enclosed within the photosynthetic lamellae. As mentioned by Titu, et al. (20), these granules are the most prevalent structural elements and are uniformly distributed in the region between the nucleoplasm and the cell wall. SEM micrographs revealed the steric configuration of the spiral structures. The structure of *Spirulina* resembles that of other Oscillatoriae and possesses all typical organelles found in procaryotic cells. The growth conditions affected the morphology and the dimensions of some organelles.

The morphological changes of *Chlorella* and *Spirulina* that occurred during spray-drying were investigated by SEM. Spray-dried algal powders consisted of globular particles (Figs. 6 & 7) with cavities in their centers (Figs. 8 & 9); this phenomenon was previously observed by Lee and Rha (8) in their study of spray-dried yeast powders. These void spaces had diameters of approximately one-half of the entire particle when viewed sectionally (Fig. 10). Considering that each granule has a diameter of approximately 50–80 μm (excluding the void space), it is estimated that each granule is composed of 3,000 to 7,000 *Chlorella* cells. The method used to determine this calculation was reported in a previous paper (9). Several entwined strips of cells comprised the granules of the *Spirulina* powder (Fig. 7); they were 40–100 μm in diameter, and ranged from a few to about a dozen trichomes, depending on the drying conditions. There was a striking difference between the two kinds of algal powders. The *Chlorella* powder granule appeared to be an aggregate of discrete cells adhered to one another (Fig. 6). The *Spirulina* powder particle, however, appeared to be a more amorphous structure of cells fused together; the boundaries between the original helical structures were no longer clearly defined (Fig. 7). This phenomenon may be related to the different chemical compositions of the cell walls of these algae. *Chlorella* is a eucaryotic cell and the surface layer is predominantly composed of cellulose (15). *Spirulina* is a procaryotic cell and the cell surface is mainly composed of mucopolysaccharide (5).

In most cases, the resulting spray-dried particle was spherical and hollow, with debris attached to the surface. Changing the feed conditions (inlet and outlet temperatures, time, and extent of atomization) led to changes in the appearance of the granules. For example, shrunken particles were observed when powders were dried at an elevated temperature (>160°C) for 6 sec or dried for a prolonged period of time (>10 sec) at 160°C. The *Spirulina* particles dried under these conditions were misshapen (Fig. 11), and the *Chlorella* particles also lost their spherical shape when improperly dried (Fig. 12); both showed an increase of smaller irregular fragments which vary considerably in size. The irregular formation of particles is attributed to the formation of a case-hardened outer surface on each particle which

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![Fig. 1. Schematic diagram of a cryogenic process used to fracture spray-dried microalgal powders so that the internal structure could be studied in detail.](image)
Fig. 2. Transmission electron micrograph of mixotrophically grown *Chlorella* cells, showing typical green algal characteristics. Note the cell wall (CW), the cell contains a cup-like chloroplast (C), a nucleus (N), mitochondrion (M), and starch grains (S). Bar = 1 μm.

Fig. 3. Scanning electron micrograph of *Chlorella* cells, showing spherical shape. Bar = 5 μm.

Fig. 4. Scanning electron micrograph of helical trichome of *Spirulina* showing the pitch (P) and the outer diameter of the helix. Bar = 50 μm.

Fig. 5. Transmission electron micrograph of a *Spirulina* cell, showing typical characteristics of blue-green algae. Note the cell wall (CW), and the septum (S), the photosynthetic lamellae (PL), and polyhedral bodies (PB). Bar = 1 μm.

Prevented liquid from reaching the surface from the particle interior. Because of high heat transfer rates in the droplets, the liquid at the center of the droplet vaporized, causing the outer shell to expand and form a hollow sphere. Sometimes the rate of vapor generation within the droplet was sufficient to blow a hole through the wall of the spherical shell, as shown in Figs. 8 & 9; the entire structure then often collapsed (Fig. 12).

The ideal cell concentration was 10% (w/v), and the range from 5 to 15% was found to be acceptable. Higher concentrations produced dried particles with a thicker wall and a smaller void space. Lower concentrations produced dried particles with a thinner wall and a larger void space. If the cell concentration of the algal cells was over 20% it tended to stick in the atomizer disc of the spray drier. The rate at which the solution was fed into the drier also affected the size of the particles. The faster the atomizer disc of the spray drier spun, the smaller the particles became, but if it spun too fast, the particles stuck to the sides of the drier and burned. The shape of the holes in the atomizing disc also affected the shape of the dried particle.

Freeze-dried algal powders were non-spherical and instead resembled three-dimensional networks or sheet-like clusters (Figs. 13, 14, & 15). Slow freezing rates between −30 to −60°C and freeze-drying resulted in the development of large ice crystals in the intercellular spaces and the displacement of the constituent parts (Fig. 14). These ice crystals could exert pressure on the cells to the extent that dehydration of the cells would occur.
If the cells were rapidly frozen in liquid nitrogen (LN$_2$) at $-196^\circ$C, it was found that smaller intercellular ice crystals formed and the cell membranes were left intact. The degree of mechanical damage caused by ice crystal formation was thus reduced, and sheet-like clusters of the cells were observed. As a result, the original shape of the sample was preserved (Fig. 15).

Bacteriological and chemical analysis of the Chiarella powders (Table 1) showed that the total chlorophyll content was higher in the spray-dried than in the freeze-dried materials. In addition, no coliforms were found in the spray-dried powders. The total bacterial count ranged from $10^3$ to $10^4$ cells per gram, which reflected the non-sterile, mixed-culture conditions of the algal production (6,7). Sinsky and Silverman (18) reported that the average proportion of Escherichia coli cells which survived freeze-drying in 2% gelatin was 0.6% of the frozen cells; 28% of the cells which survived freeze-drying under these conditions incurred damage. A pronounced effect caused by freeze-drying and rehydration of E. coli was altered permeability of the cell wall and cytoplasmic membrane. The toxic component phaeophorbid, which is considered to be an undesirable component of Chlorella, was slightly higher in the freeze-dried samples.

Spray-dried algae formed individual spheres that were hollow and not aggregated. When freeze-dried, however, the algal particles adhered together in the form of a sheet; they were not spherical as were the spray dried particles, but were joined in
Microstructure of Microalgal Powders

Fig. 10. A cross-sectional view of a spray-dried Chlorella particle, showing hollow structure. Bar = 5 μm.
Fig. 11. Scanning electron micrograph of spray-dried Spirulina powder which was formed after spray-drying at an elevated temperature, showing that most particles exhibited collapsed forms. Bar = 50 μm.
Fig. 12. Scanning electron micrograph of spray-dried Chlorella powder which was submitted to an elevated temperature, showing the shrunken and collapsed forms. Bar = 50 μm.
Fig. 13. Scanning electron micrograph of slowly frozen (−30°C) dried cells of Chlorella, showing fused appearances. Bar = 5 μm.
Fig. 14. Freeze-dried cells of Spirulina, showing cluster-like structure. Bar = 50 μm.
Fig. 15. Rapidly frozen (−196°C) cells of Chlorella, showing sheet-like arrangement. Bar = 5 μm.

a three dimensional network. In the case of Chlorella, slow freeze-drying and ice formation often fused cells together, thus making the distinction between separate cells difficult (Fig. 13). Fast freeze-drying, and the formation of smaller ice crystals, resulted in groups of discrete cells whose contents remained intact (Figs. 14 & 15). Freeze-drying appeared to be an effective method of preserving these algal strains (14).

Scanning electron microscopy is particularly well suited for a wide range of routine analyses of algal powdered foods. The process of dehydration is a major economic factor in the production of dried algal powders. It may constitute approximately 30% of the total production cost. By studying the structure of the particle, the most economical drying conditions may be determined and waste may be reduced. The method of dehydration also affects the food value, taste, color, texture, and digestibility of the final product. Spray-drying destroys toxic substances much more effectively than freeze-drying, and spray-drying produces a powder that is very hard (3.5–5.5 kg), while freeze-drying produces a powder almost half as hard (2.5 kg max). This is important for the production of hard tablets without using cementing materials.

The spray-drying of commercial quantities of algal powders is currently being practiced only in Taiwan. This study was the first to examine the microstructure of such algae in detail. A suitable cryogenic process was devised for that purpose. Both spray-drying and freeze-drying are expensive processes, but after an overall analysis of microstructure, bacterial and chemical content, they were found to be superior to conventional methods such as drum drying.

Acknowledgements

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References


Table 1. Results of bacteriological and chemical analyses of dried Chlorella powders.1

<table>
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<tr>
<th></th>
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<th>Freeze-dried</th>
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<td>Water content (%)</td>
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<td>3–4</td>
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<tr>
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<tr>
<td>Protein (%)</td>
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<td>66.5</td>
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<td>Total bacteria (×10³)</td>
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<td>3×10³</td>
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<tr>
<td>Total coliforms (×10²)</td>
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<td>1×10²</td>
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<tr>
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<td>Pheophorbide (mg%)</td>
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<td>50–60</td>
</tr>
<tr>
<td>Chlorophyllase (mg%)</td>
<td>90–160</td>
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<tr>
<td>Color</td>
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</table>

1 The initial concentration of Chlorella suspension was 10–12% (w/v), as determined by dry weight.
2 The total bacterial count in the Chlorella suspension was 10⁶–10⁷ colonies per mL. The majority were photosynthetic bacteria.
3 Approximately 100 colonies appeared on deoxycholate media.
4 The enzyme activity was converted to mg% for convenience, as described by Wickliff and Arnoff (21).
5 Hardness was determined by testing 250 mg tablets with the Monsanto type hardness meter.


**Discussion with Reviewers**

**K. Saio:** What shape is ideal when *Chlorella* or *Spirulina* are used for health foods? Do the shape and the hollowness of the particle affect its solubility in hot and cold water?

**Author:** A spherical spray-dried algal powder appears to be most suitable for the health food industry. It is much easier to produce than droplet-shaped or ellipsoid particles, and the thickness of the particle shell in relation to the void space is more easily regulated when the particle is spherical. If the void space is too large or too small, or the particle wall is too thin or too thick, the particle will be undesirable. If the wall is too thin, good tablets cannot be made; it will also dissolve too quickly in the human digestive system. If the wall is too thick, the particle is not as economical to produce. Of course, hot water will dissolve these particles much faster than cold water.

**R.A. Holley:** Do you agree with Pabst (1975) who felt that drum dried or boiled *Chlorella* were more digestible than powders produced by other methods? Why? (Pabst, W. (1975) Feeding trials for the determination of the nutritional value of microalgae. In Symposium Microbial Production of Protein 1975, F. Wagner (Ed.), Verlag Chemie, Weinheim, W. Germany (German)).

**Author:** The research done by Pabst mainly concerned *Scenedesmus*, while this study dealt with *Chlorella*. For human consumption, *Chlorella* is generally preferred because many feel that *Scenedesmus* does not contain a sufficient amount of "Growth Factor". Apart from this difference, the cellular structures of these algae are very different, and a spray-dried *Scenedesmus* particle may have a different microstructure than the spray-dried *Chlorella* particle.

In regard to digestibility, boiling or infusion will increase digestibility because it breaks down the cell wall of the algae, but this process may also cause soluble materials to leach out, causing the total nutritional value to change. Drum-drying and spray-drying are similar heating processes and thus the digestibility of both products is probably very similar as well.

**M.V. Taranto:** In order to study the internal structure of the dried algal cells, you encapsulated them in agar and then exposed the cells to water. This surely caused the cells to hydrate which more than likely altered the cell morphology. In addition, you fixed, froze, fractured and redried the cells. This surely caused morphological distortion. If your objective was to study the effect of the spray drying and freeze drying process, why did you not embed the dry cells in epoxy to immobilize the cells and then fracture the cured block? This procedure may have yielded fractured cells in the dry state which would more clearly demonstrate the effect of the drying process on cell morphology.

**Author:** The purpose of embedding the cells in the agar and cracking it open was to study the void space in the center of the sphere. Individual cells showed some distortion due to the freeze drying and freeze drying process, why did you not embed the dry cells in epoxy to immobilize the cells and then fracture the cured block? This procedure may have yielded fractured cells in the dry state which would more clearly demonstrate the effect of the drying process on cell morphology.