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Comparison of Rheological Properties of Powder *Chlorella* sp. Cultivated in Fermentor and Pond

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Abstract The current study was conducted to identify the differences in the rheological properties of *Chlorella* sp. powder cultured in a fermentor and in a pond-like environment. Cells cultured in the same media were harvested and spray dried. The biomass yield from the fermentor culture was 4.7% (dry basis), while that from the pond was 4.3% (dry basis). Measurements of the loose bulk density, tapping test, Hausner's ratio, and compressibility test all revealed differences between the rheological properties of the *Chlorella* sp. from the two cultivation systems. Although both the fermentor and pond cultured *Chlorella* sp. showed the same angle of repose, the mean size of the cells was 2.26 μm and 2.89 μm, respectively. The weight of the *Chlorella* sp. tablets cultured in the fermentor and pond was 0.663 g/tablet and 0.593 g/tablet, respectively, while the friability of the tablets was 21% and 41%, respectively. Observation by Transmission Electron Microscope (TEM) showed that the cell wall of the *Chlorella* sp. cultured in the fermentor was thinner and more spherical than that cultured in the pond, thereby providing the main characteristic rheological properties of the powder.

Key words: Powder rheology, *Chlorella* sp., loose bulk density, compressibility test

Chlorella sp. is a microalgae that exists in both fresh and marine water, and undergoes asexual reproduction [1, 2, 10, 16]. Due to its high protein content and ability for lipid accumulation [16], *Chlorella* has become an attractive contender for functional food. In particular, *Chlorella* is composed of many essential amino acids and is particularly rich in leucine, threonine, and valine [1, 5, 12]. However, the main reason for the interest in *Chlorella* as a health food is due to its sulfur nucleopeptide known as the *Chlorella* Growth Factor (CGF). The molecular weight of CGF is 5,000–10,000 and it is capable of promoting the growth of lactobacillus bacteria [1, 5]. The addition of 1% *Chlorella* powder results in a 3-fold increase in the activity of *Lactobacillus delbruckii* to produce lactic acid, whereas the addition of 2% *Chlorella* powder can also generate a 9-fold increase in *Bacillus subtilis* amylase production [5]. Furthermore, *Chlorella* is also known to have therapeutic effects on gastric ulcers, duodenal ulcers, and chronic gastric ulcers [18], and to improve the nutritional content of animal feeds [8].

Reducing the water activity in the commercial production of *Chlorella* is necessary to extend its shelf life and inactivate pheophorbide, a chemical that can cause photosensitivity [1, 6, 14]. The rheological characteristics of dried *Chlorella* powder are attributed to its physical properties. Powders are usually described at two levels; as individual particles and as powder in bulk. Although it is self-evident that the bulk properties are primarily influenced by the particles' properties, the relationship between the two is by no means simple and involves various external factors, such as the system geometry and mechanical and thermal histories of the powder [3, 4, 11, 13, 15].

Based on the above considerations, the current study was undertaken to investigate how *Chlorella* sp. cultured under different conditions can affect the physical properties and shape of the powder form. The rheological properties of the resultant *Chlorella* sp. powders were also compared with observed morphological characteristics of the cells.

Materials and Methods

Materials

The *Chlorella* sp. (Korean Collection for Type Cultures no. AG 10002) was obtained from Korea Research Institute of Bioscience and Biotechnology (KRIBB), Korea. The basal growth medium for *Chlorella* sp. was composed of peptone (2.00 g/l), yeast extract (2.00 g/l), glucose (2.00 g/l),

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$MgSO_4 \cdot 7H_2O$ (0.25 g/l), KH_2PO_4 (0.25 g/l), KCl (0.12 g/l), $Ca(NO_3)_2$ (1.00 g/l), and PIV metal solution (1.00 ml). The PIV metal solution was prepared with $FeCl_3 \cdot 6H_2O$ (97.00 mg/l), $MnCl_2 \cdot 4H_2O$ (41.00 mg/l), $ZnCl_2$ (5.00 mg/l), $CoCl_2 \cdot 6H_2O$ (2.00 mg/l), $Na_2MoO_4 \cdot 2H_2O$ (4.00 mg/l), and Na_2EDTA (0.75 mg/l). The pH of the medium was 6.0. The medium was sterilized by autoclaving at 121°C for 15 min.

Chlorella sp. Culture

Cells were activated by inoculating into 100 ml of broth culture of the growth medium in a 250-ml flask and pre-cultivated with continuous illumination at 25°C and 110 rpm for 3 days under a fluorescent lamp. For the fermentor culture, 500 ml of the precultivated chlorella was placed in a 5 l fermentor jar (KF-5L, Korea Fermentor Co., Ltd., Korea) and cultured at 0.5 vvm of aeration, with stirring at 20 rpm under an incandescent lamp at a constant temperature of 25°C. The pond culture was prepared in an acrylic box (35 cm×25 cm×35 cm) [7]. Under sterile conditions, 3 l of the growth medium was added to well-grown *Chlorella*, which had absorbency of 0.5–0.6 at 430 nm (Uvikon 922A, Kontron Instruments, Italy) [9].

Spray Drying

The concentration of *Chlorella* sp. was adjusted by diluting 10-fold. The diluted *Chlorella* sp. suspension was then pasteurized at 100°C for 3 min and cooled rapidly to inactivate the pheophorbide. Next, the *Chlorella* sp. was spray dried (SD-1000, EYELA, Japan) under the following conditions. The inlet temperature was set at 150°C and the outlet temperature at 70°C. The rate of blowing was controlled at 0.33 m^3/min and then atomized at 15×10 kPa. The prepared powder was stored in a desiccator.

Rheological Properties of Powder *Chlorella*

The prepared powder was poured into a test cell, and the original bulk density was determined by dividing the net weight of the powder by the known volume of the cells [3, 4, 11, 13, 15]. The tapping test [3, 15] was performed by placing a known weight of the powder in a graduated cylinder and then tapping against the cylinder 200 times. The tapping distance between each tap was maintained at 50 mm. The relationship between the *Chlorella* powder and the volume of the reduction fraction (γ_n) and number of taps (n) is given by the following equation,

$$\gamma_n = \frac{(V_0 - Vn)}{V_0} = \frac{abn}{(1+bn)} \quad (1)$$

in which V_0 is the initial volume, Vn is the volume after n taps, a is the constant of the asymptotic level of the volume change, and b is the constant of the rate at which compaction is achieved, in which 1/b is the number of vibrations necessary to reach half of the asymptotic change. Hausner's ratio is the ratio between the tapped and loose bulk density, which was calculated based on the ratio between the asymptotic bulk density (ρ_T) and the initial density (ρ_o).

$$HR = \frac{\rho_T}{\rho_o} \quad (2)$$

The angle of repose was measured using the rotating drum method [3]. The evaporator was modulated to a horizontal direction, and the powder was then placed in the evaporator and the instant spinning angle was measured. The compressibility test [4, 15] was performed in a 30 mm deep testing cell with an internal diameter of 45 mm. The clearance between the cover plate and the cell wall was about 1 mm. The testing cell was mounted on a Texture Analyser (XT-XT2, England), and the powder was compressed at a crosshead speed of 3.0 mm/sec at a 17% strain. The bulk density [ρ_B-pressure (σ_N) relationship] was calculated using the following equation, in which c and d are the constants of the slope of the semi-logarithmic relationship representing the compressibility of the powder.

$$\rho_B = c + d \log \sigma_N \quad (3)$$

A particle size analyzer (CILAS, France) was used to measure the particle size distribution of the *Chlorella* sp. Due to pigmentation of *Chlorella* in water, the *Chlorella* was suspended in cold methanol for the measurement.

Electron Microscopic Observation

The microstructure of *Chlorella* was investigated using a Transmission Electron Microscope (TEM, H-600, Hitachi, Japan). The *Chlorella* sp. was pre-fixed with 2.5% glutaraldehyde (4°C, pH 7.4, phosphate buffer) followed by the addition of 1% agar. The mixture was then washed with the same buffer, fixed in 1% OsO_4 (4°C, pH 7.4, phosphate buffer) for 2 h, and dehydrated by alcohol. The embedding was performed using poly/Bed 812 solutions, and then sliced into 1-mm thick sections using an Ultramicrotome (LK13, 2088, Japan). The tissue was stained by 0.5% toluidine and observed under an optical microscope. The samples were also sliced into 60–90 μm-thick sections and double stained with uranyl acetate and lead citrate for TEM observation.

Preparation of *Chlorella* sp. Tablets and Physical Measurement

The *Chlorella* sp. powder (moisture content, 15% w/w) was mixed with dextrin, and tablets were then prepared using a single-punch (12 mm) tablet-manufacturing machine (ERWEKA type Ku-1, Germany). The weight of the prepared tablets was measured using a chemical balance. The hardness of the tablets was measured with a hardness tester (ERWEKA type TB24, Germany), and the friability test was carried out at 25 rpm for 1 min using a Friabilator (ERWEKA type TAP, Germany).

RESULTS AND DISCUSSION

Growth Curve

The growth curves of the *Chlorella* sp. cultured in a fermentor and pond are presented in Fig. 1. It took 26 h and 260 h for the *Chlorella* sp. cultured in the fermentor and pond to reach the end of the log phase, respectively. Decrease in absorbance after 100 h, 190 h, and 240 h of the *Chlorella* sp. growth in the pond was due to replacement of the new growth media. In the case of the fermentor culture, the generation time, i.e. the time taken to double the initial cell number, was 14 h, while it was 22 h for the pond culture. The yield from the fermentor culture was 4.7% (dry basis) and that from the pond culture was 4.3% (dry basis).

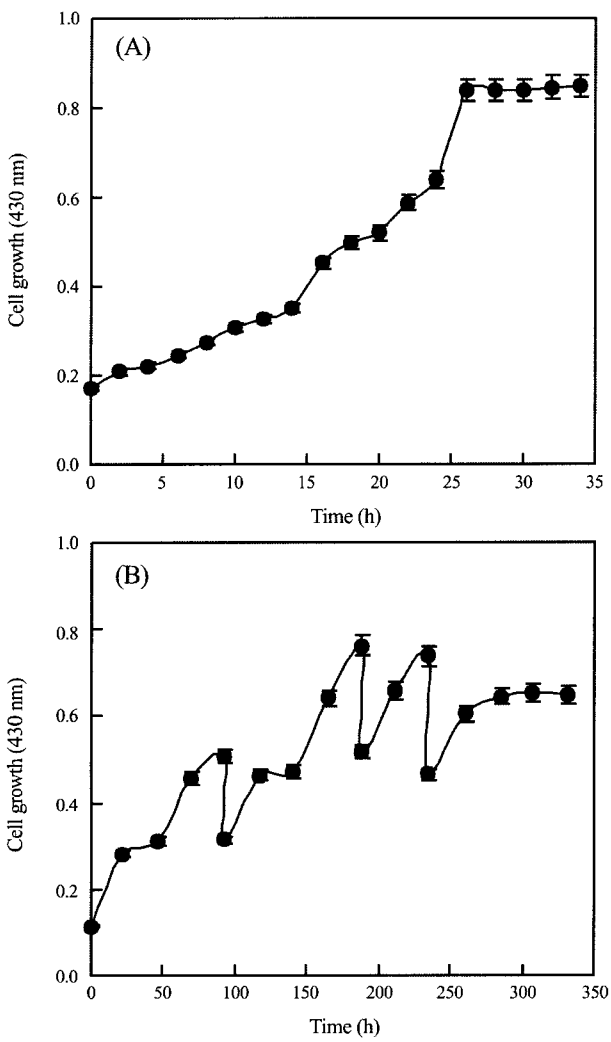

Fig. 1. Growth curves of *Chlorella* sp. cultured in fermentor (A) and pond (B).
Error bars for each data points indicate mean±SD of triplicate measurements. The medium used to grow the *Chlorella* sp. in the pond was renewed after 100 h, 190 h, and 240 h.

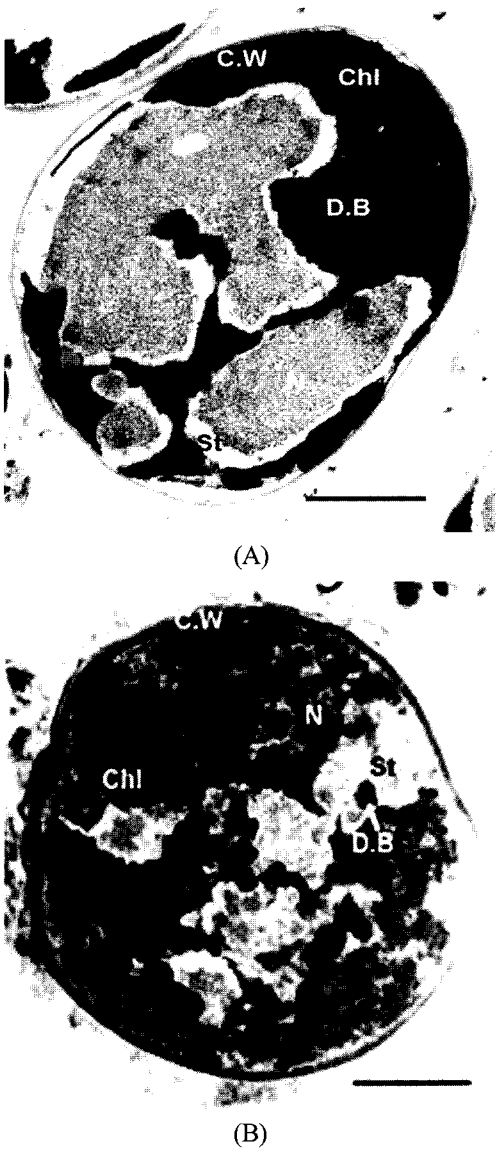

Fig. 2. Transmission Electron Microscope photography of *Chlorella* sp. cultured in the fermentor (A) and pond (B).
The annotations of the cells indicate C.W: cell wall; N: nucleus; Chl: chloroplast; D.B: dense body; and St: starch. The bar length is equivalent to 1 μm.

Morphology

Figure 2 shows photographs of the *Chlorella* sp. cells taken by TEM. The *Chlorella* sp. powder cultured in the fermentor was more spherical in shape and had a thinner cell wall than that cultured in the pond. These cell properties are important determinants for the rheological properties of the powder food [17]. Furthermore, the amount of starch stored in the cells grown in the fermentor was much higher than that in the cells grown in the pond. In contrast, more chloroplast was distributed in the *Chlorella* sp. cells grown in the pond than in the cells grown in the fermentor. These differences were most likely

Table 1. Comparison of the rheological properties of powder *Chlorella* cultivated in the fermentor and the pond.

Rheological parameter	Fermentor	Pond
Yield	4.7%	4.3%
Bulk density	0.250±0.004 g/cm^3	0.150±0.002 g/cm^3
Tapping test	28%	41%
Hausner's ratio	1.5	1.7
Angle of repose	40°	40°
Particle size	2.26±0.02 μm	2.89±0.01 μm
Moisture content	8.9%	7.5%
Tablet weight	0.663 g/tablet	0.593 g/tablet
Hardness test	0.6 kg_f	0.5 kg_f
Friability test	21%	41%

due to the length of the light exposure, which was longer for the *Chlorella* sp. cultivated in the pond, thus giving a longer period of photosynthesis.

The mean particle diameter of the spray-dried *Chlorella* sp. was 2.26±0.02 μm for those cultured in the fermentor and 2.89±0.01 μm for the pond culture (Table 1). When compared with the cells cultured in the pond, the range of size for the *Chlorella* cells grown in the fermentor was more regular (Fig. 3). In general, the range of cell size was very broad for the *Chlorella* sp. grown in the pond (0.3–25.0 μm diameter) compared to that grown in the fermentor (0.3–12.0 μm). The population density also indicated that the majority of the *Chlorella* sp. cells grown in the fermentor had a diameter of 0.3–2.8 μm, while those grown in the pond had a diameter of 0.3–4.3 μm. In the current study, the *Chlorella* sp. with smaller particle diameter exhibited higher loose bulk density. It has earlier been shown that bulk density has some relationship with the powder's porosity, wherein a low density has a high porosity [15]. In addition, Peleg [15] indicated that the more moist or cohesive the powders, the lower the bulk density. This is due to formation of an open bed structure supported by interparticle forces and liquid bridges. However, since such open structures are weak and unstable, they are likely to collapse under relatively small stress. Yet, such a situation is different in fine powders, which are cohesive when the open bed structure is at its maximum and where low moisture content makes it difficult to lower the density. Moreover, a high moisture level can result in liquefaction of the powder, thereby increasing the density. Furthermore, observation of the *Chlorella* sp. cells by TEM clearly showed that those cultured in the fermentor, which had higher bulk density than those cultured in the pond, were more spherical in shape and had a thinner cell wall.

Powder Rheology

Tapping test is an efficient method for measuring the packing property of dried powders. Furthermore, the additional tapping contributes to consolidating the compactness of the powder (Fig. 4). The asymptotic levels of the volume change from the initial volume of the *Chlorella* sp. powder cultured in the fermentor and pond were 28% and 41% after tapping, respectively (Table 1). This indicated that more of the *Chlorella* sp. powder cultured in the pond could be packed in a given container. As explained above, the fermentor cultured *Chlorella* sp. powder appeared to have small interparticle porosity due to its spherical shape, thereby explaining the low volume change after tapping. Also, the distribution of the cell size for those cells grown in the fermentor was more regular than for those grown in the ponds, further supporting the compactness of the cells after tapping.

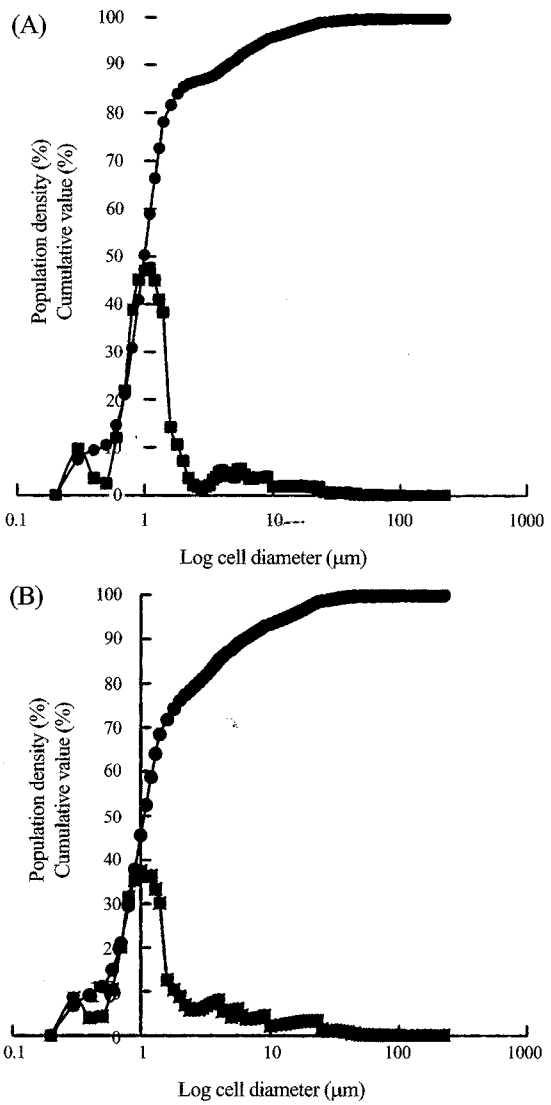

Fig. 3. Cell size distribution of *Chlorella* sp. cultured in fermentor (A) and pond (B).
The cumulative value is annotated by (■) and the population density by (●).

Hausner's ratio indicates a volume change due to external stress or vibration and is also an index of stability [3, 11, 15]. The *Chlorella* sp. powder cultured in the fermentor was more stable and showed smaller volume change with external stress or vibration than the powder grown in the pond culture. As such, Hausner's ratio exhibited a very strong correlation with the results of the tapping test. The initial volume of the powder cultured in the pond was reduced by 41% in the tapping test, indicating that the powder was unstable when exposed to stress.

Based on the measurement of the angles of repose, the flow rate was the same for both culture systems at 40°. It has previously been reported that an angle of repose of up to about 35° indicates free flowability, 35–45° some cohesiveness, 45–55° cohesiveness, and 55° and above very high cohesiveness and very limited flowability [15]. Thus, the *Chlorella* sp. powders prepared in this experiment showed some cohesiveness and flowability.

The constant for compressibility is a sensitive index of a powder's cohesiveness. It has previously been reported that a powder that is cohesive and moist has a high "d" constant value [Eq. (3)] and poor flowability [3, 11, 15], thus in agreement with the measurements made in the current study (Fig. 5). The value of the *Chlorella* sp. powder cultured in the fermentor was higher than that cultured in the pond. Corresponding to the results explained above, the fermentor cultured powder was heavier at a given volume and external pressure, indicating high compressibility because of a direct correlation between the *Chlorella* sp. density and force ($p<0.01$). It was highly likely that the high compactness of the *Chlorella* sp. cells grown in the fermentor was due to their thin cell wall and small regular size.

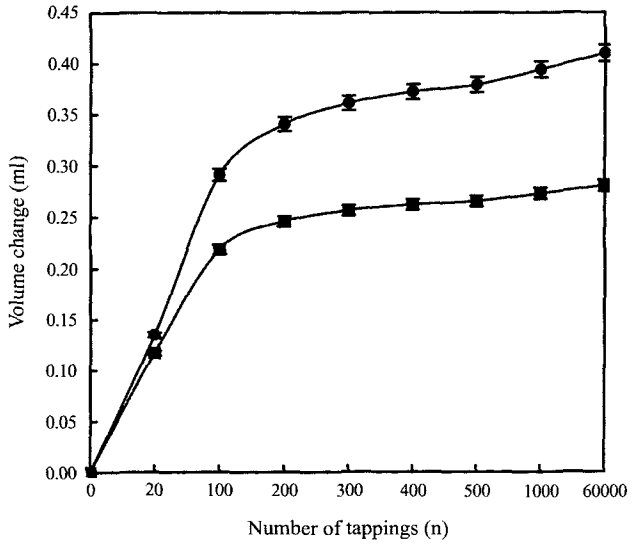

Fig. 4. Tapping test of *Chlorella* powder. Compaction curves of *Chlorella* sp. cultured in fermentor (●) and pond (■). The tapping value n is derived from Eq. (1).
The curve equation calculated was $\gamma_n=(-0.28/(1+n/28)+0.28$ for the *Chlorella* sp. cultured in the fermentor and $\gamma_n=(-0.41/(1+n/41)+0.41$ for the *Chlorella* sp. cultured in the pond.

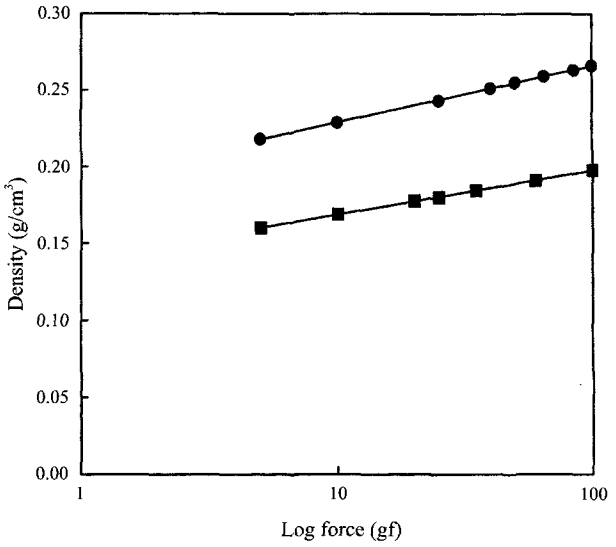

Fig. 5. Compressibility test.
The compressibility is expressed as the relationship between the bulk density and the logarithm force (gf) of the *Chlorella* sp. cultured in the fermentor (●) and pond (■). The logarithmic curve equation calculated was $Y=0.037\log X+0.192$, $r^2=0.997$ for the *Chlorella* sp. cultured in the fermentor and $Y=0.029\log X+0.140$, $r^2=0.997$ for the *Chlorella* sp. cultured in the pond.

Tablet Property

The weights of the tablets are related to the bulk density and "c" values used in the equation for compressibility [Eq. (3)]. The "c" values, which were used to measure the density under stress in the compressibility test, were higher for the *Chlorella* sp. powder cultured in the fermentor than for the pond culture, and the weight of the fermentor cultured tablets was higher than that of pond culture tablets (Table 1). The friability of the fermentor cultured and pond cultured *Chlorella* sp. tablets was 21% and 41% pond, respectively (Table 1). This indicated that the heavier fermentor cultured tablets were also less friable due to high compactness of the cells. The reason for this was likely due to the smaller and regular size of the cells compared to those grown in the pond.

In conclusion, the current study clearly showed the effect of different cultivation conditions on the rheological properties of *Chlorella* sp. powder. An uncontrolled long cultivation of *Chlorella* sp., as in the pond cultivation, produced unfavorable rheological properties when compared to the fermentor, in which the cultivation was controlled. Thus, the two cultivation conditions demonstrated that a small regular size and thin wall of the *Chlorella* sp. cells grown in the fermentor appeared to be the major factor for the differences in these rheological properties.

REFERENCES

1. Atsushi, M. 2001. *Chlorella* as a functional food in Japan. The 3rd *Chlorella* International Symposium. Konkuk University, Seoul, Korea.
2. Bold, H. C. 1985. *Introduction to the Algae*, pp. 17–33. 2nd ed. Prentice Hall, Englewood Cliffs, New Jersey, U.S.A.
3. Bhandri, B. R., N. Datta, and T. Howes. 1997. Problems associated with spray drying of sugar-rich foods. *Drying Technology* **15:** 671–684.
4. Chang, K. S., D. W. Kim, and S. S. Kim. 1996. Bulk flow properties of selected food powders at different water activity levels. *Food Biotech.* **5:** 76–84.
5. Choi, S. 1968. *Algae*, pp. 411–458. Society of Korean *Chlorella* Research.
6. Fellows, P. 1988. *Food Processing Technology - Principle and Technology.* VCH Publishers, New York, New York, U.S.A.
7. Hwang, H. G. and D. H. Ryu. 1984. A study on the N^{15}-labelling and path way of *Chlorella* in the course of culture. *J. Korean Soc. Food Nutr.* **13:** 403–405.
8. Lee, J. S., D. K. Kim, J. P. Lee, S. C. Park, J. H. Koh, and S. J. Oh. 2001. CO_2 fixation by *Chlorella* KR-1 using flue gas and its utilization as a feedstuff for chicks. *J. Microbiol. Biotechnol.* **11:** 772–775.
9. Lee, K. S., K. S. Min, and N. D. Kim. 1986. Optimal culture conditions of wild *Chlorella ellipsoidea* for tapioca alcohol distillation waste treatment. *J. Kor. Wat. Pollut. Res. Contr.* **2:** 35–42.
10. Liu, B. H., D. H. Zhang, and Y. K. Lee. 2000. Effects of nutrient levels on cell growth and secondary carotenoids formation in the fresh water green alga, *Chlorococcum* sp. *J. Microbiol. Biotechnol.* **10:** 201-207.
11. Malave, J., G. V. Barbosa-Canovas, and M. Peleg. 1985. Comparison of the compaction characteristics of selected food powders by vibration, tapping and mechanical compression. *J. Food Sci.* **50:** 1473–1476.
12. Mitsuda, H. 1973. Protein isolates from *Chlorella* algae, torula yeasts, and hydrocarbon-assimilating microoraganisms. *J. Nutr. Sci. Vitaminol.* **19:** 1–13.
13. Moreyra, R. and M. Peleg. 1980. Compressive deformation patterns of selected food powders. *J. Food Sci.* **45:** 864–868.
14. Park, K. H., D. I. Kim, and C. G. Lee. 2000. Effect of flashing light on oxygen production rates in high-density algal cultures. *J. Microbiol. Biotechnol.* **10:** 817–822.
13. Peleg, M. 1983. Physical properties of food powders, pp. 293–323. *In*: M. Peleg and E. B. Bagley (eds.), *Physical Properties of Foods,* AVI Publishing Co., Inc. Westport, U.S.A.
14. Shi, X. M., H. J. Liu, X. W. Zhang, and F. Chen. 1999. Production of biomass and luten by *Chlorella protothecoides* at various glucose concentrations in heterotrophic cultures. *Process Biochem.* **34:** 341–347.
15. Wayne, R. and M. P. Staves. 1991. The density of the cell sap and endoplasm of nitellopsis and chara. *Plant Cell Physiol.* **32:** 1137–1144.
16. Yamagishi, Y., S. Y. Hasuda, and V. Mito. 1970. Experience in taking *Chlorella* for healing less curable wound. *In*: C. J. Hwang (ed.), *Application of Chlorella Medicine and Food,* Technical Bulletin, *Chlorella* Manufacture Co., Ltd., Taipei, Taiwan.