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Effects of Different Light Intensities and Nutrient Source on Growth Rate and Crude Fat Content in *Chlorella vulgaris*

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Abstract

The aim of the present work was to study the effects of light intensities and culture medium prepared with two different types of feed on the growth rate and crude fat content of *Chlorella vulgaris*. Two different light wavelengths 796 µmol m^{-2} s^{-1} or 90% red light-emitting diodes (LEDs) and 129 µmol$m^{-2}s^{-1}$ (10% red LEDs) in combination with two different nutrient sources: 40/10 (40% crude protein and 10% crude lipid) and 32/6 diet as the nutrient source in the culture medium were employed to explore the effects of the treatments. From the experimental results, 90% red LEDs in combination with 40/10 diet exhibited the highest relative growth rate at the beginning of culture medium (day 1 and 3), while the 10% red LEDs yielded significantly higher growth rate at the end of culture medium (day 6). The light saturation could affect the growth of algae reared under 90% red LEDs, while gradual increase of light intensities in 10% red LEDs promotes better carrying capacity to support the growth of algae during the culture period. However, no significant differences in terms of crude fat content among all treatments. In comparing the growth rate and fat content, the use of 40/10 diet gave the most effective performance for the algae cultivation as the alternative feed source for aquaculture.

Keywords: *Chlorella vulgaris*; Light intensity; Culture medium; Growth rate; Cell density; Crude fat

Introduction

It has been suggested that the proper use and nutritional composition of microalgae provides several benefits in an aquaculture food chain, especially to boost the fatty acid content of live food [1,2] or as a direct food sources and feed additive for some species of fish [3,4]. In addition, microalgae also play a role in enhancing the quality of fish by providing natural sources of pigments, such as astaxanthin and carotenoids to achieve the required flesh color [5]. Recently, the application of green-water technique, to describe the presence of high level of microalgae in the larviculture environment, led to the lower bacterial species diversity compared to clear water system [6] and able to support the growth of beneficial bacteria [7]. Based on these findings, the establishment of more efficient and reliable production of microalgae is needed to increase the aquaculture productivity.

The recognition of microalgae as a good source of protein and lipid rich in polyunsaturated fatty acids (PUFA) is increasingly being taken into account [8,9]. General composition of protein and lipid in different microalgae compiled by Becker [10] indicated that several species commonly used in aquaculture production, such as *Chlorella vulgaris* and *Dunaliella salina* even contain with high protein level 51% to 58% and 57%, respectively, and lipids at the range of 14% to 22% and 6%, respectively. However, the nutritional value may vary significantly and change under different culture conditions [11]. Several factors such as minerals, carbon dioxide, temperature and particularly light could become the major limitation [12]. A number of studies even specifically indicated that changes in light intensity and nitrogen concentration within the culture media have a considerable effect on the formation of lipids [13,14]. Since sun light did not penetrate beyond a depth of 5 cm with high density population [12] and nutrient supply constitutes a substantial cost [15], the use of light-emitting diodes (LEDs) which offer variable wavelength spectra in combination with culture medium of Nile tilapia *Oreochromis niloticus* fed with different nutrient characteristics seems to be practical to effectively increase the density and lipid content of microalgae. Discharge of organic waste and inorganic nutrients generated from feeding practices within fish farming system could be significant. Study from Chatvijitkul et al. [16] illustrated that the amount of nitrogen and phosphorus released to the culture environment per one metric ton of feed applied in tilapia culture system would be around 29.3 and 7.4 kg, respectively. Indeed, this amount sufficient enough to induce the growth of microalgae, including the green algae *C. vulgaris*. Interestingly, tilapia feeds naturally on microalgae and could obtain around 30% of their nutritional needs from this natural food [17]. As a result, the later author also points out that globally, around 2.5 million metric ton of microalgae directly consumed by tilapia reared in green water system. Although the development of practical diet has shown their beneficial effect [18], nutritional benefits from microalgae could significantly reduce the food conversion ratio, production cost and improve profitability for the tilapia industry [19]. Thus, the purpose of the present study was to evaluate the growth rate and lipid content of *C. vulgaris* reared in different light intensities and nutrient characteristics as an alternative food source for farmed fish production system.

Materials and Methods

Algae and culture medium

Chlorella vulgaris was selected due to its abundance in tilapia *O. niloticus* culture tanks located at the E.W Shell Auburn University Fisheries Research Unit, Auburn, AL, USA. In this study, *Chlorella vulgaris* UTEX 259 from the culture collection of algae at the University of Texas (Austin, TX, USA) was purchased from Suncoast Marine Aquaculture, FL, USA. Prior to the initiation of experiment, *C. vulgaris* was scaled up in the 2000 ml Erlenmeyer flasks containing 1000 ml of

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distilled water and f/2 medium based on Guillard [20] and manufacture protocol. The composition of nutrient, trace metals and vitamins used in the f/2 medium are presented in Table 1. The algae was cultured and grown at 28 ± 2°C under a continuous cool-white fluorescent light with 14:10 light-dark cycle. The solution was hand shaken three to five times daily to avoid sticking. Incubation was carried out for 5 days where late exponential growth of the algae was used for the trial. Prior to use, the cell density of *C. vulgaris* was counted using hemocytometer and observed under microscope (ECLIPSE 80i, Nikon, Japan).

Artificial culture media preparation

The purpose of artificial culture medium is to mimic the culture environment of farmed fish by using prepared diet as the primary nutrient source. The fresh water used for the artificial culture was sterilized first in the autoclave at the 121^{0}C for 15 min. After sterilization, two 75 L culture tanks was filled with this sterile water and four tilapia (average weight 11.2 ± 1.2 g) were stocked into each tank. To mimic the culture environment, tilapia were fed with two different type of commercial diet that commonly used in tilapia grow-out production system, 40/10 diet (Cargill Inc., Franklinton, LA, USA with 40% crude protein (CP), 10% crude lipid (CL), and 3 mm in size) and 32/6 diet (Southfresh feed mill, Demopolis, AL, USA with 32% CP, 6% CL and 6 mm in size), as much as 3 g per day to serve as the nutrient source to the growth of algae. The culture manipulation was performed for 5 days.

Water characteristics of artificial medium

After 5 days of culture medium preparation, water samples were collected per each tank to determine the pH, dissolved oxygen (DO) and salinity (Electrometri, ProPlus, YSI Inc, Yellow Springs, USA), total ammonia nitrogen (TAN) [21] and nitrate-nitrogen (NO_3-N) (colorimetric test kit, La Motte Chemicals, Chestertown, MD, USA). Water samples were processed instantaneously to prevent sample degradation due to the preservation.

Microalgae cultivation condition

Indoor experiment was carried out under laboratory condition using eighteen 250 ml Erlenmeyer flask, consist with two different nutrient sources (40/10 and 32/6 diet) compared with normal nutrition medium (f/2 algae culture medium) for cultivation of *C. vulgaris* in three replicates. Two different light intensity, 796 μmolm^{-2}s^{-1} or 90% red light-emitting diodes (LEDs) and 129 μmolm^{-2}s^{-1} (10% red LEDs) measured by using a quantum flux meter probe (LI-250 Light Meter and LI-190 Quantum Sensor, LI-COR Biosciences, Lincoln, Nebraska, USA) were applied constantly to induce the growth of algae grown in different culture medium. The characteristics of light source used in this trial described as housing size with 208 pcs diode count, single 3 w and 5 w chips for the type of LED, 50/60 Hz feeding frequency and 640 mA for current input. 50 ml of pre-determined density of the microalgae (4 × 10^4 cells L^{-1}) were introduced to each Erlenmeyer flask with 150 mL of deionized water. Then the flask was aerated with compressed air and illuminated (Table 1).

Growth measurement

The relative growth of *C. vulgaris* (day^{-1}) was determined spectrophotometrically (Spectronic Genesys 2 spectrophotometer, Spectronic instruments, Rochester, NY USA) daily as an increase in 540 nm [22]. The relative growth rate (μ) was measured by using formulae described below:

$$relative\ growth\ rate = \frac{\ln\left(\frac{A_2}{A_1}\right)}{t_2 - t_1}$$

Where A_1 and A_2 are defined as absorbance at time t_1 and t_2, respectively. In addition, number of cells also counted on daily basis until the end of incubation. Sub-samples were taken aseptically, diluted with distilled water and number of cells in Haemocytometer was counted under the microscope.

Lipid extraction

Samples of *C. vulgaris* was centrifuged in an Eppendorf centrifuge (5810, Germany, 3000 rpm for 10 min) to obtain the concentrated biomass of the algae and then subjected to extraction process by using hexane based on the method described by Bligh and Dyer (1959).

Statistical analysis

Mean results for growth rate and lipid fraction were expressed as a mean ± standard deviation (SD) and subjected to two-way analysis of variance (ANOVA) with interaction using light and the nutrient source for the culture medium as the independent variables. Student's *t* test was applied to assess any difference in growth rate and lipid fraction between two different treatments. Statistical significance was defined at $p<0.05$ and analyzed using the General Linear Model procedure in the SAS system (V9.4, SAS Institute, Cary, NC, USA).

Results

Water quality

In this trial, pH, DO and salinity at the range of 7.75 ± 0.24, 7.31 ± 0.35 and 0 ‰ in 32/6 diet culture medium, respectively and 7.78 ± 0.33, 7.24 ± 0.52 and 0 ‰ for 40/10 diet culture medium, respectively. Numerical value of TAN in 40/10 diet culture medium was slightly higher 0.059 ± 0.010 mg L^{-1} compared to 0.052 ± 0.008 in 32/6 diet culture medium. The concentration of NO_3-N in 40/10 diet was higher 3.442 ± 0.429 mg L^{-1} compared to 2.873 ± 0.534 mg L^{-1} in 32/6 diet culture medium.

Growth rate

Level of crude protein and light intensity significantly affect the relative growth of *C. vulgaris* in day 3, day 4 and Day 6. However, no significant interaction between these two variables on relative growth of *C. vulgaris* in day 1. In day 1 and day 2, the growth of algae cultured in 796 μmolm^{-2}s^{-1} or 90% red LEDs in combination with addition of 40/10 diet was significantly higher compared to other treatments. Interestingly, at day 4 and 6 the relative growth rate of *C. vulgaris* cultured in 90% red LEDs tend to decline and significantly lower compared to the growth of algae cultured in 10% red LEDs fed with 40/10 diet. Overall, during the observation period, *C. vulgaris* cultured in control medium always yield the lowest relative growth rate compared to the artificial culture medium (Table 2).

Values are mean of three replicates. Means within columns with different superscript letter are significant different (p <0.05) based on analysis of variance followed by Tukey multiple comparison test

1. CP = Crude protein

2. Int. = Intensity

Cell density

The cell density of *C. vulgaris* cultured in control medium under 10% red LEDs was constantly lower compared to other treatment. However, in 90% red LEDs, even though showed similar trend from day 1 to day 4, at the final day of culture period, the cell density of algae

cultured in control medium was higher compared to algae cultured in 32/6 medium. Under 90% red LEDs regime, the cell density of algae cultured in 40/10 culture medium continuously increase from day 1 to day 4 and tend to decline in Day 6. The constant higher cell density in 40/10 culture medium reared under both 90% and 10% red LEDs indicated that the nutrient capacity in 40/10 diet was higher compared to 32/6 and control medium (Figure 1).

Crude fat content

The percentage of crude fat (CF) content in *C. vulgaris* is presented in Table 3 and ranged from 5.79% to 7.22%. No significant differences were observed across all treatments both under 10% and 90% red LEDs (p=0.0811). The two-way ANOVA indicated that the CF percentage was not significantly affected by light intensity (p=0.9435). On the contrary, the culture medium seems to have a significant effect to the CF content (p=0.0406).

Discussion

The water characteristics clearly showed that the 40/10 diet was the most favorable nitrogen source compared to 32/6 diet to increase the nitrogen concentration in the culture environment. Refers to the illustration provided by Chatvijitkul et al. [13], higher protein content will generate higher nitrogen load entering the culture environment. Numerous study reveal that the estimates of nitrogen value excreted from feeding activities could be significant [23-25] and lead to the organic enrichment of the culture environments [26]. Given the productivity, it is clear that the *C. Vulgaris* can take the advantage from this situation to enhance the relative growth rate and cell density.

Results also suggest that changes in light regimes have been shown to bring about differences in the optimal growth rate of *C. vulgaris*. Our results indicated that at day 1 and day 3, 90% red LEDs (796 µmol m^{-2} s^{-1}) generate faster growth rate compared to the 10% red LEDs (129 µmol m^{-2} s^{-1}) for the *C. vulgaris* cultured in 40/10 diet. However, at day 4 and 6, within similar culture medium, the 10% red LEDs could yield faster growth rate compared to the 90% red LEDs. According to Nielsen et al. [27] high light intensity could induce the culture medium reach saturation condition and eventually influence the growth rate. Therefore, with additional reduction of carrying capacity in the culture medium reared under high light intensity due to the frequent sampling regime, light saturation will accelerate the algae to reach the declining phase. On the other hand, at the later day, growth of algae in low light intensity become higher than algae cultured in high light intensity. This probably due to the carrying capacity of culture medium reared under low light intensity still not reach saturation levels and able to support the optimum growth of *C. vulgaris*, even frequent sampling regime also performed in this treatment and gradually reduce the environment carrying capacity.

It has been recognized that the light intensity and culture medium play a major role in determining the quantity of fatty acids produced by microalgae [28,29]. Our results on crude fat content showed that there is no significant differences between algae cultured in low and high light intensity in combination with low and high nutrient content. Furthermore, in comparison with control treatment, the change in light intensity and culture medium also did not cause any significant effect to the crude fat content. However, the concentration of crude fat derived from these microalgae still able to meet the requirement level for tilapia proposed by NRC [30]. Despite no significant differences in the

Chemical component	Mass ($gmol^{-1}$)	Final concentration (M)	Final concentration (gL^{-1})
$NaNO_3$	84.98	8.82×10^{-4}	0.075
$NaH_2PO_4.H_2O$	137.97	3.62×10^{-5}	0.005
$FeCl_3.6H_2O$	270.3	1.17×10^{-5}	0.0032
$MnCl_2.4H_2O$	197.01	9.10×10^{-7}	1.79×10^{-4}
$ZnSO_4.7H_2O$	186	7.65×10^{-8}	2.19×10^{-5}
$CoCl_2.6H_2O$	237	4.20×10^{-8}	9.95×10^{-6}
$CuSO_4.5H_2O$	249	3.93×10^{-8}	9.79×10^{-6}
$Na_2MoO_4.2H_2O$	237.88	2.60×10^{-8}	6.18×10^{-6}
Thiamine-HCL (Vitamin B1)	333.27	2.96×10^{-7}	1.00×10^{-4}
Biotin (Vitamin H)	242.45	2.05×10^{-9}	5.00×10^{-7}
Cyanocobalamin (Vitamin B12)	1355.4	3.69×10^{-10}	5.00×10^{-7}
$Na_2SiO_3.9H_2O$	284.04	1.06×10^{-4}	0.03
$Na_2EDTA.2H_2O$	374.24	1.17×10^{-5}	0.0044

Table 1: Chemical component of f/2 algae food adopted [20].

Treatment	Relative growth rate (day^{-1})			
	Day 1	Day 3	Day 4	Day 6
Control+10% red LEDs	0.4427^{b}	0.6317^{c}	0.4843^{c}	0.0488^{b}
32/6 diet+10% red LEDs	0.4797^{b}	0.7045^{bc}	0.6944^{b}	0.1157^{a}
40/10 diet+10% red LEDs	0.5976^{ab}	0.7035^{bc}	0.8842^{a}	0.1340^{a}
Control+90% red LEDs	0.4655^{b}	0.6540^{c}	0.2271^{d}	0.0447^{b}
32/6+90% red LEDs	0.5373^{ab}	0.7603^{b}	0.4900^{c}	0.0546^{b}
40/10+90% red LEDs	0.7131^{a}	0.8840^{a}	0.4811^{c}	0.0379^{b}
PSE				
Two-way ANOVA				
Model	0.0147	<0.0001	<0.0001	0.0001
Light intensity	0.1203	<0.0001	<0.0001	<0.0001
Crude protein level	0.0034	<0.0001	<0.0001	0.0063
Light*Crude protein	0.6299	0.0019	0.0196	0.0049

Table 2: Effect of two different light intensity and nutrient sources on the relative growth rate of *Chlorella vulgaris* for 6 days of growth culture.

Treatment	Crude fat (%)
Control + 10% red LEDs	5.8957
32/6 diet + 10% red LEDs	6.5622
40/10 diet + 10% red LEDs	7.5005
Control + 90% red LEDs	6.8762
32/6 + 90% red LEDs	5.7950
40/10 + 90% red LEDs	7.2197
PSE	
Two-way ANOVA	***p*-value**
Model	0.0811
Light intensity	0.9435
Crude protein level	0.0406
Light * Crude protein	0.1296

Table 3: Crude fat content as the effect of different light intensity and culture medium.

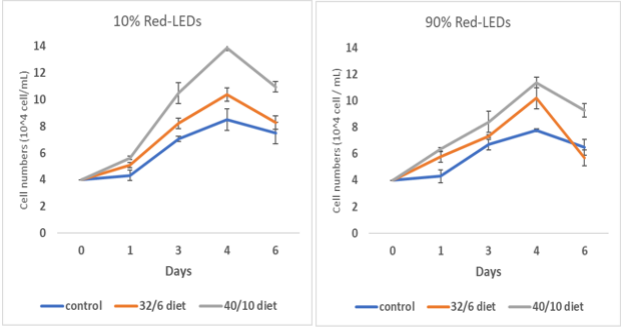

Figure 1: Effects of 10% red LEDs (A) or 90% red LEDs (B) in combination with two different feed sources (40/10 and 32/6) on cell density of *C. vulgaris* (cells mL^{-1}). Error bars represent standard error (n=3). Initial density= 4×10^4 cells mL^{-1}.

statistical outcome, in biological response stand point, it is interesting to note the fact that the utilization of better nutrient sources leads to the increased level of crude fat in *C. vulgaris*, which was even similar with the crude fat content in commercial freeze-dried *Chlorella spp* [31].

Conclusion

Artificial culture medium prepared by utilizing 40/10 diet produced much higher yields of cell density and faster growth rate of *C. vulgaris* than culture medium prepared by using 32/6 diet. A change in light intensity and culture medium did not cause any significant effect to the crude fat content of *C. vulgaris*. The proper application of light intensity and feeding regime were expected to be a potential strategy to improve the feed efficiency, lowering the production cost and increase the profitability of aquaculture production system, especially for the fish that utilize *C. vulgaris* as one of the feed sources.

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Referances

1. Reitan KI, Rainuzzo JR, Øie G, Olsen Y (1997) A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155: 207-221.
2. Chakraborty RD, Chakraborty K, Radhakrishnan EV (2007) Variation in fatty acid composition of Artemia salina nauplii enriched with microalgae and baker's yeast for use in larviculture. J Agric Food Chem 55: 4043-4051.
3. Stanley JG, Jones JB (1976) Feeding algae to fish. Aquaculture 7: 219-223.
4. Roy SS, Pal R (2015) Microalgae in aquaculture: A review with special references to nutritional value and fish dietetics. Microalgae in aquaculture: A review with special references to nutritional value and fish dietetics. Proc Zool Soc 68: 1-8.
5. Johnson E, Schroeder W (1995) Astaxanthin from the yeast Phaffia rhodozyma. Studies in Mycology 39: 81-90.
6. Nicolas JL, Corre S, Cochard JC (2004) Bacterial population association with phytoplankton cultured in a bivalve hatchery. Microb Eco 48: 400-413.
7. Gil GB, Roque A, Blanco VG (2002) Culture of vibrio alginolyticus C7b, a potential probiotic bacterium, with the microalga Chaetoceros muelleri. Aqu aculture 211: 43-48.
8. Yan C, Fan J, Xu C (2013) Analysis of oil droplets in Microalgae. Methods Cell Biol 116: 71-82.
9. Solana M, Rizza C, Bertucco A (2014) Exploiting microalgae as a source of essential fatty acids by supercritical fluid extraction of lipids: Comparison between *Scenedesmus obliquus*, *Chlorella protothecoides* and Nannochloropsis salina. J Supercrit Fluids 92: 311-318.
10. Becker EW (2007) Micro-algae as a source of protein. Biotechnol Adv 25: 207-210.
11. Brown MR, Jeffrey SW, Volkman JK, Dunstan GA (1997) Nutritional properties of microalgae for mariculture. Aquaculture 151: 315-331.
12. Richmond A, Vonshak A, Arad SM (1980) Environmental limitations in outdoor production of algal biomass. Algae biomass: Production and use/[sponsored by the National Council for Research and Development, Israel and the Gesellschaft fur Strahlen-und Umweltforschung (GSF), Munich, Germany]; editors, Gedaliah Shelef, Carl J Soeder 2: 1.
13. Piorreck M, Pohl P (1984) Formation of biomass, total protein, chlorophylls, lipids and fatty acids in green and blue-green algae during one growth phase. Phytochemistry 23: 217-223.
14. Yeesang C, Cheirsilp B (2011) Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. Bioresour Techno 102: 3034-3040.
15. Stephens E, Ross IL, Mussgnug JH, Wagner LD, Borowitzka MA, et al. (2010) Future prospects of microalgal biofuel production systems. Trends Plant Sci 15: 554-564.
16. Chatvijitkul S, Boyd EC, Davis DA (2017) Nitrogen, phosphorus, and carbon concentrations in some common aquaculture feeds. J World Aquac Soc 49: 477-483.
17. Neori A (2011) "Green water" microalgae: The leading sector in world aquaculture. J Appl Phycol 23: 143-149.
18. Trosvik KA, Webster CD, Thompson KR, Metts LA, Gannam A, et al. (2013) Effects on growth performance and body composition in Nile tilapia, Oreochromis niloticus, fry fed organic diets containing yeast extract and soyabean meal as a total replacement of fish meal without amino acid supplementation. Biol Agric Hortic 29: 173-185.
19. Popma TJ, Lovshin LL (1996) Worldwide prospects for commercial production of tilapia, International Center for Aquaculture and Aquatic Environments 2: 1.
20. Guillard RR (1975) Culture of phytoplankton for feeding marine invertebrates. Culture marine invertebrate animals pp: 29-60.
21. Callaway J (1992) Ammonia and nitrite. Standard methods for the examination of water and wastewater pp: 75-87.
22. Xiong W, Li X, Xiang J, Wu Q (2008) High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production. Appl Microbiol Biotechno 78: 29-36.
23. Liao PB, Mayo RD (1974) Intensified fish culture combining water reconditioning with pollution abatement. Aquaculture 3: 61-85.
24. Bromley PG, Smart G (1981) The effects of the major food categories on growth, composition and food conversion in rainbow trout (Salmo gairdneri Richardson). Aquaculture 23: 325-336.
25. Penczak T, Galicka W, Molinski M, Kusto E, Zalewski M (1982) The enrichment of a mesotrophic lake by carbon, phosphorus and nitrogen from the cage aquaculture of rainbow trout, Salmo gairdneri. J Appl Ecol 19: 371.
26. Islam MS (2005) Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: Review and analysis towards model development. Marine Poll Bull 50: 48-61.

27. Nielsen ES, Hansen VK, Jorgensen EG (1962) The adaptation to different light intensities in *Chlorella vulgaris* and the time dependenee on transfer to a new light intensity. Physiol Plant 15: 505-517.

28. Renaud S, Parry D, Thinh LV, Kuo C, Padovan A, et al. (1991) Effect of light intensity on the proximate biochemical and fatty acid composition of *Isochrysis* sp. and *Nannochloropsis oculata* for use in tropical aquaculture. J Appl Phycol 3: 43-53.

29. Solovchenko A, Goldberg KI, Cohen DS, Cohen Z, Merzlyak M (2008) Effects of light intensity and nitrogen starvation on growth, total fatty acids and arachidonic acid in the green microalga *Parietochloris incisa*. J Appl Phycol 20: 245-251.

30. NRC (National Research Council), (2011) Nutrient requirements of fish and shrimp. National Academy Press. Washington, D.C, US.

31. Lubitz JA (1963) The protein quality, digestibility, and composition of algae, Chlorella 71105. J Food Sci 28: 229-232.