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〈Original Article〉

Growth and changes in the biochemical composition of Isochrysis galbana under different light-emitting diode conditions

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The marine microalgae *Isochrysis galbana* was cultured under various light–emitting diode (LED) light conditions with different wavelengths to examine changes in growth and in amino acid and fatty acid profiles. The culture conditions for the microalgae were Conway medium, salinity of 33 psu, temperature of 24°C, and a 16/8 h light/dark photoperiod. Six light sources, including 5 units of 180W LED lamps (peak wavelength: blue [LB] 470 nm; green [LG] 525 nm; yellow [LY] 595 nm; red [LR] 636 nm; white [LW] 442 nm) and 1 unit of a 175W metal halide (MH) lamp, were used for the experiment. The dry cell weights (gL^{-1}) of *I. galbana* under different light conditions were in the order of LW>LB≥MH>LR>LG>LY. Levels of essential amino acids were revealed to be significantly higher under LW, LG, and MH than under the other wavelengths ($P<0.05$). The fatty acid, unsaturated fatty acid, and DHA contents of *I. galbana* were higher under MH, LW, and LG. In addition, the carotenoid content was higher under MH, LW, and LG than under the other wavelengths ($P<0.05$). The fucoxanthin content was highest under MH (0.28%) and lowest under LY (0.2%), and it was 0.26% under LW and LG. The results indicate that the combined use of LW and LG is effective when using LED lamps for *I. galbana* cultivation.

Key words: *Isochrysis galbana*, LED lamp, fatty acid, carotenoid, fucoxanthin

Introduction

Investigations into the industrial use of microalgae were initiated in Germany in the 1940s, in order to produce vegetable fat and oil through mass cultures of Bacillariophyceae. Biotechnology based on microalgae is a rapidly growing field (Cook 1951; Gummert et al. 1953). Various microalgal components have been used as natural pigments, drug materials, biochemical substances, feed materials, alternative energy sources, and health supplements (Oh et al. 2003). The efficient use of microalgae requires nutritional evaluation; thus, previous studies have reported on the use of microalgal proteins as health foods or animal feed (Fuentes et al. 2000); microalgal carbohydrates as emulsifiers and stabilizers in food (Brown and Jeffrey 1992); and microalgal fatty acids in relation to arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahex-

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aenoic acid (DHA) in medicines (Belarbi et al. 2000; Carvalho and Malcata 2000; Shamsudin 1992). Moreover, chlorophylls and carotenoids derived from microalgae have been widely used as ancillary pigments in food, for the protection of materials against oxidation, and as antioxidants in cosmetics; they have also been approved by the US Food and Drug Administration as anti–cancer medicines and for skin disease treatments (Oh et al. 2003).

When microalgae are used as live food for larval breeding, they must be nutritionally balanced, and the content of highly unsaturated fatty acids (HUFA) compared to other fatty acids must be particularly high (Langdon and Walcock 1981; Chu and Webb 1984). In addition, the mass culture must be easy to perform, in order to supply the target organism as feed (Lid and Abalde 1992). Thus, because *Isochrysis galbana* has a high HUFA content, it has been widely used as feed for farmed fish and shellfish (Volkman et al. 1989) and as a health supplement in the food industry due to its high nutritional value (Sun and Wang 2009).

The wavelength of light is an important factor in microalgal photosynthesis (Sanchez–Saavedra and Voltolina 1994). The optical specificity of monochromatic light sources has been evaluated for productivity enhancement in the mass culture of microalgae supplied for live feed in marine farming (Flaak and Epifanio 1978; Sanchez–Saavedra and Voltolina 1994). Currently, fluorescent light is mainly used for photosynthesis; however, light–emitting diodes (LEDs) have recently been developed to enhance profitability and efficiency. LEDs are long–lasting and have excellent properties of low power dissipation and fast reaction times (Oh et al. 2007). Therefore, LEDs have recently been highlighted as a next–generation energy–saving light source in the fishing industry (Choi 2008).

The present study showed that LEDs of different wavelengths can be used to cultivate *I. galbana* microalgae with different effects on growth, as well as on amino acid, fatty acid, and pigment contents. Thus, the use of LEDs enhances the production of this useful material for the aquaculture, food, and medical industries by altering its biochemical characteristics.

Materials and Methods

Cultivation of microalgae under different LED light sources

I. galbana was obtained from the Korea Marine Microalgae Culture Center (KMMCC) and batch–cultured in a 2–L flask in Conway medium (Walne 1979) at 24°C and 33 psu. When the stagnation period was reached, the microalgae were harvested and used in the experiment.

Six light sources, including five units of 180W LED lamps (WFLL180, Wisepower, Korea; peak wavelengths: blue [LB] 470 nm; green [LG] 525 nm; yellow [LY] 595 nm; red [LR] 636 nm; white [LW] 442 nm) and 1 unit of a 175W metal halide (MH) lamp (HSL–MH175W, Hwasung, Korea) were used for the experiment (Fig. 1, Table 1).

Table 1. Specification of light sources used in the experiment

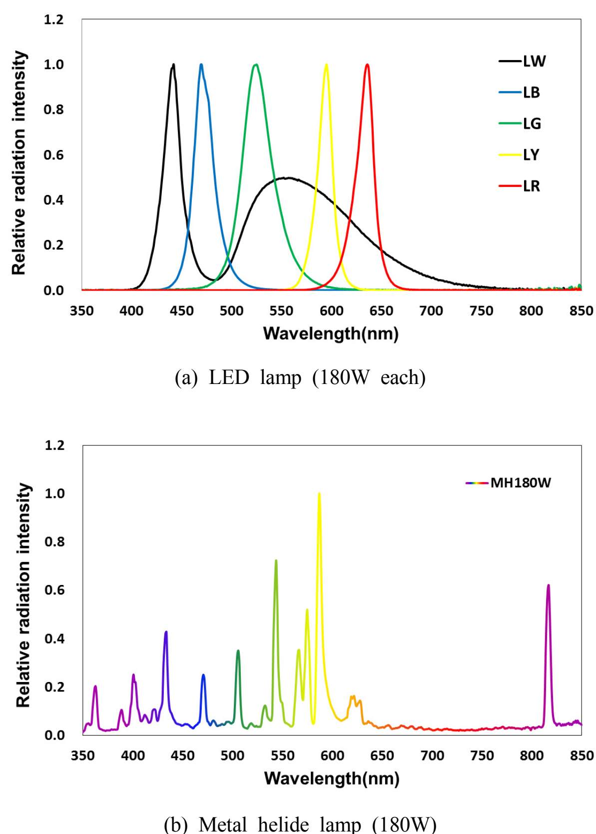
Light source	Peak wavelength (nm)	Voltage (V)	Current (A)	Irradiant flux (mW/㎡)	Photon flux density (μ ㏖/s/㎡)
LB	470	26.4V	6.6	32.92	130.45
LG	525	26.4V	6.6	11.89	52.64
LY	595	26.4V	6.6	5.68	27.98
LR	636	26.4V	6.6	19.34	102.19
LW	442	26.4V	6.6	28.40	128.95
MH	587	AC 220V	0.8	23.20	105.99

* LB, led blue; LG, led green; LY, led yellow; LR, led red; LW, led white; MH, metal halide lamp.

The dry weight (DW), which indicated growth, was measured daily after 50 mL of the culture fluid was filtered using a GF/C filter paper (47 mm, Whatman^{TM}, UK) and the remaining salt was dissolved in distilled water, then dried for 1 h at 100°C. The DW was calculated according to the following formula:

$$DW = \frac{W_2 - W_1}{t_2 - t_1} \quad (1)$$

where W_1 and W_2 are the DW (g/L) at t_1 and t_2, respectively.



(a) LED lamp (180W each)

(b) Metal helide lamp (180W)

Fig. 1. Relative radiation intensity curve of different light sources used in the experiment.

(LB, LED blue; LG, LED green; LY, LED yellow; LR, LED red; LW, LED white)

Biochemical composition analysis

To analyze the amino acids, 10 mg of each freeze–dried sample was placed in a 15–mL glass tube and hydrolyzed for 22 h at 120°C by adding 6N HCl. After the 6 N HCl was completely evaporated, 5 mL of 0.02 N HCl was added, and the amino acids were melted. Then, using a 0.45–μm filter, the melted amino acids were placed in a 1.8–mL vial, filling as much of the vial as possible, and they were then analyzed using an amino acid analyzer (Hitachi L–8800, Japan).

Microalgae were harvested and kept at −80°C after centrifuging at 7,000 rpm for 15 min (SORVALL ST16R, Thermo, Germany). In the fatty acid analysis, lipids were extracted according to the method of Parrish (1987), and the fatty acids were extracted using BF3–methanol with the fatty acid methylation method of Morrison and Smith (1964). The extracted fatty acids were analyzed by gas chromatography (HP 6,890plus, Agilent, USA), in which a capillary column (OMEGAWAX 250, Supelco^{TM}, USA) was used. The carrier gas was nitrogen (30 mL min^{-1}), and the oven temperature was increased by 10°C min^{-1} from 200°C to 235°C. The injector temperature and detector (FID) temperature were set at 210°C and 250°C, respectively. Each fatty acid peak on gas chromatography was identified using a Supelco 37 Component FAME mix (Supelco^{TM}, 100 mg Nr at 18919–1AMP, USA) as the reference material.

Carotenoids were analyzed using the method of Lichtenthaler and Wellburn (1983). First, a test piece of *I. galbana* was cultivated in each plot, then placed in a 2–mL tube and centrifuged at 3,000 rpm for 15 min (Labogene, GYROZEN Inc., Korea) for desalination. The supernatant was then removed. After resuspension in 2 mL of methanol and mixing by vortexing, the sample was kept cold for 24 h. The supernatant was again centrifuged at 3,000 rpm for 15 min, and the absorbance was measured at 470 nm, 653 nm, and 666 nm with a spectrophotometer (V–550, JASCO, Japan).

Fucoxanthin was prepared based on the method of Zapata et al. (2000). *I. galbana* cultivated in each plot was filtered through a GF/C filter paper and placed in 95% methanol, then its pigment was extracted at 4°C for 24 h. A syringe filter (MFS, 0.2 μm pore size) was then used to remove the GF/C filter pieces present in the extracted materials. The filtered extract was injected into HPLC (Alliance 2695, Agilent, USA), and the pigment was separated. The peaks for each index pigment were determined by a comparison with the standard pigment and according to the retention time. The amount of standard pigment was calculated as described by Park and Park (1997), using the coefficient of extinction (Jeffrey et al. 1997).

Statistical analysis

The average values for the amino acid, fatty acid, and pigment contents of the microalgae were compared using a one–way ANOVA test with a significance level of 95%, using SPSS software version 14.0 (SPSS Inc., Chicago, IL, USA), with Duncan's test for post hoc analysis (1955).

Results

Cultivation of microalgae according to the LED light source

The DW values, i.e. the cell growth, of *I. galbana* under different LED wavelengths are shown in Fig. 2. MH produced rapid growth up to 0.59 gL^{-1} by the sixth day of cultivation, and growth gradually decreased after the stagnation period. LW induced growth to an extent similar to that of MH until the sixth day of cultivation, but then induced continuous growth, which was different from that of MH after the seventh day; a maximum value of 0.68 $gL^{-}1$ was observed on the ninth day of cultivation. LB induced growth to an extent similar to that of LW, but at a rate that was lower and more reliable than that induced by LW. LR and LG produced DW values of 0.36 gL^{-1} and 0.34 gL^{-1}, respectively, by the sixth day of cultivation, which increased to 0.47 gL^{-1} and 0.44 gL^{-1} by the eighth day and then began to decrease. LY induced the formation of 0.16 $gL^{-}1$ on the fifth day and 0.18 gL^{-1} on the ninth day for cultivation. These results showed that cell growth varied according to the light source (P<0.05).

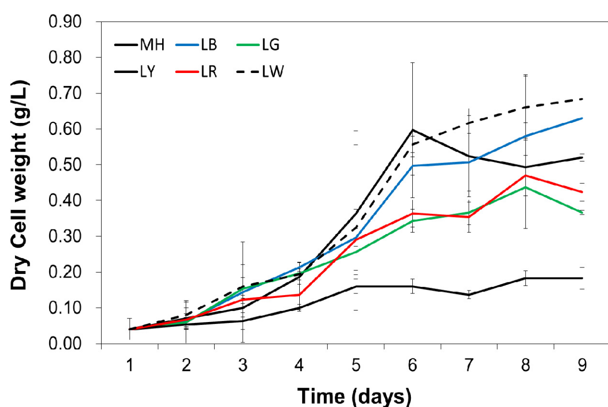


Fig. 2. Dry cell weight of *I. galbana* cultured for nine days under various light conditions. Data are expressed as mean±SD. LB, LED blue; LG, LED green; LY, LED yellow; LR, LED red; LW, LED white; MH, metal halide lamp.

Amino acid composition

Table 2 shows the amino acid composition of *I. galbana* according to wavelength of light used. Among the essential amino acids, the amounts of threonine, valine, isoleucine, leucine, phenylalanine, and lysine did not significantly differ among the different light sources (P>0.05). However, the amount of methionine under LY (5.7 mg mg^{-1}) was lower than that under the other wavelengths, and the overall level of essential amino acids (3.2 mg mg^{-1}) was thus lower under LY than under LG, LW, and MH (P<0.05) and higher under LW, LG, and MG than under other wavelengths (P<0.05). HN3 showed a high content of 26.0 mg mg^{-1} under LG and of 23.5–26.0 mg mg^{-1} under LR, LW, and MH, but LY resulted in a significantly lower value of 22.4 mg mg^{-1} (P<0.05).

The fatty acid composition of *I. galbana* grown under different wavelengths is shown in Table 3. Regarding the content of C20:5n3 (EPA) and C22:6n3 (DHA) among unsaturated fatty acids, the EPA content under LG (1.1 mg mg^{-1}) was significantly higher than under other wavelengths (P<0.05). The DHA content under LW and LG (65.7 mg mg^{-1} and 62.5 mg mg^{-1}, respectively) was also significantly increased (P<0.05); thus, n–6 HUFA levels were significantly increased under LG and LR (P<0.05). However, DHA and n–3 HUFA levels under LY were significantly lower than under other wavelengths.

The pigment composition according to wavelength is shown in Figs. 3–5. The content of chlorophyll–a under MH light (14.5 mg g^{-1}) was significantly increased (P<0.05), but that under LW, LG, and LR did not differ significantly. In contrast, LY resulted in the lowest value, 10.5 mg g^{-1} (P<0.05). In addition, the content of chlorophyll–c was highest under MH and LW (4.4 mg g^{-1} and 4.2 mg g^{-1}, respectively), which represented a significant increase over the content under other wavelengths (P<0.05).

With regard to the changes in carotenoid contents, MH resulted in the highest value at 6.4 mg g^{-1}, whereas LY resulted in the lowest value, 4.2 mg g^{-1}, thus representing a significant difference among the wavelengths (P<0.05). In addition, for fucoxanthin, MH light resulted in the highest value at 0.28%, but this value was not significantly different from those obtained using LW or LG light. LY resulted in the lowest value, 0.2%, and the values were thus significantly different from each other under the different wavelengths (P<0.05).

Table 2. Amino acid composition (ug/mg dry matter) of *I. galbana* under various light conditions

Amino acid	Light source					
	LB	LG	LY	LR	LW	MH
Aspartic acid	99.2 ± 10.57[a]	105.1 ± 2.96[a]	89.5 ± 5.18[a]	100.7 ± 7.68[a]	106.3 ± 5.01[a]	105.0 ± 1.42[a]
Threonine	54.3 ± 6.02[a]	58.1 ± 1.38[a]	48.7 ± 2.74[a]	55.6 ± 4.21[a]	58.2 ± 2.56[a]	56.3 ± 0.73[a]
Serine	54.1 ± 5.59[a]	56.9 ± 1.26[a]	49.2 ± 2.78[a]	56.4 ± 5.31[a]	57.9 ± 3.25[a]	55.4 ± 0.14[a]
Glutamic acid	122.6 ± 10.45[a]	124.6 ± 2.87[a]	111.8 ± 6.22[a]	121.8 ± 10.17[a]	130.7 ± 6.97[a]	128.5 ± 1.95[a]
Glycine	61.1 ± 6.77[a]	65.0 ± 2.46[a]	54.6 ± 3.06[a]	61.8 ± 5.46[a]	65.4 ± 3.20[a]	64.4 ± 0.68[a]
Alanine	85.4 ± 10.01[a]	88.4 ± 1.68[a]	73.8 ± 3.84[a]	86.7 ± 7.59[a]	91.8 ± 4.13[a]	85.1 ± 1.71[a]
Cysteine	5.0 ± 1.04[a]	4.4 ± 0.61[a]	2.6 ± 2.31[a]	4.0 ± 0.97[a]	4.7 ± 0.34[a]	1.7 ± 2.94[a]
Valine	46.3 ± 5.89[a]	51.7 ± 2.32[a]	42.3 ± 3.64[a]	47.5 ± 2.34[a]	50.6 ± 3.10[a]	50.6 ± 2.56[a]
Methionine	6.6 ± 1.76[ab]	10.8 ± 1.78[b]	5.7 ± 0.30[a]	9.5 ± 1.25[ab]	8.7 ± 0.39[ab]	8.6 ± 0.92[ab]
Isoleucine	34.8 ± 4.60[a]	39.0 ± 3.25[a]	31.1 ± 1.73[a]	35.2 ± 1.63[a]	37.7 ± 2.21[a]	38.1 ± 1.95[a]
Leucine	93.8 ± 10.78[a]	100.2 ± 5.03[a]	84.5 ± 5.18[a]	97.4 ± 11.96[a]	100.8 ± 4.53[a]	98.5 ± 3.78[a]
Tyrosine	26.6 ± 5.32[a]	25.7 ± 2.43[a]	21.8 ± 0.87[a]	23.9 ± 2.85[a]	27.0 ± 0.42[a]	26.4 ± 1.03[a]
Phenylalanine	56.4 ± 6.08[a]	59.4 ± 2.98[a]	49.9 ± 2.69[a]	55.9 ± 5.01[a]	60.2 ± 2.53[a]	58.9 ± 2.10[a]
Lysine	59.8 ± 7.06[a]	63.4 ± 0.76[a]	53.1 ± 2.44[a]	60.1 ± 5.35[a]	63.9 ± 2.42[a]	60.4 ± 1.81[a]
HN3	23.5 ± 1.86[ab]	26.0 ± 2.66[b]	22.4 ± 1.74[a]	25.8 ± 0.37[ab]	26.0 ± 3.31[ab]	23.5 ± 1.32[ab]
Histidine	26.5 ± 1.67[a]	28.1 ± 0.72[a]	24.3 ± 1.61[a]	27.7 ± 1.61[a]	28.8 ± 2.00[a]	27.9 ± 1.57[a]
Arginine	63.6 ± 7.66[a]	68.2 ± 0.69[a]	57.7 ± 3.31[a]	66.6 ± 5.32[a]	68.3 ± 3.58[a]	65.3 ± 1.35[a]
Proline	62.4 ± 8.06[a]	66.8 ± 2.79[a]	55.7 ± 2.53[a]	64.5 ± 6.78[a]	67.4 ± 2.92[a]	64.2 ± 2.00[a]
Hyproxyproline	23.1 ± 0.48[a]	27.0 ± 1.38[a]	21.3 ± 2.08[a]	28.4 ± 2.28[a]	26.0 ± 1.27[a]	25.0 ± 0.79[a]
Essential AA(%)	35.2 ± 4.19[ab]	38.3 ± 1.73[b]	31.5 ± 1.82[a]	36.1 ± 3.14[ab]	38.0 ± 1.65[b]	37.1 ± 0.98[b]

*Significantly different ($P<0.05$) by ANOVA and Duncan's multiple comparison.
*LB, LED blue; LG, LED green; LY, LED yellow; LR, LED red; LW, LED white; MH, metal halide lamp.

Table 3. Fatty acid composition (ug/mg dry matter) of *I. galbana* under various light conditions*

Fatty acid	Light source					
	LB	LG	LY	LR	LW	MH
C18:1n9	11.3 ± 1.47[a]	13.2 ± 0.70[a]	10.4 ± 0.69[a]	13.2 ± 1.22[a]	10.8 ± 1.36[a]	10.9 ± 2.37[a]
C18:2n6	3.8 ± 0.72[a]	10.4 ± 0.26[c]	6.5 ± 0.73[b]	8.9 ± 0.66[c]	6.6 ± 1.31[b]	6.8 ± 1.90[b]
C18:3n6	0.8 ± 0.12[a]	4.7 ± 0.32[c]	2.5 ± 0.25[b]	4.8 ± 0.41[c]	2.1 ± 0.28[b]	2.0 ± 0.42[b]
C18:3n3	3.3 ± 0.14[abc]	3.5 ± 0.06[bc]	2.6 ± 0.27[a]	3.0 ± 0.14[ab]	3.9 ± 0.63[cd]	4.4 ± 0.86[d]
C20:1n9	1.8 ± 0.49[c]	1.7 ± 0.28[bc]	1.1 ± 0.04[a]	1.1 ± 0.31[ab]	1.4 ± 0.28[abc]	1.5 ± 0.12[abc]
EPA	0.8 ± 0.16[a]	1.1 ± 0.09[b]	0.8 ± 0.21[a]	0.9 ± 0.07[ab]	0.9 ± 0.09[ab]	0.9 ± 0.13[ab]
DHA	47.4 ± 1.13[ab]	62.5 ± 6.76[c]	37.2 ± 9.79[a]	54.6 ± 7.23[bc]	65.7 ± 7.41[c]	57.8 ± 6.05[bc]
n–3 HUFA	51.5 ± 3.87[ab]	67.1 ± 6.69[c]	40.6 ± 9.96[a]	58.5 ± 7.23[bc]	70.6 ± 8.06[c]	63.0 ± 6.45[bc]
n–6 HUFA	4.6 ± 2.15[a]	15.1 ± 0.39[c]	8.9 ± 0.97[b]	13.7 ± 1.04[c]	8.7 ± 1.59[b]	8.8 ± 2.31[b]
n–9 HUFA	13.1 ± 0.31[ab]	14.8 ± 0.76[b]	11.5 ± 0.66[a]	14.3 ± 1.24[ab]	12.1 ± 1.60[a]	12.4 ± 2.42[a]
n–6/n–9	0.3 ± 0.37[a]	1.0 ± 0.03[c]	0.8 ± 0.04[b]	1.0 ± 0.05[c]	0.7 ± 0.04[b]	0.7 ± 0.05[b]
UI	326.1 ± 15.94	446.0 ± 41.32	271.9 ± 61.79	391.9 ± 44.29	447.3 ± 51.99	401.8 ± 44.68

*Significantly different ($P<0.05$) by ANOVA and Duncan's multiple comparison.
*LB, LED blue; LG, LED green; LY, LED yellow; LR, LED red; LW, LED white; MH, metal halide lamp.

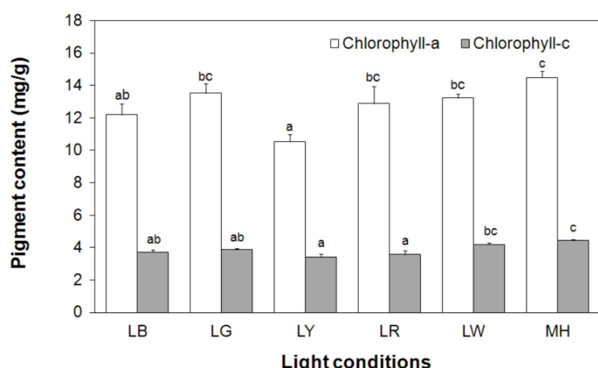


Fig. 3. Comparison of the chlorophyll–a and chlorophyll–c content of *I. galbana* under various light conditions. The letters "a" through "d" on the bars indicate significant differences as non–order symbols by Duncan's multiple–range test. Different letters (such as a:b or a:c) between different light conditions indicate significant differences (P<0.05), while an identical superscript (for example, a:ab or b:ab) indicates non–significant differences (P>0.05).

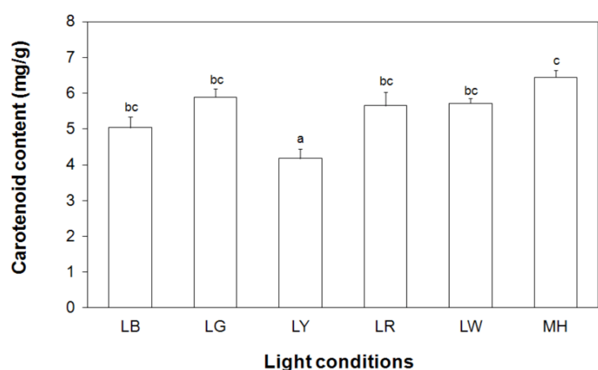


Fig. 4. Comparison of carotenoid content of *I. galbana* under various light conditions. The letters "a" through "d" on the bars indicate significant differences as non–order symbols by Duncan's multiple–range test.

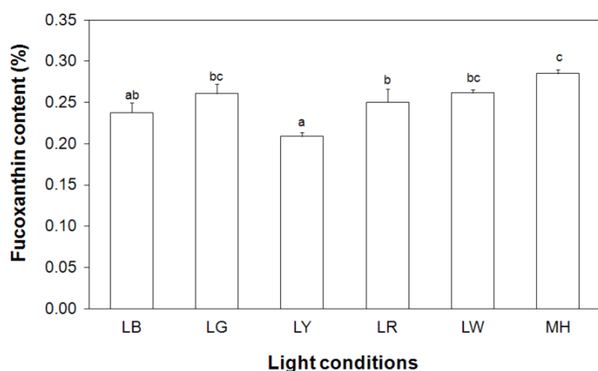


Fig. 5. Comparison of fucoxanthin content of *I. galbana* under various light conditions. The letters "a" through "d" on the bars indicate significant differences as non–order symbols by Duncan's multiple–range test.

Discussion

Growth of microalgae under different LED light sources

Photo–autotrophic microalgae are affected by aspects of light quality, such as wavelength, because they use light as their energy source for cell growth (Wang et al. 2007). Thus, LEDs can provide microalgae with the most suitable light because the necessary wavelength can be targeted; furthermore, LED lights have long lives, low power dissipation, and fast reaction times (Oh et al. 2007). Thus, the present study was performed to determine the effect of specific wavelengths of LED lamps on the growth of *I. galbana*.

Different wavelengths of light had different effects on growth; in particular, the dry weight was highest under LW light. In LW, the colors of three wavelengths are mixed in the RGB combination (red, green, and blue) (Bang and Kim 2012). Similarly, Schofield et al. (1990) showed improved growth and photosynthesis of *Chaetoceros gracilis* when it was cultivated using a white light source, and Chung et al. (1999) reported that the growth rate of *Dunaliella bardawil* was high in white light. However, in the marine environment, the cyan wavelength typically predominates (Wallen and Geen 1971), and microalgae exposed to a blue wavelength thus show a tendency toward increased chlorophyll levels and growth (Jeffrey and Vesk 1997). In contrast, there was no difference in growth rates between the blue wavelength and other wavelengths for some demersal Bacillariophyceae (Correa–Reyes et al. 2001), the dinoflagellate *Heterocapsa pygmaea* (Nelson and Prezelin 1990), and the Bacillariophyceae *Skeletonema costatum* (Tremblin et al. 2000). Thus, the effects of LED wavelength are species–specific (Oh et al. 2007). Wavelengths in the range of 400–700 nm, used for LED light sources in agriculture, are blue, green, and red (Bang and Kim 2012), and the LB and LR light in the present study thus induced increased growth. In addition, in the case of *D. bardawil*, blue light produced increased stress in the cell, because blue light has a short wavelength similar to that of ultraviolet rays. Thus, mass production of β–carotene was promoted to protect the cell, and cell growth was reported to be obstructed (Ben et al. 1989). It

has also been reported that white light is very effective for inducing a high growth rate (Chung et al. 1999).

In contrast, LY resulted in the lowest growth rate of *I. galbana*, because the yellow wavelength is outside the absorption range of chlorophyll (Oh et al. 2007), resulting in reduced photosynthesis (Blanchard and Montagna 1992).

Changes in amino acid and fatty acid contents

The nutritional properties of materials used as protein sources are related to the constituent amino acid composition and contents. Utilization of microalgae as a protein source is more efficient when the content of essential amino acids is high (Webb and Chu 1983). In this study, the content of the essential amino acids (threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine) was high under LW, LG, and MH light ($P<0.05$). Wallen and Geen (1971) reported that the amino acid content was highest under the green wavelength in *C. nana* and *D. tertiolecta* cultivated under white, blue, or green wavelengths, because of enhanced stimulation under short wavelengths, such as green and blue light. Similar results were obtained in the present study. In addition, among the amino acids, the content of glutamic acid (10.0%–11.9%) was found to be the highest in *Isochrysis* sp., *Pavlova lutheri*, and *Nannochloropsis oculata* (Brown et al. 1993). Furthermore, Brown (1991) reported that the most prevalent amino acids were glutamic acid (11.2%), aspartic acid (8.6%), leucine (7.7%), and alanine (1.9%).

The fatty acid composition of microalgae used for feed plays an important role in viability and growth. The ratio of EPA to DHA content has been used as an index for nutritional evaluation (Watanabe et al. 1983). Yoshioka et al. (2012) emphasized the importance of fatty acid composition and the effects of the light source on fatty acid content. The content of EPA was 0.8–1.1 $mgmg^{-1}$ and was only slightly different among most light sources in this study; however, the EPA content under LG light was significantly increased ($P<0.05$). Volkman et al. (1989) observed a low content of EPA in *Isochrysis* sp. In the present study, the content of DHA under LW and LG light was significantly higher than under other wavelengths ($P<0.05$). Thus, levels of n–3 HUFA tended to be similar, whereas those of n–6 HUFA and n–9 HUFA were significantly increased under LG light ($P<0.05$). Similarly, Das et al. (2011) reported that the EPA content for *Nannochloropsis* sp. was higher under white light than under blue light.

Change of pigment composition

The pigment content and photosynthetic capability of microalgae are affected by environmental factors, such as the wavelength of light (Rocha et al. 2003, Mougeter al. 2004). In the present study, the carotenoid content was high under MH light and low under LY light, which represented a significant difference among the wavelengths ($P<0.05$). Fucoxanthin demonstrated a similar tendency ($P<0.05$). Thus, MH light resulted in a higher dry weight, i.e., greater cell growth, than did LW light, probably based on a difference in chlorophyll accumulation. Chlorophyll biosynthesis was higher under MH light than under LW light; a previous study showed that rapid cell growth in *Chlorella vulgaris* involved a reduction of chlorophyll–a levels resulting from a lack of space for pigment accumulation (Seyfabadi et al. 2011). In addition, biosynthesis and accumulation of β–carotene was approximately 80%–90%, depending on the light –wavelength conditions, which resulted in decreased growth rates and reduced cell numbers under considerable stress from incident light (Lamers et al. 2010). Fucoxanthin is a type of carotenoid that is easily oxidized and destroyed by optical irradiation, peroxidase, and chemicals, and it has a specific absorption band of 300–500 nm (Mascio et al. 1989). The cell growth and the chlorophyll and carotenoid levels for *I. galbana* were significantly increased under multiple wavelengths and in the 300–500 nm band.

In the present study, the microalgal growth rate and content of amino acids, fatty acids, and pigments (carotenoids and chlorophyll a and c) varied according to the wavelength of LED light. Thus, wavelengths should be selected according to cultivation purposes; in particular, *I. galbana* will achieve optimal growth and biochemical composition when LW and LG light are used.

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