DETERMINATION OF SOME BENEFICIAL HEALTH CHEMICAL COMPOSITION AND NITRATE RESIDUAL IN NOSTOC COMMUNE UNDER RED LIGHT CONDITION AT CLOSED SYSTEM

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Abstract - The edible blue green algae, Nostoc commune Vaucher was used to determine some beneficial health chemical composition (Carotenoid and Phycocynin) and Nitrate residual. The effect condition was using BG-11 with (+N) and without (-N) $NaNO_3$ in 3 litre media with 5 litre plastic bowl container under 5 levels of irradiance, 10, 20, 30, 90, 100 and 120 µmol $/m^{-2}/s^{-1}$, with illumination provided by Red light LED lamps. 21 days culturing. The samples were yield to find biomass as the term of fresh weight, dry weight and %dry weight. The results biomass as fresh weight have shown the highest at C120+N or 120 µmol $/m^{-2}/s^{-1}$ with the value of 47.392 $g.L^{-1}$ and the lowest value was 1.580 $g.L^{-1}$ at C10+N. Dry weight have shown the highest at C120+N have the value of 1.099 $g.L^{-1}$ and the lowest value was 0.067 $g.L^{-1}$ at C10-N. The %dry weight was shown the maximum value at C100-N (4.277%) and the minimum value at C10-N was record of 1.595%. The results carotenoids content in Nostoc commune in BG-11 media -N have increase apparel to the Red- light ingredient from C10-N to C100-N and then lower at C120-N. The carotenoids content have shown the highest at C100-N with the value of 0.4±0.08 $mg.G^{-1}$ and the lowest value was 0.07±0.02 $mg.G^{-1}$ at C10-N. The result of carotenoids content was decrease at C120-N with the value of 0.2±0.02 $mg.G^{-1}$. The Phycocyanin value of Nostoc commune in form of %Crude C-Phycocyanin, %Pure C-Phycocyanin were the highest value at C10 and the lowest at C100 for all condition. The Nitrate value of Nostoc commune using BG-11 with $NaNO_3$ (+N) and without $NaNO_3$ (-N) media under Red-light condition were shown the highest value at C30-N (37.818 µg.20mL^{-1}) and the lowest at C30+N, C90+N and C120-N with the value of zero. This can be exploited to enhance the nutritional content of the Nostoc commune and safety food from nitrate contamination.

Index terms - Beneficial health chemical composition, Nitrate residual, Nostoc commune, red light, closed system

I. INTRODUCTION

Cyanobacteria are a group of Gram negative photoautotrophic microorganisms capable of oxygenic photosynthesis [1]. These ancient organisms [2,3,4] have minimal nutrient requirements. They are capable of fixing CO_2 and utilizing light as an energy source and water as an electron donor [5]. In addition, many strains possess the ability to fix atmospheric nitrogen [1,5]. It have been proven to be an extremely valuable source of natural drugs and agents for developing not only medicines, but also functional foods for disease prevention and health promotion [6,7] and antioxidant [8,9]. Nostoc commune contain rich an amino acids, fatty acids, flavonoids polysaccharide, vitamins, and many kinds of minerals [10,11]. It is also rich in carbohydrates, vitamins, minerals, phenolic, pigments (chlorophyll, carotenoids and phycobilins) and poly-unsaturated fatty acids [12,13]. Carotenoids have also the ability to stimulate the immune-system, thus being potentially involved in more than 60 life-threatening diseases—including various form of cancer, coronary heart diseases, premature ageing and arthritis [14]. Carotenoid production appears to be one of the most successful case studies of blue biotechnology. The rising market demand for pigments from natural sources has promoted large-scale cultivation of microalgae for synthesis of such compounds, so significant decreases in production costs are expected in coming years.[15]. The control and optimization of light intensity and light wavelength is regarded as one of the most important parameters for the culture of photosynthetic microorganisms [16]. In the past years, nitrate level in foods has increased. Basically, potential reduce from nitrate to nitrite is known as harmful factors on human and animals. In fact, nitrite is able to reacts Amino Acids in toxic form and converts to carcinogenic nitrosamine. In addition, nitrite is known as Methemoglobinemia (Anoxia) factor in children. Nitrite and nitrate in water drinking and foods are environmental factor to lead upper gastrointestinal cancer [17,18,19]. Nitrosamine is derived from nitrate and it seems as one of the factors and causes of gastrointestinal cancer in adults and Methemoglobinemia (blue baby syndrome). 80% of nitrate enters to the body through vegetables and fruits [20]. However, wild type N. commune has been decreasing in quantity as a result of ever-growing market demand and environmental pollution. Therefore, artificial culture of N. commune is important as it can bring great social and economic benefits. [10]. Nowadays, light emitting diodes (LED) has been increasingly used as a source of artificial light in controlled environmental system due to its energy-efficiency. LED light quality and intensity have an effect to the growth of plant in semi-closed system. [21].

The previous of our study was compare the light quantity between red light and white light condition. The result was shown that the red light was exhibit the higher phycocyanin content than white light. [22]. In this study to investigated the quality of red light intensity by adjust the treatment of light between the previous red light intensity. To find out the influence of red light intensity on beneficial health chemical composition like phycocyanin and carotenoid accumulation and Nitrate residual in this stain.

II. CULTIVATION OF NOSTOC COMMUNE

The Nostoc commune stain was isolated from natural Mae Taeng clean and shallow-river. Located in Chiang Mai province. The culture media was using BG-11 with (+N) and without (-N) $NaNO_3$ and was cultured in 3 litre media in 5 litre plastic bowl under 5 levels of irradiance, 10, 20, 30, 90, 100 and 120 µmol /m^{-2} /s^{-1}, with illumination provided by Red light LED lamps (Figure.1). The irradiance was measured on the surface of the media using light meter. The pH was measured using a 7.5 pH meter. Temperature was adjusted the air condition to 25 + 2 °C [23]. CO_2 was used to increase the source for photosynthesis and the source for mass production. (Manigandam, 2014) Light 12:12 Turn off switch was used to control the growth.

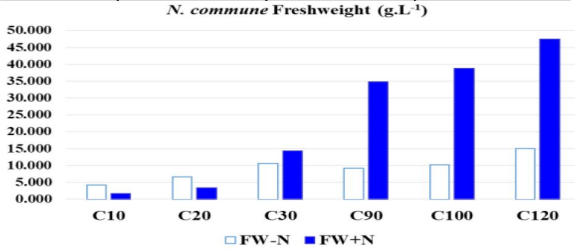
Biomass measurements

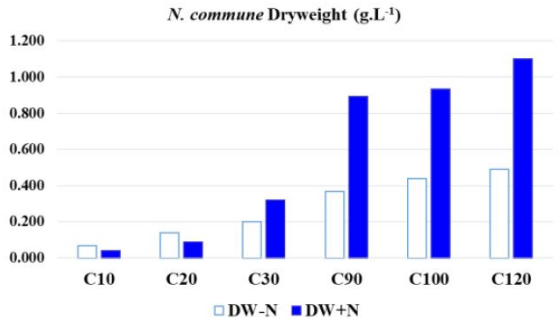
21 days after culturing,Nostoc commune. were yield to find biomass as the term of fresh weight dry weight and %dry weight. The results biomass as fresh weight have shown the highest at C120+N or 120 µmol /m^{-2}/s^{-1} with the value of 47.392 g.L^{-1} and the lowest value was 1.580 g.L^{-1} at C10+N. Biomass as dry weight have shown the highest at C120+N have the value of 1.099 g.L^{-1} and the lowest value was 0.067 g.L^{-1} at C10-N. (Table 1, Figure.2).

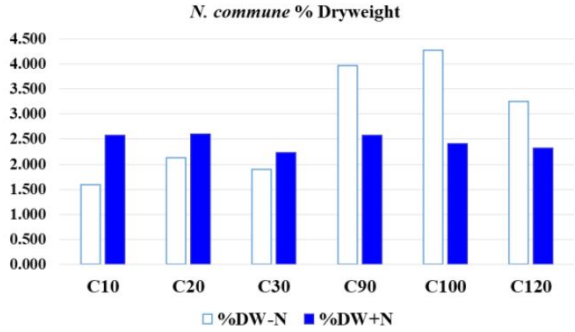
The %dry weight was shown the maximum value at C100-N was record of 4.277% and the minimum value at C10-N was record of 1.595% (Table 1,Figure2).

Table 1 Nostoc commune Biomass

	Biomass (g.L^{-1})					
Treatment	Freshweight-N		Dryweight-N		%Dryweight-N	
N. commune	FW-N	FW+N	DW-N	DW+N	%DW-N	%DW+N
C10	4.200	1.580	0.067	0.041	1.595	2.574
C20	6.592	3.402	0.140	0.088	2.124	2.597
C30	10.620	14.355	0.201	0.320	1.893	2.229
C90	9.215	34.761	0.366	0.894	3.975	2.571
C100	10.232	38.784	0.438	0.934	4.277	2.409
C120	15.050	47.392	0.490	1.099	3.258	2.320







C= LED Red-light at 10, 20, 30, 100 and 120 µmol m^{-2} s^{-1}

Figure 2 Biomass and %dry weight of Nostoc commune at difference light intensity

III. BENEFICIAL HEALTH CHEMICAL COMPOSITION

1. Carotenoid content

Carotenoid production appears to be one of the most successful case studies of blue biotechnology. The rising market demand for pigments from natural sources has promoted large-scale cultivation of microalgae for synthesis of such compounds, so significant decreases in production costs are expected in coming years [15]. In these study we point to the safety Nitrate residue in culture product, so the determination of Nitrate was done only the media without $NaNO_3$ (-N) in media of BG-11

2. Determination of Carotenoids content

The carotenoids content was analytical performed with spectrophotometer at the wavelength of 450 nm. for carotenoid follow [24] method.

3. Carotenoids content in Nostoc commune

The results carotenoids content in Nostoc commune in BG-11 media without (-N) have increase apparel to the Red- light ingredient from C10-N to C100-N and then lower at C120-N (Figure 4). The carotenoids content have shown the highest at C100-N or 100 µmol m^{-2} s^{-1} with the value of 0.4±0.08 mg.G^{-1} and the lowest value was 0.07±0.02 mg.G^{-1} at C10-N. The result of carotenoids content was decrease at C120-N with the value of 0.2±0.02 mg.G^{-1} (Table 2, Figure 4).

Table 2 The carotenoids value of Nostoc commune

Treatment	Carotenoid
N. commune	(mg.G^{-1} cell dry weight)
C10-N	0.07±0.02
C20-N	0.06±0.03
C30-N	0.19±0.04
C90-N	0.36±0.13
C100-N	0.4±0.08
C120-N	0.2±0.02

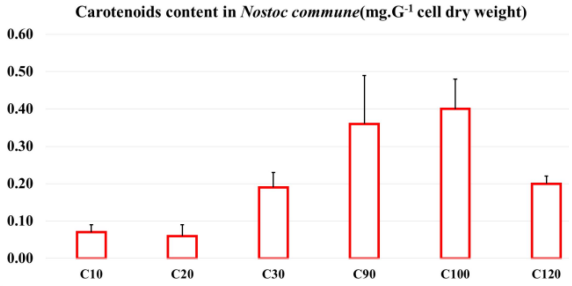


Figure 4. Comparison of Carotenoid at difference Red-light intensity in Nostoc commune

2. Phycocyanin content

C-phycocyanin (C-PC) could be extracted from cyanobacteria such as Spirulina platensis, which has been widely used in commercial applications in the food and cosmetic industry as a natural blue dye [25], anti-inflammatory [9,25,261] and antioxidant [8,9]. Some papers report C-PC extraction from cyanobacterium [9].

1. Determination of C-Phycocyanin content

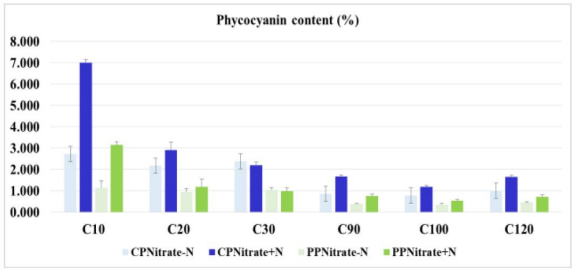
Phycocyanin was extracted from the dry biomass of algae by using Low temperature method, this is a spectrophotometry method adapted to extracted and quantify a relatively pure C-Phycocyanin fraction [27]. Calculated percent C-Phycocyanin using equation. The pure C-phycocyanin concentration was present higher than others at LD30 with the value of 0.32±0.09 % and most lower at C120 with the value of 0.17±0.01%.

2. C-Phycocyanin content in Nostoc commune

Table 3 The Phycocyanin value of Nostoc commune

	Phycocyanin content (%)			
Treatment	**% crude C-phycoyanin**		**% pure C-phycoyanin**	
N. commune	CPNitrate-N	CPNitrate+N	PPNitrate-N	PPNitrate+N
C10	2.732±0.34	7.004±0.22	1.152±0.34	3.147±0.16
C20	2.179±0.15	2.918±0.21	0.946±0.16	1.183±0.36
C30	2.374±0.08	2.196±0.22	1.046±0.12	0.973±0.16
C90	0.865±0.01	1.667±0.17	0.394±0.02	0.769±0.08
C100	0.782±0.09	1.194±0.04	0.355±0.05	0.528±0.06
C120	0.994±0.05	1.651±0.05	0.455±0.03	0.728±0.08

The Phycocyanin value of Nostoc commune in form of %Crude C-Phycocyanin, %Pure C-Phycocyanin were the highest value at C10 and lowest at C100 for condition. (Table 3,Figure 5). Those seen to be low light intensity was suitable to induce the high Phycocyanin content.



C= LED Red-light at 10, 20, 30, 100 and 120 µmol /m^{-2} /s^{-1}

Figure 5 Comparison of C-Phycocyanin at difference Red-light intensity

3. Nitrate residual

Standard limit for nitrate value in vegetables, standard limit per weight kilogram is less than 3.65 Milligram [28] (Tabatabaei et al 2005)I

1. Determination of Nitrate residual

The nitrate concentration was determine using Salicylic Acid method [29].

2. Nitrate residual in Nostoc commune

The Nitrate value of Nostoc commune using BG-11 with $NaNO_3$ (+N) and without $NaNO_3$ (-N) media under Red-light condition were shown the highest value at C30-N (37.818 µg.20mL^{-1}) and the lowest at C30 +N, C90+N and C120-N with the value of zero.(Table 4,Figure 7). The results was the effect of the media with nitrate concentration which could be interfere the nitrate residue in organism. (Figure 8). The consumption of nitrates is assumed to be the primary contributor in increasing the pH since pH increase did not occur in BG-N medium. [Kevin J. Horn. 2008]. Nostoc commune where heterocyst had formed indicating nitrogen fixation had become the source of nitrogen. Cultures of Nostoc commune that went to 61 days in the same medium were at pH 9.5-10 indicating that this range is the maximum pH induced by cell growth. The data also suggest that the primary factor in increasing the pH was the consumption of nitrate [30]. The nitrate content in Nostoc commune was low at all treatments,

Table 4 The Nitrate value of Nostoc commune

Treatment	Nitrate concentration(µg.20mL^{-1})	
N. commune	Nitrate-N	Nitrate+N
C10	20.909	4.909
C20	13.091	24.545
C30	37.818	0.000
C90	16.182	0.000
C100	16.727	13.455
C120	0.000	0.000

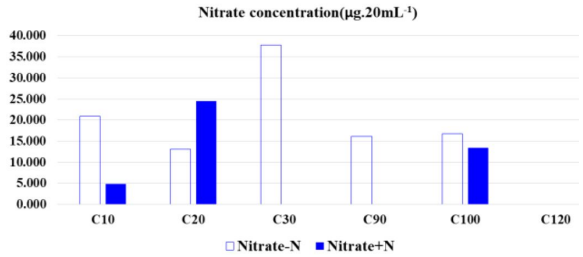


Figure 7 Comparison of Nitrate content at difference Red-light intensity in Nostoc commune

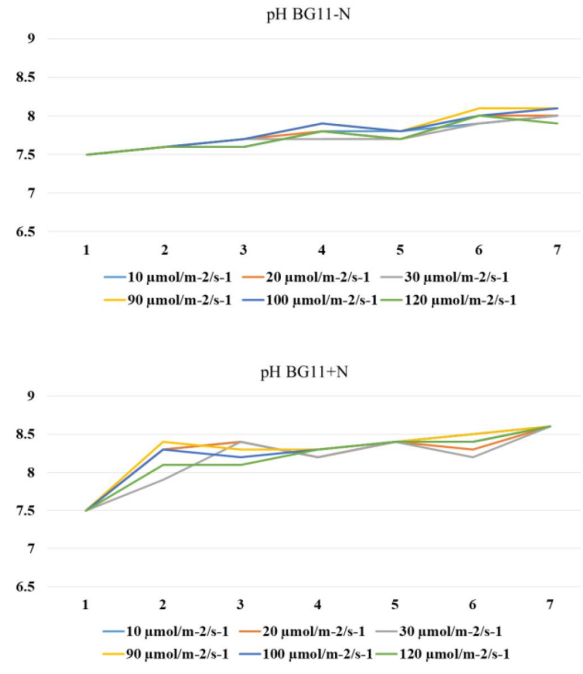


Figure 8 pH value in media with Nitrate and without Nitrate at difference Red-light intensity in Nostoc commune

CONCLUSION

The use of red-light condition and BG-11 media with (+N) and without (-N) $NaNO_3$ could be effect on the growth of Nostoc commune and the exhibit of chemical content. The optimum condition for biomass was 120 µmol /m^{-2} /s^{-1} with the BG-11 media with $NaNo_3$ and Carotenoid was 120 µmol /m^{-2} /s^{-1} with the BG-11 media without $NaNo_3$. The Phycocyanin was shown high concentration in low light intensity. The results of Nitrate residue was an effect from the media with nitrate concentration which also alternate the high pH in media. This study could be the idea to increase the some beneficial health chemical composition, such as Carotenoid and phycocyanin content and reduce nitrate residual in Nostoc commune to have the valuable food.

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