A Simple Method for Efficient Extraction and Separation of C-phycocyanin from Spirulina platensis

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Abstract :

Stroke is the third leading cause of death after cardiovascular disease and cancer. At present, there is no registered neuroprotective drug used in clinical practice that may prevent or limit cerebral tissue lesions occurring in the areas with decreased blood flow. C-Phycocyanin (C-PC) is a biliprotein pigment that plays an essential light-harvesting role in cyanobacteria with multiple applications in industry. Furthermore, phycocyanin has been proven to have therapeutic properties including antioxidant, neuroprotective agent, anti-inflammatory and anti-cancer activities. In this study, the C-PC was extracted by using sodium dihidrogen phophat (phosphate buffer). In this study, the effect of temperature and ratio of biomass and solven were also investigated. Extracted C-PC showed absorbance maximum at 30°C, however extracted C-PC showed absorbance minimum at 70°C. The use of biomass and solvent ratio, 1:50, could extract the C-PC as well as the use of ratio 1:75, 1:100. Meanwhile, extraction process by using ratio of biomass : solven, 1:125, gave the lowest absorbance value of C-PC. Pure C-PC was finally obtained from Spirullina with purity ratio 1,19 – 1,42, and is potential to be applicated in cosmetic industries.

Keywords: C-phycocyanin, extraction, phosphate buffer, spirulina platensis

1. Introduction

Stroke is a major cause of long-term disability in industrialized and also in some developing countries. It is third leading cause of death after cardiovascular disease and cancer **[1]**. The economic and social consequences of stroke are huge **[2]**. A 12% overall stroke incidence increase has been predicted over the next decade **[1]**, showing clearly its enormous impact on the society.

The treatment strategy of acute cerebral ischemia has been focused in two directions: the restoration of cerebral blood flow and the interruption of the molecular events that eventually produce the neuronal cell death (neuroprotection) **[3]**. There is no registered neuroprotective drug used in clinical practice that may prevent or limit cerebral tissue lesions occurring in the areas with decreased blood flow, at present **[4]**.

C-Phycocyanin (C-PC) plays an essential light-harvesting role in cyanobacteria, rodophytes and cryptophytes with multiple applications in industry **[5]**. C-PC accounts for around 15% of the total dry weight of spirulina **[6]**. C-phycocyanin is the major phycobiliprotein in Spirulina and may constitute up to 20% of the dry weight of Spirulina **[7]**. Phycocyanin has highly commercial uses, with a market value of around 10–50 million US$ per annum **[8,9]**. Phycocyanin is a natural blue colorant, has uses as a food colorant for chewing gum, ice sherbets, soft drinks, candies and cosmetics including lipstick and eyeliners. Small quantities are also used as biochemical tracers in immunoassays due to its fluorescent properties **[10,11]**. Furthermore, phycocyanin has been proven to have therapeutic properties including antioxidant, anti-inflammatory and anti-cancer activities **[12]**. The cost of phycocyanin products varies widely and is dependent on the purity ratio, which is defined as the relationship of absorbance at 620 nm and 280 nm (A_{620}/A_{280}). The cost of food grade phycocyanin (purity higher than 0.7) is around 0.13 US$ mg/l, whereas the cost of analytical grade (purity higher than 4.0) can be as high as 15 US$ mg/l **[13]**.

Many research previously have been done to obtain C- Phycocyanin from microalgae **[14,15,16,17,18]**. Some methods have been used to ectracti and purify C- Phycocyanin from microalgae **[14,16,18,19]**. The quality of C-Phycocyanin depends upon the cultivation and ratio of biomass and solvent. This aims were to investigate the effect of biomass and solvent ratio on the quantitiy and purity of C-phycocyanin. The profile of microalgae cultivation was also investigated.

2. Material and Methods

2.1. *Material*

Spirulina platensis was cultured in open box pond (20L). Mixing was provided by aerator. The nutrition was given to the biomass every two days to establish a fed batch culture. Spirulina biomass cultivated as describe, was subjected to oven drying process.

2.2. *C-Phycocyanin extraction and purification*

Samples from oven dried were subjected to extraction process. Extraction was done using phosphate buffer at the various ratio (w/v), under continuous mixing by stirerr at 250 rpm for an hour at various temperature. After extraction, the cell residue was removed by centrifugation at 4800xg for 15 min. Crude extract from all samples were analyzed for C-phycocyanin as well as purity.

2.3. *Analyzes*

The phycocyanin content in Spirulina was analyzed after extraction in phosphate buffer (pH 7). The absirbance of cell fragments in the crude extract was measured by spectrophotometry at a wavelength 560 nm.

3. Result and Discussion.

3.1. *Microalgae growth profile*

Figure 1 shows the microalage growth profile, where five reasonably well defined growth phases can be recognized: (1) lag phase; (2) exponential growth phase, representing themaximum growth rate under the specific conditions; (3) linear growth phase; (4) stationary growth phase; (5) regrowth phase. Fresh nutrition which fed into open box pond every two days, gave the positive growth profile. Generally algal cultures in the exponential growth phase contain more protein, while cultures in the stationary phase have more carbohydrates and glycogen **[20]**.

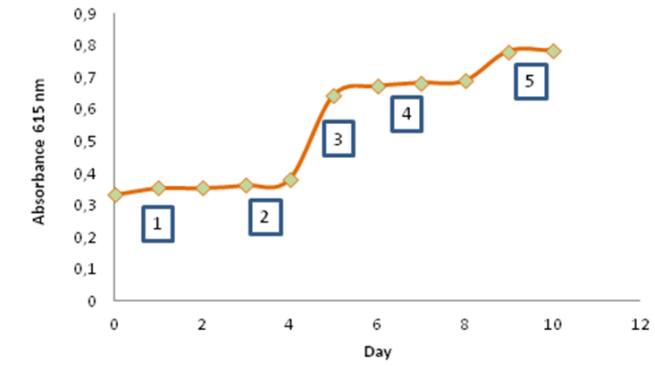
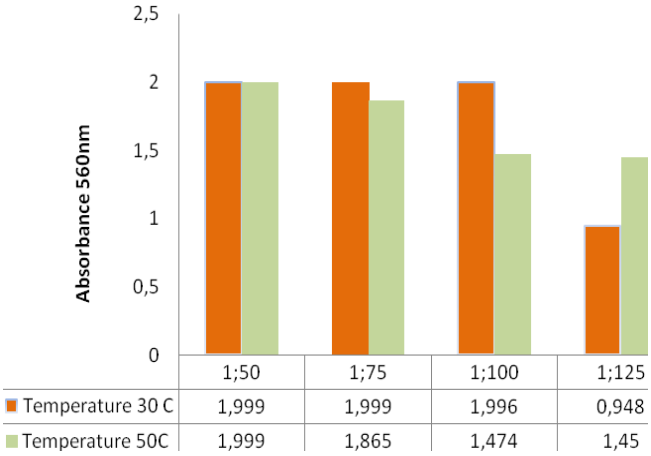


Figure 1. microalgae growth profile in fed batch culture

Under suitable climatic conditions and sufficient nutrients, microalgae can grow profusely. Commonly they double their biomass within 24 h or within 3.5 h during the exponential growth phase **[21].** There are several factors influencing algae growth: abiotic factors such as light (quality, quantity), temperature, nutrient concentration, O_2, CO_2, pH, salinity, and toxic chemicals; biotic factors such as pathogens (bacteria, fungi, viruses) and competition by other algae; operational factors such as shear produced by mixing, dilution rate, depth, harvest frequency, and addition of bicarbonate.

3.2. *The effect of temperature*

Figure 2 shows the effect of temperature (30°C and 50°C) to absorbance of phycocyanin at ratio of biomass:solvent (%w/v) 1:50, 1: 75, 1:100, 1:125. From the Figure, we realized that operation condition at 30°C gave better result than 50°C in both ratio. C-phycocyanin is very sensitive to temperature, as shown in Figure 2, that at higher temperatures (50°C), the absorbance obtained less than the extraction at low temperature (30°C). C-phycocyanin can be easily damaged and less quality product, at higher temperature.



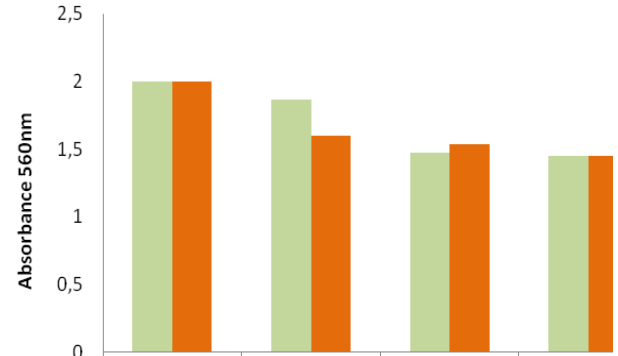
	1;50	1;75	1;100	1;125
Temperature 30 C	1,999	1,999	1,996	0,948
Temperature 50C	1,999	1,865	1,474	1,45

Figure 2. Effect of temperature

Some previous researches showed that temperature is a one of factors that can influence the quality of c-phyccocyanin. Similiar studies conducted by Doke (2005) reported that Spirullina biomass, showed a 5-7% loss of phycocyanin, when dried at 25°C under shading with air-circulation **[22]**. Oliviera et al (2009) also reported that Spirulina showed an approximately 21% loss of phycocyanin when dried in a thin layer with an air temperature of 60°C **[23]**. However, Sarada et al (1999)reported that using spray drying, cross drying, resulted in an approximately 50% loss of phycocyanin **[24]**.

3.3. The effect of biomass and solvent ratio

Effect of biomass-solvent ratio on the production of CPC is shown in Figure 3. Generally, the production of CPC at a ratio of 1:50 gave absorbance better than the 1:100 ratio. Neither the influence of the ratio of 1:50 and 1:100 were no significant influence on the temperature of 30°C. Instead, the influence of the ratio at higher temperatures showed significant changes. However, the previous study, Chaikalahan, showed that utilizing ratio 1:100 gave the best result **[14]**.



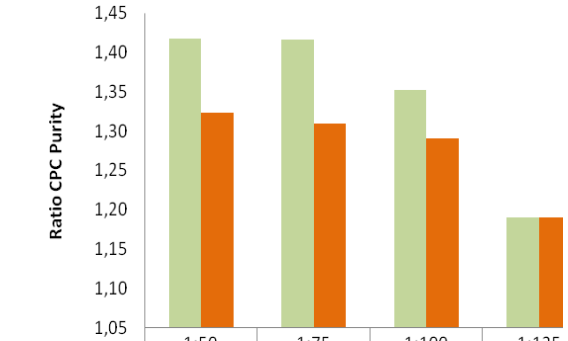
	30 C	40 C	50 C	70 C
Ratio 1:50	1,999	1,865	1,474	1,45
Ratio 1:100	1,996	1,596	1,538	1,445

Figure 3. Effect of ratio biomass : solvent

3.4. Purity of C-phycocyanin

Figure 4 shows the effect of temperature to C-phycocyanin purity. When fresh or dried *Spirulina* biomass were subjected to the process of phycocyanin extraction, it was found that the quantity of phycocyanin in the raw material had a significant influence in the purity ratio of the crude extract. In this study, the highest purity ratio

was approximately 1.42 at temperature 30°C ratio biomass and solvent 1:50. C-phycocyanin with purity ratio < 1 is considered a low purity protein that can be used in food and cosmetic industries, while purity ratio ≥ 4 is of high purity grade with pharmaceutical use **[13]**.



	1;50	1;75	1;100	1;125
Temperature 30 C	1,42	1,42	1,35	1,19
Temperature 50 C	1,32	1,31	1,29	1,19

Figure 4. The effect of temperature to C-phycocyanin purity

4. Conclusion

Extracted C-PC showed absorbance maximum at 30°C, however extracted C-PC showed absorbance minimum at 70°C. The use of biomass and solvent ratio, 1:50, could extract the C-PC as well as the use of ratio 1:75, 1:100. Meanwhile, extraction process by using ratio of biomass : solven, 1:125, gave the lowest absorbance value of C-PC. Pure C-PC was finally obtained from Spirullina with purity ratio 1,19 – 1,42, and is potential to be applicated in cosmetic industries.

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