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SEPARATION AND PURIFICATION OF PHYCOCYANIN FROM *SPIRULINA PLATENSIS* USING DIFFERENT DRYING METHODS UNDER INDOOR AND OUTDOOR CONDITION

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Abstract

The blue-green alga *Spirulina platensis* is a source of phycocyanin pigment which has highly commercial uses in food colorant, cosmetic and pharmaceutical industries. This paper describes the isolation and extraction of phycocyanin from *Spirulina platensis* using an alkaline buffer and different drying methods under indoor and outdoor conditions.

Spirulina platensis was cultivated in an open outdoor photo-bioreactor and in two glass aquarium (10 L) with Zarrouk's medium using different sources of nitrogen. The results showed that ; the highest mass production and purity ratio of phycocyanin and the lowest percentage of phycocyanin loss were conducted under outdoor condition in large scale low-cost photo-bioreactor and the fresh biomass was the best for large amount of phycocyanin (0.097 g/L) with the highest purity ratio (1.06) and the loss percentage of (3%). In addition, there was a low-cost synthetic medium by using urea as commercial fertilizer instead of analytical grade sodium nitrate as nitrogen source in Zarrouk's medium and commercial grade sodium bicarbonate instead of analytical grade. This high amount of phycocyanin with high purity and low-cost can be used in food, cosmetic and pharmaceutical industries.

Keywords: Phycocyanin; *Spirulina platensis*; blue-green algae; drying methods; purity ratio.

INTRODUCTION

Spirulina platensis is a blue-green microalga, it represents the most important commercial microalga for the production of biomass as health food (Vonshak and Tomaselli; 2000). It can produce large quantities of high value products such as phycobiliproteins (Chen and Zhang, 19997). Phycocyanin is an accessory photosynthetic pigment of the phycobiliprotein in *Spirulina* and may reach 20% of the dry weight of *Spirulina* (Vonshak, 1997). Phycocyanin is a natural blue colorant in food industries replacing the toxic synthetic pigments (Leema *et al*. 2010). It is used also in cosmetics (Sarada *et al*., 1999).Recently it has been observed that phycocyanin used in pharmaceutical industries (Reddy *et al*., 2003).

Several factors can effect on the amount of phycocyanin and its purity ratio. The drying methods, culture media and culture conditions all play important roles in the yield of phycocyanin and its purity ratio (Ratana *et al*., 2011). The cost of phycocyanin products varies widely and depends on the purity ratio (Ratana *et al*., 2011).The medium is also important for the cost involved in mass production of *Spirulina* (Raoof *et al*., 2006). This study investigated the effect of raw material quality through different methods of drying and medium composition, on the yield and purity ratio of phycocyanin under indoor and outdoor conditions, in terms of low-cost production.

MATERIAL AND METHODS

Culture conditions of *Spirulina platensis.*

(A) Indoor mass culture.

The culture experiments were carried out in two glass aquarium; each containing 10 L Zarrouk's *Spirulina* medium (Zarrouk, 1966). The first (no. one) has 2.5 (g/L) $NaNo_3$ (Analytical grade). While, the second (no. two) contains 2.5 (g/L) K_2NO_3 as nitrogen source. The pH was 9, and temperature was 25-30C°. Stirring by glass road was done once a day in the aquarium. The alga was harvested from medium solution by plankton cloth mesh, 20 diameter.

(B) Outdoor mass culture.

Using commercial synthetic medium in which the urea add as nitrogen source instead of nitrate and using commercial grade sodium bicarbonate instead of analytical grade, for low-cost mass production of *Spirulina*, in a large scale with constant aeration, pH was 9 and the temperature was 30-35C° during the end of the summer.

Extraction and spectroscopic measurement of phycocyanin.

Samples from fresh, freeze dried, sun dried and oven dried *Spirulina platensis* were subjected to extraction process. 0.1 g Dry weight of .*Spirulina platensis* was suspended into 10 ml of 0.15 M phosphate buffer (pH=7.0), leaved at 4 C° for 24 h. The cells residue of *Spirulina platensis* samples were removed by centrifugation at 13.000 rpm for 15 min. The supernatant of each sample (in blue color) was obtained.and the absorbance was measured at wave lengths 615 and 652 nm.The phycocyanin concentration was calculated according to Equation (1) (Patel *et al*., 2005).

$$pc(g/L) = \frac{OD_{615} - 0.474 OD_{652}}{5.34} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where PC is the phycocyanin concentration (g/L)

OD_{615} is the optical density of the sample at 615 nm

OD_{652} is the optical density of the sample at 652 nm

Phycocyanin purity ratio.

The purity ratio of the phycocyanin extract was determined spectrophotometrically by the A_{615}/A_{280} ratio (Abalde *at al*., 1998). Absorbance at 615 nm indicates the phycocyanin concentration, while that at 280 nm is due to the total concentration of proteins in the solution (Liu *et al*., 2005). So, the purity of phycocyanin was calculated according to Eqation (2).

$$p = \frac{OD_{615}}{OD_{280}} \quad \text{………………………………………………….(2)}$$

Where, P is the purity ratio of the phycocyanin extract.

OD_{615} is the optical density of the sample at 615 nm,

OD_{280} is the optical density of the sample at 280 nm.

The percentage loss of phycocyanin.

The percentage of phycocyanin loss for each sample was calculated from the relation between the amount of phycocyanin with different drying methods and its amount in fresh biomass.

RESULTS AND DISCUSSION

Effect of raw material on the production and purity of phycocyanin under indoor condition (1).

The present investigation aimed to exploit the high phycocyanin content of *Spirulina platensis* for using in food, cosmetic and pharmaceutical industries with low-cost mass production of *spirulina*. The results indicated that, only 4% loss of phycocyanin was observed in freeze dried biomass and 14% loss in sun dried biomass whereas around 57% loss was observed in oven dried biomass (Table 1). Similar studies conducted by Sarada *et al*. (1999) reported that oven drying, resulted in an approximately 50% loss of phycocyanin. Also Oliveira *et al*. (2009); Desmorieux and Hernandez, (2004) reported that drying methods for *Spirulina* resulted in an approximately 50% loss of phycocyanin. However, Doke (2005) reported that *Spirulina* biomass, when dried at 25 C^0 under shading with air-circulation, showed 5-7 % loss of phycocyanin. Oliveira *et al*. (2009) also reported that *Spirulina* dried at 60 C^0 showed an approximately 21% loss of phycocyanin as compared with the value of phycocyanin in fresh *Spirulina*. The highest phycocyanin content (0.073 g/L) was observed when fresh biomass was used as raw material. The amount of phycocyanin from freeze and sun dried biomass were 0.07 and

0.063 g/L, respectively. While oven dried biomass, contains the lowest amount of phycocyanin (Table 1, Fig. 1).

Table 1. Drying conditions in fresh and dried *Spirulina* biomass, Purity ratio, amount and loss (%) of phycocyanin under indoor conditions (1).

Drying methods	Phycocyanin in crude extract (g/L)	Purity ratio of crude extract	The (%)loss of phycocyanin
Fresh	0.073	0.78	0%
Freeze dried	0.07	0.75	4%
Sun dried	0.063	0.73	14%
Oven dried	0.031	0.51	57%

The highest purity ratio of crude extract was approximately 0.78, which was observed when fresh biomass was used as raw material. For dried biomass, the purity ratio of phycocyanin from freeze dried and sun dried biomass were 0.75 and 0.73 respectively. Oven dried biomass showed the lowest purity 0.19 (Table 1, Fig. 2). So the use of fresh biomass was the best for production of phycocyanin. Similar studies conducted by Ratana *et al*., (2011) reported that, the use of fresh biomass was considered suitable for the extraction of phycocyanin on the basis of purity, phycocyanin content and operating cost for drying.

Effect of raw material on the production and purity of phycocyanin under indoor condition (2).

There is no big difference between the results when using K_2NO_3 as nitrogen source instead of $NaNo_3$. The results indicated that, only 6% loss of phycocyanin was observed in freez dried biomass and 17% loss in sun dried biomass whereas around 55% loss in oven dried biomass (Table 2, Fig. 3). The highest purity ratio and crude phycocyanin were 0.72 and 0.066 g/L respectively(Table 2, Fig. 1&2). The purity ratio and amount of phycocyanin from freeze and sun dried biomass were 0.7, 0.6 and 0.062, 0.055 g/L respectively. Oven dried biomass showed the lowest purity 0.39 and the lowest amount of phycocyanin 0.03 g/L (Table 2, Fig. 1&2).

This data agree with Sarada *et al*., (1999) who reported that drying of *Spirulina* using cross-flow drying, spray drying and oven drying, resulted in an approximately 50% loss of phycocyanin. As the result, using fresh biomass was the best for phycocyanin production.

Table 2. Drying conditions in fresh and dried *Spirulina* biomass, Purity ratio, amount and loss (%) of phycocyanin under indoor conditions (2).

Drying methods	Phycocyanin in crude extract (g/L)	Purity ratio of crude extract	The (%) loss of phycocyanin
Fresh	0.066	0.72	0%
Freeze dried	0.062	0.7	6%
Sun dried	0.055	0.61	17%
Oven dried	0.03	0.39	55%

Effect of raw material on the production and purity of phycocyanin under outdoor condition.

The highest purity and phycocyanin were recorded at fresh biomass under outdoor condition. They were 1.06 and 0.097 g/L respectively (Table 3, Fig. 1&2). While the lowest purity and phycocyanin were 0.46 and 0.043 g/L respectively, which recorded at oven dried biomass (Table 3 and Fig. 1&2). This agree with, Ratana *et al*., (2011) who found that the highest purity ratio was 1.04, which was observed when fresh biomass was used as raw material. While the lowest purity and phycocyanin were recorded at oven dried biomass. Only 3% loss of phycocyanin was observed in freeze dried biomass where as 18% and 56% loss were observed in sun and oven dried biomass respectively (Table 3 and Fig. 3).

Table 3. Drying conditions in fresh and dried *Spirulina* biomass, Purity ratio, amount and loss (%) of phycocyanin under outdoor conditions.

Drying methods	Phycocyanin in crude extract (g/L)	Purity ratio of crude extract	The (%) loss of phycocyanin
Fresh	0.097	1.06	0%
Freeze dried	0.094	1.03	3%
Sun dried	0.079	0.78	18%
Oven dried	0.043	0.46	56%

So, the use of fresh biomass was suitable for commercial production of phycocyanin specially under outdoor condition in large scale production of *Spirulina platensis*, in which using commercial Zarrouk medium with urea as a nitrogen source instead of nitrate, on the basis of saving in the operating cost of *Spirulina platensis* production. Thus the use of fresh outdoor biomass was considered suitable for the extraction of phycocyanin on the basis of purity ratio, phycocyanin amount and operating cost for the production.

Fig. 1
The relationship between the different drying methods and the phycocyanin con. (g/L) under indoor 1 (a), indoor 2 (b) and outdoor (c) conditions respectively.

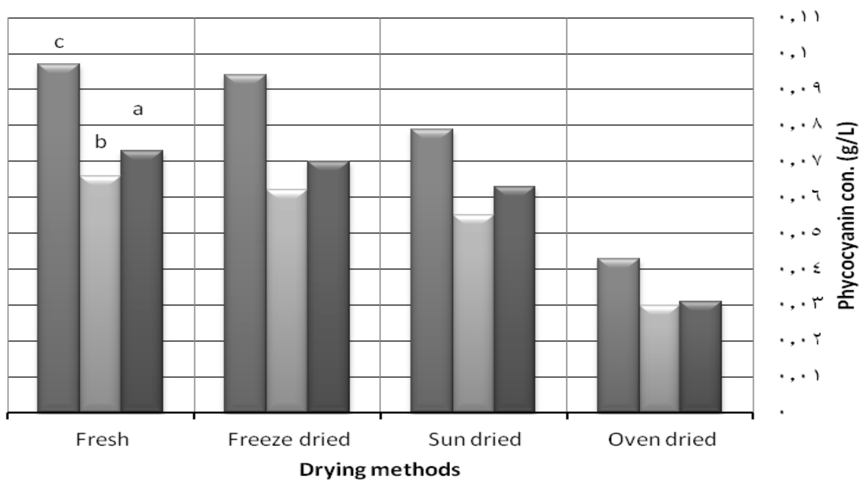


Fig. 2
The relationship between the different drying methods and the purity ratio under indoor 1 (a), indoor 2 (b) and outdoor (c) conditions respectively.

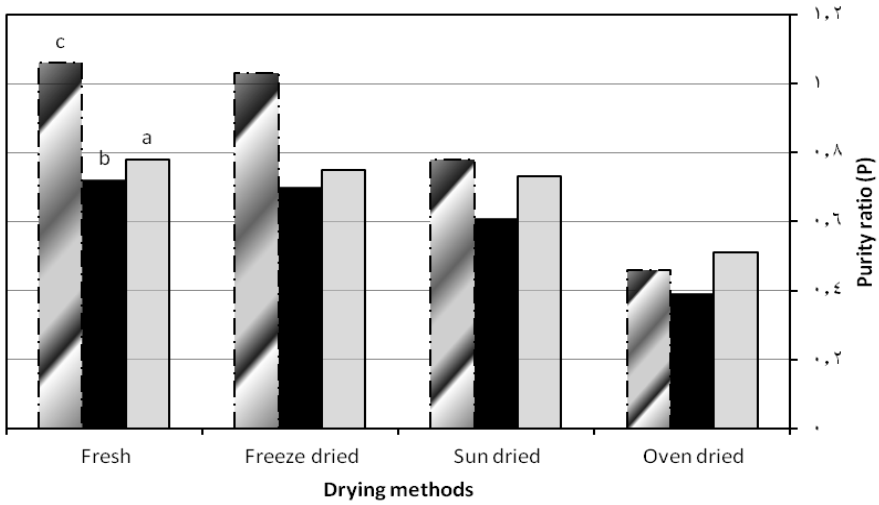
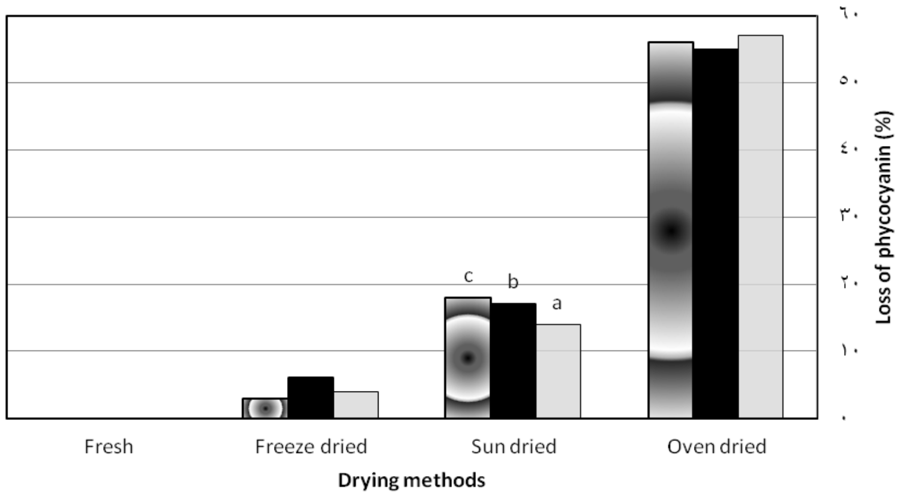


Fig.3
The relationship between the different drying methods and the loss (%) of phycocyanin under indoor 1 (a), indoor 2 (b) and outdoor (c) conditions respectively.



CONCLUSION

This work aimed to describe, a suitable method for the mass production of phycocyanin from blue-green alga *Spirulina platensis*. The highest mass production and purity ratio of phycocyanin and the lowest percentage of phycocyanin loss were conducted under outdoor condition in large scale low-cost photo-bioreactor and the fresh biomass was the best for large amount of phycocyanin (0.097 g/L) with the highest purity ratio (1.06) and the loss percentage of (3%). In addition, there was a low-cost synthetic medium by using urea as commercial fertilizer instead of analytical grade sodium nitrate as nitrogen source in Zarrouk's medium and commercial grade sodium bicarbonate instead of analytical grade. This high amount of phycocyanin with high purity and low-cost can be used in food, cosmetic and pharmaceutical industries.

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عزل وتنقية الفيكوسيانين من طحلب الاسبيرولينا بلاتنسس باستخدام طرق مختلفة للتجفيف تحت ظروف معملية و خارجية

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الملخص العربي

يعتبر طحلب الاسبيرولينا بلاتنسس من الطحالب الخضراء المزرقة وهو مصدر لصبغ الفيكوسيانين الذي له استخدامات تجاريه واسعه في مجال تلوين الاغذيه ومستحضرات التجميل وصناعات الادوية.

استهدف هذا البحث عزل واستخلاص الفيكوسيانين من الاسبيرولينا بلاتنسس باستخدام عازل قلوي وطرق مختلفه للتجفيف داخل المعمل وخارجة. وقد تم استزراع الاسبيرولينا خارج المعمل في حوض اسمنتي كبير وداخل المعمل في حوضين زجاج (١٠لتر) باستخدام وسط زروق مع مصادر مختلفه للنيتروجين. واوضحت النتائج ان اعلي انتاجية واعلي نسبة نقاوة واقل نسبة فقد في الفيكوسيانين كانت بدون تجفيف تحت ظروف خارجية (خارج المعمل) علي مستوي واسع بوسط تجاري قليل التكلفة باستخدام سماد اليوريا التجاري بدلاً من نترات الصوديوم المعملي كمصدر للنيتروجين وبيكربونات الصوديوم التجاري بدلاً من المعملي في وسط زروق. وكانت النتائج بالترتيب: ٠.٠٩٧ جم/لتر فيكوسيانين، نسبة نقاوة ١.٠٦، ٣ % نسبة الفقد في الفيكوسيانين .

وهذة الكمية الهائلة من الفيكوسيانين بنقاوتها العالية وتكلفتها الاقل يمكن استخدامها في مجال الصناعات الغذائية وصناعة مستحضرات التجميل والادوية.