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*Full Length Research Paper*

# Standardization of rapid and economical method for neutraceuticals extraction from algae

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***Spirulina* (cyanobacterium) posses a wide range of components useful in food and pharmaceutical industries. Pigments are intracellular compounds whose extraction involves cell disruption. Traditional extraction methods have several drawbacks like slow, time consuming utilize large quantity of solvents and less yield. So a systemic and economical approach was performed for maximum extraction of pigments using ultrasound-assistance. Phycocyanin concentration in the extract was calculated spectrophotometrically by measuring the absorbance at 615 and 652 nm. The purity of phycocyanin is evaluated based on the absorbance ratio of $A_{620}/A_{280}$. The optimized results obtained from both the solvents were analyzed. The result shows extraction with 1% calcium chloride solution of volume 45.27 ml, time 9.3 min and amplitude 79.72% gave the maximum yield of phycocyanin (0.20244 mg/ml). The maximum phycocyanin purity ratio of 0.62 was obtained in calcium chloride extract. The crude protein content of extracted samples has also been estimated by Kjeldahl method and it was found to vary from 18.23 to 63.63%, the highest being in distilled water extract.**

**Key words:** *Spirulina*, phycocyanin.

## INTRODUCTION

*Spirulina platensis* is a multifilamentous prokaryotic cyanobacterium and can be easily monocultured and harvested. C-phycocyanin (C-PC) is the major phycobiliprotein in *Spirulina*. This blue colored red fluorescing biliprotein of algae was first reported in 1928 by Lemberg (Eocha, 1963). The pigments mainly phycocyanin and chlorophyll has wide range of importance. So their estimation extraction has become the present need. Several factors can influence the phycocyanin extraction. The most important factors affecting phycocyanin yield are cellular disruption method, type of solvent and extraction time (Abalde et al., 1998; Reis et al., 1998).

Traditional methods such as water extraction in which *Spirulina* biomass were suspended and the pigments leached out were estimated spectrophotometrically. In view of the multiple uses of phycocyanin, we have used a new procedure for extraction of the pigments. Ultrasound is being used for disruption of cell and the disrupted cells are centrifuged and analyzed spectrophotometrically. The wet biomass is immediately utilized by bacteria and starts degradation because of its nutritional composition. Hence to avoid these problems, use of dried biomass is suitable and convenient (Jayant, 2005). In this work, the best solvent for phycocyanin extraction from *S. platensis* was first investigated. Subsequently, the effects of temperature and biomass-solvent ratio on the phycocyanin concentration and extract purity were evaluated to establish the optimum conditions for phycocyanin extraction.

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## MATERIALS AND METHODS

Spirulina was grown in batch culture in Erlenmeyer flask containing standard Zarrouk's medium (Zarrouk, 1966). At the end of cultivation, the biomass was recovered by filtration, dried at 40°C for 48 h.

### Experimental design

A statistical method, central composite rotate design (CCRD) with three independent variables (volume, time and amplitude) and two dependent variables (phycocyanin and chlorophyll concentrations) was used for designing the experiment. The statistical software

**Table 1.** Actual independent variables designed using Design Expert 7.0 software.

| Experiment no | Volume (ml) | Time (min) | Amplitude (%) |
|---|---|---|---|
| 1 | 45.27 | 9.3 | 36.22 |
| 2 | 104.73 | 9.3 | 36.22 |
| 3 | 45.27 | 20.57 | 36.22 |
| 4 | 104.73 | 20.57 | 36.22 |
| 5 | 45.27 | 9.3 | 83.78 |
| 6 | 104.73 | 9.3 | 83.78 |
| 7 | 45.27 | 20.57 | 83.78 |
| 8 | 104.73 | 20.57 | 83.78 |
| 9 | 25.00 | 15.00 | 60.00 |
| 10 | 125.00 | 15.00 | 60.00 |
| 11 | 75.00 | 5.00 | 60.00 |
| 12 | 75.00 | 25.00 | 60.00 |
| 13 | 75.00 | 15.00 | 20.00 |
| 14 | 75.00 | 15.00 | 100.00 |
| 15 | 75.00 | 15.00 | 60.00 |
| 16 | 75.00 | 15.00 | 60.00 |
| 17 | 75.00 | 15.00 | 60.00 |
| 18 | 75.00 | 15.00 | 60.00 |
| 19 | 75.00 | 15.00 | 60.00 |
| 20 | 75.00 | 15.00 | 60.00 |

Design Expert 7.0 has been used for designing and analyzing the experiment. A total of 20 experiments were performed. The independent variables were added in the design with 5 levels with equal difference (volume in range of 25 to 125 ml, time 5 to 25 min and amplitude 20 to 100%). Table 1 show the experimental design obtained through Design Expert 7.0 software.

#### Phycocyanin extraction with different solvents

Phycocyanin extraction was evaluated in terms of phycocyanin concentration using distilled water and 1% $CaCl_2$ solution (Abalde et al., 1998; Bermejo et al., 2003). The extraction was carried out by mixing 0.1 g of dried biomass with amount of the solvent, time and amplitude according to the experimental design. The samples were exposed to ultrasounds using a UP 50 H sonicator (Hielscher), followed by centrifugation for 15 min at 13500 rpm using refrigerated centrifuge model number BL156R (Biolab). The optical density of the supernatant was measured at 615 and 652 nm using an UV-VIS spectrophotometer (Elico). Phycocyanin concentration (PC), according to Bennett and Bogorad (1973), was defined as

$$\text{Phycocyanin} = \frac{(OD_{615} - 0.474(OD_{652})}{5.34}$$

The purity of C-PC is generally evaluated using the absorbance ratio of $A_{620}/A_{280}$ "Optimization experiments was carried out using CCRD with varied levels of independent variables (volume, time and amplitude)"wherein a purity of 0.7 is considered as food grade, 3.9 as reactive grade and greater than 4.0 as analytical grade (Rito-Palomares et al., 2001).

The purity of the optimized experimental sample was monitored spectrophotometrically by the A615/A280 ratio (Abalde et al., 1998). This relationship is indicative of the extract purity of phycocyanin with respect to most forms of contaminating proteins.

## RESULTS AND DISCUSSION

### Influence of different solvents on extraction of pigments

The phycocyanin content was expressed in terms of mg/ml, which was determined from the equation described by Bennett and Bogorad (1973). The protein content was measured by using Kjeldahl method and expressed as percentage. The phycocyanin and protein content during the extraction with different solvents were calcium chloride and distilled water investigated.

The maximum amount of phycocyanin, 0.3116 mg/ml was obtained in calcium chloride solution followed by 0.299 mg/ml in distilled water. The maximum amount of protein, 63.63% was obtained in distilled water solvent and 54.69% in calcium chloride solution. So $CaCl_2$ solution was found to the best solvent for extraction of phycocyanin with ultrasound assistance.

### Optimization of pigment extraction

Statistical analysis of all the 20 experiments was performed for both the solvent using the same software (Design Expert 7.0). The optimized result for the maximum yield of phycocyanin was derived. The input criteria for software, volume and time of extraction, should be minimum and amplitude of Sonicator should be in range with these criteria. The analysis was made and the result displayed in the Table 2.

Maximum phycocyanin concentration was obtained in

**Table 2.** Optimized phycocyanin concentration.

| Criteria | Distilled water | Calcium chloride |
|---|---|---|
| Volume (ml) | 45.27 | 45.27 |
| Time (min) | 9.3 | 9.3 |
| Amplitude (%) | 73.865 | 79.72 |
| Phycocyanin (mg/ml) | 0.20168 | 0.20244 |
| Desirability | 1.000 | 1.000 |
| Purity ratio | 0.50 | 0.62 |

**Table 3.** ANOVA for the phycocyanin extraction using distilled water.

| Source of variation | Sum of squares | Degree of freedom | Mean square | F-Value |
|---|---|---|---|---|
| Model | 0.057 | 9 | $6.366\times10^{-3}$ | 119.32(Significant) |
| Residual | $5.335\times10^{-4}$ | 10 | $5.335\times10^{-5}$ | |
| Lack of fit | $4.431\times10^{-4}$ | 5 | $8.861\times10^{-5}$ | 4.90(not significant) |
| Pure error | $9.045\times10^{-5}$ | 5 | $1.809\times10^{-5}$ | |
| Total | 0.058 | 19 | | |

**Table 4.** ANOVA for the phycocyanin extraction using 1% calcium chloride solution.

| Source of variation | Sum of squares | Degree of freedom | Mean square | F-Value |
|---|---|---|---|---|
| Model | 0.062 | 9 | $6.907\times10^{-3}$ | 18.96 (Significant) |
| Residual | $3.642\times10^{-3}$ | 10 | $3.642\times10^{-4}$ | |
| Lack of fit | $1.402\times10^{-3}$ | 5 | $2.805\times10^{-4}$ | 0.63 (not significant) |
| Pure error | $2.240\times10^{-3}$ | 5 | $4.480\times10^{-4}$ | |
| Total | 0.066 | 19 | | |

calcium chloride solution (0.20244 mg/ml) and followed by distilled water (0.20168 mg/ml). The result obtained with this method was compared with other traditional methods as described by Silveria et al. (2006) for *Spirulina* biomass taken in rotary shaker along with solvent, followed by centrifugation and spectrophotometric analysis. The result obtained with ultrasound shows the better yield of phycocyanin than the traditional method.

The analysis of variance made for both water and calcium chloride extracted sample results. The result for water and calcium chloride are presented in Tables 3 and 4 respectively. The results show that the model is best fit with lack of fit not significant.

The quadratic equation for results was made using the statistical software for phycocyanin. Equations (1) and (2) show the quadratic model for distilled water and calcium chloride solution results, respectively.

$$PC = 0.12 - 0.057A + 9.033 \times 10^{-4} B + 1.388 \times 10^{-3} C + 3.431 \times 10^{-4} AB - 1.094 \times 10^{-3} AC + 1.394 \times 10^{-3} BC + 0.029 A^2 - 1.908 \times 10^{-3} B^2 - 3.339 \times 10^{-3} C^2 \quad (1)$$

$$PC = 0.12 - 0.062A + 2.393 \times 10^{-3} B + 3.547 \times 10^{-3} C + 1.513 \times 10^{-3} AB - 3.087 \times 10^{-3} AC + 7.625 \times 10^{-3} BC + 0.024 A^2 - 5.096 \times 10^{-3} B^2 - 6.209 \times 10^{-3} C^2 \quad (2)$$

where PC is phycocyanin concentration in mg/ml; A = volume of solvent in ml; B = time of exposure to ultrasound in minutes, and C = amplitude of the sonicator in percent.

The purity of phycocyanin was determined by using the method as described by Liu et al. (2005) both the water and calcium chloride solution extracted sample were analyzed for all the experiments conducted. Of all, the 9$^{th}$ experiment of calcium chloride solvent extract shows the highest purity of phycocyanin of 0.682. Distilled water extracted sample has shown less purity than as prescribed for food grade purpose (0.6)

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