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Optimization of Carotenoids Production from Rhototorula Rubra Using Central Composite Design

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Abstract-The red yeast, *Rhodotorula rubra* produced 161 µg/g cell dry weight of carotenoids with 15.0 g glucose, 2.0 g $(NH_4)_2SO_4$, 1.0 g yeast extract (YE), pH 5.5, and 37 ^{0}C. (per litre). The carbon source (glucose ; c), nitrogen source (ammonium sulfate ; n), growth factor (yeast extract ; GF), pH and temperature (TEMP) were the factors affected on improving carotenoids production. The optimization was predicted the best condition for carotenoids production using central composite design and quadratic model analysis. The predicted maximum carotenoid of 259.14 µg/g cell dry weight could be estimated at 23.77 g glucose, 3.19 g $(NH_4)_2SO_4$, 3.19 g yeast extract (YE), pH 6.69, and 37.13 ^{0}C (per litre)., while that was 235.84 µg/g cell dry weight in practical.

Keywords-*carotenoid; Rhodotorula rubra;, red yeast; quadratic model analysis; central composite design.*

I. INTRODUCTION

Carotenoids are natural pigments responsible for the pleasing colors of many foods and have important biological activities. Some carotenoids are precursors of vitamin A which have beneficial effects on human health including enhancement of the immune system and reduction of the risk for degenerative diseases such as cancer, cardiovascular diseases and cataract [1,2]. Production of carotenoids using carotenoids-produced microorganisms has been taken into considerations owing to its highly efficient and easy manipulation in processing schemes [3]. The commercial utilization of microorganisms with biotechnological potential to produce carotenoids is presently limited by the high cost of production. However, the cost of carotenoids production by fermentation can be minimized by optimizing its process, using highly pigment-producing microorganisms cultured in cheap industrial by-products as nutrient sources [4].

II. MATERIAL AND METHOD

A. Microorganism and Carotenoid Production

The *Rhodotorula rubra* was used in this study, which was kindly provided from Maejo University, was maintained on agar salant. For carotenoid production, the strain was cultivated in liquid medium containing 10.0 g glucose, 1.0 g $(NH_4)_2SO_4$, 2.0 g KH_2PO_4, 1.0 g $MgSO_4.7H_2O$ and 1.0 g yeast extract (YE) (per litre) with 250 rpm , and 30 ^{0}C for 72 h.

B. Measurement of Carotenoid Content

Yeast cells were separated from the liquid medium by centrifugation at 5,000 rpm. 10 min, and rinsed twice with deionized water, and then freeze dried. The carotenoid content was extracted from the yeast and determined for carotenoid content by Foss method. [5].

C. Central Composite Design (CCD)

The central composite design (CCD) was use to evaluate the coefficients for prediction of possible condition in carotenoid production. The CCD was applied with five design factors, namely the carbon source (c), the nitrogen source (n), the growth factor (GF), pH and temperature (TEMP). The coded levels and the natural values of the factors set in this statistical experiment are shown in Table 1. The selected optimization parameters was carotenoid content (Y1). The *number of trials* (N) was based on the number of the design factors (k=5-1) as follows:

$$N = 2^k + 2k + 4 = 30 \text{ tials} \quad (1)$$

The experimental results of the central composite design were fitted with a second-order polynomial equation by a multiple regression technique. The quadratic model for predicting the optimal point was expressed as follows :

$$Y = C_0 + \sum_{i=1}^{5} C_i X_i + \sum_{i=1}^{5} C_{ii} X_i^2 + \sum_{\iota=1}^{5} \sum_{j<i} C_{ij} X_i X_j \qquad (2)$$

III. RESULT

A. Submerge Fermentation and Central Composite Design

Rhodotorula rubra produced 161 µg/g cell dry weight carotenoid with 15.0 g glucose, 2.0 g $(NH_4)_2SO_4$, 1.0 g yeast extract (YE), pH 5.5, and 37 ^{0}C. (per litre). Least square linear regression is summarized in Table 1. The ρ value of its experiment was 0.002 indicated the model significant. Using the Minitab version 16 program to find out the quadratic mathematical model, it was showed the results as the following equation ;

$$Y_1 = 2617.24–37.75C–161.77N+7.7.82GF-208.76pH109.57TEMP+0.18C^2+14.52pH^2+1.61TEMP^2+1.03CTEMP+28.69NpH \qquad (3)$$

The maximum carotenoid content of 259.14 µg/g cell dry weight was expected to obtain by using 23.77 g glucose, 3.19 g $(NH_4)_2SO_4$, and 3.19 g yeast extract (YE) (per litre) with pH 6.69, and 37.13 ^{0}C.

TABLE I. THE LEAST SQUARE REGRESSION ANALYSIS

Variables	Data		
	Coefficient	T-value	P-value
Constant	2617.24	**2.609**	**0.017**
C	-37.75	-1.714	0.103
N	-161.77	-0.502	0.621
GF	7.81	0.994	0.333
pH	-208.76	1.060	0.303
TEMP	-109.57	1.919	0.070
C*C	0.18	1.602	0.126
pH*pH	14.52	1.270	0.219
TEMP*TEMP	1.61	5.072	0.000
C*TEMP	1.03	3.008	0.007
N*pH	28.70	1.396	0.179
Coefficient of correlation (R^2) = 0.8856			F =4.62
Coefficient of determination (adj. R^2) = 0.8949			P =0.002

B. Optimal Cultivation

The optimized medium obtained from the predicted result that consisted of 23.77 g glucose, 3.19 g $(NH_4)_2SO_4$, 3.19 g yeast extract (YE), pH 6.69, and 37.13 ^{0}C. (per litre). The maximum carotenoid content of 235.84 µg/g cell dry weight was obtained after cultivated with optimized medium at 37.13 ^{0}C for 72 h. Glucose and ammonium sulfate were the most suitable carbon source and inorganic nitrogen source, respectively, for carotenoids production by *Rhodotorula rubra*. This study found that the optimization of medium has increased the carotenoids production by 1.46 times.

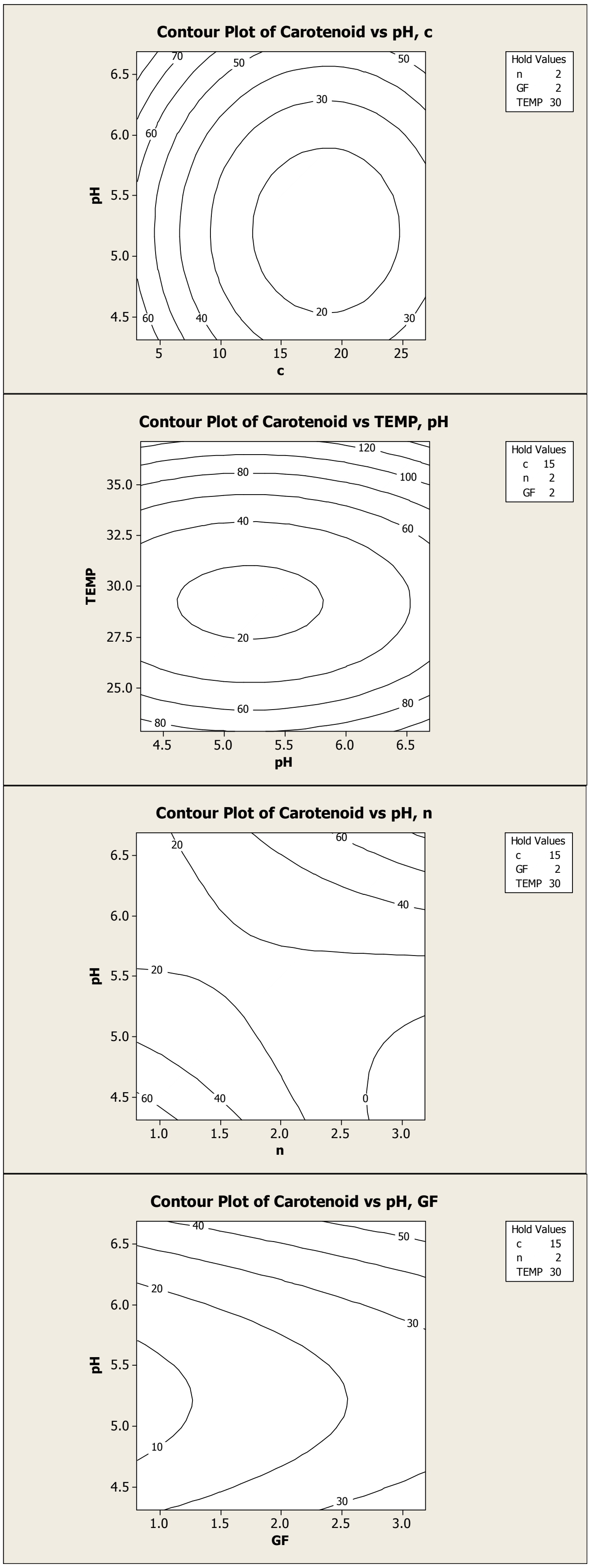

Figure 1. The result of response surface methodology

IV. Conclusion

The statistically based experimental design was applied to assess the influences of selected major nutrients and culture conditions on carotenoid production of the yeast. In particular, the central composite design scheme was used to evaluate the influences of carbon and nitrogen sources, growth factor, pH and temperature on carotenoid content. Second-order polynomial models were calculated and reduced equations were designed by neglecting non-significant ($P < 0.05$) regression coefficients. Reduced equations were used to calculate the optimal concentration of major nutrient and culture conditions in view of maximizing the level of carotenoid content. It was found that, after optimization, average final values total carotenoids (235.83 µg/g cell dry weight) of the central composite design scheme. Under the same condition, average final values of other responses were: carbon source (glucose) = 23.77 g/l, nitrogen source (ammonium sulfate) = 3.19 g/l, growth factor (yeast extract) = 3.19 g/l, pH = 6.69 and temperature = 37.13 °C.

After validate the optimum point of the factors, experimental rechecking was carried out using conditions representing those optimal factors. The above experimental data are in good agreement with calculated ones, thus confirming the reliability of the proposed empirical model in describing carotenoid production by *R. rubra* as a function of major nutrient and culture conditions.

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