# 당밀로부터 *Rhodotorula glutinis*의 카로티노이드생산을 위한 통계적인 응용

박 평규, 김 의용
서울시립대학교 화학공학과

# Application of a statistical technique to the production of carotenoid from sugar cane molasses by *Rhodotorula glutinis*

Pyoung Kyu Park, and Eui Yong Kim
[1]Dept. of Chemical Engineering, University of Seoul

## INTORDUCTION

In microorganism, carotenoids acts to important properties (anti-oxidant, free radical scanvengers etc). Algae such as *Dunaliella* and *Haematococcus*, yeasts of the genra *Phaffia*, *Rhodotorula* and *Sporobomyces*, fungi like *Blakeslea trispora* and bacteria such as *Flavobacterium* and *Micrococcus* are reported to produce carotenoids. Yeasts are more convenient than algae or fungi for large-scale production in fermentors, due to thier unicellular nature and high growth rate. *P. rhodozyma* is known to produce astaxanthin normally(1).

Culture conditions are traditionally optimized by the one-at-time strategy, i.e., varying one factor while keeping all others constant. Although the strategy is simple and easy, without the need of statistical analysis, it involves a relatively large number of experiments and the interaction among factors is often ignored. In contrast, statistically based experimental designs are more efficient approaches that can deal with a large number of variables simultaneously. Moreover, the interaction among different variables can be estimated. To date, this technique has been used by some researchers to optimized medium components and environmental factors. Literature reports little about the use of experimental design in optimizing the culture conditions of carotenoid production microorganisms(2).

During the last decade, there has been used industrial product production. In this study, the statistical approach was used to develop mathematical functions describing the relationships between environmental factors and both cell mass and volumetric carotenoid concentration. In a classical approach was to hold all variables but one variable as constant while methodically changing one at a time. Although the stratergy is generally time-consuming and requires a large number of experiments to be carried out. Since the effects of interactions between the variables are ignored. Hence, statistically based experimental designs are more efficient approaches that can deal with a large number of variables simultaneously. Moreover, the interaction among different variables can be estimated. To date, statistical method has been used by some researchers to optimize medium components. This design is a powerful tool for this purpose.

In this study, to maximize carotenoid production by *R. glutinis*, determing the optimal culture conditons (including medium components and environmental factors) is needed. The first design (factorial design) analyzes the main factors which can be influencing volumetric carotenoid production. The second (central composite design) determines the optimum condition

for the carotenoid production. Little information exists about the effects caused by the composition of the defined culture media on the carotenogenesis. The aim of this investigation was to examine the effect of culture condition on the carotenoid production in shake flask fermentation. In order to optimise the production of carotenoid by yeast we studied the influence of the sugar cane molasses, urea, $KH_2PO_4$, NaCl and pH on the cellular carotenoid and volumetric carotenoid. In the first step, screening the variable by using fractional factorial design (FFD), this methodology allows one to verify the individual factor effects and their interactions for the response. and central composite design (CCD) was employed to optimize the screened variables. Furthermore, we investigated the relationship between growth and carotenoid production. In addition, a mathematical model was proposed to determine the optimum levels of culture conditions in order to obtain the maximum volumetric carotenoid.

## MATERIAL AND METHODS

### Strains

The microorganism used was *Rhodotorula glutinis* KCTC 7989 isolated from soil in our laboratory (3).

**Analytical methods** Cell density was measured spectrophotometrically at 660 nm wavelength using UV/Vis spectrophotometer (HP 8452, USA). determined by turbidity measurements at 660 nm and correlated to dry cell weight. For the determination of dry cell weight, cells were washed twice with distilled water, and dried for 48 h at 110 ℃. Total reducing sugars in molasses were determined after inversion of sucrose with 2M HCl using the dinitrosalicylic acid method. The carotenoid analysis was carried out according to the method of Park et al (4). The carotenoid extracted from cells harvested by centrifugation, washed twice with distilled water, and frozen at 48 ℃. One milliliter each of DMSO (55 ℃), acetone, petroleum ether and sodium chloride 20% (w/v) solution was then added serially with vortex in 0.1g of lyophilized cells. The upper petroleum ether layer containing the carotenoids was collected and analyzed by thin layer chromatography.

### Experimental Design

The purpose of the first experimental design was to screened which component of this industrial medium have a significant effect on the carotenoid production of *R. glutinis* KCTC 7989. Fractional factorial design is very useful in screening the important variables and interaction between two or more nutrients in relatively few experiments as compared to the one-factor-at-time approach. The experimental design of this first screening step, the factors and the levels at which the experiments were carried out are given in Table 1, 2. Each variable is represented at three levels, a high level denoted by (+), a center point denoted by (0) and a low level designated by (-). The response variables ( cell mass, cellular carotenoid and volumetric carotenoid ) were performed in duplicated.

Carotenoid production of *R. glutinis* can be written as a function of the levels of the variables with a significant influence on the carotenoid production. The nature of this function is unknown but usually this kind of responses can be approximated by a second order polynomial :

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2$$

where $Y$ is the response calculated by the model; $x_1$, $x_2$, the level of the variables. The above equation was solved using SAS to estimate the responses of the dependent variables. Only the estimates of coefficients with significant levels higher than 95% (i.e., $p < 0.05$) were included in the final models. The $F$-test was used to evaluate the significance of the models. The next step in medium optimization was to determine the optimum level of each of key independent variable as identified by the factorial design. An experimental design such as the response surface methodology ( RSM ), which is a fraction of the full factorial was used. This design evaluated the quadratic effects and two-way interactions among the variables.

## RESULT AND DISCUSSIONS

Table 1 was shown that the variable with largest effect was SCM, urea and $KH_2PO_4$ at the cell mass concentration and SCM, urea at the volumetric carotenoid production. cell mass play a key role in volumetric carotenoid production. It can be seen that the variable with largest effect was SCM, urea and $KH_2PO_4$ at the cell mass concentration and SCM, urea at the volumetric carotenoid production. Cell mass play a key role in volumetric carotenoid production. To investigate further how the variables act on the cell mass, cellular carotenoid content and volumetric carotenoid production, we examined the effect of different variables such as sugar cane molasses (SCM), urea, $KH_2PO_4$, NaCl and pH. These variables have the greatest influence on the cell mass, cellular carotenoid content and volumetric carotenoid.

## REFERENCE

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Table 1. Experimental fractional factorial design for the cell mass, cellular carotenoid and volumetric carotenoid[a].

| Run | A | B | C | D | E | Cell mass (g/L) | Volumetric carotenoid (mg/L) |
|---|---|---|---|---|---|---|---|
| 1 | - | + | - | - | - | 1.7 | 0.49 |
| 2 | - | + | + | - | + | 2.6 | 1.03 |
| 3 | - | - | + | + | + | 4.4 | 1.18 |
| 4 | + | + | + | + | + | 8.0 | 1.28 |
| 5 | + | - | + | - | + | 11.9 | 2.59 |
| 6 | - | - | - | + | - | 3.9 | 0.78 |
| 7 | + | - | - | - | - | 11.3 | 2.27 |
| 8 | + | + | - | + | - | 7.3 | 1.59 |
| 9 | + | - | + | + | - | 9.8 | 2.46 |
| 10 | - | - | + | - | - | 5.7 | 1.45 |
| 11 | + | + | - | - | + | 4.7 | 1.66 |
| 12 | - | - | -- | - | + | 7.5 | 1.27 |
| 13 | - | + | - | + | + | 4.3 | 0.68 |
| 14 | - | + | + | + | - | 2.3 | 0.70 |
| 15 | + | + | + | - | - | 3.6 | 1.58 |
| 16 | + | - | - | + | + | 9.5 | 2.92 |
| 17 | 0 | 0 | 0 | 0 | 0 | 5.6 | 0.96 |
| 18 | 0 | 0 | 0 | 0 | 0 | 5.8 | 0.99 |
| 19 | 0 | 0 | 0 | 0 | 0 | 5.7 | 1.08 |
| 20 | 0 | 0 | 0 | 0 | 0 | 5.4 | 0.95 |

[a] A = sugar cane molasses, B = urea, C = $KH_2PO_4$, D = NaCl, E = pH.