

# EFFECTS OF NUTRIENTIAL AND ENVIRONMENTAL CONDITIONS ON CAROTENOID BIOSYNTHESIS BY *RHODOTORULA* SP.

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## ABSTRACT

Carotenoid compounds are popular natural antioxidants which are commonly isolated from the plants. Recently, there have been many researches on carotenoid biosynthesis towards low cost products. In this study, *Rhodotorula* sp. was grown on an agricultural byproduct (corncoobs) as a matrix in solid-state fermentation. Essential nutrients were added with different concentrations to optimize condition for the carotenoid biosynthesis. Effects of other environmental factors such as moisture content and fermentation time on the yield were also characterized. The optimal nutrient composition for the yeast's growth and carotenoid biosynthesis is a compound of 500 $\mu$ g nitrogen and 16mg carbon in 100g matrix. Additionally, the moisture content of 80% is the best for producing carotenoid by this yeast strain. The fermentation time for the highest carotenoid yield is observed after 8 days.

**Keywords:** Biosynthesis; Carotenoid; *Rhodotorula*.

## 1. Introduction

Carotenoids such as  $\beta$ -carotene in carrots or lycopene in tomatoes are natural bioactive compounds which were often isolated from the plants. These compounds are not only antioxidants but also antimutagens. Currently, the medical products containing carotenoids have become more and more popular in Vietnam and over the world.

Apart from the plants, strains of the genus *Rhodotorula* are also able to synthesize carotenoids. Researches on carotenoid biosynthesis by *Rhodotorula* began in the early 2000s. *Rhodotorula* yeasts can ferment sugar (glucose, xylose, saccharose,...), glycerol (Easterling et al. (2009), Kot et al. (2009), Gientka et al. (2017)) and industrial byproducts such as grape must (Buzzini et al., 2000), sugarcane molass (Bhosale et al., 2001), cassava water (Silva et al., 2016), sugar beet molass (Taskin et al., 2016), potato wastewater (Kot et al., 2017).

These yeasts can biosynthesize both lipid and carotenoid. Some researches optimized the carbon/nitrogen (C/N) ratio which obtained the highest yield of the lipid and carotenoid. Depending on the species of *Rhodotorula*, the optimal C/N ratio has been different. Production of carotenoid by *R. gracilis* reached its peak at 26mg/g dry biomass weight with the C/N ratio in the medium of 10:1 (Somasheta et al., 2000). Braunwald et al. (2013) studied on the effect of C/N ratios on carotenoid and lipid production of *R. glutinis* and showed that the increase of this ratio in the growth medium led to increased lipid and carotenoid yield.

Apart from C/N ratios, other parameters in fermentation were concerned by researchers. Buzzini et al. (2000) determined that the pH 5.78 and yeast autolysate concentration 4.67g/L were the most appropriate for *R. glutinis* carotenoid production. In Cong's report (2000), the optimum parameters for the

growth of *R. sp. hidai* were the medium containing 4g sucrose, 1.5g yeast extract, 0.1g MgSO<sub>4</sub> and 100mL sea water at pH 6.0 and 30°C at which the carotenoid yield reached 603.93µg/g dry cell weight. Study of parameters for carotenoid biosynthesis by *R. gracilis* indicated that maximum carotenogenesis (0.09%) achieved at 8% glucose level, pH 7.5 and 6.0mL/ 100mL of inoculum for an incubation period of 12 days at 24°C.

In this study, we characterized the effects of some nutritional and environmental parameters on the carotenoid production by *Rhodotorula* sp. through solid-state fermentation on corncobs in order to maximize the carotenoid production by the *Rhodotorula* sp. strain isolated from rice leaves.

## 2. Materials and Methods

### Microorganism and culture conditions

*Rhodotorula* sp. (MN12) which was isolated from rice leaves in Long An province was provided by the Institute of Biotechnology and Food Technology, Industrial University of HCM City.

The yeast was maintained on malt agar (200g ground malt, 20g agar and 1L distilled water) by subculturing every two weeks.

Cells grown for 48 hours in 250mL shaken flasks containing 150mL malt broth, harvested by centrifugation (3000 rpm for 20 mins), washed and resuspended in 10mL of

sterile distilled water were used as inocula for fermentation studies.

### Fermentation conditions

Corncoobs were cut into small pieces and stored at -18°C until using. Corncob (30g) was thawed and put into thermostable plastic boxes (volume of 0.5L). Moreover, nutrient solution consisting of glucose, NaNO<sub>3</sub>, KH<sub>2</sub>PO<sub>4</sub> and MgSO<sub>4</sub> was supplemented. After being sterilized at 121°C for 20 min, the boxes were added inoculum and then fermentation was begun and lasted for 5-10 days at 30°C.

### Carotenoid extraction and determination

After incubation, the corncod (30g) was shaken with distilled water (150mL) in the 250mL Erlenmeyer flask. The biomass was harvested by centrifugation and ground with glass powder (1:1 w/w). Mixed solvent (acetonitrile: 2-propanol: ethyl acetate 4/4/2) was used to extract carotenoids. Cell density was determined by turbidity measurement using UV/Vis spectrophotometer at 610nm. Quantitative determination of carotenoids was carried out spectrophotometrically at 454nm.

## 3. Results and Discussion

### Effect of the C/N ratio on carotenoid biosynthesis

The influence of the different C/N ratios of the medium (8:1, 16:1; 24:1; 32:1 and 40:1) on carotenoid production by the red yeast *Rhodotorula* sp. has been assessed and shown on Table 1.

**Table 1**

Carotenoid concentration was obtained in the mediums with different C/N ratios.

Carbon/nitrogen ratio	Carotenoid concentration (µg/g matrix)
8:1	23.36 <sup>bc</sup>
16:1	18.12 <sup>c</sup>
24:1	24.83 <sup>bc</sup>
32:1	34.31 <sup>ab</sup>
40:1	41.74 <sup>a</sup>

Note: the different letters (a, b, c) in the same column showed the significant difference of the carotenoid concentration.

In this experiment, the increase of C/N ratio enhanced carotenoid production. Specifically, the carotenoid yield of *Rhodotorula* sp. at C/N ratios of 8:1, 16:1; 24:1; 32:1 and 40:1 were 23.36, 18.12, 24.83, 34.31 and 41.74  $\mu\text{g}$  carotenoid /g dry matrix weight, respectively (Table 1). The carotenoid concentrations at C/N ratios of 32:1 and 40:1 were not significantly different from each other, but higher than those at lower C/N ratios. Many studies proved a correlation between C/N ratio and carotenoid production. In a previous report, *Rhodotorula gracilis* gained the maximum carotenoid production in the medium with the C/N ratio of 10:1 (Somasheka et al., 2000). Moreover, Libkind et al. (2004) achieved the highest level of carotenoid biosynthesis by *R. mucilaginosa* at

the C/N ratio of 5. Therefore, both of 32: and 40:1 C/N ratios are appropriate for our solid-state fermentation medium.

#### **Effect of the nitrogen concentration on carotenoid biosynthesis**

The carotenoid yields which were obtained in the mediums with six different nitrogen concentration (100 - 600 $\mu\text{g/g}$  dry matrix weight) were compared with each other. In the range from 100 to 500  $\mu\text{g/g}$  dry matrix weight, carotenoid content produced by *Rhodotorula* sp. increased with nitrogen concentration. At higher concentration of nitrogen (600  $\mu\text{g/g}$  dry matrix weight), the carotenoid content remained the same as at 500  $\mu\text{g/g}$  dry matrix weight (Table 2). Therefore, the nitrogen concentration of 500  $\mu\text{g/g}$  dry matrix weight was chosen for further studies.

**Table 2**

Carotenoid concentration in the mediums with different nitrogen concentration

Nitrogen concentration ( $\mu\text{g/g}$ matrix)	Carotenoid concentration ( $\mu\text{g/g}$ matrix)
100	28.99 <sup>a</sup>
200	28.14 <sup>a</sup>
300	31.03 <sup>a</sup>
400	30.05 <sup>a</sup>
500	54.71 <sup>b</sup>
600	53.96 <sup>b</sup>

Note: the different letters (a, b, c) in the same column showed the significant difference of the carotenoid concentration.

#### **Effect of the inoculum on carotenoid biosynthesis**

The range of inoculum from 10 to 10<sup>6</sup> CFU/g dry matrix weight was used to characterize the effect of initial yeast density

on carotenoid production by *Rhodotorula* sp. The data on carotenoid concentration which were obtained according to initial yeast density were showed in Table 3.

**Table 3**

Carotenoid concentration in the mediums with different initial yeast density

Initial yeast density (CFU/g matrix)	Carotenoid concentration ( $\mu\text{g/g}$ matrix)
10	27.00 <sup>a</sup>
10 <sup>2</sup>	24.80 <sup>a</sup>
10 <sup>3</sup>	27.85 <sup>a</sup>
10 <sup>4</sup>	36.23 <sup>b</sup>
10 <sup>5</sup>	38.68 <sup>b</sup>
10 <sup>6</sup>	37.16 <sup>b</sup>

Note: the different letters (a, b, c) in the same column showed the significant difference of the carotenoid concentration.

Yeast fermentations starting from 10<sup>4</sup> to 10<sup>6</sup> CFU/g dry matrix weight resulted in a higher carotenoid accumulation than those starting from 10 to 10<sup>3</sup> CFU/g dry matrix weight. When the initial yeast density was in the range of 10<sup>4</sup> to 10<sup>6</sup> CFU/g gram of dry matrix weight, the carotenoid yield obtained remained unchanged. Hence, the optimal inoculum for carotenoid production by *Rhodotorula* sp. is 10<sup>4</sup> CFU/g dry matrix weight.

#### Effect of matrix moisture on carotenoid

#### biosynthesis

The solid-state fermentation has been a method of incubating microorganisms requiring oxygen for their growth. Some agricultural byproducts can be utilized as nutrient supplies and biomass carriers, especially for yeasts and molds.

In this fermentation method, water contents in matrices have been an important parameter. In this study, the matrix moisture between 50% and 90% was studied.

**Table 4**

Carotenoid concentration in the mediums with different moisture conditions

Matrix moisture (%)	Carotenoid concentration ( $\mu\text{g/g}$ dry matrix weight)
50	17.06 <sup>a</sup>
60	14.96 <sup>a</sup>
70	26.22 <sup>a</sup>
80	69.38 <sup>b</sup>
90	73.99 <sup>b</sup>

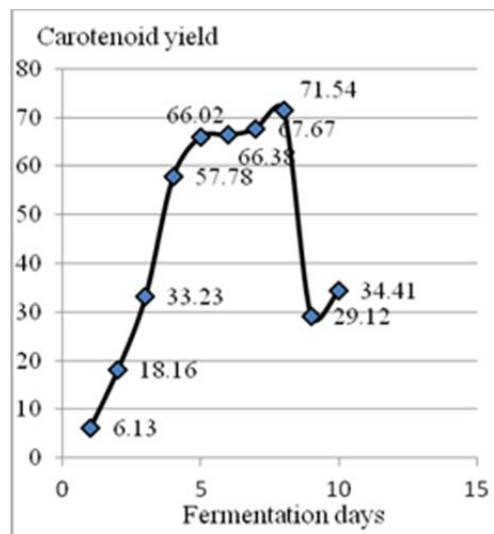
Note: the different letters (a, b, c) in the same column showed the significant difference of the carotenoid concentration.

As shown in Table 4, the matrix moisture significantly influenced the carotenoid yield. When the water content changed from 50 to 70%, the difference of carotenoid synthesis was not clearly. The yield of carotenoid increased significantly at the moisture content of 80% and the carotenoid yield stably remained when the moisture content was risen to 90%.

#### The carotenoid production during in 10-day incubation period

With the optimal parameters in the previous experiments, the fermentation was carried out after 10 days. The carotenoid yields were collected and determined every

day. As described in the Figure 1, the carotenoid production can be divided into three phases. In the first phase (the first 5 days), the carotenoid production significantly increased from 0 to 66.02  $\mu\text{g/g}$  dry matrix weight. In the second phase (the next 3 days), the carotenoid yield continued increasing but at a lower rate than in the first phase. In the last phase, the carotenoid production decreased to 34.41  $\mu\text{g/g}$  dry matrix weight. Therefore, in this solid-state fermentation conditions, the time for carotenoid biosynthesis is around 6 to 8 days, when the carotenoid yield reaches the maximum level.



**Figure 1.** Carotenoid yield during 10-day fermentation period

Our study is in line with literature data on fermentation conditions that have been reported. When using rice bran as a sole substrate for *Rhodotorula* cultivation, the carotenoid accumulation reached the highest level at the moisture content of 70% (Roadjanakamolson et al., 2008). Additionally, Hernandez-Almaza (2014) used ground polyurethane foam as the support for another strain of *Rhodotorula* which produced carotenoid. In that work, some ranges of fermentation conditions were studied in order to find out the optimized parameters of moisture (70 – 90%), fermentation time (48 –

72h) and inoculum ( $10^6 - 10^8$ ).

#### 4. Conclusion

The *Rhodotorula* strain, which was isolated from rice leaves in this study, could synthesize carotenoid with the C/N ratio of 32:1; nitrogen concentration of 500  $\mu\text{g/g}$  dry matrix weight; matrix moisture of 80% and the initial inoculum of  $10^4$  CFU/g dry matrix weight. The biomass harvested after 8-day fermentation reached the highest yield of carotenoids (71.54  $\mu\text{g/g}$  dry matrix weight). Our study would provide a basic assessment for optimization of nutritional and environmental conditions on carotenoid biosynthesis by *Rhodotorula sp.* ■

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