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Bioconversion of Shrimp By-Product into Carotenoids Using Pigmented Yeast *Sporidiobolus Pararoseus* Q

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Abstract

Chitin, a natural polysaccharide, is the second most abundant biopolymer in the world after cellulose. It consists of N-acetylglucosamine (NAG) monomers and finds widespread applications in the food, cosmetics, and pharmaceutical industries. In this study, we selected pigmented yeast strains to synthesize carotenoids from NAG and optimized the conditions for carotenoid production. The results demonstrated that the selected strain Sporidiobolus pararoseus Q could accumulate β-carotene and carotenoids, reaching 518.84 µg/g dry biomass and 595.48 µg/g dry biomass, respectively, after 96 hours of fermentation with 30 g/L NAG at pH 5. The two-step fermentation first with 80 g/L of glucose and then 50 g/L of NAG increased the carotenoids and β-carotene yields by 41% and 35%, achieving 786.69 µg/g and 632.19 µg/g, respectively. The ability to use NAG as the feedstock for carotenoid production not only adds value to shrimp by-products but also contributes to controlling environmental pollution.

Keywords: N-acetylglucosamine, carotenoids, β-carotene, Sporidiobolus pararoseus.

1. Introduction

Chitin is the second most abundant polysaccharide in nature after cellulose. It plays a crucial role in the structural composition of crustaceans (such as shrimp and crabs), fungal cell walls, and the exoskeletons of soft-bodied animals like squids and octopuses, as well as other marine soft tissues of fish [1]. Chitin is mainly generated as waste or by-product during the processing of crustaceans, primarily shrimp and crabs. This by-product consists of N-acetylglucosamine (NAG) units linked together via β-1,4-glycosidic bonds. NAG is used as a raw material to produce nitrogen-containing chemicals, such as heterocyclic compounds (Pyrrole, Pyridine, 5-hydroxymethylfurfural...), acids (formic acid, acetic acid, levulinic acid...), alcohols (N-acety lmonoethanolamine, erythritol...), and amino sugars (glucosamine) [2].

Some microorganisms including *Candida albicans* and *Escherichia coli* can utilize NAG as a carbon source for growth, and produce the metabolites [3]. NAG was used as feedstock for ethanol production by the yeast strain *Scheffersomyces stipitis* and by certain *Mucor* fungal species [4]; or for lipid production by *Cryptococcus curvacus* [5].

Carotenoids are terpenoids consisting of 40 carbon atoms and can be divided into carotenes and xanthophylls. They act as antioxidants and immunity enhancers; among them, β-carotene, a precursor of vitamin A, is commonly used as a supplement in the food and feed industry. Carotenoids are also common colorants used in food and cosmetic industries [1].

Production of β-carotene and carotenoids by microorganisms is gaining attention due to lower production costs and shorter accumulation times compared to extraction methods from plants [1].

In this study, NAG was used as the nutrient source for the biosynthesis of carotenoids using a pigmented yeast strain *Sporidiobolus pararoseus*. The effects of NAG concentration and cultivation conditions on carotenoids and β-carotene synthesis were examined and discussed. To our knowledge, this is the first report regarding the utilization of NAG as the feedstock for carotenoid synthesis.

2. Materials and Methods

2.1. Micro-Organism and Culture Conditions

The microbial strains used in this study were obtained from the collection of the Center of Research and Development in Biotechnology - School of Biotechnology and Food Technology - Hanoi University of Science and Technology (Table 1). The strains have been preserved in glycerol at -80 °C.

Microorganisms from glycerol were first streaked in Petri dishes containing Yeast Extract Peptone Dextrose (YPD) agar and incubated for 2-3 days. A colony was then picked up and transferred to a 250 mL baffled flask containing 50 mL of YPD medium and cultured for 24 h.

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Table 1. List of yeast strains used in the study

No	Strains	No	Strains
1	*Rhodotorula mucilaginosa* HL1	10	*Sporidiobolus pararoseus* Q
2	*Rhodotorula mucilaginosa* HLU1	11	*Sporidiobolus pararoseus* DH1
3	*Rhodotorula mucilaginosa* HU	12	*Sporidiobolus pararoseus* LN1
4	*Rhodosporidiobolus ruineniae* HHD	13	*Rhodosporidium toruloides* DC2
5	*Rhodosporidiobolus ruineniae* NH3	14	*Rhodosporidium toruloides* KLB1
6	*Cystobasidium slooffiae* HD2	15	*Rhodosporidium toruloides* KLB1
7	*Sporidiobolus pararoseus* NH2	16	*Rhodosporidium paludigenum* RDD
8	*Sporidiobolus pararoseus* DC6	17	*Rhodosporidium paludigenum* DG
9	*Sporidiobolus pararoseus* O1	18	*Rhodosporidium paludigenum* DL3

The fermentations were carried out in 250 baffled flasks containing 50 mL medium, at 25 ºC, 125 rpm. All experiments were duplicated and performed in the dark chamber of Bio-Shaker RB-3000 LF. The fermentation medium is composed of 20 g/L NAG, 10 g/L peptone, and 10 g/L yeast extract.

The effect of inoculation rate, pH, and concentration of NAG on carotenoid accumulation was examined in this research. The inoculum ratio was varied by changing the initial inoculum volume to achieve the initial OD_{660nm} ranging from 0.5 to 2.

For the pH control experiments, the pH of the culture media was adjusted to the desired value (varying from 4 to 10) by supplement of 4 M H_3PO_4. The adjustment was performed every 4-5 hours during the log phase or every 24 hours during the station phase.

The two-step cultivation was carried out by cultivating the yeast first in 80 g/L glucose, 10 g/L peptone, and 10 g/L yeast extract for 96 h. NAG was added to reach the concentration of 30 g/L and 50 g/L when glucose was fully consumed.

2.2. Analysis Methods

NAG concentration

The NAG and reducing sugar concentration were determined using the DNS (3,5-dinitrosalicylic acid) method according to Miller *et al*. [6]. A standard curve using NAG as a reagent in a range of 0.1 to 1.0 g/L, and the optical density were measured at a wavelength of 540 nm.

Dry biomass determination

Cells were harvested by centrifugation at $8000 \times g$ for 10 minutes then washed twice with distilled water and dried at 50 ºC to constant weight.

β-carotene and carotenoid measurements

Spectrophotometric measurement: β-carotene and carotenoids were extracted from the dried yeast biomass according to Michelon *et al.* [7]. Briefly, 0.1g of dry biomass was added to 1 mL of 4M HCl and maintained at 65 °C for 1 hour, then, centrifuged at $8000 \times g$ for 10 minutes. The supernatant was discarded, followed by adding 1.5 mL acetone for extraction. The yeast cells were separated by centrifugation at $8000 \times g$ for 10 minutes. The total carotenoids and β-carotene in the extraction were measured using a UV-VIS spectrophotometer at wavelengths of 470 nm and 450 nm, respectively.

A standard curve of β-carotene concentration from 1 to 10 µg/mL was used for β-carotene determination.

The total carotenoid content was calculated using the following equation [8]:

$$Carotenoids = \frac{A \times V \times 10^4}{\varepsilon \times m} (\mu g/g) \quad (1)$$

where A: absorbance at 470 nm; V: total volume (mL); m: weight of the sample (g); ε: extinction coefficient for carotenoids in acetone 2140 [9].

For HPLC measurement: β-carotene and carotenoids were analyzed using High-performance liquid chromatography analysis (HPLC), Agilent 1200 system, equipped with a C18 column (250 x 4.6 mm, 5 µm, Phenomenex, USA), and a Diode Array Detector (DAD). The mobile phase was acetone as solvent A and water as solvent B. The solvents were used as 70% solvent A at 0-15 min, 100% solvent A at 15-25 min, and 70% solvent A at 25-30 min with a flow rate of 1 mL/min.

The carotenoid composition analysis (β-carotene and γ-carotene, torulene, and torularhodin) was according to Pham *et al.* [10].

2.3. Calculation Methods

Specific growth rate

The specific growth rate in log phase was determined by the equation:

$$\mu = \frac{lnX - lnX_0}{t - t_0} \quad (2)$$

where μ: specific growth rate (1/h); X: biomass at time t (g/L); X_0: biomass at time t_0 (g/L); t, t_0: time (h)

Yield coefficient

The yield coefficient is the amount of biomass formed per substrate consumed and was given by:

$$Y_{X/S} = -\frac{\Delta X}{\Delta S} \quad (3)$$

where $Y_{X/S}$: yield coefficient (g/g); ΔX: difference in biomass (g); ΔS: difference in the substrate.

Substrate consumption rate

The specific substrate consumption rate was determined by the following equation:

$$q_s = \frac{\mu}{Y_{X/S}} \quad (4)$$

where q_s: specific substrate consumption rate (g/g.h); μ: specific growth rate (1/h); $Y_{X/S}$: yield coefficient (g/g).

Yield of β-carotene and carotenoid accumulations:

$$Y_{\beta-carotene} = \beta - carotene\ content \times DW \quad (5)$$

$$Y_{carotenoids} = Carotenoid\ content \times DW \quad (6)$$

where Y: yield of β-carotene or carotenoids (μg/L); carotenoid or β-carotene accumulation (μg/g); DW: dried biomass weight (g/L)

3. Results and Discussion

3.1. Screening Carotenoids Producing Yeast on NAG

Eighteen yeast strains were cultivated in the culture medium containing 20 g/L NAG (Table 1). The yeast biomass was harvested after 5 days, and the concentration of carotenoids and β-carotene accumulated in the biomass were evaluated (Fig. 1). All 18 selected yeast strains accumulated both carotenoids and β-carotene, with the concentrations ranging from 45.95 to 472.07 μg/g, and from 41.59 to 428.78 μg/g, respectively. Mata-Gomez *et al*. [11] divided carotenoid-producing strains into three groups based on the concentration of carotenoids accumulated: low (less than 100 μg/g), medium (101-500 μg/g), and high (over 500 μg/g). In this study, carotenoid-producing yeast was categorized into two groups: low (33.33%) and medium (66.67%) carotenoid-produced groups

Strain *Sporidiobolus pararoseus* Q shows the highest accumulation of β-carotene and carotenoids on NAG-based medium, corresponding to 428.78 μg/g, and 472.07 μg/g, respectively. This is the first publication on the biosynthesis of β-carotene and carotenoids from yeast using the NAG as the carbon source.

The majority of carotenoids can be observed by spectrophotometric measurement in the visible region between 400-500 nm. Therefore, the spectrophotometric measurement of β-carotene which is based on the maximal absorption at 450 nm could be influenced by other carotenoids [12]. Therefore, the extractions of the five highest β-carotene accumulating yeast were analyzed by HPLC (Fig. 2). The results showed that strain *S. pararoseus* Q has the highest accumulation of β-carotene corresponding to 257.85 μg/g. Its β-carotene accumulations were approximately 3.2, 6.31, 1.69, and 2.34-fold higher than that of strain *S. pararoseus* NH2, LN1, DC6, and DH1, respectively.

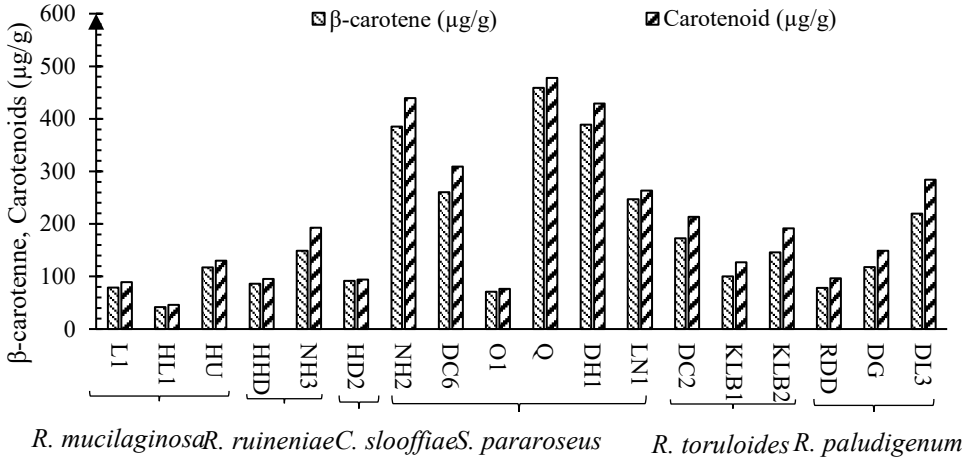

Fig. 1. β-carotene and carotenoid accumulation of eighteen strains using NAG of 20 g/L as the carbon source.

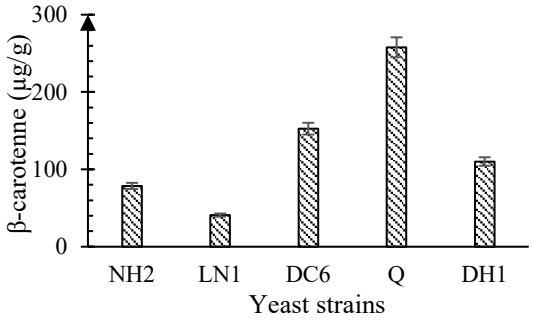

Fig. 2. HPLC analysis of 5 highest β-carotene accumulating strains

Thus, the strain *S. pararoseus* Q was selected for further examination.

3.2. Effect of Inoculum Ratio to Carotenoid Accumulation

The yeast strain *S. pararoseus* Q was cultured at various inoculum ratios to achieve the initial OD_{660nm} ranging from 0.5 to 2 (Fig. 3).

The results indicated that there was no significant difference in OD_{660nm} values in all cultures within the first 24 hours (Fig. 3a). The maximum OD_{660nm} value of 62.6 was achieved in the culture with the inoculum ratio of 0.5 after 48 h. This result was higher than those of the cultures with initial OD of 1, 1.5, and 2.

However, it could be observed that at an initial OD of 1, the yeast exhibited the highest ability to accumulate β-carotene and carotenoids (Fig. 3b). The carotenoids at initial OD_{660nm} 1 were 1.77, 1.33, and 1.43-fold higher than that with initial OD_{660nm} 0.5, 1.5 and 2, respectively.

3.3. Effect of pH Medium to Carotenoid Accumulation

The pH of the culture directly influences the growth of the micro-organism. In this study, during the fermentation, the pH of the fermentation broth tended to increase, due to the release of NH_3 group from NAG, similar to the report by Wu *et al.* [5]. Therefore, the pH during the fermentation was manually adjusted to the desired pH using H_3PO_4 acid. The results regarding the growth curve and the ability to synthesize carotenoids were shown in Fig. 4.

At neutral to alkaline pH ranging from 7 to 10, *S.pararoseus* Q grew slowly, with specific growth rates of 0.126, 0.114, 0.115, and 0.117 1/h, respectively. These cultures achieved maximum biomass at 24 hours of fermentation, then went into the decline phase (Fig. 4a). On the other hand, in acidic conditions (pH from 4 to 6), *S. pararoseus* Q exponentially grew until 34 h with the specific growth rates of 0.161, 0.147 and 0.135 1/h, respectively. Then, the OD_{660nn} continuously increased at a lower rate until 74 h before declining. The accumulations of carotenoids and β-carotene at pH ranging from 4 to 6 were higher compared to pH from 7 to 10, with the highest accumulation observed at pH 5 (Fig. 4b). In the study by Han *et al*. [13], *S. pararoseus* exhibited the highest carotenoid synthesis capacity during fermentation using glucose and corn-steep liquor at pH 6.

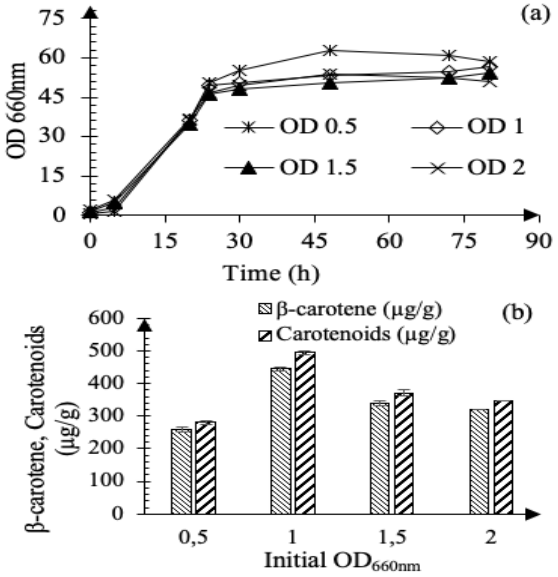

Fig. 3. Effect of inoculum ratio to the growth (a), and carotenoid accumulation at 80 h of cultivation (b).

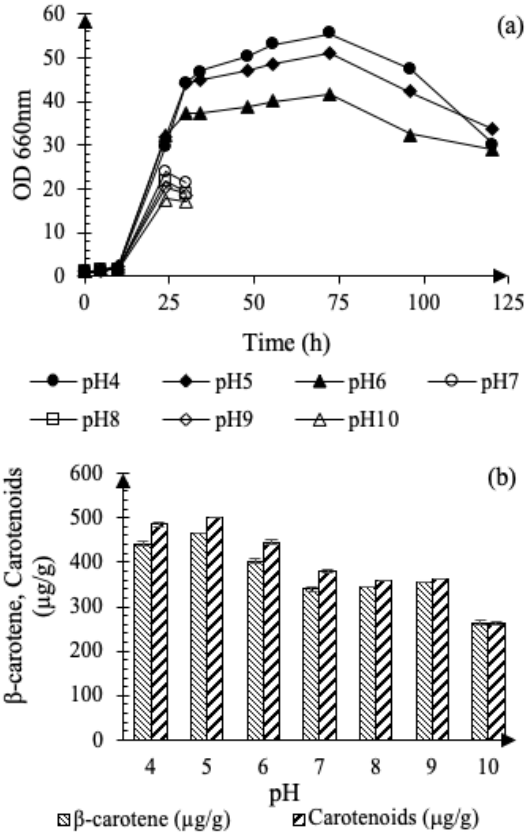

Fig. 4. Effect of pH on yeast growth (a); and carotenoid accumulations at 30 h (pH 7; 8; 9; 10) and 120 h (pH 4; 5; 6) (b) of Q strain.

3.4. Effect of NAG Concentration to Carotenoid Accumulation

The type and concentration of C sources significantly influence the growth and metabolism of the yeast [14]. In addition, it had been previously reported that the accumulation of carotenoids is driven by nitrogen sources and their concentration in the medium [15].

NAG is a nutrient source with a high carbon content, but it can also serve as a nitrogen source in the meantime. In this study, the NAG concentration in the medium was varied from 10 to 50 g/L for cultivation of *S. pararoseus* Q at pH 5 (Fig. 5). In the first 24 hours of growth, the *S. pararoseus* Q exponentially grew in all cultures (Fig. 5a) without a significant consumption of NAG. (Fig. 5b).

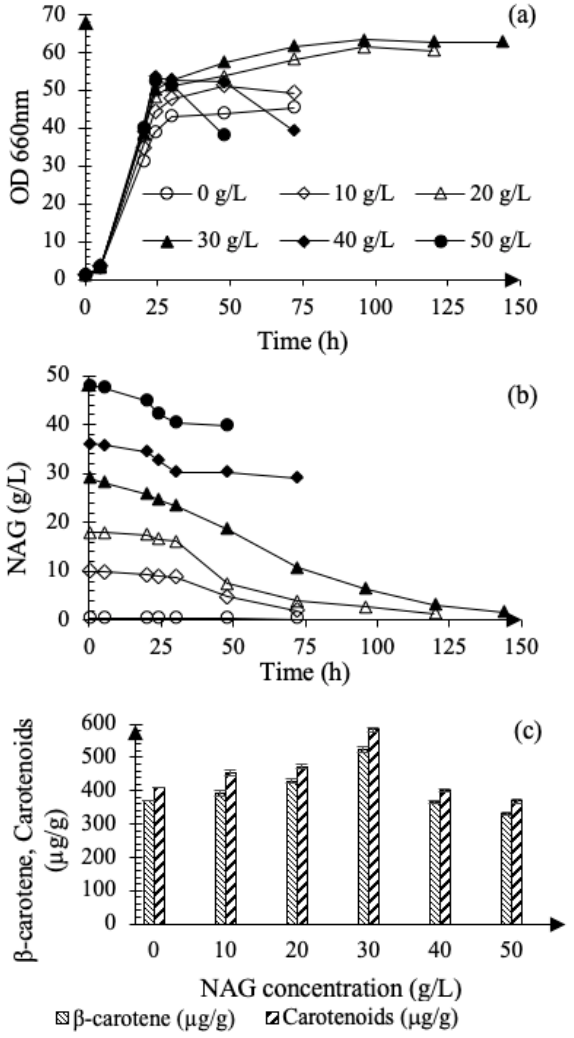

Fig. 5. Effect of NAG concentration on *S. pararoseus* Q culture. Yeast growth (a); NAG consumption (b); carotenoid and β-carotene accumulations (remarked at NAG was fully consumed) (c).

It can be inferred that for the first 24 hours, the yeast growth was supported by yeast extract and peptone in the medium. After 24 hours, these sources may be depleted, the strain started to use NAG to maintain growth in the 10-30 g/L NAG cultures but with a much lower growth rate. However, in the cultures with 40 g/L and 50 g/L NAG, NAG was not utilized. Their OD_{660nm} abruptly decreased after 24 hours of fermentation.

The accumulation of β-carotene and carotenoids of Q strain are shown in Fig. 5c. The β-carotene, and carotenoids produced in the biomass increased with the increase of NAG concentration from 0 to 30 g/L and then decreased at higher NAG concentration of 40 and 50 g/L. At the NAG concentration of 30 g/L, the strain exhibited the highest carotenoid accumulation, which was 1.42, 1.28, 1.23, 1.43, and 1.58-fold higher than the cultures without NAG or with 10, 20, 40, and 50 g/L of NAG, respectively.

3.5. Kinetic of Carotenoid Accumulation in NAG Medium of S. pararoseus Q

To determine the kinetics of the growth and carotenoid accumulation in NAG medium, the yeast strain *S. pararoseus* Q was cultivated in the optimal condition (inoculum ratio at initial OD_{660nm} 1, pH 5, NAG concentration 30 g/L, temperature of 25 °C, and agitation at 125 rpm), samples were daily collected for analysis of biomass, remaining NAG concentration, accumulated carotenoid, and β-carotene concentration.

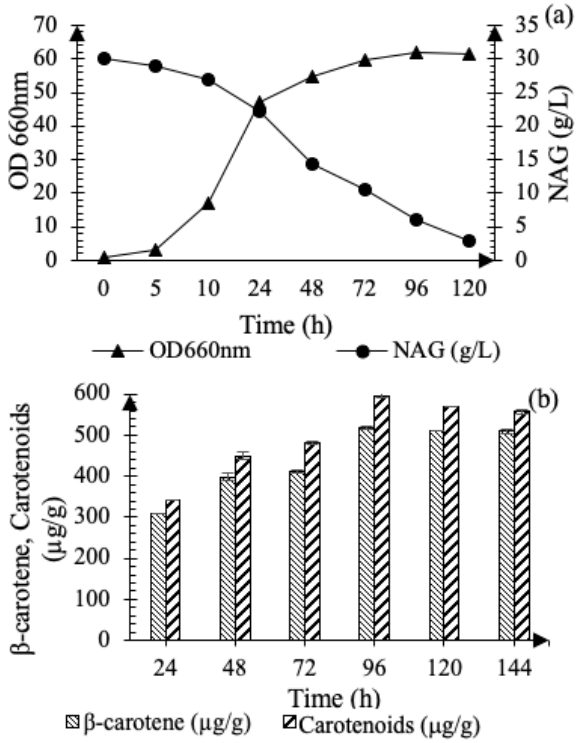

Fig. 6. Kinetic of carotenoid accumulation in NAG medium of *S. pararoseus* Q. Yeast growth (a); carotenoid accumulations (b)

The results indicated that the *S. pararoseus* Q strain took 5 hours to adapt to the culture medium (Fig. 6), and then it rapidly grew up to 24 hours with a specific growth rate of 0.145 1/h. However, only 22.2% of NAG was consumed during this period corresponding to the substrate consumption rate of 0.026 g/g.h. From 24 hours to 72 hours, the strain's growth was slowed down (the specific growth rate reduced to 0.005 1/h), while the NAG consumption rate increased up to 0.158 g/g.h. After 96 hours, the strain entered the stationary phase, but NAG was continuously consumed with a consumption rate of 0.018 g/g.h (Fig. 6a). The kinetic study indicated that the yeast strain *S. pararoseus* Q consumed NAG sparingly during the growth phase and extensively during the stationary phase. Nagaraj *et al*. [16] showed that β-carotene and carotenoid accumulations were highest at the end of the logarithmic phase, therefore, in the study, β-carotene and carotenoid accumulations were analyzed from 24 h. The analysis of β-carotene and carotenoids (Fig. 6b) showed that the production of β-carotene and carotenoids started during the logarithmic phase and increased over time along with the strain's development, peaking at the middle of the stationary phase. The highest levels of β-carotene and carotenoids were observed at 96 hours of cultivation, with a concentration of 518.84 µg/g and 595.48 µg/g, respectively. This result was higher than the findings of Cabral *et al*., [15], who cultured the *S. pararoseus* strain on 60 g/L glucose and obtained a carotenoid content of 350 µg/g dry biomass.

3.6. Two-Step Cultivation of S. pararoseus Q

The kinetic study mentioned above revealed that NAG was consumed in small quantities during the growth phase, while it was consumed more significantly during the stationary phase. Whereas, carotenoids were accumulated and reached highest during the stationary phase. Therefore, in this study, the yeast *S. pararoseus* Q was cultured in two phases. In the initial phase, the strain was cultured in a medium containing 80 g/L glucose to promote biomass growth. Then, in the subsequent phase, NAG was added to the fermentation broth to enhance the carotenoid accumulation.

Two-step cultivation using 30 g/L NAG

In this study, NAG was added to the fermentation broth to reach the concentration of 30 g/L when glucose was fully consumed at 96 h (Fig. 7a). Following the addition of NAG, the decrease in OD_{660nm} was observed, and it could be attributed to dilution effects. The NAG consumption rate reached 0.11 g/g.h during the first day after NAG supplementation. Then, it decreased to 0.06 g/g.h after 3 days of NAG supplementation. During this process, the increase in biomass was not substantial, similar to the results obtained when culturing with a one-step of 30 g/L NAG.

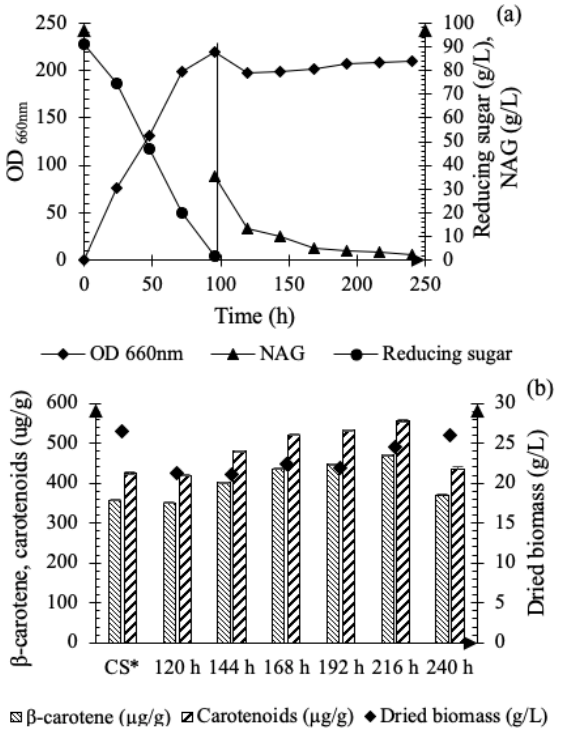

Fig. 7. *S. pararoseus* Q growth (a) and accumulation of carotenoids (b) in two-step cultivation using 30 g/L NAG. (*) control sample with 80 g/L glucose, collected at 96 h

The accumulation of carotenoids and β-carotene steadily increased from the 2nd day to the 6th day after NAG supplementation, and the highest reaching approximately 556.4 µg/g and 469.7 µg/g, respectively (Fig. 7b). Compared to the results of culturing one-step of 30 g/L NAG, the two-step cultivation showed an excessive increase in biomass, however carotenoid and β-carotene accumulations decreased 1.07 and 1.1-fold, respectively. These phenomena could be attributed to high biomass during the two-step fermentation, leading to a reduced ratio of NAG/microbial cells, thus resulting in a decrease in carotenoids and β-carotene accumulation.

Due to increased yeast biomass, the total carotenoid and β-carotene accumulations achieved 14498.96 µg/L and 12231.77 µg/L, respectively, which represented an increase of 4.39 and 4.25-fold compared to single-step culturing with 30 g/L NAG.

Two-step cultivation using 50 g/L NAG

To improve the carotenoid accumulation, the cultivation process was conducted in two steps with a NAG concentration of up to 50 g/L. The results indicated that upon adding 50 g/L NAG to the second step, the strain's biomass decreased from OD_{660nm} 235 at 120 h to OD_{660nm} 174 at 288 h (Fig. 8a).

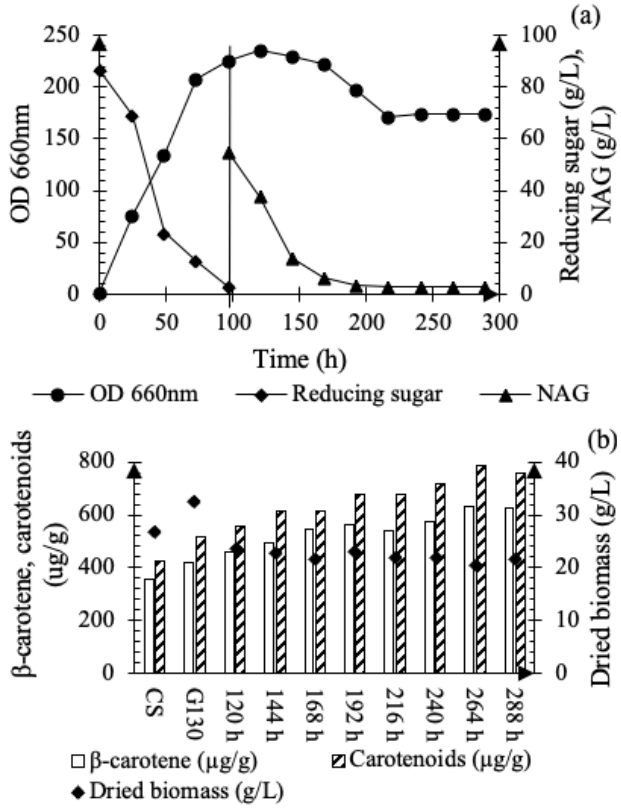

Fig. 8. *S. pararoseus* Q growth (a) and accumulation of carotenoids (b) in two-step cultivation using 50 g/L NAG. (CS collected at 96h)

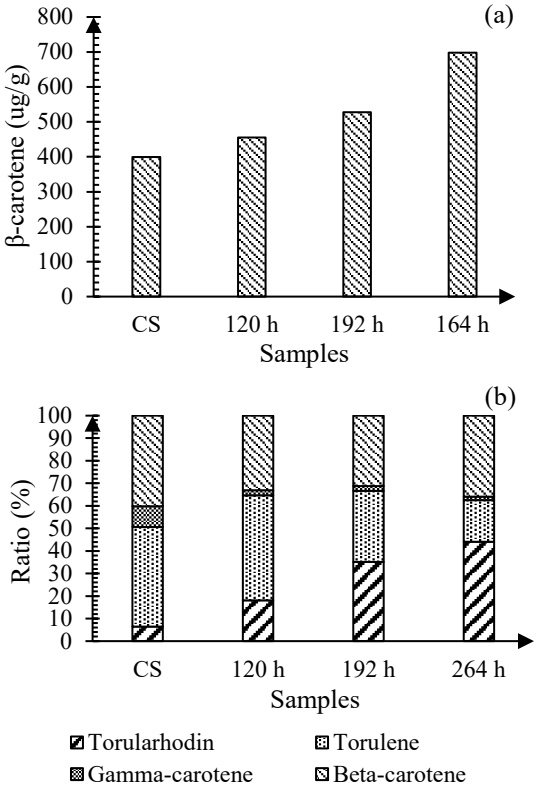

Fig. 9. HPLC analysis of carotenoids in two-step cultivation using 50 g/L NAG. β-carotene concentration (a), and carotenoids composition (b). (CS collected at 96 h)

This negative effect could be the inhibition of high NAG concentrations. The NAG consumption rate was high during the first 2 days, reaching 0.09 g/g.h, which was slightly lower than that at two-step cultivation using 30 g/L NAG. Then, the consumption rate gradually decreased to 0.03 g/g.h on the 4th day after NAG supplementation.

The accumulation of carotenoids improved from 1st day after NAG supplementation until the 7th day (Fig. 8b). The highest carotenoids and β-carotene accumulations reached 786.69 µg/g and 632.29 µg/g, respectively. They were 1.41 and 1.35-fold higher than these accumulations at two-step cultivation using a concentration of 30 g/L NAG.

The β-carotene analysis using HPLC showed a similar trend with the optical measurement (Fig. 9a). The β-carotene content gradually increased and reached its peak on the 7th day at 698.2 µg/g. This amount is 2.57-fold higher than the initial value.

The carotenoids composition showed in the Fig. 9b indicated that *S. pararoseus* Q synthesized 4 carotenoids including β-carotene, γ-carotene, torulene and torularhodin. Especially, the torularhodin, a potent antioxidant compound, was observed to steadily increase during the fermentation process using NAG. The torularhodin content within the carotenoids mixture increased from 6.4% in the control sample using 80 g/L glucose to 44.07% on the 7th day after adding 50 g/L NAG. The highest yield of torularhodin reached was 13.43-fold higher than that of the control sample (Fig. 9b).

4. Conclusion

In this study, N-acetylglucosamine (NAG) was used as the nutrient source for culturing the yeast strain *Sporidiobolus pararoseus* Q to synthesize carotenoids and β-carotene. The research demonstrates that at a maintained pH of 5, with an initial inoculum of OD_{660nm} 1, at a two-step cultivation with 80 g/L glucose and 50 g/L NAG, the highest production of carotenoids and β-carotene was achieved, reaching 786.69 and 632.19 µg/g, respectively. This is the first report on carotenoids synthesis using NAG. This research also opens up possibilities for valorization of chitin waste streams from the seafood processing industry.

Acknowledgments

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References

[1] R. V. Singh and K. Sambyal, An overview of beta carotene production: Current status and future prospects, Food Biosci., vol. 47, p. 101717, Jun. 2022. https://doi.org/10.1016/j.fbio.2022.101717

[2] C. H. M. van der Loo, M. L. G. Borst, K. Pouwer, and A. J. Minnaard, The dehydration of N-acetylglucosamine (GlcNAc) to enantiopure dihydroxyethyl acetamidofuran (Di-HAF), Org. Biomol. Chem., vol. 19, no. 46, pp. 10105-10111, Dec. 2021. https://doi.org/10.1039/D1OB02004H

[3] L. I. Alvarez-Añorve, M. L. Calcagno, and J. Plumbridge, Why does *Escherichia coli* grow more slowly on glucosamine than on N-acetylglucosamine? Effects of enzyme levels and allosteric activation of GlcN6P deaminase (NagB) on growth rates, J. Bacteriol., vol. 187, no. 9, pp. 2974-2982, May 2005. https://doi.org/10.1128/JB.187.9.2974-2982.2005

[4] K. Inokuma, M. Takano, and K. Hoshino, Direct ethanol production from N-acetylglucosamine and chitin substrates by *Mucor* species, Biochem. Eng. J., vol. 72, pp. 24-32, Mar. 2013. https://doi.org/10.1016/j.bej.2012.12.009

[5] S. Wu, C. Hu, X. Zhao, and Z. K. Zhao, Production of lipid from N-acetylglucosamine by *Cryptococcus curvatus*, Eur. J. Lipid Sci. Technol., vol. 112, no. 7, pp. 727-733, Jul. 2010 https://doi.org/10.1002/ejlt.201000005

[6] G. L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem., vol. 31, no. 3, pp. 426-428, Mar. 1959. https://doi.org/10.1021/ac60147a030

[7] M. Michelon, T. De Matos De Borba, R. Da Silva Rafael, C. A. V. Burkert, and J. F. De Medeiros Burkert, Extraction of carotenoids from *Phaffia rhodozyma*: A comparison between different techniques of cell disruption, Food Sci. Biotechnol., vol. 21, no. 1, pp. 1-8, Feb. 2012. https://doi.org/10.1007/s10068-012-0001-9

[8] L. M. J. de Carvalho *et al*., Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (Cucurbita moschata Duch): A preliminary study, Food Res. Int., vol. 47, no. 2, pp. 337-340, Jul. 2012. https://doi.org/10.1016/j.foodres.2011.07.040

[9] H. K. Lichtenthaler, Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes, in Methods in Enzymology, vol. 148, Elsevier, 1987, pp. 350-382. https://doi.org/10.1016/0076-6879(87)48036-1

[10] K. D. Pham *et al*., Effect of light on carotenoid and lipid production in the oleaginous yeast *Rhodosporidium toruloides*, Biosci. Biotechnol. Biochem., vol. 84, no. 7, pp. 1501-1512, Jul. 2020. https://doi.org/10.1080/09168451.2020.1740581

[11] Mata-Gosmez *et al*., , Biotechnological production of carotenoids by yeasts: an overview, Microbial Cell Factories, vol. 13, pp. 12-23, Jan. 2014. https://doi.org/ 10.1186/1475-2859-13-12.

[12] C. Li *et al*., Increased torulene accumulation in red yeast *Sporidiobolus pararoseus* NGR as stress response to high salt conditions, Food Chem., vol. 237, pp. 1041-1047, Dec. 2017. https://doi.org/10.1016/j.foodchem.2017.06.033

[13] M. Han, Q. He, and W.-G. Zhang, Carotenoids production in different culture conditions by *Sporidiobolus pararoseus*, Prep. Biochem. Biotechnol., vol. 42, no. 4, pp. 293-303, Jul. 2012. https://doi.org/10.1080/10826068.2011.583974

[14] G. Minyuk, R. Sidorov, and A. Solovchenko, Effect of nitrogen source on the growth, lipid, and valuable carotenoid production in the green microalga *Chromochloris zofingiensis*, J. Appl. Phycol., vol. 32, no. 2, pp. 923-935, Apr. 2020. https://doi.org/10.1007/s10811-020-02060-0

[15] M. M. S. Cabral et al., Carotenoids production from a newly isolated *Sporidiobolus pararoseus* strain by submerged fermentation, Eur. Food Res. Technol., vol. 233, no. 1, pp. 159-166, Jul. 2011. https://doi.org/10.1007/s00217-011-1510-0

[16] Y. N. Nagaraj *et al*., Identification, Quantification and Kinetic Study of Carotenoids and Lipids in *Rhodotorula toruloides* CBS 14 Cultivated on Wheat Straw Hydrolysate, Fermentation, vol. 8, no. 7, Jul. 2022. https://doi.org/10.3390/fermentation8070300