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EFFECT OF LIGHT ON CAROTENOID YIELD IN FED CULTURES OF *PHAFFIA RHODOZYMA* CBS 5626

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Key words: *Phaffia rhodozyma/Xanthophylomyces dendrorhous*, carotenogenesis, photoregulated pigmentation

The aim of this study was to evaluate the influence of light on the production of carotenoids by *Phaffia rhodozyma* CBS 5626 yeast. The fed-cultures at different illuminance were conducted: in the dark, at constant illuminance of 400 lux and 600 lux, at variable illuminance. It was found that illuminance and time had a significant effect on the level of carotenoids synthesized by *Phaffia rhodozyma* CBS 5626. But these agents had no a significant effect on yeast dry matter yield. Light stimulated carotenogenesis in cells of the tested yeast; the highest yields of carotenoids were obtained in cultures run at illuminance of 400 lux, while the lowest in cultures run in the dark.

INTRODUCTION

Phaffia rhodozyma (syn. *Xanthophyllomyces dendrorhous*) yeast produces carotenoids pigments, among them astaxanthin constitutes as much as 83- 87% [Flen *et al.*, 1999]. Astaxanthin is used in commercial salmon, rainbow trout and shrimp cultures. It is responsible for the characteristic reddish pink colour of their tissues [Gil-Hwan & Eui-Sung, 2003]. Astaxanthin also exhibits high antioxidant activity, higher than all other carotenoids [Naguib, 2000]. This makes it a bioactive substance, *i.e.* nutraceutic, readily consumed in food within a normal diet. Furthermore, astaxanthin may exert antitumor activities through the enhancement of immune responses [Wang *et al.*, 2006].

At present, numerous scientific works are conducted in order to reduce astaxanthin production costs using *Phaffia rhodozyma* strains. They are connected with selection of culture media composition and optimization of environmental agents influencing carotenogenesis [Flores-Cotera *et al.*, 2001; Ramirez *et al.*, 2001]. There is scarce information in available literature on the role of lighting in the production of astaxanthin by *Phaffia rhodozyma* strains. It is generally believed that it has no effect on growth nor carotenoid synthesis in cells of that yeast. However, studies by Meyer & du Preez [1994] and Vázquez [2001] showed that illuminance and light wavelength have a significant effect on the course of carotenogenesis in *Phaffia rhodozyma* yeast.

The aim of this study was to evaluate the influence of light on the production of carotenoids by *Phaffia rhodozyma* CBS 5626 yeast.

MATERIALS AND METHODS

Microorganisms. The yeast of *Phaffia rhodozyma* CBS 5626 was used. The yeast culture was run on the YM medium containing glucose [Calo *et al.*, 1995]. Initial medium pH was 6.0. Yeast was stored on agar slants at 4°C.

Cultivation. Test cultures were run in 250 mL Erlenmayer flasks for 192 h, at 22°C, on a shaker (150 rpm), at different illuminance: variant I – in the dark, variant II – at illuminance of 400 lux, variant III – at illuminance of 600 lux, variant IV – at variable illuminance (96 h in the dark, next 96 h at illuminance of 600 lux). Since the 48th h, pH of the culture was adjusted to the initial level (6.0) and glucose was supplemented to the concentration 10 g/L in the medium. Each culture variant was run in triplicate.

Analyses. The pH of the medium, glucose concentration [Höstettler *et al.*, 1951], yield of yeast dry matter and total carotenoids were controlled every 24 h. The method described by Sedmak *et al.* [1990] was applied to isolate carotenoids from cells of the analysed yeasts. In the obtained extract the amount of carotenoids was determined by spectrophotometry (λ=484 nm). Results were verified statistically using Microsoft Excel 97 software.

RESULTS AND DISCUSSION

Carotenoid and yeast dry matter yields obtained in the test cultures are presented in Figures 1-3.

A significant effect (α=0.05) of illuminance and time on carotenoid synthesis by yeast of *Phaffia rhodozyma* CBS 5626 was shown in the experiment, both in terms of yeast dry mat-

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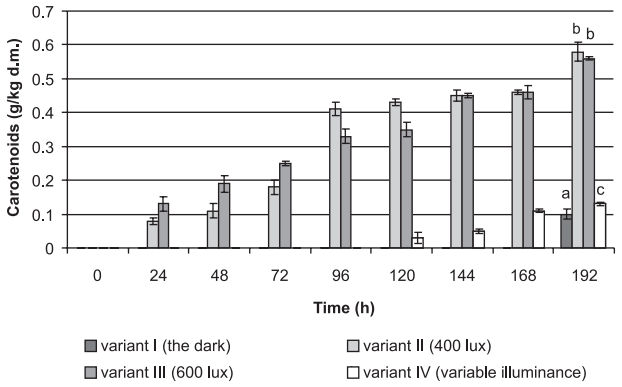

FIGURE 1. The effect of time and light on carotenoid yield in cultures of *Phaffia rhodozyma* CBS 5626 (g/kg dry matter of yeast; *values with different letters differ significantly at α=0.05).

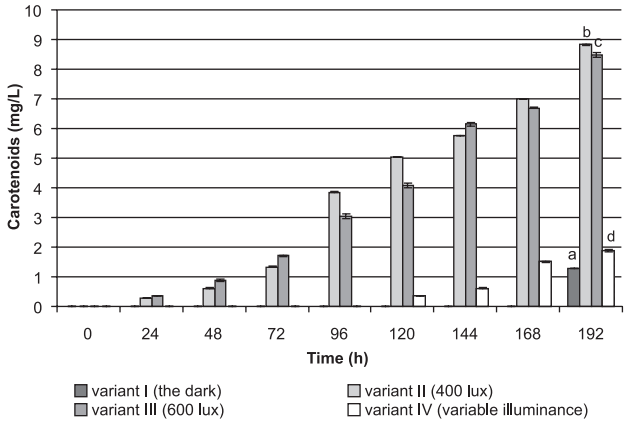

FIGURE 2. The effect of time and light on carotenoid yield in cultures of *Phaffia rhodozyma* CBS 5626 (mg/L; *values with different letters differ significantly at α=0.05).

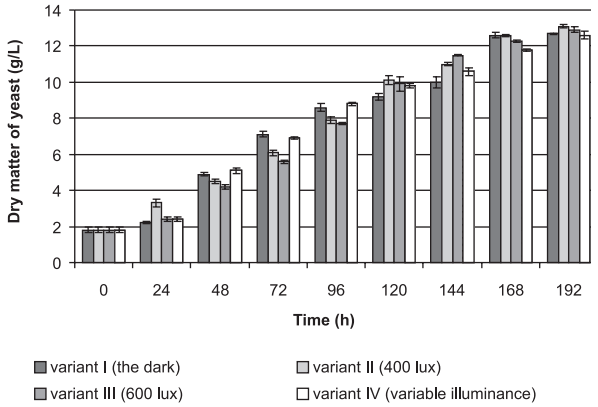

FIGURE 3. The effect of time and light on yield of yeast dry matter in cultures of *Phaffia rhodozyma* CBS 5626.

ter and a culture volume unit (1 L). Irrespective of the culture variant, the highest yields of pigments were obtained on the last day of experiment.

The results obtained indicate that the constant presence of light during culture stimulates carotenogenesis in cells of the analysed yeast. The highest pigment yields were obtained in cultures run at illuminance of 400 and 600 lux. In these cultures the maximum carotenoid yields in terms of yeast dry matter did not differ significantly (α=0.05) and were approx. 0.57 g/kg (Figure 1). But in the case of maximum yields in terms of a culture unit (1 L), significant differences were shown (α=0.05). On the 8th day of experiment in culture run at illuminance of 400 lux (variant II) the content of carotenoids accounted for 8.83 mg/L whereas the in case of culture run at illuminance of 600 lux (variant III) – for 8.49 mg/L, which lower by 4% (Figure 2).

In culture run in the dark, the presence of pigment (0.1 g/kg dry matter of yeast and 1.65 mg/L of culture) was noted only on the last day of experiment (Figures 1-2). The yields obtained were by above 80% lower in comparison to the culture variants run at constant illumination. Similar results were reported by Vázquez [2001] when running cultures of six different strains of *Phaffia rhodozyma* at illuminance of 500 lux and in the dark. Irrespective of the strain, higher carotenoid yields were obtained in cultures run in the presence of light than those run in the dark, with the amount of synthesized pigments depending on the yeast strain applied.

In culture run at variable illuminance carotenoid synthesis by the analysed yeast was found at 120 h, *i.e.* after yeast cells exposition on illumination of 600 lux (Figures 1-2). This fact indicates that carotenogenesis in *Phaffia rhodozyma* CBS 5626 is presumably photoregulated and may be a defense response to environmental stress cells are exposed to, in this case light stress. For majority of microorganisms the light stimulates carotenoid production in their cells [Vázquez, 2001]. But in the case of culture run at variable illuminance the highest pigment yields were only 0.13 g/kg dry matter of yeast and 1.89 mg/L culture. They were approx. by 80% lower in comparison to the cultures run at illumination of 400 and 600 lux in spite of significant differences between these yields and these obtained from culture run in the dark. This fact indicates that constant light stress the yeast cells are exposed to enhances carotenogenesis to a greater extent than single light treatment.

A lack of a significant effect of illuminance and time on the synthesis of yeast biomass was found in the experiment (α=0.05). In all cultures a constant increase of yeast dry matter yield from 1 L culture was observed. On the last day 12.6-13.1 g dry matter of yeast /L depending of culture variants was obtained (Figure 3).

CONCLUSIONS

1. Illuminance and time had a significant effect on the level of carotenoids synthesized by yeasts *Phaffia rhodozyma* CBS 5626.

2. Light stimulated carotenogenesis in cells of the tested yeast. The highest yields of carotenoids were obtained in cultures run at constant illuminance of 400 lux and 600 lux, while the lowest in culture run in the dark.

3. Synthesis of carotenoids by *Phaffia rhodozyma* CBS 5626 yeast may be a defense response to light stress. But constant light stress the yeast cells are exposed to enhances carotenogenesis to a greater extent than one-time light treatment.

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WPŁYW ŚWIATŁA NA WYDAJNOŚĆ KAROTENOIDÓW W HODOWLACH ZASILANYCH *PHAFFIA RHODOZYMA* CBS 56 26

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Celem pracy była ocena wpływu światła na produkcję karotenoidów przez drożdże *Phaffia rhodozyma* CBS 5626. Przeprowadzono cztery warianty hodowli zasilanych przy różnym natężeniu oświetlenia: I – 400, II - 600 lux, III - zmiennym i IV - w ciemności. W doświadczeniu wykazano istotny wpływ czasu i natężenia światła na poziom syntetyzowanych karotenoidów przez *Phaffia rhodozyma* CBS 5626. Natomiast oba czynniki nie miały istotnego wpływu na wydajność suchej substancji drożdżowej. Światło stymulowało proces karotenogenezy w komórkach badanych drożdży; najwyższe wydajności barwników uzyskano w hodowlach prowadzonych przy stałym natężeniu oświetlenia 400 lux, najniższe w hodowlach prowadzonych w ciemności.