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Production of Astaxanthin Rich Feed Supplement for Animals from *Phaffia Rhodozyma* Yeast at Low Cost

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Abstract. Dietary nutrients such as amino acids, vitamins, minerals and antioxidants can play a significant role in determining meat quality and also the growth rate of poultry or animal. *Phaffia rhodozyma* was grown on waste from brewery industry to produce astaxanthin rich feed supplements at a very low cost. *Phaffia rhodozyma* is yeast specie that has ability to produce carotenoids and approximately 80% of its total carotenoid content is astaxanthin, which is highly valuable carotenoid for food, feed and aquaculture industry. This study was carried out to test yeast extract of spent yeast from brewing industry waste (residual yeast) as potential nitrogen source for growth of *Phaffia rhodozyma*. Cultivation was carried out in liquid media prepared by yeast extracts and other components (glucose and peptone). Carotenoids from the biomass were released into biomass by suspending cells in DMSO for destruction of cells followed by extraction with petroleum ether. The extracted carotenoids were studied by spectrophotometry to identify and quantify astaxanthin and other carotenoids produced.

INTRODUCTION

Astaxanthin is an extraordinary antioxidant, colorant and health-promoting compound, hence very important in food, feed and pharmaceutical industry [1]. Astaxanthin is known to possess an antioxidant activity 10 times higher than β-carotene and 1000 times higher than vitamin E [2]. Astaxanthin is present in some marine animals including salmon and lobster. Farms raised marine animals like salmon lack this pigment because they do not feed on natural seaweeds unlike those grown in fresh water so, astaxanthin should be included into their diet to raise the consumer acceptability for farm-raised species. It is estimated that 10 % of the total cost of salmon feed is from astaxanthin [3]. Astaxanthin has large commercial market still growing day by day; almost 90 % of this huge demand is feed by synthetic astaxanthin [4]. Biotechnological production of astaxanthin at industrial scale is still challenging because of uneconomical production methods.

Phaffia rhodozyma was initially isolated by Hermen Phaffia in 1960 as a natural source of carotenoids [5]. This yeast specie was paid attention, importance due to its ability to produce astaxanthin as its primary carotenoid, its rapid growth pattern and most prominently because of being approved as GRAS (Generally Recognized as Safe) by FDA [6]. Various attempts have been carried out to improve the growth of *Phaffia rhodozyma*. Another way to make the production process economically feasible at industrial scale is to reduce the cost of production. Taking advantage of the *Phaffia rhodozyma*`s ability to utilize the wide range of saccharides scientists has tried some by-products from industries as low-cost nutrient media. Coconut milk [7], sugar molasses, grape juice [8], cassava [9], etc. has been successfully utilized as the cheap carbon source for *Phaffia rhodozyma* growth. Using inexpensive substrate is an important consideration so, in this study we try to replace the essential components of growth media with cheap industrial by-products to cut-off the production cost. There are many of brewing industries in the world. Utilizing brewery residual yeast could serve as cheap and nutritious source for *Phaffia rhodozyma* as well as can overcome the problem of discarding residual yeast waste from brewery industry.

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This study is aimed to reduce the production cost of astaxanthin enriched feed supplement. A series of experiments have been carried out in order to produce and to test yeast extract from brewery residual yeast as a potential nitrogen source for growth of *Phaffia rhodozyma*, because yeast extract is usually used for cell cultures as it is a source of amino acids, water soluble vitamins, peptides and carbohydrates [9].

MATERIALS AND METHODS

Microorganisms

The wild strain *Phaffia rhodozyma* Y1655 was purchased from National Collection of Industrial Microorganisms, Scientific Center of Russian Federation Research Institute for Genetics and Selection of Industrial Microorganisms, Moscow, Russia. The yeast maintained on yeast/malt (YM) agar plates containing (per litter of tap water): 1 g yeast extract, 10 g peptone, 20 g glucose, 20 g agar.

Materials

Residual yeast was provided by craft brewery "BeersFan" (Yekaterinburg, Russian Federation), glucose and peptone were obtained from "Chemreaktivsnab" (Ufa, Bashkortostan, Russian Federation). Ethyl acetate and petroleum ether were purchased from Ecos Co., Moscow, Russian Federation. Acetone was obtained from Tikkurila Co., Minsk, Byelorussia.

Yeast Extract

The modified Man-Jin method was used for yeast extract preparation [10]. 10 g of bakery yeast was added to 0.5 L of tap water. The yeast suspension was transferred into a 1L heat resistant conical flask, which was placed into a thermostat and heated up to 50°C for 60 minutes. The reaction mixture then was boiled for 30 minutes and cooled down up to room temperature. Yeast residue was removed by filtration. Obtained yeast extract was used for preparation of YM broth (0.5L yeast extract, 10 g peptone, 20 g glucose) and for peptone free YM broth (0.5L yeast extract, 20 g glucose).

Residual-Brewery Yeast Extract

The modified Man-Jin method for residual-brewery yeast extract preparation was used as well [10]. 40 g of residual-brewery yeast was added to 0.5 L of tap water. The yeast suspension was transferred into a 1L heat resistant conical flask, which was placed in thermostat and heated up to 50°C for 60 minutes. The reaction mixture then was boiled for 30 minutes and cooled down to room temperature. Yeast residue was separated from liquid medium by filtration. The obtained yeast extract was used for preparation of the modified YM broth (0.5L yeast extract, 10 g peptone, 20 g glucose) and for peptone free modified YM broth (0.5L yeast extract, 20 g glucose).

Inoculum Preparation

Two cell inoculums were prepared using the modified YM and YM broth. They were grown in 100-ml Erlenmeyer flask containing 10 ml of medium at a constant temperature (18°C) and rotation at 150 rpm for 48 hours. Experiments were carried in triplicate. 90 ml of fresh media were inoculated with 10 ml of seed cultures. The yeast was grown at the same conditions for 120 hours.

ANALYTICAL METHODS

Dry Residue Determination

Ph. rhodozyma culture samples (1 ml) were centrifuged at 5000 g for 5 min. Cell pellets were rinsed twice with distilled water. Then pellets were dried at 50°C for 24 hours.

Growth Determination

Ph. rhodozyma cell growth was assessed daily by the cell microscopic counter by Goryaev's chamber in triplicate.

Pigment Extraction and Determination

A modified method was used for pigment extraction [11]: 1 ml of cell suspension was centrifuged and washed twice with distilled water and frozen for 2 hours, followed by adding 1 ml acetone up to the solvent evaporated. For cell distraction 1 ml DMSO was used. At this stage, in order to enhance cell wall disruption cells were ultra-sonicated for 3 minutes. To this 2 ml of suspension petroleum ether was added and mixed intensively. Finally, to separate petroleum ether containing pigments the samples were centrifuged at 5000 g for 5 min. Pigments were determined by spectrophotometry at 474 nm. Astaxanthin was quantified using a calibration curve.

RESULTS AND DISCUSSION

The main aim of this study was to investigate a possibility of applying residual brewery yeast extract as one of medium component for *Ph. rhodozyma* biomass growth. Growth cells and astaxanthin yield were compared in two media, modified YM and YM broth. Results demonstrated that modified YM medium is more suitable to produce astaxanthin rich biomass.

In the study we performed the cultivation of wild-type strain of *Ph. rhodozyma* Y 1655 in different media modifications. In particular, we used modified YM broth, modified YM broth without peptone, YM broth, YM broth without peptone.

On the one hand, the cell density in YM was found to be 1.63E+8 CFU/ml, whereas the maximal astaxanthin yield was 80 µg/l for 96 hour. On the other hand, the cell density in the modified YM was 1.53 E+8 CFU/ml and the maximal astaxanthin yield was higher (99 µg/l) for 96 hour (Fig. 1). For other media types used the cell density was less than 1.3E+8 CFU/ml as well astaxanthin yield was less than 30 µg/l.

It was clear that both YM medium and modified YM medium were more preferable for cultivation than those without peptone, where growth of yeast was found to be less by 20 %. As it was expected, astaxanthin yield was higher by 20 % in modified YM medium than in YM medium. In contrast, in peptone free media astaxanthin accumulation was two times less (Fig. 2).

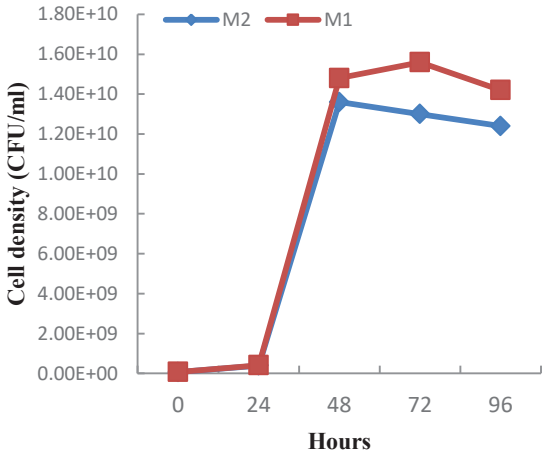


FIGURE 1. Growth response of *Phaffia rhodozyma* in different types of media, where M1 – YM, M2 – modified YM

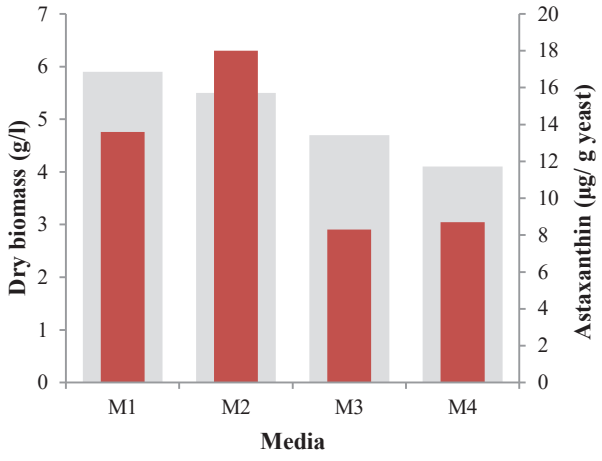


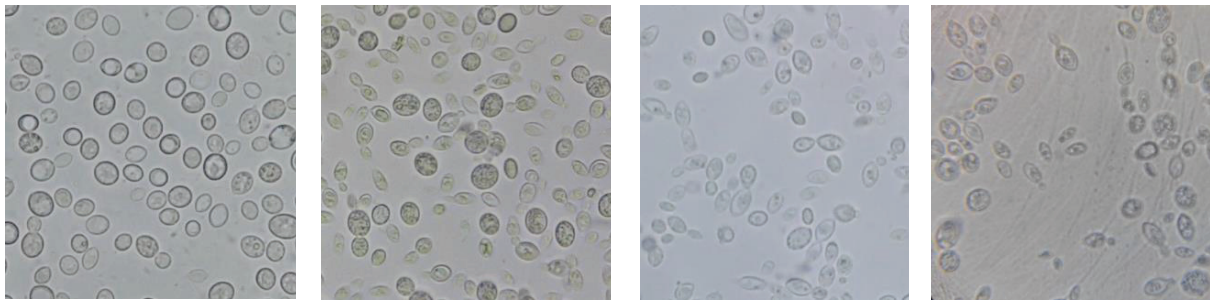
FIGURE 2. Dry residue biomass (filled squares) and astaxanthin content in cells (open squares) of *Ph. rhodozyma* in different types of media in 5-day cultures, where M1 – YM, M2 – modified YM, M3 – YM without peptone, M4 – modified YM without peptone

The results demonstrated that the most considerable growth was observed in YM media, whereas in YM media without peptone the growth was the lowest. In contrast, the highest astaxanthin yield was observed in modified YM media, while the lowest one was done in YM media without peptone.

We suppose that brewery yeast extract provides sufficient growth and higher astaxanthin yield because it contains beer residuals and vitamins, which are additional nutritious supplements for yeast growth.

Turning to shape and size of cells it was obviously that cells cultivated in modified YM medium were bigger, than ones in other types of media (Fig. 3) due to a more nutritious environment.

Thus, we have demonstrated that brewery residual yeast can be successfully used in biotechnological production of feed supplements from *Phaffia rhodozyma* yeast biomass.



M1 M2 M3 M4

FIGURE 3. Cells of yeast *Phaffia rhodozyma* in different type of media (×400), where M1 – YM, M2 – modified YM, M3 – YM without peptone, M4 – modified YM without peptone

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