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Extended Abstract

Effect of *Saccharomyces cerevisiae* Live Cell and Treated with Heat and Acid on Citrinin and Pigments of *Monascus purpureus*

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Abstarct

Monascus purpureus can produce a wide range of natural pigments so attract the attention of food producers as substitute of synthetic colors. Monascus pigments usually accompanied with citrinin contamination which limits their wide application in foods. The usage of *Saccharomyces cerevisiae* is known as a useful biological way for citrinin elimination. In this study, the effect of *Saccharomyces cerevisiae* cells in two modes of treatment (heat treatment at 121 °C and acid treatment in 2M HCL) and live at three different concentrations (10^3, 10^4, 10^5 CFU/mL) were investigated on the amount of citrinin and yellow, orange and red pigments produced by *Monascus purpureus*. The results showed that live yeast cell and yeast cells treated by heat and acid significantly decreased the citrinin in culture, so that citrinin content decreased from 4.43 mg/L in control sample to 0.9, 0.10 and 0.07 mg/L (at 10^5 CFU/mL yeast suspension) respectively. The maximum amount of yellow, orange and red extracellular and intracellular measured pigments belong to the samples with live yeast, while the amount of this pigments in the samples containing yeast treated with heat and acid reduced respectively. Among the treatments, the use of live yeast at concentration of 10^4 CFU/mL per milliliter provided the best conditions to achieve the desired goal.

Keywords: Acid treatment, Heat treatment, Pigment, *Saccharomyces cerevisiae* Citrinin, *Monascus purpureus*

Introduction

The color of a food is one of the most important factors which is immediately received by the optical senses of humans (Malik, Tokkas, & Goyal, 2012). Synthetic colorants are one of the most important colors widely used in industries to give an attractive color to the food (Nadzri, 2012). In recent years, the number of permitted synthetic colorants in foods has gradually decreased, since they have a potential for carcinogenicity or teratogenicity (Kurniawati & Zubaidah, 2014). This problem has increased demands for natural colors as an alternative

(Kongruang, 2011). Indeed, many efforts have been made to find natural sources for producing edible pigments.

Monascus pigments were traditionally used in Asian countries as food colorant and preservation of fermented foods such as alcoholic beverage, red soybean curd, meat, and vegetables (Nadzri, 2012; Suh & Shin, 2000a). However, their application is greatly limited as food additive and colorant in food industries by the organization for regulation of food safety such as European Union and FDA because of coproduction of mycotoxin citrinin (Duraklı-Velioğlu, Boyacı, Şimşek, & Gümüş, 2013). Therefore, the level of citrinin should be strictly controlled during the production process.

Biological control using useful microorganisms has been proposed for a long time as a good option to decontamination of mycotoxins in foods and feeds. Among different decontaminating microorganisms, *Saccharomyces cerevisiae* is one of the most potent biocontrol agents given their biology and nontoxic properties (Armando *et al*., 2012). Extensive research has been conducted on increasing production of pigments by *M. purpureus*, but there is no sufficient data on reduction of citrinin in this case which is produced along with pigments. The purpose of this work was to study the effect of different concentrations of *S. cerevisiae* in form of live and treated by acid and heat on adsorption of citrinin and pigment produced by *M. purpureus* and finding the best condition for pigment production and citrinin reduction in the medium.

Materials and methods

Microorganism

Monascus purpureus ATCC 16362 and *Saccharomyces cerevisiae* PTCC 5052 were obtained from Iranian Research Organization for Science and Technology (IROST).

Preparation of the Yeast Suspension

One loop of *S. cerevisiae* was inoculated in to 10mL of potato dextrose broth medium (Quelab, UK) and incubated at 25 °C for 24 h (Kurniawati & Zubaidah, 2014). The cells were harvested by centrifugation at 7200 rpm for 10 min, washed twice with phosphate-buffered saline (pH 6). The turbidity of the suspension was standardized to match that of a 0.5 McFarland standard. The working suspension was prepared through diluting the stock suspension in sterile distilled to yield 1×10^4, 10^5 and 10^6 cell/mL. The suspension of activated yeast was treated with heat at 121 °C (20 min) or acid hydrochloric 2M (1 h, 25 °C) (Karazhiyan, Mehraban Sangatash, Karazhyan, Mehrzad, & Haghighi, 2016; Shetty, Hald, & Jespersen, 2007).

Adition of *S. cerevisiae* suspention to Monascus fermentation

M. purpureus was grown on YPSS culture at 30 °C for 1 week until they were sporulated. The spores were scraped from the surface of plates. Then, the sterile PBS was added to adjust spore suspension to 1×10^6 cell/mL. Next, a 250-mL flask containing 40 mL of YES medium was inoculated with 400 μL of spore suspension (1×10^6 cell/ mL) and cultivated for 14 days on a rotary shaker at 30 °C and 60 rpm (Wang, Chen, Wang, Zhang, & Ban, 2009; Wang, Ju, & Zhou, 2005). The media were inoculated with 4 mL of live and treated yeast suspension at different initial concentrations (1×10^4, 10^5 and 10^6 cell/mL) on day 13 of Monascus fermentation whereby the final concentration of yeast suspensions in the culture reached 1×10^3, 10^4 and 10^5 cell/mL. The fermentation process ended on day 14 (Kurniawati & Zubaidah, 2014; Wang, Zhao, Mu, Sun, & Chen, 2014).

Pigment Analysis

To measure the amount of extracellular pigment, the fermentation broth was passed through a filter paper (Whatmann No. 42, 125 mm) to remove the mycelia (Dikshit & Tallapragada, 2011). The filtered solution was then centrifuged at 8800 rpm for 15 min. For intracellular pigment, the mycelia were washed twice by distilled water and then collected and crushed. Intracellular pigments were extracted from the broken mycelia with 10 mL of ethanol aqueous

solution 70% (V/V) (pH=2) for 2 h in shaker at 120 rpm. The mycelia were separated from extracted intracellular pigment via centrifugation at 8800 rpm for 15 min. The concentration of extracellular and intracellular pigments was determined by UV/VIS spectrophotometer (WPA, UK) at wavelengths of 400, 460, and 500 nm for yellow, orange, and red pigments, respectively (Chen, Shi, Song, Quan, & Wu, 2015).

Measurement of Citrinin

The pigment solution was filtered through a 0.45 μm membrane (Millipore, USA) filter and analyzed for citrinin content by HPLC-FLD. Detection was performed by an Agilent 1200 fluorescence detector with excitation and emission wavelengths at 331 nm and 500 nm, respectively (Chen, Xue, Chen, Li, & Wang, 2016; Chen *et al*., 2015; Wang, Zhang, Lu, Huang, & Xu, 2013).

Statistical Analysis

The data were analyzed by a completely randomized design. Each experiment was performed in triplicate and the results expressed as mean ± standard deviation. The data were compared using Bonferroni test (P<0.05) and Analysis of variance was performed using Minitab version 16, with the significance considered at 5% level.

Results and discussion

Citrinin

The results of this study showed (Table 1) that both treatments were able to reduce the citrinin in the medium significantly, so that the amount of citrinin decreased from 4.43 mg/L in control sample (without adding yeast) to 0.1 mg/L in the heat treatment and 0.07 mg/L in the acid treatment at concentration of 10^5 cells/mL of yeast suspension. The results of study on the effect of live yeast on the reduction of citrinin produced by Monscus showed that live yeast was able to significantly eliminate citrinin, but its effect was much less than the treated yeast. similar results were reported by Shetty *et al*. (2007) and Rahaie, Emam-Djomeh, Razavi, & Mazaheri (2010) on biological reduction of aflatoxin by *S. cerevisiae*.

Table 1. Effect of live and treated *S. cerevisiae* in three different concentrations on citrinin reduction

Yeast cell concentration	Citrinin content (mg L^{-1})		
Treatment	10^3 (cell ml^{-1})	10^4 (cell ml^{-1})	10^5 (cell ml^{-1})
121 °C	0.17 ± 0.04^{Ca}	0.14 ± 0.02^{Cab}	0.10 ± 0.02^{Cb}
Hydrochloric acid	0.12 ± 0.01^{Ca}	0.10 ± 0.01^{Da}	0.07 ± 0.01^{Cb}
Live cell	2.35 ± 0.12^{Ba}	1.50 ± 0.10^{Bb}	0.90 ± 0.10^{Bc}
control	4.43 ± 0.54^{A}	4.43 ± 0.54^{A}	4.43 ± 0.54^{A}

Different superscript with capital letter in column and lowercase letter in row represent significant differences at P< 0.05. Values are mean±standard deviation (n=3).

Pigment

Figure 1a shows that the amount of yellow extracellular pigments in acid and heat treatment is less than the control sample (without yeast), which indicates that the treatment of yeast cells not only absorb citrinin, but also absorbs some of the extracellular pigments; Also, with increasing the concentration of yeast suspension in acid and heat treatments, the amount of extracellular pigment decreases, which is due to the increase in the number of yeast cells and the increase in receptor positions at the cell surface and removal of protective factors from receptor sites in acid and heat treatments which increases the absorption of extracellular pigments. The highest extracellular pigment reduction was related to the yeast treated with acid with a concentration of 10^5 cell/mL, followed by heat treated and live yeast. This results were agreement with research of Suh & Shin (2000a) and Kurniawati & Zubaidah (2014).

Comparison of samples showed that the amount of intracellular pigments production in all three concentrations of live yeast as well as extracellular pigments in two concentrations of live yeast 10^4 and 10^3 cell/mL was higher than the samples treated with Heat, acid and control samples (Fig. 1).

Production of metabolites by *S. cerevisiae* in *Monascus* fermentation can considerably influence the growth process and pigment production. The co-culture of *S. cerevisiae* cells with *Monascus* had two effects on the cells, stimulation of reproduction followed by cell proliferation, and enhancement of pigment production (Suh & Shin, 2000b).

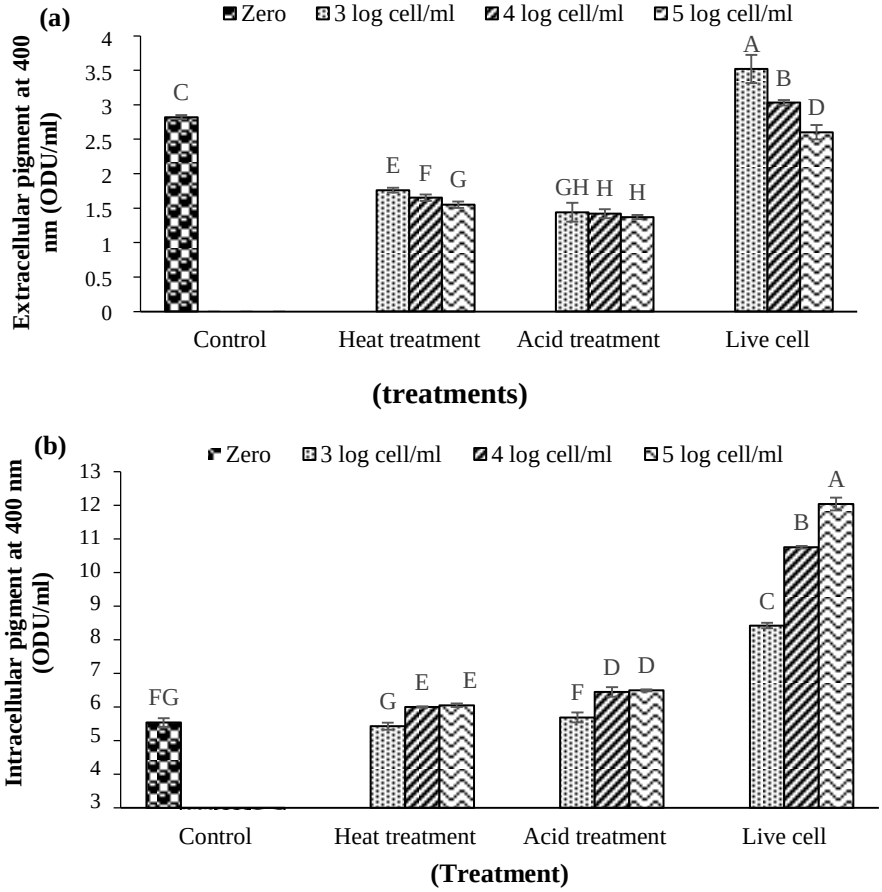

Fig 1. Effect of *Saccharomyces cerevisiae* on yellow extracellular and intracellular pigments produced by *Monascus purpureus*. Values are mean ± standard deviation (n=3). Data with the same letter are not significantly different (P<0.05) according to Bonferroni test.

Conclusion

In this study, the effect of live and inactive yeast in different concentrations for toxin reduction was investigated. Based on the results, it can be concluded that the use of live yeast at concentration of 10^4 cell/mL is the best treatment because increases the production of intracellular and extracellular pigments and reduced the citrinin lower than the European standard. Also, the simultaneous use of live and treated yeast will have a greater effect on increasing pigment production and reducing citrinin, which requires further investigation.

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