The Production of Coloring Nata Colored by *Monascus purpureus* J1 Pigments as Functional Food

**Key words:** colored-nata, *Monascus purpureus*, monacolin K

Functional food is currently getting more popular as a food that is delicious as well as nutritious. It is expected to contain substances for both health and sickness prevention. Nata is a kind of functional food that contains bacterial cellulose from *Acetobacter xylinum*. It is jelly-like, white, and chewy. Cellulose is important for water absorption in the colon. Nata is produced in coconut water, and can be expected to contain substances for both health and sickness prevention.

**RESULTS**

**Isolation of *Monascus purpureus* from Angkak.** Based on the growth rate and color formation, strain TP-1 is the best isolate. Further isolation revealed that the yellow and orange pigments were produced by a separate colony grown from one spore, namely the strains M1, J1, and P1. *Monascus purpureus* J1 was selected for further experiment due to its high growth rate and color production of the orange and red pigments.

Red was observed in the supernatant of *M. purpureus* J1 when grown in the liquid of PS medium, while orange and yellow were observed in the liquid of SG medium. The expressed color in the culture supernatant came from the yellow pigments, as yellow-orange, and red (Fig 1 and 2). The optimum concentrations of yellow, orange, and red pigments in the supernatant of PS medium with and without nata were observed after 5 days of incubation (Fig 1a and b). The pigmentation concentrations were higher in the culture supernatant with nata addition than those with nata addition. In the supernatant of *M. purpureus* J1 grown in PS medium with and without nata addition, the yellow and orange pigments (31.1 and 30.8 μg mL⁻¹) were relatively similar and higher than the red pigments (12.4 μg mL⁻¹), while in that without nata addition, the orange and red pigments (31.1 and 30.8 μg mL⁻¹) were higher than the yellow ones (26.4 μg mL⁻¹).

The yellow pigments appeared dominant in the culture of SG medium, while orange and red were less dominant. The optimum pigmentation production in the culture supernatant of SG medium was observed on the 12th day (Fig 2b), while in the media with added nata the pigment production was still increasing on the 14th day (Fig 2a). The pigments in the SG medium with and without nata sharply increased on the 12th day. Pigment concentrations in SG medium without nata addition increased from 8.6 to 43.6 μg mL⁻¹ in the nata added supernatant (Fig 1). On the 12th day, the orange pigments in the supernatant of SG medium without nata addition reached 23.9 μg mL⁻¹, while in the nata added supernatant it was 12.4 μg mL⁻¹.

**Production of Coloring Nata and Pigments in Liquid Medium with *M. purpureus* J1.** Two kinds of liquid media, PS and SG were conducted to observe the pattern of pigment production. The media were prepared for 50 mL in 250 mL flasks, 6 flasks for each medium. The PS medium was prepared like PSA but without agar. The SG medium was prepared following Ng and Shyu (2004), which was 5% (w/v) glucose, 0.5% (w/v) KCl, 0.01% (w/v) MgSO₄ 7H₂O, 0.001% (w/v) ZnSO₄, and 0.05% (w/v) CaCl₂ at pH 7, modified with addition of 1.5% (w/v) NH₄Cl and 0.05% (w/v) yeast extract. Long nata contains 3.0 x 10⁻³ mg mL⁻¹ monacolin K and 3.5 x 10⁻³ mg mL⁻¹ of pigments in 3 flasks of each medium. Every treatment was repeated three times. All flasks were autoclaved at 121 °C for 15 minutes, cooled and inoculated with *M. purpureus* J1 grown on PS. The culture was incubated at 30 °C for 150 rpm, 30°C for 14 days. Pigments in the culture supernatant were measured at 2, 3, 5, 7, 12 and 14 days of incubation time.

The first experiment was conducted to observe the monacolin K production. The media, inoculum and nata were prepared by following the first experiment, except that the analyses were conducted on the 5th day of incubation. For the 2nd experiment, monacolin K was determined in the mixture culture of monacolin K, while biomass content was only determined in the culture supernatant. The culture supernatant was centrifuged at 12,000 x g for 10 minutes. Pigment concentrations were only calculated in the culture supernatant, while dry biomass content was determined in the culture supernatant. The yellow and orange pigments were observed in the culture supernatant of PS medium. The expressed color in the culture supernatant came from the mixture of three pigments: yellow, orange, and red (Fig 1a and b). The optimum concentrations of yellow, orange, and red pigments in the supernatant of PS medium with and without nata were observed after 5 days of incubation (Fig 1a and b). The pigmentation concentrations were higher in the culture supernatant with nata addition than those with nata addition. In the supernatant of *M. purpureus* J1 grown in PS medium with and without nata addition, the yellow and orange pigments (31.1 and 30.8 μg mL⁻¹) were relatively similar and higher than the red pigments (12.4 μg mL⁻¹), while in that without nata addition, the orange and red pigments (31.1 and 30.8 μg mL⁻¹) were higher than the yellow ones (26.4 μg mL⁻¹).

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The nata fermented in the PS medium appeared red (Fig 3a), while in the SG medium the nata was orange (Fig 3b). In the supernatant of SG medium, the yellow pigments were high (33.3 μg mL⁻¹), while the red pigments were low (9.4 μg mL⁻¹). In the supernatant of the PS medium, the yellow pigment content (26.4 μg mL⁻¹) was relatively similar to the red pigment content (30.8 μg mL⁻¹) (Table 1).

Monacolin K concentration in the supernatant of PS medium (4.6 μg mL⁻¹) was lower than that of the SG medium (14.6 μg mL⁻¹). The monacolin K concentration of nata in PS medium (0.6 μg mL⁻¹) was lower than that in SG medium (3.2 μg mL⁻¹), while monacolin K concentrations of the nata in both media were lower than their supernatant (Table 1).

Isolation of mold from one spore should be conducted to obtain pure isolate that produces stable pigments and monacolin K. In the preliminary experiment it was difficult to have homogeneity in the repeated samples (unpublished data). *M. purpureus* J1 appeared red when grown in PS medium, and orange in SG medium. The same colors were also visualized both in the supernatant and fermented nata. It is possible that the pigments produced by the mold in the liquid medium were infused into the nata. Another possibility is that the mold mycelia in the nata pores produced similar pigments depending on the type of carbon source (Ng et al. 2004). Even though the culture showed only red and orange, the spectrophotometer data showed the observed color originated from a mixture of three pigments: yellow, orange and red (Juzlava et al. 1996; Damuri 2008). The orange pigment content (30.8 μg mL⁻¹) while red pigments were low (9.4 μg mL⁻¹), while the yellow pigments were high (33.3 μg mL⁻¹) and red pigments 13.5 μg mL⁻¹.

During visual observation, the color of nata did not change in almost all treatments, such as washing, freezing and soaking at different pHs, except after boiling which showed a lighter color. However, the spectrophotometric data of the δH₂O nata extract showed different results (Table 2). All treatments decreased the pigment and monacolin K concentrations. The monacolin K concentrations were more greatly reduced than those of the pigment concentrations. Washing and freezing destroyed almost all monacolin K concentrations. The lowest value due to highest decrease of pigment concentrations was observed after soaking at pH 3, which gave 73.1, 71.4 and 67.1% reduction for yellow, orange and red pigments respectively. Other treatments reduced the pigment concentration by 37-50%. The pigments were the least decreased after freezing and soaking at pH 12; however, freezing destroyed monacolin K. The highest monacolin K concentration (the least reduction) was observed after soaking at pH 7; however, the reduction reached 83%.

Soaking in pH 12 did almost not reduce pigment concentration, and Ng et al. (2004) reported an instability factor on the monacolin K concentration which was visually not different with the color of the control. The color of nata is visually quite stable, thus pigments from *M. purpureus* J1 is good for coloring nata products. (Miyake et al. 2006), a different result was observed in our experiment. Our data agreed with Damuri’s (2008), who reported that better pigment production came from rice which contains higher amylase than amylopectin. The amylosrich rice is more degradable in producing glucose than amylopectin is. Addition of minerals also influenced the production of cells, pigments, and monacolin K. Higher concentration of pigments in the GS medium produced higher monacolin K due to similar synthesis pathway of these molecules. Both molecules have the same precursor, monoketide (Hajjaj et al. 1997).

The nata in SG medium produced a yellow color on the third day of incubation, and appeared orange on the fourth day of incubation. (Fig 2b) This result did not agree with Ng and Shyú’s report (2004), which showed that the red nata was obtained in the glucose medium. Monacolin K concentration in colored nata from this experiment was much lower compared with the nata from Ng et al. (2004), which was 3.2 vs 157 μg mL⁻¹. These might be related to the different *Monascus* strains and different media compositions used.

**DISCUSSION**

The amylosrich rice is more degradable in producing glucose than amylopectin is. Addition of minerals also influenced the production of cells, pigments, and monacolin K. Higher concentration of pigments in the GS medium produced higher monacolin K due to similar synthesis pathway of these molecules. Both molecules have the same precursor, monoketide (Hajjaj et al. 1997).

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and soaked at neutral pH was caused by the solubility of the pigment transfer from the nata to the buffer. Beside the solubility, soaking in acid and alkali conditions (pH 3 and 12, respectively) might damage the molecules. This experiment implied that nata could be boiled for a limited time, and ultraheat treatment might be more protective for the monacolin K. Boiling treatment is in fact a necessity to preserve the colored nata. The boiled nata will contain cellulose, angkak pigments, and monacolin K, and therefore, could be referred to as functional food. The colored nata should better be stored in moderate temperature of 4-25°C, and does not need to be washed.

Monascus purpureus J1 isolated from commercial angkak makes the nata red when fermented in potato sucrose medium and orange in synthetic glucose medium. The color does not only come from the mycelia grown in the gel, but may have resulted from the diffusion of the pigment compounds from the fermentation liquid into the nata.

The red and orange nata are functional food containing cellulose, organic pigments, and monacolin K. The functions of cellulose and monacolin K in human have been discussed, while the function of pigments is not reported. The pigments of angkak are also polyketides that might be easily oxidized and function as an antioxidant.

Monacolin K concentration of the orange nata was higher than that of the red nata. Pigment and monacolin K production are influenced by media composition and Monascus strain. The high monacolin K content might be correlated with high yellow pigments and cell biomass. These results also gave more information than that reported in Ng et al. (2004) which only produced red nata.

Although the colored nata visually looks stable, the concentrations of the pigments are decreased when washed, boiled and soaked in acid and alkaline pHs. In addition, monacolin K is not stable to boiling and freezing, which reduced monacolin K significantly. Washing and soaking in pH 3, 7 and 12 also reduced the monacolin K concentrations. Boiling nata still makes it red which contains the three pigments, and monacolin K; therefore, it can be applied in the production of this functional food.

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REFERENCES


