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STUDIES ON FERMENTATION OF *Monascus purpureus* TISTR 3090 WITH BACTERIAL CELLULOSE FROM *Acetobacter xylinum* TISTR 967

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ABSTRACT

Bacterial cellulose produced by *Acetobacter xylinum* TISTR 967 was fermented by *Monascus purpureus* TISTR 3090 in culture medium on a rotary shaker at 150 rpm, 30 °C for 12 days. Sucrose concentration of 5% (50 g/l), ammonium nitrate concentration of 1.5%, initial pH at 5.5 and a temperature at 30 °C resulted in the production of an appealing bright red color. The color stability study was carried out using a medium composed of 5% sucrose, 1.5% ammonium nitrate and initial pH at 5.5 for fermentation at 30 °C for 12 days. Products demonstrated good resistance to washing, heat, freezing, acidification and irradiation.

KEYWORDS: Bacterial cellulose**,** Fermentation, *Monascus purpureus*

1. INTRODUCTION

Bacterial cellulose (Nata) is produced by *Acetobacter xylinum* through fermentation of fruit juices. Bacterial cellulose is considered one of the traditional foods among Philippine people and is also popular in other Asian countries, including Indonesia, Japan and Taiwan, due to its distinctly soft texture and high fiber content [1-2] The biogenesis and fermentation for bacterial cellulose production have been well studied [1, 3-5]. However, few studies have reported on the coloring of bacterial cellulose. The coloring of bacterial cellulose can improve its appearance and provide more variety for application as a food stuff.

The best known of the microbial colorants are produced by *Monascus* group, specifically *Monascus anka* and *M. purpureus*. Traditionally grown on rice in the Orient, *Monascus* species produce a red mass which may be incorporated into foods as is or dried powdered, and added as desired. Much research has been carried out on the general culture conditions and substrates. Lin and Lizuka [6] studied rice meal, wheat bran, wheat meal, bread meal, corn meal and a number of other substrates. Kim *et al*. [7] studied the use of molasses and corn. Shepherd and Carels [8] developed a two-stage process optimized first for growth and then for pigment production. Tadao *et al*. [9] studied the growth process to optimize the type and amount of pigment. Lin [10] and Su *et al*. [11] also studied strain selection for pigment production. Wong and Koehler [12], Wong and Bau [13] found the antibacterial effect of *Monascus* cultures to be present, but low. Monascus pigments have been used in the Orient for hundreds of years as a general food colorant, as a colorant for wine and bean curd and as a medicine [6]. They can be made water soluble or oil soluble and are stable in the pH range between 2·10. They are heat stable and can be autoclaved. These

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properties, together with a color range from yellow to red, should make them good candidates for food colorants. The objective of this study was to develop a new foodstuff by using *M. purpureus* to ferment bacterial cellulose. The parameters affecting the fermentation and production of color by *M. purpureus* were studied. The color stability of the product was also examined.

2. MATERIALS AND METHODS

2.1 Organisms and cultivation

Acetobacter xylinum TISTR 967 was obtained from TISTR, Thailand. The culture medium was prepared by dissolving 50 g of sucrose, 1 g of $(NH_4)_2SO_4$, 10 ml of glacial acetic acid in 1,000 ml of coconut water. The seed broth was prepared by adding a loopful of stock culture to 10 ml of culture medium and incubating at room temperature for 3-4 days. Culture for producing cellulose was carried out in inoculating plastic trays by 10% (v/v) of the seed broth into the culture medium and incubating under the same conditions for 7 days. Bacterial cellulose thus obtained was cut into 2 cm x 2 cm x 1.0 cm pieces and then washed with running water for 1–2 h at room temperature to remove the acetic acid residue before fermentation.

Monascus purpureus TISTR 3090 was obtained from TISTR, Bangkok, Thailand, and maintained on potato dextrose agar (PDA) slopes at 4 °C. A distilled water suspension from a 7 days old PDA slope of *M. purpureus* TISTR 3090 grown at 30°C was used for inoculation.

2.2 Fermentation

Fermentation medium described by Lin and Demain [14] was used with a maltose concentration of 50 g/l. When required, glucose, sucrose, rice powder or soybean powder (50 g/l) was substituted for maltose, whereas peptone, ammonium nitrate or ammonium chloride (15 g/l) was substituted for monosodium glutamate (MSG). The pH of the medium was adjusted using 1 M HCl or 1 M NaOH (pH 3.5, 4.5, 5.5, 6.5 and 7.5). Seven bacterial cellulose pieces were put into the medium (150 ml) in a 500 ml flask. After sterilization at 121°C for 20 min, each flask was inoculated with 1.5 ml of *M. purpureus* TISTR 3090 in distilled water. Fermentation was carried out on a rotary shaker (150 rpm) at various temperatures (15, 20, 25, 30 and 35 °C) for 12 days.

2.3 Color determination

A CIELAB colorimetry system was used for color determination. Coloration was determined with a Chroma meter (Minolta, CR-300), which measured the spectrum of reflected light and converted it to a set of color coordinated (L, a and b) values. The C value, calculated from Equation 1, is a measure of the saturation or purity of the color.

$$C = (a^2 + b^2)^{1/2} \quad (1)$$

where C = chroma
a = redness/greenness (+ = red, - = green)
b = yellowness/blueness (+ = yellow, - = blue)

The percentage (%) of discoloration was calculated from the relationship

$$\text{The percentage (\%)} = (\text{C after treatment} / \text{C before treatment}) \times 100 \quad (2)$$

2.4 Statistical analysis

The experiments were designed by Completely Randomized Design (CRD) and analyzed with Analysis of Variance (ANOVA). The results were determined and compared different between test group by DMRT (Duncan's Multiple Range Test).

2.5 Determination of the color stability

The color resistance of *Monascus* – bacterial cellulose complex to washing, heat, freezing, acidification, alkalization and UV treatments was compared. For washing resistance evaluation, samples were washed under running tap water without any detergent at a constant flow rate of 10 liters/h for 8 h. To examine the thermal stability of the color, the samples were autoclaved at 121 °C for 1 h or frozen at –20 °C for 5 days before analysis. For acidification and alkalization experiments, the samples were soaked in acetic acid and ammonium hydroxide solutions at pH 2.5 or pH 12.5, respectively for 5 days. To study the UV resistance, samples were exposed to ultraviolet light (365 nm) for 36 h. The residual coloration on the surface of each sample was determined.

3. RESULTS AND DISCUSSION

3.1 Fermentation conditions for coloration

Carbon source, nitrogen source and pH have been shown to influence pigment production by *Monascus purpureus* [14]. Therefore, the effect of these compounds on coloration of *Monascus* – bacterial cellulose complex was studied. When glucose, rice powder, maltose or sucrose was used as a sole carbon source (5%), the most appealing and bright red color was observed with the sucrose medium in 12 days (Figure 1). In the nitrogen source experiment, 1.5% ammonium nitrate medium produced an appealing red appearance while other nitrogen sources produced faint or foggy reds. (Figure 2). Although the difference of red colors in Figure 2 is difficult to distinguish by eyes, it was clearly identified by CIELAB colorimetry. Additionally, initial pH of 5.5 and fermentation at 30°C had significant effect ($P > 0.05$) on coloration (Figures 3 and 4). From the results above, further experiments on color stability were carried out using a medium composed of 5% sucrose and 1.5% ammonium nitrate, and initial pH 5.5 for fermentation at 30°C for 12 days.

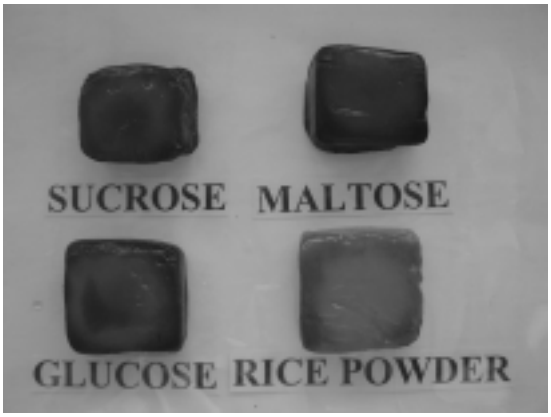

Figure 1 Influence of different carbon sources on the coloration of *Monascus*-bacterial cellulose complex (MSG was used as a nitrogen source)

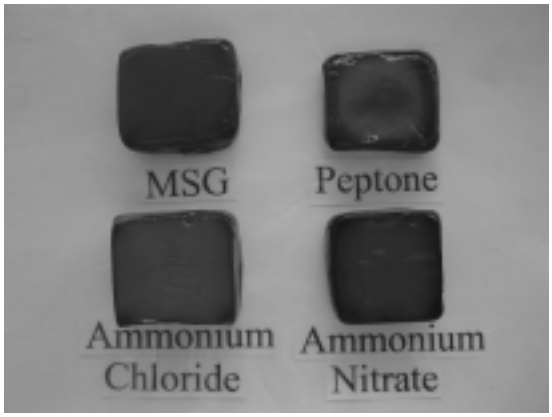

Figure 2 Influences of different nitrogen sourc on the coloration of *Monascus*-bacterial cellulose complex (sucrose was used as a carbon source)

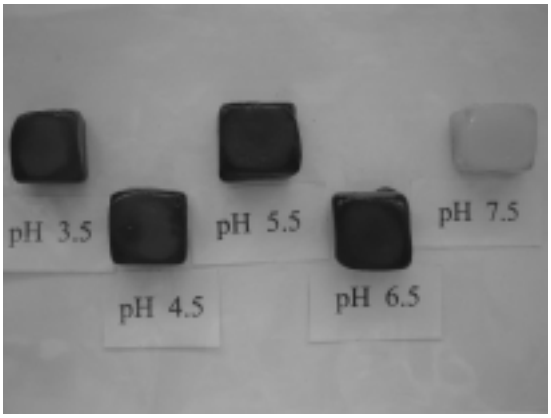

Figure 3 Influence of different initial pH on the coloration of *Monascus*-bacterial cellulose complex

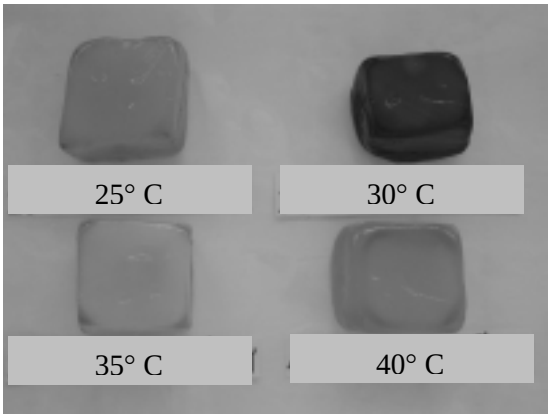

Figure 4 Influence of fermentation temperature on the coloration of *Monascus*-bacterial cellulose complex

3.2 Color stability

The color stability of *Monascus* – bacterial cellulose was examined. The complex showed good resistance to washing decoloration, 90.92% coloration was retained after 8 h of washing. There was no appreciable color change of any sample after autoclaving, -20 °C freezing or pH 2.5 acidity. However, at pH 12.5, the complex of *Monascus* – bacterial cellulose was completely decolorized. This result was the same as a study by Sheu *et al*. [15]. Fading of *Monascus*-bacterial cellulose complex was observed under irradiation with 365 nm ultraviolet light for 36 h. A 21.03% decoloration of the complex was observed (Figure 5). Therefore, packaging of the complex is important to avoid the UV decoloration by sunlight.

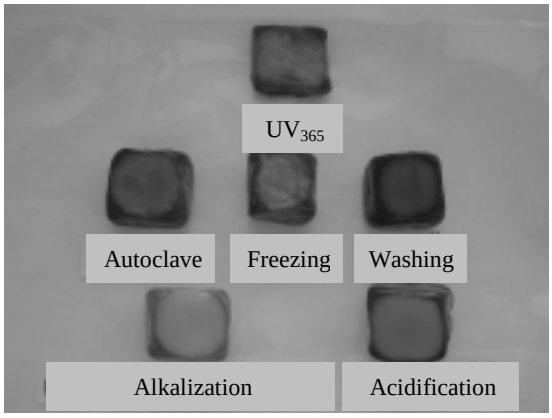

Figure 5 The color stability of *Monascus*-bacterial cellulose after thermal, UV, acid and alkali treatment

The coloration of this complex was influenced by the carbon and nitrogen sources, temperature and initial pH during fermentation. In addition, the complex showed good resistance to washing, autoclaving, acidification and some resistance to UV treatment.

Monascus-bacterial cellulose complex, which combined the properties of bacterial cellulose and *Monascus* fungi, showed the potential to be a new foodstuff as vegetarian meat or seafood replacement. Growth of *Monascus* mycelium did not impart a flavor to this new product creating it a good base as a flavor-added food. The color and texture of the complex were like liver or lean meat. It also provides high fiber content, limited calories and healthy nutrients. Moreover, the waste broth from the fermentation could be further used as a source of water-soluble pigments.

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