Advance Journal of Food Science and Technology 6(1): 56-59, 2014
DOI:10.19026/ajfst.6.3030
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: August 21, 2013 Accepted: September 16, 2013 Published: January 10, 2014

Research Article

Effect Addition of Rice Bran on Fermentation Process to Increasing Lovastatin and Intensity of Red Pigment Angkak

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Abstract: Red yeast rice, known as Angkak, is a natural dye product of rice fermented by mold *Monascus purpureus*. Red yeast rice contains lovastatin compounds as secondary metabolites that have been shown to lower triglycerides and cholesterol. Increase secondary metabolites can be done by the addition of a source of vitamin B1 and zinc. Rice bran is a good source of vitamin B1 and zinc. This study aims to increase levels of lovastatin and red pigments are produced during the fermentation of red yeast rice. The study uses a completely randomized design consisting of 6 treatments: the addition of rice bran on the fermentation medium (2.5, 5, 7.5, 10, 12.5 and 15%, respectively). The best treatment obtained from treatment adding with 5% rice bran where has the following characteristics: intensity of red pigment 3.574, 102.040 ppm lovastatin levels, the level of redness (a+) 15.40. The best treatment resulting red pigment solubility at a temperature of 25, 60, 80 and 100°C range from 1,149 to 2,552.

Keywords: Angkak, lovastatin, red pigmen, rice bran

INTRODUCTION

Angkak, rice fermented with *M. purpureus* (in form of dried powder), is one of the natural dyes are produced by mold (Fabre *et al*., 1993). Dye from red yeast rice has a non-toxic nature and is not to be carcinogenic (Fardiaz and Zakaria, 1996). In addition to producing the red pigment, during fermentation of red yeast rice is also produced compound lovastatin. Lovastatin is a statin class of compounds that have been shown to lower triglycerides and cholesterol in the blood. Lovastatin can lower blood cholesterol levels by 11-32% and triglycerides by 12-19% and did not show any adverse effects (Heber and Ashley, 1999). Lovastatin works as a competitive inhibitor of HMG CoA reductase is a key enzyme in cholesterol synthesis support liver (Erdogrul and Azirak, 2004). Presence of lovastatin would compete with HMG CoA (3-hydroxyl-3-metilglutaril-coenzyme A) which is a substrate for the formation so that levels of LDL cholesterol (Low Density Lipoprotein) in the blood will decrease. One of the factors that influence the production of fermented red yeast rice is a type of medium used. Angkak fermentation medium is a medium with a high content of amylose and amylopectin were lower. IR36 rice is a type of rice that has a high amylose content (>20%) is as much as 27% (Suprihatno *et al*., 2009).

To increase the intensity of pigments and lovastatin content of red yeast rice is fermented red yeast rice with modifying media. One of the factors that influence the production of secondary metabolites (pigments and lovastatin) on the fermentation of red yeast rice is the presence of vitamin B1 and zinc (Danuri, 2008). Vitamin B1 acts as a coenzyme that catalyzes the formation of acetyl CoA will be required to produce secondary metabolites, while zinc will inhibit the growth of *M. purpureus* but will encourage the formation of secondary metabolites.

Rice bran is the inner layer of the rice grain (aleron /epidermis) and the starch fraction. Rice bran contains a high vitamin B complex, vitamin B1 which amounted to 2,753 mg/100 g rice bran (USDA, 2012) and zinc was 6.04 mg/100 g. This study aims to increase levels of lovastatin and red pigments are produced during the fermentation of red yeast rice. The addition of rice bran on the fermentation media IR 36 could be expected to increase the intensity of the pigment and the resulting levels of lovastatin red yeast rice.

MATERIALS AND METHODS

The experimental design used was a Randomized Block Design (RBD) with the addition of rice bran, 6 levels: 2.5, 5, 7.5, 10, 12.5, 15 and 0%, respectively as control (without rice bran). Preparation of liquid starter, rice flour dissolving water 4% (w/v), NH_4NO_3 0.15% (w/v), KH_2PO_4 0.25% (w/v), $MgSO_4$.7H2O 0.1% (w/v) in 100 mL of distilled water and then the pH of the medium was adjusted to achieve a solution pH of 6.0 with 0.25 M KOH or 0.1 M HCl. Media were sterilized

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by autoclaving at 121°C for 15 min and then cooled to a temperature of 35°C and then inoculated 2 ose spores *M. purpureus* and incubated for 7 days (Danuri, 2008).

Rice soaked in water for 8 h, then add distilled water with a ratio of 1:1 (w/v) and supplemented with rice bran, consisting of 6 levels: 2.5, 5, 7.5, 10, 12.5, 15 and 0%, respectively as control and then added 4% rice flour (w/v), 0.15% NH_4NO_3 (w/v), KH_2PO_4 0.25% (w/v), 0.10% $MgSO_4.7H_2O$ (w/v) MSG and 0.10% (w/v) in 12.5 mL of distilled water and adjusted to pH 6.0 with 0.25 M KOH or 0.1 M HCl. Fermentation medium sterilized by autoclaving 121°C for 15 min and cooled to a temperature of 35°C medium, inoculated with 4 mL starter *M. purpureus* that containing 2.08×10^8 cells/mL and followed by the incubation for 14 days at a temperature of 30°C. Fermented red yeast rice is then dried in a dry oven temperature of 70°C for 24 h and pulverized in a blender to obtain a powder of dried red yeast rice (Modified Danuri, 2008).

Red color intensity analysis: (0.05 g) of red yeast rice powder was extracted with 10 mL of 96% methanol. The solution is then inserted shaker at 120 rpm for 24 h. The solution was then separated from the residue by filtration using filter paper. The filtrate was then measured with spektofotemeter absorbance at a wavelength of 500 nm (Kasim *et al*., 2005).

Lovastatin content analysis: Five g of red yeast rice powder dissolved in 25 mL ethyl acetate and stored in an incubator shaker with agitation speed of 180 rpm at a temperature of 70°C for 90 min. The mixture was then centrifuged at 3000 g for 8 min and then filtered with a membrane FTPE, to obtain extracts of lovastatin (Panda *et al*., 2009). Lovastatin red yeast rice extract solution was injected into the LC/MS MS. Mobile phase used in the form A = 0.1 formic acid in distilled water and B = 0.1 formic acid in acetonitrile. The flow rate is set at a speed of 250 microliters/min at 100°C using a Hypersil Gold column (50×2.1×1.9 Lm).

Resistance of red pigment angkak to temperature: Six hundred mg of red yeast rice powder dissolved in 100 mL of water and stirred for 1 min. The solution is then filtered. The filtrate was then transferred into 4 test tubes in which each filled with 10 mL of the filtrate. Each tube is then heated in an oven at 25, 70, 121, 180°C, respectively for 1 h and absorbance was measured using a spectrophotometer at a wavelength of 500 nm (Jennie, 1997).

RESULTS AND DISCUSSION

The intensity of the red pigment increases with the addition of rice bran, but decreased on the addition ofmorethan5% rice bran. The mean intensity of the red pigment of red yeast rice produced is presented in Fig. 1.

The higher the addition of rice bran, increasing the intensity of the red pigment. The addition of rice bran up to 10% increased the intensity of the red pigment

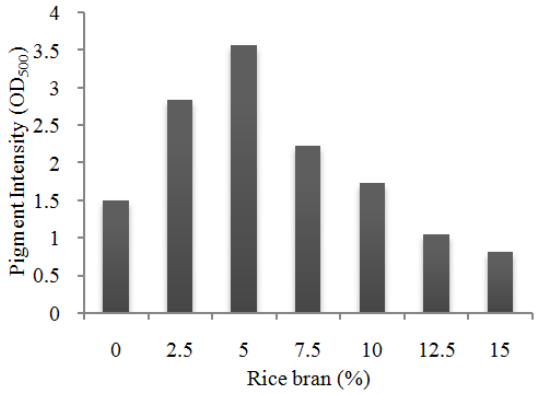

Fig. 1: Effect of rice bran addition to the intensity red pigment

produced by *M. purpureus* compared with controls without the addition of rice bran (0%). This is presumably because rice bran has many micronutrients that can be utilized by *M. purpureus* for the growth and formation of primary and secondary metabolites. Jenie *et al*. (1994) states that *M. purpureus* requires minerals such as magnesium, potassium, phosphate and iron for growth for pigment production.

Increase in the intensity of the pigment in red yeast rice powder enriched with rice bran compare to control (0%) presumable caused by the presence of vitamin B1, amino acids and zinc in rice bran. Vitamin B1 (Thiamine pyrophosphate) is a coenzyme or prosthetic group in the enzyme pyruvate dehydrogenation complex that catalyzes the conversion of pyruvate to acetyl-CoA in glucose metabolism. Pigments *M. purpureus* produced via polyketide pathway and requires acetyl-CoA. Therefore, indirectly influence vitamin B1 in the production of pyruvic acid from glucose during the process of pigment biosynthesis (Danuri, 2008). Research conducted by Lee *et al*. (2001) on mineral additions in red yeast rice substrate showed that the addition of Fe element provides a major influence on the formation of pigment from red yeast rice.

Decrease in the intensity of pigment in the treatment of rice bran addition of more than 5% presumable caused by a number of minerals that too many in the media fermented red yeast rice that can inhibit the growth of *M. purpureus*. This is supported by Jenie *et al*. (1997) statement that in addition to vitamin B1, in the process fementation angkak, the element iron and copper are also needed in very small amounts for the activation of enzymes and pigment biosynthesis. In large amounts, these elements will inhibit the growth of *M. purpureus*. Research conducted by Lee *et al*. (2001) showed that the addition of zinc in a certain amount of substrate for *M. purpureus* able to reduce the production of pigment. This is supported by research conducted by the Bau and Wong (1979) stated that zinc at a concentration of 2×10-3M and 3×10-3M

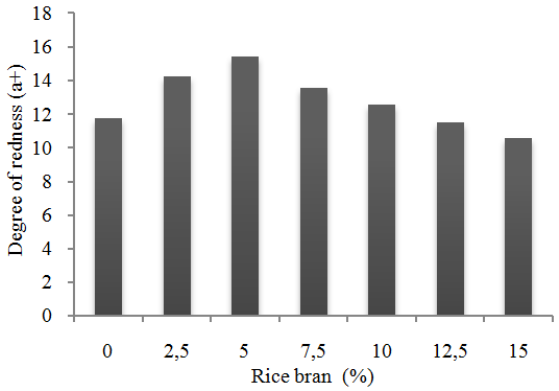

Fig. 2: Effect of rice bran addition to the degree of redness (a+)

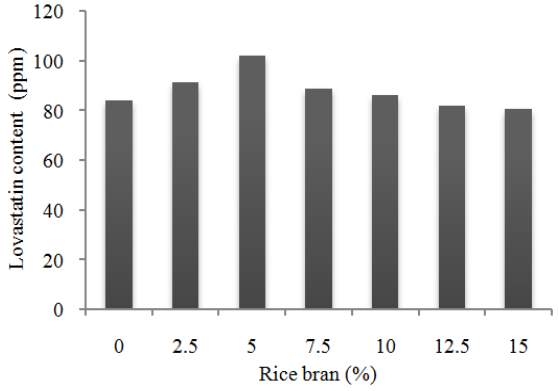

Fig. 3: Effect of rice bran addition to the lovastatin content

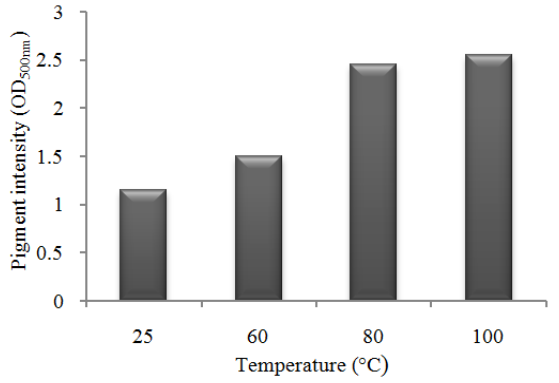

Fig. 4: Red pigment angkak solubility in water in different temperature

virtually stop the growth and pigment production by *M. purpureus*. The addition of zinc in the growth medium unzur *M. purpureus* can act as a growth inhibitor and simultaneously as a stimulant for glucose absorption and for the synthesis of metabolites.

Effect addition of rice bran on the level of redness (a+) presented in Fig. 2.

It is correlate with the level of intensity of the redness of red pigment in red yeast rice pigment where the higher intensity, the higher a+ values.

Monascorubramine and rubropunctamine pigment produced during fermentation and that gives the red color in the final product. Decreasing concentration of the red pigment will make the reddish color of the lower (Isnaini, 2010). Presence of vitamin B1 which is a coenzyme or prosthetic group in the enzyme pyruvate dehydrogenation complex that catalyzes the conversion of pyruvate to acetyl-CoA in glucose metabolism indirectly affect the formation of pigment (Danuri, 2008) increased levels of pigments in fermented red yeast rice, the level of redness (a+) participating has increased.

The higher the addition of rice bran, increasing the lovastatin content. The addition of rice bran up to 10% increased the lovastatin content produced by *M. purpureus* compared with controls without the addition of rice bran (0%). Increase in the intensity of the red pigment of red yeast rice has a positive correlation with levels of lovastatin produced. This is because the pigments and lovastatin precursor which is subsequently synthesized into tetraketida pigments and lovastatin (Kasim *et al*., 2005). Levels of lovastatin red yeast rice powder produced can be seen in Fig. 3.

Figure 3 shows that the levels of lovastatin increased with the addition of rice bran, but decreased on the addition of more than 5% rice bran. Increased levels of lovastatin in red yeast rice powder enriched with rice bran fermentation media presumable caused by the presence of amino acids and zinc in rice bran. Specific for amino acids, amino acids methionin is essential for the biosynthesis of lovastatin as an immediate precursor in the Diels-Alders reaction to the formation of lovastatin (Stocking and Williams, 2003). In research conducted by Stocking and Williams (2003) by providing a fermentation medium methionin in mind that the two methyl groups on lovastatin is a derivative of the compound S-Adenosyl-Methionin (SAM). Presence of vitamin B1 (Thiamine pyrophosphate) catalyzes the conversion of pyruvate to acetyl-CoA which is the precursor of secondary metabolites resulting in increased production of lovastatin in red yeast rice powder produced (Danuri, 2008). However, the addition of more than 5% rice bran in the fermentation medium led to decreased levels of lovastatin red yeast rice red yeast rice powder produced. This is presumably due to the minerals that are inhibiting the growth of *M. purpureus*.

Angkak with the best treatment outcomes (adding with rice bran 5%), tested solubility in water at various temperatures (25, 60, 80 and 100°C, respectively). Solubility of red yeast rice powder is determined by the value of OD (Optical Density) of red yeast rice powder solution at a wavelength of 500 nm. It appears that red yeast rice pigment solubility increases with increasing temperature (Fig. 4).

Increase the solubility of the pigment in line with the increase in temperature. It can be caused by an increase in kinetic energy of particles in the event of heating. Particles have greater kinetic energy will cause more collisions so that the solubility increases. Angkak

pigment soluble in methanol, ethanol, chloroform, benzene, acetic acid and acetone, but slightly soluble in water and petroleum ether (Chulyoung, 2006). Yamaguchi *et al*. (1973) reported that the pigment of red yeast rice can be made into a water soluble pigment by reacting it with water-soluble protein, soluble peptides, amino acids.

CONCLUSION

The addition of rice bran can increase the intensity of the pigments and lovastatin content of red yeast rice powder in which the intensity of the pigment and the highest levels of lovastatin obtained from the fermentation of red yeast rice bran with the addition of as much as 5%, where has the following characteristics intensity of red pigment 3.574, 102.040 ppm lovastatin levels, the level of redness (a+) 15.40. The best treatment resulting red pigment solubility at a temperature of 25, 60, 80 and 100°C, respectively range from 1,149 to 2,552.

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