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Contributed Paper

Growth, Glucoamylase, Pigments and Monacolin K Production on Rice Solid Culture in Flask and Koji Chamber Using *Monascus* sp. KB9

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ABSTRACT

Monascus sp. KB9, with capability to promote high production of natural red pigment and anti-cholesterol agent, monacolin K, was used for solid rice culture in static flask and koji chambers. The effect of various factors on the optimal fermentation condition for 15 days incubation was determined using 500ml-flask containing 100 g rice substrate. The results indicated that incubation temperature of 30°C and 38% (w/w) initial moisture content of rice at neural pH gave the highest red pigments and monacolin K production at 3,571.97 A_{420}U/gdw, 2,697.71 A_{500}U/gdw, and 13,536.61 mg/kgdw, respectively. While the highest glucoamylase activity and glucosamine content of 189,685.66 unit/gdw and 4.438 mg/gdw were obtained under 35°C incubation, with 38% (w/w) initial moisture content of rice substrate at initial pH 5. The secondary metabolites were drastically reduced (80-90%) at 41-43% (w/w) initial moisture content although the biomass was similar in content. Remarkably, scaling up of fermentation in the semi-automated koji chambers of 10 and 50 kg rice substrate by using information from flask level showed that *Monascus* sp. KB9 could grow and produce higher pigments and monacolin K in a shorter time. Fermented rice of 10 kg and 50 kg koji chambers produced 2 times higher pigments and 2 and 4 time higher monacolin K than those obtained from flask scale cultivation. Ready-to-use product of fermented red rice was examined for subsequent processing of storage stability. The results showed that the amount of pigments and monacolin K in red rice were stable by steaming at 100°C 30 min for growth inactivation, drying at 50°C overnight and storing in the refrigerator (2-4°C) for 1.5 months.

Keywords: *Monascus* sp., growth, glucoamylase, monacolin K, red pigments, fermented red rice, koji chamber

1. INTRODUCTION

Red yeast rice or Ang-kak, a traditionally fermented product, was produced by fermenting *Monascus* sp. on steamed rice and has been applied widely in many countries. It is a dietary staple and is used as colorants, medicinal, and health

supplements [1,2,3]. *Monascus* spp. could produce red pigment and monacolin K content in solid state fermentation. Monacolin K, commercially known as lovastatin is a secondary metabolite which is a regulatory and rate-limiting enzyme of cholesterol biosynthesis [4]. Glucoamylase is an exo-acting carbohydrolase which liberates glucose units from the non-reducing end of starch. Glucose was thus used as precursor for growth and secondary metabolites production. However, the conditions of fermentation were crucial in controlling the quality of fermented red rice [5] and also affected the secondary metabolites production. A wild type strain of *M. kaoliang* KB9, which was toxicity safety proof by rat feeding trial, chromosome aberration test on human lymphocyte and chicken embryo bioassay [6], was studied on rice solid culture and found that initial moisture content of rice affected glucoamylase activity [7]. Many studies reported the pigments and monacolins production by solid state fermentation of *Monascus* sp. at laboratory scale [8-10]. The understanding of relationship between growth, glucoamylase activity and secondary metabolites in rice solid culture was necessary for scaling up fermented red rice production. Koji chamber is a simple bioreactor for scaling up rice solid culture in a tray with a perforated bed while the fermentation temperature of rice was controlled by a self-compressor. Our fermented red rice has been applied as supplement of feed in laying hens and the results showed that 0.5% (w/w) of fermented red rice could significantly reduce 20% of serum and egg yolk cholesterol while enhance 20% of egg yolk pigmentation [11], The results could lead to cholesterol and triglyceride reduction in human trials. Our aims are to find the optimum conditions of these functional rice products at flask scale and thus subsequently used in scaling up to 10 kg and 50 kg rice solid culture in koji chamber to provide basic information for the feasibility in industrial scale to higher production to meet the functional food market demands.

2. MATERIALS AND METHODS

2.1 Microorganism and Cultivation

A wild type strain, *Monascus* sp. KB9 was used. The stock culture was maintained on MY agar.

2.2 Inoculum

Inoculum preparation: *Monascus* sp. KB9 was cultured on C-medium slant culture at 30°C for 7 days. Conidia or asexual spores were collected and suspended in sterilized 0.1% tween 80. The spores were scrapped under aseptic conditions. The spore suspension was used (2% v/w) as the inoculums (10^6 spores/ml) in flask scale and the fermented red rice powder (7 days age) (10^6 spores/ml) was used as the inoculums in koji scale.

2.3 Solid-rice Fermentation of *Monascus* sp. KB9

A local, non-sticky rice, Hom-mali cultivar was used throughout the experiments. The preparation of rice substrate is as follow: broken-milled rice was soaked in tap water for 3 h. After the water was removed, the soaked rice was drained for 10 min and then a 500-ml Erlenmeyer flask containing 100 g rice was autoclaved at 121°C for 15 min and cooled to room temperature.

2.3.1 Effect of temperature on growth, glucoamylase, red pigment and monacolin K production

Two milliliters of inoculum was inoculated in prepared flask. The pigments

and monacolin K production were examined at different temperatures 20, 25, 30, 35 and 40°C for 15 days.

2.3.2 Effect of initial moisture on growth, glucoamylase, red pigment and monacolin K production

Samples were examined at different moisture by adding sterile water of 0, 5, 10, 15 and 20 ml into the flasks to adjust initial rice moisture content. Levels of initial rice moisture contents were 32%, 35%, 38%, 41% and 43%, respectively. The sample was incubated at 30□C for 15 days. The pigments and monacolin K were investigated.

2.3.3 Effect of initial pH on growth, glucoamylase, red pigment and monacolin K production

To avoid any acid hydrolysis to the rice substrate, steamed rice was examined at different pH adjusted water (pH 3 to 11) which was prior adjusted by 0.5 N HCl or 0.5 N NaOH and then added about 10 ml/flask to 100 g rice for proper moisture content of 38%. The sample was incubated at 30□ C for 15 days. Growth, glucoamylase activity, the pigments and monacolin K content were investigated.

2.4 Koji Preparation

The size (w×l×h) of koji chamber 10 and 50 kg were (0.46×0.46×0.12 m) and (0.99×1.52×0.18 m), respectively. The diagram of koji chamber was shown in Figure 5a. Polished rice (10 kg) and (50 kg) were washed and then soaked in water for 3 h. After the water was drained 1 h, the rice was steamed at 100-121°C for 60 min in 200 liters autoclave and subsequently cooled to about 40°C. Red-koji mold starter (2% w/w) was inoculated onto the steamed rice, mixed in horizontally rotating drum and incubated in koji chamber for 48 h at 35°C. During the preparation of koji, the temperature of the inoculated rice was measured with a thermometer and temperature probe of incubator at a central point. The fermented rice was sprayed with water twice daily to keep moisture content about 50% and cultivated for 15 days at a temperature of 30-35°C.

2.5 Extraction of Pigment

The pigment of fermented rice was extracted by the method described. One gram of fermented rice was blended with 39 ml of 70% ethanol in a 250 ml flask. Forty milliliters of sample was extracted on a rotary shaker at 300 rpm for 3 h. The extract was filtered by No.4 of Whatman filter to remove suspended solids. The pigment concentration was measured by spectrophotometry at 420 and 500 nm for orange and red pigments, respectively by using a spectrophotometer (UV-240, Shimadzu, Kyoto) against a 70% ethanol blank. The moisture content of rice samples was determined by weight loss after drying the fermented rice in a hot air oven overnight at 105°C

2.6 Extraction of Monacolin K [8]

The fermented rice was steamed at 100°C for 30 min, dried at 50°C for 24 h and then ground by mortar and pestle to powder of which 0.5 g was extracted with 25 ml of 70% ethanol at 50°C for 2 h, followed by filtration through a 0.2 μm filter membrane and then measured by an HPLC system. The HPLC system consisted of a Shimadzu LC-10AT VP liquid chromatograph, a FCV-10AL VP pump, an LDC analytical spectromonitor 3100 detector set at 238 nm and an LDC analytical CI-4100 integrator. Chromatography columns persuit C18, 5 μm, 250×4.6 mm were

connected to a guard column (MetaGuard pursuit 4.6 mm 5µ C18) and 20 µl loop injector. An isocratic mobile phase of acetonitrile: water at the ratio of 65:35 (v/v) was used. The flow rate was 1.0 ml/min at a temperature of 28°C. Monacolin K (Sigma) was dissolved in methanol to prepare the standard monacolin K (100-500 ppm).

2.7 Glucoamylase Activity [7]

The glucoamylase activity was routinely assayed by the determination of reducing sugars liberated in the reaction mixture in 20 min at 55°C. The reaction mixture contained 0.5 ml of the enzyme solution and 0.5 ml of boiled 1% soluble starch in 0.05 M final concentration acetate buffer pH 5.5. The reducing sugar liberated was determined using UV-240 spectrophotometer at 520 nm absorbance. Glucose was used as a standard. One unit of the glucoamylase was defined as the amount of enzyme to liberate reducing sugar equivalent to 1 g of glucose/ml in min at 55°C.

2.8 Biomass Estimation [12]

Fermented rice (1 g) was extracted with 5 ml of HCl at room temperature for 20 h. The mixture was cooled and filtrated. Two milliliters of extract was mixed with one milliliter of distilled water. The mixture was boiled at 100°C for 2 hours and neutralized as pH7 with 30% NaOH. The mixture was filled to 50 ml volume with distilled water and filtered with No.4 of Whatman filter. The mixture was assayed for glucosamine. One milliliter of acetyl acetone reagent and boiled at 100°C for 20 min. The mixture was added with 10 ml of ethanol and 1 ml of Erhlick's reagent. The mixture was mixed well at static condition for 30 min. The glucosamine content was determined using UV-1700 spectrophotometer at 530 nm absorbance. Glucosamine concentration was estimated from a standards curve with 20-100 µg/ml glucosamine HCl.

3. RESULTS AND DISCUSSION

3.1 Flask Cultivation

Effect of temperature on growth, glucoamylase, red pigments and monacolin K production.

Temperature is an important factor as it influences metabolic activities and microbial growth. The *Monascus* spp. could generally grow between 18-45°C and the optimum temperature between 30-38°C [13]. Our strain *Monascus* sp. KB9 could grow (0.013-0.047 gdw/ml) in the range of 17.5-47°C and produced red pigments (0.15-10.70 A_{420}U/ml and 0.12-12.30 A_{500}U/ml) incubation for 7 days in MY broth using the temperature gradient techniques (machine). The temperature requirements in growth and development (production of cleistothecia and conidia) and other culture characteristics on agar media have already been reported by Chayawat *et al*. [10]. The Wort agar gave the best mycelium growth while good mycelium as well as sexual structure were found on GYP agar. Moreover, multi amount of conidia as well as ascospore within cleistothecia were found under the light microscope. The fermented rice of *Monascus* sp. KB9 with 32% initial moisture content was incubated at 20, 25, 30, 35 and 40°C for 15 days cultivation. Fungal growth in this study was actually detected as glucosamine concentration presented in solid rice culture. Figure 1 indicated that the fermentation at 20°C incubation gave the poorest growth and product formation. While at 30°C incubation was most favorable for secondary metabolites formation such as pigments and

anti-cholesterol at 2,229.15 A_{420}U/gdw, 1,119.95 A_{500}U/gdw and 5,164.38 monacolin K mg/kgdw, respectively. The different incubated temperature also affected the glucosamine content as well as glucoamylase activity. Result showed that both formations increased rapidly in the range of 30-35°C incubation, the highest growth and glucoamylase were 3.420 mg/gdw and 45,998.02 U/gdw, respectively, at 35°C incubation. These results correspond to the previous data showing that 35°C temperature of fermentation effected on mycelium growth of *Monascus* sp. [2] while the maximum red pigment production of *M. purpureus* LBP97 was obtained at 30°C [14]. Teng and Feldheim [1] also found that anka in flask fermentation at 30°C, the yield of pigments occurred between the fifth and fifteenth days and the fermented rice should be least incubated for 10 days and not longer than 15 days. The temperature of fermented rice of KB9 strain should then be maintained at 30-35°C in favor of the best propagation, mycelium growth and red pigmentation. On the beginning days of fermentation, the rice would appear a pink color and been stirred and shaken to distribute the moisture for growth and pigments production. A regular fermentation procedure took 7 days to reach the deep red colored at the center of the rice [2]. Ganrong *et al*. [8] and Gailing [15] reported the optimal temperature for monacolins production was 26°C while that of Tsukahara *et al*. [16] was 23°C. Chayawat *et al*. [10] showed that room temperature (28-32°C) was the best temperature for monacolins production of strain KB9. Our study on this strain found that slow production of monacolins occurred at the 3-5 days of incubation with the yield below 1,000 mg/kgdw. After that, the productivity increased rapidly till the end of 15 days incubation giving the yields of 5,164.38 mg/kgdw while that production at 35°C gave only 4,613.85 mg/kgdw. Incubation temperature at 30°C was thus used in the subsequent experiments.

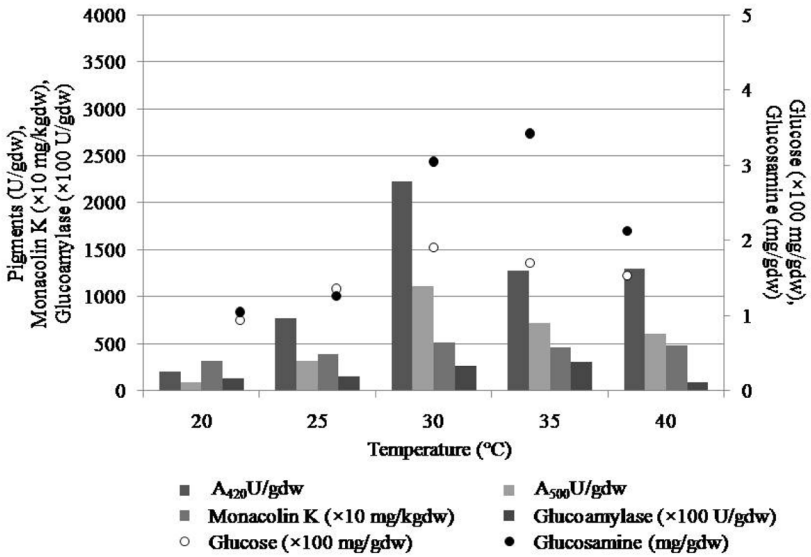

Figure 1. Effect of temperature (20-40°C) on growth, glucoamylase, pigments and monacolin K production by *Monascus* sp. KB9 in flask scale. Experiments were carried out with 32% (w/w) initial moisture of rice substrate for 15 days incubation.

3.2 Effect of Initial Moisture on Growth, Glucoamylase, Red Pigments and Monacolin K Production

Initial moisture content is a critical factor for growth and enzyme production of solid state fermentation because it is necessary for new cell synthesis [14]. The effect of initial rice moisture content at 32-43% was found to be one of the key factors affecting glucoamylase activity as well as pigmentation of *Monascus* [7]. In this study, 32% initial moisture content of rice showed low growth and low product formation with rather dry rice appearance. The glucosamine as well as glucoamylase activity increased at 35-38% initial moisture content while both reached their maximum at 3.448 mg/gdw and 182,947.00 U/gdw, respectively, from the 38% initial moisture content which could also accumulate highest yields of secondary metabolites of pigments and monacolin K at 3,571.97 A_{420}, 2,697.71 A_{500}U/gdw and 13,536.41 mg/kgdw, respectively. For those having the higher initial moisture content of 41-43%, the rice grain looked wet but still promoted growth and glucoamylase activity whereas pigments and monacolin K were 80% and 98% decreased remarkably (Figure 2). Following the fermentation time course in details as shown in Figure 3, it is confirmed that initial rice moisture content of 41-43%, the fungus grew well and reached its early stationary phase at 5 days incubation where rice was hydrolyzed very fast and the amount of glucose liberation had high concentration at 413.50 and 402.42 mg/gdw at 7 days of incubation. Pigments and monacolins production could thus be inhibited to some extent by such high glucose content. Furthermore, higher moisture content (41% and 43%) not only resulted in lower pigments and monacolin K production but also gave the agglomeration of the substrate which leads to reduced oxygen transfer and minimizes heat exchange. In addition, the air ventilation was low, the CO_2 accumulated and promoted 0.6% ethanol synthesis [7]. It could then be a possible explanation for reduction in pigment and monacolin K production at high initial moisture content. Generally, at 32-38% initial moisture content gave the highest glucoamylase at 9 days incubation and the glucose was decreased after 7 days incubation to be consumed up for secondary metabolites where the pigments and monacolin K were continuously increased. Surprisingly, the high initial moisture content (41-43%) of rice, *Monascus* sp. KB9 rapidly grew and gave also earlier but lower enzyme activity. While the glucose was highly liberation but it was slowly used up with increasing moisture content up to 71% would inhibit secondary metabolites production. Occurred "air tight" phenomena obtained from such high moisture content as well as high glucose liberation importantly affected on the production of secondary metabolites, pigments [17] or monacolins [18]. Lee *et al*. [5] reported that the initial moisture content of approximately 50% was good for red pigment production of *M. purpureus* ATCC 16362 but the initial moisture content at 60% decreased the red pigment production drastically. However, Chen and Hu [9] concluded that cultivation of a mutant strain *M. pilosus* M12-69 yield the best monacolin K/citrinin ratio when the moisture contents was 55-75%. The red pigment and monacolin K production were high when the moisture about 50-70% and this moisture was traditionally used for rice fermentation [19]. Other substrate such as jack fruit seed, Babitha *et al*. [14] reported that *M. purpureus* LPB 97 could produce the higher alpha amylase and glucoamylase activity at 50% initial moisture content.

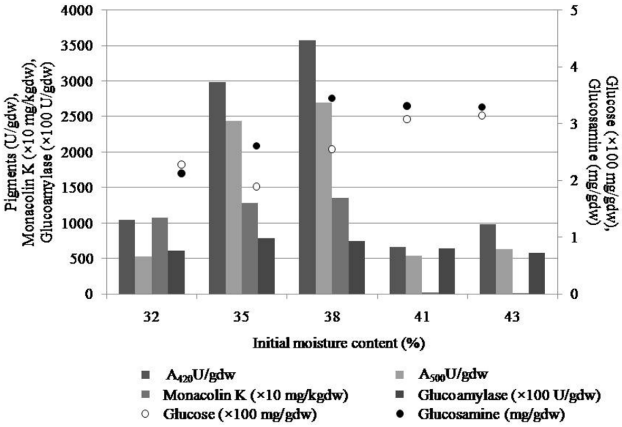

Figure 2. Effect of initial moisture content (32-43% w/w) on growth, glucoamylase, pigments and monacolin K production by *Monascus* sp. KB9 in flask scale. Experiments were carried out at room temperature (28-30°C) for 15 days incubation.

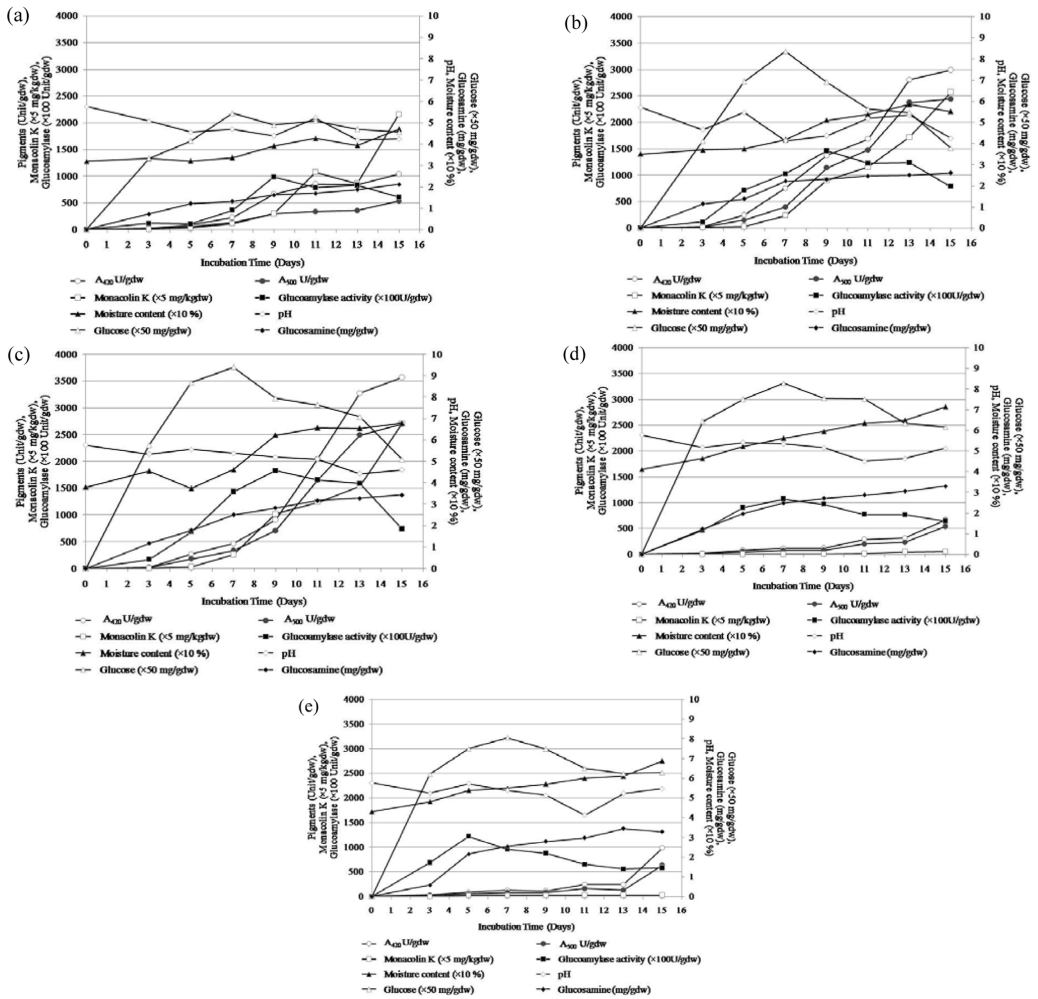

Figure 3. Fermentation time course of growth, glucoamylase, pigments and monacolin K production by *Monascus* sp. KB9 on rice substrates with various initial moisture content (32-43% w/w) in flask scale. Experiments were carried out at room temperature (28-30°C) for 15 days incubation. (a) 32%,(b) 35%, (c) 38%, (d) 41% and (e) 43%.

In our study the 38% (w/w) initial moisture content at 30°C incubation were used in the subsequent experiments.

3.3 Effect of Initial pH on Growth, Glucoamylase, Red Pigments and Monacolin K Production

Monascus sp. KB9 could grow in all initial pH adjusted water (pH 3, 5, 7, 9 and 11) to solid rice culture, though the highest growth was obtained from the initial pH of 5.0. The initial pH also affected the red pigment formation. A pH range of 5 to7 gave favorable red pigment production similar to those observed by Lee *et al*. [5] who showed that the highest level of red pigment were obtained at a pH of 6.0. Pigment color tones of ethanol extract solution changed correspond to pH variation indicated as: pH 3-4 orange yellow, pH 5 orange red, pH 6 red and pH 7-10 purple red [20]. The red pigment was found more stable in neutral (pH 7) or alkaline (pH 9.5) than acidic condition (pH 3) [2]. However, the actual initial pH of rice, which sterile pH adjusted water (pH 3, 5, 7, 9 and 11) addition after sterilization was changed as 5.422, 5.942, 6.201, 6.358 and 7.128, respectively. The results from the solid-state flask culture performed at various initial pH adjusted water to steriled rice were presented in Figure 4 and showed that during cultivation, the actual initial neutral pH (around 6.201) of rice affected the red pigments formation but the lower pH (around 5.942) was optimal condition for growth, glucoamylase activity and monacolin K content. The highest glucoamylase activity, monacolin K, and glucosamine at 189,685.66 U/gdw, 8,557.62 mg/kgdw and 4.438 mg/gdw respectively were obtained at actual pH 5.942. The results of neutral actual pH (6.201) sample were best on pigments production of 2,991.34 A_{420}U/gdw and 2,009.33 A_{500}U/gdw at the 38% initial moisture content. Initial actual pH of rice at 5-7 of this study played no influence to *Monascus* fermentation of primary or secondary metabolite synthesis.

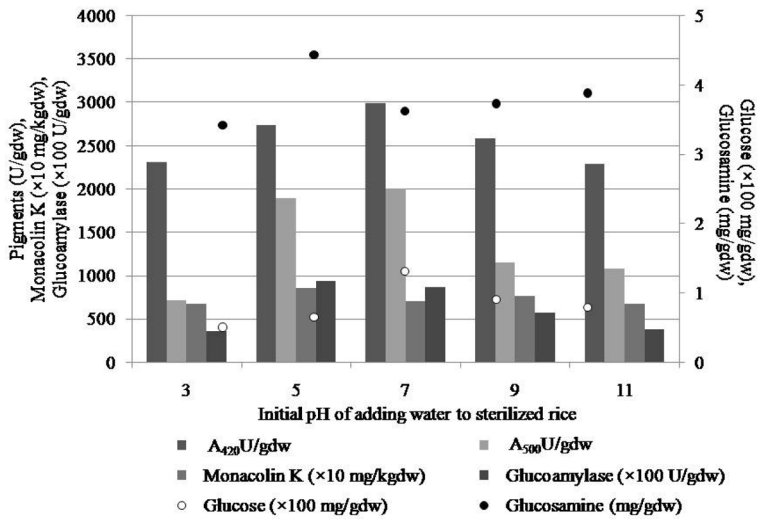

Figure 4. Effect of pH adjustment on growth, glucoamylase, pigments and monacolin K production by *Monascus* sp. KB9 in flask scale. Sterile water at pH (3, 5, 7, 9 and 11) was added to rice substrate of 38% (w/w) initial moisture content and incubated at 30°C for 15 days.

3.4 Scaling Up of Red Rice Fermentation in Koji Chamber

In previous study, optimum conditions in flask scale fermentation such as 30-35°C incubation temperature, 38% initial moisture content and initial pH 5-7 were applied in 10, 50 koji scale for growth, glucoamylase and secondary metabolites. *Monascus* spp. is generally quite slow-growing mold, which its rice culture is easily contaminated under non-fully aseptic conditions by fast-growing *Aspergillus* or *Rhizopus* spp. Scaling up of red rice fermentation to 10 kg and 50 kg capacity were carried out in the koji chambers, shown in Figure 5. Steamed rice was thus prepared and then mixed with 2% red rice inoculums (10^6spores/g). Red rice powder inoculum was conveniently used at the higher scale of 10-50 kg rice substrates for it could be steadily mixed with steamed rice while initial moisture content of rice in koji chamber of 10 and 50 kg were 43.65% and 42.97%, respectively. Incubation in koji chamber was carried out with the lid closed for 2 days incubation. The ability of *Monascus* sp. KB9 to create volatile compounds such as alcohol or esters was activated in koji chamber (10 kg and 50 kg) by controlling the high initial temperature at 35°C. During fermentation, the rice grains were flipped daily to release the heat generated from metabolism so fermented rice might need an adequate amount of water to compensate for the moisture lost during incubation. The fungus mycelium grew and covered the surface of the grains while the moisture content of rice was decreased. After 2 day incubation, distilled water was added in fermented rice (2 times/day) in order to increase more moisture content to be within 50% level and then mixed throughly everyday. Strain KB9 could grow and produce red pigment within 5 day and the color gradually changed to dark red. *Monascus* sp. produced volatile compounds such as alcohol, esters, or volatile fatty acids to inhibit other fungi under increased temperature (not exceed 45°C) in 5 days but all was disappeared after 7 days of incubation. Glucose in koji scale was highly produced more than that in flask scale but readily consumed by the fungus. At the 9 days, the fungal biomass had covered the entire rice surface and penetrated into the rice. Fermented rice then became soft while moisture content increased. At for glucoamylase in the capacities of 100 g, 10 and 50 kg rice were produced at 9,525.50, 83,979.07 and 296,625.19 U/gdw while glucose residues were 225.81, 354.08 and 365.45 mg/gdw. The glucoamylase activity in 50 kg of fermented rice was higher than 10 kg of fermented rice but the glucose residue was quite similar in amount. The catabolite repression was not occurred due to the higher glucoamylase should accumulate more glucose but such glucose content was subsequent used as carbon source for growth and was transformed in glycolysis to acetyl CoA and enter the TCA cycle and polyketide pathway for pigmentation and monacolin K production [21]. *Monascus* growth as glucosamine content was still gradually increased until the end of fermentation (15 days) while continuously accumulated pigments and monacolin K content. The optimum temperature for maximum secondary metabolites production is usually lower than that required for growth [22]. The temperature in koji was firstly adjusted at 35°C for growth within 3 days incubation and then the temperature was controlled to 30°C in order to release heat and promote pigment and monacolins production. Temperature-shift cultivation has been applied in koji scale [16]. The monacolin K content was increased by

shifting the temperature from 30 to 23°C at 4 days incubation. The monacolin K content was nearly 20 times higher than having the constant temperature of cultivation at 30°C.

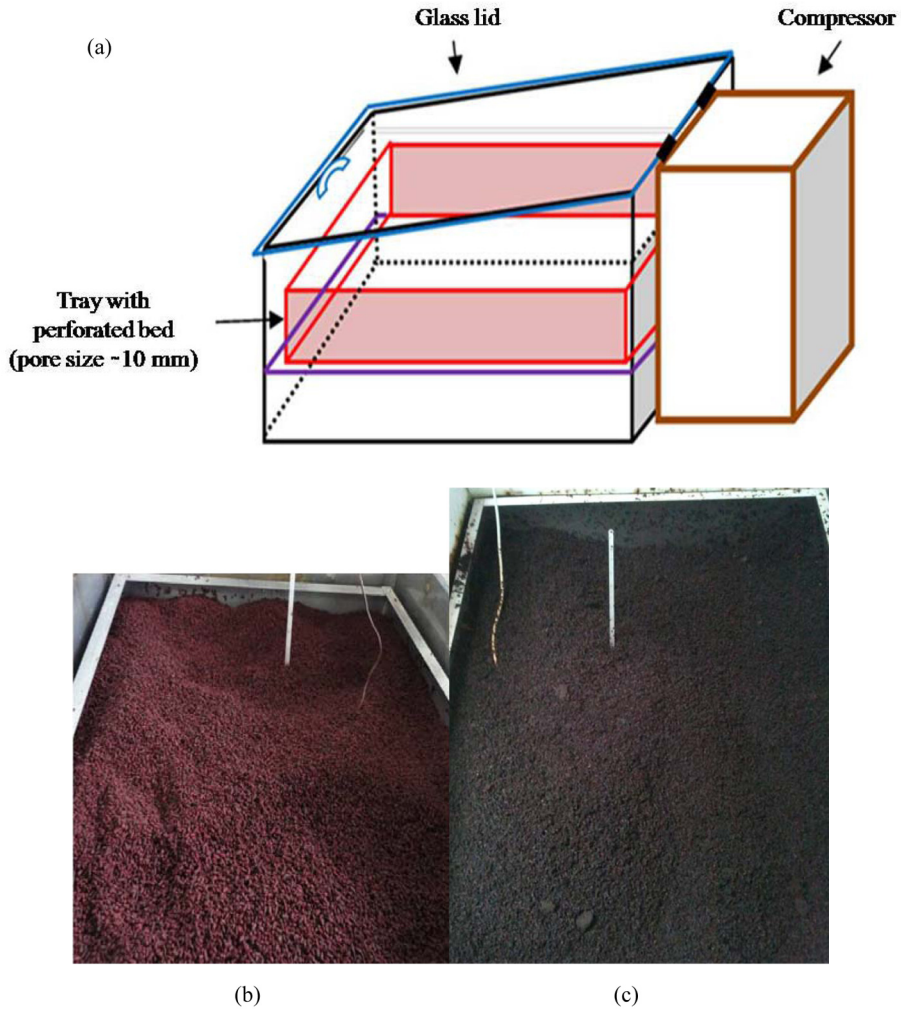

Figure 5. Fermented rice by *Monascus* sp. KB9 in koji chamber, (a) diagram of koji chamber, (b) 10 kg rice, (c) 50 kg rice for 15 days incubation.

Finally, glucoamylase activity of fermented red rice in flask and koji scales at 15 days incubation containing 100 g, 10, 50 kg were 8,069.27, 104,850.72 and 251,395.65 U/gdw while the glucosamine were 2.935, 8.244 and 20.049 mg/gdw, respectively. The pigment production achieved best results using the fermentation of 10 kg and 50 kg koji chambers and produced 2 times greater pigments than those in flask. The pigments in 100 g rice flask scale were 1,820.48 A_{420} U/gdw and 1,898.78 A_{500}U/gdw while the pigments in 10 kg rice koji scale 10 were 3,538.42 A_{420} U/gdw and 3,613.70 A_{500}U/gdw and 50 kg rice koji scale were 3,942.86 A_{420}U/gdw and 4,206.48 A_{500}U/gdw, respectively. Monacolin K in the similar 100 g rice flask and koji

scales 10 and 50 kg rice were produced at 7,183.75, 14,580.45 and 27,871.66 mg/kgdw respectively (Figure 6). Remarkably, the scaling up of 10 and 50 kg rice in koji chambers succeeded in giving 2 and 4 times higher monacolin K than that in flask scale. Many researchers reported monacolin K production of 0.53 mg/g by *M. perpureus* NTU601 [23] 2.52 mg/g by *M. pilosus* [9] and 2.584 mg/g by *M. purpureus* NTU301 [24]. Strain of *Monascus* is an important factor for specification which could produce different amount of pigment and monacolin K. We selected *M. kaoliang* KB9 because it could rapidly grow and produce high secondary metabolites. The aeration system influenced on pigments formation while the amount of pigment was directly proportional to the biomass produced [25]. Chiu *et al*. [26] reported temperature at 37-38°C was effective for reproduction and growth but the final stage of fermentation has to be kept at 34°C while we kept fermented rice at 30°C. After downstreaming process, only half biomass has been obtained. The fermented rice was changed to dark red pigment while monacolin K content was lower so we must choose conditions for fungal inactivation, temperature of drying process and preservative method in order to keep most metabolites in fermented rice.

3.5 Stability of Pigments and Monacolin K in Red Fermented Rice

The downsteaming process was examined for storage stability of fermented rice. The resistance of pigments and monacolin K content were found optimum using steaming at 100°C for 30 min for fungal growth and metabolism inactivation. Pigments and monacolin K of fermented

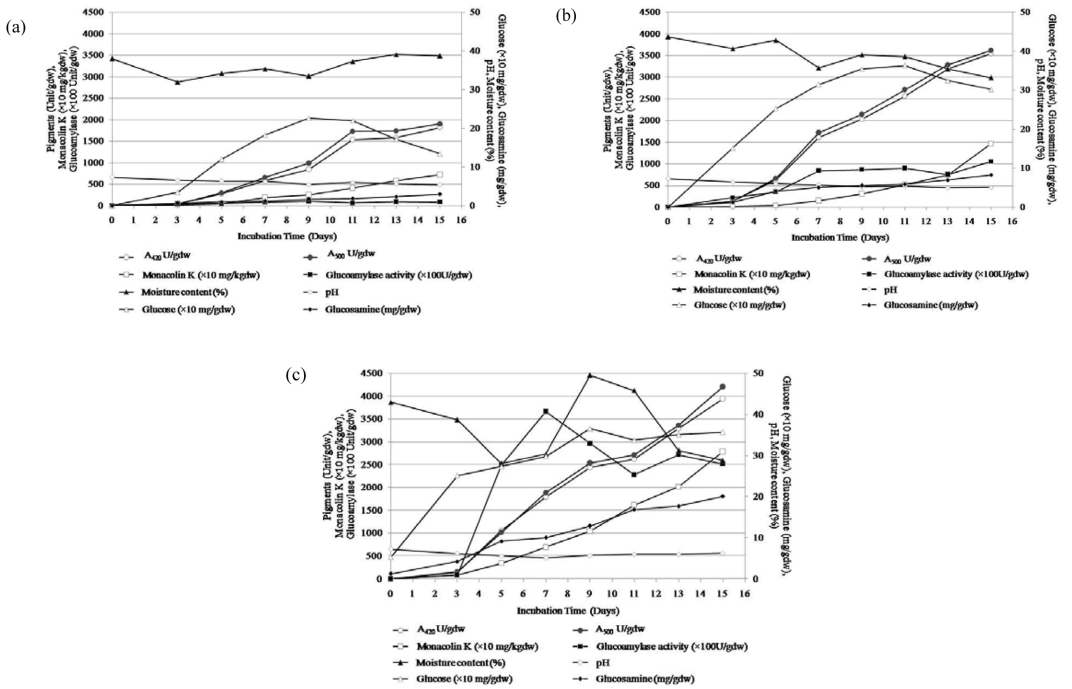

Figure 6. Fermentation time course of growth, glucoamylase, pigments and monacolin K production by *Monascus* sp. KB9 on fermented rice (a) 100 g in flask; (b) 10 kg rice in koji chamber; (c) 50 kg rice in koji chamber.

rice was remained at 93%, 93% and 90% while the sterilization (121 C for 15 min) removed most pigments and monacolin K. Moreover, the pigments and monacolin K in fermented rice was further destroyed by dry heating (50°C overnight) and remained at 55%, 55% and 75%. The fermented rice thus became dark red rice and giving typical odor red rice after drying. Fermented rice was stable in the refrigerator (2-4°C) at least for 1.5 month while the pigments and monacolin K were still remained at 37%, 38% and 41% (Figure 7). Li *et al*. [27] found that the monacolins decreased significantly under the conditions of high humidity at high temperature (75% RH, 60°C) and sunlight.

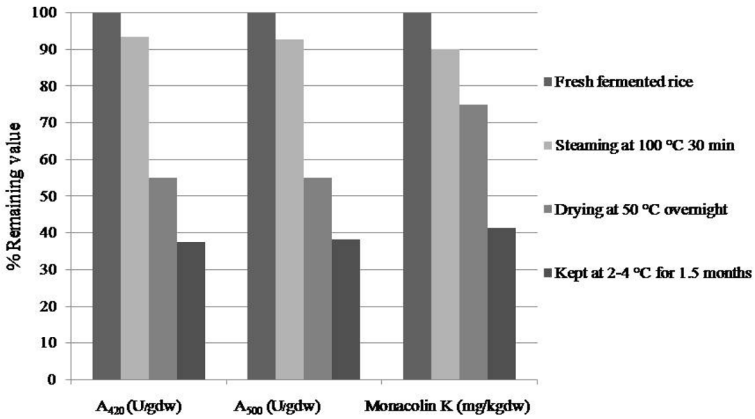

Figure 7. Percentage of remaining value of pigments and monacolin K in downstream processing.

4. CONCLUSIONS

The results of solid state fermentation of *Monascus* sp. KB9 in flask and koji chamber showed that both temperature and initial moisture content significantly affected red pigment and monacolin K production. The initial moisture content was the most influencing factor for growth and activity of enzyme of rice fermentation of this strain as well as for pigments and monacolin K production. In flask, the initial moisture content of rice at 38% (w/w) and incubated at 30°C was best for both red pigment and monacolin K production while at 35°C was good for growth and glucoamylase formation. Initial moisture content (41-43% w/w) reduced pigment as well as monacolin K symthesis because it facilitated *Monascus* sp. KB9 growth and glucoamylase activity so fast. High glucose liberation under high moisture content (70% w/w) accumulation could give the rice substrate agglomeration which leads to decreasing oxygen transfer and minimizes heat exchange, resulted in both secondary metabolites reduction. Scaling up to 10 and 50 kg rice culture in koji chamber could be time and area saving for fermentation. In koji scale, the red pigments and monacolin K content were higher than flask level. Water spraying was important for maintaining moisture loss due to fermentation heat generated from growth and glucoamylase activity. In addition, temperature must be controlled in the range of 30-35°C for pigment and monacolin K production. Glucose was controlled by metabolism of fungi which affected secondary metabolite production. It is not necessary to use aseptic technique

in koji chambers scale because *Monascus* sp. KB9 could produce alcohol at the early period of fermentation which inhibited other microorganisms. Fermented red rice with good odor, deep red pigment and higher monacolin K content in koji chambers had been obtained in a shorter time. Downstreaming of fermented *Monascus* rice was carried out by steaming at 100°C for 30 min to inactivate fungal biomass, and then drying at 50°C in hot air oven and kept in closed package in 2-4°C refrigerator. Further study was suggested to develop more automatic control system for facilating the higher quality consistency of red rice product to meet the needs of the functional food market.

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